# Wild Birds as a Reservoir of Antimicrobial Resistance (AMR) in the Environment

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# Declaration

I hereby declare that the work presented in this thesis has not been submitted for any other degree or professional qualification, and that it is the result of my own independent work under the guidance and advice of my supervisors.

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# Abstract

Antimicrobial resistance (AMR) is one of the greatest public health threats and has been conceptualised as a slowly emerging disaster. Studies have suggested that wild birds contribute as a reservoir and dispersal route of AMR, and proximity to anthropogenic activity has been associated with higher prevalence of AMR. This study was conducted to determine the prevalence and diversity of extended-spectrum β-lactam (ESBL) resistant coliforms in wild bird populations in Scotland across a gradient of anthropogenic activity.

Two bird taxa with functional differences were selected: gulls and geese and sampled across areas of different anthropogenic activity: urban and rural. A total of 226 bird faecal samples (47 gull faeces from a waste water treatment works (WWTW), 50 gull faeces from a rural site, 77 goose faeces from city lochs and 52 goose faeces from a rural site) were screened using a selective media. To determine diversity of isolates, up to 10 resistant and sensitive colonies from each sample were selected and tested using REP-PCR. One isolate from each REP type was selected for further characterisation including *E. coli* strain diversity, phylo-groups, sequence type (ST), susceptibility testing and resistance genes.

This study found a significantly higher prevalence of ESBL-producer *E. coli* (57% urban vs 2% rural) and ESBL-producer non-*E. coli* coliforms (32% urban vs 4% rural) in gulls at the urban site compared to the rural site. The difference in the prevalence of ESBL-producer non-*E. coli* coliforms in geese in the urban (5%) and rural (17%) sites was not statistically significant. Of 33 *E. coli* REP types identified, 88% were distinctly carried by birds. Six phylo-groups and one cryptic clade were detected, and the phylo-group B1 was the most prevalent. Multi-drug resistant (resistance ≥ 3 antibiotic classes) *E. coli* were only found in gulls at the WWTW (urban site). The most common ESBL-producer gene in this study was $bla_{CTX\text{-}M}$ group 1. This study suggests that gulls feeding on a WWTW site have a major role as a reservoir of AMR, whereas geese in urban lochs and migratory geese have a minor role.

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# Table of Contents

Declaration ..... i
Abstract ..... ii
Acknowledgements ..... iii
Table of Contents ..... iv
List of Tables ..... vi
List of Figures ..... viii
Abbreviations ..... ix
1. Introduction ..... 2
1.1 Background of Study ..... 2
1.2 Extended-Spectrum β-Lactamases (ESBLs)-Producer *E. coli* ..... 4
1.3 Studies of ESBL-Producer E. coli in Wild Bird Populations ..... 6
1.4 Knowledge Gaps Addressed in The Present Study ..... 8
2. Aim and Objectives ..... 9
2.1 Aim ..... 9
2.2 Objectives ..... 9
3. Methods ..... 10
3.1 Study Populations ..... 10
3.2 Faecal Sampling ..... 11
3.3 Enumeration of Total and ESBL-Producer Coliforms in Faeces ..... 14
3.4 Enrichment of ESBL-Producer Coliforms ..... 15
3.5 Prevalence, Number and Proportion of ESBL-Producer Coliforms ..... 16
3.6 Selection of Isolates for Further Characterisation ..... 17
3.7 Phenotypic Identification of Coliforms ..... 17
3.8 Antibiotic Susceptibility Testing (AST) ..... 18
3.9 PCR Assays ..... 21
3.10 16S rRNA Sequencing ..... 32
3.11 Pulsed-Field Gel Electrophoresis (PFGE) ..... 33
4. Results ..... 35
4.1 Bird Faecal Sample Collections ..... 35
4.2 Enumeration of Presumptive Total and ESBL-Producer Coliforms in Birds ..... 36
4.3 Selection of Isolates for Further Characterisation ..... 40
4.4 Comparison of Rep-PCR Primers ..... 42
4.5 Selection of The Representative Isolates using REP-PCR Typing ..... 44
4.6 Species Identification of Presumptive *E. coli* ..... 52

4.7 Species Identification of Presumptive ESBL-Producer Non-*E. coli* Coliforms....61
4.8 Strain Diversity of *E. coli*....64
4.9 Clonal Analysis of *E. coli* Strains/Types....67
4.10 Characterisation of Resistance Profile....69
5. Discussion....74
References....92
Appendix 1. Bird Faecal Sample Collections....116
Appendix 2. Enumeration of Presumptive ESBL-Producer Coliforms in Birds....121
Appendix 3. Selection of Isolates for Further Characterisation....148
Appendix 4. Comparison of REP and $(GTG)_5$ Primers for the Rep-PCR Assay....155
Appendix 5. DNA Fingerprint Pattern of the 175 Isolates from Bird Samples by REP-PCR assay....159
Appendix 6. Species Identification of Presumptive ESBL-Producer Non-*E. coli* Coliforms....177
Appendix 7. 16S rRNA Chromatography....178
Appendix 8. Strain Diversity of *E. coli*....286
Appendix 9. Clonal Analysis of *E. coli* Strains/Types....293
Appendix 10. Antibiotic Susceptibility Testing of ESBL-Producer Isolates and ESBL Sensitive *E. coli*....297

# List of Tables

Table 1. Details of study design showing sites and species sampled……. 10
Table 2. Clinical breakpoints table for interpretation of zone diameters in antibiotic susceptibility testing…….…….. 20
Table 3. Primer sequences used in the duplex (*lacZ3* and *yaiO*) PCR assay……. 22
Table 4. Primer sequences used in the *gadA* PCR assay…… 22
Table 5. Primer sequences used in the *uidA* PCR assay……. 23
Table 6. Primer sequences used in the quadruplex phylo-group assay…….. 24
Table 7. Interpretation of the quadruplex phylo-group PCR assay…… 25
Table 8. Primer sequences used in the phylo-group C-specific PCR assay……... 25
Table 9. Primer sequences used in the phylo-group E-specific PCR assay……... 26
Table 10. Primer sequences used in the cryptic clade PCR assay…….. 27
Table 11. Primer sequences used in the PCR-based MLST assay……. 27
Table 12. Primer sequences used in the CTX-M group PCR assay……... 28
Table 13. Primer sequences used in the SHV, TEM and OXA multiplex PCR assay... 29
Table 14. Primer sequences used in the rep-PCR assay……. 30
Table 15. Universal bacterial primers used in the 16S rRNA sequencing……. 32
Table 16. Number of faecal samples collected in each urban and rural site……. 35
Table 17. Prevalence of total and ESBL-producer coliforms in gulls within each urban and rural site……..... 37
Table 18. Prevalence of total and ESBL-producer coliforms in geese within each urban and rural site……..... 38
Table 19. The median (CFU/g) of total *E. coli* and ESBL-producer coliforms in gulls…… 39
Table 20. The median (CFU/g) of total *E. coli* and ESBL-producer coliforms in geese……..... 40
Table 21. Details of presumptive ESBL-producer coliforms and ESBL sensitive *E. coli* from gulls and geese selected for further characterisation…… 41
Table 22. Representative isolates of ESBL-producer *E. coli* selected for further characterisation……... 50
Table 23. Representative isolates of ESBL sensitive *E. coli* selected for further characterisation……... 51

Table 24. Representative isolates of ESBL-producer non-*E. coli* coliforms selected for further characterisation……... 52
Table 25. Confirmation of species identification of presumptive *E. coli*……... 59
Table 26. 16S rRNA sequencing of "non-*E. coli*" blue colonies on Coliform *ChromoSelect* agar……...…….. 61
Table 27. Confirmation of species identification of presumptive ESBL-producer non-*E. coli* coliforms…….. 63
Table 28. 16S rRNA sequencing of ESBL-producer "non-*E. coli*" red colonies on Coliform *ChromoSelect* agar……..…… 64
Table 29. Strain-richness of confirmed *E. coli* within the birds…… 66
Table 30. Phylo-grouping results of *E. coli* from gull and goose faeces…… 68
Table 31. Resistance antibiogram of ESBL-producer isolates and ESBL sensitive *E. coli* isolated from gulls and geese……...…….. 71
Table 30. ESBL-producer genes harboured in ESBL-producer coliforms isolates from gulls…… 73

# List of Figures

Figure 1. Maps of the study sites…… 11
Figure 2. An example of the band intensity from the normalised gel image……. 31
Figure 3. A comparison of small colony variants (SCVs) presumptive *E. coli* on Eosin Methylene Blue agar……...42
Figure 4. Comparison of rep-PCR with $(GTG)_5$ primer (lane 2 – 4) and REP primers (lane 6 – 8)…….. 44
Figure 5. The dendrogram of REP-PCR assay of presumptive ESBL-producer *E. coli* generated by BioNumerics……. 46
Figure 6. The dendrogram of REP-PCR assay of presumptive ESBL sensitive *E. coli* generated by BioNumerics……. 48
Figure 7. The dendrogram of REP-PCR assay of presumptive ESBL-producer non-*E. coli* coliforms generated by BioNumerics…….. 49
Figure 8. The *lacZ3* and *yaiO* PCR assay of presumptive ESBL-producer *E. coli*….... 54
Figure 9. The *lacZ3* and *yaiO* PCR assay of presumptive ESBL sensitive *E. coli*…… 54
Figure 10. The *gadA* PCR assay of presumptive ESBL-producer *E. coli*…..……... 55
Figure 11. The *gadA* PCR assay of presumptive ESBL sensitive *E. coli*…….. 56
Figure 12. The *uidA* PCR assay of presumptive ESBL-producer *E. coli*……..…… 57
Figure 13. The *uidA* PCR assay of presumptive ESBL sensitive *E. coli*……... 57
Figure 14. The *lacZ3* and *yaiO* PCR assay on presumptive non-*E. coli* coliforms…… 62
Figure 15. The dendrogram of confirmed *E. coli* isolates (resistant and sensitive) in gulls and geese……..…….. 65
Figure 16. PFGE analysis of ESBL-producer *E. coli*……..…….. 67
Figure 17. E. coli phylo-groups in gulls vs geese and resistant vs sensitive…….. 69
Figure 18. Antibiotic susceptibility testing of ESBL-producer coliforms and ESBL sensitive E. coli from gulls and geese…… 70
Figure 19a. The PCR detection of CTX-M group genes on ESBL-producer isolates… 72
Figure 19b. The multiplex PCR detection of SHV, TEM and OXA genes on ESBL-producer isolates..……... 72

# Abbreviations

1. AMR = Antimicrobial Resistance
2. ARB = Antibiotic Resistant Bacteria
3. ARG = Antibiotic Resistance Genes
4. CFU/g = Colony-forming units per gram
5. ESBL = Extended Spectrum β-Lactam
6. ESBLs = Extended Spectrum β-Lactamase
7. GD = Goose Duddingston Loch
8. G. Dun. = Goose Dunsapie Loch
9. GL = Goose Lochend Loch
10. GS = Goose St Margaret's Loch
11. LSA = Laridae (Gull) St Abbs
12. LSW = Laridae (Gull) Seafield WWTW
13. MDR = Multi-drug resistant
14. MLST = Multi-locus Sequence Typing
15. PFGE = Pulsed-field Gel Electrophoresis
16. Rep = Repetitive Element Palindromic
17. REP-PCR = REP primers used in the rep-PCR assay
18. REPNE = REP type of ESBL-producer non-*E. coli* coliforms
19. REPR = REP type of ESBL-producer *E. coli*
20. REPS = REP type of ESBL sensitive *E. coli*
21. SCV = Small Colony Variant
22. SPA = Special Protection Area
23. SSSI = Site of Special Scientific Interest
24. ST = Sequence Type
25. TMTC = Too many to count
26. UPGMA = Unweighted Pair Group Method with Arithmetic mean
27. WWTW = Waste Water Treatment Works

# 1. Introduction

## 1.1 Background of Study

In recent years, antimicrobial resistance (AMR) has become one of the greatest public health threats in the world (Leonard *et al.*, 2018). Public Health England (PHE, 2016) estimated more than 25,000 people annually die in England due to multidrug-resistant bacteria. In the UK's five-year national action plan published in 2019, AMR is stated to cause 700,000 deaths each year globally (HM Government, 2019). By 2050, AMR is predicted to be the main cause of deaths globally, with 10 million deaths annually (de Kraker, *et al.,* 2016; Leonard *et al.*, 2018). World Health Organization Global Antimicrobial Surveillance System (GLASS) data stated a high level of resistant *Escherichia coli* (*E. coli*) and resistant *Klebsiella pneumoniae* (*K. pneumoniae*) are among the most commonly reported resistant bacteria globally (Mayor, 2018). These antibiotic-resistant bacteria (ARB) are a major concern as they are harder to treat and can spread to a healthy human through other humans, livestock animals and the natural environment (Huijbers *et al.*, 2015; Leonard *et al.*, 2015). The latter reservoir of AMR is the focus of this study.

Studies suggested that human and animal sectors contribute in the development of resistance. The overuse and inappropriate prescription of antibiotics in humans as well as the extensive use of antibiotics in agricultural sectors, such as livestock, play an important role in the development and prevalence of antibiotic resistance (Ventola, 2015). An important recent development is the linking of AMR with the natural environment. Studies have implicated that antibiotic residues from both sources were introduced into the environment through urine and faeces of livestock, as well as human waste in Waste Water Treatment Works (WWTW) (Martinez, 2009; Ventola, 2015). This is a major concern as these residues may affect the environmental microbiome, resulting in both the emergence of novel resistance and the enhancement of resistance in bacteria (Larsson *et al.*¸2018; Ventola, 2015).

The natural environment has recently been the focus of several studies to understand its important role as a reservoir and route of dispersal of ARB and antibiotic resistance genes (ARG) (Larsson *et al.*, 2018). Huijbers *et al.* (2015) reviewed 241 publications from 1994 to 2014 regarding AMR in the environment. They divided the environment into three different main areas: contamination sources (waste water and manure), human exposure-relevant sites (beach sand, recreational water, drinking water, ambient air,

shellfish, and irrigation water) and environmental compartments (soil, water, air/dust, and wildlife). ARB can be introduced to humans and animals through these exposure-relevant sites. Bengtsson-Palme *et al.* (2018) also suggested an important role of the natural environment in the dispersal routes and reservoirs of ARB: maintaining resistant pathogens and recruitment of the novel resistance genes to human. ARBs were detected in 100% of contamination sources, 92% of human exposure-relevant sites and 89% of environmental (Huijbers *et al.*, 2015). Within the 89% of environmental compartments, ARBs, including extended-spectrum β-lactamase (ESBLs)-producing *Enterobacteriaceae*, methicillin-resistant *S. aureus* (MRSA) and vancomycin-resistant *Enterococcus* spp. (VRE), were detected in the studies of wildlife (85%) (Huijbers *et al.*, 2015). The number of AMR studies in wildlife was lower compared to other environmental compartments (>90%), and more research is needed to obtain more understanding. Wild birds as potential reservoirs and spreaders of ARB are the focus of this study.

Several external factors, including sources of contamination, anthropogenic factors, human density/population and remoteness of an area, have been suggested to impact the level of resistance in the natural environment (Atterby *et al.*, 2016; Bonnedahl & Järhut, 2014; Ramey *et al.*, 2018; Vredenburg *et al.*, 2014). Among these factors, AMR has been strongly linked with anthropogenic activity. Anthropogenic activities in various environmental compartments, such as animal feeding operations and WWTW, have been suggested as pathways of AMR dissemination (Pruden *et al.*, 2012). To obtain more understanding of how anthropogenic activities could impact the level of resistance in the environment, a One Health approach must be taken into consideration. This approach was introduced as a global effort to minimise the emergence and spread of AMR by implementing multisectoral approach through human, animal and environmental sectors (Larsson *et al.*, 2018; Lebov *et al.*, 2017). The idea behind the One Health approach is the understanding that AMR does not recognise any borders; many infections in animals and humans are caused by the same microbes (WHO, 2017). ARB and ARG also have the ability to move in any direction, between all the sectors (Larsson *et al.*, 2018; Lebov *et al.*, 2017). This means that human, animals and the environment are interconnected (healthy human = healthy animals = healthy environment). In terms of the dispersal of resistance, it is expected that antibiotic resistance can be effectively combatted and prevented, and better public health outcomes can be achieved in the future by implementing this approach in research studies (WHO, 2017). Understanding the impact

of anthropogenic activity to the prevalence of ARB in wild birds is one of the main objectives of this study.

### 1.2 Extended-Spectrum β-Lactamases (ESBLs)-Producer *E. coli*

Based on data from NHS England Hospital Trusts in 2014, 107,000 cases of bacteraemia were reported across laboratories in England, Wales and Northern Ireland, and estimated 57.5% were caused by Gram-negative bacteria (PHE, 2016). Resistant *E. coli*, belonging to family *Enterobacteriaceae*, have been reported to be the cause of 15,183 cases of bacteraemia, which were associated with 2,712 excess deaths and 120,065 excess hospital days in Europe (PHE, 2016). In Scotland, 4,802 cases of bacteraemia in 2016 were reported to be caused by *E. coli*, and they were consistently found to be resistant towards ciprofloxacin (18.8%), gentamicin (10.4%), piperacillin/tazobactam (11.9%) and even carbapenems (HPS, 2017).

Phylogenetic analyses using multi-locus sequence typing (MLST) have been used to understand the diversity of *E. coli* clones, and were previously used to establish a phylogenetic tree of *E. coli* (Clermont *et al.*, 2013). A phylogenetic tree is a very useful tool to visualise the evolutionary relationships between species/strains based on their genetics by involving the comparison of homologous sequences (Choudhuri, 2014). Thus, the evolutionary relatedness between *E. coli* strains can be understood (Sangal *et al.*, 2014). Clermont *et al*. (2013) developed a simplified characterisation of *E. coli* phylo-groups by using PCR assay, targeting three genes (*arpA*, *chuA* and *yjaA*) and DNA fragment TspE4.C2. There are seven recognised phylo-groups *E. coli sensu stricto* (A, B1, B2, C, D, E, F) and one corresponding to *Escherichia* clades (I to V) (Clermont *et al.*, 2013). MLST has also been used to divide *E. coli* into sequence types (ST), including ST131, ST69, ST95 and ST73 which are clinically associated with causing urinary tract infections and bloodstream infections (Riley, 2014). Other than MLST, different typing methods can be used to distinguish between *E. coli* strains, such as PFGE used by CDC PulseNet (2017) and PCR assays – a more rapid, low cost and effective method.

*E. coli* can be distinguished into three families: commensal *E. coli*, intestinal pathogenic *E. coli* (IPEC) and extraintestinal pathogenic *E. coli* (ExPEC) (Poolman, 2017). However, the distinction between IPEC and ExPEC are not clear and there is considerably overlap as the substantial genetic diversity within the pathovars is complex (Poolman, 2017). Duriez *et al.* (2001) in their study found phylo-group A and B1 were

the most common phylo-groups in human commensal *E. coli* strains. In clinical settings, pathogenic *E. coli* phylo-group A, B1 and E associated with IPEC, while pathogenic *E. coli* phylo-group B2 and D associated with ExPEC. *E. coli* phylo-group C was associated with the phylo-group B1, while *E. coli* phylo-group F was associated with phylo-group D and B2 (Donnenberg, 2013). In animals, phylo-group B1 has been suggested as the most frequently isolated commensal *E. coli* (Higgins *et al.*, 2007; Ishii *et al.*, 2007; Carlos *et al.*¸2010).

*Escherichia coli* have developed extreme genetic mechanisms to survive antibiotics (Vranic & Uzunovic, 2016). This can be done, for instance, by producing β-lactamases (due to spontaneous mutation or plasmid/DNA transfer), up-regulated impermeability and target modification (Rawat & Nair, 2010). Extended-spectrum β-lactamase (ESBLs)-producing *E. coli* is a particular concern (Leonard *et al.*, 2015). Based on the PHE latest annual data in Gram-negative bacteraemia, it is suggested that around 4,900 bacteraemia cases annually in England are caused by ESBL-producer *E. coli*, and more across the UK (Day *et al.*, 2019). ESBL-*E. coli* were also detected in 11% of human faecal samples from five regions of the UK (East Anglia, London, Northwest, Scotland and Wales) (Day *et al.*, 2019). ESBLs cause the major resistance towards β-lactam drugs, particularly third-generation cephalosporins - the most commonly prescribed drug classes in the treatment of Gram-negative infections (Hawkey & Jones, 2009). ESBL genes are typically acquired by horizontal gene transfer, with plasmids as the major vector (Hawkey & Jones, 2009). Based on the amino acid sequence, β-lactamases can be classified into four different molecular groups: A, B, C and D (Ambler, 1980). Bush & Jacoby (2010) further divided β-lactamases into different groups based on the structural and functional classification. ESBL CTX-M, TEM and SHV types were classified into class 2be and ESBL OXA type was classified into class 2de. The class 2be was defined to hydrolyse oxyimino-β-lactams, including cefotaxime, ceftazidime and aztreonam. The class 2de was defined to hydrolyse cloxacillin or oxacillin and oxyimino- β-lactams (Bush & Jacoby, 2010).

Each ESBL enzyme has many variants, including 223 TEM type variants, 193 SHV type variants, 498 OXA type variants and 172 CTX-M type variants (Rahman *et al.*, 2018). Of the 172 CTX-M type variants, they have been group into five different groups of CTX-M type: group 1, 2, 8, 9 and 25 based on the amino acid sequence similarities (Bonnet, 2004). Notably, ESBL CTX-M type is known to have spread widely among resistant *E. coli* in recent years, and has become the most prevalent ESBL enzyme in Europe, the UK and worldwide (Amos *et al.*, 2014; Bevan *et al.*, 2017; Woodford *et al.*,

2005). It is further stated that CTX-M Group 1 (CTX-M-15) and CTX-M Group 9 (CTX-M-14) are the most prevalent genotypes worldwide, including Scotland (Bevan *et al.*, 2017; Cantón *et al.*, 2012; Hawkey & Jones, 2009). ESBL-producing *E. coli* CTX-M-15 type (CTX-M Group 1) was also described to be associated with pathogenic extraintestinal pathogenic *E. coli* (ExPEC) Sequence Type (ST) 131, which ST 131 has increased extensively in the prevalence of resistant *E. coli* (Amos *et al.*, 2014; Banerjee & Johnson, 2014).

### 1.3 Studies of ESBL-Producer E. coli in Wild Bird Populations

Leclerc *et al.* (2001) and Curutiu *et al.* (2019) in their review of microbial water safety and microbiological pollutants suggested the suitability of coliform group as a marker of faecal contamination in the environment. Coliform group is defined as a group of β-galactosidase-positive *Enterobacteriaceae*, including *E. coli*, *K. pneumoniae*, *Enterobacter cloacae* and *Citrobacter freundii* which originated from human and animal intestine/faecal matters (Leclerc *et al.*, 2001). *E. coli* constitutes about 1% of the total bacterial biomass inhabiting the large intestine of humans and warm-blooded mammals (Leclerc *et al*., 2001). The presence of *E. coli* in the environment has exclusively and uniquely indicated faecal pollution by several studies, which to a certain extent, also indicates the presence of other pathogens (Lecler *et al*., 2001; Ortega *et al.*, 2009; Price & Wildeboer, 2017).

Other than water samples, wild birds have been suggested as a useful environmental indicator for ecosystem health and their habitat quality, environmental pollution and biodiversity (Hill, 2015; Radhouani *et al.*¸2012). Studies have implied that wild birds act as a source, reservoir and a spreader of ARB through the ability of migration over long distances, in which they can transmit the ARB into other parts of the world and introduce ARB to remote natural ecosystems across their migratory range (Agnew *et al*., 2016; Bonnedahl & Järhult, 2014; McFadzean, 2015).

Studies of AMR have been conducted in different species of wild birds and have used particularly *E. coli* as indicator bacteria (Alcalá *et al.*, 2015; Dotto *et al.*¸2016, Bonnedahl *et al.*, 2009; 2010). To date, different resistance genes have been found in wild birds, including beta-lactamase (*bla*) genes (CTX-M, SHV, TEM, AmpC, CMY, IMP) and vancomycin resistance genes (*vanA, vanC-1*) (Alcalá *et al.*, 2015; Dolejska *et al*., 2016; 2018; Jamborova *et al.*, 2017, 2018; Poirel *et al*., 2012; Roberts *et al.*, 2016; Silva *et al.*,

2011; Veldman *et al.*, 2013; Vergara *et al.*, 2017). The most recent finding of ARG in wild birds was colistin resistance gene (*mcr-1*) which carried by ring-billed gulls (*Larus delawarensis*) (Franklin *et al.*, 2020). Among these resistance genes, ESBL-producer *E. coli* CTX-M type is frequently carried by wild birds, and have been reported in several areas, including Europe, South America, North America, Africa and Asia (Bonnedahl & Järhult, 2014; McFadzean, 2015; Mohsin *et al.*, 2017; Wang *et al.*, 2017). In this project, ESBL-producer coliforms, in particular *E. coli*, are used as indicators of AMR in wild birds.

Vredenburg *et al.* (2014) suggested the role of gulls as a bioindicator of clinically important ARG in the environment. ESBL-producer *E. coli* have been isolated in different species of gulls (i.e. Franklin's gulls - *Leucophaeus pipixcan*, black-headed gulls - *Larus ridibundus*, wild kelp gulls - *Larus dominicanus*, yellow-legged gulls - *Larus michahellis*) from South America, Sweden and France (Báez *et al.*, 2015; Bonnedahl *et al.*, 2009; 2010; Liakopoulos *et al.*, 2016). Another potential bioindicator of ARB in the environment has also been demonstrated in geese. The role of geese as a reservoir and vector of ARB in the natural environment has been suggested by the isolation of resistant *Enterobacteriaceae* from Canada geese (*Branta canadensis*) and long-distance migratory East Canadian High Arctic (ECHA) light bellied Brent geese (*Branta bernicla hrota*) in Canada, Ireland and USA (Agnew *et al.*, 2016; Cole *et al.*, 2005; Middleton & Ambrose, 2005; Vogt *et al.*, 2018).

Studies have suggested that gulls living in proximity to anthropogenic environments, such as wastewaters, might represent important sources, reservoirs and vectors of antibiotic resistance due to their scavening habit and ability to travel over large distances (Nelson *et al.*, 2008; Vredenburg *et al.*, 2014). This relation between AMR in wild birds and anthropogenic activity has been suggested by Atterby *et al.* (2016), which showed more resistant *E. coli* isolated from an urban area (55% antibiotics resistance) compared to a remote location (8% resistance) in Southcentral Alaska. A low level of resistant *E. coli* (2% resistance) was also found among migratory birds inhabiting remote Alaska, suggesting that anthropogenic activity influenced the prevalence of ARB in the environment (Ramey *et al.*, 2018).

To the best of the investigator's knowledge, only two studies of *E. coli* in wild birds had been conducted in Scotland. A longitudinal study by Foster *et al.* (2006) successfully isolated a verocytotoxin-producing *E. coli* O157 from one (0.4%) out of 231 wild-bird samples in a garden feeding station in Dumfries and Galloway, southwest Scotland. The

source of sample was not specified; however, members of the Passeriformes order: blackbirds (*Turdus merula*), house sparrows (*Passer domesticus*), greenfinches (*Chloris chloris*) and chaffinches (*Fringilla coelebs*) were observed to visit the bird table where the positive sample was obtained (Foster *et al.*, 2006). The most recent study found ESBL-producing *E. coli* in 43% out of 30 dead gulls. This was an unpublished report of a preliminary study on AMR in Scotland released by Scotland's Rural College (McFadzean, 2015).

### 1.4 Knowledge Gaps Addressed in The Present Study

A leading panel of researchers on AMR in the environment has proposed four major research questions to be answered: (1) What is the relative contribution of different sources of ARB into the environment? (2) What is the role of the environment, particularly anthropogenic input, on the evolution of antibiotic resistance? (3) How significant is the exposure of humans to ARB via different environmental routes, and what is the impact on public health? (4) What are the interventions that effectively could mitigate the emergence and spread of antibiotic resistance via the environment? (JPIAMR, 2017; Larsson *et al.*, 2018). This study is conducted to answer the knowledge gap on the contribution of the environment to the AMR by specifically addressing the role of wild birds in the dispersal of AMR in the environment (The European Commission, 2017). To elucidate their role and how different sources of ARB contributes into the environment, prevalence of ESBL-producer coliforms in gulls and geese and the impact of anthropogenic input to their prevalence are investigated.

In 2019, five different objectives for surveillance of antibiotics and antibiotic resistance in the environment were defined (Huijbers *et al.*, 2019). One of these objectives: the risk of transmission of already antibiotic-resistant pathogens between humans, animals and the environment is addressed in this study by characterising the diversity and resistance profile of total and ESBL-producer coliforms isolated from gulls and geese (JPIAMR, 2017). Two markers for environmental surveillance which provide strong evidence for risks were chosen: (1) the absolute number and (2) the proportion of ARB within species (Huijbers *et al.*, 2019). The absolute number could provide a valuable information regarding the risk of pathogen transmission, while the proportion of bacteria could provide a valuable information regarding the selection pressure driving the resistance evolution (Huijbers *et al.*, 2019).

# 2. Aim and Objectives

## 2.1 Aim

To determine the prevalence and diversity of ESBL-producer coliforms in wild bird populations in Scotland across a gradient of anthropogenic activity.

## 2.2 Objectives

2.2.1 Compare the prevalence of ESBL-producer coliforms in gull and goose populations in a high and a low anthropogenic activity site.

2.2.2 Determine the diversity of *E. coli* isolates in gull and goose populations.

2.2.3 Determine the resistance profile of ESBL-producer coliforms in gull and goose populations.

# 3. Methods

## 3.1 Study Populations

The study was designed to obtain an understanding of how anthropogenic activity affects the prevalence and diversity of ESBL-producer coliforms in populations of gulls and geese (Table 1). The high anthropogenic activity areas are represented in the present study by urban sites, and the low anthropogenic activity areas represented by rural sites.

***Table 1. Details of study design showing sites and species sampled.***

| Areas | Bird taxa | | | |
|---|---|---|---|---|
| | Gulls (Laridae) | | Geese (Anatidae) | |
| Urban | Site: | Seafield waste water treatment works, City of Edinburgh | Site(s): | Urban lochs, City of Edinburgh |
| | Species: | Black-headed gulls (*Chroicicephalus ridibundus*) and herring gulls (*Larus argentatus*) | Species: | Canada geese (*Branta canadensis*) and greylag geese (*Anser anser*) |
| Rural | Site: | St Abbs Village, Scottish Borders | Site: | Agricultural fields, Slamannan Plateau, Falkirk District |
| | Species: | Herring gulls | Species: | Taiga bean geese (*Anser fabalis fabalis*) |

Four sites across the central belt of Scotland were chosen to represent urban and rural populations of two bird taxa (Fig. 1). Urban areas were defined as areas with a settlement of 3,000 or more people, and rural areas as areas with a population of fewer than 3,000 people (Scottish Government, 2018). Seafield Waste Water Treatment Works (WWTW) and four urban lochs in the City of Edinburgh were selected as the representatives of urban sites. St Abbs village in the Scottish Borders and Slamannan Plateau in the Falkirk district were selected as the representatives of rural sites. Further information about each site is given in Sections 3.2.1 and 3.2.2, below.

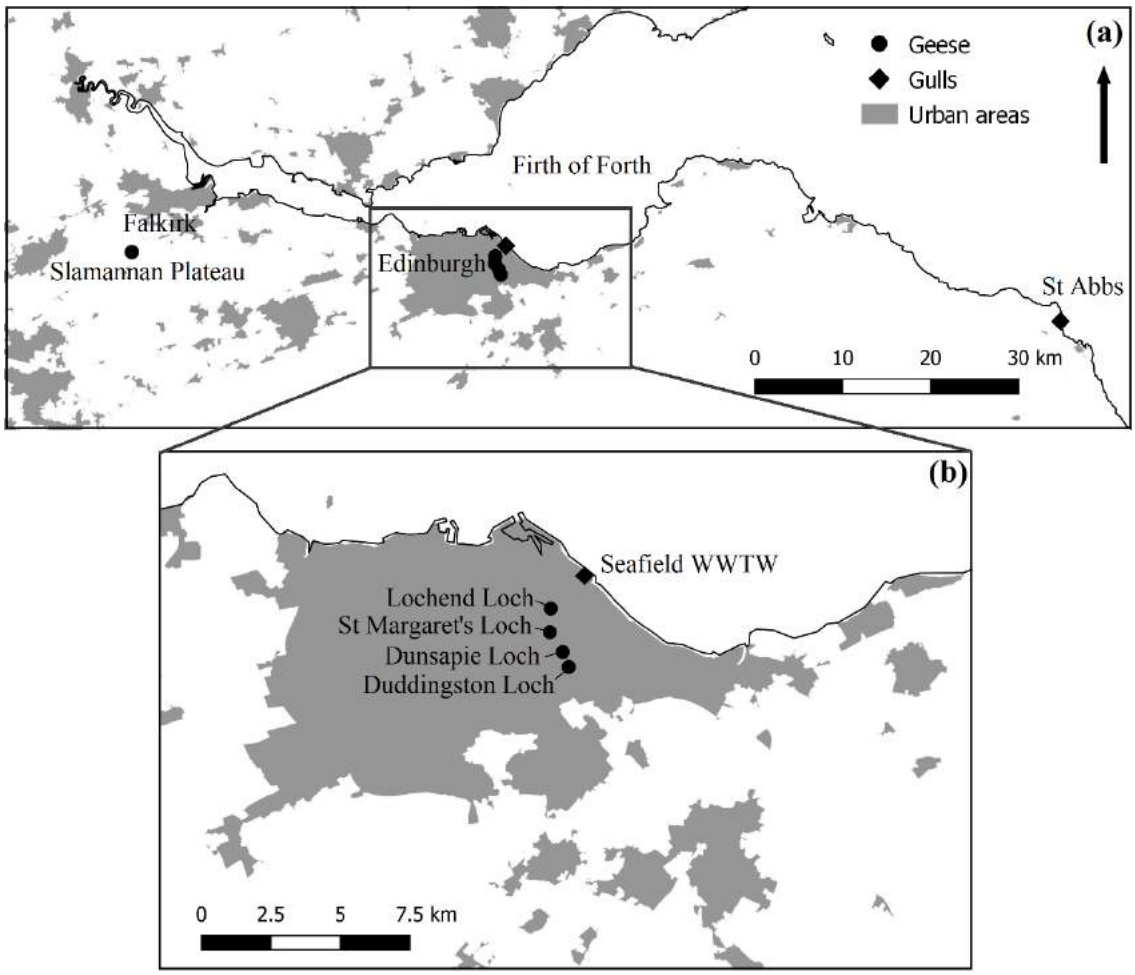


***Figure 1. Maps of the study sites.*** *(a) All the study sites in the present study. The data of urban areas (shown in grey) was provided by National Records of Scotland (NRS, 2018). (b) An enlarged map of the City of Edinburgh showing the location of urban lochs and Seafield WWTW. Maps were generated by QGIS 3.2 software. Urban areas are defined as per Scottish Government (2018).*

## 3.2 Faecal Sampling

Faecal samples were collected into 30 ml sterile plastic containers (SLS7500, Scientific Labs) using a rubber glove to avoid contamination from skin. Care was also taken during the sampling to avoid contamination from soil or water. Sampling was undertaken between January and April 2019. Due to differences in the accessibility of sampling on the sites, different sampling approaches at each site were implemented as described below.

### 3.2.1 Gull Faeces

**Urban Population**. Edinburgh is an urban area located on the Firth of Forth's southern shore. It is the capital city of Scotland with an estimated population of 518,500 in mid-2018 (NRS, 2019). Seafield WWTW, situated 4 km to the Northeast of Edinburgh city centre, is the largest waste water treatment works in Scotland (Figure 1b). It is owned by Scottish Water and run by Veolia Water (Scottish Water, 2019). Seafield WWTW processes about 300 million litres of urban wastewater every day (Scottish Water, 2019). The anthropogenic activity in the site includes the treatment of urban wastewater from residential and industrial areas, including food waste, sewage water, chemical solvents and agricultural wastes. Various anthropogenic activities (high anthropogenic activity) were observed in the surrounding areas of the waste plant, such as housing, offices, family recreation areas (recreation ground and beaches) as well as other industries. Multiple potential sources of contamination and wastewaters from these activities might affect the prevalence of ESBL-producer coliforms in gulls. During the sampling day on 4$^{th}$ February 2019, black-headed gulls and herring gulls were observed scavenging for food from the open primary settling tanks containing untreated sewage. Droppings that appeared relatively fresh were sampled around the primary settling tanks where large numbers of gulls were observed perching. Samples were collected and labelled as LSW (Laridae Seafield WWTW). Samples were stored at -20°C within two hours post-sampling, with lack of cold chain, and processed within six weeks.

**Rural Population**. St Abbs is a rural small fishing village with an estimated population of 1,081 in 2010 (Scottish Government, 2018; Scottish Borders Council, 2011). It is situated approximately 4 km northwest of the town of Eyemouth, Scottish Borders (Figure 1a). Adjacent to the village is St Abb's Head National Nature Reserve, an area designated as a Special Protection Area (SPA) and Site of Special Scientific Interest (SSSI) for its breeding seabird colonies, including herring gulls (Scottish Natural Heritage, 2009). Compared to Edinburgh, St Abbs generally has a lower anthropogenic activity, including housing, fishing and seasonal tourism. The site is popular for recreation activities (e.g. walking, bird watching). People are attracted to visit the site due to its landscape and wildlife, particularly between April and October. Regular sightseeing boat trips run from the St Abbs harbour during the summer holiday, depending on the weather and sea conditions. The anthropogenic activities in the site include walking, birdwatching, diving and sea angling. Local fishing boats were also observed to moor on the berths around the harbour. Wastewaters from this area is treated by a septic tank and

are pumped to a local WWTW at Eyemouth (SEPA, 2001). Short outflows containing raw sewage (located west of the harbour mouth) are visible though are small, serving individual households (SEPA, 2001). Faecal samples were collected on 15th April 2019. Herring gulls were observed and considered as the predominant gull species in the site. Fresh gull droppings were obtained by luring gulls with bread on the car parking area next to the shore. In addition, relatively fresh gull droppings (defecation was not observed) were obtained from a known breeding colony of herring gulls around steep cliffs behind St Abbs Marine Station, rocky cliffs behind St Abbs Visitor Centre and in the harbour (including the car park) area. Samples were collected and labelled as LSA (Laridae St Abbs). Samples were stored at -20°C within two hours post-sampling, with lack of cold chain, and processed within six weeks.

### 3.2.2 Goose Faeces

**Urban Population**. Four urban lochs in Edinburgh were selected based on their nature as a habitat for different species of wild birds. These include Duddingston Loch, St Margaret's Loch, Dunsapie Loch and Lochend Loch (Figure 1b). This area is regarded as high anthropogenic activity relative to Slamannan Plateau mainly due to the urban area and hence impacted by a relatively higher population. The urban lochs are surrounded by housing, schools, offices, family recreation and tourism activity. Canada geese were observed to be the predominant goose species in Duddingston Loch, Dunsapie Loch and Lochend Loch, whereas greylag geese were observed to be the predominant goose species in St Margaret's Loch. Both geese species were sampled on 17th January 2019, 28th February 2019 and 2nd April 2019. Fresh geese faecal sample were collected on the bank by waiting for them to defecate. Where geese were in the water, bird-food consisting of mixed grains was also used to lure them onto the bank. Samples were collected and labelled as GD (Goose Duddingston Loch), G. Dun. (Goose Dunsapie Loch), GS (Goose St Margaret's Loch) and GL (Goose Lochend Loch). Samples were stored at -20°C within two hours post-sampling, with lack of cold chain, and processed within six weeks.

**Rural Population**. Slamannan Plateau is located approximately 4 km east of Cumbernauld and 5 km southwest of Falkirk on an upland area (Figure 1a), 170 m above sea level (Falkirk Council, 2015). It lies in the headwaters of the River Avon, and is surrounded by low undulating hills of peatlands, improved grasslands, rough pasture and two small lochs within Fannyside Muir (Perks, 2000). Scottish Natural Heritage (2016) also stated the site consists rushes and peatlands (bogs). Slamannan Plateau is described as a rural site with small settlements and numerous small farms (Perks, 2000). Similar to

the other rural area studied, it has been designated as a SPA for the conservation of the wintering Taiga bean goose population (Falkirk Council, 2015). Compared to Edinburgh, Slamannan Plateau generally has a lower anthropogenic activity, including agricultural activity by the locals and family recreation (e.g. walking, cycling and horse riding) (Scottish Natural Heritage, 2016). To avoid disturbance to this species in the protected area, samples were obtained by an experienced member of the Bean Goose Action Group (A. MacIvor). Detailed instructions and sampling equipment were personally given to minimise differences in sample collection procedure. Relatively fresh faecal samples (within 24 h after defecation) were collected on 27$^{th}$ January 2019 across the field at grid reference NS862752 while keeping the disturbance to the population and habitat to a minimum. As the university closes on the weekend, samples were stored at 4°C overnight prior to storage at -20°C, with lack of cold chain from sampling to storage, and were processed within six weeks.

### 3.3 Enumeration of Total and ESBL-Producer Coliforms in Faeces

Enumeration of ESBL-producer coliforms was undertaken by a spread plate method, the most common and accurate method for the enumeration of microorganisms (Szermer-Olearnik *et al.*, 2014). Samples were thawed at room temperature for 2 h prior to processing. Samples were diluted in 0.85% NaCl (Bauer *et al*., 1966), and a vortex (VELP Scientifica, Italy) was used to mix each sample with the diluent for 1 min. Samples were then left to stand for 30 s. **Gull faeces**. Gull samples were weighed and processed in a serial dilution of 1:3, 1:9 and 1:18 in a single test. These low dilutions were used to avoid false-negatives due to the possibility of low presence of ESBL-producer coliforms in bird samples (Veldman *et al.*, 2013). One hundred µl of each dilution of gull samples was placed on Coliform *ChromoSelect* agar (81938, Sigma-Aldrich) with and without 4 mg/l cefotaxime. **Goose faeces**. One gram of each goose sample was processed in two different sets of serial dilutions: a serial dilution of $10^{-1}$ - $10^{-5}$, and a serial dilution of 1:4, 1:16 and 1:48 for later samples in a single test. The change of dilution was implemented as the results from earlier samples showed that many samples had low levels of ESBL-producer coliforms. Fifty µl (10-fold serial dilution) and 100 µl (1:4, 1:16 and 1:48 serial dilution) of each dilution of goose samples were placed on Coliform *ChromoSelect* agar with and without 4 mg/l cefotaxime. Cefotaxime has been suggested as an effective marker of resistance in *Enterobacteriaceae*, particularly when resistance was caused by ESBLs (Robinson *et al*., 2016). A stringent concentration of 4 mg/l cefotaxime, based on the

Clinical and Laboratory Standards Institute (CLSI) standards, was used to select for ESBL-producer coliforms in the samples (CLSI, 2020). The dilutions were spread to one-third of the plates with a 10 µl wire loop (VWR, UK). The plates were then incubated at 37°C for two days. Other researchers in Donald Morrison's laboratory found that incubation for two days was more reliable and gave higher numbers, thus two-days incubation-time was used.

Coliform *ChromoSelect* agar is selective for coliform bacteria, which includes *E. coli* (Lange *et al.*, 2013). Coliform is a group of Gram-negative, non-spore-forming, rod-shaped and β-D-galactosidase-positive bacteria which ferment lactose with gas and acid formation within 48 h at 35°C (APHA *et al.*, 1998; Rompré *et al.*, 2002). The interpretation of the colonies on Coliform *ChromoSelect* agar is based on their ability to cleave two chromogenic substrates: Salmon-GAL and X-glucuronide (Sigma-Aldrich, 2013). *E. coli* and non-*E. coli* coliforms produce the enzyme β-D-galactosidase that cleaves Salmon-GAL. However, only *E. coli* produce the enzyme β-D-glucuronidase which cleaves the X-glucuronide. The cleavage of both chromogenic substrates results in dark blue to violet colony. These were regarded as presumptive *E. coli*. Non-*E. coli* coliform colonies appear as salmon to red colony due to the cleavage of Salmon-GAL alone. These were regarded as presumptive non-*E. coli* coliforms. Colourless colonies were regarded as non-coliforms due to the non-cleavage of both chromogenic substrates and not processed further. All presumptive coliform (blue and red) colonies that grew on Coliform *ChromoSelect* agar with 4 mg/l cefotaxime were regarded as presumptive ESBL-producer. In this study, four categories of coliform isolates were studied: total *E. coli* (ESBL-producer and sensitive), ESBL-producer *E. coli*, ESBL-producer non-*E. coli* coliforms and ESBL sensitive *E. coli*. Blue colonies on Coliform *ChromoSelect* agar without 4 mg/l cefotaxime were regarded as presumptive total *E. coli*. Presumptive ESBL sensitive *E. coli* were then selected from blue colonies on Coliform *ChromoSelect* agar without 4 mg/l cefotaxime and confirmed using cefotaxime (5 µg) disk.

### 3.4 Enrichment of ESBL-Producer Coliforms

All samples with no growth of ESBL-producer coliforms on Coliforms *ChromoSelect* agar with 4 mg/l cefotaxime (in the initial enumeration test) were retested by enrichment based on PHE enrichment protocol (PHE, 2014). Tryptic soy broth (TSB, CM0129, Oxoid) was used as it contains more nutritive components, including a pancreatic digest

of casein and enzymatic digest of soya bean, which beneficial for the growth of stressed cells (Dalynn Biologicals, 2014). **Gull samples** were enriched by adding TSB with 4 mg/l cefotaxime to the 1:3 diluted gull samples. The reason of using the diluted gull samples was due to the low amount of gull faeces available. **Goose samples** were enriched by adding TSB with 4 mg/l cefotaxime to 1 g of the undiluted faecal sample. The enrichment broths were incubated at 37°C overnight. A wire loop was used to streak each overnight culture onto four quadrants of the Coliform *ChromoSelect* agar plate with 4 mg/l cefotaxime and the plates were incubated at 37°C for two days. The amount of growth observed on each plate was crudely measured on a scale of '+++++' to '+'. '+++++' as heavy (growth over three quadrants of the plate), '++++' as medium (growth over two quadrants of the plate), '+++' as up to 50 colonies, '++' as up to 20 colonies and '+' as up to five colonies.

### 3.5 Prevalence, Number and Proportion of ESBL-Producer Coliforms

The prevalence of ESBL-producer coliforms in gulls and geese was defined as the percentage of samples positive for ESBL-producer coliforms in a population. This provided an indication of the overall ESBL-producer within the population during the study period (NIH, 2017). The prevalence was determined by dividing the number of positive samples for ESBL-producer (using data from both the enumeration and the enrichment process) with the total number of samples tested, multiplied by 100. The significance of differences in prevalence between urban and rural sites was assessed using a Chi-squared test with Yates' continuity correction, separately for gulls and geese. The analysis was carried out in 2 x 2 frequency tables (urban vs. rural, resistant vs. non-resistant) using R (R Core Team, 2019).

A countable range of 8 - 83 colonies per 100 µl sample on the one-third of the plate (25 – 250 colonies per plate) was considered as satisfactory and used to determine the colony-forming units per gram (CFU/g) in the original faeces (Sutton, 2011). The mean number of ESBL-producer coliforms and total coliforms was determined by counting the number of colonies (either total coliforms or ESBL-producer coliforms) on the enumeration plates, multiplied by the dilution factor and by 'n' (multiplied by 10 for 100 µl volume and by 20 for 50 µl volume). 'TMTC' (too many too count) was reported when more than 83 colonies were observed. The proportion of ESBL-producer coliforms (*E.*

*coli* and non-*E.* coli coliforms) per total coliforms was calculated using the counts on Coliforms *ChromoSelect* agar with and without 4 mg/l cefotaxime.

### 3.6 Selection of Isolates for Further Characterisation

To assess the diversity of coliforms in gulls and geese, up to 10 colonies were selected from each sample. The selection was based on the colour (different shade of blue and red, as described in Section 3.3) and morphology (round and irregular) of colonies on the Coliform *ChromoSelect* agar. If no different colonies were observed, then up to 10 similar colonies were chosen. Each selected colony was given a reference isolate number based on the initials of the investigator (BAD- Bimo Andrianus Djuwanto) and a number (starting from 50 upwards). A two-step purity plate was used to ensure the purity of the culture. A single colony from the Coliform *ChromoSelect* agar plate was selected and plated on either Eosin Methylene Blue agar (70186, Sigma-Aldrich) or MacConkney agar (CM0115, Oxoid), and incubated at 37°C. Subsequently, a distinct single colony was selected from this initial purity plate and plated out on a fresh Eosin Methylene Blue or MacConkey agar plate. Eosin Methylene Blue and MacConkey agars are both recognised as selective and differential plates for coliforms (Wanger *et al.*, 2017). Both of the agars were used in this study. Fresh cultures of each isolate were stored at -20°C in 1 ml of 20% glycerol broth (20 ml of glycerol in 80 ml TSB) (ATCC, 2015; Tonoyan *et al.*, 2019).

### 3.7 Phenotypic Identification of Coliforms

#### 3.7.1 Growth on Eosin Methylene Blue and MacConkey Agars

Coliforms generally form dark violet colonies on Eosin Methylene Blue agar due to lactose fermentation which lowers the pH. In the low pH, Eosin Y and Methylene Blue as the pH indicators combine to form the dark violet colour. The appearance of a green metallic sheen on dark violet colonies indicates *E. coli* due to the formation of a dye complex in the low pH, which differentiated them from other coliform colonies (MacFadden, 1985). On MacConkey agar, coliforms appear in an intense red colour due to lactose fermentation and the presence of neutral red as the pH indicator dye. Non-coliforms appear as a straw-colour or colourless on both Eosin Methylene Blue and MacConkey agars due to peptone fermentation alone (no lactose fermentation) and hence no change of the pH.

### 3.7.2 Gram Staining

The UK Standards for Microbiology Investigations for Gram staining was followed (PHE, 2019). The Gram staining method was performed to divide the isolates into the two main bacterial categories based on the Gram staining reaction and the cell shape. Gram-negative bacteria were indicated by a pink/red stain due to the inability to retain the methyl/crystal violet in their thin cell walls (PHE, 2019). The cells are stained by safranin, a counterstain used in the present study. A pink/red stain and a single *bacillus* shape (rod-shaped) were indicative of coliforms (Rompré *et al*., 2002). Gram-positive bacteria were indicated by a deep blue/purple stain due to a thicker and denser peptidoglycan layers in their cell walls (PHE, 2019). The intact cell-wall is penetrated by iodine, and the function of blue dye to inhibit its diffusion through the cell wall during decolourisation is altered (PHE, 2019).

### 3.7.3 Indole Test

The indole test was conducted to determine the ability of isolates to produce indole from the hydrolysis of tryptophan (PHE, 2018). The amino acid tryptophan is hydrolysed by tryptophanase to produce three end-products: indole, pyruvate and ammonium ion (PHE, 2018). A loopful of overnight-grown cultures was inoculated into a sterile test tube containing 5 ml TSB broth, and incubated at 37°C overnight (PHE, 2018). Three hundred µl of the Kovac's reagent was added into the overnight culture, which was then shaken gently. A positive result was indicated by the formation of a pink to red coloured ring in the upper layer of liquid within a few seconds. A negative result was indicated by no colour change of TSB with a yellow or slightly cloudy layer in the upper layer of liquid (PHE, 2018). Coliforms such as *E. coli*, *K. oxytoca*, *C. freundii* and *C. braakii* are indole-positive, while other coliforms such as *Enterobacter* sp., *K. pneumoniae* and *Serratia* sp. are indole-negative (Janda *et al.*, 1994; MacWilliams, 2009; Niemi *et al.*, 2003).

## 3.8 Antibiotic Susceptibility Testing (AST)

The disk diffusion antibiotic susceptibility testing method of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) was followed (Matuschek *et al.*, 2014). Twelve antibiotics from seven different antibiotics classes were tested: ampicillin (10 µg), amoxicillin-clavulanic acid (20-10 µg), cefoxitin (30 µg), cefotaxime (5 µg), ceftazidime (10 µg), meropenem (10 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg),

gentamicin (10 µg), tigecycline (15 µg), tetracycline (15 µg) and trimethoprim-sulfamethoxazole (1.25-23.75 µg) (Table 2). The antibiotics were selected based on two UK surveillance reports and the annual report of The Scottish One Health Antimicrobial Use and Antimicrobial Resistance Report (SONAAR) in human and veterinary sectors (HPS, 2019b; O'Dwyer, 2017; Veterinary Medicines Directorate, 2018; 2019b). This selection will further allow "One Health" comparison of resistance data.

The inoculum was made in 0.85% NaCl to a density of a McFarland 0.5 standard (approx. 1.5 x $10^8$ CFU/ml) by visual comparison (Matuschek *et al.*, 2014). The suspension was evenly spread onto Mueller-Hinton agar (CM0337, Oxoid) in at least three directions using a sterile cotton swab. Six antibiotic disks were placed on each plate within 15 min of inoculation, and the plates were incubated at 35 ± 1°C for 18 ± 2 h. The recommended clinical breakpoints from EUCAST (version 9.0) were used for interpretation of zone-inhibition diameters (Table 2) (EUCAST, 2019). Isolates were classified using Magiorakos *et al.* (2012) as multidrug-resistant (MDR), extensively drug-resistant (XDR) and pandrug-resistant (PDR). MDR is defined as resistance to at least one agent in three or more classes of antibiotics, XDR as resistance to at least one agent in all but two or fewer classes of antibiotics and PDR as resistance to all antibiotic agents in all classes of antibiotics.

***Table 2. Clinical breakpoints table for interpretation of zone diameters in antibiotic susceptibility testing.*** *Classification of class, subclass and breakpoints in the table based on EUCAST version 9.0 (EUCAST, 2019). S = Sensitive, I = Intermediate, R = Resistant.*

| Class | Sub class | Antimicrobial agent | Abbreviation | Disc content (µg) | Zone diameter breakpoints (mm) | | |
|---|---|---|---|---|---|---|---|
| | | | | | S ≥ | I | R < |
| β-lactam | Penicillins | Ampicillin | AMP | 10 | 14[B] | - | 14[B] |
| β-lactam | Penicillins | Amoxicillin-clavulanic acid | AMC | 20-10 | 19[B] | - | 19[B] |
| β-lactam | Cephalosporins | Cefoxitin | FOX | 30 | 19 | - | 19 |
| β-lactam | Cephalosporins | Cefotaxime | CTX | 5 | 20 | 17-19 | 17 |
| β-lactam | Cephalosporins | Ceftazidime | CAZ | 10 | 22 | 19-21 | 19 |
| β-lactam | Carbapenems | Meropenem | MEM | 10 | 22 | 16-21 | 16 |
| Chloramphenicol | | Chloramphenicol | C | 30 | 17 | - | 17 |
| Fluoroquinolones | | Ciprofloxacin | CIP | 5 | 25 | 22-24 | 22 |
| Aminoglycosides | | Gentamicin | CN | 10 | 17 | 14-16 | 14 |
| Glycylcyclines* | | Tigecycline | TGC | 15 | 18[A] | - | 18[A] |
| Tetracyclines | | Tetracycline | TE | 30 | 19 | - | - |
| Miscellaneous agents** | | Trimethoprim-sulfamethoxazole | SXT | 1.25-23.75 | 14 | 11-13 | 11 |

**Classification based on Magiorakos et al. (2012). **Miscellaneous agent used in the present study was a combination drug of trimethoprim and sulphonamides class. [A]Breakpoints validated for E. coli only. [B]Growth that may appear as a thin inner zone on MH agars should be ignored*

## 3.9 PCR Assays

### 3.9.1 DNA Extraction for PCR Assay

DNA extraction using the standard boilate method was initially used (Grant *et al.*, 2001); however, inconsistent results were obtained. Therefore, DNA was extracted using a Chelex method based on Kariyama *et al.* (2000) and a Bio-Rad protocol. The bacterial lysate (DNA extraction) with Chelex® 100 Resin has been shown to be as reliable as a DNA extraction kit (InstaGene Matrix) method (Kariyama *et al.*, 2000). An initial study by Singer-Sam *et al.* (1989) stated the addition of Chelex® 100 might prevent the degradation of DNA with its high affinity for polyvalent metal ions, hence, prevented the likely catalyst process (chelating metal ions) in the breakdown of DNA in low ionic strength solutions at high temperatures.

An isolate was inoculated into 9 ml TSB broth and incubated overnight at 37°C. Twenty-five µl of the overnight culture was added into a 0.2 ml microtube and centrifuged (Eppendorf, UK) for 1 min at 12,000 rpm. The supernatant was removed, and 100 µl 7.5% Chelex® 100 Resin (Bio-Rad, UK; dissolved in sterile distilled water) was added. The mixture was vortexed at 2,000 rpm for 10 s and boiled for 10 min at 100°C in a heating block (Flowgen Bioscience, UK). The mixture was further vortexed for 10 s at 2,000 rpm and centrifuged at 12,000 rpm for 3 min. This supernatant was used as the DNA template for the PCR assays in Section 3.9.2-3.9.9.

### 3.9.2 *lacZ3* and *yaiO* PCR Assay

This duplex PCR assay was performed to identify coliforms (*lacZ3* primer) and *E. coli* (*yaiO* primer). The *lacZ3* and *yaiO* primers were developed by Molina *et al*. (2015). *LacZ3* was designed from a conserved region of *lacZ* gene, and *yaiO* (located in *E. coli* orphan open reading frames) was designed as an alternative to the *uidA* gene and was claimed to have a higher specificity for *E. coli in silico* than the *uidA* gene. Molina *et al.* (2015) further stated that both primers have a superior-detection ability than other *lacZ* and *uidA* primers for confirming lactose fermenter strains.

The PCR reaction mix contained 2 µl DNA template, 12.5 µl MyTaq$^{TM}$ Mix (Bioline), 7.5 µl distilled water and 3 µl primer mix with a final concentration of each primer 0.12 µM (Table 3).

***Table 3. Primer sequences used in the duplex (lacZ3 and yaiO) PCR assay.***

| Target | Primer sequences | PCR product (bp) |
|---|---|---|
| *lacZ3* | F: 5' TTGAAAATGGTCTGCTGCTG 3' | 234 |
| | R: 5' TATTGGCTTCATCCACCACA 3' | |
| *yaiO* | F: 5' TGATTTCCGTGCGTCTGAATG 3' | 115 |
| | R: 5' ATGCTGCCGTAGCGTGTTTC 3' | |

PCR amplification was performed with a Prime Thermal Cycler as follows: initial denaturation at 95°C for 3 min, 35 cycles of denaturation at 95°C for 30 s, annealing at 58°C for 30 s and extension at 72°C for 1 min, followed by a last extension at 72°C for 10 min. *E. coli* ATCC® 47055 and *K. pneumoniae* NCIMB 8805 strains were used as positive controls. Sterile distilled water was used as a negative control. Gels were made and visualised as in Section 3.9.10, with modification of running time to 30 min. The interpretation of results was based on the absence/presence of the targeted band sizes. *lacZ3* (+) and *yaiO* (+) indicating *E. coli*, and *lacZ3* (+) and *yaiO* (-) indicating non-*E. coli* coliforms (Molina *et al.*, 2015).

3.9.3 *gadA* PCR Assay

The *gadA* PCR assay was performed based on Doumith *et al.* (2012), which specifically target the *E. coli* glutamate decarboxylase-alpha gene. The expression of *gadA* gene generally maintains a near-neutral intracellular pH of bacteria, including *E. coli*, *Shigella flexneri* and *Listeria monocytogenes* in extremely acidic conditions (Masuda & Church, 2003).

The PCR mixture contained 2 µl DNA template, 12.5 µl MyTaq$^{TM}$ Mix (Bioline), 9.5 µl sterile distilled water and 1µl primer mix with final concentration of 0.4 µM *gadA* primers (Table 4).

***Table 4. Primer sequences used in the gadA PCR assay.***

| Target | Primer sequences | PCR product (bp) |
|---|---|---|
| *gadA* | F: 5' GATGAAATGGCGTTGGCGCAAG 3' | 373 |
| | R: 5' GGCGGAAGTCCCAGACGATATCC 3' | |

PCR amplification was conducted as follows: initial denaturation at 94°C for 4 min, followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 65°C for 30 s,

extension at 72°C for 30 s and a final extension at 72°C for 5 min. *E. coli* ATCC® 47055 strain was used as a positive control. Sterile distilled water was used as a negative control. The amplified products were visualised as in Section 3.9.10. *E. coli* was confirmed by the presence of a 373 bp band.

3.9.4 *uidA* PCR Assay

The *uidA* PCR assay was performed based on Bej *et al.* (1991). The *uidA* gene is unique and conserved in *E. coli* as well as *Shigella* spp., encoding an enzyme named β-glucuronidase (Bej *et al.*, 1991). The β-glucuronidase hydrolyses β-glucuronic acid residues from the non-reducing termini of glycosaminoglycans (Arul *et al.*, 2008). It is of note that this enzyme also cleaves β-glucuronic acid from X-glucuronide substrate in Coliform *ChromoSelect* agar, producing the blue colour.

The PCR reaction mix was made as previously described in the *gadA* PCR assay (Section 3.9.3), with final concentration of 2µM *uidA* primers (Table 5).

***Table 5. Primer sequences used in the uidA PCR assay.***

| Target | Primer sequences | PCR product (bp) |
|---|---|---|
| *uidA* | F: 5' TGGTGATTACCGACGAAAACGGC 3' | 162 |
| | R: 5' ACGCGTGGTTACAGTCTTGCG 3' | |

PCR amplification was performed as follows: initial denaturation at 95°C at 3 min with 25 cycles of denaturation at 94°C for 1 min and primer annealing and extension at 50°C for 1 min. *E. coli* ATCC® 47055 strain was used as a *uidA*-positive control, and *K. pneumoniae* NCIMB 8805 strain was used as a *uidA*-negative control. The amplified products were visualised as in Section 3.9.10, with modification of running time to 30 min. *E. coli* was confirmed by the presence of a 162 bp band.

3.9.5 Phylo-group PCR Assay

The phylo-group PCR assay was performed based on Clermont *et al*. (2013). Based on the absence/ presence of the targeted genes, *E. coli* was assigned to one of the eight recognised phylo-groups- seven belongs to *E. coli sensu stricto* (A, B1, B2, C, D, E, F) and *Escherichia* cryptic clade (I-V) (Clermont *et al.*, 2013).

3.9.5.1 Quadruplex Phylo-group PCR Assay

The quadruplex PCR was conducted in 20 µl PCR reaction mix containing 3 µl DNA template, 10 µl MyTaq$^{TM}$ Mix, 5 µl sterile distilled water and 2 µl primer mix containing 1 µM of each primer (Table 6). PCR amplification was performed as follows: initial denaturation at 94°C for 4 min, followed by 30 cycles of denaturation at 94°C for 5 s and annealing at 59°C for 20 s, and a final extension step at 72°C for 5 min.

***Table 6. Primer sequences used in the quadruplex phylo-group assay.***

| Target | Primer sequences | PCR product (bp) |
|---|---|---|
| *arpA* | F: 5' AACGCTATTCGCCAGCTTGC 3' | 400 |
| | R: 5' TCTCCCCATACCGTACGCTA 3' | |
| *chuA* | F: 5' ATGGTACCGGACGAACCAAC 3' | 288 |
| | R: 5' TGCCGCCAGTACCAAAGACA 3' | |
| *yjaA* | F: 5' CAAACGTGAAGTGTCAGGAG 3' | 211 |
| | R: 5' AATGCGTTCCTCAACCTGTG 3' | |
| TspE4.C2 | F: 5' CACTATTCGTAAGGTCATCC 3' | 152 |
| | R: 5' AGTTTATCGCTGCGGGTCGC 3' | |

The control for this PCR was provided by Donald Morrison's laboratory: ERI 39 for phylo-group B2 (has all products, except *arpA*). Sterile distilled water was used as a negative control. The expected band sizes of the amplified PCR product and presence/absence of targeted genes were used to interpret the results (Table 7). PCR-specific assays (E-specific, C-specific, cryptic clade) were undertaken as a "next step" to further characterise these isolates (Table 7). Gels were made and visualised as in Section 3.9.10.

***Table 7. Interpretation of the quadruplex phylo-group PCR assay.*** *The table was adapted from Clermont et al. (2013). Isolates showing two possible phylo-group assignments and cryptic clades in quadruplex PCR assay were screened with phylo-group specific and cryptic clade PCR assays.*

| Quadruplex genotype | | | | Phylo-group | Next step |
|---|---|---|---|---|---|
| *arpA* (400 bp) | *chuA* (288 bp) | *yjaA* (211 bp) | TspE4.C2 (152 bp) | | |
| + | - | - | - | A | |
| + | - | - | + | B1 | |
| - | + | - | - | F | |
| - | + | + | - | B2 | |
| - | + | + | + | B2 | |
| + | - | + | - | B2 | |
| + | + | - | - | A or C | Screen using C-specific PCR assay. If C+ then C, else A |
| + | + | - | + | D or E | Screen using E-specific PCR assay. If E+ then E, else D |
| + | + | + | - | D or E | Screen using E-specific PCR assay. If E+ then E, else D |
| - | - | + | - | E or clade I | Screen using E-specific PCR assay. If E- then clade I, confirm using cryptic clade PCR assay |
| - | (476 bp) | - | - | Clade III, IV or V | Confirm using cryptic clade PCR assay |
| - | - | - | + | Unknown | Confirm using cryptic clade PCR assay |
| - | - | + | + | Unknown | |
| + | - | + | + | Unknown | |
| + | + | + | + | Unknown | |
| - | - | - | - | Unknown | |

3.9.5.2 Phylo-group C-specific PCR Assay

The phylo-group C-specific PCR assay was conducted in 20 µl PCR reaction mix and PCR conditions as for the quadruplex PCR (Section 3.9.5.1), with primer mix containing 1 µM of each t*rpA* C-specific primers and 0.6 µM of each *trpA* primers as an internal control (found in all *E. coli* strains) (Table 8).

***Table 8. Primer sequences used in the phylo-group C-specific PCR assay.***

| Target | Primer sequences | PCR product (bp) |
|---|---|---|
| *trpA* | F: 5' CGGCGATAAAGACATCTTCAC 3' | 489 |
| | R: 5' GCAACGCGGCCTGGCGGAAG 3' | |
| *trpA* C-specific | F: 5' AGTTTTATGCCCAGTGCGAG 3' | 219 |
| | R: 5' TCTGCGCCGGTCACGCCC 3' | |

A phylo-group C control was provided by Donald Morrison's laboratory: *E. coli* ATCC® 47055. Sterile distilled water was used as a negative control. Gels were made and visualised as in Section 3.9.10. The expected band sizes of the amplified fragment were used to interpret the results.

3.9.5.3 Phylo-group E-specific PCR Assay

The phylo-group E-specific PCR was conducted in 20 µl PCR reaction mix as for the quadruplex PCR (Section 3.9.5.1), with primer mix contained 1 µM of each *arpA* E-specific primers and 0.6 µM of each *trpA* primers as an internal control (Table 9).

***Table 9. Primer sequences used in the phylo-group E-specific PCR assay.***

| Target | Primer sequences | PCR product (bp) |
|---|---|---|
| *trpA* | F: 5' CGGCGATAAAGACATCTTCAC 3' | 489 |
| | R: 5' GCAACGCGGCCTGGCGGAAG 3' | |
| *arpA* E-specific | F: 5' GATTCCATCTTGTCAAAATATGCC 3' | 301 |
| | R: 5' GAAAAGAAAAAGAATTCCCAAGAG 3' | |

PCR amplification was performed as mentioned in the quadruplex PCR, with modified primers annealing temperature at 57°C for 20 s. A phylo-group E control was provided by Donald Morrison's laboratory: ERI 40. Sterile distilled water was used as a negative control. Gels were made and visualised as in Section 3.9.10. The expected band sizes of the amplified fragment were used to interpret the results.

3.9.5.4 Cryptic Clade PCR Assay

The cryptic clade PCR assay was performed based on Clermont *et al*. (2011b). A study by Walk *et al.* (2009) previously suggested the existence of five different clades of cryptic species (I to V) based on genetic polymorphisms observed with multi-locus sequence typing (MLST). *Escherichia* cryptic lineages are genetically distinct yet phenotypically indistinguishable from *E. coli* (Clermont *et al.*, 2013). *Escherichia* cryptic clade I is described to be closely related to *E. coli* (Walk *et al*, 2009). Clade II is rarely isolated and remains largely uninvestigated (Shen *et al.*, 2019). Clade III and IV are known to be sister group, branching between *E. coli* and clade V which is the most divergent clade (Clermont *et al.*, 2011).

The PCR assay was conducted in 20 µl PCR reaction mix as for the quadruplex PCR (Section 3.9.5.1), with a modified primer mix and final concentrations of 2 µM for each primer (Table 10). PCR amplification was performed as follows: initial denaturation at 94°C for 4 min, followed by 30 cycles of denaturation at 94°C for 5 s, annealing at 63°C for 30 s and a final extension at 72°C for 5 min. Sterile distilled water was used as a negative control. No positive controls were available for this assay; hence, the expected

band sizes of the amplified fragment were used to interpret the results. Gels were made and visualised as in Section 3.9.10.

***Table 10. Primer sequences used in the cryptic clade PCR assay.***

| Target | Primer sequences | PCR product (bp) |
|---|---|---|
| Clade I | F: 5' CCTCTACTCACCCAAAAGTC 3' | 315 |
| | R: 5' ATCACGTAACCACAACGCAC 3' | |
| Clade II | F: 5' CGCCTGTTGTCACTTCCACG 3' | 125 |
| | R: 5' GTTTATCACGCAGCCACAAG 3' | |
| Clade III | F: 5' GTGTTGAGATTGTCCGTGGG 3' | 183 |
| | R: 5' CAAAAGCACTGGCGCCCAG 3' | |
| Clade IV | F: 5' CTGGCGAAAGGAACCTGGA 3' | 461 |
| | R: 5' GTTATCTCATCTTGCAGCCAA 3' | |
| Clade V | F: 5' ACTGTATGGCAGTGGCGCAT 3' | 600 |
| | R: 5' GCAAAACTATCGGCAAACAGC 3' | |

3.9.6 PCR-based Multi-Locus Sequence Typing (MLST) Assay

Four clinically important sequence types (STs): ST69, ST73, ST95 and ST131 were detected using a PCR-based MLST assay based on Doumith *et al.* (2015). The final PCR mixture of 25 µl contained 2 µl DNA template, 12.5 µl MyTaq$^{TM}$ Mix, 8.5 µl sterile distilled water and 2 µl primer mix at final concentrations of 0.8 µM for each primer (Table 11).

***Table 11. Primer sequences used in the PCR-based MLST assay.***

| MLST type | Primer sequences | PCR product (bp) |
|---|---|---|
| ST 73 | F: 5' TGGTTTTACCATTTTGTCGGA 3' | 490 |
| | R: 5' GGAAATCGTTGATGTTGGCT 3' | |
| ST 131 | F: 5' GACTGCATTTCGTCGCCATA 3' | 310 |
| | R: 5' CCGGCGGCATCATAATGAAA 3' | |
| ST 95 | F: 5' ACTAATCAGGATGGCGAGAC 3' | 200 |
| | R: 5' ATCACGCCCATTAATCCAGT 3' | |
| ST 69 | F: 5' ATCTGGAGGCAACAAGCATA 3' | 104 |
| | R: 5' AGAGAAAGGGCGTTCAGAAT 3' | |

PCR amplification was performed with conditions as follows: initial denaturation at 94°C for 3 min, 30 cycles of denaturation at 94°C for 30 s, annealing at 60°C for 30 s and extension at 72°C for 30 s, followed by a final extension at 72°C for 5 min. Sterile distilled water was used as a negative control. No positive controls were available for this assay;

hence, the expected band sizes of the amplified fragment were used to interpret the results. Amplified genomic fragments were visualised as in Section 3.9.10.

### 3.9.7 PCR Detection of CTX-M Group Resistance Genes

Detection of clinically important CTX-M extended-spectrum β-lactamase genes in a multiplex PCR assay was performed based on Woodford *et al.* (2005). The 25 µl PCR mixture contained 2 µl DNA template, 12.5 µl MyTaq$^{TM}$ Mix, 9.5 µl sterile distilled water and 1 µl primer mix at final concentrations of 0.4 µM for each primer (Table 12). Groups 8 and 25 were amplified with a shared reverse primer. The amplification was performed with conditions as follows: initial denaturation at 94°C for 5 min, 30 cycles of denaturation at 94°C for 25 s, annealing at 52°C for 40 s and extension at 72°C for 50 s, followed by a final extension at 72°C for 6 min.

Controls for *bla*$_{\text{CTX-M}}$ group 1 and *bla*$_{\text{CTX-M}}$ group 9 were provided by Donald Morrison's laboratory: AT 1.3 and AT 4.2, respectively. Sterile distilled water was used as a negative control. No controls for *bla*$_{\text{CTX-M}}$ group 2, 8 and 25 were available for this assay, hence, the expected band sizes of the amplified fragment were used to interpret the results. Gels were made and visualised as in Section 3.9.10, with modification of running time to 40 min.

***Table 12. Primer sequences used in the CTX-M group PCR assay.***

| Target | Primer sequences | PCR product (bp) |
|---|---|---|
| *bla* $_{\text{CTX-M}}$ group 1 | F: 5' AAAAATCACTGCGCCAGTTC 3' | 415 |
| | R: 5' AGCTTATTCATCGCCACGTT 3' | |
| *bla* $_{\text{CTX-M}}$ group 2 | F: 5' CGACGCTACCCCTGCTATT 3' | 552 |
| | R: 5' CCAGCGTCAGATTTTTCAGG 3' | |
| *bla* $_{\text{CTX-M}}$ group 9 | F: 5' CAAAGAGAGTGCAACGGATG 3' | 205 |
| | R: 5' ATTGGAAAGCGTTCATCACC 3' | |
| *bla* $_{\text{CTX-M}}$ group 8 | F: 5' TCGCGTTAAGCGGATGATGC 3' | 666 |
| Shared reverse primer group 8 and 25 | R: 5' AACCCACGATGTGGGTAGC 3' | |
| *bla* $_{\text{CTX-M}}$ group 25 | F: 5' GCACGATGACATTCGGG 3' | 327 |

### 3.9.8 PCR Detection of TEM, SHV and OXA Resistance Genes

Three further ESBL genes (TEM, SHV and OXA) were detected using a multiplex PCR assay based on Dallenne *et al.* (2010). The 25 µl PCR mixture contained 2 µl DNA template, 12.5 µl MyTaq$^{TM}$ Mix, 9.5 µl sterile distilled water and 1 µl primer mix at final concentrations of 0.4 µM for each primer (Table 13).

***Table 13. Primer sequences used in the SHV, TEM and OXA multiplex PCR assay.***

| Target | Primer sequences | PCR product (bp) |
|---|---|---|
| *bla* $_{TEM}$ | F: 5' CATTTCCGTGTCGCCCTTATTC 3' | 800 |
| | R: 5' CGTTCATCCATAGTTGCCTGAC 3' | |
| *bla* $_{SHV}$ | F: 5' AGCCGCTTGAGCAAATTAAAC 3' | 713 |
| | R: 5' ATCCCGCAGATAAATCACCAC 3' | |
| *bla* $_{OXA}$ | F: 5' GGCACCAGATTCAACTTTCAAG 3' | 564 |
| | R: 5' GACCCCAAGTTTCCTGTAAGTG 3' | |

PCR amplification was performed with conditions as follows: initial denaturation at 94°C for 10 min, 30 cycles of denaturation at 94°C for 40 s, annealing at 60°C for 40 s and extension at 72°C for 1 min, followed by a final extension at 72°C for 7 min. Controls were provided by Donald Morrison's laboratory: AT 1.2 for TEM, SHV and OXA genes, and AT 1.3 for TEM genes. Sterile distilled water was used as a negative control. Gels were made and visualised as in Section 3.9.10. The expected band sizes of the amplified fragment were used to interpret the results.

### 3.9.9 Repetitive Element Palindromic PCR (rep-PCR)

#### 3.9.9.1 Rep-PCR Assay

Rep-PCR assay was performed due to its ability to distinguish bacteria at the subspecies/strain level (Versalovic *et al.*, 1998). This PCR-based genomic fingerprinting uses oligonucleotide primers that amplify short repetitive sequences in diverse regions throughout the genome, leading to amplicon patterns that are specific for an individual strain (Versalovic *et al*., 1991; Rademaker & de Bruijn, 1997).

The rep-PCR assay was performed using two sets of primers: REP and $(GTG)_5$. Rep-PCR with REP primers was based on Malathum *et al*. (1998), and rep-PCR with $(GTG)_5$ primer was based on Mohapatra *et al.* (2007). To compare these two primers, the generated DNA fingerprint patterns were visually compared and assessed using the performance and convenience criteria for microbial typing methods (van Belkum, *et al.*, 2007). The performance criteria includes typeability (the ability to assign isolates to a type), discriminatory power (the ability to assign a different type of two unrelated strains) and reproducibility (the ability to assign the same type to an isolated tested on different occasions). The convenience criteria include ease of analyse and interpretation of the DNA fingerprint.

The rep-PCR reaction mix contained 2 µl DNA template, 12.5 µl MyTaq$^{TM}$ Hot Start (HS) Mix (Bioline, UK), 9.5 µl sterile distilled water and 1 µl primer mix with final concentrations of 2 µM for each primer (Table 14). HS mix polymerase was used in this assay as previously optimised in Donald Morrison's laboratory.

***Table 14. Primer sequences used in the rep-PCR assay.*** *N = A, C, G or T. I = Inosine (a universal base).*

| Primer | Primer sequences |
|---|---|
| Rep1R-Dt | 5' IIINCGNCGNCATCNGGC 3' |
| Rep2-Dt | 5' NCGNCTTATCNGGCCTAC 3' |
| $(GTG)_5$ | 5' GTGGTGGTGGTGGTG 3' |

REP PCR was performed as follows: initial denaturation at 95°C for 7 min, 35 cycles of denaturation at 90°C for 30 s, annealing at 40°C for 1 min and extension at 65°C for 8 min, followed by a final extension at 65°C for 16 min. PCR amplification for $(GTG)_5$ PCR was performed as follows: initial denaturation at 95°C for 2 min, 30 cycles of denaturation at 94°C for 3 s and 92°C for 30 s, annealing at 40°C for 1 min and extension at 65°C for 8 min, followed by a final extension at 65°C for 16 min.

*E. coli* ATCC® 47055 and *K. pneumoniae* NCIMB 8805 patterns were used as the rep-PCR reference patterns. These allowed the DNA fingerprint pattern of isolates from the same and different gels to be compared, and they were also used as the reference patterns for cluster analysis in the BioNumerics software (Section 3.9.9.2 below). The rep-PCR agarose gels were made and visualised as in Section 3.9.10, with the modification of the running conditions at 70V for 1 h 50 min. HyperLadder$^{TM}$ 1 kb (Bioline, UK) was used as both a size-reference ladder and an external reference standard for the normalisation of the gels (using BioNumerics). The image files were saved in TIFF format for the analysis in BioNumerics.

3.9.9.2 Analysis of Rep-PCR Profiles

BioNumerics v.7.6.1 (Applied Maths, Belgium) was used to analyse the DNA fingerprint data generated by the rep-PCR assay. Gel images with .tif format were imported and processed as follows. Gel lanes and densitometric curve were manually defined by entering the number of lanes in the gel image, adjusting the width of the lanes and adjusting the thickness of 'bands curve' for the curve extraction. Lanes containing

the 1 kb DNA ladder were used as an external reference standard with a defined range from 400 to 3000 bp, which allowed the gels to be normalised by the software. Because the band intensity varied, the bands were manually defined. The band intensity was visually distinguished into three general categories: strong (+++), medium (++) and faint (+) (Fig. 2). In the present study, only strong and medium bands were selected for the analysis as faint bands are not regarded as reproducible (Garaizar *et al.*, 2000).

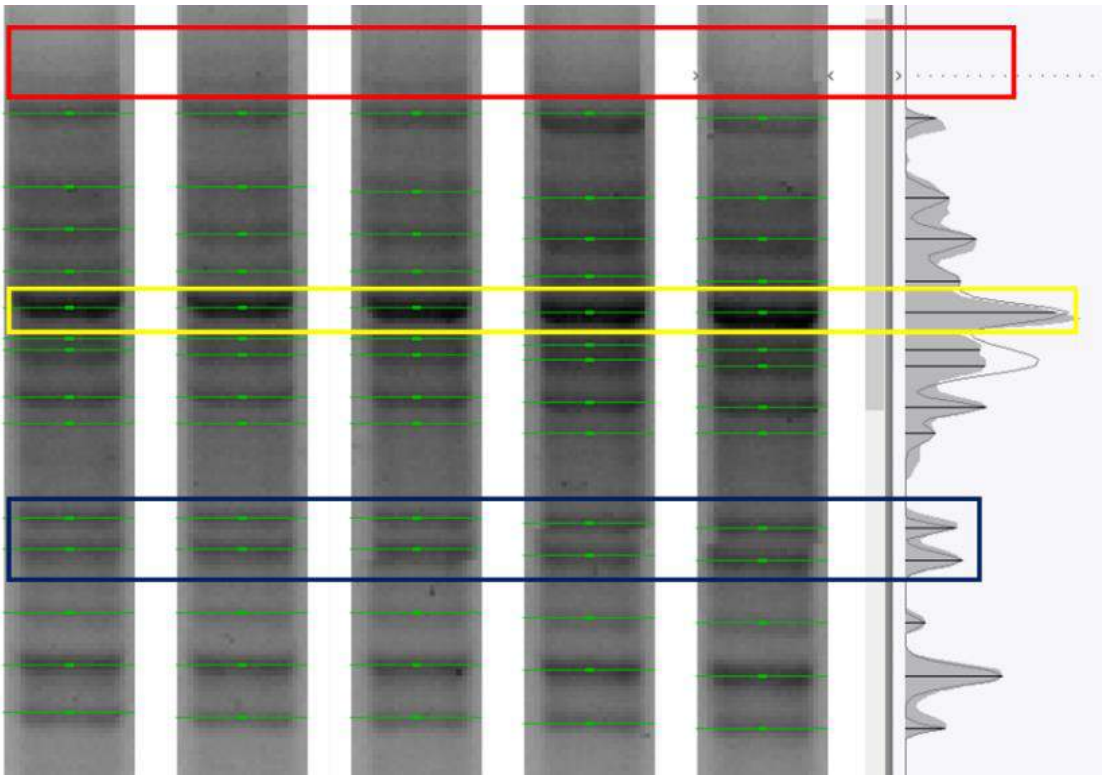

***Figure 2. An example of the band intensity from the normalised gel image.*** *The densitometric curve is shown on the right side. Bands are indicated by green lines. Examples of strong bands (+++) are shown in the yellow rectangle, medium bands (++) in the dark blue rectangle and faint bands (+) in the red rectangle.*

A simple band-based similarity coefficient (number of different bands) with the tolerance set to 2.5% for band matching and Unweighted Pair Group Method with Arithmetic mean (UPGMA) were selected for cluster analysis. This comparison generated a dendrogram. “Distance” (indicates band difference) was selected to show the nodes information. A less than three bands difference rule was applied to define a REP-type (Spigaglia & Mastrantonio, 2003). This meant that all isolates with a DNA fingerprint pattern which differed by less than three bands were assigned the same REP-type number. By changing the setting for nodes information from “distance” to “similarity

value", the band different could be expressed in a similarity value (%). In this study, isolates assigned to the same REP-type were considered to be the same strain.

### 3.9.10 PCR Gel Electrophoresis and Visualisation

A 2% agarose gel was made by mixing 2 g of agarose molecular grade (BIO-41025, Bioline, UK) with 100 ml Tris-acetate-EDTA (1x, Sigma-Aldrich, UK) buffer and 5 µl SafeView stain (NBS Biologicals LTD., Cambridgeshire, UK). Six microlitres of DNA loading buffer blue (5x, BIO-37045, Bioline, UK) was added to the 20 - 25 µl PCR products, and 15 µl of the mixture was loaded into the gel. The gel was run at 100 V for 35 min, except as indicated in each assay described below. HyperLadder™ 100 bp (Bioline, UK) was used as a size marker, except for the rep-PCR assay where HyperLadder™ 1 kb was used and appropriately placed on the first, middle and last lane of the gel. Gels were visualised by ChemiDoc™ XRS+ using Image Lab™ v.6.0.1 software (Bio-Rad, UK). The gel images were captured and saved in .jpg format.

## 3.10 16S rRNA Sequencing

16S rRNA sequencing has been recommended for the precise identification of poorly described, rarely isolated or phenotypically aberrant strains (Clarridge, 2004). The 16S rRNA sequencing method was based on the laboratory protocol from Microbiology Society (2016) used in Patricia Gonzalez-Iglesias' laboratory (Edinburgh Napier University), with primer sequences obtained from Lane *et al.* (1991). The DNA extraction method and DNA purification kit were modified from the reference method. DNA was extracted using the Boilate Method (a loopful of colonies was suspended in 200 µl sterile distilled water and boiled at 100°C for 10 min using a heating block). The 25 µl PCR reaction mix contained only 5 µl DNA template and 10 µl of each universal primer with a final concentration of 0.4 µM for each primer (Table 15).

***Table 15. Universal bacterial primers used in the 16S rRNA sequencing.***

| Target | Primer sequences | PCR product (bp) |
|---|---|---|
| 27F | 5' AGAGTTTGATCCTGGCTCAG 3' | 1484 |
| 1492R | 5' GGTTACCTTGTTACGACTT 3' | |

The amplification was performed as follows: denaturation at 94°C for 2 min, 30 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min and extension at 72°C for

2 min, followed by a final extension at 72°C for 2 min. The amplified products were separated by 2% agarose gel (Section 3.9.10) at 110 V for 1 h 30 min. HyperLadder™ 1 kb (Bioline) was used as a band size ladder. Gels were visualised under UV to confirm the presence of a 1,484 bp band. The 1,484 bp band was carefully cut from the gel using a sterile scalpel (70% alcohol and flamed), and the DNA was purified using the ISOLATE II PCR and Gel Kit (Bioline) following the manufacture's protocol. The purity of the DNA product was checked using NanoDrop™ 2000 Spectrophotometer (ThermoScientific, UK). The DNA concentration and the purity ratio were calculated at 260/280 wavelength. Samples with DNA concentration of 15-40 ng and purity ratio of ~1.8 was deemed acceptable and were used for Sanger sequencing (Thermo Fisher Scientific, 2012).

Five microlitres of the purified DNA were mixed with 1 µl of 27F primer (stock concentration of 6.4 µM) in a 0.2 ml strip tube to generate the forward sequence. Another 5 µl of the purified DNA was mixed with 1 µl of 1492R primer (stock concentration of 6.4 µM) in a different 0.2 ml strip tube to generate the reverse sequence. The tubes were appropriately labelled and sent to Edinburgh Genomics (Scotland) for Sanger sequencing. The chromatogram files (.ab1) were analysed using Chromas 2.6.6 (Technelysium, Australia) software as follows. The low-quality sequences at both ends from forward and reverse primers were trimmed with Chromas 2.6.6 default settings to avoid poor-sequence alignment. "Reverse" option was selected to obtain the reverse complement sequence from the reverse sequence. The FASTA sequences were then exported from both sequences. Both forward and reverse nucleotide sequences were merged and compared with the 16S ribosomal RNA sequences (Bacteria and Archaea) database from Basic Local Alignment Search Tool NCBI (BLASTn, blast.ncbi.nlm.nih.gov). The interpretation of the 16S rRNA sequencing was as follows: ≥ 99% sequence-similarity regarded as the same species (Johnson *et al.*, 2019), 97% to < 99% similarity corresponded to genus identification (Han, 2006) and < 97% similarity corresponded to poor-quality sequence (Welinder-Olsson *et al.*, 2007).

## 3.11 Pulsed-Field Gel Electrophoresis (PFGE)

PFGE was performed based on the PulseNet PFGE method for *E. coli* O157:H7 (CDC PulseNet, 2017). Several colonies from MacConkey or EMB agar plate were resuspended in 2 ml Cell Suspension Buffer (100 mM Tris:100 mM EDTA, pH 8.0). The optical

density (OD) of the cell suspension was measured and adjusted to 0.8-1.0. Agarose plugs were cast in a mold containing 200 µl adjusted cell suspensions, 10 µl Proteinase K (20 mg/ml, P2308, Sigma-Aldrich, UK) and 200 µl of 2% Certified™ Megabase Agarose (Bio-Rad, UK) in Tris-EDTA buffer (TE buffer, 10 mM Tris:1 mM EDTA, pH 8.0).

Cell Lysis Buffer/Proteinase K mix was made by mixing 5 ml Cell Lysis Buffer (50 mM Tris:50 mM EDTA, pH 8.0 + 1% Sarcosyl) and 25 µl Proteinase K (20 mg/ml) per tube. Five millilitres of the mix were then transferred to each labelled 50 ml falcon tube. The plugs were pushed out of the molds into the falcon tubes containing the Cell Lysis Buffer/Proteinase K mix. The tubes were placed on a shaking incubator at 55°C for 2 h with constant and vigorous agitation (200 rpm). The agarose plugs were washed three times with 10 ml preheated (to 55°C) sterile Ultrapure Laboratory Grade Water (ULGW, ReAgent, UK), and then four times with preheated (to 55°C) 10 ml TE buffer. All washes were incubated at 55°C for 15 min. Following the washing, a 2.0 x 2.5 mm portion of the agarose plug was cut and digested in a 1.5 ml microtube with 200 µl mix containing 20 µl restriction enzyme *XbaI* (New England Biolabs, 20 units/µl) and 180 µl sterile ULGW for 2 h in 37°C water bath.

An agarose gel was cast, containing 1% Pulsed Field Certified Agarose (Bio-Rad, UK) in 200 ml 0.5x Tris-Borate EDTA (Sigma-Aldrich, UK) buffer. Three to five millilitres of the agarose gel was stored in a universal bottle at 60°C incubator for later use to seal the plugs. The restricted plug slices were then added to the wells of the gel, and up to 200 µl of the 1% Pulsed Field Certified Agarose was used to fill in the wells. The fragmented DNA in the plugs was separated using CHEF-DR® II System (Bio-Rad, UK) at 14°C, the flow rate of 1 l/min, 6 V/cm, for 20 h with initial switch time 6.8 s and final switch time 35.4 s. *Salmonella braenderup* was used as a size standard on the first lane. The gel was stained in 3x GelRed™ (Biotium, USA) for 30 min at room temperature, and the gels were visualised by ChemiDoc™ XRS+ using Image Lab™ v.6.0.1 software (Bio-Rad, UK). The gel images were captured and saved in .jpg format.

# 4. Results

## 4.1 Bird Faecal Sample Collections

A total of 226 samples from gulls and geese was collected between January and April 2019 across both the urban (high anthropogenic activity) and the rural (low anthropogenic activity) sites (Table 16, Appendix 1).

***Table 16. Number of faecal samples collected in each urban and rural site.*** *Species of gulls and geese, specific location and month(s) of sample collections are provided. Appendix 1 provides further sampling details and processing dates.*

| Bird taxa | Area | | Collection month | Species | Number of samples |
|---|---|---|---|---|---|
| Gulls | Urban | Seafield WWTW, Edinburgh | Feb | Black-headed gulls and herring gulls | 47 |
| | Rural | St Abbs village, Scottish Borders | Apr | Herring gulls | 50 |
| Geese | Urban | Urban Lochs, Edinburgh | Jan, Feb, Apr | Canada geese, greylag geese | 77 |
| | Rural | Agricultural fields, Slamannan Plateau | Jan | Taiga bean geese | 52 |

**Gull faeces - urban site**. Forty-seven gull faecal samples were collected at Seafield wastewater treatment works (WWTW). On the sampling day, a high anthropogenic activity was indicated as the waste plant was treating wastewater across Edinburgh and the surrounding area. During the sampling, more than 100 gulls in this urban site were observed to feed on the open primary settling tanks containing untreated sewage.

**Gull faeces - rural site**. Fifty gull faecal samples were collected at St Abbs. A low anthropogenic activity was observed on the day of sampling, with only small number (<15) of locals/visitors. Gulls in this rural site were observed to scavenge for bread and chips, and presumably other foods on the shore such as small crabs and fish. Fish scales pellets were visually observed in some gull faecal samples.

**Goose faeces - urban site**. A total of 77 goose faecal samples (Canada and greylag geese) were collected around urban lochs in Edinburgh. Forty-seven Canada goose faecal samples were collected from Duddingston Loch, 12 from Dunsapie Loch and one from Lochend Loch. In addition, 17 greylag goose faecal samples were collected only from one site (St Margaret's Loch). Grass fibre was visually observed in all goose samples, indicating this is an important component of their diet. A relatively high anthropogenic

activity was also observed during the sampling day. Close-interaction between humans and geese in this urban site was observed through direct and indirect wild-bird feeding. Locals and/or visitors were observed carrying out recreational activities (e.g. daily exercise, walking, cycling) around the lochs.

**Goose faeces - rural site**. Fifty-two bean goose faecal samples were collected in an agricultural field in Slamannan Plateau. Undigested residue of plant matter was observed in all samples. Geese in this rural site feed on agricultural grasses and waste grains (grain spilled during agricultural activity), with limited contact with humans but likely contact with agricultural waste, including livestock droppings.

## 4.2 Enumeration of Presumptive Total and ESBL-Producer Coliforms in Birds

### 4.2.1 Prevalence of Total and ESBL-Producer Coliforms

To understand how anthropogenic activity within a site affects the prevalence of resistant bacteria, the prevalence of ESBL-producer coliforms in the four bird populations was determined. Prevalence was defined as the percentage of bird faecal samples that were positive for coliform bacteria. Colonies that grew on Coliform *ChromoSelect* agar were regarded as presumptive coliforms, and these were further divided into two groups depending on the colour - *E. coli* (blue to violet colonies) and non-*E. coli* coliforms (salmon to red colonies). "Total" indicates colonies that grew on Coliform *ChromoSelect* agar without 4 mg/l cefotaxime, hence indicating both ESBL-producer and sensitive colonies.

**Enrichment Process**. A total of 192 out of 226 faecal samples (85%) showed no growth of ESBL-producer coliforms on the spread plate "enumeration" method. To confirm that these were true negatives, these samples underwent an enrichment process. The enrichment process was not undertaken for samples that showed no coliforms growth on Coliform *ChromoSelect* agar without 4 mg/l cefotaxime. The enrichment process enables injured or stressed cells to grow (Özkanca *et al.*, 2009). Cells might be injured or stressed (metabolic injury) due to external factors, including low heat, chemicals, freezing and frozen storage, which resulting in the inability to multiply in selective media thus the need for additional nutrients for growth and multiplication (Ray, 1978). Twenty-five of the 192 negative samples (13%, gulls = 10 and geese = 15) grew on Coliform *ChromoSelect* agar with 4 mg/l cefotaxime following the enrichment in TSB with 4 mg/l

cefotaxime. Overall, 26% (59 out of 226) of bird faeces were positive for ESBL-producer coliforms. Both the enrichment and enumeration positive samples were used in the calculation of the prevalence of ESBL-producer coliforms below.

**Gull faeces**. Overall, 80% [78 (urban = 38 and rural = 40) of 97 faeces] of gulls carried total *E. coli* (Table 17). The percentage of gulls carrying total *E. coli* in the urban (81%) and rural (80%) sites was similar. The prevalence of ESBL-producer *E. coli* in the urban site (57%) was significantly higher ($X^2_1 = 33.6$, $P < 0.001$) than in the rural site (2%). Overall, 74% [72 (urban = 43 and rural = 29) of 97 faeces] of gulls carried total non-*E. coli* coliforms, with significantly higher ($X^2_1 = 12.5$, $P < 0.001$) in the urban site (91%) compared to the rural site (58%). The difference in the prevalence of ESBL-producer non-*E. coli* coliforms in gulls from the urban (32%) and rural (4%) sites was also significant ($X^2_1 = 11.2$, $P = 0.001$). The significant differences of ESBL-producer coliforms indicate that anthropogenic activity impacts the prevalence of resistant bacteria in gulls within the respective sites.

***Table 17. Prevalence of total and ESBL-producer coliforms in gulls within each urban and rural site.*** *The percentage was determined by a calculation (number of positive samples/total number of samples (n) multiplied by 100)*

| Bird taxon | Sites | n | Number of samples positive | | | |
|---|---|---|---|---|---|---|
| | | | Total *E. coli* | ESBL-producer *E. coli* | Total non-*E. coli* coliforms | ESBL-producer non-*E. coli* coliforms |
| Gulls | Urban | 47 | 38 (81%) | 27 (57%) | 43 (91%) | 15 (32%) |
| | Rural | 50 | 40 (80%) | 1 (2%) | 29 (58%) | 2 (4%) |

**Goose faeces**. Compared to the gulls, *E. coli* was isolated from only 18% [24 (urban = 19 and rural = 5) out of 129 goose faeces, and the difference between the urban (25%) and rural (10%) sites was not significant (Table 18; $X^2_1 = 3.7$, $P = 0.054$). The difference in the prevalence of ESBL-producer *E. coli* from geese between the urban (3%) and rural (0%) sites could not be statistically tested because the expected values violated the $X^2$ test's assumption: each expected value should be at least 5 (R Core Team, 2019). Nonetheless, the percentage of geese carried ESBL-producer *E. coli* in the urban site was lower (3%) compared to the gulls (57%). Interestingly, the prevalence of goose samples with total non-*E. coli* coliforms in the rural site (81%) was significantly higher than the urban site (60%; $X^2_1 = 5.4$, $P < 0.05$), and in contrast to the finding in gulls. The difference

in the prevalence of ESBL-producer non-*E. coli* coliforms between geese in the urban (5%) and rural (17%) sites was not statistically significant ($X^2_1$ = 3.8, P = 0.052). In contrast with gulls, this data of ESBL-producer coliforms suggests that anthropogenic activity does not influence the prevalence of resistant bacteria in geese within the respective sites.

***Table 18. Prevalence of total and ESBL-producer coliforms in geese within each urban and rural site.*** *The percentage was determined by a calculation (number of positive samples/total number of samples (n) multiplied by 100)*

| Bird taxon | Sites | n | Number of samples positive | | | |
|---|---|---|---|---|---|---|
| | | | Total *E. coli* | ESBL-producer *E. coli* | Total non-*E. coli* coliforms | ESBL-producer non-*E. coli* coliforms |
| Geese | Urban | 77 | 19 (25%) | 2 (3%) | 46 (60%) | 4 (5%) |
| | Rural | 52 | 5 (10%) | 0 (0%) | 42 (81%) | 9 (17%) |

4.2.2 Abundance and Proportion of Total and ESBL-producer *E. coli*

Huijbers *et al.* (2019) proposed the use of abundance/number (CFU/g) of bacteria to understand the risk of transmission, and the use of proportion of resistant bacteria to investigate the resistance evolution. Only the samples positive for coliforms were used in the calculation of median (CFU/g). Due to problems with determining the optimum dilution ranges for the different samples, gull and goose faeces gave extremely variable data ('zero' CFU/g to 'TMTC' predominating) in this study (Appendix 2.1, Table S.8 – S.11). Plates with colony numbers below the standard countable range of 8 colonies on the one-third of the plate (Sutton, 2011) were included in the calculation of median (CFU/g), hence the calculations were regarded as 'estimated' (ASTM, 1998). The median (CFU/g) was calculated with different number of samples due to the variability of data. When two dilutions showed countable colonies within the range, colonies on the smaller dilution were used to calculate the numbers (CFU/g) to avoid errors in the estimates increase with increasing serial dilutions (Sutton, 2011). Samples with 'TMTC' count on all dilutions were calculated by multiplying the upper limit of the counting range per plate (250) with the highest dilution factor (1:18 for gulls and 1:48 for geese), and reported as $> 4.5 \times 10^3$ CFU/g and $> 1.2 \times 10^4$ CFU/g for gulls and geese, respectively (ASTM, 1998). When no visible colonies were observed, the numbers (CFU/g) were reported as less than the limit of detection (LOD) (ASTM, 1998). The LOD of gull faeces was 3 CFU/g, and

the LOD of goose faeces was 4 CFU/g for geese with the 1:4 dilution and 10 CFU/g for geese with the 10-fold dilution. Hence, the numbers (CFU/g) were reported as < 3 CFU/g for gulls, < 4 CFU/g for geese with the 1:4 dilution and < 10 CFU/g for geese with the 10-fold dilution. However, these numbers (CFU/g) from 'TMTC' counts and "zero" count were not included in the calculation of the medians (CFU/g).

**Gull faeces**. The median (CFU/g) of total *E. coli* in gulls in the urban site ($6.2 \times 10^2$ CFU/g) was double than those in the rural site ($3.5 \times 10^2$ CFU/g; Table 19; Appendix 2.2, Table S.12 – S.13). The median (CFU/g) of ESBL-producer coliforms in the rural site cannot be estimated as only one sample was used. The number of ESBL-producer *E. coli* and the number (CFU/g) of ESBL-producer non-*E. coli* coliforms in gulls in the rural site were $9.2 \times 10^3$ CFU/g and $3.2 \times 10^1$ CFU/g, respectively. Of 13 gull faeces, the proportion of ESBL-producer *E. coli* ranged from 0.6 - 76.4%. The proportion of ESBL-producer non-*E. coli* coliforms of 3 gull faeces ranged from 2.2 - 26.3%. These numbers (CFU/g) and proportions need to be treated with caution as they were based on a small number of samples. The confidence and reliability of the data were not achieved.

***Table 19. The median (CFU/g) of total E. coli and ESBL-producer coliforms in gulls.*** *The median (CFU/g) was calculated from samples which were positive for coliforms (see Appendix 2.2, Table S.12 – S.13 for the numbers (CFU/g) of each sample). Different numbers of samples were used to calculate the median (CFU/g). Numbers (CFU/g) in the bracket indicates only one sample was used.*

| Bird taxa | Sites (n = total number of samples from the site) | "n" for the calculation | | Median (CFU/g) | Range (CFU/g) |
|---|---|---|---|---|---|
| Gulls | Urban (n=47) | 31 | total *E. coli* | $6.2 \times 10^2$ | $4.9 \times 10^1$ - $2.5 \times 10^4$ |
| | | 20 | ESBL-producer *E. coli* | $4.1 \times 10^2$ | $1.3 \times 10^1$ - $1.4 \times 10^5$ |
| | | 11 | ESBL-producer non-*E. coli* coliforms | $3.9 \times 10^2$ | $3.1 \times 10^1$ - $9.1 \times 10^3$ |
| | Rural (n=50) | 10 | total *E. coli* | $3.5 \times 10^2$ | $1.7 \times 10^1$ - $2.5 \times 10^3$ |
| | | 1 | ESBL-producer *E. coli* | [$9.2 \times 10^2$] | - |
| | | 1 | ESBL-producer non-*E. coli* coliforms | [$3.2 \times 10^1$] | - |

**Goose faeces**. Compared to gulls in the urban site, the median (CFU/g) of total *E. coli* in geese in the urban site ($2.0 \times 10^2$ CFU/g) was three times lower (Table 20; Appendix 2.2, Table S.14 – S.15). Interestingly, the median (CFU/g) of total *E. coli* in geese in the rural site ($2.0 \times 10^3$ CFU/g) was hundredfold than those in the urban site. However, the rural data was biased by one sample which had a much higher count ($1.5 \times 10^8$ CFU/g) than other samples (ranging from $2 \times 10^2$ – $4.2 \times 10^4$). It is likely that this was an outlier

sample as the possibility of sampling error by A. MacIvor cannot be outlined. No goose samples with countable data for ESBL-producer coliforms were available. The number (CFU/g) of ESBL-producer coliforms in geese in the urban site were reported as < 4 CFU/g, whereas the number (CFU/g) of ESBL-producer coliforms in geese in the rural site were reported as < 10 CFU/g. In contrast to gulls, the proportion of ESBL-producer coliforms in geese cannot be calculated.

***Table 20. The median (CFU/g) of total E. coli and ESBL-producer coliforms in geese.*** *The median (CFU/g) was calculated from samples which were positive for coliforms (see Appendix 2.2, Table S.14 – S.15 for the numbers (CFU/g) of each sample). Different numbers of samples were used to calculate the median (CFU/g).*

| Bird taxa | Sites (n = total number of samples from the site) | "n" for the calculation | | Median (CFU/g) | Range (CFU/g) |
|---|---|---|---|---|---|
| Geese | Urban (n=77) | 17 | total *E. coli* | $2.0 \times 10^2$ | $4.0 \times 10^1$ - $2.4 \times 10^4$ |
| | Rural (n=52) | 5 | total *E. coli* | $2.0 \times 10^3$ | $2.0 \times 10^2$ - $1.5 \times 10^8$ |

## 4.3 Selection of Isolates for Further Characterisation

**Selection of Isolates**. To investigate the diversity of ESBL-producer coliform isolates in these two bird populations, up to 10 colonies (ranging between 1 and 10) were selected from the 59 ESBL-producer positive samples. In addition, to understand the overall diversity of *E. coli* population within these birds, up to five colonies (ranging between 1 and 5) of ESBL sensitive *E. coli* were selected from the 102 *E. coli* positive samples (Kim *et al.*, 2016). A total of 175 isolates from different sites and bird taxa were selected - 88 presumptive ESBL-producer coliforms (51 *E. coli* and 37 non-*E. coli* coliforms) and a comparison set of 87 presumptive ESBL sensitive *E. coli* (Table 21; Appendix 3, Table S.16 – S.18).

***Table 21. Details of presumptive ESBL-producer coliforms and ESBL sensitive E. coli from gulls and geese selected for further characterisation.*** *The number of isolates from each site and the samples are shown. Appendix 3 provides further details.*

| | Number of isolates | Details of isolates | | |
|---|---|---|---|---|
| | | Number of isolates | Samples | Sites |
| Presumptive ESBL-producer *E. coli* | 51 | 33 | LSW | Gulls- urban |
| | | 10 | Enriched LSW | Gulls- urban |
| | | 5 | LSA | Gulls- rural |
| | | 1 | Enriched LSA | Gulls- rural |
| | | 1 | Enriched GD | Geese- urban |
| | | 1 | Enriched GS | Geese- urban |
| Presumptive ESBL-producer non- *E. coli* coliforms | 37 | 5 | LSW | Gulls- urban |
| | | 5 | LSA | Gulls- rural |
| | | 5 | Enriched LSA | Gulls- rural |
| | | 12 | Enriched GD | Geese- urban |
| | | 10 | Enriched GSP | Geese- rural |
| Presumptive ESBL sensitive *E. coli* | 87 | 33 | LSW | Gulls- urban |
| | | 21 | LSA | Gulls- rural |
| | | 7 | GD | Geese- urban |
| | | 4 | GS | Geese- urban |
| | | 3 | G. Dun | Geese- urban |
| | | 3 | GL | Geese- urban |
| | | 16 | GSP | Geese- rural |

During the process of subculturing isolates from the original (enumeration or enrichment) plates, two unexpected colony morphologies were observed.

(1) On the first subculture, 40 out of the 175 selected isolates (14 *E. coli* and 26 non-*E. coli* coliforms) grew as what appeared to resemble "small colony variants" (SCV) (Fig. 3; Appendix 3). The SCVs were observed to be approximately one-seventh the diameter of the typical size of the colonies. The green metallic sheen of SCV *E. coli* on Eosin Methylene Blue was not apparent and needed to be observed in a certain angle to really see the colour. Upon the second subculture, none of these reverted to normal size. However, thirteen SCV isolates (13/40) reverted to normal size after several sub-cultivation, which would suggest some of the SCV are not stable (nonstable SCVs). Further investigation of these isolates was not possible within the study time frame.

(2) Twenty-two out of 37 non-*E. coli* coliforms (red colonies on Coliform *ChromoSelect* agar) appeared on the first subculture plate as SCV with a faint green metallic sheen on Eosin Methylene Blue agar, suggesting they were *E. coli* (Appendix 3,

Table S.17). The appearance of these SCVs on Eosin Methylene Blue agar was exactly the same as the SCV *E. coli* (picture was not taken). This was the first indication in this study that the Coliform *ChromoSelect* agar and/or Eosin Methylene Blue agar may give discrepant results.

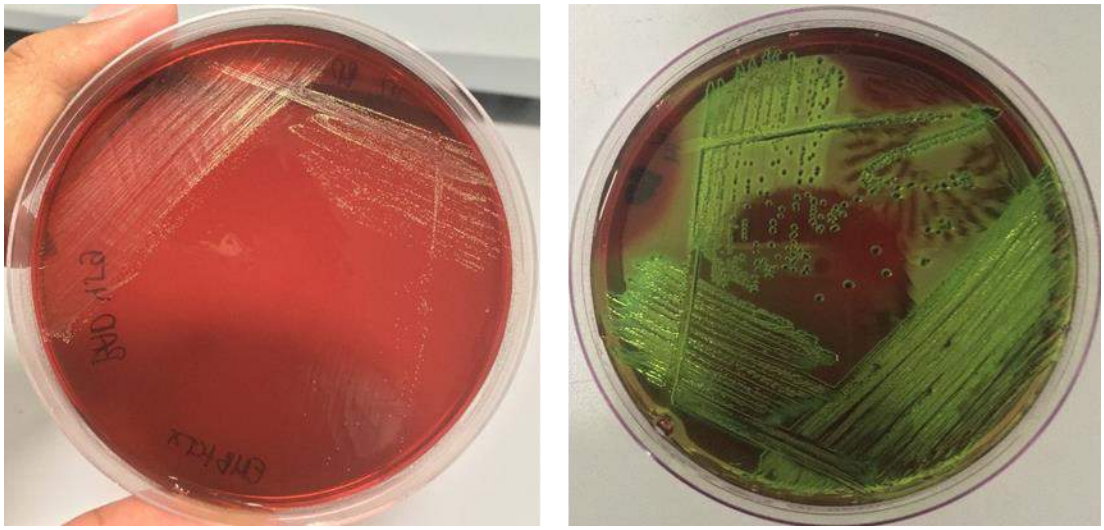

***Figure 3. A comparison of small colony variants (SCVs) presumptive E. coli on Eosin Methylene Blue agar.*** *Left - SCV colonies, tiny colonies (approx. 0.3 mm) with less (almost none) green metallic sheen. Right - typical E. coli colonies, typical size of colonies (approx. 1 mm), dark violet with a green metallic sheen. The appearance of SCV non-E. coli coliforms were the same as SCV E. coli (picture was not taken).*

In order that this project would be manageable in the time frame available, in depth characterisation of all 175 selected isolates was not possible. Rep-PCR assay, a time and cost-effective method with a good level of discrimination, was undertaken on all 175 isolates to reduce the number of isolates tested further. A similar strategy has been used by dos Anjos Borges *et al.* (2003) and Scheirlinck *et al.* (2007) while studying the diversity of *E. coli* and lactic acid bacteria, respectively.

## 4.4 Comparison of Rep-PCR Primers

Rep-PCR assay uses primers which target repetitive sequence elements throughout the bacterial genome (Versalovic *et al.*, 1991). There are several different primers available to be used of which five (BOX, REP, ERIC, ERIC2 and $(GTG)_5$) have been compared by

Mohapatra *et al*. (2007). They found the $(GTG)_5$ primer the most suitable for discrimination of faecal and environmental *E.* coli. To investigate the suitability of the $(GTG)_5$ primer for this project, a comparison was undertaken with the REP primers which have been used extensively in Donald Morrison's lab. Another reason of doing this comparison was that differences in the PCR cycler and PCR reagents used in different labs can affect the rep-PCR typing method. Each method was compared on the basis of the performance and convenience criteria devised for microbial typing methods (van Belkum *et al.*, 2007).

Three strains of *E. coli* (ERI 40, ATCC 47055, AT 1.3) which were previously known to have distinct REP-PCR DNA fingerprint pattern in Donald Morrison's lab were typed using both primers and visually compared side by side. The assay was undertaken in triplicate. Both the $(GTG)_5$ and REP primers gave 100% typeability. The $(GTG)_5$ primer produced 13 – 17 bands (faint to strong) ranging from 400 bp to 3000 bp (Fig. 4, lane 2 – 4). For the REP primers, 20 – 23 bands (faint to strong) was observed with a good spread of bands ranging from 400 bp to 5000 bp (Fig. 4, lane 6 – 8). Although both primers showed the same typeability and were high reproducibility (100%), the REP primers showed a superior discriminatory power (10 - 12 medium - strong bands difference between unrelated isolates) compared to $(GTG)_5$ primer (5 - 7 medium - strong bands difference) (Appendix 4, Table S.20 – REP primers, Table S.22 – $(GTG)_5$ primer). In addition, the band patterns were easier to be read and visually analysed with REP primers (Appendix 4.1.1). Therefore, REP primers were selected to be used for the rep-PCR assay in this study. A reference strain is important for intergel comparison and is required by the BioNumerics software. ATCC 47055 was used as the reference strain of *E. coli* to be included on every gel as it showed a good spread of clearly separated bands. For non-*E. coli* coliforms, *K. pneumoniae* NCIMB 8805 was used as the reference strain. To compensate for the known reproducibility issues in the rep-PCR assay (Foxman *et al.*, 2005) in interpreting the banding patterns, only strong and medium bands were used in this study.

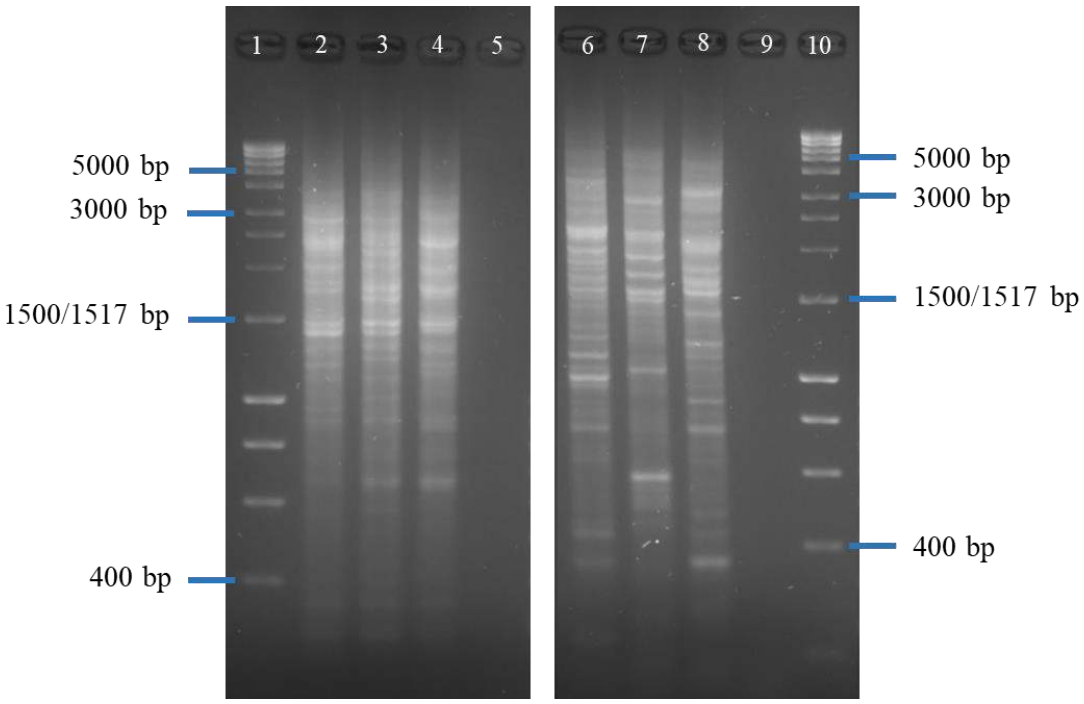


***Figure 4. Comparison of rep-PCR with $(GTG)_5$ primer (lane 2 - 4) and REP primers (lane 6 - 8).*** *Lane 1 and 10: Ladder 1 kb. Lane 2 and 6: E. coli ERI 40. Lane 3 and 7: E. coli ATCC 47055. Lane 4 and 8: E. coli AT 1.3. Lane 5 and 9: 7.5% Chelex® water for negative control. Bands (faint to strong) are indicated by yellow lines. Appendix 4 provides the assessment of reproducibility and discriminatory power of both rep-PCR primers in details. The gaps indicate that the gel pictures have been cut out from different gels for the figure. The ladder run on each gel has been used to match up the different gels in the figure (this applies to other PCR gels below with gaps in the figure).*

## 4.5 Selection of The Representative Isolates using REP-PCR Typing

REP-PCR assay was performed to select a small representative group of the 175 selected isolates for further characterisation. All the 175 selected isolates were typed using the REP primers (All gels can be seen in Appendix 5). Although DNA fingerprint patterns on the same gel can be compared visually by eye, this is not possible when the isolates are run on a large number of gels. To compare the isolates on the 15 gels, BioNumerics, a commercial software package was used to process and analyse the fingerprints. A simple band-based similarity coefficient (number of different bands) and UPGMA method were used in the present study for the cluster analysis Bands were manually curated to avoid errors in the automatic band assignment, and isolates within a less than three bands difference were clustered into a same REP type and considered as

the same strain (Carriço *et al.*, 2005; Spigaglia & Mastrantonio, 2003). In BioNumerics, this equated to clustering at > 97% similarity (Appendix 5 - Fig. S.8, Fig. S.15, Fig. S.20). A > 97% similarity cut-off has been used in other studies using rep-PCR assay (Anderson *et al.*, 2015; Thomson *et al.*, 2014).

The REP-PCR assay was performed on three isolate groups: (1) ESBL-producer *E. coli*, (2) ESBL sensitive *E. coli* and (3) ESBL-producer non-*E. coli* coliforms. To distinguish the REP types for each group, they were assigned slightly differed labels: ESBL-producer *E. coli* - REPR type; ESBL sensitive *E. coli* - REPS type and ESBL-producer non-*E. coli* coliforms - REPNE type.

### 4.5.1 Presumptive ESBL-producer *E. coli*

The REP-PCR assay of the 51 ESBL-producer *E. coli* isolates from 16 different samples generated 18 unique patterns (gels can be seen in Appendix 5.1.1, Fig. S.3 – Fig. S.7). By using a less than three bands difference rule, 12 REP types were identified (Fig. 5). These REP types differed from each other by 3 - 10 bands.

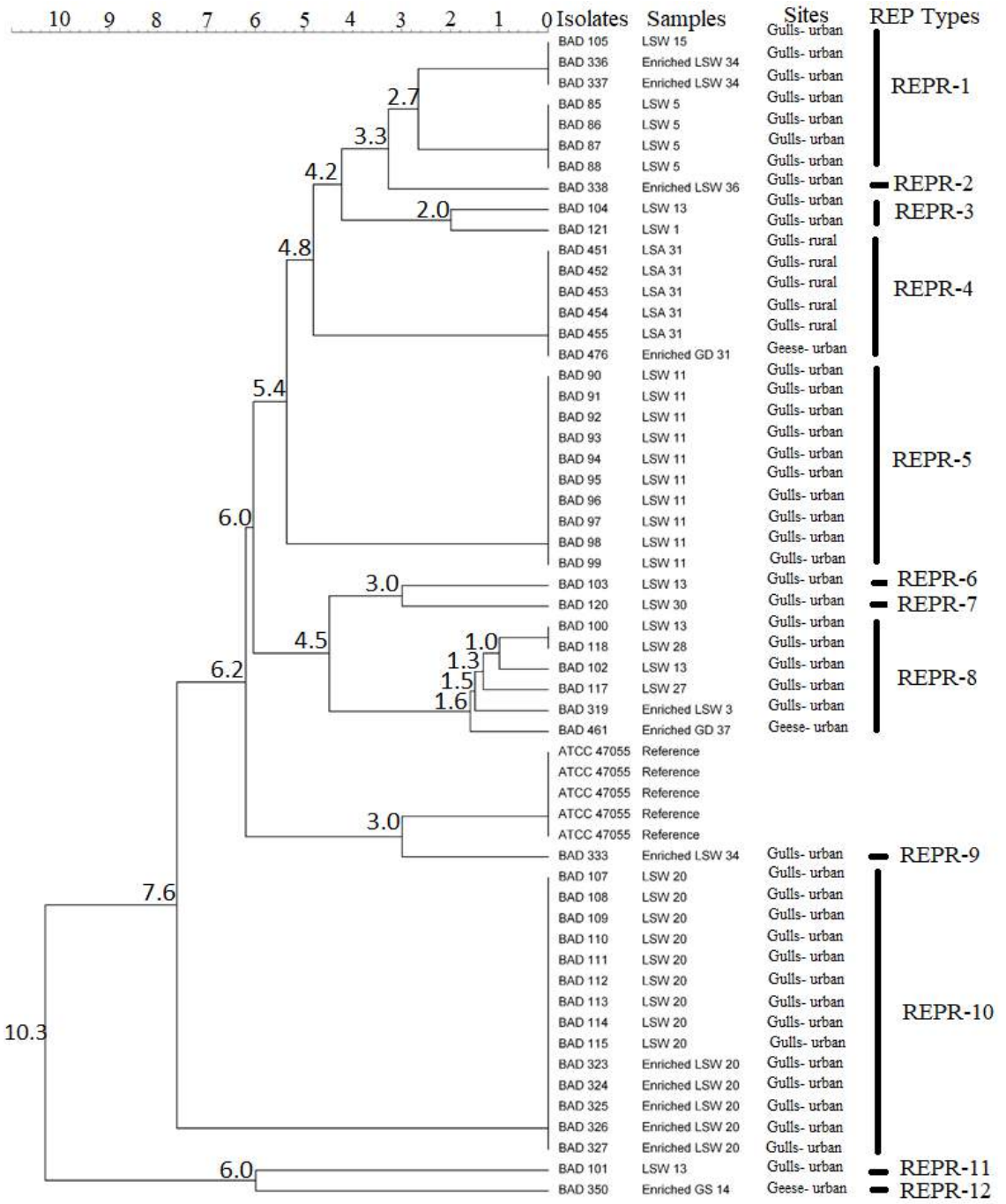


***Figure 5. The dendrogram of REP-PCR assay of presumptive ESBL-producer E. coli generated by BioNumerics.*** *Cluster analysis was done using the number of band difference coefficient and UPGMA method. Numbers at the nodes show the band difference. The equated similarity (%) is shown in Appendix 5.1.2 - Fig. S.8. The samples (isolate numbers and sites) and REP types (REPR) are shown in the dendrogram. The axis line at the top (0-10) indicates the number of band difference. REP types have been assigned based on <3 bands difference rule.*

### 4.5.2 Presumptive ESBL Sensitive *E. coli*

The 87 ESBL sensitive *E. coli* from 26 different samples were divided into 46 unique patterns by the REP-PCR assay (Appendix 5.2.1, Fig. S.9 – Fig. S.14). Thirty-four REP types were clustered using a less than three bands difference rule (Fig. 6). A difference by 3-10 bands from each other was observed from these REP types. Interestingly, one isolate (BAD 777) showed an indistinguishable pattern with the reference strain (ATCC 47055).

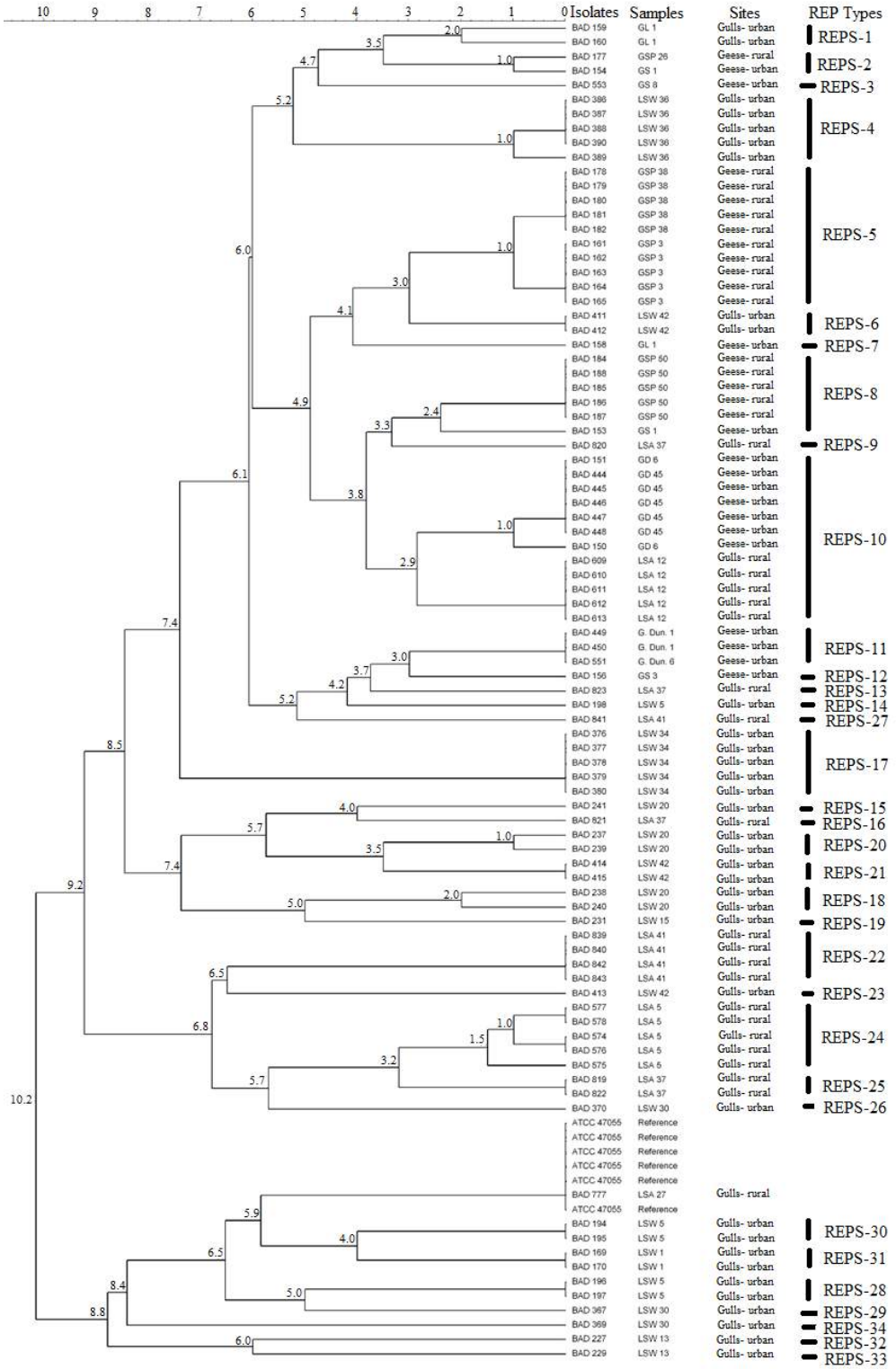


***Figure 6. The dendrogram of REP-PCR assay of presumptive ESBL sensitive E. coli generated by BioNumerics.*** *Cluster analysis was done using the number of band difference coefficient and UPGMA method. Numbers at the nodes show the band difference. The equated similarity (%) is shown in Appendix 5.2.2 - Fig. S.15. The samples (isolate numbers and sites) and REP types (REPS) are shown in the dendrogram. The axis line at the top (0-10) indicates the number of band difference. REP types have been assigned based on <3 bands difference rule.*

4.5.3 Presumptive ESBL-producer Non-*E. coli* Coliforms

The REP-PCR assay of 37 ESBL-producer non-*E. coli* coliforms from 11 different samples generated 20 unique patterns (Appendix 5.3.1, Fig. S.16 – Fig. S.19). Fourteen REP types were observed using a less than three bands difference rule (Fig.7). These REP types were different from each other by 3-9 bands. Notably, one isolate (BAD 106) showed three-bands difference with the reference strain (*K. pneumoniae* NCIMB 8805).

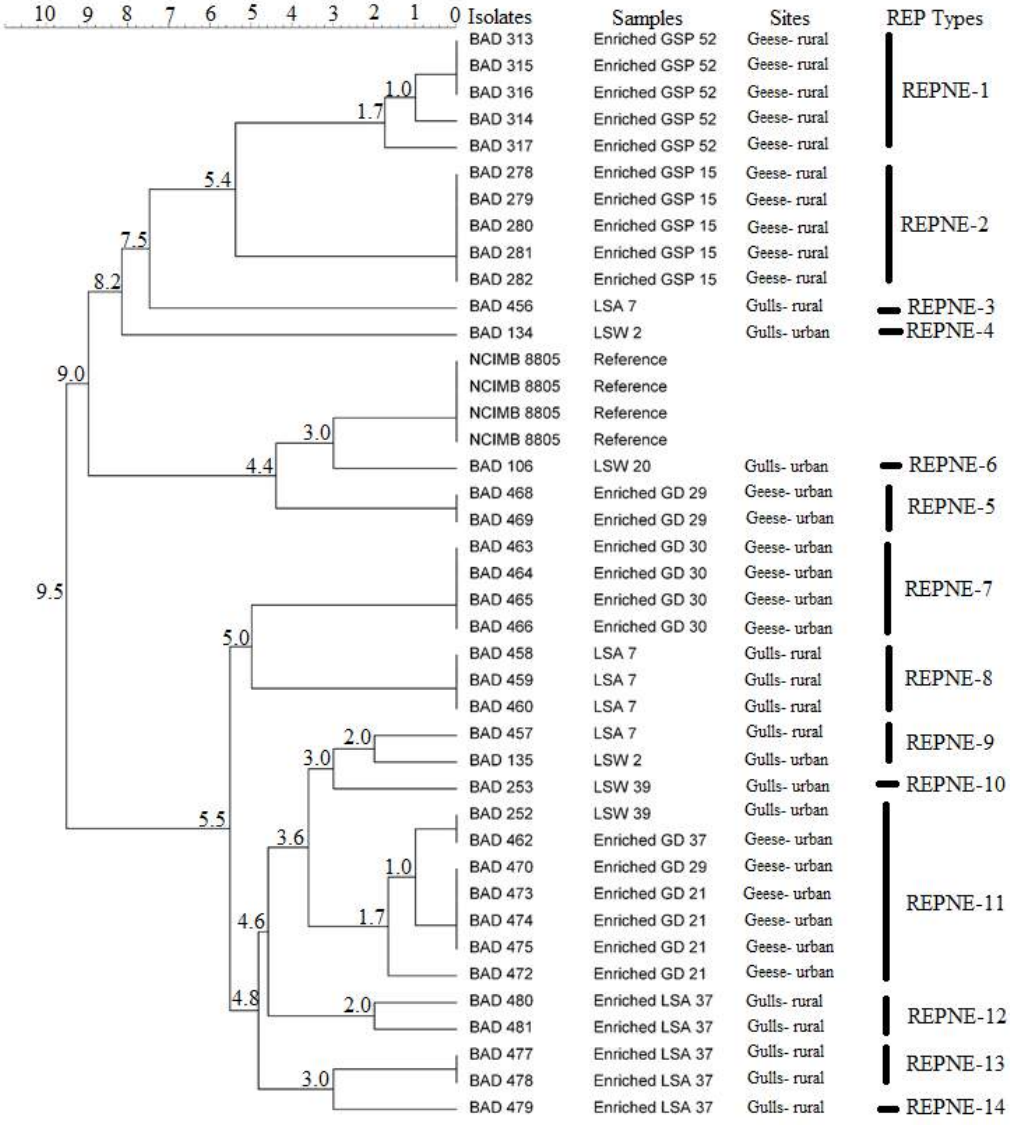


***Figure 7. The dendrogram of REP-PCR assay of presumptive ESBL-producer non-E. coli coliforms generated by BioNumerics.*** *Cluster analysis was done using the number of band difference coefficient and UPGMA method. Numbers at the nodes show the band difference. The equated similarity (%) is shown in Appendix 5.3.2 - Fig. S.20. The samples (isolate numbers and sites) and REP types (REPNE) are shown in the dendrogram. The axis line at the top (0-10) indicates the number of band difference. REP types have been assigned based on <3 bands difference rule.*

4.5.4 Representative REP Types Selected for Further Characterisation

Based on the REP-PCR typing data, a total of 60 isolates representing all REP types identified were selected for further characterisation. One isolate representing each REP type was selected from different bird samples and different sites, when possible. These included 12 REP types (designated REPR-1 to -12) of presumptive ESBL-producer *E. coli* (Table 22), 34 REP types (designated REPS-1 to -34) of presumptive ESBL sensitive *E. coli* (Table 23) and 14 REP types (designated REPNE-1 to -14) of presumptive ESBL-producer non-*E. coli* coliforms (Table 24).

***Table 22. Representative isolates of ESBL-producer E. coli selected for further characterisation.***

| Isolate reference numbers | Samples | Sites | REP types |
|---|---|---|---|
| BAD 86 | Enriched LSW 34 | Gull- urban | REPR-1 |
| BAD 338 | Enriched LSW 36 | Gull- urban | REPR-2 |
| BAD 121 | LSW 1 | Gull- urban | REPR-3 |
| BAD 453 | LSA 31 | Gulls- rural | REPR-4 |
| BAD 95 | LSW 11 | Gulls- urban | REPR-5 |
| BAD 103 | LSW 13 | Gulls- urban | REPR-6 |
| BAD 120 | LSW 30 | Gulls- urban | REPR-7 |
| BAD 100 | LSW 13 | Gulls- urban | REPR-8 |
| BAD 333 | Enriched LSW 34 | Gulls- urban | REPR-9 |
| BAD 110 | LSW 20 | Gulls- urban | REPR-10 |
| BAD 101 | LSW 13 | Gulls- urban | REPR-11 |
| BAD 350 | Enriched GS 14 | Geese- urban | REPR-12 |

***Table 23. Representative isolates of ESBL sensitive E. coli selected for further characterisation.***

| Isolate reference numbers | Samples | Sites | REP types |
|---|---|---|---|
| BAD 160 | GL 1 | Geese- urban | REPS-1 |
| BAD 154 | GS 1 | Geese- urban | REPS-2 |
| BAD 553 | GS 8 | Geese- urban | REPS-3 |
| BAD 387 | LSW 36 | Gulls- urban | REPS-4 |
| BAD 163 | GSP 3 | Geese- rural | REPS-5 |
| BAD 412 | LSW 42 | Gulls- urban | REPS-6 |
| BAD 158 | GL 1 | Geese- urban | REPS-7 |
| BAD 186 | GSP 50 | Geese- rural | REPS-8 |
| BAD 820 | LSA 37 | Gulls- rural | REPS-9 |
| BAD 446 | GD 45 | Geese- urban | REPS-10 |
| BAD 450 | G. Dun. 1 | Geese- urban | REPS-11 |
| BAD 156 | GS 3 | Geese- urban | REPS-12 |
| BAD 823 | LSA 37 | Gulls- rural | REPS-13 |
| BAD 198 | LSW 5 | Gulls- urban | REPS-14 |
| BAD 241 | LSW 20 | Gulls- urban | REPS-15 |
| BAD 821 | LSA 37 | Gulls- rural | REPS-16 |
| BAD 378 | LSW 34 | Gulls- urban | REPS-17 |
| BAD 238 | LSW 20 | Gulls- urban | REPS-18 |
| BAD 231 | LSW 15 | Gulls- urban | REPS-19 |
| BAD 237 | LSW 20 | Gulls- urban | REPS-20 |
| BAD 414 | LSW 42 | Gulls- urban | REPS-21 |
| BAD 842 | LSA 41 | Gulls- rural | REPS-22 |
| BAD 413 | LSW 42 | Gulls- urban | REPS-23 |
| BAD 576 | LSA 5 | Gulls- rural | REPS-24 |
| BAD 819 | LSA 37 | Gulls- rural | REPS-25 |
| BAD 370 | LSW 30 | Gulls- urban | REPS-26 |
| BAD 841 | LSA 41 | Gulls- rural | REPS-27 |
| BAD 196 | LSW 5 | Gulls- urban | REPS-28 |
| BAD 367 | LSW 30 | Gulls- urban | REPS-29 |
| BAD 194 | LSW 5 | Gulls- urban | REPS-30 |
| BAD 170 | LSW 1 | Gulls- urban | REPS-31 |
| BAD 227 | LSW 13 | Gulls- urban | REPS-32 |
| BAD 229 | LSW 13 | Gulls- urban | REPS-33 |
| BAD 369 | LSW 30 | Gulls- urban | REPS-34 |

***Table 24. Representative isolates of ESBL-producer non-E. coli coliforms selected for further characterisation.***

| Isolate reference numbers | Samples | Sites | REP types |
|---|---|---|---|
| BAD 315 | Enriched GSP 52 | Geese- rural | REPNE-1 |
| BAD 280 | Enriched GSP 15 | Geese- rural | REPNE-2 |
| BAD 456 | LSA 7 | Gulls- rural | REPNE-3 |
| BAD 134 | LSW 2 | Gulls- urban | REPNE-4 |
| BAD 469 | Enriched GD 29 | Geese- urban | REPNE-5 |
| BAD 106 | LSW 20 | Gulls- urban | REPNE-6 |
| BAD 465 | Enriched GD 30 | Geese- urban | REPNE-7 |
| BAD 459 | LSA 7 | Gulls- rural | REPNE-8 |
| BAD 135 | LSW 2 | Gulls- urban | REPNE-9 |
| BAD 253 | LSW 39 | Gulls- urban | REPNE-10 |
| BAD 252 | LSW 39 | Gulls- urban | REPNE-11 |
| BAD 480 | Enriched GD 21 | Geese- urban | REPNE-12 |
| BAD 478 | Enriched LSA 37 | Gulls- rural | REPNE-13 |
| BAD 479 | Enriched LSA 37 | Gulls- rural | REPNE-14 |

## 4.6 Species Identification of Presumptive *E. coli*

Forty-six presumptive *E. coli* (resistant = 12 and sensitive = 34) isolates from 23 different samples were identified to species level by genotypic (PCR assays and 16S rRNA) and phenotypic (Gram staining and indole tests) methods. Up till this point of the study, isolates have been given a presumptive species identification based on the colour of the colonies on Coliform *ChromoSelect* agar – blue colonies representing *E. coli*.

### 4.6.1 Genotypic Species Identification

The strategy for the genotypic species identification was first to use a duplex PCR assay which targets coliform-specific and *E. coli*-specific genes by Molina *et al.* (2015). However, the results were unexpected. Hence, two *E. coli*-specific (*gadA* and *uidA*) PCR assays were undertaken.

#### 4.6.1.1 Coliforms and *E. coli*-specific PCR assay - *lacZ3* and *yaiO* PCR Assay

This duplex PCR assay targets *lacZ* and *yaiO* genes. A primer named *lacZ3* was designed by Molina *et al*. (2015) to target the *lacZ* gene and is coliform-specific, whereas the *yaiO* primer is *E. coli*-specific gene. The *lacZ* gene encodes an enzyme named β-galactosidase which cleaves lactose to form glucose and galactose (Juers *et al.*, 2012),

while the *yaiO* gene encodes a protein found to be expressed and localised in the outer membrane of *E. coli* (a bona fide gene; Molina *et al.*, 2015)

Eighteen (resistant = 9, sensitive = 9) out of the 46 presumptive *E. coli* were either *lacZ3* negative, *yaiO* negative or both negative, indicating that a large number (39%) of the selected dark blue-to-violet colonies on Coliform *ChromoSelect* agar were not *E. coli* (Fig. 8 - ESBL-producer *E. coli*, Fig.9 - ESBL sensitive *E. coli*, Table 25). The other 28 isolates were all *lacZ3* and *yaiO* positive, indicating *E. coli*. Interestingly, one **ESBL-producer *E. coli*** (REPR-10) was *lacZ3* positive and *yaiO* negative, indicating it is a non-*E. coli* coliform (Fig. 8, lane 10). Two **ESBL sensitive *E. coli*** (Fig. 10, lane 27 and 44) were non-*E. coli* coliforms, and one ESBL sensitive *E. coli* was indicating *Shigella* sp. (*lacZ3* -, *yaiO* +) by this PCR assay (Fig.9, lane 18). Another two ESBL sensitive *E. coli* were *lacZ3* positive but had a very faint band at the expected band size of *yaiO* (Fig. 9, lane 20 and 21). This very faint band (*yaiO*) was not accepted as positive at this point of the study as the control *yaiO* band showed no faint bands. However, the two *E. coli*-specific PCR assays (section 4.6.1.2 and 4.6.1.3 below) confirmed these isolates were *E. coli*. The faint band at the expected band size of *lacZ3* (Fig. 9, lane 11 and 19) was accepted as positive because the isolates showed *yaiO* positive, indicating *E. coli*, and in addition this isolate was positive for the *E.coli*-specific PCR assays (*gadA* and *uidA* PCR assays, section 4.6.1.2 and 4.6.1.3 below).

Nonspecific bands (ranging in size from ~700 to more than 1000 bp) were observed in two presumptive ESBL-producer *E. coli* (Fig. 8, lane 9 and 14) and three presumptive ESBL sensitive *E. coli* (Fig. 9, lane 24, 26 and 40). These nonspecific bands were also seen on several other gels and PCR assays in this study (see below). The most probable reason for this is that the primers and PCR cycle protocol has been used as in the published paper without being optimised for the DNA extraction method, PCR reagent and equipment used in this study due to time limitation. Other than that, the nonspecific bands might be resulted from a poor integrity of the DNA templates as crude DNA extraction was used rather than the use of DNA extraction kits which yield a purer DNA sample (Korvin *et al.*, 2014). Different DNA extraction method affects the quality of DNA (Videnska *et al.*, 2019).

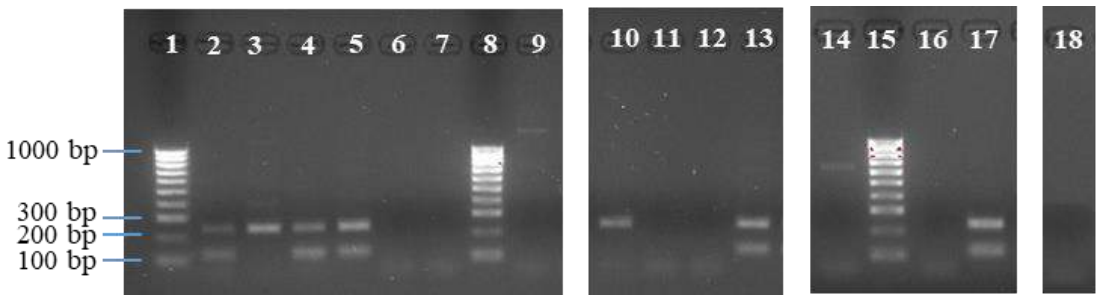


***Figure 8. The lacZ3 and yaiO PCR assay of presumptive ESBL-producer E. coli.*** *Lane 1, 8 and 15: Ladder 100 bp. Lane 2: E. coli ATCC® 47055 for E. coli control (lacZ3 - 234 bp and yaiO - 115 bp). Lane 3: K. pneumoniae NCIMB 8805 for coliform control. Lane 18: Sterile distilled water for negative control. Lane 4-7: REPR-1, REPR-5, REPR-8, REPR-11. Lane 9-14: REPR-6, REPR-10, REPR-7, REPR-3, REPR-9, REPR-2. Lane 16-17: REPR-12 and REPR-4.*

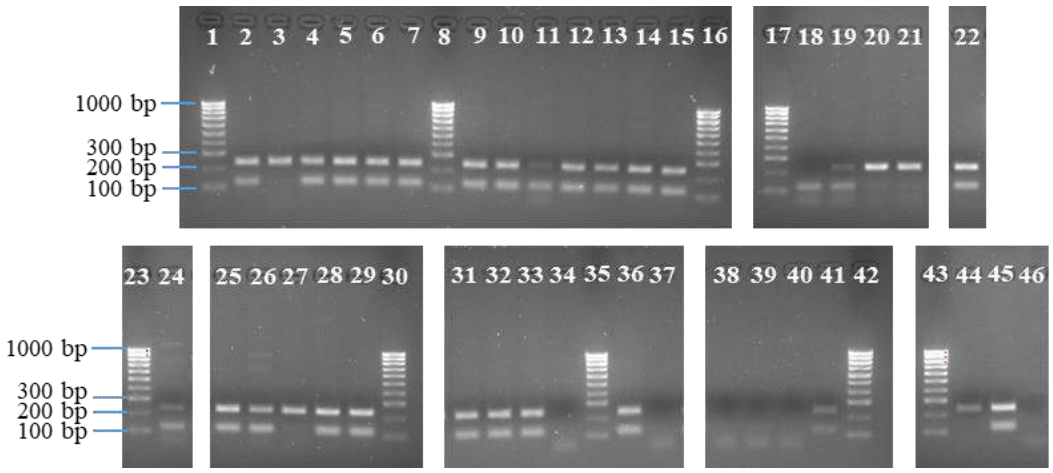


***Figure 9. The lacZ3 and yaiO PCR assay of presumptive ESBL sensitive E. coli.*** *Lane 1, 8, 16, 17, 23, 30, 35, 42 and 43: Ladder 100 bp. Lane 2: E. coli ATCC® 47055 for E. coli control (lacZ3 - 234 bp and yaiO - 115 bp). Lane 3: K. pneumoniae NCIMB 8805 for coliform control. Lane 46: Sterile distilled water for negative control. Lane 4-7: REPS-18, REPS-19, REPS-20, REPS-21. Lane 9-15: REPS-27, REPS-11, REPS-12, REPS-14, REPS-3, REPS-15, REPS-16. Lane 18-22: REPS-5, REPS-6, REPS-7, REPS-8, REPS-9. Lane 24-29: REPS-10, REPS-1, REPS-2, REPS-4, REPS-17, REPS-22. Lane 31-34: REPS-23, REPS-24, REPS-25, REPS-26. Lane 36-41: REPS-28, REPS-29, REPS-34, REPS-32, REPS-33, REPS-30. Lane 44-45: REPS-31 and REPS-13.*

4.6.1.2 *E. coli*-specific PCR assays

As a high number of the blue colonies (presumptive *E. coli*) were not *E. coli* by the duplex *lacZ3* and *yaiO* PCR assay, the species identification of *E. coli* isolates was confirmed with two *E. coli*-specific PCR assays- *gadA* and *uidA* PCR assays. Each PCR assay amplified different genes in *E. coli*, which could support or might contradict the findings from the *lacZ3* and *yaiO* PCR assay. The *gadA* PCR assay is targeting the *E. coli* glutamate decarboxylase-alpha gene, and the *uidA* PCR assay is targeting the *uidA* gene which encodes β-glucuronidase and relatively specific to *E. coli*.

***gadA* PCR Assay**. The 28 *E. coli* confirmed by the duplex PCR assay were *gadA* positive, indicating *E. coli* (Fig.10 – ESBL-producer, Fig.11 – ESBL sensitive, Table 25). An additional of five isolates (1 resistant and 4 sensitive) which indicated as non-*E. coli* in the duplex PCR assay were confirmed as *E. coli* by *gadA* positive, adding the number of confirmed *E. coli* to 33.

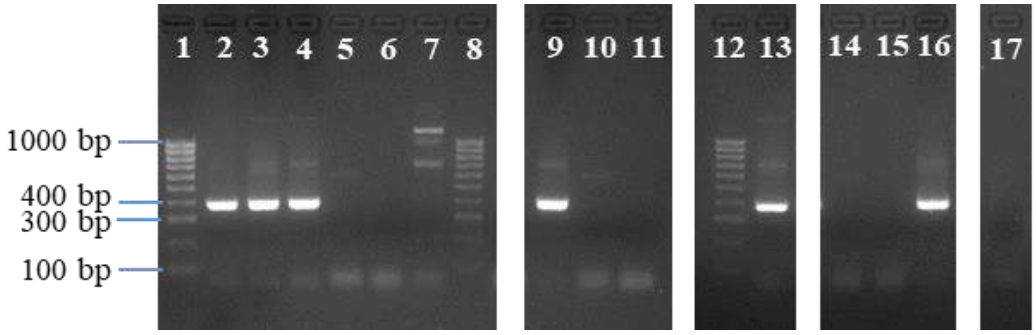


***Figure 10. The gadA PCR assay of presumptive ESBL-producer E. coli.*** *Lane 1, 8 and 12: Ladder 100 bp. Lane 2: E. coli ATCC® 47055 for positive control (gadA – 373 bp). Lane 17: Sterile distilled water for negative control. Lane 3-7: REPR-1, REPR-5, REPR-8, REPR-11, REPR-6. Lane 9-11: REPR-10, REPR-7, REPR-3. Lane 13-16: REPR-9, REPR-2, REPR-12, REPR-4.*

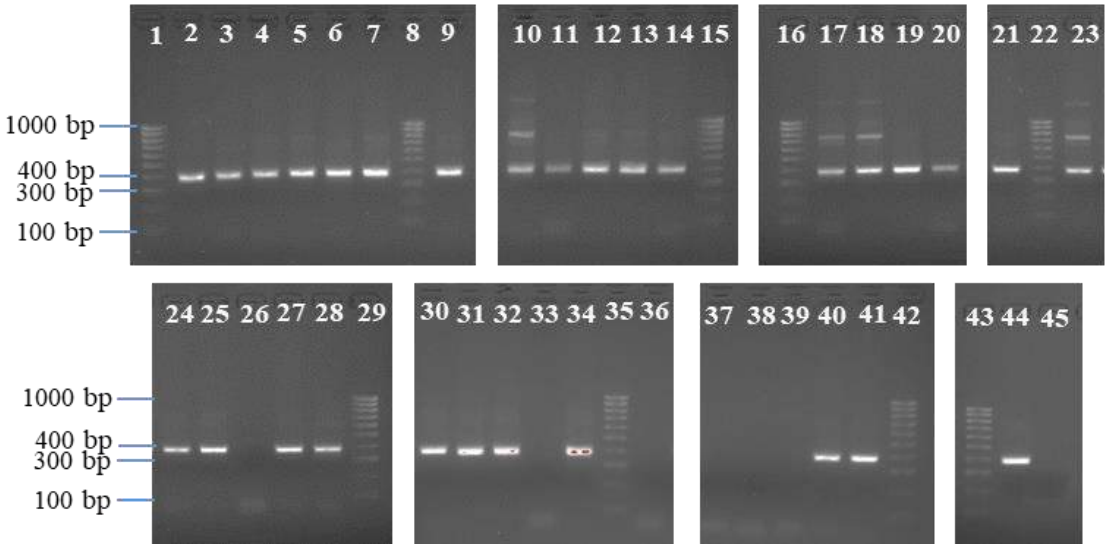


***Figure 11. The gadA PCR assay of presumptive ESBL sensitive E. coli.*** *Lane 1, 8, 15, 16, 22, 29, 35, 42 and 43: Ladder 100 bp. Lane 2: E. coli ATCC® 47055 for positive control (gadA – 373 bp). Lane 45: Sterile distilled water for negative control. Lane 3-7: REPS-18, REPS-19, REPS-20, REPS-21, REPS-27. Lane 9-14: REPS-11, REPS-12, REPS-14, REPS-3, REPS-15, REPS-16. Lane 17-21: REPS-5, REPS-6, REPS-7, REPS-8, REPS-9. Lane 23-28: REPS-10, REPS-1, REPS-2, REPS-4, REPS-17, REPS-22. Lane 30-34: REPS-23, REPS-24, REPS-25, REPS-26, REPS-28. Lane 36-41: REPS-29, REPS-34, REPS-32, REPS-33, REPS-30, REPS-31. Lane 44: REPS-13.*

***uidA* PCR Assay**. The findings from the second *E. coli*-specific PCR assays (*uidA* PCR assay) aligned with the finding from the *gadA* PCR assay, in which 33 out of 46 presumptive *E. coli* (72%) were confirmed as *E. coli* (Fig.12 – ESBL-producer, Fig.13 – ESBL sensitive, Table 25). Multiple nonspecific bands were observed in one ESBL-producer *E. coli*, with one very faint band at the expected band size of *uidA* (Fig. 12, lane 9). This faint band was not accepted as positive due to the presence of multiple bands, and this was confirmed by the duplex and *gadA* PCR assays as non-*E. coli* and confirmed by 16S rRNA sequencing below as *Enterococci* (section 4.6.3).

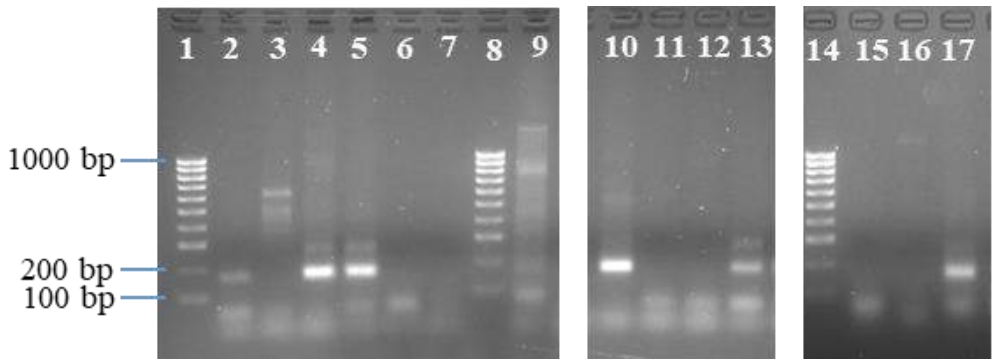


***Figure 12. The uidA PCR assay of presumptive ESBL-producer E. coli.*** *Lane 1, 8 and 14: Ladder 100 bp. Lane 2: E. coli ATCC® 47055 for E. coli control (uidA – 162 bp). Lane 3: K. pneumoniae NCIMB 8805 for negative control strain. Lane 4-7: REPR-1, REPR-5, REPR-8, REPR-11. Lane 9-13: REPR-6, REPR-10, REPR-7, REPR-3, REPR-9. Lane 15-17: REPR-2, REPR-12, REPR-4.*

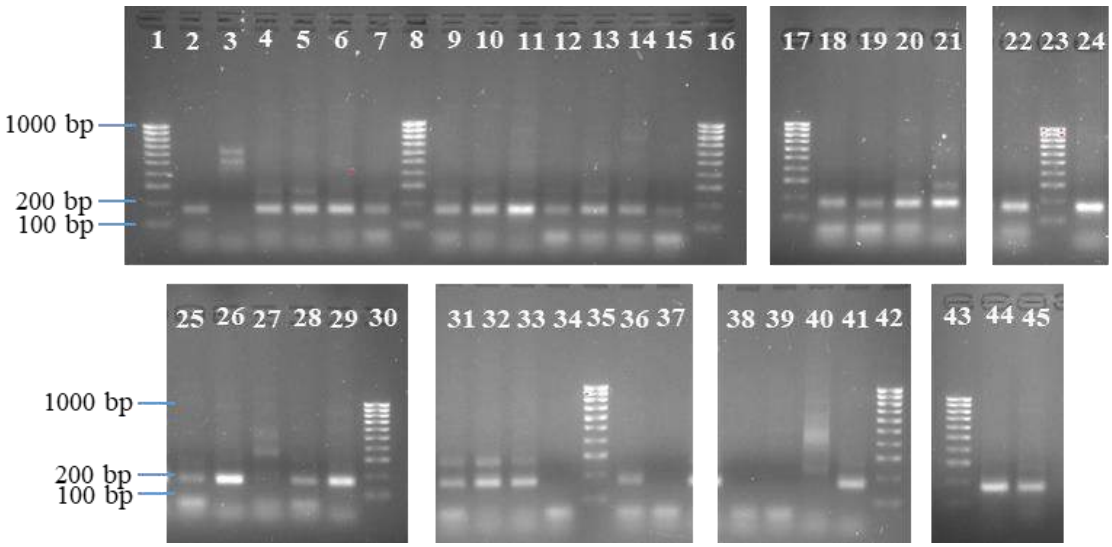


***Figure 13. The uidA PCR assay of presumptive ESBL sensitive E. coli.*** *Lane 1, 8, 16, 17, 23, 30, 35, 42 and 43: Ladder 100 bp. Lane 2: E. coli ATCC® 47055 for E. coli control (uidA - 162 bp). Lane 3: K. pneumoniae NCIMB 8805 for negative control strain. Lane 4-7: REPS-18, REPS-19, REPS-20, REPS-21. Lane 9-15: REPS-27, REPS-11, REPS-12, REPS-14, REPS-3, REPS-15, REPS-16. Lane 18-22: REPS-5, REPS-6, REPS-7, REPS-8, REPS-9. Lane 24-29: REPS-10, REPS-1, REPS-2, REPS-4, REPS-17, REPS-22. Lane 31-34: REPS-23, REPS-24, REPS-25, REPS-26. Lane 36-41: REPS-28, REPS-29, REPS-34, REPS-32, REPS-33, REPS-30. Lane 44-45: REPS-31, REPS-13.*

### 4.6.2 Phenotypic Species Identification

The PCR assays showed interesting findings, which only 33 out of the 46 blue colonies on Coliform *ChromoSelect* agar were confirmed as *E. coli* (Table 25). To investigate this, the Gram staining and the indole test were undertaken. The Gram staining was used to confirm that the isolates were Gram-negative, rod shaped, arranged singly or in pairs as expected of *E. coli* (PHE, 2019; Rompré *et al.*, 2002). The Gram staining found that 41 of the 46 presumptive *E. coli* (89%) were as expected. The other five (11%), which were *lacZ3*, *yaiO*, *gadA* and *uidA* negative, were Gram stained purple, indicating as Gram-positive bacteria. These five isolates were also cocci (round-shaped) and arranged in the form of short chains. To further identify the coliform bacteria, the indole test, one of the IMViC tests (Indole production, Methyl-red reaction, the Voges-Proskauer reaction and utilisation of citrate; Parr, 1936) was performed as it is relatively easy to undertake. In the indole test, which measures the ability of isolates to produce indole from tryptophan, most of the *E. coli* strains (96 – 98%) are indole positive whereas other coliforms vary. In this study, 25 (76%) of the 33 confirmed *E. coli* were as expected, with the other 8 (24%) were indole negative.

***Table 25. Confirmation of species identification of presumptive E. coli.*** *Positive results are highlighted in yellow colour. Gram positive are highlighted in purple colour. REPR type indicates ESBL-producer E. coli. REPS type indicates ESBL sensitive E. coli.*

| Isolate reference numbers | Samples | Sites | REP types | Gram stain | Indole test | *lacZ3* | *yaiO* | *gadA* | *uidA* |
|---|---|---|---|---|---|---|---|---|---|
| BAD 86 | LSW 5 | Gulls- urban | REPR-1 | Negative | + | + | + | + | + |
| BAD 453 | LSA 31 | Gulls- rural | REPR-4 | Negative | - | + | + | + | + |
| BAD 95 | LSW 11 | Gulls- urban | REPR-5 | Negative | + | + | + | + | + |
| BAD 333 | Enriched LSW 34 | Gulls- urban | REPR-9 | Negative | + | + | + | + | + |
| BAD 110 | LSW 20 | Gulls- urban | REPR-10 | Negative | - | + | - | + | + |
| BAD 160 | GL 1 | Geese- urban | REPS-1 | Negative | + | + | + | + | + |
| BAD 154 | GS 1 | Geese- urban | REPS-2 | Negative | + | + | + | + | + |
| BAD 553 | GS 8 | Geese- urban | REPS-3 | Negative | + | + | + | + | + |
| BAD 163 | GSP 3 | Geese- rural | REPS-5 | Negative | - | - | + | + | + |
| BAD 412 | LSW 42 | Gulls- urban | REPS-6 | Negative | + | + | + | + | + |
| BAD 158 | GL 1 | Geese- urban | REPS-7 | Negative | + | + | - | + | + |
| BAD 186 | GSP 50 | Geese- rural | REPS-8 | Negative | + | + | - | + | + |
| BAD 820 | LSA 37 | Gulls- rural | REPS-9 | Negative | + | + | + | + | + |
| BAD 446 | GD 45 | Geese- urban | REPS-10 | Negative | - | + | + | + | + |
| BAD 450 | G. Dun. 1 | Geese- urban | REPS-11 | Negative | + | + | + | + | + |
| BAD 156 | GS 3 | Geese- urban | REPS-12 | Negative | - | + | + | + | + |
| BAD 823 | LSA 37 | Gulls- rural | REPS-13 | Negative | + | + | + | + | + |
| BAD 198 | LSW 5 | Gulls- urban | REPS-14 | Negative | + | + | + | + | + |
| BAD 241 | LSW 20 | Gulls- urban | REPS-15 | Negative | + | + | + | + | + |
| BAD 821 | LSA 37 | Gulls- rural | REPS-16 | Negative | + | + | + | + | + |
| BAD 378 | LSW 34 | Gulls- urban | REPS-17 | Negative | + | + | + | + | + |
| BAD 238 | LSW 20 | Gulls- urban | REPS-18 | Negative | + | + | + | + | + |
| BAD 231 | LSW 15 | Gulls- urban | REPS-19 | Negative | + | + | + | + | + |
| BAD 237 | LSW 20 | Gulls- urban | REPS-20 | Negative | + | + | + | + | + |
| BAD 414 | LSW 42 | Gulls- urban | REPS-21 | Negative | + | + | + | + | + |
| BAD 842 | LSA 41 | Gulls- rural | REPS-22 | Negative | + | + | + | + | + |
| BAD 413 | LSW 42 | Gulls- urban | REPS-23 | Negative | - | + | + | + | + |
| BAD 576 | LSA 5 | Gulls- rural | REPS-24 | Negative | + | + | + | + | + |
| BAD 819 | LSA 37 | Gulls- rural | REPS-25 | Negative | - | + | + | + | + |
| BAD 841 | LSA 41 | Gulls- rural | REPS-27 | Negative | + | + | + | + | + |
| BAD 196 | LSW 5 | Gulls- urban | REPS-28 | Negative | + | + | + | + | + |
| BAD 194 | LSW 5 | Gulls- urban | REPS-30 | Negative | - | + | + | + | + |
| BAD 170 | LSW 1 | Gulls- urban | REPS-31 | Negative | + | + | - | + | + |
| BAD 387 | LSW 36 | Gulls- urban | REPS-4 | Negative | - | + | - | - | - |
| BAD 338 | Enriched LSW 36 | Gulls- urban | REPR-2 | Negative | + | - | - | - | - |
| BAD 121 | LSW 1 | Gulls- urban | REPR-3 | Negative | + | - | - | - | - |
| BAD 120 | LSW 30 | Gulls- urban | REPR-7 | Negative | + | - | - | - | - |
| BAD 100 | LSW 13 | Gulls- urban | REPR-8 | Negative | + | - | - | - | - |
| BAD 101 | LSW 13 | Gulls- urban | REPR-11 | Negative | + | - | - | - | - |
| BAD 350 | Enriched GS 14 | Geese- urban | REPR-12 | Negative | + | - | - | - | - |
| BAD 229 | LSW 13 | Gulls- urban | REPS-33 | Negative | + | - | - | - | - |
| BAD 103 | LSW 13 | Gulls- urban | REPR-6 | Positive | - | - | - | - | - |
| BAD 370 | LSW 30 | Gulls- urban | REPS-26 | Positive | - | - | - | - | - |
| BAD 367 | LSW 30 | Gulls- urban | REPS-29 | Positive | - | - | - | - | - |
| BAD 227 | LSW 13 | Gulls- urban | REPS-32 | Positive | - | - | - | - | - |
| BAD 369 | LSW 30 | Gulls- urban | REPS-34 | Positive | - | - | - | - | - |

### 4.6.3 16S rRNA Sequencing of "non-*E. coli*" Blue Colonies

Based on the PCR assays, Gram staining and indole test, 13 (28%) of the 46 presumptive *E. coli* were identified as non-*E. coli* isolates. Five of these 13 "non-*E. coli*"

blue colonies on Coliform *ChromoSelect* agar were identified as Gram-positive bacteria, and the other 8 were identified as Gram-negative bacteria. Hence, these 13 isolates were tested by 16S rRNA sequencing for a definitive identification (Petti *et al.*¸2005). 16S rRNA sequencing is a "gold standard" for species identification as it is accurate and far superior method for bacterial identification (Abayasekara *et al.*¸2017; Clarridge, 2004). In this study, ≥ 99% sequence-similarity indicates the species (Johnson *et al.*, 2019), 97 to < 99% similarity indicates the genus (Han, 2006) and < 97% similarity indicates a poor-quality sequence and regarded as not reliable (Welinder-Olsson *et al.*, 2007). Chromas software was used to trim the low-quality sequence using their default parameters.

Three Gram-positive bacteria were identified as *Enterococcus* bacteria (i.e. *E. mundtii*, *E. hirae* and *E. faecalis*; Table 26). Although the poor-quality bases were trimmed at both ends using Chromas default parameters prior to the comparison of the database, two isolates (REPS-26 and -34) still showed a low similarity (91.19% and 94.54%). Based on their chromatograms, the poor-quality sequence might be resulted from the baseline noise which was not trimmed by the software (REPS-26 - Appendix 7.9, Fig. S.51 and S.53, REPS-34 – Appendix 7.13, Fig. S.67 and S.69). This was also observed in all low sequence-similarity below. These findings from 16S rRNA sequencing were not reliable to be reported (Welinder-Olsson *et al.*, 2007), and it was unfortunately not possible to redo the 16S rRNA sequencing on isolates with low similarity due to time constrains. Hence, the finding from the Gram staining was used instead.

Seven of the 8 Gram-negative bacteria were identified as non-*E. coli Escherichia* sp. (n = 5, i.e. *E. fergusonii*), *Salmonella* sp. (n = 1) and *Buttiauxella* sp. (n = 1), whereas the other one (REPR-11) showed a low similarity (95.10%; Appendix 7.6); hence, it was reported as "Gram-negative" instead. As the 16S rRNA sequencing identified these 13 "non-*E. coli*" blue colonies as non-*E. coli*, this confirmed the findings from the *gadA* and *uidA* PCR assay. The *E. coli*-specific assays and 16S rRNA sequencing also confirmed that Coliform *ChromoSelect* agar gave discrepant result, with 28% of false-positive.

***Table 26. 16S rRNA sequencing of "non-E. coli" blue colonies on Coliform ChromoSelect agar.*** *Analysis was performed using BLASTn algorithm and 16S ribosomal RNA sequences (Bacteria and Archaea) database. Species identification and similarity (%) of the isolates are highlighted in yellow. Similarity (%) to E. coli strain is also shown. Appendix 7 provides the chromatograms and the FASTA sequences. REPR = resistant isolates, REPS = sensitive isolates, "-" = poor-quality sequence.*

| Isolate reference numbers | Samples | Sites | REP types | 16S Species Identification | Similarity (%) |
|---|---|---|---|---|---|
| BAD 103 | LSW 13 | Gulls- urban | REPR-6 | *Enterococcus mundtii* | 99.15 |
| BAD 367 | LSW 30 | Gulls- urban | REPS-29 | *Enterococcus hirae* | 99.18 |
| BAD 227 | LSW 13 | Gulls- urban | REPS-32 | *Enterococcus faecalis* | 99.12 |
| BAD 370 | LSW 30 | Gulls- urban | REPS-26 | - | 94.54 |
| BAD 369 | LSW 30 | Gulls- urban | REPS-34 | - | 91.19 |
| BAD 100 | LSW 13 | Gulls- urban | REPR-8 | *Escherichia fergusonii* | 99.28 |
| | | | | Similarity to *E. coli* : 99.10% | |
| BAD 120 | LSW 30 | Gulls- urban | REPR-7 | *Escherichia* sp. | 98.83 |
| | | | | Similarity to *E. coli* : 98.48% | |
| BAD 121 | LSW 1 | Gulls- urban | REPR-3 | *Escherichia* sp. | 98.76 |
| | | | | Similarity to *E. coli* : 98.41% | |
| BAD 338 | Enriched LSW 36 | Gulls- urban | REPR-2 | *Escherichia* sp. | 97.95 |
| | | | | Similarity to *E. coli* : 97.42% | |
| BAD 350 | Enriched GS 14 | Geese- urban | REPR-12 | *Escherichia* sp. | 97.74 |
| | | | | Similarity to *E. coli* : 97.57% | |
| BAD 101 | LSW 13 | Gulls- urban | REPR-11 | - | 95.10 |
| | | | | Similarity to *E. coli* : 94.72% | |
| BAD 387 | LSW 36 | Gulls- urban | REPS-4 | *Buttiauxella* sp. | 97.94 |
| | | | | Similarity to *E. coli* : 94.38% | |
| BAD 229 | LSW 13 | Gulls- urban | REPS-33 | *Salmonella* sp. | 98.02 |
| | | | | Similarity to *E. coli* : 97.97% | |

## 4.7 Species Identification of Presumptive ESBL-Producer Non-*E. coli* Coliforms

In addition to the 46 blue colonies on Coliform *ChromoSelect* agar (presumptive *E. coli*) selected for further identified and characterised above (section 4.6), 14 red colonies from 11 samples on Coliform *ChromoSelect* agar (presumptive non-*E. coli* coliforms) were selected and identified in this section. Similar approach was undertaken, with only the duplex PCR assay was performed. This duplex PCR assay, phenotypic species identification and 16S rRNA sequencing would also confirm the species of 13 of the 14 presumptive non-*E. coli* coliforms which grew with metallic green sheen on Eosin Methylene Blue agar (indicating *E. coli*, section 4.3).

4.7.1 Genotypic Species Identification

4.7.1.1 Coliforms and *E. coli*-specific PCR assay - *lacZ3* and *yaiO* PCR Assay

Only one (REPNE-6; Fig. 14, lane 17) out of the 14 presumptive non-*E. coli* coliform isolates (7%) was identified as a coliform (*lacZ3* positive and *yaiO* negative). This indicates that almost all (93%) of the selected red colonies on Coliform *ChromoSelect* agar were not as expected (Fig. 14, Table 27). Nonetheless, all the 14 red colonies were confirmed as non-*E. coli* by *yaiO* negative, as expected.


***Figure 14. The lacZ3 and yaiO PCR assay on presumptive non-E. coli coliforms.*** *Lane 1, 7, 15 and 16: Ladder 100 bp. Lane 2: E. coli ATCC® 47055 for E. coli control (lacZ3 - 234 bp and yaiO - 115 bp). Lane 3: K. pneumoniae NCIMB 8805 for coliform control. Lane 21: Sterile distilled water for negative control. Lane 4-6: REPNE-2, REPNE-1, REPNE-3. Lane 8-14: REPNE-8, REPNE-7, REPNE-5, REPNE-11, REPNE-12, REPNE-13, REPNE-14. Lane 17-20: REPNE-6, REPNE-4, REPNE-9, REPNE-10.*

4.7.2 Phenotypic Species Identification

As performed on the presumptive *E. coli* isolates, the Gram staining was used to confirm that the isolates were Gram-negative and rod shaped as also expected of non-*E. coli* coliforms (PHE, 2019; Rompré *et al.*, 2002). Three (21%) out of the 14 presumptive non-*E. coli* coliforms with *lacZ3* and *yaiO* negative were Gram-positive cocci (round-shaped) and arranged in short chains (Table 27). As found above in section 4.6, this finding was unexpected as Coliform *ChromoSelect* agar is reported to be selective for coliforms. The other 11 isolates were Gram-negative rods, as expected. From the indole test, all the ESBL-producer non-*E. coli* coliforms were indole negative.

***Table 27. Confirmation of species identification of presumptive ESBL-producer non-E. coli coliforms.*** *Positive results are highlighted in yellow colour. Gram positive are highlighted in purple colour.*

| Isolate Reference Number | Samples | Sites | REP types | Gram stain | Indole test | *lacZ3* | *yaiO* |
|---|---|---|---|---|---|---|---|
| BAD 315 | Enriched GSP 52 | Geese- rural | REPNE-1 | Positive | - | - | - |
| BAD 134 | LSW 2 | Gulls- urban | REPNE-4 | Positive | - | - | - |
| BAD 478 | Enriched LSA 37 | Gulls- rural | REPNE-13 | Positive | - | - | - |
| BAD 106 | LSW 20 | Gulls- urban | REPNE-6 | Negative | - | + | - |
| BAD 469 | Enriched GD 29 | Geese- urban | REPNE-5 | Negative | - | - | - |
| BAD 465 | Enriched GD 30 | Geese- urban | REPNE-7 | Negative | - | - | - |
| BAD 459 | LSA 7 | Gulls- rural | REPNE-8 | Negative | - | - | - |
| BAD 135 | LSW 2 | Gulls- urban | REPNE-9 | Negative | - | - | - |
| BAD 253 | LSW 39 | Gulls- urban | REPNE-10 | Negative | - | - | - |
| BAD 252 | LSW 39 | Gulls- urban | REPNE-11 | Negative | - | - | - |
| BAD 480 | Enriched GD 21 | Geese- urban | REPNE-12 | Negative | - | - | - |
| BAD 479 | Enriched LSA 37 | Gulls- rural | REPNE-14 | Negative | - | - | - |
| BAD 280 | Enriched GSP 15 | Geese- rural | REPNE-2 | Negative | - | - | - |
| BAD 456 | LSA 7 | Gulls- rural | REPNE-3 | Negative | - | - | - |

### 4.7.3 16S rRNA Sequencing of Non-*E. coli* Red Colonies

16S rRNA sequencing was performed on all the 14 non-*E. coli* red colonies on Coliform *ChromoSelect* agar for a definitive identification. The same thresholds to analyse the results were used (section 4.6.3). Two of the three Gram-positive bacteria were identified as *Enterococcus* sp., whereas the other one (REPNE-4) showed a low similarity (94.82%; Table 28; Appendix 7.17); hence it was reported as "Gram-positive" instead. Nine of the 11 Gram-negative bacteria were identified as non-*E. coli Escherichia* sp. (n = 8) and *Serratia* sp. (n = 1). The other two (REPNE-6 and -14) were reported as "Gram-negative" bacteria due to low similarity (Appendix 7.19 and 7.27, respectively). *Klebsiella* sp. was the closest identified genus for the REPNE-6 isolate (0.03% difference to the 97% cut-off) and non-*E. coli Escherichia* sp. was the closest identified genus for the REPNE-14 isolate (0.31% difference to the 97% cut-off). The 16S rRNA sequencing confirmed that Eosin Methylene Blue agar gave discrepant results of non-*E. coli* coliform isolate; all the 11 REPNE isolates that grew with metallic green sheen on Eosin Methylene Blue agar (suggesting *E. coli*) were confirmed as non-*E. coli* (Appendix 6 - Table S.23).

***Table 28. 16S rRNA sequencing of ESBL-producer "non-E. coli" red colonies on Coliform ChromoSelect agar.*** *Analysis was performed using BLASTn algorithm and 16S ribosomal RNA sequences (Bacteria and Archaea) database. Species identification and similarity (%) of the isolates are shown. Appendix 7 provides the chromatograms and the FASTA sequences.*

| Isolate reference numbers | Samples | Sites | REP types | 16S Species Identification | Similarity (%) |
|---|---|---|---|---|---|
| BAD 478 | Enriched LSA 37 | Gulls- rural | REPNE-13 | *Enterococcus* sp. | 98.86 |
| BAD 315 | Enriched GSP 52 | Geese- rural | REPNE-1 | *Enterococcus* sp. | 98.79 |
| BAD 134 | LSW 2 | Gulls- urban | REPNE-4 | - | 94.82 |
| BAD 253 | LSW 39 | Gulls- urban | REPNE-10 | *Escherichia* sp. | 98.91 |
| BAD 469 | Enriched GD 29 | Geese- urban | REPNE-5 | *Escherichia* sp. | 98.84 |
| BAD 456 | LSA 7 | Gulls- rural | REPNE-3 | *Escherichia* sp. | 98.73 |
| BAD 480 | Enriched GD 21 | Geese- urban | REPNE-12 | *Escherichia* sp. | 98.60 |
| BAD 135 | LSW 2 | Gulls- urban | REPNE-9 | *Escherichia* sp. | 98.48 |
| BAD 252 | LSW 39 | Gulls- urban | REPNE-11 | *Escherichia* sp. | 98.47 |
| BAD 465 | Enriched GD 30 | Geese- urban | REPNE-7 | *Escherichia* sp. | 98.13 |
| BAD 459 | LSA 7 | Gulls- rural | REPNE-8 | *Escherichia* sp. | 98.05 |
| BAD 280 | Enriched GSP 15 | Geese- rural | REPNE-2 | *Serratia* sp. | 98.86 |
| BAD 479 | Enriched LSA 37 | Gulls- rural | REPNE-14 | - | 96.69 |
| BAD 106 | LSW 20 | Gulls- urban | REPNE-6 | - | 96.97 |

## 4.8 Strain Diversity of *E. coli*

The second objective of this study was to determine the diversity of *E. coli* in bird populations. In this section, *E. coli* strain diversity between and within bird populations was assessed. The strain diversity was measured by two DNA fingerprinting methods: REP-PCR assay and Pulsed-Field Gel Electrophoresis (PFGE).

### 4.8.1 REP-PCR Assay Strain Diversity

By the species identification PCR assays, 33 of the 46 blue colonies on Coliform *ChromoSelect* agar were confirmed as *E. coli*. These 33 *E. coli* (resistant = 5, sensitive = 28) REP patterns were re-analysed using BioNumerics software to re-assess the strain diversity of *E. coli* within and between bird samples as in section 4.5 above. All the confirmed non-*E. coli* isolates were excluded from this re-analysis. To differentiate the analysis of these confirmed *E. coli* isolates to the previous analysis (section 4.5, presumptive *E. coli*), rep types were differently labelled as *E. coli* REP type (A-AG).

Thirty-three *E. coli* REP types were observed from this re-analysis (Fig.15; Appendix 8 – Fig. S.127 shows nodes as % similarity). The dendrogram shows no identical REP types (based on ≤ 3 bands difference rule) between *E. coli* resistant (n = 5) and sensitive

(n = 28) isolates. Between bird populations, four (12%) of the 33 *E. coli* REP type were shared between isolates from geese in the urban site and gulls in the rural site (n =2, type N and M) and between isolates from geese in the urban and rural sites (n = 2, type J and L) (Appendix 8, Table S.24). The other 29 (88%) shared no REP types between birds and sites.

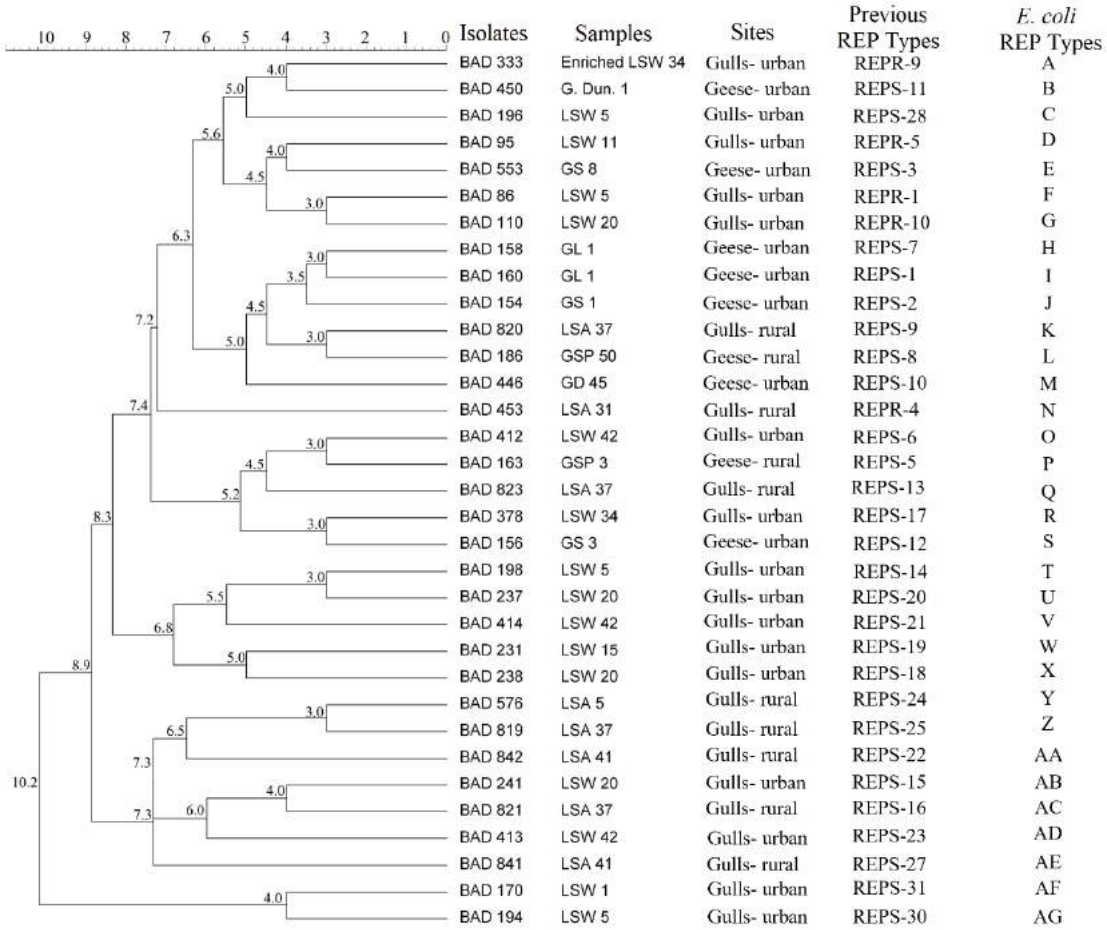


***Figure 15. The dendrogram of the confirmed E. coli isolates (resistant and sensitive) in gulls and geese.*** *Cluster analysis was done using the number of band difference coefficient and UPGMA method. Numbers at the nodes show the band difference (Appendix 8 – Fig. S.127 shows the nodes as % similarity). The REP types from these confirmed E. coli were labelled type A – AG to differentiate the analysis of presumptive E. coli (section 4.5, REPR and REPS types). The samples (isolate numbers and sites), previous analysis REP types (section 4.5) and E. coli REP types are shown in the dendrogram. The axis line at the top (0-10) indicates the number of band difference. REP types have been assigned based on <3 bands difference rule.*

The strain-richness (different *E. coli* REP types within sample) within 25 bird faeces (gulls = 12 and geese = 13) was moderate, with 64% of the samples exhibited one *E. coli* REP types and 12% exhibited four types (Table 29). None of the bird samples exhibited five *E. coli* REP types within one sample. From 12 gull faecal samples (urban = 7 and rural= 5), the strain-richness was high, with 42% of gull faeces exhibited one *E. coli* REP types, 17% each exhibited two and three types and 25% exhibited four types (Appendix 8.1 - Table S.25). Within the gull sites, more richness was observed from the urban site (29% each exhibited one, three and four types, and 14% exhibited two types) compared to the rural site (60% exhibited one types, and 20% exhibited two and four types) (Appendix 8.1 - Table S.26).

Compared to the gulls, the strain-richness in 13 goose faeces (urban = 9 and rural = 4) was lower, with 85% of them exhibited only one *E. coli* REP types and 15% exhibited two types (Appendix 8.2 - Table S.27). Similar to the findings on the gull sites, more strain-richness was observed from geese in the urban site (78% exhibited one type and 22% exhibited two types) compared to the rural site (100% exhibited one type) (Appendix 8.2 - Table S.28).

***Table 29. Strain-richness of confirmed E. coli within the birds.*** *Gulls = 12 faecal samples, geese = 13 faecal samples. The percentage was determined by a calculation (number of samples/total number of samples multiplied by 100). Appendix 8 provides details of the strain-richness within each sample.*

| Strain-richness within sample | Number of samples |
|---|---|
| 1 *E. coli* REP type | 16 (64%) |
| 2 *E. coli* REP types | 4 (16%) |
| 3 *E. coli* REP types | 2 (8%) |
| 4 *E. coli* REP types | 3 (12%) |
| 5 *E. coli* REP types | - |

4.8.2 Pulsed-Field Gel Electrophoresis (PFGE) of ESBL-producer *E. coli*

PFGE has been described as a strain typing method with moderate to high discriminatory power and a high rate of relative repeatability and reproducibility (Foxman *et al.*, 2005). Herrero-Fresno *et al.* (2017) has suggested that a rep-PCR typing followed by PFGE analysis is a good approach to assessing diversity and to study the relationship between rep-PCR patterns. Due to time limitation, PFGE was undertaken only on five of the *E. coli* REP types (Resistant strains, type A, D, F, G and N). PFGE gave similar results

in dividing the five REP types into five PFGE types (Fig. 16). These unique PFGE types were different from each other by 6-13 bands.

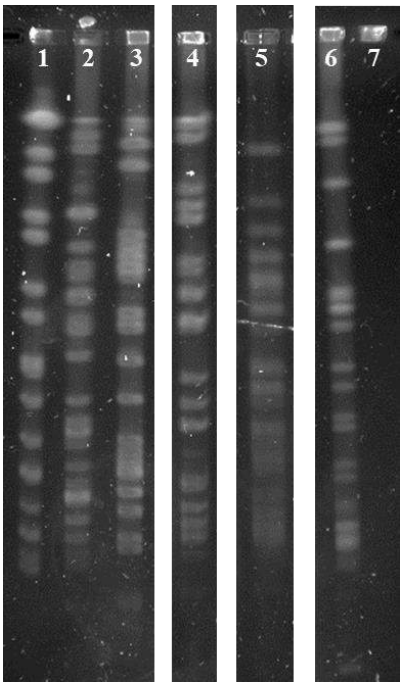


***Figure 16. PFGE analysis of ESBL-producer E. coli.*** *Lane 1: Salmonella braenderup reference ladder. Lane 7: Sterile distilled water for negative control. Lane 2-6: REPR-1, REPR-5, REPR-10, REPR-9 and REPR-4.*

## 4.9 Clonal Analysis of *E. coli* Strains/Types

The extensive MLST data sets of *E.* coli have improved the understanding of the genetic substructure of *E. coli*, and the MLST provides further insight of clonal complexes. This has resulted in the development and validation of a PCR assay which rapidly assigns *E. coli* isolates into different phylo-groups based on the presence and/or absence of four fragment markers (*arpA*, *chuA*, *yjaA* and TspE4.C2). Each defined phylo-group comprises numerous individual STs (Clermont *et al.*, 2013). The phylogenetic analyses have also established the link between phylogenetic group and virulence (Picard *et al.*, 1999). The clonal analysis of isolates from wild birds (as environmental compartment) in this study would allow the comparison to clinical setting data.

### 4.9.1 *E. coli* Phylo-grouping

To understand further the diversity of *E. coli* in bird samples, the Clermont phylo-typing method (PCR assay) was used to assign the 33 confirmed *E. coli* isolates (resistant = 5, sensitive = 28) into one of the recognised *E. coli sensu stricto* phylo-groups (A, B1,

B2, C, D, E, F) or the *Escherichia* cryptic clades (I to V). Four different PCR assays (quadruplex, E-specific, C-specific and cryptic clade) were undertaken (All the gels can be seen in Appendix 9.1 - Fig. S.128 – S.132). Nine isolates showed the presence of *arpA* and TspE4.C2, which assigned them into phylo-group B1 (Table 30). Five isolates were assigned to phylo-group E-specific PCR assays and positive for E-specific gene. Three isolates showed the presence of *arpA* gene only, indicating phylo-group A. Two isolates were assigned to phylo-group C-specific PCR assays and one belonged to phylo-group C, and the other one belonged to phylo-group A. Four isolates were assigned to phylo-group B2, which two showed the presence of all fragment markers but *arpA* and two showed the presence of only *arpA* and *yjaA*. One isolate belonged to phylo-group F due to the presence of *chuA* only. Four isolates showed no presence of the four fragment markers but a band ~476 bp, indicating them as cryptic clades. Five isolates were belonged to unknown group.

***Table 30. Phylo-grouping results of E. coli from gull and goose faeces.*** *Unknown groups are highlighted in yellow colour. Species confirmation using gadA is shown. The gels can be seen in Appendix 9.1 - Fig. S.128 – S.132.*

| Isolate reference numbers | Samples | Sites | ESBL *E. coli* | *E. coli* REP types | *gadA* | *arpA* | *chuA* | *yjaA* | $TspE_4.C2$ | Phylo-groups |
|---|---|---|---|---|---|---|---|---|---|---|
| BAD 450 | G. Dun. 1 | Geese- urban | Sensitive | B | + | + | - | - | + | B1 |
| BAD 553 | GS 8 | Geese- urban | Sensitive | E | + | + | - | - | + | B1 |
| BAD 154 | GS 1 | Geese- urban | Sensitive | J | + | + | - | - | + | B1 |
| BAD 237 | LSW 20 | Gulls- urban | Sensitive | U | + | + | - | - | + | B1 |
| BAD 576 | LSA 5 | Gulls- rural | Sensitive | Y | + | + | - | - | + | B1 |
| BAD 241 | LSW 20 | Gulls- urban | Sensitive | AB | + | + | - | - | + | B1 |
| BAD 821 | LSA 37 | Gulls- rural | Sensitive | AC | + | + | - | - | + | B1 |
| BAD 413 | LSW 42 | Gulls- urban | Sensitive | AD | + | + | - | - | + | B1 |
| BAD 841 | LSA 41 | Gulls- rural | Sensitive | AE | + | + | - | - | + | B1 |
| BAD 196 | LSW 5 | Gulls- urban | Sensitive | C | + | + | + | - | + | E |
| BAD 86 | LSW 5 | Gulls- urban | Resistant | F | + | + | + | - | + | E |
| BAD 186 | GSP 50 | Geese- rural | Sensitive | L | + | + | + | + | - | E |
| BAD 842 | LSA 41 | Gulls- rural | Sensitive | AA | + | + | + | - | + | E |
| BAD 170 | LSW 1 | Gulls- urban | Sensitive | AF | + | + | + | - | + | E |
| BAD 231 | LSW 15 | Gulls- urban | Sensitive | W | + | + | - | - | - | A |
| BAD 238 | LSW 20 | Gulls- urban | Sensitive | X | + | + | - | - | - | A |
| BAD 819 | LSA 37 | Gulls- rural | Sensitive | Z | + | + | + | - | - | A |
| BAD 194 | LSW 5 | Gulls- urban | Sensitive | AG | + | + | - | - | - | A |
| BAD 820 | LSA 37 | Gulls- rural | Sensitive | K | + | - | + | + | + | B2 |
| BAD 378 | LSW 34 | Gulls- urban | Sensitive | R | + | - | + | + | + | B2 |
| BAD 198 | LSW 5 | Gulls- urban | Sensitive | T | + | + | - | + | - | B2 |
| BAD 110 | LSW 20 | Gulls- urban | Resistant | G | + | + | - | + | - | B2 |
| BAD 453 | LSA 31 | Gulls- rural | Resistant | N | + | + | + | - | - | C |
| BAD 414 | LSW 42 | Gulls- urban | Sensitive | V | + | - | + | - | - | F |
| BAD 446 | GD 45 | Geese- urban | Sensitive | M | + | - | (~476) | - | - | Clade V |
| BAD 412 | LSW 42 | Gulls- urban | Sensitive | O | + | - | (~476) | - | - | Clade V |
| BAD 163 | GSP 3 | Geese- rural | Sensitive | P | + | - | (~476) | - | - | Clade V |
| BAD 156 | GS 3 | Geese- urban | Sensitive | S | + | - | (~476) | - | - | Clade V |
| BAD 333 | Enriched LSW 34 | Gulls- urban | Resistant | A | + | + | - | + | + | Unknown |
| BAD 95 | LSW 11 | Gulls- urban | Resistant | D | + | + | - | + | + | Unknown |
| BAD 158 | GL 1 | Geese- urban | Sensitive | H | + | - | - | - | - | Unknown |
| BAD 160 | GL 1 | Geese- urban | Sensitive | I | + | + | + | + | + | Unknown |
| BAD 823 | LSA 37 | Gulls- rural | Sensitive | Q | + | - | - | - | - | Unknown |

*E. coli* phylo-group B1 was found as the most prevalent phylo-group in this study (9/33, 27%), and phylo-group C (1/33, 3%) and phylo-group F (1/33, 3%) were the least (Figure 17). None of the *E. coli* isolates belong to phylo-group D. *E. coli* phylo-group B1 was also the most prevalent phylo-group in gull faeces (6/24, 25%) and ESBL sensitive *E. coli* (9/28, 32%).

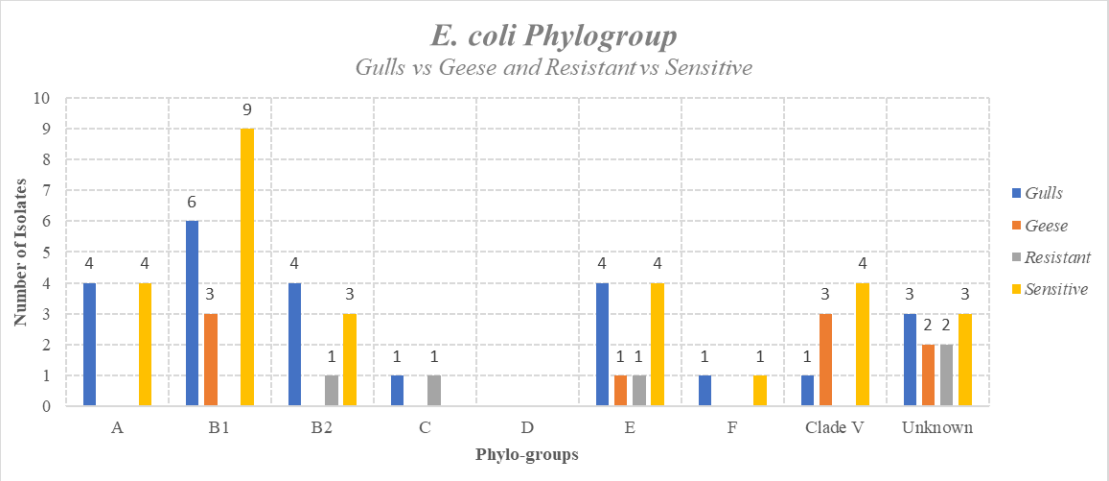


***Figure 17. E. coli* phylo-groups in gulls vs geese and resistant vs sensitive**. *The gels can be seen in Appendix 9.1 - Fig. S.128 – S.132.*

### 4.9.2 Identification of Clinically Important Sequence Types (STs)

One of the ways to understand the risk of ESBL-producer *E. coli* to public health is to understand if clinically important *E. coli* are circulating in the environment. If clinical clones are detected in the environment, then there is a risk they can spread to humans at exposure relevant sites as described by Huijbers *et al.* (2015). In this project, four clinically important sequence-types (ST 69, 73, 95 and 131) causing urinary tract infections were targeted by PCR-based MLST assay. Of 33 *E. coli* isolates (resistant = 5, sensitive = 28), only one of these ST types was detected (Appendix 9.2 – Fig. S.133). ST69 was identified in an ESBL-producer *E. coli* (type N) isolated from a gull sample in the rural site. None of these STs were detected in ESBL sensitive *E. coli* (Appendix 9.2 – Fig. S.134).

## 4.10 Characterisation of Resistance Profile

The emergence of multi-drug resistant (MDR) and ‘superbug’ bacteria in the animal, human and environmental sectors pose a high global threat and is a cause of a concern

(Aslam *et al.*, 2018). Moreover, it is suggested that there is an interconnected sharing between these three sectors (Aslam *et al.*, 2018). The third objective of this study was to determine the resistance profile of ESBL-producer isolates in birds. The resistance profile was assessed phenotypically (antibiotic susceptibility testing) and genotypically (resistance genes PCR assay). These characterisations would allow the resistance profile of isolates from wild birds (as environmental compartment) to be compared to human and animal surveillance data. Thirty-three *E. coli* (resistant = 5, sensitive = 28) were characterised. From the ESBL-producer non-*E. coli* coliform group, only one identified resistant isolate closest to *Klebsiella* sp. (REPNE-6, section 4.7.3) was decided to be characterised in this section as *Klebsiella* sp. is one of the clinically important resistant-bacteria worldwide, including in the UK (Woodford *et al.*¸2004).

### 4.10.1 Antibiotic Susceptibility Testing (AST)

Thirty-three *E. coli* and one resistant isolate closest to *Klebsiella* sp. were tested against 12 clinically important antibiotics. All the ESBL-producer *E. coli* isolates (100%) and one ESBL-producer isolate closest to *Klebsiella* sp. were resistant to ampicillin and cefotaxime (Figure 18; Appendix 10.1 – Table S.29). None of these ESBL-producer isolates were resistant to cefoxitin, meropenem, chloramphenicol, gentamicin and tigecycline. Twenty-seven out of the 28 **ESBL sensitive *E. coli*** (96%) were susceptible to all the tested antibiotics. Only one ESBL sensitive *E. coli* (4%) found to be resistant to ampicillin, tetracycline and trimethoprim-sulfamethoxazole.

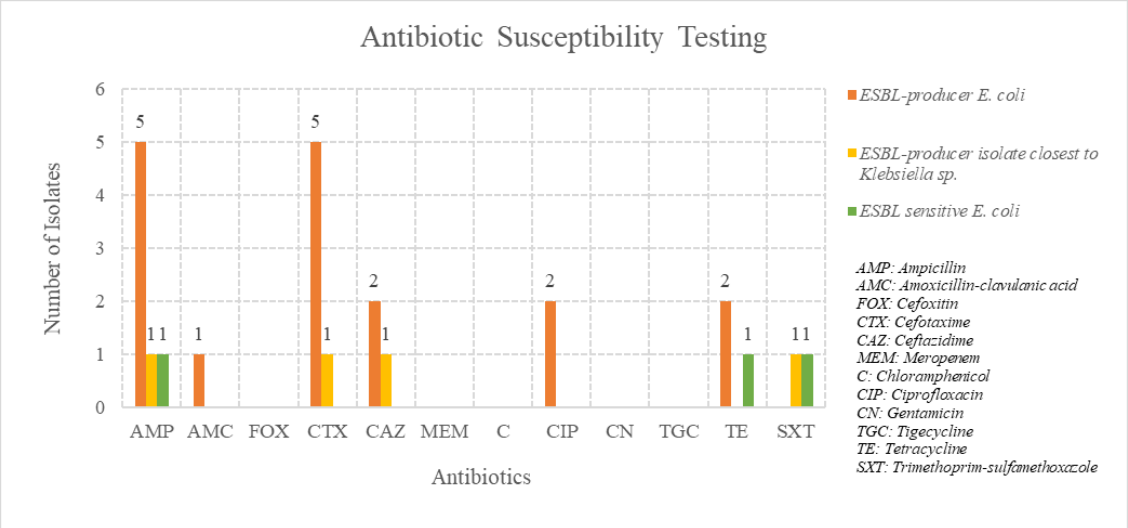


***Figure 18. Antibiotic susceptibility testing of ESBL-producer isolates and ESBL sensitive E. coli from gulls and geese****. Appendix 10 – Table S.29 and S.30 provide the zone of inhibitions (mm) of each isolate towards each tested antibiotic.*

Multi-drug resistant (MDR) *E. coli* were regarded to one ESBL-producer *E. coli* (1/5, type D) and one ESBL sensitive *E. coli* (1/28, type AD), and shared a resistance profile to ampicillin and tetracycline (Table 31). No isolates were regarded as extensively drug-resistant (resistance to at least one antibiotic agent in all but two or fewer class) and pandrug-resistant (resistance to all antibiotic agents in all classes).

***Table 31. Resistance antibiogram of ESBL-producer isolates and ESBL sensitive E. coli isolated from gulls and geese.*** *MDR (resistance ≥ 3 antibiotic classes) isolates are highlighted in yellow colour. AMP = ampicillin, AMC = amoxicillin-clavulanic acid, CTX = cefotaxime, CAZ = ceftazidime, CIP = ciprofloxacin, TE = Tetracycline, SXT = trimethoprim-sulfamethoxazole. N/A = Not applicable. Appendix 10 – Table S.29 and S.30 provide the zone of inhibitions (mm) of each isolate towards each tested antibiotic.*

| Isolate reference numbers | Samples | Sites | Species | *E. coli* REP types | Resistance antibiogram | Note |
|---|---|---|---|---|---|---|
| BAD 95 | LSW 11 | Gulls- urban | Resistant *E. coli* | D | AMP/AMC/CTX/CIP/TE | MDR |
| BAD 413 | LSW 42 | Gulls- urban | Sensitive *E. coli* | AD | AMP/SXT/TE | MDR |
| BAD 333 | Enriched LSW 34 | Gulls- urban | Resistant *E. coli* | A | AMP/CTX/CAZ/CIP | |
| BAD 453 | LSA 31 | Gulls- rural | Resistant *E. coli* | N | AMP/CTX/CAZ/TE | |
| BAD 86 | LSW 5 | Gulls- urban | Resistant *E. coli* | F | AMP/CTX | |
| BAD 110 | LSW 20 | Gulls- urban | Resistant *E. coli* | G | AMP/CTX | |
| BAD 106 | LSW 20 | Gulls- urban | Resistant closest to *Klebsiella* sp. | N/A | AMP/CTX/CAZ/SXT | |

### 4.10.2 Detection of ESBLs Genes

ESBL-producer *E. coli* can harbour multiple resistance genes on their plasmids, including CTX-M, TEM, SHV, OXA, FOX, CMY, IMI, VIM, etc (Giske *et al.*, 2009). In this section, five ESBL-producer *E. coli* and one ESBL-producer *Klebsiella* sp. were tested for four of the major ESBLs genes (CTX-M, SHV, TEM and OXA genes). The CTX-M Group PCR assay which detects five distinct CTX-M enzyme groups, showed $bla_{\text{CTX-M}}$ group 1 as the most prevalent ESBL gene in this study (Fig. 19a, Table 32). Multiplex PCR assay to detect $bla_{\text{TEM}}$, $bla_{\text{SHV}}$ and $bla_{\text{OXA}}$ genes also showed two *E. coli* isolates and one isolate closest to *Klebsiella* sp. from gulls in the urban site carried multiple ESBL genes (CTX-M/TEM/OXA, CTX-M/TEM/SHV and CTX-M/TEM, respectively).

A faint band at ~300 bp in AT 4.2 control for $bla_{\text{CTX-M}}$ group (Fig 19a, lane 3), at ~564 bp in AT 1.2 control for $bla_{\text{TEM}}$, $bla_{\text{SHV}}$ and $bla_{\text{OXA}}$ (Fig 19b, lane 2) and at 800 bp in AT 1.3 control for $bla_{\text{TEM}}$ (Fig 17b, lane 3) were observed. A very faint band at the expected band size for $bla_{\text{TEM}}$ gene (800 bp) were observed in type F and type N (Fig. 19b, lane 4

and 8, respectively). This very faint band at the expected band size was not regarded as a 'true' band as it was too faint compared to the controls and might be resulted from the crude DNA extraction and non-fully optimised PCR assay. However, the faint bands at the expected band size for *bla*$_{TEM}$ and *bla*oxa genes observed in type A (Fig.19b, lane 7) were accepted as shown by AT 1.2 and AT 1.3 controls.

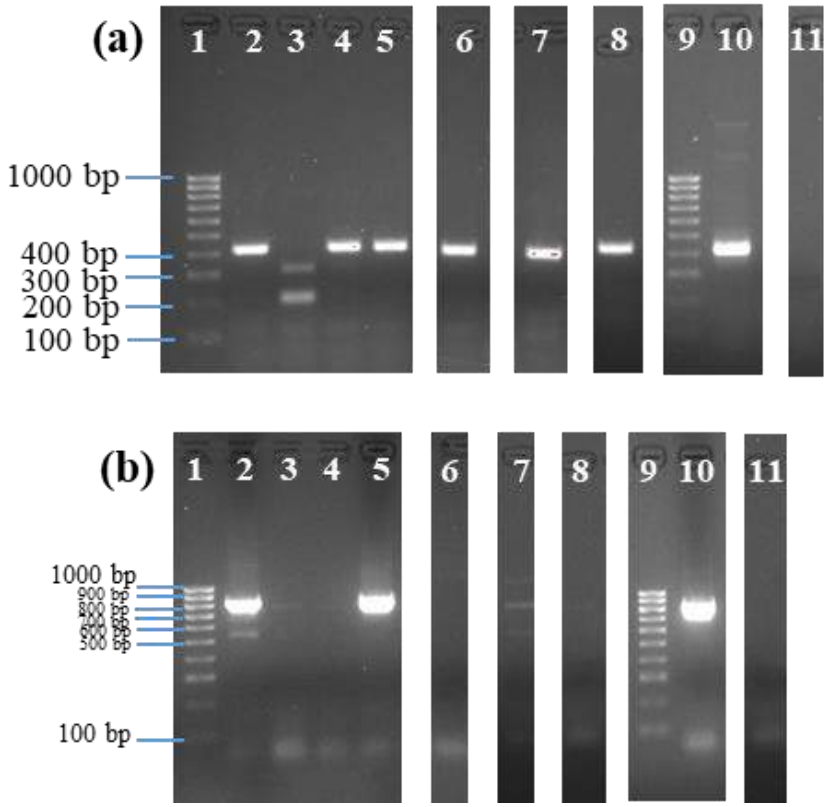


***Figure 19. (a) The PCR detection of CTX-M group genes on ESBL-producer isolates.*** *Lane 1 and 9: Ladder 100 bp. Lane 2: AT 1.3 for CTX-M Group 1 (415 bp). Lane 3: AT 4.2 for CTX-M Group 9 (205 bp). Lane 11: Sterile distilled water for negative control. Lane 4-8: E. coli type F, type D, type G, type A, type N. Lane 10: Klebsiella sp.* ***(b) The multiplex PCR detection of SHV, TEM and OXA genes on ESBL-producer isolates.*** *Lane 1 and 9: Ladder 100 bp. Lane 2: AT 1.2 for TEM (800 bp), SHV (713 bp) and OXA (564 bp). Lane 3: AT 1.3 for TEM. Lane 11: Sterile distilled water for negative control. Lane 4-8: E. coli type F, type D, type G, type A, type N. Lane 10: Klebsiella sp.*

***Table 32. ESBL-producer genes harboured in ESBL-producer isolates from gulls.*** *N/A = Not applicable.*

| Isolate reference numbers | Samples | Sites | Species | *E. coli* REP types | Resistance genes |
|---|---|---|---|---|---|
| BAD 333 | Enriched LSW 34 | Gulls- urban | Resistant *E. coli* | A | CTX-M Group 1/TEM/OXA |
| BAD 95 | LSW 11 | Gulls- urban | Resistant *E. coli* | D | CTX-M Group 1/TEM/SHV |
| BAD 86 | LSW 5 | Gulls- urban | Resistant *E. coli* | F | CTX-M Group 1 |
| BAD 110 | LSW 20 | Gulls- urban | Resistant *E. coli* | G | CTX-M Group 1 |
| BAD 453 | LSA 31 | Gulls- rural | Resistant *E. coli* | N | CTX-M Group 1 |
| BAD 106 | LSW 20 | Gulls- urban | Resistant closest to *Klebsiella* sp. | N/A | CTX-M Group 1/TEM |

# 5. Discussion

The presence of antimicrobial resistance (AMR) in the environment has been demonstrated in several environmental compartments (Huijbers *et al.*, 2015), and several of these studies have suggested that wildlife, especially wild small mammals and wild birds, may play a role as sentinels for AMR transmission in the environment (Arnold *et al.*, 2016; Bonnedahl *et al.*, 2015; Furness *et al.*, 2017; Vittecoq *et al.*, 2016). As an environmental compartment, wild birds have been suggested as a useful indicator for ecosystem health and a good environmental indicator for their habitat quality, environmental pollution and biodiversity as they are sensitive to changes in the environment, easy to survey (compile and interpret data) and at relatively high trophic levels (Egwumah *et al.*, 2017; Hill, 2015). The present study was conducted to understand the role of gulls and geese as a reservoir, and hence a potential pathway for transmission of ESBL-producer coliforms in the environment, and to understand how different spectrum of anthropogenic activity at various sites impact the prevalence and diversity of these resistant bacteria. Gulls were chosen because as scavengers living in close contact to humans and feeding readily on waste, they would be expected, of all taxa, to be reservoirs of AMR bacteria. Geese on the other hand are primarily herbivores and thus their potential role as reservoirs or vectors would be less expected, though they can also live close to humans. This comparison hopefully allows the difference in the prevalence of AMR in birds as a function of their ecology to be somewhat discerned.

One of the objectives of the present study was to determine the prevalence of ESBL-producer coliforms in two functionally different wild bird taxa at two sites with different levels of anthropogenic activity. Prevalence is 'the proportion of a population who have a specific characteristic in a given time period, regardless of when they first developed the characteristic' (NIH, 2017). To measure and report the prevalence, point prevalence (at a specific point in time) was used. Prevalence in this study was defined as the number of bird faecal samples with ESBL-producer coliforms at each site. In this study, coliforms were studied as two groups: *E. coli* as the major species of coliform group, and non-*E. coli* coliforms.

One of the taxa selected for this study was gulls, of which many species are opportunistic-omnivores (Trapp, 1979). Gulls in urban areas have previously been observed to scavenge foods from sewage works (Vernon, 1972), and hence Seafield WWTW was chosen as a site of high anthropogenic activity (urban area). During the sampling, many black-headed gulls and herring gulls were observed scavenging from the

open primary tanks containing untreated sewage. The low anthropogenic activity site (rural area) chosen for the gull sampling was St Abbs, a small fishing village adjacent to a Special Protection Area (SPA) site. Herring gulls in this area were observed to scavenge for bread, chips and other foods from the shore (e.g. small crabs, fish).

This study found a significantly higher prevalence of ESBL-producer *E. coli* (57% urban vs 2% rural) and ESBL-producer non-*E. coli* coliforms (32% urban vs 4% rural) in the urban site compared to the rural site. The significantly higher prevalence of ESBL-producer coliforms in gulls in the urban site than the rural site indicates the impact of high anthropogenic activity at the urban site. The present study concurred with that of Atterby *et al*. (2016), in which a high prevalence of resistant bacteria in large-bodied gulls (i.e. glaucous-winged gulls – *Larus glaucescens*, herring and potentially hybrid gulls) was associated with urban environment and influenced by anthropogenic activity (sampling site in close proximity to high-populated community landfill and mouth of the largest waterway in the city) in Southcentral Alaska. The finding in this study is also consistent with the premise that anthropogenic activity of the local environment impacts the prevalence of resistant bacteria among different species of birds inhabiting the area (Bonnedahl & Järhult, 2014; Ramey *et al*., 2018). The significant prevalence of ESBL-producer coliforms in gulls in this study also indicates their role as a reservoir of antibiotic resistant bacteria (ARB) in the environment. With their ability to migrate long distances, it is a concern that they may act as a vector of ARB. Coulson and Butterfield (1985) previously studied the movements of British herring gulls in the UK, and found that herring gulls made some large movements across the Britain, particularly extensive in northern Britain (i.e. Scotland, Orkney and Shetland) with 54% of the studied population moving more than 200 km. Hence, there is a possibility that gulls in the urban site (Seafield WWTW) may act as a spreader of ARB in the Britain.

The much higher prevalence in Seafield WWTW may be explained by the fact that the site collects untreated human wastes, which will include enteric bacteria and faecal coliforms such as *E. coli*, *Klebsiella*, *Ctirobacter*, *Salmonella*, *Shigella* and *Enterococcus* (Leclerc *et al.*, 2001; Mudge & Ball, 1964). The urban waste may also contain biocides/antibiotic residues, which may lead to the emergence of resistant bacteria within the urban site (Atterby *et* al., 2016). ESBL-producer coliforms might be introduced to gulls in the urban site from this feeding process, causing the prevalence to be significantly higher than in the rural site. Nelson *et al*. (2008) confirmed that gulls picked-up resistant

bacteria from wastewater by finding identical genotypes of *E. coli* in wastewater and in herring gulls.

As a comparison of different bird taxa to gulls, geese were chosen due to their dietary habits as non-scavengers. Geese are obligate herbivores (Fox *et al.*, 2017). Faeces of Canada geese and greylag geese were sampled from urban lochs in City of Edinburgh, a high anthropogenic activity site (urban), and with close-interaction between humans and geese (feeding activity) being observed during the sampling. Reed (1976) stated that Canada goose and greylag goose feed by stripping seeds, grazing and rooting from standing grasses. This explains the observation of grass fibre in all their faecal samples. A pasture field in Slamannan Plateau, a low anthropogenic activity (rural) and a SPA site, was selected to sample faeces from Taiga bean geese, a species within the same family (Anatidae) as Canada and greylag geese. Taiga bean geese are migratory birds, with the main UK population wintering on the Slamannan Plateau. The sampling was done during their wintering period in the UK. The Royal Society for the Protection of Birds also stated the diet of bean goose consists of grass, cereals, potatoes and other crops, similar to Canada and greylag geese.

This study found a statistically insignificant difference of ESBL-producer *E. coli* (3% urban vs 0% rural) and ESBL-producer non-*E. coli* coliforms (5% urban vs 17% rural) between geese in the urban and rural sites. Compared to the gulls, the findings from geese suggest a minor role of geese as a reservoir of ESBL-producer coliforms. Different findings between gulls and geese in the urban sites may arise from different levels of anthropogenic activity between urban lochs and Seafield WWTW where they feed on. Seafield WWTW is a much higher anthropogenic activity site, processing urban wastewaters from the City of Edinburgh and surrounding area. Blaak *et al.* (2015) demonstrated a higher number of ESBL-producer *E. coli* in WWTW influents ($8.2 \times 10^5$ CFU/l) and in WWTW effluents ($1.5 \times 10^3$ CFU/l) compared to surface waters (1.5 CFU/l, i.e. river, lake, canals, rivulet) in the Netherlands. Hence, gulls as scavengers (feeding on WWTW) have a higher risk to be exposed to AMR in the site as they are directly feeding on human wastes. On the other hand, geese as non-scavenger (feeding on grass, seeds, etc) at the urban lochs where the site is separated from any large-scale waste sources might explain the lower number of ESBL-producer coliforms than the gulls (Grond *et al.*, 2018).

Nonetheless, the presence of ESBL-producer coliforms found in geese from the urban and rural sites in this supports the potential role of geese as a reservoir of resistant bacteria

as several other studies found this (Agnew *et al.*, 2016; Cole *et al.*, 2005; Middleton & Ambrose, 2005; Vogt *et al.*, 2018). Moreover, the presence of ESBL-producer non-*E. coli* coliforms in taiga bean goose (migratory goose) from rural site indicate the possible role of taiga bean geese as an international vector/reservoir of resistant bacteria. In spring, the studied population of taiga bean geese migrate from the Slamannan Plateau (Scotland) to Dalarna county (Sweden) to breed, with several stop-over sites where they rest and feed, including in Norway and Denmark (Mitchell *et al.*, 2016). During their migration, their preferred habitats are grass pasture and arable stubbles, and they roost primarily in areas such as on the sea, fresh-water bodies, wetlands, flooded fields and agricultural fields near farmland where they could potentially introduce ARB through their faeces.

In this study, the numbers (CFU/g) of total *E. coli* (all *E. coli*, both sensitive and resistant) and ESBL-producer coliforms are an estimation, and have to be seen in light of several limitations. (1) The numbers (CFU/g) were calculated based on a small number of samples due to an extreme variability of data from the spread plate method. This variability of data was expected as it is known that environmental samples are extremely variable compared to controlled lab studies, and are completely out of the control of investigator (Sutton, 2011). Sutton (2011) also stated that the numbers (CFU/g) from environmental samples do not conform to a normal distribution. As observed in this study, sporadic counts of < 3 CFU/g, < 4 CFU/g, < 10 CFU/g and > 4.5 x $10^3$ CFU/g are more predominant (Appendix 2). For instance, 73 (75%) of 97 gull faecal samples were reported as < 3 CFU/g due to 'zero' counts of ESBL-producer *E. coli* on the spread plate, with the other three (3%) were reported as > 4.5 x $10^3$ CFU/g due to 'TMTC' counts and only 21 (22%) of 97 gull faeces were used to calculate the numbers. (2) The numbers (CFU/g) were also calculated based on Coliform *ChromoSelect* agar and subject to bias as this study has shown that coliforms identified by this media are not as accurate as expected. Nine (15%) of 60 isolates with coliforms appearance (blue and red) on Coliform *ChromoSelect* agar were identified as non-coliforms, and 13 (39%) of the 46 blue colonies identified as non-*E. coli*. (3) The serial dilutions in this study were determined based on trial-and-error. Ten-fold serial dilution was chosen as several studies in wild birds have demonstrated the use of 10-fold serial dilutions for bird faecal samples and gave countable colonies, including for yellow-legged gulls, Marabou stork (*Leptoptilos crumenifer*) and Chinese egret (*Egretta eulophotes*) samples (Araújo *et al.*, 2014; Nyakundi & Mwangi, 2011; Wu *et al.*, 2018). Other studies demonstrated the use of 1:10 dilution alone to process the faecal samples from Canada geese and other bird samples (Jamali *et al.*, 2015;

Middleton & Ambrose, 2005). However, low yield of total *E. coli* and ESBL-producer coliforms along the progress of the study caused the suspicion of improper dilutions to arise. Samples were then diluted using a lower dilution (1:3, 1:9, 1:18 for gulls and 1:4, 1:16, 1:48 for geese), which caused the 'TMTC' counts of total *E. coli*, and these counts could not be used to calculate the numbers (CFU/g). (4) Coliform *ChromoSelect* agar plates for the spread plate were incubated for 2 days. The colonies count was only done after 2-days incubation and might affect the counting (Brown *et al.*, 2011). Brown *et al.* (2011) found that reading plates after 48 h incubation resulted in more difficult countable plates due to luxuriant growth of colonies and condensate inside the plate. (5) Due to the small weight of samples obtained and time constraints, it was not possible to obtain more samples and do the spread plate in biological and technical replicates. Wille *et al.* (1996) demonstrated that the replicate plating from the same sample (biological replicate) was no more accurate than single plating. However, the investigator acknowledged that these numbers (CFU/g), from a single plate, had not the same confidence of reliability as in triplicate. The single technical and biological plate therefore may underestimate the actual number of total *E. coli* and ESBL-producer *E. coli* in gulls and geese and subject results to bias.

The present study calculated an almost double number of total *E. coli* in gulls in the urban site ($6.2 \times 10^2$ CFU/g) than in the rural site ($3.5 \times 10^2$ CFU/g). These numbers (CFU/g) are low compared to the other studies of Fogarty *et al*. (2003) and Meerburg *et al.* (2011), which found a range of total *E. coli* of $1.4 \times 10^7 – 4.9 \times 10^8$ CFU/g in gulls (i.e. Herring, black-backed and black-headed gulls). Other than the limitations above, different techniques in the sample collection and handling were observed from these two studies. Fogarty *et al.* (2003) used a sterile swab to sample the faeces, stored on ice and processed within 24-48 hours with unspecific serial dilutions, while Meerburg *et al*. (2011) immediately processed the sample and used 10-fold serial dilutions (to $10^{-7}$). In comparison, bird faecal samples were stored at -20°C and processed within 6 weeks (not immediately processed), and were diluted in a low dilution. These might affect the viability of *E. coli*, resulting in a low yield of *E. coli* in this study as several studies stated that sample handling and storage affected the microbiota composition of the faeces (Foster *et al.*, 2006; Gratton *et al.*, 2016; Tedjo *et al.*, 2015).

Compared to the gulls in the urban site ($6.2 \times 10^2$ CFU/g), the number (CFU/g) of total *E. coli* in geese in the urban site was three-times lower ($2.0 \times 10^2$ CFU/g). This finding aligned with the study of Alderisio and DeLuca (1999), which found a higher

concentration of faecal coliforms (which *E. coli* as the major species) in ring-billed gulls ($3.7 \times 10^8$ CFU/g) than Canada geese ($1.5 \times 10^4$ CFU/g) in Westchester County, USA (an urban area). Interestingly, the number (CFU/g) of total *E. coli* in geese in the rural site ($2.0 \times 10^3$ CFU/g) was higher than in gulls in the rural site ($3.5 \times 10^2$ CFU/g); however, it was likely due to bias in geese in the rural site data by one sample, which had a much higher count ($1.5 \times 10^8$ CFU/g) than other samples (ranging from $2 \times 10^2 - 4.2 \times 10^4$ CFU/g). To the best of the investigator's knowledge, no studies of the numbers (CFU/g) of ESBL-producer coliforms in gulls and geese were available; hence the comparison of the numbers (CFU/g) in this study cannot currently be made. Other studies of ESBL-producer isolates focused on determining the prevalence and characterising the resistance profile, rather than determining the number (CFU/g) of resistant *E. coli* in gulls and geese.

For the purpose of understanding AMR, the environment can be divided into four compartments: water, soil, air/dust and wildlife (Huijbers *et al.*, 2015). Compared to other environmental compartments, the prevalence of ESBL-producer *E. coli* in gulls in the urban site (57%) was higher than that found in a study of 86 designated Scottish bathing waters (8%) (Morrison, 2019). Similarly, the number (CFU/g) of ESBL-producer *E. coli* obtained in this study was 30 times higher than the number of ESBL-producer *E. coli* (1.3 CFU/100 ml) calculated in four recreational water areas in the Netherlands (Blaak *et al.*, 2014). This indicates that one gram of gull faeces in the urban site (Seafield WWTW) poses a greater risk than 100 ml of recreational water. Other than surface water, urban wastewaters have also been identified as an AMR compartment in the environment. In this study, Seafiled WWTW as the urban site processes urban wastewaters across the City of Edinburgh. The number (CFU/g) of ESBL-producer *E. coli* calculated from gulls in the urban site ($4.1 \times 10^2$ CFU/g) was slightly lower compared to urban wastewater ($7.5 \times 10^2$ CFU/ml) in Besançon city, France (Bréchet *et al.*, 2014). This comparison indicates that one gram of gull faeces in the urban site poses a similarly risk as one millilitre of urban wastewater.

The other main objectives of this study were to determine the diversity of *E. coli* and resistance profile of ESBL-producer coliforms in birds. Up to 10 resistant coliforms were selected from each sample to obtain the diversity of ESBL-producer coliforms in gulls and geese. In addition, up to five 'sensitive' *E. coli* isolates were selected to determine the overall diversity of *E. coli* population within these birds. During the subculturing of these isolates, atypical coliform morphology was observed on Eosin Methylene Blue and/or MacConkey agar plates. Forty out of 175 isolates (23%) grew as small colony

variant (SCV) on the first sub-culture. SCV coliforms were isolated from both gull and goose samples. The present study appears to be the first to report the presence of SCV coliforms in wild bird samples. Clinically, SCVs are a manifestation of persisters (subpopulations, growth arrested bacteria) which results from a long lag time (Vulin *et al.*, 2018). SCVs grow slowly which confers antibiotic tolerance. Bacteria can gain tolerance to antibiotics in a state of growth arrest (e.g. stationary/lag phase), and the tolerance is lost once the growth is initiated (Conlon *et al.*, 2016; Proctor *et al.*, 2006; Vulin *et al.*, 2018).

Small Colony Variants have been reported in several Gram-positive and Gram-negative species, including *S. aureus, P. aeruginosa, Shigella* spp., *S. marcescens* and *E. coli* (Proctor *et al*., 2006). Proctor *et al*. (2006) found that SCV were nearly one-tenth the diameter of the actual size of the wild-type bacteria. SCV can also revert to normal growth (Musher *et al.*, 1977; Lewis *et al.*, 1991) yet limited information is available about the genetic events responsible for the reappearance of larger colonies (Neut *et al.*, 2007). This reversion was observed in this study where 13 of the 40 SCV isolates (32%) reverted to normal size upon further sub-cultivation. These nonstable SCVs are challenging to characterise due to their growth dynamics (Vulin *et al.*, 2018). The significant finding of these ESBL-producer coliforms in birds was not further investigated due to time limitation of the project.

To study the diversity and resistance profile of coliforms isolated from birds, a total of 175 isolates from 41 bird faecal samples (gulls = 22, geese = 19) was obtained from the selection of up to 10 colonies per sample. To ensure the characterisation of isolate was manageable during the given time, rep-PCR assay was undertaken. This strategy of using the rep-PCR assay to reduce the number of isolates to be characterised has been used in several other studies (Bora, 2015; dos Anjos Borges *et al.*, 2003; Scheirlinck *et al.*, 2007). The rep-PCR can be performed using different set of primers, such as ERIC, ERIC2, BOX, REP and $(GTG)_5$ (Mohapatra *et al.*, 2007). In this study, a comparison was made of two primers: REP which used in Donald Morrison's lab, and $(GTG)_5$ which regarded as the most suitable primer by Mohapatra *et al.* (2007). Although rep-PCR has been described as having low-to-moderate discriminatory power, medium repeatability as well as low reproducibility, it is less time-consuming, efficient, low-cost and reliable bacterial typing technique (Olive & Bean, 1999; Abdollahzadeh & Zolfaghari, 2014; Foxman *et al.*, 2005). Therefore, rep-PCR was more suitable to be performed in this study within the time-frame available compared to PFGE, which prior to Whole Genome Sequencing

(WGS) was considered the 'gold standard' for third-generation bacteria typing (Neoh *et al.*¸2019).

The comparison of REP and $(GTG)_5$ primers showed a contrasting finding with Mohapatra *et al*. (2007). Mohapatra *et al.* (2007) found that $(GTG)_5$ primer to be the most suitable primer for the discrimination of faecal and environmental *E. coli* isolates. However, in this study, the REP primers used in Donald Morrison's lab showed a higher discriminatory power, and band pattern was easier to be read and visually analysed compared to $(GTG)_5$ primer. Other study also showed that REP primers displayed a high resolution and clear fingerprint patterns of *S. maltophilia*, compared to BOX primers (Lin *et al.*, 2008), and several studies of *E. coli* have also used REP-PCR assay (Herrero-Fresno *et al.*, 2017; McLellan *et al.*, 2003; McLellan, 2004)

Of the 175 isolates from 41 bird faeces, 60 distinct REP types (ESBL-producer *E. coli* = 12 types, ESBL-producer non-*E. coli* coliforms = 14 types, ESBL sensitive *E. coli* = 34 types) were found using the REP-PCR assay. Isolates with a less than three bands difference were assigned to the same REP type, and one isolate per REP type was selected for further characterisation. Of the 46 presumptive *E. coli*, 33 isolates were confirmed as *E. coli* by the three species identification PCR assays used in this study. By the indole test, eight of the 33 confirmed *E. coli* (24%) were indole-negative, which likely belong to 2-4% indole-negative *E. coli* (Rezwan *et al.*¸2004; Schets *et al.*, 2002). Han *et al.* (2011) also demonstrated that environmental factors (pH, temperature, presence of antibiotics) affects the indole production in *E. coli*. The other 13 "non-*E. coli*" blue colonies on Coliform *ChromoSelect* agar were identified as Gram negative (n = 8) and Gram-positive (n = 5) bacteria by Gram staining. By 16S rRNA sequencing, six of the eight Gram-negative bacteria were confirmed as coliform isolates, with five identified as non-*E. coli Escherichia* (i.e. *E. fergusonii*) and one identified as *Buttiauxella* sp.. One Gram-negative bacterium was identified as non-coliform *Salmonella* sp. *Salmonella* sp. isolate (REPS-33) in the phenotypic species identification showed a production of indole, which in contrast to their common metabolism as indole negative (Percival & Williams, 2014). Three of the five Gram-positive were identified as *Enterococci* (i.e. *E. mundtii, E. hirae, E. faecalis*).

All the 14 red colonies on Coliform *ChromoSelect* agar were confirmed as non-*E. coli* by the duplex species identification PCR assay in this study. Gram staining of these 14 isolates showed that 11 isolates were Gram-negative bacteria, and the other three were Gram-positive bacteria. By 16S rRNA sequencing, eight of the 11 Gram-negative bacteria

were identified as coliform bacteria, with eight identified as non-*E. coli Escherichia* sp. and one identified as *Serratia* sp. This finding further showed the inability of Molina *et al*. (2015) duplex PCR assay to detect non-*E. coli Escherichia* and *Serratia* genus as coliform bacteria (Guentzel, 1996; Leclerc *et al.*, 2001). This might happen as non-*E. coli Escherichia* and *Serratia* sp. were underrepresented in their study (only *S. marcescens* which is slow/weak lactose-fermenter), and the *lacZ3* primers which they designed are likely not broad enough to cover the *lacZ* consensus sequence. Two of the three Gram-positive bacteria were identified as *Enterococcus* sp.

Notably, analysis of 16S rRNA sequencing result was generally varied. There is no clear-cut consensus definition of bacterial genus or species by 16S rRNA gene sequence comparisons, which resulted in different assumptions of results in a different laboratory (Clarridge, 2004). Six isolates (3 blue colonies and 3 red colonies) in this study showed a low percentage of similarity (< 97%) due to the poor quality of the sequences, and this finding cannot be used to report the species identification due to the unreliability of the result (Welinder-Olsson *et al.*, 2007). Different methods on how to produce an accurate and adequate sequence, e.g. using just the forward or reverse sequence or using multiple overlap sequence, also can cause a problem in generating a sequence (Clarridge, 2004). The present study used forward and reverse sequences as has been suggested by Lane *et al.* (1991).

The species identification of these isolates confirmed that Coliform *ChromoSelect* agar, as selective media, gave discrepant results in this study though Lange *et al.* (2013) validated high sensitivity (94% and 91%) and specificity (97% and 94%) of Coliform *ChromoSelect* agar for the detection of *E. coli* and non-*E. coli* coliforms, respectively. However, these high sensitivity and specificity were likely obtained as they used naturally contaminated water samples and pure cultures with known bacterial strains (laboratory samples). The other selective media for *E. coli* (e.g. mTEC, m-FC) have the same issue of selectivity and specificity (ranging from 85 - 92%) (Pagel *et al.*, 1982). False-positive rate of Coliform *ChromoSelect* agar to detect *E. coli* ranging from 6.2 - 6.7% and to detect coliforms ranging from 5.1 - 18.7% (González *et al.*, 2003; Lange *et al.*, 2013). The false-positive in this study (28% for *E. coli* and 21% for non-*E. coli* coliforms) was higher compared to these previous studies. The presence of eight Gram-positive bacteria, with five identified as *Enterococci* (*E. mundtii*, *E. hirae*, *E. faecalis*) by 16S rRNA sequencing, also showed that sodium lauryl sulphate in Coliform *ChromoSelect* agar does not always inhibit the growth of Gram-positive bacteria (Sigma-Aldrich, 2013).

On Eosin Methylene Blue agar plate, which was used to sub-culture the isolates, coliforms appear as dark violet colonies due to lactose fermentation, in which dark violet colonies with the appearance of a green metallic sheen indicates *E. coli*. However, atypical phenotype of non-*E. coli* coliforms on Eosin Methylene Blue agar were observed in this study on the first sub-culture of several isolates. Twenty-two of the 37 selected red colonies from Coliform *ChromoSelect* agar (indicating presumptive non-*E. coli* coliforms) in the present study appeared with green metallic sheen on Eosin Methylene Blue agar, suggesting they were *E. coli* (Appendix 3). Following the analysis using the REP-PCR assay (section 4.5.3), these 22 isolates were grouped into 11 different REP types. The species identification of their representative isolates confirmed them as non-*E. coli Escherichia* (9) and Gram-positive (2), with one of the latter identified as *Enterococcus* sp. (Appendix 6 - Table S.23). Antony *et al.* (2016) studied the phenomenon, in which both *E. coli* and non-*E. coli* from natural samples produced a green metallic sheen. The false-positive and false-negative of Eosin Methylene Blue were found high (40% and 15.75%, respectively), with sensitivity of 68.5% and low specificity of 20% (Antony *et al*., 2016). Non-*E. coli* species that were reported to form green metallic sheen on Eosin Methylene Blue agar including *Citrobacter*, *Enterobacter*, *Hafnia*, *Klebsiella*, *Serratia* and non-*E. coli Escherichia* (Antony *et al.*, 2016; Kim *et al.*, 2015).

Isolation of *Enterobacteriaceae* have previously been isolated from cloacal samples of four different species of gulls (herring, black-headed, great black-backed - *Larus marinus*, Caspian - *Larus cachinnans*) in Kaunas city dump (Lithuania) using Next-Generation Sequencing (Merkeviciene *et al.*, 2017), and also from greylag and Canada geese (Middleton & Ambrose, 2005; Wang *et al.*, 2018). From the species identification, species diversity in wild birds were determined. In gulls, isolated coliforms include *E. coli*, non-*E. coli Escherichia* (i.e. *E. fergusonii*) and *Buttiauxella* sp.. Non-*E. coli Escherichia* (i.e. *E. fergusonii*, *E. hermanii* and *E. vulneris*) are clinically associated with opportunistic pathogens (Leclerc *et al.*, 2001). In birds, multi-drug resistant *E. vulneris* has also been isolated from nine non-migrating and six migrating birds at Taif province, Saudi Arabia (Shobrak & Abo-Amer, 2015). Leclerc *et al*. (2001) stated that *Buttiauxella* is a coliform originated from aquatic source- obligate parasites in the intestines of slugs, snails and other molluscs, which might present in Seafield WWTW where the gulls fed on. In birds, *Buttiauxella agrestis* was reported to be isolated from East Canadian High Arctic light-bellied Brent goose (*Branta bernicla hrota*) in North Bull Island, Dublin (Agnew *et al.*, 2016). In this study, one isolate from a gulls sample showed a closest

identification to *Klebsiella* sp.. *Klebsiella* is a coliform originated from faecal source and predominate in sewage; hence, it was expected to be isolated from gull samples in Seafield WWTW (Leclerc *et al.*, 2001). Resistant *Klebsiella* has also been detected in Franklin gulls (Bonnedahl *et al.*, 2014). In geese, *E. coli*, non-*E. coli Escherichia* and *Serratia* sp. from the coliform group bacteria were isolated. *Serratia* (*S. fonticola*) is a frequently isolated coliform from fresh water supplies (Leclerc *et al.*, 2001). To the best of the investigator's knowledge, the present study appears to be the first to identify resistant *Serratia* in geese population.

Following the species identification, the strain and clonal diversity of ESBL-producer coliforms were determined. Strain diversity was undertaken by rep-PCR assay and PFGE, and clonal diversity was undertaken by Clermont phylo-grouping and PCR-based MLST assay. In further characterising the diversity, a term of 'clonal' was preferred to be used rather than 'strain' because clone indicates monophyly, which means all the cells characterised have the same ancestor and descendants of the progenitor (Dijkshoorn *et al.*, 2000). Strains of bacteria may change overtime due to mutations and plasmid lost (Dijkshoorn *et al.*, 2000).

It was challenging to determine the cut-off rules to define a strain by rep-PCR assay in this study. Different rules can be used, either by number of bands difference or similarity percentage. Several studies have used similarity cut-off between 90% and 97% (Anderson *et al.*, 2015; Babouee *et al.*, 2011; Herrero-Fresno *et al.*, 2017; Kon *et al.*, 2009; Mohapatra *et al.*, 2007; Thomson *et al.*, 2014). In this study, criterion of < 3 bands difference was equated to > 97% similarity and was used to define a strain (Spigaglia & Mastrantonio, 2003) as has previously been defined (Reboli *et al.*¸1994; Rodriguez-Barradas *et al.*, 1995; Woods *et al.*, 1992).

Thirty-three *E. coli* REP types were observed from the re-analysis of the 33 confirmed *E. coli* (resistant = 5, sensitive = 28). Although this finding was expected as the isolates were the representatives of each REP type in the previous analysis of presumptive *E. coli* (section 4.5), this re-analysis of the confirmed *E. coli* indicates there was no shared REP types between resistant and sensitive isolates. Four (12%) of the 33 *E. coli* REP type were shared between birds and sites, whereas the other 29 (88%) showed no shared REP types between birds and sites. A high diversity of *E. coli* in birds has also been demonstrated by McLellan (2004), with 50.4% unique strain types (> 85% similarity cut-off) in ring-billed gull (*Larus delawarensis*) faecal samples using rep-PCR assay. Other than their dietary habits, a high diversity of *E. coli* strains within bird populations might also be

explained due to their environment, local food resources and their migration behaviour (Grond *et al.*, 2018). The environmental conditions (e.g. close to contamination sources) in their preferred habitats for feeding, ingestion of different microorganisms from their local food resources (e.g. WWTW and a pasture field) and exposure of different microbial environments during their migration (i.e. Taiga bean geese) can be the reason such diversity was obtained in gulls and geese populations in different sites sampled (Bonnedahl & Järhult, 2014; Grond *et al.*, 2018).

In this study, each isolate from gulls in the urban and rural sites showed different *E. coli* REP types, indicating a high diversity of *E. coli* within gulls. Within gull samples, more richness was observed from the urban site compared to the rural site. *E. coli* diversity in gulls in the urban and rural sites resulted from their scavenging habits and the food sources, which affected the composition of their gut microbiome (Fuirst *et al.*, 2018; Grond *et al.*, 2018). Gulls in the urban site scavenged around the opened primary tanks of untreated sewage from multiple sources in urban area (e.g. housing) in Seafiled WWTW. These multiple sources of wastewater contain different *E. coli* types, including those that resistant to antibiotics (Anastasi *et al.*, 2012; Mahfouz *et al.*, 2018). Hence, gulls may pick-up different *E. coli* types from this feeding process (Nelson *et al.*, 2008). Gulls in the rural site scavenged around a harbour, on the shore and likely on untreated local sewage outflows where they may pick-up different *E. coli* types from those areas. From the PFGE analysis, five resistant isolates from gulls showed that they belonged to five different PFGE type, indicating that PFGE and rep-PCR assay showed the same result and appropriate to analyse the genetic diversity (Ahmed *et al.*, 2017; Neoh *et al*., 2019).

Compared to gulls, two shared REP types (type J and L) between geese in the urban and rural sites were observed. Two other *E. coli* REP types (type M and N) were also shared between isolates from gulls in the rural site and geese in the urban site. This suggests the presence of *E. coli* type J and L in geese in both sites, and the presence of *E. coli* type M and N in gulls and geese. Within geese, more richness was also observed from geese in the urban site compared to the rural site. These diversities might be explained due to their dietary habits (basic vegetation), food sources as well as their environment, including contamination from surrounding area (i.e. surface run-off). The urban lochs are surrounded by hills, housing areas, parks and public roads. During rain-fall, surface run-off occurred and excess water might flow to the lochs. This can be the way different microbes present in surface run-off are introduced to the lochs. Ibekwe *et*

*al*. (2011) performed PFGE analysis on *E. coli* isolated from 19 different locations throughout watershed and found surface run-off in areas dominated by urban development or anthropogenic activity was the source of the greatest variety of different types of *E. coli*. The diversity in geese in the rural site more likely happened due to the intrinsic factors, including their diet which affects the gut microbiome (Grond *et al.*, 2018). More isolates from taiga bean goose faecal samples is needed to consider the external factors as the cause (e.g. their migration behaviour).

Results showed a diverse number of phylo-groups were found among these *E. coli* (six phylo-groups: A, B1, B2, C, E, F and one clade: clade V). Phylo-group B1 was found to be the most prevalent *E. coli* phylo-group in this study. This finding was aligned with Smith *et al*. (2014) study in herring gulls in a suburb of Dublin City, with 7 of 10 samples were phylo-group B1. The phylo-group B1 has also been suggested as the most frequently isolated commensal *E. coli* in human and animal sectors (Duriez *et al.*, 2001; Higgins *et al.*, 2007; Ishii *et al.*, 2007). Four (12%) of the *E. coli* isolates in this study belonged to clade V. This finding is aligned with Clermont *et al.* (2011), which found 8-28% of the cryptic clade in birds (not specified), in both France and Australia, and found the clade V to be the most abundant. Cryptic clade V has also been associated with *Escherichia marmotae* (Gonzalez-Alba *et al.*, 2019). Although the cryptic clades are phenotypically and biochemically indistinguishable from *E. coli*, they are genetically divergent from *E. coli* (Clermont *et al.*¸2011b; Kallonen *et al.*, 2016). Interestingly, the PCR assays in the species identification showed that these four isolates (belonged to clade V) were *gadA* and *yaiO* positive, indicating *E. coli*. Further investigation of this interesting finding was not possible due to time constraint.

The extended method of Clermont *et al*. (2013) used in the present study provided a new perspective in assessing *E. coli* phylo-group in birds, compared to other gull studies that only used the triplex PCR assay (Alves *et al.*, 2014; Bonnedahl *et al.*, 2010). Triplex PCR assay targets two genes (*chuA* and *yjaA*) and DNA fragment TspE4.C2 to assign isolate into one of four phylo-groups (A, B1, B2 and D) (Clermont *et al.*, 2000). In triplex PCR assay by Clermont *et al*. (2000), phylo-group F was misidentified as phylo-group D, and phylo-group E was a set of unassigned strains, indicating the diversity from the previous studies (Alves *et al.*, 2014; Bonnedahl *et al.*, 2010) might be underestimated. The extended method of Clermont *et al.* (2013), however, is subject to one acknowledged limitation. The PCR assay cannot assign the *E. coli* isolates in this study into a recently confirmed phylo-group G (Gonzalez-Alba *et al.*, 2019).

MLST of ExPEC lineages has advanced the understanding of the predominance of ST69, ST73, ST95 and ST131 in human infections (Doumith *et al.*¸2015). Regional study of ST among ExPEC in the UK showed these four STs to be consistently prevalent in urinary and bloodstream infections (Doumith *et al.*¸2015). *E. coli* ST69 and ST131 are also among the multi-drug resistant high-risk clones of *Enterobacteriaceae,* and play a role in the spread of resistance to significant antibiotics in clinical setting (Mathers *et al.*, 2015; Wang *et al*., 2017). Therefore, PCR-based MLST assay was performed by targeting these four clinically important STs in the UK, to see if there is any of these clinically important clones in the wild birds.

Results of PCR-based MLST found one ESBL-producer *E. coli* from gulls in the rural site belonged to ST69. The present study did not detect ST131, though it is the most commonly detected clone in wildlife, including wild birds (Wang *et al.*, 2017). The finding of ST69 from gulls in the present study aligned with studies by Bonnedahl *et al.* (2015) and Hernandez *et al*. (2013), in which ST69 was identified in ESBL-producer *E. coli* isolated from Franklin's gulls in Canada and Chile, respectively. The finding of ST 69 from this study was novel, indicating the presence of clinically important pathogenic *E. coli* in gulls in the rural site. The possibility was that gulls might carry ESBL-producer *E. coli* ST69 from the clinical setting/urban area due to their ability to migrate long distances, and might introduce it to the rural site (Ahlstrom *et al.*, 2019; Smith *et al.*, 2014). Ahlstrom *et al.* (2019) combined animal tracking and molecular epidemiology approaches on their study, and found that gulls on the Kenai Peninsula, Alaska, frequently moved around between local areas and impacted the AMR *E. coli* prevalence of the areas. Another plausible explanation was the environment was contaminated with ESBL-producer *E. coli* ST69 from a certain source of contamination (presumably urban/domestic wastewaters), in which in this site might come from the untreated local sewage outflows located west of the harbour mouth of St Abbs, and gulls picked-up *E. coli* ST69 as their scavenging around the area (Nelson *et al.*, 2008).

The third objective of this study was to determine the resistance profile of the isolated ESBL-producer coliforms from gull and goose populations. Five ESBL-producer *E. coli* and one ESBL-producer identified closest to *Klebsiella* sp. from gulls were characterised. Susceptibility testing was performed to check if these ESBL-producer isolates from bird faecal samples were multi-resistant towards other clinically important antibiotics in human and animal sectors.

Antibiotic susceptibility testing (AST) in the present study found all ESBL-producer isolates (five *E. coli* and one identified closest to *Klebsiella* sp.) were resistant towards ampicillin 10 mcg and also cefotaxime 5 mcg. It was expected to get this resistance profile as ESBLs also break down antibiotics belonging to the penicillin group (in this study represented by ampicillin) and cefotaxime was used as the selective agent in this study (Shaikh *et al.*, 2015). This also indicates that the screening method using 4 mg/l cefotaxime was specific. If this resistance profile of gulls is compared to the animal sector, high resistance (> 50%) of *E. coli* isolates to ampicillin was found in other animals, including cattle (*Bos taurus*), chickens (*Gallus gallus domesticus*) and turkeys (*Meleagris gallopavo*) in the UK's Veterinary Antimicrobial Resistance and Sales Surveillance annual antibiotic report in 2015-2018 (Veterinary Medicines Directorate, 2018; 2019b). A resistance towards cefotaxime was also found in *E. coli* isolated from broilers (any chicken that is bred and raised specifically for meat production), though the resistance level was low (1.6%) (Veterinary Medicines Directorate, 2019). Aligned to this study, all *E. coli* isolated from gulls at the Soldotna landfill in southcentral Alaska and wild kelp gulls in South America were also resistant towards ampicillin antibiotics (Ahlstrom *et al.*, 2018; Liakopoulos *et al.*, 2016). Similar resistance profile towards ampicillin and cefotaxime was also observed in human clinical settings. A high resistance to ampicillin was observed in patients with UTIs in Sarajevo, Bosnia and Herzegovina, due to high prescription of ampicillin in the empirical treatment of UTIs (Vranic & Uzunovic, 2016). In the clinical setting in the UK, cefotaxime-resistant *E. coli* were also found in 5% of healthy individuals in the UK (Kirchner *et al.*, 2013).

Resistance of ESBL-producer *E. coli* from gulls (urban = 2 and rural = 1) towards amoxicillin-clavulanic acid, ceftazidime, ciprofloxacin and tetracycline were also observed in this study. A similar resistance profile was previously observed in both clinical and animal sectors. In clinical settings, 60.9 and 52.8% of *E. coli* isolated from blood and urine, respectively, were resistant to amoxicillin (HPS, 2018). Resistance to ciprofloxacin was reported from *E. coli* isolated from blood (19.7%) and urine (16.9%) (HPS, 2018). In animal sector in Scotland, the Scottish One Health Antimicrobial Use and Antimicrobial Resistance Annual Report in 2016 (HPS, 2017) reported 15.8 and 28.4% of *E. coli* isolated from sheep (*Ovis aries*) and cattle, respectively, were resistant to tetracycline. Other than ampicillin and cefotaxime, the isolate closest to *Klebsiella* sp. in this study was resistant to ceftazidime and trimethoprim-sulfamethoxazole. This resistance profile of *Klebsiella* sp. was also found in other bird study. *Klebsiella* sp. which

were resistant to ampicillin (84.3%) and trimethoprim-sulfamethoxazole (18.7%) were isolated from passerine (Passeriformes) and psittacine (Psittaciformes) birds in São Paulo, Brazil (Davies *et al.*, 2016). In comparison to the clinical setting, a similar resistance profile (resistant to cefotaxime, ceftazidime and trimethoprim-sulfamethoxazole) was observed in *Klebsiella* sp. isolated from blood, urine and other human samples from community settings in Taiwan (Lin *et al.*, 2016). In the UK and Ireland, resistant to cefotaxime (> 50%) was observed from a collection of 250 *Klebsiella pneumoniae* isolates causing bacteraemia from a systematic bacteraemia surveillance program between 2001 and 2011 (Moradigaravand, *et al.*, 2017). In Scotland, the resistance of *K. pneumoniae* causing bacteraemia was generally stable, with 20.4% of isolates were resistant to trimethoprim in 2018 (HPS, 2019b).

From this AST, two *E. coli* isolates (2/33, 6%) isolated from gulls in the urban site were regarded as multi-drug resistant (MDR) bacteria. MDR indicates that the isolates resistant to at least one antibiotic agents in ≥ 3 antibiotic classes (Magiorakos *et al.*, 2012) MDR *E. coli* has previously been isolated from wild birds in Northern Italy (Dotto *et al.*, 2016), in migratory birds in Pakistan (Mohsin *et al.*, 2017) and in wild birds in Northern Spain (Alcalá *et al.*, 2015). It is a concern as gulls in the urban site might transfer the MDR *E. coli* to other environmental sources thus pose a potential threat to human and animal health (Shobrak & Abo-Amer, 2015). Gulls in the urban site might acquire MDR *E. coli* due to their feeding time in the WWTW site and also the food source itself (Alm *et al.*, 2018). Alm *et al.* (2018) found that gulls traveling around human waste sites (i.e. landfill and wastewater lagoons) were positive for human-associated bacteria. Several studies stated that WWTW provides a suitable place for horizontal gene transfer (transfer of resistance genes) across the bacteria by the production of plasmid-mediated ESBL genes, thus allows the further development and spread of resistance (Amos *et al.*, 2014; Rizzo *et al.*, 2013). This transfer of resistance genes between bacteria in WWTW causes the rapid development of MDR bacteria as they do not have to rely on self-adaptive mutation (Sun *et al.*, 2019). Other than that, the development of MDR bacteria might be due to constant exposure to residues of antimicrobials/antibiotics in WWTW and natural environment, causing the spontaneous mutation (Sun *et al.*, 2019; Tamhankar & Lundborg, 2019).

The present study also contributed to understand the dissemination of ESBL-producer genes in the environment, particularly in gulls and geese. Result of ESBL-producer genes in this study indicates that $bla_{\text{CTX-M}}$ group 1 is the most prevalent ESBL-producing genes

in the gulls. This finding is aligned with several studies, including by Bonnedahl *et al.* (2010), Báez *et al.* (2015), Stedt *et al.* (2015) and Mohsin *et al.* (2017), in which found *bla*$_{\text{CTX-M}}$ group 1 was the dominated *bla*$_{\text{CTX-M}}$ groups harboured by different species of gulls and migratory birds in different areas (nine European countries, city of Kalmar-southeast coast of Sweden, Antofagasta- North of Chile Pakistan and Pakistan). Among the *bla*$_{\text{CTX-M}}$ groups, *bla*$_{\text{CTX-M}}$ group 1 has also been associated with the dissemination of *E. coli* ST131. However, the correlation between *bla*$_{\text{CTX-M}}$ group 1 and ST131 was not observed in the present study, contradicting Amos *et al.* (2014) and Banerjee & Johnson (2014) studies.

ESBL-producing bacteria have also been demonstrated to often harbour two or three different types of ESBL genes (Dallenne *et al.*, 2010). In this study, three ESBL-producer coliforms (2 *E. coli* and 1 *Klebsiella* sp.) from gulls in the urban site harboured more than one ESBL-producing genes (up to three genes). The finding is aligned with studies by Bonnedahl *et al.* (2010) and Stedt *et al.* (2015), which found that ESBL-producer coliforms from gulls carried multiple ESBL genes (*bla*$_{\text{TEM}}$ and *bla*$_{\text{SHV}}$). The harbouring of multiple ESBL genes in gulls has also been demonstrated in wild kelp gulls (*bla*$_{\text{TEM}}$ and *bla*$_{\text{SHV}}$ genes) in South America (Liakopoulos *et al.*, 2016) and yellow-legged gulls (*bla*$_{\text{CTX-M}}$ group 1 and *bla*$_{\text{TEM}}$) in South of France (Bonnedahl *et al.*, 2009). Other than gulls, several species of wild birds (carried *bla*$_{\text{TEM}}$ and *bla*$_{\text{SHV}}$ genes) in Northern Spain (Alcalá *et al.*, 2015) and a passerine species (carried *bla*$_{\text{CTX-M}}$ and *bla*$_{\text{SHV}}$) in Azores Archipelago, Portugal (Silva *et al.*, 2011) were found to harbour multiple ESBL genes. One *E. coli* isolated from a gull sample in the urban site also harboured *bla*$_{\text{OXA}}$ gene. Harbouring of *bla*$_{\text{OXA}}$ gene in *E. coli* isolate from gulls was previously found by Ahlstrom *et al*. (2019) in gulls in Kenai Peninsula, Alaska. The finding of multiple resistance genes in the isolate closest to *Klebsiella* sp. from gulls in the urban site was also supported the finding by Bonnedahl *et al.* (2015), which found *K. pneumoniae* isolated from gulls residing close to a landfill site in Alaska harboured *bla*$_{\text{CTX-M,}}$ *bla*$_{\text{SHV}}$ and *bla*$_{\text{TEM}}$.

The present study demonstrated an important role of gulls and a minor role of geese as a reservoir of ESBL-producer coliforms in Scotland. Food sources, dietary habits and/or anthropogenic activity within the sites appears to affect the prevalence and diversity of ESBL-producer coliforms in birds. A longitudinal study of gulls and geese in the same sites with this study could support this study about the role of wild birds as a reservoir of resistant bacteria in Scotland. A different sampling method (faecal swab technique), whole genome sequencing (WGS) for species confirmation, MLST for

characterising other STs and the variants of ESBLs would be beneficial to characterise isolates even more and could give a different perspective of relative abundance of coliforms in wild birds. Addition of environmental samples, including wastewater samples (influents and effluents) from Seafield WWTW, sand and/or water samples from St Abbs, water samples from the urban lochs and soils/manure samples from the pasture field in Slamannan Plateau in the future study of gulls and geese in these sites should be collected. These samples and the use of specific markers (for birds and human) in Microbial Source Tracking (MST) study could beneficially support the impact of anthropogenic activity to the prevalence and diversity of ESBL-producer coliforms and would give an insight on how ESBL-producer coliforms from other environmental compartments are introduced to gulls and geese and vice versa.

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# Appendix 1. Bird Faecal Sample Collections

## 1.1 Gull Faeces

***Table S.1. Samples from gulls in the urban site (Seafield WWTW, LSW).*** *Species, date of sampling and date of process are shown.*

| Samples | Species | Date of sampling | Date of Processed |
|---|---|---|---|
| LSW 1 | Herring gulls and black-headed gulls | 04-Feb-19 | 05-Feb-19 |
| LSW 2 | | 04-Feb-19 | 05-Feb-19 |
| LSW 3 | | 04-Feb-19 | 05-Feb-19 |
| LSW 4 | | 04-Feb-19 | 20-Feb-19 |
| LSW 5 | | 04-Feb-19 | 20-Feb-19 |
| LSW 6 | | 04-Feb-19 | 20-Feb-19 |
| LSW 7 | | 04-Feb-19 | 20-Feb-19 |
| LSW 8 | | 04-Feb-19 | 20-Feb-19 |
| LSW 9 | | 04-Feb-19 | 20-Feb-19 |
| LSW 10 | | 04-Feb-19 | 20-Feb-19 |
| LSW 11 | | 04-Feb-19 | 25-Feb-19 |
| LSW 12 | | 04-Feb-19 | 25-Feb-19 |
| LSW 13 | | 04-Feb-19 | 25-Feb-19 |
| LSW 14 | | 04-Feb-19 | 25-Feb-19 |
| LSW 15 | | 04-Feb-19 | 25-Feb-19 |
| LSW 16 | | 04-Feb-19 | 25-Feb-19 |
| LSW 17 | | 04-Feb-19 | 25-Feb-19 |
| LSW 18 | | 04-Feb-19 | 25-Feb-19 |
| LSW 19 | | 04-Feb-19 | 25-Feb-19 |
| LSW 20 | | 04-Feb-19 | 25-Feb-19 |
| LSW 21 | | 04-Feb-19 | 25-Feb-19 |
| LSW 22 | | 04-Feb-19 | 25-Feb-19 |
| LSW 23 | | 04-Feb-19 | 25-Feb-19 |
| LSW 24 | | 04-Feb-19 | 25-Feb-19 |
| LSW 25 | | 04-Feb-19 | 25-Feb-19 |
| LSW 26 | | 04-Feb-19 | 25-Feb-19 |
| LSW 27 | | 04-Feb-19 | 25-Feb-19 |
| LSW 28 | | 04-Feb-19 | 25-Feb-19 |
| LSW 29 | | 04-Feb-19 | 25-Feb-19 |
| LSW 30 | | 04-Feb-19 | 25-Feb-19 |
| LSW 31 | | 04-Feb-19 | 26-Feb-19 |
| LSW 32 | | 04-Feb-19 | 26-Feb-19 |
| LSW 33 | | 04-Feb-19 | 26-Feb-19 |
| LSW 34 | | 04-Feb-19 | 26-Feb-19 |
| LSW 35 | | 04-Feb-19 | 26-Feb-19 |
| LSW 36 | | 04-Feb-19 | 26-Feb-19 |
| LSW 37 | | 04-Feb-19 | 26-Feb-19 |
| LSW 38 | | 04-Feb-19 | 26-Feb-19 |
| LSW 39 | | 04-Feb-19 | 26-Feb-19 |
| LSW 40 | | 04-Feb-19 | 26-Feb-19 |
| LSW 41 | | 04-Feb-19 | 26-Feb-19 |
| LSW 42 | | 04-Feb-19 | 26-Feb-19 |
| LSW 43 | | 04-Feb-19 | 26-Feb-19 |
| LSW 44 | | 04-Feb-19 | 26-Feb-19 |
| LSW 45 | | 04-Feb-19 | 26-Feb-19 |
| LSW 46 | | 04-Feb-19 | 26-Feb-19 |
| LSW 47 | | 04-Feb-19 | 26-Feb-19 |

***Table S.2. Samples from gulls in the rural site (St Abbs, LSA).*** *Species, date of sampling and time of process are shown.*

| Samples | Species | Date of sampling | Date of Processed |
|---|---|---|---|
| LSA 1 | Herring gulls | 15-Apr-19 | 23-Apr-19 |
| LSA 2 | | 15-Apr-19 | 23-Apr-19 |
| LSA 3 | | 15-Apr-19 | 23-Apr-19 |
| LSA 4 | | 15-Apr-19 | 23-Apr-19 |
| LSA 5 | | 15-Apr-19 | 23-Apr-19 |
| LSA 6 | | 15-Apr-19 | 23-Apr-19 |
| LSA 7 | | 15-Apr-19 | 23-Apr-19 |
| LSA 8 | | 15-Apr-19 | 23-Apr-19 |
| LSA 9 | | 15-Apr-19 | 23-Apr-19 |
| LSA 10 | | 15-Apr-19 | 23-Apr-19 |
| LSA 11 | | 15-Apr-19 | 23-Apr-19 |
| LSA 12 | | 15-Apr-19 | 23-Apr-19 |
| LSA 13 | | 15-Apr-19 | 23-Apr-19 |
| LSA 14 | | 15-Apr-19 | 23-Apr-19 |
| LSA 15 | | 15-Apr-19 | 23-Apr-19 |
| LSA 16 | | 15-Apr-19 | 23-Apr-19 |
| LSA 17 | | 15-Apr-19 | 23-Apr-19 |
| LSA 18 | | 15-Apr-19 | 23-Apr-19 |
| LSA 19 | | 15-Apr-19 | 23-Apr-19 |
| LSA 20 | | 15-Apr-19 | 23-Apr-19 |
| LSA 21 | | 15-Apr-19 | 23-Apr-19 |
| LSA 22 | | 15-Apr-19 | 23-Apr-19 |
| LSA 23 | | 15-Apr-19 | 23-Apr-19 |
| LSA 24 | | 15-Apr-19 | 23-Apr-19 |
| LSA 25 | | 15-Apr-19 | 23-Apr-19 |
| LSA 26 | | 15-Apr-19 | 23-Apr-19 |
| LSA 27 | | 15-Apr-19 | 23-Apr-19 |
| LSA 28 | | 15-Apr-19 | 23-Apr-19 |
| LSA 29 | | 15-Apr-19 | 23-Apr-19 |
| LSA 30 | | 15-Apr-19 | 23-Apr-19 |
| LSA 31 | | 15-Apr-19 | 23-Apr-19 |
| LSA 32 | | 15-Apr-19 | 23-Apr-19 |
| LSA 33 | | 15-Apr-19 | 23-Apr-19 |
| LSA 34 | | 15-Apr-19 | 23-Apr-19 |
| LSA 35 | | 15-Apr-19 | 23-Apr-19 |
| LSA 36 | | 15-Apr-19 | 23-Apr-19 |
| LSA 37 | | 15-Apr-19 | 23-Apr-19 |
| LSA 38 | | 15-Apr-19 | 23-Apr-19 |
| LSA 39 | | 15-Apr-19 | 23-Apr-19 |
| LSA 40 | | 15-Apr-19 | 23-Apr-19 |
| LSA 41 | | 15-Apr-19 | 23-Apr-19 |
| LSA 42 | | 15-Apr-19 | 23-Apr-19 |
| LSA 43 | | 15-Apr-19 | 23-Apr-19 |
| LSA 44 | | 15-Apr-19 | 23-Apr-19 |
| LSA 45 | | 15-Apr-19 | 23-Apr-19 |
| LSA 46 | | 15-Apr-19 | 23-Apr-19 |
| LSA 47 | | 15-Apr-19 | 23-Apr-19 |
| LSA 48 | | 15-Apr-19 | 23-Apr-19 |
| LSA 49 | | 15-Apr-19 | 23-Apr-19 |
| LSA 50 | | 15-Apr-19 | 23-Apr-19 |

1.2 Goose Faeces

1.2.1 Urban Site

***Table S.3. Samples from geese in Duddingston loch (GD).*** *Species, date of sampling and date of process are shown.*

| Samples | Species | Date of sampling | Date of Processed |
|---|---|---|---|
| GD 1 | Hybrid* | 17-Jan-19 | 17-Jan-19 |
| GD 2 | Canada geese | 17-Jan-19 | 17-Jan-19 |
| GD 3 | | 17-Jan-19 | 17-Jan-19 |
| GD 4 | | 17-Jan-19 | 17-Jan-19 |
| GD 5 | | 17-Jan-19 | 17-Jan-19 |
| GD 6 | | 17-Jan-19 | 17-Jan-19 |
| GD 7 | | 17-Jan-19 | 17-Jan-19 |
| GD 8 | | 17-Jan-19 | 17-Jan-19 |
| GD 9 | | 28-Feb-19 | 12-Apr-19 |
| GD 10 | | 28-Feb-19 | 12-Apr-19 |
| GD 11 | | 28-Feb-19 | 12-Apr-19 |
| GD 12 | | 28-Feb-19 | 12-Apr-19 |
| GD 13 | | 28-Feb-19 | 12-Apr-19 |
| GD 14 | | 28-Feb-19 | 12-Apr-19 |
| GD 15 | | 28-Feb-19 | 12-Apr-19 |
| GD 16 | | 28-Feb-19 | 12-Apr-19 |
| GD 17 | | 28-Feb-19 | 12-Apr-19 |
| GD 18 | | 28-Feb-19 | 12-Apr-19 |
| GD 19 | | 28-Feb-19 | 12-Apr-19 |
| GD 20 | | 28-Feb-19 | 12-Apr-19 |
| GD 21 | | 28-Feb-19 | 12-Apr-19 |
| GD 22 | | 28-Feb-19 | 12-Apr-19 |
| GD 23 | | 28-Feb-19 | 12-Apr-19 |
| GD 24 | | 28-Feb-19 | 12-Apr-19 |
| GD 25 | | 28-Feb-19 | 12-Apr-19 |
| GD 26 | | 28-Feb-19 | 12-Apr-19 |
| GD 27 | | 28-Feb-19 | 12-Apr-19 |
| GD 28 | | 28-Feb-19 | 12-Apr-19 |
| GD 29 | | 28-Feb-19 | 12-Apr-19 |
| GD 30 | | 28-Feb-19 | 12-Apr-19 |
| GD 31 | | 28-Feb-19 | 12-Apr-19 |
| GD 32 | | 28-Feb-19 | 12-Apr-19 |
| GD 33 | | 28-Feb-19 | 12-Apr-19 |
| GD 34 | | 28-Feb-19 | 12-Apr-19 |
| GD 35 | | 28-Feb-19 | 12-Apr-19 |
| GD 36 | | 28-Feb-19 | 12-Apr-19 |
| GD 37 | | 28-Feb-19 | 12-Apr-19 |
| GD 38 | | 28-Feb-19 | 12-Apr-19 |
| GD 39 | | 28-Feb-19 | 12-Apr-19 |
| GD 40 | | 28-Feb-19 | 12-Apr-19 |
| GD 41 | | 28-Feb-19 | 12-Apr-19 |
| GD 42 | | 28-Feb-19 | 12-Apr-19 |
| GD 43 | | 02-Apr-19 | 04-Apr-19 |
| GD 44 | | 02-Apr-19 | 04-Apr-19 |
| GD 45 | | 02-Apr-19 | 04-Apr-19 |
| GD 46 | | 02-Apr-19 | 04-Apr-19 |
| GD 47 | | 02-Apr-19 | 04-Apr-19 |

** Hybrid of Canada and greylag goose*

***Table S.4. Samples from geese in Dunsapie loch (G.Dun.).*** *Species, date of sampling and date of process are shown.*

| Samples | Species | Date of sampling | Date of Processed |
|---|---|---|---|
| G. Dun 1 | Canada geese | 28-Feb-19 | 09-Apr-19 |
| G. Dun 2 | | 28-Feb-19 | 09-Apr-19 |
| G. Dun 3 | | 28-Feb-19 | 09-Apr-19 |
| G. Dun 4 | | 28-Feb-19 | 09-Apr-19 |
| G. Dun 5 | | 28-Feb-19 | 09-Apr-19 |
| G. Dun 6 | | 28-Feb-19 | 09-Apr-19 |
| G. Dun 7 | | 28-Feb-19 | 09-Apr-19 |
| G. Dun 8 | | 28-Feb-19 | 09-Apr-19 |
| G. Dun 9 | | 28-Feb-19 | 09-Apr-19 |
| G. Dun 10 | | 28-Feb-19 | 09-Apr-19 |
| G. Dun 11 | | 28-Feb-19 | 09-Apr-19 |
| G. Dun 12 | | 28-Feb-19 | 09-Apr-19 |

***Table S.5. Samples from geese in St Margaret's loch (GS).*** *Species, date of sampling and date of process are shown.*

| Samples | Species | Date of sampling | Date of Processed |
|---|---|---|---|
| GS 1 | Greylag geese | 17-Jan-19 | 17-Jan-19 |
| GS 2 | | 17-Jan-19 | 17-Jan-19 |
| GS 3 | | 17-Jan-19 | 17-Jan-19 |
| GS 4 | | 17-Jan-19 | 17-Jan-19 |
| GS 5 | | 28-Feb-19 | 10-Apr-19 |
| GS 6 | | 28-Feb-19 | 10-Apr-19 |
| GS 7 | | 28-Feb-19 | 10-Apr-19 |
| GS 8 | | 28-Feb-19 | 10-Apr-19 |
| GS 9 | | 28-Feb-19 | 10-Apr-19 |
| GS 10 | | 28-Feb-19 | 10-Apr-19 |
| GS 11 | | 28-Feb-19 | 10-Apr-19 |
| GS 12 | | 28-Feb-19 | 10-Apr-19 |
| GS 13 | | 28-Feb-19 | 10-Apr-19 |
| GS 14 | | 28-Feb-19 | 10-Apr-19 |
| GS 15 | | 28-Feb-19 | 10-Apr-19 |
| GS 16 | | 28-Feb-19 | 10-Apr-19 |
| GS 17 | | 28-Feb-19 | 10-Apr-19 |

***Table S.6. Samples from goose in Lochend loch (GL).*** *Species, date of sampling and date of process are shown.*

| Sample | Species | Date of sampling | Date of Processed |
|---|---|---|---|
| GL 1 | Canada goose | 17-Jan-19 | 17-Jan-19 |

1.2.2 Rural Site

***Table S.7. Samples from geese in Slamannan Plateau (GSP).*** *Species, date of sampling and date of process are shown.*

| Samples | Species | Date of sampling | Date of Processed |
|---|---|---|---|
| GSP 1 | Bean geese | 27-Jan-19 | 31-Jan-19 |
| GSP 2 | | 27-Jan-19 | 31-Jan-19 |
| GSP 3 | | 27-Jan-19 | 31-Jan-19 |
| GSP 4 | | 27-Jan-19 | 31-Jan-19 |
| GSP 5 | | 27-Jan-19 | 31-Jan-19 |
| GSP 6 | | 27-Jan-19 | 31-Jan-19 |
| GSP 7 | | 27-Jan-19 | 31-Jan-19 |
| GSP 8 | | 27-Jan-19 | 31-Jan-19 |
| GSP 9 | | 27-Jan-19 | 31-Jan-19 |
| GSP 10 | | 27-Jan-19 | 31-Jan-19 |
| GSP 11 | | 27-Jan-19 | 31-Jan-19 |
| GSP 12 | | 27-Jan-19 | 31-Jan-19 |
| GSP 13 | | 27-Jan-19 | 31-Jan-19 |
| GSP 14 | | 27-Jan-19 | 31-Jan-19 |
| GSP 15 | | 27-Jan-19 | 31-Jan-19 |
| GSP 16 | | 27-Jan-19 | 04-Feb-19 |
| GSP 17 | | 27-Jan-19 | 04-Feb-19 |
| GSP 18 | | 27-Jan-19 | 04-Feb-19 |
| GSP 19 | | 27-Jan-19 | 04-Feb-19 |
| GSP 20 | | 27-Jan-19 | 04-Feb-19 |
| GSP 21 | | 27-Jan-19 | 05-Feb-19 |
| GSP 22 | | 27-Jan-19 | 05-Feb-19 |
| GSP 23 | | 27-Jan-19 | 05-Feb-19 |
| GSP 24 | | 27-Jan-19 | 05-Feb-19 |
| GSP 25 | | 27-Jan-19 | 05-Feb-19 |
| GSP 26 | | 27-Jan-19 | 18-Feb-19 |
| GSP 27 | | 27-Jan-19 | 18-Feb-19 |
| GSP 28 | | 27-Jan-19 | 18-Feb-19 |
| GSP 29 | | 27-Jan-19 | 18-Feb-19 |
| GSP 30 | | 27-Jan-19 | 18-Feb-19 |
| GSP 31 | | 27-Jan-19 | 18-Feb-19 |
| GSP 32 | | 27-Jan-19 | 18-Feb-19 |
| GSP 33 | | 27-Jan-19 | 18-Feb-19 |
| GSP 34 | | 27-Jan-19 | 18-Feb-19 |
| GSP 35 | | 27-Jan-19 | 18-Feb-19 |
| GSP 36 | | 27-Jan-19 | 18-Feb-19 |
| GSP 37 | | 27-Jan-19 | 18-Feb-19 |
| GSP 38 | | 27-Jan-19 | 18-Feb-19 |
| GSP 39 | | 27-Jan-19 | 18-Feb-19 |
| GSP 40 | | 27-Jan-19 | 18-Feb-19 |
| GSP 41 | | 27-Jan-19 | 19-Feb-19 |
| GSP 42 | | 27-Jan-19 | 19-Feb-19 |
| GSP 43 | | 27-Jan-19 | 19-Feb-19 |
| GSP 44 | | 27-Jan-19 | 19-Feb-19 |
| GSP 45 | | 27-Jan-19 | 19-Feb-19 |
| GSP 46 | | 27-Jan-19 | 19-Feb-19 |
| GSP 47 | | 27-Jan-19 | 19-Feb-19 |
| GSP 48 | | 27-Jan-19 | 19-Feb-19 |
| GSP 49 | | 27-Jan-19 | 19-Feb-19 |
| GSP 50 | | 27-Jan-19 | 19-Feb-19 |
| GSP 51 | | 27-Jan-19 | 19-Feb-19 |
| GSP 52 | | 27-Jan-19 | 19-Feb-19 |

# Appendix 2. Enumeration of Presumptive ESBL-Producer Coliforms in Birds

## 2.1 The Counts of Colonies on the Spread Plate

### 2.1.1 Gull Faeces

***Table S.8. The count of colonies of gull samples from the urban site (LSW).*** *Three dilutions (1:3, 1:9 and 1:18) were used. The counting of colonies was undertaken after 48 h of incubation. The colour of colonies observed on Coliform ChromoSelect agar and the weight of faeces used are shown. '-' = no colonies observed. 'TMTC' = Too many to count.*

| Samples | Colifrom *Chromoselect* agar | | | Coliform *Chromoselect* agar + cefotaxime | | | Weight (g) |
|---|---|---|---|---|---|---|---|
| | Dilutions | Number of colonies | Colour | Dilutions | Number of colonies | Colour | |
| LSW 1 | 1:3 | TMTC | Red | 1:3 | TMTC | Blue | 0.42 |
| | | TMTC | Blue | | TMTC | Colourless | |
| | | TMTC | Colourless | | | | |
| | 1:9 | TMTC | Blue | 1:9 | 66 | Blue | |
| | | TMTC | Red | | TMTC | Colourless | |
| | | TMTC | Colourless | | | | |
| | 1:18 | 58 | Blue | 1:18 | 15 | Blue | |
| | | TMTC | Red | | TMTC | Colourless | |
| | | TMTC | Colourless | | | | |
| LSW 2 | 1:3 | TMTC | Red | 1:3 | 66 | Blue | 0.26 |
| | | TMTC | Blue | | 79 | Red | |
| | | TMTC | Colourless | | TMTC | Colourless | |
| | 1:9 | 121 | Blue | 1:9 | 46 | Blue | |
| | | TMTC | Red | | 31 | Red | |
| | | TMTC | Colourless | | TMTC | Colourless | |
| | 1:18 | 15 | Blue | 1:18 | 28 | Blue | |
| | | TMTC | Colourless | | 20 | Red | |
| | | TMTC | Red | | TMTC | Colourless | |
| LSW 3 | 1:3 | 19 | Red | 1:3 | 8 | Blue | 0.4 |
| | | 2 | Blue | | 19 | Colourless | |
| | | TMTC | Colourless | | 5 | Red | |
| | 1:9 | 3 | Blue | 1:9 | 1 | Blue | |
| | | 9 | Red | | 12 | Colourless | |
| | | TMTC | Colourless | | | | |
| | 1:18 | 4 | Blue | 1:18 | 7 | Colourless | |
| | | 5 | Red | | | | |
| | | TMTC | Colourless | | | | |
| LSW 4 | 1:3 | TMTC | Red | 1:3 | - | - | 0.82 |
| | | 12 | Blue | | | | |
| | 1:9 | 6 | Blue | 1:9 | 6 | Colourless | |
| | | TMTC | Red | | | | |
| | | 1 | Colourless | | | | |
| | 1:18 | 49 | Red | 1:18 | 3 | Colourless | |
| | | 1 | Colourless | | | | |
| LSW 5 | 1:3 | TMTC | Blue | 1:3 | 3 | Colourless | 1.27 |
| | | 12 | Red | | 4 | Blue | |
| | 1:9 | TMTC | Blue | 1:9 | 1 | Colourless | |
| | | 1 | Red | | | | |
| | 1:18 | 20 | Blue | 1:18 | 1 | Colourless | |
| | | 5 | Violet | | | | |
| LSW 6 | 1:3 | TMTC | Blue | 1:3 | 1 | Blue | 2.23 |
| | | 26 | Red | | 4 (big colonies) | Colourless | |
| | | 7 | Violet | | - | - | |
| | 1:9 | 32 | Blue | 1:9 | 9 | Colourless | |
| | | 4 | Red | | | | |
| | | 2 | Violet | | | | |
| | | 45 | Colourless | | | | |
| | 1:18 | 10 | Blue | 1:18 | 2 | Colourless | |
| | | 2 | Violet | | | | |
| | | 2 | Red | | | | |
| | | 14 | Colourless | | | | |

*(Continued)*

| Samples | Colifrom *Chromoselect* agar | | | Coliform *Chromoselect* agar + cefotaxime | | | Weight (g) |
|---|---|---|---|---|---|---|---|
| | Dilutions | Number of colonies | Colour | Dilutions | Number of colonies | Colour | |
| LSW 7 | 1:3 | TMTC | Colourless | 1:3 | TMTC | Colourless | 0.77 |
| | | 23 | Red | | | | |
| | | 8 | Blue | | | | |
| | 1:9 | 8 | Red | 1:9 | 18 | Colourless | |
| | | TMTC | Colourless | | | | |
| | 1:18 | 17 | Colourless | 1:18 | 3 | Colourless | |
| | | 2 | Red | | | | |
| LSW 8 | 1:3 | TMTC | Blue | 1:3 | 10 | Colourless | 1.28 |
| | | Uncountable | Red | | | | |
| | 1:9 | 40 | Red | 1:9 | 3 | Colourless | |
| | | 101 | Blue | | | | |
| | | 11 | Violet | | | | |
| | 1:18 | Uncountable | Blue | 1:18 | - | - | |
| | | 7 | Violet | | | | |
| LSW 9 | 1:3 | TMTC | Blue | 1:3 | 72 | Colourless | 0.56 |
| | | 74 | Red | | | | |
| | | TMTC | Colourless | | | | |
| | 1:9 | TMTC | Colourless | 1:9 | 20 | Colourless | |
| | | 38 | Red | | | | |
| | | TMTC | Blue | | | | |
| | 1:18 | 53 | Blue | 1:18 | 22 | Colourless | |
| | | 12 | Red | | | | |
| | | TMTC | Colourless | | | | |
| LSW 10 | 1:3 | 2 | Blue | 1:3 | - | - | 0.67 |
| | | 38 | Red | | | | |
| | | Uncountable | Colourless | | | | |
| | 1:9 | 10 | Red | 1:9 | - | - | |
| | | 22 | Colourless | | | | |
| | 1:18 | 5 | Colourless | 1:18 | - | - | |
| LSW 11 | 1:3 | 6 | Red | 1:3 | TMTC | Blue | 2.43 |
| | | 1 | Violet | | | | |
| | | TMTC | Blue | | | | |
| | 1:9 | 2 | Red | 1:9 | TMTC | Blue | |
| | | TMTC | Blue | | | | |
| | | 1 | Violet | | | | |
| | 1:18 | 2 | Violet | 1:18 | 102 | Blue | |
| | | 110 | Blue | | | | |
| | | 3 | Red | | | | |
| LSW 12 | 1:3 | 2 | Red | 1:3 | - | - | 0.13 |
| | | 10 | Colourless | | | | |
| | 1:9 | 12 | Colourless | 1:9 | - | - | |
| | 1:18 | 3 | Colourless | 1:18 | - | - | |
| LSW 13 | 1:3 | TMTC | Colourless | 1:3 | TMTC | Colourless | 0.91 |
| | | TMTC | Red | | 55 | Blue | |
| | | 90 | Blue | | 59 | Pink | |
| | 1:9 | TMTC | Colourless | 1:9 | TMTC | Colourless | |
| | | TMTC | Red | | 19 | Blue | |
| | | 24 | Blue | | 23 | Pink | |
| | 1:18 | TMTC | Colourless | 1:18 | TMTC | Colourless | |
| | | 5 | Blue | | 2 | Blue | |
| | | 109 | Red | | 4 | Pink | |
| LSW 14 | 1:3 | 16 | Colourless | 1:3 | 5 | Colourless | 5.34 |
| | | 6 | Pink | | | | |
| | 1:9 | 6 | Colourless | 1:9 | - | - | |
| | | 3 | Pink | | | | |
| | 1:18 | 3 | Colourless | 1:18 | - | - | |
| | | 2 | Pink | | | | |
| LSW 15 | 1:3 | TMTC | Red | 1:3 | TMTC | Colourless | 2.23 |
| | | TMTC | Colourless | | 1 | Blue | |
| | | TMTC | Blue | | 10 | Red | |
| | 1:9 | TMTC | Red | 1:9 | TMTC | Colourless | |
| | | TMTC | Colourless | | 1 | Blue | |
| | | 52 | Blue | | | | |
| | 1:18 | TMTC | Red | 1:18 | TMTC | Colourless | |
| | | TMTC | Colourless | | | | |
| | | 11 | Blue | | | | |
| LSW 16 | 1:3 | 2 | Colourless | 1:3 | 1 | Colourless | 0.46 |
| | 1:9 | 2 | Colourless | 1:9 | - | - | |
| | 1:18 | - | - | 1:18 | - | - | |
| LSW 17 | 1:3 | 18 | Red | 1:3 | - | - | 0.44 |
| | 1:9 | 1 | Blue | 1:9 | - | - | |
| | | 2 | Red | | | | |
| | 1:18 | - | - | 1:18 | - | - | |

*(Continued)*

| Samples | Colifrom *Chromoselect* agar | | | Coliform *Chromoselect* agar + cefotaxime | | | Weight (g) |
|---|---|---|---|---|---|---|---|
| | Dilutions | Number of colonies | Colour | Dilutions | Number of colonies | Colour | |
| LSW 18 | 1:3 | - | - | 1:3 | - | - | 0.99 |
| | 1:9 | - | - | 1:9 | - | - | |
| | 1:18 | - | - | 1:18 | - | - | |
| LSW 19 | 1:3 | - | - | 1:3 | - | - | 0.59 |
| | 1:9 | - | - | 1:9 | - | - | |
| | 1:18 | - | - | 1:18 | - | - | |
| LSW 20 | 1:3 | TMTC | Blue | 1:3 | TMTC | Blue | 5.73 |
| | | TMTC | Red | | 2 | Violet | |
| | | TMTC | Colourless | | | | |
| | 1:9 | TMTC | Blue | 1:9 | TMTC | Blue | |
| | | TMTC | Red | | 3 | Violet | |
| | | TMTC | Colourless | | | | |
| | 1:18 | TMTC | Blue | 1:18 | 1 | Red | |
| | | TMTC | Colourless | | 1 | Violet | |
| | | 45 | Red | | 188 | Blue | |
| LSW 21 | 1:3 | TMTC | Red | 1:3 | TMTC | Colourless | 0.39 |
| | | TMTC | Colourless | | | | |
| | | 9 | Blue | | | | |
| | 1:9 | TMTC | Red | 1:9 | TMTC | Colourless | |
| | | TMTC | Colourless | | | | |
| | | 3 | Blue | | | | |
| | 1:18 | TMTC | Red | 1:18 | 1 | Blue | |
| | | TMTC | Colourless | | 45 | Colourless | |
| | | 2 | Blue | | | | |
| LSW 22 | 1:3 | 2 | Colourless | 1:3 | - | - | 0.36 |
| | 1:9 | 1 | Colourless | 1:9 | - | - | |
| | 1:18 | - | - | 1:18 | - | - | |
| LSW 23 | 1:3 | 2 | Blue | 1:3 | TMTC | Colourless | 1.23 |
| | | TMTC | Colourless | | | | |
| | | TMTC | Red | | | | |
| | 1:9 | 21 | Colourless | 1:9 | 29 | Colourless | |
| | | TMTC | Red | | | | |
| | 1:18 | 64 | Red | 1:18 | 20 | Colourless | |
| | | 18 | Colourless | | | | |
| LSW 24 | 1:3 | 36 | Blue | 1:3 | 57 | Colourless | 1.03 |
| | | TMTC | Colourless | | | | |
| | | 20 | Red | | | | |
| | | 7 | Yellow | | | | |
| | 1:9 | 5 | Blue | 1:9 | 27 | Colourless | |
| | | 26 | Red | | | | |
| | | TMTC | Colourless | | | | |
| | 1:18 | 1 | Blue | 1:18 | 10 | Colourless | |
| | | 10 | Red | | | | |
| | | 48 | Colourless | | | | |
| LSW 25 | 1:3 | 10 | Colourless | 1:3 | 4 | Colourless | 0.39 |
| | | 2 | Red | | | | |
| | 1:9 | 2 | Colourless | 1:9 | - | - | |
| | | 3 | Red | | | | |
| | 1:18 | 2 | Colourless | 1:18 | - | - | |
| LSW 26 | 1:3 | TMTC | Blue | 1:3 | 43 | Colourless | 3.08 |
| | | TMTC | Red | | | | |
| | | TMTC | Colourless | | 2 | Blue | |
| | | 1 | Violet | | | | |
| | 1:9 | TMTC | Blue | 1:9 | 34 | Colourless | |
| | | TMTC | Red | | | | |
| | | TMTC | Colourless | | | | |
| | | 1 | Violet | | | | |
| | 1:18 | 88 | Blue | 1:18 | 24 | Colourless | |
| | | 70 | Red | | | | |
| | | TMTC | Colourless | | | | |
| LSW 27 | 1:3 | TMTC | Red | 1:3 | 1 | Blue | 0.58 |
| | | TMTC | Colourless | | 31 | Colourless | |
| | | 1 | Blue | | | | |
| | 1:9 | TMTC | Red | 1:9 | 12 | Colourless | |
| | | TMTC | Colourless | | | | |
| | | 2 | Blue | | | | |
| | 1:18 | TMTC | Red | 1:18 | 1 | Blue | |
| | | TMTC | Colourless | | 5 | Colourless | |
| LSW 28 | 1:3 | 2 | Blue | 1:3 | 1 | Colourless | 0.25 |
| | | TMTC | Colourless | | | | |
| | | TMTC | Red | | | | |
| | 1:9 | 5 | Blue | 1:9 | 14 | Colourless | |
| | | TMTC | Red | | | | |
| | | TMTC | Colourless | | | | |
| | 1:18 | TMTC | Red | 1:18 | 1 | Blue | |
| | | TMTC | Colourless | | | | |

*(Continued)*

| Samples | Colifrom *Chromoselect* agar | | | Coliform *Chromoselect* agar + cefotaxime | | | Weight (g) |
|---|---|---|---|---|---|---|---|
| | Dilutions | Number of colonies | Colour | Dilutions | Number of colonies | Colour | |
| LSW 29 | 1:3 | 4 | Blue | 1:3 | 34 | Colourless | 1.11 |
| | | 10 | Pink | | | | |
| | 1:9 | 1 | Blue | 1:9 | 13 | Colourless | |
| | | 2 | Pink | | | | |
| | 1:18 | 2 | Pink | 1:18 | 7 | Colourless | |
| LSW 30 | 1:3 | TMTC | Red | 1:3 | 3 | Blue | 0.25 |
| | | TMTC | Colourless | | 2 | Red | |
| | | 12 | Blue | | 22 | Colourless | |
| | 1:9 | TMTC | Red | 1:9 | 20 | Colourless | |
| | | TMTC | Colourless | | | | |
| | | 4 | Blue | | | | |
| | 1:18 | TMTC | Red | 1:18 | 21 | Colourless | |
| | | TMTC | Colourless | | | | |
| | | 4 | Blue | | | | |
| LSW 31 | 1:3 | 43 | Red | 1:3 | - | - | 1.86 |
| | | TMTC | Colourless | | | | |
| | 1:9 | 26 | Red | 1:9 | - | - | |
| | | TMTC | Colourless | | | | |
| | 1:18 | 9 | Red | 1:18 | - | - | |
| | | TMTC | Colourless | | | | |
| LSW 32 | 1:3 | 1 | Red | 1:3 | - | - | 0.39 |
| | | 2 | Colourless | | | | |
| | 1:9 | 2 | Colourless | 1:9 | - | - | |
| | 1:18 | 1 | Colourless | 1:18 | - | - | |
| LSW 33 | 1:3 | TMTC | Colourless | 1:3 | 11 | Blue | 3.02 |
| | | TMTC | Blue | | TMTC | Red | |
| | | | | | TMTC | Colourless | |
| | 1:9 | TMTC | Colourless | 1:9 | TMTC | Red | |
| | | 12 | Blue | | TMTC | Colourless | |
| | | TMTC | Red | | | | |
| | 1:18 | TMTC | Colourless | 1:18 | 48 | Red | |
| | | 6 | Blue | | TMTC | Colourless | |
| | | TMTC | Red | | | | |
| LSW 34 | 1:3 | 54 | Red | 1:3 | 4 | Red | 1.03 |
| | | 10 | Blue | | 19 | Colourless | |
| | | 14 | Colourless | | | | |
| | 1:9 | 5 | Blue | 1:9 | 1 | Red | |
| | | 52 | Red | | 16 | Colourless | |
| | | 19 | Colourless | | | | |
| | 1:18 | 1 | Blue | 1:18 | 4 | Colourless | |
| | | 20 | Red | | | | |
| | | 13 | Colourless | | | | |
| LSW 35 | 1:3 | TMTC | Colourless | 1:3 | 11 | Blue | 0.69 |
| | | 18 | Blue | | | | |
| | | 24 | Red | | | | |
| | 1:9 | TMTC | Colourless | 1:9 | 4 | Blue | |
| | | 3 | Blue | | | | |
| | | 23 | Red | | | | |
| | 1:18 | TMTC | Colourless | 1:18 | 6 | Blue | |
| | | 3 | Blue | | | | |
| | | 8 | Red | | | | |
| LSW 36 | 1:3 | 12 | Blue | 1:3 | TMTC | Colourless | 1.41 |
| | | TMTC | Red | | | | |
| | | TMTC | Colourless | | | | |
| | 1:9 | 5 | Blue | 1:9 | TMTC | Colourless | |
| | | 75 | Red | | | | |
| | | TMTC | Colourless | | | | |
| | 1:18 | 3 | Blue | 1:18 | 153 | Colourless | |
| | | 47 | Red | | | | |
| | | TMTC | Colourless | | | | |
| LSW 37 | 1:3 | TMTC | Blue | 1:3 | TMTC | Colourless | 2.42 |
| | 1:9 | TMTC | Blue | 1:9 | 57 | Colourless | |
| | 1:18 | 265 | Blue | 1:18 | 26 | Colourless | |
| | | 13 | Red | | | | |
| LSW 38 | 1:3 | TMTC | Blue | 1:3 | 56 | Blue | 3.17 |
| | | TMTC | Red | | TMTC | Colourless | |
| | 1:9 | TMTC | Blue | 1:9 | 4 | Blue | |
| | | TMTC | Red | | TMTC | Colourless | |
| | | | | | 33 | Red | |
| | 1:18 | TMTC | Blue | 1:18 | 10 | Red | |
| | | TMTC | Red | | 1 | Blue | |
| | | | | | TMTC | Colourless | |

*(Continued)*

| Samples | Colifrom *Chromoselect* agar | | | Coliform *Chromoselect* agar + cefotaxime | | | Weight (g) |
|---|---|---|---|---|---|---|---|
| | Dilutions | Number of colonies | Colour | Dilutions | Number of colonies | Colour | |
| LSW 39 | 1:3 | TMTC | Blue | 1:3 | 57 | Blue | 1.89 |
| | | TMTC | Colourless | | 25 | Red | |
| | | TMTC | Red | | TMTC | Colourless | |
| | 1:9 | 13 | Blue | 1:9 | 10 | Blue | |
| | | TMTC | Colourless | | 4 | Red | |
| | | TMTC | Red | | 112 | Colourless | |
| | 1:18 | 7 | Blue | 1:18 | 2 | Blue | |
| | | TMTC | Colourless | | 46 | Colourless | |
| | | TMTC | Red | | | | |
| LSW 40 | 1:3 | 5 | Blue | 1:3 | 1 | Blue | 1.87 |
| | | TMTC | Red | | 21 | Colourless | |
| | | TMTC | Colourless | | | | |
| | 1:9 | 4 | Blue | 1:9 | 16 | Colourless | |
| | | TMTC | Red | | | | |
| | | TMTC | Colourless | | | | |
| | 1:18 | 5 | Blue | 1:18 | 6 | Colourless | |
| | | TMTC | Red | | | | |
| | | TMTC | Colourless | | | | |
| LSW 41 | 1:3 | 18 | Colourless | 1:3 | - | - | 3.48 |
| | 1:9 | 8 | Colourless | 1:9 | - | - | |
| | | 2 | Red | | | | |
| | 1:18 | 2 | Colourless | 1:18 | 1 | Colourless | |
| LSW 42 | 1:3 | 8 | Blue | 1:3 | 37 | Colourless | 0.42 |
| | | 25 | Colourless | | | | |
| | | TMTC | Red | | | | |
| | 1:9 | 9 | Blue | 1:9 | 13 | Colourless | |
| | | 31 | Colourless | | | | |
| | | TMTC | Red | | | | |
| | 1:18 | 4 | Blue | 1:18 | 10 | Colourless | |
| | | 33 | Colourless | | | | |
| | | TMTC | Red | | | | |
| LSW 43 | 1:3 | TMTC | Red | 1:3 | 3 | Blue | 2.13 |
| | | TMTC | Blue | | TMTC | Colourless | |
| | | TMTC | Colourless | | | | |
| | 1:9 | TMTC | Blue | 1:9 | TMTC | Colourless | |
| | | TMTC | Red | | | | |
| | | TMTC | Colourless | | | | |
| | 1:18 | 74 | Blue | 1:18 | TMTC | Colourless | |
| | | TMTC | Red | | | | |
| | | TMTC | Colourless | | | | |
| LSW 44 | 1:3 | TMTC | Colourless | 1:3 | 11 | Colourless | 0.26 |
| | | Uncountable | Blue | | | | |
| | 1:9 | TMTC | Colourless | 1:9 | 14 | Colourless | |
| | | 10 | Blue | | | | |
| | 1:18 | TMTC | Colourless | 1:18 | 4 | Colourless | |
| | | 11 | Blue | | | | |
| LSW 45 | 1:3 | Uncountable | Blue | 1:3 | TMTC | Red | 2.15 |
| | | 16 | Red | | TMTC | Colourless | |
| | 1:9 | Uncountable | Red | 1:9 | 52 | Red | |
| | | 4 | Violet | | 2 | Blue | |
| | | 13 (big) | Blue | | 67 | Colourless | |
| | 1:18 | 13 | Red | 1:18 | 13 | Red | |
| | | Uncountable | Blue | | 26 | Colourless | |
| | | | | | | | |
| LSW 46 | 1:3 | 22 | Red | 1:3 | - | - | 0.2 |
| | | 2 | Blue | | | | |
| | | Uncountable | Colourless | | | | |
| | 1:9 | 16 | Colourless | 1:9 | - | - | |
| | 1:18 | 1 | Blue | 1:18 | - | - | |
| | | 5 | Colourless | | | | |
| LSW 47 | 1:3 | 21 | Blue | 1:3 | 10 | Blue | 0.49 |
| | | TMTC | Red | | 11 | Colourless | |
| | | TMTC | Colourless | | | | |
| | 1:9 | 9 | Blue | 1:9 | 5 | Blue | |
| | | TMTC | Red | | 4 | Colourless | |
| | | TMTC | Colourless | | | | |
| | 1:18 | 3 | Blue | 1:18 | 2 | Blue | |
| | | TMTC | Red | | | | |
| | | TMTC | Colourless | | | | |

***Table S.9. The count of colonies of gull samples from the rural site (LSA).*** *Three dilutions (1:3, 1:9 and 1:18) were used. The counting of colonies was undertaken after 48 h of incubation. The colour of colonies observed on Coliform ChromoSelect agar and the weight of faeces used are shown. '-' = no colonies observed. 'TMTC' = Too many to count.*

| Samples | Colifrom *Chromoselect* agar | | | Coliform *Chromoselect* agar + cefotaxime | | | Weight (g) |
|---|---|---|---|---|---|---|---|
| | Dilutions | Number of colonies | Colour | Dilutions | Number of colonies | Colour | |
| LSA 1 | 1:3 | TMTC | Blue | 1:3 | 2 | Colourless | 3.82 |
| | 1:9 | TMTC | Blue | 1:9 | - | - | |
| | 1:18 | TMTC | Blue | 1:18 | 1 | Colourless | |
| LSA 2 | 1:3 | TMTC | Blue | 1:3 | - | - | 4.12 |
| | | TMTC | Red | | | | |
| | 1:9 | TMTC | Blue | 1:9 | - | - | |
| | | TMTC | Red | | | | |
| | 1:18 | TMTC | Blue | 1:18 | - | - | |
| | | TMTC | Red | | | | |
| LSA 3 | 1:3 | TMTC | Blue | 1:3 | - | - | 3.62 |
| | | TMTC | Red | | | | |
| | 1:9 | TMTC | Blue | 1:9 | - | - | |
| | | TMTC | Red | | | | |
| | 1:18 | TMTC | Blue | 1:18 | - | - | |
| | | TMTC | Red | | | | |
| LSA 4 | 1:3 | TMTC | Blue | 1:3 | - | - | 3.82 |
| | | TMTC | Red | | | | |
| | 1:9 | TMTC | Blue | 1:9 | - | - | |
| | | TMTC | Red | | | | |
| | 1:18 | TMTC | Blue | 1:18 | - | - | |
| | | TMTC | Red | | | | |
| LSA 5 | 1:3 | TMTC | Blue | 1:3 | TMTC | Colourless | 2.02 |
| | 1:9 | TMTC | Blue | 1:9 | TMTC | Colourless | |
| | 1:18 | TMTC | Blue | 1:18 | 68 | Colourless | |
| LSA 6 | 1:3 | TMTC | Blue | 1:3 | 2 | Colourless | 2.32 |
| | 1:9 | TMTC | Blue | 1:9 | 6 | Colourless | |
| | 1:18 | TMTC | Blue | 1:18 | - | - | |
| LSA 7 | 1:3 | TMTC | Blue | 1:3 | 4 | Red | 3.72 |
| | | TMTC | Red | | TMTC | Colourless | |
| | 1:9 | TMTC | Blue | 1:9 | TMTC | Colourless | |
| | | TMTC | Red | | | | |
| | 1:18 | TMTC | Blue | 1:18 | TMTC | Colourless | |
| | | TMTC | Red | | | | |
| LSA 8 | 1:3 | TMTC | Blue | 1:3 | 2 | Colourless | 1.82 |
| | 1:9 | TMTC | Blue | 1:9 | - | - | |
| | | 3 | Red | | | | |
| | 1:18 | TMTC | Blue | 1:18 | 1 | Colourless | |
| LSA 9 | 1:3 | TMTC | Blue | 1:3 | - | - | 6.42 |
| | | TMTC | Red | | | | |
| | 1:9 | TMTC | Blue | 1:9 | - | - | |
| | | TMTC | Red | | | | |
| | 1:18 | 147 | Blue | 1:18 | - | - | |
| | | TMTC | Red | | | | |
| LSA 10 | 1:3 | TMTC | Blue | 1:3 | 2 | Colourless | 2.02 |
| | 1:9 | TMTC | Blue | 1:9 | 1 | Colourless | |
| | 1:18 | TMTC | Blue | 1:18 | - | - | |
| LSA 11 | 1:3 | TMTC | Blue | 1:3 | - | - | 3.02 |
| | 1:9 | 123 | Blue | 1:9 | - | - | |
| | 1:18 | 15 | Blue | 1:18 | - | - | |
| LSA 12 | 1:3 | TMTC | Blue | 1:3 | 51 | Colourless | 4.22 |
| | | TMTC | Red | | | | |
| | 1:9 | TMTC | Blue | 1:9 | 30 | Colourless | |
| | | TMTC | Red | | | | |
| | 1:18 | TMTC | Blue | 1:18 | 13 | Colourless | |
| | | TMTC | Red | | | | |
| LSA 13 | 1:3 | TMTC | Blue | 1:3 | 20 | Colourless | 3.82 |
| | | TMTC | Red | | | | |
| | 1:9 | TMTC | Blue | 1:9 | 10 | Colourless | |
| | | TMTC | Red | | | | |
| | 1:18 | TMTC | Blue | 1:18 | 2 | Colourless | |
| | | TMTC | Red | | | | |
| LSA 14 | 1:3 | TMTC | Red | 1:3 | - | - | 1.12 |
| | | 10 | Blue | | | | |
| | 1:9 | TMTC | Red | 1:9 | 5 | Colourless | |
| | 1:18 | TMTC | Red | 1:18 | - | - | |
| LSA 15 | 1:3 | TMTC | Blue | 1:3 | - | - | 4.72 |
| | 1:9 | TMTC | Blue | 1:9 | - | - | |
| | 1:18 | TMTC | Blue | 1:18 | - | - | |

*(Continued)*

| Samples | Colifrom *Chromoselect* agar | | | Coliform *Chromoselect* agar + cefotaxime | | | Weight (g) |
|---|---|---|---|---|---|---|---|
| | Dilutions | Number of colonies | Colour | Dilutions | Number of colonies | Colour | |
| LSA 16 | 1:3 | TMTC | Blue | 1:3 | 1 | Colourless | 7.52 |
| | | TMTC | Red | | | | |
| | 1:9 | TMTC | Blue | 1:9 | - | - | |
| | | TMTC | Red | | | | |
| | 1:18 | TMTC | Blue | 1:18 | - | - | |
| | | TMTC | Red | | | | |
| LSA 17 | 1:3 | TMTC | Blue | 1:3 | - | - | 5.82 |
| | | TMTC | Red | | | | |
| | 1:9 | TMTC | Blue | 1:9 | - | - | |
| | | TMTC | Red | | | | |
| | 1:18 | TMTC | Blue | 1:18 | - | - | |
| | | TMTC | Red | | | | |
| LSA 18 | 1:3 | 1 | Blue | 1:3 | 3 | Colourless | 1.72 |
| | | 25 | Red | | | | |
| | | TMTC | Colourless | | | | |
| | 1:9 | 52 | Red | 1:9 | - | - | |
| | | TMTC | Colourless | | | | |
| | 1:18 | 22 | Red | 1:18 | - | - | |
| | | TMTC | Colourless | | | | |
| LSA 19 | 1:3 | TMTC | Blue | 1:3 | - | - | 1.92 |
| | 1:9 | TMTC | Blue | 1:9 | - | - | |
| | 1:18 | TMTC | Blue | 1:18 | - | - | |
| LSA 20 | 1:3 | TMTC | Blue | 1:3 | - | - | 5.32 |
| | | TMTC | Red | | | | |
| | 1:9 | TMTC | Blue | 1:9 | - | - | |
| | | TMTC | Red | | | | |
| | 1:18 | 147 | Blue | 1:18 | - | - | |
| | | TMTC | Red | | | | |
| LSA 21 | 1:3 | 44 | Blue | 1:3 | 8 | Colourless | 3.02 |
| | | 18 | Red | | | | |
| | | TMTC | Colourless | | | | |
| | 1:9 | 23 | Blue | 1:9 | 7 | Colourless | |
| | | 60 | Colourless | | | | |
| | 1:18 | 13 | Blue | 1:18 | - | - | |
| | | 1 | Red | | | | |
| | | 29 | Colourless | | | | |
| LSA 22 | 1:3 | TMTC | Blue | 1:3 | 6 | Colourless | 2.22 |
| | 1:9 | TMTC | Blue | 1:9 | 2 | Colourless | |
| | 1:18 | TMTC | Blue | 1:18 | - | - | |
| LSA 23 | 1:3 | TMTC | Blue | 1:3 | 15 | Colourless | 2.62 |
| | | TMTC | Red | | | | |
| | 1:9 | TMTC | Blue | 1:9 | - | - | |
| | | TMTC | Red | | | | |
| | 1:18 | TMTC | Blue | 1:18 | - | - | |
| | | TMTC | Red | | | | |
| LSA 24 | 1:3 | 4 | Blue | 1:3 | - | - | 2.72 |
| | | TMTC | Red | | | | |
| | | TMTC | Colourless | | | | |
| | 1:9 | 3 | Blue | 1:9 | - | - | |
| | | TMTC | Red | | | | |
| | | TMTC | Colourless | | | | |
| | 1:18 | TMTC | Red | 1:18 | - | - | |
| | | TMTC | Colourless | | | | |
| LSA 25 | 1:3 | TMTC | Red | 1:3 | - | - | 0.62 |
| | 1:9 | TMTC | Red | 1:9 | - | - | |
| | 1:18 | TMTC | Red | 1:18 | - | - | |
| LSA 26 | 1:3 | TMTC | Blue | 1:3 | - | - | 3.62 |
| | | TMTC | Red | | | | |
| | 1:9 | TMTC | Blue | 1:9 | - | - | |
| | | TMTC | Red | | | | |
| | 1:18 | TMTC | Blue | 1:18 | - | - | |
| | | TMTC | Red | | | | |
| LSA 27 | 1:3 | TMTC | Colourless | 1:3 | - | - | 3.82 |
| | | TMTC | Blue | | | | |
| | 1:9 | TMTC | Colourless | 1:9 | - | - | |
| | | 30 | Blue | | | | |
| | 1:18 | TMTC | Colourless | 1:18 | - | - | |
| | | 3 | Blue | | | | |
| LSA 28 | 1:3 | TMTC | Red | 1:3 | - | - | 1.52 |
| | | 26 | Blue | | | | |
| | 1:9 | TMTC | Red | 1:9 | - | - | |
| | | 8 | Blue | | | | |
| | 1:18 | TMTC | Red | 1:18 | - | - | |
| | | 3 | Blue | | | | |

*(Continued)*

| Samples | Colifrom *Chromoselect* agar | | | Coliform *Chromoselect* agar + cefotaxime | | | Weight (g) |
|---|---|---|---|---|---|---|---|
| | Dilutions | Number of colonies | Colour | Dilutions | Number of colonies | Colour | |
| LSA 29 | 1:3 | TMTC | Blue | 1:3 | 12 | Colourless | 1.92 |
| | | TMTC | Red | | | | |
| | 1:9 | TMTC | Blue | 1:9 | - | - | |
| | | TMTC | Red | | | | |
| | 1:18 | TMTC | Blue | 1:18 | 2 | Colourless | |
| | | TMTC | Red | | | | |
| LSA 30 | 1:3 | TMTC | Blue | 1:3 | 8 | Colourless | 6.42 |
| | | TMTC | Red | | | | |
| | 1:9 | TMTC | Blue | 1:9 | - | - | |
| | | TMTC | Red | | | | |
| | 1:18 | TMTC | Blue | 1:18 | - | - | |
| | | TMTC | Red | | | | |
| LSA 31 | 1:3 | Shade | Blue | 1:3 | TMTC | Blue | 1.52 |
| | | | | | TMTC | Colourless | |
| | 1:9 | Shade | Blue | 1:9 | 134 | Blue | |
| | | | | | TMTC | Colourless | |
| | 1:18 | Shade | Blue | 1:18 | 78 | Blue | |
| | | | | | TMTC | Colourless | |
| LSA 32 | 1:3 | Shade | Blue | 1:3 | 55 | Colourless | 2.82 |
| | 1:9 | Shade | Blue | 1:9 | 20 | Colourless | |
| | 1:18 | Shade | Blue | 1:18 | 2 | Colourless | |
| LSA 33 | 1:3 | TMTC | Blue | 1:3 | 20 | Colourless | 2.92 |
| | | TMTC | Colourless | | | | |
| | 1:9 | TMTC | Blue | 1:9 | - | - | |
| | | TMTC | Colourless | | | | |
| | 1:18 | 197 | Blue | 1:18 | - | - | |
| | | TMTC | Colourless | | | | |
| LSA 34 | 1:3 | TMTC | Blue | 1:3 | - | - | 8.52 |
| | | TMTC | Red | | | | |
| | 1:9 | TMTC | Blue | 1:9 | - | - | |
| | | TMTC | Red | | | | |
| | 1:18 | 86 | Blue | 1:18 | - | - | |
| | | TMTC | Red | | | | |
| LSA 35 | 1:3 | TMTC | Blue | 1:3 | - | - | 2.32 |
| | | TMTC | Red | | | | |
| | 1:9 | TMTC | Blue | 1:9 | - | - | |
| | | TMTC | Red | | | | |
| | 1:18 | TMTC | Blue | 1:18 | - | - | |
| | | TMTC | Red | | | | |
| LSA 36 | 1:3 | TMTC | Blue | 1:3 | 3 | Colourless | 6.02 |
| | 1:9 | TMTC | Blue | 1:9 | 4 | Colourless | |
| | 1:18 | TMTC | Blue | 1:18 | - | - | |
| LSA 37 | 1:3 | 10 | Blue | 1:3 | TMTC | Colourless | 6.82 |
| | | 27 | Red | | | | |
| | 1:9 | 20 | Red | 1:9 | TMTC | Colourless | |
| | 1:18 | 4 | Blue | 1:18 | 66 | Colourless | |
| | | 1 | Red | | | | |
| LSA 38 | 1:3 | 13 | Blue | 1:3 | - | - | 3.12 |
| | | 5 | Red | | | | |
| | 1:9 | 9 | Blue | 1:9 | - | - | |
| | | 1 | Red | | | | |
| | 1:18 | 5 | Blue | 1:18 | - | - | |
| | | 1 | Red | | | | |
| LSA 39 | 1:3 | TMTC | Red | 1:3 | TMTC | Colourless | 1.52 |
| | | TMTC | Blue | | | | |
| | 1:9 | TMTC | Red | 1:9 | TMTC | Colourless | |
| | | TMTC | Blue | | | | |
| | 1:18 | TMTC | Red | 1:18 | TMTC | Colourless | |
| | | 211 | Blue | | | | |
| LSA 40 | 1:3 | TMTC | Blue | 1:3 | - | - | 3.52 |
| | | TMTC | Red | | | | |
| | 1:9 | 97 | Blue | 1:9 | - | - | |
| | | TMTC | Red | | | | |
| | 1:18 | 50 | Blue | 1:18 | - | - | |
| | | TMTC | Red | | | | |
| LSA 41 | 1:3 | TMTC | Red | 1:3 | 8 | Colourless | 4.42 |
| | | TMTC | Blue | | | | |
| | 1:9 | TMTC | Red | 1:9 | 6 | Colourless | |
| | | TMTC | Blue | | | | |
| | 1:18 | 263 | Red | 1:18 | - | - | |
| | | 121 | Blue | | | | |

*(Continued)*

| Samples | Colifrom *Chromoselect* agar | | | Coliform *Chromoselect* agar + cefotaxime | | | Weight (g) |
|---|---|---|---|---|---|---|---|
| | Dilutions | Number of colonies | Colour | Dilutions | Number of colonies | Colour | |
| LSA 42 | 1:3 | TMTC | Red | 1:3 | 3 | Colourless | 2.62 |
| | | TMTC | Blue | | | | |
| | 1:9 | TMTC | Red | 1:9 | - | Colourless | |
| | | TMTC | Blue | | | | |
| | 1:18 | TMTC | Red | 1:18 | - | - | |
| | | TMTC | Blue | | | | |
| LSA 43 | 1:3 | - | - | 1:3 | - | - | 0.52 |
| | 1:9 | - | - | 1:9 | - | - | |
| | 1:18 | - | - | 1:18 | - | - | |
| LSA 44 | 1:3 | - | - | 1:3 | - | - | 0.82 |
| | 1:9 | - | - | 1:9 | - | - | |
| | 1:18 | - | - | 1:18 | - | - | |
| LSA 45 | 1:3 | 1 | Colourless | 1:3 | - | - | 1.02 |
| | | Shade | Blue | | | | |
| | 1:9 | Shade | Blue | 1:9 | Shade | Blue | |
| | 1:18 | Shade | Blue | 1:18 | Shade | Blue | |
| LSA 46 | 1:3 | - | - | 1:3 | Shade | Blue | 1.01 |
| | 1:9 | - | - | 1:9 | - | - | |
| | 1:18 | - | - | 1:18 | - | - | |
| LSA 47 | 1:3 | - | - | 1:3 | - | - | 0.42 |
| | 1:9 | - | - | 1:9 | - | - | |
| | 1:18 | - | - | 1:18 | - | - | |
| LSA 48 | 1:3 | - | - | 1:3 | - | - | 0.42 |
| | 1:9 | - | - | 1:9 | - | - | |
| | 1:18 | - | - | 1:18 | - | - | |
| LSA 49 | 1:3 | - | - | 1:3 | - | - | 0.42 |
| | 1:9 | - | - | 1:9 | - | - | |
| | 1:18 | - | - | 1:18 | - | - | |
| LSA 50 | 1:3 | - | - | 1:3 | - | - | 0.42 |
| | 1:9 | - | - | 1:9 | - | - | |
| | 1:18 | - | - | 1:18 | - | - | |

### 2.1.2 Goose Faeces

***Table S.10. The count of colonies of goose samples from the urban site (GD, GS, G. Dun., GL).*** *Two sets of dilutions (10-fold dilutions and 1:4, 1:16 and 1:48) were used. The counting of colonies was undertaken after 48 h of incubation. The colour of colonies observed on Coliform ChromoSelect agar and the weight of faeces used are shown. '-' = no colonies observed. 'TMTC' = Too many to count.*

| Samples | Colifrom *Chromoselect* agar | | | Coliform *Chromoselect* agar + cefotaxime | | | Weight (g) |
|---|---|---|---|---|---|---|---|
| | Dilutions | Number of colonies | Colour | Dilutions | Number of colonies | Colour | |
| GD 1 | $10^{-1}$ | - | - | $10^{-1}$ | - | - | 1 |
| | $10^{-2}$ | - | - | $10^{-2}$ | - | - | |
| | $10^{-3}$ | - | - | $10^{-3}$ | - | - | |
| | $10^{-4}$ | - | - | $10^{-4}$ | - | - | |
| | $10^{-5}$ | - | - | $10^{-5}$ | - | - | |
| GD 2 | $10^{-1}$ | 5 | Colourless | $10^{-1}$ | 4 | Colourless | 1 |
| | $10^{-2}$ | 1 | Pink | $10^{-2}$ | - | - | |
| | $10^{-3}$ | - | - | $10^{-3}$ | - | - | |
| | $10^{-4}$ | - | - | $10^{-4}$ | - | - | |
| | $10^{-5}$ | - | - | $10^{-5}$ | - | - | |
| GD 3 | $10^{-1}$ | - | - | $10^{-1}$ | - | - | 1 |
| | $10^{-2}$ | - | - | $10^{-2}$ | - | - | |
| | $10^{-3}$ | - | - | $10^{-3}$ | - | - | |
| | $10^{-4}$ | - | - | $10^{-4}$ | - | - | |
| | $10^{-5}$ | - | - | $10^{-5}$ | - | - | |

*(Continued)*

| Samples | Colifrom *Chromoselect* agar | | | Coliform *Chromoselect* agar + cefotaxime | | | Weight (g) |
|---|---|---|---|---|---|---|---|
| | Dilutions | Number of colonies | Colour | Dilutions | Number of colonies | Colour | |
| GD 4 | $10^{-1}$ | 1 | Pink | $10^{-1}$ | 2 | Colourless | 1 |
| | | 9 | Colourless | | | | |
| | $10^{-2}$ | - | - | $10^{-2}$ | - | - | |
| | $10^{-3}$ | - | - | $10^{-3}$ | - | - | |
| | $10^{-4}$ | - | - | $10^{-4}$ | - | - | |
| | $10^{-5}$ | - | - | $10^{-5}$ | - | - | |
| GD 5 | $10^{-1}$ | - | - | $10^{-1}$ | - | - | 1 |
| | $10^{-2}$ | - | - | $10^{-2}$ | - | - | |
| | $10^{-3}$ | - | - | $10^{-3}$ | - | - | |
| | $10^{-4}$ | - | - | $10^{-4}$ | - | - | |
| | $10^{-5}$ | - | - | $10^{-5}$ | - | - | |
| GD 6 | $10^{-1}$ | 5 | Red | $10^{-1}$ | 7 | Colourless | 1 |
| | | 25 | Blue | | | | |
| | | 1 | Colourless | | | | |
| | $10^{-2}$ | 2 | Blue | $10^{-2}$ | - | - | |
| | $10^{-3}$ | 1 | Blue | $10^{-3}$ | - | - | |
| | $10^{-4}$ | - | - | $10^{-4}$ | - | - | |
| | $10^{-5}$ | - | - | $10^{-5}$ | - | - | |
| GD 7 | $10^{-1}$ | 7 | Red | $10^{-1}$ | 7 | Colourless | 1 |
| | | 1 | Blue | | | | |
| | | 10 | Colourless | | | | |
| | $10^{-2}$ | 4 | Red | $10^{-2}$ | 4 | Colourless | |
| | | 2 | Colourless | | | | |
| | $10^{-3}$ | - | - | $10^{-3}$ | - | - | |
| | $10^{-4}$ | - | - | $10^{-4}$ | - | - | |
| | $10^{-5}$ | - | - | $10^{-5}$ | - | - | |
| GD 8 | $10^{-1}$ | - | - | $10^{-1}$ | 1 | Colourless | 1 |
| | $10^{-2}$ | - | - | $10^{-2}$ | - | - | |
| | $10^{-3}$ | - | - | $10^{-3}$ | - | - | |
| | $10^{-4}$ | - | - | $10^{-4}$ | - | - | |
| | $10^{-5}$ | - | - | $10^{-5}$ | - | - | |
| GD 9 | 1:4 | 1 | Red | 1:4 | - | - | 1 |
| | 1:16 | - | - | 1:16 | - | - | |
| | 1:48 | - | - | 1:48 | - | - | |
| GD 10 | 1:4 | TMTC | Red | 1:4 | - | - | 1 |
| | | 1 | Blue | | | | |
| | 1:16 | 102 | Red | 1:16 | - | - | |
| | 1:48 | 22 | Red | 1:48 | - | - | |
| GD 11 | 1:4 | 4 | Red | 1:4 | 30 | Colourless | 1 |
| | | 49 | Colourless | | | | |
| | 1:16 | 11 | Red | 1:16 | - | - | |
| | 1:48 | - | - | 1:48 | - | - | |
| GD 12 | 1:4 | 35 | Red | 1:4 | - | - | 1 |
| | 1:16 | 9 | Colourless | 1:16 | - | - | |
| | 1:48 | - | - | 1:48 | - | - | |
| GD 13 | 1:4 | 2 | Straw | 1:4 | 15 | Colourless | 1 |
| | | TMTC | Colourless | | | | |
| | 1:16 | 3 | Straw | 1:16 | 4 | Colourless | |
| | | 41 | Colourless | | | | |
| | 1:48 | 3 | Colourless | 1:48 | - | - | |
| GD 14 | 1:4 | - | - | 1:4 | - | - | 1 |
| | 1:16 | - | - | 1:16 | - | - | |
| | 1:48 | - | - | 1:48 | - | - | |
| GD 15 | 1:4 | 5 | Red | 1:4 | - | - | 1 |
| | 1:16 | - | - | 1:16 | - | - | |
| | 1:48 | - | - | 1:48 | - | - | |

*(Continued)*

| Samples | Colifrom *Chromoselect* agar | | | Coliform *Chromoselect* agar + cefotaxime | | | Weight (g) |
|---|---|---|---|---|---|---|---|
| | Dilutions | Number of colonies | Colour | Dilutions | Number of colonies | Colour | |
| GD 16 | 1:4 | 55 | Red | 1:4 | - | - | 1 |
| | | 9 | Colourless | | | | |
| | 1:16 | - | - | 1:16 | - | - | |
| | 1:48 | - | - | 1:48 | - | - | |
| GD 17 | 1:4 | 8 | Red | 1:4 | - | - | 1 |
| | 1:16 | - | - | 1:16 | - | - | |
| | 1:48 | - | - | 1:48 | - | - | |
| GD 18 | 1:4 | 1 | Blue | 1:4 | 19 | Colourless | 1 |
| | | 5 | Red | | | | |
| | | 10 | Yellow | | | | |
| | | 7 | Colourless | | | | |
| | 1:16 | 11 | Red | 1:16 | - | - | |
| | | 7 | Colourless | | | | |
| | 1:48 | 1 | Red | 1:48 | - | - | |
| GD 19 | 1:4 | 7 | Colourless | 1:4 | 2 | Colourless | 1 |
| | | 1 | Red | | | | |
| | 1:16 | 1 | Colourless | 1:16 | - | - | |
| | 1:48 | - | - | 1:48 | - | - | |
| GD 20 | 1:4 | - | - | 1:4 | - | - | 1 |
| | 1:16 | - | - | 1:16 | - | - | |
| | 1:48 | - | - | 1:48 | - | - | |
| GD 21 | 1:4 | 23 | Blue | 1:4 | - | - | 1 |
| | 1:16 | 6 | Blue | 1:16 | - | - | |
| | 1:48 | 1 | Blue | 1:48 | - | - | |
| GD 22 | 1:4 | - | - | 1:4 | - | - | 1 |
| | 1:16 | - | - | 1:16 | - | - | |
| | 1:48 | - | - | 1:48 | - | - | |
| GD 23 | 1:4 | - | - | 1:4 | - | - | 1 |
| | 1:16 | - | - | 1:16 | - | - | |
| | 1:48 | - | - | 1:48 | - | - | |
| GD 24 | 1:4 | - | - | 1:4 | - | - | 1 |
| | 1:16 | - | - | 1:16 | - | - | |
| | 1:48 | - | - | 1:48 | - | - | |
| GD 25 | 1:4 | 2 | Red | 1:4 | - | - | 1 |
| | 1:16 | - | - | 1:16 | - | - | |
| | 1:48 | - | - | 1:48 | - | - | |
| GD 26 | 1:4 | - | - | 1:4 | 2 | Colourless | 1 |
| | 1:16 | - | - | 1:16 | - | - | |
| | 1:48 | - | - | 1:48 | - | - | |
| GD 27 | 1:4 | 25 | Red | 1:4 | - | - | 1 |
| | 1:16 | - | - | 1:16 | - | - | |
| | 1:48 | - | - | 1:48 | - | - | |
| GD 28 | 1:4 | - | - | 1:4 | - | - | 1 |
| | 1:16 | - | - | 1:16 | - | - | |
| | 1:48 | - | - | 1:48 | - | - | |
| GD 29 | 1:4 | 1 | Red | 1:4 | - | - | 1 |
| | 1:16 | - | - | 1:16 | - | - | |
| | 1:48 | - | - | 1:48 | - | - | |
| GD 30 | 1:4 | - | - | 1:4 | - | - | 1 |
| | 1:16 | - | - | 1:16 | - | - | |
| | 1:48 | - | - | 1:48 | - | - | |
| GD 31 | 1:4 | TMTC | Colourless | 1:4 | 4 | Colourless | 1 |
| | | 43 | Red | | | | |
| | 1:16 | TMTC | Colourless | 1:16 | 2 | Colourless | |
| | 1:48 | TMTC | Colourless | 1:48 | - | - | |
| GD 32 | 1:4 | - | - | 1:4 | 3 | Colourless | 1 |
| | 1:16 | - | - | 1:16 | - | - | |
| | 1:48 | - | - | 1:48 | - | - | |

*(Continued)*

| Samples | Colifrom *Chromoselect* agar | | | Coliform *Chromoselect* agar + cefotaxime | | | Weight (g) |
|---|---|---|---|---|---|---|---|
| | Dilutions | Number of colonies | Colour | Dilutions | Number of colonies | Colour | |
| GD 33 | 1:4 | TMTC | Red | 1:4 | - | - | 1 |
| | | 1 | Blue | | | | |
| | 1:16 | 8 | Red | 1:16 | - | - | |
| | 1:48 | - | - | 1:48 | - | - | |
| GD 34 | 1:4 | 19 | Colourless | 1:4 | - | - | 1 |
| | 1:16 | - | - | 1:16 | - | - | |
| | 1:48 | 1 | Red | 1:48 | - | - | |
| GD 35 | 1:4 | 10 | Colourless | 1:4 | - | - | 1 |
| | 1:16 | - | - | 1:16 | - | - | |
| | 1:48 | - | - | 1:48 | - | - | |
| GD 36 | 1:4 | 3 | Red | 1:4 | 1 | Colourless | 1 |
| | | 4 | Colourless | | | | |
| | 1:16 | 1 | Colourless | 1:16 | - | - | |
| | 1:48 | 1 | Colourless | 1:48 | - | - | |
| GD 37 | 1:4 | TMTC | Red | 1:4 | 14 | Colourless | 1 |
| | 1:16 | TMTC | Red | 1:16 | - | - | |
| | 1:48 | 38 | Red | 1:48 | - | - | |
| GD 38 | 1:4 | - | - | 1:4 | 1 | Colourless | 1 |
| | 1:16 | - | - | 1:16 | - | - | |
| | 1:48 | - | - | 1:48 | - | - | |
| GD 39 | 1:4 | 14 | Red | 1:4 | 5 | Colourless | 1 |
| | | 17 | Colourless | | | | |
| | 1:16 | 5 | Red | 1:16 | - | - | |
| | | 9 | Colourless | | | | |
| | 1:48 | 1 | Colourless | 1:48 | - | - | |
| GD 40 | 1:4 | - | - | 1:4 | - | - | 1 |
| | 1:16 | - | - | 1:16 | - | - | |
| | 1:48 | - | - | 1:48 | - | - | |
| GD 41 | 1:4 | 15 | Colourless | 1:4 | - | - | 1 |
| | 1:16 | - | - | 1:16 | - | - | |
| | 1:48 | 1 | Colourless | 1:48 | - | - | |
| GD 42 | 1:4 | 20 | Red | 1:4 | - | - | 1 |
| | 1:16 | - | - | 1:16 | - | - | |
| | 1:48 | - | - | 1:48 | - | - | |
| GD 43 | 1:4 | - | - | 1:4 | - | - | 1 |
| | 1:16 | - | - | 1:16 | - | - | |
| | 1:48 | - | - | 1:48 | - | - | |
| GD 44 | 1:4 | 1 | Blue | 1:4 | - | - | 1 |
| | | 5 | Red | | | | |
| | | 4 | Colourless | | | | |
| | 1:16 | - | - | 1:16 | - | - | |
| | 1:48 | - | - | 1:48 | - | - | |
| GD 45 | 1:4 | 4 | Colourless | 1:4 | - | - | 1 |
| | 1:16 | - | - | 1:16 | - | - | |
| | 1:48 | - | - | 1:48 | - | - | |
| GD 46 | 1:4 | - | - | 1:4 | - | - | 1 |
| | 1:16 | - | - | 1:16 | - | - | |
| | 1:48 | - | - | 1:48 | - | - | |
| GD 47 | 1:4 | 12 | Blue | 1:4 | - | - | 1 |
| | | 25 | Colourless | | | | |
| | 1:16 | 2 | Blue | 1:16 | - | - | |
| | | 3 | Red | | | | |
| | 1:48 | 1 | Red | 1:48 | - | - | |
| G.Dun.1 | 1:4 | 1 | Blue | 1:4 | - | - | 1 |
| | | 4 | Colourless | | | | |
| | 1:16 | 2 | Blue | 1:16 | - | - | |
| | 1:48 | - | - | 1:48 | - | - | |
| G.Dun. 2 | 1:4 | 4 | Colourless | 1:4 | 5 | Colourless | 1 |
| | 1:16 | 1 | Colourless | 1:16 | - | - | |
| | 1:48 | - | - | 1:48 | - | - | |

*(Continued)*

| Samples | Colifrom *Chromoselect* agar | | | Coliform *Chromoselect* agar + cefotaxime | | | Weight (g) |
|---|---|---|---|---|---|---|---|
| | Dilutions | Number of colonies | Colour | Dilutions | Number of colonies | Colour | |
| G.Dun. 3 | 1:4 | - | - | 1:4 | - | - | 1 |
| | 1:16 | - | - | 1:16 | - | - | |
| | 1:48 | - | - | 1:48 | - | - | |
| G.Dun. 4 | 1:4 | 3 | Colourless | 1:4 | 2 | Colourless | 1 |
| | 1:16 | - | - | 1:16 | - | - | |
| | 1:48 | - | - | 1:48 | - | - | |
| G.Dun. 5 | 1:4 | 1 | Red | 1:4 | - | - | 1 |
| | 1:16 | - | - | 1:16 | - | - | |
| | 1:48 | - | - | 1:48 | - | - | |
| G.Dun. 6 | 1:4 | 1 | Blue | 1:4 | - | - | 1 |
| | | 3 | Colourless | | | | |
| | 1:16 | 2 | Colourless | 1:16 | - | - | |
| | 1:48 | - | - | 1:48 | - | - | |
| G.Dun. 7 | 1:4 | 1 | Colourless | 1:4 | 1 | Colourless | 1 |
| | 1:16 | - | - | 1:16 | - | - | |
| | 1:48 | - | - | 1:48 | - | - | |
| G.Dun. 8 | 1:4 | - | - | 1:4 | 1 | Colourless | 1 |
| | 1:16 | - | - | 1:16 | - | - | |
| | 1:48 | - | - | 1:48 | - | - | |
| G.Dun. 9 | 1:4 | 82 | Red | 1:4 | - | - | 1 |
| | | 2 | Colourless | | | | |
| | 1:16 | 29 | Red | 1:16 | - | - | |
| | 1:48 | 15 | Red | 1:48 | - | - | |
| G.Dun. 10 | 1:4 | 9 | Colourless | 1:4 | 1 | Colourless | 1 |
| | 1:16 | - | - | 1:16 | - | - | |
| | 1:48 | - | - | 1:48 | - | - | |
| G.Dun. 11 | 1:4 | 1 | Blue | 1:4 | - | - | 1 |
| | 1:16 | - | - | 1:16 | - | - | |
| | 1:48 | - | - | 1:48 | - | - | |
| G.Dun. 12 | 1:4 | - | - | 1:4 | - | - | 1 |
| | 1:16 | - | - | 1:16 | - | - | |
| | 1:48 | - | - | 1:48 | - | - | |
| GS 1 | $10^{-1}$ | 9 | Blue | $10^{-1}$ | 5 | Colourless | 1 |
| | | 20 | Red | | | | |
| | | 3 | Colourless | | | | |
| | $10^{-2}$ | 10 | Red | $10^{-2}$ | 1 | Colourless | |
| | | 2 | Colourless | | | | |
| | $10^{-3}$ | - | - | $10^{-3}$ | - | - | |
| | $10^{-4}$ | - | - | $10^{-4}$ | - | - | |
| | $10^{-5}$ | - | - | $10^{-5}$ | - | - | |
| GS 2 | $10^{-1}$ | 2 | Blue | $10^{-1}$ | 3 | Colourless | 1 |
| | | 9 | Red | | | | |
| | | 2 | Colourless | | | | |
| | $10^{-2}$ | - | - | $10^{-2}$ | - | - | |
| | $10^{-3}$ | - | - | $10^{-3}$ | - | - | |
| | $10^{-4}$ | - | - | $10^{-4}$ | - | - | |
| | $10^{-5}$ | - | - | $10^{-5}$ | - | - | |
| GS 3 | $10^{-1}$ | 1 | Blue | $10^{-1}$ | - | - | 1 |
| | | 32 | Red | | | | |
| | | 3 | Colourless | | | | |
| | $10^{-2}$ | 4 | Red | $10^{-2}$ | - | - | |
| | $10^{-3}$ | - | - | $10^{-3}$ | - | - | |
| | $10^{-4}$ | - | - | $10^{-4}$ | - | - | |
| | $10^{-5}$ | - | - | $10^{-5}$ | - | - | |
| GS 4 | $10^{-1}$ | 1 | Blue | $10^{-1}$ | - | - | 1 |
| | | 1 | Red | | | | |
| | $10^{-2}$ | - | - | $10^{-2}$ | - | - | |
| | $10^{-3}$ | - | - | $10^{-3}$ | - | - | |
| | $10^{-4}$ | - | - | $10^{-4}$ | - | - | |
| | $10^{-5}$ | - | - | $10^{-5}$ | - | - | |

*(Continued)*

| Samples | Colifrom *Chromoselect* agar | | | Coliform *Chromoselect* agar + cefotaxime | | | Weight (g) |
|---|---|---|---|---|---|---|---|
| | Dilutions | Number of colonies | Colour | Dilutions | Number of colonies | Colour | |
| GS 5 | 1:4 | 1 | Red | 1:4 | - | - | 1 |
| | | 1 | Colourless | | | | |
| | 1:16 | - | - | 1:16 | - | - | |
| | 1:48 | - | - | 1:48 | - | - | |
| GS 6 | 1:4 | 4 | Red | 1:4 | - | - | 1 |
| | 1:16 | 1 | Colourless | 1:16 | - | - | |
| | 1:48 | - | - | 1:48 | - | - | |
| GS 7 | 1:4 | 21 | Red | 1:4 | 4 | Colourless | 1 |
| | | 24 | Colourless | | | | |
| | 1:16 | 5 | Red | 1:16 | - | - | |
| | | 8 | Colourless | | | | |
| | 1:48 | - | - | 1:48 | - | - | |
| GS 8 | 1:4 | 1 | Blue | 1:4 | 1 | Colourless | 1 |
| | | 77 | Red | | | | |
| | | 3 | Colourless | | | | |
| | 1:16 | 19 | Red | 1:16 | - | - | |
| | | 4 | Colourless | | | | |
| | 1:48 | 10 | Red | 1:48 | - | - | |
| | | 2 | Colourless | | | | |
| GS 9 | 1:4 | TMTC | Red | 1:4 | 14 | Colourless | 1 |
| | | 24 | Colourless | | | | |
| | 1:16 | TMTC | Red | 1:16 | 6 | Colourless | |
| | | 10 | Colourless | | | | |
| | 1:48 | 88 | Red | 1:48 | 1 | Colourless | |
| | | 1 | Colourless | | | | |
| GS 10 | 1:4 | 2 | Red | 1:4 | - | - | 1 |
| | 1:16 | - | - | 1:16 | - | - | |
| | 1:48 | - | - | 1:48 | - | - | |
| GS 11 | 1:4 | 75 | Red | 1:4 | 9 | Colourless | 1 |
| | | 2 | Colourless | | | | |
| | 1:16 | 32 | Red | 1:16 | - | - | |
| | | 1 | Colourless | | | | |
| | 1:48 | 10 | Red | 1:48 | - | - | |
| | | 2 | Colourless | | | | |
| GS 12 | 1:4 | 1 | Red | 1:4 | - | - | 1 |
| | 1:16 | - | - | 1:16 | - | - | |
| | 1:48 | - | - | 1:48 | - | - | |
| GS 13 | 1:4 | 3 | Red | 1:4 | - | - | 1 |
| | | 1 | Colourless | | | | |
| | 1:16 | 2 | Red | 1:16 | - | - | |
| | 1:48 | - | - | 1:48 | - | - | |
| GS 14 | 1:4 | TMTC | Red | 1:4 | TMTC | Colourless | 1 |
| | | TMTC | Colourless | | | | |
| | 1:16 | 69 | Red | 1:16 | 28 | Colourless | |
| | | 39 | Colourless | | | | |
| | 1:48 | 26 | Red | 1:48 | 15 | Colourless | |
| | | 17 | Colourless | | | | |
| GS 15 | 1:4 | - | - | 1:4 | - | - | 1 |
| | 1:16 | - | - | 1:16 | - | - | |
| | 1:48 | - | - | 1:48 | - | - | |
| GS 16 | 1:4 | 8 | Red | 1:4 | 2 | Colourless | 1 |
| | | 3 | Colourless | | | | |
| | 1:16 | 1 | Red | 1:16 | - | - | |
| | | 1 | Colourless | | | | |
| | 1:48 | 1 | Red | 1:48 | - | - | |
| GS 17 | 1:4 | TMTC | Red | 1:4 | 3 | Colourless | 1 |
| | | 4 | Colourless | | | | |
| | 1:16 | TMTC | Red | 1:16 | - | - | |
| | | 2 | Colourless | | | | |
| | 1:48 | 57 | Red | 1:48 | - | - | |
| | | 2 | Colourless | | | | |

*(Continued)*

| Samples | Colifrom *Chromoselect* agar | | | Coliform *Chromoselect* agar + cefotaxime | | | Weight (g) |
|---|---|---|---|---|---|---|---|
| | Dilutions | Number of colonies | Colour | Dilutions | Number of colonies | Colour | |
| GL 1 | $10^{-1}$ | TMTC | Blue | $10^{-1}$ | - | - | 1 |
| | | 2 | Red | | | | |
| | | 4 | Violet | | | | |
| | | 6 | Colourless | | | | |
| | $10^{-2}$ | 12 | Blue | $10^{-2}$ | - | - | |
| | | 2 | Red | | | | |
| | $10^{-3}$ | - | - | $10^{-3}$ | - | - | |
| | $10^{-4}$ | - | - | $10^{-4}$ | - | - | |
| | $10^{-5}$ | - | - | $10^{-5}$ | - | - | |

***Table S.11. The count of colonies of goose samples from the rural site (GSP).*** *Two sets of dilutions (10-fold dilutions and 1:4, 1:16 and 1:48) were used. The counting of colonies was undertaken after 48 h of incubation. The colour of colonies observed on Coliform ChromoSelect agar and the weight of faeces used are shown. '-' = no colonies observed. 'TMTC' = Too many to count.*

| Samples | Colifrom *Chromoselect* agar | | | Coliform *Chromoselect* agar + cefotaxime | | | Weight (g) |
|---|---|---|---|---|---|---|---|
| | Dilutions | Number of colonies | Colour | Dilutions | Number of colonies | Colour | |
| GSP 1 | $10^{-1}$ | 2 | Red | $10^{-1}$ | - | - | 1 |
| | $10^{-2}$ | - | - | $10^{-2}$ | - | - | |
| | $10^{-3}$ | - | - | $10^{-3}$ | - | - | |
| | $10^{-4}$ | - | - | $10^{-4}$ | - | - | |
| | $10^{-5}$ | - | - | $10^{-5}$ | - | - | |
| GSP 2 | $10^{-1}$ | 8 | Red | $10^{-1}$ | 19 | Colourless | 1 |
| | | 6 | Colourless | | | | |
| | $10^{-2}$ | 1 | Colourless | $10^{-2}$ | 1 | Colourless | |
| | $10^{-3}$ | - | - | $10^{-3}$ | - | - | |
| | $10^{-4}$ | - | - | $10^{-4}$ | - | - | |
| | $10^{-5}$ | - | - | $10^{-5}$ | - | - | |
| GSP 3 | $10^{-1}$ | TMTC | Blue | $10^{-1}$ | - | - | 1 |
| | $10^{-2}$ | TMTC | Blue | $10^{-2}$ | - | - | |
| | $10^{-3}$ | TMTC | Blue | $10^{-3}$ | - | - | |
| | $10^{-4}$ | TMTC | Blue | $10^{-4}$ | - | - | |
| | $10^{-5}$ | 77 | Blue | $10^{-5}$ | - | - | |
| GSP 4 | $10^{-1}$ | 11 | Red | $10^{-1}$ | - | - | 1 |
| | | 1 | Colourless | | | | |
| | $10^{-2}$ | 37 | Colourless | $10^{-2}$ | - | - | |
| | | 1 | Red | | | | |
| | $10^{-3}$ | 1 | Colourless | $10^{-3}$ | - | - | |
| | $10^{-4}$ | - | - | $10^{-4}$ | - | - | |
| | $10^{-5}$ | - | - | $10^{-5}$ | - | - | |
| GSP 5 | $10^{-1}$ | TMTC | Red | $10^{-1}$ | 17 | Colourless | 1 |
| | $10^{-2}$ | 55 | Red | $10^{-2}$ | 2 | Colourless | |
| | $10^{-3}$ | 14 | Red | $10^{-3}$ | - | - | |
| | $10^{-4}$ | 1 | Red | $10^{-4}$ | - | - | |
| | $10^{-5}$ | - | - | $10^{-5}$ | - | - | |
| GSP 6 | $10^{-1}$ | 27 | Red | $10^{-1}$ | 1 | Colourless | 1 |
| | | 4 | Colourless | | | | |
| | $10^{-2}$ | 6 | Red | $10^{-2}$ | - | - | |
| | $10^{-3}$ | 7 | Colourless | $10^{-3}$ | - | - | |
| | $10^{-4}$ | - | - | $10^{-4}$ | - | - | |
| | $10^{-5}$ | - | - | $10^{-5}$ | - | - | |

*(Continued)*

| Samples | Colifrom *Chromoselect* agar | | | Coliform *Chromoselect* agar + cefotaxime | | | Weight (g) |
|---|---|---|---|---|---|---|---|
| | Dilutions | Number of colonies | Colour | Dilutions | Number of colonies | Colour | |
| GSP 7 | $10^{-1}$ | 15 | Red | $10^{-1}$ | - | - | 1 |
| | | 4 | Colourless | | | | |
| | $10^{-2}$ | 3 | Colourless | $10^{-2}$ | - | - | |
| | | 3 | Red | | | | |
| | $10^{-3}$ | - | - | $10^{-3}$ | - | - | |
| | $10^{-4}$ | - | - | $10^{-4}$ | - | - | |
| | $10^{-5}$ | - | - | $10^{-5}$ | - | - | |
| GSP 8 | $10^{-1}$ | TMTC | Red | $10^{-1}$ | 21 | Colourless | 1 |
| | $10^{-2}$ | 23 | Red | $10^{-2}$ | 3 | Colourless | |
| | | 6 | Colourless | | | | |
| | $10^{-3}$ | 2 | Red | $10^{-3}$ | - | - | |
| | | 3 | Colourless | | | | |
| | $10^{-4}$ | - | - | $10^{-4}$ | - | - | |
| | $10^{-5}$ | - | - | $10^{-5}$ | - | - | |
| GSP 9 | $10^{-1}$ | 3 | Red | $10^{-1}$ | 12 | Colourless | 1 |
| | | 9 | Colourless | | | | |
| | $10^{-2}$ | 2 | Red | $10^{-2}$ | 3 | Colourless | |
| | | 92 | Colourless | | | | |
| | $10^{-3}$ | 7 | Colourless | $10^{-3}$ | - | - | |
| | $10^{-4}$ | 3 | Colourless | $10^{-4}$ | - | - | |
| | $10^{-5}$ | - | - | $10^{-5}$ | - | - | |
| GSP 10 | $10^{-1}$ | 36 | Colourless | $10^{-1}$ | 52 | Colourless | 1 |
| | $10^{-2}$ | 7 | Colourless | $10^{-2}$ | 9 | Colourless | |
| | $10^{-3}$ | 1 | Colourless | $10^{-3}$ | 2 | Colourless | |
| | $10^{-4}$ | - | - | $10^{-4}$ | - | - | |
| | $10^{-5}$ | - | - | $10^{-5}$ | - | - | |
| GSP 11 | $10^{-1}$ | 4 | Red | $10^{-1}$ | - | - | 1 |
| | | 1 | Colourless | | | | |
| | $10^{-2}$ | - | - | $10^{-2}$ | - | - | |
| | $10^{-3}$ | - | - | $10^{-3}$ | - | - | |
| | $10^{-4}$ | - | - | $10^{-4}$ | - | - | |
| | $10^{-5}$ | - | - | $10^{-5}$ | - | - | |
| GSP 12 | $10^{-1}$ | TMTC | Red | $10^{-1}$ | 4 | Colourless | 1 |
| | $10^{-2}$ | TMTC | Red | $10^{-2}$ | - | - | |
| | $10^{-3}$ | 44 | Red | $10^{-3}$ | - | - | |
| | $10^{-4}$ | 10 | Red | $10^{-4}$ | - | - | |
| | $10^{-5}$ | - | - | $10^{-5}$ | - | - | |
| GSP 13 | $10^{-1}$ | 10 | Red | $10^{-1}$ | - | - | 1 |
| | $10^{-2}$ | 2 | Red | $10^{-2}$ | - | - | |
| | $10^{-3}$ | - | - | $10^{-3}$ | - | - | |
| | $10^{-4}$ | - | - | $10^{-4}$ | - | - | |
| | $10^{-5}$ | - | - | $10^{-5}$ | - | - | |
| GSP 14 | $10^{-1}$ | TMTC | Red | $10^{-1}$ | 27 | Colourless | 1 |
| | $10^{-2}$ | 37 | Red | $10^{-2}$ | 3 | Colourless | |
| | $10^{-3}$ | 1 | Red | $10^{-3}$ | - | - | |
| | $10^{-4}$ | - | - | $10^{-4}$ | - | - | |
| | $10^{-5}$ | - | - | $10^{-5}$ | - | - | |
| GSP 15 | $10^{-1}$ | 21 | Red | $10^{-1}$ | 5 | Colourless | 1 |
| | $10^{-2}$ | 3 | Red | $10^{-2}$ | - | - | |
| | $10^{-3}$ | - | - | $10^{-3}$ | - | - | |
| | $10^{-4}$ | - | - | $10^{-4}$ | - | - | |
| | $10^{-5}$ | - | - | $10^{-5}$ | - | - | |
| GSP 16 | $10^{-1}$ | 9 | Red | $10^{-1}$ | - | - | 1 |
| | | 12 | Colourless | | | | |
| | $10^{-2}$ | 1 | Red | $10^{-2}$ | - | - | |
| | | 3 | Colourless | | | | |
| | $10^{-3}$ | - | - | $10^{-3}$ | - | - | |
| | $10^{-4}$ | - | - | $10^{-4}$ | - | - | |
| | $10^{-5}$ | - | - | $10^{-5}$ | - | - | |

*(Continued)*

| Samples | Colifrom *Chromoselect* agar | | | Coliform *Chromoselect* agar + cefotaxime | | | Weight (g) |
|---|---|---|---|---|---|---|---|
| | Dilutions | Number of colonies | Colour | Dilutions | Number of colonies | Colour | |
| GSP 17 | $10^{-1}$ | TMTC | Colourless | $10^{-1}$ | TMTC | Colourless | 1 |
| | $10^{-2}$ | TMTC | Colourless | $10^{-2}$ | 81 | Colourless | |
| | $10^{-3}$ | 25 | Colourless | $10^{-3}$ | 10 | Colourless | |
| | $10^{-4}$ | 2 | Colourless | $10^{-4}$ | 1 | Colourless | |
| | $10^{-5}$ | 1 | Colourless | $10^{-5}$ | - | - | |
| GSP 18 | $10^{-1}$ | 8 | Red | $10^{-1}$ | - | - | 1 |
| | $10^{-2}$ | 3 | Red | $10^{-2}$ | - | - | |
| | $10^{-3}$ | 1 | Red | $10^{-3}$ | - | - | |
| | $10^{-4}$ | - | - | $10^{-4}$ | - | - | |
| | $10^{-5}$ | - | - | $10^{-5}$ | - | - | |
| GSP 19 | $10^{-1}$ | TMTC | Red | $10^{-1}$ | TMTC | Colourless | 1 |
| | | 10 | Pink | | | | |
| | $10^{-2}$ | 67 | Red | $10^{-2}$ | TMTC | Colourless | |
| | $10^{-3}$ | 5 | Red | $10^{-3}$ | 11 | Colourless | |
| | $10^{-4}$ | - | - | $10^{-4}$ | - | - | |
| | $10^{-5}$ | - | - | $10^{-5}$ | - | - | |
| GSP 20 | $10^{-1}$ | 1 | Red | $10^{-1}$ | - | - | 1 |
| | | 11 | Colourless | | | | |
| | $10^{-2}$ | 1 | Red | $10^{-2}$ | - | - | |
| | $10^{-3}$ | - | - | $10^{-3}$ | - | - | |
| | $10^{-4}$ | - | - | $10^{-4}$ | - | - | |
| | $10^{-5}$ | - | - | $10^{-5}$ | - | - | |
| GSP 21 | $10^{-1}$ | TMTC | Colourless | $10^{-1}$ | 66 | Colourless | 1 |
| | $10^{-2}$ | 5 | Colourless | $10^{-2}$ | 7 | Colourless | |
| | $10^{-3}$ | - | - | $10^{-3}$ | - | - | |
| | $10^{-4}$ | - | - | $10^{-4}$ | - | - | |
| | $10^{-5}$ | - | - | $10^{-5}$ | - | - | |
| GSP 22 | $10^{-1}$ | 4 | Red | $10^{-1}$ | 60 | Colourless | 1 |
| | | TMTC | Colourless | | | | |
| | $10^{-2}$ | 1 | Red | $10^{-2}$ | 10 | Colourless | |
| | | 11 | Colourless | | | | |
| | $10^{-3}$ | 4 | Colourless | $10^{-3}$ | - | - | |
| | $10^{-4}$ | - | - | $10^{-4}$ | - | - | |
| | $10^{-5}$ | - | - | $10^{-5}$ | - | - | |
| GSP 23 | $10^{-1}$ | TMTC | Red | $10^{-1}$ | - | - | 1 |
| | $10^{-2}$ | TMTC | Red | $10^{-2}$ | - | - | |
| | $10^{-3}$ | 59 | Red | $10^{-3}$ | - | - | |
| | $10^{-4}$ | 11 | Red | $10^{-4}$ | - | - | |
| | $10^{-5}$ | 1 | Red | $10^{-5}$ | - | - | |
| GSP 24 | $10^{-1}$ | 10 | Red | $10^{-1}$ | TMTC | Colourless | 1 |
| | | TMTC | Colourless | | | | |
| | $10^{-2}$ | 1 | Red | $10^{-2}$ | TMTC | Colourless | |
| | | TMTC | Colourless | | | | |
| | $10^{-3}$ | 71 | Colourless | $10^{-3}$ | 28 | Colourless | |
| | $10^{-4}$ | 7 | Colourless | $10^{-4}$ | - | - | |
| | $10^{-5}$ | - | - | $10^{-5}$ | - | - | |
| GSP 25 | $10^{-1}$ | TMTC | Red | $10^{-1}$ | TMTC | Colourless | 1 |
| | | TMTC | Colourless | | | | |
| | $10^{-2}$ | 25 | Pink | $10^{-2}$ | 40 | Colourless | |
| | | 23 | Colourless | | | | |
| | $10^{-3}$ | 4 | Red | $10^{-3}$ | - | - | |
| | | 6 | Colourless | | | | |
| | $10^{-4}$ | - | - | $10^{-4}$ | - | - | |
| | $10^{-5}$ | - | - | $10^{-5}$ | - | - | |

*(Continued)*

| Samples | Colifrom *Chromoselect* agar | | | Coliform *Chromoselect* agar + cefotaxime | | | Weight (g) |
|---|---|---|---|---|---|---|---|
| | Dilutions | Number of colonies | Colour | Dilutions | Number of colonies | Colour | |
| GSP 26 | $10^{-1}$ | 1 | Blue | $10^{-1}$ | TMTC | Colourless | 1 |
| | | 19 | Red | | | | |
| | | TMTC | Colourless | | | | |
| | $10^{-2}$ | 6 | Red | $10^{-2}$ | 60 | Colourless | |
| | | 28 | Colourless | | | | |
| | $10^{-3}$ | - | - | $10^{-3}$ | 7 | Colourless | |
| | $10^{-4}$ | - | - | $10^{-4}$ | - | - | |
| | $10^{-5}$ | - | - | $10^{-5}$ | - | - | |
| GSP 27 | $10^{-1}$ | TMTC | Colourless | $10^{-1}$ | - | - | 1 |
| | $10^{-2}$ | 28 | Colourless | $10^{-2}$ | - | - | |
| | $10^{-3}$ | - | - | $10^{-3}$ | - | - | |
| | $10^{-4}$ | - | - | $10^{-4}$ | - | - | |
| | $10^{-5}$ | - | - | $10^{-5}$ | - | - | |
| GSP 28 | $10^{-1}$ | 22 | Colourless | $10^{-1}$ | - | - | 1 |
| | $10^{-2}$ | 2 | Colourless | $10^{-2}$ | - | - | |
| | $10^{-3}$ | - | - | $10^{-3}$ | - | - | |
| | $10^{-4}$ | - | - | $10^{-4}$ | - | - | |
| | $10^{-5}$ | - | - | $10^{-5}$ | - | - | |
| GSP 29 | $10^{-1}$ | 20 | Pink | $10^{-1}$ | 15 | Colourless | 1 |
| | | 9 | Colourless | | | | |
| | $10^{-2}$ | 1 | Colourless | $10^{-2}$ | 1 | Colourless | |
| | $10^{-3}$ | - | - | $10^{-3}$ | - | - | |
| | $10^{-4}$ | - | - | $10^{-4}$ | - | - | |
| | $10^{-5}$ | - | - | $10^{-5}$ | - | - | |
| GSP 30 | $10^{-1}$ | 6 | Pink | $10^{-1}$ | - | - | 1 |
| | $10^{-2}$ | - | - | $10^{-2}$ | - | - | |
| | $10^{-3}$ | - | - | $10^{-3}$ | - | - | |
| | $10^{-4}$ | - | - | $10^{-4}$ | - | - | |
| | $10^{-5}$ | - | - | $10^{-5}$ | - | - | |
| GSP 31 | $10^{-1}$ | 10 | Pink | $10^{-1}$ | - | - | 1 |
| | $10^{-2}$ | - | - | $10^{-2}$ | - | - | |
| | $10^{-3}$ | - | - | $10^{-3}$ | - | - | |
| | $10^{-4}$ | - | - | $10^{-4}$ | - | - | |
| | $10^{-5}$ | - | - | $10^{-5}$ | - | - | |
| GSP 32 | $10^{-1}$ | 44 | Red | $10^{-1}$ | TMTC | Colourless | 1 |
| | | TMTC | Colourless | | | | |
| | $10^{-2}$ | 2 | Pink | $10^{-2}$ | 54 | Colourless | |
| | | 59 | Colourless | | | | |
| | $10^{-3}$ | 11 | Colourless | $10^{-3}$ | 5 | Colourless | |
| | $10^{-4}$ | - | - | $10^{-4}$ | - | - | |
| | $10^{-5}$ | - | - | $10^{-5}$ | - | - | |
| GSP 33 | $10^{-1}$ | TMTC | Colourless | $10^{-1}$ | TMTC | Colourless | 1 |
| | $10^{-2}$ | TMTC | Colourless | $10^{-2}$ | TMTC | Colourless | |
| | $10^{-3}$ | 82 | Colourless | $10^{-3}$ | 72 | Colourless | |
| | $10^{-4}$ | 10 | Colourless | $10^{-4}$ | 6 | Colourless | |
| | $10^{-5}$ | - | - | $10^{-5}$ | - | - | |
| GSP 34 | $10^{-1}$ | 2 | Red | $10^{-1}$ | TMTC | Colourless | 1 |
| | | TMTC | Colourless | | | | |
| | $10^{-2}$ | TMTC | Colourless | $10^{-2}$ | TMTC | Colourless | |
| | $10^{-3}$ | - | - | $10^{-3}$ | 13 | Colourless | |
| | $10^{-4}$ | 72 | Colourless | $10^{-4}$ | - | - | |
| | $10^{-5}$ | 12 | Colourless | $10^{-5}$ | - | - | |
| GSP 35 | $10^{-1}$ | TMTC | Colourless | $10^{-1}$ | 1 | Colourless | 1 |
| | $10^{-2}$ | TMTC | Colourless | $10^{-2}$ | - | - | |
| | $10^{-3}$ | 99 | Colourless | $10^{-3}$ | - | - | |
| | $10^{-4}$ | 11 | Colourless | $10^{-4}$ | - | - | |
| | $10^{-5}$ | - | - | $10^{-5}$ | - | - | |

*(Continued)*

| Samples | Colifrom *Chromoselect* agar | | | Coliform *Chromoselect* agar + cefotaxime | | | Weight (g) |
|---|---|---|---|---|---|---|---|
| | Dilutions | Number of colonies | Colour | Dilutions | Number of colonies | Colour | |
| GSP 36 | $10^{-1}$ | 12 | Red | $10^{-1}$ | 3 | Colourless | 1 |
| | $10^{-2}$ | 1 | Red | $10^{-2}$ | - | - | |
| | $10^{-3}$ | - | - | $10^{-3}$ | - | - | |
| | $10^{-4}$ | - | - | $10^{-4}$ | - | - | |
| | $10^{-5}$ | - | - | $10^{-5}$ | - | - | |
| GSP 37 | $10^{-1}$ | 48 | Red | $10^{-1}$ | 1 | Colourless | 1 |
| | $10^{-2}$ | 5 | Red | $10^{-2}$ | - | - | |
| | $10^{-3}$ | - | - | $10^{-3}$ | - | - | |
| | $10^{-4}$ | - | - | $10^{-4}$ | - | - | |
| | $10^{-5}$ | - | - | $10^{-5}$ | - | - | |
| GSP 38 | $10^{-1}$ | 85 | Blue | $10^{-1}$ | TMTC | Colourless | 1 |
| | | 5 | Red | | | | |
| | | 8 | Colourless | | | | |
| | $10^{-2}$ | 21 | Blue | $10^{-2}$ | TMTC | Colourless | |
| | | 5 | Colourless | | | | |
| | $10^{-3}$ | 2 | Blue | $10^{-3}$ | TMTC | Colourless | |
| | $10^{-4}$ | - | - | $10^{-4}$ | TMTC | Colourless | |
| | $10^{-5}$ | - | - | $10^{-5}$ | 6 | Colourless | |
| GSP 39 | $10^{-1}$ | 87 | Red | $10^{-1}$ | TMTC | Colourless | 1 |
| | $10^{-2}$ | 19 | Red | $10^{-2}$ | TMTC | Colourless | |
| | $10^{-3}$ | 3 | Red | $10^{-3}$ | 24 | Colourless | |
| | | TMTC | Colourless | | | | |
| | $10^{-4}$ | 42 | Colourless | $10^{-4}$ | 1 | Colourless | |
| | $10^{-5}$ | - | - | $10^{-5}$ | - | - | |
| GSP 40 | $10^{-1}$ | 17 | Red | $10^{-1}$ | 8 | Colourless | 1 |
| | | 10 | Colourless | | | | |
| | $10^{-2}$ | 2 | Red | $10^{-2}$ | 2 | Colourless | |
| | | 1 | Colourless | | | | |
| | $10^{-3}$ | - | - | $10^{-3}$ | - | - | |
| | $10^{-4}$ | - | - | $10^{-4}$ | - | - | |
| | $10^{-5}$ | - | - | $10^{-5}$ | - | - | |
| GSP 41 | $10^{-1}$ | TMTC | Red | $10^{-1}$ | 29 | Colourless | 1 |
| | $10^{-2}$ | 17 | Red | $10^{-2}$ | 1 | Colourless | |
| | | 1 | Blue | | | | |
| | $10^{-3}$ | 1 | Blue | $10^{-3}$ | - | - | |
| | $10^{-4}$ | - | - | $10^{-4}$ | - | - | |
| | $10^{-5}$ | - | - | $10^{-5}$ | - | - | |
| GSP 42 | $10^{-1}$ | TMTC | Colourless | $10^{-1}$ | 4 | Colourless | 1 |
| | $10^{-2}$ | TMTC | Colourless | $10^{-2}$ | - | - | |
| | $10^{-3}$ | 20 | Colourless | $10^{-3}$ | - | - | |
| | $10^{-4}$ | - | - | $10^{-4}$ | - | - | |
| | $10^{-5}$ | - | - | $10^{-5}$ | - | - | |
| GSP 43 | $10^{-1}$ | TMTC | Colourless | $10^{-1}$ | TMTC | Colourless | 1 |
| | | TMTC | Red | | | | |
| | $10^{-2}$ | 20 | Red | $10^{-2}$ | TMTC | Colourless | |
| | | TMTC | Colourless | | | | |
| | $10^{-3}$ | 4 | Red | $10^{-3}$ | 33 | Colourless | |
| | | 29 | Colourless | | | | |
| | $10^{-4}$ | 4 | Colourless | $10^{-4}$ | 2 | Colourless | |
| | $10^{-5}$ | 2 | Colourless | $10^{-5}$ | - | - | |
| GSP 44 | $10^{-1}$ | TMTC | Colourless | $10^{-1}$ | - | - | 1 |
| | $10^{-2}$ | TMTC | Colourless | $10^{-2}$ | - | - | |
| | $10^{-3}$ | 32 | Colourless | $10^{-3}$ | - | - | |
| | $10^{-4}$ | 4 | Colourless | $10^{-4}$ | - | - | |
| | $10^{-5}$ | - | - | $10^{-5}$ | - | - | |

*(Continued)*

| Samples | Colifrom *Chromoselect* agar | | | Coliform *Chromoselect* agar + cefotaxime | | | Weight (g) |
|---|---|---|---|---|---|---|---|
| | Dilutions | Number of colonies | Colour | Dilutions | Number of colonies | Colour | |
| GSP 45 | $10^{-1}$ | TMTC | Colourless | $10^{-1}$ | 20 | Colourless | 1 |
| | $10^{-2}$ | 90 | Colourless | $10^{-2}$ | - | - | |
| | $10^{-3}$ | 9 | Colourless | $10^{-3}$ | - | - | |
| | $10^{-4}$ | - | - | $10^{-4}$ | - | - | |
| | $10^{-5}$ | - | - | $10^{-5}$ | - | - | |
| GSP 46 | $10^{-1}$ | 33 | Red | $10^{-1}$ | - | - | 1 |
| | $10^{-2}$ | 3 | Red | $10^{-2}$ | - | - | |
| | | 5 | Colourless | | | | |
| | $10^{-3}$ | - | - | $10^{-3}$ | - | - | |
| | $10^{-4}$ | - | - | $10^{-4}$ | - | - | |
| | $10^{-5}$ | - | - | $10^{-5}$ | - | - | |
| GSP 47 | $10^{-1}$ | TMTC | Colourless | $10^{-1}$ | - | - | 1 |
| | $10^{-2}$ | TMTC | Colourless | $10^{-2}$ | - | - | |
| | $10^{-3}$ | 10 | Colourless | $10^{-3}$ | - | - | |
| | $10^{-4}$ | - | - | $10^{-4}$ | - | - | |
| | $10^{-5}$ | - | - | $10^{-5}$ | - | - | |
| GSP 48 | $10^{-1}$ | 1 | Red | $10^{-1}$ | 27 | Colourless | 1 |
| | | TMTC | Colourless | | | | |
| | $10^{-2}$ | TMTC | Colourless | $10^{-2}$ | 2 | Colourless | |
| | $10^{-3}$ | 68 | Colourless | $10^{-3}$ | - | - | |
| | $10^{-4}$ | 5 | Colourless | $10^{-4}$ | - | - | |
| | $10^{-5}$ | - | - | $10^{-5}$ | - | - | |
| GSP 49 | $10^{-1}$ | 2 | Red | $10^{-1}$ | 2 | Colourless | 1 |
| | | TMTC | Colourless | | | | |
| | $10^{-2}$ | 38 | Colourless | $10^{-2}$ | - | - | |
| | $10^{-3}$ | - | - | $10^{-3}$ | - | - | |
| | $10^{-4}$ | - | - | $10^{-4}$ | - | - | |
| | $10^{-5}$ | - | - | $10^{-5}$ | - | - | |
| GSP 50 | $10^{-1}$ | 6 | Blue | $10^{-1}$ | 2 | Colourless | 1 |
| | $10^{-2}$ | 2 | Blue | $10^{-2}$ | - | - | |
| | $10^{-3}$ | - | - | $10^{-3}$ | - | - | |
| | $10^{-4}$ | - | - | $10^{-4}$ | - | - | |
| | $10^{-5}$ | - | - | $10^{-5}$ | - | - | |
| GSP 51 | $10^{-1}$ | TMTC | Colourless | $10^{-1}$ | - | - | 1 |
| | $10^{-2}$ | 23 | Colourless | $10^{-2}$ | - | - | |
| | $10^{-3}$ | 2 | Colourless | $10^{-3}$ | - | - | |
| | $10^{-4}$ | - | - | $10^{-4}$ | - | - | |
| | $10^{-5}$ | - | - | $10^{-5}$ | - | - | |
| GSP 52 | $10^{-1}$ | 30 | Red | $10^{-1}$ | | | 1 |
| | | 48 | Colourless | | | | |
| | $10^{-2}$ | 3 | Red | $10^{-2}$ | | | |
| | | 4 | Colourless | | | | |
| | $10^{-3}$ | - | - | $10^{-3}$ | | | |
| | $10^{-4}$ | - | - | $10^{-4}$ | | | |
| | $10^{-5}$ | - | - | $10^{-5}$ | | | |

2.2 The Numbers (CFU/g) of Total *E. coli* and ESBL-Producer Coliforms in Gulls and Geese

2.2.1 Gull Faeces

***Table S.12. The numbers (CFU/g) of total E. coli and ESBL-producer coliforms in gulls in the urban site****. The number (CFU/g) of colony was calculated from samples with visible E. coli colonies on the spread plate within the countable range (8 - 83). When the counts were below the lower limit (1 - 7), the numbers (CFU/g) were regarded as 'estimated' (ASTM, 1998). These numbers (CFU/g) have been marked with an asterisk (*) in the table. Samples with 'TMTC' counts were reported as '> 4500 CFU/g'. Samples with 'zero' counts were reported as '< 3 CFU/g' as the limit of detection (LOD) of gull faeces was 3 CFU/g. Both the numbers (CFU/g) from 'TMTC' and 'zero' counts were excluded from the calculation of the median (CFU/g). The median (CFU/g), maximum (CFU/g) and minimum (CFU/g) of the numbers (CFU/g) are shown at the bottom of the table.*

| **Samples** | **Number of colony (CFU/g)** | | |
|---|---|---|---|
| | **Total *E. coli*** | **Resistant *E. coli*** | **Resistant non-*E. coli* coliforms** |
| LSW 1 | 24857 | 141423 | < 3 |
| LSW 2 | 10385 | 7615 | 9115 |
| LSW 3 | 150* | 600 | 375* |
| LSW 4 | 439 | < 3 | < 3 |
| LSW 5 | 2835 | 94* | < 3 |
| LSW 6 | 1372 | 13* | < 3 |
| LSW 7 | 312 | < 3 | < 3 |
| LSW 8 | > 4500 | < 3 | < 3 |
| LSW 9 | 17036 | < 3 | < 3 |
| LSW 10 | 89* | < 3 | < 3 |
| LSW 11 | > 4500 | > 4500 | < 3 |
| LSW 12 | < 3 | < 3 | < 3 |
| LSW 13 | 2373 | 1813 | 1945 |
| LSW 14 | < 3 | < 3 | < 3 |
| LSW 15 | 2099 | 13* | 134 |
| LSW 16 | < 3 | < 3 | < 3 |
| LSW 17 | 204* | < 3 | < 3 |
| LSW 18 | < 3 | < 3 | < 3 |
| LSW 19 | < 3 | < 3 | < 3 |
| LSW 20 | > 4500 | > 4500 | 31 |
| LSW 21 | 693 | 461* | < 3 |
| LSW 22 | < 3 | < 3 | < 3 |
| LSW 23 | 49* | < 3 | < 3 |
| LSW 24 | 1048 | < 3 | < 3 |
| LSW 25 | < 3 | < 3 | < 3 |
| LSW 26 | > 4500 | 19* | < 3 |
| LSW 27 | 52* | 52* | < 3 |
| LSW 28 | 240* | 720* | < 3 |
| LSW 29 | 108* | < 3 | < 3 |
| LSW 30 | 1440 | 360* | 240* |

*(Continued)*

| Samples | Number of colony (CFU/g) | | |
|---|---|---|---|
| | **Total *E. coli*** | **Resistant *E. coli*** | **Resistant non-*E. coli* coliforms** |
| LSW 31 | < 3 | < 3 | < 3 |
| LSW 32 | < 3 | < 3 | < 3 |
| LSW 33 | 358 | 109 | 2861 |
| LSW 34 | 291 | < 3 | 116* |
| LSW 35 | 783 | 478 | < 3 |
| LSW 36 | 255 | < 3 | < 3 |
| LSW 37 | > 4500 | < 3 | < 3 |
| LSW 38 | > 4500 | 530 | 937 |
| LSW 39 | 619 | 904 | 397 |
| LSW 40 | 80* | 16* | < 3 |
| LSW 41 | < 3 | < 3 | < 3 |
| LSW 42 | 571 | < 3 | < 3 |
| LSW 43 | 6253 | 42* | < 3 |
| LSW 44 | 3461 | < 3 | < 3 |
| LSW 45 | 712 | 84* | 2177 |
| LSW 46 | 300* | < 3 | < 3 |
| LSW 47 | 1286 | 612 | < 3 |
| Number of samples | 31/47 | 20/47 | 11/47 |
| Total | 80750 | 155958 | 18328 |
| Median | 619 | 410.5 | 397 |
| Maximum | 24857 | 141423 | 9115 |
| Minimum | 49 | 13 | 31 |

***Table S.13. The numbers (CFU/g) of total E. coli and ESBL-producer coliforms in gulls in the rural site****. The number (CFU/g) of colony was calculated from samples with visible E. coli colonies on the spread plate within the countable range (8 - 83). When the counts were below the lower limit (1 - 7), the numbers (CFU/g) were regarded as 'estimated' (ASTM, 1998). These numbers (CFU/g) have been marked with an asterisk (*) in the table. Samples with 'TMTC' counts were reported as '> 4500 CFU/g'. Samples with 'zero' counts were reported as '< 3 CFU/g' as the LOD of gull faeces was 3 CFU/g. Both the numbers (CFU/g) from 'TMTC' and 'zero' counts were excluded from the calculation of the median (CFU/g). The median (CFU/g), maximum (CFU/g) and minimum (CFU/g) of the numbers (CFU/g) are shown at the bottom of the table.*

| Samples | Number of colony (CFU/g) | | |
|---|---|---|---|
| | **Total *E. coli*** | **Resistant *E. coli*** | **Resistant non-*E. coli* coliforms** |
| LSA 1 | > 4500 | < 3 | < 3 |
| LSA 2 | > 4500 | < 3 | < 3 |
| LSA 3 | > 4500 | < 3 | < 3 |
| LSA 4 | > 4500 | < 3 | < 3 |
| LSA 5 | > 4500 | < 3 | < 3 |
| LSA 6 | > 4500 | < 3 | < 3 |
| LSA 7 | > 4500 | < 3 | 32* |
| LSA 8 | > 4500 | < 3 | < 3 |

*(Continued)*

| Samples | Number of colony (CFU/g) | | |
|---|---|---|---|
| | **Total *E. coli*** | **Resistant *E. coli*** | **Resistant non-*E. coli* coliforms** |
| LSA 9 | > 4500 | < 3 | < 3 |
| LSA 10 | > 4500 | < 3 | < 3 |
| LSA 11 | 894 | < 3 | < 3 |
| LSA 12 | > 4500 | < 3 | < 3 |
| LSA 13 | > 4500 | < 3 | < 3 |
| LSA 14 | 268 | < 3 | < 3 |
| LSA 15 | > 4500 | < 3 | < 3 |
| LSA 16 | > 4500 | < 3 | < 3 |
| LSA 17 | > 4500 | < 3 | < 3 |
| LSA 18 | 17* | < 3 | < 3 |
| LSA 19 | > 4500 | < 3 | < 3 |
| LSA 20 | > 4500 | < 3 | < 3 |
| LSA 21 | 437 | < 3 | < 3 |
| LSA 22 | > 4500 | < 3 | < 3 |
| LSA 23 | > 4500 | < 3 | < 3 |
| LSA 24 | 44* | < 3 | < 3 |
| LSA 25 | < 3 | < 3 | < 3 |
| LSA 26 | > 4500 | < 3 | < 3 |
| LSA 27 | 707 | < 3 | < 3 |
| LSA 28 | 513 | < 3 | < 3 |
| LSA 29 | > 4500 | < 3 | < 3 |
| LSA 30 | > 4500 | < 3 | < 3 |
| LSA 31 | > 4500 | 9237 | < 3 |
| LSA 32 | > 4500 | < 3 | < 3 |
| LSA 33 | > 4500 | < 3 | < 3 |
| LSA 34 | > 4500 | < 3 | < 3 |
| LSA 35 | > 4500 | < 3 | < 3 |
| LSA 36 | > 4500 | < 3 | < 3 |
| LSA 37 | 44 | < 3 | < 3 |
| LSA 38 | 125 | < 3 | < 3 |
| LSA 39 | > 4500 | < 3 | < 3 |
| LSA 40 | 2557 | < 3 | < 3 |
| LSA 41 | > 4500 | < 3 | < 3 |
| LSA 42 | > 4500 | < 3 | < 3 |
| LSA 43 | < 3 | < 3 | < 3 |
| LSA 44 | < 3 | < 3 | < 3 |
| LSA 45 | > 4500 | < 3 | < 3 |
| LSA 46 | < 3 | > 4500 | < 3 |
| LSA 47 | < 3 | < 3 | < 3 |
| LSA 48 | < 3 | < 3 | < 3 |
| LSA 49 | < 3 | < 3 | < 3 |
| LSA 50 | < 3 | < 3 | < 3 |
| Number of samples | 10/50 | 1/50 | 1/50 |
| Total | 5606 | 9237 | 32 |
| Median | 352.5 | - | - |
| Maximum | 2557 | 9237 | 32 |
| Minimum | 17 | - | - |

2.2.2 Goose Faeces

***Table S.14. The numbers (CFU/g) of total E. coli and ESBL-producer coliforms in geese in the urban site****. The number (CFU/g) of colony was calculated from samples with visible E. coli colonies on the spread plate within the countable range (8 - 83). When the counts were below the lower limit (1 - 7), the numbers (CFU/g) were regarded as 'estimated' (ASTM, 1998). These numbers (CFU/g) have been marked with an asterisk (*) in the table. Samples with 'TMTC' counts were reported as '> 4500 CFU/g'. As goose samples were diluted in two different sets of dilutions, samples with 'zero' counts in 1:4 dilution were reported as '< 4 CFU/g' as the LOD of goose faeces in this dilution was 4 CFU/g, and samples with 'zero' counts in 10-fold dilution were reported as '< 10 CFU/g' as the LOD of goose faeces in this dilution was 10 CFU/g . Both the numbers (CFU/g) from 'TMTC' and 'zero' counts were excluded from the calculation of the median (CFU/g). The median (CFU/g), maximum (CFU/g) and minimum (CFU/g) of the numbers (CFU/g) are shown at the bottom of the table.*

| **Samples** | **Number of colony (CFU/g)** | | |
|---|---|---|---|
| | **Total *E. coli*** | **Resistant *E. coli*** | **Resistant non-*E. coli* coliforms** |
| GD 1 | < 10 | < 10 | < 10 |
| GD 2 | < 10 | < 10 | < 10 |
| GD 3 | < 10 | < 10 | < 10 |
| GD 4 | < 10 | < 10 | < 10 |
| GD 5 | < 10 | < 10 | < 10 |
| GD 6 | 5000 | < 10 | < 10 |
| GD 7 | 200* | < 10 | < 10 |
| GD 8 | < 10 | < 10 | < 10 |
| GD 9 | < 4 | < 4 | < 4 |
| GD 10 | 40* | < 4 | < 4 |
| GD 11 | < 4 | < 4 | < 4 |
| GD 12 | < 4 | < 4 | < 4 |
| GD 13 | < 4 | < 4 | < 4 |
| GD 14 | < 4 | < 4 | < 4 |
| GD 15 | < 4 | < 4 | < 4 |
| GD 16 | < 4 | < 4 | < 4 |
| GD 17 | < 4 | < 4 | < 4 |
| GD 18 | 40* | < 4 | < 4 |
| GD 19 | < 4 | < 4 | < 4 |
| GD 20 | < 4 | < 4 | < 4 |
| GD 21 | 920 | < 4 | < 4 |
| GD 22 | < 4 | < 4 | < 4 |
| GD 23 | < 4 | < 4 | < 4 |
| GD 24 | < 4 | < 4 | < 4 |
| GD 25 | < 4 | < 4 | < 4 |
| GD 26 | < 4 | < 4 | < 4 |
| GD 27 | < 4 | < 4 | < 4 |
| GD 28 | < 4 | < 4 | < 4 |

*(Continued)*

| Samples | Number of colony (CFU/g) | | |
|---|---|---|---|
| | **Total *E. coli*** | **Resistant *E. coli*** | **Resistant non-*E. coli* coliforms** |
| GD 29 | < 4 | < 4 | < 4 |
| GD 30 | < 4 | < 4 | < 4 |
| GD 31 | < 4 | < 4 | < 4 |
| GD 32 | < 4 | < 4 | < 4 |
| GD 33 | 40* | < 4 | < 4 |
| GD 34 | < 4 | < 4 | < 4 |
| GD 35 | < 4 | < 4 | < 4 |
| GD 36 | < 4 | < 4 | < 4 |
| GD 37 | < 4 | < 4 | < 4 |
| GD 38 | < 4 | < 4 | < 4 |
| GD 39 | < 4 | < 4 | < 4 |
| GD 40 | < 4 | < 4 | < 4 |
| GD 41 | < 4 | < 4 | < 4 |
| GD 42 | < 4 | < 4 | < 4 |
| GD 43 | < 4 | < 4 | < 4 |
| GD 44 | 40* | < 4 | < 4 |
| GD 45 | < 4 | < 4 | < 4 |
| GD 46 | < 4 | < 4 | < 4 |
| GD 47 | 480 | < 4 | < 4 |
| G. Dun. 1 | 40* | < 4 | < 4 |
| G. Dun. 2 | < 4 | < 4 | < 4 |
| G. Dun. 3 | < 4 | < 4 | < 4 |
| G. Dun. 4 | < 4 | < 4 | < 4 |
| G. Dun. 5 | < 4 | < 4 | < 4 |
| G. Dun. 6 | 40* | < 4 | < 4 |
| G. Dun. 7 | < 4 | < 4 | < 4 |
| G. Dun. 8 | < 4 | < 4 | < 4 |
| G. Dun. 9 | < 4 | < 4 | < 4 |
| G. Dun. 10 | < 4 | < 4 | < 4 |
| G. Dun. 11 | 40* | < 4 | < 4 |
| G. Dun. 12 | < 4 | < 4 | < 4 |
| GS 1 | 1800 | < 10 | < 10 |
| GS 2 | 400* | < 10 | < 10 |
| GS 3 | 200* | < 10 | < 10 |
| GS 4 | 200* | < 10 | < 10 |
| GS 5 | < 4 | < 4 | < 4 |
| GS 6 | < 4 | < 4 | < 4 |
| GS 7 | < 4 | < 4 | < 4 |
| GS 8 | 40* | < 4 | < 4 |
| GS 9 | < 4 | < 4 | < 4 |
| GS 10 | < 4 | < 4 | < 4 |
| GS 11 | < 4 | < 4 | < 4 |
| GS 12 | < 4 | < 4 | < 4 |
| GS 13 | < 4 | < 4 | < 4 |
| GS 14 | < 4 | < 4 | < 4 |
| GS 15 | < 4 | < 4 | < 4 |
| GS 16 | < 4 | < 4 | < 4 |

*(Continued)*

| Samples | Number of colony (CFU/g) | | |
|---|---|---|---|
| | **Total *E. coli*** | **Resistant *E. coli*** | **Resistant non-*E. coli* coliforms** |
| GS 17 | < 4 | < 4 | < 4 |
| GL 1 | 24000 | < 10 | < 4 |
| Number of samples | 17/77 | 0/77 | 0/77 |
| Total | 33520 | 0 | 0 |
| Median | 200 | - | - |
| Maximum | 24000 | - | - |
| Minimum | 40 | - | - |

***Table S.15. The numbers (CFU/g) of total E. coli and ESBL-producer coliforms in geese in the urban site****. The number (CFU/g) of colony was calculated from samples with visible E. coli colonies on the spread plate within the countable range (8 - 83). When the counts were below the lower limit (1 - 7), the numbers (CFU/g) were regarded as 'estimated' (ASTM, 1998). These numbers (CFU/g) have been marked with an asterisk (*) in the table. Samples with 'TMTC' counts were reported as '> 4500 CFU/g'. Samples with 'zero' counts were reported as '< 10 CFU/g' as the LOD of goose faeces was 10 CFU/g . Both the numbers (CFU/g) from 'TMTC' and 'zero' counts were excluded from the calculation of the median (CFU/g). The median (CFU/g), maximum (CFU/g) and minimum (CFU/g) of the numbers (CFU/g) are shown at the bottom of the table.*

| Samples | Number of colony (CFU/g) | | |
|---|---|---|---|
| | **Total *E. coli*** | **Resistant *E. coli*** | **Resistant non-*E. coli* coliforms** |
| GSP 1 | < 10 | < 10 | < 10 |
| GSP 2 | < 10 | < 10 | < 10 |
| GSP 3 | 154000000 | < 10 | < 10 |
| GSP 4 | < 10 | < 10 | < 10 |
| GSP 5 | < 10 | < 10 | < 10 |
| GSP 6 | < 10 | < 10 | < 10 |
| GSP 7 | < 10 | < 10 | < 10 |
| GSP 8 | < 10 | < 10 | < 10 |
| GSP 9 | < 10 | < 10 | < 10 |
| GSP 10 | < 10 | < 10 | < 10 |
| GSP 11 | < 10 | < 10 | < 10 |
| GSP 12 | < 10 | < 10 | < 10 |
| GSP 13 | < 10 | < 10 | < 10 |
| GSP 14 | < 10 | < 10 | < 10 |
| GSP 15 | < 10 | < 10 | < 10 |
| GSP 16 | < 10 | < 10 | < 10 |
| GSP 17 | < 10 | < 10 | < 10 |
| GSP 18 | < 10 | < 10 | < 10 |
| GSP 19 | < 10 | < 10 | < 10 |
| GSP 20 | < 10 | < 10 | < 10 |

*(Continued)*

| Samples | Number of colony (CFU/g) | | |
|---|---|---|---|
| | **Total *E. coli*** | **Resistant *E. coli*** | **Resistant non-*E. coli* coliforms** |
| GSP 21 | < 10 | < 10 | < 10 |
| GSP 22 | < 10 | < 10 | < 10 |
| GSP 23 | < 10 | < 10 | < 10 |
| GSP 24 | < 10 | < 10 | < 10 |
| GSP 25 | < 10 | < 10 | < 10 |
| GSP 26 | 200* | < 10 | < 10 |
| GSP 27 | < 10 | < 10 | < 10 |
| GSP 28 | < 10 | < 10 | < 10 |
| GSP 29 | < 10 | < 10 | < 10 |
| GSP 30 | < 10 | < 10 | < 10 |
| GSP 31 | < 10 | < 10 | < 10 |
| GSP 32 | < 10 | < 10 | < 10 |
| GSP 33 | < 10 | < 10 | < 10 |
| GSP 34 | < 10 | < 10 | < 10 |
| GSP 35 | < 10 | < 10 | < 10 |
| GSP 36 | < 10 | < 10 | < 10 |
| GSP 37 | < 10 | < 10 | < 10 |
| GSP 38 | 42000 | < 10 | < 10 |
| GSP 39 | < 10 | < 10 | < 10 |
| GSP 40 | < 10 | < 10 | < 10 |
| GSP 41 | 2000 | < 10 | < 10 |
| GSP 42 | < 10 | < 10 | < 10 |
| GSP 43 | < 10 | < 10 | < 10 |
| GSP 44 | < 10 | < 10 | < 10 |
| GSP 45 | < 10 | < 10 | < 10 |
| GSP 46 | < 10 | < 10 | < 10 |
| GSP 47 | < 10 | < 10 | < 10 |
| GSP 48 | < 10 | < 10 | < 10 |
| GSP 49 | < 10 | < 10 | < 10 |
| GSP 50 | 1200* | < 10 | < 10 |
| GSP 51 | < 10 | < 10 | < 10 |
| GSP 52 | < 10 | < 10 | < 10 |
| Number of samples | 5/52 | 0/52 | 0/52 |
| Total | 154045400 | 0 | 0 |
| Median | 2000 | - | - |
| Maximum | 154000000 | - | - |
| Minimum | 200 | - | - |

# Appendix 3. Selection of Isolates for Further Characterisation

***Table S.16. Presumptive ESBL-producer E. coli.*** *Up to 10 colonies (ranging from 1 to 10) were selected from Coliform ChromoSelect agar (CCA) and given a unique isolate reference number (BAD). A two-step purity plate (indicates as 'first' and 'second' in the table) was undertaken on either MacConkey (Mac) or Eosin Methylene Blue (EMB) agar to ensure the purity of the culture. SCVs are coloured in blue. SCVs that reverted to a normal colony size upon subsequent subcultures (regarded as nonstable SCVs) are coloured in red.*

| Isolate Reference Numbers | Samples | Selected Colonies on CCA | | First Subculture | | | | Second Subculture | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Colonies on Mac | | Colonies on EMB | | Colonies on Mac | | Colonies on EMB | |
| | | Colour | Morphology | Colour | Morphology | Colour | Morphology | Colour | Morphology | Colour | Morphology |
| BAD-85 | LSW 5 | Blue | 1 big colony, Irregular, flat | | | Metallic green | Round, flat | | | Metallic green | Round, flat |
| BAD-86 | LSW 5 | Blue | 1 big colony, Irregular, flat | | | Metallic green | Round, flat | | | Metallic green | Round, flat |
| BAD-87 | LSW 5 | Blue | 1 big colony, Irregular, flat | | | Metallic green | Round, flat | | | Metallic green | Round, flat |
| BAD-88 | LSW 5 | Blue | 1 big colony, Irregular, flat | | | Metallic green | Round, flat | | | Metallic green | Round, flat |
| BAD-90 | LSW 11 | Blue | Round, flat | Red | Round, flat | | | | | Metallic green | Round, flat |
| BAD-91 | LSW 11 | Blue | Round, flat | Red | Round, flat | | | | | Metallic green | Round, flat |
| BAD-92 | LSW 11 | Blue | Round, flat | Red | Round, flat | | | | | Metallic green | Round, flat |
| BAD-93 | LSW 11 | Blue | Round, flat | Red | Round, flat | | | | | Metallic green | Round, flat |
| BAD-94 | LSW 11 | Blue | Round, flat | Red | Round, flat | | | | | Metallic green | Round, flat |
| BAD-95 | LSW 11 | Blue | Round, flat | Red | Round, flat | | | | | Metallic green | Round, flat |
| BAD-96 | LSW 11 | Blue | Round, flat | Red | Round, flat | | | | | Metallic green | Round, flat |
| BAD-97 | LSW 11 | Blue | Round, flat | Red | Round, flat | | | | | Metallic green | Round, flat |
| BAD-98 | LSW 11 | Blue | Round, flat | Red | Round, flat | | | | | Metallic green | Round, flat |
| BAD-99 | LSW 11 | Blue | Round, flat | Red | Round, flat | | | | | Metallic green | Round, flat |
| BAD-100 | LSW 13 | Blue | Round, flat | | | Metallic green | Round, flat | | | Violet | Round, flat |
| BAD-101 | LSW 13 | Blue | Round, flat | | | Metallic green | Round, flat | | | Metallic green | Round, flat |
| BAD-102 | LSW 13 | Blue | Round, flat | | | Metallic green | Round, flat | | | Violet | Round, flat |
| BAD-103 | LSW 13 | Blue | Round, flat | | | Mix metallic green and violet | Round, flat | | | Violet | Round, flat |
| BAD-104 | LSW 13 | Blue | Round, flat | | | Metallic green | Round, flat | | | Metallic green | Round, flat |
| BAD-105 | LSW 15 | Blue | Round, flat | Red | Round, flat | | | Red | Round, flat | | |
| BAD-107 | LSW 20 | Violet | Round, flat | Red | Round, flat | | | Red | Round, flat | | |

*(Continued)*

| Isolate Reference Numbers | Samples | Selected Colonies on CCA | | First Subculture | | | | Second Subculture | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Colonies on Mac | | Colonies on EMB | | Colonies on Mac | | Colonies on EMB | |
| | | Colour | Morphology | Colour | Morphology | Colour | Morphology | Colour | Morphology | Colour | Morphology |
| BAD-108 | LSW 20 | Blue | Round, flat | Red | Round, flat | | | Red | Round, flat | | |
| BAD-109 | LSW 20 | Blue | Round, flat | Red | Round, flat | | | Red | Round, flat | | |
| BAD-110 | LSW 20 | Blue | Round, flat | Red | Round, flat | | | Red | Round, flat | | |
| BAD-111 | LSW 20 | Blue | Round, flat | Red | Round, flat | | | Red | Round, flat | | |
| BAD-112 | LSW 20 | Blue | Round, flat | Red | Round, flat | | | Red | Round, flat | | |
| BAD-113 | LSW 20 | Blue | Round, flat | Red | Round, flat | | | Red | Round, flat | | |
| BAD-114 | LSW 20 | Blue | Round, flat | Red | Round, flat | | | Red | Round, flat | | |
| BAD-115 | LSW 20 | Blue | Round, flat | Red | Round, flat | | | Red | Round, flat | | |
| BAD-117 | LSW 27 | Blue | Round, flat | | | Mix metallic green and violet | Round, flat | | | Violet | Round, flat |
| BAD-118 | LSW 28 | Blue | Round, flat | | | Mix metallic green and violet | Round, flat | | | Violet | Round, flat |
| BAD-120 | LSW 30 | Blue | Round, flat | | | Metallic green | Round, flat | | | Metallic green | Round, flat |
| BAD-121 | LSW 1 | Blue | Round, flat | | | Violet | Round, flat | Red-Colourless | Round, flat | | |
| BAD-319 | Enriched LSW 3 | Blue | Round, flat | Red | Round, flat | | | Red | Round,flat | | |
| BAD-323 | Enriched LSW 20 | Blue | Round, flat | Red | Round, flat | | | Red | Round, flat | | |
| BAD-324 | Enriched LSW 20 | Blue | Round, flat | Red | Round, flat | | | Red | Round, flat | | |
| BAD-325 | Enriched LSW 20 | Blue | Round, flat | Red | Round, flat | | | Red | Round, flat | | |
| BAD-326 | Enriched LSW 20 | Blue | Round, flat | Red | Round, flat | | | Red | Round, flat | | |
| BAD-327 | Enriched LSW 20 | Blue | Round, flat | Red | Round, flat | | | Red | Round, flat | | |
| BAD-333 | Enriched LSW 34 | Blue | Round, flat | Red | Round, flat | | | Red | Round, flat | | |
| BAD-336 | Enriched LSW 34 | Violet | Round, flat | Red | Round, flat | | | Red | Round, flat | | |
| BAD-337 | Enriched LSW 34 | Violet | Round, flat | Red | Round, flat | | | Red | Round, flat | | |
| BAD-338 | Enriched LSW 36 | Blue | Round, flat | Red | Round, flat | | | Red | Round, flat | | |
| BAD-350 | Enriched GS 14 | Blue | Round, flat | | | Metallic green | Round, flat | | | Metallic green | Round, flat |
| BAD-451 | LSA 31 | Blue | Round, flat | | | Metallic green | Round, flat | | | Metallic green | Round, flat |
| BAD-452 | LSA 31 | Blue | Round, flat | | | Metallic green | Round, flat | | | Metallic green | Round, flat |
| BAD-453 | LSA 31 | Blue | Round, flat | | | Metallic green | Round, flat | | | Metallic green | Round, flat |
| BAD-454 | LSA 31 | Blue | Round, flat | | | Metallic green | Round, flat | | | Metallic green | Round, flat |
| BAD-455 | LSA 31 | Blue | Round, flat | | | Metallic green | Round, flat | | | Metallic green | Round, flat |
| BAD-461 | Enriched GD 37 | Blue | Irregular, flat | | | Mix metallic green and colourless | Round, flat | | | Metallic green | Round, flat |
| BAD-476 | Enriched LSA 31 | Blue | Round, flat | | | Metallic green | Round, flat | | | Metallic green | Round, flat |

***Table S.17. Presumptive ESBL-producer non-E. coli coliforms.*** *Up to 10 colonies (ranging from 1 to 10) were selected from Coliform ChromoSelect agar (CCA) and given a unique isolate reference number (BAD). A two-step purity plate (indicates as 'first' and 'second' in the table) was undertaken on either MacConkey (Mac) or Eosin Methylene Blue (EMB) agar to ensure the purity of the culture. SCVs are coloured in blue. SCVs that reverted to a normal colony size upon subsequent subcultures (regarded as nonstable SCVs) are coloured in red. Selected red colonies on Coliform ChromoSelect agar which appeared as SCV with a faint green metallic sheen on Eosin Methylene Blue agar (suggesting they were E. coli) are highlighted in green.*

| Isolate Reference Numbers | Samples | Selected Colonies on CCA | | First Subculture | | | | Second Subculture | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Colonies on Mac | | Colonies on EMB | | Colonies on Mac | | Colonies on EMB | |
| | | Colour | Morphology | Colour | Morphology | Colour | Morphology | Colour | Morphology | Colour | Morphology |
| BAD-106 | LSW 20 | Red | Round, flat | Red | Round, flat | | | Red-Colourless | Round, flat | | |
| BAD-134 | LSW 2 | Red | Round, flat | | | Metallic green | Round, flat | Red-Colourless | Round, flat | | |
| BAD-135 | LSW 2 | Red | Round, flat | | | Metallic green | Round, flat | Red-Colourless | Round, flat | | |
| BAD-252 | LSW 39 | Red | Round, flat | | | Metallic green | Round, flat | Red-Colourless | Round, flat | | |
| BAD-253 | LSW 39 | Red | Round, flat | | | Metallic green | Round, flat | Red-Colourless | Round, flat | | |
| BAD-278 | Enriched GSP 15 | Red | Round, flat | Red | Round, flat | | | Red | Round, flat | | |
| BAD-279 | Enriched GSP 15 | Red | Round, flat | Red | Round, flat | | | Red | Round, flat | | |
| BAD-280 | Enriched GSP 15 | Red | Round, flat | Red | Round, flat | | | Red | Round, flat | | |
| BAD-281 | Enriched GSP 15 | Red | Round, flat | Red | Round, flat | | | Red | Round, flat | | |
| BAD-282 | Enriched GSP 15 | Red | Round, flat | Red | Round, flat | | | Red | Round, flat | | |
| BAD-313 | Enriched GSP 52 | Red | Round, flat | Red-Colourless | Round, flat | | | Red-Colourless | Round, flat | | |
| BAD-314 | Enriched GSP 52 | Red | Round, flat | Red-Colourless | Round, flat | | | Red-Colourless | Round, flat | | |
| BAD-315 | Enriched GSP 52 | Red | Round, flat | Red-Colourless | Round, flat | | | Red-Colourless | Round, flat | | |
| BAD-316 | Enriched GSP 52 | Red | Round, flat | Red-Colourless | Round, flat | | | Red-Colourless | Round, flat | | |
| BAD-317 | Enriched GSP 52 | Red | Round, flat | Red-Colourless | Round, flat | | | Red-Colourless | Round, flat | | |
| BAD-456 | LSA 7 | Red | Round, flat | | | Metallic green | Round, flat | | | Metallic green | Round, flat |
| BAD-457 | LSA 7 | Red | Round, flat | | | Metallic green | Round, flat | | | Metallic green | Round, flat |
| BAD-458 | LSA 7 | Red | Round, flat | | | Metallic green | Round, flat | | | Metallic green | Round, flat |
| BAD-459 | LSA 7 | Red | Round, flat | | | Metallic green | Round, flat | | | Metallic green | Round, flat |
| BAD-460 | LSA 7 | Red | Round, flat | | | Metallic green | Round, flat | | | Metallic green | Round, flat |
| BAD-462 | Enriched GD 37 | Red | Round, flat | | | Mix metallic green and colourless | Round, flat | | | Metallic green | Round, flat |
| BAD-463 | Enriched GD 30 | Red | Round, flat | | | Metallic green | Round, flat | | | Metallic green | Round, flat |

*(Continued)*

| Isolate Reference Numbers | Samples | Selected Colonies on CCA | | First Subculture | | | | Second Subculture | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Colonies on Mac | | Colonies on EMB | | Colonies on Mac | | Colonies on EMB | |
| | | Colour | Morphology | Colour | Morphology | Colour | Morphology | Colour | Morphology | Colour | Morphology |
| BAD-464 | Enriched GD 30 | Red | Round, flat | | | Metallic green | Round, flat | | | Metallic green | Round, flat |
| BAD-465 | Enriched GD 30 | Red | Round, flat | | | Metallic green | Round, flat | | | Metallic green | Round, flat |
| BAD-466 | Enriched GD 30 | Red | Round, flat | | | Metallic green | Round, flat | | | Metallic green | Round, flat |
| BAD-468 | Enriched GD 29 | Red | Irregular, flat | | | Mix metallic green and colourless | Round, flat | | | Metallic green | Round, flat |
| BAD-469 | Enriched GD 29 | Red | Irregular, flat | | | Mix metallic green and colourless | Round, flat | | | Metallic green | Round, flat |
| BAD-470 | Enriched GD 29 | Red | Irregular, flat | | | Mix metallic green and colourless | Round, flat | | | Metallic green | Round, flat |
| BAD-472 | Enriched GD 21 | Red | Round, flat | | | Mix metallic green and colourless | Round, flat | | | Metallic green | Round, flat |
| BAD-473 | Enriched GD 21 | Red | Round, flat | | | Mix metallic green and colourless | Round, flat | | | Metallic green | Round, flat |
| BAD-474 | Enriched GD 21 | Red | Round, flat | | | Mix metallic green and colourless | Round, flat | | | Metallic green | Round, flat |
| BAD-475 | Enriched GD 21 | Red | Round, flat | | | Mix metallic green and colourless | Round, flat | | | Metallic green | Round, flat |
| BAD-477 | Enriched LSA 37 | Red | Round, flat | | | Mix metallic green and colourless | Round, flat | | | Metallic green | Round, flat |
| BAD-478 | Enriched LSA 37 | Red | Round, flat | | | Mix metallic green and colourless | Round, flat | | | Metallic green | Round, flat |
| BAD-479 | Enriched LSA 37 | Red | Round, flat | | | Mix metallic green and colourless | Round, flat | | | Metallic green | Round, flat |
| BAD-480 | Enriched LSA 37 | Red | Round, flat | | | Mix metallic green and colourless | Round, flat | | | Metallic green | Round, flat |
| BAD-481 | Enriched LSA 37 | Red | Round, flat | | | Mix metallic green and colourless | Round, flat | | | Metallic green | Round, flat |

***Table S.18. Presumptive ESBL Sensitive E. coli.*** *Up to 10 colonies (ranging from 1 to 10) were selected from Coliform ChromoSelect agar (CCA) and given a unique isolate reference number (BAD). A two-step purity plate (indicates as 'first' and 'second' in the table) was undertaken on either MacConkey (Mac) or Eosin Methylene Blue (EMB) agar to ensure the purity of the culture. SCVs are coloured in blue.*

| Isolate Reference Numbers | Samples | Selected Colonies on CCA | | First Subculture | | | | Second Subculture | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Colonies on Mac | | Colonies on EMB | | Colonies on Mac | | Colonies on EMB | |
| | | Colour | Morphology | Colour | Morphology | Colour | Morphology | Colour | Morphology | Colour | Morphology |
| BAD-150 | GD 6 | Blue | Round, flat | | | Metallic green | Round, flat | | | Metallic green | Round, flat |
| BAD-151 | GD 6 | Blue | Round, flat | | | Metallic green | Round, flat | | | Metallic green | Round, flat |
| BAD-153 | GS 1 | Blue | Round, flat | | | Metallic green | Round, flat | | | Metallic green | Round, flat |
| BAD-154 | GS 1 | Blue | Round, flat | | | Metallic green | Round, flat | | | Metallic green | Round, flat |
| BAD-156 | GS 3 | Blue | Irregular, flat | | | Metallic green | Round, flat | | | Metallic green | Round, flat |

*(Continued)*

| Isolate Reference Numbers | Samples | Selected Colonies on CCA | | First Subculture | | | | Second Subculture | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Colonies on Mac | | Colonies on EMB | | Colonies on Mac | | Colonies on EMB | |
| | | Colour | Morphology | Colour | Morphology | Colour | Morphology | Colour | Morphology | Colour | Morphology |
| BAD-158 | GL 1 | Blue | Irregular, flat | | | Metallic green | Round, flat | | | Metallic green | Round, flat |
| BAD-159 | GL 1 | Blue | Round, flat | | | Metallic green | Round, flat | | | Metallic green | Round, flat |
| BAD-160 | GL 1 | Blue | Round, flat | | | Metallic green | Round, flat | | | Metallic green | Round, flat |
| BAD-161 | GSP 3 | Blue | Irregular, flat | | | Metallic green | Round, flat | Red | Round, flat | | |
| BAD-162 | GSP 3 | Blue | Irregular, flat | | | Metallic green | Round, flat | Red | Round, flat | | |
| BAD-163 | GSP 3 | Blue | Irregular, flat | | | Metallic green | Round, flat | Red | Round, flat | | |
| BAD-164 | GSP 3 | Blue | Irregular, flat | | | Metallic green | Round, flat | Red | Round, flat | | |
| BAD-165 | GSP 3 | Blue | Irregular, flat | | | Metallic green | Round, flat | Red | Round, flat | | |
| BAD-169 | LSW 1 | Blue | Round, flat | | | Metallic green | Round, flat | | | Metallic green | Round, flat |
| BAD-170 | LSW 1 | Blue | Round, flat | | | Metallic green | Round, flat | | | Metallic green | Round, flat |
| BAD-177 | GSP 26 | Blue | Irregular, flat | | | Metallic green | Round, flat | Red | Round, flat | | |
| BAD-178 | GSP 38 | Blue | Round, flat | | | Metallic green | Round, flat | Red | Round, flat | | |
| BAD-179 | GSP 38 | Blue | Round, flat | | | Metallic green | Round, flat | Red | Round, flat | | |
| BAD-180 | GSP 38 | Blue | Round, flat | | | Metallic green | Round, flat | Red | Round, flat | | |
| BAD-181 | GSP 38 | Blue | Round, flat | | | Metallic green | Round, flat | Red | Round, flat | | |
| BAD-182 | GSP 38 | Blue | Round, flat | | | Metallic green | Round, flat | Red | Round, flat | | |
| BAD-184 | GSP 50 | Blue | Round, flat | | | Metallic green | Round, flat | Red | Round, flat | | |
| BAD-185 | GSP 50 | Blue | Round, flat | | | Metallic green | Round, flat | | | Metallic green | Round, flat |
| BAD-186 | GSP 50 | Blue | Round, flat | | | Metallic green | Round, flat | | | Metallic green | Round, flat |
| BAD-187 | GSP 50 | Blue | Round, flat | | | Metallic green | Round, flat | | | Metallic green | Round, flat |
| BAD-188 | GSP 50 | Blue | Round, flat | | | Metallic green | Round, flat | | | Metallic green | Round, flat |
| BAD-194 | LSW 5 | Blue | Round, flat | | | Metallic green | Round, flat | Red | Round, flat | | |
| BAD-195 | LSW 5 | Blue | Round, flat | | | Metallic green | Round, flat | Red | Round, flat | | |
| BAD-196 | LSW 5 | Blue | Round, flat | Red | Irregular, flat | | | Red | Round, flat | | |
| BAD-197 | LSW 5 | Blue | Round, flat | Red | Irregular, flat | | | Red | Round, flat | | |
| BAD-198 | LSW 5 | Blue | Round, flat | Red | Round, flat | | | Red | Round, flat | | |
| BAD-227 | LSW 13 | Blue | Round, flat | | | Mix metallic green and violet | Round, flat | Red-Colourless | Round, flat | | |
| BAD-229 | LSW 13 | Blue | Round, flat | | | Mix metallic green and violet | Round, flat | Red-Colourless | Round, flat | | |

*(Continued)*

| Isolate Reference Numbers | Samples | Selected Colonies on CCA | | First Subculture | | | | Second Subculture | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Colonies on Mac | | Colonies on EMB | | Colonies on Mac | | Colonies on EMB | |
| | | Colour | Morphology | Colour | Morphology | Colour | Morphology | Colour | Morphology | Colour | Morphology |
| BAD-231 | LSW 15 | Blue | Round, flat | Red | Irregular, flat | | | Red | Round, flat | | |
| BAD-237 | LSW 20 | Blue | Round, flat | Red | Irregular, flat | | | | | Metallic green | Round, flat |
| BAD-238 | LSW 20 | Blue | Round, flat | Red | Irregular, flat | | | | | Metallic green | Round, flat |
| BAD-239 | LSW 20 | Blue | Round, flat | Red | Round, flat | | | | | Metallic green | Round, flat |
| BAD-240 | LSW 20 | Blue | Round, flat | Red | Round, flat | | | | | Metallic green | Round, flat |
| BAD-241 | LSW 20 | Blue | Round, flat | Red | Round, flat | | | | | Metallic green | Round, flat |
| BAD-367 | LSW 30 | Blue | Round, flat | | | Metallic green | Round, flat | | | Metallic green | Round, flat |
| BAD-369 | LSW 30 | Blue | Round, flat | | | Metallic green | Round, flat | | | Metallic green | Round, flat |
| BAD-370 | LSW 30 | Blue | Round, flat | | | Metallic green | Round, flat | | | Metallic green | Round, flat |
| BAD-376 | LSW 34 | Blue | Round, flat | | | Metallic green | Round, flat | Red | Round, flat | | |
| BAD-377 | LSW 34 | Blue | Round, flat | | | Metallic green | Round, flat | Red | Round, flat | | |
| BAD-378 | LSW 34 | Blue | Round, flat | | | Metallic green | Round, flat | Red | Round, flat | | |
| BAD-379 | LSW 34 | Blue | Round, flat | | | Metallic green | Round, flat | Red | Round, flat | | |
| BAD-380 | LSW 34 | Blue | Round, flat | | | Metallic green | Round, flat | Red | Round, flat | | |
| BAD-386 | LSW 36 | Blue | Round, flat | | | Metallic green | Round, flat | Red | Round, flat | | |
| BAD-387 | LSW 36 | Blue | Round, flat | | | Metallic green | Round, flat | Red | Round, flat | | |
| BAD-388 | LSW 36 | Blue | Round, flat | | | Metallic green | Round, flat | Red | Round, flat | | |
| BAD-389 | LSW 36 | Blue | Round, flat | | | Metallic green | Round, flat | Red | Round, flat | | |
| BAD-390 | LSW 36 | Blue | Round, flat | | | Metallic green | Round, flat | Red | Round, flat | | |
| BAD-411 | LSW 42 | Blue | Irregular, flat | | | Metallic green | Round, flat | Red | Round, flat | | |
| BAD-412 | LSW 42 | Blue | Irregular, flat | | | Metallic green | Round, flat | Red-Colourless | Round, flat | | |
| BAD-413 | LSW 42 | Blue | Irregular, flat | | | Metallic green | Round, flat | Red | Round, flat | | |
| BAD-414 | LSW 42 | Blue | Irregular, flat | | | Metallic green | Round, flat | Red | Round, flat | | |
| BAD-415 | LSW 42 | Blue | Irregular, flat | | | Metallic green | Round, flat | Red | Round, flat | | |
| BAD-444 | GD 45 | Blue | Round, flat | | | Metallic green | Round, flat | | | Metallic green | Round, flat |
| BAD-445 | GD 45 | Blue | Round, flat | | | Metallic green | Round, flat | | | Metallic green | Round, flat |
| BAD-446 | GD 45 | Blue | Round, flat | | | Metallic green | Round, flat | | | Metallic green | Round, flat |

*(Continued)*

| Isolate Reference Numbers | Samples | Selected Colonies on CCA | | First Subculture | | | | Second Subculture | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Colonies on Mac | | Colonies on EMB | | Colonies on Mac | | Colonies on EMB | |
| | | Colour | Morphology | Colour | Morphology | Colour | Morphology | Colour | Morphology | Colour | Morphology |
| BAD-447 | GD 45 | Blue | Round, flat | | | Metallic green | Round, flat | | | Metallic green | Round, flat |
| BAD-448 | GD 45 | Blue | Round, flat | | | Metallic green | Round, flat | | | Metallic green | Round, flat |
| BAD-449 | G.Dun. 1 | Blue | Round, flat | | | Metallic green | Round, flat | | | Metallic green | Round, flat |
| BAD-450 | G.Dun. 1 | Blue | Round, flat | | | Metallic green | Round, flat | | | Metallic green | Round, flat |
| BAD-551 | G.Dun. 6 | Blue | Round, flat | | | Metallic green | Round, flat | | | Metallic green | Round, flat |
| BAD-553 | GS 8 | Blue | Round, flat | | | Metallic green | Round, flat | | | Metallic green | Round, flat |
| BAD-574 | LSA 5 | Blue | Round, flat | | | Metallic green | Round, flat | | | Metallic green | Round, flat |
| BAD-575 | LSA 5 | Blue | Round, flat | | | Metallic green | Round, flat | | | Metallic green | Round, flat |
| BAD-576 | LSA 5 | Blue | Round, flat | | | Metallic green | Round, flat | | | Metallic green | Round, flat |
| BAD-577 | LSA 5 | Blue | Round, flat | | | Metallic green | Round, flat | | | Metallic green | Round, flat |
| BAD-578 | LSA 5 | Blue | Round, flat | | | Metallic green | Round, flat | | | Metallic green | Round, flat |
| BAD-609 | LSA 12 | Blue | Round, flat | | | Metallic green | Round, flat | | | Metallic green | Round, flat |
| BAD-610 | LSA 12 | Blue | Round, flat | | | Metallic green | Round, flat | | | Metallic green | Round, flat |
| BAD-611 | LSA 12 | Blue | Round, flat | | | Metallic green | Round, flat | | | Metallic green | Round, flat |
| BAD-612 | LSA 12 | Blue | Round, flat | | | Metallic green | Round, flat | | | Metallic green | Round, flat |
| BAD-613 | LSA 12 | Blue | Round, flat | | | Metallic green | Round, flat | | | Metallic green | Round, flat |
| BAD-777 | LSA 27 | Blue | Round, flat | | | Metallic green | Round, flat | | | Metallic green | Round, flat |
| BAD-819 | LSA 37 | Blue | Round, flat | | | Metallic green | Round, flat | | | Metallic green | Round, flat |
| BAD-820 | LSA 37 | Blue | Round, flat | | | Metallic green | Round, flat | | | Metallic green | Round, flat |
| BAD-821 | LSA 37 | Blue | Round, flat | | | Metallic green | Round, flat | | | Metallic green | Round, flat |
| BAD-822 | LSA 37 | Blue | Round, flat | | | Metallic green | Round, flat | | | Metallic green | Round, flat |
| BAD-823 | LSA 37 | Blue | Round, flat | | | Metallic green | Round, flat | | | Metallic green | Round, flat |
| BAD-839 | LSA 41 | Blue | Round, flat | | | Metallic green | Round, flat | | | Metallic green | Round, flat |
| BAD-840 | LSA 41 | Blue | Round, flat | | | Metallic green | Round, flat | | | Metallic green | Round, flat |
| BAD-841 | LSA 41 | Blue | Round, flat | | | Metallic green | Round, flat | | | Metallic green | Round, flat |
| BAD-842 | LSA 41 | Blue | Round, flat | | | Metallic green | Round, flat | | | Metallic green | Round, flat |
| BAD-843 | LSA 41 | Blue | Round, flat | | | Metallic green | Round, flat | | | Metallic green | Round, flat |

# Appendix 4. Comparison of REP and $(GTG)_5$ Primers for the Rep-PCR Assay

## 4.1 REP Primers

### 4.1.1 Reproducibility

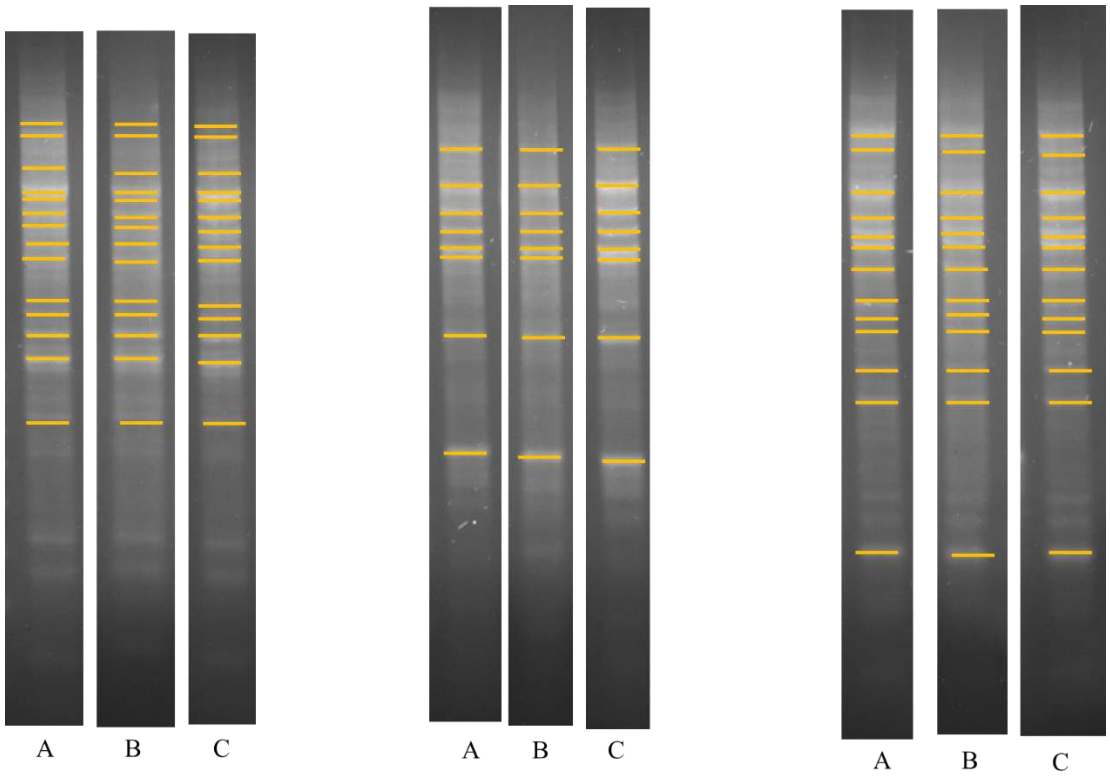



***Figure S.1. Reproducibility of ERI 40 (left), ATCC 47055 (middle) and AT 1.3 (right) using REP primers.*** *Three strains of E. coli (ERI 40, ATCC 47055, AT 1.3) with distinct REP-PCR DNA fingerprint pattern were typed using REP primers in triplicate and visually compared side by side. Bands (medium and strong) are indicated by orange lines. The reproducibility of medium and strong bands produced by REP primers was assessed (Table S.19).*

***Table S.19. Reproducibility of REP primers.*** *The reproducibility of medium (▥) and strong (▨)bands produced by REP primers was noted for each isolate.*

| Reproducibility of REP primer | Bands | Reproducibility % |
|---|---|---|
| **ERI 40** | | 100% |
| Repeat | 1 2 3 4 5 6 7 8 9 10 11 12 13 14 | |
| A | | |
| B | | |
| C | | |
| **ATCC 47055** | | 100% |
| Repeat | 1 2 3 4 5 6 7 8 | |
| A | | |
| B | | |
| C | | |
| **AT 1.3** | | 100% |
| Repeat | 1 2 3 4 5 6 7 8 9 10 11 12 13 | |
| A | | |
| B | | |
| C | | |
| **Mean** | | **100%** |

4.1.2 Discriminatory Power

***Table S.20. Discriminatory power (Band differences) of REP primers.*** *The discriminatory power (the ability to assign a different type of two unrelated strains) was assessed by visually comparing the band patterns between isolates.*

| | Controls | ERI 40 | ATCC 47055 | AT 1.3 |
|---|---|---|---|---|
| **Repeat A** | ERI 40 | | 10 | 11 |
| | ATCC 47055 | | | 12 |
| | AT 1.3 | | | |
| **Repeat B** | Controls | ERI 40 | ATCC 47055 | AT 1.3 |
| | ERI 40 | | 10 | 11 |
| | ATCC 47055 | | | 12 |
| | AT 1.3 | | | |
| **Repeat C** | Controls | ERI 40 | ATCC 47055 | AT 1.3 |
| | ERI 40 | | 10 | 11 |
| | ATCC 47055 | | | 12 |
| | AT 1.3 | | | |

4.2 $(GTG)_5$ Primer

4.2.1 Reproducibility

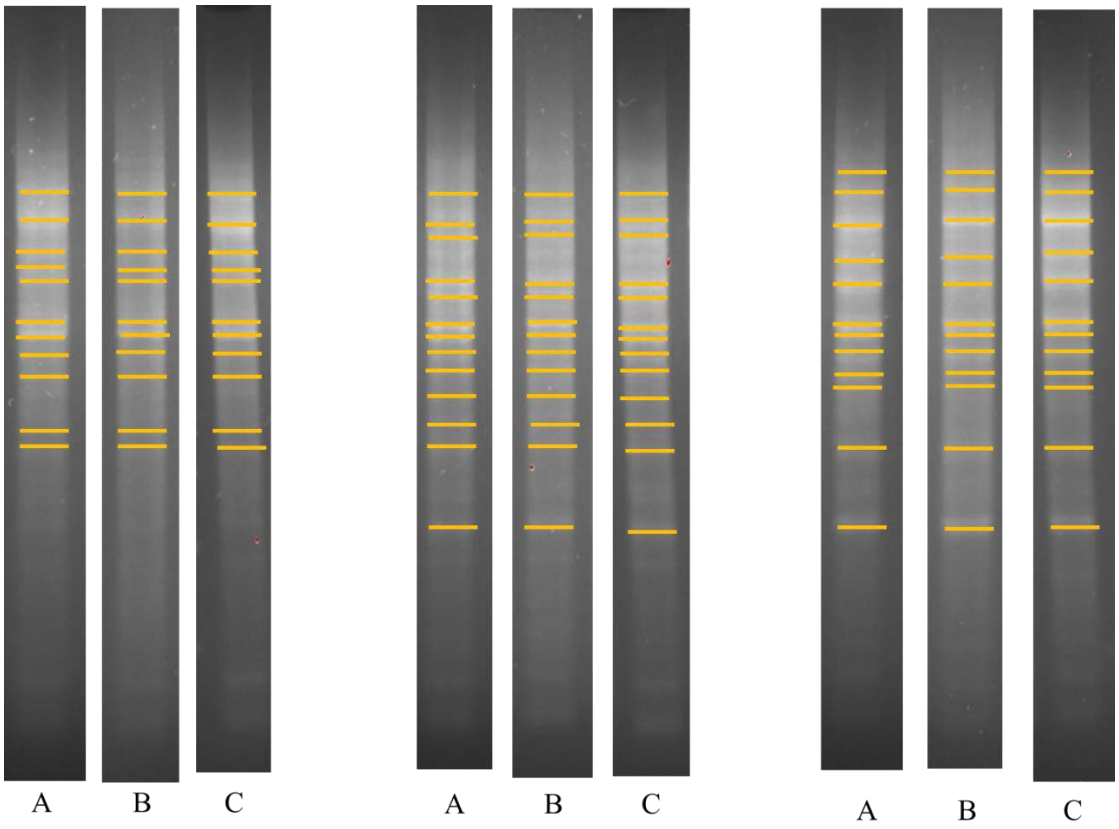
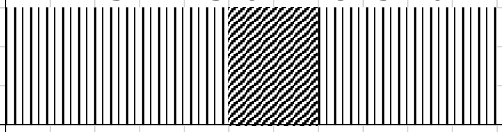
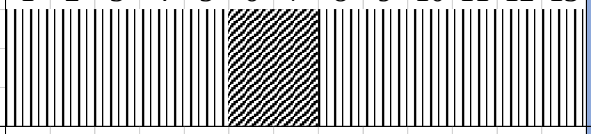
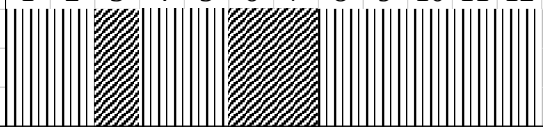



***Figure S.2. Reproducibility of ERI 40 (left), ATCC 47055 (middle) and AT 1.3 (right) using $(GTG)_5$ primer*** *Three strains of E. coli (ERI 40, ATCC 47055, AT 1.3) with distinct REP-PCR DNA fingerprint pattern were typed using $(GTG)_5$ primer in triplicate and visually compared side by side. Bands (medium and strong) are indicated by orange lines. The reproducibility of medium and strong bands produced by $(GTG)_5$ primer was assessed (Table S.21).*

***Table S.21. Reproducibility of $(GTG)_5$ primer.*** *The reproducibility of medium (▥) and strong (▨)bands produced by $(GTG)_5$ primer was noted for each isolate.*

| **Reproducibility of $(GTG)_5$ primer** | **Bands** | **Reproducibility %** |
|---|---|---|
| **ERI 40** | | 100% |
| Repeat | 1 2 3 4 5 6 7 8 9 10 11 | |
| A |  | |
| B | | |
| C | | |
| **ATCC 47055** | | 100% |
| Repeat | 1 2 3 4 5 6 7 8 9 10 11 12 13 | |
| A |  | |
| B | | |
| C | | |
| **AT 1.3** | | 100% |
| Repeat | 1 2 3 4 5 6 7 8 9 10 11 12 | |
| A |  | |
| B | | |
| C | | |
| **Mean** | | **100%** |

4.2.2 Discriminatory Power

***Table S.22. Discriminatory power (Band differences) of $(GTG)_5$ primer.*** *The discriminatory power (the ability to assign a different type of two unrelated strains) was assessed by visually comparing the band patterns between isolates.*

| | Controls | ERI 40 | ATCC 47055 | AT 1.3 |
|---|---|---|---|---|
| **Repeat A** | ERI 40 | | 6 | 5 |
| | ATCC 47055 | | | 7 |
| | AT 1.3 | | | |
| | Controls | ERI 40 | ATCC 47055 | AT 1.3 |
| **Repeat B** | ERI 40 | | 6 | 5 |
| | ATCC 47055 | | | 7 |
| | AT 1.3 | | | |
| | Controls | ERI 40 | ATCC 47055 | AT 1.3 |
| **Repeat C** | ERI 40 | | 6 | 5 |
| | ATCC 47055 | | | 7 |
| | AT 1.3 | | | |

# Appendix 5. DNA Fingerprint Pattern of the 175 Isolates from Bird Samples by REP-PCR assay

5.1 Presumptive ESBL-Producer *E. coli*

5.1.1 Gels of REP-PCR DNA Fingerprinting

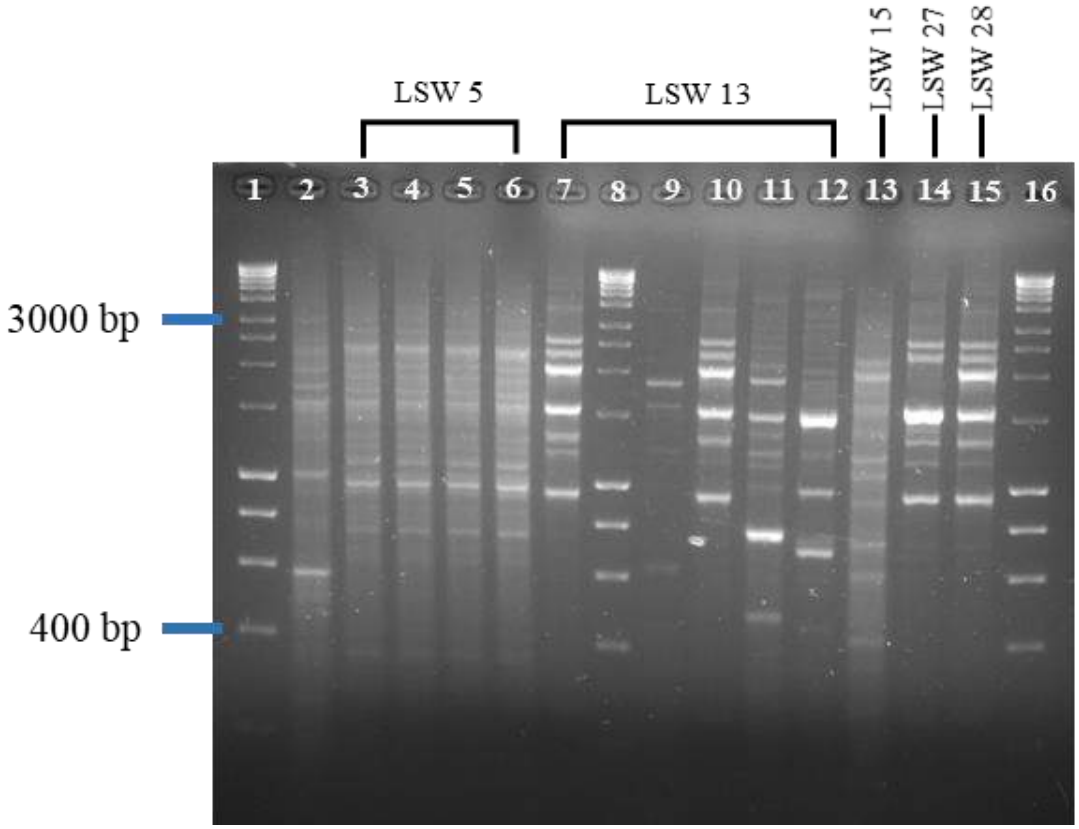



***Figure S.3. REP-PCR of presumptive ESBL-producer E. coli (1).*** *Lane 1, 8 and 16: Ladder 1 kb. Lane 2: E. coli ATCC® 47055 strain for E. coli reference. Lane 3-7: BAD 85, BAD 86, BAD 87, BAD 88, BAD 100. Lane 9-15: BAD 101, BAD 102, BAD 103, BAD 104, BAD 105, BAD 117, BAD 118. Sample reference (LSW - gulls in Seafield WWTW) is shown and indicates the samples from which isolates were selected.*

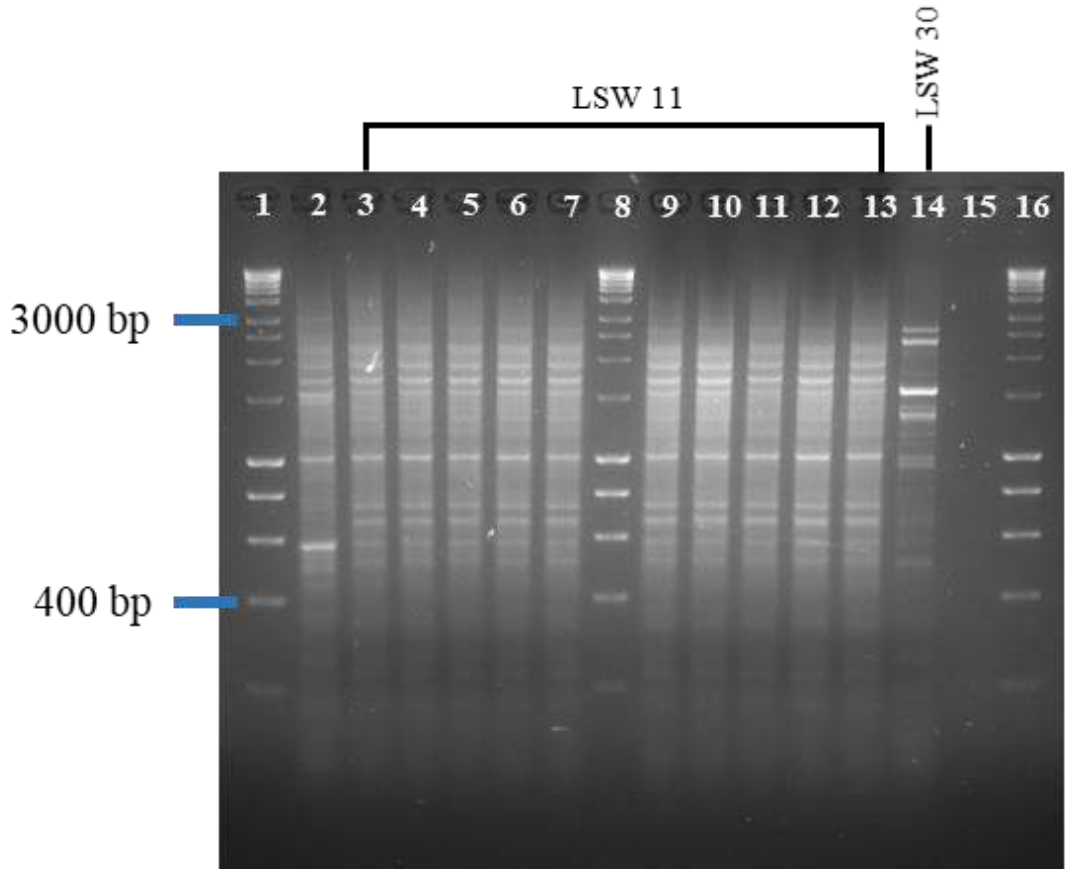



***Figure S.4. REP-PCR of presumptive ESBL-producer E. coli (2).*** *Lane 1, 8 and 16: Ladder 1 kb. Lane 2: E. coli ATCC® 47055 strain for E. coli reference. Lane 3-7: BAD 90, BAD 91, BAD 92, BAD 93, BAD 94. Lane 9-14: BAD 95, BAD 96, BAD 97, BAD 98, BAD 99, BAD 120. Sample reference (LSW - gulls in Seafield WWTW) is shown and indicates the samples from which isolates were selected.*

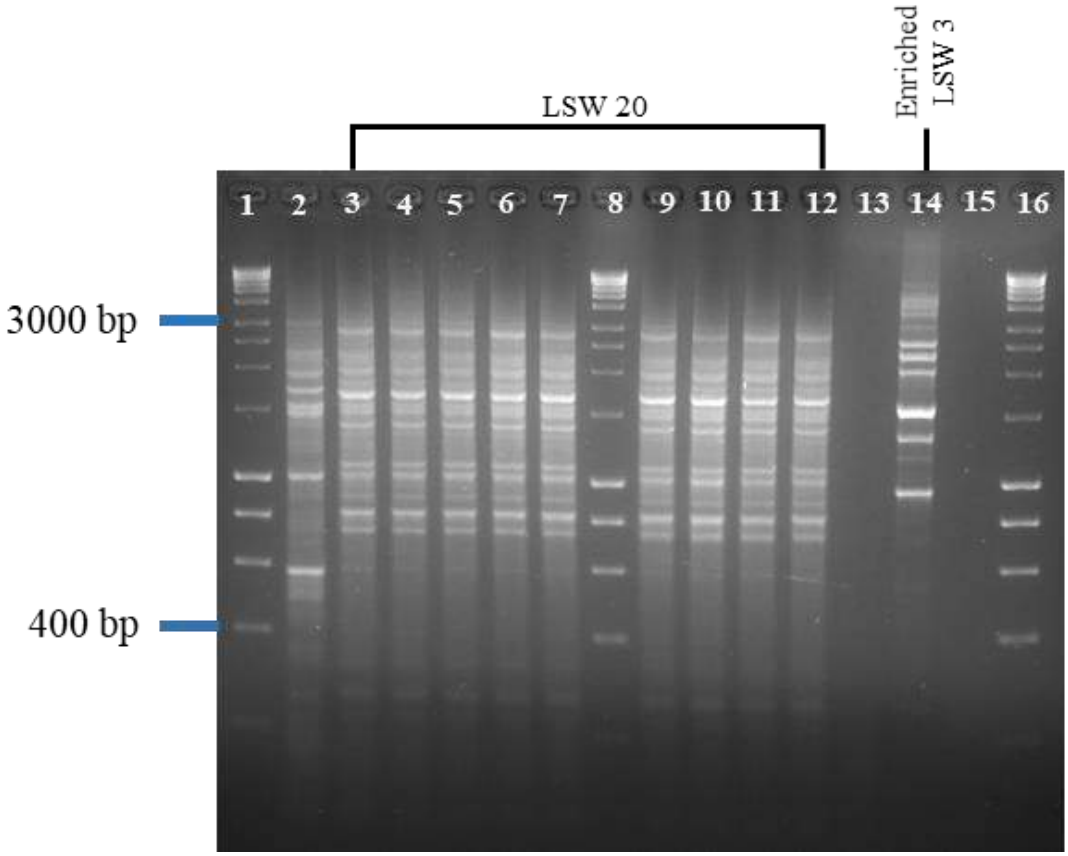



***Figure S.5. REP-PCR of presumptive ESBL-producer E. coli (3).*** *Lane 1, 8 and 16: Ladder 1 kb. Lane 2: E. coli ATCC® 47055 strain for E. coli reference. Lane 3-7: BAD 107, BAD 108, BAD 109, BAD 110, BAD 111. Lane 9-14: BAD 112, BAD 113, BAD 114, BAD 115, -, BAD 319. Sample reference (LSW - gulls in Seafield WWTW) is shown and indicates the samples from which isolates were selected.*

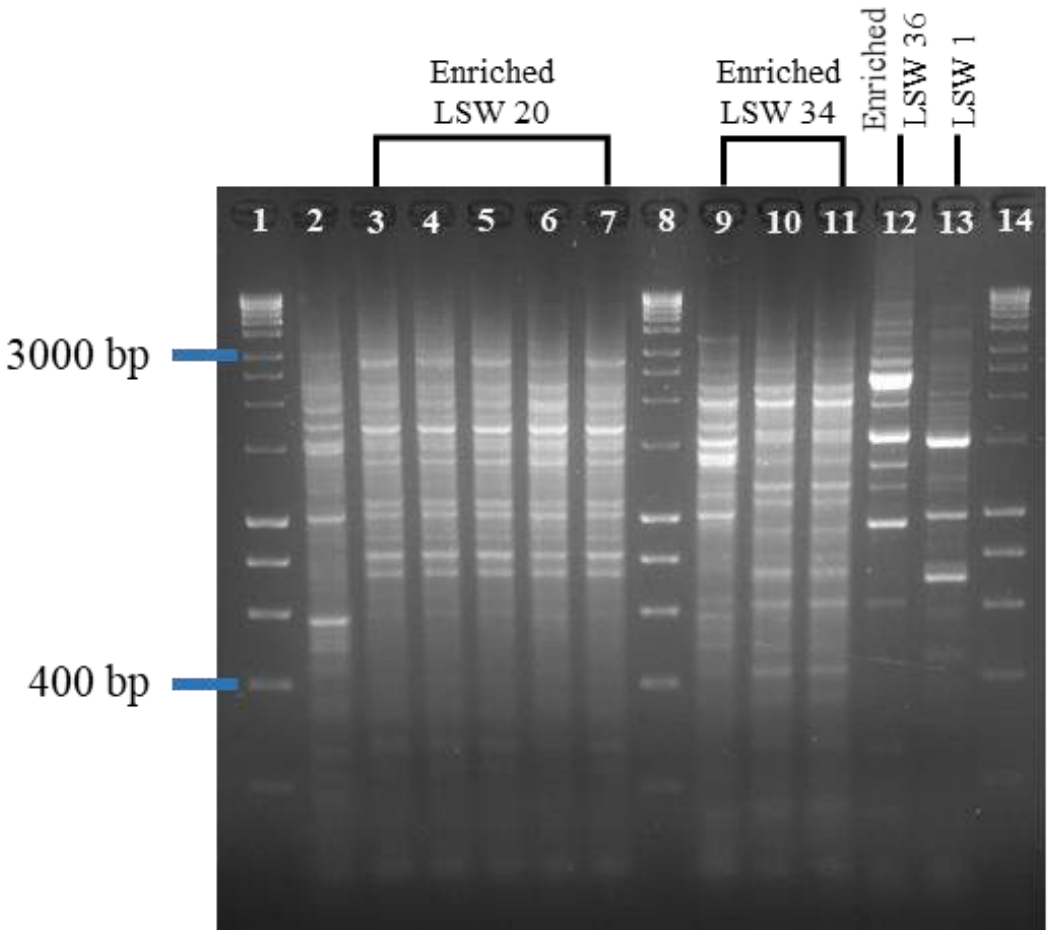



***Figure S.6. REP-PCR of presumptive ESBL-producer E. coli (4).*** *Lane 1, 8 and 14: Ladder 1 kb. Lane 2: E. coli ATCC® 47055 strain for E. coli reference. Lane 3-7: BAD 323, BAD 324, BAD 325, BAD 326, BAD 327. Lane 9-13: BAD 333, BAD 336, BAD 337, BAD 338, BAD 121. Sample reference (LSW - gulls in Seafield WWTW) is shown and indicates the samples from which isolates were selected.*

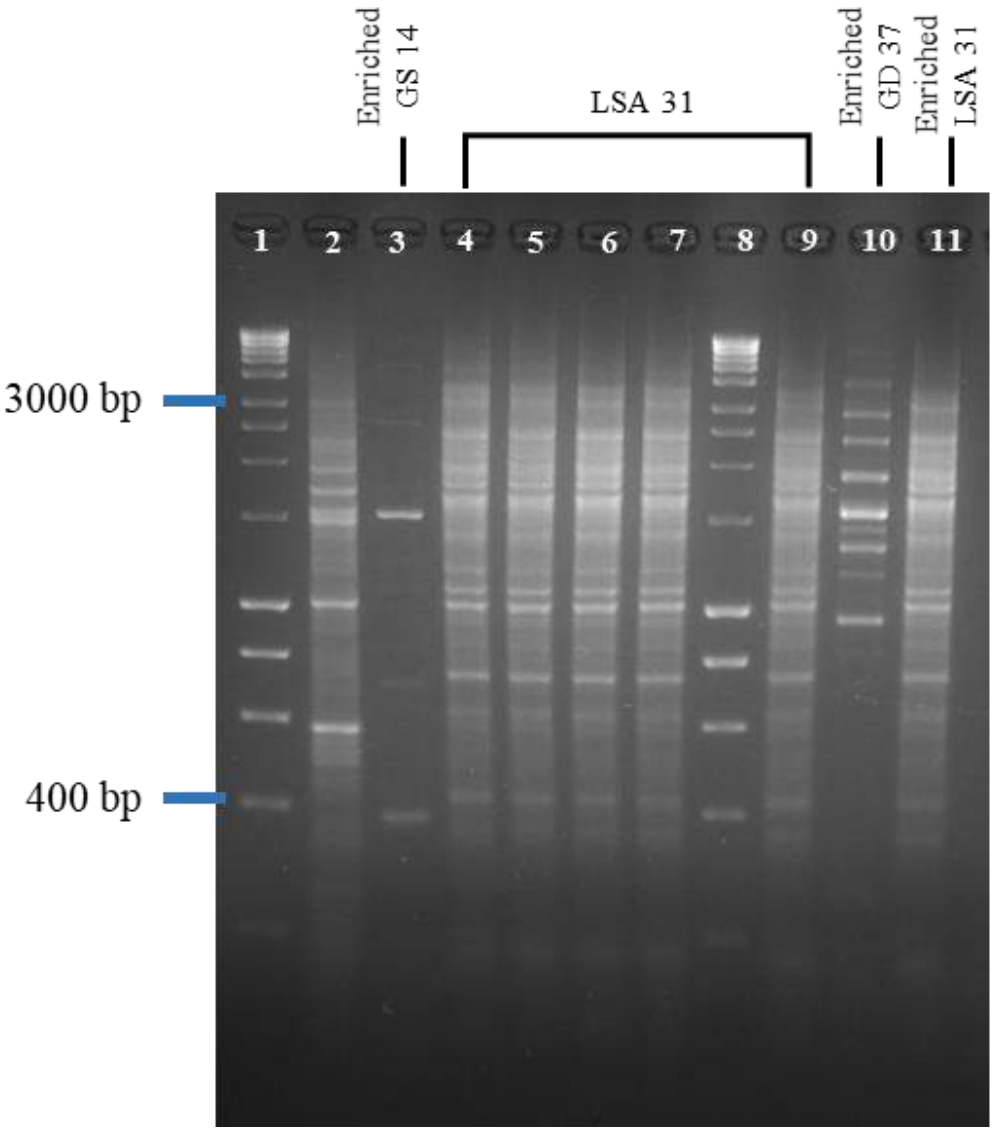



***Figure S.7. REP-PCR of presumptive ESBL-producer E. coli (5).*** *Lane 1 and 8: Ladder 1 kb. Lane 2: E. coli ATCC® 47055 strain for E. coli reference. Lane 3-7: BAD 350, BAD 451, BAD 452, BAD 453, BAD 454. Lane 9-11: BAD 455, BAD 461, BAD 476. Sample reference (GS – geese in St Margaret's Loch, GD – geese in Duddingston Loch, LSA – gulls in St Abbs) is shown and indicates the samples from which isolates were selected.*

5.1.2 Dendrogram of All DNA Fingerprints showing REP Types (REPR) Identified

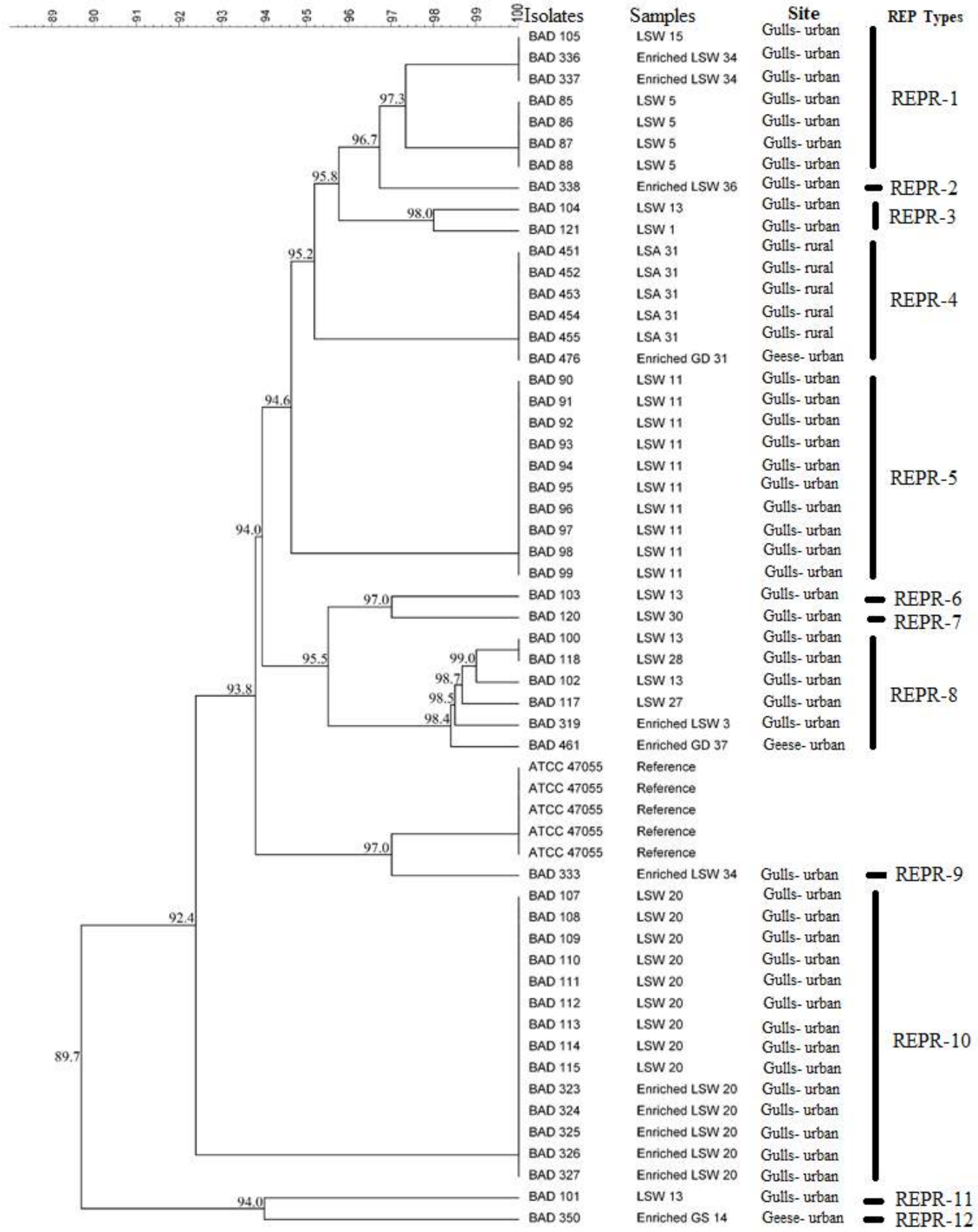



***Figure S.8. The dendrogram of REP-PCR assay of presumptive ESBL-producer E. coli generated by BioNumerics.*** *Cluster analysis was done using the number of band difference coefficient and UPGMA method. Numbers at the nodes show the equated similarity (%) of the band difference in Fig.5 (section 4.5.1). The samples (isolate numbers and sites) and REP types (REPR) are shown in the dendrogram.. The axis line at the top (89-100) indicates the similarity (%). REP types have been assigned based on > 97% similarity (equated to <3 bands difference rule).*

5.2 Presumptive ESBL Sensitive *E. coli*

5.2.1 Gels of REP-PCR DNA Fingerprinting

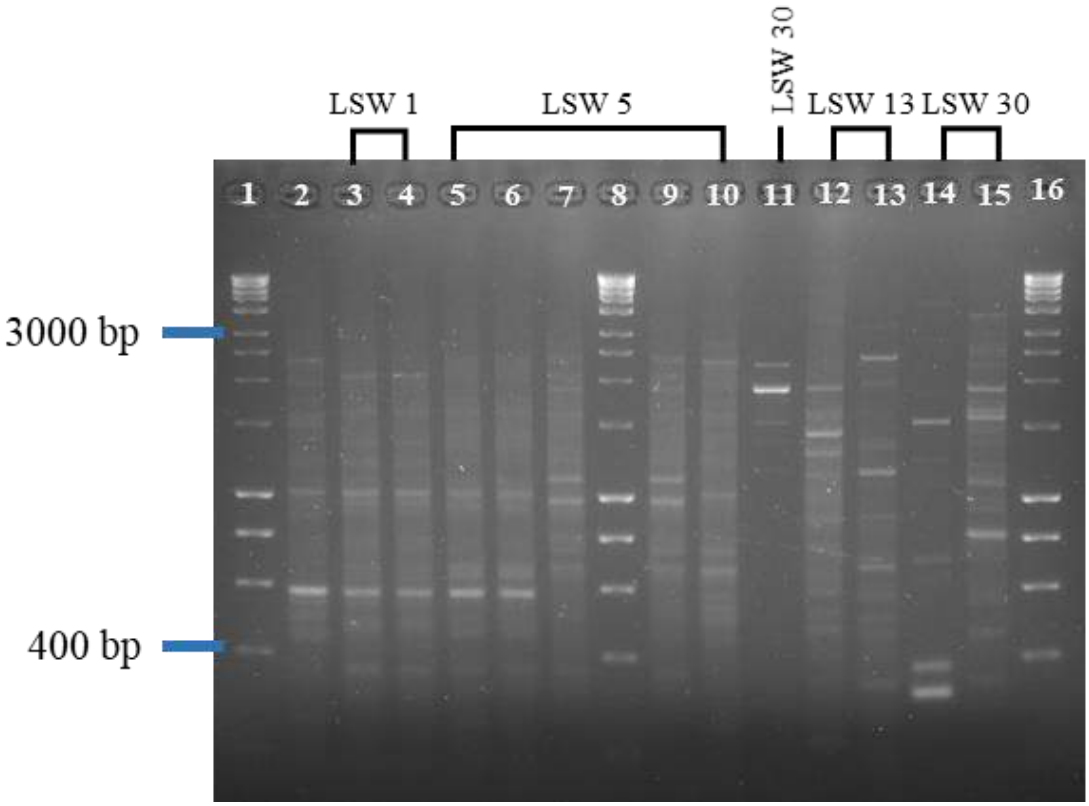



***Figure S.9. REP-PCR of presumptive ESBL sensitive E. coli (1).*** *Lane 1, 8 and 16: Ladder 1 kb. Lane 2: E. coli ATCC® 47055 strain for E. coli reference. Lane 3-7: BAD 169, BAD 170, BAD 194, BAD 195, BAD 196. Lane 9-15: BAD 197, BAD 198, BAD 367, BAD 227, BAD 229, BAD 369, BAD 370. Sample reference (LSW – gulls in Seafield WWTW) is shown and indicates the samples from which isolates were selected.*

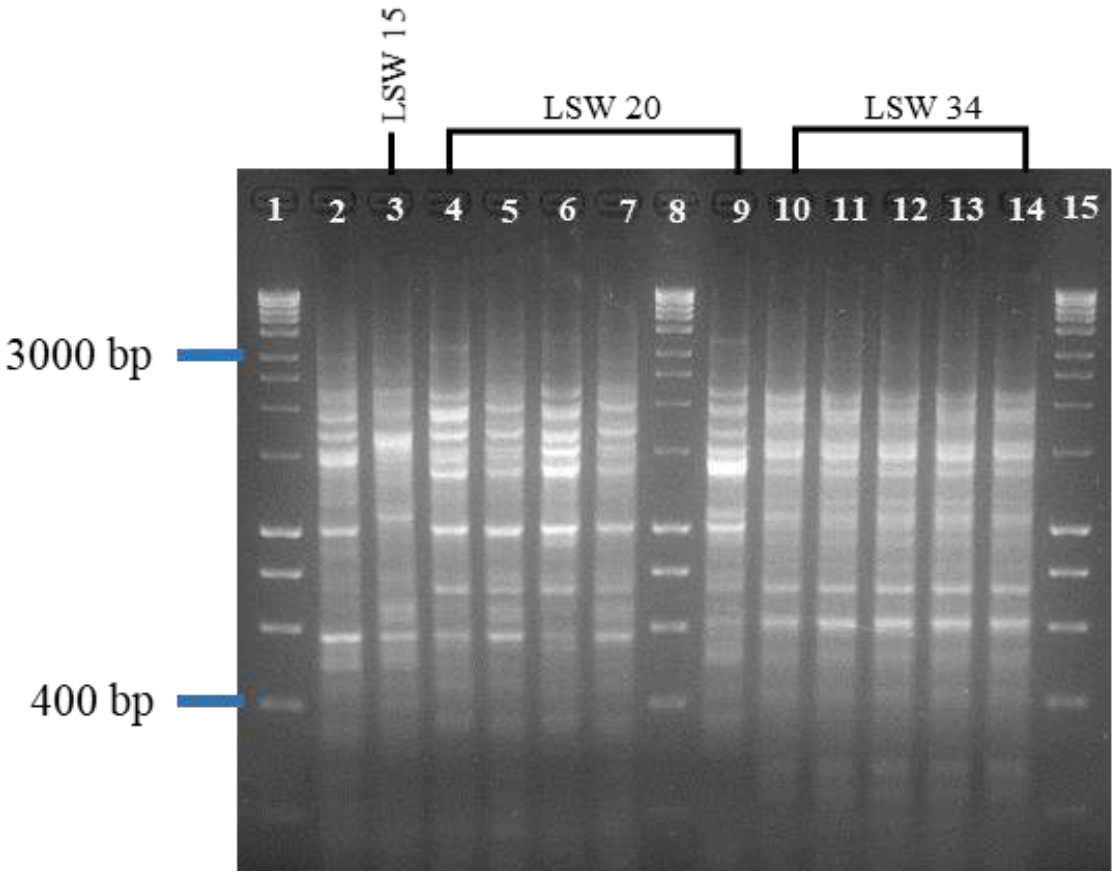



***Figure S.10. REP-PCR of presumptive ESBL sensitive E. coli (2).*** *Lane 1, 8 and 15: Ladder 1 kb. Lane 2: E. coli ATCC® 47055 strain for E. coli reference. Lane 3-7: BAD 231, BAD 237, BAD 238, BAD 239, BAD 240. Ladder 9-14: BAD 241, BAD 376, BAD 377, BAD 378, BAD 379, BAD 380. Sample reference (LSW – gulls in Seafield WWTW) is shown and indicates the samples from which isolates were selected.*

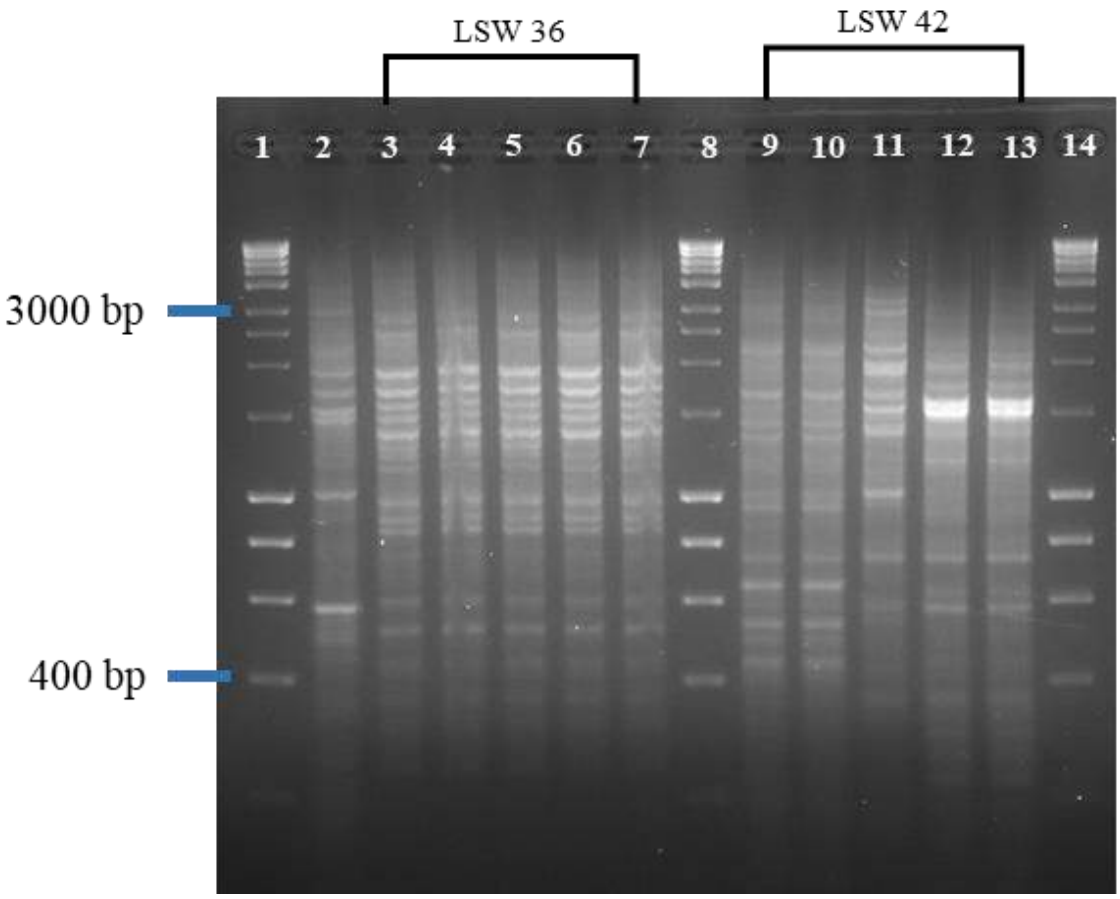



***Figure S.11. REP-PCR of presumptive ESBL sensitive E. coli (3).*** *Lane 1, 8 and 14: Ladder 1 kb. Lane 2: E. coli ATCC® 47055 strain for E. coli reference. Lane 3-7: BAD 386, BAD 387, BAD 388, BAD 389, BAD 390. Lane 9-13: BAD 411, BAD 412, BAD 413, BAD 414, BAD 415. Sample reference (LSW – gulls in Seafield WWTW) is shown and indicates the samples from which isolates were selected.*

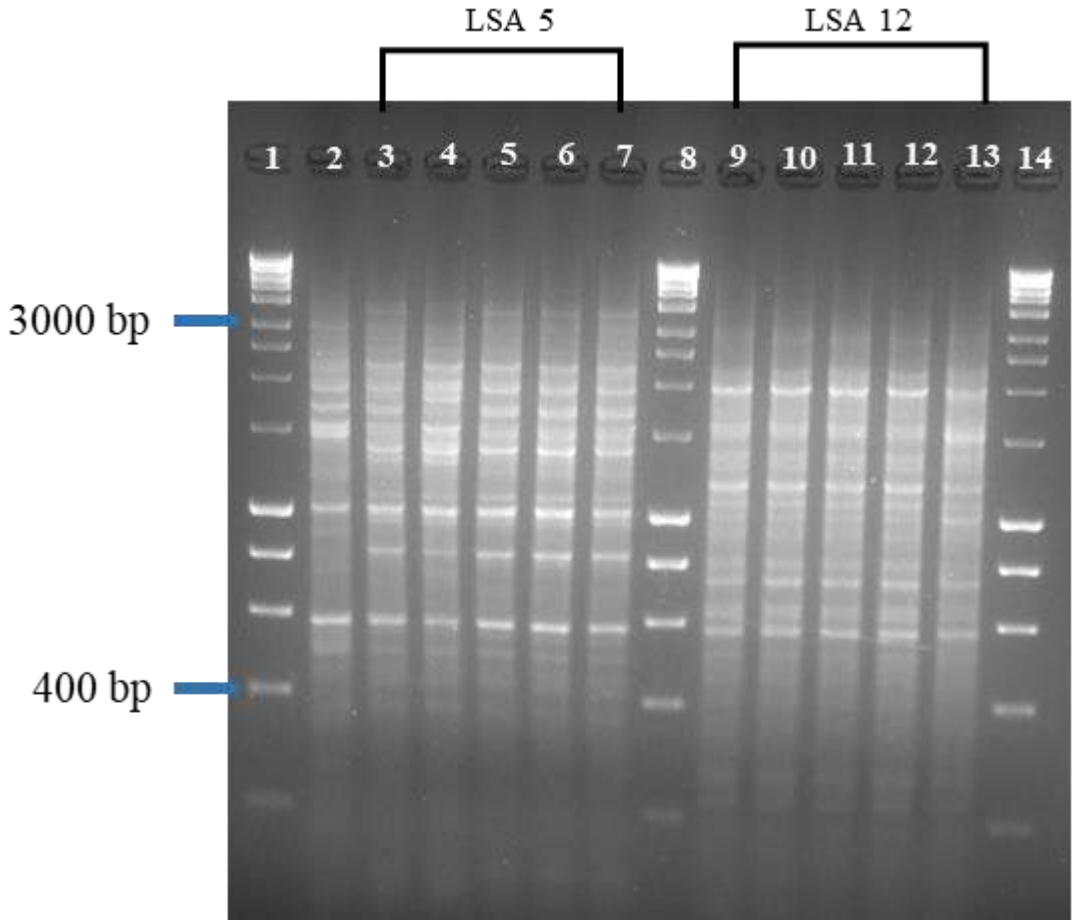



***Figure S.12. REP-PCR of presumptive ESBL sensitive E. coli (4).*** *Lane 1, 8 and 14: Ladder 1 kb. Lane 2: E. coli ATCC® 47055 strain for E. coli reference. Lane 3-7: BAD 574, BAD 575, BAD 576, BAD 577, BAD 578. Lane 9-13: BAD 609, BAD 610, BAD 611, BAD 612, BAD 613. Sample reference (LSA – gulls in St Abbs) is shown and indicates the samples from which isolates were selected.*

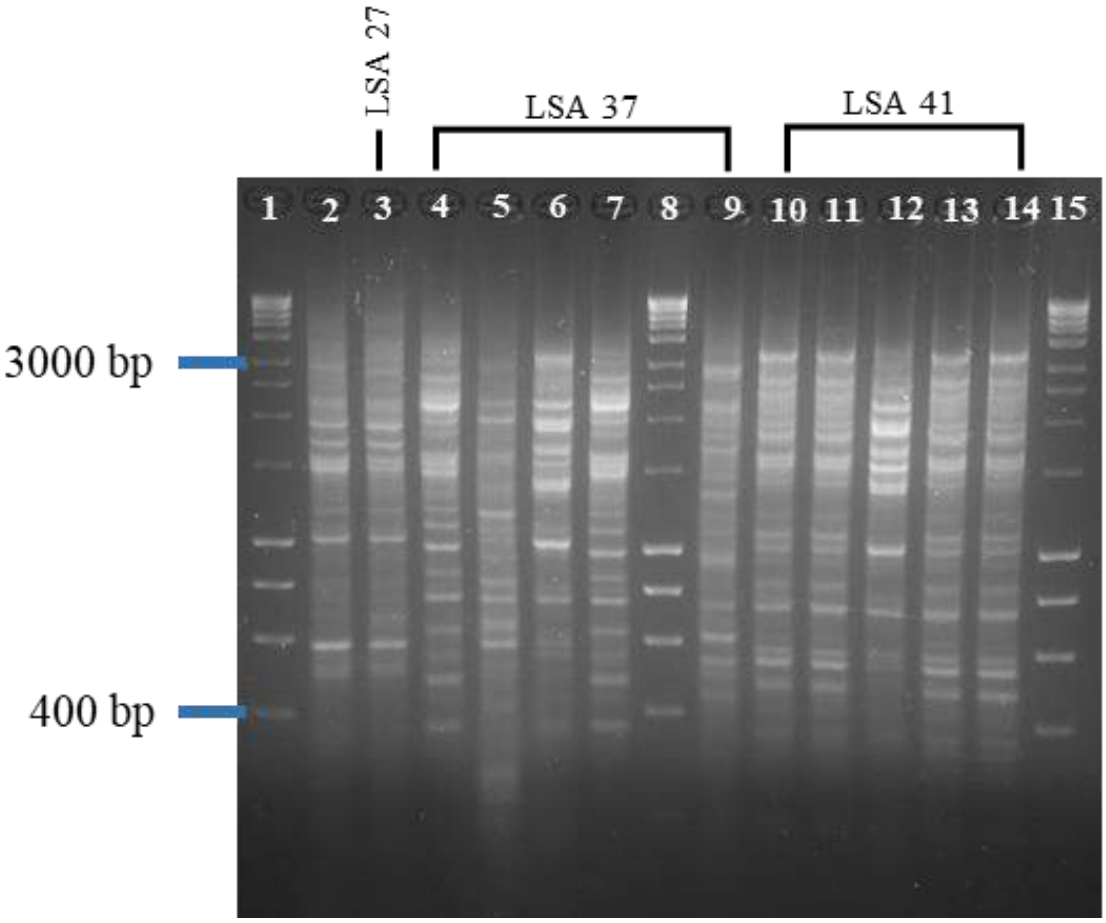



***Figure S.13. REP-PCR of presumptive ESBL sensitive E. coli (5).*** *Lane 1, 8 and 15: Ladder 1 kb. Lane 2: E. coli ATCC® 47055 strain for E. coli reference. Lane 3-7: BAD 777, BAD 819, BAD 820, BAD 821, BAD 822. Lane 9-14: BAD 823, BAD 839, BAD 840, BAD 841, BAD 842, BAD 843. Sample reference (LSA – gulls in St Abbs) is shown and indicates the samples from which isolates were selected.*

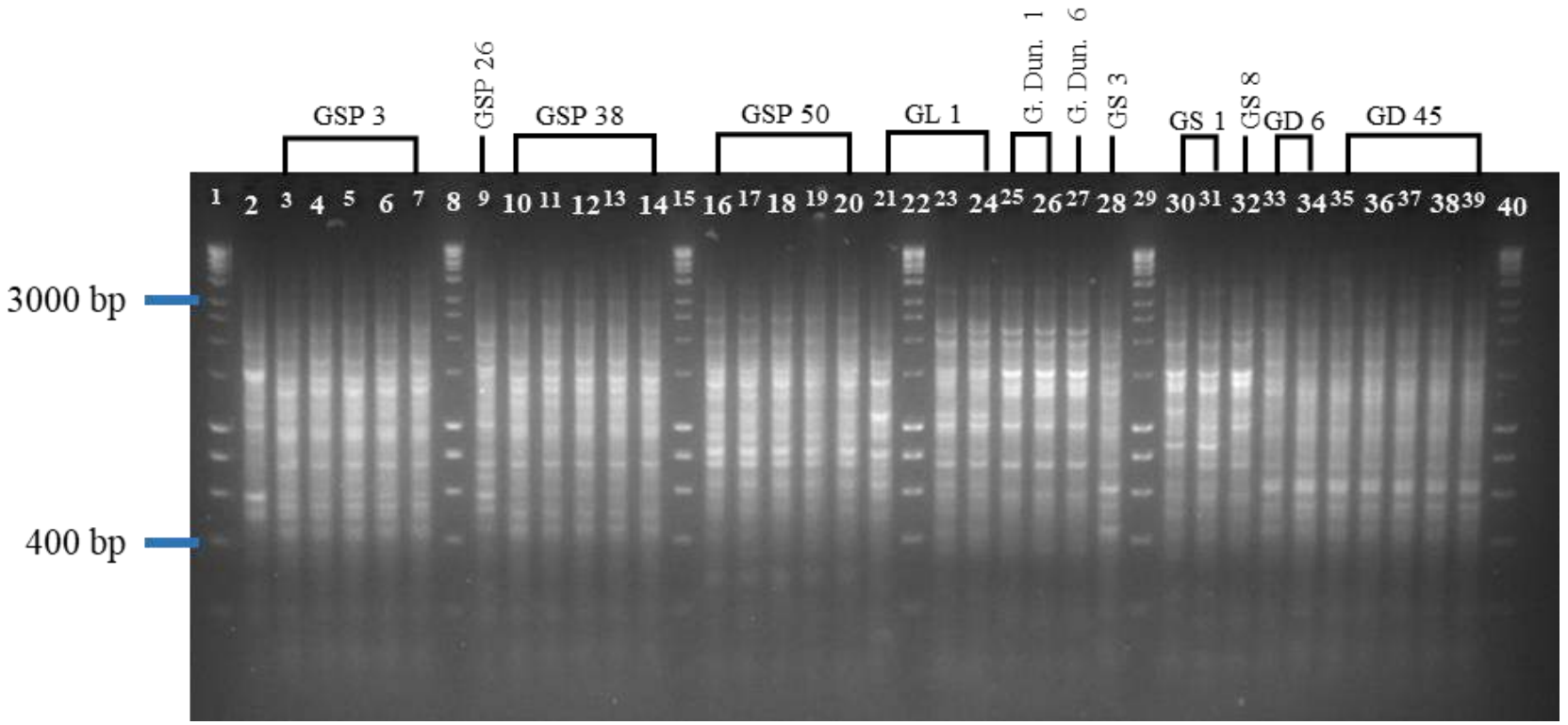



***Figure S.14. REP-PCR of presumptive ESBL sensitive E. coli (6).*** *Lane 1, 8, 15, 22, 29 and 40: Ladder 1 kb. Lane 2: E. coli ATCC® 47055 strain for E. coli reference. Lane 3-7: BAD 161, BAD 162, BAD 163, BAD 164, BAD 165. Lane 9-14: BAD 177, BAD 178, BAD 179, BAD 180, BAD 181, BAD 182. Lane 16-21: BAD 184, BAD 185, BAD 186, BAD 187, BAD 188, BAD 158. Lane 23-28: BAD 159, BAD 160, BAD 449, BAD 450, BAD 551, BAD 156. Lane 30-39: BAD 153, BAD 154, BAD 553, BAD 150, BAD 151, BAD 444, BAD 445, BAD 446, BAD 447, BAD 448. Sample reference (GD – geese in Duddingston Loch, GS – geese in St Margaret's Loch, G. Dun. – geese in Dunsapie Loch, GL – geese in Lochend Loch, GSP – geese in Slamannan Plateau) is shown and indicates the samples from which isolates were selected.*

5.2.2 Dendrogram of All DNA Fingerprints showing REP Types (REPS) Identified

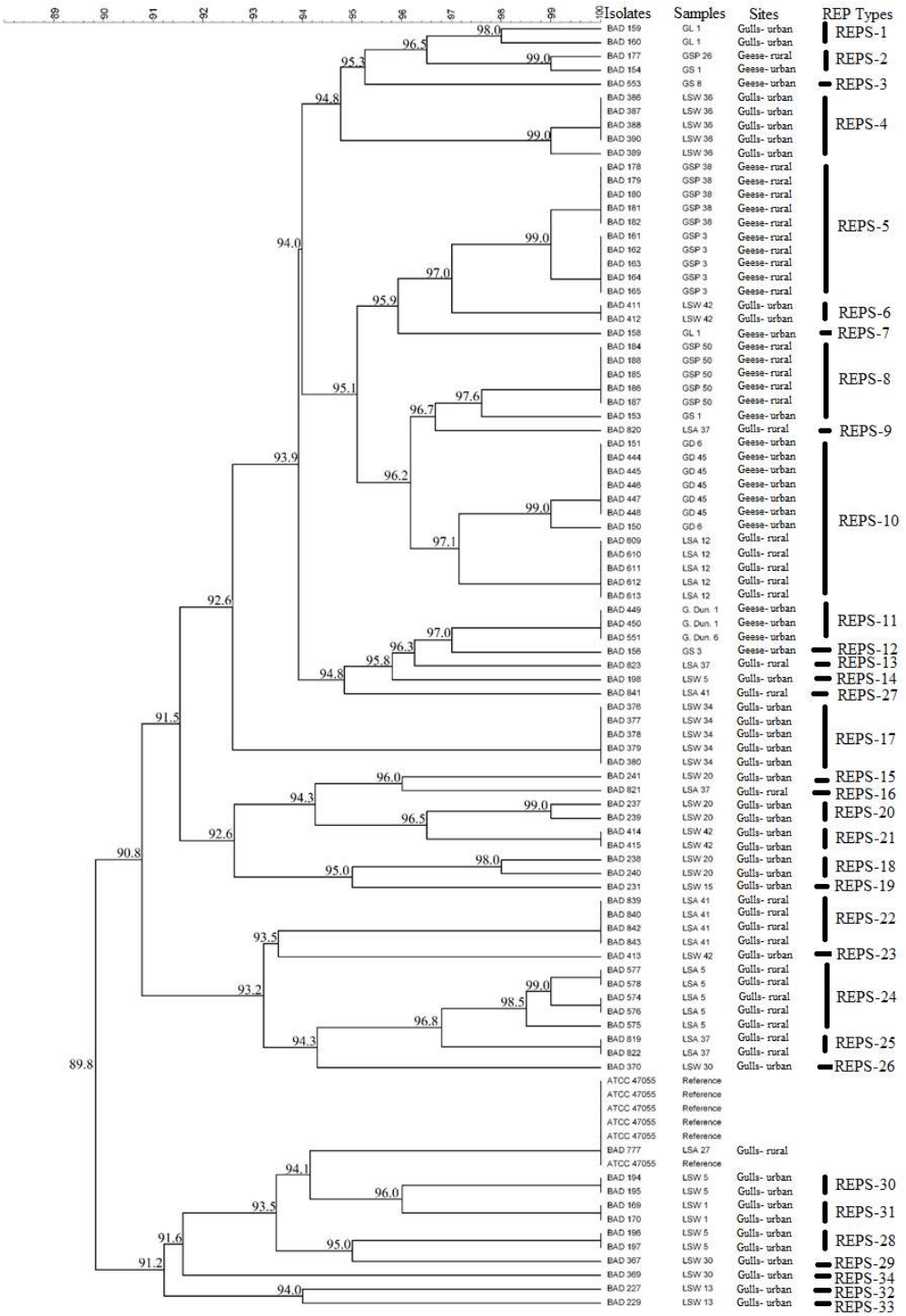



***Figure S.15. The dendrogram of REP-PCR assay of presumptive ESBL sensitive E. coli generated by BioNumerics.*** *Cluster analysis was done using the number of band difference coefficient and UPGMA method. Numbers at the nodes show the equated similarity (%) of the band difference in Fig.6 (section 4.5.2). The samples (isolate numbers and sites) and REP types (REPR) are shown in the dendrogram. The axis line at the top (89-100) indicates the similarity (%). REP types have been assigned based on > 97% similarity (equated to <3 bands difference rule).*

5.3 Presumptive ESBL-Producer Non-*E. coli* Coliforms

5.3.1 Gels of REP-PCR DNA Fingerprinting

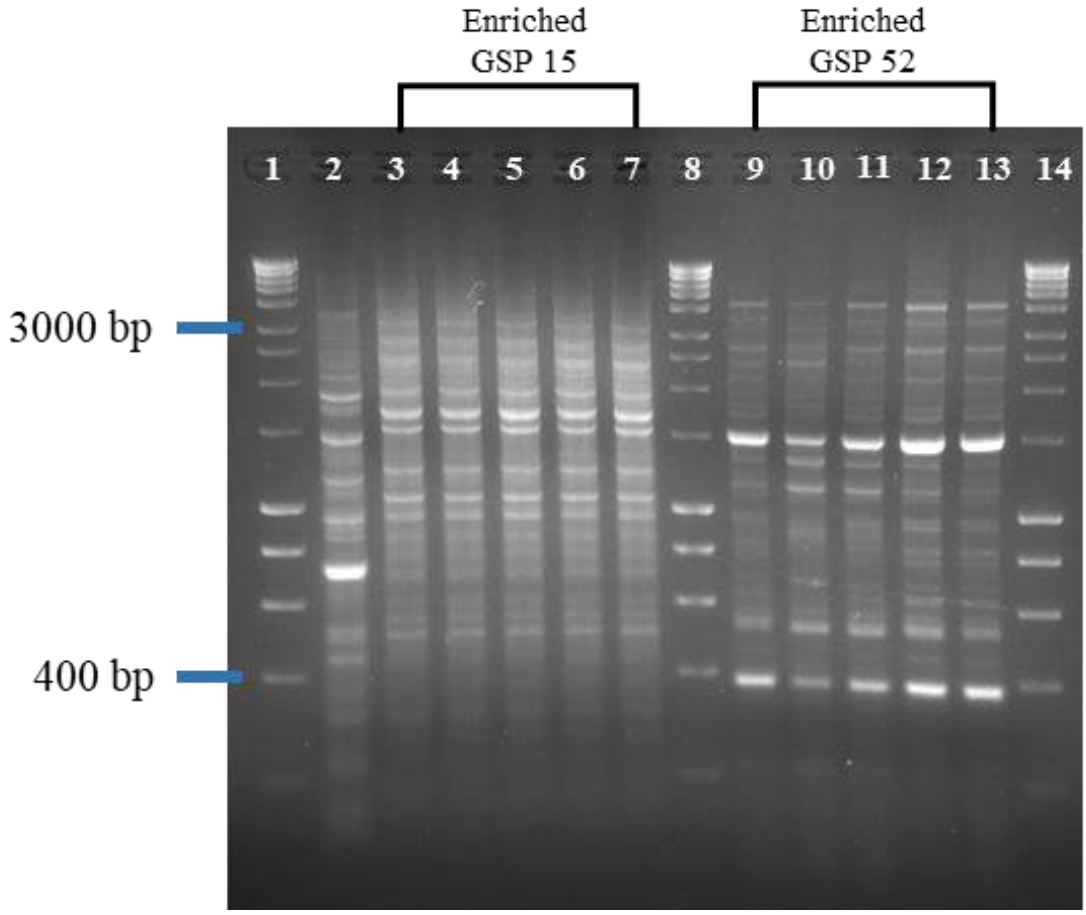



***Figure S.16. REP-PCR of presumptive ESBL-producer non-E. coli coliforms (1).*** *Lane 1, 8 and 14: Ladder 1 kb. Lane 2: K. pneumoniae NCIMB 8805 strain for non-E. coli coliforms reference. Lane 3-7: BAD 278, BAD 279, BAD 280, BAD 281, BAD 282. Lane 9-13: BAD 313, BAD 314, BAD 315, BAD 316, BAD 317. Sample reference (GSP – geese in Slamannan Plateau) is shown and indicates the samples from which isolates were selected.*

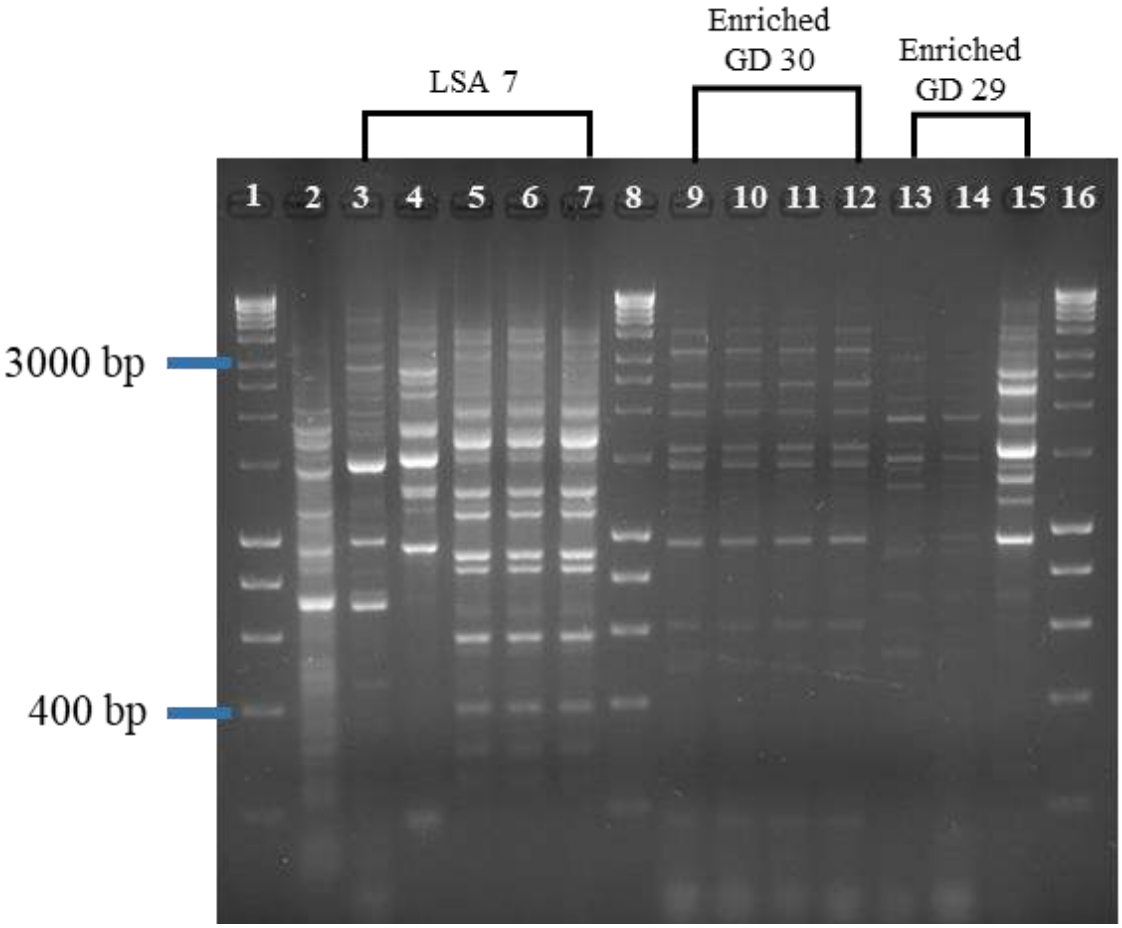



***Figure S.17. REP-PCR of presumptive ESBL-producer non-E. coli coliforms (2).*** *Lane 1, 8 and 16: Ladder 1 kb. Lane 2: K. pneumoniae NCIMB 8805 strain for non-E. coli coliforms reference. Lane 3-7: BAD 456, BAD 457, BAD 458, BAD 459, BAD 460. Lane 9-15: BAD 463, BAD 464, BAD 465, BAD 466, BAD 468, BAD 469, BAD 470. Sample reference (GD – geese in Duddingston Loch, LSA – gulls in St Abbs) is shown and indicates the samples from which isolates were selected.*

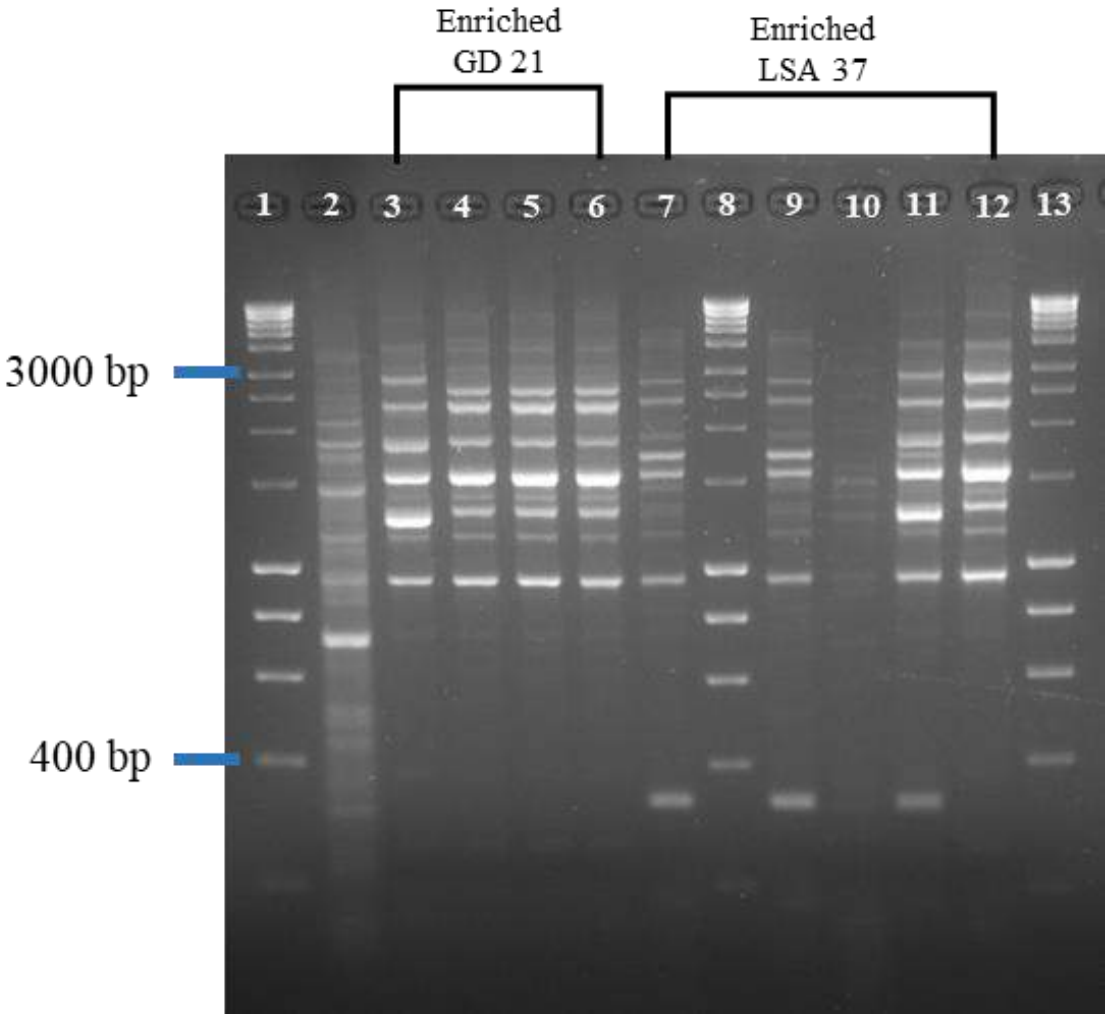



***Figure S.18. REP-PCR of presumptive ESBL-producer non-E. coli coliforms (3).*** *Lane 1, 8 and 13: Ladder 1 kb. Lane 2: K. pneumoniae NCIMB 8805 strain for non-E. coli coliforms reference. Lane 3-7: BAD 472, BAD 473, BAD 474, BAD 475, BAD 477. Lane 9-12: BAD 478, BAD 479, BAD 480, BAD 481. Sample reference (GD – geese in Duddingston Loch, LSA – gulls in St Abbs) is shown and indicates the samples from which isolates were selected.*

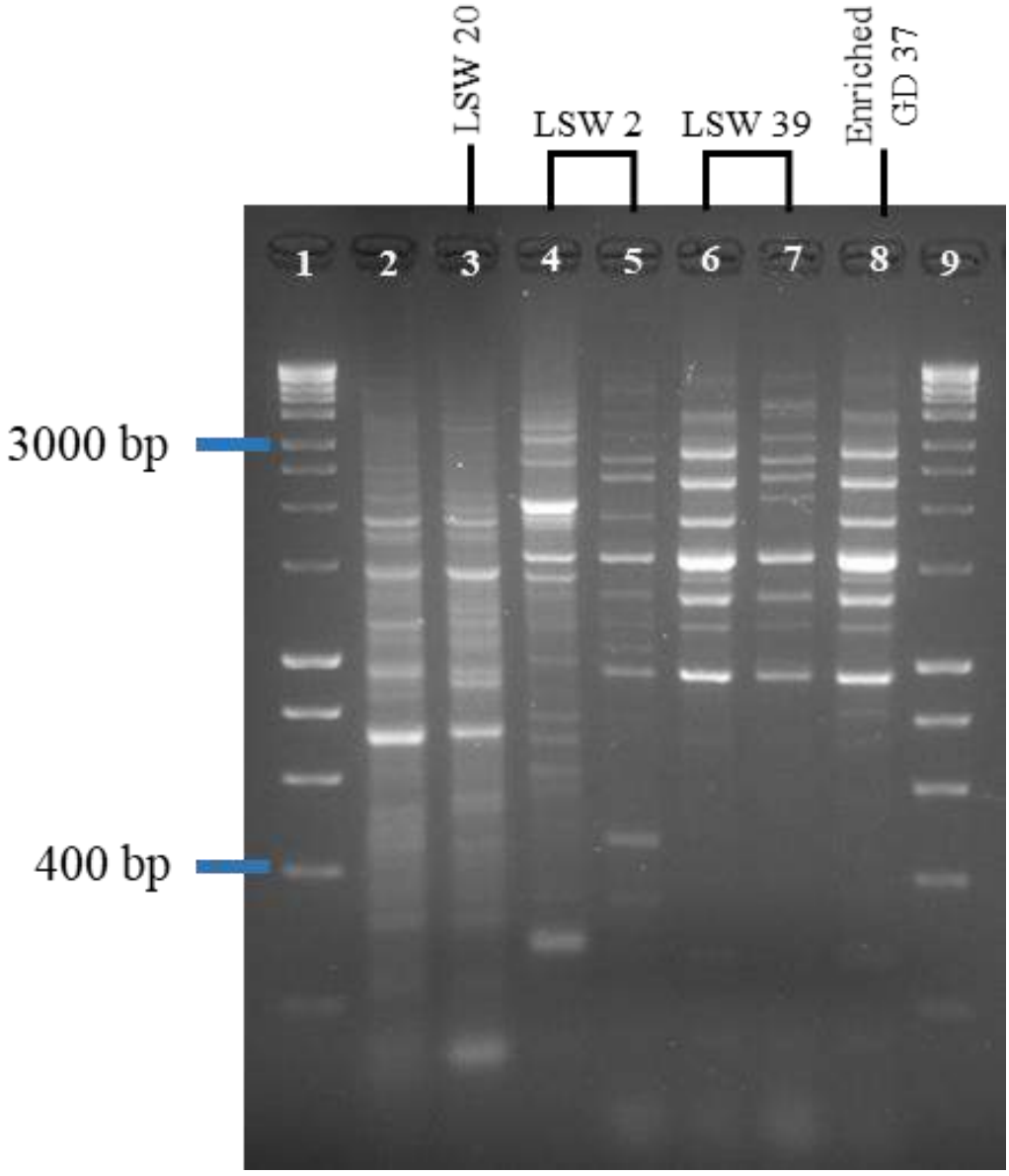



***Figure S.19. REP-PCR of presumptive ESBL-producer non-E. coli coliforms (4).*** *Lane 1 and 9: Ladder 1 kb. Lane 2: K. pneumoniae NCIMB 8805 strain for non-E. coli coliforms reference. Lane 3-8: BAD 106, BAD 134, BAD 135, BAD 252, BAD 253, BAD 462. Sample reference (GD – geese in Duddingston Loch, LSW – gulls in Seafield WWTW) is shown and indicates the samples from which isolates were selected.*

5.3.2 Dendrogram of All DNA Fingerprints showing REP Types (REPNE) Identified

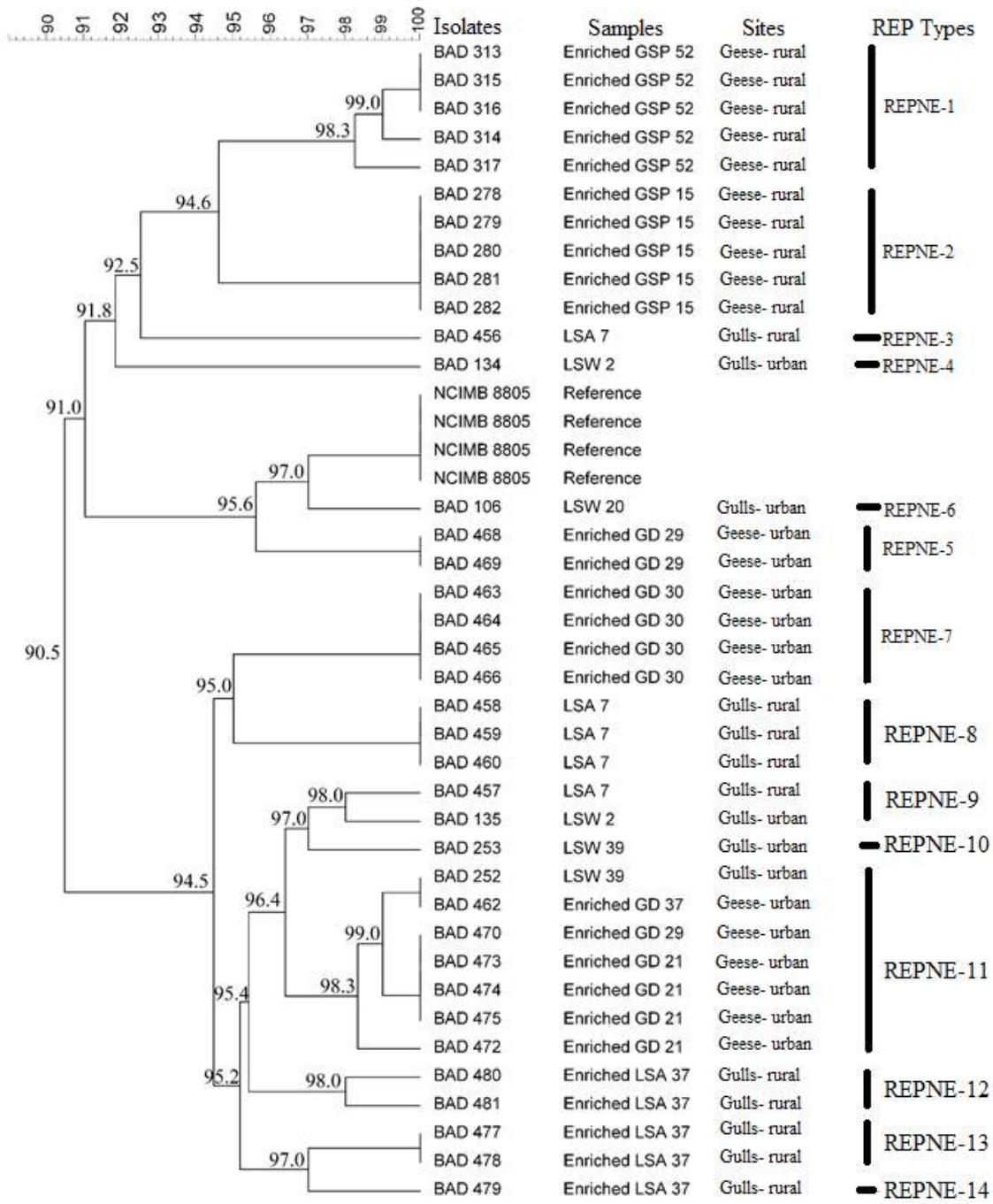



***Figure S.20. The dendrogram of REP-PCR assay of presumptive ESBL-producer non-E. coli coliforms generated by BioNumerics.*** *Cluster analysis was done using the number of band difference coefficient and UPGMA method. Numbers at the nodes show the equated similarity (%) of the band difference in Fig.7 (section 4.5.3). The samples (isolate numbers and sites) and REP types (REPNE) are shown in the dendrogram. The axis line at the top (90-100) indicates the similarity (%). REP types have been assigned based on > 97% similarity (equated to <3 bands difference rule).*

# Appendix 6. Species Identification of Presumptive ESBL-Producer Non-*E. coli* Coliforms

***Table S.23. Result of species identification of presumptive non-E. coli isolates [red colony on Coliform ChromoSelect agar (CCA)] with E. coli-like appearance (green metallic sheen) on Eosin Methylene Blue agar (EMB).*** *Results were obtained from Gram staining, species identification PCR assays and 16S rRNA sequencing. SCVs are coloured in blue. SCVs that reverted to a normal colony size upon subsequent subcultures (regarded as nonstable SCVs) are coloured in red.*

| Isolate reference numbers | Samples | Selected Colonies on CCA | | Appearance of colonies on EMB | | REP types | Species identification |
|---|---|---|---|---|---|---|---|
| | | Colour | Morphology | Colour | Morphology | | |
| BAD-456 | LSA 7 | Red | Round, flat | Metallic green | Round, flat | REPNE-3 | Non-*E. coli Escherichia* sp. |
| BAD-134 | LSW 2 | Red | Round, flat | Metallic green | Round, flat | REPNE-4 | Gram-positive |
| BAD-468 | Enriched GD 29 | Red | Irregular, flat | Metallic green | Round, flat | REPNE-5 | Non-*E. coli Escherichia sp.* |
| BAD-469 | Enriched GD 29 | Red | Irregular, flat | Metallic green | Round, flat | | |
| BAD-463 | Enriched GD 30 | Red | Round, flat | Metallic green | Round, flat | REPNE-7 | Non-*E. coli Escherichia sp.* |
| BAD-464 | Enriched GD 30 | Red | Round, flat | Metallic green | Round, flat | | |
| BAD-465 | Enriched GD 30 | Red | Round, flat | Metallic green | Round, flat | | |
| BAD-466 | Enriched GD 30 | Red | Round, flat | Metallic green | Round, flat | | |
| BAD-458 | LSA 7 | Red | Round, flat | Metallic green | Round, flat | REPNE-8 | Non-*E. coli Escherichia sp.* |
| BAD-459 | LSA 7 | Red | Round, flat | Metallic green | Round, flat | | |
| BAD-460 | LSA 7 | Red | Round, flat | Metallic green | Round, flat | | |
| BAD-135 | LSW 2 | Red | Round, flat | Metallic green | Round, flat | REPNE-9 | Non-*E. coli Escherichia sp.* |
| BAD-457 | LSA 7 | Red | Round, flat | Metallic green | Round, flat | | |
| BAD-253 | LSW 39 | Red | Round, flat | Metallic green | Round, flat | REPNE-10 | Non-*E. coli Escherichia* sp. |
| BAD-252 | LSW 39 | Red | Round, flat | Metallic green | Round, flat | REPNE-11 | Non-*E. coli Escherichia sp.* |
| BAD-462 | Enriched GD 37 | Red | Round, flat | Metallic green | Round, flat | | |
| BAD-470 | Enriched GD 29 | Red | Irregular, flat | Metallic green | Round, flat | | |
| BAD-472 | Enriched GD 21 | Red | Round, flat | Metallic green | Round, flat | | |
| BAD-473 | Enriched GD 21 | Red | Round, flat | Metallic green | Round, flat | | |
| BAD-474 | Enriched GD 21 | Red | Round, flat | Metallic green | Round, flat | | |
| BAD-475 | Enriched GD 21 | Red | Round, flat | Metallic green | Round, flat | | |
| BAD-480 | Enriched LSA 37 | Red | Round, flat | Metallic green | Round, flat | REPNE-12 | Non-*E. coli Escherichia sp.* |
| BAD-481 | Enriched LSA 37 | Red | Round, flat | Metallic green | Round, flat | | |
| BAD-477 | Enriched LSA 37 | Red | Round, flat | Metallic green | Round, flat | REPNE-13 | *Enterococcus* sp. |
| BAD-478 | Enriched LSA 37 | Red | Round, flat | Metallic green | Round, flat | | |
| BAD-479 | Enriched LSA 37 | Red | Round, flat | Metallic green | Round, flat | REPNE-14 | Gram-negative |

# Appendix 7. 16S rRNA Chromatography

## 7.1 REPR-2 Isolate

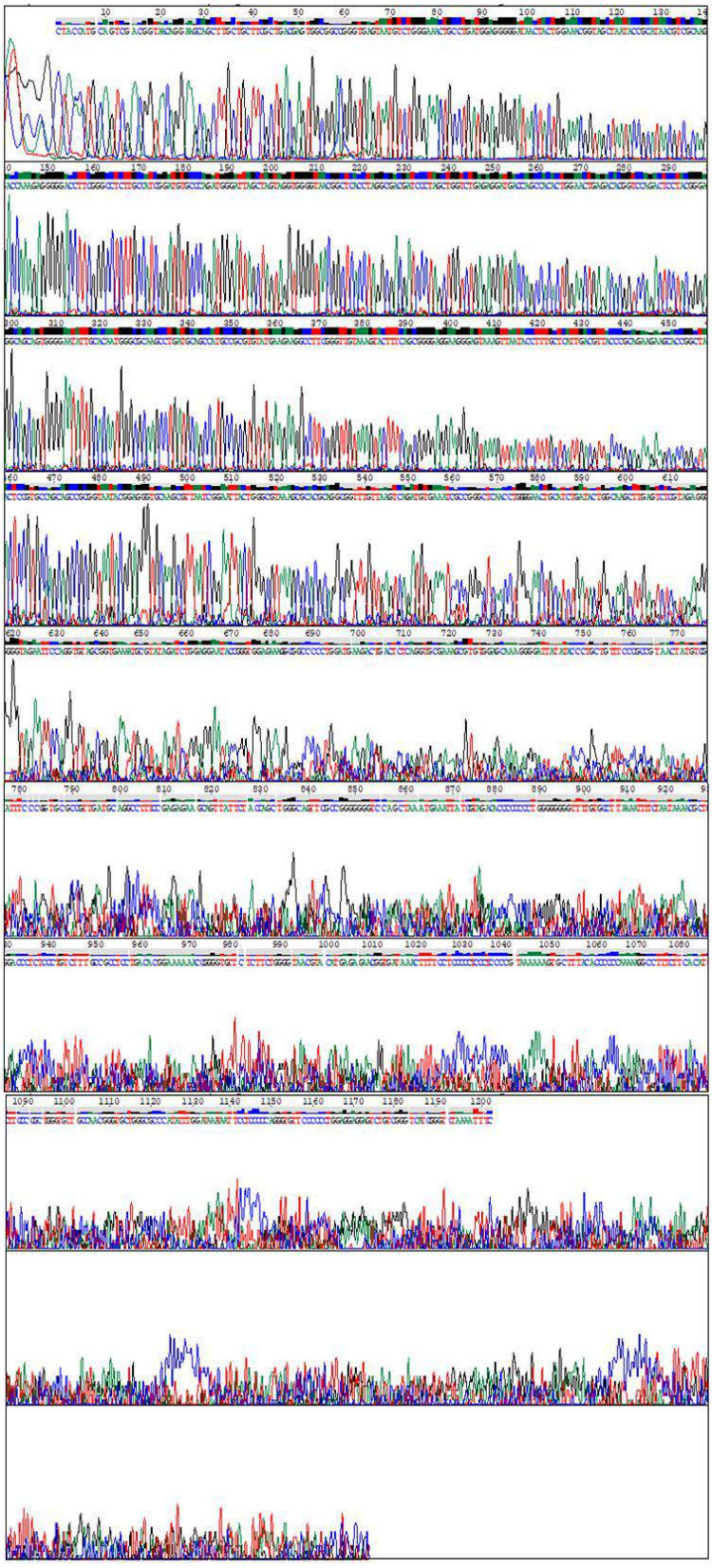


***Figure S.21. Chromatogram of forward sequence of REPR-2 isolate.*** *Low-quality bases at both ends of the sequence were trimmed using Chromas software with default setting.*

CTACCATGCAGTCGACGGTAACAGGAAGCAGCTTGCTGCTTCGCTGACGAGTGGCG
GCCGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGAAA
CGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGAGGGGGACCTTCGGGCCTCT
TGCCATCGGATGTGCCCAGATGGGATTAGCTAGTAGGTGGGGTAACGGCTCACCTA
GGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGACA
CGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAG
CCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTC
AGCGGGGAGGAAGGGAGTAAAGTTAATACCTTTGCTCATTGACGTTACCCGCAGAA
GAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGT
TAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTTTGTTAAGTCAGATGTGA
AATCGCCGGGCTCAACCTGGGGAACTGCATCTGATACTGGCAAGCTTGAGTCTCGT
AGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAAATGCGTATAGATCTGGAGGAA
TACCGGGTGGAGAAAGGCGGCCCCCTGGATGAAGACTGACTCTCAGGTGCGAAAG
CGTGTGGAGCAAAGGGGATTATATACCCTGCTGTTTCCCGCCGTAACTATGTCGATT
TCCCGGTGCGCCGTTGATGCAGGCCTTTCCGAGAGAAGCAGTTATTCTACCAGCTG
GGCAGTTCGCCGGGGGGGTCCAGCTAAATGAAATTATCGTAGACACCCCCCCCTGG
GGGGGGTTTTGTGCCTTAAAATTTTCTAATAAAACGCTGGACCCTCTCCCTGTCTTT
GCCGCCTCCTGACACGGAAAAAACCGGGGTGTTCTCTTCTGGGGTAACGTACATGA
GAGAACGGTGATAAACTTTTCCTCCCCCTCCCTCCCCGTAAAAAAGTGCTTTACACC
CCCCAAAAAGGCCTTTCTTCACATCTTCCCCGCTGGGTGTTGCCAACGGGTGCTGGG
CGCCCATATTTGGATAAATAATTCCTCCCCCAGGGTGTTCCCCCCTGGAGGAGGAG
TCTGTCGGGTCATCGGGTCTAAAATTTC

***Figure S.22. Forward sequence of REPR-2 isolate.*** *The FASTA sequence were exported from the chromatogram of forward sequence of REPR-2 isolate after the trim of low-quality bases at both ends.*

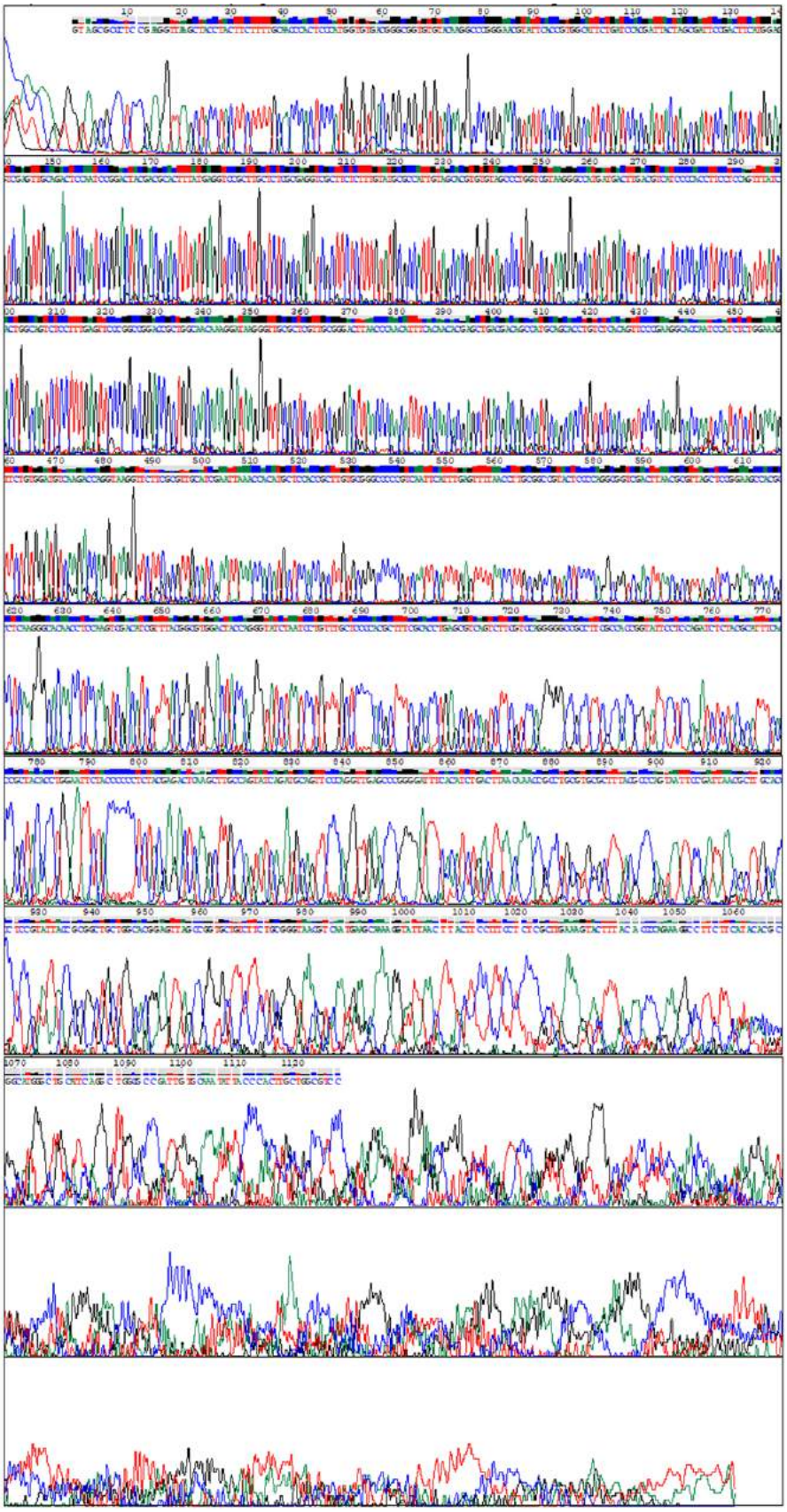


***Figure S.23. Chromatogram of reverse sequence of REPR-2 isolate.*** *Low-quality bases at both ends of the sequence were trimmed using Chromas software with default setting.*

GGACGCCAGCAAGTGGGTAATATTTGCACAATCGGCGCCAGCCTGAATGCAGCCCA
TGCCGCGTGTATGAAGAAGGCCTTTCTGGGGTGTAAAGTACTTTCAAGCGAGAGGA
AAGGAAGTAAGTTAATACCTTTTGCTTCATTGACGTTACCCGCAGAAGACAGCACC
GGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGA
ATTACTGGGCGTAAAGCGCACGCAGGCGGTTTGTTAAGTCAGATGTGAAATCCCCG
GGCTCAACCTGGGAACTGCATCTGATACTGGCAAGCTTGAGTCTCGTAGAGGGGGG
TAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGC
GAAGGCGGCCCCCTGGACGAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAA
ACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGAGGTTGT
GCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTAC
GGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGC
ATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGTCTTGACATCCACAGA
ACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTGTGAGACAGGTGCTGCATGGCT
GTCGTCAGCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTT
ATCCTTTGTTGCCAGCGGTCCGGCCGGGAACTCAAAGGAGACTGCCAGTGATAAAC
TGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGACCAGGGCTACAC
ACGTGCTACAATGGCGCATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCT
CATAAAGTGCGTCGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGA
ATCGCTAGTAATCGTGGATCAGAATGCCACGGTGAATACGTTCCCGGGCCTTGTAC
ACACCGCCCGTCACACCATGGGAGTGGGTTGCAAAAGAAGTAGGTAGCTTAACCTT
CGGAGGGCGCTAC

***Figure S.24. Reverse sequence of REPR-2 isolate.*** *The FASTA sequence were exported from the chromatogram of reverse sequence of REPR-2 isolate after the trim of low-quality bases at both ends.*

7.2 REPR-3 Isolate

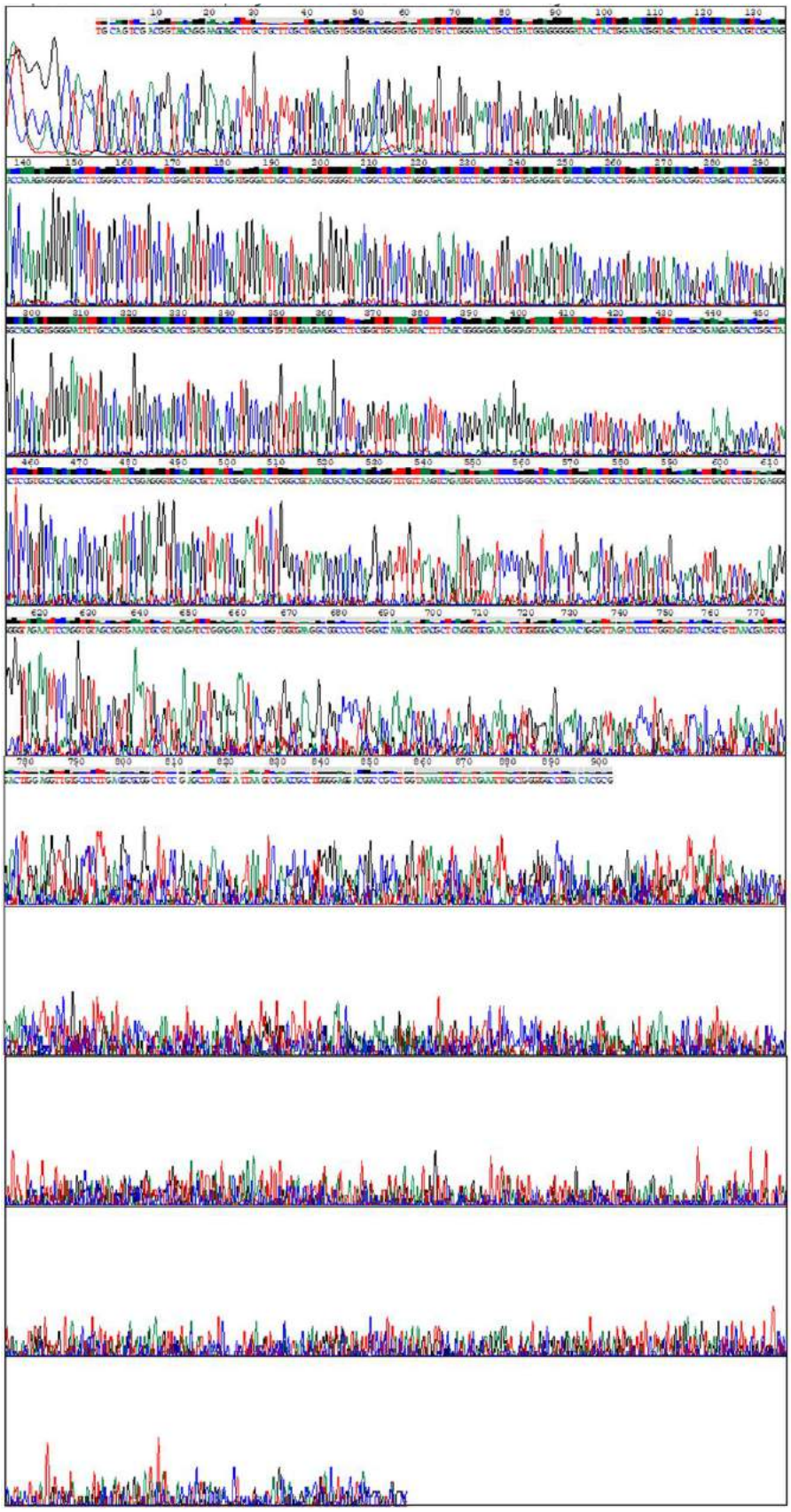


***Figure S.25. Chromatogram of forward sequence of REPR-3 isolate.*** *Low-quality bases at both ends of the sequence were trimmed using Chromas software with default setting.*

TGCAGTCGACGGTAACAGGAAGCAAGCTTGCTGCTTCGCTGACGAGTGGCGGCACG
GGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGAAACGGT
AGCTAATACCGCATAACGTCGCAAGACCAAAGAGGGGGACCTTCGGGCCTCTTGCC
ATCGGATGTGCCCAGATGGGATTAGCTAGTAGGTGGGGTAACGGCTCACCTAGGCG
ACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGACACGGTC
CAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGA
TGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGG
GGAGGAAGGGAGTAAAGTTAATACCTTTGCTCATTGACGTTACCCGCAGAAGAAGC
ACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCG
GAATTACTGGGCGTAAAGCGCACGCAGGCGGTTTGTTAAGTCAGATGTGAAATCCC
CGGGCTCAACCTGGGAACTGCATCTGATACTGGCAAGCTTGAGTCTCGTAGAGGGG
GGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTG
GTGAAGGCGGCCCCCTGGACCAAAAACTGACGCTCAGGTTGCGAAATCGTTGTGGG
AGCAAACAGGATTAGATACCCCTGGTAGTCCCACGCCGTTAAACGATGTCGACTTG
GAGGTTGTGCCTCTTGACGCGCGGCTTCCGAGCTTACCGTATTAAGTCGAACCGCCT
TGGGGAGGACGGCCGCCTGGTAAAAATCTCATATGAAATTTAGCTGGGTGGCCTCG
CACACGCG

***Figure S.26. Forward sequence of REPR-3 isolate.*** *The FASTA sequence were exported from the chromatogram of forward sequence of REPR-3 isolate after the trim of low-quality bases at both ends.*

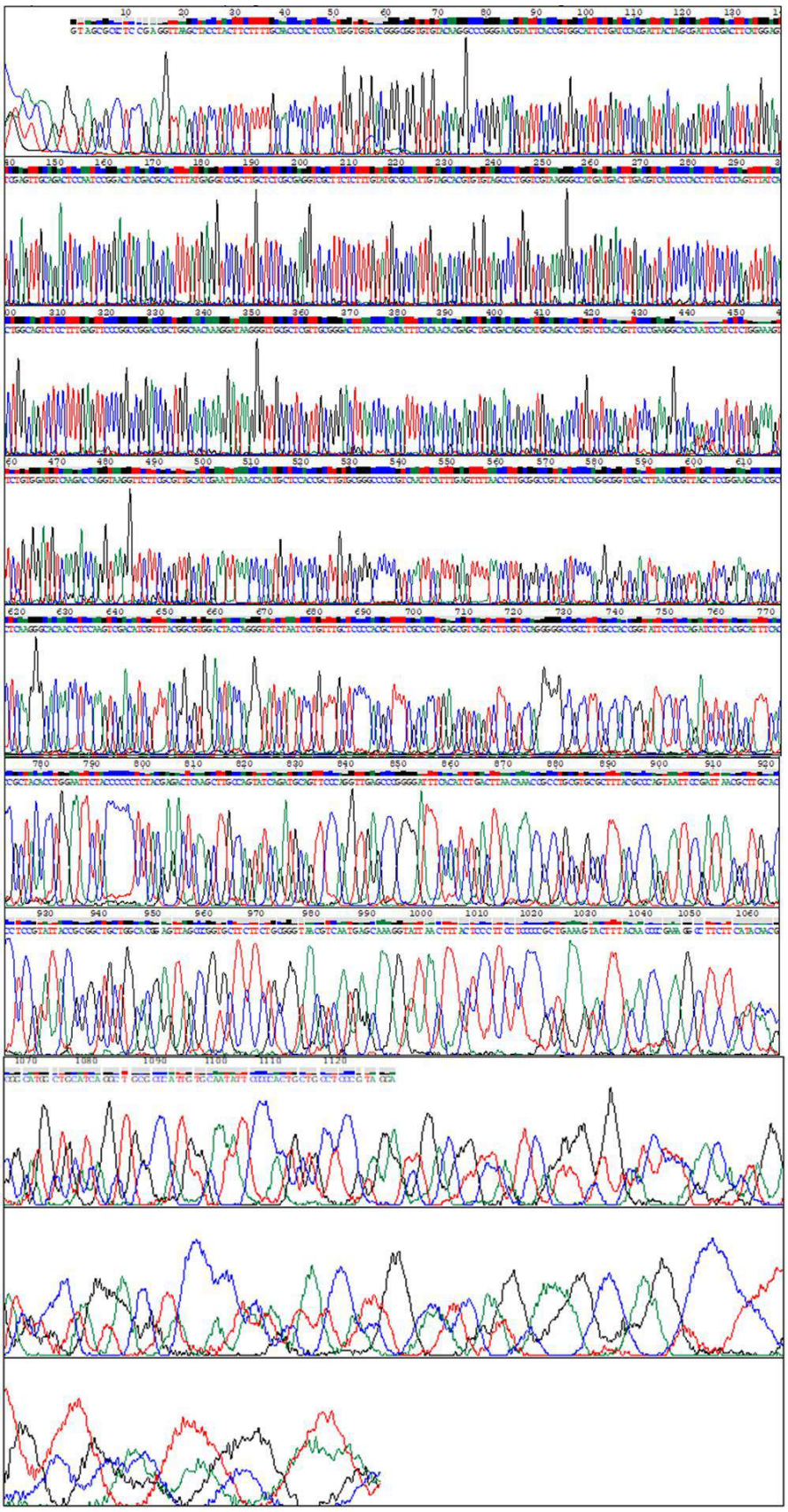


***Figure S.25. Chromatogram of reverse sequence of REPR-3 isolate.*** *Low-quality bases at both ends of the sequence were trimmed using Chromas software with default setting.*

TCCTACGGGAGGCAGCAGTGGGGGAATATTGCACAATGGGCGCAGCCTGATGCAG
CCATGCCGCGTTGTATGAAGAAGGCCTTTCGGGGTTGTAAAGTACTTTCAGCGGGG
GAGGAAGGGAGTAAAGTTAATACCTTTGCTCATTGACGTTACCCGCAGAAGAAGCA
CCGGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCG
GAATTACTGGGCGTAAAGCGCACGCAGGCGGTTTGTTAAGTCAGATGTGAAATCCC
CGGGCTCAACCTGGGAACTGCATCTGATACTGGCAAGCTTGAGTCTCGTAGAGGGG
GGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTG
GCGAAGGCGGCCCCCTGGACGAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGC
AAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGAGGTT
GTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGT
ACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGA
GCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGTCTTGACATCCACA
GAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTGTGAGACAGGTGCTGCATGG
CTGTCGTCAGCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCC
TTATCCTTTGTTGCCAGCGGTCCGGCCGGGAACTCAAAGGAGACTGCCAGTGATAA
ACTGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGACCAGGGCTAC
ACACGTGCTACAATGGCGCATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGAC
CTCATAAAGTGCGTCGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCG
GAATCGCTAGTAATCGTGGATCAGAATGCCACGGTGAATACGTTCCCGGGCCTTGT
ACACACCGCCCGTCACACCATGGGAGTGGGTTGCAAAAGAAGTAGGTAGCTTAACC
TCGGAGGGCGCTAC

***Figure S.26. Reverse sequence of REPR-3 isolate.*** *The FASTA sequence were exported from the chromatogram of reverse sequence of REPR-3 isolate after the trim of low-quality bases at both ends.*

7.3 REPR-6 Isolate

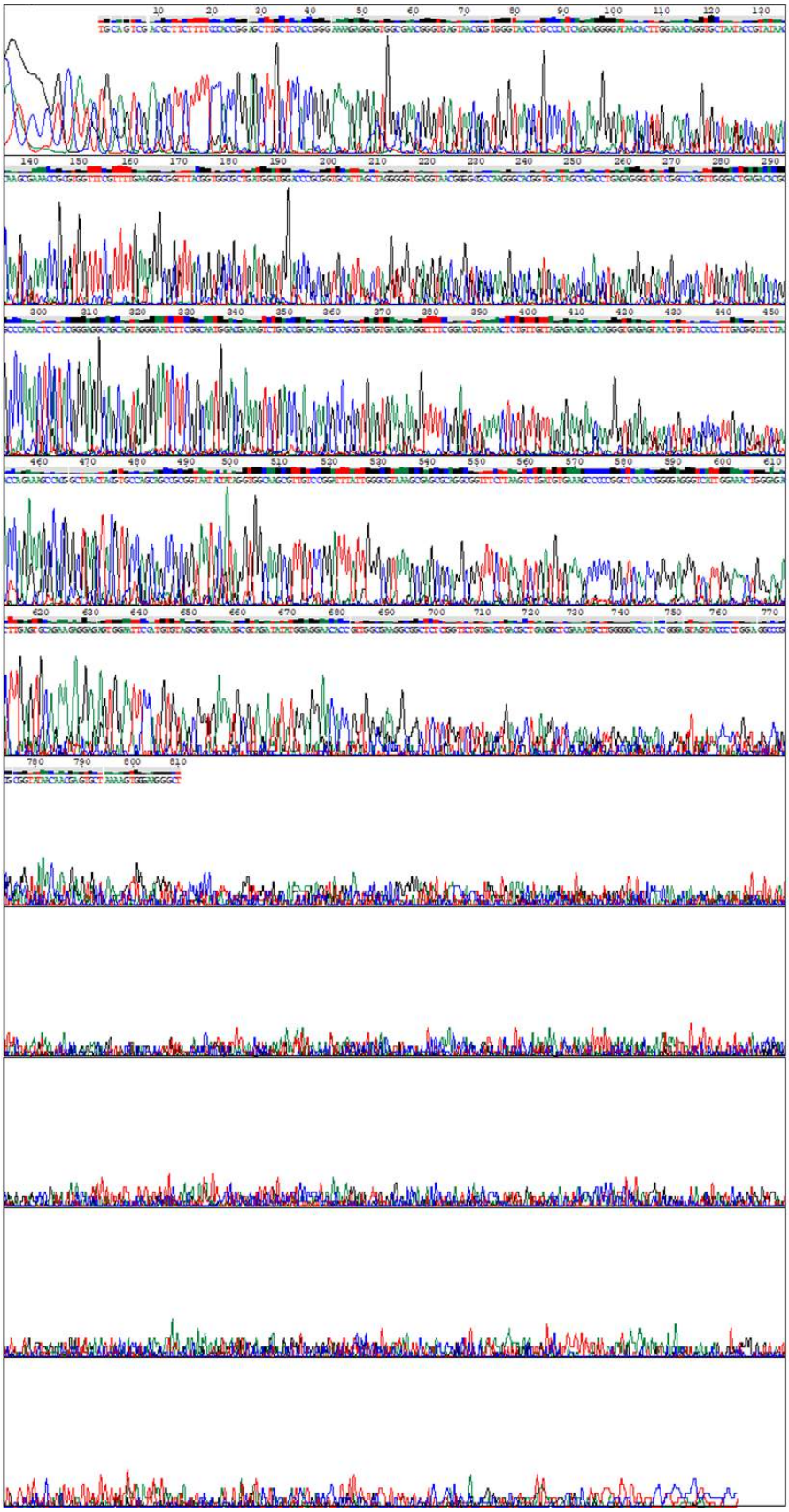


***Figure S.27. Chromatogram of forward sequence of REPR-6 isolate***. *Low-quality bases at both ends of the sequence were trimmed using Chromas software with default setting.*

TGCAGTCGACGCTTCTTTTCCCACCGGAGCTTGCTCCACCGGGAAAAGAGGAGTGG
CGAACGGGTGAGTAACGCGTGGGTAACCTGCCCATCAGAAGGGGATAACACTTGG
AAACAGGTGCTAATACCGTATAACAAGCGAAACCGCGTGGTTTCGTTTTGAAGGGC
GGTTTACGGTGGCGCTGATGGATGGACCCGCGGTGCATTAGCTAGGGGGTGAGGTA
ACGGGGCGCCAAGGGCACGGTGCATAGCCGACCTGAGAGGGTGATCGGCCACGTT
GGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTTCGGC
AATGGACGAAAGTCTGACCGAGCAACGCCGCGTGAGTGAAGAAGGTTTTCGGATC
GTAAAACTCTGTTGTTAGAGAAGAACAAGGGTGAGAGTAACTGTTCACCCCTTGAC
GGTATCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACTAT
AGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTCTT
AAGTCTGATGTGAAAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGAG
ACTTGAGTGCAGAAGAGGAGAGTGGAATTCCATGTGTAGCGGTGAAATGCGTAGA
TATATGGAGGAACACCGTTGGCGAAGGCGGCTCTCTGGTTCTGTGACTGACGCTGA
AGGCTCGAAATGCTTGGGGGACCAACGGGAGTAGTAACCCCTGGAGGCCCGCGCG
GTATAACAACGAGTGCTAAAAGTGGGAAGGGCT

***Figure S.28. Forward sequence of REPR-6 isolate.*** *The FASTA sequence were exported from the chromatogram of forward sequence of REPR-6 isolate after the trim of low-quality bases at both ends.*

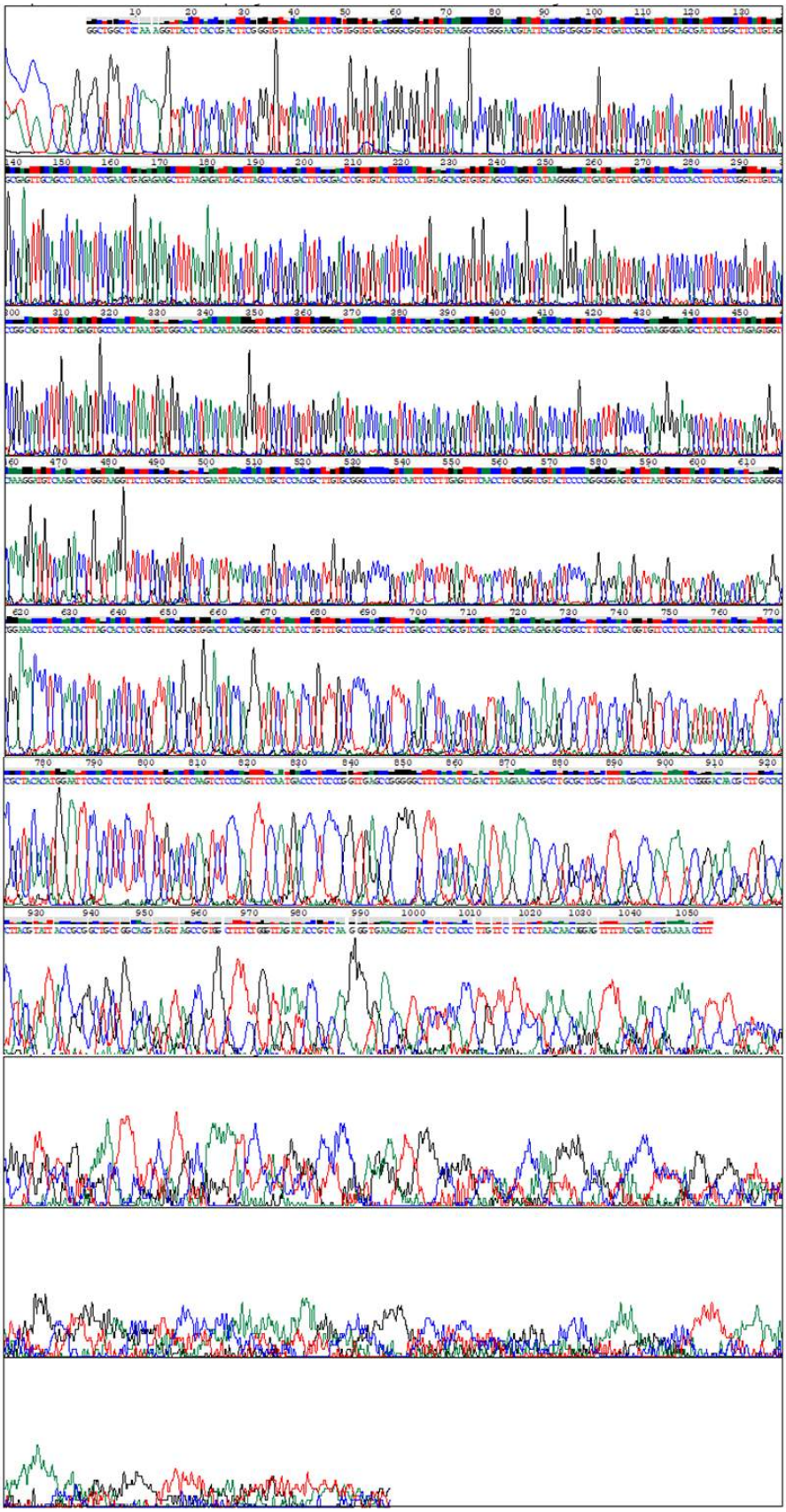


***Figure S.29. Chromatogram of reverse sequence of REPR-6 isolate***. *Low-quality bases at both ends of the sequence were trimmed using Chromas software with default setting.*

AAAGGTTTTCGGATCGTAAAAACTCCTGTTGTTAGAGAAGAACAAGGGTGAGAGTA
ACTGTTCACCCTTGACGGTATCTAACCCAGAAAAGCCACGGCTAACTACGTGCCAG
CAGCCGCGGTAATACGTAAGGTGGCAAGCGTTGTCCCGGATTTATTGGGCGTAAAG
CGAGCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCCCGGCTCAACCGGGGAGG
GTCATTGGAAACTGGGAGACTTGAGTGCAGAAGAGGAGAGTGGAATTCCATGTGT
AGCGGTGAAATGCGTAGATATATGGAGGAACACCAGTGGCGAAGGCGGCTCTCTG
GTCTGTAACTGACGCTGAGGCTCGAAAGCGTGGGGAGCAAACAGGATTAGATACC
CTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTGGAGGGTTTCCGCCCTTC
AGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGA
AACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCG
AAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTTTGACCACTCTAGAGATAG
AGCTTCCCCTTCGGGGGCAAAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGT
CGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATTGTTAGTTGCCATC
ATTTAGTTGGGCACTCTAGCAAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGA
TGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGGA
AGTACAACGAGTCGCGAAGTCGCGAGGCTAAGCTAATCTCTTAAAGCTTCTCTCAG
TTCGGATTGTAGGCTGCAACTCGCCTACATGAAGCCGGAATCGCTAGTAATCGCGG
ATCAGCACGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACC
ACGAGAGTTTGTAACACCCGAAGTCGGTGAGGTAACCTTTGGAGCCAGCC

***Figure S.30. Reverse sequence of REPR-6 isolate.*** *The FASTA sequence were exported from the chromatogram of reverse sequence of REPR-6 isolate after the trim of low-quality bases at both ends.*

7.4 REPR-7 Isolate

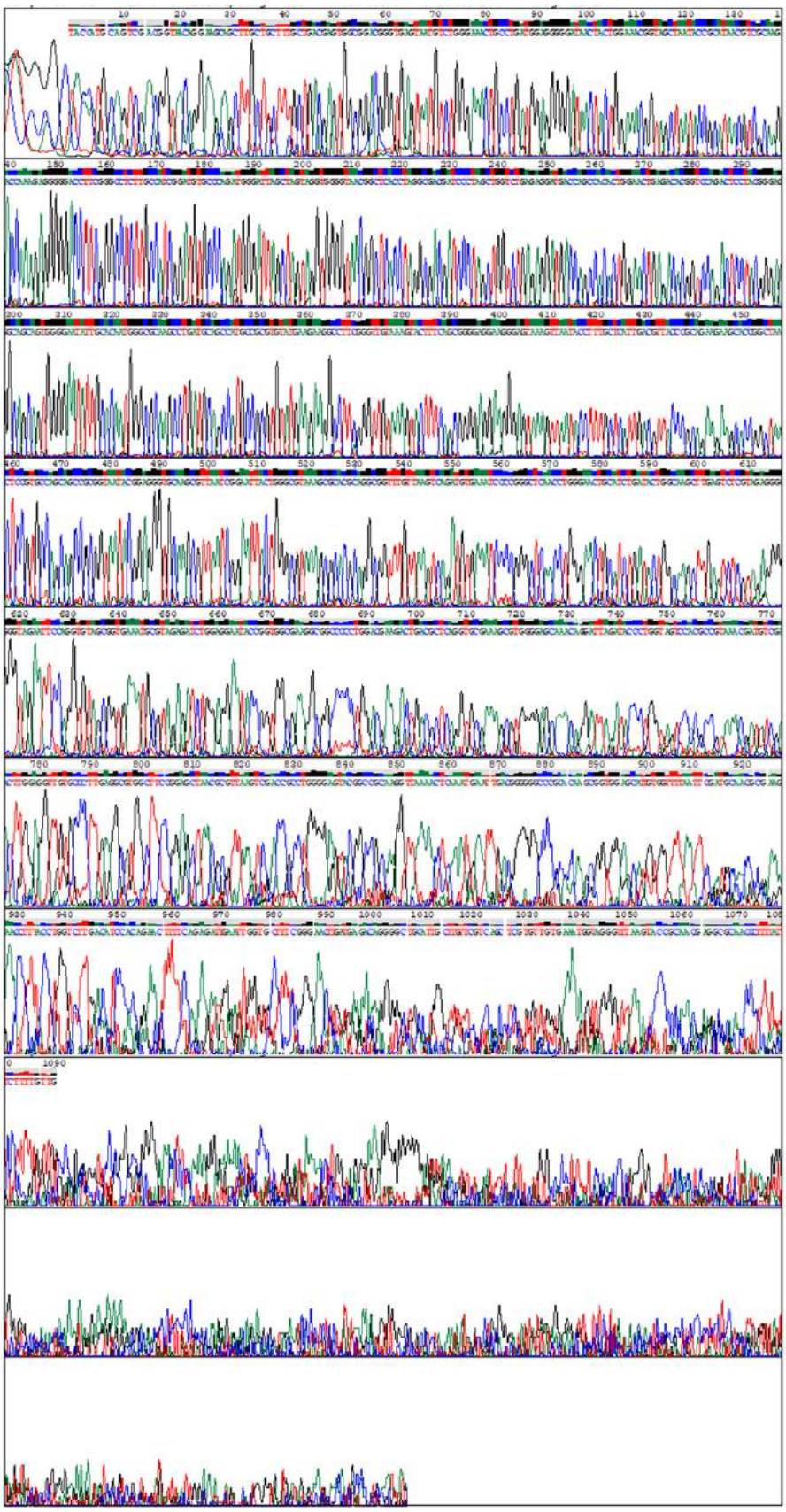


***Figure S.31. Chromatogram of forward sequence of REPR-7 isolate.*** *Low-quality bases at both ends of the sequence were trimmed using Chromas software with default setting.*

TACCATGCAGTCGACGGTAACAGGAAGCAGCTTGCTGCTTTGCTGACGAGTGGCGG
ACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGAAAC
GGTAGCTAATACCGCATAACGTCGCAAGACCAAAGAGGGGGACCTTCGGGCCTCTT
GCCATCGGATGTGCCCAGATGGGATTAGCTAGTAGGTGGGGTAACGGCTCACCTAG
GCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGACAC
GGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGC
CTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCA
GCGGGGAGGAAGGGAGTAAAGTTAATACCTTTGCTCATTGACGTTACCCGCAGAAG
AAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTT
AATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTTTGTTAAGTCAGATGTGAA
ATCCCCGGGCTCAACCTGGGAACTGCATCTGATACTGGCAAGCTTGAGTCTCGTAG
AGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATAC
CGGTGGCGAAGGCGGCCCCCTGGACGAAGACTGACGCTCAGGTGCGAAAGCGTGG
GGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGG
AGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGG
GGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGGCCCGCACAAGC
GGTGGAGCATTGTGGTTTTAATTCGATGCAACGCGAAGAACCTTTACCTGGTCTTGA
CATCCACAGAACTTTTCAGAGATTGAATTGGTGCTTTCGGGAACTTGATGAGACAG
GGGCTGCATTGCTTGTTCGTCAGCTCGTGTTGTGAAATGGTAGGGTTTAAGTACCGC
AACGAGGCGCAACCCTTTTATTCTTTTGTTG

***Figure S.32. Forward sequence of REPR-7 isolate.*** *The FASTA sequence were exported from the chromatogram of forward sequence of REPR-7 isolate after the trim of low-quality bases at both ends.*

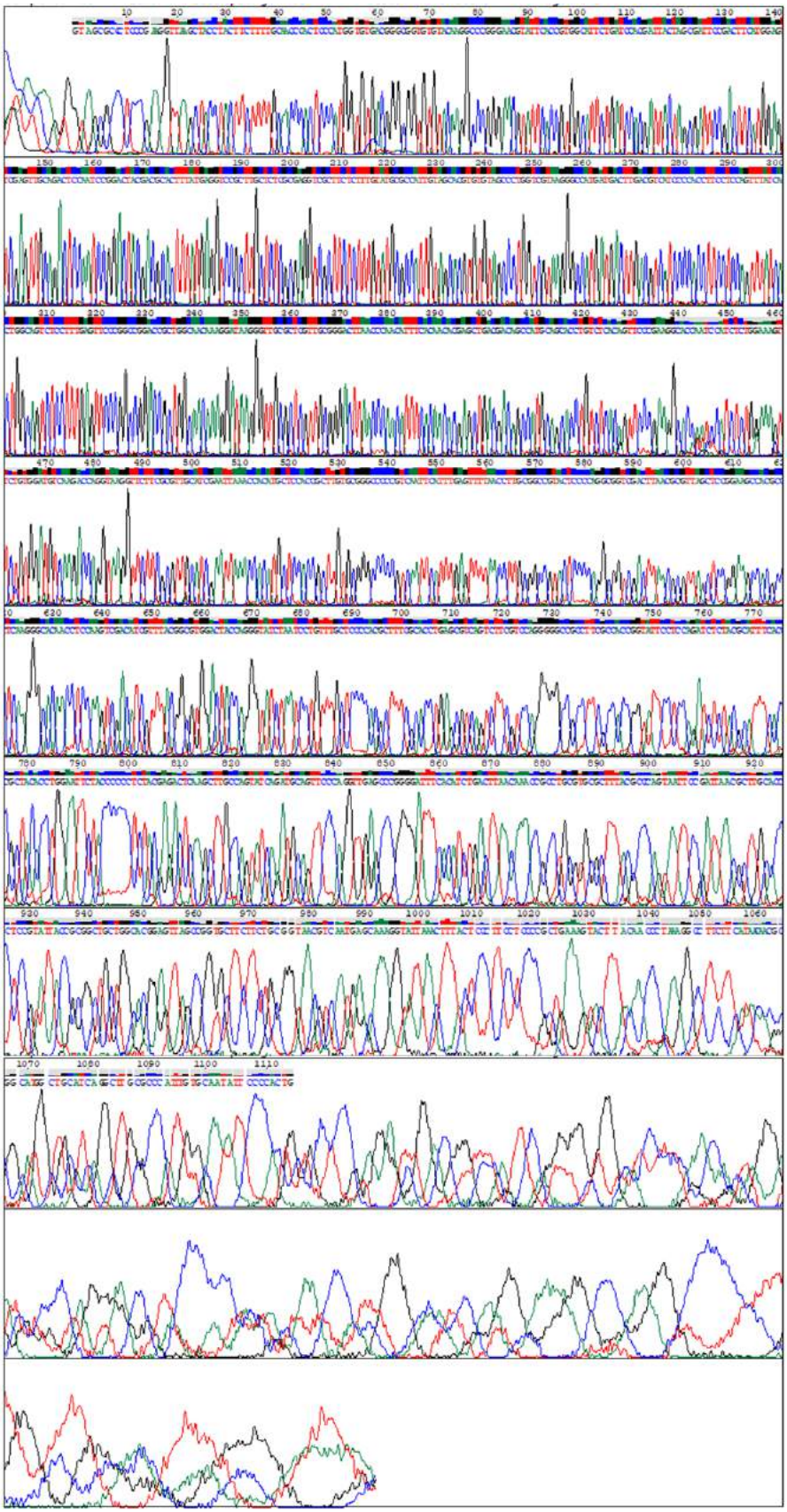


***Figure S.33. Chromatogram of reverse sequence of REPR-7 isolate.*** *Low-quality bases at both ends of the sequence were trimmed using Chromas software with default setting.*

CAGTGGGGAATATTGCACAAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTTGTT
ATGAAGAAGGCCTTTAGGGTTGTAAGTACTTTCAGCGGGGAGGAAGGGAGTAAAG
TTTAATACCTTTGCTCATTGACGTTACCGCAGAAGAAGCACCGGCTAACTCCGTGCC
AGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAA
GCGCACGCAGGCGGTTTGTTAAGTCAGATGTGAAATCCCCGGGCTCAACCTGGGAA
CTGCATCTGATACTGGCAAGCTTGAGTCTCGTAGAGGGGGGTAGAATTCCAGGTGT
AGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTG
GACGAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACC
CTGGTAGTCCACGCCGTAAACGATGTCGACTTGGAGGTTGTGCCCTTGAGGCGTGG
CTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAA
ACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGA
TGCAACGCGAAGAACCTTACCTGGTCTTGACATCCACAGAACTTTCCAGAGATGGA
TTGGTGCCTTCGGGAACTGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTG
TGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCG
GTCCGGCCGGGAACTCAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGGA
TGACGTCAAGTCATCATGGCCCTTACGACCAGGGCTACACACGTGCTACAATGGCG
CATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTGCGTCGTA
GTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGTG
GATCAGAATGCCACGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACAC
CATGGGAGTGGGTTGCAAAAGAAGTAGGTAGCTTAACCTTCGGGAGGGCGCTAC

***Figure S.34. Reverse sequence of REPR-7 isolate.*** *The FASTA sequence were exported from the chromatogram of reverse sequence of REPR-7 isolate after the trim of low-quality bases at both ends.*

7.5 REPR-8 Isolate

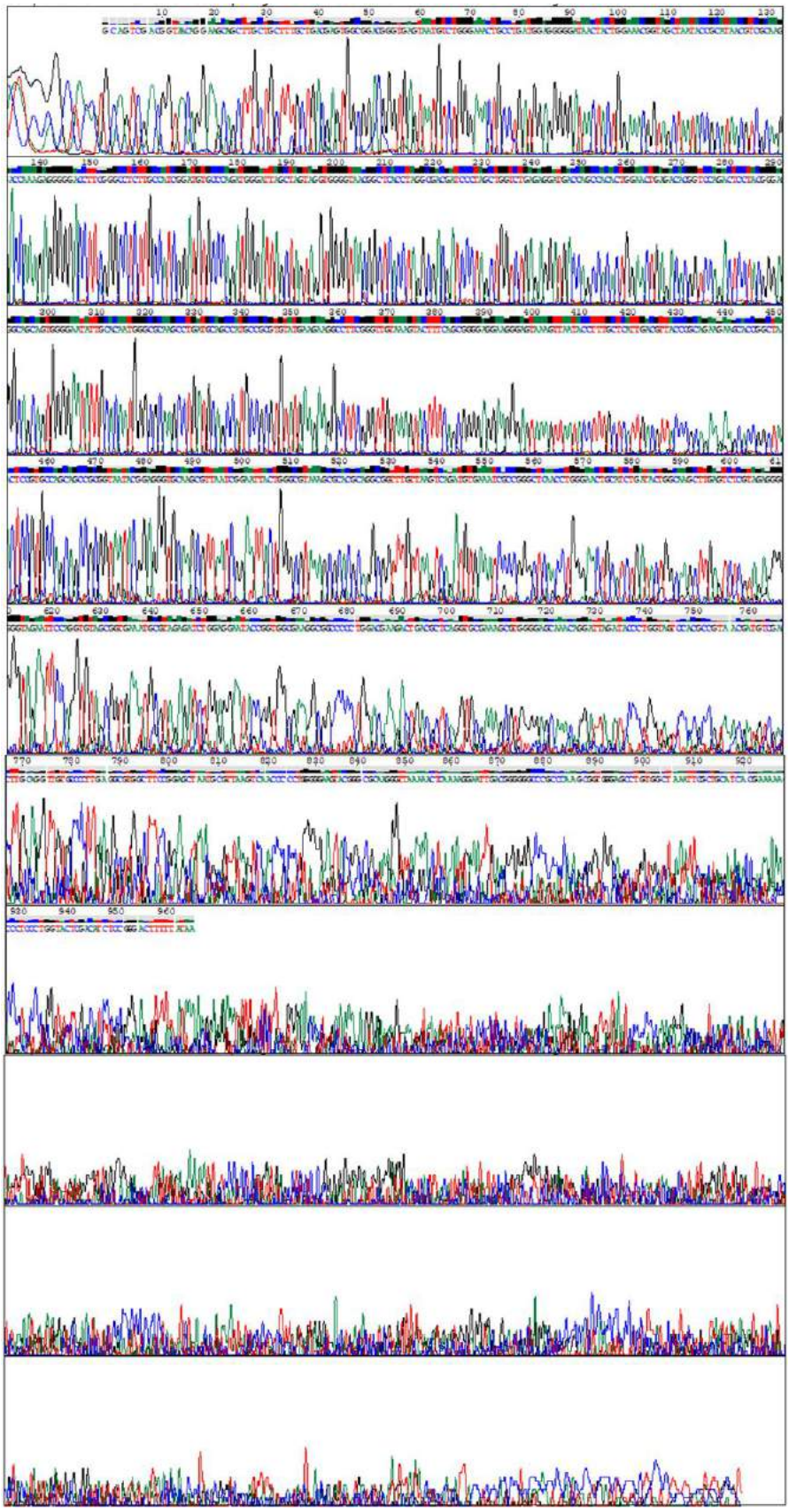


***Figure S.35. Chromatogram of forward sequence of REPR-8 isolate.*** *Low-quality bases at both ends of the sequence were trimmed using Chromas software with default setting.*

GCAGTCGACGGTAACAGGAAGCAGCTTGCTGCTTTGCTGACGAGTGGCGGACGGGT
GAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGAAACGGTAGC
TAATACCGCATAACGTCGCAAGACCAAAGAGGGGGACCTTCGGGCCTCTTGCCATC
GGATGTGCCCAGATGGGATTAGCTAGTAGGTGGGGTAACGGCTCACCTAGGCGACG
ATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGACACGGTCCAG
ACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGC
AGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGA
GGAAGGGAGTAAAGTTAATACCTTTGCTCATTGACGTTACCCGCAGAAGAAGCACC
GGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGA
ATTACTGGGCGTAAAGCGCACGCAGGCGGTTTGTTAAGTCAGATGTGAAATCGCCG
GGCTCAACCTGGGAACTGCATCTGATACTGGCAAGCTTGAGTCTCGTAGAGGGGGG
TAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGC
GAAGGCGGCCCCCTGGACGAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAA
ACAGGATTAGATACCCTGGTAGTCCACGCCGTAACGATGTCGACTTGCAGGTTGTG
CCCCTTGAGGCGTGGCTTCCGGAGCTAATGCGTTAAGTCAACCCCCTGGGGGAAGT
ACGGGCGCAAGGGTTAAAAACTCAAAAGGAATTGACGGGGGGCCCGCCCAAGCGG
TGGGAGCCTGTGGCTAAATTCGCTGCATCACGAAAAACCCTCCCTGGTACTCGACA
TCTCCGGGACTTTTTATAA

***Figure S.36. Forward sequence of REPR-8 isolate.*** *The FASTA sequence were exported from the chromatogram of forward sequence of REPR-8 isolate after the trim of low-quality bases at both ends.*

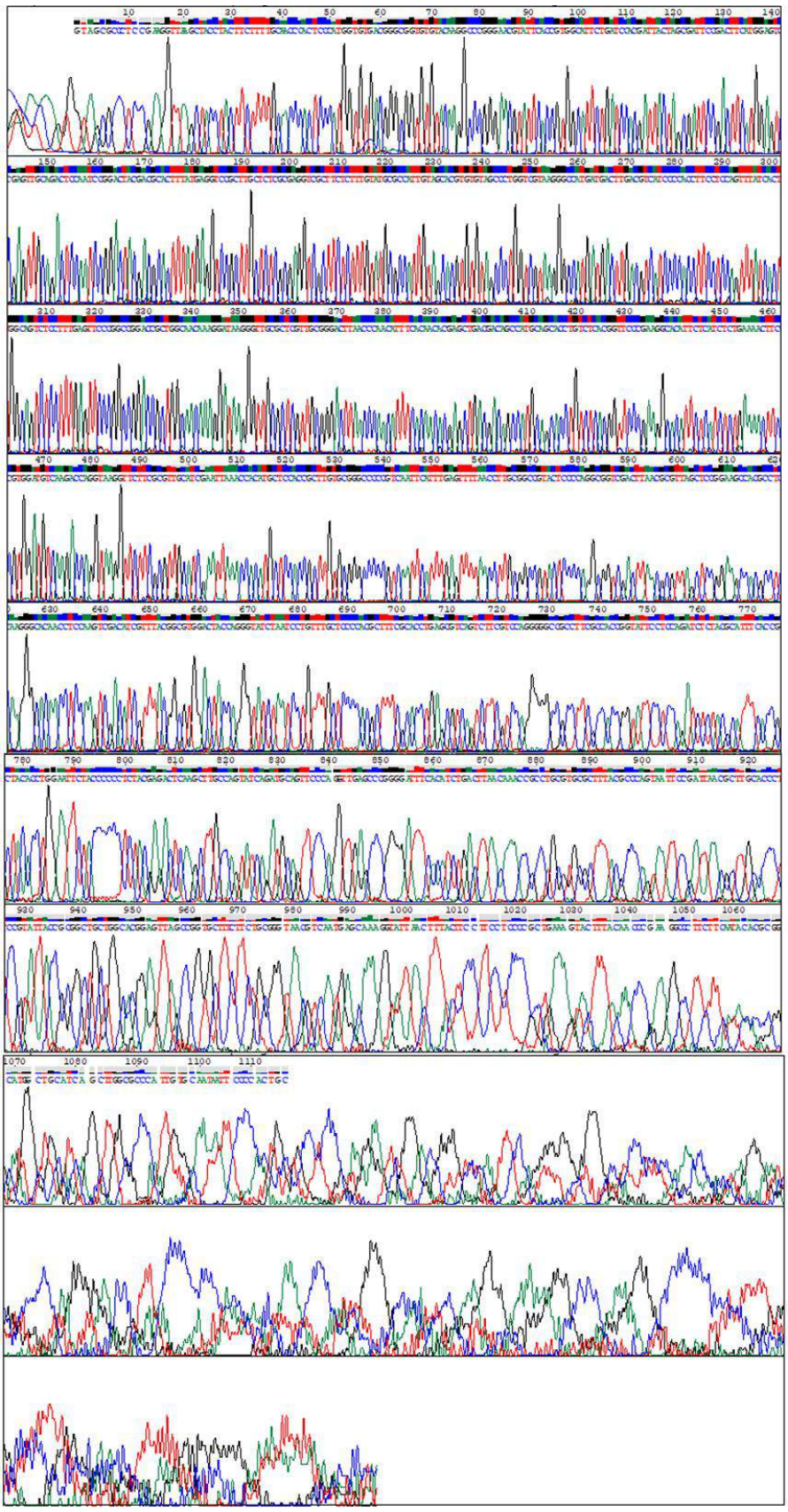


***Figure S.37. Chromatogram of reverse sequence of REPR-8 isolate.*** *Low-quality bases at both ends of the sequence were trimmed using Chromas software with default setting.*

GCAGTGGGGAATTATTGCACAATGGGCGCCAAGCTGATGCAGCCATGCCGCGTGTA
TTGAAGAAGGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGAAGTAAA
GTTAATACCTTTGCTCATTGACGTTACCCGCAGAAGAAAGCACCGGCTAACTCCGT
GCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGT
AAAGCGCACGCAGGCGGTTTGTTAAGTCAGATGTGAAATCCCCGGGCTCAACCTGG
GAACTGCATCTGATACTGGCAAGCTTGAGTCTCGTAGAGGGGGGTAGAATTCCAGG
TGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCC
CTGGACGAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGAT
ACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGAGGTTGTGCCCTTGAGGCG
TGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTT
AAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATT
CGATGCAACGCGAAGAACCTTACCTGGTCTTGACATCCACGGAAGTTTTCAGAGAT
GAGAATGTGCCTTCGGGAACCGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGT
GTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCA
GCGGTCCGGCCGGGAACTCAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGG
GGATGACGTCAAGTCATCATGGCCCTTACGACCAGGGCTACACACGTGCTACAATG
GCGCATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTGCGTC
GTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATC
GTGGATCAGAATGCCACGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCA
CACCATGGGAGTGGGTTGCAAAAGAAGTAGGTAGCTTAACCTTCGGAGGGCGCTAC

***Figure S.38. Reverse sequence of REPR-8 isolate.*** *The FASTA sequence were exported from the chromatogram of reverse sequence of REPR-8 isolate after the trim of low-quality bases at both ends.*

7.6 REPR-11 Isolate

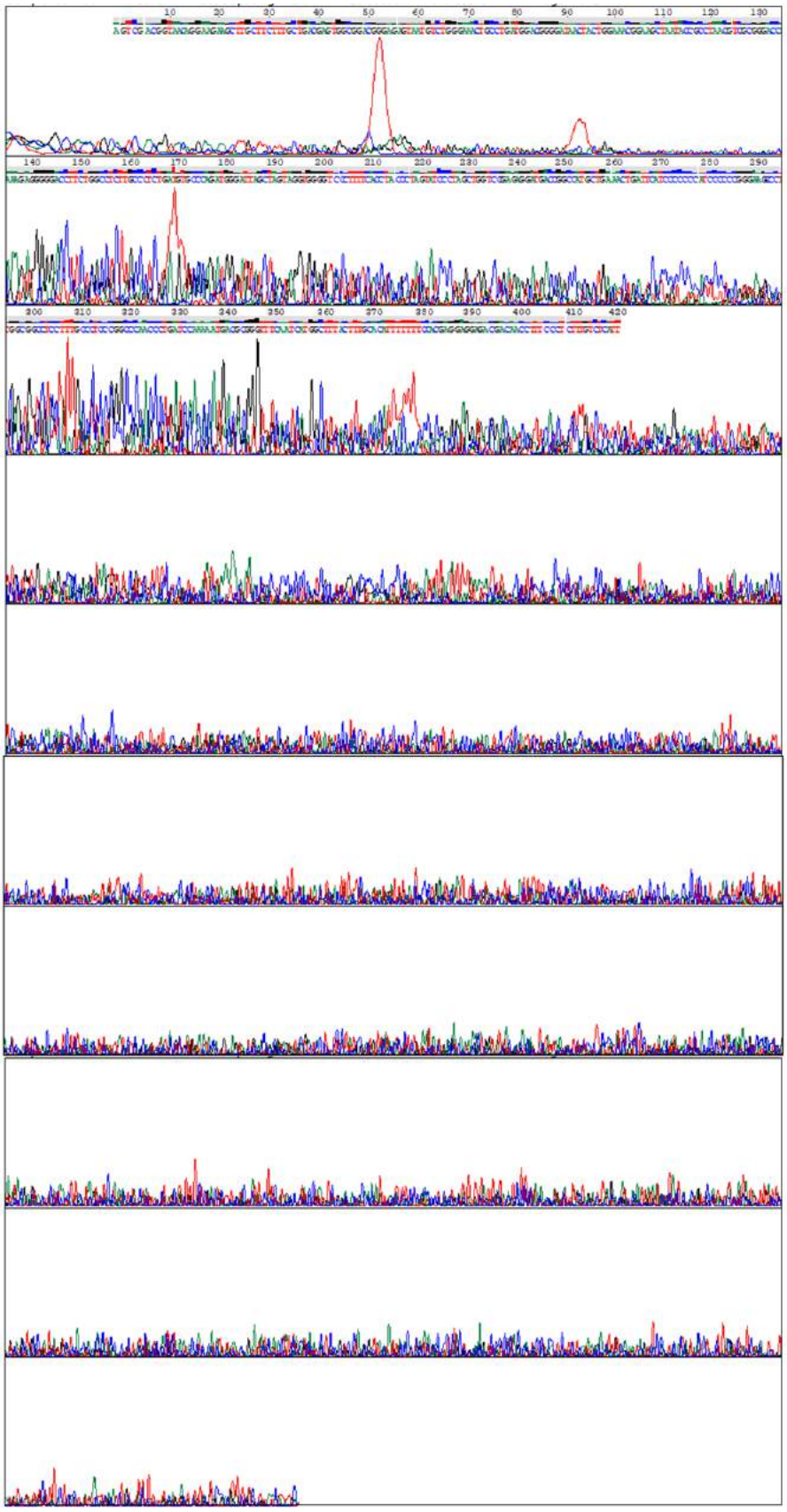


***Figure S.39. Chromatogram of forward sequence of REPR-11 isolate.*** *Low-quality bases at both ends of the sequence were trimmed using Chromas software with default setting.*

AGTCGACGGTAACAGGAAGAAGCTTGCTTCTTTGCTGACGAGTGGCGGACGGGAG
AGTAATGTCTGGGAAACTGCCTGATGGACGGGGATAACTACTGGAAACGGAAGCT
AATACCGCCTAACGTCGCGGGACCAAAGAGGGGGACCTTCTGGCCTCTTGCCCTCT
GATGTGCCCAGATGGGATTAGCTAGTAGGTGGGGTCCCTTTTCACCTACCCTAGTAT
CCCTAGCTGGTCCGAGAGGATGACCGGCCATGCTGAAACTGATTCATCCCCCCCAT
CCCCCCGGGAAGCCCTGGCGGCCTCCTTTGCCCTCCCGGCCCAACCCTGATCCAAA
AATGACGCGGGTTTCAATCATGGCTTTACTTTGCACATTTTTTTCCACGAGGAGGAG
ACGACAACCTTTCCCTCTTTGTCTCATT

***Figure S.40. Forward sequence of REPR-11 isolate.*** *The FASTA sequence were exported from the chromatogram of forward sequence of REPR-11 isolate after the trim of low-quality bases at both ends.*

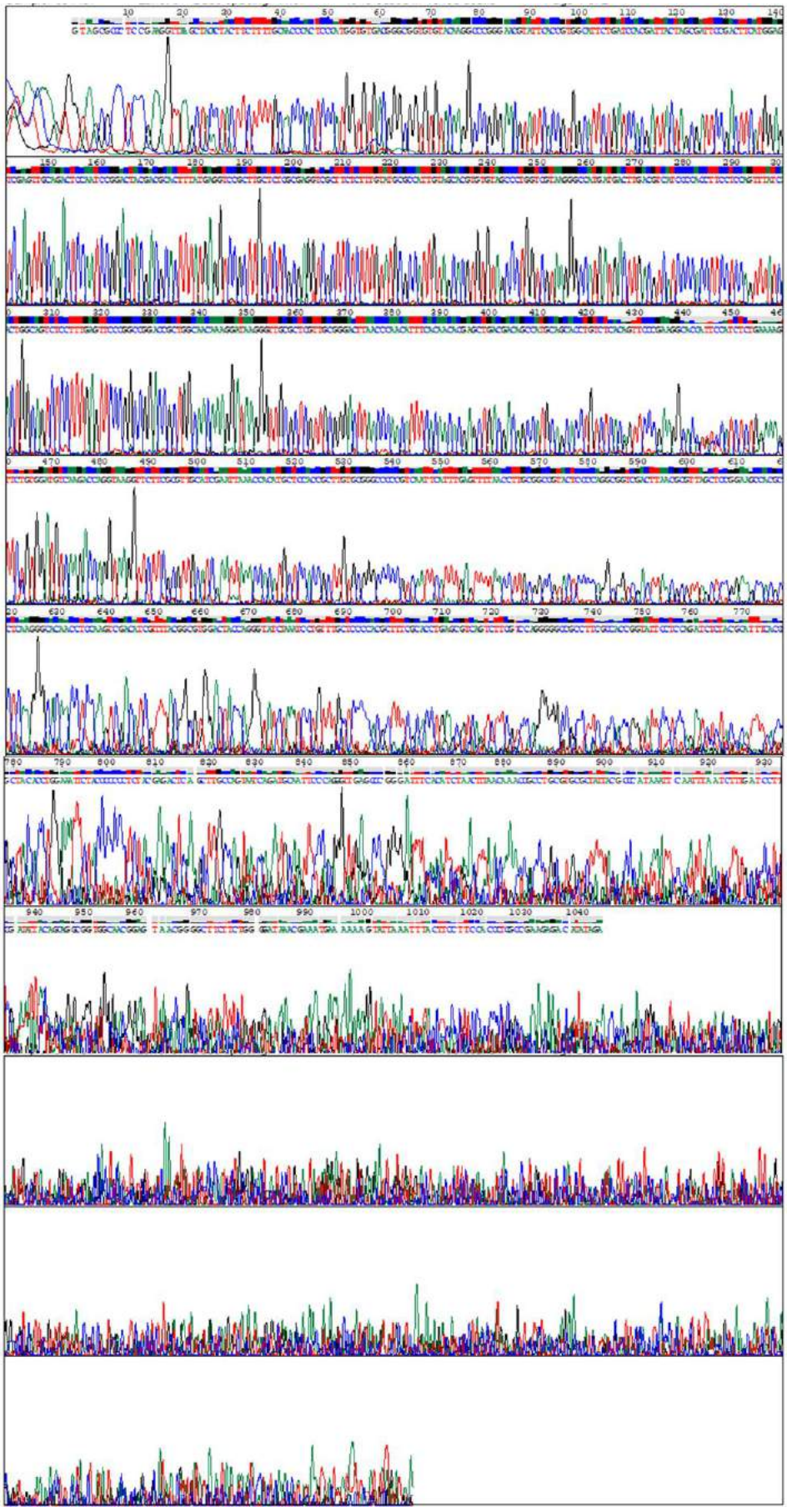


***Figure S.41. Chromatogram of reverse sequence of REPR-11 isolate.*** *Low-quality bases at both ends of the sequence were trimmed using Chromas software with default setting.*

TCTATATGTCTCTTCGGCGAGGGTGGAAGGAAGTAAATTTAATACTTTTTTCATTTC
GTTTATCCCCAGAAGAAGCCCCGTTACTCCGTTGCCACCGCCTGCTGTAATATCGAA
GGATCAAAGATTAAATTGAATTTATGGGCGTAATAGCGCACGCAGGCGGTTTGTTA
AAGTTAGATGTGAAATCCCGGGCTCAACCCTGGGAATTGCATCTGATTACTGGCAA
GCTGAGTCTCGTAGAGGGGGGGTAGAATTTCCAGGTGTAGCGGTGAAATGCGTAGA
GATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACGAAGACTGACGCTCA
GGTGCGAAAGCGTGGGGAGCAAACAGGATTTAGATACCCTGGTAGTCCACGCCGT
AAAACGATGTCGACTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGC
GTTAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGAC
GGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAAC
CTTACCTGGTCTTGACATCCACAGAACTTTTCAGAGATGGAATGGTGCCTTCGGGA
ACTGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGTT
AAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTCCGGCCGGGAAC
TCAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGGATGACGTCAAGTCAT
CATGGCCCTTACGACCAGGGCTACACACGTGCTACAATGGCGCATACAAAGAGAA
GCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTGCGTCGTAGTCCGGATTGGAGT
CTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGTGGATCAGAATGCCAC
GGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTT
GCAAAAGAAGTAGTGTAGCTTAACCTTCGGAGGGCGCTAC

***Figure S.42. Reverse sequence of REPR-11 isolate.*** *The FASTA sequence were exported from the chromatogram of reverse sequence of REPR-11 isolate after the trim of low-quality bases at both ends.*

7.7 REPR-12 Isolate

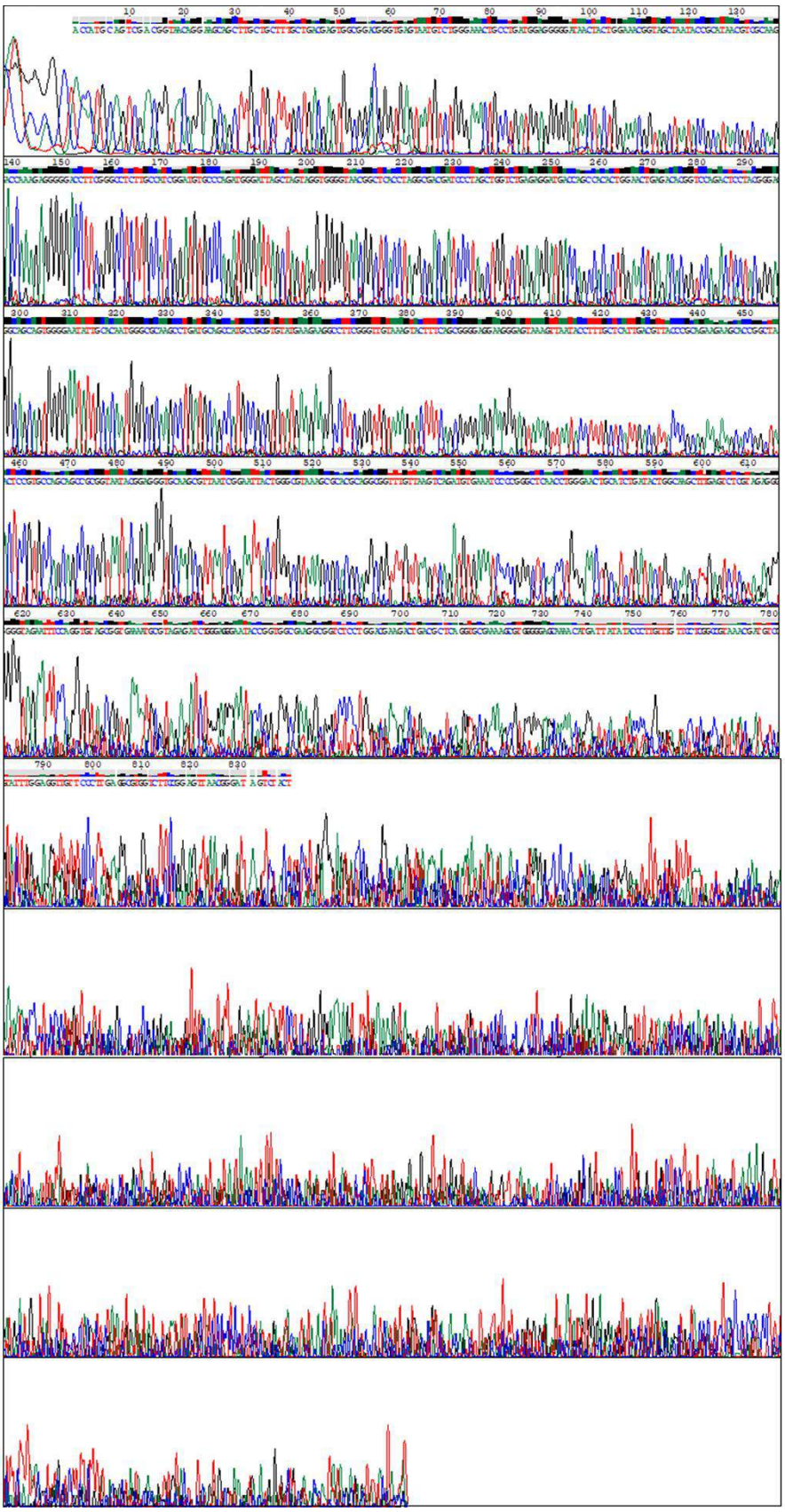


***Figure S.43. Chromatogram of forward sequence of REPR-12 isolate.*** *Low-quality bases at both ends of the sequence were trimmed using Chromas software with default setting.*

ACCATGCAGTCGACGGTAACAGGAAGCAGCTTGCTGCTTTGCTGACGAGTGGCGGA
CGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGAAACG
GTAGCTAATACCGCATAACGTCGCAAGACCAAAGAGGGGGACCTTCGGGCCTCTTG
CCATCGGATGTGCCCAGATGGGATTAGCTAGTAGGTGGGGTAACGGCTCACCTAGG
CGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGACACG
GTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCC
TGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAG
CGGGGAGGAAGGGAGTAAAGTTAATACCTTTGCTCATTGACGTTACCCGCAGAAGA
AGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTA
ATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTTTGTTAAGTCAGATGTGAAA
TCCCCGGGCTCAACCTGGGAACTGCATCTGATACTGGCAAGCTTTGAGTCTCGTAG
AGGGGGGTAGAATTTCCAGGTGTAGCGGTGAAAATGCGTAGAGATCTGGGAGGGA
ATACCGGTGGCGAAGGCGGTCTCCTGGACGAAGACTGACGCTCAGGTGCGAAAAG
CGTGGGGGAGCAAAACATGATTATATACCCTTGTTTGTTCCTCGGCCGTAAACGAT
GTCGTATTTGGAGGTTGTTCCCTTGAGGCGTGGTCTTCCGGAGTTAACGGGATAGTC
TACT

***Figure S.44. Forward sequence of REPR-12 isolate.*** *The FASTA sequence were exported from the chromatogram of forward sequence of REPR-12 isolate after the trim of low-quality bases at both ends.*

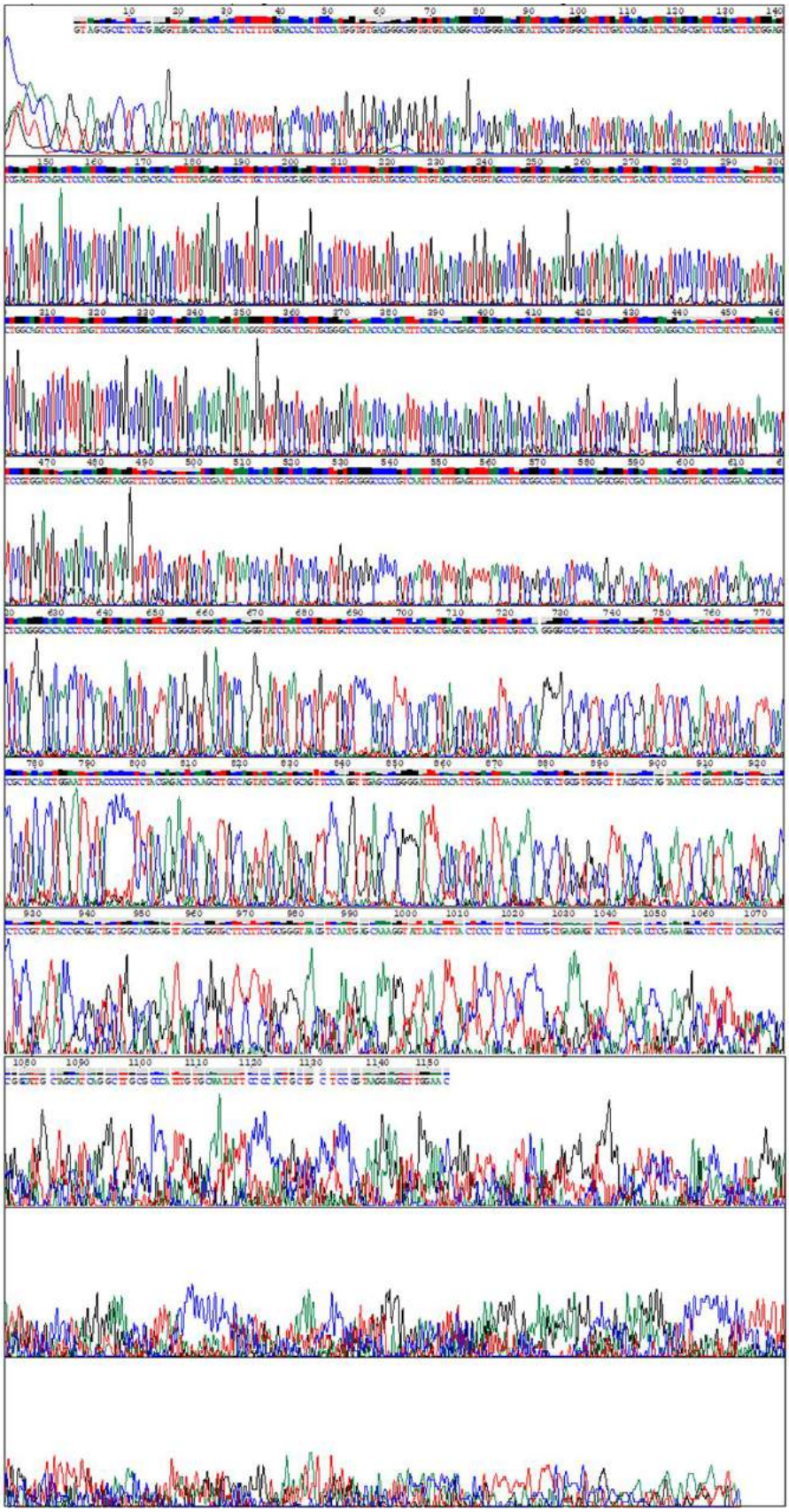


***Figure S.45. Chromatogram of reverse sequence of REPR-12 isolate.*** *Low-quality bases at both ends of the sequence were trimmed using Chromas software with default setting.*

GTTCCAAGACTTCCTTACGGGAGCAGCAGTGGGGAATATTTGCACAAATGGGCGCA
AGCCTGATGCTAGCAATGCCGGCGTTGATATGAAGAAGGGCCTTTCGAGGTCGTAA
AGGTACTTCTTCAGCGGGGGAGGAAGGGAGTAAAGGTTAATACCTTTGCTCATTGA
CGTTACCCGCAGAAAGAAGCACCGGGCTAACTCCGTGCCAGCAGCCGCGGTAATAC
GGAGGGTGCAAGCGTTAATCGGAATTTACTGGGCGTAAGCGCACGCAGGCGGTTTG
TTAAGTCAGATGTGAAAATCCCCGGGCTCAACCTGGGAACTGCATCTGATACTGGC
AAGCTTGAGTCTCGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAG
AGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCTGGACGAAGACTGACGCTCA
GGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTA
AACGATGTCGACTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGT
TAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACG
GGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCT
TACCTGGTCTTGACATCCACGGAAGTTTTCAGAGATGAGAATGTGCCTTCGGGAAC
CGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGTTAA
GTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTCCGGCCGGGAACTC
AAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGGATGACGTCAAGTCATCA
TGGCCCTTACGACCAGGGCTACACACGTGCTACAATGGCGCATACAAAGAGAAGC
GACCTCGCGAGAGCAAGCGGACCTCATAAAGTGCGTCGTAGTCCGGATTGGAGTCT
GCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGTGGATCAGAATGCCACGG
TGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGC
AAAAGAAGTAGGTAGCTTAACCTTCGGGAGGGCGCTAC

***Figure S.46. Reverse sequence of REPR-12 isolate.*** *The FASTA sequence were exported from the chromatogram of reverse sequence of REPR-12 isolate after the trim of low-quality bases at both ends.*

7.8 REPS-4 Isolate

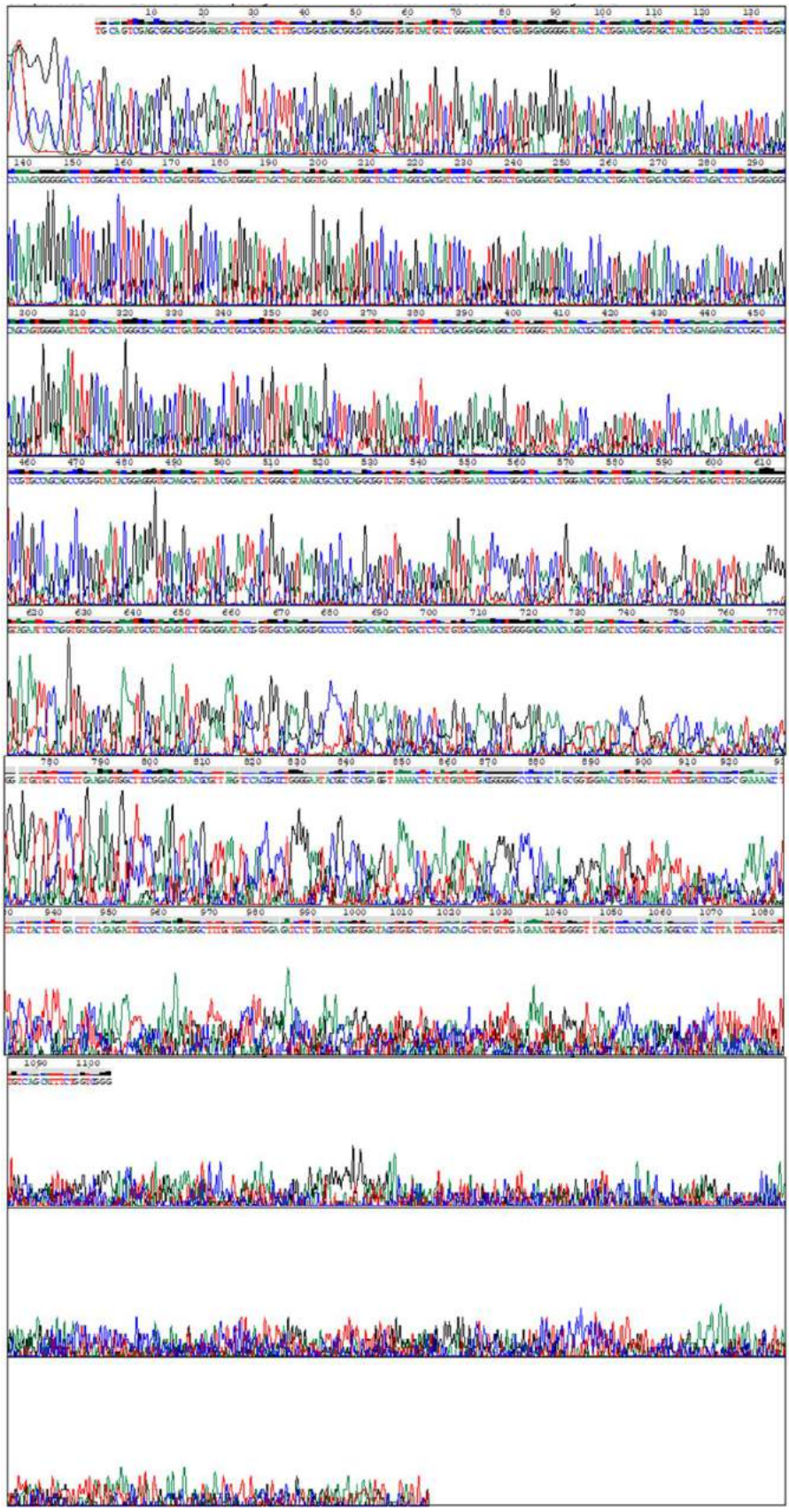


***Figure S.47. Chromatogram of forward sequence of REPS-4 isolate.*** *Low-quality bases at both ends of the sequence were trimmed using Chromas software with default setting.*

TGCAGTCGAGCGGCAGCGGGAAGTAGCTTGCTACTTTGCCGGCGAGCGGCGGACG
GGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGAAACGGT
AGCTAATACCGCATAACGTCTTCGGACCAAAGAGGGGGACCTTCGGGCCTCTTGCC
ATCAGATGTGCCCAGATGGGATTAGCTAGTAGGTGAGGTAATGGCTCACCTAGGCG
ACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGACACGGTC
CAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGA
TGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGA
GGAGGAAGGCATTGGGGTTAATAACCGCAGTGATTGACGTTACTCGCAGAAGAAG
CACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATC
GGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTCAAGTCGGATGTGAAATCC
CCGGGCTCAACCTGGGAACTGCATTCGAAACTGGCAGGCTAGAGTCTTGTAGAGGG
GGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGT
GGCGAAGGCGGCCCCCTGGACAAAGACTGACTCTCATGTGCGAAAGCGTGGGGAG
CAAACAAGATTAGATACCCTGGTAGTCCACGCCGTAAACTATGTCGACTGGATGTT
GTTCCCTTGAAGAGTGGCTTCCGGAGCTAACGCGTTAAGTCCACCGCCCTGGGGAA
TACGGCCGCGAGGTAAAAACTCATATGATATTTGACGGGGGGCCCGCACAGCGGTG
GAACATGTGGTTTAATTTCTGATGCCACCGCGAAAAACCTTACCTACTCTTGACTTC
AGAAGATTTCCGCAGAGATGGCTTTGTTGTCCTTGGAGATCTCTGATAACAGGTGG
ATACGTGTGCTGTTGCACAGCTTGTGTTGAGAAATGTTGGGGTTAGTCCCCACCACG
AGGCGCCACCTTATTCCTTTTTGTTGTCAGCATTTCTGGTCGGG

***Figure S.48. Forward sequence of REPS-4 isolate.*** *The FASTA sequence were exported from the chromatogram of forward sequence of REPS-4 isolate after the trim of low-quality bases at both ends.*

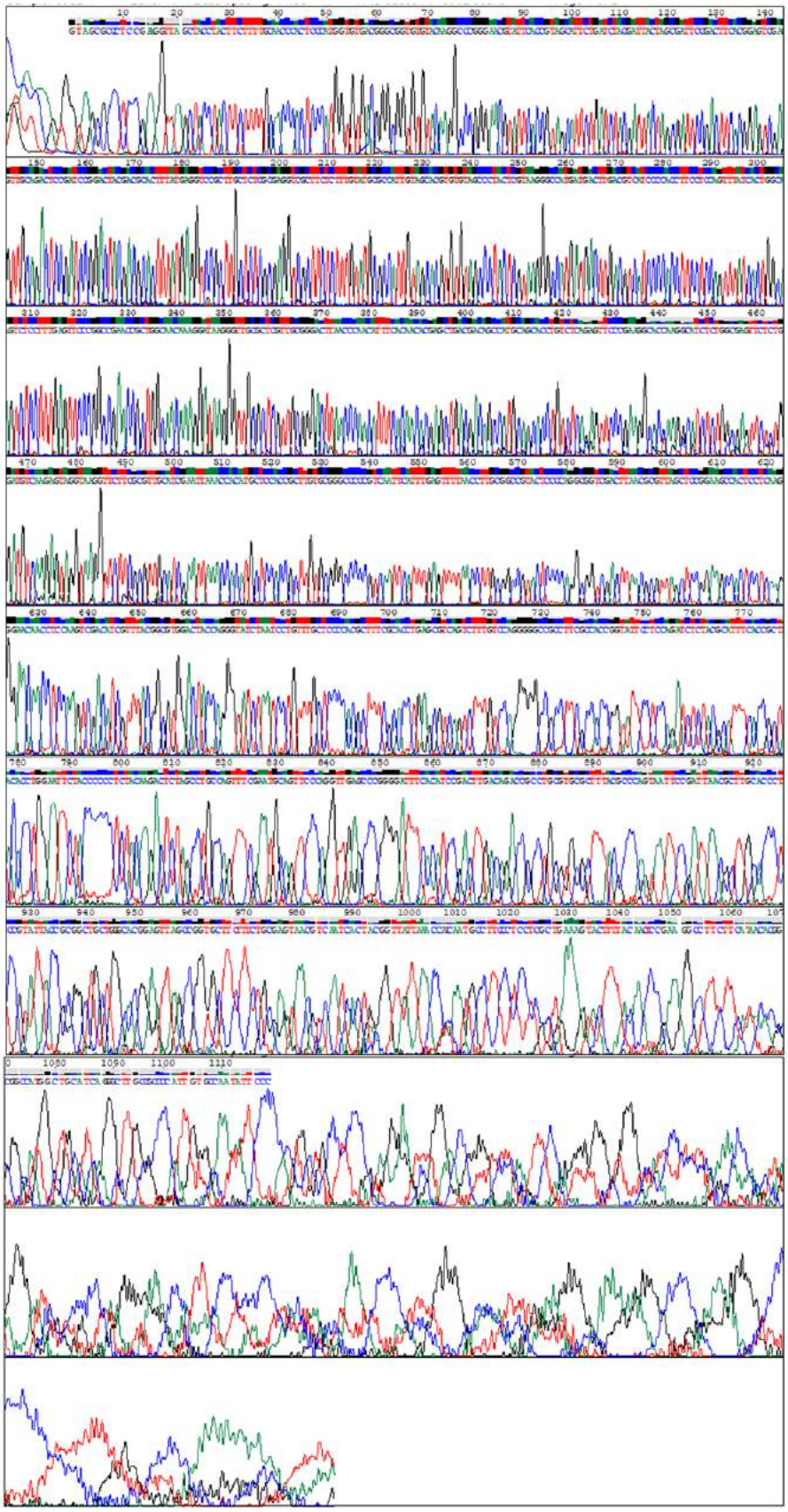


***Figure S.49. Chromatogram of reverse sequence of REPS-4 isolate.*** *Low-quality bases at both ends of the sequence were trimmed using Chromas software with default setting.*

GGGAATATTGGCACAATGGGGCGGCAAGCCCTGATGCAGCCATGGCCGCCGTGTTA
TGAAGAAGGCCTTCGGAGTTGTAAAAGTACTTTCAGCGAGGAGGGAAGGCATTGAT
GGTTTAATAACCGTAGTGATTGACGTTACTCGCAGAAAGAAGCACCGGCTAACTCC
GTGCCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGC
GTAAAGCGCACGCAGGCGGTCTGTCAAGTCGGATGTGAAATCCCCGGGCTCAACCT
GGGAACTGCATTCGAAACTGGCAGGCTAGAGTCTTGTAGAGGGGGGTAGAATTCCA
GGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCC
CCCTGGACAAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAG
ATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGAGGTTGTTCCCTTGAGG
AGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCCGCAAGG
TTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAA
TTCGATGCAACGCGAAGAACCTTACCTACTCTTGACATCCAGAGAACTCGCCAGAG
ATGCCTTGGTGCCTTCGGGAACTCTGAGACAGGTGCTGCATGGCTGTCGTCAGCTC
GTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGC
CAGCGGTTCGGCCGGGAACTCAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGT
GGGGATGACGTCAAGTCATCATGGCCCTTACGAGTAGGGCTACACACGTGCTACAA
TGGCGCATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTGCG
TCGTAGTCCGGATCGGAGTCTGCAACTCGACTCCGTGAAGTCGGAATCGCTAGTAA
TCGTAGATCAGAATGCTACGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGT
CACACCATGGGAGTGGGTTGCAAAAGAAGTAGGTAGCTAACCTTCGGAGGGCGCT
AC

***Figure S.50. Reverse sequence of REPS-4 isolate.*** *The FASTA sequence were exported from the chromatogram of reverse sequence of REPS-4 isolate after the trim of low-quality bases at both ends.*

7.9 REPS-26 Isolate

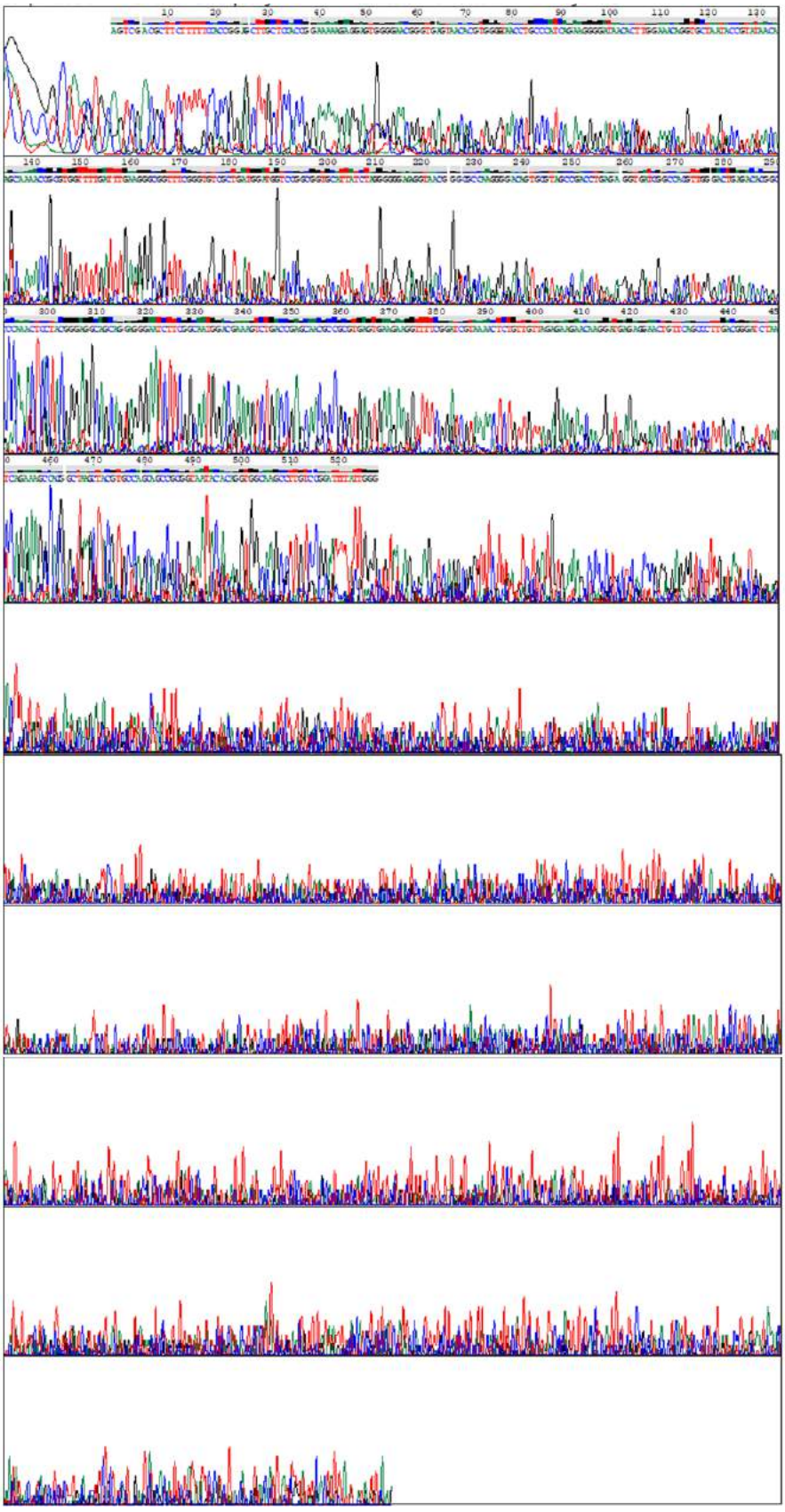


***Figure S.51. Chromatogram of forward sequence of REPS-26 isolate.*** *Low-quality bases at both ends of the sequence were trimmed using Chromas software with default setting.*

AGTCGACGCTTCTTTTTCCACCGGAGCTTGCTCCACCGGAAAAAGAGGAGTGGGGA
ACGGGTGAGTAACACGTGGGGTAACCTGCCCATCAGAAGGGGATAACACTTGGAA
ACAGGTGCTAATACCGTATAACAAGCAAAACCGCGTGGTTTTGATTTGAAGGGCGG
TTTCGGGTGTCGCTGATGGATGGTCCGGCGGTGCATTATCTAGGGGGGAGAGGTAA
CGGGCGCCAAGGGGACAGTGCGTAGCCGACCTGAGAGGTGATCGGCCACGTTGGG
ACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGGAGGGAATCTTCGGCAAT
GGACGAAAGTCTGACCGAGCAACGCCGCGTGAGTGAAGAAGGTTTTCGGATCGTA
AAACTCTGTTGTTAGAGAAGAACAAGGATGAGAGGAACTGTTCAGCCCTTGACGGG
ATCTAATCAGAAAGCCACGGCTAAGCTACGTGCCAGCAGCCGCGGTAATACACAG
GTGGCAAGCCTTGTCCGGATTTTATTGGG

***Figure S.52. Forward sequence of REPS-26 isolate.*** *The FASTA sequence were exported from the chromatogram of forward sequence of REPS-26 isolate after the trim of low-quality bases at both ends.*

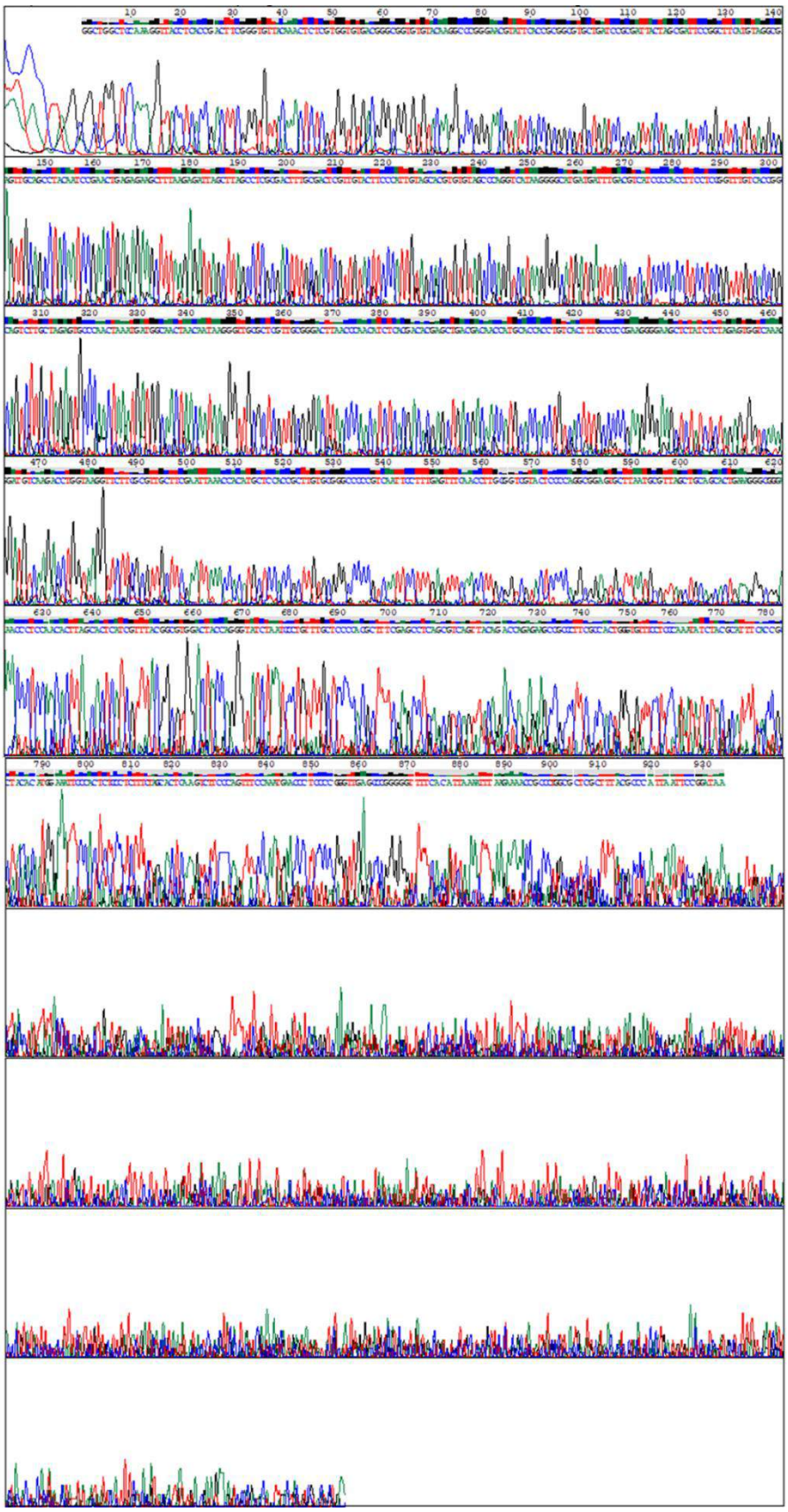


***Figure S.53. Chromatogram of reverse sequence of REPS-26 isolate.*** *Low-quality bases at both ends of the sequence were trimmed using Chromas software with default setting.*

TTATCCGGAATTAATGGGCGTAAAGCGAGCGCCAGGGCGGTTTTCTTAAATTTTAA
TGTGAAAACCCCCGGGCTCAACCCGGGGAGGGTTCATTTGGAAACTGGGAAGACTT
GAGTGCTAGAAAGAGGGAGAGTGGGAATTTCCATGTGTAGCGGTGAAATGCGTAG
ATATTTGGGAGGAAACACCCAGTGGCGAAGGGCGGCTTCTCTGGTCTGTAACTGAC
GCTGAGGCTCGAAAGCGTGGGGAGCAAACAGGGATTAGATACCCTGGTAGTCCAC
GCCGTAAACGATGAGTGCTAAGTGTTGGAGGGTTTCCCGCCCTTTCAGTGCTGCAG
CTAACGCATTAAGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGG
AATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGC
GAAGAACCTTACCAGGTCTTGACATCCTTTGACCACTCTAGAGATAGAGCTTCCCCT
TCGGGGGCAAAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGT
TGGGTTAAGTCCCGCAACGAGCGCAACCCTTATTGTTAGTTGCCATCATTTAGTTGG
GCACTCTAGCAAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAA
TCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGGAAGTACAACGA
GTCGCAAAGTCGCGAGGCTAAGCTAATCTCTTAAAGCTTCTCTCAGTTCGGATTGTA
GGCTGCAACTCGCCTACATGAAGCCGGAATCGCTAGTAATCGCGGATCAGCACGCC
GCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCACGAGAGTTTG
TAACACCCGAAGTCGGTGAGGTAACCTTTGGAGCCAGCC

***Figure S.54. Reverse sequence of REPS-26 isolate.*** *The FASTA sequence were exported from the chromatogram of reverse sequence of REPS-26 isolate after the trim of low-quality bases at both ends.*

7.10 REPS-29 Isolate

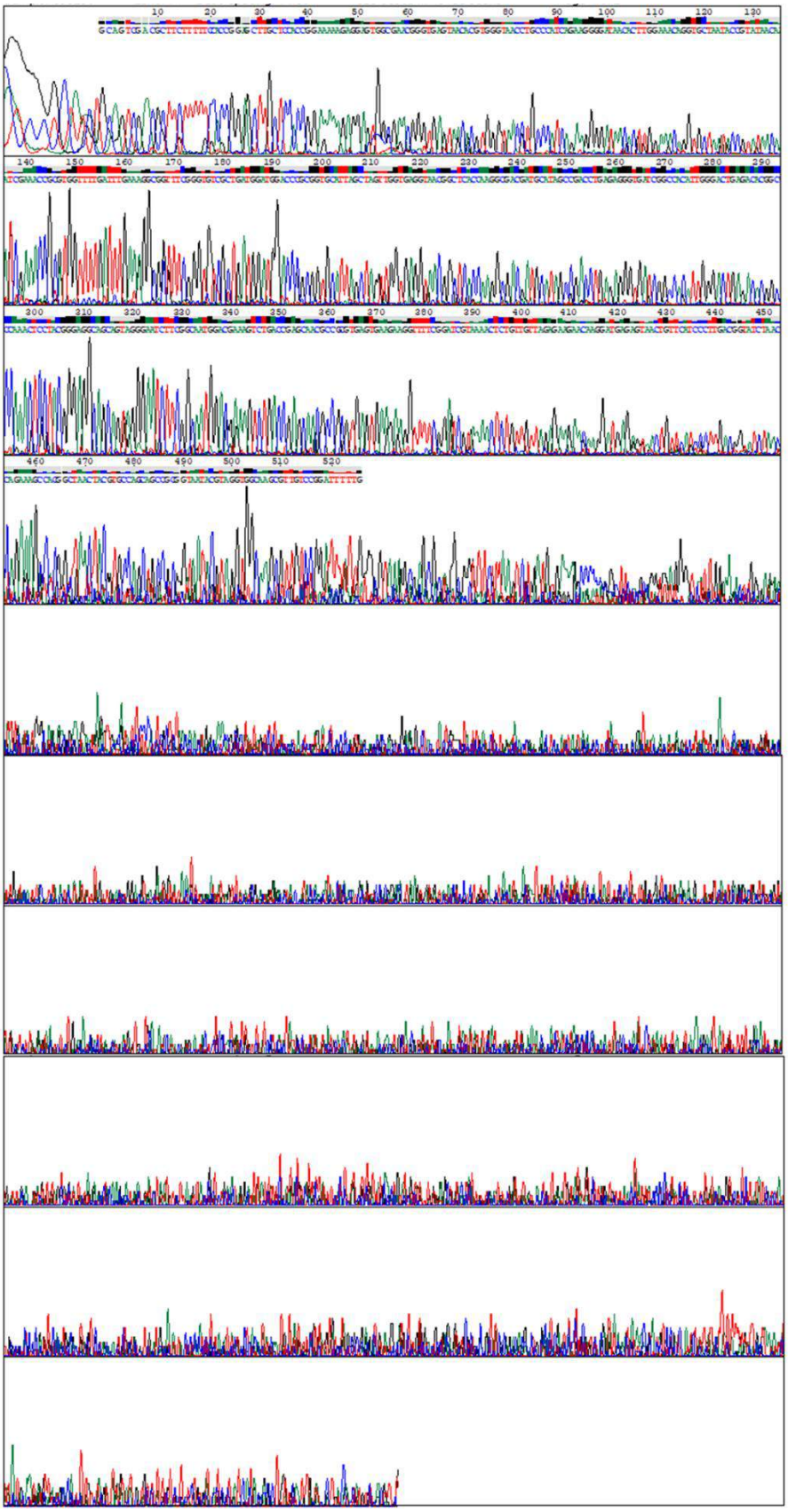


***Figure S.55. Chromatogram of forward sequence of REPS-29 isolate.*** *Low-quality bases at both ends of the sequence were trimmed using Chromas software with default setting.*

GCAGTCGACGCTTCTTTTTCCACCGGAGCTTGCTCCACCGGAAAAAGAGGAGTGGC
GAACGGGTGAGTAACACGTGGGTAACCTGCCCATCAGAAGGGGATAACACTTGGA
AACAGGTGCTAATACCGTATAACAATCGAAACCGCGTGGTTTTGATTTGAAAGGCG
GTTTCGGGTGTCGCTGATGGATGGACCCGCGGTGCATTAGCTAGTTGGTGAGGTAA
CGGCTCACCAAGGCGACGATGCATAGCCGACCTGAGAGGGTGATCGGCCACATTG
GGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTTCGGCA
ATGGACGAAAGTCTGACCGAGCAACGCCGCGTGAGTGAAGAAGGTTTTCGGATCGT
AAAACTCTGTTGTTAGAGAAGAACAAGGATGAGAGTAACTGTTCATCCCTTGACGG
TATCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGG
TGGCAAGCGTTGTCCGGATTTTTG

***Figure S.56. Forward sequence of REPS-29 isolate.*** *The FASTA sequence were exported from the chromatogram of forward sequence of REPS-29 isolate after the trim of low-quality bases at both ends.*

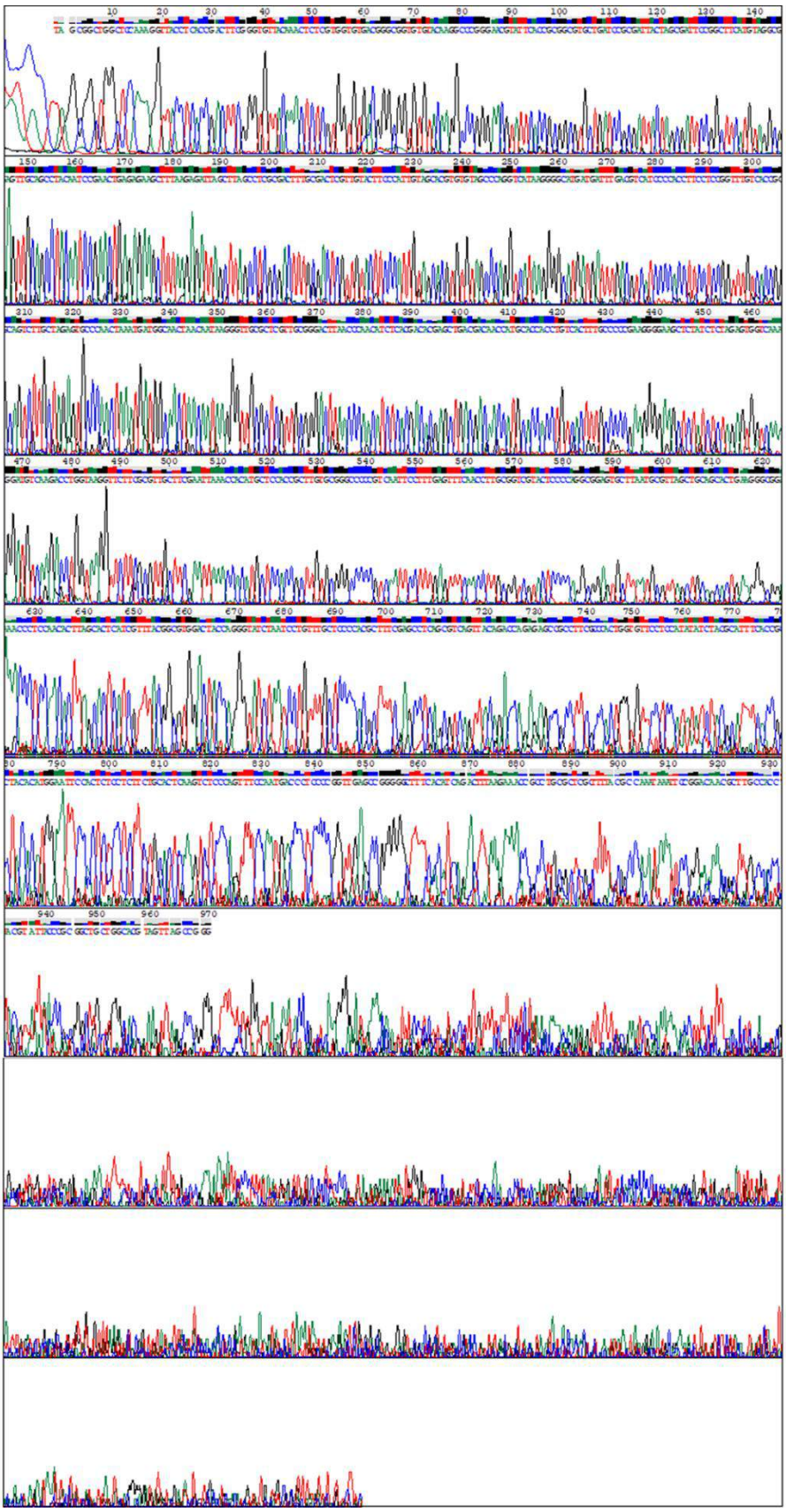


***Figure S.57. Chromatogram of reverse sequence of REPS-29 isolate.*** *Low-quality bases at both ends of the sequence were trimmed using Chromas software with default setting.*

CCCGGCTAACTACGTGCCAGCAGCCGCGGGTAATACGTAGGTGGCAAGCGTTGTCC
GGAATTTATTTGGCGTAAAAGCGAGCGCAGGCGGTTTCTTAAAGTCTGATGTGAAA
GCCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGAGACTTGAGTGCAGAAG
AGGAGAGTGGAATTCCATGTGTAGCGGTGAAATGCGTAGATATATGGAGGAACAC
CAGTGGGCGAAGGCGGCTCTCTGGTCTGTAACTGACGCTGAGGCTCGAAAGCGTGG
GGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAA
GTGTTGGAGGGTTTCCGCCCTTCAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTG
GGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGC
GGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACA
TCCTTTGACCACTCTAGAGATAGAGCTTCCCCTTCGGGGGCAAAGTGACAGGTGGT
GCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCG
CAACCCTTATTGTTAGTTGCCATCATTTAGTTGGGCACTCTAGCAAGACTGCCGGTG
ACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGG
CTACACACGTGCTACAATGGGAAGTACAACGAGTCGCAAAGTCGCGAGGCTAAGC
TAATCTCTTAAAGCTTCTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAG
CCGGAATCGCTAGTAATCGCGGATCAGCACGCCGCGGTGAATACGTTCCCGGGCCT
TGTACACACCGCCCGTCACACCACGAGAGTTTGTAACACCCGAAGTCGGTGAGGTA
ACCTTTGGAGCCAGCCGCTA

***Figure S.58. Reverse sequence of REPS-29 isolate.*** *The FASTA sequence were exported from the chromatogram of reverse sequence of REPS-29 isolate after the trim of low-quality bases at both ends.*

7.11 REPS-32 Isolate

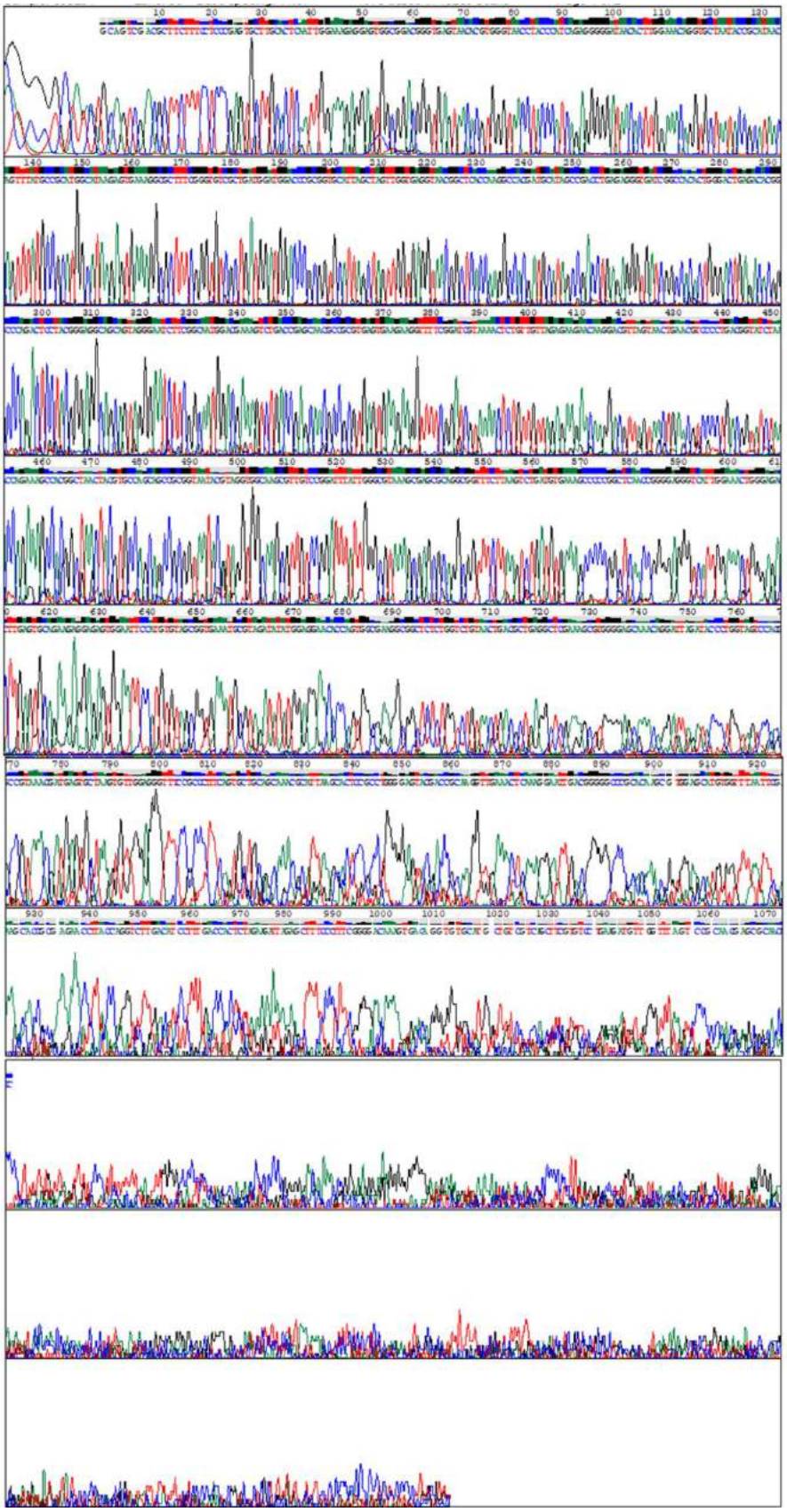


***Figure S.59. Chromatogram of forward sequence of REPS-32 isolate.*** *Low-quality bases at both ends of the sequence were trimmed using Chromas software with default setting.*

GCAGTCGACGCTTCTTTCCTCCCGAGTGCTTGCACTCAATTGGAAAGAGGAGTGGC
GGACGGGTGAGTAACACGTGGGTAACCTACCCATCAGAGGGGGATAACACTTGGA
AACAGGTGCTAATACCGCATAACAGTTTATGCCGCATGGCATAAGAGTGAAAGGCG
CTTTCGGGTGTCGCTGATGGATGGACCCGCGGTGCATTAGCTAGTTGGTGAGGTAA
CGGCTCACCAAGGCCACGATGCATAGCCGACCTGAGAGGGTGATCGGCCACACTG
GGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCGGCA
ATGGACGAAAGTCTGACCGAGCAACGCCGCGTGAGTGAAGAAGGTTTTCGGATCGT
AAAACTCTGTTGTTAGAGAAGAACAAGGACGTTAGTAACTGAACGTCCCCTGACGG
TATCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGG
TGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTCTTAAG
TCTGATGTGAAAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGAGACTT
GAGTGCAGAAGAGGAGAGTGGAATTCCATGTGTAGCGGTGAAATGCGTAGATATA
TGGAGGAACACCAGTGGCGAAGGCGGCTCTCTGGTCTGTAACTGACGCTGAGGCTC
GAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGA
TGAGTGCTAAGTGTTGGAGGGTTTCCGCCCTTTCAGTGCTGCAGCAAACGCATTAA
GCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGG
GCCCGCACAAGCGTGGAGCATGTGGTTTAATTCGAAGCACCGCGAGAACCTTACCA
GGTCTTGACATCCTTTGACCACTCTAGAAGATTAGAGCTTTCCCTTTCGGGGACAAA
GTGACAGGTGTGCATGCTGTCGTCAGCTTCGTGTCCTGAAGATGTTGGTTTAGTCCG
CAACGAGCGCAACCC

***Figure S.60. Forward sequence of REPS-32 isolate.*** *The FASTA sequence were exported from the chromatogram of forward sequence of REPS-32 isolate after the trim of low-quality bases at both ends.*

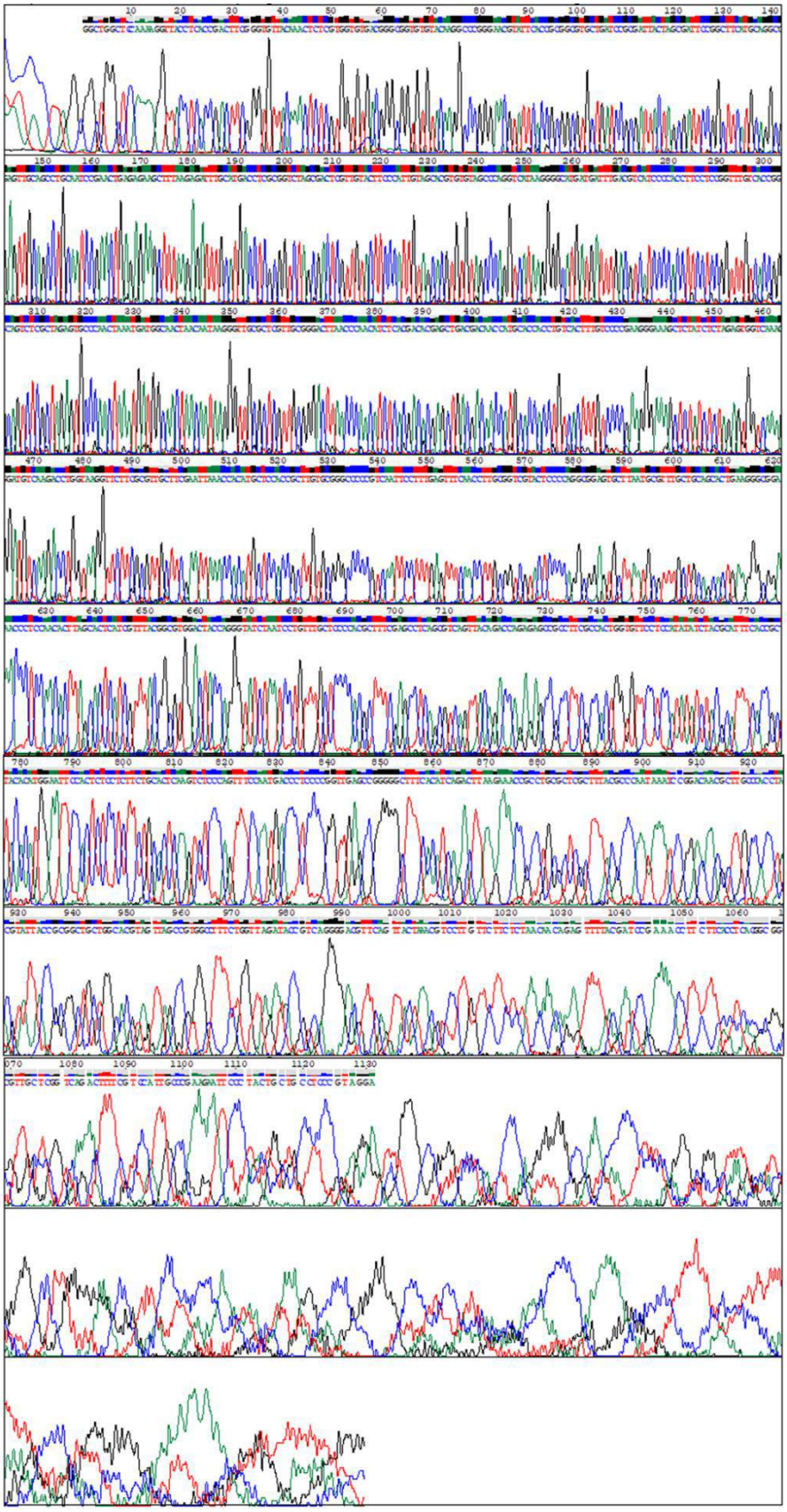


***Figure S.61. Chromatogram of reverse sequence of REPS-32 isolate.*** *Low-quality bases at both ends of the sequence were trimmed using Chromas software with default setting.*

TCCTACGGGAGGCAGCAGTAGGGAATTCTTCGGGCAATGGACGAAAAGTCTGACC
GAGCAACGCCGCCGTGAGGTGAAGAAGGTTTCGGATCGTAAAACTCTGTTGTTAGA
GAAGAACAAGGACGTTTAGTAACTGAACGTCCCCTGACGGTATCTAACCAGAAAG
GCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGGCAAGCGTTGT
CCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTCTTAAGTCTGATGTGAAAG
CCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGAGACTTGAGTGCAGAAGA
GGAGAGTGGAATTCCATGTGTAGCGGTGAAATGCGTAGATATATGGAGGAACACC
AGTGGCGAAGGCGGCTCTCTGGTCTGTAACTGACGCTGAGGCTCGAAAGCGTGGGG
AGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTG
TTGGAGGGTTTCCGCCCTTCAGTGCTGCAGCAAACGCATTAAGCACTCCGCCTGGG
GAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGG
TGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATC
CTTTGACCACTCTAGAGATAGAGCTTTCCCTTCGGGGACAAAGTGACAGGTGGTGC
ATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCA
ACCCTTATTGTTAGTTGCCATCATTTAGTTGGGCACTCTAGCGAGACTGCCGGTGAC
AAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCT
ACACACGTGCTACAATGGGAAGTACAACGAGTCGCTAGACCGCGAGGTCATGCAA
ATCTCTTAAAGCTTCTCTCAGTTCGGATTGCAGGCTGCAACTCGCCTGCATGAAGCC
GGAATCGCTAGTAATCGCGGATCAGCACGCCGCGGTGAATACGTTCCCGGGCCTTG
TACACACCGCCCGTCACACCACGAGAGTTTGTAACACCCGAAGTCGGTGAGGTAAC
CTTTTGGAGCCAGCC

***Figure S.62. Reverse sequence of REPS-32 isolate.*** *The FASTA sequence were exported from the chromatogram of reverse sequence of REPS-32 isolate after the trim of low-quality bases at both ends.*

7.12 REPS-33 Isolate

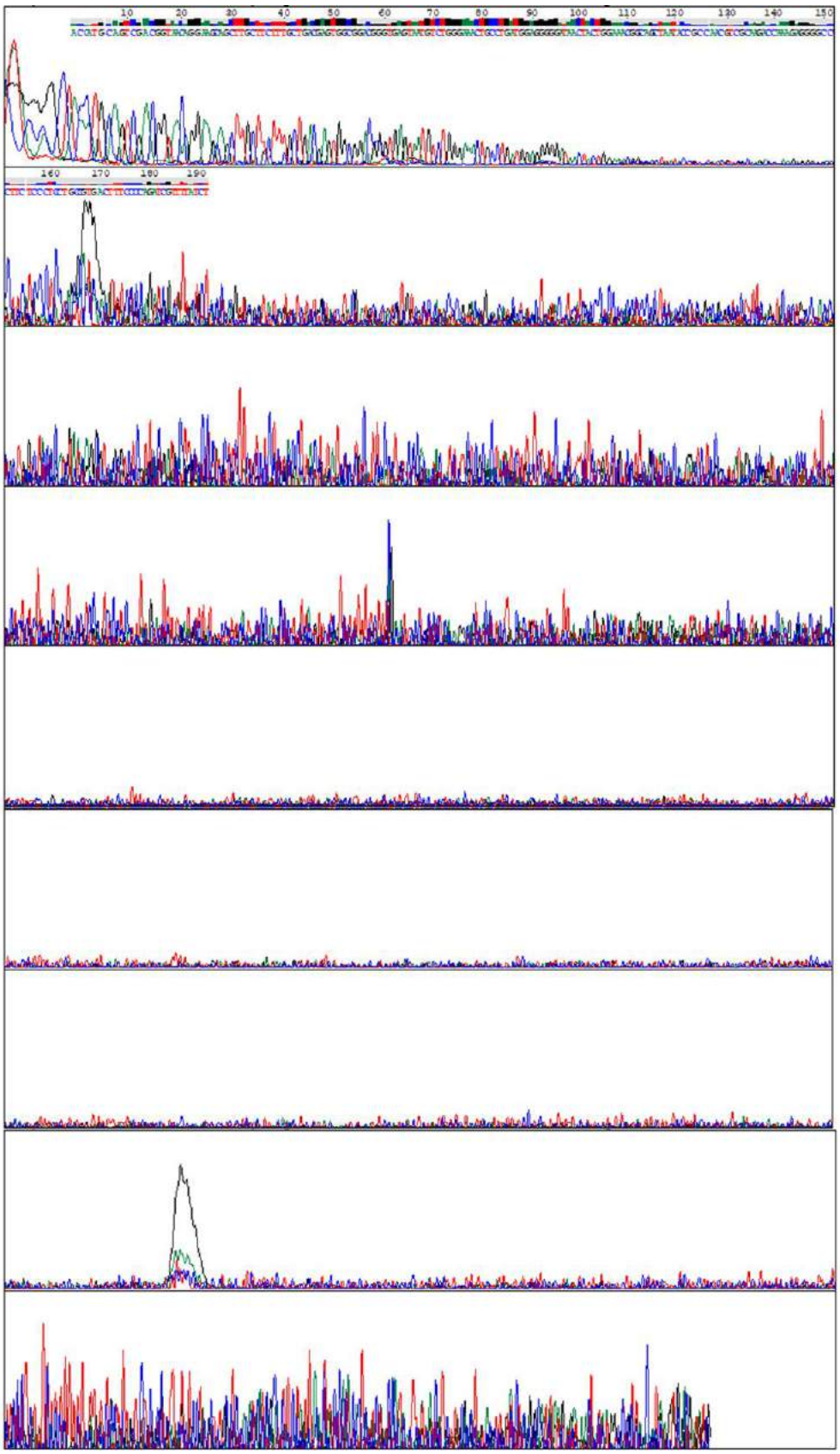


***Figure S.63. Chromatogram of forward sequence of REPS-33 isolate.*** *Low-quality bases at both ends of the sequence were trimmed using Chromas software with default setting.*

ACCATGCAGTCGACGGTAACAGGAAGCAGCTTGCTTCTTTGCTGACGAGTGGCGGA
CGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGAAACG
GCAGCTAATACCGCCAACGTCGCAAGACCAAAGAGGGGCCCTTCTCCCTCCTGCCG
TGACTTTCCCCAGATCGTTTTATCT

***Figure S.64. Forward sequence of REPS-33 isolate.*** *The FASTA sequence were exported from the chromatogram of forward sequence of REPS-33 isolate after the trim of low-quality bases at both ends.*

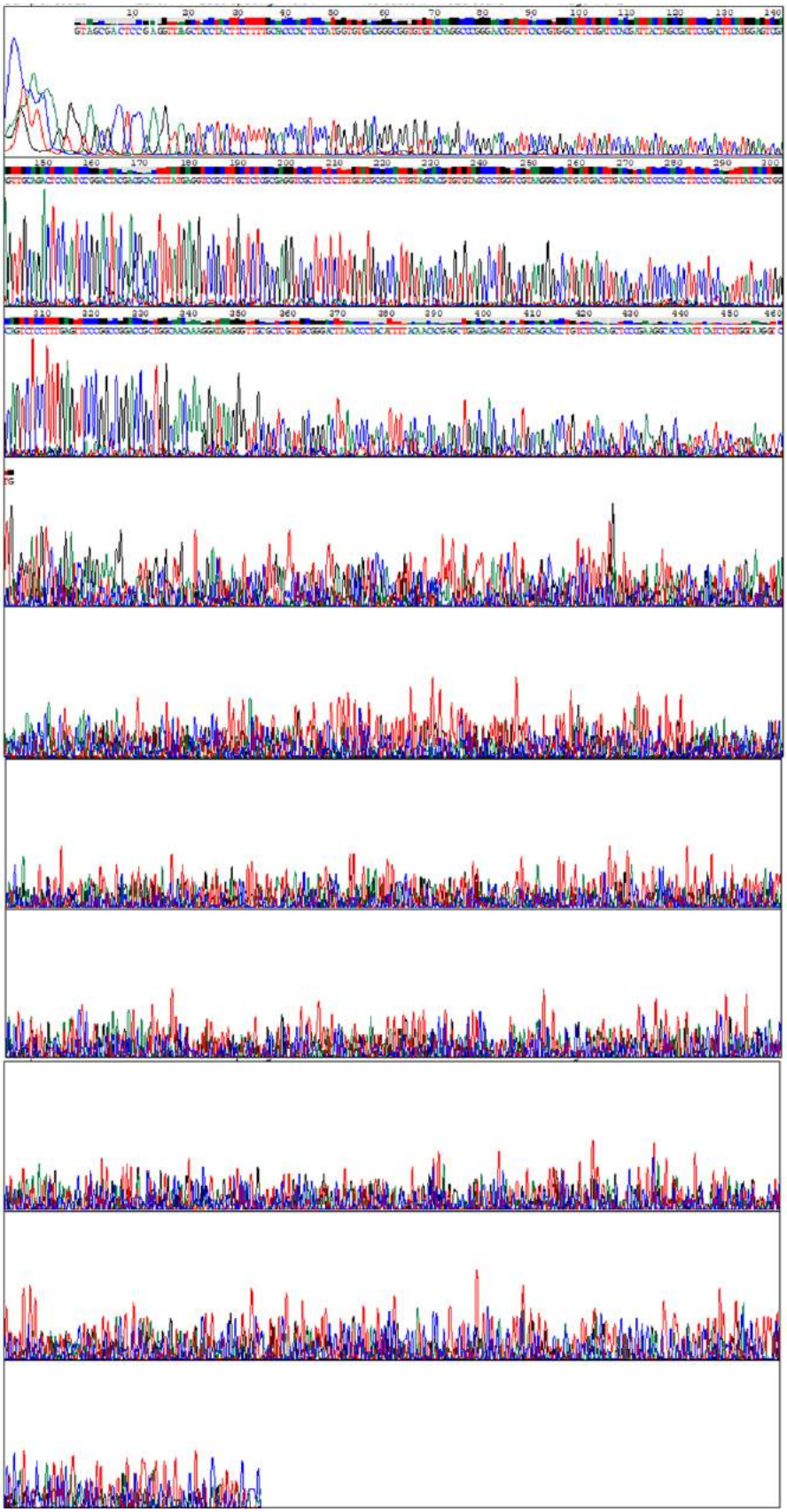


***Figure S.65. Chromatogram of reverse sequence of REPS-33 isolate.*** *Low-quality bases at both ends of the sequence were trimmed using Chromas software with default setting.*

CAGACCTTACCAAGAGATGAATTGGTGCCTTCGGGAGCTGTGAGACAGGTGCTGCA
TGACTGTCGTCAGCTCGTGTTGTAAAATGTAGGGTTAAGTCCCGCAACGAGCGCAA
CCCTTATCCTTTGTTGCCAGCGGTCCGGCCGGGAACTCAAAGGAGACTGCCAGTGA
TAAACTGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGACCAGGG
CTACACACGTGCTACAATGGCGCATACAAAGAGAAGCGACCTCGCGAGAGCAAGC
GGACCTCATAAAGTGCGTCGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAA
GTCGGAATCGCTAGTAATCGTGGATCAGAATGCCACGGTGAATACGTTCCCGGGCC
TTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCAAAAGAAGTAGGTAGCTT
AACCTCGGAGTCGCTAC

***Figure S.66. Reverse sequence of REPS-33 isolate.*** *The FASTA sequence were exported from the chromatogram of reverse sequence of REPS-33 isolate after the trim of low-quality bases at both ends.*

7.13 REPS-34 Isolate

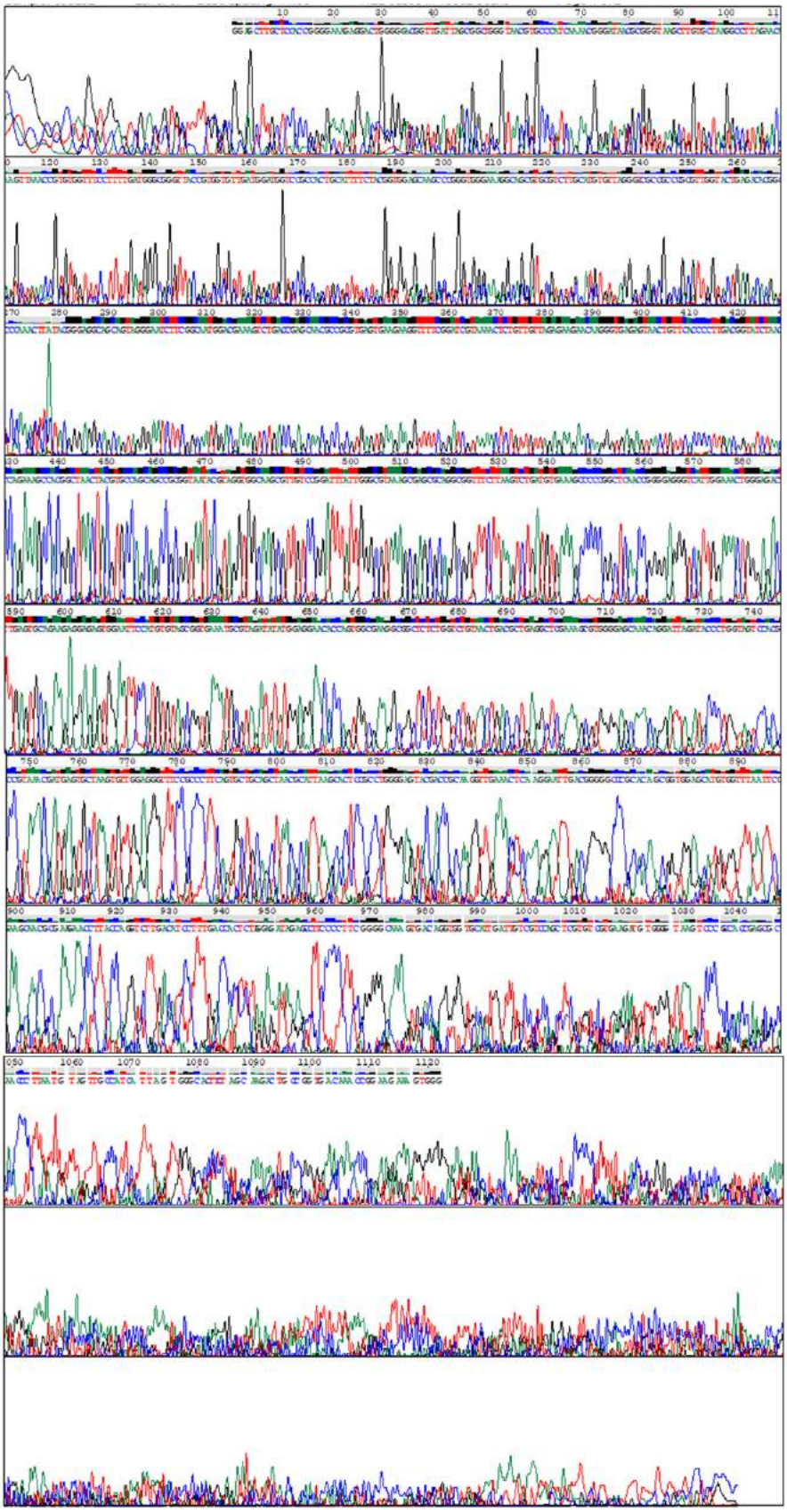


***Figure S.67. Chromatogram of forward sequence of REPS-34 isolate.*** *Low-quality bases at both ends of the sequence were trimmed using Chromas software with default setting.*

GGAGCTTGCTCCACCGGGGAAAGAGGACTGGGGGACGGTTGATTAGCGGCTGGGT
AACGTGCCCATCAAAACGGGATAACGCGGGTAAGCTTGTGCTAAGGCCTTAGAACA
AGTTAAACCGTGTGGTTTCCTTTTGATGGGCGGGTTACCGTGGTGTTGATGGATGGT
CCGCCACTGCATTTTCTACGGTGGAGCAAGCCCGGGTGGGAAAGGCAGCGTGCGTC
TTGCATGTGTTAGGGTCGCCGCCCGCGTTGGTACTGAGACACGGCCCAAACTTATA
CGGGAGGCAGCAGTAGGGAATCTTCGGCAATGGACGAAAGTCTGACCGAGCAACG
CCGCGTGAGTGAAGAAGGTTTTCGGATCGTAAAACTCTGTTGTTAGAGAAGAACAA
GGGTGAGAGTAACTGTTCACCCCTTGACGGTATCTAACCAGAAAGCCACGGCTAAC
TACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTATTGG
GCGTAAAGCGAGCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCCCGGCTCAAC
CGGGGAGGGTCATTGGAAACTGGGAGACTTGAGTGCAGAAGAGGAGAGTGGAATT
CCATGTGTAGCGGTGAAATGCGTAGATATATGGAGGAACACCAGTGGCGAAGGCG
GCTCTCTGGTCTGTAACTGACGCTGAGGCTCGAAAGCGTGGGGAGCAAACAGGATT
AGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTGGAGGGTTTCC
GCCCTTCAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGACCGCA
AGGTTGAAACTCAAGGAATTGACGGGGGCCCGCACAGCGGTGGAGCATGTGGTTT
AATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTTTGACCACTCTAG
GAGATAGAGCCTTCCCCTTCGGGGCAAAGTGACAGGTGGTGCATTGATTGTCGTCC
AGCTCGTGTCGTGAAGATGTGGGGTAAGTCCCGCCACCGAGCGCAACCCTTAATGT
AGTTGCCATCATTAGTGGGCACTTCTAGCAAGACTGCCGGTGACAAACCGGAAGAA
AGTGGG

***Figure S.68. Forward sequence of REPS-34 isolate.*** *The FASTA sequence were exported from the chromatogram of forward sequence of REPS-34 isolate after the trim of low-quality bases at both ends.*

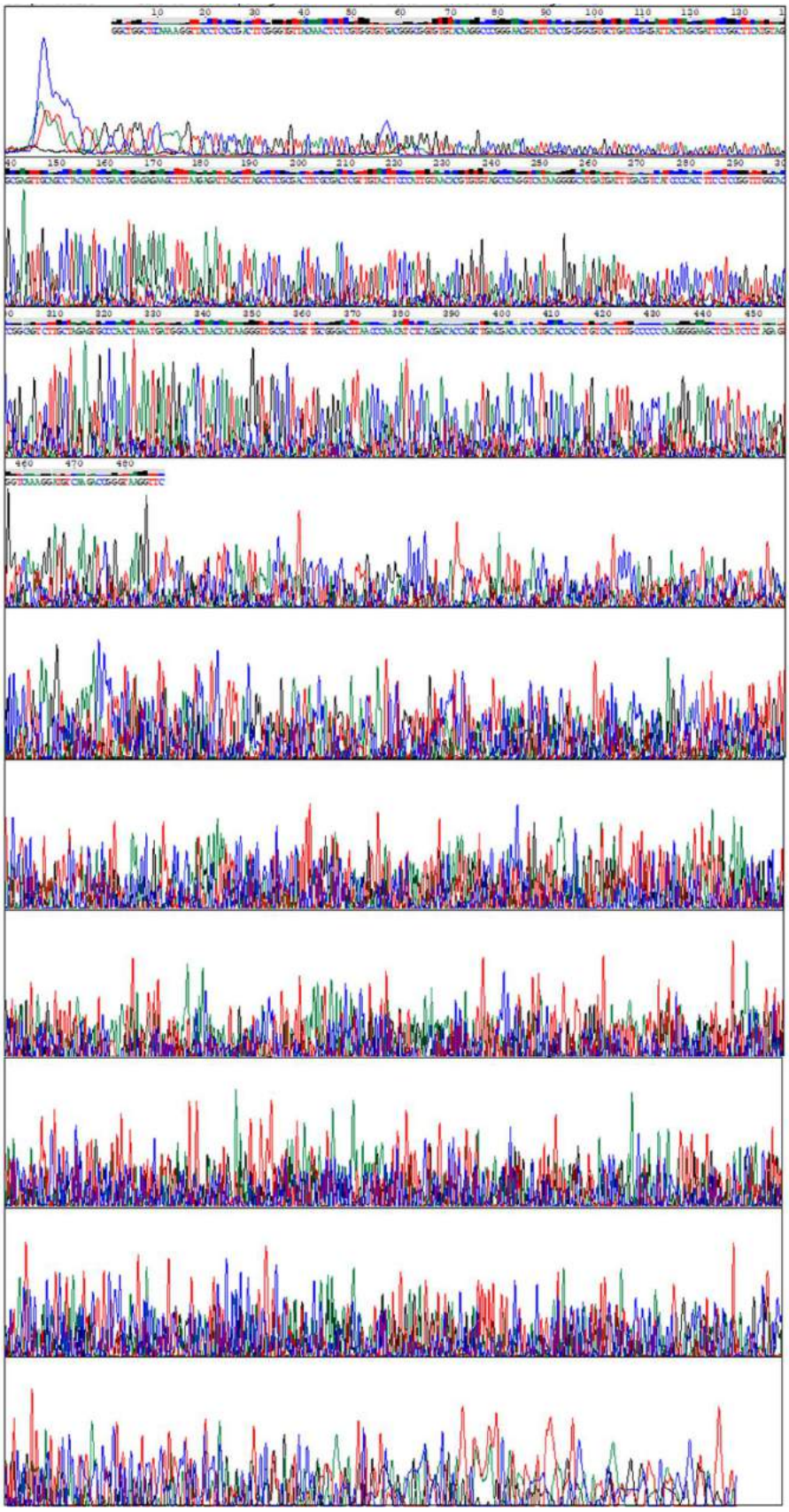


***Figure S.69. Chromatogram of reverse sequence of REPS-34 isolate.*** *Low-quality bases at both ends of the sequence were trimmed using Chromas software with default setting.*

GAACCTTACCCGGTCTTGACATCCTTTGACCACTCTAGAGATAGAGCTTCCCCTTGG
GGGGCAAAGTGACAGGTGGTGCATGGTTGTCGTCAGCTGGTGTCGTGAGATGTTGG
GTTAAGTCCCGCAACGAGCGCAACCCTTATTGTTAGTTGCCATCATTTAGTTGGGCA
CTCTAGCAAGACTGCCGGTGCCAAACCGGAGGAAGGTGGGGATGACGTCAAATCA
TCATGCCCCTTATGACCTGGGCTACACACGTGTTACAATGGGAAGTACAACGAGTC
GCGAAGTCGCGAGGCTAAGCTAATCTCTTAAAGCTTCTCTCAGTTCGGATTGTAGG
CTGCAACTCGCCTACATGAAGCCGGAATCGCTAGTAATCGCGGATCAGCACGCCGC
GGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCACGAGAGTTTGTA
ACACCCGAAGTCGGTGAGGTAACCTTTTGGAGCCAGCC

***Figure S.70. Reverse sequence of REPS-34 isolate.*** *The FASTA sequence were exported from the chromatogram of reverse sequence of REPS-34 isolate after the trim of low-quality bases at both ends.*

7.14 REPNE-1 Isolate

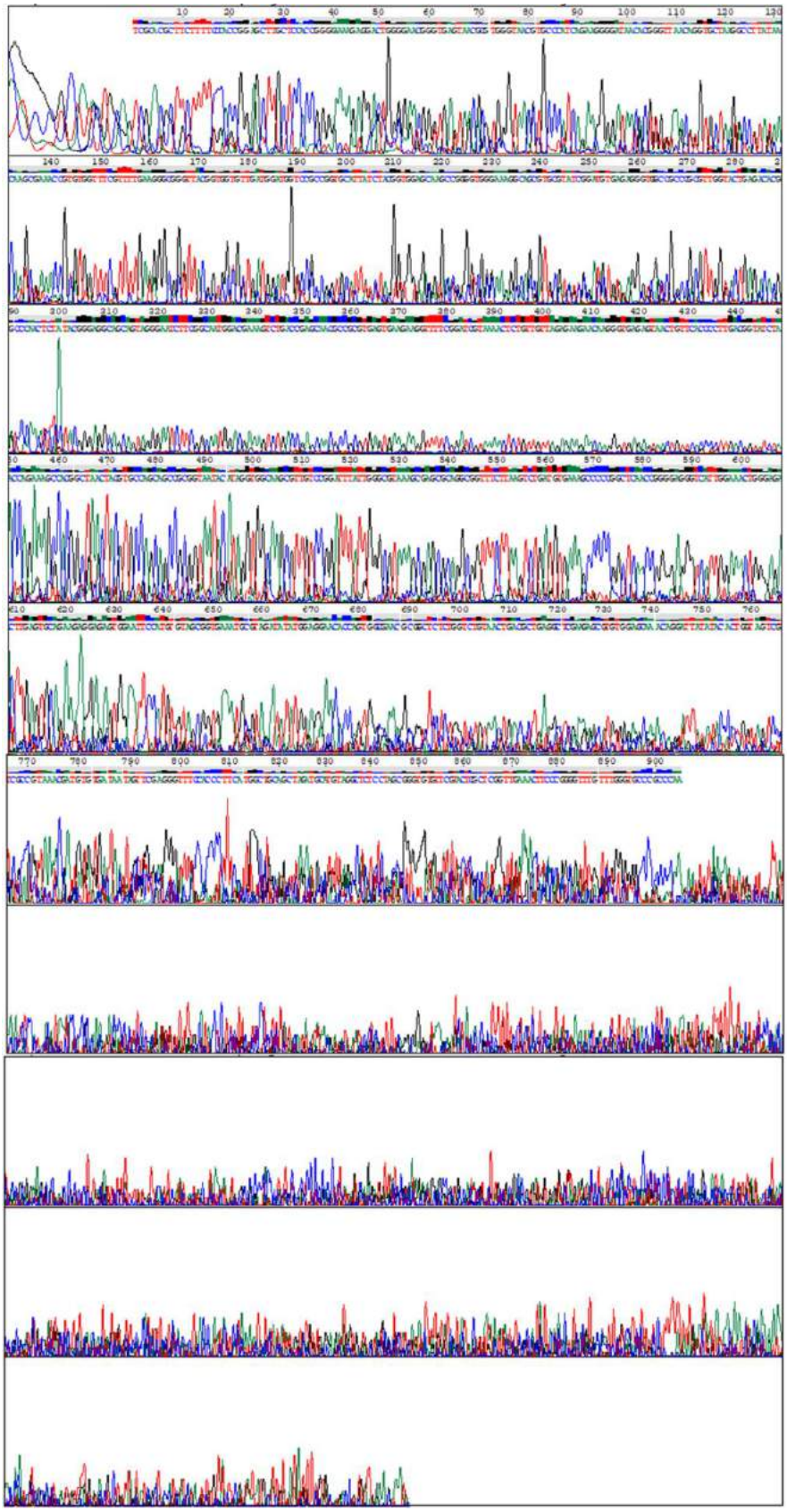


***Figure S.71. Chromatogram of forward sequence of REPNE-1 isolate.*** *Low-quality bases at both ends of the sequence were trimmed using Chromas software with default setting.*

TCGCACGCTTCTTTTCCCACCGGAGCTTGCTCCACCGGGGAAAAGAGGACTGGGGA
ACGGGTGAGTAACGCGTGGGTAACGTGCCCATCAGAAGGGGATAACACGGGTTAA
CAGGTGCTAAGGCCTTATAACAAGCGAAACCGTGTGGTTTCGTTTTGAAGGGCGGG
TTACGGTGGTGTTGATGGATGGTCCGCCGGTGCATTATCTACGGTGGAGCAAGCCG
GGGTGGGAAAGGCAGCGTGCGTATCGGATGTGAGAGGGTGGCCGCCCGCGTTGGT
ACTGAGACACGGCCCAACTCTATACGGGAGGCAGCAGTAGGGAATCTTCGGCAAT
GGACGAAAGTCTGACCGAGCAACGCCGCGTGAGTGAAGAAGGTTTTCGGATCGTA
AAACTCTGTTGTTAGAGAAGAACAAGGGTGAGAGTAACTGTTCACCCCTTGACGGT
ATCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACATAGGT
GGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTCTTAAGT
CTGATGTGAAAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGAGACTTG
AGTGCAGAAGAGGAGAGTGGAATTCCATGTGTAGCGGTGAAATGCGTAGATATAT
GGAGGAACACCAGTGGCGAACGCGGCTCTCTGGTCTGTAACTGACGCTGAGGCTCG
AAGAGCGTGTGGAGCAAACAGGATTATATACACTGGTAGTCGTCGCCGTAAACGAT
GTGTGATAATAGTTCGAGGGTTTCCACCCTTCATGGCTGCAGCTAGATGCATGTAG
GCTCTCCTAGCGGGTGTGTTCGTACTCGCTCGGTTGAAACTTCCCGGGGTTTGTTTG
GGTGCCCGCCCAA

***Figure S.72. Forward sequence of REPNE-1 isolate.*** *The FASTA sequence were exported from the chromatogram of forward sequence of REPNE-1 isolate after the trim of low-quality bases at both ends.*

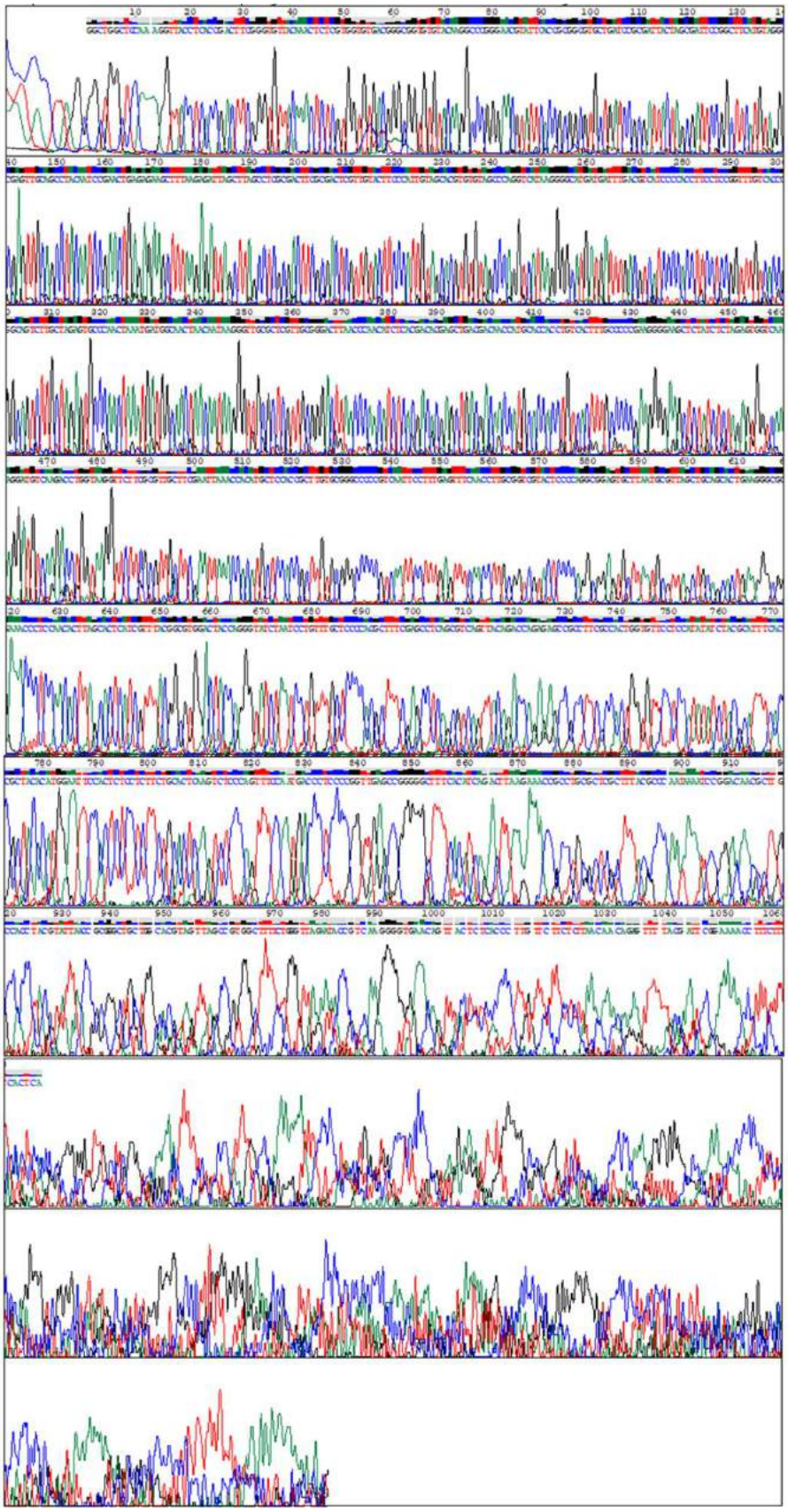


***Figure S.73. Chromatogram of reverse sequence of REPNE-1 isolate.*** *Low-quality bases at both ends of the sequence were trimmed using Chromas software with default setting.*

TGAGTGAAAGAAAGGTTTTCCGAATCGTAAAACTCTGTTGTTAAGAGAAGAACAAG
GGTGAGAGTAACTGTTCACCCCTTGACGGTATTCTAACCCAGAAAAGCCACGGCTA
ACTACGTGCCAGCAGCCCGCGGTTAATACGTAGGTGGCAAGCGTTGTCCGGATTTT
ATTGGGCGTAAAGCGAGCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCCCGGC
TCAACCGGGGAGGGTCATTGGAAACTGGGAGACTTGAGTGCAGAAGAGGAGAGTG
GAATTCCATGTGTAGCGGTGAAATGCGTAGATATATGGAGGAACACCAGTGGCGA
AGGCGGCTCTCTGGTCTGTAACTGACGCTGAGGCTCGAAAGCGTGGGGAGCAAACA
GGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTGGAGGG
TTTCCGCCCTTCAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGA
CCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCAT
GTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTTTGACC
ACTCTAGAGATAGAGCTTCCCCTTCGGGGGCAAAGTGACAGGTGGTGCATGGTTGT
CGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTAT
TGTTAGTTGCCATCATTTAGTTGGGCACTCTAGCAAGACTGCCGGTGACAAACCGG
AGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACG
TGCTACAATGGGAAGTACAACGAGTCGCGAAGTCGCGAGGCTAAGCTAATCTCTTA
AAGCTTCTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAGCCGGAATCG
CTAGTAATCGCGGATCAGCACGCCGCGGTGAATACGTTCCCGGGCCTTGTACACAC
CGCCCGTCACACCACGAGAGTTTGTAACACCCGAAGTCGGTGAGGTAACCTTTGGA
GCCAGCC

***Figure S.74. Reverse sequence of REPNE-1 isolate.*** *The FASTA sequence were exported from the chromatogram of reverse sequence of REPNE-1 isolate after the trim of low-quality bases at both ends.*

7.15 REPNE-2 Isolate

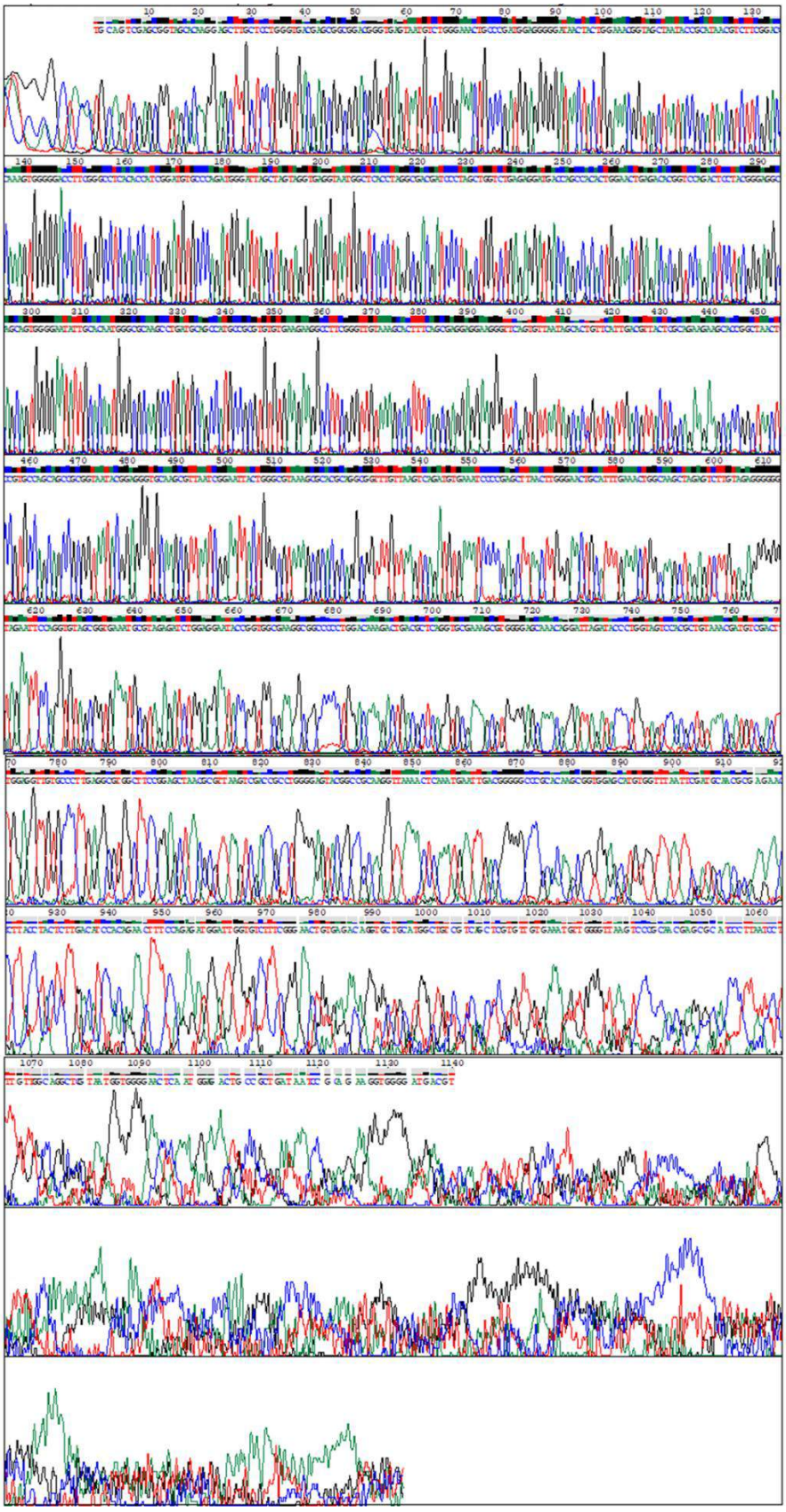


***Figure S.75. Chromatogram of forward sequence of REPNE-2 isolate.*** *Low-quality bases at both ends of the sequence were trimmed using Chromas software with default setting.*

TGCAGTCGAGCGGTAGCACAAGGAGCTTGCTCCTGGGTGACGAGCGGCGGACGGG
TGAGTAATGTCTGGGAAACTGCCCGATGGAGGGGGATAACTACTGGAAACGGTAG
CTAATACCGCATAACGTCTTCGGACCAAAGTGGGGGACCTTCGGGCCTCACACCAT
CGGATGTGCCCAGATGGGATTAGCTAGTAGGTGAGGTAATGGCTCACCTAGGCGAC
GATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGACACGGTCCA
GACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATG
CAGCCATGCCGCGTGTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTTTCAGCGAGG
AGGAAGGGTTCAGTGTTAATAGCACTGTTCATTGACGTTACTCGCAGAAGAAGCAC
CGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGA
ATTACTGGGCGTAAAGCGCACGCAGGCGGTTTGTTAAGTCAGATGTGAAATCCCCG
AGCTTAACTTGGGAACTGCATTTGAAACTGGCAAGCTAGAGTCTTGTAGAGGGGGG
TAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGC
GAAGGCGGCCCCCTGGACAAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAA
ACAGGATTAGATACCCTGGTAGTCCACGCTGTAAACGATGTCGACTTGGAGGTTGT
GCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTAC
GGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGC
ATGTGGTTTAATTCGATGCAACGCGAGAACCTTACCTACTCTTGACATCCACAGAA
CTTTCCAGAGATGGATTGGTGTCTTTCGGGAACTGTGAGACAGGTGCTGCATGGCT
GTCGTCAGCTCGTGTTGTGAAATGTTGGGGTTAAGTCCCGCAACGAGCGCATCCCTT
AATCCTTTGTTGGCAGGCTCGTAATGGTGGGGAACTCAATGGAGACTGCCGCTGAT
AATCCGCAGAAGGTGGGGATGACGT

***Figure S.76. Forward sequence of REPNE-2 isolate.*** *The FASTA sequence were exported from the chromatogram of forward sequence of REPNE-2 isolate after the trim of low-quality bases at both ends.*

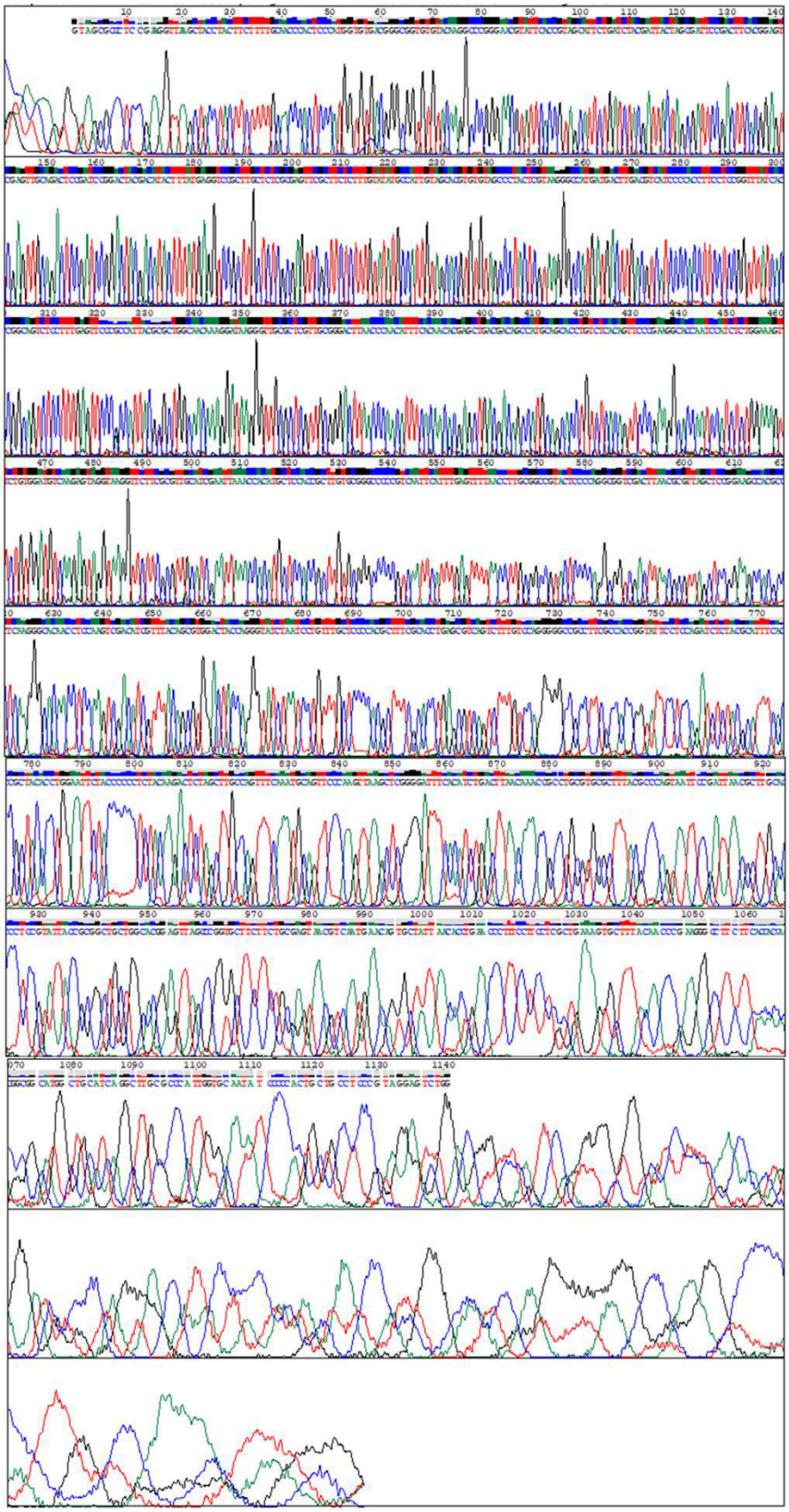


***Figure S.77. Chromatogram of reverse sequence of REPNE-2 isolate.*** *Low-quality bases at both ends of the sequence were trimmed using Chromas software with default setting.*

CCAGACTCCTACGGGAGGCAGCAGTGGGGGATATTGCACCAATGGGCGCAAGCCT
GATGCAGCCATGCCGCCGTGGTGGTGAAGAAGGCCCTTCGGGTTGTAAAGCACTTT
CAGCGAGGAAGGAAAGGGTTCAGGTGTTAATAGCACTGTTCATTGACGTTACTCGC
AGAAGAAGCACCGGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCA
AGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTTTGTTAAGTCAGA
TGTGAAATCCCCGAGCTTAACTTGGGAACTGCATTTGAAACTGGCAAGCTAGAGTC
TTGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAG
GAATACCGGTGGCGAAGGCGGCCCCCTGGACAAAGACTGACGCTCAGGTGCGAAA
GCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCTGTAAACGATGTCG
ACTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAGTCGACC
GCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCA
CAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTACTCT
TGACATCCACAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTGTGAGACAG
GTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACG
AGCGCAACCCTTATCCTTTGTTGCCAGCGCGTAATGGCGGGAACTCAAAGGAGACT
GCCGGTGATAAACCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTAC
GAGTAGGGCTACACACGTGCTACAATGGCATATACAAAGAGAAGCGAACTCGCGA
GAGCAAGCGGACCTCATAAAGTATGTCGTAGTCCGGATCGGAGTCTGCAACTCGAC
TCCGTGAAGTCGGAATCGCTAGTAATCGTAGATCAGAATGCTACGGTGAATACGTT
CCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCAAAAGAAGTA
GGTAGCTTAACCTTCGGAGGGCGCTAC

***Figure S.78. Reverse sequence of REPNE-2 isolate.*** *The FASTA sequence were exported from the chromatogram of reverse sequence of REPNE-2 isolate after the trim of low-quality bases at both ends.*

7.16 REPNE-3 Isolate

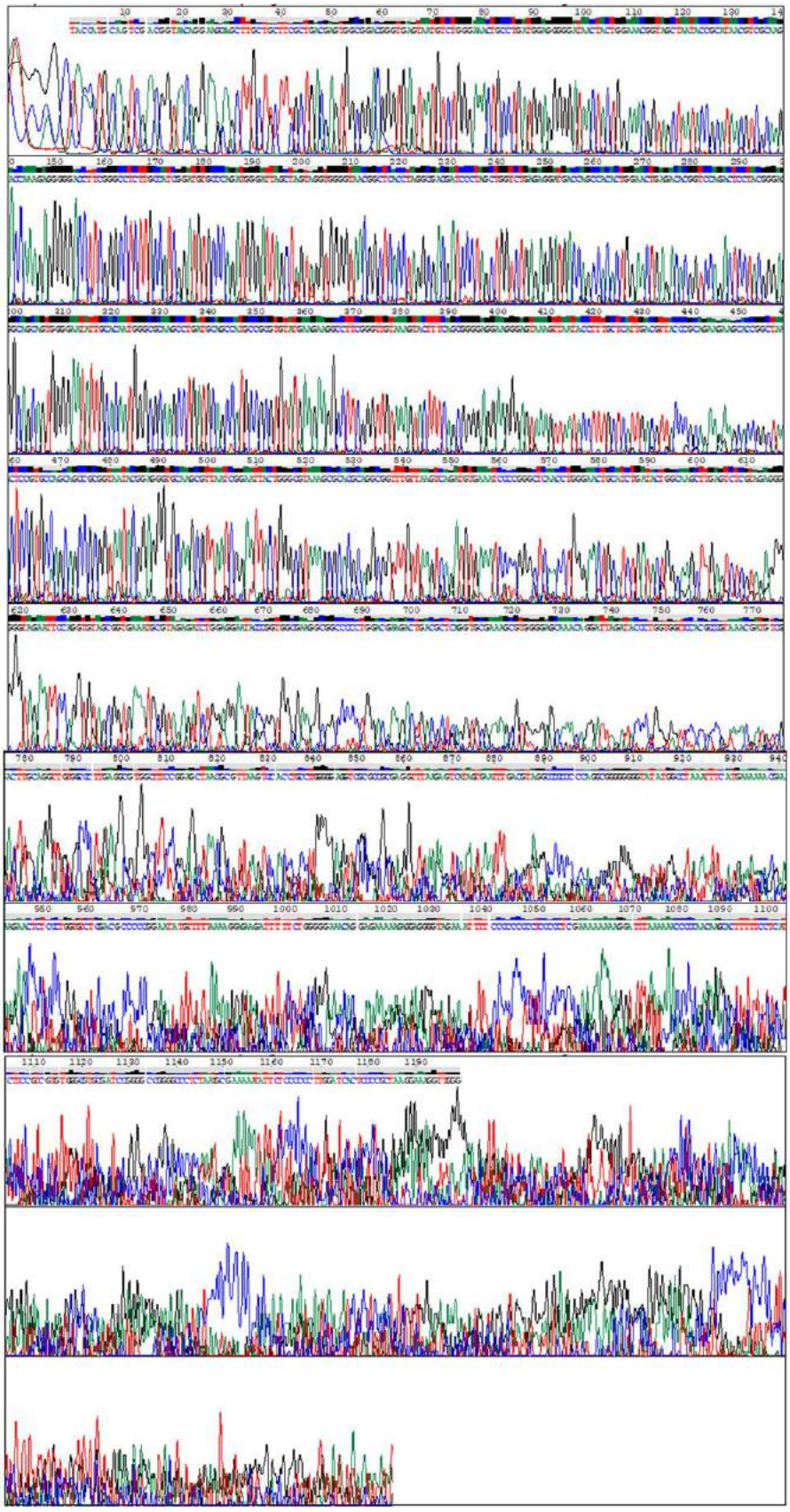


***Figure S.79. Chromatogram of forward sequence of REPNE-3 isolate.*** *Low-quality bases at both ends of the sequence were trimmed using Chromas software with default setting.*

TACCATGCAGTCGACGGTAACAGGAAGCAAGCTTGCTGCTTCGCTGACGAGTGGCG
GACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGAAA
CGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGAGGGGGACCTTCGGGCCTCT
TGCCATCGGATGTGCCCAGATGGGATTAGCTAGTAGGTGGGGTAACGGCTCACCTA
GGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGACA
CGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAG
CCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTC
AGCGGGGAGGAAGGGAGTAAAGTTAATACCTTTGCTCATTGACGTTACCCGCAGAA
GAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGT
TAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTTTGTTAAGTCAGATGTGA
AATCCCCGGGCTCAACCTGGGAACTGCATCTGATACTGGCAAGCTTGAGTCTCGTA
GAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATA
CCCGGTGGCGAAGGCGGCCCCCTGGACGAAGACTGACGCTCAGGTGCGAAAGCGT
GGGGAGCAAACAGGATTAGATACCCTGGTGGTTCCACGCCCGTAAACGATGTCGAC
TTGCAGGTTGTGGCCCTTGAGGCGTGGCTTTCCGGAGCTAACGCGTTAAGTCCACCT
GCCTTGGGGGAGGTCGCGCCGCGAGGTTTAAGAGTCATAGTGAATTTGACGTAGGC
CCCCCCCCAGGCGGGGGGGGTATATGGCCTAAATTTCATGAAAAAACGAAAAGAA
CTCTCCCTGGTGTCTCGACGCCCCCGGAATATGTTTTTAAAAGGAGAAGATTTTTCT
GGGGGAAACAGGAGAAAAAGAGGAGGGGTAGAAATTTTCCCCCCCCCTCCCCCTC
GAAAAAAAAGGATTTAAAAAACCCCCAACAAGCACTTTTTCCTCATCTTCCGTCGT
GTGGGCGTGCGATCCGGGGCCGGGGCCCTCTAATGCGAAAAATATTCTCCCCCCTT
GGATCACTCCCCGCTAAGGAAAGGTTGGG

***Figure S.80. Forward sequence of REPNE-3 isolate.*** *The FASTA sequence were exported from the chromatogram of forward sequence of REPNE-3 isolate after the trim of low-quality bases at both ends.*

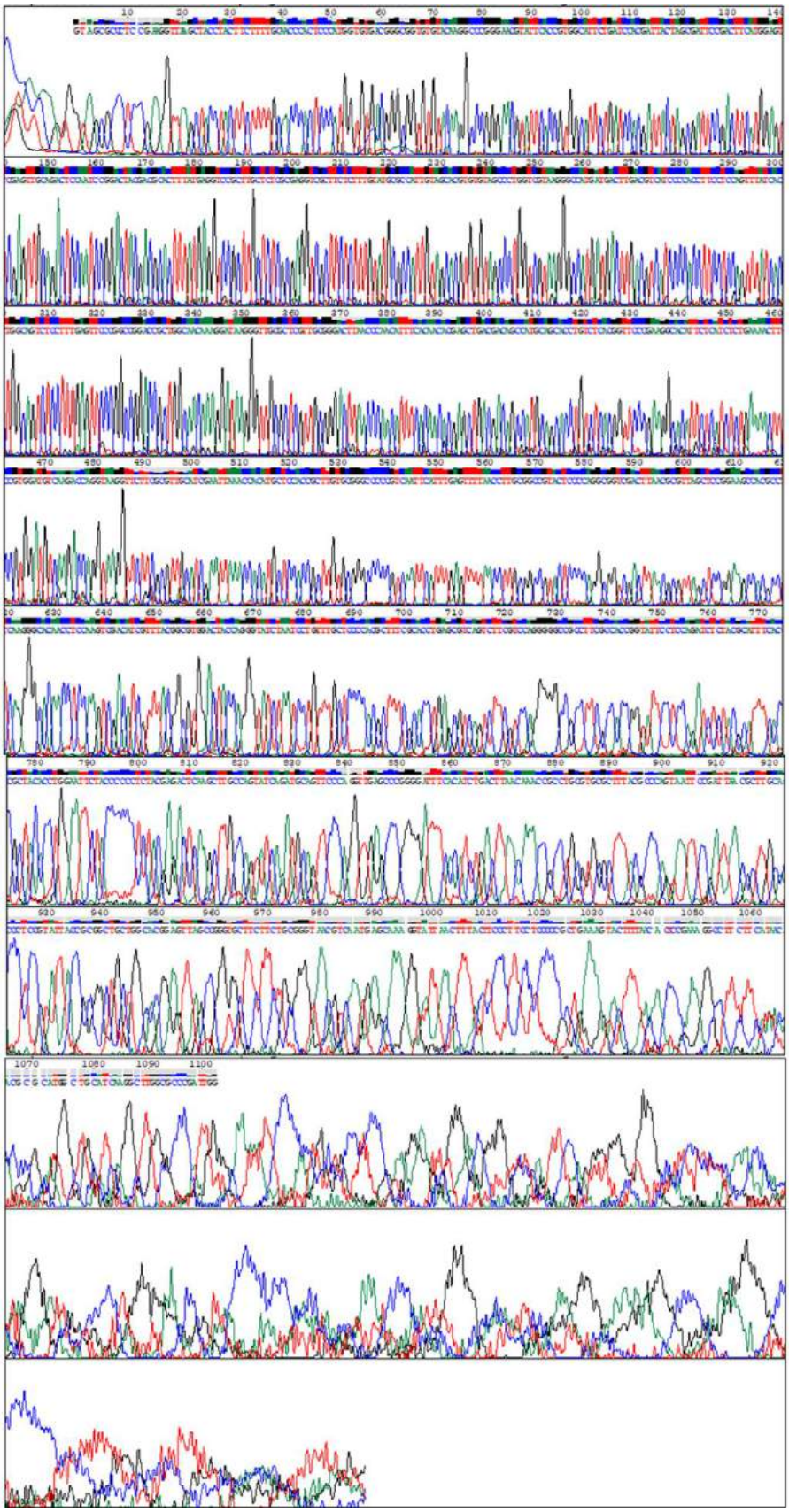


***Figure S.81. Chromatogram of reverse sequence of REPNE-3 isolate.*** *Low-quality bases at both ends of the sequence were trimmed using Chromas software with default setting.*

CCAATCGGGCGCCAAGCCTTGATGCAGCCATGCGCGTGTTATGAAGAAGGCCTTTC
GGAGTGTTAAAAGTACTTTCAGCGGGGGAGGAAGGGAAGTAAAGTTAATACCTTTG
CTCATTGACGTTACCCGCAGAAGAAGCACCCGGCTAACTCCGTGCCAGCAGCCGCG
GTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAG
GCGGTTTGTTAAGTCAGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATCTGA
TACTGGCAAGCTTGAGTCTCGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAA
TGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACGAAGACT
GACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCC
ACGCCGTAAACGATGTCGACTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGC
TAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGA
ATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGA
AGAACCTTACCTGGTCTTGACATCCACGGAAGTTTTCAGAGATGAGAATGTGCCTT
CGGGAACCGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGAAATGTT
GGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTCCGGCCG
GGAACTCAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGGATGACGTCAA
GTCATCATGGCCCTTACGACCAGGGCTACACACGTGCTACAATGGCGCATACAAAG
AGAAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTGCGTCGTAGTCCGGATT
GGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGTGGATCAGAAT
GCCACGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGT
GGGTTGCAAAAGAAGTAGGTAGCTTAACCTTCGGAGGGCGCTAC

***Figure S.82. Reverse sequence of REPNE-3 isolate.*** *The FASTA sequence were exported from the chromatogram of reverse sequence of REPNE-3 isolate after the trim of low-quality bases at both ends.*

7.17 REPNE-4 Isolate

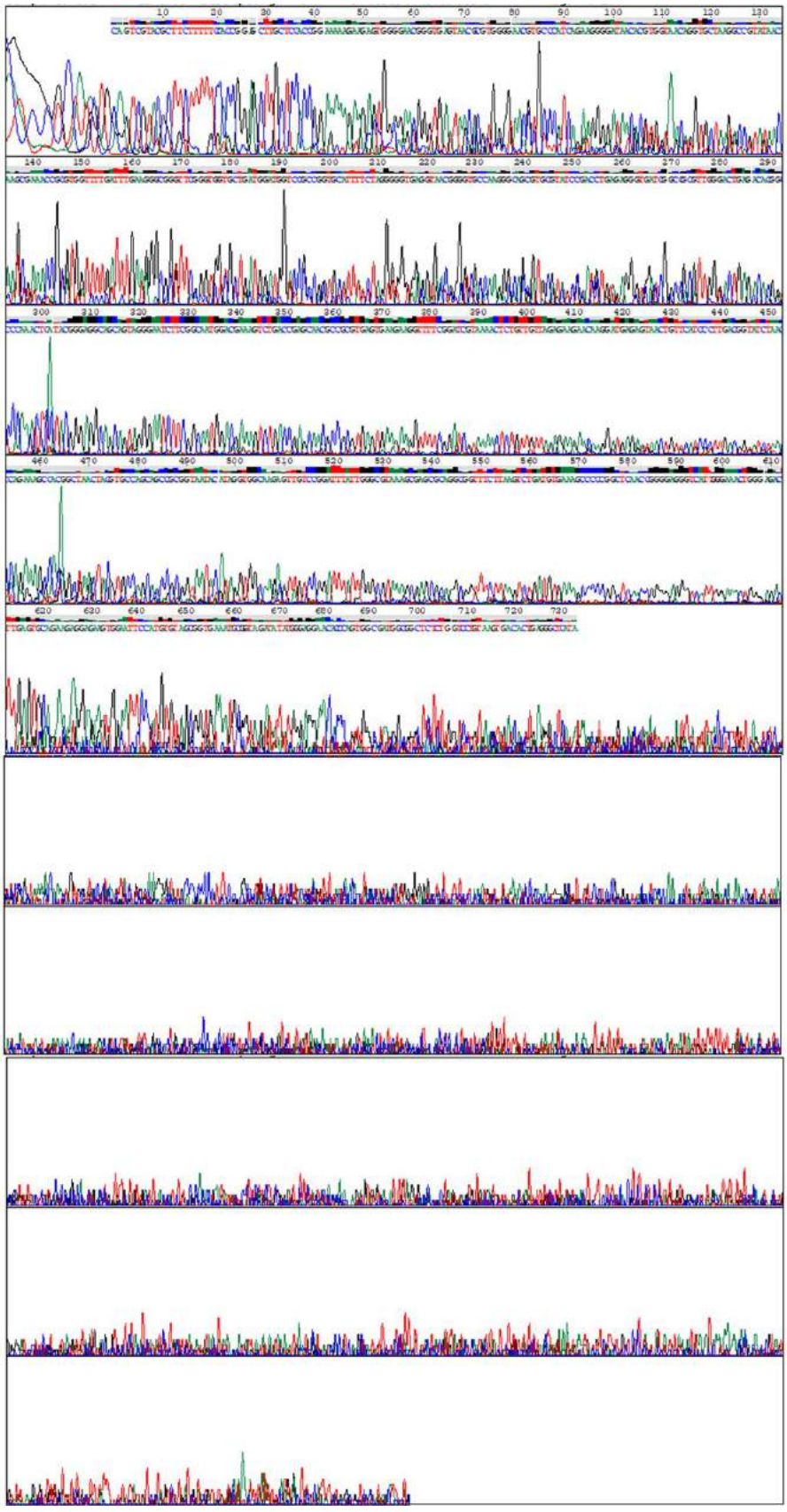


***Figure S.83. Chromatogram of forward sequence of REPNE-4 isolate.*** *Low-quality bases at both ends of the sequence were trimmed using Chromas software with default setting.*

CAGTCGTACGCTTCTTTTTCCACCGGAGCTTGCTCCACCGGAAAAAGAAGAGTGGG
GAACGGGTGAGTAACGCGTGGGGAACGTGCCCATCAGAAGGGGATAACACGTGGT
AACAGGTGCTAAGGCCGTATAACAAGCGAAACCGCGTGGTTTTGATTTGAAGGGCG
GGTTCGGGTGGTGCTGATGGATGGTCCGCCGGTGCATTTTCTAGGGGGTGAGGTAA
CGGGGTGCCAAGGGCAGCGTGCGTATCCGACCTGAGAGGGTGATCGGCCGCGTTG
GGACTGAGACACGGCCCAAACTCATACGGGAGGCAGCAGTAGGGAATCTTCGGCA
ATGGACGAAAGTCTGACCGAGCAACGCCGCGTGAGTGAAGAAGGTTTTCGGATCGT
AAAACTCTGTTGTTAGAGAAGAACAAGGATGAGAGTAACTGTTCATCCCTTGACGG
TATCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACATAGG
TGGCAAGAGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTCTTAAG
TCTGATGTGAAAGCCCCCGGCTCAACCGGGGAGGGTCATTGGGAAACTGGGAGACT
TGAGTGCAGAAGAGGAGAAGTGGAATTCCATGTGTAGCGGTGAAATGCGGTAGAT
ATATGGGAGGAACACCCAGTGGCGATGGCGGCTCTCTGGTCCTGTAAGTGACACTG
AGGGCTCATA

***Figure S.84. Forward sequence of REPNE-4 isolate.*** *The FASTA sequence were exported from the chromatogram of forward sequence of REPNE-4 isolate after the trim of low-quality bases at both ends.*

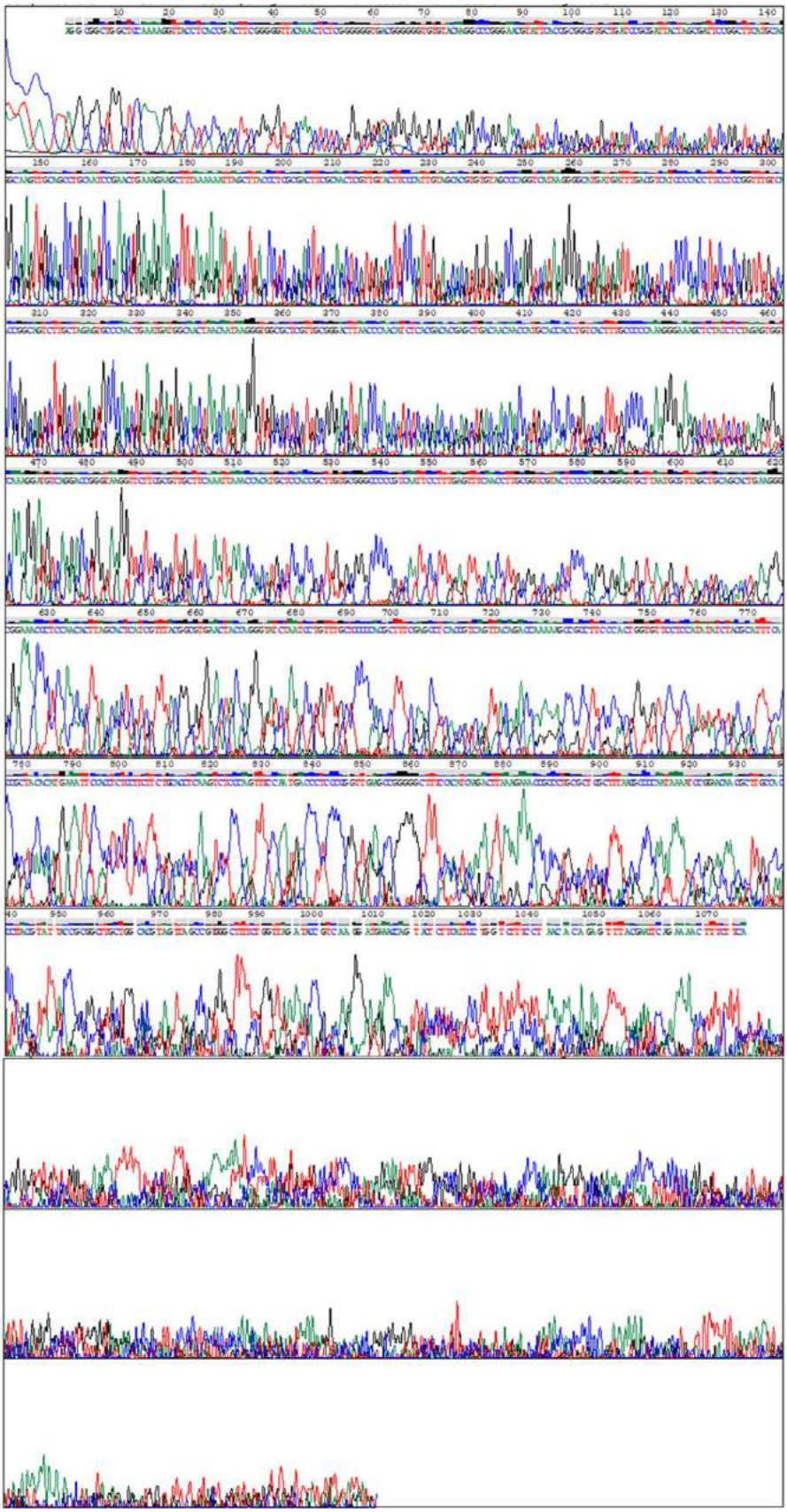


***Figure S.85. Chromatogram of reverse sequence of REPNE-4 isolate.*** *Low-quality bases at both ends of the sequence were trimmed using Chromas software with default setting.*

TGAAGAAAGTTTTCTGAATTCGTAAACTCTGTGTTAGGAAAGACCAGGAATGAAGA
GTACTGGTTTCATCCTTGACGGTATCTAACCAGAAAAGCCCACGGCTAACTACGTG
CCAGCAAGCCGCGGTAATACGTAAGGGTGGCAAGCGTTGTTCCGGATTTTATTGGG
GCGTTAAAGCGAGCGCAGGGCGGTTTCTTTAAGTCTTGATGTGGAAAGCCCCCGGC
TTCAACCGGGGAGGGTCATTGGAAACTGGGAGACTTGAGGTGCAGAAGAAGGAGA
GGTGGAATTTCATGTGTAGCGGTGAAATGCGTAGATATATGGAGGAACACCAGTGG
GAAGGCGGCTTTTTGGTCTGTAACTGACGGTGAGGCTCGAAAGCGTGGGGGAGCAA
ACAGGATTAGATACCCTGGTAGTTCACGCCGTAAACGATGAGTGCTAAGTGTTGGA
GGGTTTCCGCCCTTCAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTA
CGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAG
CATGTGGTTTAATTTGAAGCAACGCGAAGAACCTTACCCGGTCCTGACATCCTTTGA
CCACTCTAGAGATAGAGCTTTCCCTTTGGGGGCAAAGTGACAGGTGGTGCATGGTT
GTTGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCCACCCTT
ATTGTTAGTTGCCATCATTCAGTTGGGCACTCTAGCAAGACTGCCGGTGACAAACC
GGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACA
CGTGCTACAATGGGAAGTACAACGAGTTGCGAAGTCGCGAGGGTAAGCTAATTTTT
TAAAGCTTCTTTCAGTTCGGATTGCAGGCTGCAACTTGCCTGCATGAAGCCGGAATC
GCTAGTAATCGCGGATCAGCACGCCGCGGTGAATACGTTCCCGGGCCTTGTACACA
CCCCCCGTCACCCCCCGAGAGTTTGTAACCCCCGAAGTCGGTGAGGTAACCTTTTG
GAGCCAGCCGCCT

***Figure S.86. Reverse sequence of REPNE-4 isolate.*** *The FASTA sequence were exported from the chromatogram of reverse sequence of REPNE-4 isolate after the trim of low-quality bases at both ends.*

7.18 REPNE-5 Isolate

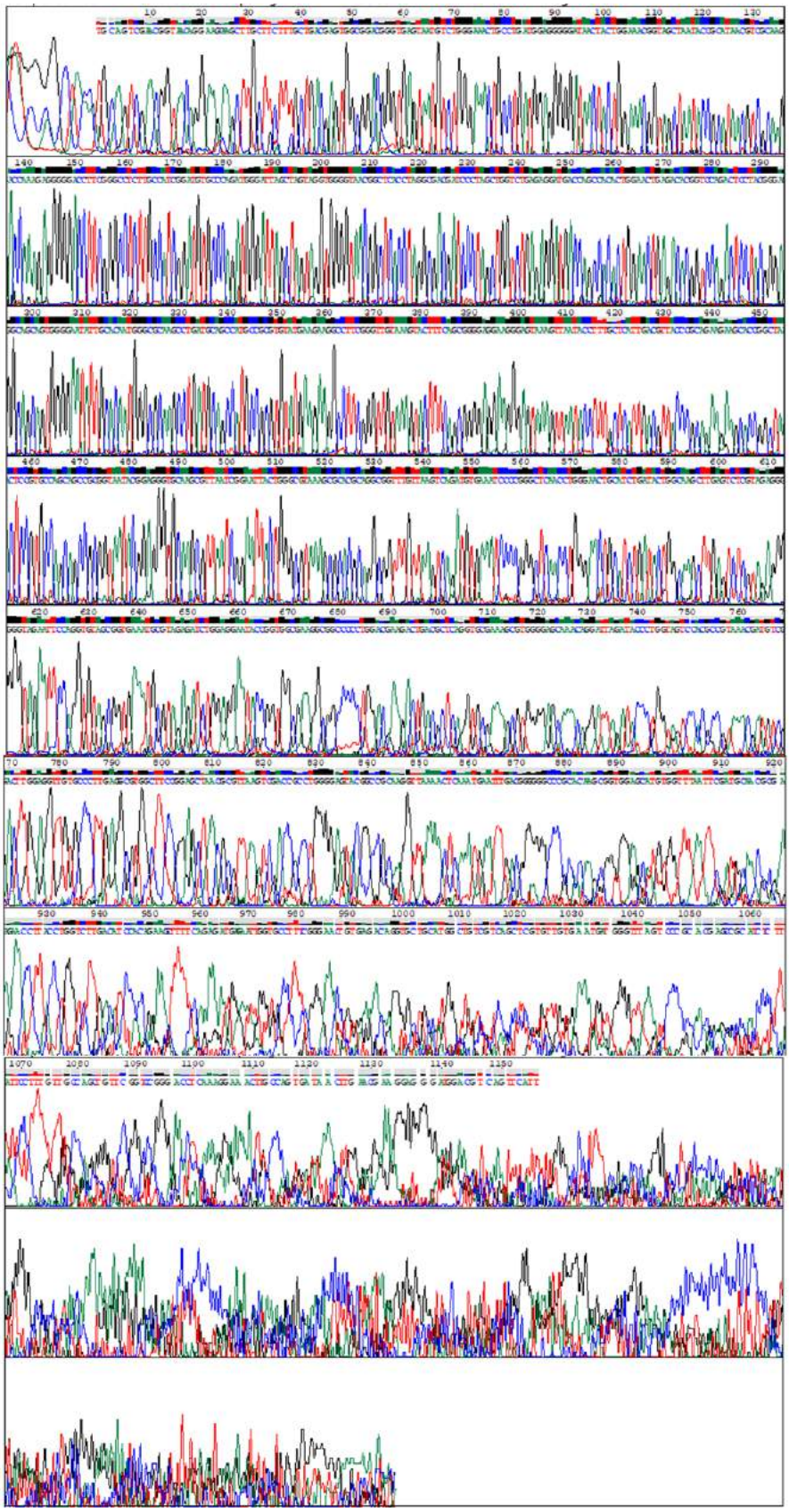


***Figure S.87. Chromatogram of forward sequence of REPNE-5 isolate.*** *Low-quality bases at both ends of the sequence were trimmed using Chromas software with default setting.*

TGCAGTCGAACGGTAACAGGAAGCAAGCTTGCTTCTTTGCTGACGAGTGGCGGACG
GGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGAAACGGT
AGCTAATACCGCATAACGTCGCAAGACCAAAGAGGGGGACCTTCGGGCCTCTTGCC
ATCGGATGTGCCCAGATGGGATTAGCTAGTAGGTGGGGTAACGGCTCACCTAGGCG
ACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGACACGGTC
CAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGA
TGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGG
GGAGGAAGGGAGTAAAGTTAATACCTTTGCTCATTGACGTTACCCGCAGAAGAAGC
ACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCG
GAATTACTGGGCGTAAAGCGCACGCAGGCGGTTTGTTAAGTCAGATGTGAAATCCC
CGGGCTCAACCTGGGAACTGCATCTGATACTGGCAAGCTTGAGTCTCGTAGAGGGG
GGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTG
GCGAAGGCGGCCCCCTGGACGAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGC
AAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGAGGTT
GTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGT
ACGGCCGCAAGGTTAAAACTCAAATGAATTTGACGGGGGGCCCGCACAAGCGGTG
GAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGTCTTGACATCCA
CAGAAGCTTTTCAGAGATGAGAATTGGTGCCTTTCGGGAACTGTGAGACAGGTGCT
GCATGGCTGTCGTCAGCTCGTGTTGTGAATGATGGGTTTAGTCCCGCACGAGCCGC
ATCTCTTATTCCTTTGTTGCCAGCTGTTCGGTCCGGGACCTCAAAGGAAACTTGCCA
GTGATAACTTGAACGAAGGAGGGATGGACGTCAGTTCATT

***Figure S.88. Forward sequence of REPNE-5 isolate.*** *The FASTA sequence were exported from the chromatogram of forward sequence of REPNE-5 isolate after the trim of low-quality bases at both ends.*

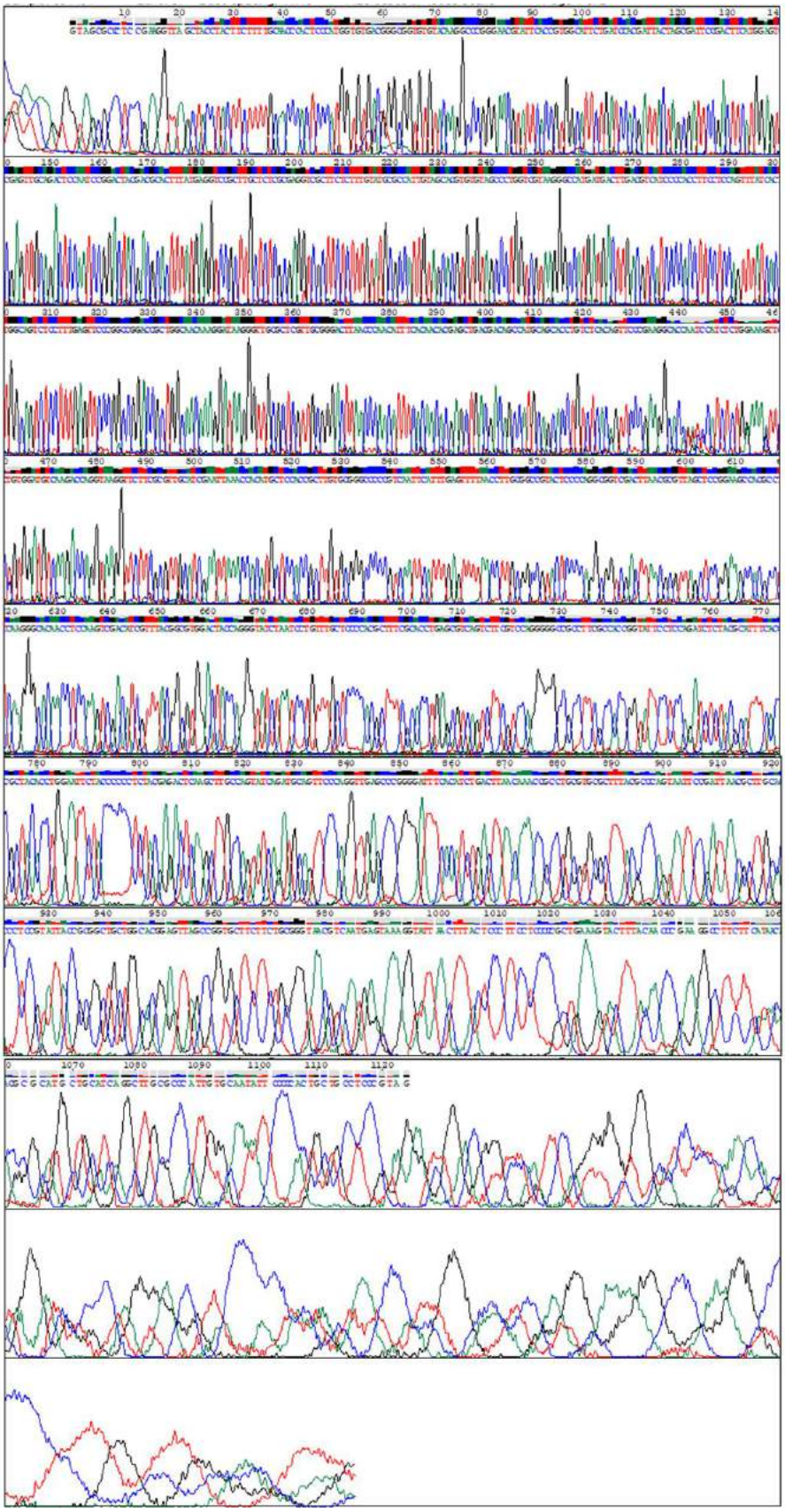


***Figure S.89. Chromatogram of reverse sequence of REPNE-5 isolate.*** *Low-quality bases at both ends of the sequence were trimmed using Chromas software with default setting.*

CTACGGGAGGCAGCAGTGGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGC
ATGCGCGTGTTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGGAGGA
AGGGAGTAAAGTTAATACCTTTACTCATTGACGTTACCCGCAGAAGAAGCACCGGC
TAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTA
CTGGGCGTAAAGCGCACGCAGGCGGTTTGTTAAGTCAGATGTGAAATCCCCGGGCT
CAACCTGGGAACTGCATCTGATACTGGCAAGCTTGAGTCTCGTAGAGGGGGGTAGA
ATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAG
GCGGCCCCCTGGACGAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAG
GATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGAGGTTGTGCCC
TTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCC
GCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGT
GGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGTCTTGACATCCACAGAACTTT
CCAGAGATGGATTGGTGCCTTCGGGAACTGTGAGACAGGTGCTGCATGGCTGTCGT
CAGCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTT
TGTTGCCAGCGGTCCGGCCGGGAACTCAAAGGAGACTGCCAGTGATAAACTGGAG
GAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGACCAGGGCTACACACGTG
CTACAATGGCGCATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCTCATAA
AGTGCGTCGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGC
TAGTAATCGTGGATCAGAATGCCACGGTGAATACGTTCCCGGGCCTTGTACACACC
GCCCGTCACACCATGGGAGTGGGTTGCAAAAGAAGTAGGTAGCTAACCTTCGGAG
GGCGCTAC

***Figure S.90. Reverse sequence of REPNE-5 isolate.*** *The FASTA sequence were exported from the chromatogram of reverse sequence of REPNE-5 isolate after the trim of low-quality bases at both ends.*

7.19 REPNE-6 Isolate

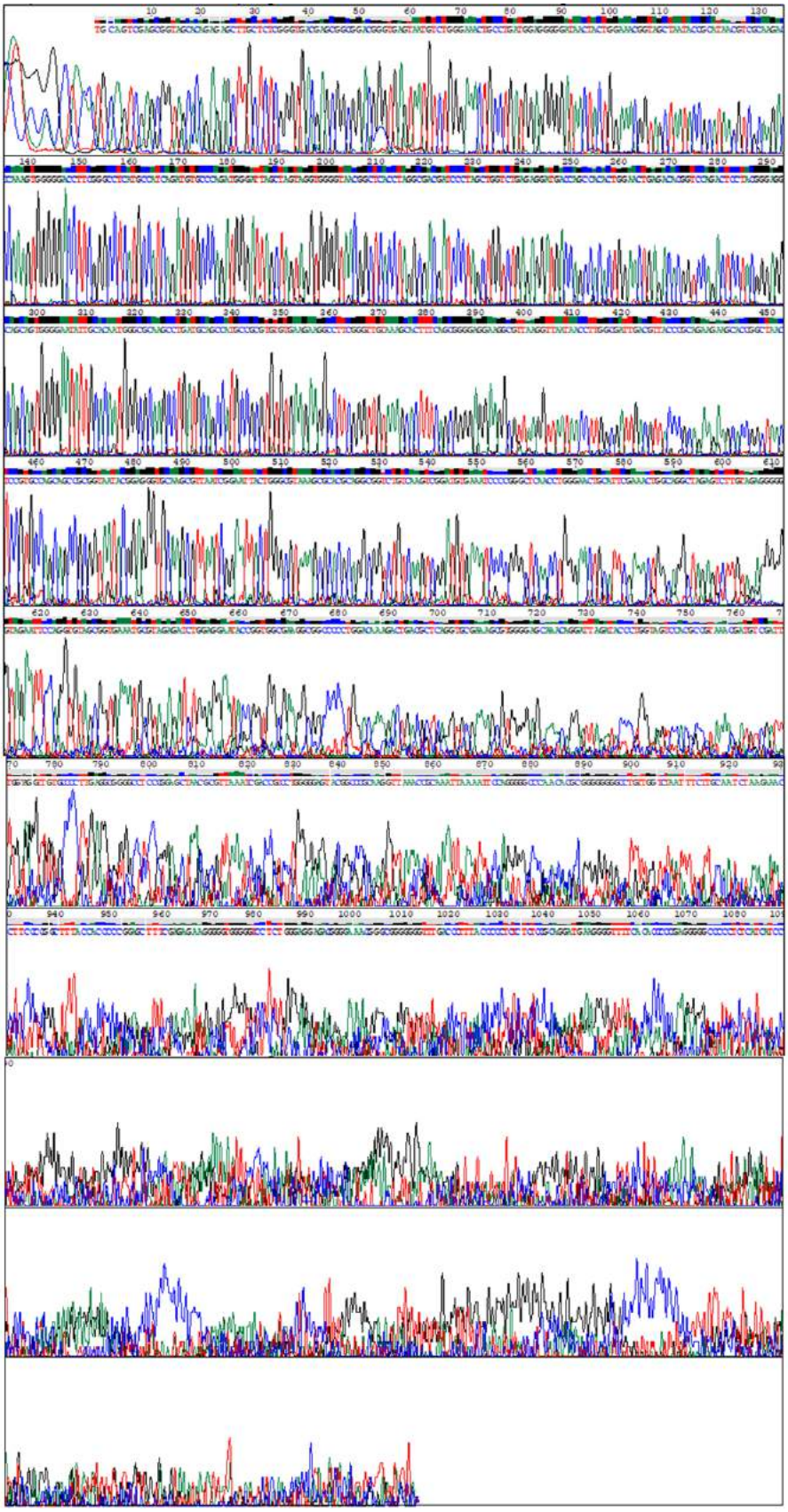


***Figure S.91. Chromatogram of forward sequence of REPNE-6 isolate.*** *Low-quality bases at both ends of the sequence were trimmed using Chromas software with default setting.*

TGCAGTCGAGCGGTAGCACAGAGAGCTTGCTCTCGGGTGACGAGCGGCGGACGGG
TGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGAAACGGTAG
CTAATACCGCATAACGTCGCAAGACCAAAGTGGGGGACCTTCGGGCCTCATGCCAT
CAGATGTGCCCAGATGGGATTAGCTAGTAGGTGGGGTAACGGCTCACCTAGGCGAC
GATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGACACGGTCCA
GACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATG
CAGCCATGCCGCGTGTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTTTCAGCGGGG
AGGAAGGCGTTAAGGTTAATAACCTTGGCGATTGACGTTACCCGCAGAAGAAGCAC
CGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGA
ATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTCAAGTCGGATGTGAAATCCCCG
GGCTCAACCTGGGAACTGCATTCGAAACTGGCAGGCTAGAGTCTTGTAGAGGGGGG
TAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGC
GAAGGCGGCCCCCTGGACAAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAA
ACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGATTTGGTAGGTTG
TGCCCCTTGAGGCGGGGCCTCCCGGAGCTAACGCGTTAAATCGACCGTCCTGGGGG
AGTACGGCCCGCAAGGTTAAACCGCAAATTAAAATTCCAGGGGGCCCAACACGCG
GGGGGGGCCTGTTGGTCTAATTTCTTGCAATCTAAGAAACCTTCCCCGGCTTTACCA
CCCCCGGAGCTTTCGAGAGAAGGGGGTGGGGGTCCTCTGGGAGGAGACGGGGAAA
CGGGCGGGGGGGTTTGACCCTTTACCCCCTCTCTCTCCGCAGGATGAAGGGGTTTTC
ACACCCCCGAGGGGGCCCCCTCTCATCATCC

***Figure S.92. Forward sequence of REPNE-6 isolate.*** *The FASTA sequence were exported from the chromatogram of forward sequence of REPNE-6 isolate after the trim of low-quality bases at both ends.*

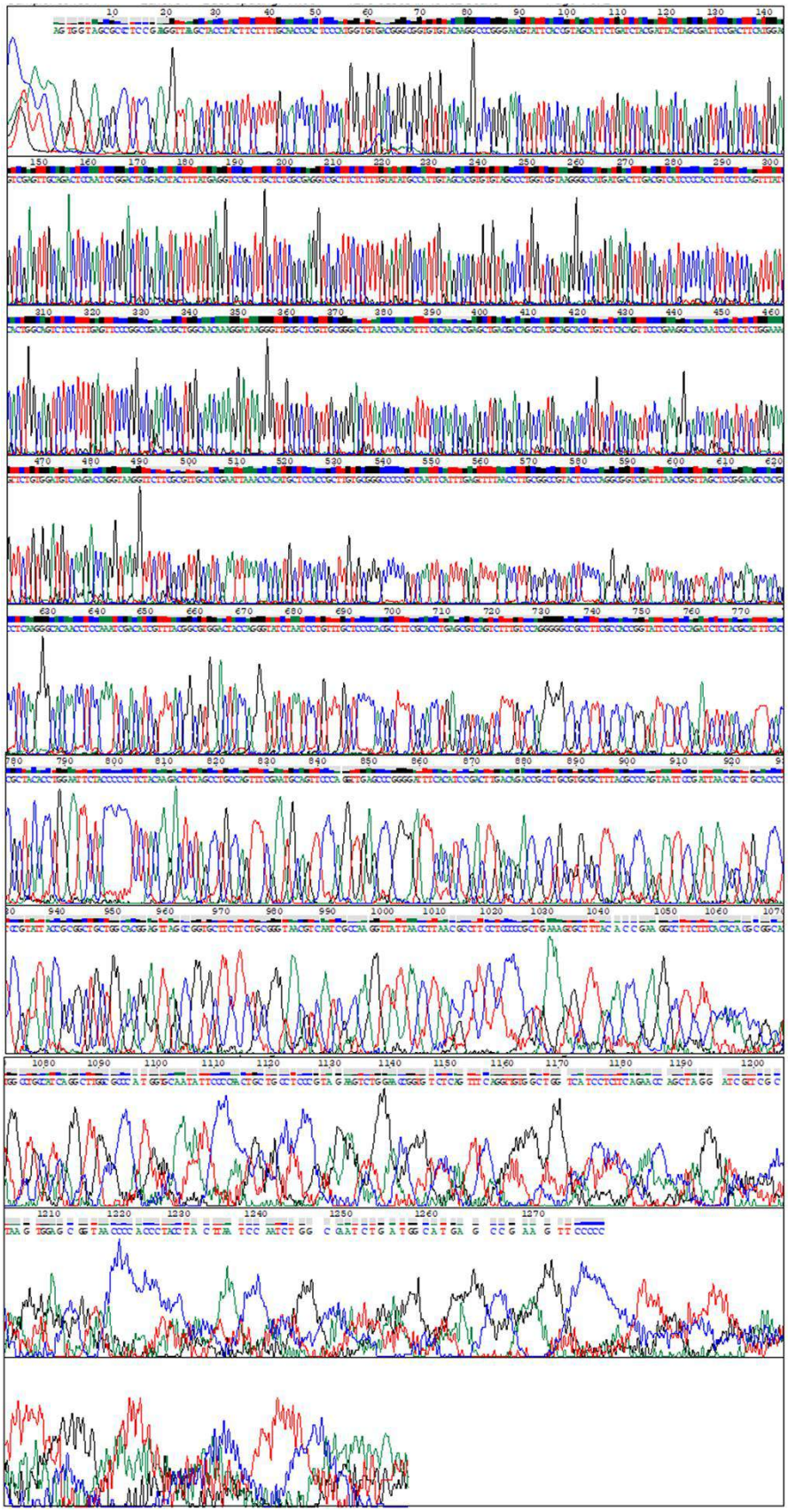


***Figure S.93. Chromatogram of reverse sequence of REPNE-6 isolate.*** *Low-quality bases at both ends of the sequence were trimmed using Chromas software with default setting.*

GGGGGAACTTCGGCTCATGCCATCAGATTGGCCAGATTGGATTAAGTAGGTAGGGT
GGGGTTACCGCTCCACTTAGCGAACGATCCTAGCTGGTTCTGAAGAGGATGACCAG
CCACAACCTGAAACTGAGACACCGGTTCCAGACTTCTACGGGAGGCAGCAGTTGGG
GAATATTGCACCATGGGCGCCAAGCCTGATGGCAGGCCATGCCGCGTGTGTGAAAG
AAGGCCTTCGGTGTAAAGCACTTTCAGCGGGGGAGGAAGGCGTTAAGGTTAATAAC
CTTGGCGATTGACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCC
GCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACG
CAGGCGGTCTGTCAAGTCGGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATT
CGAAACTGGCAGGCTAGAGTCTTGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGT
GAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACAAA
GACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTA
GTCCACGCCGTAAACGATGTCGATTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCG
GAGCTAACGCGTTAAATCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCA
AATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAA
CGCGAAGAACCTTACCTGGTCTTGACATCCACAGAACTTTCCAGAGATGGATTGGT
GCCTTCGGGAACTGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGAA
ATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTTCG
GCCGGGAACTCAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGGATGACG
TCAAGTCATCATGGCCCTTACGACCAGGGCTACACACGTGCTACAATGGCATATAC
AAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTATGTCGTAGTCCG
GATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGTAGATCA
GAATGCTACGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGG
GAGTGGGTTGCAAAAGAAGTAGGTAGCTTAACCTTCGGAGGGCGCTACCACT

***Figure S.94. Reverse sequence of REPNE-6 isolate.*** *The FASTA sequence were exported from the chromatogram of reverse sequence of REPNE-6 isolate after the trim of low-quality bases at both ends.*

7.20 REPNE-7 Isolate

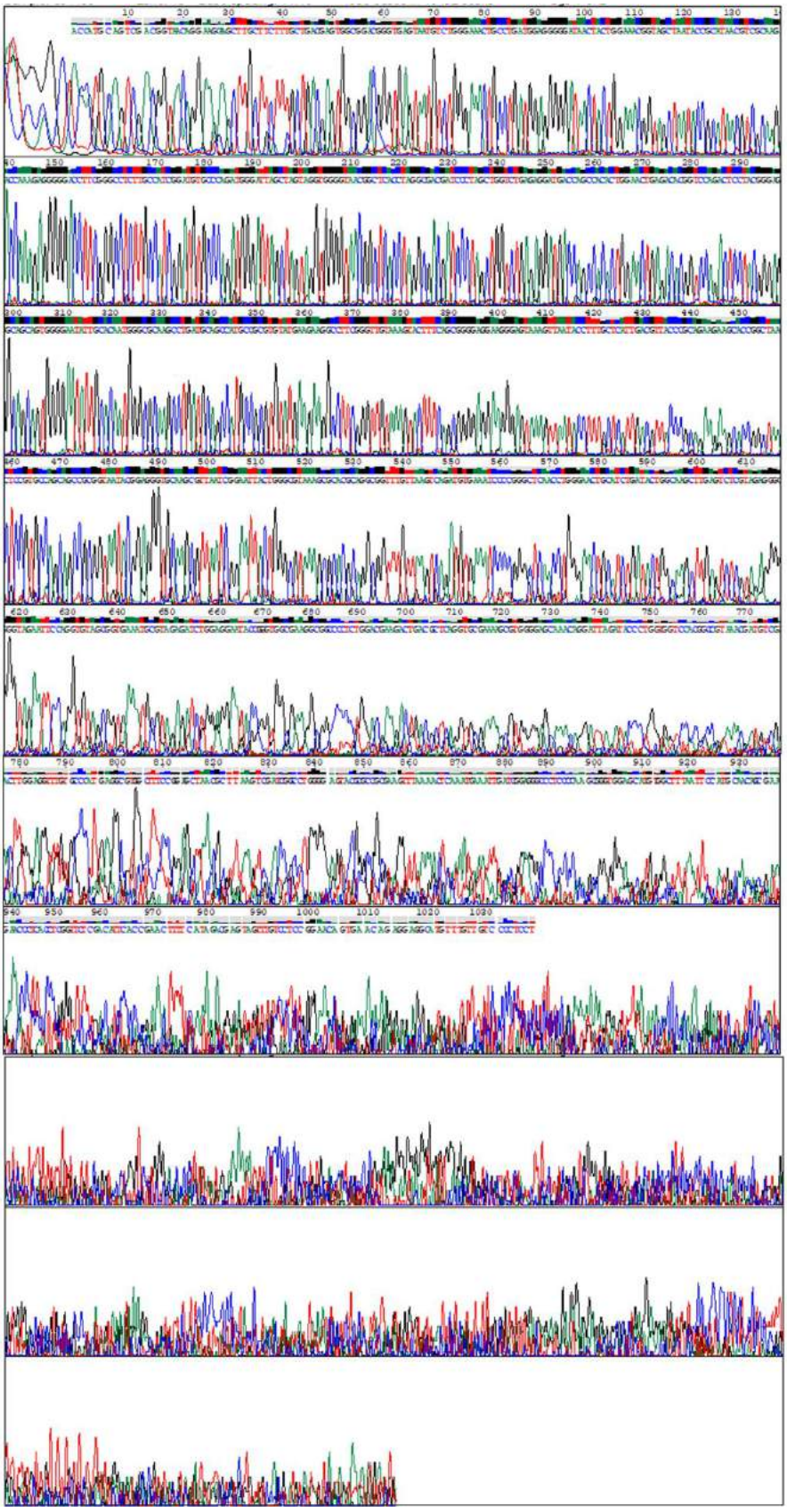


***Figure S.95. Chromatogram of forward sequence of REPNE-7 isolate.*** *Low-quality bases at both ends of the sequence were trimmed using Chromas software with default setting.*

ACCATGCAGTCGACGGTAACAGGAAGCAAGCTTGCTTCTTTGCTGACGAGTGGCGG
ACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGAAAC
GGTAGCTAATACCGCATAACGTCGCAAGACCAAAGAGGGGGACCTTCGGGCCTCTT
GCCATCGGATGTGCCCAGATGGGATTAGCTAGTAGGTGGGGTAACGGCTCACCTAG
GCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGACAC
GGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGC
CTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCA
GCGGGGAGGAAGGGAGTAAAGTTAATACCTTTGCTCATTGACGTTACCCGCAGAAG
AAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTT
AATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTTTGTTAAGTCAGATGTGAA
ATCCCCGGGCTCAACCTGGGAACTGCATCTGATACTGGCAAGCTTGAGTCTCGTAG
AGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATAC
CGGGTGGCGAAGGCGGCCCCTCTGGACGAAGACTGACGCTCAGGTGCGAAAAGCG
TGGGGAGCAAACAGGATTAGATACCCTGGTGGTCCACGGCCGTAAACGATGTCGAC
TTGGAGGTTTGTGCCCATGAGGCGTGGCTTTCCGGAGCTAACGCTTAAGTCGATCG
GCCTGGGGAGTACGCGCCGCGAAGTTTAAAACTCAAATGAAATTGATCGGAGGGC
CCTCCCCAAGCGGGTGGAGCATGTGGCTTTAATTCCATGCAACAGCGAAGAACCCT
CACCTCGGTTCTCGACATTCACCGAACTTTTCATAGACGAGTAGTTTGTCCTCCGGA
ACAGTGAACAGAGGAGGCATGTTTGTTGTCCCCTCCT

***Figure S.96. Forward sequence of REPNE-7 isolate.*** *The FASTA sequence were exported from the chromatogram of forward sequence of REPNE-7 isolate after the trim of low-quality bases at both ends.*

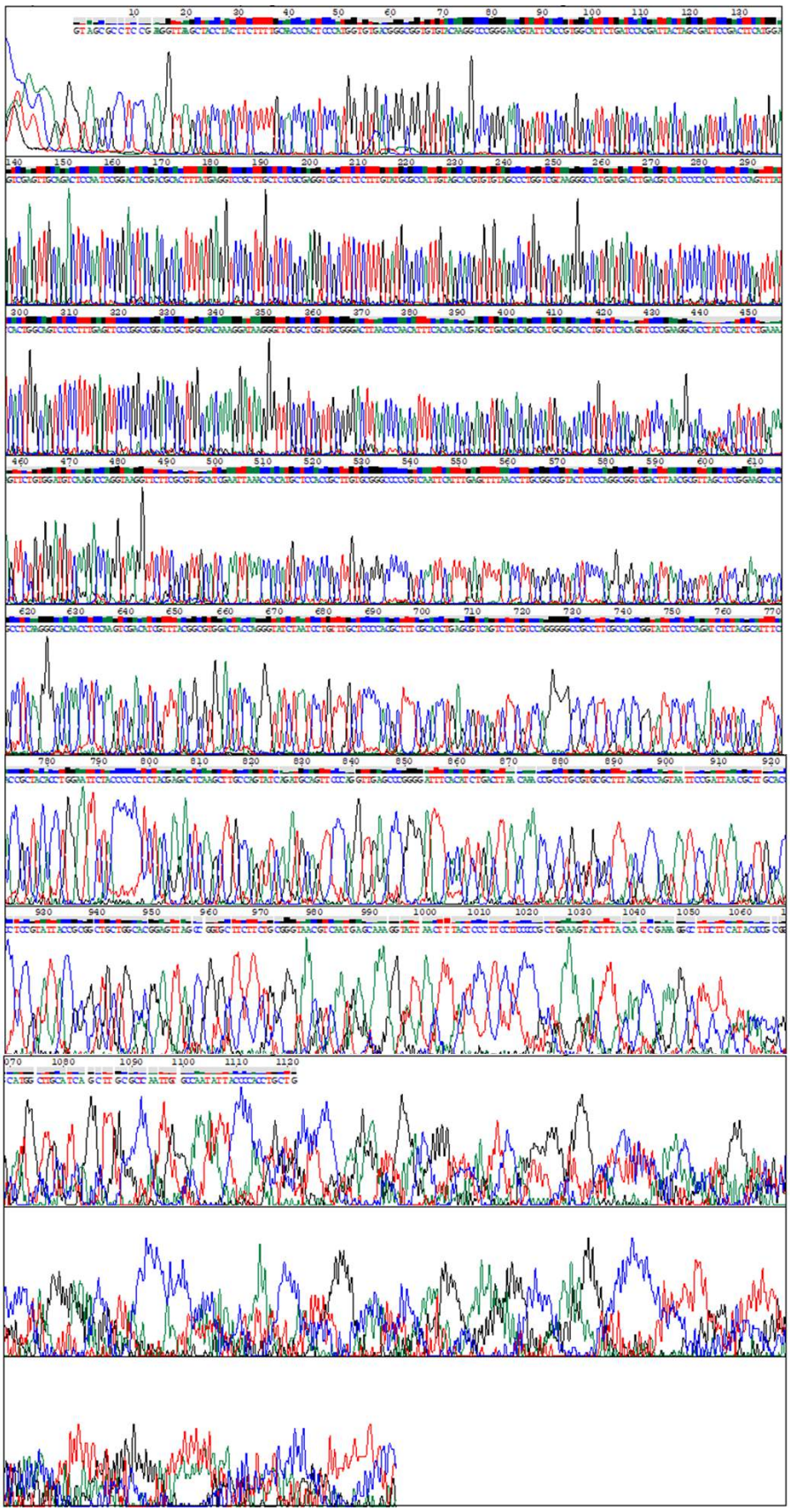


***Figure S.97. Chromatogram of reverse sequence of REPNE-7 isolate.*** *Low-quality bases at both ends of the sequence were trimmed using Chromas software with default setting.*

CAGCAGGTGGGGTAATATTGGCACAATTGAGCGCAAGCTGATGCAAGCCATGCCGC
GGTGTATGAAGAAAGGCCTTTCGAGTTGTAAAGTACTTTCAGCGGGGGAAGGAAG
GGAGTAAAGTTAATACCTTTGCTCATTGACGTTACCCGCAGAAGAAGCACCGGCTA
ACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACT
GGGCGTAAAGCGCACGCAGGCGGTTTGTTAAGTCAGATGTGAAATCCCCGGGCTCA
ACCTGGGAACTGCATCTGATACTGGCAAGCTTGAGTCTCGTAGAGGGGGGTAGAAT
TCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGC
GGCCCCCTGGACGAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGA
TTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGAGGTTGTGCCCTTG
AGGCGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCCGCA
AGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGT
TTAATTCGATGCAACGCGAAGAACCTTACCTGGTCTTGACATCCACAGAACTTTTCA
GAGATGGATAGGTGCCTTCGGGAACTGTGAGACAGGTGCTGCATGGCTGTCGTCAG
CTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGT
TGCCAGCGGTCCGGCCGGGAACTCAAAGGAGACTGCCAGTGATAAACTGGAGGAA
GGTGGGGATGACGTCAAGTCATCATGGCCCTTACGACCAGGGCTACACACGTGCTA
CAATGGCGCATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGT
GCGTCGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAG
TAATCGTGGATCAGAATGCCACGGTGAATACGTTCCCGGGCCTTGTACACACCGCC
CGTCACACCATGGGAGTGGGTTGCAAAAGAAGTAGGTAGCTTAACCTTCGGAGGCG
CTAC

***Figure S.98. Reverse sequence of REPNE-7 isolate.*** *The FASTA sequence were exported from the chromatogram of reverse sequence of REPNE-7 isolate after the trim of low-quality bases at both ends.*

7.21 REPNE-8 Isolate

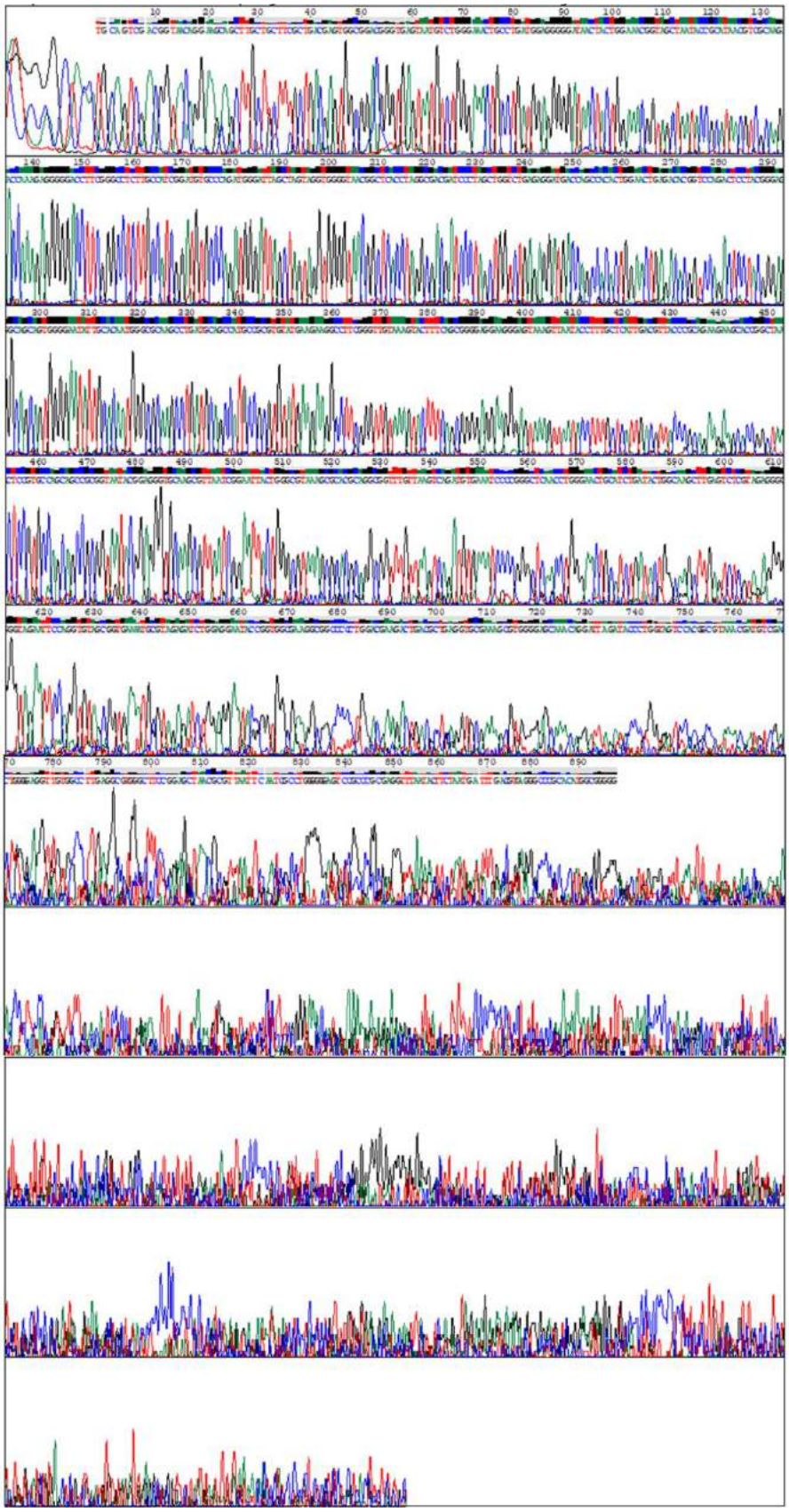


***Figure S.99. Chromatogram of forward sequence of REPNE-8 isolate.*** *Low-quality bases at both ends of the sequence were trimmed using Chromas software with default setting.*

TGCAGTCGACGGTAACAGGAAGCAGCTTGCTGCTTCGCTGACGAGTGGCGGACGGG
TGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGAAACGGTAG
CTAATACCGCATAACGTCGCAAGACCAAAGAGGGGGACCTTCGGGCCTCTTGCCAT
CGGATGTGCCCAGATGGGATTAGCTAGTAGGTGGGGTAACGGCTCACCTAGGCGAC
GATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGACACGGTCCA
GACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATG
CAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGG
AGGAAGGGAGTAAAGTTAATACCTTTGCTCATTGACGTTACCCGCAGAAGAAGCAC
CGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGA
ATTACTGGGCGTAAAGCGCACGCAGGCGGTTTGTTAAGTCAGATGTGAAATCCCCG
GGCTCAACCTGGGAACTGCATCTGATACTGGCAAGCTTGAGTCTCGTAGAGGGGGG
TAGAATTCCAGGTGTAGCGGTGAAAATGCGTAGAGATCTGGAGGAATACCGGTGG
CGAAGGCGGCCCCCTGGACGAAGACTGACGCTGAGGTGCGAAAGCGTGGGGAGCA
AACAGGATTAGATACCCTGGTAGTCCACGGCGTAAACGATGTCGACTGGGGAGGTT
GTGGCCTTGAGGCGTGGGCTTCCGGAGCTAACGCGTTAATTCAATCGCCTGGGGGG
AGTCCGCCCGCGAGGTTTAATACTTCTAATGATTTGACGTGAGGGCCCGCACATGG
CGGGGG

***Figure S.100. Forward sequence of REPNE-8 isolate.*** *The FASTA sequence were exported from the chromatogram of forward sequence of REPNE-8 isolate after the trim of low-quality bases at both ends.*

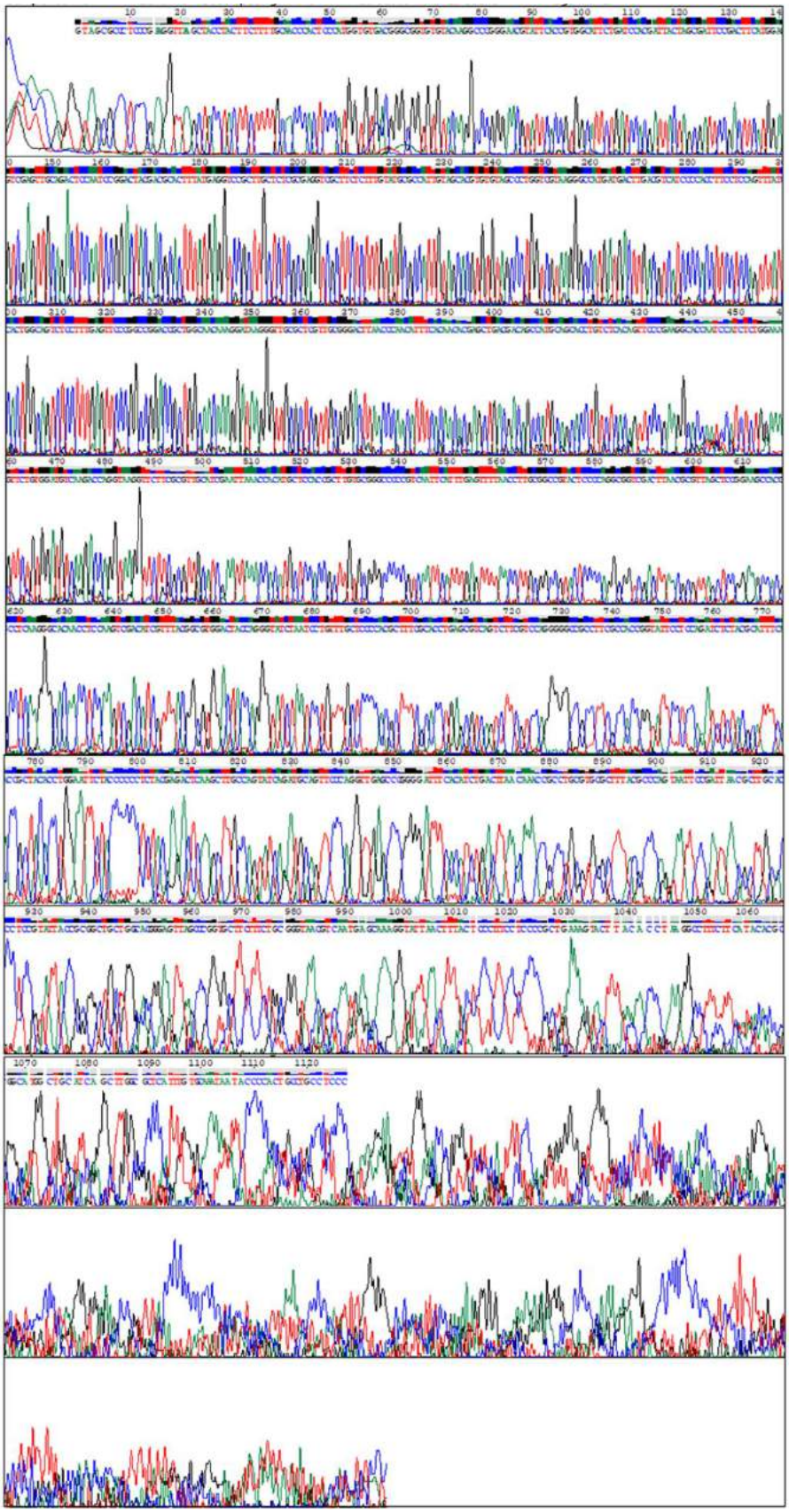


***Figure S.101. Chromatogram of reverse sequence of REPNE-8 isolate.*** *Low-quality bases at both ends of the sequence were trimmed using Chromas software with default setting.*

GGGAGGCAGGCAGTGGGGTATTATTTGCACAAATGAGCGCCAAGCTGATGCAGCC
ATGCCGCGTGTATGAAGAAAGGCCTTAGGTGTAAGTACTTTCAGCGGGGAGGAAA
GGGAGTAAAGTTAATACCTTTGCTCATTGACGTTACCCGCAGAAAGAAGCACCGGG
CTAACTCCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAAT
TACTGGGCGTAAAGCGCACGCAGGCGGTTTGTTAAGTCAGATGTGAAATCCCCGGG
CTCAACCTGGGAACTGCATCTGATACTGGCAAGCTTGAGTCTCGTAGAGGGGGGTA
GAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGA
AGGCGGCCCCCTGGACGAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAAC
AGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGAGGTTGTGC
CCTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGG
CCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCAT
GTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGTCTTGACATCCACAGAAC
TTTCCAGAGATGGATTGGTGCCTTCGGGAACTGTGAGACAGGTGCTGCATGGCTGT
CGTCAGCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTAT
CCTTTGTTGCCAGCGGTCCGGCCGGGAACTCAAAGGAGACTGCCAGTGATAAACTG
GAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGACCAGGGCTACACA
CGTGCTACAATGGCGCATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCTC
ATAAAGTGCGTCGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAA
TCGCTAGTAATCGTGGATCAGAATGCCACGGTGAATACGTTCCCGGGCCTTGTACA
CACCGCCCGTCACACCATGGGAGTGGGTTGCAAAAGAAGTAGGTAGCTTAACCTTC
GGGAGGGCGCTAC

***Figure S.102. Reverse sequence of REPNE-8 isolate.*** *The FASTA sequence were exported from the chromatogram of reverse sequence of REPNE-8 isolate after the trim of low-quality bases at both ends.*

7.22 REPNE-9 Isolate

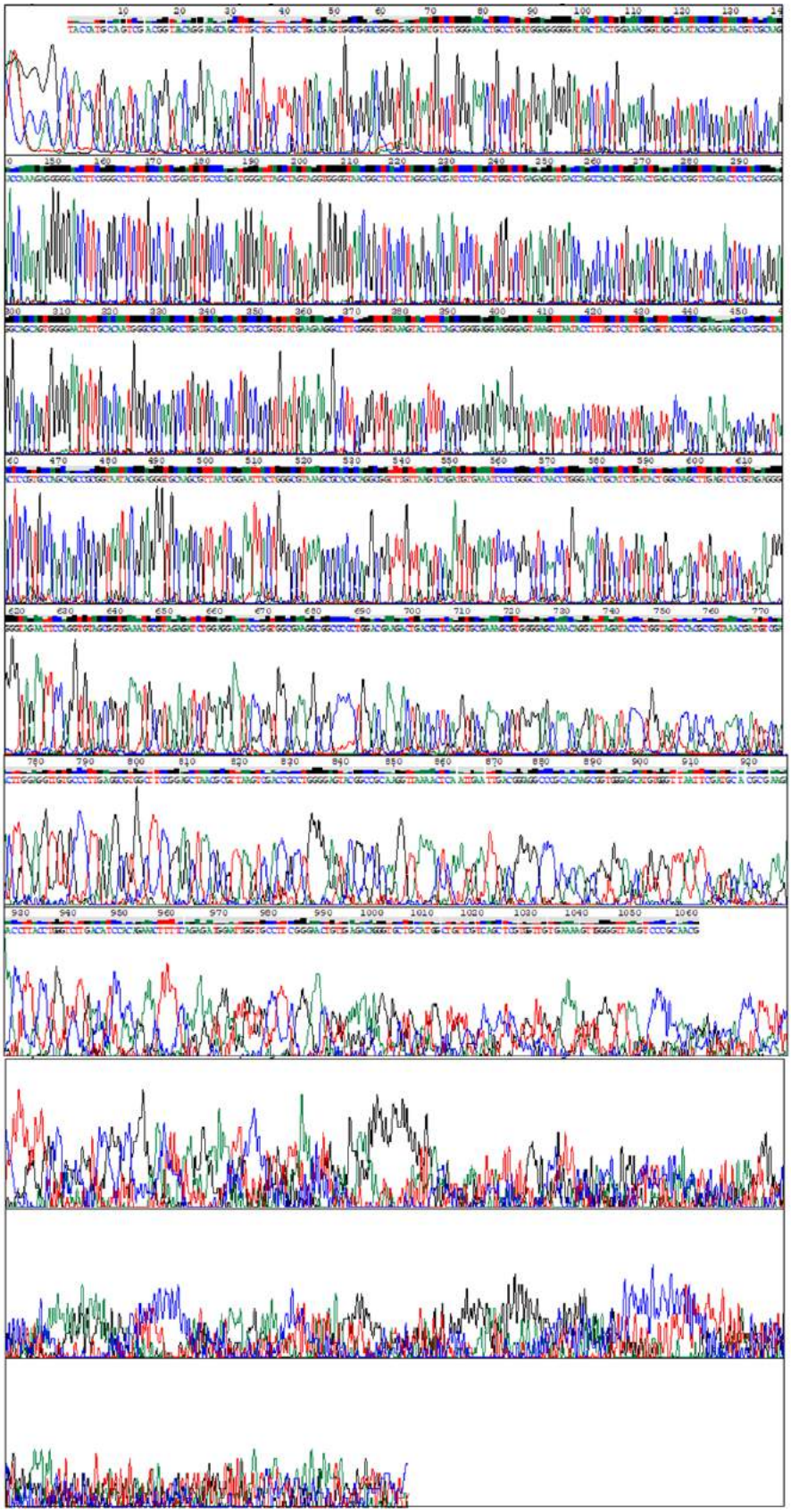


***Figure S.103. Chromatogram of forward sequence of REPNE-9 isolate.*** *Low-quality bases at both ends of the sequence were trimmed using Chromas software with default setting.*

TACCATGCAGTCGACGGTAACAGGAAGCAGCTTGCTGCTTCGCTGACGAGTGGCGG
CACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGAAA
CGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGAGGGGGACCTTCGGGCCTCT
TGCCATCGGATGTGCCCAGATGGGATTAGCTAGTAGGTGGGGTAACGGCTCACCTA
GGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGACA
CGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAG
CCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTC
AGCGGGGAGGAAGGGAGTAAAGTTAATACCTTTGCTCATTGACGTTACCCGCAGAA
GAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGT
TAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTTTGTTAAGTCAGATGTGA
AATCCCCGGGCTCAACCTGGGAACTGCATCTGATACTGGCAAGCTTGAGTCTCGTA
GAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATA
CCGGTGGCGAAGGCGGCCCCCTGGACGAAGACTGACGCTCAGGTGCGAAAGCGTG
GGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTG
GAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTG
GGGAGTACGGCCGCAAGGTTAAAACTCAATTGAATTGACGGGAGGCCCGCACAAG
CGGTGGGAGCATGTGGGTTAATTCGATGCACGCGAAGAACCTTACCTGGGTCTTGA
CATCCACAGAAACTTTTCAGAGATGGAATTGGTGCCTTCGGGAACTGTTGAGACAG
GGTGCTGCATGGCTGTTCGTCAGCTCGTGGTTGTGAAAAGTTGGGGTTAAGTCCCG
CAACG

***Figure S.104. Forward sequence of REPNE-9 isolate.*** *The FASTA sequence were exported from the chromatogram of forward sequence of REPNE-9 isolate after the trim of low-quality bases at both ends.*

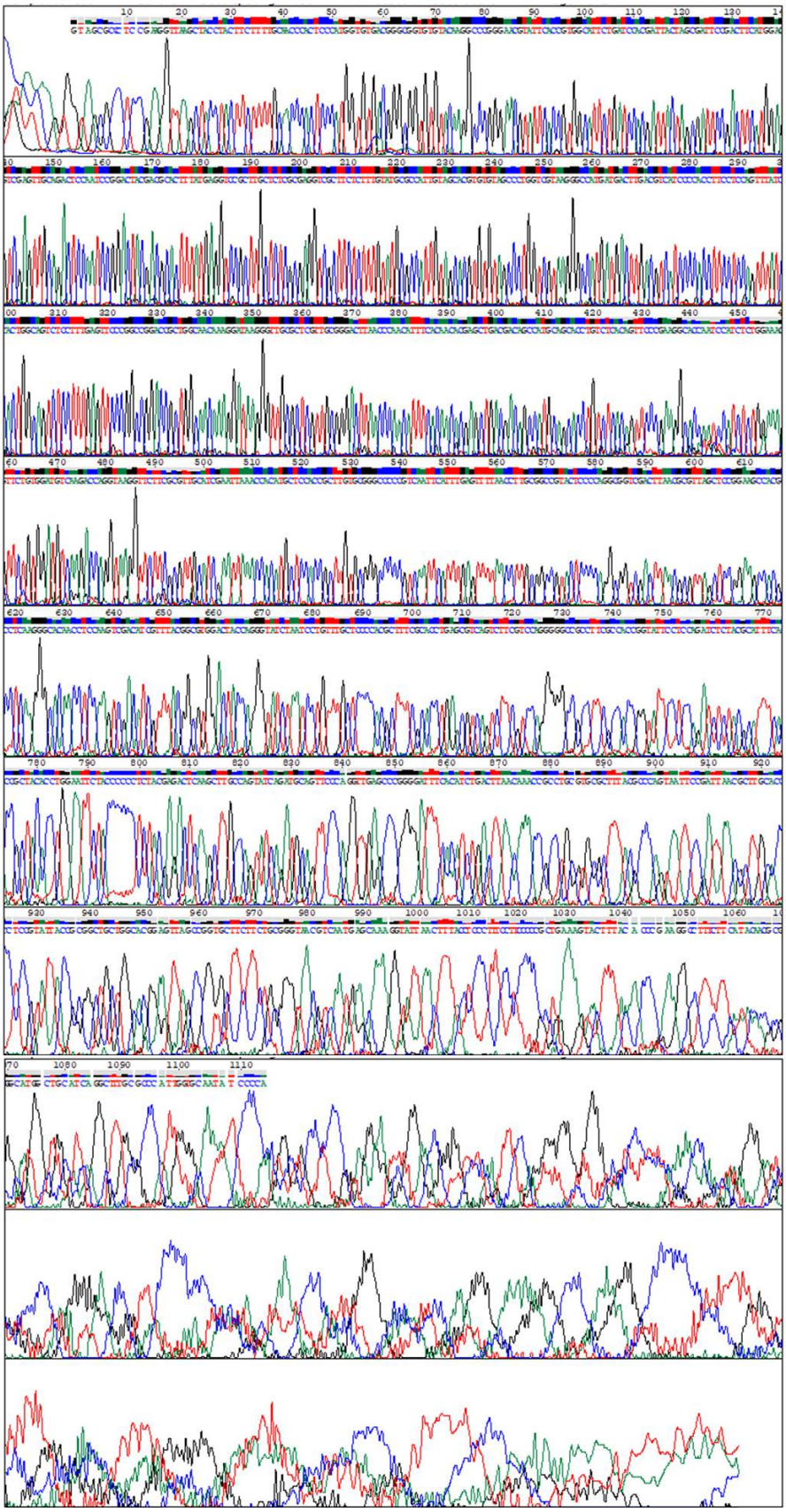


***Figure S.105. Chromatogram of reverse sequence of REPNE-9 isolate.*** *Low-quality bases at both ends of the sequence were trimmed using Chromas software with default setting.*

TGGGGATATTGCACCAATGGGCGCAAAGCCTGATGCAGCCATGCCCGCGTTGTATG
AAGAAAGGCCTTCGGGTGTAAAGTACTTTCAGCGGGGGAAGGAAAGGGAGGTAAA
GTTAATACCTTTGCTCATTGACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTG
CCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTA
AAGCGCACGCAGGCGGTTTGTTAAGTCAGATGTGAAATCCCCGGGCTCAACCTGGG
AACTGCATCTGATACTGGCAAGCTTGAGTCTCGTAGAGGGGGGTAGAATTCCAGGT
GTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCC
TGGACGAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATA
CCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGAGGTTGTGCCCTTGAGGCGT
GGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTA
AAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTC
GATGCAACGCGAAGAACCTTACCTGGTCTTGACATCCACAGAACTTTCCAGAGATG
GATTGGTGCCTTCGGGAACTGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTG
TTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAG
CGGTCCGGCCGGGAACTCAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGG
GATGACGTCAAGTCATCATGGCCCTTACGACCAGGGCTACACACGTGCTACAATGG
CGCATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTGCGTCG
TAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCG
TGGATCAGAATGCCACGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCAC
ACCATGGGAGTGGGTTGCAAAAGAAGTAGGTAGCTTAACCTTCGGAGGGCGCTAC

***Figure S.106. Reverse sequence of REPNE-9 isolate.*** *The FASTA sequence were exported from the chromatogram of reverse sequence of REPNE-9 isolate after the trim of low-quality bases at both ends.*

7.23 REPNE-10 Isolate

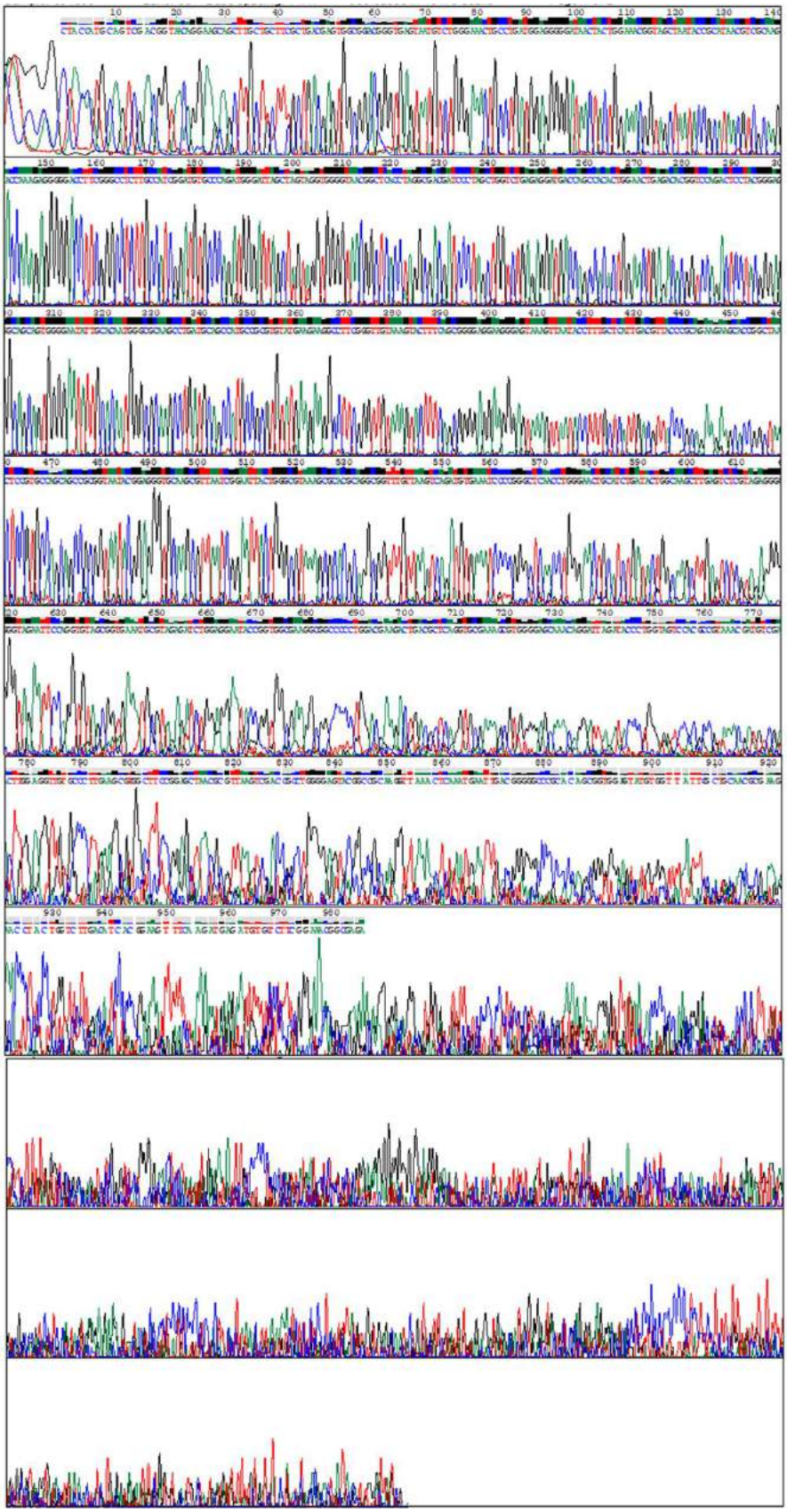


***Figure S.107. Chromatogram of forward sequence of REPNE-10 isolate.*** *Low-quality bases at both ends of the sequence were trimmed using Chromas software with default setting.*

CTACCATGCAGTCGACGGTAACAGGAAGCAGCTTGCTGCTTCGCTGACGAGTGGCG
GCACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGAA
ACGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGAGGGGGACCTTCGGGCCT
CTTGCCATCGGATGTGCCCAGATGGGATTAGCTAGTAGGTGGGGTAACGGCTCACC
TAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGAC
ACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAA
GCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTT
CAGCGGGGAGGAAGGGAGTAAAGTTAATACCTTTGCTCATTGACGTTACCCGCAGA
AGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCG
TTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTTTGTTAAGTCAGATGTG
AAATCCCCGGGCTCAACCTGGGAACTGCATCTGATACTGGCAAGCTTGAGTCTCGT
AGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAAT
ACCGGTGGCGAAGGCGGCCCCCTGGACGAAGACTGACGCTCAGGTGCGAAAGCGT
GGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTT
GGAGGTTGTGCCCTTGAAGCGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCT
GGGGAGTACGGCCGCAAGGTTAAACTCAAATGAATTGACGGGGGCCCGCACAGCG
GTGGAGTATGTGGTTATTCGCTGCAACGCGAAGAACCTACTGGTCTTGACATCACG
GAAGTTTCAAGATGAGATGTTGCTCTTCGGAAACGGCGAGA

***Figure S.108. Forward sequence of REPNE-10 isolate.*** *The FASTA sequence were exported from the chromatogram of forward sequence of REPNE-10 isolate after the trim of low-quality bases at both ends.*

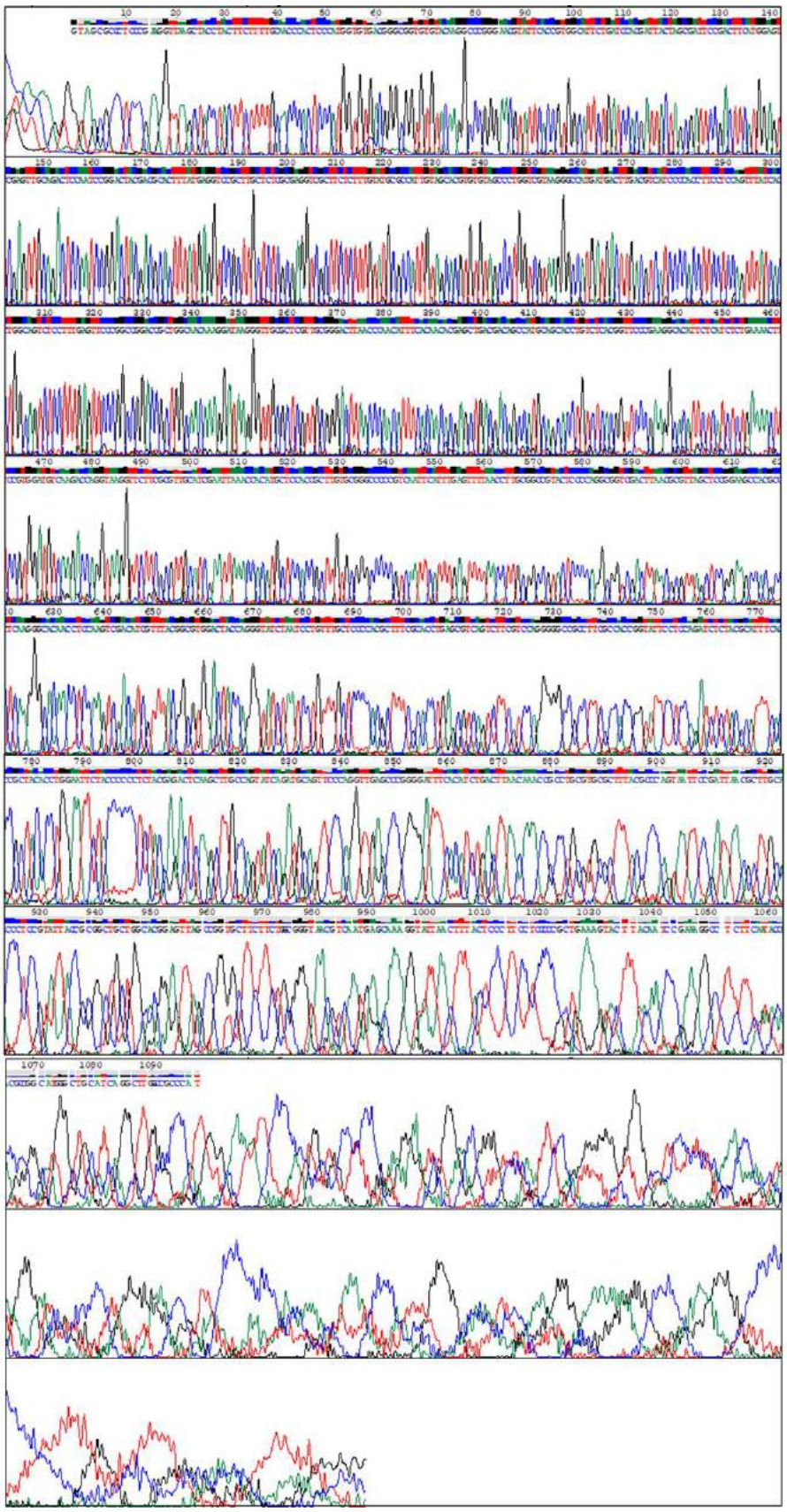


***Figure S.109. Chromatogram of reverse sequence of REPNE-10 isolate.*** *Low-quality bases at both ends of the sequence were trimmed using Chromas software with default setting.*

ATGGGCGGCCAAGCCTGATGCAGCCCATGCCGGCGTGGTATTGAAGAGGCCTTTCG
GATTGTAAGTACTTTCAGCGGGGGAGGAAGGGAGTAAAGTTAATACCTTTGCTCAT
TGACGTTACCCGCCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAAT
ACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGT
TTGTTAAGTCAGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATCTGATACTG
GCAAGCTTGAGTCTCGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGT
AGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACGAAGACTGACGC
TCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCC
GTAAACGATGTCGACTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACG
CGTTAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGA
CGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAA
CCTTACCTGGTCTTGACATCCACGGAAGTTTTCAGAGATGAGAATGTGCCTTCGGG
AACCGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGT
TAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTCCGGCCGGGAA
CTCAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGGATGACGTCAAGTCA
TCATGGCCCTTACGACCAGGGCTACACACGTGCTACAATGGCGCATACAAAGAGAA
GCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTGCGTCGTAGTCCGGATTGGAGT
CTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGTGGATCAGAATGCCAC
GGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTT
GCAAAAGAAGTAGGTAGCTTAACCTTCGGGAGGGCGCTAC

***Figure S.110. Reverse sequence of REPNE-10 isolate.*** *The FASTA sequence were exported from the chromatogram of reverse sequence of REPNE-10 isolate after the trim of low-quality bases at both ends.*

7.24. REPNE-11 Isolate

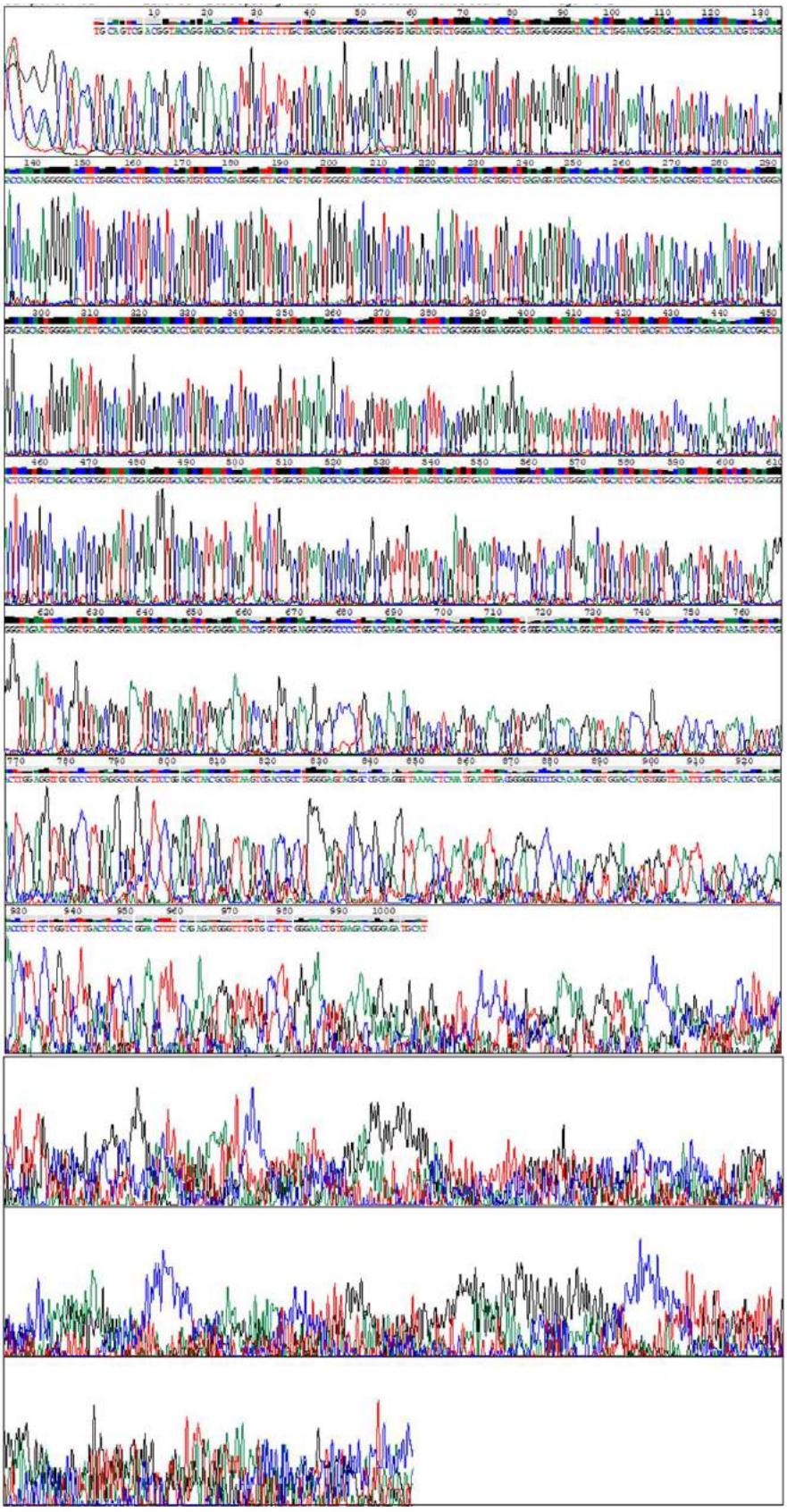


***Figure S.111. Chromatogram of forward sequence of REPNE-11 isolate.*** *Low-quality bases at both ends of the sequence were trimmed using Chromas software with default setting.*

TGCAGTCGACGGTAACAGGAAGCAGCTTGCTTCTTTGCTGACGAGTGGCGGACGGG
TGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGAAACGGTAG
CTAATACCGCATAACGTCGCAAGACCAAAGAGGGGGACCTTCGGGCCTCTTGCCAT
CGGATGTGCCCAGATGGGATTAGCTAGTAGGTGGGGTAACGGCTCACCTAGGCGAC
GATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGACACGGTCCA
GACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATG
CAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGG
AGGAAGGGAGTAAAGTTAATACCTTTGCTCATTGACGTTACCCGCAGAAGAAGCAC
CGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGA
ATTACTGGGCGTAAAGCGCACGCAGGCGGTTTGTTAAGTCAGATGTGAAATCCCCG
GGCTCAACCTGGGAACTGCATCTGATACTGGCAAGCTTGAGTCTCGTAGAGGGGGG
TAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGC
GAAGGCGGCCCCCTGGACGAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAA
ACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGAGGTTGT
GCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTAC
GGCCGCGAGGGTTAAAACTCAAATGAATTTGACGGGAGGGCCCCGCACAAGCGGT
GGAGCATGTGGGTTTAATTTCGATGCAACGCGAAGAACCCTTCCTGGTCTTGACAT
CCACGGAACTTTTCAGAGATGGGTTTGTGCCTTCGGGAACTGTGAAGACAGGGAGA
TGCAT

***Figure S.112. Forward sequence of REPNE-11 isolate.*** *The FASTA sequence were exported from the chromatogram of forward sequence of REPNE-11 isolate after the trim of low-quality bases at both ends.*

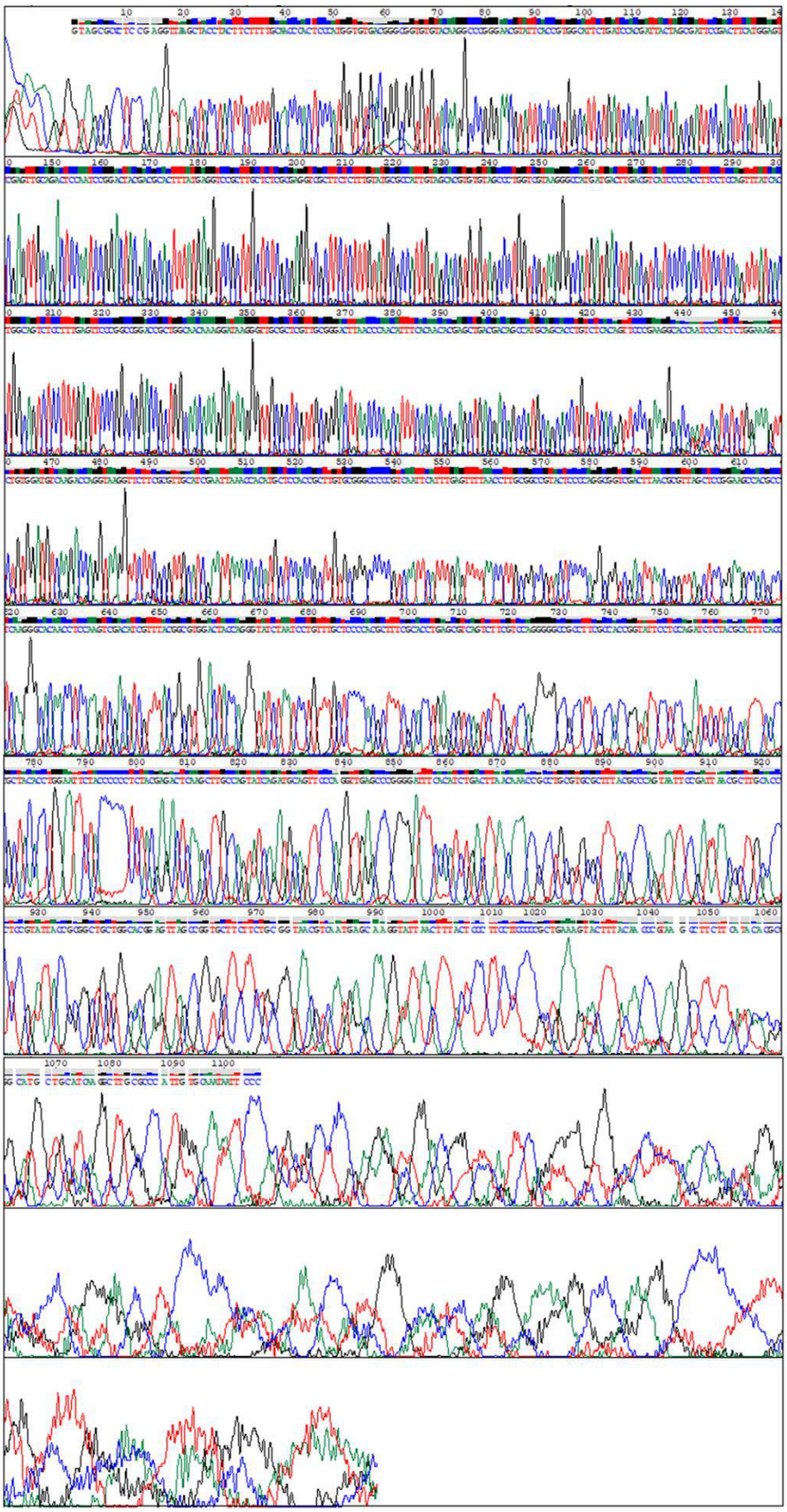


***Figure S.113. Chromatogram of reverse sequence of REPNE-11 isolate.*** *Low-quality bases at both ends of the sequence were trimmed using Chromas software with default setting.*

GGGAATTATTTGCACAATGGGCGCAAGCCTTGATGCAGCATGCCGCGTGTATGAAG
AAGGCTTACGGGTTGTAAAGTACTTTCAGCGGGGGAAGGAAGGGAGTAAAGTTAA
TACCTTGCTCATTGACGTTACCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAG
CCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCA
CGCAGGCGGTTTGTTAAGTCAGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCA
TCTGATACTGGCAAGCTTGAGTCTCGTAGAGGGGGGTAGAATTCCAGGTGTAGCGG
TGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACGA
AGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGT
AGTCCACGCCGTAAACGATGTCGACTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCC
GGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTC
AAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCA
ACGCGAAGAACCTTACCTGGTCTTGACATCCACAGAACTTTCCAGAGATGGATTGG
TGCCTTCGGGAACTGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGA
AATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTCC
GGCCGGGAACTCAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGGATGAC
GTCAAGTCATCATGGCCCTTACGACCAGGGCTACACACGTGCTACAATGGCGCATA
CAAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTGCGTCGTAGTCC
GGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGTGGATC
AGAATGCCACGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATG
GGAGTGGGTTGCAAAAGAAGTAGGTAGCTTAACCTCGGAGGGCGCTAC

***Figure S.114. Reverse sequence of REPNE-11 isolate.*** *The FASTA sequence were exported from the chromatogram of reverse sequence of REPNE-11 isolate after the trim of low-quality bases at both ends.*

7.25 REPNE-12 Isolate

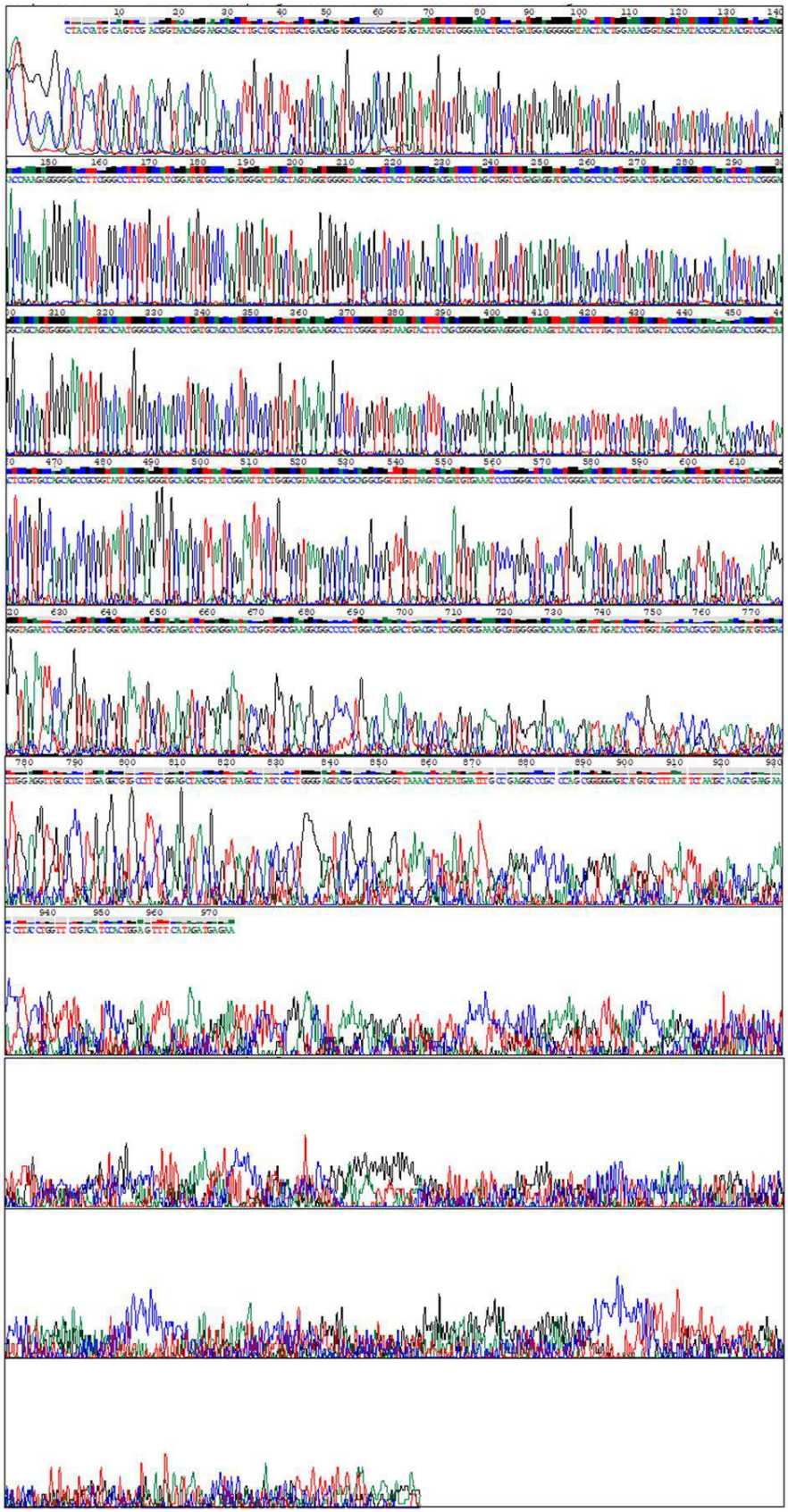


***Figure S.115. Chromatogram of forward sequence of REPNE-12 isolate.*** *Low-quality bases at both ends of the sequence were trimmed using Chromas software with default setting.*

CTACCATGCAGTCGACGGTAACAGGAAGCAGCTTGCTGCTTCTGCTGACGAGTGGC
GGCCGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGAA
ACGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGAGGGGGACCTTCGGGCCT
CTTGCCATCGGATGTGCCCAGATGGGATTAGCTAGTAGGTGGGGTAACGGCTCACC
TAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGAC
ACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAA
GCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTT
CAGCGGGGAGGAAGGGAGTAAAGTTAATACCTTTGCTCATTGACGTTACCCGCAGA
AGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCG
TTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTTTGTTAAGTCAGATGTG
AAATCCCCGGGCTCAACCTGGGAACTGCATCTGATACTGGCAAGCTTGAGTCTCGT
AGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAAT
ACCGGTGGCGAAGGCGGCCCCCTGGACGAAGACTGACGCTCAGGTGCGAAAGCGT
GGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTT
GGAGGTTGTGCCCTTGAGGCGTGCCTTCCGGAGCTAACGCGTTAAGTCCATCGCCT
GGGGAGTACGGCCGCGAGGTTAAAACTCTATATGAATTTGCCGAGGCCCGCCCAGC
GGTGGGAGTCATGTGCTTTAATTCTAATGCACAGCGAAGAACCTTACCTGGTTCTG
ACATCCACTGGAGTTTCATAGATGAGAA

***Figure S.116. Forward sequence of REPNE-12 isolate.*** *The FASTA sequence were exported from the chromatogram of forward sequence of REPNE-12 isolate after the trim of low-quality bases at both ends.*

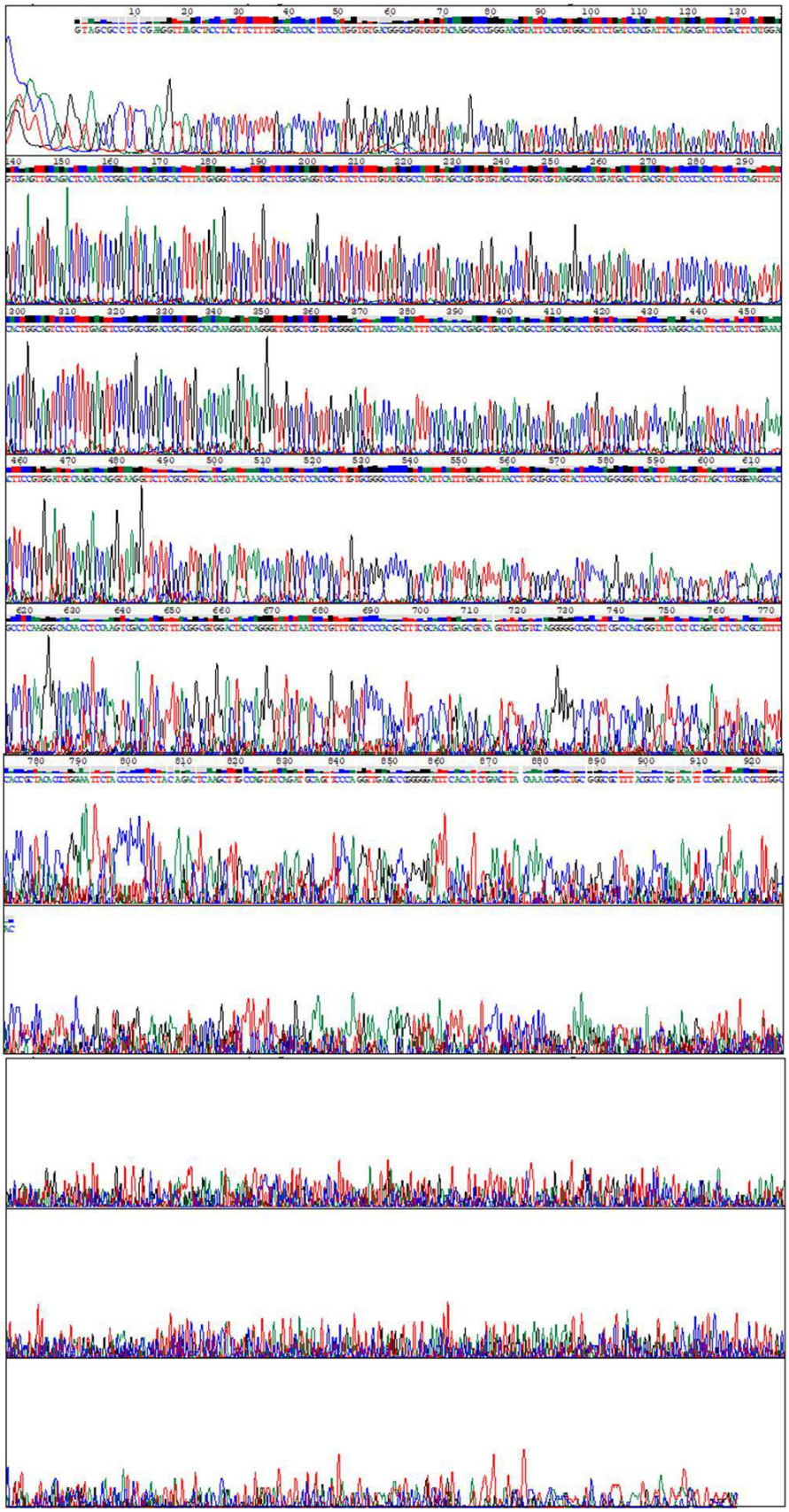


***Figure S.117. Chromatogram of reverse sequence of REPNE-12 isolate.*** *Low-quality bases at both ends of the sequence were trimmed using Chromas software with default setting.*

GTGCCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCCCGCAGGCGGTTTGTAAG
TTCAGATGTGAAATCCCCCGGGCTCAACCTGGGAACTGCATCTGATACTGGCAAGC
TTGAGTCTGTAGAGGGGGGTAGAATTTCCAGGGTGTAGCGGTGAAAATGCGTAGAG
ATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACGAAAGACTGACGCTCA
GGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTA
AACGATGTCGACTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCCGGAGCTAACGCG
TTAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACG
GGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCT
TACCTGGTCTTGACATCCACGGAAGTTTTCAGAGATGAGAATGTGCCTTCGGGAAC
CGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGTTAA
GTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTCCGGCCGGGAACTC
AAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGGATGACGTCAAGTCATCA
TGGCCCTTACGACCAGGGCTACACACGTGCTACAATGGCGCATACAAAGAGAAGC
GACCTCGCGAGAGCAAGCGGACCTCATAAAGTGCGTCGTAGTCCGGATTGGAGTCT
GCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGTGGATCAGAATGCCACGG
TGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGC
AAAAGAAGTAGGTAGCTTAACCTTCGGAGGCGCTAC

***Figure S.118. Reverse sequence of REPNE-12 isolate.*** *The FASTA sequence were exported from the chromatogram of reverse sequence of REPNE-12 isolate after the trim of low-quality bases at both ends.*

7.26 REPNE-13 Isolate

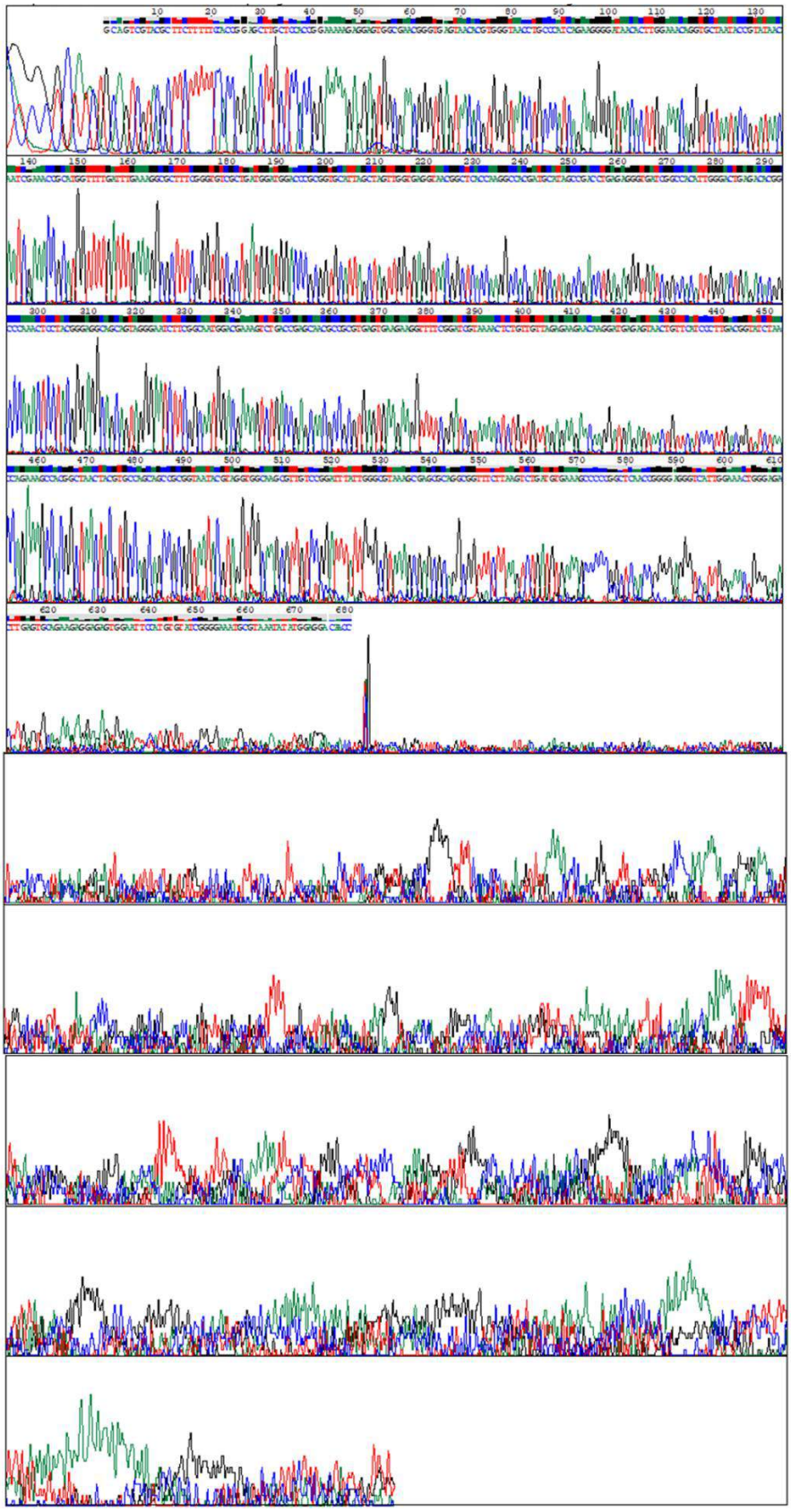


***Figure S.119. Chromatogram of forward sequence of REPNE-13 isolate.*** *Low-quality bases at both ends of the sequence were trimmed using Chromas software with default setting.*

GCAGTCGTACGCTTCTTTTTCCACCGGAGCTTGCTCCACCGGAAAAAGAGGAGTGG
CGAACGGGTGAGTAACACGTGGGTAACCTGCCCATCAGAAGGGGATAACACTTGG
AAACAGGTGCTAATACCGTATAACAATCGAAACCGCATGGTTTTGATTTGAAAGGC
GCTTTCGGGTGTCGCTGATGGATGGACCCGCGGTGCATTAGCTAGTTGGTGAGGTA
ACGGCTCACCAAGGCCACGATGCATAGCCGACCTGAGAGGGTGATCGGCCACATTG
GGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTTCGGCA
ATGGACGAAAGTCTGACCGAGCAACGCCGCGTGAGTGAAGAAGGTTTTCGGATCGT
AAAACTCTGTTGTTAGAGAAGAACAAGGATGAGAGTAACTGTTCATCCCTTGACGG
TATCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGG
TGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTCTTAAG
TCTGATGTGAAAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGAGACTT
GAGTGCAGAAGAGGAGAGTGGAATTCCATGTGTATCGGGGAAATGCGTAAATATA
TGGAGGACAACC

***Figure S.120. Forward sequence of REPNE-13 isolate.*** *The FASTA sequence were exported from the chromatogram of forward sequence of REPNE-13 isolate after the trim of low-quality bases at both ends.*

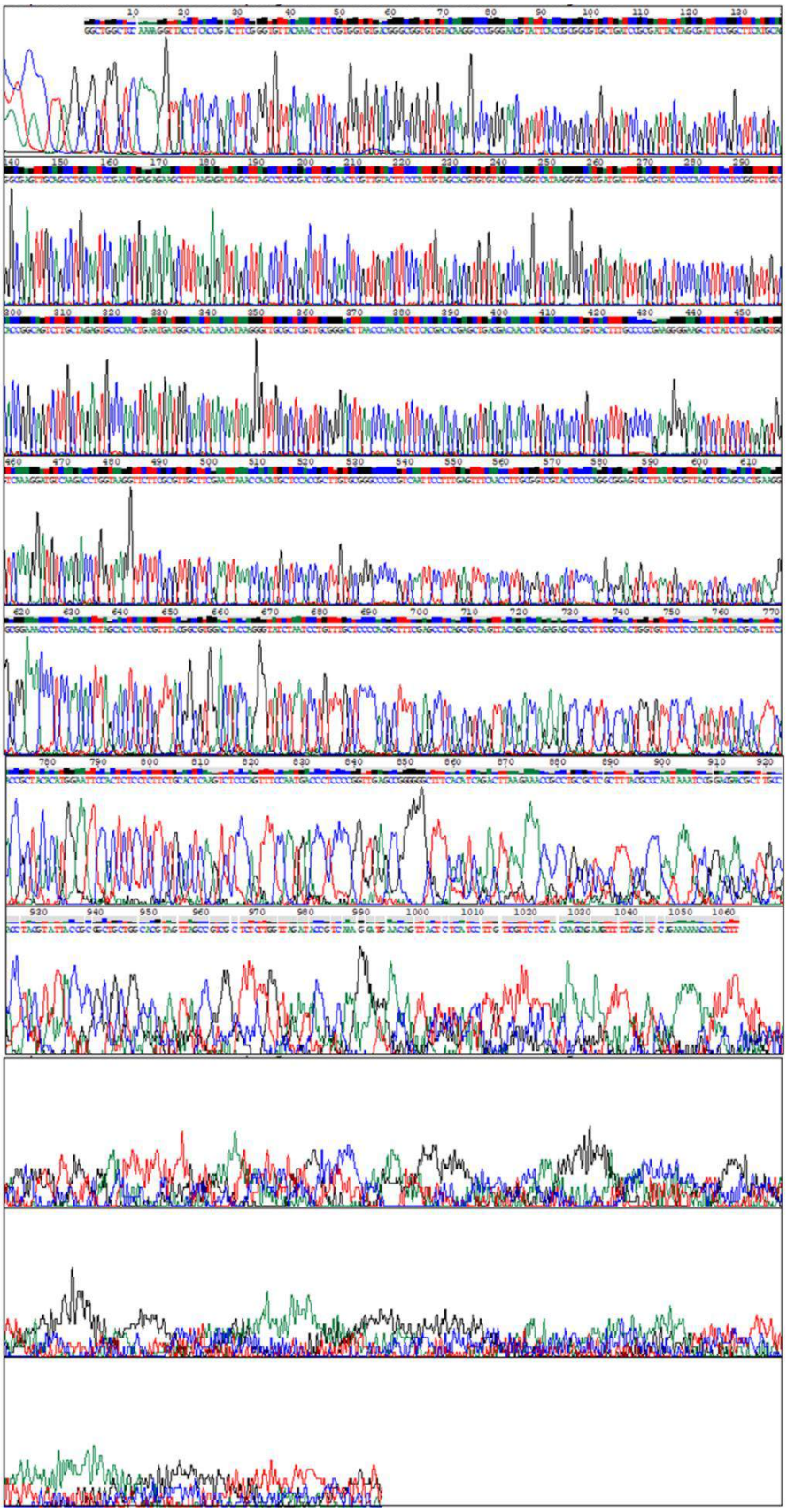


***Figure S.121. Chromatogram of reverse sequence of REPNE-13 isolate.*** *Low-quality bases at both ends of the sequence were trimmed using Chromas software with default setting.*

AAAGTATTGTTTTTTCTGATCGTAAAAACTTCTGCTTGTAGAGAACGAACAAGGAT
GAGAGTAACTGTTCATCCTTTGACGGTATCTAACCAAGAGAGCGACGGCTAACTAC
GTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTCGTCCGGATTTATTGGGC
GTAAAGCGAGCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCCCCGGCTCAACC
GGGGAGGGTCATTGGAAACTGGGAGACTTGAGTGCAGAAGAGGAGAGTGGAATTC
CATGTGTAGCGGTGAAATGCGTAGATATATGGAGGAACACCAGTGGCGAAGGCGG
CTCTCTGGTCTGTAACTGACGCTGAGGCTCGAAAGCGTGGGGAGCAAACAGGATTA
GATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTGGAGGGTTTCCG
CCCTTCAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGACCGCAA
GGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTT
TAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTTTGACCACTCTA
GAGATAGAGCTTCCCCTTCGGGGGCAAAGTGACAGGTGGTGCATGGTTGTCGTCAG
CTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATTGTTAGT
TGCCATCATTCAGTTGGGCACTCTAGCAAGACTGCCGGTGACAAACCGGAGGAAGG
TGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACA
ATGGGAAGTACAACGAGTTGCGAAGTCGCGAGGCTAAGCTAATCTCTTAAAGCTTC
TCTCAGTTCGGATTGCAGGCTGCAACTCGCCTGCATGAAGCCGGAATCGCTAGTAA
TCGCGGATCAGCACGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGT
CACACCACGAGAGTTTGTAACACCCGAAGTCGGTGAGGTAACCTTTTGGAGCCAGC
C

***Figure S.122. Reverse sequence of REPNE-13 isolate.*** *The FASTA sequence were exported from the chromatogram of reverse sequence of REPNE-13 isolate after the trim of low-quality bases at both ends.*

7.27 REPNE-14 Isolate

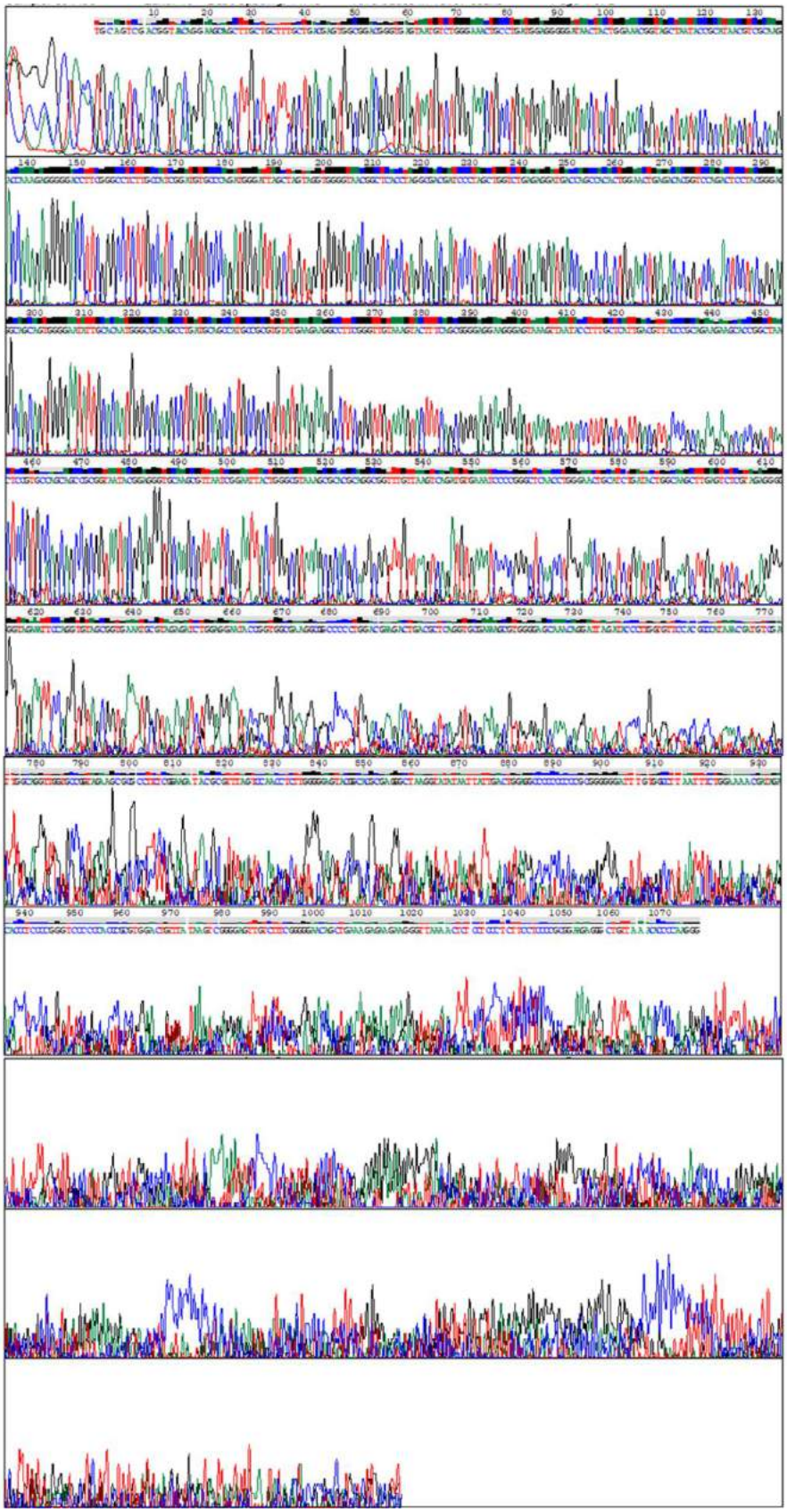


***Figure S.123. Chromatogram of forward sequence of REPNE-14 isolate.*** *Low-quality bases at both ends of the sequence were trimmed using Chromas software with default setting.*

TGCAGTCGACGGTAACAGGAAGCAAGCTTGCTGCTTTGCTGACGAGTGGCGGACGG
GTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGAAACGGTA
GCTAATACCGCATAACGTCGCAAGACCAAAGAGGGGGACCTTCGGGCCTCTTGCCA
TCGGATGTGCCCAGATGGGATTAGCTAGTAGGTGGGGTAACGGCTCACCTAGGCGA
CGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGACACGGTCC
AGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGAT
GCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGG
GAGGAAGGGAGTAAAGTTAATACCTTTGCTCATTGACGTTACCCGCAGAAGAAGCA
CCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGG
AATTACTGGGCGTAAAGCGCACGCAGGCGGTTTGTTAAGTCAGATGTGAAATCCCC
GGGCTCAACCTGGGAACTGCATCTGATACTGGCAAGCTTGAGTCTCGTAGAGGGGG
GTAGAAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTG
GCGAAGGCGGCCCCCTGGACGAAGACTGACGCTCAGGTGCGAAAAGCGTGGGGAG
CAAACAGGATTAGATACCCTTGGTGTTCCACGCCCATAAACGATGTCGATTGGCAG
GTTGGTGCCGGTAGAAGCGCGCCTTCTCGGAAGATACGCGTTTAGTCCAACCTCTT
GGGGGAGTACGGCACGCGAGGGCTAAGGTATATAATTATTGACTGGAGGCCCCCCC
CCCGCGGGGGGATTTGTGGCCTTAATTTCTGGAAAACGATAGACACCCTCCCCGGG
TCCCCCCACCCGCGTGGACTGTTTATAAGTCGGGGAGTTGTCTTTCGGGGGAACAG
CTGAAAGAGAAGAAGGGTTAAAACTCTCCTCCCTCTTCCTCCCCGCGGAAGAGGGC
TGTTAAACACCCCAAGGG

***Figure S.124. Forward sequence of REPNE-14 isolate.*** *The FASTA sequence were exported from the chromatogram of forward sequence of REPNE-14 isolate after the trim of low-quality bases at both ends.*

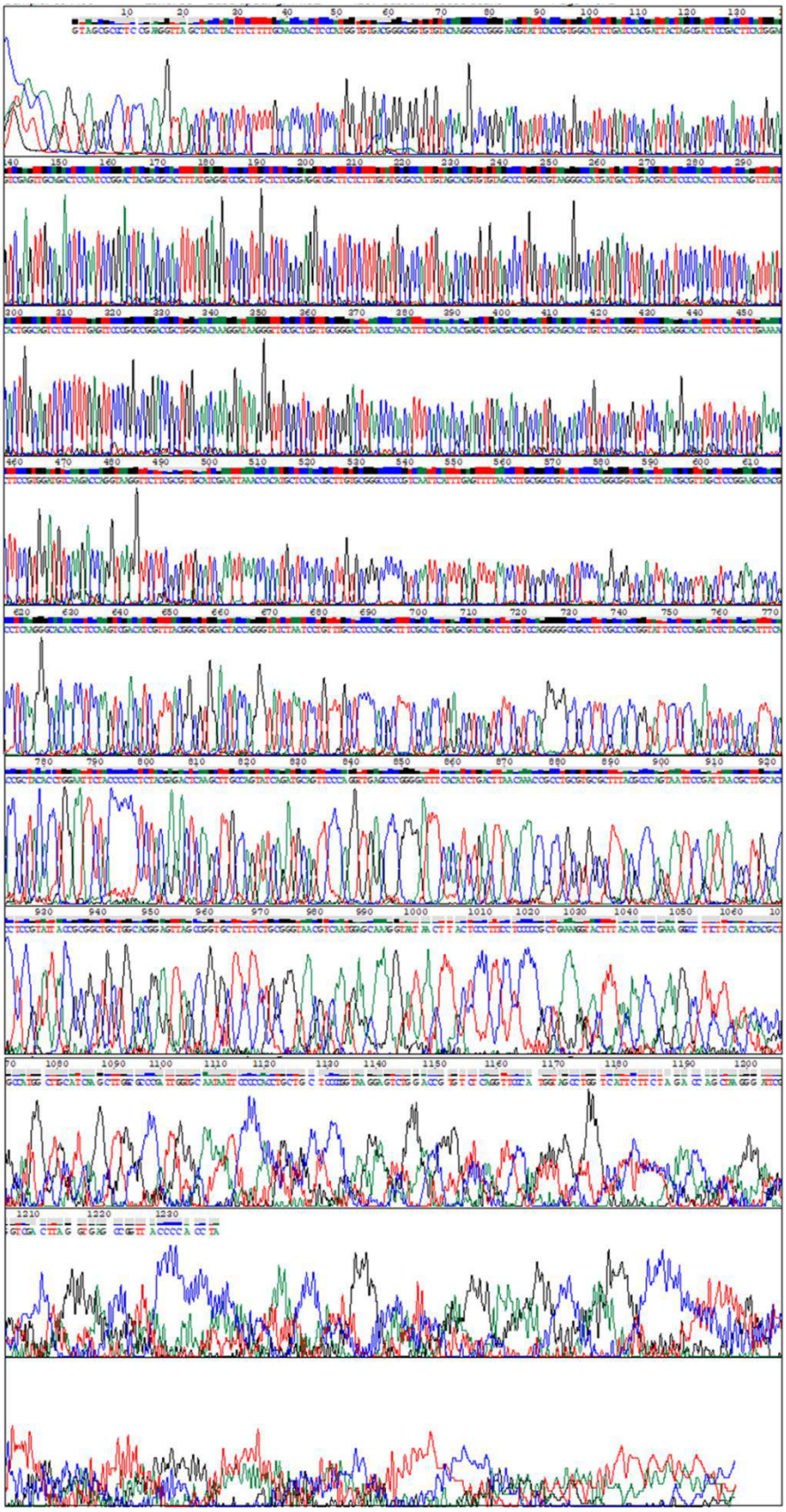


***Figure S.125. Chromatogram of reverse sequence of REPNE-14 isolate.*** *Low-quality bases at both ends of the sequence were trimmed using Chromas software with default setting.*

TAGGTGGGGTAACCGGCTCACCTAAGTCGACCGAATCCCTTAGCTGGTCTAGAAGA
ATGACCAGGCTACCATGGGAACCTGAGACACGGTCCAGACTCCTTACCGGGGAGCA
GCAGGTGGGGGAATTATTGCACCAATCGGGCGCCAAGCTTGATGCAAGCCATGGCA
GCGTGGTATGAAGAAGGGCCTTTCGGGTTGTAAAGTACCTTTTCAGCGGGGGAGGA
AAGGGAGTAAGTTATTACCTTTGCTCCATTGACGTTACCCGCAGAAGAAGCACCGG
CTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATT
ACTGGGCGTAAAGCGCACGCAGGCGGTTTGTTAAGTCAGATGTGAAATCCCCGGGC
TCAACCTGGGAACTGCATCTGATACTGGCAAGCTTGAGTCTCGTAGAGGGGGGTAG
AATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGAA
GGCGGCCCCCTGGACGAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACA
GGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGAGGTTGTGCC
CTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGC
CGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATG
TGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGTCTTGACATCCACGGAAGT
TTTCAGAGATGAGAATGTGCCTTCGGGAACCGTGAGACAGGTGCTGCATGGCTGTC
GTCAGCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATC
CTTTGTTGCCAGCGGTCCGGCCGGGAACTCAAAGGAGACTGCCAGTGATAAACTGG
AGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGACCAGGGCTACACAC
GTGCTACAATGGCGCATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCTCA
TAAAGTGCGTCGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAAT
CGCTAGTAATCGTGGATCAGAATGCCACGGTGAATACGTTCCCGGGCCTTGTACAC
ACCGCCCGTCACACCATGGGAGTGGGTTGCAAAAGAAGTAGGTAGCTAACCTTCGG
AGGGCGCTAC

***Figure S.126. Reverse sequence of REPNE-13 isolate.*** *The FASTA sequence were exported from the chromatogram of reverse sequence of REPNE-13 isolate after the trim of low-quality bases at both ends.*

# Appendix 8. Strain Diversity of *E. coli*

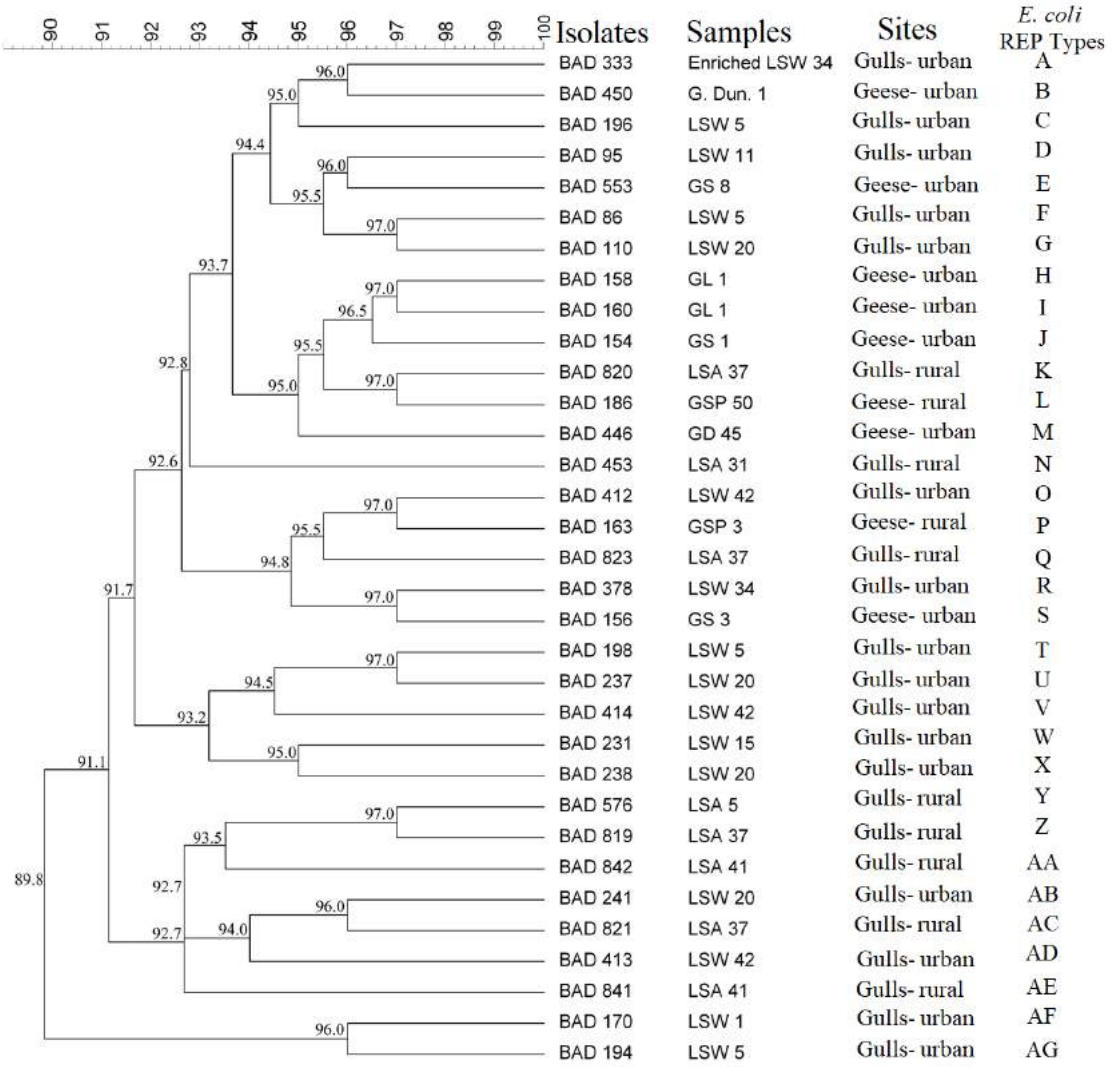



***Figure S.127. The dendrogram of the confirmed representative-E. coli isolates (resistant and sensitive) in gulls and geese.*** *Cluster analysis was done using the number of band difference coefficient and UPGMA method. Numbers at the nodes show the equated similarity (%) of the band difference in Fig.15 (section 4.8.1). The samples (isolate numbers and sites) and E. coli REP types are shown in the dendrogram. The axis line at the top (90-100) indicates the similarity (%). REP types have been assigned based on > 97% similarity (equated to <3 bands difference rule).*

***Table S.24. Diversity of E. coli REP types between birds and sites***. *Shared REP types between bird taxa are highlighted in yellow. Shared REP types between isolates from different sites within the same taxon are highlighted in yellow.*

| Isolate reference numbers | Samples | Sites | *E. coli* REP Types |
|---|---|---|---|
| BAD 333 | Enriched LSW 34 | Gulls- urban | A |
| BAD 449 | G. Dun. 1 | Geese- urban | B |
| BAD 450 | G. Dun. 1 | Geese- urban | |
| BAD 551 | G. Dun. 6 | Geese- urban | |
| BAD 196 | LSW 5 | Gulls- urban | C |
| BAD 197 | LSW 5 | Gulls- urban | |
| BAD 90 | LSW 11 | Gulls- urban | D |
| BAD 91 | LSW 11 | Gulls- urban | |
| BAD 92 | LSW 11 | Gulls- urban | |
| BAD 93 | LSW 11 | Gulls- urban | |
| BAD 94 | LSW 11 | Gulls- urban | |
| BAD 95 | LSW 11 | Gulls- urban | |
| BAD 96 | LSW 11 | Gulls- urban | |
| BAD 97 | LSW 11 | Gulls- urban | |
| BAD 98 | LSW 11 | Gulls- urban | |
| BAD 99 | LSW 11 | Gulls- urban | |
| BAD 553 | GS 8 | Geese- urban | E |
| BAD 85 | LSW 5 | Gulls- urban | F |
| BAD 86 | LSW 5 | Gulls- urban | |
| BAD 87 | LSW 5 | Gulls- urban | |
| BAD 88 | LSW 5 | Gulls- urban | |
| BAD 105 | LSW 15 | Gulls- urban | |
| BAD 336 | Enriched LSW 34 | Gulls- urban | |
| BAD 337 | Enriched LSW 34 | Gulls- urban | |
| BAD 107 | LSW 20 | Gulls- urban | G |
| BAD 108 | LSW 20 | Gulls- urban | |
| BAD 109 | LSW 20 | Gulls- urban | |
| BAD 110 | LSW 20 | Gulls- urban | |
| BAD 111 | LSW 20 | Gulls- urban | |
| BAD 112 | LSW 20 | Gulls- urban | |
| BAD 113 | LSW 20 | Gulls- urban | |
| BAD 114 | LSW 20 | Gulls- urban | |
| BAD 115 | LSW 20 | Gulls- urban | |
| BAD 323 | Enriched LSW 20 | Gulls- urban | |
| BAD 324 | Enriched LSW 20 | Gulls- urban | |
| BAD 325 | Enriched LSW 20 | Gulls- urban | |
| BAD 326 | Enriched LSW 20 | Gulls- urban | |
| BAD 327 | Enriched LSW 20 | Gulls- urban | |
| BAD 158 | GL 1 | Geese- urban | H |
| BAD 159 | GL 1 | Geese- urban | I |
| BAD 160 | GL 1 | Geese- urban | |

*(Continued)*

| Isolate reference numbers | Samples | Sites | *E. coli* REP Types |
|---|---|---|---|
| BAD 177 | GSP 26 | Geese- rural | J |
| BAD 154 | GS 1 | Geese- urban | |
| BAD 820 | LSA 37 | Gulls- rural | K |
| BAD 184 | GSP 50 | Geese- rural | L |
| BAD 185 | GSP 50 | Geese- rural | |
| BAD 186 | GSP 50 | Geese- rural | |
| BAD 187 | GSP 50 | Geese- rural | |
| BAD 188 | GSP 50 | Geese- rural | |
| BAD 153 | GS 1 | Geese- urban | |
| BAD 150 | GD 6 | Geese- urban | M |
| BAD 151 | GD 6 | Geese- urban | |
| BAD 444 | GD 45 | Geese- urban | |
| BAD 445 | GD 45 | Geese- urban | |
| BAD 446 | GD 45 | Geese- urban | |
| BAD 447 | GD 45 | Geese- urban | |
| BAD 448 | GD 45 | Geese- urban | |
| BAD 609 | LSA 12 | Gulls- rural | |
| BAD 610 | LSA 12 | Gulls- rural | |
| BAD 611 | LSA 12 | Gulls- rural | |
| BAD 612 | LSA 12 | Gulls- rural | |
| BAD 613 | LSA 12 | Gulls- rural | |
| BAD 451 | LSA 31 | Gulls- rural | N |
| BAD 452 | LSA 31 | Gulls- rural | |
| BAD 453 | LSA 31 | Gulls- rural | |
| BAD 454 | LSA 31 | Gulls- rural | |
| BAD 455 | LSA 31 | Gulls- rural | |
| BAD 476 | Enriched GD 31 | Geese- urban | |
| BAD 411 | LSW 42 | Gulls- urban | O |
| BAD 412 | LSW 42 | Gulls- urban | |
| BAD 161 | GSP 3 | Geese- rural | P |
| BAD 162 | GSP 3 | Geese- rural | |
| BAD 163 | GSP 3 | Geese- rural | |
| BAD 164 | GSP 3 | Geese- rural | |
| BAD 165 | GSP 3 | Geese- rural | |
| BAD 178 | GSP 38 | Geese- rural | |
| BAD 179 | GSP 38 | Geese- rural | |
| BAD 180 | GSP 38 | Geese- rural | |
| BAD 181 | GSP 38 | Geese- rural | |
| BAD 182 | GSP 38 | Geese- rural | |
| BAD 823 | LSA 37 | Gulls- rural | Q |
| BAD 376 | LSW 34 | Gulls- urban | R |
| BAD 377 | LSW 34 | Gulls- urban | |
| BAD 378 | LSW 34 | Gulls- urban | |
| BAD 379 | LSW 34 | Gulls- urban | |
| BAD 380 | LSW 34 | Gulls- urban | |

*(Continued)*

| Isolate reference numbers | Samples | Sites | *E. coli* REP Types |
|---|---|---|---|
| BAD 156 | GS 3 | Geese- urban | S |
| BAD 198 | LSW 5 | Gulls- urban | T |
| BAD 237 | LSW 20 | Gulls- urban | U |
| BAD 239 | LSW 20 | Gulls- urban | |
| BAD 414 | LSW 42 | Gulls- urban | V |
| BAD 415 | LSW 42 | Gulls- urban | |
| BAD 231 | LSW 15 | Gulls- urban | W |
| BAD 238 | LSW 20 | Gulls- urban | X |
| BAD 240 | LSW 20 | Gulls- urban | |
| BAD 574 | LSA 5 | Gulls- rural | Y |
| BAD 575 | LSA 5 | Gulls- rural | |
| BAD 576 | LSA 5 | Gulls- rural | |
| BAD 577 | LSA 5 | Gulls- rural | |
| BAD 578 | LSA 5 | Gulls- rural | |
| BAD 819 | LSA 37 | Gulls- rural | Z |
| BAD 822 | LSA 37 | Gulls- rural | |
| BAD 839 | LSA 41 | Gulls- rural | AA |
| BAD 840 | LSA 41 | Gulls- rural | |
| BAD 842 | LSA 41 | Gulls- rural | |
| BAD 843 | LSA 41 | Gulls- rural | |
| BAD 241 | LSW 20 | Gulls- urban | AB |
| BAD 821 | LSA 37 | Gulls- rural | AC |
| BAD 413 | LSW 42 | Gulls- urban | AD |
| BAD 841 | LSA 41 | Gulls- rural | AE |
| BAD 169 | LSW 1 | Gulls- urban | AF |
| BAD 170 | LSW 1 | Gulls- urban | |
| BAD 194 | LSW 5 | Gulls- urban | AG |
| BAD 195 | LSW 5 | Gulls- urban | |

8.1 Gull Faeces

***Table S.25. Strain-richness within gull faecal samples.*** *The percentage was determined by a calculation (number of samples/total number of samples multiplied by 100).*

| Strain-richness within sample | Number of samples |
|---|---|
| 1 *E. coli* REP type | 5 (42%) |
| 2 *E. coli* REP types | 2 (17%) |
| 3 *E. coli* REP types | 2 (17%) |
| 4 *E. coli* REP types | 3 (25%) |
| 5 *E. coli* REP types | - |

***Table S.26. Details of the strain-richness within each gull faecal sample.***

| Isolate reference numbers | Samples | Sites | *E. coli* REP Types | Strain-richness |
|---|---|---|---|---|
| BAD 169 | LSW 1 | Gulls- urban | AF | 1 |
| BAD 170 | LSW 1 | Gulls- urban | | |
| BAD 85 | LSW 5 | Gulls- urban | F | 4 |
| BAD 86 | LSW 5 | Gulls- urban | | |
| BAD 87 | LSW 5 | Gulls- urban | | |
| BAD 88 | LSW 5 | Gulls- urban | | |
| BAD 194 | LSW 5 | Gulls- urban | AG | |
| BAD 195 | LSW 5 | Gulls- urban | | |
| BAD 196 | LSW 5 | Gulls- urban | C | |
| BAD 197 | LSW 5 | Gulls- urban | | |
| BAD 198 | LSW 5 | Gulls- urban | T | |
| BAD 90 | LSW 11 | Gulls- urban | D | 1 |
| BAD 91 | LSW 11 | Gulls- urban | | |
| BAD 92 | LSW 11 | Gulls- urban | | |
| BAD 93 | LSW 11 | Gulls- urban | | |
| BAD 94 | LSW 11 | Gulls- urban | | |
| BAD 95 | LSW 11 | Gulls- urban | | |
| BAD 96 | LSW 11 | Gulls- urban | | |
| BAD 97 | LSW 11 | Gulls- urban | | |
| BAD 98 | LSW 11 | Gulls- urban | | |
| BAD 99 | LSW 11 | Gulls- urban | | |
| BAD 105 | LSW 15 | Gulls- urban | F | 2 |
| BAD 231 | LSW 15 | Gulls- urban | W | |
| BAD 107 | LSW 20 | Gulls- urban | G | 4 |
| BAD 108 | LSW 20 | Gulls- urban | | |
| BAD 109 | LSW 20 | Gulls- urban | | |
| BAD 110 | LSW 20 | Gulls- urban | | |
| BAD 111 | LSW 20 | Gulls- urban | | |
| BAD 112 | LSW 20 | Gulls- urban | | |
| BAD 113 | LSW 20 | Gulls- urban | | |
| BAD 114 | LSW 20 | Gulls- urban | | |
| BAD 115 | LSW 20 | Gulls- urban | | |
| BAD 238 | LSW 20 | Gulls- urban | X | |
| BAD 240 | LSW 20 | Gulls- urban | | |
| BAD 237 | LSW 20 | Gulls- urban | U | |
| BAD 239 | LSW 20 | Gulls- urban | | |
| BAD 241 | LSW 20 | Gulls- urban | AB | |
| BAD 323 | Enriched LSW 20 | Gulls- urban | G | |
| BAD 324 | Enriched LSW 20 | Gulls- urban | | |
| BAD 325 | Enriched LSW 20 | Gulls- urban | | |
| BAD 326 | Enriched LSW 20 | Gulls- urban | | |
| BAD 327 | Enriched LSW 20 | Gulls- urban | | |
| BAD 333 | Enriched LSW 34 | Gulls- urban | A | 3 |
| BAD 336 | Enriched LSW 34 | Gulls- urban | F | |
| BAD 337 | Enriched LSW 34 | Gulls- urban | | |
| BAD 376 | LSW 34 | Gulls- urban | R | |
| BAD 377 | LSW 34 | Gulls- urban | | |
| BAD 378 | LSW 34 | Gulls- urban | | |
| BAD 379 | LSW 34 | Gulls- urban | | |
| BAD 380 | LSW 34 | Gulls- urban | | |

*(Continued)*

| Isolate reference numbers | Samples | Sites | *E. coli* REP Types | Strain-richness |
|---|---|---|---|---|
| BAD 411 | LSW 42 | Gulls- urban | O | 3 |
| BAD 412 | LSW 42 | Gulls- urban | | |
| BAD 413 | LSW 42 | Gulls- urban | AD | |
| BAD 414 | LSW 42 | Gulls- urban | V | |
| BAD 415 | LSW 42 | Gulls- urban | | |
| BAD 574 | LSA 5 | Gulls- rural | Y | 1 |
| BAD 575 | LSA 5 | Gulls- rural | | |
| BAD 576 | LSA 5 | Gulls- rural | | |
| BAD 577 | LSA 5 | Gulls- rural | | |
| BAD 578 | LSA 5 | Gulls- rural | | |
| BAD 609 | LSA 12 | Gulls- rural | M | 1 |
| BAD 610 | LSA 12 | Gulls- rural | | |
| BAD 611 | LSA 12 | Gulls- rural | | |
| BAD 612 | LSA 12 | Gulls- rural | | |
| BAD 613 | LSA 12 | Gulls- rural | | |
| BAD 451 | LSA 31 | Gulls- rural | N | 1 |
| BAD 452 | LSA 31 | Gulls- rural | | |
| BAD 453 | LSA 31 | Gulls- rural | | |
| BAD 454 | LSA 31 | Gulls- rural | | |
| BAD 455 | LSA 31 | Gulls- rural | | |
| BAD 820 | LSA 37 | Gulls- rural | K | 4 |
| BAD 821 | LSA 37 | Gulls- rural | AC | |
| BAD 819 | LSA 37 | Gulls- rural | Z | |
| BAD 822 | LSA 37 | Gulls- rural | | |
| BAD 823 | LSA 37 | Gulls- rural | Q | |
| BAD 839 | LSA 41 | Gulls- rural | AA | 2 |
| BAD 840 | LSA 41 | Gulls- rural | | |
| BAD 842 | LSA 41 | Gulls- rural | | |
| BAD 843 | LSA 41 | Gulls- rural | | |
| BAD 841 | LSA 41 | Gulls- rural | AE | |

8.2 Goose Faeces

***Table S.27. Strain-richness within goose faecal samples.*** *The percentage was determined by a calculation (number of samples/total number of samples multiplied by 100).*

| Strain-richness within sample | Number of samples |
|---|---|
| 1 *E. coli* REP type | 11 (85%) |
| 2 *E. coli* REP types | 2 (15%) |
| 3 *E. coli* REP types | - |
| 4 *E. coli* REP types | - |
| 5 *E. coli* REP types | - |

***Table S.28. Details of the strain-richness within each goose faecal sample.***

| Isolate reference numbers | Samples | Sites | *E. coli* REP Types | Strain Richness |
|---|---|---|---|---|
| BAD 161 | GSP 3 | Geese- rural | P | 1 |
| BAD 162 | GSP 3 | Geese- rural | | |
| BAD 163 | GSP 3 | Geese- rural | | |
| BAD 164 | GSP 3 | Geese- rural | | |
| BAD 165 | GSP 3 | Geese- rural | | |
| BAD 177 | GSP 26 | Geese- rural | J | 1 |
| BAD 178 | GSP 38 | Geese- rural | P | 1 |
| BAD 179 | GSP 38 | Geese- rural | | |
| BAD 180 | GSP 38 | Geese- rural | | |
| BAD 181 | GSP 38 | Geese- rural | | |
| BAD 182 | GSP 38 | Geese- rural | | |
| BAD 184 | GSP 50 | Geese- rural | L | 1 |
| BAD 185 | GSP 50 | Geese- rural | | |
| BAD 186 | GSP 50 | Geese- rural | | |
| BAD 187 | GSP 50 | Geese- rural | | |
| BAD 188 | GSP 50 | Geese- rural | | |
| BAD 158 | GL 1 | Geese- urban | H | 2 |
| BAD 159 | GL 1 | Geese- urban | I | |
| BAD 160 | GL 1 | Geese- urban | | |
| BAD 449 | G. Dun. 1 | Geese- urban | B | 1 |
| BAD 450 | G. Dun. 1 | Geese- urban | | |
| BAD 551 | G. Dun. 6 | Geese- urban | B | 1 |
| BAD 153 | GS 1 | Geese- urban | L | 2 |
| BAD 154 | GS 1 | Geese- urban | J | |
| BAD 156 | GS 3 | Geese- urban | S | 1 |
| BAD 553 | GS 8 | Geese- urban | E | 1 |
| BAD 150 | GD 6 | Geese- urban | M | 1 |
| BAD 151 | GD 6 | Geese- urban | | |
| BAD 476 | Enriched GD 31 | Geese- urban | N | 1 |
| BAD 444 | GD 45 | Geese- urban | M | 1 |
| BAD 445 | GD 45 | Geese- urban | | |
| BAD 446 | GD 45 | Geese- urban | | |
| BAD 447 | GD 45 | Geese- urban | | |
| BAD 448 | GD 45 | Geese- urban | | |

# Appendix 9. Clonal Analysis of *E. coli* Strains/Types

## 9.1 *E. coli* Phylo-group

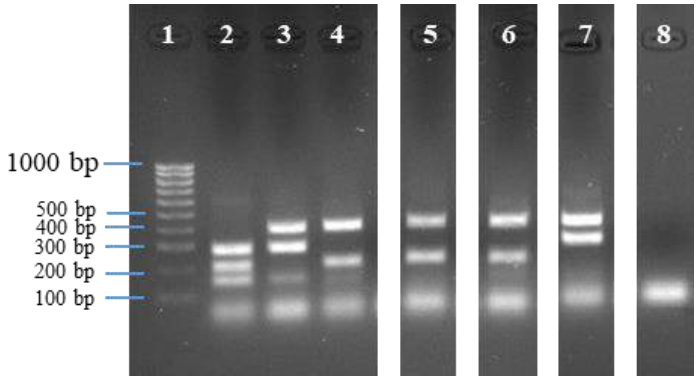



***Figure S.128. The quadruplex phylo-group PCR assay of ESBL-producer E. coli.*** *Lane 1: Ladder 100 bp. Lane 2: ERI 39 for phylo-group B2 (arpA -, chuA +, yjaA +, TspE4.C2 +). Lane 8: Sterile distilled water for negative control. Lane 3-7: E. coli type F, type D, type G, type A, type N.*

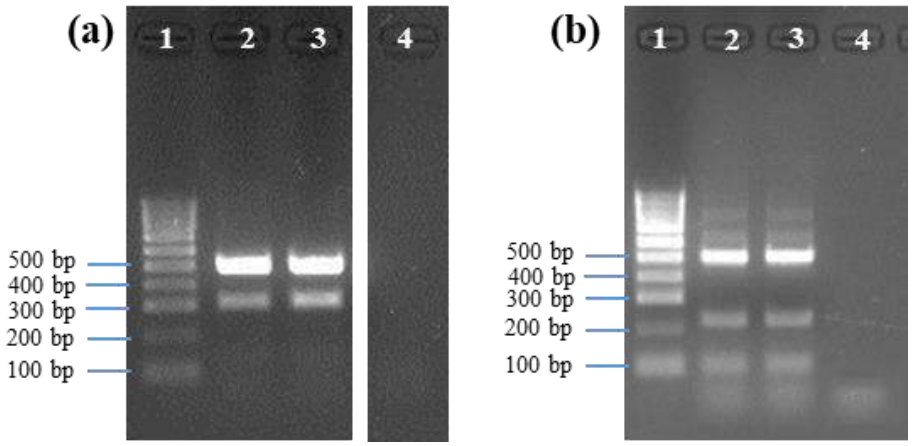



***Figure S.129. (a) The phylo-group E-specific PCR assay of ESBL-producer E. coli.*** *Lane 1: Ladder 100 bp. Lane 2: ERI 40 for phylo-group E (trpA – 489 bp, arpA E-specific – 301 bp). Lane 5: Sterile distilled water for negative control. Lane 3: E. coli type F.* ***(b) The phylo-group C-specific PCR assay of ESBL-producer E. coli.*** *Lane 1: Ladder 100 bp. Lane 2: ATCC® 47055 for phylo-group C (trpA – 489 bp, trpA C-specific – 219 bp). Lane 4: Sterile distilled water for negative control. Lane 3: E. coli type N.*

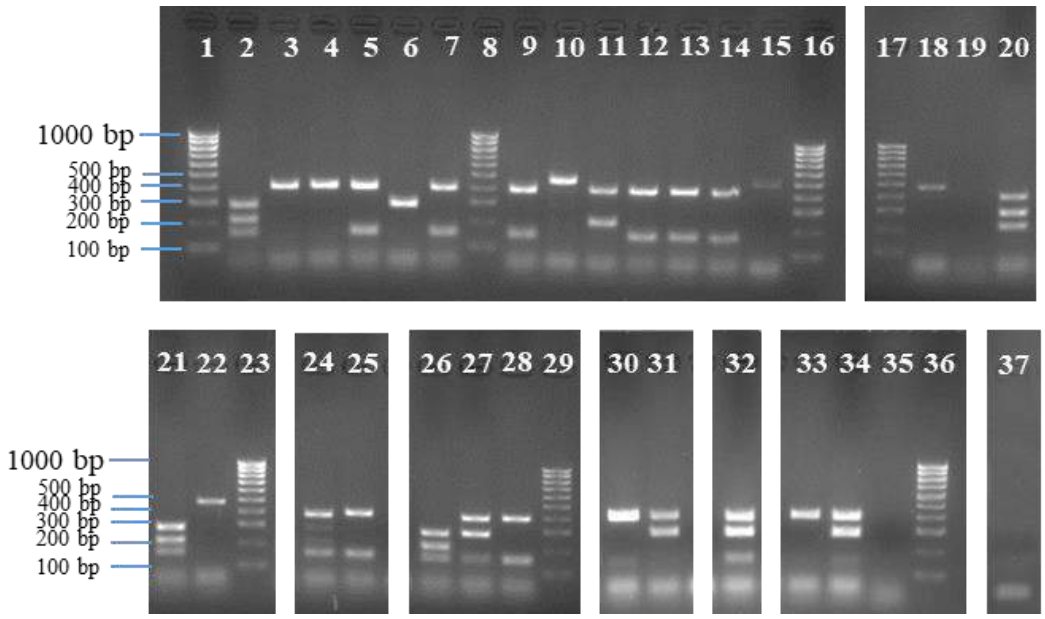



***Figure S.130. The quadruplex phylo-group PCR assay of ESBL-producer E. coli.*** *Lane 1, 8, 16, 17, 23, 29 and 36: Ladder 100 bp. Lane 2: ERI 39 for phylo-group B2 (arpA -, chuA +, yjaA +, TspE4.C2 +). Lane 37: Sterile distilled water for negative control. Lane 3-7: E. coli type X, type W, type U, type V, type AE. Lane 9-15: Type B, type S, type T, type E, type AB, type AC, type P. Lane 18-22: Type O, type H, type L, type K, type M. Lane 24-28: Type I, type J, type R, type AA, type AD. Lane 30-35: Type Y, type Z, type C, type AG, type AF, type Q.*

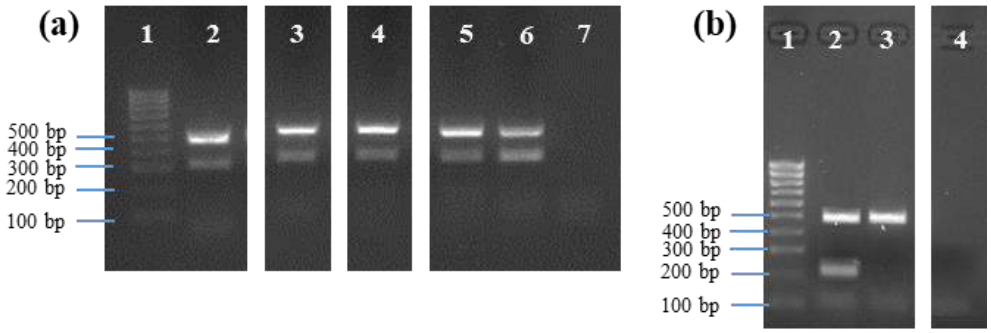



***Figure S.131. (a) The phylo-group E-specific PCR assay on ESBL sensitive E. coli.*** *Lane 1: Ladder 100 bp. Lane 2: ERI 40 for phylo-group E (trpA – 489 bp, arpA E-specific – 301 bp). Lane 7: Sterile distilled water for negative control. Lane 3-6: E. coli type L, type AA, type C, type AF.* ***(b) The phylo-group C-specific PCR assay on ESBL sensitive E. coli.*** *Lane 1: Ladder 100 bp. Lane 2: ATCC® 47055 for phylo-group C (trpA – 489 bp, trpA C-specific – 219 bp). Lane 4: Sterile distilled water for negative control. Lane 3: E. coli type Z.*

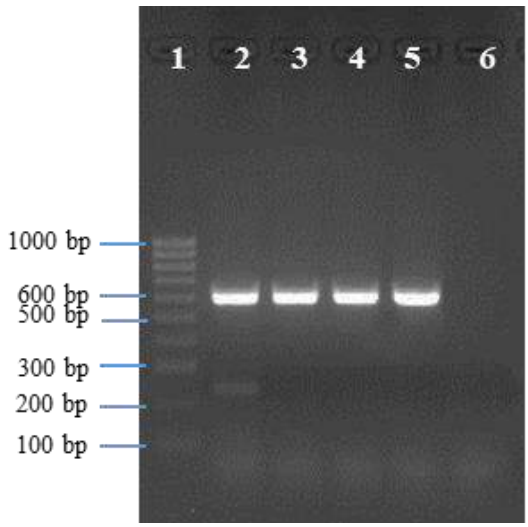



***Figure S.132. The cryptic clade PCR assay of ESBL sensitive E. coli shows Clade V (600 bp).*** *Lane 1: Ladder 100 bp. Lane 6: Sterile distilled water for negative control. Lane 2-5: E. coli type S, type P, type O, type M.*

9.2 Identification of Clinically Important STs

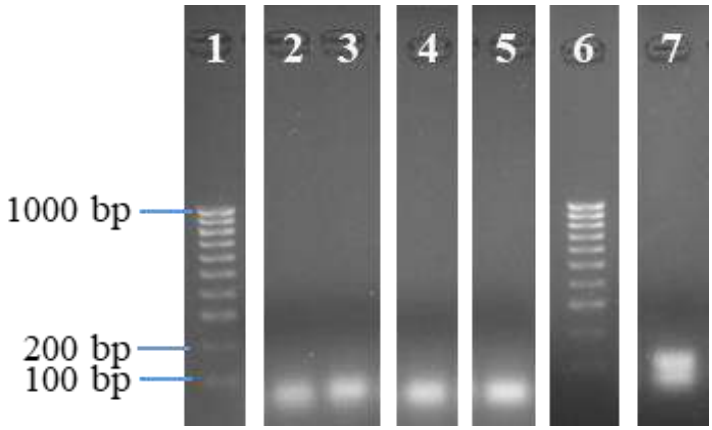



***Figure S.133. PCR-based MLST assay of ESBL-producer E. coli.*** *Lane 1 and 6: Ladder 100 bp. Lane 2-5: E. coli type F, type D, type G, type A. Lane 7: Type N, showing positive for ST 69 (104 bp).*

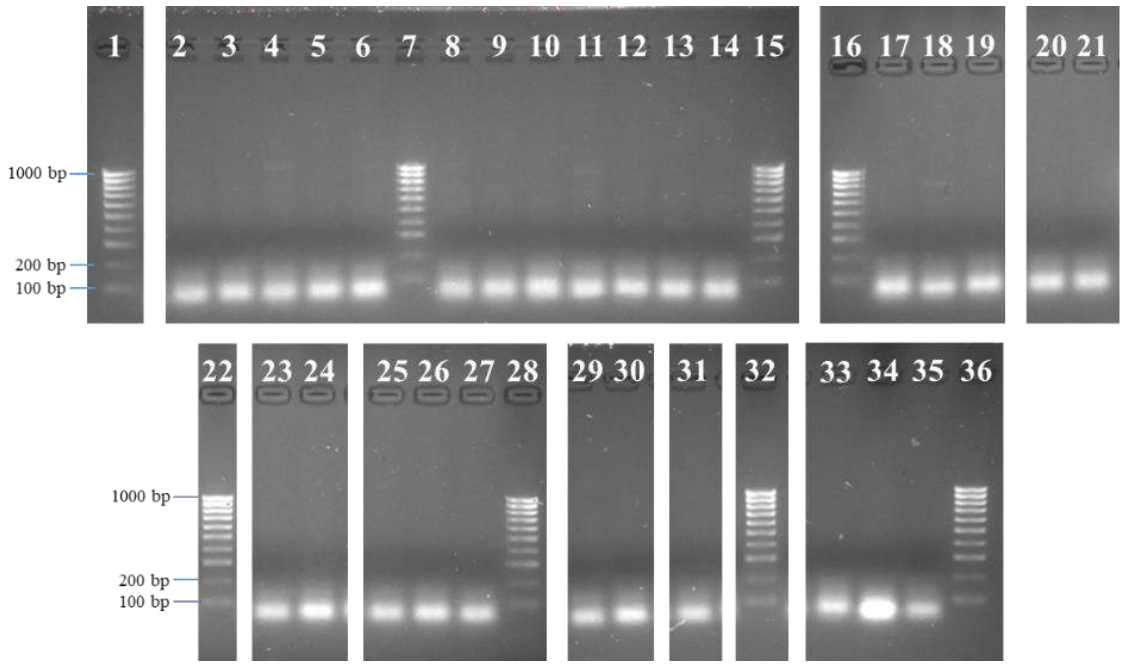



***Figure S.134. The PCR-based MLST assay of ESBL sensitive E. coli****. Lane 1, 7, 15, 16, 22, 28, 32 and 36: Ladder 100 bp. Lane 2-6: E. coli type X, type W, type U, type V, type AE. Lane 8-14: Type B, type S, type T, type E, type AB, type AC, type P. Lane 17-21:Type O, type H, type L, type K, type M. Lane 23-27: Type I, type J, type R, type AA, type AD. Lane 29-31: Type Y, type Z, type C. Lane 33-35: Type AG, type AF, type Q.*

# Appendix 10. Antibiotic Susceptibility Testing of ESBL-Producer Isolates and ESBL Sensitive *E. coli*

## 10.1 ESBL-producer Coliforms

***Table S.29. Zone of inhibition and interpretation of ESBL-producer isolates towards 12 antibiotic discs.*** *Disk diffusion AST method by EUCAST was followed (Matuschek et al.,* 2014*). Clinical breakpoints from EUCAST version 9.0 were used to interpret zone-inhibition diameters (EUCAST, 2019). [R]: Resistant. [I]: Intermediate. [S]: Sensitive. FOX = cefoxitin, MEM = meropenem, AMP = ampicillin, AMC = amoxicillin-clavulanic acid, CTX = cefotaxime, CAZ = ceftazidime, , CIP = ciprofloxacin, SXT = trimethoprim-sulfamethoxazole, TE = tetracycline, CN = gentamicin, C = chloramphenicol, TGC = tigecycline. N/A = Not applicable. MDR isolate (resistance ≥ 3 antibiotic classes) is highlighted in yellow.*

| Isolate reference numbers | Samples | Sites | Species | E. coli REP types | Zone of Inhibitions (mm) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | FOX | MEM | AMP | AMC | CTX | CAZ | CIP | SXT | TE | CN | C | TGC |
| BAD 95 | LSW 11 | Gulls- urban | *E. coli* | D | 26 [S] | 33 [S] | 0 [R] | 10 [R] | 0 [R] | 23 [S] | 0 [R] | 22 [S] | 0 [R] | 21 [S] | 26 [S] | 22 [S] |
| BAD 333 | Enriched LSW 34 | Gulls- urban | *E. coli* | A | 23 [S] | 32 [S] | 0 [R] | 22 [S] | 0 [R] | 14 [R] | 0 [R] | 30 [S] | 23 [S] | 23 [S] | 30 [S] | 23 [S] |
| BAD 86 | LSW 5 | Gulls- urban | *E. coli* | F | 23 [S] | 34 [S] | 0 [R] | 20 [S] | 0 [R] | 19 [I] | 30 [S] | 31 [S] | 21 [S] | 21 [S] | 23 [S] | 22 [S] |
| BAD 110 | LSW 20 | Gulls- urban | *E. coli* | G | 29 [S] | 34 [S] | 0 [R] | 26 [S] | 10 [R] | 21 [I] | 27 [S] | 30 [S] | 23 [S] | 20 [S] | 27 [S] | 22 [S] |
| BAD 453 | LSA 31 | Gulls- rural | *E. coli* | N | 25 [S] | 34 [S] | 0 [R] | 25 [S] | 0 [R] | 18 [R] | 29 [S] | 32 [S] | 0 [R] | 21 [S] | 28 [S] | 24 [S] |
| BAD 106 | LSW 20 | Gulls- urban | closest to *Klebsiella* sp. | N/A | 25 [S] | 30 [S] | 0 [R] | 20 [S] | 0 [R] | 14 [R] | 23 [I] | 0 [R] | 22 [S] | 20 [S] | 22 [S] | 19 [S] |

10.2 ESBL Sensitive *E. coli*

***Table S.30. Zone of inhibition and interpretation of ESBL sensitive E. coli towards 12 antibiotic discs.*** *Disk diffusion AST method by EUCAST was followed (Matuschek et al.,* 2014*). Clinical breakpoints from EUCAST version 9.0 were used to interpret zone-inhibition diameters (EUCAST, 2019). [R]: Resistant. [I]: Intermediate. [S]: Sensitive. FOX = cefoxitin, MEM = meropenem, AMP = ampicillin, AMC = amoxicillin-clavulanic acid, CTX = cefotaxime, CAZ = ceftazidime, , CIP = ciprofloxacin, SXT = trimethoprim-sulfamethoxazole, TE = tetracycline, CN = gentamicin, C = chloramphenicol, TGC = tigecycline. MDR isolate (resistance ≥ 3 antibiotic classes) is highlighted in yellow.*

| Isolate Reference Numbers | Samples | Sites | Species | E. coli REP types | Zone of Inhibitions (mm) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | FOX | MEM | AMP | AMC | CTX | CAZ | CIP | SXT | TE | CN | C | TGC |
| BAD 413 | LSW 42 | Gulls- urban | *E. coli* | AD | 27 [S] | 33 [S] | 0 [R] | 21 [S] | 28 [S] | 27 [S] | 31 [S] | 0 [R] | 0 [R] | 20 [S] | 23 [S] | 20 [S] |
| BAD 450 | G. Dun. 1 | Geese- urban | *E. coli* | B | 27 [S] | 35 [S] | 21 [S] | 23 [S] | 29 [S] | 29 [S] | 35 [S] | 30 [S] | 23 [S] | 21 [S] | 25 [S] | 21 [S] |
| BAD 196 | LSW 5 | Gulls- urban | *E. coli* | C | 27 [S] | 37 [S] | 22 [S] | 25 [S] | 31 [S] | 31 [S] | 36 [S] | 36 [S] | 24 [S] | 25 [S] | 31 [S] | 24 [S] |
| BAD 553 | GS 8 | Geese- urban | *E. coli* | E | 23 [S] | 33 [S] | 16 [S] | 21 [S] | 26 [S] | 26 [S] | 32 [S] | 30 [S] | 23 [S] | 20 [S] | 26 [S] | 21 [S] |
| BAD 158 | GL 1 | Geese- urban | *E. coli* | H | 27 [S] | 34 [S] | 22 [S] | 25 [S] | 29 [S] | 28 [S] | 33 [S] | 30 [S] | 22 [S] | 21 [S] | 30 [S] | 22 [S] |
| BAD 160 | GL 1 | Geese- urban | *E. coli* | I | 25 [S] | 31 [S] | 19 [S] | 21 [S] | 27 [S] | 26 [S] | 22 [I] | 30 [S] | 23 [S] | 19 [S] | 24 [S] | 23 [S] |
| BAD 154 | GS 1 | Geese- urban | *E. coli* | J | 25 [S] | 32 [S] | 20 [S] | 22 [S] | 28 [S] | 26 [S] | 31 [S] | 28 [S] | 24 [S] | 20 [S] | 26 [S] | 22 [S] |
| BAD 820 | LSA 37 | Gulls- rural | *E. coli* | K | 26 [S] | 34 [S] | 21 [S] | 23 [S] | 28 [S] | 28 [S] | 31 [S] | 30 [S] | 23 [S] | 20 [S] | 25 [S] | 21 [S] |
| BAD 186 | GSP 50 | Geese- rural | *E. coli* | L | 27 [S] | 33 [S] | 21 [S] | 24 [S] | 28 [S] | 28 [S] | 36 [S] | 29 [S] | 25 [S] | 22 [S] | 27 [S] | 24 [S] |
| BAD 446 | GD 45 | Geese- urban | *E. coli* | M | 25 [S] | 32 [S] | 21 [S] | 24 [S] | 29 [S] | 27 [S] | 40 [S] | 30 [S] | 23 [S] | 21 [S] | 28 [S] | 22 [S] |
| BAD 412 | LSW 42 | Gulls- urban | *E. coli* | O | 26 [S] | 34 [S] | 22 [S] | 24 [S] | 28 [S] | 27 [S] | 31 [S] | 29 [S] | 22 [S] | 21 [S] | 25 [S] | 22 [S] |
| BAD 163 | GSP 3 | Geese- rural | *E. coli* | P | 25 [S] | 33 [S] | 23 [S] | 25 [S] | 30 [S] | 28 [S] | 32 [S] | 29 [S] | 23 [S] | 20 [S] | 28 [S] | 21 [S] |
| BAD 823 | LSA 37 | Gulls- rural | *E. coli* | Q | 26 [S] | 34 [S] | 22 [S] | 26 [S] | 29 [S] | 28 [S] | 35 [S] | 29 [S] | 22 [S] | 18 [S] | 26 [S] | 21 [S] |
| BAD 378 | LSW 34 | Gulls- urban | *E. coli* | R | 28 [S] | 33 [S] | 22 [S] | 24 [S] | 30 [S] | 28 [S] | 32 [S] | 29 [S] | 24 [S] | 20 [S] | 28 [S] | 22 [S] |
| BAD 156 | GS 3 | Geese- urban | *E. coli* | S | 26 [S] | 33 [S] | 23 [S] | 24 [S] | 29 [S] | 28 [S] | 35 [S] | 28 [S] | 24 [S] | 22 [S] | 25 [S] | 21 [S] |
| BAD 198 | LSW 5 | Gulls- urban | *E. coli* | T | 25 [S] | 34 [S] | 24 [S] | 25 [S] | 27 [S] | 27 [S] | 29 [S] | 26 [S] | 21 [S] | 20 [S] | 27 [S] | 19 [S] |
| BAD 237 | LSW 20 | Gulls- urban | *E. coli* | U | 24 [S] | 33 [S] | 20 [S] | 22 [S] | 26 [S] | 27 [S] | 33 [S] | 29 [S] | 22 [S] | 20 [S] | 27 [S] | 21 [S] |
| BAD 414 | LSW 42 | Gulls- urban | *E. coli* | V | 23 [S] | 32 [S] | 18 [S] | 22 [S] | 27 [S] | 27 [S] | 30 [S] | 30 [S] | 24 [S] | 21 [S] | 25 [S] | 22 [S] |
| BAD 231 | LSW 15 | Gulls- urban | *E. coli* | W | 26 [S] | 33 [S] | 21 [S] | 23 [S] | 28 [S] | 28 [S] | 30 [S] | 28 [S] | 23 [S] | 20 [S] | 25 [S] | 20 [S] |

*(Continued)*

| Isolate Reference Numbers | Samples | Sites | Species | E. coli REP types | Zone of Inhibitions (mm) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | FOX | MEM | AMP | AMC | CTX | CAZ | CIP | SXT | TE | CN | C | TGC |
| BAD 238 | LSW 20 | Gulls- urban | *E. coli* | X | 23 [S] | 33 [S] | 20 [S] | 23 [S] | 27 [S] | 26 [S] | 32 [S] | 31 [S] | 27 [S] | 20 [S] | 26 [S] | 21 [S] |
| BAD 576 | LSA 5 | Gulls- rural | *E. coli* | Y | 27 [S] | 32 [S] | 20 [S] | 24 [S] | 30 [S] | 28 [S] | 42 [S] | 30 [S] | 25 [S] | 24 [S] | 27 [S] | 22 [S] |
| BAD 819 | LSA 37 | Gulls- rural | *E. coli* | Z | 25 [S] | 36 [S] | 23 [S] | 24 [S] | 30 [S] | 29 [S] | 34 [S] | 33 [S] | 20 [S] | 22 [S] | 26 [S] | 24 [S] |
| BAD 842 | LSA 41 | Gulls- rural | *E. coli* | AA | 25 [S] | 32 [S] | 22 [S] | 24 [S] | 29 [S] | 27 [S] | 36 [S] | 32 [S] | 23 [S] | 21 [S] | 22 [S] | 23 [S] |
| BAD 241 | LSW 20 | Gulls- urban | *E. coli* | AB | 25 [S] | 34 [S] | 20 [S] | 24 [S] | 29 [S] | 29 [S] | 31 [S] | 31 [S] | 24 [S] | 21 [S] | 26 [S] | 21 [S] |
| BAD 821 | LSA 37 | Gulls- rural | *E. coli* | AC | 25 [S] | 32 [S] | 21 [S] | 23 [S] | 29 [S] | 28 [S] | 34 [S] | 29 [S] | 24 [S] | 20 [S] | 25 [S] | 22 [S] |
| BAD 841 | LSA 41 | Gulls- rural | *E. coli* | AE | 25 [S] | 33 [S] | 19 [S] | 22 [S] | 27 [S] | 26 [S] | 38 [S] | 31 [S] | 23 [S] | 20 [S] | 29 [S] | 22 [S] |
| BAD 170 | LSW 1 | Gulls- urban | *E. coli* | AF | 26 [S] | 34 [S] | 19 [S] | 23 [S] | 27 [S] | 27 [S] | 35 [S] | 29 [S] | 23 [S] | 20 [S] | 24 [S] | 23 [S] |
| BAD 194 | LSW 5 | Gulls- urban | *E. coli* | AG | 23 [S] | 32 [S] | 21 [S] | 24 [S] | 25 [S] | 25 [S] | 29 [S] | 30 [S] | 21 [S] | 21 [S] | 24 [S] | 22 [S] |