Microbial Diversity of Antarctic Dry Valley Mineral Soil

By

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Declaration

"I declare that, Microbial Diversity of Antarctic Dry Valley Mineral Soil, is my own work, that it has not been submitted for any degree or examination in any university and that all the sources I have used or quoted have been indicated and acknowledged by means of complete references".

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December 2004

Abstract

Antarctica provides some of the most extreme environments on Earth. Low temperatures, low water availability and nutrient deficiency are contributing factors to the limited colonisation of Antarctic biotopes, particularly in the continental Dry Valleys. The survival of microorganisms in this harsh continent provides the basis for the significance of this study. In this study we aim to explore microbial phylotypic diversity across a 500m altitudinal transect in the Miers Dry Valley, Ross Desert, East Antarctica. We also attempt to infer from phylogenetic data, the possible presence of indicative phenotypes which might contribute to a functional microbial community.

Total genomic DNA was isolated from 12 soil samples and 16S rDNA PCR was performed with primers designed to target the conserved regions of the bacterial 16S rRNA gene. A preliminary analysis of bacterial diversity across the transect was conducted via Denaturing Gradient Gel Electrophoresis (DGGE). It was observed that essentially similar phylotypes were present in every level. The vertical transect of 500m in the Miers Dry Valley was shown to have little effect on microbial diversity, as DGGE indicated that few phylotypes appeared to be altitudinal dependent. Due to the similarity between the transect samples, 16S rDNA clone libraries of transect samples 1, 5, 7 and 9 were prepared. A total of 121 clones were sequenced and similarity searches with known bacterial 16S rDNA sequences in public databases were evaluated. 115 were =90% identical to their respective matches in the database, 2 sequences were 89% identical and 4 sequences were 88% identical. Approximately 500 base pairs of the 16S sequences were being compared to those on the database. Major taxonomic groups represented by the genera included: a, ß, ? Proteobacteria, Actinobacteria, Verrucomicrobia, Planctomycetes and several uncultured environmental and Antarctic samples. Genera which can be assigned with some confidence included, *Opitutis*, *Clostridium*, *Rhodoglobus*, *Brevundimonas*, *Lysobacter*, *Nocardia*, *Kribella*, *Sphingomonas*, *Rubrobacter*, *Stenotrophomonas*, and *Janthinobacterium*.

Molecular evidence did not support the presence of an established trophic community structure in the Miers Valley transect as most of the phylotypes and genera identified in the present investigation were heterotrophs. The possibility of autotrophs inhabiting the Miers Dry Valley cannot be eliminated as a large portion of the phylotypes were uncultured and there was

evidence for the possible presence of autotrophs in the Miers Dry Valley. Exogenous heterotrophic substrates are thought to be negligible in the Dry Valley mineral soils and the present investigation supports this statement as ~80% of the identified phylotypes were heterotrophs. For this reason heterotrophs depend on other sources of organic matter such as aerial dispersion.

Phylogenetic studies have shown that most of the clones clustered with their respective matches obtained from the database and also displaying bootstrap values of 100. Some Antarctic isolates clustered together whilst others exhibited high similarity to environmental samples. This suggests that most Antarctic microorganisms are common to other soil environments, but may have adapted to the extreme psychrophilic habitat. A relatively small proportion (~10%) of Antarctic phylotypes appeared to be novel.

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Table of Contents

Declaration	i
Abstract	ii
Acknowledgements	iv
List of Figures	vii
List of Tables	viii
List of Abbreviations	ix
1. Introduction and Aims	1
1.1. General introduction	1
1.2. Extremophiles in biotechnology	1
1.3. Why study Antarctica?	3
1.4. Molecular Techniques	4
1.5. Dissertation	5
1.6. References	6
2. Literature Review	8
2.1. Antarctica	8
2.1.1. Maritime Antarctica	8
2.1.2. Terrestrial Antarctica	10
2.1.3. Antarctic Lakes	12
2.2. Molecular Techniques	13
2.2.1. DNA extraction from soil	13
2.2.2. 16S rDNA PCR	14
2.2.3. Denaturing gradient gel electrophoresis (DGGE)	17
2.2.4. Phylogenetic Analyses	18
2.3. References	21
3. Methodology	26
3.1. Retrieval and storage of soil samples	26
3.2. DNA Extraction	27
3.3. PCR	28

		3.3.1. 16S rDNA PCR	28
		3.3.2. DGGE specific Touchdown PCR	28
		3.3.3. M13 PCR	29
	3.4.	Denaturing gradient gel electrophoresis (DGGE)	29
	3.5.	Cloning	30
		3.5.1. Calculation of the amount of insert required	30
		3.5.2. Phosphokinase reaction	30
		3.5.3. Ligation	31
		3.5.4. Transformation	31
		3.5.5. Direct colony PCR screening	31
	3.6.	Amplified rDNA restriction analysis (ARDRA)	31
	3.7.	Plasmid isolation	32
	3.8.	Sequencing	32
	3.9.	Phylogenetic analyses	32
	3.10.	References	33
4.	**Results and Discussion**		34
	4.1.	Introduction	34
	4.2.	gDNA Isolation	34
	4.3.	16S rDNA PCR	36
	4.4.	Denaturing gradient gel electrophoresis (DGGE)	36
	4.5.	16S Clone Libraries and Phylogenetic Analyses	37
		4.5.1. MVT 1	40
		4.5.2. MVT 5	42
		4.5.3. MVT 7	46
		4.5.4. MVT 9	48
		4.5.5. MVT 12	50
	4.6.	References	56
5.	**Conclusion**		63
6.	**Appendix**		66

List of Figures

Pg. No.

Figure 1.1. Terms used to study extremophiles. 3

Figure 2.1. Picture of the Miers Dry Valley. 11

Figure 2.2. Illustration of 16S rRNA gene of *E. coli*. 15

Figure 2.3. A Cladogram and a Phylogram showing a branch and a node. 19

Figure 3.1. Picture of the Miers Dry Valley showing 500m altitudinal transect. 26

Figure 4.1. gDNA isolations of MVT samples 1 to 12. 35

Figure 4.2. Products of 16S rDNA PCR amplification. 35

Figure 4.3. Denaturing gradient gel showing bacterial phylotypic diversity across the MVT transect. 37

Figure 4.4. Phylogenetic tree of MVT 1. 52

Figure 4.5. The percentage of different phyla in MVT 1. 42

Figure 4.6. The percentage of different phyla in MVT 5. 44

Figure 4.7. The percentage of different phyla in MVT 7. 46

Figure 4.8. Phylogenetic tree of MVT 7. 53

Figure 4.9. Phylogenetic tree of MVT 9. 54

Figure 4.10. The percentage of different phyla in MVT 9. 48

Figure 4.11. Phylogenetic tree of MVT 12. 55

Figure 4.12. The percentage of different phyla in MVT 12. 50

List of Tables

Pg. No.

Table 3.1. Summary of the Miers Valley Transect (MVT) and the different levels. 27

Table 4.1. Summary of MVT 1 Blast results. 43

Table 4.2. Summary of MVT 5 Blast results. 45

Table 4.3. Summary of MVT 7 Blast results. 47

Table 4.4. Summary of MVT 9 Blast results. 49

Table 4.5. Summary of MVT 12 Blast results. 51

List of Abbreviations

16S rDNA PCR	16S ribosomal DNA Polymerase Chain Reaction
V	Variable regions
Tm	Melting temperature
DGGE	Denaturing gradient gel electrophoresis
TGGE	Temperature gradient gel electrophoresis
BLAST	Basic Local Alignment Search Tool
NJ	Neighbour Joining
MP	Maximum Parsimony
ML	Maximum Likelihood
MVT	Miers Valley Transect

Chapter 1

Introduction and Aims

1.1 General introduction

The term 'extreme' pertains to conditions that would be uncomfortable for the normal functioning of man. An organism which has adapted to extreme environmental conditions; e.g. high or low temperature, pH or salt concentration, low nutrient content and water availability is termed an extremophile. The use of the term 'extremophile' first appeared in 1974 in a paper by MacElroy.[1] Ever since, the extremophile research field has progressed to the extent that the first International Congress on extremophiles was convened in Portugal in June 1996, with the establishment of the scientific journal "Extremophiles" in February the following year.[2] Terms used to study some extremophiles are included in Fig. 1.1. Additional extremophiles include, endoliths (organisms that live in rocks), oligotrophs (organisms that are capable of surviving in nutrient limited environments)[3] and toxitolerant organisms which thrive on toxic compounds.

1.2. Extremophiles in biotechnology

Extremophile research displays great potential for applications in biotechnology. Extremozymes, enzymes isolated from extremophiles, generally have a similar mode of action as their homologous mesophilic enzymes. However, extremozymes have adapted to function in extreme conditions which make them very valuable for applications in biotechnology.[4] For example, psychrophilic enzymes function optimally at low temperatures. The potential of psychrophilic bacteria for applications in biotechnology have been reported in a number of articles and reviews. A recent review discusses the definition of psychrophilic bacteria, description of their habitats and focuses on the adaptive changes in proteins and lipids particularly those explored for biotechnological purposes.[5] One particular study investigated

the ability of certain psychrophilic yeast strains to produce novel pectinolytic enzymes that are capable of degrading pectin compounds at low temperatures. The study investigated the application of cold-active pectinolytic enzymes in the food industry, for the clarification of fruit juice below 5°C.[6] The use of psychrophilic microorganisms for applications in biotechnology are presently being employed, for example the application of eurythermal polar cyanobacteria for wastewater treatment in cold climates[7] and the incorporation of proteases, lipases and cellulases into detergents to improve its mode of action in cold water. The use of nucleating proteins in psychrophiles are currently being investigated for manufacturing synthetic snow as well as freeze-dried food.[8] Research developments with respect to isolation of novel bacteria, culture collections, bioactivity screening, taxonomy, production of polyunsaturated fatty acids (PUFA's), cold adapted enzymes and bioremediation permits for the exploitation of these and other findings for the possibility of new biotechnological products from Antarctic microorganisms.[9]

Thermophilic enzymes are thermostable, resistant to denaturation (e.g. in organic solvents) and are active at high temperatures. Whole thermophilic microorganisms also display advantages for uses in biotechnology in processes like fermentation. With the use of thermophilic microorganisms this process could be conducted at high temperatures, destroying or repressing the growth of pathogenic microorganisms. These properties render thermophiles and their enzymes very useful in biotechnology. Certain thermophilic enzymes are currently being used in industry including for example, amylases to produce glucose (as a sweetener) and xylanases for paper bleaching.[10]

Other applications of extremophiles include the use of hypersaline organisms for the treatment of hypersaline waste, modification of food flavours by halophilic microorganisms and the production of antibiotics from alkaliphilic microorganisms.[2] It is evident that extremophile research displays great potential for applications in biotechnological processes.

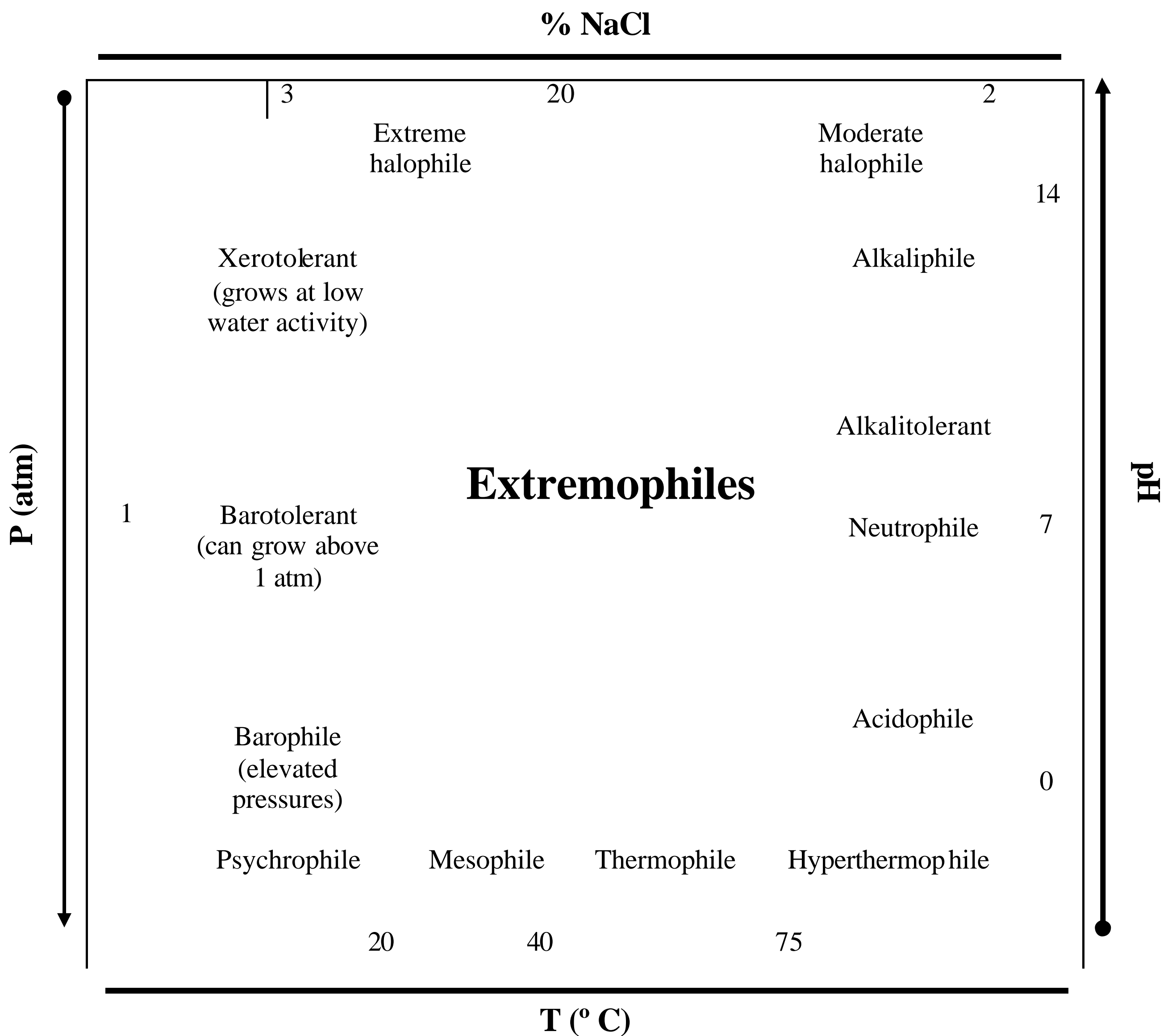

Figure 1.1. Terms used to study extremophiles.

1.3. Why study Antarctica?

The biology of Antarctica, more than any other continent, is dominated by microorganisms.[3] Antarctic microbial habitats have remained relatively preserved for many years as compared to other invaded habitats. Hence, there exists unique opportunities for studying microbial evolution and microbial endemism (genotypes of microorganisms specific to a geographical region).[11, 12] In addition, Antarctic food webs are relatively reduced in

complexity as compared to the invaded habitats, where there is continuous interference with higher plants and animals.[11]

The Dry Valleys of Antarctica also serve as an exobiological model. Evidence has shown that the dry cold saline soils of the ice free Antarctic Dry Valleys probably offer one of the best possible analogues on Earth for understanding the Martian climate and the possible disappearance of life on Mars.[13] In addition, trace fossils of cryptoendolithic microbial communities are an easier target for life detection systems as compared to fossils of cellular structures.[14]

The potential of psychrophiles for applications in biotechnology have already been discussed in section 1.2.

1.4. Molecular techniques

The study of prokaryotic biodiversity has been hindered for many years due to the difficulty of accessing true diversity by culture dependent methods. It has been estimated that less than 0.1% of the total microbial population can be successfully isolated in pure culture.[15] This occurs because bacteria are highly selective in their growth requirements. Hence, a variety of media need to be utilised to obtain diverse microbial populations. This can become extremely laborious and time consuming.

Over the last decade, advances in molecular biology have facilitated the analysis of bacterial diversity. Major developments in this area of research have circumvented problems that may arise from the isolation and culturing of bacteria. For example, the isolation of bacterial nucleic acids directly from a soil sample, PCR, sequencing and a variety of molecular techniques have become useful tools for the detection of bacteria that cannot be cultured. Continuous advancements in this area of research have permitted rapid and effective analyses of prokaryotic biodiversity from almost any environmental sample.

1.5. Dissertation

Low temperatures, low carbon content and arid mineral soils render the Antarctic Dry Valleys an extreme habitat. The survival of microorganisms in this hostile habitat provides the basis for the significance of this study. This study aims to investigate bacterial phylotypic diversity in the Miers Dry Valley (Ross Desert, East Antarctica), specifically:

(i) if altitude has an effect on microbial diversity.

(ii) the presence of a putative community structure in terms of autotrophs and heterotrophs.

(iii) the uniqueness of Antarctic isolates as compared to other microorganisms.

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Chapter 2

Literature Review

2.1 Antarctica

Antarctica covers an area of nearly 4 million square kilometres and is considered the largest and most pristine wilderness on earth. It is the coldest, windiest and highest continent on Earth. Less than 1% of this desert-like continent is ice-free. Containing about 70% of the world's freshwater and 90% of the world's ice, Antarctica is not only a scientific curiosity but is also a key component of planet Earth, as processes occurring there affect the worlds climate.[1, 2]

Only a few insects can survive the harsh terrestrial conditions but numerous animal species that thrive in the surrounding waters include fish, krill, penguins, seals, whales and various kinds of sea birds. Plant cover is dominated by lichens and in maritime Antarctica (sea-ice environment excluding Ross Dessert) lichen growth is more conspicuous on rock surfaces and soil whilst in terrestrial Antarctica (Ross Dessert), lichens grow between rock surfaces where temperature stability is greater.[1] However, despite the abundance of these eukaryotes, prokaryotic diversity is more prominent in Antarctica.

2.1.1 Maritime Antarctica (sea-ice environment)

Sea ice is the major nutrient source in Maritime Antarctica. Although ice may appear to be an uncongenial habitat to support microbial growth, brine inclusions, interstices within ice floes (sheets of floating ice) and the ice-water interface support the survival of rich microbial populations.[3] Brine inclusions are formed through the accumulation of dissolved salts exuded from ice crystals, when ice forms from sea water at -1.9°C.[4] Since the salinity of enclosed brine is affected by the temperature[5] *in situ*, the volume of ice occupied by brine

varies directly as a function of temperature. These low temperature high salinity extremes within ice may be important in determining the survival of organisms as well as in controlling the rate of biological processes.

An extraordinary feature of Antarctic ice is the occurrence of oxygen at supersaturated levels in certain portions of the water column. Research has shown oxygenation in sediments down to fifteen and in some cases twenty-five centimetres and it was concluded that this might be typical for perennial ice cover,[6] (due to difficulty of diffusion through ice). These high oxygen levels undoubtedly contribute to the microbial diversity.

A range of morphological types of bacteria has been found to be associated with sea-ice. Light and scanning electron microscopy studies have shown the presence of rods, cocci, straight and branching filamentous, fusiform and prosthecate bacteria. In addition, it was found that approximately 70% of the bacteria in a sea-ice community at McMurdo Sound were free-living, whereas 30% were attached to either detritus or active algal cells.[7] Microorganisms in the Antarctic ice include non-spore and spore forming bacteria. The best studied genus of non-spore bacterium is *Pseudomonas*.[8] Two strains that have been isolated and have displayed psychrophilic properties include *P. fluorescens* and *P. alcaligenes*.[9] Spore forming bacteria included a variety of *Bacillus* species.[8] Actinomycetes have also been found in the ice and soil and the two representative genera include *Streptomyces* and *Nocardia.*[10]

It has been shown by microautoradiography that heterotrophic bacteria in sea-ice are able to take up ^{3}H-amino acids, ^{3}H-glucose and ^{3}H-thymidine, under *in situ* conditions.[11] Chemoautotrophs were also shown to be present in sea-ice assemblages in the form of ammonia-oxidizing bacteria.[12] Pigmented and gas vacuolated bacteria have also been isolated from sea-ice in McMurdo Sound. Although some of the red to orange pigmented, filamentous bacteria contained gas vacuoles, it is uncertain whether the gas vacuoles confer an adaptive advantage for the sea-ice habitat.[13] Plasmids have also appeared to be pervasive in sea-ice bacteria. Of 79 bacterial isolates from sea-ice in McMurdo Sound, 30% contained at least one plasmid.[14] Certain plasmids may contain genetic elements such as integrase genes. These genes enable bacteria to acquire and express foreign DNA molecules and thus facilitate increased genetic diversity in sea-ice bacteria.[15]

There are several factors that contribute to the growing interest in Antarctic sea-ice biota. For example, the high estimates of phytoplankton production and evidence of significant bacterial productivity suggests the presence of a microbial food web.[1] In addition, the association of the Antarctic krill, *Euphausia superba*, with sea-ice has suggested that sea-ice biota serve as a resource for this key pelagic consumer in the Antarctic marine food web.[16] However, microbial food webs in polar waters have not been extensively documented as compared to the lower latitude-latitude marine ecosystems.[17]

Studies on Antarctic sea-ice biota can be dated back to 1847[18] where the research was focused largely on systematic studies of the ice microflora (primarily diatoms). Recent studies have encompassed an increased range of organisms and their ecological roles as members of complex assemblages in close association with the ice.[19]

2.1.2 Terrestrial Antarctica

The Ross Desert is characterised as the 'true desert' of the Antarctic continent. It occupies less than 2% of continental Antarctica, and covers an area of over 7000 km^2.[20] Environmental properties such as, mean annual temperature is -20°C, average wind speed is 100km/h, water content is 0.2-0.5% H_2O/g soil, mean relative humidities are 50% or less and the solar flux (available energy) is generally less than 100W.m, render the Antarctic Dry Valleys an extreme habitat[21] (Fig. 2.1. is a picture of the Miers Dry Valley and the description of a typical valley). The Dry Valley regions comprise alternating mountain ranges and glacial valleys. Some of the lowest temperatures on earth have been recorded here, which in conjunction with the very low precipitation and humidity levels means that the Ross Desert is among the driest deserts on the planet.[22] When viewed from South to North along the coast of McMurdo Sound, the major ice-free valleys include the Taylor, Wright, McKelvey, Balham, Victoria and Barwick Valleys. Structurally similar but smaller valleys include the Miers, Marshall, Garwood and Salmon Stream Valley, occurring to the south of Taylor Valley.[20]

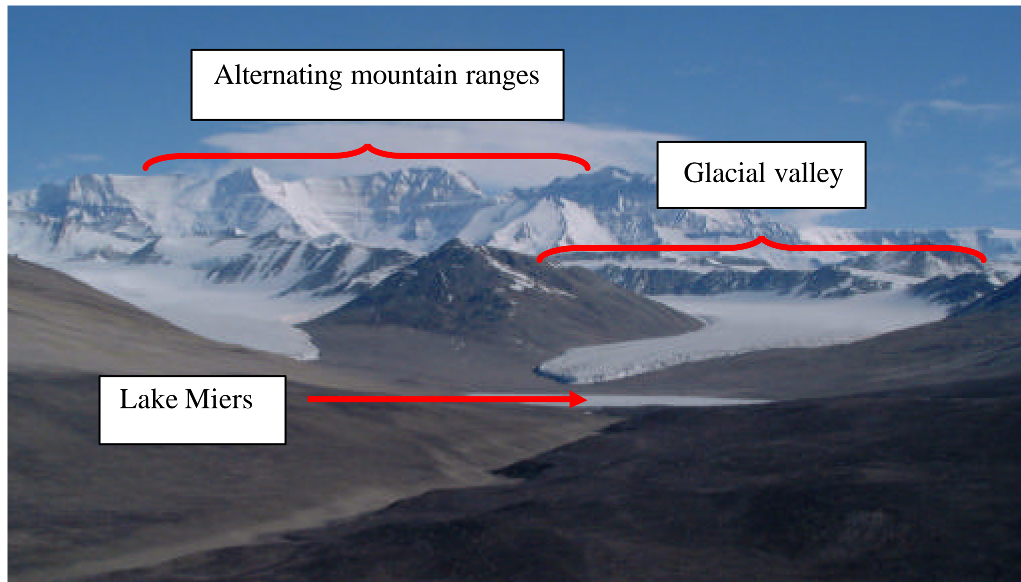

Figure 2.1. Picture of the Miers Dry Valley

The ice-free dry valleys of McMurdo Sound are the largest and highest in elevation in Antarctica and are termed "oases", defined as ice-free areas that are kept free from ice by the process of ablation (erosive processes that reduce the size of glaciers due to a higher absorption than reflection of incident light).[23] There exist several explanations for the origin of these ice-free areas and one of the earliest and most widely accepted explanations is the orographic (science of mountains) and global climatic change hypothesis, proposed in 1969.[24] It was proposed that ice-free areas are formed when ice sheets attenuate. The ice thinning may be associated with the general warming trends over the past ten thousand years and the positive radiation balance between the dark soils and the rocks maintains the ice-free areas. Lake formation can be attributed to summer meltwater that collects in the catchment basins. A similar concept was proposed in 1970,[22] which also explained the formation of ice-free areas on the basis of the ratio of precipitation to evaporation balance. It may also be important to note that most Antarctic ice-free areas are relatively ancient as present-day examples may have formed as much as four to five million years ago.[25]

The environmental properties of ice-free areas provide a hostile habitat for the colonization of microorganisms. Antarctic soils are highly aerobic therefore anaerobic bacteria is considered very rare. In previous studies characterising Antarctic soil bacteria using culture based approaches, seventy-one percent of bacteria were related to coryneform bacteria, within the genera *Arthrobacter, Brevibacterium, Cellulomonas, Corynebacterium* and *Kurthia*.[26].

Pseudomonas, Flavobacterium and other gram negative aerobic rods like *Alcaligenes* and *Arthrobacter* were also identified.[26] Recent studies employing molecular analysis, has detected the presence of anaerobic, gram positive *Clostridium* sp.[27] These data indicate that culture based methods alone remain inadequate for providing an accurate reflection of the microbial diversity in an environment. In Ross Dessert soils, coryneforms are also prominent whilst *Bacillus* and *Pseudomonas* are rare.[10] Of five hundred and sixty eight isolates, twenty three percent were ascribed to *Corynebacterium* and fifty-six percent to members of the "coryneform-related group". Other lower percentages included *Bacillus* (7%), *Micrococcus* (20%), *Nocardia* (3%), *Streptomyces* (3%), *Flavobacterium* and *Pseudomonas* (6%).[10]

2.1.3 Antarctic lakes

For a description of an Antarctic lake refer to Fig. 2.1. The major Antarctic lakes are found in glacial valleys. During winter the lakes remains frozen but may thaw for a few weeks during summer.[28]

Sediment deposition, high occurrence of certain gases and light, are some of the major regulators of microbial activity in Antarctic lakes. The glacial melt streams, which flow only for a restricted period during summer and the lake margins which thaw briefly, contribute minimally to sediment deposition.[29] Ice cover of perennial lakes, has shown to be the major contributor of sediments. It was proposed that ice traps wind blown sediments and provides a plane for the movement of these sediment particles (either by saltation or rolling and drifting on the ice), to the middle of the lake.[30] The first observation of sediment deposition occurring through ice cover was reported in 1983.[30] Cracks present on the ice surface of Lake Vanda facilitated the production of gas bubbles to escape from the water below to the atmosphere. Any sediment deposited on the ice surface would eventually make its way down through the ice cover into the water column and the lack of sediment deposits of the surface of Lake Vanda, supported this conclusion.[30]

Certain gases such as O_2 and N_2 occur at elevated levels at the bottom of the ice in a lake.[31] Supersaturation with nitrous oxide in an Antarctic lake was reported at a depth of 54m. The nitrous oxide concentration of more than 200 times that of air saturation was believed to be produced by a narrow band of nitrifying bacteria at a depth of about 52-55m.[32]

Perhaps the most important environmental regulator in any ecosystem is light. Light provides energy for photosynthesis and thus serves as the pioneer source of energy at the base of any food web. Light is a major limiting factor for the development of microbial communities in Antarctic lakes as most Antarctic lakes are covered by ice. However, studies of light attenuation by snow and ice showed that about 99% of light striking a lakes surface is absorbed. These results suggest that microbial communities present in a lake are very well adapted to low light conditions.[33]

Microbial mats in Antarctic lakes are composed primarily of Cyanobacteria, diatoms and heterotrophic bacteria.[34] Culture based studies found *Phormidium frigidum* and *Lyngbya martensiana* to be the dominant filamentous Cyanobacteria present in microbial mats.[34] Morphological and molecular analyses of Cyanobacterial diversity of microbial mats in Lake Fryxell, were conducted. Results revealed the presence of *Nostoc* sp. and *Schizothrix* sp. and morphotypes such as *Hydrocoryn* cf. *spongiosa, Nodularia* cf. *harveyana* and *Phormidium* cf. *autumnale*.[35] Other autotrophic bacteria include photosynthetic green sulphur bacteria such as *Chlorobium vibrioforme* and *Chlorobium limnicola*.[36] The majority of isolates from five habitats in Vestfold Hills belonged to *Pseudomonas* sp. followed by pigmented *Flavobacterium* and non-pigmented *Moraxella*.[37]

2.2 Molecular techniques

2.2.1 DNA extraction from soil

Bacteria form essential agents of soil microflora, due to their abundance (~10^9 cells per gram of soil), their species diversity (minimum of 4000-7000 different bacterial genomes per gram of soil)[38] and the combinational effects of their metabolic activities. Hence, when investigating soil microbial diversity, both rapid and comprehensive means of analyses need to be employed.

A protocol for extracting DNA directly from a soil sample generally involves three steps:- (i) cell extraction / cell lysis, (ii) removal of cell fragments and debris and (iii) nucleic acid precipitation and purification.[39] Cell extraction involves the isolation of microbial cells from their environmental matrix, prior to cell lysis. A typical cell extraction procedure consists of successive cycles of blending and centrifugation to recover the microbial cells present in the

sample.[38] However, two major limitations of this procedure is that it is time consuming and may not fully represent the microbial diversity of a particular environment as a fewer number of cells are obtained.[39] Direct lysis is a more popular method, as it does not require a preliminary cell extraction step. With direct lysis a larger number of microorganisms are exposed to the lysis procedure hence, exposure to a wider range of genomes.[40] The major problem associated with direct lysis is that there is a higher chance of co-extracting contaminants, requiring a more extensive purification procedure.[39]

Cell lysis procedures can be chemical, mechanical or enzymatic. A combination of mechanical lysis (usually bead beating) and chemical lysis (use of detergents) usually produces DNA of good quality and purity.[41] In a comparison of 5 different soil DNA extraction procedures, the Zhou method[42] and the Ultra clean soil DNA isolation kit (MoBio Inc., Solana, CA, USA) produced the best purity and yield of DNA.[43] It was also suggested that an increase in the bead beating time increases shearing of DNA and reduces the DNA fragment size. Shearing can be reduced by bead beating prior to the addition of SDS or other chemical denaturants. Extraction buffers containing SDS increase DNA yields but also increase humic acid contamination.[43]

2.2.2 16S rDNA PCR

16S rDNA PCR forms the basis for analyses of microbial diversity. C-type cytochromes, globins and other common proteins could be used for mapping phylogenetic relationships but these molecules are limited to the 'higher' eukaryotic systems.[44] Since prokaryotic and eukaryotic systems are so biochemically diverse, the use of homologous proteins would prove to be inadequate for studying prokaryotes. In addition, rRNA studies are much less complicated than the analysis of homologous proteins. For these and many other reasons (elaborated below) rRNA analysis has become the method of choice for determining phylogeny as well as understanding microbial diversity.[45]

(i) rRNA`s are fundamental elements for synthesising proteins and are therefore functionally and evolutionary homologous in all organisms.

(ii) rRNA`s are ancient molecules and their overall structure and nucleotide sequences are conserved. Certain nucleotide stretches are highly conserved in the rRNA gene across all 3 'primary kingdoms' while other portions are variant. The

conserved regions are essential as they provide primer directed sites for PCR as well as convenient hybridization targets for the cloning of rRNA genes.

(iii) rRNA genes lack artifacts of lateral transfer between similar organisms and are therefore thought to reflect the direct evolutionary pathway of organisms.[45]

Finally, with the proper primer design to target the rRNA gene conserved regions, sufficient sequence information can be obtained to facilitate significant comparisons between organisms.[46]

Prokaryotes contain 3 rRNA`s, 5S (~ 120 nucleotides), 16S (~1600 nucleotides) and 23S (~3000 nucleotides). By the late 1960`s, the 5S rRNA was the most popular of the three for phylogenetic analysis as it was small and very convenient for sequence analysis. However, the paucity of varying nucleotide positions limited its use for phylogenetic studies. Initially, the 16S rRNA was too large for phylogenetic analysis. However, the development of DNA cloning and sequencing protocols has facilitated the sequencing of the full 16S gene, which has become important for the analysis of microbial diversity as well as for phylogenetic studies.[45]

Fig. 2.2. shows the nucleotide sequence of the 16S gene from *E. coli*.[47] Regions that remain totally conserved (blue) as well as conserved (red) are depicted. These regions are the targets for primer binding sites where the variable regions V1 – V9 can be efficiently amplified by PCR. Commonly utilised primers for 16S gene analysis include E9F [48] and U1510R. [49]

KEY: totally conserved conserved variable highly variable >75% variable

variable regions priming sites

10 E8F E9F 30 40 50 60

AAATTGAAGAGTTTGATCATGGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATGCAAGTCGAACG**GT**

80 90 100 110 120 130

AACAGGAAGAAGCTTGCTTCTTTGCTGACGAGTGGCGGACGGGTGAGTAATGTCTGGGAAACTGCC**TGAT**

150 V_1 160 170 180 190 200

GGAGGGGGATAACTACTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGAGGGGGACCTTC

220 230 240 V_2 250 260 270

GGGCCTCTTGCCATCGGATGTGCCCAGATGGGATTAGCTAGTAGGTGGGGTAACGGCTCACCTAGGCGAC

290 300 310 320 330 340

GATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAC

360 370 380 390 400 410 E334F/341FGC

GCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCT

E334F-conti. 430 440 450 460 470 480

TCGGGTTGTAAAGTACTTT**CAGCGGGGAGGAAGGGAGTAAAGTTAATACCTTTGCTCATTGACGTTACCC**

500 510 520 530 *V*$_3$ 540 550

GCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAA

570 580 590 U529/34/E535R/534R/519F 610 620

TTACTGGGCGTAAAGCGCACGCAGGCGGT**TTGTTAAGTCAGATGTGAAATCCCCGGGCTCAACCTGGGAA**

640 650 660 670 680 *V*$_4$ 690

CTGCATCTGATACTGGCAAGCTTGAGTCTCGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCG

710 720 730 740 750 760

TAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACGAAGACTGACGCTCAGGTGCGAAAGC

780 790 800 810 820 830

GTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTT**GGAGGTTGTGCC**

850 860 E786F 870 880 890 900

CTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAAC

V$_5$ 920 930 940 950 960 970 U926R

TCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACC

990 E939R 1000 1010 1020 1030 1040

TTACCTGGTCTTGACATCC**ACGGAAGTTTTCAGAGATGAGAATGTGCCTTCGGGAA**CCGTGAGACAGGTG

1060 1070 1080 *V*$_6$ 1090 1100 1110

CTGCATGGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCC

U1053F 1130 1140 1150 1160 1170 U1115R/U1098F

TTTGTTGCCAGCGGTCCGGCCGGGAACTCAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGGATG

1200 *V*$_7$ 1210 1220 1230 1240 1250

ACGTCAAGTCATCATGGCCCTTACGACCAGGGCTACACACGTGCTACAATGG**CGCATACAAAGAGAAGCG**

1270 1280 1290 1300 1310 1320

ACCTCGCGAGAGCAAGCGGACCTCATAAAGTGCGTCGTAGTCCGGATTGGAGTCTGCAACTCGACTCCAT

1340 V_8 1350 1360 1370 1380 1390

GAAGTCGGAATCGCTAGTAATCGTGGATCAGAATGCCACGGTGAATACGTTCCCGGGCCTTGTACACACC

1410 1420 1430 1440 1450 1460 U1406/15

GCCCGTCACACCATGGGAGTGGGTTGCAAAAGAA**GTAGGTAGCTTAACCTTCGGGAGGGCGCTTA**CCACT

U1406/15conti 1480 1490 1500 1510 V_9 1520 1530

TTGTGATTCATGACTGGGGTGAAGTCGTAACAAGGTAACCGTAGGGGAACCTGCGGTTGGATCACCTCCTTA

U1510R E1541R

Figure 2.2: Illustration of 16S rRNA gene of *E. coli*

2.2.3. Denaturing gradient gel electrophoresis (DGGE)

Electrophoretic separation of PCR products in a polyacrylamide gel containing a denaturing chemical or physical gradient is a relatively recent technique.[50] The former method utilises urea and formamide in a process commonly known as denaturing gradient gel electrophoresis,[51] whilst the latter method utilises temperature in a process commonly known as temperature gradient gel electrophoresis (TGGE).[52] Whilst both techniques have been reported to produce consistent results,[53] DGGE is a much cheaper but a slightly more tedious technique to perform. DGGE is capable of separating DNA fragments of similar sizes but different nucleotide sequences and is based on the principle that since A-T rich fragments have a lower T_m than G-C rich fragments and melted DNA fragments migrate more slowly through the gel matrix, fragments that melt first are separated from those that melt subsequently.[51, 54]

DGGE is considered a very powerful and sensitive technique,[55] as it is capable of detecting up to 96% of all mutations or single base pair substitutions in fragments up to 500 base pairs in length.[55, 56] Primers 534R and 341FGC[57] (see Fig. 2.2.) were utilised in this study for the amplification of a 193 base pair amplicon from the 16S rRNA gene. These primers ensure amplification of the V_3 variable region of the 16S gene, which is sufficient for discriminating between different microbial species.

The use of a G-C clamp (40 bp of G-C nucleotides) in DGGE analysis is important as it imparts melting stability to the fragments. The most convenient way of obtaining a G-C clamp

is by attaching it to one of the primers.[51] Double stranded DNA molecules denature more slowly than single stranded molecules therefore they migrate with greater stability during electrophoresis. Single stranded fragments tend to supercoil. They may therefore denature incorrectly and migrate more quickly through the gel. Since A-T sequences have lower T_m values than G-C rich sequences, the G-C clamp imparts melting stability by preventing the fragments from becoming completely single stranded and electrophoresed off the gel.[56,58]

2.2.4. Phylogenetic analyses

The construction of phylogenetic trees has become a very useful tool for the analysis of evolutionary processes and the historical relationships between different organisms.[59]

Phylogenetic trees enable one to:

(i) characterise unknown proteins
(ii) Obtain biological function of proteins
(iii) Examine how closely or distantly a particular DNA sequence relates to other sequences, as an indication of functions assigned to DNA sequences
(iv) Characterise organisms on the basis of sequence similarity[60]

A tree is simply an illustration of evolutionary relationships or similarities between a variety of sequences. It is made up of nodes and branches (Fig. 2.3.), where a branch is a line that connects two nodes. The nodes can either be external (the tips of the tree where the taxa are being considered) or internal (points that represent a common ancestor of two other nodes). The two basic styles of a phylogenetic tree is a cladogram or a phylogram (Fig. 2.3.). A cladogram merely represents the branching order of the nodes whereby, the branch lengths convey no information. A cladogram can either be slanted or in the more popular rectangular fashion. A phylogram displays both the branching order and distance information of the sequences. The branch distance is a representation of the sequence changes between two sequences; i.e., the longer the branch the greater the difference between sequences. A distance value which can be displayed on the tree will represent the number of substitutions that have occurred between two sequences.[60]

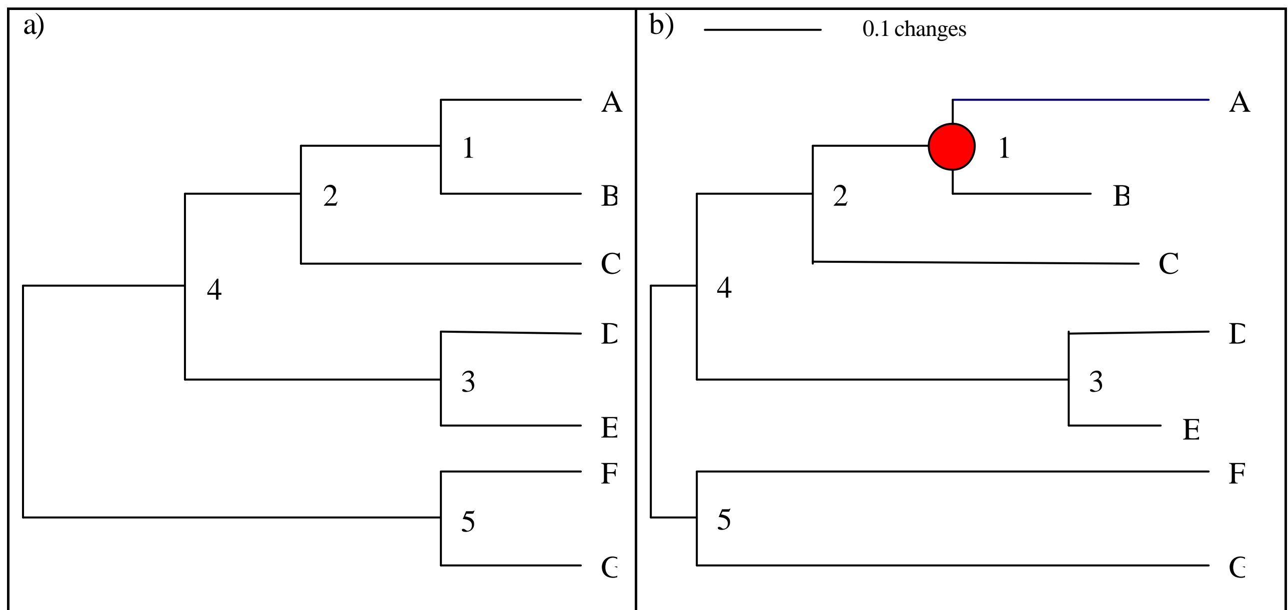

Figure 2.3. a) Cladogram and b) Phylogram showing a branch __ and a node.

The basic steps involved in constructing phylogenetic trees from molecular sequence data include:

(i) Obtain the DNA or protein sequence of interest

(ii) With the use of the BLAST database, search for sequence similarity and obtain electronic files of the similar sequences

(iii) With use of appropriate computer programs, create an alignment of the sequences

(iv) Create phylogenetic trees with the aligned sequences

When constructing a tree it is important to provide a root for the tree as this will be a representation of the common ancestor of all the taxa being considered. Unrooted trees only specify the relationships among the taxa and not the evolutionary pathways. A tree is rooted when there is a unique directional path that leads to each taxon. The easiest solution for assigning a root to the tree would be to choose the sequence that is derived from the organism which is known to be one of the earliest lineages in a particular kingdom. The topology of a tree is the order in which the different sequences diverge and by assigning a boostrap value, the order would be obtained more reliably. A bootstrap value could range from 100 to 1000 and pending the value chosen, sequence comparisons would occur that number of times respectively. Hence, a bootstrap value provides a measure of the reliability of the phylogenetic tree.[59, 60]

Additional methods for tree construction would be a choice between an algorithmic and a tree searching approach. The former approach uses an algorithm to construct a tree from the data provided whilst the latter constructs many trees and utilises certain criteria to decide which is the best tree.[60] The algorithmic approach has two advantages in that it is fast and produces one tree for every given data set. The most common method employed is Neighbour Joining (NJ).[60] The tree searching methods include Maximum Parsimony (MP), Maximum Likelihood (ML) and the Bayesian method. Maximum Parsimony looks for the tree with the minimum number of evolutionary changes that explains the entire sequence evolution. Maximum Likelihood utilises a log likelihood value (chosen by the user) and the tree that displays the highest value is chosen as the ML tree. The Bayesian method is a variant of the ML method however, instead of producing a single tree, a set of trees of roughly equal likelihoods are produced from which the user can decide.[61] All methods produce consistent results and have proven to be reliable however, preference is given to the Neighbour Joining method as it is fast and produces one tree.[60, 61]

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Chapter 3

Methodology

3.1 Retrieval and storage of soil samples

Samples were acquired from the Miers Dry Valley, along a 500m vertical transect of the McMurdo Dry Valleys, east Antarctica, during the 2002 UWC/University of Waikato field expedition. Details of the various levels of the sampling site are given in Fig. 3.1. and Table 3.1. Samples were recovered under aseptic conditions by removal of a 1-2cm surface layer from a 20cm x 20cm grid. Samples were stored at below 0°C until transport to the Scott Base for storage at -18°C. During subsequent transport to UWC, Cape Town, samples were maintained at <0°C. Samples were preserved at -80°C until required for further use.

Figure 3.1. Picture of the Miers Dry Valley showing the 500m vertical transect with ascending samples 1 to 12. White arrow indicates sample 1 and red arrow indicates sample 12.

Table 3.1: Summary of the Miers Valley Transect (MVT) and the different levels.

Transect sample	Description of soil	Temp.	GPS	Elevation
MVT 1	Wet gravel from flood plain	-0.2 ºC	79° 05.679 163° 48.271	554 feet
MVT 2	Coarse gravels	-3.9 ºC air -0.5 ºC soil	78° 05.670 163° 48.285	553 feet
MVT 3	Sorted gravels on moraine below valley slope	-3.2 ºC air -0.3 ºC soil	78° 05.582 163° 48.324	582 feet
MVT 4	Gravels at base of Northern slope	-0.4 ºC soil	78° 05.541 163° 48.310	601 feet
MVT 5	Lower Northern slope, fine gravels	-0.4 ºC soil	78° 05.480 163° 48.370	662 feet
MVT 6	Northern slope 20m upslope from flat rock	-4.7 ºC air -0.2 ºC soil	78° 05.398 163° 48.462	768 feet
MVT 7	Northern slope	-0.4 ºC soil	78° 05.324 163° 48.520	860 feet
MVT 8	Dry fine gravels	-4.9 ºC air -0.2 ºC soil	78° 05.184 163° 48.690	1094 feet
MVT 9	Dry fine gravels along Northern slope	-4.7 ºC air +0.8 ºC soil	78° 04.904 163° 48.853	1400 feet
MVT 10	Fine and coarse gravels	-4.0 ºC air +1.1 ºC soil	78° 04.685 163° 49.178	1698 feet
MVT 11	Fine gravels	-7.2 ºC air -0.3 ºC soil	78° 04.503 163° 49.297	2001 feet
MVT 12	Dry fine gravels, ~50ft below Miers Valley/Snowy lake, Marshall Valley	-6.9 ºC air -3.9 ºC ground	78° 03.968 163° 52.083	2689 feet

3.2 DNA Extraction

Total genomic DNA was extracted directly from the soil samples using the BIO 101 Kit (Qbiogene). Direct cell lysis was achieved by mechanical (bead beating) and chemical (sodium phosphate and sodium lauryl sulphate) lysis. After centrifugation, each supernatant was transferred to a clean tube and proteins were then removed using a protein precipitating agent (Potassium acetate and glacial acetic acid). The supernatant was mixed with a binding matrix solution (silica gel suspension with guanidine thiocyanate) then passed through a spin filter column for elution of DNA.

3.3 PCR

3.3.1 16S rDNA PCR

Universal primers E9F[1] (5'– GAGTTTGATCCTGGCTCAG –3') and U1510R[2] (5'-GGTTACCTTGTTACGACTT -3'), designed to target the conserved regions of the rRNA gene, were utilized. Reagents of the PCR mix included, 5µl of 10X buffer, 3µl of 25mM $MgCl_2$, 5µl of 5µm E9F, 5µl of 5µm U1510R, 10µl of 1mM DNTP's, 0.5µl of *Taq* polymerase enzyme (Fermentas) and 1µl of gDNA (50ng/µl). Each reaction was adjusted to a final volume of 50µl with sterile super quality (super Q) water and amplified in an automated thermal cycler (Thermo Hybaid system). The PCR conditions were as follows:-

Initial denaturation:	94 °C for 2 mins	
Denaturation:	94 °C for 30 s	× 30 cycles
Annealing:	50 °C for 45 s	
Extension:	72 °C for 60 s	
Final extension:	72 °C for 10 mins	

3.3.2 DGGE specific Touchdown PCR

341FGC[3] (5'-CGCCCGCCGCGCGGCGGGCGGGGCGGGGGCACGGGGGG CCTACGGGAGGCAGCAG –3') and 534R[3] (5'- ATTACCGCGGCTGCTGG –3') (refer to Fig. 2.2. for positions on the 16S gene) are primers designed to target the conserved regions of the rRNA gene. The amplification results in the production of smaller PCR amplicons (encompassing the V3 region) that are more suitable for DGGE analysis. Reagents of the PCR mix included, 5µl of 10X buffer, 3µl of 25mM $MgCl_2$, 5µl of 5µm 341FGC, 5µl of 5µm 534R, 10µl of 1mM DNTP's, 0.5µl of *Taq* polymerase enzyme (Fermentas) and 1µl of gDNA (50ng/µl). Each reaction was adjusted to a final volume of 50µl with sterile super Q water and amplified in a Gene Amp PCR 2700 system. To increase the specificity of the PCR reaction, a touchdown PCR protocol was employed whereby the annealing temperature was set 10°C above the required temperature and decreased by 1°C every cycle until the required temperature was attained.[4] The annealing temperature was set initially at 65°C and then

decreased by 1°C every cycle to 55°C, where the temperature was held for the next 20 cycles. The PCR conditions were as follows:-

Initial denaturation:	94 °C for 5 mins	
Denaturation:	94 °C for 1 min	x 30 cycles
Annealing:	65°C - 55°C* for 1 min	
Extension:	72 °C for 2 mins	
Final extension:	72 °C for 10 mins	

* Annealing temperature decreases by 1 °C every cycle.

3.3.3 M13 PCR

M13 R (5'- CAGGAAACAGCTATGAC –3') and M13 F (5'- GTTTTCCCAGTCACGAC –3') primers designed to target M13 cloning sites in the pMOS vector, were utilized. Reagents of the PCR mix included, 5µl of 10X buffer, 3µl of 25mM $MgCl_2$, 5µl of 5µm M13F, 5µl of 5µm M13R, 10µl of 1mM DNTP's, 0.5µl of Taq polymerase enzyme and 1µl of gDNA (50ng/µl). Each reaction was adjusted to a final volume of 50µl with sterile super Q water and amplified in an automated thermal cycler (Thermo Hybaid system). The PCR conditions were as follows:-

Initial denaturation:	94 °C for 5 mins	
Denaturation:	94 °C for 30 s	x 30 cycles
Annealing:	65 °C for 45 s	
Extension:	72 °C for 60 s	
Final extension:	72 °C for 10 mins	

3.4 Denaturing gradient gel electrophoresis (DGGE)

Glass DGGE plates were thoroughly washed with methanol and potassium hydroxide solution, with a final 70% ethanol rinse to ensure a dust free surface. "C thru" solution was spread on one of the plates to prevent the gel from adhering to the glass and to allow for easy handling when viewing the gel. DGGE was performed with a 10% (wt/vol) polyacrylamide gel (37.5:1, acrylamide:bisacrylamide). A 30% – 60% low to high gradient was used for DNA

analysis. Usually, 100% denaturant corresponds to 7M urea and 40% (vol/vol) acrylamide. The 30% gradient contained 5ml of 40% polyacrylamide, 1ml of 10× TAE, 2.4ml of formamide, 2.5g urea and was adjusted to a final volume of 20ml with distilled water. The 60% gradient contained 5ml of 40% polyacrylamide, 1ml of 10× TAE, 4.8ml of formamide, 5.0g of urea and was adjusted to a final volume of 20ml with distilled water. 180μl of 10% ammonium persulfate and 18μl of TEMED were added to catalyse the polymerization process. Samples were electrophorised for 16h at 100V at 60°C, with the Scie-plas Bio-rad system. 0.5X TAE (20mM Tris-acetate pH 7.4, 10mM sodium acetate and 0.5 mM EDTA pH 7.4) was used as electrophoresis buffer.[5 - 7]

3.5. Cloning

3.5.1 Calculation of the amount of insert required

Samples were cloned using the pMOS*Blue* blunt ended cloning kit (Amersham Pharmacia Biotech). The PCR insert to be cloned was gel purified using the GFX PCR DNA and Gel Band Purification Kit (Amersham Biosciences), to ensure optimal cloning efficiency. The insert concentration was estimated by comparison to DNA standards on an agarose gel. The amount of insert required to obtain a molar vector:insert ratio of 1:2.5, when using 50ng of vector, was calculated with the following equation:-

$$\left(\frac{(Z).50}{2887} x \frac{2.5}{1}\right) = \text{ng of insert}$$

where Z is the size of insert in bp

2887 is the size of the vector in bp

50 is the concentration of the vector (ng)

3.5.2 Phosphokinase reaction

Each phosphokinase reaction was carried out with 1μl of 10x phosphokinase buffer, 0.5μl of 100mM DTT, 1μl of phosphokinase enzyme and xμl of PCR product (calculated as above). Final volume was adjusted to 10μl with super Q water. The production of blunt

ended, phosphorylated PCR products in a one step reaction was obtained after a 22°C incubation for 40 mins.

3.5.3 Ligation

A 75°C incubation for 10 mins. was used to inactivate the phosphokinase.. The reaction was cooled on ice for 2 mins to prevent the ligase from being inactivated. 10µl of the phosphorylated PCR product (the entire pk reaction), 1µl of pMOS*Blue* vector (50ng/µl) and 1µl of T_4 DNA ligase were then incubated overnight at 22°C to allow for a more efficient ligation of the insert in the vector.

3.5.4 Transformation

1µl of the ligation mix was transformed into 20µl of pre-chilled chemically competent *E. coli* cells via heat shock transformation. After incubation for 1 hour at 37°C, the cells were then plated onto LB agar ampicillin plates containing 35µl of 50mg/ml X-gal and 20µl of 100mM IPTG, for blue/white screening of recombinant cells.

3.5.5 Direct colony PCR screening

Master plates of each colony were initially constructed and detection of the insert was then carried out via direct colony PCR screening. Selected colonies, approximately 1mm in diameter, were transferred to a 1.5ml tube containing 40µl of sterile distilled water. Tubes were then placed in boiling water for 5mins to lyse the cells and denature DNases. After centrifugation, 10µl of each supernatant was transferred to a clean eppendorf tube for M13 PCR analysis.

3.6. Amplified rDNA restriction analysis (ARDRA)

ARDRA, which was conducted on samples that showed an insert during M13 PCR analysis, facilitates the comparison of insert sequences and eliminates need for sequencing of multiple common inserts. *Eco* R1 (8u/µl) was used to screen all inserts. 5µl of DNA was incubated with 0.8µl of *Eco* R1, 2µl of buffer and 12.2µl of water, overnight. *Alu*1, a 4bp

restriction endonuclease was used to obtain more detailed banding patterns of the inserts (similar sequences displayed similar bands). 5µl of DNA were incubated with 0.8µl of *Alu*1, 2µl of buffer (Y+ Tango, Fermentas) and 12.2µl of water, overnight.

3.7. Plasmid isolation

Clones containing the inserts selected for sequencing were inoculated into LB medium and incubated overnight. Plasmids were isolated using the GFX Micro Plasmid Prep Kit (Amersham Biosciences). The above procedure makes use of the modified alkaline lysis procedure as well as a glass fiber matrix to produce high yields of DNA.

3.8. Sequencing

Sequencing reactions were conducted at the University of Cape Town, using the Sanger Dideoxy sequencing method. Sequencing reactions were conducted by Di James, the senior technical officer at the Department of Molecular and Cell Biology at UCT.

3.9. Phylogenetic analysis

Alignments were conducted with Clustal W multiple alignments featured in Bioedit Sequence Alignment Editor version 6.0.5 (Copyright 1997-2001 Tom Hall Isis Pharmaceuticals Inc. Department of Microbiology, North Carolina State University). The software package TREECON was used for the construction and drawing of the phylogenetic trees, based on evolutionary distances computed from nucleic acid sequences.[8] The Galtier and Gouy[9] distance based method was used for constructing neighbour joining phylogenetic trees with a bootstrap value of 100.

3.10. References:

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Chapter 4

Results and Discussion

4.1. Introduction

Documented reports on the molecular investigations of microbial communities in the McMurdo Dry Valleys are few.[1] Previous studies have focused on microscopy and culture dependent methods but such approaches do not reflect the true diversity of a microbial community.[1] This study utilised 16S rDNA PCR and phylogenetic analysis to obtain qualitative data on the bacterial phylotypes that inhabit the Miers Dry Valley of McMurdo Sound. No previous studies have attempted to establish the presence of a putative community structure in Dry Valley mineral soils. Hence, this investigation has employed molecular phylogenetics in an attempt to infer the presence of one of the key components of a stable functional community, whereby carbon acquisition is either heterotrophic or autotrophic. In the Dry Valley mineral soils, exogenous heterotrophic substrates are thought to be negligible[2] it is therefore suggested that any putative community must inevitably be based on autotrophy (photoautotrophy/chemoautotrophy). In this investigation we attempt to infer from phylogenetic data, the possible presence of indicative phenotypes which might contribute to a functional microbial community.

4.2. gDNA Isolation

gDNA obtained with the BIO 101 Kit was of a higher purity and less sheared (Fig. 4.1.) than that of the modified Zhou method. All samples showed the presence of DNA with a size range of between 11 and 14kb. However, samples 1 to 5, 10 and 12 displayed weak signals indicating low biomass, whereas samples 6 to 9 and 11 showed strong signals, indicative of a higher biomass. These relative biomass levels might be attributed to the physical

characteristics of the mineral soils. For example, the gravels of samples 1 to 5 were coarser in nature while, with an increase in elevation, the gravels became finer (D. A. Cowan pers. comm.). Hence, the smaller the soil granules the greater the available surface area for microbial growth.

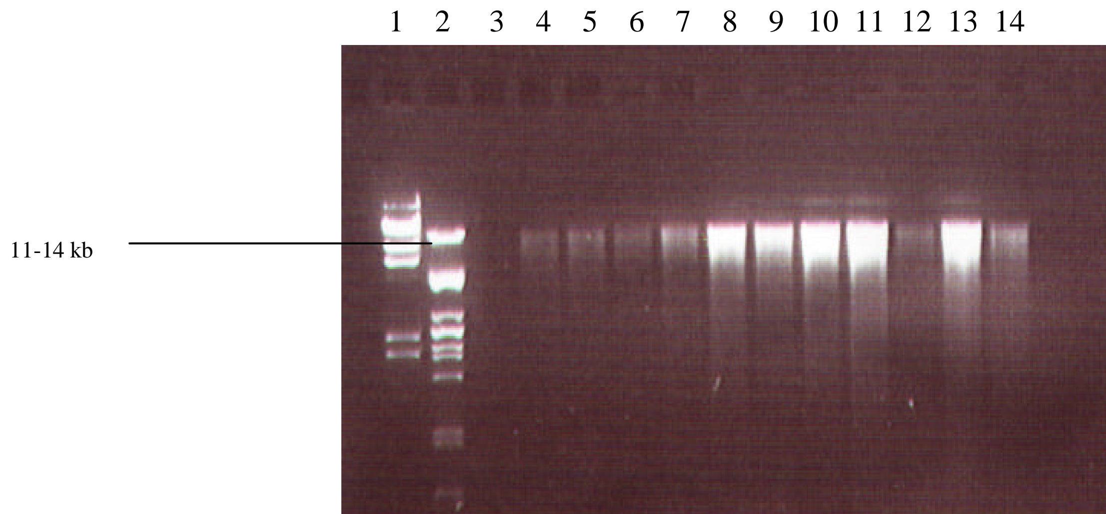

Figure 4.1. gDNA isolations of MVT samples 1 to 12. Lane 1 is ? DNA cut with *Hind* III, lane 2 ? DNA cut with *Pst* I and lanes 3 to 14 show DNA extractions from MVT samples 1 to 12, respectively.

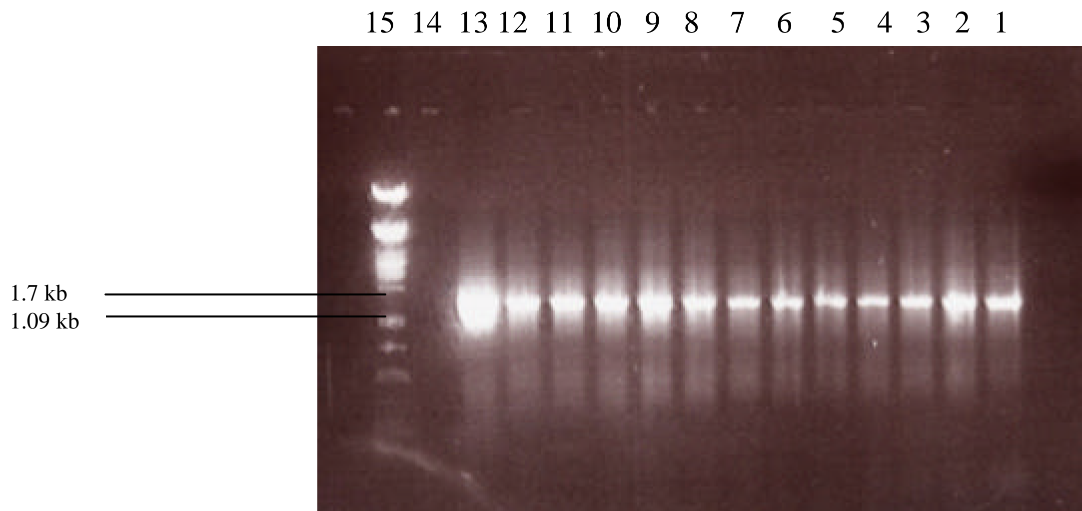

Figure 4.2. Products of 16S rDNA PCR amplification. Lanes 1 to 12 are MVT samples 1 to 12 respectively, lane 13 is the positive control (16S PCR of *E. coli* gDNA*)*, lane 14 is the negative control and lane 15 is ? DNA cut with *Pst* I.

4.3. 16S rDNA PCR

16S rDNA PCR, using the universal bacterial 16S primers E9F[3] and U1510R,[4] was successful with all 12 samples. In all cases amplicons of approximately 1500 bp (the expected size) were obtained (Fig. 4.2.).

4.4. Denaturing gradient gel electrophoresis (DGGE)

DGGE, conducted to obtain an overview of the bacterial diversity across the 12 samples, showed that many of the transect samples displayed similar banding patterns (Fig. 4.3.). It was seen that each sample displayed significant bacterial diversity (each band represents one or more different microorganisms). With the exception of sample 7 many of the bacterial phylotypes were common in every sample. However, certain bacterial phylotypes appeared to be site specific (see arrows Fig. 4.3.). DGGE is capable of detecting up to 96% of all mutations or single base pair substitutions in fragments up to 500 base pairs in length and was also shown to be reproducible.[5,6] The sensitivity of DGGE was evident in the present investigation as results were consistent when DGGE was conducted repeatedly. The consistency of phylotypes present in every sample, reflected by DGGE, was also indicated in the 16S rDNA clone libraries.

A vertical transect of 500m in the Miers Dry Valley has little effect on microbial diversity, as DGGE has indicated that few phylotypes appeared to be altitude-dependent. Arrow **A** in Fig. 4.3. shows phylotypes that are common in samples 5 to 12 and arrow **B** shows phylotypes common in samples 8 to 12. Due to the similarity between the various samples clone libraries for samples 1, 5, 7, 9 and 12 were constructed.

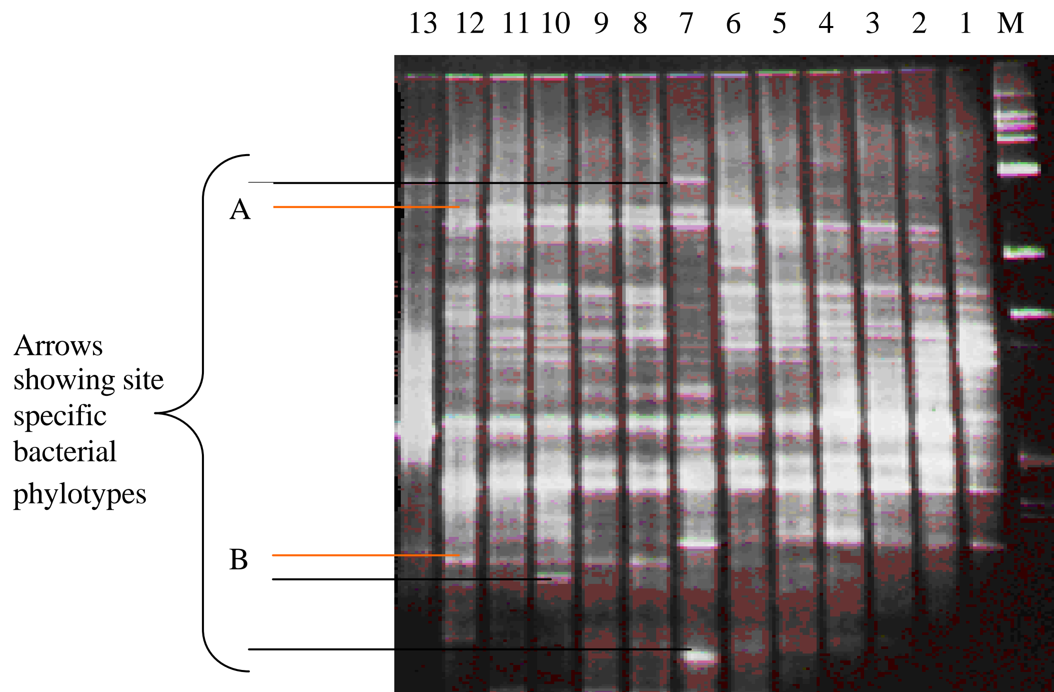

Figure 4.3. Denaturing gradient gel showing bacterial phylotypic diversity across the MVT transect. (M) is marker ? DNA cut with *Pst* I, lanes 1 to 12 is MVT samples 1 to 12 respectively and lane 13 is the positive control (*E. coli* partial 16S sequence).

4.5. 16S Clone Libraries and Phylogenetic Analyses

Previous studies conducted on microbial communities in the McMurdo Dry Valleys have largely utilised microscopy and culture-based methods.[7] However, it is now widely accepted that these approaches do not reflect the true microbial diversity of an environment. For example, an investigation utilising morphological and molecular approaches to explore cyanobacterial diversity in Lake Fryxell (McMurdo Dry Valleys) showed a substantial discrepancy between the two techniques.[8] Microscopy identified eight morphotypes whilst molecular analyses revealed fifteen different phylotypes.[8] The report provided evidence for the molecular diversity of Cyanobacteria, which was shown to be greater than the previously known diversity based on culture and microscopy methods.[8] Previous culture based methods investigating the microbiology of Antarctic Dry Valley mineral soils showed the presence of predominately gram negative aerobic rods such as *Bacillus*, *Micrococcus* and *Streptomyces*.[9] The present investigation discovered a wider range of phylotypes encompassing gram positive aerobic and anaerobic genera such as *Clostridium* (anaerobic), *Rhodoglobus* and *Rubrobacter.*

In the present investigation partial 16S gene amplicons of each sample were sequenced with the E9F[3] primer via the Sanger dideoxy sequencing method. Sequences of approximately 500bp, encompassing the variable regions V1, V2 and V3, were obtained. A total of 121 clones were sequenced and similarity searches with known bacterial 16S rDNA sequences in public databases were evaluated. Of the 121 sequences, 115 were =90% identical to their respective matches in the database, 2 sequences were 89% identical and 4 sequences were 88% identical. These high percentage homology values would confirm the phylum and in some cases (=95%) the genus level of the sequences.[7] A sequence identity value of =98% may correspond to species designation.[7] However, confirmatory biochemical, physiological and morphological testing should be conducted (pending the culturable state of the isolates).

The partial 16S rDNA sequences of each library and their reference sequences from the database were aligned with Clustal W featured in Bioedit version 6.0.5. Neighbour joining phylogenetic trees were constructed with Treecon,[10] employing the Galtier and Gouy distance based method.[11] The scale bar of all trees represents a 0.1% difference in nucleotide sequences. Bootstrap values provide a measure of the reliability of the phylogenetic analysis and values of 65 and above for the nodes are displayed on the trees.

All trees showed that most of the clones clustered with their respective matches obtained from the database and also displaying bootstrap values of 100. For most of these clusters, small differences in the branch distance indicated a high nucleotide similarity between the sequences. These results suggest the high probability of phylotypes in the present study being considered closely related to those in the database. Some Antarctic isolates clustered together whilst others exhibited high similarity to environmental samples. This suggests that most Antarctic genera are common to other soil environments, but may have adapted to the extreme psychrophilic habitat. A relatively small proportion (~10%) of Antarctic phylotypes appeared to be novel.

16S rDNA PCR and phylogenetic analyses have been previously used to investigate the microbial diversity of cryptoendolithic communities of the McMurdo Dry Valleys, in a previous study.[7] Results showed that Actinobacteria, a and ?-Proteobacteria and Planctomycetes were among the dominant phylotypes present.[7] Results correlated with the

present investigation as the major taxonomic groups represented by the genera included a-, ß- and ?-Proteobacteria, Actinobacteria, Verrucomicrobia, Planctomycetes and several uncultured environmental and Antarctic samples.

Proteobacteria, considered the most diverse group of bacteria, are the second largest group (Firmicutes being the first), consisting of 429 named genera in 72 named families.[12] All species are gram negative with their diversity ranging from purple phototrophs to chemoautotrophs and chemoheterotrophs.[13] Each library varied in the composition of a, ß and ?-Proteobacteria. The a-Proteobacteria contain genera that are mostly pathogenic in nature, either to humans or plants. Other genera are chemoautotrophs and some are capable of fixing nitrogen.[13] a-Proteobacteria are mostly digotrophic bacteria, that occupy nutrient limiting environments and they are also common in pristine soils.[12] ß-Proteobacteria consist either of chemoautotrophs (capable of oxidising elemental sulfur) or chemoheterotrophs (capable of utilising organic sulfur).[13] ?-Proteobacteria are autotrophs either using light (purple sulfur bacteria, photoautotrophs) or H_2S as a source of energy (chemoautotrophs). This group also contains chemoheterotrophs.[13]

Actinobacteria are a group of aerobic, gram positive bacteria with high GC content genomes. They constitute some of the most common soil microbiota, which play important roles in decomposition and humus formation.[7,14] Planctomycetes are a group of budding peptidoglycan-less bacteria[15] that are capable of growing anaerobically and autotrophically via the oxidation of ammonium.[16] Verrucomicrobia, also termed prosthecobacteria (having multiple appendages on cell surfaces) are heterotrophic, gram negative non-motile bacteria. Their common environments include eutrophic lakes and ponds. The prostheca enable attachment to various surfaces.[17]

Cyanobacteria were shown to be the dominant phototrophs in many moist Antarctica habitats, such as lakes, ponds, endolithic and sublithic communities.[18,19] However, the limited detection of cyanobacteria throughout the Miers Dry Valley Transect may reflect the low precipitation levels of the valley.

4.5.1. MVT 1

Based on homology values of =95% and phylogenetic studies, five sequences can be assigned to specific genera with some assurance. These included the sequences from clones 18, 19, 37, 54 and 60 (Table 4.1., Fig. 4.4.). Sequence from clone 18 showed a 96% homology to an *Opitutis* sp.[17] and sequence from clone 19 was 97% identical to a *Clostridium* sp. These genera were also confirmed through phylogenetic analyses with respect to the high bootstrap values (100) and the small difference in branch lengths. The inhabitance of *Clostridia* (anaerobic, gram positive and spore forming) in Antarctica may be unexpected due to the highly aerobic nature of the environment.[20] However, a previous study conducted on the microbial community of a mat sample from Lake Fryxell, Antarctica reported that more than 10% of the cloned 16S rRNA gene sequences and five of the isolates belonged to the genera *Clostridium*.[21] More specifically, most of the 16S rRNA gene sequences and four of the five isolates were phylogenetically related to *Clostridium estertheticum,* a psychrophilic species originally isolated from spoiled vacuum-packed refrigerated beef.[21]

The other confirmed genera included *Rhodoglobus* (clone 37) (Table 4.1.), an Antarctic isolate from the McMurdo Dry Valleys.[22] Phylogenetic studies have illustrated that sequence from clone 37 has closely clustered with *Rhodoglobus*, with a bootstrap value of 100 and a small difference in the branch lengths (Fig. 4.4.). This microorganism was characterised as a psychrophilic, gram positive, aerobic bacterium that forms red pigmented colonies when grown at 18ºC. *Rhodoglobus vestali* belongs to the family Microbacteriaceae and shows a very high similarity to the genus *Leifsonia*, a cryobacterium.[22]

The sequence from clone 41 was 98% identical to a bacterium that contained a gene that was shown to code for a diooxygenase enzyme capable of breaking down naphthalene[23] (Table 4.1.). The bacterium, referred to as strain CJ2, displayed a number of characteristics that made it a likely candidate to be of Antarctic origin.[23] In the present investigation phylogenetic analyses has indicated that sequence 41 and the uncultured sample cluster closely to other Antarctic and Arctic sequences (Fig. 4.4.). Strain CJ2 is a gram negative coccus that grows in the presence of naphthalene, at temperatures only under 20ºC (optimally at 10ºC) and it was also incapable of growing in rich media.[23]

The remaining two genera that can be assigned with some confidence, included sequence from clone 54 which was 99% identical to a *Brevundimonas* sp.,[24] and sequence from clone 60 which was 98% identical to a *Lysobacter* sp. (Table 4.1.). *Brevundimonas* is an a-Proteobacterium that resides in low nutrient freshwater or soil habitats.[24] Both these genera are chemoheterotrophs. Evidence for the presence of *Brevundimonas* sp. in Antarctic soils has been provided in other reports.[25]

Microbial community structures are dependent on the relationships between photo- and chemoautotrophs as well as photo- and chemoheterotrophs. The former, being primary producers, generate organic compounds like starch and sugars through the oxidation of inorganic material, which are then utilised by the photoheterotrophs. Organic forms of carbon together with sunlight are used by the photoheterotrophs to produce other complex compounds and nutrients. These compounds are then metabolised by the chemoheterotrophs such as saprobes, which release the inorganic material back into the environment.

The five confirmed genera in the MVT 1 16S clone library are all heterotrophs. Species belonging to the genera *Opitutis* and *Clostridium* are chemoheterotrophs that require organic compounds for growth and are strictly fermentative.[17,20] The genera *Brevundimonas* and *Lysobacter* are also chemoheterotrophs.[24] *Rhodoglobus* is an Actinobacterium which is known to be a saprophytic heterotroph.[22] Previous studies using culture based methods have shown the presence of photoautotrophs in the form of cyanobacteria, no chemoautotrophs were reported.[2] The use of molecular techniques in the present investigation cannot confirm the presence of chemoautotrophs. However, their possible presence cannot be eliminated as a large portion of the sequences remained uncultured (Fig. 4.5.). Photoautotrophs in the form of cyanobacteria accounted for 3% of the phylotypic diversity (Fig. 4.5.) in MVT 1, but this could be attributed to the aerial transport of dry cyanobacterial mats as site 1 resides close to the margins of Lake Miers.

Photo- and chemoautotrophic activity have shown to be deficient in the arid, highly aerobic soils of the Miers Dry Valley.[2] Heterotrophic activity is therefore highly dependent on imported organic matter.[2] Organic matter originates in the aquatic environments and in

cyanobacterial communities within cryptoendolithic habitats and is aerially dispersed across the Dry Valleys.[2]

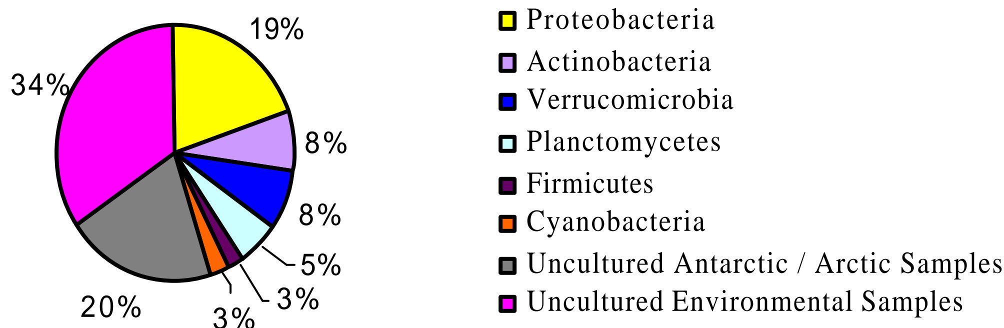

Figure 4.5. The percentage of different phyla in MVT 1.

4.5.2. MVT 5

Actinobacteria in MVT 5 accounted for 13% of the phylotypic diversity (Fig. 4.6.). These included *Nocardia* sp. (clone 2),[36] *Kribbella* sp. (clone 5) and possibly *Rubrobacter radiotolerans* (clone 8)[37] (Table 4.2.). *Rubrobacter radiotolerans* is a radiation resistant bacterium which is frequently isolated from thermal environments. It is also known that the DNA of *Rubrobacter* sp. is frequently isolated from desert soils.[37] Studies have shown that highly radiation resistant bacteria have the ability to repair DNA that is damaged by radiation and this may also be an adaptation to repair DNA that is damaged by desiccation.[7] In another study investigating cryptoendolithic communities from the McMurdo Dry Valleys, BLAST results of bacterial 16S sequences resulted in homology to members of the *Thermus-Deinococcus* phylogenetic group.[7] *Deinococcus* spp. have also shown to be possible inhabitants of granite outcrops in Antarctica[38] and they are also very similar to *Rubrobacter radiotolerans* especially with its radiation resistant ability.[7] This provides evidence for the possibility of *Rubrobacter* spp. inhabiting Antarctica.

Table 4.1. Summary of MVT 1 Blast results

Clone No.	Size	Phylogenetic Group	Organism	%Identity/ %Similarity	E Value	Accession Number	Ref.
3	89-437	Uncultured environmental sample	Uncultured bacterium clone cRI32d	344/350 (98%)	0	AY364069	26
4	198-683	Uncultured environmental sample	Uncultured bacterium clone KD4-108	448/486 (92%)	0	AY218624	Unpublished
6	47-692	Uncultured environmental sample	Uncultured bacterium clone ARKIA-43	600/647 (92%)	0	AF468297	27
7	82-656	? proteobacteria	Uncultured Xanthomonadaceae bacterium clone M10Ba23	524/577 (90%)	0	AY360613	28
8	182-638	Actinobacteria	*Sphaerobacter thermophilus* strain DSM 20745T	405/457 (88%)	e^{-136}	AJ420142	Unpublished
10	42-663	Uncultured environmental sample	Uncultured bacterium clone a13115	604/623 (96%)	0	AY102322	29
12	50-718	Uncultured environmental sample	Uncultured bacterium clone ARKMP-16	663/671 (98%)	0	AF468326	Unpublished
18	85-682	Verrucomicrobia	*Opitutus* sp. strain VeCb1	580/598 (96%)	0	X99391	17
19	83-696	Clostridia	*Clostridium estertheticum* A-1/C-an/C1	600/614 (97%)	0	AJ297442	Unpublished
24	22-535	Uncultured environmental sample	Uncultivated soil bacterium clone C102	497/515 (96%)	0	AF013529	30
29	76-543	Planctomycetes	Uncultured Planctomy cetales bacterium clone M10Ba61	435/468 (92%)	0	AY360649	28
30	53-666	Verrucomicrobia	Bacterium Ellin5102	571/614 (92%)	0	AY234519	31
32	179-704	Planctomycetes	Uncultured Planctomycetales bacterium clone SM1A02	464/526 (88%)	e^{-154}	AF445645	Unpublished
34	215-607	Cyanobacteria	Uncultured cyanobacterium clone TAF-B69	373/393 (94%)	e^{-179}	AY038727	32
36	58-448	Uncultured environmental sample	Uncultured Antarctic bacterium LB3-30	378/391 (96%)	0	AF173822	Unpublished
37	65-610	Actinobacteria	*Rhodoglobus vestalii*, strain LV3	520/546 (95%)	0	AJ459101	22
41	82-627	Uncultured environmental sample	Uncultured bacterium clone 61	537/546 (98%)	0	AY250101	23
47	110-286	Uncultured environmental sample	Uncultured bacterium clone CBF2	168/177 (94%)	$2e^{-80}$	AF392790	Unpublished
48	83-501	Uncultured environmental sample	Uncultured soil bacterium clone Tc120-141	391/419 (93%)	0	AY242634	33
49	70-644	Uncultured environmental sample	Unidentified bacterium, strain BD5-13	528/575 (91%)	0	AB015569	34
54	81-681	a proteobacteria	*Brevundimonas* sp., strain FWC04	596/601 (99%)	0	AJ227793	24
55	97-584	Uncultured environmental sample	Uncultured gold mine bacterium D33	457/489 (93%)	0	AF337887	Unpublished
57	149-537	a proteobacteria	*R. capsulatus*	365/390 (93%)	e^{-180}	AY128090	Unpublished
60	105-630	? proteobacteria	*Lysobacter* sp. Dae16	518/526 (98%)	0	AB166878	Unpublished
61	75-505	Uncultured environmental sample	Uncultured Crater Lake bacterium CL0-56	414/431 (96%)	0	AF316782	35

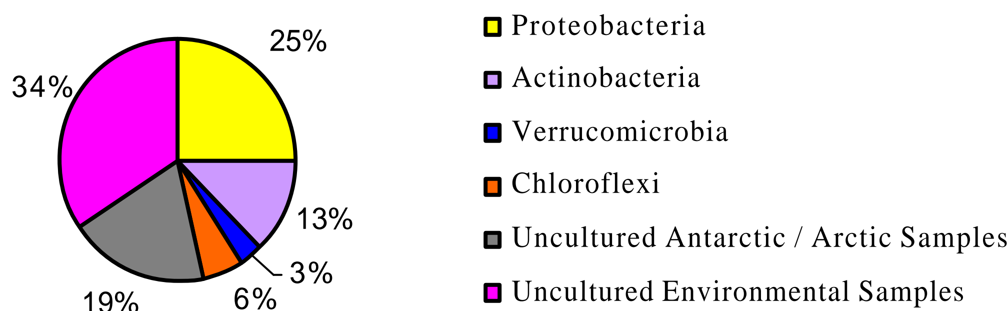

Figure 4.6. The percentage of different phyla in MVT 5.

The genera or family of five sequences in MVT 5 can be assigned with confidence, based on homology values of =95%. These included sequences from clones 1, 2, 5, 24 and 68 (Table 4.2.). The sequence from clone 1 displayed an identity value of 97% to a Comamonadaceae bacterium.[28] Comamonadaceae are chemoheterotrophic, gram negative, aerobic bacteria that are frequently used for the treatment of activated sludge.[28] The sequence from clone 2 was 95% homologous to a *Nocardia* sp.[36] This genus comprises filamentous, chemoheterotrophic and facultatively anaerobic bacteria that are frequently used in foaming and wastewater treatment plants.[36] The sequence from clone 24 was 97% identical to a *Sphingomonas* spp.[39] and sequence from clone 68 was 98 % homologous to a *Lysobacter* sp. Both these genera are chemoheterotrophs. Supporting evidence for the presence of *Sphingomonas* sp. in Antarctic soils is provided in other reports.[25] Blast results in MVT 5 16S clone library did not support the presence of autotrophs. The apparent lack of autotrophic activity means that these heterotrophs have to rely on other sources of organic compounds and the dominant available form of organic compounds is through aerial dispersion as discussed previously.

Blast results have shown predominantly heterotrophs to be present in the Miers Dry Valley. However, the possibility of autotrophs being present in this environment cannot be eliminated as a large portion of the sequences showed homology to uncultured Antarctic and environmental samples (Fig. 4.6.). The sequence from clone 9 showed a 94% homology to *Methylobacterium nodulans*[40] (Table 4.2.), a chemoautotroph capable of oxidising methane to

Table 4.2. Summary of MVT 5 Blast results

Clone No.	Size	Phylogenetic Group	Organism	%Identity / %Similarity	E Value	Accession Number	Ref.
1	10 - 679	ß Proteobacteria	Uncultured Comamonadaceae bacterium clone M3Ba22	655/670 (97%)	0	AY360686	28
2	1437- 1087	Actinobacteria	*N. uniformis*	336/351 (95%)	e^{-159}	Z46752	36
5	1471- 821	Actinobacteria	*Kribbella antibiotica*	632/651 (97%)	0	AY082063	Unpublished
8	15 - 646	Actinobacteria	*Rubrobacter radiotolerans*	596/632 (94%)	0	U65647	37
9	1410- 762	α proteobacteria	*Methylobacterium nodulans* strain ORS2060	611/649 (94%)	0	AF220763	40
10	13-681	uncultured environmental samples	Uncultured bacterium clone D138	656/669 (98%)	0	AY274144	41
11	1408 - 762	α proteobacteria	*X. flavus* strain JW/KR-E1	609/651 (93%)	0	X94206	42
12	204 - 687	uncultured environmental samples	Uncultured Crater Lake bacterium CL500-48	450/486 (92%)	0	AF316757	35
13	1362 - 799	uncultured environmental samples	Uncultured Antarctic bacterium LB3-92	531/566 (93%)	0	AF173824	Unpublished
14	1420 - 838	uncultured environmental samples	Uncultured bacterium clone ARKMP-14	572/583 (98%)	0	AF468332	Unpublished
18	1103 - 411	uncultured environmental samples	Uncultured bacterium clone KD7-88	631/694 (90%)	0	AY218718	Unpublished
21	1311-750	ß Proteobacteria	Glacier bacterium FJI10	554/563 (98%)	0	AY315180	Unpublished
24	1 - 586	α proteobacteria	*Sphingomonas* sp. SIA181-1A1	572/588 (97%)	0	AF395032	39
26	1-626	Acidobacteria	Uncultured Acidobacteria bacterium clone 351B	622/626 (99%)	0	AY571792	Unpublished
29	1-436	uncultured environmental samples	Uncultured bacterium, clone JG34-KF-314	432/436 (99%)	0	AJ532726	43
30	744 - 510	Chloroflexi	Uncultured Chloroflexi bacterium clone s02wfb8	219/235 (93%)	e^{-87}	AY184460	Unpublished
34	1451-819	? -Proteobacteria	Uncultured beta proteobacterium clone B-BH93	633/633 (100%)	0	AY622261	Unpublished
37	1517-890	uncultured environmental samples	Bacterial species, clone RB41	585/632 (92%),	0	Z95722	Unpublished
39	2-585	uncultured environmental samples	Uncultured bacterium clone C-F-12	546/585 (93%)	0	AF443578	44
40	1 - 627	uncultured environmental samples	Uncultured soil bacterium clone Tc123-C09	582/629 (92%)	0	AY242727	33
51	180-641	Actinobacteria	*Sphaerobacter thermophilus* strain DSM 20745T	405/457 (88%)	e^{-136}	AJ420142	Unpublished
54	44-660	Uncultured environmental sample	Uncultured bacterium clone a13115	604/623 (96%)	0	AY102322	29
62	55-656	Verrucomicrobia	Bacterium Ellin5102	571/614 (92%)	0	AY234519	31
65	47-692	Uncultured environmental sample	Uncultured bacterium clone ARKIA-43	600/647 (92%)	0	AF468297	27
68	107-631	? proteobacteria	*Lysobacter* sp. Dae16	518/526 (98%)	0	AB166878	Unpublished

methanol. Although the percentage homology may not be sufficient to confirm the genus of the organism, it indicated the possible presence of chemoautotrophs in these samples.

4.5.2. MVT 7

MVT 7 displays a different banding pattern as compared to the other samples, when viewed by DGGE (Fig. 4.3.). MVT 7 seems to lack some of the phylotypes that are common in the other samples. This sample is at the centre of the transect and it is the driest, as the bottom samples may experience contact with flowing water from Lake Miers whilst the upper levels experience some moisture due to fog effects and cloud cover. Hence, a dominancy of Actinobacteria would be expected in this sample. This is consistent with the results obtained as the uncultured Antarctic and environmental samples accounted for the highest percentage of phylotypes, followed by Actinobacteria (Fig. 4.7.).

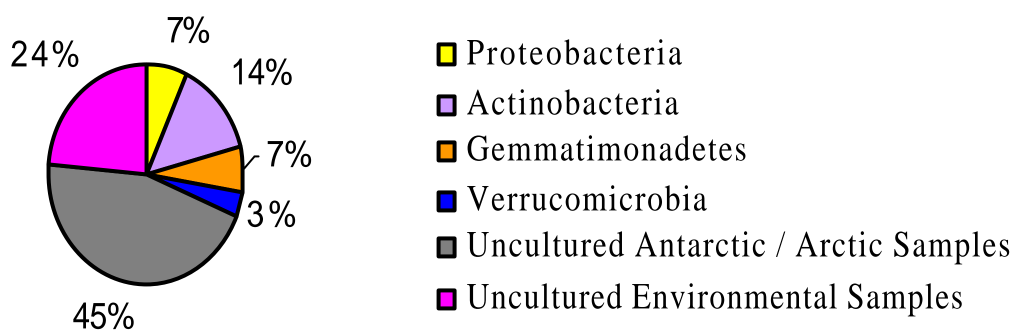

Figure 4.7. The percentage of different phyla in MVT 7.

The sequence from clone 94 showed a 95% homology to *Rubrobacter radiotolerans*[37] (Table 4.3.) and the high bootstrap value (100) and small difference in branch lengths shown in the phylogenetic studies (Fig. 4.8.), confirmed the genus. *Rubrobacter radiotolerans* is an extremely radiation resistant bacterium and the ability to repair DNA that is damaged by radiation may also be an adaptation to repair DNA that is damaged by desiccation[7,37] (sec. 4.5.2.). Previous studies have postulated the possible presence of this microorganism in Antarctic mineral soils[38] and the above analyses confirm these hypotheses.

Table 4.3. Summary of MVT 7 Blast results

Clone No.	Size	Phylogenetic Group	Organism	%Identity / %Similarity	E Value	Accession Number	Ref.
11	78-423	Gemmatimonadetes	Uncultured Gemmatimonadetes bacterium clone SL2-1-C8	311/347 (89%)	e^{-105}	AY214645	46
13	215-564	Gemmatimonadetes	Uncultured candidate division BD bacterium clone GR12	331/351 (94%)	e^{-149}	AF545640	47
18	103-511	uncultured environmental samples	Uncultured bacterium clone D121	376/410 (91%)	e^{-163}	AY274130	41
24	49-660	Actinobacteria	Uncultured *Pseudonocardia* sp. clone 343G	599/613 (97%)	0	AY571815	Unpublished
29	53-536	uncultured environmental samples	Uncultured bacterium clone C-F-15	439/486 (90%)	e^{-168}	AF443586	44
31	120-359	Actinobacteria	Uncultured actinobacterium clone SMS9.6WL	220/241 (91%)	$2e^{-83}$	AY043904	48
37	33-626	Actinobacteria	Uncultured actinobacterium clone FBP234	564/594 (94%)	0	AY250866	7
49	50-661	Verrucomicrobia	Bacterium Ellin5102	571/614 (92%)	0	AY234519	31
52	44-687	Uncultured environmental sample	Uncultured bacterium clone ARKIA-43	600/647 (92%)	0	AF468297	27
58	55-444	Uncultured environmental sample	Uncultured Antarctic bacterium LB3-30	378/391 (96%)	0	AF173822	Unpublished
61	85-499	Uncultured environmental sample	Uncultured soil bacterium clone Tc120-141	391/419 (93%)	0	AY242634	33
67	77-501	Uncultured environmental sample	Uncultured Crater Lake bacterium CL0-56	414/431 (96%)	0	AF316782	35
74	1411-759	α proteobacteria	*X. flavus* strain JW/KR-E1	609/651 (93%)	0	X94206	42
79	1453-817	?-Proteobacteria	Uncultured beta proteobacterium clone B-BH93	633/633 (100%)	0	AY622261	Unpublished
82	37-657	Actinobacteria	*Arthrobacter* sp. I4	573/622 (92%)	0	AY177353	49
84	86-684	Actinobacteria	Kineococcus-like bacterium AS3187	562/601 (93%)	0	AF060689	Unpublished
85	22-608	uncultured environmental samples	Uncultured bacterium clone ARKCH2Br2-66	544/588 (92%)	0	AF468240	Unpublished
90	748-65	uncultured environmental samples	Uncultured bacterium clone D11	631/684 (92%)	0	AY268337	50
92	104-611	uncultured environmental samples	Uncultured bacterium clone KD1-79	475/509 (93%)	0	AY218566	Unpublished
94	58-646	Actinobacteria	*Rubrobacter radiotolerans*	561/590 (95%)	0	U65647	37
98	37-709	Uncultured environmental sample	Uncultured earthworm intestine bacterium clone ew57	664/678 (97%)	0	AY154521	51
104	143-560	uncultured environmental samples	uncultivated soil bacterium clone S007	395/418 (94%)	0	AF013544	30

4.5.4. MVT 9

The sequence from clone 21 was 95% identical to an uncultured environmental sample isolated from a heavy metal contaminated site, a part of a study of integron diversity[41] (Table 4.4.). This sequence was present throughout all MVT samples. Integrons are horizontal gene transfer systems, which contain elements that are necessary for site-specific recombination and the expression of foreign DNA.[41] The study found 14 previously undescribed integrase genes.[41] As integrons are important agents for gene transfer particularly in response to selective pressure, their possible existence in Antarctic isolates may be pivotal as a means of acquiring genes that could provide a selective advantage under adverse conditions.

Phylogenetic analysis (bootstrap value of 100 and a small difference in branch lengths) (Fig. 4.9.) of the sequence from clone 58 and an identity value of 99% confirmed the genus to be the ?-Proteobacterium *Stenotrophomonas*[52] (Table 4.4.). *Stenotrophomonas* is a rod shaped, multi drug resistant human pathogen. The microorganism can be found in a variety of environments and displays antifungal and antibiotic properties.[52] This is an extremely evolved bacterium with high intraspecies diversity, which was determined by physiological parameters[53] and genotypic studies.[54] This microorganism was also shown to be present in Antarctica soils in a previous study.[25]

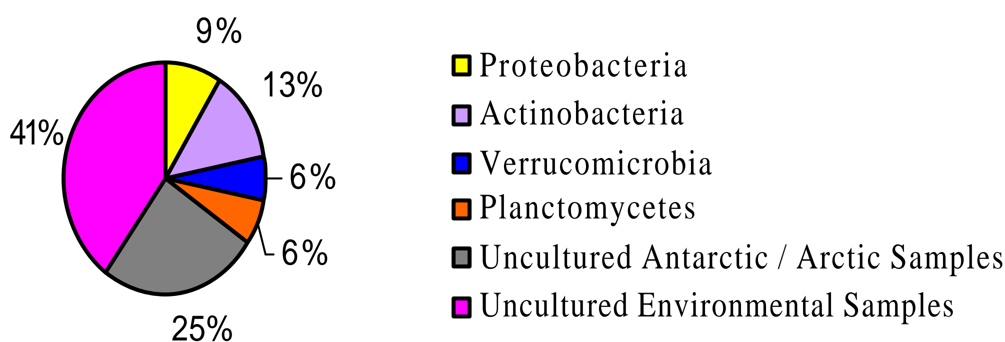

Figure 4.10. The percentage of different phyla in MVT 9.

Table 4.4. Summary of MVT 9 Blast results

Clone No.	Size	Phylogenetic Group	Organism	%Identity/ %Similarity	E Value	Accession Number	Ref.
4	74-584	Actinobacteria	Uncultured *Rubrobacterium* #0319-7H2	485/517 (93%)	0	AF234151	55
10	181-690	Planctomycetes	*Nostocoida limicola* III strain Ben223	481/512 (93%)	0	AF244750	Unpublished
13	207-647	Uncultured environmental sample	Uncultured soil bacterium clone S0202	416/441 (94%)	0	AF507699	56
14	183-689	Planctomycetes	Uncultured Planctomycetales bacterium clone M10Ba61	470/509 (92%)	0	AY360649	28
17	58-683	Actinobacteria	Uncultured Pseudonocardia sp. clone 343G	619/627 (98%)	0	AY571815	Unpublished
19	207-685	uncultured environmental samples	Uncultured Crater Lake bacterium CL500-48	450/486 (92%)	0	AF316757	35
21	96-537	Uncultured environmental sample	Uncultured bacterium clone D116	421/443 (95%)	0	AY274126	41
23	49-689	Uncultured environmental sample	Uncultured bacterium clone ARKIA-43	600/647 (92%)	0	AF468297	27
26	93-401	Uncultured environmental sample	Uncultured bacterium clone C-F-15	286/311 (91%)	e^{-106}	AF443586	44
28	62-467	Verrucomicrobium	Uncultured Verrucomicrobia bacterium clone NMW3.42WL	385/414 (92%)	e^{-151}	AY043923	48
37	50-663	Verrucomicrobia	Bacterium Ellin5102	571/614 (92%)	0	AY234519	31
41	91-435	Uncultured environmental sample	Uncultured bacterium clone cRI32d	344/350 (98%)	0	AY364069	26
43	199-679	Uncultured environmental sample	Uncultured bacterium clone KD4-108	448/486 (92%)	0	AY218624	Unpublished
44	73-307	Uncultured environmental sample	Uncultured soil bacterium clone Tc135-228	221/243 (90%)	$2e^{-63}$	AY242765	33
46	37-300	Acidobacteria	Uncultured Acidobacteria bacterium clone 351B	261/271 (96%)	e^{-104}	AY571792	Unpublished
48	61-445	Uncultured environmental sample	Uncultured Antarctic bacterium LB3-30	378/391 (96%)	0	AF173822	Unpublished
50	24-121	Actinobacteria	Uncultured actinobacterium clone SMS9.30WL	96/100 (96%)	$2e^{-31}$	AY043899	48
51	47-660	Uncultured environmental sample	Uncultured bacterium clone a13115	604/623 (96%)	0	AY102322	29
55	53-755	α proteobacteria	*Sphingomonas sp*. SIA181-1A1	696/708 (98%)	0	AF395032	39
58	69-751	γ proteobacteria	*Stenotrophomonas maltophilia* strain e-a21	687/688 (99%)	0	AJ293470	52
61	89-603	Uncultured environmental sample	Uncultured Acidobacteria bacterium clone 351B	514/520 (98%)	0	AY250867	7
62	37-710	Uncultured environmental sample	Uncultured earthworm intestine bacterium clone ew57	664/678 (97%)	0	AY154521	51
63	69-724	Uncultured environmental sample	Uncultured soil bacterium clone 460	633/661 (95%)	0	AY493946	Unpublished
68	1425-835	uncultured environmental samples	Uncultured bacterium clone ARKMP-14	572/583 (98%)	0	AF468332	Unpublished
70	1455-817	? -Proteobacteria	Uncultured beta proteobacterium clone B-BH93	633/633 (100%)	0	AY622261	Unpublished

4.5.5. MVT 12

The sequence from clone 56 was 98% homologous to the bacterium *Janthinobacterium agaricidamnosum* (Table 4.5.). Phylogenetic analysis showed a close association between sequence 56 and *Janthinobacterium agaricidamnosum* (Fig. 4.11.). The presence of *Janthinobacterium* spp. in Antarctica has also been previously reported.[1] Little is known about this microorganism, except that it causes a soft rot disease of the cultivated mushroom, *Agaricus bisporus.*[57] The putative genera of three sequences fom the MVT 12 sample, that were assigned with some assurance included sequence 3 (*Sphingomonas*),[39] sequence 6 (*Stenotrophomonas*),[52] and sequence 56 (*Janthinobacterium)* (Table 4.5.). All these genera are chemoheterotrophs.

All samples have indicated that heterotrophs are more prevalent in Dry Valley mineral soils as compared to autotrophs. The presence of chemoautotrophs in Dry Valley mineral soils could not be confirmed. However, there was evidence for their possible presence as indicated by the identification of phylotypes showing homology with known chemoautotrophic genera (section 4.5.2.). Photoautotrophs in the form of cyanobacteria were evident in MVT samples 1 and 12. Cyanobacteria accounted for 3% of the phylum diversity in MVT 12 (Fig. 4.12.). This sample was obtained from the top of the Miers Valley Transect where water availability, resulting from occasional snowfall, fog effects and cloud cover, is considered to be higher than mid-slope regions. Although it is likely that the identification of cyanobacterial phylotypic signals in sample MVT 1 (lowest transect sample) was due to the aerial transport of dry cyanobacterial mats from the adjacent margins of Lake Miers, this data indicates the possibility of photoautotrophic activity in the higher altitude mineral soils.

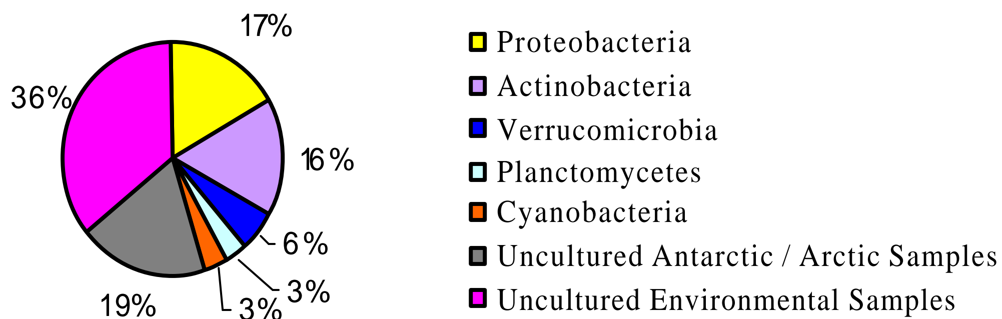

Figure 4.12. The percentage of different phyla in MVT 12.

Table 4.5. Summary of MVT 12 Blast results

Clone No.	Size	Phylogenetic Group	Organism	%Identity/ %Similarity	E Value	Accession Number	Ref.
3	50 - 757	α proteobacteria	*Sphingomonas sp*. SIA181-1A1	696/708 (98%)	0	AF395032	39
5	38 - 699	Uncultured environmental sample	Uncultured Antarctic bacterium LB3-30	634/665 (95%)	0	AF173822	Unpublished
6	67 - 754	γ proteobacteria	*Stenotrophomonas maltophilia* strain e-a21	687/688 (99%)	0	AJ293470	52
8	70 - 652	Uncultured environmental sample	Uncultured bacteriumclone C-F-1	563/585 (96%)	0	AF443581	44
13	569 - 66	Uncultured environmental soil bacterium	Uncultured soil bacterium clone S092	451/504 (89%)	e^{-165}	AF507523	56
15	64 - 728	Actinobacteria	*Rubrobacter radiotolerans*	619/665 (93%)	0	U65647	37
16	87 - 606	Acidobacteria	Uncultured Acidobacteria bacterium clone 351B	514/520 (98%)	0	AY571792	Unpublished
19	211 - 725	Uncultured Actinobacteria	Uncultured bacterium ARFS-13	498/515 (96%)	0	AJ277692	60
20	22 - 679	Uncultured environmental sample	Uncultured bacterium clone Tc2	638/662 (96%)	0	AF445086	33
21	231 - 701	Actinobacteria	*Sphaerobacter thermophilus* strain DSM 20745T	419/471 (88%)	e^{-146}	AJ420142	Unpublished
27	87-606	Uncultured environmental sample	Uncultured Acidobacteria bacterium clone 351B	514/520 (98%)	0	AY250867	7
29	55 - 741	Cyanobacteria	Uncultured Antarctic cyanobacterium clone BGC-Fr054	665/688 (96%)	0	AY151722	8
33	62-722	Uncultured environmental sample	Uncultured bacterium clone D138	605/661 (91%)	0	AY274144	41
42	490-77	Uncultured environmental sample	Uncultured bacterium clone D11	402/414 (97%)	0	AY268337	50
43	69 - 730	uncultured soil bacterium	Uncultured soil bacterium clone G7-1465-5	610/664 (91%)	0	AF525836	61
50	56 - 424	Verrucomicrobia	Uncultured Verrucomicrobia bacterium clone SMW4.44WL	339/369 (91%)	e^{-136}	AY043931	48
52	34 - 711	Uncultured environmental sample	Uncultured earthworm intestine bacterium clone ew57	664/678 (97%)	0	AY154521	51
53	204 - 687	Uncultured environmental sample	Uncultured Crater Lake bacterium CL500-48	450/486 (92%)	0	AF316757	35
55	55-692	Actinobacteria	*Modestobacter multiseptatus*	591/638 (92%)	0	Y18646	62
56	40 - 668	ß proteobacteria	*Janthinobacterium agaricidamnosum* strain SAFR-022	619/631 (98%)	0	AY167838	Unpublished
58	61 - 539	Uncultured environmental sample	Uncultured bacterium clone KD6-15	461/479 (96%)	0	AY218754	Unpublished
60	68 - 727	Uncultured environmental sample	Uncultured soil bacterium clone 460	633/661 (95%)	0	AY493946	Unpublished
62	183-694	Planctomycetes	*Nostocoida limicola* III strain Ben223	481/512 (93%)	0	AF244750	Unpublished
65	51-687	Uncultured environmental sample	Uncultured bacterium clone ARKIA-43	600/647 (92%)	0	AF468297	27

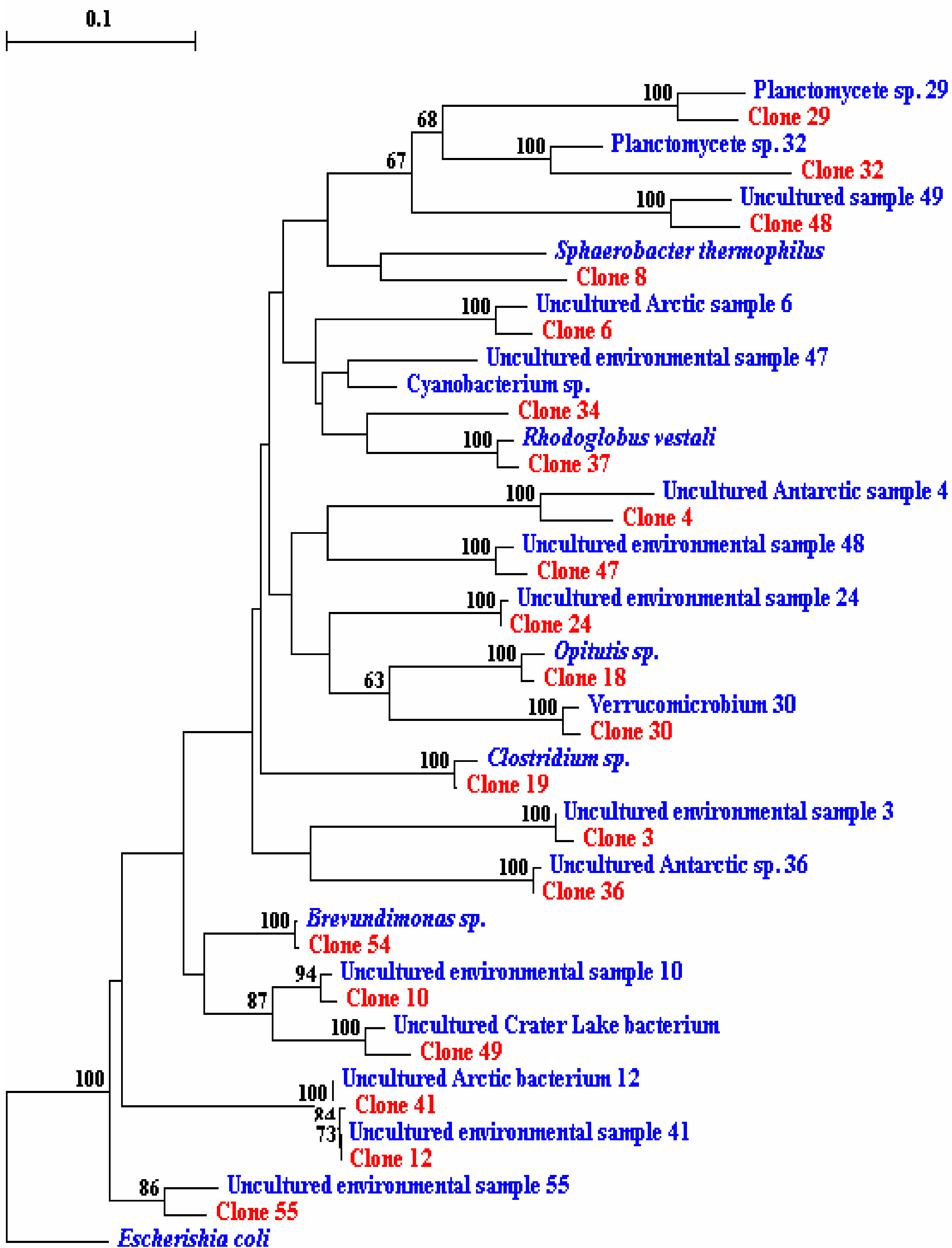

Figure 4.4. Phylogenetic tree of MVT 1 containing partial 16S clone sequences (red) and their reference sequences obtained from Genbank (blue), rooted with *E. coli.*

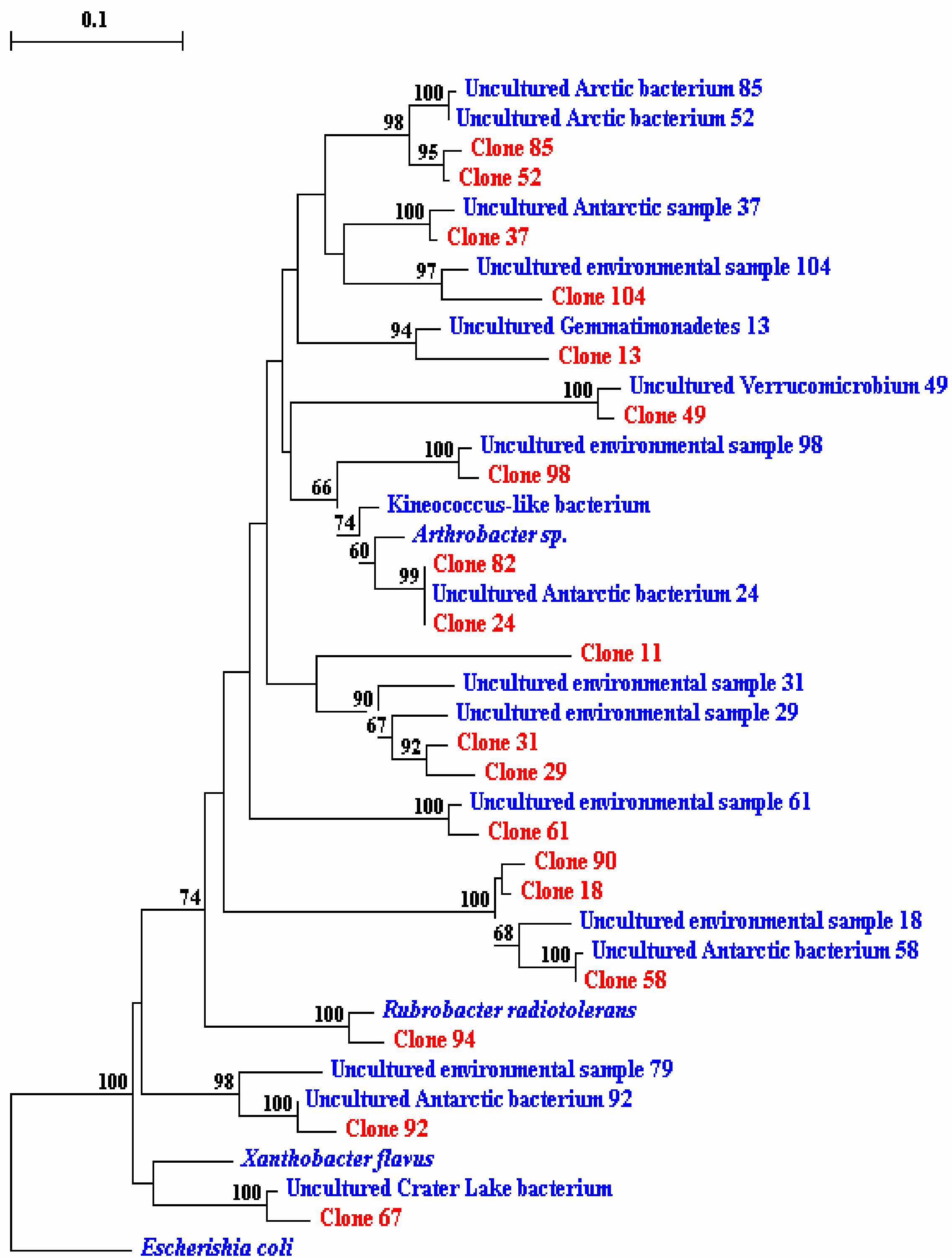

Figure 4.8. Phylogenetic tree of MVT 7 containing partial 16S clone sequences (red) and their reference sequences obtained from Genbank (blue), rooted with *E. coli.*

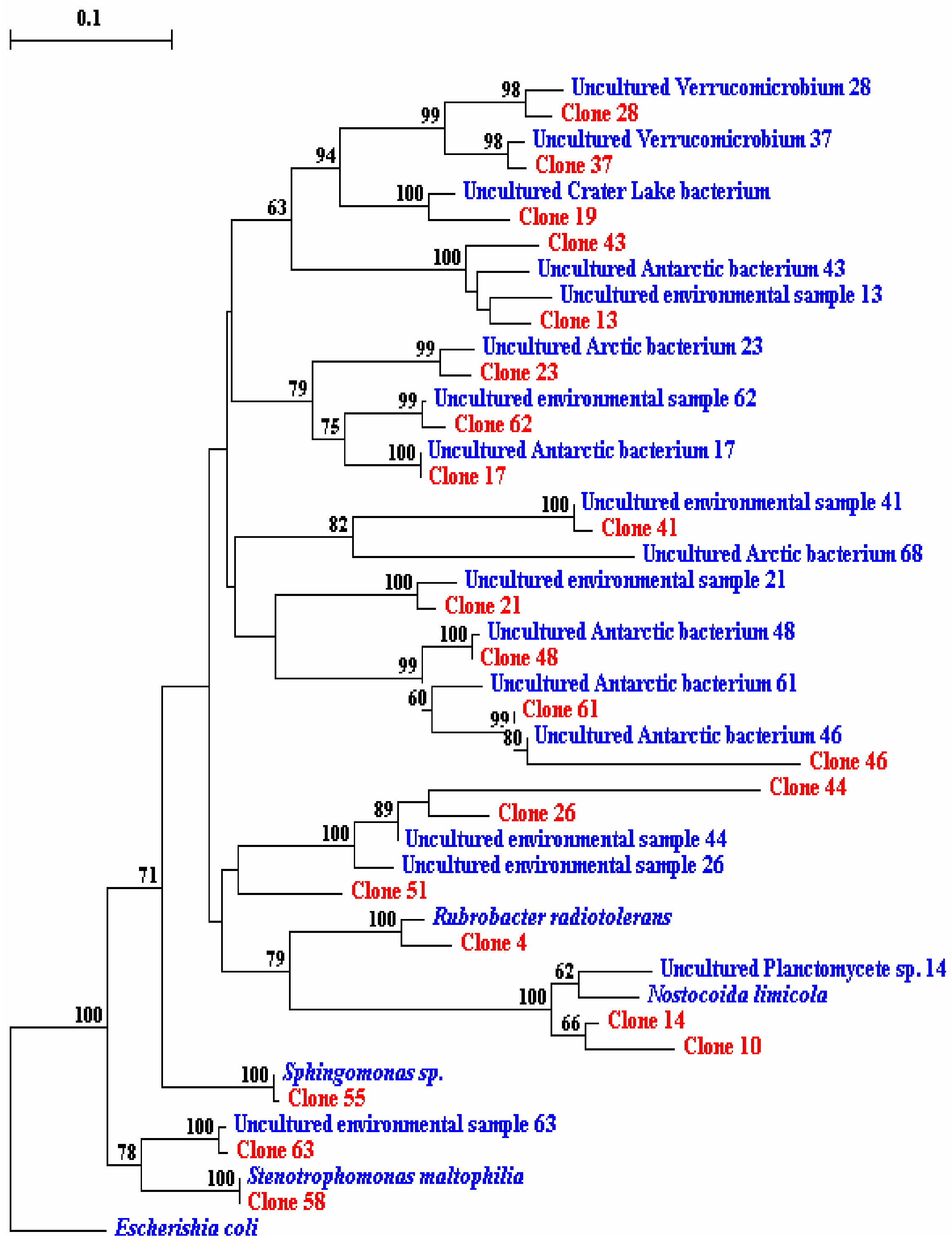

Figure 4.9. Phylogenetic tree of MVT 9 containing partial 16S clone sequences (red) and their reference sequences obtained from Genbank (blue), rooted with *E. coli.*

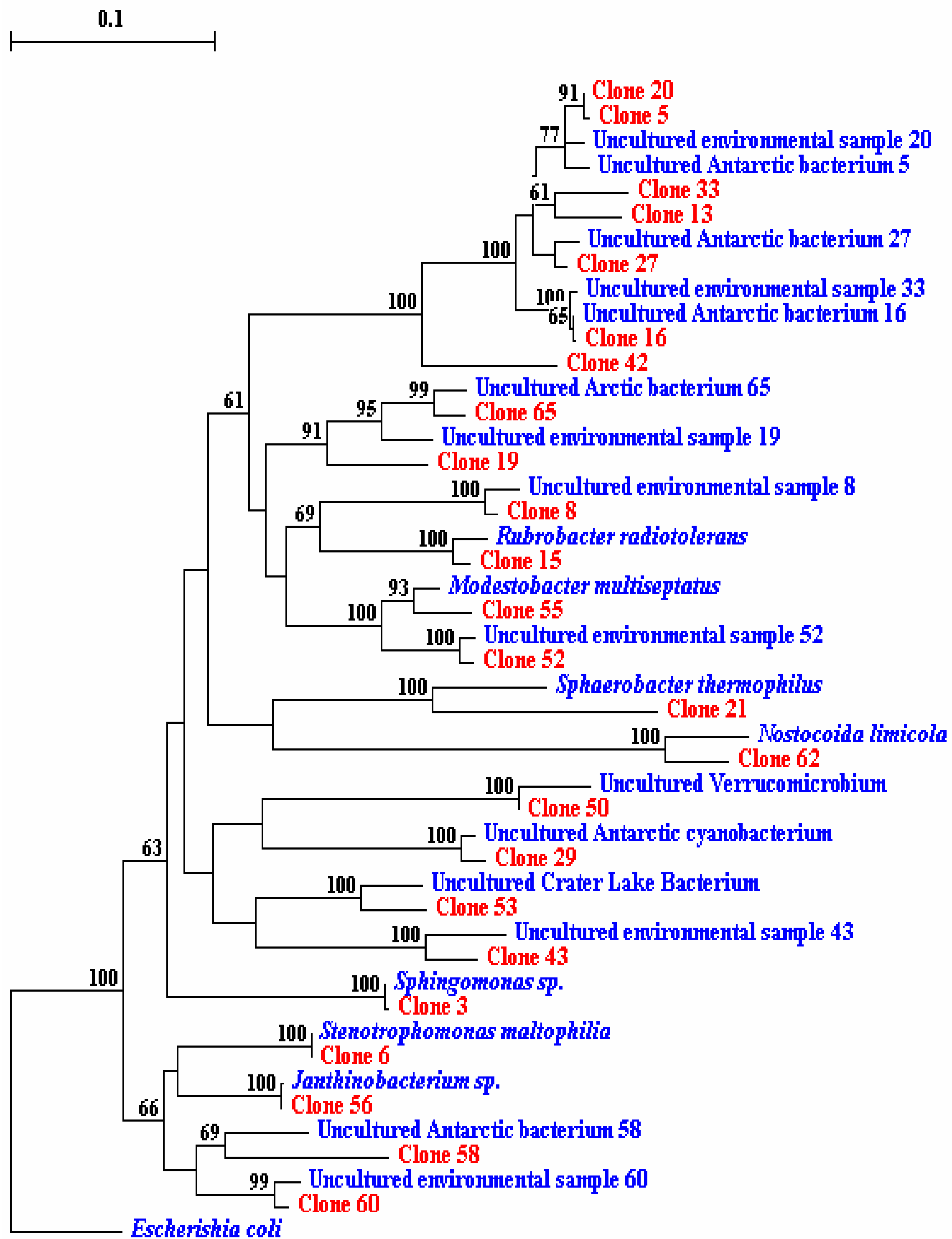

Figure 4.11. Phylogenetic tree of MVT 12 containing partial 16S clone sequences (red) and their reference sequences obtained from Genbank (blue), rooted with *E. coli.*

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Chapter 5

Conclusion

The use of molecular techniques for the analyses of microbial diversity in the Miers Dry Valley proved to be successful as genera which were not previously detected by culture based studies, were evident in the present investigation. For example, previous culture-dependent studies showed the presence of predominantly gram negative aerobic rods such as *Bacillus*, *Micrococcus* and *Streptomyces*, in Antarctic Dry Valley mineral soils.[1] However, the present investigation encompassed a wider range of phylotypes including gram positive anaerobic genera such as *Clostridium.* The major taxonomic groups identified from phylotypic analyses included a-, ß- and ?-Proteobacteria, Actinobacteria, Verrucomicrobia, Planctomycetes and several uncultured environmental and Antarctic samples. These results correlated with a previous investigation of Antarctic Dry Valley mineral soils utilising molecular techniques.[2]

Proteobacteria and Actinobacteria, were shown to be the two dominant phylogenetic groups. a-Proteobacteria are predominantly oligotrophic bacteria[3] and this provides a logical explanation for their dominance in the nutrient-poor Antarctic Dry Valley mineral soils. Actinobacteria are considered saprophytic heterotrophs and also constitute some of the most common soil microbiota.[4] They are dominant in dry soil environments hence, the abundance in the Antarctic Dry Valley mineral soils. Genera which can be assigned with some confidence included, *Opitutis*, *Clostridium*, *Rhodoglobus*, *Brevundimonas*, *Lysobacter*, *Nocardia*, *Kribbella*, *Sphingomonas*, *Rubrobacter*, *Stenotrophomonas*, and *Janthinobacterium*.

DGGE has shown that most of the observed phylotypes were common to all samples. Analysis of microbial diversity across the 500m vertical transect in the Miers Dry Valley suggested that few phylotypes appeared to be altitude-dependent. It should be noted however, that the altitudinal change is relatively small, and unlikely to be directly responsible for major changes in environmental parameters. Indirect effects, such as differences in Aeolian dispersal

patterns and varying water availability are more likely to be implicated in observed variations in microbial diversity.

Molecular evidence did not support the presence of an established trophic community structure in most samples across the Miers Valley Transect. With the exception of the cyanobacteria, virtually all of the phylotypes which could be assigned to putative genera with any confidence were heterotrophs. The possibility of autotrophs inhabiting the Miers Dry Valley cannot be eliminated due to a large portion of the samples being unassignable (i.e., low BLAST homology values) or falling within the 'uncultured' phylotypic group. An established trophic community structure would require a balance between the presence of autotrophs and heterotrophs. In a trophic community structure the sustainance of heterotrophs would depend on organic matter derived from autotrophic activity. However, ±80% of the phylotypes in the present investigation was shown to be putative heterotrophs. The low abundance of autotrophs may be insufficient to support the activity of heterotrophs and an established trophic community structure. It is for this reason that the present investigation supports the derivation of organic matter through aerial dispersion, (as discussed by previous studies)[5] instead of through the activity of autotrophs.

Phylogenetic data have correlated with the 16S rDNA studies as most of the clones clustered with their respective matches obtained from the database and also displayed bootstrap values of 100 (the higher the bootstrap value the more reliable the phylogenetic analysis, with 100 being the maximum). Phylogenetic lineages were difficult to establish due to the large number of uncultured samples. The high nucleotide similarity between the sequences indicated the high probability of phylotypes in the present study being considered closely related to those in the database. Some Antarctic isolates clustered together whilst others exhibited high similarity to environmental samples. A relatively small proportion (~10%) of Antarctic phylotypes appeared to be novel. This suggests that most Antarctic microorganisms are common to other soil environments, but may have adapted to the extreme psychrophilic habitat.

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APPENDIX

Sequences from MVT 1 16S rDNA clone library

Sequence from clone 3

```
1       TATTTGGTGG CGACCGKCAA ACGGGTGCGG AACACGTACA GAACCTTCCT
51      TTAAGTGGGG GATAGCCCAG AGAAATTTGG ATTAATACCC CGTAACATTA
101     TGAAGTGGCA TCACCTTATA ATTATAGATT TATCGCTTAG AGATGGCTGT
151     GCGGCTGATT AGGTAGTTGG TGTGGGTAAC GGCCCACCAA GCCTTCGATC
201     AGTAACTGGT GTGAGAGCAC GACCAGTCAC ACGGGCACTG AGACACGGGC
251     CCGACTCCTA CGGGAGGCAG CAGTAAGGAA TATTGGTCAA TGGACGCAAG
301     TCTGAACCAG CCATGCCGCG TGAAGGATGA AGGTCCTCTG GATTGTAAAC
351     TTCTTTTWYG MSAAACCCC
```

Sequence from clone 4

```
1       GACTGTTACG GAGCGGCKMA CGGGTGAGTA ACACGTGAAT AACCTGCCCT
51      CACATTCTGG ATAATTCACC GAAAGGTGTT GTAATACAGG CGAGGATTCT
101     TAAGAGGCAT TTCTTGAGAA GGGAAGGCGC AAGCCGTGCG AGGAGGGGTT
151     CGCGGATTAT CAGGTAGTTG GTGAGGTAAC GGCTCACCAA GCCGACGACG
201     ATTAGCTGGT CTGAGAGGAT GGTCAGCCAC ATTGGGACTG AGACACTGCC
251     CAGACTCCTA CGGGAGGCTG CAGTCGAGAA TCTTGCACAA TGTACGAAAG
301     TATGATGCAG CGACGCCGCG TGAAGGATGA AGGCCCTCTG GGTCGTAAAC
351     TTCTTTTATG TGGGAAGAAT AAATGACGGT ACCGCATGAA TAAGCCACGG
401     CTAACTACGT GCCAGCAGCC GCGGTAATAC GTAGGTGGCA AGCGTTGTCC
451     GGATTTACTG GGCGTAAAGA GTATGTAGGC GGATGTTTAA GTAGGAAGTG
501     AAAGGTTGGA GCTCAACTCC GACACTGCTC CCTATACTGG GCATCTTGAG
551     GGCCGGAGAG GAAAGCGGAA CGACACGTGT AGCGGTGAAA TGCGTTGATA
601     TGTGTCG
```

Sequence from clone 6

```
1       AGCTCCTGAA GATCTAGTKC CGAACGGGTG CRWAACACGT GAGAAACCTG
51      TCCCGAACTT GGGAATAACA GCCGAAAACS ACTGCTAATA CCGAATATCT
101     TCGTAACGTC GCATGGCGAT TCGAAGAAAG CTTTATGCGG TTTGGGAGGG
151     TCTCGCGGCC TATCAGCTTG TTGGTGAGGT AATGGCTCAC CAAGGCATCG
201     ACGGGTAGCT GGTCTGAGAG GATGATCAGC CACACTGGGA CTGAGACACG
251     GCCCAGACTC CTACGGGAGG CAGCAGTGGG GAATATTGCA CAATGGGCGA
301     AAGCCTGATG CAGCGATGCC GCGTGCGGGA AGAAGGCCCT AGGGTTGTAA
351     ACCGCTTTCA GTAGGGAAGA AAATGACGGT ACCTACAGAA GAAGGTGCGG
401     CCAACTACGT GCCAGCAGCC GCGGTGACAC GTAGGCACCA AGCGTTGTCC
451     GGATTTATTG GGCGTAAAGA GCTCGTAGGC GGTTTGGTAA GTCGGGTGTG
501     AAAACTCTGG GCTCAACCCA GAGAGGCCAC TCGATACTGC CATGACTTGA
551     GTACGGTAGG GGAGTGGGGA ATTTCTAGTG TAGCGGTGAA ATGCGCAGAT
601     ATTAGAAGGA ACACCAGTGG CGAAGGCGCC ACTCTGGGCC GTAACTGACG
651     CT
```

Sequence from clone 7

```
1       TTGCTCTGTG GGTGGCGWST GGCGGACGGG CGAGGAATAC GTCGGAATCT
51      GCCCTGTTGT GGGGGATAAC GTAGGGAAAC TTACGCTAAT ACCGCATAAG
101     ACGGTGACGT GAAAGCGGGG GATCCGTAAG GACCTCGCGC GATGGGATGA
151     GCCGACGTCG GATTAGCTTG TTGGTGGGGT AAAGGCCTAC CAAGGCGACG
201     ATCCGTAGCT GGTCTGAGAG GATGATCAGC CACACTGGGA CTGAGACACG
251     GCCCAGACTC CCACGGGAGG CAGCAGTGGG GAATATTGGA CAATGGGCGC
301     AAGCCTGATC CAGCAATGCC GCGTGTGTGA AGAAGGCCTT CGGGTTGTAA
351     AGCACTTTTA TCAGGAACGA AAAGGTGTCG GCGAATACCC GGCACTGCTG
401     ACGGTACCTG AGGAATAAGC ACCGGCTAAC TTCGTGCCAG CAGCCGCGGT
451     AATACGAAGG GTGCAAGCGT TAATCGGAAT TACTGGGCGT AAAGGGTGTG
501     TAGGTGGCCT GTTAAGTCTG TCGTGAAAGC CCTGGGCTCA ACCTGGGAAT
551     GGCGGTGGAT ACTGGCGGGC TCGAGTACGG TA
```

Sequence from clone 8

```
1       GAGGAACACG TAGCTAACCT GCCCAACAGA GGGGGATAAC CTCGGGAAAC
51      CGAGGCTAAT ACCGCATACG CTCATTTTTG GGGACGAGGA TGAGGAAACG
101     GAGCAATCCG CTGATGGAGG GGGCTGCGGC CGATTAGCTA GTTGGTGGGG
151     TAAAAGCCTA CCGAGGCGGT GATCGGTAGC TGGTCTGAGA GGACGATCAG
201     CCACACGGGG ACTGAGACAC GGCCCCGACT CCTACGGGAG GCAGCAGCAA
251     GGAATTTTCC ACAATGGGCG CAAGCCTGAT GGAGCAACGC CGCGTGGGGG
301     ATGACGCTTT TCGGAGTGTA AACCCCTTTT CGAGAGGACG AAGCTAATGA
351     CGGTACTCTC GGAATAAGGA CCGGCTAACT ACGTGCCAGC AGCCGCGGTA
401     AGACGTAGGG TCCGAGCGTT GTCCGGAGTT ACTGGGCGTA AAGCGCGCGC
451     AGGCGGTTAG ACACGTCGGG TGTGAAAGCC CCCCGCTCAA CGGGGGAGGG
501     TCATTCGAAA CGGTCAGACT GGAGGCAGGG AGAGGTCGGT GGAATTCCCG
551     GTGTAGTGGT GAAATGCGTA GATAT
```

Sequence from clone 10

```
1       CAATACATCA GCGGCAGACG GGAGAGTAAC ACGTGGGAAC GCGCCCTTCG
51      GTTCGGAATA ACTCAGGGAA ACTTGAGCTA ATACCGGATA CGCCCTTACG
101     GGGAAAGATT TATTGCCGAA GGAACGGCCC GCGTCGGATT AGCTAGTTGG
151     TGAGGTAATG GCTCACCAAG GCAACGATCC GTAGCTGGTC TAAGAGGATG
201     ATCAGCCTCA CTGGGACTGA GACACGGCCC AGACTCCTAC GGGAGGCAGC
251     AGTGGGGAAT ATTGGACAAT GGGCGAAAGC CTGATCCAGC CATGCCGCGT
301     GGATGATGAA GGCCTTAGGG TTGTAAAGTC CTTTTAACGG GGAAGATAAT
351     GACGGTACCC GTAGAATAAG CCCCGGCTAA CTTCGTGCCA GCAGCCGCGG
401     TAATACGAAG GGGGCTAGCG TTGCTCGGAA TTACTGGGCG TAAAGCGCAC
451     GTAGGCGGAT TGTTAAGTCG GGGGTGAAAT CCTGGAGCTC AACTCCAGAA
501     CTGCCTTCGA AACTGGCGAT CTTGAGTCCG GGAGAGGTGA GTGGAACTGC
551     GAGTGTAGAG GTGAAATTCG TAGATATTCG CAAGAACACC AGTGGCGAAG
601     GCGGCTCACT GGCCCGGTAC TGACGCTGAG G
```

Sequence from clone 12

```
1       CTGGTGGCGA GTGGCKCACG GGTGAGTAAT ATATCGGAAC GTGCCCAGTC
51      GTGGGGGATA ACGTAGAGAA ATTTACGCTA ATACCGCATA CGATCTAAGG
101     ATGAAAGCGG GGGACTCGCA AGGGCCTCGC GCGATTGGAG CGGCTGATAT
151     CAGATTAGGT TGTTGGTGAG GTAAAAGCTC ACCAAGCCGA CGATCTGTAG
201     CTGGTTTGAG AGAACGACCA GCCACACTGG GACTGAGACA CGGCCCAGAC
251     TCCTACGGGA GGCAGCAGTG GGGAATTTTG GACAATGGGC GAAAGCCTGA
301     TCCAGCAATG CCGCGTGCAG GAAGAAGGCC TTCGGGTTGT AAACTGCTTT
351     TGTACGGAAC GAAAAGGTCT GCCCTAATAC GGCGGGCCCA TGACGGTACC
401     GTAAGAATAA GCACCGGCTA ACTACGTGCC AGCAGCCGCG GTAATACGTA
451     GGGTGCGAGC GTTAATCGGA ATTACTGGGC GTAAAGCGTG CGCAGGCGGT
501     GATGTAAGAC AGTTGTGAAA TCCCCGGGCT CAACCTGGGA ATTGCATCTG
551     TGACTGCATC GCTAGAGTAC GGTAGAGGGG GATGGAATTC CGCGTGTAGC
601     AGTGAAATGC GTAGATATGC GGAGGAACAC CGATGGCGAA GGCAATCCCC
651     TGGACCTGTA MTGACGCTCA T
```

Sequence from clone 18

```
1       GCGTAACACG TGAACAATCT ACCTTCAAAT GGGGAATAGC TCGCCGAAAG
51      GCGAATTAAT ACCGCATGTG GTTGCTTCTC GCATGAGAGG CATATCAAAG
101     TCAGGGACCG CAAGGCCTGA CGTTAGAAGA GGAGTTCGCG GCCTATCAGC
151     TAGTTGGCGA GGTAACGGCT CACCAAGGCT AAGACGGGTA GCTGGTCTGA
201     GAGGATGATC AGCCACACTG GAACTGAGAC ACGGTCCAGA CACCTACGGG
251     TGGCAGCAGT TTCGAATTAT TCACAATGGG CGAAAGCCTG ATGGTGCGAC
301     GCCGCGTGAG GGATGAAGGC CTTCGGGTTG TAAACCTCTG TCACCGGGGA
351     AGAAACGCTT CAAGTTAACA ACTTGAAACC TGACTTAACC CGGAGAGGAA
401     GCAGTGGCTA ACTCTGTGCC AGCAGCCGCG GTAATACAGA GACTGCAAGC
451     GTTATTCGGA TTCACTGGGC GTAAAGGGTG CGCAGGCGGC CGAGTGTGTG
501     AGGCGTGAAA GCCCGGAGCT TAACTCCGGA ATTGCACCTC AAACTACACG
551     GCTAGAGCAT TGGAGAGGGT AGCAGAATTC ACGGTGTAGC AGTGAAAT
```

Sequence from clone 19

```
1       GGGTAACCTG CCTCAAAGAG GGGAATAGCC TTCCGAAAGG AAGATYAATA
51      CCGCATAATA TGTTTTGGTC GCATGACCGA GATATCAAAG GAGTAATCCG
101     CTTTGAGATG GACCCGCGGC GCATTAGCTA GTTGGTGAGG TAACGGCTCA
151     CCAAGGCGAC GATGCGTAGC CGACCTGAGA GGGTGATCGG CCACATTGGA
201     ACTGAGACAC GGTCCAGACT CCTACGGGAG GCAGCAGTGG GGAATATTGC
251     GCAATGGGGG AAACCCCGAC GCAGCAACGC CGCGTGAATG ATGAAGGCCT
301     TCGGGTTGTA AAGTTCTGTC TTCTGGGACG ATAATGACGG TACCAGAGGA
351     GGAAGCCACG GCTAACTACG TGCCAGCAGC CGCGGTAATA CGTAGGTGGC
401     AAGCGTTGTC CGGATTTACT GGGCGTAAAG GATGCGTAGG CGGACATTTA
451     AGTCAGATGT GAAATACCCG AGCTTAACTT GGGTGCTGCA TTTGAAACTG
501     GGTGTCTAGA GTGCAGGAGA GGTAAGTGGA ATTCCTAGTG TAGCGGTGAA
551     ATGCGTAGAG ATTAGGAAGA ACACCAGTGG CGAAGGCGAC TTACTGGACT
601     GTAACTGACG CTGA
```

Sequence from clone 24

```
1       GGGCAAGTAG AGTGGCAWCC GGGTGAGTAA CACGTGGGTG ACCTGCCTTC
51      GAGCGGGGGA TAACGTCCCG AAAGGGACGC TAATACCGCA TAACATCCTG
101     CCTTTGAAGA GGTGGAGATC AAAGCTGGGG ATCGCAAGAC CCGGCACTTG
151     AAGAGGGGCC CGCGTCTGAT TAGCTAGTTG GTGGGGTAAT GGCCTACCAA
201     GGCAACGATC AGTATCCGGC CTGAGAGGGC GGACGGACAC ACTGGGACTG
251     AGACACGGCC CAGACTCCTA CGGGAGGCAG CAGTGGGGAA TTGTTCGCAA
301     TGGGCGCAAG CCTGACGACG CAACGCCGCG TGGAGGATGA AGATTTTCGG
351     ATCGTAAACT CCTGTCGAAT GGGACGAACA GACTGCGGGT TAACAGCCCA
401     TAGTCCTGAC GGTACCGTTA AAGGAAACCC CGGCTAACTC CGTGCCAGCA
451     GCCGCGGTAA TACGTAGGGT CCGAGCGTTG TCCGGAATTA TTGGGCGTAA
501     AGGGCTCGTA GGCGGTTTGT CGCGTCGGGA GTGAAAACTC AGGGCTCAAC
551     CCTGAGCGTG CTTCCGATAC GGGCAGACTA GAGGTATGCA
```

Sequence from clone 29

```
1       AGGCGCAAGG GTGAGTAWGG CGGAGGCAAC CAACCCCACA CTTGGGTATA
51      GCCGCGGGAA ACTGCGGGTA ATCCCCAGCG ACGTCGCGAG GAGACATCTC
101     CTTGCGACCA AAGGTGTGAT TCCGGTGTGG GACGGGCCTC CGTGGTATCA
151     GGTTGTTGGT GAGGTAATGG CTCACCAAGC CGATGACGCC TACCGGGCGT
201     GCGAGCGTGG CCCGGCACAC TGGGACTGAG ACACTGCCCA GACTCCTACG
251     GGAGGCTGCA GTCGAGAATC TTCGGCAATG GGCGCAAGCC TGACCGAGCG
301     ACGCCGCGTG GAGGACGAAG GCCTTCGGGT TGTAAACTCC TGTCGAGGGG
351     GAGGAAGGCG GCGCGAAGAG CGTCGCTTGA CCGATCCCTG GARGAAGCAC
401     GGGCTAAGTT CGTGCCAGCA GCCGCGGTAA GACGAACCGT GCGAACGTTA
451     TTCCGAATCA CTGGGCTTT
```

Sequence from clone 30

```
1       CAATATTCTT GGGTTGGMCC GGCGCAAGGG TGCGTAACAC GTGGGTAATT
51      TGCCATGAAG TCTGGAATAA CTTGCTGAAA GGCGAGCTAA TGCCGGATGT
101     GATTTTCGGG AAGCATTTCT TGAAACTCAA AGTTGGGGAC CGCAAGGCCT
151     GACGCTTCTT GATAAGCCCG CGGCCTATCA GCTAGTTGGT GAGGTAATGG
201     CTCACCAAGG CTAAGACGGG TAGCTGGTCT GAGAGGACGA CCAGCCACAC
251     TGGAACTGAG ACACGGTCCA GACACCTACG GGTGGCAGCA GTCGAGAATT
301     TTTCACAATG GGCGAAAGCC TGATGGAGCG ACGCCGCGTG GGGGATGAAT
351     GGCTTCGGCC CGTAAACCCC TGTCATTTGC GAACAAACCT TACCGGTTAA
401     CAACCGTTGA GCTGATTGTA GCGGAAGAGG AAGGGACGGC TAACTCTGTG
451     CCAGCAGCCG CGGTAATACA GAGGTCCCAA GCGTTGTTCG GATTCACTGG
501     GCGTAAAGGG TGCGTAGGTG GTGGGGTAAG TCGGATGTGA AATCTCCGGG
551     CTCAACCCGG AAATGGCATT GGAAACTACC TTGCTAGAGG ATTTGAGGGG
601     GGATTGGAAT ACTTGGTGTA GCAGTG
```

Sequence from clone 32

```
1       TGGCGAAAGG GTCAKYNATA CGATCGAACG TACCCTGAGG TGGAGGATAG
51      GCACGGGAAA CTGTGCGTAA TACTCCATGT GCACCAAGGT GGAAAGCCGC
101     AAGGCGCCTT TGGAGCGGCG ATCGTCCTAT CAGGTAGTTG GCGGGGTAAA
151     GGCCCACCAA GCCTTCGACG GGTAGCGGGT GTGAGAGCAC GACCCGCGAC
201     ATCGGGACTG AGACACTGCC CGGACTCCTA CGGGAGGCTG CAGCGACGAA
251     TCTTCCGCAA TGGGCGAAAG CCTGACGGAG CAATGCCGCG TGCAGGATGA
301     AGCGGCTACG CCGTGTAAAC TGCTGTCAGG GGGTAGAAAC ACTGATCACC
351     CCCAAAGGAA GAGCCGGCTA ACCCTGTGCC AGCAGCCGCG GTAATACAGG
401     GGGCTCGAGC GTTAATCGGA ATCATTGGGC TTAAAGGGTG CGTAGGCGGG
451     TTGCGAAGTG TCTTGTGAAA TCCCTCGGCT CAACCGGGGA ATCGCAAGGC
501     ATACTGGCAA CCTTGAGGCA TGTAGGGGCG GACGGAACTG TAGGTGGAGC
551     GGTGAAATGC GTAGATATCT ACAGGAACGC CGATGGTGAA GACGGTCCGC
601     TGGGCATGTC CTGACGCTGA GGCACGAAAG
```

Sequence from clone 34

```
1       GGGGGTACAC GAGCGGCCNA CGGGTGAGTA ACACGTGAGT AATCTGCCCT
51      TCACTCTAGG ATAAGCCTCA GAAATGGGGT CTAATACTGG ATATGACTCG
101     TCCCTGCATG GGGGTGGGTG GAGAGATTTA TCGGTGGGGG ATGTGCTCGC
151     GGCCTATCAG CTTGATGGTG GGGTAATGGC CTACCAAGGC GACGATCGGT
201     AGCTGGTCTG AGAGGACGAT CAGCCACACT GGGACTGAGA CACGGCCCAG
251     ACTCCTACGG GAGGCAGCAG TGGGGAATTT TCCGCAATGG GCGAAAGCCT
301     GACGGAGCAA TACCGCGTGA GGGAAGAAGG CTCTTGGGTT GTAAACCTCT
351     TTTCTTAGGG AAGAAAAAAA TGACGGTACC TAAGGAATAA GCATCGGCTA
401     ACTCCGTGCC AGCAGCCGCG GTAATACGGA GGATGCAAGC GTTATCCGGA
451     ATGATTGGGC GTAAAGCGTC CGCAGGTGGC AAGTCAAGTT TGCGGTTAAA
501     GGCTCTGGCT CAACCAGAGA CAGGCCGTGA AAACTGACTA GCTAGAGTAT
551     GGTAGGGG
```

Sequence from clone 36

```
1       GAAAGATATA AAGTGKCGCA CGGGTGAGTA ACACGTAGGT AATCTACCTT
51      TGAGTGGGGA ATAACGTTCG GAAACGAACG CTAATACCGC ATAATGCAGC
101     GGCACCGCAA GGTGACAGTT GTTAAAGGAG CAATCCGCTT AAAGAGGAGC
151     CTGCGGCAGA TTAGCTAGTT GGTAAGGTAA TGGCTTACCA AGGCTACGAT
201     CTGTAACCGA CCTGAGAGGG TGGTCGGTCA CACTGACACT GAATAACGGG
251     TCAGACTCCT ACGGGAGGCA GCAGTCGGGA ATTTTGGGCA ATGGGCGAAA
301     GCCTGACCCA GCAACGCCGC GTGAAGGATG AAGTATTTCG GTATGTAAAC
351     TTCGAAAGAA TAGGAAGAAT AAATGACGGT ACTATTTATA ARGTCCG
```

Sequence from clone 37

```
1       AACACGTGAG TAACCTGCCC TTGACTCTGG GATAAGCGTT GGAAACSACG
51      TCTAATACCG GATACGAGCT TCAGCCGCAT GGCTAGGAGT TGGAAAGAAT
101     TTTGGTCAAG GATGGACTCG CGGCCTATCA GGTAGTTGGT GAGGTAATGG
151     CTCACCAAGC CGACGACGGG TAGCCGGCCT GAGAGGGTGA CCGGCCACAC
201     TGGGACTGAK TCACGGCCCA GACTCCTACG GGAGGCAGCA GTGGGGAATA
251     TTGCACAATG GGCSAAAGCC TGATGCAKCA ACGCCGCGTG AGGGACGACG
301     GCCTTCGGGT TGTAAACCTC TTTTAGCAGG GAAGAAGCGA TGTGCTTGTC
351     ATGTCATGAC GGTACCTGCA GAAAAAGCAC CGGCTAACTA CGTGCCAGCA
401     GCCGCGGTAA TACGTAGGGT GCAAGCGTTG TCCGGAATTA TTGGGCGTAA
451     AGAGCTCGYA YGCGGTTTGC CGCGTCTGCT GTGAAAACGC GARGCTCAAC
501     CTCGCGCCTG CAGTGGGTAC GGGCAGACTA GAGTGCGGTA GGGGAG
```

Sequence from clone 41

```
1      ATATCGGAAC GTGCCCAGTC GTGGGGGATA ACGTAGAGAA WTTTCCGCTA
51     ATACCGCATA CGATCTAAGG ATGAAAGCGG GGGACTCGCA AGAGCCTCGC
101    GCGATTGGAG CGGCTGATAT CAGATTAGGT TGTTGGTGAG GTAAAAGCTC
151    ACCAAGCCGA CGATCTGTAG CTGGTTTGAG AGAACGACCA GCCACACTGG
201    GACTGAGACA CGGCCCAGAC TCCTACGGGA GGCAGCAGTG GGGAATTTTG
251    GACAATGGGC GAAAGCCTGA TCCAGCAATG CCGCGTGCAG GAAGAAGGCC
301    TTCGGGTTGT AAACTGCTTT TGTACGGAAC GAAAAGGTCT GCCCTAATAC
351    GGCGGGCCCA TGACGGTACC GTAAGAATAA GCACCGGCTA ACTACGTGCC
401    AGCAGCCGCG GTAATACGTA GGGTGCGAGC GTTAATCGGA ATTACTGGGC
451    GTAAAGCGTG CGCARGCSGY GATGTAAGAC AGTTGTGAAA TCCCCGGGCT
501    CAACCTGGGA ATTGCATCTG TGACTGCATC GCTAGAGTAC GGTAGA
```

Sequence from clone 47

```
1      CTTTAGGAGG GGGATAACAA CTGGAAACGG TTGCTAATAY CCCCTATGCT
51     TTCGAGWGAA ATGGATTTTC CGCCTAAAGA GAAGCTTGCG GCTGATTAGC
101    TTGKTGGTGA GGTAAGAGCT CACCAAGGSG ACGATCAGTA TCTGGTTTGA
151    GAGGACGATC MGACACACTG GAACTGA
```

Sequence from clone 48

```
1      AGGAACATGA CCTTCGGCGG GGGATAGCCG GCCCAACGGC YKGCCAATAC
51     CGCGTACGAM CACATGGGGA CATCCCTGAG TGGTGAAAGC AGCAATGCGC
101    CGATGGAGTG CCTCGCGGCC TATCAGCTAG TTGGTGAGGT AACGGCTCAC
151    CAAGGCAACG ACGGGTAGCT GGTCTGAGAG GATGGCCAGC CACATTGGGA
201    CTGAGACWYK GCCCAGACTC CTACGGGAGG CAGCAGTGGG GAATATTGCG
251    CAMTGGACGA AAGTCTGACG CATCKWYKCC GCGTGTGGGA TGACGGTCTT
301    CGGATTGTAA ACCACTGTCG GGAGGGACGA ATACGCCGYA AGGCGGGTGA
351    CGGTACCTCC AAAGGAAGCW CCGGCTAACT CCGTGCCAGC ARCCGCKGTA
401    ATACGTAGGG TGCAAGCGTY
```

Sequence from clone 49

```
1      GGCCCATGGC AGACGAGGTA GGAACACGTA GGTACGTACC CCAAAGTCAG
51     GGATAATCCG TCGAAAGACG GCACAATACT TGATGGTCTC TTCGGAGTAA
101    AGATTTATCG CTTTGGGAAC GGCCTGCGGG CTATCAGCTT GTTGGTAAGG
151    TAACGGCTTA CCAAGGCTAC GACGGCTAGG GGAGGTGAGA GCCTGACCCC
201    CACCGATGGA ACTGCGACAC GGTCCATACT CCTACGGGAG GCTGCAGTCG
251    AGAATCTTCC GCAATGGACG AAAGTCTGAC GGAGCGACGC CGCGTGGTGG
301    ATGAAGTCCC TCGGGACGTA AACACCTTTT ATGGAGGAGA AAGTTTATTG
351    ATGTTACTCC ATGAATAAGG GGCTCCCAAC TCTGTGCCAG CAGGAGCGGT
401    AATACAGAGG CCCCAAGCAT TATCCGGATT TACTGGGCGT AAAGGTTGCG
451    TAGGCGGTTA TATTAGTCAG GTGTTAAATC CCGAGGCTCA ACCTCGGAAT
501    CGCATTTGAA ACGGTATAAC TAGAATAAGT CAGAGGCAAG CAGAACTCAC
551    GGTGTAGGGG TGAAATCCGT TGATATCGTG G
```

Sequence from clone 54

```
1       GGGAACGTGC CTTTAGGTTC GGAATAGCTC CTGGAAACGG GTGGTAATGC
51      CGMATGTGCC CTTCGGGGGA AAGATTTATC GCCTTTAGAG CGGCCCGCGT
101     CTGATTAGCT TGTTGGTGGG GTAATGGCCC ACCAAGGCTA CGATCAGTAG
151     CTGGTCTGAG AGGATGACCA GCCACATTGG GACTGAGACA CGGCCCAAAC
201     TCCTACGGGA GGCAGCAGTG GGGAATCTTG CGCAATGGGC GAAAGCCTGA
251     CGCAKCCATG CCGCGTGTAT GATGAAGGTC TTAGGATTGT AAAATACTTT
301     CACCGGTGAA GATAATGACT GTAGCCGGAG AAGAAGCCCC GGCTAACTTC
351     GTGCCAGCAG CCGCGGTAAT ACGAAGGGGG CTAGCGTTGC TCGGAATTAC
401     TGGGCGTAAA GGGAGCGTAG GCGGACATTT AAGTCAGAGG TGAAATCCCG
451     GAGCTTAACT TCGGAACTGC CTTTGATACT GGGTGTCTTG AGTGTGAGAG
501     AGGTATGTGG AACTCCGAGT GTAGAGGTGA AATTCGTAGA TATTCGGAAG
551     AACAMCAGTG GCGAANGCGA CATACTGGCT CATTACTGAC GCTGAGGCTC
601     G
```

Sequence from clone 55

```
1       AACACGTGGG AACCTTCCTA GAGGTATGGA ACAACGCAGG GAAACTTGTG
51      CTAATACCGT ATACGCTCGA GAGAGGAAAG ATTTATCGCC TTTAGACGGG
101     CCCGCGTCGG ATTAGCTAGT TGGTGGGGTA ACGGCCTACC AAGGCGACGA
151     TCCGTAGCTG ATCTTAGAGG ATGATCAGCC ACACTGGGAC TGAGACAYGG
201     CCCAGACTCC TACGGGAGGC AKYWGTGGGG AATCTTGGAC AATGGGCGCA
251     AGCCTGATTC AGCCATGCCG CGTGAGTGAA GAAGGTCTTC GGATTGTAAA
301     GCTCTTTTAC CAGGGCACGA TAATGACGGT ACCTGGAGAA TAAGCCCCGG
351     CAAACTTCGT GCCAGCAGCC GCGGTAATAC GAAGGGGGCT AGCGTTGTTC
401     GGAATTACTG GGCGTAAAGC GCACGTAGGC GGGTTATTAA GTCAGGGGTG
451     AAATCCCGGA GCTCAACTCC GGAACTGCCT TTGATACTG
```

Sequence from clone 57

```
1       CCTTCGGTTC RGAATAGCCT CGGGAAACTG GGAGTAATAY YSSMMTACGG
51      TCTACGGACG AAAGATTTAT CGCCGAAGGA TTAGCCCGCG TTGGATTAGG
101     TAGTTGGTGG GGTAATGGYC TACCAAGCCG ACGATCCATA GCTGGTTTGA
151     GAGGATGACC AGCCACACTG GGACTGAKAY WYKGYCCAGA CTCCTACGGG
201     AGGCAGYWKT GGGGAATCTT AGACAATGGG GGAAACCCTG ATCTAGCCAT
251     GCCGCGTGAT CGATGAAGGC CTTAGGGTTG TAAAGCTCTT TCAGGGGGGA
301     AGGTAATGAC GGTACCCCCA GAAGAAGCCC CGGCTAACTC CGTGCCAGCA
351     GCCGCGGTAA TACGGAGGGG GCTAGCGTTA TTCGGAATTA CTGGGCGTAA
401     AAGCGCACGT ARGCGGGTCK GAAAGTCARA GGTGAAATCC CAGGGCTCA
```

Sequence from clone 60

```
1       GAAAACGTCG GAATCTGCCT ATTTGTGGGG GATAACGTAK GGNMACTTAC
51      GCTAATACCG CATACGACCT ACGGGTGAAA GCGGAGGACC TTCGGGCTTC
101     GCGCAGATAG ATGAGCCGAC GTCGGATTAG CTAGTTGGCG GGGTAAAGGC
151     CCACCAAGGC GACGATCCGT AGCTGGCCTG AKAGGATGAT CAGCCACACT
201     GGAACTGAGA CACGGTCCAG ACTCCTACGG GAGGCAGCAG TGGGGAATAT
251     TGGACAATGG GCGCAAGCCT GATCCAGCCA TGCCGCGTGT GTGAAGAAGG
301     CCTTCGGGTT GTAAAGCACT TTTGTCCGGA AAGAAAAGCA CTGGGTTAAT
351     ACCCTGGTGT CATGACGGTA CCGGAAGAAT AAGCACCGGC TAACTTCGTG
401     CCAGCAGCCG CGGTAATACG AAGGGTGCAA GCGTTACTCG GAATTACTGG
451     GCGTAAAGCG TGCGTAGGCG GTTTGTTAAG TCTGATGTGA AAGCCCTGGG
501     CTCAACCTGG GAATGGCATT GGATACTGGC
```

Sequence from clone 61

```
1       TAGTGGCGGA CGGGTGACTA ACGCGTGGGA ACATGCCCTT TGGTACGGGA
51      TAGCCTCGGG AAACTGGGTG TAATACCGTA TGTGCTCGAA AGAGGAAAGA
101     TTTATCGCCA AGGGATTGGC CCGCGTTGGA TTAGGTAGTT GGTGGGGTAA
151     TGGCCTACCA AGCCGACGAT CCATAGCTGG TTTGAGAGGA TGATCAGCCA
201     CACTGGGACT GAGACACGGC CCAGACTCCT ACGGGAGGCA GCAGTGGGGA
251     ATCTTAGACA ATGGGGGCAA CCCTGATCTA GCCATGCCGC GTGATCGATG
301     AAGGCCTTAG GGTTGTAAAG ATCTTTCAGT GGGGAAGATA ATGACGGTAC
351     CCACAGAAGA AGCCCCAGCT AACTCCGTGC CAGCAGCCGC GGTAATACGG
401     AGGGGGCTAG CGTTATTCNG AATTACTGGG C
```

Sequences from MVT 5 16S rDNA clone library

Sequence from clone 1

```
1       TCCTGGCTCA GATTGAACGC TGGCGGAMTG CTTTACACAT GCAAGTCGAA
51      CGGCAGCACG GGGGCAACCC TGGTGGCGAG TGGCGAACGG GTGAGTAATA
101     CATCGGAACG TGCCCAGTCG TGGGGGATAA CGTAGCGAAA GCTACGCTAA
151     TACCGCATAC GATCTATGGA TGAAAGCGGG GGACCGCAAG GCCTCGCGCG
201     ATTGGAGCGG CCGATGGCAG ATTAGGTAGT TGGTGGGGTA AAGGCTCACC
251     AAGCCTGCGA TCTGTAGCTG GTCTGAGAGG ACGACCAGCC ACACTGGGAC
301     TGAGACACGG CCCAGACTCC TACGGGAGGC AGCAGTGGGG AATTTTGGAC
351     AATGGGCGCA AGCCTGATCC AGCCATTCCG CGTGCAGGAT GAAGGCCTTC
401     GGGTTGTAAA CTGCTTTTGT ACGGAACGAA AAGGCCTTTT CTAATACAGA
451     GGGCTCATGA CGGTACCGTA AGAATAAGCA CCGGCTAACT ACGTGCCAGC
501     AGCCGCGGTA ATACGTAGGG TGCAAGCGTT AATCGGAATT ACTGGGCGTA
551     AAGCGTGCGC AGGCGGTGAT GTAAGACAGA TGTGAAATCC CCGGGCTCAA
601     CCTGGGAACT GCATTTGTGA CTGCATCGCT GGAGTGCGGC AGAGGGGGAT
651     GGAATTCCGC GTGTAGCAGT
```

Sequence from clone 2

```
1       TCCCAATCGC CAGTCCCACC TTCGACGGCT CCCTCCCAAA GGTTGGGCCA
51      CCGGCTTCGG GTGTTACCGA CTTTCGTGAC GTGACGGGCG GTGTGTACAA
101     GGCCCGGGAA CGTATTCACC GCAGCATTGC TGATCTGCGA TTACTAGCGA
151     CTCCAACTTC ACGGGGTCGA GTTGCAGACC CCGATCCGAA CTGAGACCGG
201     CTTTGTGAGA TTCGCTCCAC CTTGCGGATT CGCAGCCCTC TGTACCGGCC
251     ATTGTAGCAT GTGTGAAGCC CTGGACATAA GGGGCATGAT GACTTGACGT
301     CGTCCCCACC TTCCTCCGAG TTGACCCCGG CAGTCTCCCA TGGGTCCCCG
351     GCCCAGTGAC AATGTCACTG GCCGCTGGCA ACATGGAACG AGGGTTGCGC
401     TCGTTGCGGG ACTTAACCCA ACATCTCACG ACACGAGCTG ACGACAGCCA
451     TGCACCACCT GTACACCGAC CTTGCGGGGC ACCTGTCTCC AGATGTTTCC
501     GGTGTATGTC AAACCCAGGT AAGGTTCTTC GCGTTGCATC GAATTAATCC
551     ACATGCTCCG CCGCTTGTGC GGGCCCCCGT CAATTCCTTT GAGTTTTAGC
601     CTTGCGGCCG TACTCCCCAG GCGGGGCGCT TAATGCGTTA GCTGCGGCAC
```

Sequence from clone 5

```
1       TTAMGACTTC GTCCCAATCG CCAGCCCCAC CTTCGACGGC TCCCTCCACA
51      AGGGTTGGGC CACCGGCTTC GGGTGTTGCC GACTTTCGTG ACGTGACGGG
101     CGGTGTGTAC AAGGCCCGGG AACGTATTCA CCGCAGCGTT GCTGATCTGC
151     GATTACTAGC GACTCCGACT TCATGGGGTC GAGTTGCAGA CCCCAATCCG
201     AACTGAGACC GGCTTTTTGG GATTCGCTCC ACCTTGCGGT ATCGCAGCCC
251     TTTGTACCGG CCATTGTAGC ATGCGTGAAG CCCTGGGCAT AAGGGGCATG
301     ATGACTTGAC GTCATCCCCA CCTTCCTCCG AGTTGACCCC GGCAGTCTCT
351     TATGAGTCCC CACCATTACG TGCTGGCAAC ATAAGACGAG GGTTGCGCTC
401     GTTGCGGGAC TTAACCCAAC ATCTCACGAC ACGAGCTGAC GACAGCCATG
451     CACCACCTGT ATAGAGCCCG TAAGGACCTG CCATCTCTGA CAGTTTTCTC
501     CATATGTCAA ACCCAGGTAA GGTTCTTCGC GTTGCATCGA ATTAATCCGC
551     ATGCTCCGCC GCTTGTGCGG GCCCCCGTCA ATTCCTTTGA GTTTTAGCCT
601     TGCGGCCGTA CTCCCCAGGC GGGGCGCTTA ATGCGTTAGC TGCGGCACGG
651     AACTCGTGGA ATGAGTCCCA C
```

Sequence from clone 8

```
1       CTGGCTCAGG ACGAACGCTG ACGGTGTGCT TTAGGCATGC AAGTCGAACG
51      AGAAAGCCCT TCGGGGTGAG TAAAGTGGCG AACGGGTGAG TAACACGTGG
101     GCAACCTACC CCTCGCAGGG GAACAACCGG AGGAAACTCC GGCTAATACC
151     CCGTAAGCTT TCAGGGTCGC ATGGCCCTGT AAGGAAAGGT AGCTTCGGCC
201     ATCCGGCGAG GGATGGGCCC GCGGTGCATT AGCTAGTTGG TGGGGTAAAG
251     GCCCACCAAG GCGACGATGC GTAGCTGGTC TGAGAGGATG ATCAGCCACA
301     CTGGGACTGA GACACGGCCC AGACTCCTAC GGGAGGCAGC AGCCAGGAAT
351     CTTGGGCAAT GGGCGAAAGC CTGACCCAGC AACACCGTGT GGGCGATGAA
401     GGCCTTCGGG TCGTAAAGCC CTGTTGATAG GGACGAAGGG CGAAGGGTTA
451     ATAGCCCCTA GCCTGACGGT ACCTTTCGAG GAAGCCCCGG CTAACTACGT
501     GCCAGCAGCC GCGGTAATAC GTAGGGGGCG AGCGTTGTCC GGAATTATTG
551     GGCGTAAAGA GCGTGTAGGC GGTTCGGTAA GTCTGTCGTG AAATCCTGGG
601     GCTCAACCCT GGGCGTGCGA TGGATACTGC C
```

Sequence from clone 9

```
1       ACGACTTCAC CCCAGTCGCT GACCCTACCG TGGTCGCCTG CCCCCTTGCG
51      GTTGGCGCAA CGCCTTCGGG TAGAACCAAC TCCCATGGTG TGACGGGCGG
101     TGTGTACAAG GCCCGGGAAC GTATTCACCG TGGCATGCTG ATCCACGATT
151     ACTAGCGATT CCACCTTCAT GCACTCGAGT TGCAGAGTGC AATCCGAACT
201     GAGACGGCTT TTTGAGATTT GCTCAGGGTC GCCCCTTGGC ATCCCACTGT
251     CACCGCCATT GTAGCACGTG TGTAGCCCAG CCCGTAAGGG CCATGAGGAC
301     TTGACGTCAT CCCCACCTTC CTCCGGCTTA TCACCGGCAG TCTCCCTAGA
351     GTGCCCAACT GAATGATGGC AACTAAGGAC GAGGGTTGCG CTCGTTGCGG
401     GACTTAACCC AACATCTCAC GACACGAGCT GACGACAGCC ATGCAGCACC
451     TGTCTCCGCG TCCCCGAAGG GAACCTTGGG TCTCCCCAAG TAGCACGGGA
501     TGTCAAGAGC TGGTAAGGTT CTGCGCGTTG CTTCGAATTA AACCACATGC
551     TCCACCGCTT GTGCGGGCCC CCGTCAATTC CTTTGAGTTT TAATCTTGCG
601     ACCGTACTCC CCGGGCGGGA TGCTTAAAGC GTTAGCTGCG CCACTGAGAA
651     GCAAGCTTCC CAAACGGCTG
```

Sequence from clone 10

```
1       CCTGGCTCAG AATCAACGCT GGCGGCGTGC CTCAGACATG CAAGTCGAAC
51      GATTAAACTT TCCTTCGGGA AAGATATAAA GTGGCGCACG GGTGAGTAAC
101     ACGTAGGTAA TGTACCTTTG GGTGGGGAAT AACTTAGGGA AACTTAAGCT
151     AATACCGCAT AATGCAGCGG CTCCTTCGGG AGACAGTTGT TAAAGATTTA
201     TCGCCTAAAG AGCAGCCTGC GGCAGATTAG CTAGTTGGTA AGGTAACGGC
251     TTACCAAGGC TACGATCTGT ATCCGACCTG AGAGGGTGGT CGGACACACT
301     GACACTGAAT AACGGGTCAG ACTCCTACGG GAGGCAGCAG TCGGGAATTT
351     TGGGCAATGG GCGAAAGCCT GACCCAGCAA CGCCGCGTGA AGGATGAAGT
401     CTCTCGGGAT GTAAACTTCG AAAGAATAGG AAGAATAAAT GACGGTACTA
451     TTTATAAGGT CCGGCTAACT ACGTGCCAGC AGCCGCGGTA ATACGTAGGG
501     ACCAAGCGTT GTTCGGATTT ACTGGGCGTA AAGGGCGCGT AGGCGGCGTG
551     ACAAGTCAAT TGTGAAATCT CCGGGCTTAA CTCGGAACGG TCAATTGATA
601     CTGTTGTGCT AGAGTACAGA AGGGGCAATC GGAATTCTTG GTGTAGCGGT
651     GAAATGCGTA GATATCAAGA G
```

Sequence from clone 11

```
1       TACGACTTCA CCCCAGTCGC TGACCCTACC GTGGTTGGCT GCCTCCCGAT
51      TGCTCAGGTT AGCGCACCAC CTTCGGGTAG AACCAACTCC CATGGTGTGA
101     CGGGCGGTGT GTACAAGGCC CGGGAACGTA TTCACCGTGG CGTGCTGATC
151     CACGATTACT AGCGATTCCA GCTTCATGCC CTCGAGTTGC AGAGGACAAT
201     CCGAACTGAG ACGGCTTTTT GGGATTAGCT TCTCCTTGCG AAGTAGCAGC
251     CCACTGTCAC CGCCATTGTA GCACGTGTGT AGCCCAGCCC GTAAGGGCCA
301     TGAGGACTTG ACGTCATCCC CACCTTCCTC TCGGCTTATC ACCGGCAGTC
351     CCCCTAGAGT GCCCAACTGA ATGATGGCAA CTAAGGGCGA GGGTTGCGCT
401     CGTTGCGGGA CTTAACCCAA CATCTCACGA CACGAGCTGA CGACAGCCAT
451     GCAGCACCTG TGCGCAGGTC TCTTGCGAGA AGGAATCCAT CTCTGGAAGC
501     CGTCCTGCCA TGTCAAGGGC TGGTAAGGTT CTGCGCGTTG CTTCGAATTA
551     AACCACATGC TCCACCGCTT GTGCGGGCCC CCGTCAATTC CTTTGAGTTT
601     TAATCTTGCG ACCGTACTCC CCAGGCGGAA TGCTTAATGC GTTAGCTGCG
651     C
```

Sequence from clone 12

```
1       CGAACGCTTG CGGCGTGCCT AAGAAATGCA AGTCGAACGG ACATTCCAGC
51      AATGGGGTGC TAGTGGCGAA CGGTCGCGTA ACACGTAGGC AACCTGCCCT
101     GAAGTGGGGG ACAACAGCCC GAAAGGGCTG CTAATACCGC ATGTGAACAA
151     CGAATCACAT GGTTTGTTGT TCAAAGGCTA TGGCAACATG GTCGCTTTGG
201     GATGGGCTTG CGGCCTATCA GGTAGTTGGT GGGGTAATGG CCCACCAAGC
251     CGACGACGGG TAGCTGGTCT GAGAGGACGA TCAGCCGGAT TGGGACTGAG
301     ATACGGCCCA GACTCCTACG GGGGGCAGCA ATTAGGAATC TTGCGCAATG
351     GGCGAAAGCC TGACGCAGCG ACGCCGCGTG CGGGATGAAG GCCTTCGGGT
401     CGTAAACCGC TTTTAACGGG GAAGAAGAAT GTGACGGTAC CCGTTGAATA
451     AGCCCCGGCT AACTACGTGC CAGCAGCCGC GGTAATACGT AGGGGGCGAG
501     CGTTGTCCGA AGTTACTGGG CGTAAAGCGC GCGTAGGCGG TTGCCTAAGT
551     CTGGGGTGAA AGGTTCAGGG CTTAACCCGA ACAGTGCCTT GGATACTGGG
601     CGACTTGAGT GCCGAAGAGG AAAGCGGAAT TCCTGGTGTA GCGGTGAAAT
651     GCGTAGATAT CAGGAGGAAC ACCGATGGCG AAGGCARCTT
```

Sequence from clone 13

```
1       CGACTTCACC CCAATCATAA ATCATACCGT AGTAACTTGC CCCTCTTGCG
51      AGTTAGCCCA ACTACTTCTA GTACAACCTA CTTTCGTGAT GTGACGGGCG
101     GTGTGTACAA GACCCGGGAA CGTATTCACC GCGGCGTTCT GATCCGCGAT
151     TACTAGCGAT TCCAACTTCA TGAAGTCGAG TTGCAGACTT CAATCCGAAC
201     TGAGATTGGT TTTTGCGATT AGCTCACTCT TACGAGATTG CGACGTTTTG
251     TACCAACCAT TGTAGCACGT GTGTAGCCCT GAACATAAAG GCCATGATGA
301     CTTGACATCA TCCCCACCTT CCTCCGTTTT ATCAACGGCA GTCTTAACAG
351     AGTTCTCAAC ATTACTTGTT AGCAACTGTC AATAGGGGTT GCGCTCGTTG
401     CGGGACTTAA CCCAACATCT CGCGACACGA GCTGACGACA GCCATGCAGC
451     ACCTTGTTTT GGGTCCGGTT GCCCGGACGA TTGGAATTAC CCAATCTTCC
501     CTCACATTCT AGTCCAGGTA AGGTTCTTCG CGTTGCGTCG AATTAAACCA
551     CATGCTCCAC CGCTTGTGCG GGTCCCCGTC AATTCCTTTG AGTTTCACAC
601     TTGCGTGCGT ACTCCCCAGG CGGAATGCTT AAAACGTTAG CGACGG
```

Sequence from clone 14

```
1       CTAGTTACCT GTTCTACCCT AACCGGCTTC TTTTACGAGC ACCGGCTTCA
51      GGTCTACCAA ACTTCCATGG CTTGACGGGC GGTGTGTACA AGGCCCGGGA
101     ACGTATTCAC CGCGTCATTG CTGATACGCG ATTACTAGTG ATTCCAGCTT
151     CACGGAGTCG AGTTGCAGAC TCCGATCCGA ACTGAGAACG GCTTTTCGGG
201     ATTGGCGCAC CATCGCTGGT TGGCAACCCG CTGTACCGTC CATTGTAGCA
251     CGTGTGTAGC CCTAGGCGTA AGGGCCATGA TGACCTGACG TCGTCCCCGC
301     CTTCCTCACT GCTTGCGCAG GCAGTCTGTC TAGAGTCCCC GCCATTACGC
351     GCTGGCAACT AAACATAGGG GTTGCGCTCG TTGCGGGACT TAACCCAACA
401     CCTCACGGCA CGAGCTGACG ACGGCCATGC AGCACCTTGC TTTGTGTCCC
451     GAAGGAAAGG TTCATCTCTG AACCGGTCAC GCGCATTCTA GCCTAGGTAA
501     GGTTCCTCGC GTATCATCGA ATTAAACCAC ATGCTCCACC ACTTGTGCGG
551     GCCCCCGTCA ATTCTTTTGA GTTTCACTCT TGCGAGCGTA C
```

Sequence from clone 18

```
1       CGCCAGTTAC CAGCTCNACC TTCGGCGCCT GCCTCCTTGC GGTTAGCACG
51      GCGACTTCGG GTAGAACCGA TTTCCGTCAC TTGACGGGCG GTGTGTGCAA
101     GGCCCGGGAA CGTATTCACC GCAGTATTGC TGACCTGCGG TTACTAGCGA
151     TTCCAACTTC ATGGAGGCGA GTTGCAGCCT CCAATCCGAA CTGAGACCGG
201     CTTTTTGAGA TTAGCATGCC CTCGCGGGTT AGCAACTCTT TGTACCGGCC
251     ATTGTAGCAT ATGTGCAGCC CAAGATGTAA GGGGCATGAT GACTTGACGT
301     CATCCCCACC TTCCTCCTCT TTACAGAGGC AGTTTGTTCC GAGTTCCCGG
351     CATTACCCGC TGGCAACAGA ACATGAGGGT TGCGCTCGTT GCGGGACTTA
401     ACCCAACATT TCACAACACG AGCTGACGAC AGCCATGCAC CACCTGTGGA
451     TCACCCTCGA AGGCGACGAT ATTTCTACCG CTTGCAGATC CATGTCAAAC
501     CTTGGTAAGG TTCTTCGCGT TGCATCGAAT TAAGCCATAT GCTCCACCGC
551     TTGTGCGGGC CCCCGCCAAT TCCTTTGAGT TTCAACCTTG CGGCCGTAGT
601     TCCCAGGCGG TTCACTTAAT GCGTTAGCTG CGACACCGGG GCGAAGCCCC
651     GACATCTAGT GAACATCGTT TATAGCTATG ACTACCAGGG TATCTAATCC
701     TGTTCGCTAC ATAG
```

Sequence from clone 21

```
1       CCCCAGTTAC CTGTTCTACC CTAACTGGCT TCTGTGACGA GCGCCAGCTT
51      CAGGYCTACC AGACTTCCAT GGCTTGACGG GCGGTGTGTA CAAGGCCCGG
101     GAACGTATTC ACCGCGTCAT TGCTGATACG CGATTACTAG CGATTCCAAC
151     TTCATGCAGT CGAGTTGTAG ACTGCAATCC GGACTACGAT ACACTTTCTG
201     GGATTAGCTC CCCCTCGCGG GTTGGCGGCC CTCTGTATGT ACCATTGTAT
251     GACGTGTGAA GCCCTACCCA TAAGGGCCAT GAGGACTTGA CGTCATCCCC
301     ACCTTCCTCC GGTTTGTCAC CGGCAGTCTC ATTAGAGTGC TCAACTGAAT
351     GTAGCAACTA ATGACAAGGG TTGCGCTCGT TGCGGGACTT AACCCAACAT
401     CTCACGACAC GAGCTGACGA CAGCCATGCA GCACCTGTGT ACCGGCTCTC
451     TTTCGAGCAC GCCCCAATCT CTCGGGGCTT CCGACCATGT CAAGGGTAGG
501     TAAGGTTTTT CGCGTTGCAT CGAATTAATC CACATCATCC ACCGCTTGTG
551     CGGGTCCCCG TCAATTCCTT TGAGTTTTAA TCTTGCGACC GTACTCCCCA
601     GGCGGTCAAC TTCACGCGTT AGCTGCGTTA CCAAGTC
```

Sequence from clone 24

```
1       AGTTTGATCC TGGCTCAGAA CGAACGCTGG CGGCATGCCT AACACATGCA
51      AGTCGAACGA GATCCTTCGG GGTCTAGTGG CGCACGGGTG CGTAACGCGT
101     GGGAATCTGC CCTTGGGTTC GGAATAACAG TGGGAAACTA CTGCTAATAC
151     CGGATGATGT CTTCGGACCA AAGATTTATC GCCCAGGGAT GAGCCCGCGT
201     AAGATTAGCT AGTTGGTGAG GTAAAGGCTC ACCAAGGCTA CGATCTTTAG
251     CTGGTCTGAG AGGATGATCA GCCACACTGG GACTGAGACA CGGCCCAGAC
301     TCCTACGGGA GGCAGCAGTG GGGAATATTG GACAATGGGC GAAAGCCTGA
351     TCCAGCAATG CCGCGTGAGT GATGAAGGCC TTAGGGTTGT AAAGCTCTTT
401     TACCCGGGAT GATAATGACA GTACCGGGAG AATAAGCTCC GGCTAACTCC
451     GTGCCAGCAG CCGCGGTAAT ACGGAGGGAG CTAGCGTTGT TCGGAATTAC
501     TGGGCGTAAA GCGCACGTAG GCGGCTTTGT AAGTTAGAGG TGAAAGCCCG
551     GGGCTCAACT CCGGAACTGC CTTTAAGACT GCATCGCTTG AATCCAGGAG
601     A
```

Sequence from clone 26

```
1       GGGGATCCGA TGAGTTTGAT CCTGGCTCMG AATCAACGCT GGCGGCGTGC
51      CTCAGACATG CAAGTCGAAC GATTAAACTT TCCTTCGGGA AAGATATAAA
101     GTGGCGCACG GGTGAGTAAC ACGTAGGTAA TGTACCTTTG GGTGGGGAAT
151     AACTTAGGGA AACTTAAGCT AATACCGCAT AATGCAGCGG CTCCTTCGGG
201     AGACAGTTGT TAAAGATTTA TCGCCTAAAG AGCAGCCTGC GGCAGATTAG
251     CTAGTTGGTA AGGTAACGGC TTACCAAGGC TACGATCTGT ATCCGACCTG
301     AGAGGGTGGT CGGACACACT GACACTGAAT AACGGGTCAG ACTCCTACGG
351     GAGGCAGCAG TCGGGAATTT TGGGCAATGG GCGAAAGCCT GACCCAGCAA
401     CGCCGCGTGA AGGATGAAGT CTCTCGGGAT GTAAACTTCG AAAGAATAGG
451     AAGAATAAAT GACGGTACTA TTTATAAGGT CCGGCTAACT ACGTGCCAGC
501     AGCCGCGGTA ATACGTAGGG ACCAAGCGTT GTTCGGATTT ACTGGGCGTA
551     AAGGGCGCGT AGGCGGCGTG ACAAGTCAAT TGTGAAATCT CCGGGCTTAA
601     CTCGGAACGG TCAATTGATA CTGTTGTGCT AGAGTACAGA AGGGGCAATC
651     GG
```

Sequence from clone 29

```
1       GCGTGCCTAA CACATGCAAG TCGAACGGGA CCAGGGGCAA CTCTGGTTCA
51      GTGGCGGACG GGTGCGTAAC ACGTGAGGAA CATGACCTTC GGCGGGGGAT
101     AGCCGGCCCA ACGGCCGGGT AATACCGCGT ACGACCTTTC GGGGACATCC
151     CCGGATGGTG AAAGCAGCAA TGCGCCGATG GAGTGCCTCG CGGCCTATCA
201     GCTGGTTGGT GAGGTAACGG CTCACCAAGG CAACGACGGG TAGCTGGTCT
251     GAGAGGATGG CCAGCCACAT TGGGACTGAG ACACGGCCCA GACTCCTACG
301     GGAGGCAGCA GTGGGGAATA TTGCGCAATG GACGAAAGTC TGACGCAGCG
351     ACGCCGCGTG TGGGATGACG GTCTTCGGAT TGTAAACCAC TGTCGGGAGG
401     GACGAATACG CCGCAAGGCG GGTGACGGTA CCTCCAAAGG AAGCACCGGC
451     TAACTCCG
```

Sequence from clone 30

```
1       CGACCCTCGG CCGCTGCCTC GCTTGCGCGT TAGCCCACGG ACTTCAGGTC
51      TTCCCCACTC CCATGACGTG ACGGGCGGTG TGTACAAGGC CCGGGTACAG
101     ATTCACCGCC GTATGGCTGA CCGGCGATTA CTAGCAACTC CGCCTTCATG
151     GGGGCGAGTT GCAGCCCCCA ATCTGAACTG AGACCGACCT TCGAGATCCG
201     CCACATGTTA CCATGCAGCA ACCCATTCGT CCGGCCATTG TAGCGTGTGT
251     GTCGCCCTGG TCGTACGGGC CATGCGGACT TGACGTCATC CCCGCCTTCC
301     TCCGTGGTTG ACCACGGCAG TCATGTGTGA CACAAGTAAC ACACATCAGG
351     GGTTGCGCTC GTTGCGGGAC TTAACCCAAC ATCTCACGAC ACGAGCTGAC
401     GACAGCCATG CAGCACCGGT GCACCACCCT CGAAGGCAGC CATGTTTCCA
451     CGACTTGCAG GTGCATGTCA AGACCAGGTA AGGTTCTGCG CGTTGCGTCG
501     AATTAAACCA CACGCTCCGC TGCTTGTGCG GGCCCCCGTC AATTCCTTTG
551     AGTTTTAAGC TTGCGCTCGT AGTCCCCAGG CGGCATACTC AACACGTAAG
601     TTAAGGCACT GNCCTGGCTT A
```

Sequence from clone 34

```
1       TTAMCTTGTT ACGACTTCAC CCCAGTCACG AATCCTACCG TGGTAAGCGC
51      CCCCCTTGCG GTTAAGCTAC CTACTTCTGG TAAAACCCGC TCCCATGGTG
101     TGACGGGCGG TGTGTACAAG ACCCGGGAAC GTATTCACCG CGACATGCTG
151     ATCCGCGATT ACTAGCGATT CCAACTTCAT GTAGTCGAGT TGCAGACTAC
201     AATCCGGACT ACGATACACT TTCTGGGATT AGCTCCCCCT CGCGGGTTGG
251     CGGCCCTCTG TATGTACCAT TGTATGACGT GTGAAGCCCT ACCCATAAGG
301     GCCATGAGGA CTTGACGTCA TCCCCACCTT CCTCCGGTTT GTCACCGGCA
351     GTCTCATTAG AGTGCTCTTT CGTAGCAACT AATGACAAGG GTTGCGCTCG
401     TTGCGGGACT TAACCCAACA TCTCACGACA CGAGCTGACG ACAGCCATGC
451     AGCACCTGTG TTACGGCTCT CTTTCGAGCA CACCTCGATC TCTCGTGGCT
501     TCCGTACATG TCAAGGGTAG GTAAGGTTTT TCGCGTTGCA TCGAATTAAT
551     CCACATCATC CACCGCTTGT GCGGGTCCCC GTCAATTCCT TTGAGTTTTA
601     ATCTTGCGAC CGTACTCCCC AGGCGGTCTA CTTCACGCGT TAGCTGCGTT
```

Sequence from clone 37

```
1       GGGGATCCGA TGGTTAMCTT GTTACGACTT CACCCCAATC ATGAATCATA
51      CCGTTACACC ATGCCTCCCT TACGGGTTAG CTCTGGCGCT TCTAGTACAA
101     CCCACTTTCG TGATGTGACG GGCGGTGTGT ACAAGACCCG GGAACGTATT
151     CACCGCGGCG TGCTGATCCG CGATTACTAG CGATTCCAAC TTCATGAAGT
201     CGAGTTGCAG ACTTCAATCC GAACTGAGAC GAGCTTTTTC CGATTGGCTC
251     CCCATCGCTG GTTTGCAACG GTTTGTACTC GCCATTGTAG CACGTGTGTA
301     GCCCTACTCA TAAAGGCCAT GATGACTTGA CGTCGTCCCC ACCTTCCTCC
351     GTTTTGTCAA CGGCAGTCTC ACCAGAGTTC TCGGCTTAAC CCGTTAGTAA
401     CTGATGATAA GGGTTGCGCT CGTTGCGGGA CTTAACCCAA CATCTCACGA
451     CACGAGCTGA CGACAGCCAT GCAGCACCTT GCATCTCGTC CGGTTTTACC
501     CGGAAGGCTC CATCTCTGGA GTTGTCGAGA GCATTCTAGA GTAGGTAAGG
551     TTCTTCGCGT TGCGTCGAAT TAAACCACAT GCTCCACCGC TTGTGCGGGT
601     CCCCGTCAAT TCCTTTGAGT TTCATTCTTG CGAACGTACT CCCC
```

Sequence from clone 39

```
1       GGTTAMCTTG TTACGACYTC ACCCCAATCA TAAATCATAC CGTGGTAACT
51      TGCCTCCCTT GCGAGTTAGC CCAGCTACTT CTAGTACAAC CTACTTTCGT
101     GATGTGACGG GCGGTGTGTA CAAGACCCGG GAACGTATTC ACCGCAGCGT
151     TCTGATCTGC GATTACTAGC GATTCCAACT TCATGGAGTC GAGTTGCAGA
201     CTCCAATCCG AACTGAGACC GGCTTTTTAC GATTGGCTCA CTCTTGCGAG
251     TTTGCAGCGT TTTGTACCGG CCATTGTAGC ACGTGTGTAG CCCTAGTCAT
301     AAAGGCTATG AGGACTTGAC GTCATCCCCA CCTTCCTCCG TTTTATCAAC
351     GGCAGTCTCA ACCGAGTTCC CGGCATTACC CGCTGGCAAC AGTTGATAAG
401     GGTTGCGCTC GTTGCGGGAC TTAACCCAAC ATCTCACGAC ACGAGCTGAC
451     GACAGCCATG CAGCACCTTG CATCTTGCTT GGTTTTACCC AAGAAACCCT
501     ATCTCTAGGG CTGTCAAGAG CATTCTAGAC TAGGTAAGGT TCTTCGCGTT
551     GCGTCGAATT AAACCACATG CTCCACCGCT TGTGCGGGTC CCCGTCAATT
601     CCTTTGAGTT TCATGCTTGC GCACGTACTC CC
```

Sequence from clone 40

```
1       ATCCGATGAG TTTGATCCTG GCTCAGAATG AACGTTGGCG GCGTGGATTA
51      GGCATGCAAG TCGGACGGGC CGCAAGGCCA GTGGCGTAAG GGTGAGTAAG
101     GCGACGGCAA TCATCCCCAC GGTTGGGTAT AGCCGCGAGA AATCGCGGGT
151     AATCCCCAGC GACGCAGGGT GTCGGCATCG ACGCCCTGCC AAAGGCTCGC
201     CGCCGTGGGA CGAGCCGTTG TGGTATTAGG TTGTTGGCGG GGTAACGGCC
251     CACCAAGCCT GCGATGCCTA CCGGGCGTGC GAGCGTGGCC CGGCACACTG
301     GGACTGAGAC ACTGCCCAGA CTCCTACGGG AGGCTGCAGT CGAGAATCTT
351     CGGCAATGGG CGCAAGCCTG ACCGAGCGAC GCCGCGTGGA GGACGAAGGC
401     CTTCGGGTTG TAAACTCCTG TCGAGGGGAA GGAAGGGGCC GCGAGGCCCT
451     TGACCGCTCC CTGGAGGAAG CACGGGCTAA GTTCGTGCCA GCAGCCGCGG
501     TAAGACGAAC CGTGCGAACG TTATTCGGAA TCACTGGGCT TAAAGCGCGT
551     GTAGGCGGGG CGGTGCGTCG GCCGTTGAAA TCCCCCGGCT CAACCGGGGA
601     AGTGGCGCCG AAACGACCGG CCTGGAGCGA CGTAGGGGGA ACTGGAACTT
651     CCGGTGGAGC
```

Sequence from clone 51

```
1       TTGGAACACG TAGCTAACCT GCCCAACAGA GGGGGATAAC CTCGGGAAAC
51      CGAGGCTAAT ACCGCATACG CTCATTTTTG GGGACGAGGA TGAGGAAACG
101     GAGCAATCCG CTGATGGAGG GGGCTGCGGC CGATTAGCTA GTTGGTGGGG
151     TAAAAGCCTA CCGAGGCGGT GATCGGTAGC TGGTCTGAGA GGACGATCAG
201     CCACACGGGG ACTGAGACAC GGCCCCGACT CCTACGGGAG GCAGCAGCAA
251     GGAATTTTCC ACAATGGGCG CAAGCCTGAT GGAGCAACGC CGCGTGGGGG
301     ATGACGCTTT TCGGAGTGTA AACCCCTTTT CGAGAGGACG AAGCTAATGA
351     CGGTACTCTC GGAATAAGGA CCGGCTAACT ACGTGCCAGC AGCCGCGGTA
401     AGACGTAGGG TCCGAGCGTT GTCCGGAGTT ACTGGGCGTA AAGCGCGCGC
451     AGGCGGTTAG ACACGTCGGG TGTGAAAGCC CCCCGCTCAA CGGGGGAGGG
501     TCATTCGAAA CGGTCAGACT GGAGGCAGGG AGAGGTCGGT GGAATTCCCG
551     GTGTAGTGGT
```

Sequence from clone 54

```
1       CAATACATCA GCGGCAGACG GGAGAGTAAC ACGTGGGAAC GCGCCCTTCG
51      GTTCGGAATA ACTCAGGGAA ACTTGAGCTA ATACCGGATA CGCCCTTACG
101     GGGAAAGATT TATTGCCGAA GGAACGGCCC GCGTCGGATT AGCTAGTTGG
151     TGAGGTAATG GCTCACCAAG GCAACGATCC GTAGCTGGTC TAAGAGGATG
201     ATCAGCCTCA CTGGGACTGA GACACGGCCC AGACTCCTAC GGGAGGCAGC
251     AGTGGGGAAT ATTGGACAAT GGGCGAAAGC CTGATCCAGC CATGCCGCGT
301     GGATGATGAA GGCCTTAGGG TTGTAAAGTC CTTTTAACGG GGAAGATAAT
351     GACGGTACCC GTAGAATAAG CCCCGGCTAA CTTCGTGCCA GCAGCCGCGG
401     TAATACGAAG GGGGCTAGCG TTGCTCGGAA TTACTGGGCG TAAAGCGCAC
451     GTAGGCGGAT TGTTAAGTCG GGGGTGAAAT CCTGGAGCTC AACTCCAGAA
501     CTGCCTTCGA AACTGGCGAT CTTGAGTCCG GGAGAGGTGA GTGGAACTGC
551     GAGTGTAGAG GTGAAATTCG TAGATATTCG CAAGAACACC AGTGGCGAAG
601     GCGGCTCACT GGCCCGGTAC
```

Sequence from clone 62

```
1       CAGGTATTCTT GGGTTGGMCC GGCGCAAGGG TGCGTAACAC GTGGGTAATT
51      TGCCATGAAG TCTGGAATAA CTTGCTGAAA GGCGAGCTAA TGCCGGATGT
101     GATTTTCGGG AAGCATTTCT TGAAACTCAA AGTTGGGGAC CGCAAGGCCT
151     GACGCTTCTT GATAAGCCCG CGGCCTATCA GCTAGTTGGT GAGGTAATGG
201     CTCACCAAGG CTAAGACGGG TAGCTGGTCT GAGAGGACGA CCAGCCACAC
251     TGGAACTGAG ACACGGTCCA GACACCTACG GGTGGCAGCA GTCGAGAATT
301     TTTCACAATG GGCGAAAGCC TGATGGAGCG ACGCCGCGTG GGGGATGAAT
351     GGCTTCGGCC CGTAAACCCC TGTCATTTGC GAACAAACCT TACCGGTTAA
401     CAACCGTTGA GCTGATTGTA GCGGAAGAGG AAGGGACGGC TAACTCTGTG
451     CCAGCAGCCG CGGTAATACA GAGGTCCCAA GCGTTGTTCG GATTCACTGG
501     GCGTAAAGGG TGCGTAGGTG GTGGGGTAAG TCGGATGTGA AATCTCCGGG
551     CTCAACCCGG AAATGGCATT GGAAACTACC TTGCTAGAGG ATTTGAGGGG
601     GGATTGGAAT ACTTGGTGTA
```

Sequence from clone 65

```
1       TGCTCCTGAA GATCTAGTKC CGAACGGGTG CRWAACACGT GAGAAACCTG
51      TCCCGAACTT GGGAATAACA GCCGAAAACS ACTGCTAATA CCGAATATCT
101     TCGTAACGTC GCATGGCGAT TCGAAGAAAG CTTTATGCGG TTTGGGAGGG
151     TCTCGCGGCC TATCAGCTTG TTGGTGAGGT AATGGCTCAC CAAGGCATCG
201     ACGGGTAGCT GGTCTGAGAG GATGATCAGC CACACTGGGA CTGAGACACG
251     GCCCAGACTC CTACGGGAGG CAGCAGTGGG GAATATTGCA CAATGGGCGA
301     AAGCCTGATG CAGCGATGCC GCGTGCGGGA AGAAGGCCCT AGGGTTGTAA
351     ACCGCTTTCA GTAGGGAAGA AAATGACGGT ACCTACAGAA GAAGGTGCGG
401     CCAACTACGT GCCAGCAGCC GCGGTGACAC GTAGGCACCA AGCGTTGTCC
451     GGATTTATTG GGCGTAAAGA GCTCGTAGGC GGTTTGGTAA GTCGGGTGTG
501     AAAACTCTGG GCTCAACCCA GAGAGGCCAC TCGATACTGC CATGACTTGA
551     GTACGGTAGG GGAGTGGGGA ATTTCTAGTG TAGCGGTGAA ATGCGCAGAT
601     ATTAGAAGGA ACACCAGTGG CGAAGGCGCC ACTCTGGGCC GTAACT
```

Sequence from clone 68

```
1       GAAAACGTCG GAATCTGCCT ATTTGTGGGG GATAACGTAK GGNMACTTAC
51      GCTAATACCG CATACGACCT ACGGGTGAAA GCGGAGGACC TTCGGGCTTC
101     GCGCAGATAG ATGAGCCGAC GTCGGATTAG CTAGTTGGCG GGGTAAAGGC
151     CCACCAAGGC GACGATCCGT AGCTGGCCTG AKAGGATGAT CAGCCACACT
201     GGAACTGAGA CACGGTCCAG ACTCCTACGG GAGGCAGCAG TGGGGAATAT
251     TGGACAATGG GCGCAAGCCT GATCCAGCCA TGCCGCGTGT GTGAAGAAGG
301     CCTTCGGGTT GTAAAGCACT TTTGTCCGGA AAGAAAAGCA CTGGGTTAAT
351     ACCCTGGTGT CATGACGGTA CCGGAAGAAT AAGCACCGGC TAACTTCGTG
401     CCAGCAGCCG CGGTAATACG AAGGGTGCAA GCGTTACTCG GAATTACTGG
451     GCGTAAAGCG TGCGTAGGCG GTTTGTTAAG TCTGATGTGA AAGCCCTGGG
501     CTCAACCTGG GAATGGCATT GGATACTG
```

Sequences from MVT 7 16S rDNA clone library

Sequence from clone 11

```
1       TGTGCGCAAG CGCWCMCACA TCCGGAGTGG CGGACGGGTG CGTAACACGT
51      GAGCGATCTG CCCAGATGGG GGGGATACCC CGGGGAAACC CGGGTCAATC
101     CCGCATGTGG TTTTACCTCT TCATGGAGGT TCAATCAAAG ATCCTCTCAA
151     GGGATTCTGT CTGGAGGAGC TCGCGGCGTA TCAGCTAGTT GGTAGGGTAA
201     CGGCCTACCA AGGCGACGAC GCGTAGGGGG TCTGAGAGGA TGGCCCCCCA
251     CATGGGGACT GAGATACGGC CCCGACTCCT ACGGGAGGCA GCAGTGGGGA
301     ATCTTGCGCA ATGGGCGAAA GCCTGACGCA GCGACGCCGC GTGCGGGAGG
351     ACGCTTTTCG GAGTGTAAAC CGCTGTCGGG AGGGACGAAT CCTGTGAGGA
401     GGAAATGTCC CACAGTTGAC GGTACCTTCA AAGGAAGCAC CGGCTAACTC
451     TGTGCCAGCA GCCGCGGTAA TACAGAGGGT GCAAGCGTTG TTCGGAATCA
501     TTGGGCGTAA AGCGCACGTA GGCGGCCCGT TAAGTCCGAC TGTGAAAGAC
551     CGGGGCTCAA CCCCGGGGCT GCAGCGGATA CTGGCGGGCT TGAGACACGT
601     A
```

Sequence from clone 13

1 TAACACGTGG GTAACCTGCC CTCAGCTCTG GGATAAGCYY GGCCCAACTG
51 GGTCTAATAC CGGATATGAC CTCGCATCGC ATGGTGTGGG GTGGAAAGCC
101 TTGTGCGGCT GAGGATGGGC CCGCGGCCTA TCAGCTAGTT GGTGCGGTCA
151 CGGCGCACCA AGGCGACGAC GGGTAGCTGG TCTGAGAGGA TGGCCAGCCA
201 CATTGGGACT GAKAAACGGC CCAGACTCCT ACGGGAGGCA GCAGTGGGGA
251 ATCTTGCGCA ATGGCCGAAA GGCTGACGCA GCGACGCCGC GTGTGGGAGG
301 AAGCCTTTCG GGGTGTAAAC CACTGTTGCC CGGGACGAAC AGCTCCTTCG
351 TGGAGCCTGA CGGTACCGGG TGAGGAAGCA CCGGCTAACT CCGTGCCAGC
401 AGCCGCGGTA ATACGGAGGG TGCAAGCGTT GTCCGGATTT ATTGGGTTTA
451 AAGGGTGCGT AGGCGGTTTT ATAAGTCAGT GGTGAAAGAC GTCAGCTTAA
501 CTGTCGCAGT GCCATTGATA CTGTAGAACT TGARTATAGT TGAGGTAGGC

Sequence from clone 18

1 GAGTAACACG TAAGTAATCT ACCTTTGGGT GGGGGATAAY WTCCNGAAAC
51 CGATGCTAAT ACCGCATAAT GCAGCGGCAT CATATGATGA CGGTTGTTAA
101 AGCATTTATG TGCCTAAAGA GGAGCTTGCG GCAGGTTAGC TAGTTGGTAA
151 GGTAATGGCT TACCAAGGCA ACGATCTGTA GCCGACCTGA GAGGGTGGTC
201 GGTCACACTK TWYACTGAAT AACGGGTCAG ACTCCTACGG GAGGCAGCAK
251 TYGKGAATTT TGGGCAATGG GCGAAAGCCT GACCCAGCAA CGCCGCGTGA
301 AGGATGAAGT CTTTCGGGAT GTAAACTTCG GAAATATAGG AAGAATAAAT
351 GACGGTACTA TATCTAAGGT CCGGCTAACT ACGTGCCAGC AGCCGCGGTA
401 ATACGTAKGG

Sequence from clone 24

1 TAACACGTGG GTAACCTGCC CTCAGCTCTG GGATAAGCCC GGGMMACTGG
51 GTCTAATACC GGATATGACC TCGCATCGCA TGGTGTGGGG TGGAAAGCCT
101 TGTGCGGCTG AGGATGGGCC CGCGGCCTAT CAGCTTGTTG GTGGGGTAGT
151 GGCCTACCAA GGCGACGACG GGTGGCCGGC CTGAGAGGGC GACCGGCCAC
201 ACTGGGACTG AGACACGGCC CAGACTCCTA CGGGAGGCAG CAGTGGGGAA
251 TATTGCGCAA TGGGCGAAAG CCTGACGCAG CGACGCCGCG TGAGGGATGA
301 CGGCCTTCGG GTTGTAAACC TCTTTCAGCT CCGACGAAGC CTTCGGGTGA
351 CGGTAGGGGC AGAAGAAGCA CCGGCCAACT ACGTGCCAGC AGCCGCGGTA
401 ATACGTAGGG TGCAAGCGTT GTCCGGAATT ATTGGGCGTA AAGAGCTCGT
451 AGGCGGTTTG TCGCGTCGAC TGTGAAAACT CAGGGGCTCA ACTCCGAGCT
501 TGCAGTTGAT ACGGGCAGAC TAGAGTTCGG CAGGGGAGAC TGGAATTCCT
551 GGTGTAGCGG TGAAATGCGC AGATATCAGG AGGAACACCG GTGGCGAAGG
601 CGGGTCTCTG GGCCGATACT GAC

Sequence from clone 29

1 CAGTGGAGCG ACGAACCAGG CTTCGGCCTG GGGCANAGCC GCGAACGGGT
51 GAGTAACACG TGGGTGACCT GCCCCGATGA CCGGGACAAC CCGAGGGAAA
101 CTCGGGCTAA TACCGGATGC GTCCACCTCG CGACAGCGTG GCGGGCAAAG
151 GTAGCTTCGG CCTCCGCATC GGGATGGGCC CGCGGCCCAT TAGCTTGTTG
201 GTGAGGTAAC GGCTCACCAA GGCGACCATG GGTAGCTGGT CTGAGAGGAC
251 GATCAGCCAC ACTGGGACTG AGACACGGCC CAGACTCCTA CGGGAGGCAG
301 NNGTGNGGAA TCTTGCGCAA TGCGCGAAAG CGTGACGCAC CNACGCCGCN
351 TGGGGGAAGA CGGCCTTCGG GTTGTAAACC CCTTTCANGA TGNACGAAGG
401 TGTGGCGGTG ATTAGCCGAC CATACTGACG GTACCTCCAG AAGAAGNCCC
451 NGTTAACTAC NGNGCCATCA GCCGCGGTTA TACGTAGTGG GG

Sequence from clone 31

```
1       GTAACACGTG GGTGACCTGC CCCGATGACC GGGACAACYY GCCNAAACTC
51      GGGCTAATAC CGGATGCGTC CACCTCGCGA CAGCGGGACG GGCAAAGGTA
101     GCTTCGGCCT CCGCATCGGG ATGGGCCCGC GGCCCATTAG CTTGTTGGTG
151     AGGTAACGGC TCACCAAGGC GACGATGGGT AGCTGGTCTG AGAGGACGAT
201     CAGCCACWCT GGGACTGAGA CACGGCCCAG ACTCCTACGG GAGGCAGYWK
251     TGGGGAATCT TGCGCAATGC GCGAAAGCGT GACGCAGCAA CGCCGCGTGG
301     GGGAAGACGG CCTTCGGGTT GTAAACCCCT TTCAGTTGGG ACGAAGCCTC
351     GGCGGTTAAC AGCCGTTCGG GGTGACGGTA CCTTCAGAAG AAGCCCCGGC
401     TAACTACGTG CCAGCAGCCG CGGTAATACG TAGGGGGCCA GCGTTGTCCG
451     GAATCATTGG GCGTAAAGAG CGCGTAGGCG GTCCGATCAG TCCGCTGTGA
501     AAGT
```

Sequence from clone 37

```
1       CAGTCGAGCG GAACCACCAG TGGCAACACT GGGGCAGTCT GAGCGCCGAA
51      CGGGTGAGTA ACACGTGAGG AACCTGCCCC GAAGACCGGG ATAACCCTCC
101     GAAAGGAGGG CTAATACCGG ATACCCCCAT CGAGTCGCAT GGCTTGTTGA
151     GGAAATGGAT TCCGCTTCGG GAGGGCCTCG CGGCCTATCA GCTTGTTGGT
201     GAGGTAACGG CTCACCAAGG CGTCGACGGG TAGCTAGTCT GAGAGGACGA
251     TTAGCCACAC TGGGACTGAG ACACGGCCCA GACTCCTACG GGAGGCAGCA
301     GTGGGGAATC TTGCGCAATG GGCGAAAGCC TGACGCAGCA ACGCCGCGTG
351     GGGGATGAAG GCTCTCGGGT TGTAAACCCC TTTCAGCGGG GACGATTATG
401     ACGGTACCCG CAGAAGAAGG ACCGGCCAAC TACGTGCCAG CAGCCGCGGT
451     AATACGTAGG GTCCAAGCGT TGTCCGGATT TATTGGGCGT AAAGAGCTCG
501     TANGTGGCTT CGTAAGTCGG GTGTGAAAAC CCCAGGCTCA ACCTGGGGAC
551     GCCACTCGAT ACTGCGGTAG CTAGAGTCTG GTAGGGGATC TCG
```

Sequence from clone 49

```
1       GTTGGMCCGG CGCAAGGGTG CGTAACACGT GGGTAATTTG CCATGAAGTC
51      TGGAATAACT TGCTGAAAGG CGAGCTAATG CCGGATGTGA TTTTCGGGAA
101     GCATTTCTTG AAACTCAAAG TTGGGGACCG CAAGGCCTGA CGCTTCTTGA
151     TAAGCCCGCG GCCTATCAGC TAGTTGGTGA GGTAATGGCT CACCAAGGCT
201     AAGACGGGTA GCTGGTCTGA GAGGACGACC AGCCACACTG GAACTGAGAC
251     ACGGTCCAGA CACCTACGGG TGGCAGCAGT CGAGAATTTT TCACAATGGG
301     CGAAAGCCTG ATGGAGCGAC GCCGCGTGGG GGATGAATGG CTTCGGCCCG
351     TAAACCCCTG TCATTTGCGA ACAAACCTTA CCGGTTAACA ACCGTTGAGC
401     TGATTGTAGC GGAAGAGGAA GGGACGGCTA ACTCTGTGCC AGCAGCCGCG
451     GTAATACAGA GGTCCCAAGC GTTGTTCGGA TTCACTGGGC GTAAAGGGTG
501     CGTAGGTGGT GGGGTAAGTC GGATGTGAAA TCTCCGGGCT CAACCCGGAA
551     ATGGCATTGG AAACTACCTT GCTAGAGGAT TTGAGGGGGG ATTGGAATAC
601     TTGGTG
```

Sequence from clone 52

```
1       CCGAACGGGT GCRWAACACG TGAGAAACCT GTCCCGAACT TGGGAATAAC
51      AGCCGAAAAC SACTGCTAAT ACCGAATATC TTCGTAACGT CGCATGGCGA
101     TTCGAAGAAA GCTTTATGCG GTTTGGGAGG GTCTCGCGGC CTATCAGCTT
151     GTTGGTGAGG TAATGGCTCA CCAAGGCATC GACGGGTAGC TGGTCTGAGA
201     GGATGATCAG CCACACTGGG ACTGAGACAC GGCCCAGACT CCTACGGGAG
251     GCAGCAGTGG GGAATATTGC ACAATGGGCG AAAGCCTGAT GCAGCGATGC
301     CGCGTGCGGG AAGAAGGCCC TAGGGTTGTA AACCGCTTTC AGTAGGGAAG
351     AAAATGACGG TACCTACAGA AGAAGGTGCG GCCAACTACG TGCCAGCAGC
401     CGCGGTGACA CGTAGGCACC AAGCGTTGTC CGGATTTATT GGGCGTAAAG
451     AGCTCGTAGG CGGTTTGGTA AGTCGGGTGT GAAAACTCTG GGCTCAACCC
501     AGAGAGGCCA CTCGATACTG CCATGACTTG AGTACGGTAG GGGAGTGGGG
551     AATTTCTAGT GTAGCGGTGA AATGCGCAGA TATTAGAAGG AACACCAGTG
601     GCGAAGGCGC CACTCTGGGC CGTAA
```

Sequence from clone 58

```
1       AGATATAAAG TGKCGCACGG GTGAGTAACA CGTAGGTAAT CTACCTTTGA
51      GTGGGGAATA ACGTTCGGAA ACGAACGCTA ATACCGCATA ATGCAGCGGC
101     ACCGCAAGGT GACAGTTGTT AAAGGAGCAA TCCGCTTAAA GAGGAGCCTG
151     CGGCAGATTA GCTAGTTGGT AAGGTAATGG CTTACCAAGG CTACGATCTG
201     TAACCGACCT GAGAGGGTGG TCGGTCACAC TGACACTGAA TAACGGGTCA
251     GACTCCTACG GGAGGCAGCA GTCGGGAATT TTGGGCAATG GGCGAAAGCC
301     TGACCCAGCA ACGCCGCGTG AAGGATGAAG TATTTCGGTA TGTAAACTTC
351     GAAAGAATAG GAAGAATAAA TGACGGTACT ATTTATAA
```

Sequence from clone 61

```
1       TCGGCGGGGG ATAGCCGGCC CAACGGCYKG CCAATACCGC GTACGAMCAC
51      ATGGGGACAT CCCTGAGTGG TGAAAGCAGC AATGCGCCGA TGGAGTGCCT
101     CGCGGCCTAT CAGCTAGTTG GTGAGGTAAC GGCTCACCAA GGCAACGACG
151     GGTAGCTGGT CTGAGAGGAT GGCCAGCCAC ATTGGGACTG AGACWYKGCC
201     CAGACTCCTA CGGGAGGCAG CAGTGGGGAA TATTGCGCAM TGGACGAAAG
251     TCTGACGCAT CKWYKCCGCG TGTGGGATGA CGGTCTTCGG ATTGTAAACC
301     ACTGTCGGGA GGGACGAATA CGCCGYAAGG CGGGTGACGG TACCTCCAAA
351     GGAAGCWCCG GCTAACTCCG TGCCAGCARC CGCKGTAATA CGT
```

Sequence from clone 67

```
1       TGACTAACGC GTGGGAACAT GCCCTTTGGT ACGGGATAGC CTCGGGAAAC
51      TGGGTGTAAT ACCGTATGTG CTCGAAAGAG GAAAGATTTA TCGCCAAGGG
101     ATTGGCCCGC GTTGGATTAG GTAGTTGGTG GGGTAATGGC CTACCAAGCC
151     GACGATCCAT AGCTGGTTTG AGAGGATGAT CAGCCACACT GGGACTGAGA
201     CACGGCCCAG ACTCCTACGG GAGGCAGCAG TGGGGAATCT TAGACAATGG
251     GGGCAACCCT GATCTAGCCA TGCCGCGTGA TCGATGAAGG CCTTAGGGTT
301     GTAAAGATCT TTCAGTGGGG AAGATAATGA CGGTACCCAC AGAAGAAGCC
351     CCAGCTAACT CCGTGCCAGC AGCCGCGGTA ATACGGAGGG GGCTAGCGTT
401     ATT
```

Sequence from clone 74

```
1      GTCGCTGACC CTACCGTGGT TGGCTGCCTC CCGATTGCTC AGGTTAGCGC
51     ACCACCTTCG GGTAGAACCA ACTCCCATGG TGTGACGGGC GGTGTGTACA
101    AGGCCCGGGA ACGTATTCAC CGTGGCGTGC TGATCCACGA TTACTAGCGA
151    TTCCAGCTTC ATGCCCTCGA GTTGCAGAGG ACAATCCGAA CTGAGACGGC
201    TTTTTGGGAT TAGCTTCTCC TTGCGAAGTA GCAGCCCACT GTCACCGCCA
251    TTGTAGCACG TGTGTAGCCC AGCCCGTAAG GGCCATGAGG ACTTGACGTC
301    ATCCCCACCT TCCTCTCGGC TTATCACCGG CAGTCCCCCT AGAGTGCCCA
351    ACTGAATGAT GGCAACTAAG GGCGAGGGTT GCGCTCGTTG CGGGACTTAA
401    CCCAACATCT CACGACACGA GCTGACGACA GCCATGCAGC ACCTGTGCGC
451    AGGTCTCTTG CGAGAAGGAA TCCATCTCTG GAAGCCGTCC TGCCATGTCA
501    AGGGCTGGTA AGGTTCTGCG CGTTGCTTCG AATTAAACCA CATGCTCCAC
551    CGCTTGTGCG GGCCCCCGTC AATTCCTTTG AGTTTTAATC TTGCGACCGT
601    ACTCCCCAGG CGGAATGCTT A
```

Sequence from clone 79

```
1      TCACCCCAGT CACGAATCCT ACCGTGGTAA GCGCCCCCCT TGCGGTTAAG
51     CTACCTACTT CTGGTAAAAC CCGCTCCCAT GGTGTGACGG GCGGTGTGTA
101    CAAGACCCGG GAACGTATTC ACCGCGACAT GCTGATCCGC GATTACTAGC
151    GATTCCAACT TCATGTAGTC GAGTTGCAGA CTACAATCCG GACTACGATA
201    CACTTTCTGG GATTAGCTCC CCCTCGCGGG TTGGCGGCCC TCTGTATGTA
251    CCATTGTATG ACGTGTGAAG CCCTACCCAT AAGGGCCATG AGGACTTGAC
301    GTCATCCCCA CCTTCCTCCG GTTTGTCACC GGCAGTCTCA TTAGAGTGCT
351    CTTTCGTAGC AACTAATGAC AAGGGTTGCG CTCGTTGCGG GACTTAACCC
401    AACATCTCAC GACACGAGCT GACGACAGCC ATGCAGCACC TGTGTTACGG
451    CTCTCTTTCG AGCACACCTC GATCTCTCGT GGCTTCCGTA CATGTCAAGG
501    GTAGGTAAGG TTTTTCGCGT TGCATCGAAT TAATCCACAT CATCCACCGC
551    TTGTGCGGGT CCCCGTCAAT TCCTTTGAGT TTTAATCTTG CGACCGTACT
601    CCCCAGGCGG TCTACT
```

Sequence from clone 82

```
1      CCTTTCGGGG GTACACGASC GGCGAACGGG TGAGTAACAC GTGGGTAACC
51     TGCCCTCAGC TCTGGGATAA GCCCGGGAAA CTGGGTCTAA TACCGGATAT
101    GACTCCGCAT CGCATGGTGT GGGGTGGAAA GCCTTGTGCG GCTGAGGATG
151    GACCCGCGGC CTATCAGCTT GTTGGTGGGG TAGTGGCCTA CCAAGGCGAC
201    GACGGGTAGC CGGCCTGAGA GGGCGACCGG CCACACTGGG ACTGAGACAC
251    GGCCCAGACT CCTACGGGAG GCAGCAGTGG GGAATATTGC GCAATGGGCG
301    AAAGCCTGAC GCAGCGACGC CGCGTGAGGG ATGACGGCCT TCGGGTTGTA
351    AACCTCTTTC AGCTCCGACG AAGCGAGAGT GACGGTAGGA GCAGAAGAAG
401    CACCGGCCAA CTACGTGCCA GCAGCCGCGG TAATACGTAG GGTGCAAGCG
451    TTGTCCGGAA TTATTGGGCG TAAAGAGCTC GTAGGCGGTT TGTCGCGTCG
501    ACTGTGAAAA CTCAGGGGCT CAACTCCGAG CTTGCAGTTG ATACGGGCAG
551    ACTAGAGTTC GGCAGGGGAG ACTGGAATTC CTGGTGTAGC GGTGAAATGC
601    GCAGATATCA GGAGGAACAC CGATGGCGAA GGCAGGTCTC TGAGCCACTA
651    CTGAC
```

Sequence from clone 84

```
1       CAGCGGTAAG GNCCTTTCGG GGGTACACGW CCGGCGAACG GGTGAGTAAC
51      ACGTGGGTAA CCTGCCCTCA GCTCTGGGAT AAGCCCGGGA AACTGGGTCT
101     AATACCGGAT ATGACTCCGC ATCGCATGGT GTGGGGTGGA AAGCCTTGTG
151     CGGCTGAGGA TGGACCCGCG GCCTATCAGC TTGTTGGTGG GGTAGTGGCC
201     TACCAAGGCG ACGACGGGTA GCCGGCCTGA GAGGGCGACC GGCCACACTG
251     GGACTGAGAC ACGGCCCAGA CTCCTACGGG AGGCAGCAGT GGGGAATATT
301     GCGCAATGGG CGAAAGCCTG ACGCAGCGAC GCCGCGTGAG GGATGACGGC
351     CTTCGGGTTG TAAACCTCTT TCAGCTCCGA CGAAGCGAGA GTGACGGTAG
401     GAGCAGAAGA AGCACCGGCC AACTACGTGC CAGCAGCCGC GGTAATACGT
451     AGGGTGCAAG CGTTGTCCGG AATTATTGGG CGTAAAGAGC TTGTAGGCGG
501     TTTGTCGCGT CTGCTGTGAA AACTCAGGGC TTAACCCTGA GCCTGCAGTG
551     GGTACGGGCA GACTAGAGTG TGGTAGGGGA GACTGGAATT CCTGGTGTAG
601     CGGTGGAATG CGCAGATATC AGGAGGAACA CCTATGGC
```

Sequence from clone 85

```
1       ATGCAAGTCG AACGAGGTCC ATGGAGCTTG CTCCGGAAGA CCGAGTGGCG
51      AACGGGTGCG TAACACGTGA GTAACCTACC CTGAACTTGG GAATAACAGT
101     CGGAAACGAC TGCTAATACC GAATATCTTC ACGACGTCGC ATGGCGATGT
151     GAAGAAAGCT TTATGCGGTT TAGGAGGGTC TCGCGGCCTA TCAGCTTGTT
201     GGTGAGGTAA CGGCTCACCA AGGCATCGAC GGGTAGCTGG TCTGAGAGGA
251     TGATCAGCCA CACTGGGACT GAGACACGGC CCAGACTCCT ACGGGAGGCA
301     GCAGTGGGGA ATATTGCACA ATGGGCGCAA GCCTGATGCA GCGATGCCGC
351     GTGCGGGATG AAGGCCCTAG GGTTGTAAAC CGCTTTCAGT AGGGAAGAAA
401     ATGACGGTAC CTACAGAAGA AGGTGCGGCC AACTACGTGC CAGCAGCCGC
451     GGTGACACGT AGGCACCAAG CGTTGTCCGG ATTTATTGGG CGTAAAGAGC
501     TCGTAGGCGG TTCAGTTAGT CGGGTGTGAA AACTCTGGGC TCAACCCAGA
551     AACGCCACCC GATACTGCTG TGACTAGAGT ACGGTAGG
```

Sequence from clone 90

```
1       CAACGATTAA ACTTTCCTTC GGGAAAGATA TMAAGTGGCG TACGGGTGAG
51      TAACACGTAA GTAATCTACC TTTGGGTGGG GGATAACTCA GGGAAACTTG
101     AGCTAATACC GCATAATGCA GCGGCATCAT ATGATGACGG TTGTTAAAGC
151     ATTTATGTGC CTAAAGAGGA GCTTGCGGCA GATTAGCTAG TTGGTAAGGT
201     AATGGCTTAC CAAGGCAACG ATCTGTAGCC GACCTGAGAG GGTGGTCGGT
251     CACACTGACA CTGAATAACG GGTCAGACTC CTACGGGAGG CAGCAGTCGG
301     GAATTTTGGG CAATGGGCGA AAGCCTGACC CAGCAACGCC GCGTGAAGGA
351     TGAAGTCTTT CGGGATGTAA ACTTCGGAAA TATAGGAAGA ATAAATGACG
401     GTACTATATC TAAGGTCCGG CTAACTACGT GCCAGCAGCC GCGGTAATAC
451     GTAGGGACCA AGCGTTGTTC GGATTTACTG GGCGTAAAGG GTGCGTAGGC
501     GGCGTGACAA GTCACTTGTG AAATCTCCGG GCTTAACTCG GAACTGCCAA
551     GTGATACTGT CGTGCTAGAG TACAGAAAGG GTAACTGGAA TTCTTGGTGT
601     AGCGGTGAAA TGCGTAGATA TCAAGAGGAA CACCTGAGGC GAAGGCGAGT
651     TACTAGGCTG ATACTGACGC TGAGGCACGA AAGCT
```

Sequence from clone 92

```
1       GTAATACATC GGAACGTGCC CAGTAGTGGG GGATAGCTCG KCNCCCGCCG
51      GATTAATACC GCATACGACC TACGGGTGAA AGCGGGGGAT CGCAAGACCT
101     CGCGCTATTG GAGCGGCCGA TGGCAGATTA GCTTGTTGGT GGGGTAAAAG
151     CCTACCAAGG CGACGATCTG TAGCTGGTCT GAGAGGACGA CCAGCCACAC
201     TGGGACTGAG ACACGGCCCA GACTCCTACG GGAGGCAGCA GTGGGGAATT
251     TTGGACAATG GGCGCAAGCC TGATCCAGCA ATGCCGCGTG TGTGATGAAG
301     GCCTTCGGGT TGTAAAGCAC TTTTAGTGGG AACGAAACGG TCCGGGCCAA
351     TACCCTGGAT TACTGACGGT ACCCGCAGAA TAAGCACCGG CCAACTACGT
401     GCCAGCAGCC GCGGTAATAC GGAGGGTGCG AGCGTTATCC GGAATCACTG
451     GCGCGTAAAG GGCGCGTAGG CGGTTTGTCA AGTCCGATGT TAAAGACCGG
501     GGCTCAACCC CGACACGGCG TTGGATACTG ACGAGCTTGA CGACTGGAGA
551     GGGAGGTAGA ATTACCAGAG TAGCGGTGGA ATGCGTAGAT ACTGGTAGGA
601     ATACCCATAG CGAAGGCAGC CTTCTGGACA GTTAG
```

Sequence from clone 94

```
1       TCGAACGAGA AAAGCCCTTC GGGGTTAGTA AAGTGGCGAA CGGGTGCGTA
51      ACACGTGGGC AATCTGCCCC TCGCAGGGGG ACAACCGGAG GAAACTCCGG
101     CTAATACCCC GTAAGCTTTC AGGGTCGCAT GGCCTTGTAA GGAAAGGTAG
151     CTTCGGCCAT CCGGCGAGGG ATGAGCCCGC GGTACATTAG CTAGTTGGTG
201     GGGTAACGGC CTACCAAGGC GACGATGTAT AGCTGGTCTG AGAGGATGAT
251     CAGCCACACT GGGACTGAGA CACGGCCCAG ACTCCTACGG GAGGCAGCAG
301     TCGGGAATCT TGCACAATGG GCGAAAGCCT GATGCAGCAA CACCGTGTGA
351     GCGAGGAAGG CCTTCGGGTC GTAAAGCTCT GTTGTTGGGG AAGAAGGGCG
401     AAGGGTTAAT AGCCCCTAGC TTGACGGTAC CCTTCGAGGA AGCCCCAGCT
451     AACTACGTGC CAGCAGCCGC GGTAATACGT AGGGGGCGAG CGTTGTCCGG
501     AATTATTGGG CGTAAAGAGC GTGTAGGCGG TTCGGTAAGT CTGTCGTGAA
551     AACCTGGGGC TCAACCCCGG GCGTGCGATG GATACTGCCG
```

Sequence from clone 98

```
1       GTAAGGCTCC TTCGGGAGTA CACGAGCGGC GAACGGGTGA GTAACACGTG
51      AGCAATCTGC CCTTCACACG GGGATAACTT CGGGAAACCG ATGCTAATAC
101     CCGATACGAC CACTTCAGGC ATCTGATGGT GGTGGAAAGT TCCGGCGGTG
151     AAGGATGAGC TCGCGGCCTA TCAGCTTGTT GGTGGGGTAA TGGCCCACCA
201     AGGCAACGAC GGGTAGCCGG CCTGAGAGGG TGACCGGCCA CACTGGGACT
251     GAGACACGGC CCAGACTCCT ACGGGAGGCA GCAGTGGGGA ATATTGGACA
301     ATGGGCGAAA GCCTGATCCA GCAACGCCGC GTGAGGGATG ACGGCCTTCG
351     GGTTGTAAAC CTCTTTCAGC AGGGACGAAG CGAAAGTGAC GGTACCTGCA
401     GAAGAAGCAC CGGCCAACTA CGTGCCAGCA GCCGCGGTAA TACGTAGGGT
451     GCGAGCGTTG TCCGGAATTA TTGGGCGTAA AGGGCTCGTA GGCGGTTTGT
501     CACGTCGGGA GTGAAAACTC AGGGCTTAAC CCTGAGCCTG CTTCCGATAC
551     GGGCAGACTA GAGGTATGCA GGGGAGAACG GAATTCCTGG TGTAGCGGTG
601     AAATGCGCAG ATATCAGGAG GAACACCGGT GGCGAAGGCG GTTCTCTGG
```

Sequence from clone 104

```
1       ACACGTGAAG AAACCTGCCC TGCAGACCGG AATAACCACT NCCAAACTGT
51      GGCTAATGCC GGATGACCTC AGCGGTCCGC ATGGACCGCA GAGCAAATGG
101     TCAGCCGCTG CAGGATGGCC TCGCGGCCTA TCANCTTGTT GGTGGGGTAA
151     TGGCCCACCA AGGCTCCGAC GGGTAGCTGG CGTGAGAGCG CGACCAGCCA
201     CACTGGGACT GAGACACGGC CCAGACTCCT ACGGGAGGCA GCAGTGGGGA
251     ATCTTGCTCA ATGGGCGAAA GCCTGAAGCA GCGACGCCGC GTGCGGGAAG
301     AAGGCCTTCG GGTTGTAAAC CGCTTTCAGG AGGGAAGAAG CGAAAGTGAC
351     GGTACCTCCA GAAGAAGCCC CGGCCAACTA CGTGCCAGCA GCCGCNGTAT
401     ACGTANGGGG CAAGCGTTGT CCGGAATTAT TGGGCGTAAA GAGCTCGTAN
451     GCNGTCCATT AAGTCGGATG TGAATCTCAG GGCTCAACCC TGAAATTGCA
501     TCCGATACTG TT
```

Sequences from MVT 9 16S rDNA clone library

Sequence from clone 4

```
1       CGGGTGAGTA CACGTGGGCA ACCTGCCCCT CGCAGGGGAC AACCGGAGGA
51      AACTCCGGCT AATACCCCGA TACGCGTTGT TGGATCGCAT GGTCCGGCAA
101     GGAAAGGTAG CTTCGGCCAT CCGGCGAGGG ATGGGCCCGC GTTGCATTAG
151     CGTAGTTGGT GGGGTAACGG CCCACCAAGG CAACGAGTGC GTAGCTGGTC
201     TGAGAGGATG ATCAGCCAGA CTGGGACTGA GACACGGCCC AGACTCCATA
251     CGGGAGGCAG CAGCCAGGAA TCTTGGGCAA TGGGCGAAAG CCTGACCCAG
301     CAACACCGTG TGGGTGACGA AGGCCTTAGG GTCGTAAAGC CCTGTTGATA
351     GGGACGAAGG GCGAAGGGTT AATAGCCCCC AGCTTGACGG TACCTTTCGA
401     GGAAAGCCCC GGCTAACTAC GTGCCAGCAG CCGCGGTAAT ACGTAGGGGC
451     GAGCGTTGTC CGGAATTATT GGGCGTAAAG AGCGTGTAGG CGGTTCGGTA
501     AGTCTGCTGT GAAATCCTAG GGCTTCAAAC CCCTGCNGNA CNGTTGCACN
551     ANAGCGGAAT ACTGCCGGGG CTAGAGGGT
```

Sequence from clone 10

```
1       GTAAGGCGAC GGCAATCATC TCACGGTTGG GTATAGCCGC GAGAAATCGC
51      GGGTAATCCC CAGCGACGCA GGGTGTCGGC ATCGACGCCC TGCCAAAGGC
101     TCGCCGCCGT GGGACGAGCC GTCGTGGTAT TAGGTTGTTG GCGGGGTAAC
151     GGCCCACCAA GCCTGCGATG CCTACCGGGC GTGCGAGCGT GGCCCGGCAC
201     ACTGGGACTG AGACACTGCC CAGACTCCTA TGGGAGGCTG CAGTCGAGAA
251     TCTTCGGCAA TGGGCGCAAG CCTGACCGAG CGACGCCGCG TGGAGGACGA
301     AGGCCTTCGG GTTGTAAACT CCTGTCGAGG GGAAGGAAGG GGCCGCAAGG
351     CCCTTGACCG CTCCCTGGAG GAAGCACGGG CTAAGTTCGT GCCAGCAGCC
401     GCGGTAAGAC GAACCGTGCG AACGTTATTC GGAATCACTG GGCTTAAAGC
451     GCGTGTAGGC GGGTCGGTGC GTCGGCCGTT GAAATCCCCC GGCTCAACCG
501     GGGAAGTGGC GCCGATACGA CCGGCCTGGA GACGACGTAN CGGGGAACTG
551     GAACTTCCGG TGGAGCGGNG AAATGCGTTG AGATCGGAAG AACGCCGNGG
601     CGAAAGCGAG TTCCTGGACG TCGGCTG
```

Sequence from clone 13

```
1       CCGCGGCGAA CGGGTGAGTA ACACGTGAAC ATCTGTCCCT ACATTCCGGA
51      TAATTGGCCG AAAGGCCTTG TAATACGGGC GAGGATGGTG GTGAGGCATC
101     TCACTATCAG GAAGGTGAAG CGCAAGCTTT GCCGTGCAGG AGGGGTTCGC
151     GGCCTATCAG TTAGTTGGTG GGGTAACGGC CTACCAAGAC GACGACGGGT
201     AGCTGGTCTG AGAGGATGGT CAGCCACATT GGAACTGAGA CACTGTCCAG
251     ACCCCTACGG GAGGCTGCAG TCGAGAATCT TGGGCAATGC ACGAAAGTGT
301     GACCCAGCGA CGCCGCGTGG AGGATGAAGG CCCTTGGGTT GTAAACTCCT
351     TTTAGGGGAG AAGAACGCTC CTTCGGGAGC TTGACGGTAC CCCCTGAATA
401     AGCCACGGCT AACTACGTGC CAGCAGCCGC GGTAATACGT AGGTGGCAAG
451     CGTTGTCCGG ATTTACTGGG CGTAAAGCGT GTGTAGGCGG ACCTTTAAGT
501     AGAAAGTGAA AGGTCGGAGC TCAACTCCTA CACTGCTCTC TATACTGGAG
551     GTCTTGAGTG TCGGAGAGGA AGATGGA
```

Sequence from clone 14

```
1       GCAAGGCCAG TGGCCCAAGG GGTGATTTAA GGCGCCGTAA CCAACCCCAC
51      GGTCGGGGCA TAGCCGCGGG AAACCGCGGG TAATTCTCGG CGACGCCCTA
101     TTCCGGCATC GGGACGGGGC CAAAGGTGCG ATTCCTGCCG TGGGACGGGC
151     CGTCGTGGTA TTAGCTTGTT GGCGGGGTGA CGGCCCACCA AGGCTGCGAT
201     GCCTACCGGG CGTGCGAGCG TGGCCCGGCA CACTGGGACT GAGACACTGC
251     CCAGACTCCT ACGGGAGGCT GCAGTCGAGA ATCTTCGGCA ATGGGCGCAA
301     GCCTGACCGA GCGGCGCCGC GTGGAGGACG AAGGCCTTCG GGTTGTAAAC
351     TCCTGTCGAG GGGGAGGAAG GGGGCGTGCA GAGCGTTCCT TGACCGATCC
401     CTGGAGGAAG CACGGGCTAA GTTCGTGCCA GCAGCCGCGG TAAGACGAAC
451     CGTGCGAACG TTATTCGGAA TCACTGGGCT TAAAGCGTGC GTAGGCGGGC
501     CGCCGCATCG GTCGCTGAAA TCCCCCGGCT TAACCGGGGA AGTGGCGCCG
551     AGATGGGCGG TCTGGACGGG GCGTAGGGGG ATCTGGAACT CCCGGTGGAG
601     CGGTGAAATG CGTTGAGATC GGGAGGA
```

Sequence from clone 17

```
1       GTACACGAGC GGAGAACGGG TGAGTAACAC GTGGGTAACC TGCCTCAGCT
51      CTGGGATAAG CCCGGGAAAC TGGGTCTAAT ACCGGATATG ACCTCGCATC
101     GCATGGTGTG GGGTGGAAAG CCTTGTGCGG CTGAGGATGG GCCCGCGGCC
151     TATCAGCTTG TTGGTGGGGT AGTGGCCTAC CAAGGCGACG ACGGGTAGCC
201     GGCCTGAGAG GGCGACCGGC CACACTGGGA CTGAGACACG GCCCAGACTC
251     CTACGGGAGG CAGCAGTGGG GAATATTGCG CAATGGGCGA AAGCCTGACG
301     CAGCGACGCC GCGTGAGGGA TGACGGCCTT CGGGTTGTAA ACCTCTTTCA
351     GCTCCGACGA AGCCTTCGGG TGACGGTAGG GGCAGAAGAA GCACCGGCCA
401     ACTACGTGCC AGCAGCCGCG GTAATACGTA GGGTGCAAGC GTTGTCCGGA
451     ATTATTGGGC GTAAAGAGCT CGTAGGCGGT TTGTCGCGTC GACTGTGAAA
501     ACTCAGGGCT CAACTCCGAG CTTGCAGTTG ATACGGGCAG NCTAGAGTTC
551     GGCAGGGAGA CTGGAATTCC TGGTGTAGCG GTGAAATGCG CAGATATCAG
601     GAGGAACACC GGTGGCGAAG GCGGAAACGC GTGTGCTAC
```

Sequence from clone 19

1 GCTTGCGGCG TGCCTAAGAA ATGCAAGTCG AACGGACATT CCAGCAATGG
51 GGTGCTAGTG GCGAACGGTC GCGTAACACG TAGGCAACCT GCCCTGAAGT
101 GGGGGACAAC AGCCCGAAAG GGCTGCTAAT ACCGCATGTG AACAACGAAT
151 CACATGGTTT GTTGTTCAAA GGCTATGGCA ACATGGTCGC TTTGGGATGG
201 GCTTGCGGCC TATCAGGTAG TTGGTGGGGT AATGGCCCAC CAAGCCGACG
251 ACGGGTAGCT GGTCTGAGAG GACGATCAGC CGGATTGGGA CTGAGATACG
301 GCCCAGACTC CTACGGGGGG CAGCAATTAG GAATCTTGCG CAATGGGCGA
351 AAGCCTGACG CAGCGACGCC GCGTGCGGGA TGAAGGCCTT CGGGTCGTAA
401 ACCGCTTTTA ACGGGGAAGA AGAATGTGAC GGTACCCGTT GAATAAGCCC
451 CGGCTAACTA CGTGCCAGCA GCCGCGGTAA TACGTAGGGG GCGAGCGTTG
501 TCCGAAGTTA CTGGGCGTAA AGCGCGCGTA GGCGGTTGCC TAAGTCTGGG
551 GTGAAAGGTT CAGGGCTTAA CCCGAACAGT GCCTTGGATA CTGGGCGACT
601 TGAGTGCCGA AGAGGAAAGC GGAATTCCTG GTGTAGCGGT GAAATGCGTA
651 GATATCAGGA GGAACACCGA TGGCGAAGG

Sequence from clone 21

1 GGGTGAGTAA CACGTGGGTA ATCTACTCTG GGTGGGGGAT AACTCTGGGA
51 AACCGGAGCT AATACCGCAT AAGCCTGAAA AGGGAAAGGG GAAATTCGCC
101 GAGAGAGGAG CCCGCGGCCG ATTAGCTAGT TGGTGGGGTA AAGGCCTACC
151 AAGGCGACGA TCGGTAGCCG GCCTGAGAGG GCACACGGCC ACACTGGCAC
201 TGAAACACGG GCCAGACTCC TACGGGAGGC AGCAGTGGGG AATCTTGCAC
251 AATGGGGGCA ACCCTGATGC AGCGACGCCG CGTGAGCGAT TAAGCCCTTC
301 GGGGTGTAAA GCTCTTTCGG CAGGAACGAT CATGACGGTA CCTGAAGAAG
351 AAGCTGCGGC TAACTACGTG CCAGCAGCCG CGGTAATACG TAGGCAGCGA
401 GCGTTGTCGG AGTTTACTGG GCGTAAGGGT GCGTAGGCGG GTTTTCTTAA
451 GGTCTTGGTG TGAAATCTCC CGGGTCA

Sequence from clone 23

1 ACACGTGAGA AACCTGTCCC GAACTTGGGA ATAACAGCCG AAAACSACTG
51 CTAATACCGA ATATCTTCGT AACGTCGCAT GGCGATTCGA AGAAAGCTTT
101 ATGCGGTTTG GGAGGGTCTC GCGGCCTATC AGCTTGTTGG TGAGGTAATG
151 GCTCACCAAG GCATCGACGG GTAGCTGGTC TGAGAGGATG ATCAGCCACA
201 CTGGGACTGA GACACGGCCC AGACTCCTAC GGGAGGCAGC AGTGGGGAAT
251 ATTGCACAAT GGGCGAAAGC CTGATGCAGC GATGCCGCGT GCGGGAAGAA
301 GGCCCTAGGG TTGTAAACCG CTTTCAGTAG GGAAGAAAAT GACGGTACCT
351 ACAGAAGAAG GTGCGGCCAA CTACGTGCCA GCAGCCGCGG TGACACGTAG
401 GCACCAAGCG TTGTCCGGAT TTATTGGGCG TAAAGAGCTC GTAGGCGGTT
451 TGGTAAGTCG GGTGTGAAAA CTCTGGGCTC AACCCAGAGA GGCCACTCGA
501 TACTGCCATG ACTTGAGTAC GGTAGGGGAG TGGGGAATTT CTAGTGTAGC
551 GGTGAAATGC GCAGATATTA GAAGGAACAC CAGTGGCGAA GGCGCCACTC
601 TGGGCCGTAA CTGACG

Sequence from clone 26

1 GGCAGAGTTA CGAACGGGTG AGTAAAGTGG GTGACTGCCC CGATGACCGG
51 GACAACCCGA GGAAANTCGG GCTAATACCG GGATGTGTCC ACCTCGCGAC
101 AGCGGGGCGG GCAAAGGTAG CTTCGGCCTC CGCATCGGGA TGGGCCCGCG
151 GCCCATTAGC TTGTTGGTGA GGTAACGGCT CACCAAGGCG ACNATGGGTA
201 GCTGGTCTGA GAGGACGATC AGCCACACTG GGACTGAGAC ACGGCCCAGA
251 CTCCTACGGG AGGCAGCAGT GGGGAATCTT GCGCAAATGC GCGAAAGCGT
301 GACGCAGCAA CGCCGCGTTG

Sequence from clone 28

```
1       TTGGTAGCAA TCCTAAGAGT ANTTAGTGGC GAACGGGTGC GTAACACGTG
51      GCAATCTGCC GAGAAGTGGG GGATAGCTCG CCGAAAGGCG AATTAATACC
101     GCATATGACC AGAGGCGACA TCGCTTCGAA ATCAAAGGTG GCGCAAGCTA
151     CCGCTTTCCG ATGAGCCCGC GGCCTATCAG CTAGTTGGTG AAGTAACGGC
201     TCACCAAGGG CGATGACGGG TAGCTGGTCT GAGAGGACTC NACCAGTCAC
251     ACTGGAACTG AGACACGGTC CAGACACCTA CGGGTGGCAG CAGTCGAGAA
301     TTTTTCTCAA TGGGGGAAAC CCTGAAGGAG CGACGCCGCG TGGAGGATGA
351     AGGCTTTCGG GTTGTAAAAC TCCTGTCATT TTGAGAACAC GGTGCCGAAC
401     AAGTAACTAC TGTCGGGCTT GATAGTATCC GAAGAGGAAG AGACGGCTAA
451     CTCTGTGCCA GCAGCCGCGG TAATACCGAG GTCTCAAGCG TTGTTCGGAT
501     TC
```

Sequence from clone 37

```
1       TGCGTAACAC GTGGGTAATT TGCCATGAAG TCTGGAATAA CTTGCTGAAA
51      GGCGAGCTAA TGCCGGATGT GATTTTCGGG AAGCATTTCT TGAAACTCAA
101     AGTTGGGGAC CGCAAGGCCT GACGCTTCTT GATAAGCCCG CGGCCTATCA
151     GCTAGTTGGT GAGGTAATGG CTCACCAAGG CTAAGACGGG TAGCTGGTCT
201     GAGAGGACGA CCAGCCACAC TGGAACTGAG ACACGGTCCA GACACCTACG
251     GGTGGCAGCA GTCGAGAATT TTTCACAATG GGCGAAAGCC TGATGGAGCG
301     ACGCCGCGTG GGGGATGAAT GGCTTCGGCC CGTAAACCCC TGTCATTTGC
351     GAACAAACCT TACCGGTTAA CAACCGTTGA GCTGATTGTA GCGGAAGAGG
401     AAGGGACGGC TAACTCTGTG CCAGCAGCCG CGGTAATACA GAGGTCCCAA
451     GCGTTGTTCG GATTCACTGG GCGTAAAGGG TGCGTAGGTG GTGGGGTAAG
501     TCGGATGTGA AATCTCCGGG CTCAACCCGG AAATGGCATT GGAAACTACC
551     TTGCTAGAGG ATTTGAGGGG GGATTGGAAT ACTTGGTGTA
```

Sequence from clone 41

```
1       ATTTGGTGGC GACCGKCAAA CGGGTGCGGA ACACGTACAG AACCTTCCTT
51      TAAGTGGGGG ATAGCCCAGA GAAATTTGGA TTAATACCCC GTAACATTAT
101     GAAGTGGCAT CACCTTATAA TTATAGATTT ATCGCTTAGA GATGGCTGTG
151     CGGCTGATTA GGTAGTTGGT GTGGGTAACG GCCCACCAAG CCTTCGATCA
201     GTAACTGGTG TGAGAGCACG ACCAGTCACA CGGGCACTGA GACACGGGCC
251     CGACTCCTAC GGGAGGCAGC AGTAAGGAAT ATTGGTCAAT GGACGCAAGT
301     CTGAACCAGC CATGCCGCGT GAAGGATGAA GGTCCTCTGG ATTGTAAACT
351     TCTTTT
```

Sequence from clone 43

```
1       CGGGTGAGTA ACACGTGAAT AACCTGCCCT CACATTCTGG ATAATTCACC
51      GAAAGGTGTT GTAATACAGG CGAGGATTCT TAAGAGGCAT TTCTTGAGAA
101     GGGAAGGCGC AAGCCGTGCG AGGAGGGGTT CGCGGATTAT CAGGTAGTTG
151     GTGAGGTAAC GGCTCACCAA GCCGACGACG ATTAGCTGGT CTGAGAGGAT
201     GGTCAGCCAC ATTGGGACTG AGACACTGCC CAGACTCCTA CGGGAGGCTG
251     CAGTCGAGAA TCTTGCACAA TGTACGAAAG TATGATGCAG CGACGCCGCG
301     TGAAGGATGA AGGCCCTCTG GGTCGTAAAC TTCTTTTATG TGGGAAGAAT
351     AAATGACGGT ACCGCATGAA TAAGCCACGG CTAACTACGT GCCAGCAGCC
401     GCGGTAATAC GTAGGTGGCA AGCGTTGTCC GGATTTACTG GGCGTAAAGA
451     GTATGTAGGC GGATGTTTAA GTAGGAAGTG AAAGGTTGGA GCTCAACTCC
501     GACACTGCTC CCTATACTGG GCATCTTGAG GGCCGGAGAG GAAAGCGGAA
551     CGACACGTGT AGCGGTGAAA TGCGTTGATA
```

Sequence from clone 44

```
1       TGGACGCGAC GAACTAGTGC TTCGTGCCTG GTGTGCAGCA GCCTGCTGAA
51      CGTGTGTGAG TAACACGTGG GCAACCTTGC CCCGATGATT CGGGACAANC
101     CGGGGAAACT CGGGCTAAGT ACCGAATGTG CTCTCCTCAC ATCAGTGAGG
151     CGTGTAAAGG AAGCTTCGGC CTCCGCATTG GGATGGGCCC CGCAGGCCCA
201     TTAGCTTGTT GGTGAGGTAA CGGCTCACCA AGGCCGNGAA TGGGTAGCTG
251     GTCTGAGAGG ACGATCAGCC ACACTTGGGA CTTGAGACAC GGNCCAGAAA
301     CTTCCCTTAC GTGTGTATGT GNCNACGGCA GTCGNGNGTG AAACTTCTTT
351     GCTNCAATTG ACTGCCGAAA TCA
```

Sequence from clone 46

```
1       GCAGTCGAAC GATTAACTTT CCTTCGCGGA AAGATATACA AGTGGCGCAC
51      GGGTGAGTAC ACGGTAGTGT AATGTACCTT TGGNGTGGGG AATAACTTAG
101     GGAAACTTAA GCTAATACCG CATAATGCAG CGGCTCCTTC GGGAGACAGT
151     TGTTAAAGAT TTATCGCCTA AAGAGCAGCC TGCGGCAGAT TAGCTAGTTG
201     GTAAGTGTAA TGGCTTACCA AGGCTACGAT CTGTATCCGA CCTGAGAGGG
251     TGGTCGGACA CCACTGACAC TNAAATTTAA CCGGTTCCAA ATCTCCTCTN
301     TAACGGGAAA AGCGCAAACA TCTCCGGAAA ATTTGGGGGC CACCAATGGC
351     GCCGAAACC
```

Sequence from clone 48

```
1       ATATAAAGTG KCGCACGGGT GAGTAACACG TAGGTAATCT ACCTTTGAGT
51      GGGGAATAAC GTTCGGAAAC GAACGCTAAT ACCGCATAAT GCAGCGGCAC
101     CGCAAGGTGA CAGTTGTTAA AGGAGCAATC CGCTTAAAGA GGAGCCTGCG
151     GCAGATTAGC TAGTTGGTAA GGTAATGGCT TACCAAGGCT ACGATCTGTA
201     ACCGACCTGA GAGGGTGGTC GGTCACACTG ACACTGAATA ACGGGTCAGA
251     CTCCTACGGG AGGCAGCAGT CGGGAATTTT GGGCAATGGG CGAAAGCCTG
301     ACCCAGCAAC GCCGCGTGAA GGATGAAGTA TTTCGGTATG TAAACTTCGA
351     AAGAATAGGA AGAATAAATG ACGGTACTAT TTATA
```

Sequence from clone 50

```
1       GTGGAGCGAC GAACGGGCTT CGGCCCGGGG TCAAAGCCTG CGAACGGGTG
51      AGTAACACGT GGGTAACCTG CCCCGATGAC CGGGACAACC CGAGGAAACC
101     CTGGGCTNGT ACCGGATGCG CTCGGTTCAC ACCAGTGGGC CGAGCAAAGG
151     TAGGTTCGGC CGTCCGCCTC GGGATGGGCC CGCAGAGCNG CGATTAGCTT
201     GTTGGTGGGG TAACGGACTT ACCAAAGGNT AACGAANGGC CGTTAACAGC
251     CTTTGGCGCT CCTTTGAAGT
```

Sequence from clone 51

```
1       GCGGCAGACG GGAGAGTAAC ACGTGGGAAC GCGCCCTTCG GTTCGGAATA
51      ACTCAGGGAA ACTTGAGCTA ATACCGGATA CGCCCTTACG GGGAAAGATT
101     TATTGCCGAA GGAACGGCCC GCGTCGGATT AGCTAGTTGG TGAGGTAATG
151     GCTCACCAAG GCAACGATCC GTAGCTGGTC TAAGAGGATG ATCAGCCTCA
201     CTGGGACTGA GACACGGCCC AGACTCCTAC GGGAGGCAGC AGTGGGGAAT
251     ATTGGACAAT GGGCGAAAGC CTGATCCAGC CATGCCGCGT GGATGATGAA
301     GGCCTTAGGG TTGTAAAGTC CTTTTAACGG GGAAGATAAT GACGGTACCC
351     GTAGAATAAG CCCCGGCTAA CTTCGTGCCA GCAGCCGCGG TAATACGAAG
401     GGGGCTAGCG TTGCTCGGAA TTACTGGGCG TAAAGCGCAC GTAGGCGGAT
451     TGTTAAGTCG GGGGTGAAAT CCTGGAGCTC AACTCCAGAA CTGCCTTCGA
501     AACTGGCGAT CTTGAGTCCG GGAGAGGTGA GTGGAACTGC GAGTGTAGAG
551     GTGAAATTCG TAGATATTCG CAAGAACACC AGTGGCGAAG GCGGCTCACT
601     GGCCCGGTAC
```

Sequence from clone 55

```
1       GCCTTCGGGT CTAGTGGCGC ACGGGTGCGT AACGCGTGGG AATCTGCCCT
51      TGGGTTCGGG ATAACAGTTG GAAACGACTG CTAATACCGG ATGATGACTT
101     CGGTCCAAAG ATTTATCGCC CAAGGATGAG CCCGCGTAGG ATTAGCTTGT
151     TGGTGAGGTA AGAGCTCACC AAGGCGACGA TCCTTAGCTG GTCTGAGAGG
201     ATGATCAGCC ACACTGGGAC TGAGACACGG CCCAGACTCC TACGGGAGGC
251     AGCAGTGGGG AATATTGGAC AATGGGCGAA AGCCTGATCC AGCAATGCCG
301     CGTGAGTGAT GAAGGCCTTA GGGTTGTAAA GCTCTTTTAC CCGGGATGAT
351     AATGGCAGTA CCGGGAGAAT AAGCCCCGGC TAACTCCGTG CCAGCAGCCG
401     CGGTAATACG GAGGGGGCTA GCGTTGTTCG GAATTACTGG GCGTAAAGCG
451     CGCGTAGGCG GCTTTGTAAG TTAGGGGTGA AAGCCCGGAG CTCAACTCCG
501     GAATTGCCTT TAAGACTGCA TCGCTAGAAT CATGGAGAGG TGAGTGGAAT
551     TCCGAGTGTA GAGGTGAAAT TCGTAGATAT TCGGAAGAAC ACCAGTGGCG
601     AAGGCGACTC ACTGGACATG TATTGACGCT GAGGTGCGAA AGCGTGGGGA
651     GCAAACAGGA TTAGATACCC TGGTAGTCCA CGCC
```

Sequence from clone 58

```
1       GGTGGCGAGT GGCGGACGGG TGAGGAATAC ATCGGAATCT ACTCTGTCGT
51      GGGGGATAAC GTAGGGAAAC TTACGCTAAT ACCGCATACG ACCTACGGGT
101     GAAAGCAGGG GATCTTCGGA CCTTGCGCGA TTGAATGAGC CGATGTCGGA
151     TTAGCTAGTT GGCGGGGTAA AGGCCCACCA AGGCGACGAT CCGTAGCTGG
201     TCTGAGAGGA TGATCAGCCA CACTGGAACT GAGACACGGT CCAGACTCCT
251     ACGGGAGGCA GCAGTGGGGA ATATTGGACA ATGGGCGCAA GCCTGATCCA
301     GCCATACCGC GTGGGTGAAG AAGGCCTTCG GGTTGTAAAG CCCTTTTGTT
351     GGGAAAGAAA TCCAGCTGGC TAATACCCGG TTGGGATGAC GGTACCCAAA
401     GAATAAGCAC CGGCTAACTT CGTGCCAGCA GCCGCGGTAA TACGAAGGGT
451     GCAAGCGTTA CTCGGAATTA CTGGGCGTAA AGCGTGCGTA GGTGGTCGTT
501     TAAGTCCGTT GTGAAAGCCC TGGGCTCAAC CTGGGAACTG CAGTGGATAC
551     TGGGCGACTA GAGTGTGGTA GAGGGTAGCG GAATTCCTGG TGTAGCAGTG
601     AAATGCGTAG AGATCAGGAG GAACATCCAT GGCGAAGGCA GCTACCTGGA
651     CC
```

Sequence from clone 61

```
1       GAGGTAATGT ACCTTTGGGT CGGGAWTAAC YTAGGGAAAC TTAAGCTAAT
51      ACCGCATAAT GCAGCGGCTC CTTCGGGAGA CAGTTGTTAA AGATTTATCG
101     CCTAAAGAGC AGCCTGCGGC AGATTAGCTA GTTGGTAAGG TAACGGCTTA
151     CCAAGGCTAC GATCTGTATC CGACCTGAGA GGGTGGTCGG ACNYWCTGAC
201     ACTGAATAAC GGGTCAGACT CCTACGGGAG GCAGCAGTCG GGAATTTTGG
251     GCAATGGGCG AAAGCCTGAC CCAGCAACGC CGCGTGAAGG ATGAAGTCTT
301     TCGGGATGTA AACTTCGTAA GAATAGGAAG AATAAATGAC GGTACTATTT
351     GTAAGGTCCG GCTAACTACG TGCCAGCAGC CGCGGTAATA CGTAGGGACC
401     AAGCGTTGTT CGGATTTACT GGGCGTAAAG GGCGCGTAGG CGGCGTGACA
451     AGTCAATTGT GAAATCTCCG GGCTTAACTC GGAACGGTCA ATTGATACTG
501     TTGT
```

Sequence from clone 62

```
1       TCGGGAGTAC ACGAGCGGCG AACGGGTGAG TAACACGTGA GCAATCTGCC
51      CTTCACACGG GGATAACTTC GGGAAACCGA TGCTAATACC CGATACGACC
101     ACTTCAGGCA TCTGATGGTG GTGGAAAGTT CCGGCGGTGA AGGATGAGCT
151     CGCGGCCTAT CAGCTTGTTG GTGGGGTAAT GGCCCACCAA GGCAACGACG
201     GGTAGCCGGC CTGAGAGGGT GACCGGCCAC ACTGGGACTG AGACACGGCC
251     CAGACTCCTA CGGGAGGCAG CAGTGGGGAA TATTGGACAA TGGGCGAAAG
301     CCTGATCCAG CAACGCCGCG TGAGGGATGA CGGCCTTCGG GTTGTAAACC
351     TCTTTCAGCA GGGACGAAGC GAAAGTGACG GTACCTGCAG AAGAAGCACC
401     GGCCAACTAC GTGCCAGCAG CCGCGGTAAT ACGTAGGGTG CGAGCGTTGT
451     CCGGAATTAT TGGGCGTAAA GGGCTCGTAG GCGGTTTGTC ACGTCGGGAG
501     TGAAAACTCA GGGCTTAACC CTGAGCCTGC TTCCGATACG GGCAGACTAG
551     AGGTATGCAG GGGAGAACGG AATTCCTGGT GTAGCGGTGA AATGCGCAGA
601     TATCAGGAGG AACACCGGTG GCGAAGGCGG TTCTCTGGGC ATTACCTGAC
651     GCT
```

Sequence from clone 63

```
1       GCGAACGGGT GAGTAATACA TCGGAACGTA TCCTATAGCG GGGGATAACC
51      TCTCGAAAGA GAGGCTAATA CCGCATACGA CCCATGGGTG AAAGAGGGGG
101     ATCGCAAGAC CTCTCACTAT TGGAGCGGCC GATGTCGGAT TAGCTAGTTG
151     GCGGGGTAAA AGCCCACCAA GGCTACGATC CGTAGCTGGT CTGAGAGGAC
201     GACCAGCCAC ACTGGAACTG AGACACGGTC CAGACTCCTA CGGGAGGCAG
251     CAGTGGGGAA TTTTGGACAA TGGGCGCAAG CCTGATCCAG CCATGCCGCG
301     TGAGTGAAGA AGGCCTTCGG GTTGTAAAGC TCTTTCGGCG GGGACGAAAA
351     GATTCGCGTT AACACCGCGG ATCCATGACG GTACCCGCAG AAGAAGCACC
401     GGCTAACTAC GTGCCAGCAG CCGCGGTAAT ACGTAGGGTG CAGGCGTTAA
451     TCGGAATTAC TGGGCGTAAA GCGTGCGCAG GCGGTCTTTT AAGTCAGATG
501     TGAAATCCCC GGGCTTAACC TGGGAACTGC GTTTGAAACT GGAAGGCTAG
551     AGTGTGGCAG AGGGGGGTGG AATTCCACGT GTAGCAGTGA AATGCGTAGA
601     TATGTGGAGG AACAMCGATG GCGAAAGGCA GCCCCCTGGG CTAACAC
```

Sequence from clone 68

```
1       ACCCTAACCG GCTTCTTTTA CGAGCACCGG CTTCAGGTCT ACCAAACTTC
51      CATGGCTTGA CGGGCGGTGT GTACAAGGCC CGGGAACGTA TTCACCGCGT
101     CATTGCTGAT ACGCGATTAC TAGTGATTCC AGCTTCACGG AGTCGAGTTG
151     CAGACTCCGA TCCGAACTGA GAACGGCTTT TCGGGATTGG CGCACCATCG
201     CTGGTTGGCA ACCCGCTGTA CCGTCCATTG TAGCACGTGT GTAGCCCTAG
251     GCGTAAGGGC CATGATGACC TGACGTCGTC CCCGCCTTCC TCACTGCTTG
301     CGCAGGCAGT CTGTCTAGAG TCCCCGCCAT TACGCGCTGG CAACTAAACA
351     TAGGGGTTGC GCTCGTTGCG GGACTTAACC CAACACCTCA CGGCACGAGC
401     TGACGACGGC CATGCAGCAC CTTGCTTTGT GTCCCGAAGG AAAGGTTCAT
451     CTCTGAACCG GTCACGCGCA TTCTAGCCTA GGTAAGGTTC CTCGCGTATC
501     ATCGAATTAA ACCACATGCT CCACCACTTG TGCGGGCCCC CGTCAATTCT
551     TTTGA
```

Sequence from clone 70

```
1       CCCAGTCACG AATCCTACCG TGGTAAGCGC CCCCCTTGCG GTTAAGCTAC
51      CTACTTCTGG TAAAACCCGC TCCCATGGTG TGACGGGCGG TGTGTACAAG
101     ACCCGGGAAC GTATTCACCG CGACATGCTG ATCCGCGATT ACTAGCGATT
151     CCAACTTCAT GTAGTCGAGT TGCAGACTAC AATCCGGACT ACGATACACT
201     TTCTGGGATT AGCTCCCCCT CGCGGGTTGG CGGCCCTCTG TATGTACCAT
251     TGTATGACGT GTGAAGCCCT ACCCATAAGG GCCATGAGGA CTTGACGTCA
301     TCCCCACCTT CCTCCGGTTT GTCACCGGCA GTCTCATTAG AGTGCTCTTT
351     CGTAGCAACT AATGACAAGG GTTGCGCTCG TTGCGGGACT TAACCCAACA
401     TCTCACGACA CGAGCTGACG ACAGCCATGC AGCACCTGTG TTACGGCTCT
451     CTTTCGAGCA CACCTCGATC TCTCGTGGCT TCCGTACATG TCAAGGGTAG
501     GTAAGGTTTT TCGCGTTGCA TCGAATTAAT CCACATCATC CACCGCTTGT
551     GCGGGTCCCC GTCAATTCCT TTGAGTTTTA ATCTTGCGAC CGTACTCCCC
601     AGGCGGTCTA CTTCACGCGT
```

Sequences from MVT 12 16S rDNA clone library

Sequence from clone 3

```
1       AACGAGGCCT TCGGGTCTAG TGGCGCACGG GTGCGTAACG CGTGGGAATC
51      TGCCCTTGGG TTCGGGATAA CAGTTGGAAA CGACTGCTAA TACCGGATGA
101     TGACTTCGGT CCAAAGATTT ATCGCCCAAG GATGAGCCCG CGTAGGATTA
151     GCTTGTTGGT GAGGTAAGAG CTCACCAAGG CGACGATCCT TAGCTGGTCT
201     GAGAGGATGA TCAGCCACAC TGGGACTGAG ACACGGCCCA GACTCCTACG
251     GGAGGCAGCA GTGGGGAATA TTGGACAATG GGCGAAAGCC TGATCCAGCA
301     ATGCCGCGTG AGTGATGAAG GCCTTAGGGT TGTAAAGCTC TTTTACCCGG
351     GATGATAATG GCAGTACCGG GAGAATAAGC CCCGGCTAAC TCCGTGCCAG
401     CAGCCGCGGT AATACGGAGG GGGCTAGCGT TGTTCGGAAT TACTGGGCGT
451     AAAGCGCGCG TAGGCGGCTT TGTAAGTTAG GGGTGAAAGC CCGGAGCTCA
501     ACTCCGGAAT TGCCTTTAAG ACTGCATCGC TAGAATCATG GAGAGGTGAG
551     TGGAATTCCG AGTGTAGAGG TGAAATTCGT AGATATTCGG AAGAACACCA
601     GTGGCGAAGG CGACTCACTG GACATGTATT GACGCTGAGG TGCGAAAGCG
651     TGGGGAGCAA ACAGGATTAG ATACCCTGGT AGTCCACGCC GTAAACGATG
701     ATGACTAG
```

Sequence from clone 5_

```
1       AAAGCTCTCT TCGGAGAGTG YATAGAGTGG CGCACGGGTG AGTAACACGT
51      AAGTAATCTA CCTTTGAGTG GGGAATAACG TCCGGAAACG GACGCTAATA
101     CCGCATAATG CAGCGGCATC GCAAGATGAC AGTTGTTAAA GGAATTTATT
151     TCGCTTGAAG AGGAGCTTGC GGCAGATTAG CTAGTTGGTA AGGTAATGGC
201     TTACCAAGGC TACGATCTGT AACCGGTCTT AGAGGACGGT CGGTCACACT
251     GACACTGAAT AACGGGTCAG ACTCCTACGG GAGGCAGCAG TCGGGAATTT
301     TGGGCAATGG GCGAAAGCCT GACCCAGCAA CGCCGCGTGA AGGATGAAGT
351     ATTTCGGTAT GTAAACTTCG AAAGAATGGG AAGAATCAAT GACGGTACCA
401     TTTATAAGGT CCGGCTAACT ACGTGCCAGC AGCCGCGGTA ATACGTAGGG
451     ACCAAGCGTT GTTCGGATTT ACTGGGCGTA AAGGGCGCGT AGGCGGCTTG
501     TCAAGTCACT TGTGAAATCT CCGGGCTTAA CTCGGAACGG TCAAGTGAAA
551     CTGTCAAGCT AGAGTGTGGA AGGGGCAATC GGAATTCTTG GTGTAGCGGT
601     GAAATGCGTA GATATCAAGA GGAACACCTG AGGTGAAGAC GGGTTGCTGG
651     GCCAACACTG ACGC
```

Sequence from clone 6

```
1       AGAGGAGCTT GCTCCTYGGG TGGCGAGTGG CGGACGGGTG AGGAATACAT
51      CGGAATCTAC TCTGTCGTGG GGGATAACGT AGGGAAACTT ACGCTAATAC
101     CGCATACGAC CTACGGGTGA AAGCAGGGGA TCTTCGGACC TTGCGCGATT
151     GAATGAGCCG ATGTCGGATT AGCTAGTTGG CGGGGTAAAG GCCCACCAAG
201     GCGACGATCC GTAGCTGGTC TGAGAGGATG ATCAGCCACA CTGGAACTGA
251     GACACGGTCC AGACTCCTAC GGGAGGCAGC AGTGGGGAAT ATTGGACAAT
301     GGGCGCAAGC CTGATCCAGC CATACCGCGT GGGTGAAGAA GGCCTTCGGG
351     TTGTAAAGCC CTTTTGTTGG GAAAGAAATC CAGCTGGCTA ATACCCGGTT
401     GGGATGACGG TACCCAAAGA ATAAGCACCG GCTAACTTCG TGCCAGCAGC
451     CGCGGTAATA CGAAGGGTGC AAGCGTTACT CGGAATTACT GGGCGTAAAG
501     CGTGCGTAGG TGGTCGTTTA AGTCCGTTGT GAAAGCCCTG GGCTCAACCT
551     GGGAACTGCA GTGGATACTG GGCGACTAGA GTGTGGTAGA GGGTAGCGGA
601     ATTCCTGGTG TAGCAGTGAA ATGCGTAGAG ATCAGGAGGA ACATCCATGG
651     CGAAGGCAGC TACCTGGACC AACACTGACA CTGAGGCA
```

Sequence from clone 8

```
1       GGGCTTGCCC TGGGSCAGAG CCGCGAACGG GTGAGTAACA CGTGGGTAAC
51      GTGCCCCGAT GACTGGGACA ACCCGGGGAA ACCCGGGCTA ATACCGGATA
101     TGCCCCCTCA CGCGAGTGAG GTGTGTAAAG GAAGCTTCGG CCTCCGCATC
151     GGGATCGGCC CGCGGCGCAT TAGCTTGTTG GTGAGGTAAC GGCTTACCAA
201     GGCAACGATG CGTAGCTGGT CTGAGAGGAC GATCAGCCAC ACTGGGACTG
251     AGACACGGCC CAGACTCCTA CGGGAGGCAG CAGTGGGGAA TCTTGCGCAA
301     TGCGCGAAAG CGTGACGCAG CAACGCCGCG TGGGGGAAGA AGGCCTTCGG
351     GTTGTAAACC CCTTTCAGTT GGGACGAAGT GTGGGCGGTT AATAGCCGTT
401     CTGCATGACG GTACCTTCAC AAGAAGCCCC GGCTAACTAC GTGCCAGCAG
451     CCGCGGTAAT ACGTAGGGGG CAAGCGTTGT CCGGAATCAT TGGGCGTAAA
501     GAGCGTGTAG GCGGCCCGGT AAGTCCGTTG TGAAAGTCGA GGGCTCAACC
551     CTCGAATGCC GGCGGATACT GTCGGGCTAG AGTCCGGAAG AGGC
```

Sequence from clone 13

```
1       GTTTCTTCGG AAACCGASTA GAGTGGCGCA CGGGTGAGTA ACACGTGAGT
51      AATCTGCCTT TGGGTGGGGG ATACCAATCG GAAACGATTG TTAATACCGC
101     ATAACGCAGC GGCATCGCAA GATGACAGTT GTTAAAGCGG GGGAACGAAG
151     CAATTCGTCC TCGCGCCAGA AGAGGAGCTC GCGGCAGATT AGGTAGTTGG
201     TGAGGTAATG GCTCACCAAG CCTGCGATCT GTAACCGGCC TGAGAGGGCG
251     GTCGGTCACA CTGACACTTA GATACGGGTC AGACTCCTAC GGGAGGCAGC
301     AGTCGGGAAT TTTGGGCAAT GGGCGCAAGC CTGACCCAGC AACGCCGCGT
351     GAAGGATGAA GCATTTCGGT GTGTAAACTT CGCAAGAATA GGAAGAATAA
401     GAGTAAGCAA ATACCTTGCT CGATGACGGT ACTATTTGTA AGCCCCGGCT
451     AACTCCGTGC CAGCAGCCGC GGTAATACGG GGGGGGCAAG CGTTGTTCGG
501     ATTTACTGGG CGTAAAGGGT GCGTAGGCGG CACCACAAGT CACTTGTGAA
551     ATCTCCAAGC TCAACTTGGA ACGGTCAAGT GATACTGTGG AGCTAGAGTG
601     CAGAAGGGGC AACCGGAATT CTCGGTGTAG CGGTGAAATG CGTAGATATC
651     GAGAGGAACA CT
```

Sequence from clone 15

```
1       GAGAAAGCCC TTCGGGGTTA GTAAAGTGGC GAACGGGTGA GTAACACGTG
51      GGCAACCTGC CCCTCGCAGG GGGACAACCG GAGGAAACTC CGGCTAATAC
101     CCCGTACGCT TGTTGGATCG CATGGTCCGG CAAGGAAAGG TAGCTTCGGC
151     CATCCGGCGA GGGATGGGCC CGCGTTGCAT TAGCTAGTTG GTAGGGTAAC
201     GGCCTACCAA GGCTACGATG CGTAGCTGGT CTGAGAGGAT GATCAGCCAC
251     ACTGGGACTG AGACACGGCC CAGACTCCTA CGGGAGGCAG CAGCCAGGAA
301     TCTTGGGCAA TGGGCGAAAG CCTGACCCAG CAACACCGTG TGGGTGATGA
351     AGGCCTTAGG GTCGTAAAGC CCTGTTGATA GGGACGAAGG GCGAAGGGTT
401     AATAGCCCGG AGCTTGACGG TACCTTTCGA GGAAGCCCCG GCTAACTACG
451     TGCCAGCAGC CGCGGTAATA CGTAGGGGGC GAGCGTTGTC CGGAATTATT
501     GGGCGTAAAG AGCGTGTAGG CGGTTCGGTA AGTCTGCTGT GAAATCTTGG
551     GGCTCAACCC TGAGCGTGCA GCGGATACTG CCGGGCTAGA GGGTGGTAGA
601     GGCGAGTGGA ATTCCGAGTG TAGCGGTGAA ATGCGCAGAT ATTCGGAGGA
651     ACACCAGTAG CGAA
```

Sequence from clone 16

```
1       TGAGTAACAC GTAGGTAATG TACCTTTGGG TCGGGAWTAA CYTAGGGAAA
51      CTTAAGCTAA TACCGCATAA TGCAGCGGCT CCTTCGGGAG ACAGTTGTTA
101     AAGATTTATC GCCTAAAGAG CAGCCTGCGG CAGATTAGCT AGTTGGTAAG
151     GTAACGGCTT ACCAAGGCTA CGATCTGTAT CCGACCTGAG AGGGTGGTCG
201     GACNYWCTGA CACTGAATAA CGGGTCAGAC TCCTACGGGA GGCAGCAGTC
251     GGGAATTTTG GGCAATGGGC GAAAGCCTGA CCCAGCAACG CCGCGTGAAG
301     GATGAAGTCT TTCGGGATGT AAACTTCGTA AGAATAGGAA GAATAAATGA
351     CGGTACTATT TGTAAGGTCC GGCTAACTAC GTGCCAGCAG CCGCGGTAAT
401     ACGTAGGGAC CAAGCGTTGT TCGGATTTAC TGGGCGTAAA GGGCGCGTAG
451     GCGGCGTGAC AAGTCAATTG TGAAATCTCC GGGCTTAACT CGGAACGGTC
501     AATTGATACT GTTGTGCTAG
```

Sequence from clone 19

```
1       TTCGGGTCTA GTGGCGAACS GGTGAGTAAC ACGTGAGGAA CGTGCCCCAG
51      AGACCGGGAT AAGCCGAGGA AACTTGGTCT AATACCGGAT GTCCCCACCG
101     GATCGCATGG TCTGGTGAGG AAATGGATTC CGCTCTGGGA GCGCCTCGCG
151     GCCTATCAGC TAGTTGGTGA GGTAACGGCC CACCAAGGCG TCGACGGGTA
201     GCTGGTCTGA GAGGATGATC AGCCACACTG GGACTGAGAC ACGGCCCAGA
251     CTCCTACGGG AGGCAGCAGT GGGGAATCTT GCGCAATGGG CGAAAGCCTG
301     ACGCAGCAAC GCCGCGTGCG GGACGACGGC CCTCGGGTTG TAAACCGCTT
351     TCAGCAGGAA CGATGATGAC GGTACCTGCA GAAGAAGCTC CGGCCAACTA
401     CGTGCCAGCA GCCGCGGTAA TACGTAGGGA GCAAGCGTTG TCCGGATTTA
451     TTGGGCGTAA AGAGCTCGTA GGCGGTTCGG TAAGTCGGGT GTGAAAACTC
501     TGGGCTCAAC CCGGAGAGGC CACTCGATAC TGCTGTGACT TGAGTCTGGT
551     AGGGGAGCAC GGAATTCCTG GTGTAGCGGT GAAATGCACA GATATCAGGA
601     GGAACACCGG TGGCGAAGGC GGTGCTCTGG GCCAGTACTG ACGCTGAGGA
651     GCGAAAGCG
```

Sequence from clone 20

```
1       CTCTCTTCGG AGAGTGTATA GAGTGGCGCA CGGGTGAGTA ACACGTAAGT
51      AATCTACCTT TGAGTGGGGA ATAACGTCCG GAAACGGACG CTAATACCGC
101     ATAATGCAGC GGCATCGCAA GATGACAGTT GTTAAAGGAA TTTATTTCGC
151     TTGAAGAGGA GCTTGCGGCA GATTAGCTAG TTGGTAAGGT AATGGCTTAC
201     CAAGGCTACG ATCTGTAACC GGTCTAAGAG GACGGTCGGT CACACTGACA
251     CTGAATAACG GGTCAGACTC CTACGGGAGG CAGCAGTCGG GAATTTTGGG
301     CAATGGGCGA AAGCCTGACC CAGCAACGCC GCGTGAAGGA TGAAGTATTT
351     CGGTATGTAA ACTTCGAAAG AATGGGAAGA ATCAATGACG GTACCATTTA
401     TAAGGTCCGG CTAACTACGT GCCAGCAGCC GCGGTAATAC GTAGGGACCA
451     AGCGTTGTTC GGATTTACTG GGCGTAAAGG GCGCGTAGGC GGCTTGTCAA
501     GTCACTTGTG AAATCTCCGG GCTTAACTCG GAACGGTCAA GTGAAACTGT
551     CAAGCTAGAG CGTGGAAGGG GCAATCGGAA TTCTTGGTGT AGCGGTGAAA
601     TGCGTAGATA TCAAGAGGAA CACCTGAGGT GAAGACGGGT TGCTAGGCCA
651     ACACTGACGC TG
```

Sequence from clone 21

```
1       CGGGAGCTCA TTTATGAGTC GACCGTGGCG GACGGGTGAG GAACACGTAG
51      CTAACCTGCC CAGGTATGGG GGATATGCGC TGGAAACGGC GTGCAATACC
101     GCATACGTTC GGGTCACGGG AGTGACTTGA GGAAAGCCGC AAGGCGTACC
151     TGGAGGGGGC TGCGTCCGAT TAGCTAGTTG GTGTGGTAAG AGCGCACCAA
201     GGCGATGATC GGTAGCTGGT CTGAGAGGAC GATCAGCCAC ACGGGGACTG
251     AGACACGGCC CCGACTCCTA CGGGAGGCAG CAGCAAGGAA TTTTCCACAA
301     TGGGCGCAAG CCTGATGGAG CAACGCCGCG TGGGGGATGA CGCGTTTCGG
351     CGTGTAAACC CCTTTTCGAG GGGACGAAGC TAATGACGGT ACCCTCGGAA
401     TAAGGACCGG CTAACTACGT GCCAGCAGCC GCGGTAAGAC GTAGGGTCCG
451     AGCGTTGTCC GGAATTACTG GGCGTAAAGC GCGCGCAGGC GGATTCGCGC
501     ATCATCGGTG AAAGCCCCCC GCTTAACGGG GGAGGGTCCG GTGAGATGGC
551     GAGTCTGGAG GCAGGGAGAG GCGAGTGGAA TTCCGGGTGT AGTGGTGAAA
601     TGCGTAGAGA TCCGGANGAA CACCAGTGGC GAANGCGGCT CGCTGGACCT
651     GACCTGACGC TGAAGCGCGA A
```

Sequence from clone 27

```
1       CGAAAGTTTC CTTCGGGAAG CGAGTAGAGT GGCGCACGGG TGAGTAACAC
51      GTAAGTAATC TACCCTCGGG TGGGGAATAA CATCGGGAAA CCGATGCTAA
101     TACCGCATAA TGCAGCGGCT CCTTATGGAG ACAGTTGTTA AAGTATTTAT
151     ATGCCTGGGG AGGAGCTTGC GGCAGATTAG CTAGTTGGTA AGGTAATGGC
201     TTACCAAGGC TACGATCTGT AGCCGACCTG AGAGGGTGGT CGGTCACACT
251     GACACTGAAT AACGGGTCAG ACTCCTACGG GAGGCAGCAG TCGGGAATTT
301     TGGGCAATGG GCGAAAGCCT GACCCAGCAA CGCCGCGTGA AGGATGAAGT
351     CTTTCGGGAT GTAAACTTCG TAAGAATAGG AAGAATAAAT GACGGTACTA
401     TTTGTAAGGT CCGGCTAACT ACGTGCCAGC AGCCGCGGTA ATACGTAGGG
451     ACCAAGCGTT GTTCGGATTT ACTGGGCGTA AAGGGCGCGT AGGCGGCGTG
501     ACAAGTCACT TGTGAAATCT CCGAGCTTAA CTCGGAACGG TCAAGTGATA
551     CTGTTATGCT AGAGTACAGA AGGGGTAATC GGAATTCTCG GTGTAGCGGT
601     GAAATGCGTA GATATCGAGA GGAACACCAT TTCCTGG
```

Sequence from clone 29

```
1       GGGAGTGAGT GGCGYYCNGG TGAGTAACRC RTGAGGATCT GCCTACAGGA
51      TGGGGACAAC AGTGGGAAAC TGCTGCTAAA ACCCAATGTG CCGAGAGGTG
101     AAAYATTAAT AGCCCTGTAG ATGAGCTCGC GTCTGATTAG CTMGTTGGTG
151     TGGTAAAGGC ATACCAAGGC GACGATCAGT AGCTGGTCTG AGAGGACGAT
201     CAGCCACACT GGGACTGAGA CACGGCCCAG ACTCCTACGG GAGGCAGCAG
251     TGGGGAATTT TCCGCAATGG GCGAAAGCCT GACGGAGCAA CGCCGCGTGA
301     GGGAGGAAGG CCTGTGGGTT GTAAACCTCT TTTCTCAAGG AAGAAGTTCT
351     GACGGTACTT GAGGAATCAG CATCGGCTAA CTCCGTGCCA GCAGCCGCGG
401     TAAGACGGAG GATGCAAGCG TTATCCGGAA TTATTGGGCG TAAAGCGTCC
451     GTAGGCGGTT ATAAAAGTCT GTTGTTAAAG CTCACAGCTC AACTGTGAAT
501     GGGCGATGGA AACTGTATGA CTAGAGAGTG GTAGGGGTAG AGGGAATTCC
551     TAGTGTAGCG GTGAAATGCG TAGATATTAG GAAGAACACC AGTGGCGAAG
601     GCGCTCTACT GGGCCATTAC TGACGCTGAT GGACGAAAGC TAGGGGAGCG
651     AAAGGGATTA GATACCCCTG TAGTCCTAGC TGTNAACGAT GG
```

Sequence from clone 33

```
1       ATTAAACTTT CCTTCGGGAA AGATATAAAG TGGCGCACGG GTGAGTAACA
51      CGTAGGTAAT TTGCCTTTGG GTGGGGAATA ACCGTCGGAA ACGACGGCTA
101     ATACCGCATA ATGCAGCGGC TCCTTATGGA GACAGTTGTT AAAGATTTAT
151     CGCCTGAAGA GAAGCCTGCG GCAGATTAGG TAGTTGGTGA GGTAATGGCT
201     CACCAAGCCC GCGATCTGTA TCCGGTCTAA GAGGATGGTC GGACACACTG
251     ACACTGAATA ACGGGTCAGA CTCCTACGGG AGGCAGCAGT CGGGAATTTT
301     GGGCAATGGG CGAAAGCCTG ACCCAGCAAC GCCGCGTGAA GGATGAAGTA
351     TCTCGGTATG TAAACTTCGG AAGAATGGGA AGAATAAATG ACGGTACCAT
401     TTTTAAGCCC CGGCTAACTC TGTGCCAGCA GCCGCGGTAA TACAGAGGGG
451     GCAAGCGTTG TTCGGATTTA CTGGGCGTAA AGGGCGCGTA GGCGGCGTGT
501     TAAGTCACTT GTGAAATCTC TGAGCTTAAC TCAGAACGGT CAAGTGATAC
551     TGATGTGCTA GAGTGCAGAA GGGGCAACTG GAATTCTTGG TGTAGCGGTG
601     AAATGCGTAG ATATCAAGAG GAACACCTGA GGCGAANGCG GGTTGCTGGG
651     CTGACACTGA C
```

Sequence from clone 42

```
1       AAGAGGTAGT GGCGAGCGGG TGAGTAACAC GTGAGAAACC TATCCTGGTC
51      TCTGGGAYMA CAGCCGGAAA CGGCTGCTAA TACCGGATGC CGTCGGAGCG
101     TCGCATGGCG CGCTGACGAA MGGGTTACTG GATCAGGAGG GTCTCGCGGC
151     CTATCAGCTA GTTGGTGGGG TAATGGCCTA CCAAGGCATC SACGGGTWKY
201     TGGTCTGAGA GGATGATCAG CCACWCTGGG ACTGAATAAC GGGTCAGACT
251     CCTACGGGAG GCAGCAGTCG GGAATTTTGG GCAATGGGCG AAAGCCTGAC
301     CCAGCAACGC CGCGTGAAGG ATGAAGTCTT TCGGGATGTA AACTTCGTAA
351     AAATAGGAAG AATAAATGAC GGTACTATTT ATAAGGTCCG GCTAACTACG
401     TGCCAGCAGC CGCGGTAATA CGTAGGGACC AAGCGTTGTT CGGATTTACT
451     GGGCGTAAAG GGCGCGTAGG CGGCAATTCA AGTCAGTTGT GAAATCTCCG
501     AGCTTAACTC GGAACGGTCA ACTGATACTG CTTTGCTAGA GTACAGAAGG
551     GGCAATCGGA ATTCTTGGTG TAGCGGTGAA ATGCGTAGAT ATCAAGAGGA
601     ACACCTGAGG TGAAGACGGG TTGCTGGGCT GATACTGACG CTGA
```

Sequence from clone 43

```
1       GGCCCCTTCG GGGGTACACG MSCGGCGAAC GGCTGAGTAA CGCGTGGGAA
51      TCCACCCCAA AGTGAGGGAT AAGCACCGGA AACGGTGTCT AATACCGCAT
101     ATGGTCTTCG GATTAAAGTT TTATACGCTT TGGGAGGAGC CCGCSTCCGA
151     TTAGGTTGTT GGTGAGGTAA TGGCTCACCA AGCCGACGAT CGGTAGCTGG
201     TCTGAGAGGA TGATCAGCCA GACTGGAACT GAGACACGGT CCAGACTCCT
251     ACGGGAGGCA GCAGTAAGGA ATCTTCCACA ATGGGCGAAA GCCTGATGGA
301     GCAACGCCGC GTGCAGGACG AAGGCCTTCG GGTCGTAAAC TGCTTTTGTA
351     TACGAAGAAT TTGACGGTAG TATACGAATA AGGATCGGCT AACTCCGTGC
401     CAGCAGCCGC GGTCATACGG AGGATCCAAG CGTTATCCGG AGTGACTGGG
451     CGTAAAGAGT TGCGTAGGTG GTTAGTAAAG TGAATAGTGA AACCTGAAGG
501     CTCAACCTTC AGACTATTAT TCAAACTTAC TAACTCGAGA ATGGTAGAGG
551     TAGCTGGAAT TTCTAGTGTA GGAGTGAAAT CCGTAGATAT TAGAAGGAAC
601     ACCAATGGCG TAGGCAGGCT ACTGGACCAT TTCTGACACT AAGGCACGAA
651     AGCGTGGGGA GCGAACCGGA TTAGATA
```

Sequence from clone 50

```
1       AACGGGAATA TTCGCTATAG CAATATAGCG GATGTCTAGT GGCGGAAGGG
51      TGCGTAACAC GTGGGCAATC TGCCGAAAAG TGGGGAATAG CTCGCCGAAA
101     GGCGAATTAA TACCGCATAC GATTAACGAA AGCCTTTTTG TGAAATCAAA
151     GCTGGGGAAA CTTGGCGCTT TTCGATGAGC CCGCGGCCTA TCAGCTAGTT
201     GGCGAGGTAA TGGCTCACCA AGGCGATGAC GGGTAGCTGG TCTGAGAGGA
251     CGACCAGCCA CACTGGAACT GAGACACGGT CCAGACACCT ACGGGTGGCA
301     GCAGTCGAGA ATTTTTCTCA ATGGGGGAAA CCCTGAAGGA GCGACGCCGC
351     GTGGAGGATG AAGGTCTTCG GATTGTAAAC TCCTGTCATC AGAGAACAAT
401     GGGCACATTA ACCGTGTGTC TTGATAGTAC CTGAAGAGGA AGAGACGGCT
451     AACTCTGTGC CAGCAGCCGC GGTAATACGG GGGGGCAAG CGTTGTTCGG
501     ATTTACTGGG CGTAAAGGGT GCGTAGGCGG CACCACAAGT CACTTGTGAA
551     ATCTCCAAGC TCAACTTGGA ACGGTCAAGT GATACTGTGG AGCTAGAGTG
601     CAGAAGGGGC
```

Sequence from clone 52

```
1       CGAGCGGTAA GGCTCCTTCG GGAGTACACG AGCGGCGAAC GGGTGAGTAA
51      CACGTGAGCA ATCTGCCCTT CACACGGGGA TAACTTCGGG AAACCGATGC
101     TAATACCCGA TACGACCACT TCAGGCATCT GATGGTGGTG GAAAGTTCCG
151     GCGGTGAAGG ATGAGCTCGC GGCCTATCAG CTTGTTGGTG GGGTAATGGC
201     CCACCAAGGC AACGACGGGT AGCCGGCCTG AGAGGGTGAC CGGCCACACT
251     GGGACTGAGA CACGGCCCAG ACTCCTACGG GAGGCAGCAG TGGGGAATAT
301     TGGACAATGG GCGAAAGCCT GATCCAGCAA CGCCGCGTGA GGGATGACGG
351     CCTTCGGGTT GTAAACCTCT TTCAGCAGGG ACGAAGCGAA AGTGACGGTA
401     CCTGCAGAAG AAGCACCGGC CAACTACGTG CCAGCAGCCG CGGTAATACG
451     TAGGGTGCGA GCGTTGTCCG GAATTATTGG GCGTAAAGGG CTCGTAGGCG
501     GTTTGTCACG TCGGGAGTGA AAACTCAGGG CTTAACCCTG AGCCTGCTTC
551     CGATACGGGC AGACTAGAGG TATGCAGGGG AGAACGGAAT TCCTGGTGTA
601     GCGGTGAAAT GCGCAGATAT CAGGAGGAAC ACCGGTGGCG AAGGCGGTTC
651     TCTGGGCATT ACCTGACGCT GAGGAGCG
```

Sequence from clone 53

```
1       CGGAMATTCC AGCAATGGGG TGTTAGTGGC GAACGGTCGC GTAACACGTA
51      GGCAACCTGC CCTGAAGTGG GGGACAACAG CCCGAAAGGG CTGCTAATAC
101     CGCATGTGAA CAACGAATCG CATGGTTTGT TGTTCAAAGG CTATGGCAAC
151     ATGGTCGCTT TGGGATGGGC TTGCGGCCTA TCAGGTAGTT GGTGGGGTAA
201     TGGCCCACCA AGCCGACGAC GGGTAGCTGG TCTGAGAGGA CGATCAGCCG
251     GATTGGGACT GAGATACGGC CCAGACTCCT ACGGGGGGCA GCAATTAGGA
301     ATCTTGCGCA ATGGGCGAAA GCCTGACGCA GCGACGCCGC GTGCGGGATG
351     AAGGCCTTCG GGTCGTAAAC CGCTTTTAAC GGGGAAGAAG AATGTGACGG
401     TACCCGTTGA ATAAGCCCCG GCTAACTACG TGCCAGCAGC CGCGGTAATA
451     CGTAGGGGGC GAGCGTTGTC CGAAGTTACT GGGCGTAAAG CGCGCGTAGG
501     CGGTTGCCTA AGTCTGGGGT GAAAGGTTCA GGGCTTAACC CGAACAGTGC
551     CTTGGATACT GGGCGACTTG AGTGCCGAAG AGGAAAGCGG AATTCCTGGT
601     GTAGCGGTGA AATGCGTAGA TATCAGGAGG AACACCGATG GCGAAGGCAG
651     CTTTCTGGTC GGCAACTGAC G
```

Sequence from clone 55

```
1       GTGAAGCCCT TCGGGGTGGA TCASYGGCGA ACGGGTGAGT AACACGTGAG
51      CAACCTGCCC TTCACTCTGG GATAACTCCG GGAAACCGGT GCTAATACCG
101     GATACGAGTA TCGGCCTCAT GGTCTGGTGC TGGAAAGAAT TTTGGTGGGG
151     GATGGGCTCG CGGCCTATCA GCTTGTTGGT GAGGTAATGG CTCACCAAGG
201     CGACGACGGG TAGCCGGCCT GAGAGGGCGA CCGGCCACAC TGGGACTGAG
251     ACACGGGCCC GACTCCTACG GGAGGCAGCA GTAAGGAATA TTGGTCAATG
301     GGCGAAAGCC TGAAGCAGCG ACGCCGCGTG AGGGATGAAG GCCTTCGGGT
351     TGTAAACCTC TTTCAGTAGG GACGAAGCGA AAGTGACGGT ACCTACAGAA
401     GAAGCACCGG CCAACTACGT GCCAGCAGCC GCGGTAATAC GTAGGGTGCA
451     AGCGTTGTCC GGAATTATTG GGCGTAAAGA GCTCGTAGGC GGTTTGTCAC
501     GTCGGCTGTG AAATCCCGAG GCTCAACCTC GGGTCTGCAG TCGATACGGG
551     CAGACTAGAG TACTGCAGGG GAGACTGGAA TTCCTGGTGT AGCGGTGGAA
601     TGCGCAGATA TCAGGAGGAA CACCGGTGGC GAAGGCGGGT CTCTGGGCAG
651     TAACTGACGC TG
```

Sequence from clone 56

```
1       GCGGGGCAAC CTGGCGGCCA GTGGCGAACG GGTGAGTAAT ATATCGGAAC
51      GTACCCTGGA GTGGGGGATA ACGTAGCGAA AGTTACGCTA ATACCGCATA
101     CGATCTAAGG ATGAAAGTGG GGGATTCGCA AGAACCTCAT GCTCCTGGAG
151     CGGCCGATAT CTGATTAGCT AGTTGGTGGG GTAAAGGCCT ACCAAGGCAT
201     CGATCAGTAG CTGGTCTGAG AGGACGACCA GCCACACTGG AACTGAGACA
251     CGGTCCAGAC TCCTACGGGA GGCAGCAGTG GGGAATTTTG GACAATGGGC
301     GAAAGCCTGA TCCAGCAATG CCGCGTGAGT GAAGAAGGCC TTCGGGTTGT
351     AAAGCTCTTT TGTCAGGGAA GAAACGGTGA AAGCTAATAT CTTTTGCTAA
401     TGACGGTACC TGAAGAATAA GCACCGGCTA ACTACGTGCC AGCAGCCGCG
451     GTAATACGTA GGGTGCAAGC GTTAATCGGA ATTACTGGGC GTAAAGCGTG
501     CGCAGGCGGT TTTGTAAGTT TGTCGTGAAA TCCCCGGGCT CAACCTGGGA
551     ATTGCGATGA AGACTGCAAG GCTAGAATCT GGCAGAGGGG GGTAGAATTC
601     CACGTGTAGC AGTGAAATGC GTAGAGATGT G
```

Sequence from clone 58

```
1       GGCAGCACGG GAGCAATCCT GGTGGCGAGT GGCGAACGGG TGAGTAATAC
51      ATCGGAACGT GTCCATTAGT GGGGGATAAC CCGGCGAAAG CCGGACTAAT
101     ACCGCATACG ACCTAAGGGT GAAAGCGGGG GATCGCAAGA CCTCGCGCTA
151     GCGGAGCGGC CGATGTCAGA TTAGCTTGTT GGTGGGGTAA AAGCCTACCA
201     AGGCAACGAT CTGTAGCTGG TCTGAGAGGA CGACCAGCCA CACTGGGACT
251     GAGACACGGC CCAGACTCCT ACGGGAGGCA GCAGTGGGGA ATTTTGGACA
301     ATGGGCGCAA GCCTGATCCA GCCATGCCGC GTGCGGGAAG AAGGCCTTCG
351     GGTTGTAAAC CGCTTTTGTC AGGGAAGAAA AGCTCCGGGT CAACACCTCG
401     GAGTCATGAC GGTACCTGAA GAATAAGCAC CGGCTAACTC CGTGCCAGCA
451     GCCGCGGTAA TACGGAGGGT GCAAGCGTTG TCCGGATTTA TTGGGTTTAA
501     AGGGTGCGTA GGTGGCGTCT TAAGTCTGGT TTGAAAGCAG GCGGCTCAAC
551     CGTCTGATGT GGCTGGAAAC TGGGGCGCTT GAATGGGTTG GCGGTAGCCG
601     GAACGGGTCA TGTAGCGGTG AAATGCATAG ATATGACCCA GAACACCGAT
651     TGCGAAGGCA GGCTACTACG ACTTGATTGA CACTGAGGCA CGAGAGCA
```

Sequence from clone 60

```
1       GGGGGCAACC CTGGTGSCGA GTGGCGAACG GGTGAGTAAT ACATCGGAAC
51      GTATCCTATA GCGGGGGATA ACCTCTCGAA AGAGAGGCTA ATACCGCATA
101     CGACCCATGG GTGAAAGAGG GGGATCGCAA GACCTCTCAC TATTGGAGCG
151     GCCGATGTCG GATTAGCTAG TTGGCGGGGT AAAAGCCCAC CAAGGCTACG
201     ATCCGTAGCT GGTCTGAGAG GACGACCAGC CACACTGGAA CTGAGACACG
251     GTCCAGACTC CTACGGGAGG CAGCAGTGGG GAATTTTGGA CAATGGGCGC
301     AAGCCTGATC CAGCCATGCC GCGTGAGTGA AGAAGGCCTT CGGGTTGTAA
351     AGCTCTTTCG GCGGGGACGA AAAGATTCGC GTTAACACCG CGGATCCATG
401     ACGGTACCCG CAGAAGAAGC ACCGGCTAAC TACGTGCCAG CAGCCGCGGT
451     AATACGTAGG GTGCAGGCGT TAATCGGAAT TACTGGGCGT AAAGCGTGCG
501     CAGGCGGTCT TTTAAGTCAG ATGTGAAATC CCCGGGCTTA ACCTGGGAAC
551     TGCGTTTGAA ACTGGAAGGC TAGAGTGTGG CAGAGGGGGG TGGAATTCCA
601     CGTGTAGCAG TGAAATGCGT AGATATGTGG AGGAACAMCG ATGGCGAAAG
651     GCAGCCCCCT GGGCTAACAC TGACGCTCA
```

Sequence from clone 62

```
1       AATCATCTCA CGGTTGGGTA TAGCCGCGAG AAATCGCGGG TAATCCCCAG
51      CGACGCAGGG TGTCGGCATC GACGCCCTGC CAAAGGCTCG CCGCCGTGGG
101     ACGAGCCGTC GTGGTATTAG GTTGTTGGCG GGGTAACGGC CCACCAAGCC
151     TGCGATGCCT ACCGGGCGTG CGAGCGTGGC CCGGCACACT GGGACTGAGA
201     CACTGCCCAG ACTCCTATGG GAGGCTGCAG TCGAGAATCT TCGGCAATGG
251     GCGCAAGCCT GACCGAGCGA CGCCGCGTGG AGGACGAAGG CCTTCGGGTT
301     GTAAACTCCT GTCGAGGGGA AGGAAGGGGC CGCAAGGCCC TTGACCGCTC
351     CCTGGAGGAA GCACGGGCTA AGTTCGTGCC AGCAGCCGCG GTAAGACGAA
401     CCGTGCGAAC GTTATTCGGA ATCACTGGGC TTAAAGCGCG TGTAGGCGGG
451     TCGGTGCGTC GGCCGTTGAA ATCCCCCGGC TCAACCGGGG AAGTGGCGCC
501     GATACGACCG GCCTGGAGAC GACGTANCGG GGAACTGGAA CTTCCGGTGG
551     AGCGGNGAAA TGCGTTGAGA TCGGAAGAAC GCCGNGGCGA AAGCGAGTTC
601     C
```

Sequence from clone 65

```
1       TGTCCCGAAC TTGGGAATAA CAGCCGAAAA CSACTGCTAA TACCGAATAT
51      CTTCGTAACG TCGCATGGCG ATTCGAAGAA AGCTTTATGC GGTTTGGGAG
101     GGTCTCGCGG CCTATCAGCT TGTTGGTGAG GTAATGGCTC ACCAAGGCAT
151     CGACGGGTAG CTGGTCTGAG AGGATGATCA GCCACACTGG GACTGAGACA
201     CGGCCCAGAC TCCTACGGGA GGCAGCAGTG GGGAATATTG CACAATGGGC
251     GAAAGCCTGA TGCAGCGATG CCGCGTGCGG GAAGAAGGCC CTAGGGTTGT
301     AAACCGCTTT CAGTAGGGAA GAAAATGACG GTACCTACAG AAGAAGGTGC
351     GGCCAACTAC GTGCCAGCAG CCGCGGTGAC ACGTAGGCAC CAAGCGTTGT
401     CCGGATTTAT TGGGCGTAAA GAGCTCGTAG GCGGTTTGGT AAGTCGGGTG
451     TGAAAACTCT GGGCTCAACC CAGAGAGGCC ACTCGATACT GCCATGACTT
501     GAGTACGGTA GGGGAGTGGG GAATTTCTAG TGTAGCGGTG AAATGCGCAG
551     ATATTAGAAG GAACACCAGT GGCGAAGGCG CCACTCTGG
```