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**Adaptive Evolution of *Escherichia coli*:
Growth Kinetics and Genetic Changes
from 2 to 10% Halophilization**

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Abstract

Escherichia coli (*E. coli*) is a Gram negative, rod-shaped bacterium, commonly found in the lower human intestine. Salt (NaCl, sodium chloride) is present in the human diet and constantly interacts with *E. coli* in the intestine. High consumption of salt may pose adverse effects on the growth of *E. coli*. In order to adapt to the changes to its environment, *E. coli* may undergo halophilization. In this study, we observed the growth kinetics and genetic changes of *E. coli* under increasing concentrations of 3% - 10% NaCl over 80 passages. Adaptability of *E. coli* was estimated by generation time and cell density at stationary phase. Minimum Inhibitory Concentration Experiment (MIC) was used to confirm the resistance of *E. coli* to the range of salt stress. Colony MIC was conducted to observe the deviation in mutations of the *E. coli* cells more accurately. Our results demonstrated that *E. coli* adapted from 1% NaCl to 8% NaCl at the rate of about 1% increment per month. Our colony MIC results demonstrated that the area under the MIC curve where NaCl is above 7.5% increased from 5% at passage 44 (cultured in 5% NaCl) to 13% at passage 72 (cultured at 7% NaCl). Polymerase Chain Reaction and Restriction Fragment Length Polymorphism were used to analyse the adaptation and mutation of *E. coli* at a genomic level. The amplification and digestion profiles were analysed using Nei-Li Dissimilarity and demonstrated an increasing trend of genomic distance across passages suggesting that the genomes of the *E. coli* has changed over the course of the 80 passages.

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Abbreviations

AUC	Area under Curve
BSA	Bovine Serum Albumin
DI	Dissimilarity Index
DNA	Deoxyribonucleic Acid
dNTPs	Deoxyribonucleotide Triphosphates
EDTA	Ethylenediaminetetraacetic Acid
MIC	Minimum Inhibitory Concentration
NB	Nutrient Broth
STE	Sodium Chloride–Tris–EDTA

1. Introduction

Escherichia coli, a Gram negative bacterium, is commonly found in the lower intestine of warm blooded organisms. They are part of the normal flora of the gut and can produce vitamin K which is needed for clotting of blood and prevent establishment of pathogenic bacteria in the intestine.

The adaption and evolution of *E. coli* to develop a resistance to antibiotics and drugs are widely studied but the mechanisms to non-antibiotic agents, such as salt are less understood. Salt, which is used as a common food additive, is added to preserve food and inhibits growth of microorganisms by drawing water out of the cells of both microbe and food through osmosis. As *E. coli* is constantly exposed to the salt present in the faecal matter, it is important to investigate their relationship on how *E. coli* copes with the change in environment.

Our project aims to observe the adaptation of *E. coli* cultured in NaCl supplemented medium over 80 passages. Adaptability over time is estimated by generation time and cell density of the stationary phase. Polymerase Chain Reaction (PCR) and Restriction Fragments Length Polymorphism (RFLP) are also used to characterize adaptation/evolution at genomic level.

2. Literature Review

2.1 Evolutionary Studies Done on Enteric Bacteria

The enteric bacteria belong to a group of bacteria that live in the gastrointestinal tract of humans and mammals. They are of special concern because they share a symbiotic relationship to mammals and as humans are a mammalian species, it is also important for us. For example, bacteria of the *Lactobacilli* strain are able to curb the growth of other pathogenic bacteria such as *Streptococcus pyogenes* (Westbroek et al., 2010). Lactobacilli are able to inhibit the growth of pathogenic bacteria because they produce lactic acid, bacteriocins and hydrogen peroxide (Westbroek et al., 2010). Members of the enteric bacteria family are also able to inhibit the growth of *Escherichia coli O157:H7* (Toshima et al., 2007). *E. coli O157:H7* is one of the pathogenic strains of *E. coli* and is able to cause haemorrhagic colitis. *E. coli O157:H7* infects the large intestine and produces Shiga toxin that causes bloody diarrhoea. If untreated, haemorrhagic colitis can cause death; hence, the enteric bacteria are important as a defence and guarding mechanism which is able to keep the pathogenic bacteria in check. Many of which, help protect us from diseases that arise from the gastrointestinal tract. The non-pathogenic strain of *E. coli* which is found naturally in the gastrointestinal tract is able to produce Vitamin K2, a vitamin that is important in the coagulation of blood (Bently and Meganathan, 1982). Evolutionary studies (Cai et al., 2009; Lozupone et al., 2008) had shown how these enteric bacteria have adapted to the conditions within the host and how these bacteria share a symbiotic relationship with us. Comparison between the genomes of enteric bacterial species shows that various genetic convergences may arise due to the fact that the different species of bacteria are exposed to the same environment (Lozupone et al., 2008). The balance of the symbiotic relationship between these organisms and humans, which is the result of the many interactions between the bacteria and the host, can be better understood.

Evolutionary studies were also done on various strains of enteric bacteria such as on *Lactobacillus casei ATCC 334* (Cai et al., 2009) or whole population of enteric bacteria (Lozupone et al., 2008). These studies revealed that genetic interactions such as horizontal gene transfer had occurred between certain bacterial species. These genetic interactions are crucial in the evolution of the enteric bacteria as they can allow different bacterial strains to acquire genes which originate from other strains. A study (Cai et al., 2009) compared the

genomes between *Lactobacillus casei* and other members of the *Lactobacilli* family revealed that *L. casei* contains many Insertion Sequence (IS) elements that result in the expression of proteins that are involved in the metabolism of various carbohydrate substrates. The ability to metabolize a wide variety of carbohydrate compounds is essential for the survival of *L. casei*. The IS elements were also found to be similar in a genus of enteric bacteria, *Enterococcus*. The IS elements could have been transferred over to *L. casei* from *Enterococcus* by horizontal gene transfer between the two bacterial strains. Another study done on the microbes in the gastrointestinal tract revealed that the metabolic genes of the microbes have converged (Lozupone et al., 2008). The convergence is likely to be caused by horizontal gene transfer and parallel gene loss between different species of bacteria. This is because all of the microbes in the gastrointestinal tract have been exposed to the same environment. The environmental stress experienced by all of the microbes should be the same; therefore, the convergence in the metabolic genes could be because of the same stress that is experienced by the microbes.

2.2 Halophilization of Bacteria

Halophilization is the gradual adaptation of the organism to the inhibitory effects of salt (sodium chloride; NaCl) by introducing increasing concentrations of salt into the growth environment of the organism. The organism normally thrives best in environmental conditions where salt is present in low concentrations resembling the natural environment of which the organism lives. The addition of salt presents a number of stresses, including osmotic stress and ionic stress (Burg et al., 2007). These stresses can cause cell death if the cells were not adapted to growing in higher salt concentrations.

2.3 Factors Affecting the Survival of Bacteria under Salt Stress

Halophiles are a category of extremophile organisms which thrive in environments of high salt concentrations. They are generally classified as mildly, moderately and extremely halophilic (Nester et al., 2004), with a range of 0-5% NaCl tolerance for mildly halophilic, 0-6.5% NaCl tolerance for moderately halophilic, and 3-15% NaCl tolerance for extremely halophilic organisms (Garrity et al., 2003). On another hand, halotolerant organisms are not naturally salt adapted organisms, but had adapted to high salinity over the course of time.

Some microorganisms may be able to adapt to the harsh environments by expressing or repressing genes which changes their characteristics, allowing them to survive in conditions where they usually would not (Alberts et al., 2002). Other factors such as the presence of hydrogen ions, yeast autolysate, aeration and physiological conditions of the bacteria also play a key role in the bacteria's overall salt tolerance (Doudoroff, 1940).

Osmosis, osmotic pressure and water activity are also key factors which affect the ability of bacteria to survive under salt stress. High salinity in an environment can affect bacteria. A hypertonic solution of high salt concentration would cause osmosis due to the low water concentration within the bacteria. Osmosis would cause the water within the bacteria to leave, resulting in bacteria plasmolysis. However, osmosis can be countered by osmotolerant organisms. Osmotolerant organisms have the ability to withstand osmotic pressure from the environment; hence, allowing them to survive in various concentrations of NaCl (Table 2.1).

Classification	Genera	Species	NaCl Tolerance
Mildly Halophile	<i>Clostridium</i>	<i>C. botulinum</i> <i>C. sporogenes</i> <i>C. perfringens</i>	0-5%
Moderately Halophile	<i>Bacillus</i> <i>Enterococcus</i>	<i>B. cereus</i> <i>E. faecalis</i> <i>E. faecium</i> <i>E. avium</i>	0-2.8% 6.5%
Extreme Halophiles	<i>Halobacillus</i> <i>Staphylococcus</i>	<i>H. halophilus</i> <i>S. aureus</i> <i>S. epidermidis</i> <i>S. saprophyticus</i>	3-10% 15%
Osmotolerant	<i>Halococcus</i> <i>Micrococcus</i> <i>Streptococcus</i> <i>Vibrio</i> <i>Salmonella</i> <i>Leuconostoc</i>	<i>H. orrhuae</i> <i>M. luteus</i> <i>S. termophilus</i> <i>V. mimicus</i> <i>V. cholerae</i> <i>S. typhimurium</i> <i>L. mensenteroides</i> <i>L. lactis</i>	25-30% 5-15% 4- 10% 6-10% 9% 3-6.5%

Table 2.1: Salt tolerance of different bacteria (Garrity et al, 2003).

The osmotic stress can be explained by the concept of water activity. Water activity (a_w), is defined as the amount of water that is available for growth and reproduction of an organism (Baeza et al., 2008). Different bacterial strains are able to grow in different levels of salinity

and this means that they can tolerate a range of a_w in their environment (Marshall et al., 1971). a_w is calculated as a ratio of vapour pressure of a liquid in the unknown substance and pure water at the same temperature. Pure distilled water has an a_w of one. A higher a_w value is better for growth of bacteria, which normally requires at least a_w of 0.91. Organisms which are able to survive in low $a_w = 0.8$, are known as xerophiles. Halophiles often exhibit characteristics of xerotolerance as the high salt concentrations often result in low a_w .

Salt reduces the amount of water in the environment, which leads to a reduced a_w . Decreased a_w leads to osmotic stress that are experienced by the organisms which grow in the environment. When cells are exposed to salt, the influx of salt ions and the subsequent rapid efflux of water molecules out of the cells can result in a higher concentration of ions and a decrease in the concentration of water within the cells. In addition, the influx of salt ions can cause ionic stress. The stresses are able to interfere with the normal functions of the cells, causing cell death if the stresses are too much. Bacteria that are exposed to NaCl typically exhibit a prolonged lag phase and the growth rate of the cells decreases in increasing NaCl concentration (Carlucci & Pramer, 1960). This is because the bacterial cells need time to adapt to the change in environmental conditions (Liu et al., 2005). The addition of NaCl is able to cause the efflux of water out of the cells due to osmosis (Burg et al., 2007). The change in osmolarity within the cell can lead to other effects, such as cell shrinkage. Additionally, salt stress also has an adverse effect on cellular motility (Liu et al., 2005). Bacterial cells need to have certain methods of handling the detrimental effects of high NaCl. A common method is the accumulation of organic solutes known as organic osmolytes (Oren, 2008; Diamant, 2001). Examples of osmolytes are glycine betaine, glycerol and trehalose.

2.4 Past Research on *Escherichia coli*

In the study on the adaptation of *E. coli* to sodium chloride, *E. coli* was grown in ordinary fresh-water media before being transferred to saline nutrient solutions (Doudoroff, 1940). It was shown that the viable count of the bacteria remained at a constant until a certain NaCl concentration. The relationship between the percentage of viable bacteria and salinity of the medium was almost constant in a large number of the experiments, provided the bacteria were first grown in fresh-water broth (Doudoroff, 1940). Through the testing of the right conditions for carrying out the experiment, it was noted that the concentration of yeast

autolysate, affected the amount of viable bacteria when in the presence of high concentrations of NaCl. In (7% NaCl – 0.5% yeast autolysate) : (7% NaCl – 2.5% yeast autolysate), the difference of just 2% increase in yeast autolysate concentrations caused a decrease of viable organisms to 0.002 of original (7% NaCl – 0.5% yeast autolysate) mix. However, no significant change was noted with the doubling of the amount of nutrients in 6% NaCl (Doudoroff, 1940). This suggests that yeast autolysate exerted an additional inhibitory influence on the bacteria under high NaCl concentration. Observations of the reproduction of bacteria taken from a fresh-water medium and suddenly immersed into saline broth showed that aeration was a key factor in determining the reproducibility of individuals in the unfavourable saline environment (Doudoroff, 1940). Through the process of having 6 different ways of aerating and culturing the bacteria, with various degrees of stirring, agitation and storage container, it was shown that constant agitation of the culture when stored in larger bottles instead of tubes greatly improved the viable count of bacteria in 7% NaCl. It was also shown that if the salinity of the medium was raised gradually, more organisms from a 24 hour (stationary phase) culture were viable in saline broth than if the bacteria were suddenly immersed into the saline medium (Doudoroff, 1940). This suggests that the acclimatization of microorganisms to unfavourable environments and conditions by subjecting them to small doses was effective in allowing them to resist it (Doudoroff, 1940). The results obtained by (Doudoroff, 1940) on *E. coli* relation of viable count to NaCl concentration matches the findings of studies on the growth of *Bacillus megatherium* (Vaas, 1938) where the adaptability of *B. megatherium* was lowest in the early logarithmic period and greatest during the early stationary phase. The ratios of viable counts in saline broth and the ability of *E. coli* to grow in a saline environment are greatest at the early stationary and least during logarithmic phase.

The survival of *E. coli* and *Acinetobacter junii* at various concentrations of sodium chloride was studied (Hrenovic and Ivankovic, 2009). The findings of the study were that at 5% NaCl, the *E. coli* was undergoing stress and there was no significant growth of the *E. coli* at 7% NaCl. While increasing concentrations of salt reduced the survivability of the bacterial cells, there was no significant difference between sea salt and NaCl on the bacteria (Carlucci and Pramer, 1960). The study has shown that there was an absence of bacterial decay at 3.5% NaCl, which may be attributed to the use of a complex media, nutrient broth, which helped the osmotolerance and survival of *E. coli* under salt stress as opposed to nutrient-depleted media. Sodium ions were deduced to have been neutralized by binding to the organic matter

in the nutrient rich media, which reduced the negative effects of NaCl on the bacteria. *E. coli* in nutrient broth were able to reproduce actively up to 5%, with total die-off after 48 hours and 72 hours at 30% and 20% NaCl respectively (Hrenovic and Ivankovic, 2009).

It has been suggested that methods used to counter stress caused by salt can also be used to counter other forms of stress (Diamant et al., 2001), such as heat stress. A study had investigated the effects of combined salt and heat stresses on *E. coli* (Diamant et al., 2001). Two cultures of *E. coli* were used in the experiment. One *E. coli* culture was previously exposed to heat stress first and then exposed to salt stress. The other culture was exposed to salt stress directly. Their results demonstrated that the *E. coli* which was exposed to heat stress was able to adapt to the subsequent salt stress in a shorter time as compared to the other culture of *E. coli* which was exposed to salt stress only. Additionally, an osmolyte known as betaine was found in high levels in the *E. coli* culture that exposed to salt stress. The betaine was also used by the *E. coli* to adapt to heat stress (Diamant et al., 2001). This experiment suggested that the methods used by *E. coli* to counter heat stress can also be used to counter salt stress.

With improving food processing technologies, salt becomes a common food additive that is added to enhance the flavour of the food and also to help preserve the food (Shee et al., 2010). Food is digested in the gastrointestinal tract, along with the salt that is contained within it. It is plausible that the salt could end up in the lower gastrointestinal tract due to digestion and disrupt the growth of *E. coli* in the gastrointestinal tract as *E. coli* is non-halotolerant. This is especially important for individuals who frequently consume food of high salt content. The *E. coli* is constantly exposed to the salt that is present in the fecal matter. Thus, the growth of the *E. coli* will be adversely affected by the presence of salt. The *E. coli* must find out ways to survive the environment that contains salt and osmotic stresses. This would mean that the *E. coli* has to undergo halophilization in order to survive the changes in its environment.

2.5 Biofilm Formation in *Escherichia coli*

A biofilm is a made up of organisms that have aggregated together on a surface, these organisms can be bacteria or eukaryotes such as the unicellular algae and fungi. The

formation of biofilms is a virulence factor that is exhibited by pathogenic bacteria such as the *Yersinia* sp., *Vibro cholerae*, *Neisseria* sp. and certain strains of *E. coli* which are enterohemorrhagic or enteropathogenic (Karatan and Watnick, 2009). An example of an enterohemorrhagic strain of *E. coli* is *E. coli* O157:H7, which was described briefly earlier. The ability to form biofilms is important for the survival and proliferation of these pathogenic bacteria in the human host. In other words, if the formation of biofilms is inhibited, the pathogens can be eliminated before they can cause any tissue damage. The formation of biofilms can be caused by cellular stresses that are experienced by the organisms. The stress causes these organisms to aggregate together to form a biofilm, which can provide a network of which these organisms can depend on to survive. As such, many organisms might not even be able to survive if biofilms are non-existent. The stresses can be in the form of heat stress, stress caused by antibiotics, or even salt stress. Biofilm formation typically starts when extracellular polymeric substances (EPS), proteins or DNA are excreted (Kawarai et al., 2009; Karatan and Watnick, 2009). These substances form a matrix to act as protection for the bacterial cells. The matrix can also form a network to allow communication between the individual bacterial cells in the biofilm.

2.6 Adaptation Study on *Escherichia coli* ATCC 8739

First discovered by Theodor Escherich in 1885, *E. coli* is currently one of the most commonly used bacteria in the field of biotechnology. *E. coli* is able to accept and transfer plasmids to and from other bacteria which makes it highly useful to create recombinant DNA. A particularly important use of manipulating *E. coli* in laboratories is to synthesize human insulin for treatment of diabetes patients. *E. coli* also exist in pathogenic strains, such as *E. coli* O157:H7 which is responsible for infection of enteric, urinary, pulmonary and nervous systems of mammals as well as food-borne illnesses (Strockbine et al., 1986).

The *E. coli* strain ATCC 8739, also known as the *Escherichia coli* C strain, is a faecal strain, often used to evaluate the efficiency of antimicrobial agents (Copeland et al., 2008). It has an insertion element within ompC; thus, expresses ompF as the major outer membrane porin (Copeland et al., 2008). The ATCC 8739 strain of *E. coli* is often used in research because its DNA has been fully sequenced. Mutations or adaptive resistances to the salt stress, which it

will be subjected to, will then be easily detected by simply comparing the changes in the bacterial genome against the original strain.

In a previous study to observe the evolution of *E. coli* growing under different concentrations of various food additives (Lee et al., 2010), it was found that high concentrations of food additives induced more stress on the bacteria which would induce a shorter generation time eventually. It was found that the *E. coli* bacteria took at least 25 passages to stabilize and adapt to the stress-inducing environment. The eventual shortening of generation time trend indicated that the bacteria was adapting to their various environments and could thrive better. However, it was found that despite the different food additives used to culture the bacteria, the RFLP showed a converging trend, which suggested that the evolution of the *E. coli* under the different stresses mutated the same stress mechanism and DNA repair amongst them (Lee et al., 2010).

2.7 Minimum Inhibitory Concentration on *Escherichia coli*

Minimum inhibitory concentrations (MICs) are defined as the lowest concentration of an antimicrobial that will inhibit bacterial growth after a period of time. Minimum inhibitory concentrations are often used to confirm resistance of a particular microorganism (Andrews, 2001). MICs can be determined by broth dilution and agar dilution (Wiegand et al, 2007). For broth dilution, antibiotics are inserted in a liquid microbial growth medium which is inoculated with a standardized number of organisms and incubated for a period of time. The lowest concentration (highest dilution) of test agent preventing appearance of growth is considered to be the minimal inhibitory concentration (MIC). For agar dilution, it involves the addition of different concentrations of antibiotics into a nutrient agar medium accompanied by the incorporation of a standardized number of organisms to the surface of the agar plate. The zone of growth is then examined.

Studies have been carried out on *E. coli* in which MIC of carbonyl cyanide m-chlorophenylhydrazone (CCCP) was used to test on *E. coli* halotolerance and comparing it with a halophilic bacteria (Ghoul et al., 1989). A drop of *E. coli* and *Vibrio* spp. culture which was incubated in Trypticase soy broth (TSB) at 37°C for 18 hour was transferred onto agar. Minimal medium M63 (Cohen and Rickenberg, 1956) with 10mM of glucose was used as the

carbon and energy source. The osmotic strengths of the media were increased by adding sodium chloride at the following concentrations: 0.085, 0.5, 0.68, 0.85, 1.02 and 1.20 molar. Bacteria were then incubated in the presence of CCCP dissolved in dimethyl sulfoxide at the following concentrations: 0, 0.312, 0.625, 1.25, 2.5, 5, 10, 20, 40, 50, 60, 80 and 100 μ M. MICs were determined by duplicate tests and readings taken after 18 hour of incubation. Their results show that when CCCP was used to test *E. coli* against a halophilic bacteria (*Vibrio spp*), the growth-inhibitory effect of CCCP was less on *E. coli* compared to *Vibrio spp*. This shows that *E. coli* have a higher MIC than *Vibrio spp*. However, when both are grown on sodium chloride, the growth-inhibitory was more pronounced for *E. coli* but stable for *Vibrio spp* when concentration of sodium chloride increases. This let them to conclude that resistance of *Escherichia coli* was dependent on the nature of the medium (Ghoul et al., 1989).

Rowbury et al. (1994) demonstrated when *E. coli* previously grown in low-salt broth containing NaCl was cultured with the addition of more salt (NaCl), the progeny of *E. coli* from the high salt broth are markedly more acid sensitive with protein synthesis-dependent and –independent sensitization components at exposure to pH 3.0. The strains that were used are derivatives of *E. coli* K-12. Organisms were grown in low salt broth at 37°C and incubated overnight with shaking. They were then transferred to Oxoid No. 2 broth and acidified with HCl. It was then shaken at 37°C and samples were removed at intervals, diluted in pH 7.0 and plated on nutrient agar for 24 to 30 h. This was to assess for its acid sensitivity. The strains were washed and shifted to the same medium with the addition of more salt (NaCl) to compare the acid sensitivity of its progeny of the latter and the former.

Aagaard et al. (1991) attempted to find the MIC of ciprofloxacin and trimethoprim for *E. coli* at different inoculum size of *E. coli* ranging from 10^2 to 10^9 , pH values, prostatic tissue extracts and urine from humans and dogs which were used as a medium for *E. coli*. Their aim was to evaluate the potential efficacy of both drugs against *E. coli* when used under various conditions. Five different strains of *E. coli* were isolated from patients with urinary tract infections. The MIC was then determined with routine dilution method. To elucidate the effect of inoculum size on MIC, the initial 10^9 of bacteria inoculum was diluted down ten-fold down to 10^2 with MIC being determined at each step. To study the effect of tissue and body fluids on MIC, 1g of prostatic tissue from human was homogenize in 3mL of phosphate

buffer at pH 7.0. The produced supernatant along with the human urine was adjusted to pH 7.0 using sodium hydroxide and the MICs were determined at each medium.

2.8 Objectives and Hypothesis of Project

The objective is to adapt *E. coli* ATCC 8739 from 1% NaCl medium (Lee et al., 2010) to 10% NaCl. The original ATCC 8739 strain and adapted strain (Lee et al., 2010) does not differ in osmotolerance as it is below the 5% critical level and as such, would still undergo salt stress, resulting in stretched generation times at the start of every increase in salt concentration percentage. As *E. coli* adapts to the salt concentration over passages, the generation time is expected to decrease, showing an improved fitness of the *E. coli*. The *E. coli* will then be subjected to a higher concentration of salt with increasing intervals of 0.5-1% NaCl for another period of time (passages) till it has fully adapted to the new concentration. The concentration of salt is increased again when the *E. coli* is found to have adapted to the present salt concentration. *E. coli* is likely to adapt to 5–10% NaCl, however, the rate of adaptation is expected to be slower as the concentration of salt increases. The point of salt concentration whereby the *E. coli* grows best at, judging by OD_{max}, is expected to increase with respect to the highest salt concentration that it has adapted to. The osmotolerance of *E. coli* is likely a genetic effect and the DNA profile is likely to differ from the ancestor strain as it adapts to grow at higher salt concentrations.

3. Methods and Materials

3.1 Subculture

The halophilization of *Escherichia coli* was done in 4 separate samples, A, B, C and D, with each sample prepared in an individual 15ml tube. The nutrient used for the growth of *E. coli* samples are 1× nutrient broth with a fixed concentration of NaCl for acclimatization. 100 μ l *E. coli* samples are subcultured into a new tube on a 2 to 3 day basis. This passage of time was chosen such that subcultures were done on Mondays, Wednesdays and Fridays, with the following subculture on the next Monday, will be a 3 day passage. The increment of NaCl concentration was determined during the course of subculture.

3.2 Glycerol Stock

Glycerol stocks were done for the latest passage of *E. coli* which has adapted to the particular salt concentration and shown decreased generation times. 100 μ l of *E. coli* from the 4 sample tubes are inoculated respectively onto 4 separate MacConkey agar in petri dishes and incubated over 2 to 3 days. 3ml of sterile 90% (v/v) glycerol is added to the MacConkey agar in the petri dish to resuspend the colonies, which is then aliquoted into eppendorf tubes and frozen at -80°C.

3.3 Generation Time and Cell Density

Generation time was analyzed at every 3 passages. 10 μ l of *E. coli* cultures from each sample tubes (A, B, C, D) were inoculated into 1ml of 1× nutrient broth and OD600 readings were taken at intervals of up to 360 minutes.

3.4 Minimum Inhibitory Concentration

10 μ l of culture from each sample were inoculated into 1× nutrient broth supplemented with different range of salt concentration of 0% (w/v) NaCl, 1% (w/v) NaCl, 3% (w/v) NaCl, 5% (w/v) NaCl, 7% (w/v) NaCl, 9% (w/v) NaCl and 11% (w/v) NaCl and incubated for 21 to 23 hours at 37°C. OD600 readings were taken after incubation.

3.5 Colony Minimum Inhibitory Concentration

Each sample of *E. coli* was streaked on nutrient agar and incubated overnight at 37 °C. Ten colonies were randomly taken from each plate and inoculated into 1ml of 1× nutrient broth and incubated overnight at 37 °C. These colonies were then subjected to the MIC experiment.

3.6 Extraction of Total Genomic DNA from *E. coli* Cell Cultures

Genomic DNA from the *E. coli* was extracted using the Phenol-Chloroform method for Gram-negative bacteria (Cheng and Jiang, 2006). The cells were harvested by centrifugation at 4,000rpm for 15 minutes and washed twice with 400µl of STE buffer (100 mM NaCl, 10 mM Tris/HCl, 1 mM EDTA, pH 8.0). 200µl of Tris/HCl buffer was lastly added to the cell pellet and this was followed by the addition of 200µl of Phenol/Chloroform/Isoamyl Alcohol (25:24:1). The suspension of cell was vortexed and centrifuged at 13,000rpm for 5 minutes. The white interphase was first removed with a sterile toothpick before vortexing and undergoing a second centrifugation at 13,000rpm for 5 minutes. The aqueous phase was added to 200µl of chloroform, vortexed and centrifuged at 13,000rpm for 5 minutes. The aqueous phase was added into equal volume of absolute isopropanol and incubated at -20°C for at least 30 minutes for DNA precipitation. The precipitate was harvested by centrifugation at 13,000rpm for 20 minutes and air-dried at room temperature before resuspension to 100ng/ul in sterile Nanopure water and stored at -20°C until use.

3.7 Polymerase Chain Reaction

A total volume of 50µl of reaction mixture is prepared from 200ng of DNA template in 10pmoles of dNTPs, 50pmoles of primer, 1 unit of Taq Polymerase and lastly 1X standard buffer (containing 1.5mM of MgCl₂) that is provided by the supplier (New England Biolabs, Inc.). A total of 3 primers were used separately in different reactions, they are: Primer 5, CgCgCTggC; Primer 6, gCTggCggC; and Primer 7, CAggCggCg. The PCR reaction was carried out (Hybaid Limited, PCR Express) with the following procedure: initial denaturation at 95°C for 10minutes, followed by 35 cycles of amplification, with each cycle beginning at 95°C for 1 minute, 27°C for 1 minute, 72°C for 3 minutes. The final extension is at 72°C for 10 minutes. Gel electrophoresis is then conducted on the PCR products in 2% (w/v) agarose gel using 1X GelRed for analysis.

3.8 Restriction Fragment Length Polymorphism

11 μ l of the PCR product is digested using 1 unit of restriction endonuclease (MspI, HinfI or TaqI) in a total volume of 20 μ l with 1X restriction buffer and 100ng/ μ l acetylated BSA provided by the supplier. Each reaction was incubated at 37°C (65°C for TaqI) for 16 hours before gel electrophoresis is conducted in 2% (w/v) agarose gel using 1X GelRed for analysis.

3.9 Data Analysis

Five and Seven Day Cell Density. The cell density of the passages was estimated from the OD600 readings using cell size correction suggested by Sezonov et al. (2007). The size of the cells remains constant up to OD600 0.3, which is equivalent to 5×10^7 cells per milliliter. After OD600 0.3, the size of the cells decrease and the correlation between the OD600 and the cell density changes. The correction graph (Lee et al., 2010) shows the standard curve of cell density = $52137400 * \ln(\text{OD}600 \text{ Reading}) + 118718650$.

The cell density for the five and seven day cultures were compared by dividing the cell density at day seven with the cell density at five days. A ratio of 100% would indicate that there is no increase in cell density from the period of time between day five and day seven. Whereas a ratio lower than 100% would mean that the cell density at day five is higher than the cell density at day seven and vice versa for a ratio that is higher than 100%.

Minimum Inhibitory Concentration. The OD600 readings obtained from the MIC experiment were fitted to $\text{OD}600 = M_4(\% \text{NaCl})^4 + M_3(\% \text{NaCl})^3 + M_2(\% \text{NaCl})^2 + M_1(\% \text{NaCl})^1 + M_0$. The concentration of NaCl in % ($[\text{NaCl}] \%$) whereby the OD600 is at maximum, $[\text{NaCl}] \%$ whereby OD600 is at half of maximum and Area under the curve (AUC) whereby NaCl is higher than 7.5% were calculated from the fitted equations. This process of analysis is also repeated for each colony in the colony MIC experiment.

Number of Generations. OD600 readings were taken prior to subculture to determine the cell density in the passage. As 10% of the culture was used as inoculum, the number of generations occurred within each passage can be calculated. This allowed an overview of the generations formed across the various passages, allowing the analysis of generations. The

increase or decrease in generations would be able to show the adaptation of *E. coli* to the respective passage salt concentration. Data points which were abnormally high, as they affected due to the presence of red dye due to prior decontamination using MacConkey agar were removed from the tabulations to reduce inaccuracies in calculation of generations formed.

Generation Time. The first reading was not taken due to lag phase inclusion of growth. The geometric mean of the following intervals were obtained using cell density after cell count correction (Sezonov et al., 2007) to give an average generation time in minutes which was representative of the treatment. The generation time of all 4 samples having the same treatment were compared with one another across passages.

Polymerase Chain Reaction / Restriction Length Polymorphism. Migration distance of the bands for the PCR and RFLP agarose gels between the four treatments (A, B, C and D) within the same passage were tabulated and a Nei-Li Dissimilarity Index (DI) (Nei and Li, 1979) was obtained for each pair-wise comparison (6 in total) between the treatments of each passage. Nei-Li DI is a measure of the genetic differences between two organisms, according to the presence and absence of common bands before and after digestion by restriction endonucleases. The Nei-Li DI was calculated (Chay et al., 2010) where a maximum DI value of 1 means that there are no common bands between the two comparing samples while a minimum DI value of 0 means that the two comparing samples have the exact same bands. The DI for the 6 pair-wise comparisons of each passage were calculated.

4. Results

4.1 Number of Generations

The number of generations per passage is generally constant from Passage 1 – 43 with exception of passages 16 – 21 where a increase in number of generations per passage (Figure 4.1). It is seen from passage 43 that the number of generations from Passage 43 to 80 fluctuated with a gradual increasing trend.

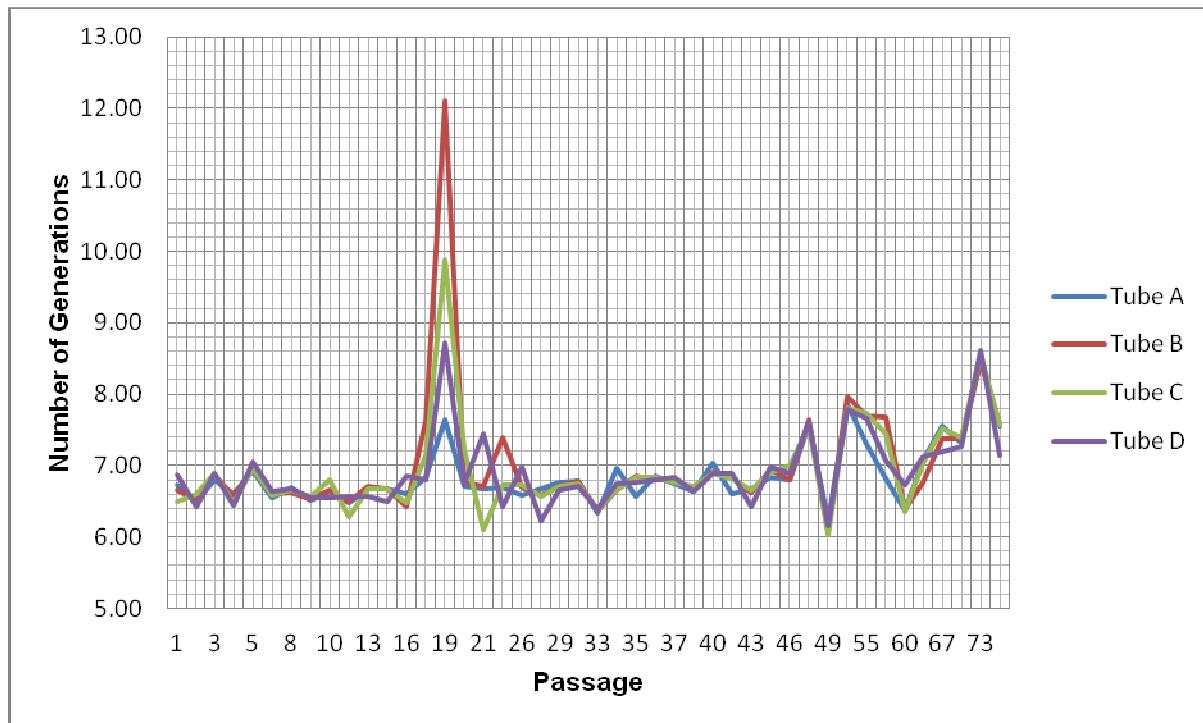


Figure 4.1: Comparison of 2-Day Generations Across Tubes A, B, C and D.

The trend lines for the four different tube cultures that the number of generations increase across passages (Figure 4.2). Tube C has the steepest gradient of 0.0172, followed by Tubes A, D and B, with gradients of 0.0168, 0.0152 and 0.0128 respectively.

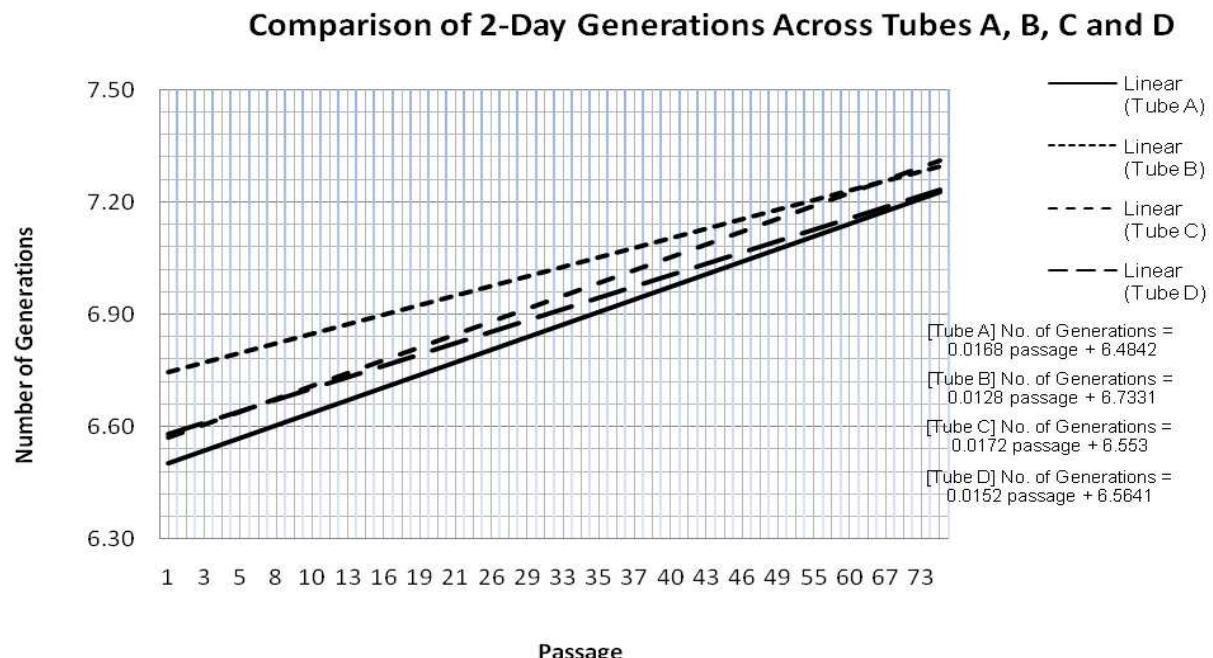


Figure 4.2: Comparison of 2-Day Generations Across Tube A, B, C and D Trend Line.

4.2 Generation Time

Analysis of the four tubes generation time over the passage had shown different rates of increasing generation times (Figure 4.3, 4.4). The steepest increase in generation time occurs in Tube D where approximately 1.18 increases in generation time per passage over 80 passages were seen. This is followed by Tube B (0.76 minutes), Tube A (0.68 minutes), Tube C (0.42 minutes). The intercept on the generation time axis (Figure 4.3) for all four tubes may be used to estimate the generation time of the cells for the first passage which is indicative of the level of initial stress (3% NaCl) on the cells.

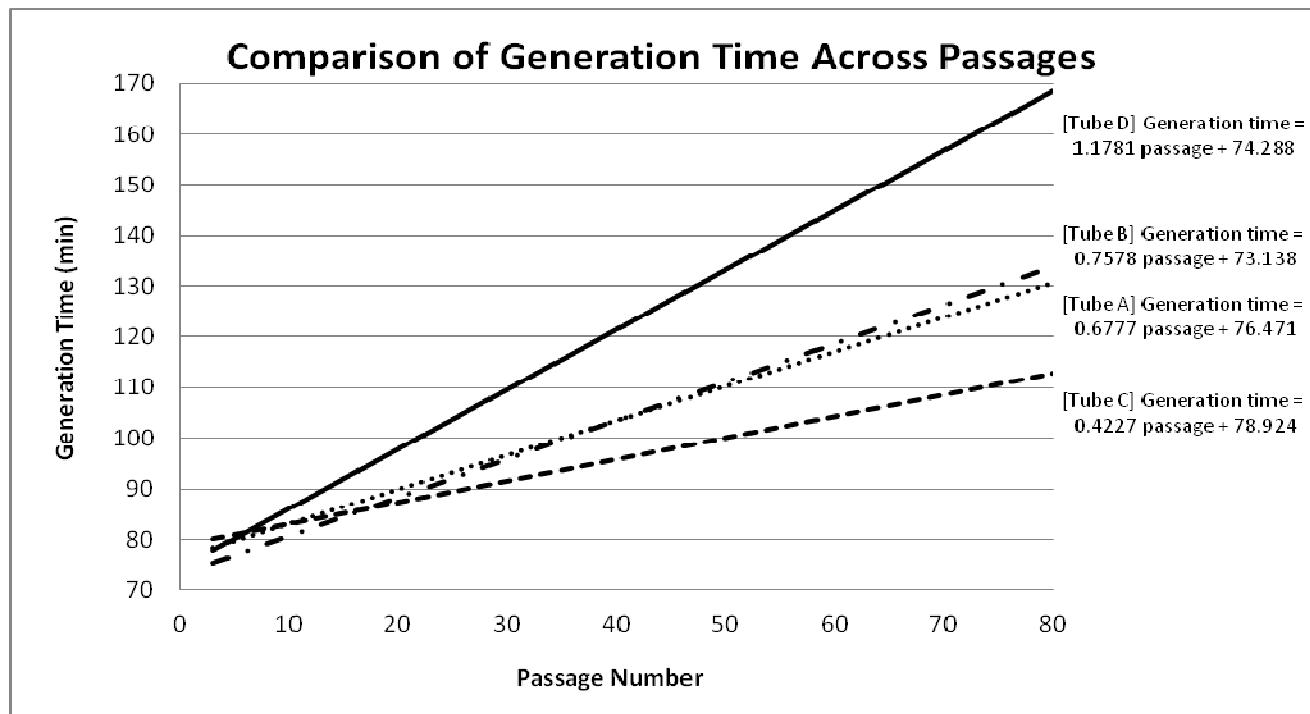
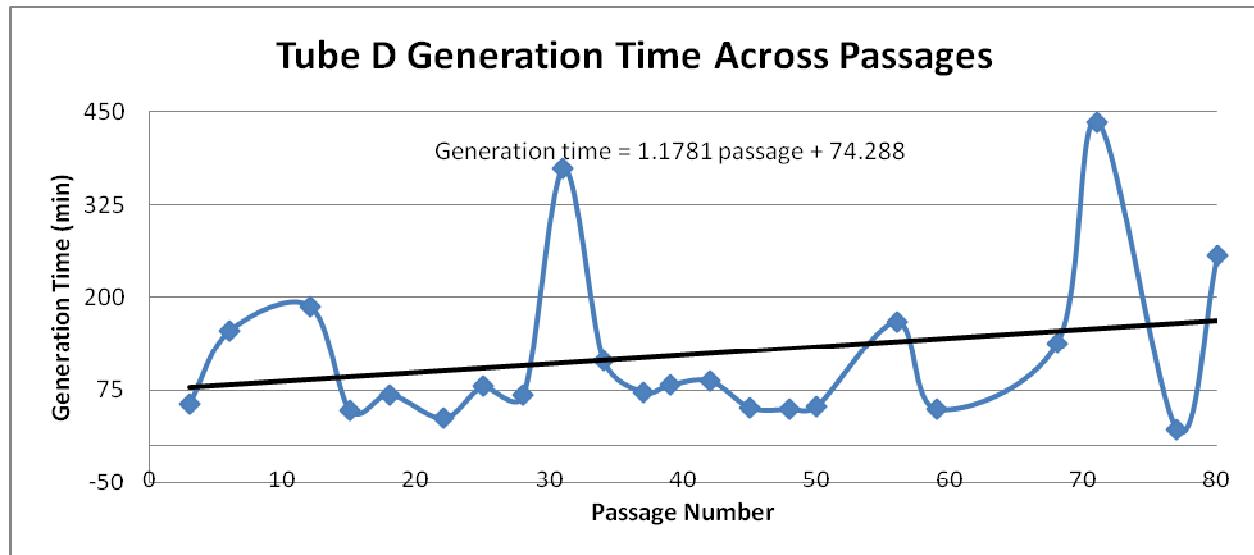
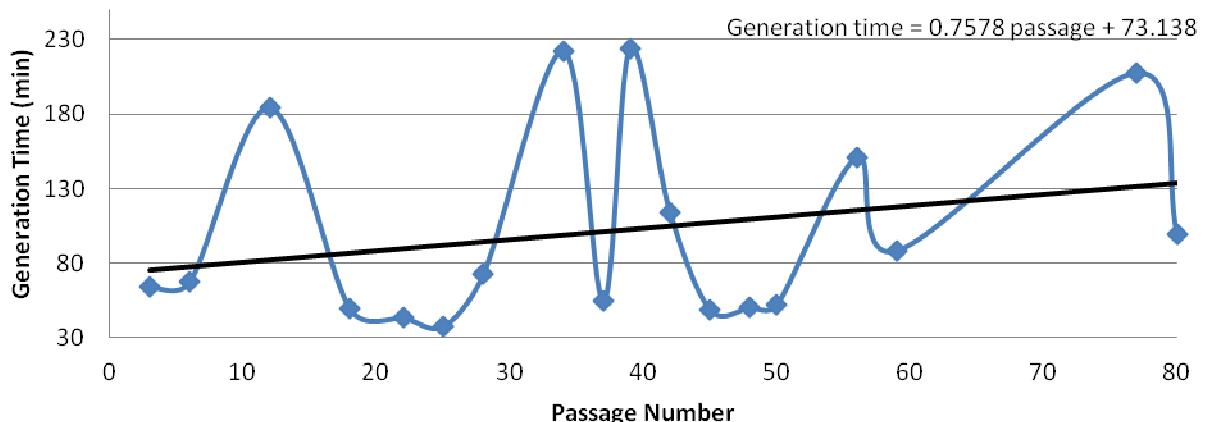


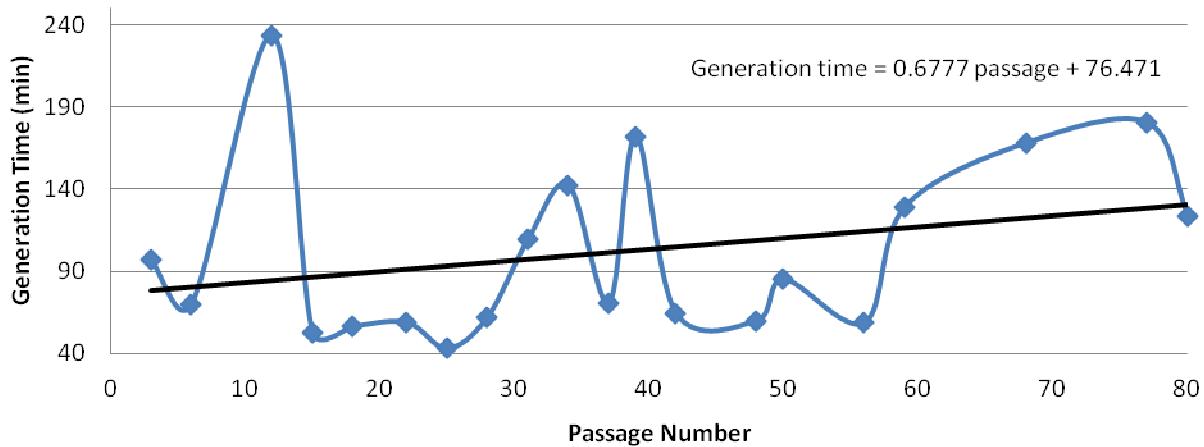
Figure 4.3: Comparing the generation time of four tubes: Tube A, Tube B, Tube C and Tube D across 80 passages.



Tube B Generation Time Across Passages



Tube A Generation Time Across Passages



Tube C Generation Time Across Passages

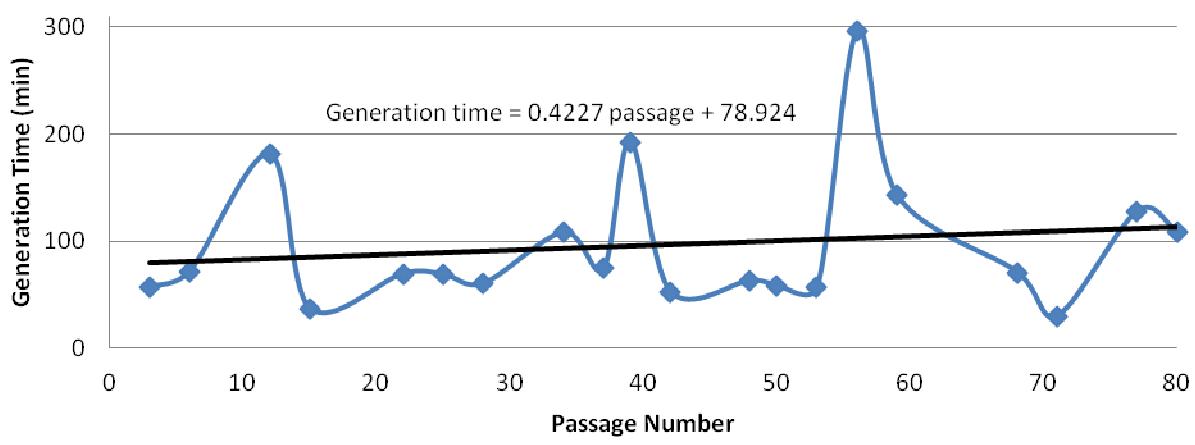


Figure 4.4: Generation time of the four tubes: Tube D, Tube B, Tube A and Tube C respectively in order across 80 passages.

Source	Type III Sum of Squares	df	Mean Square	F	Significance
Treatment	82976.186	6	13829.364	3.176	0.010
Tube	9085.138	3	3028.379	0.696	0.559
Treatment × Tube	69277.818	18	4075.166	0.936	0.539

Table 4.1: Tests of between – subjects effects in relation to generation time.

Different concentration of NaCl (treatment) and tubes (A, B, C, D) were used to analyze the significance of generation time in relation to the increasing concentration of salt (Table 4.1). Two factor analysis of variance (ANOVA) on the treatment and tube demonstrated that only the treatment is significant (p -value = 0.01).

4.3 Day 7/Day 5 Cell Density Ratio

From around P1 – P15 (3% NaCl) to P16 – P31 (4% NaCl), the coefficient of variations started to increase. There is also an increase in P40 – P50 (5% NaCl). However, the coefficient of variation starts to decrease when the NaCl is increased to 6% and above. Table 4.2 shows the coefficient of variations which are calculated using the Day 7 / Day 5 cell density ratios.

Passage (NaCl%)	Tube			
	A	B	C	D
P1 - P15 (3%)	0.66%	0.93%	0.56%	0.61%
P16 - P31 (4%)	0.83%	2.55%	1.31%	1.07%
P32 - P39 (4.5%)	0.50%	0.41%	0.70%	0.88%
P40 - P50 (5%)	1.32%	1.10%	1.30%	1.69%
P51 - P62 (6%)	0.50%	0.29%	0.57%	0.34%
P63 - P74 (7%)	0.66%	0.60%	0.71%	0.38%
P75 - P80 (8%)	0.54%	0.45%	0.73%	0.36%

Table 4.2: Table of coefficient of variation for all four treatments, from 3% NaCl to 8% NaCl.

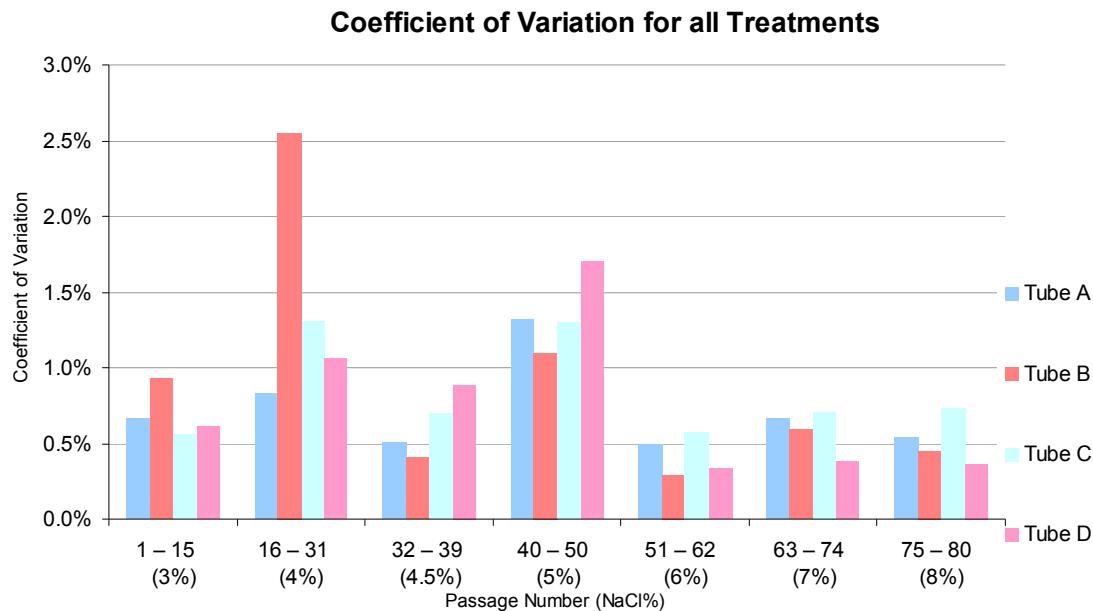
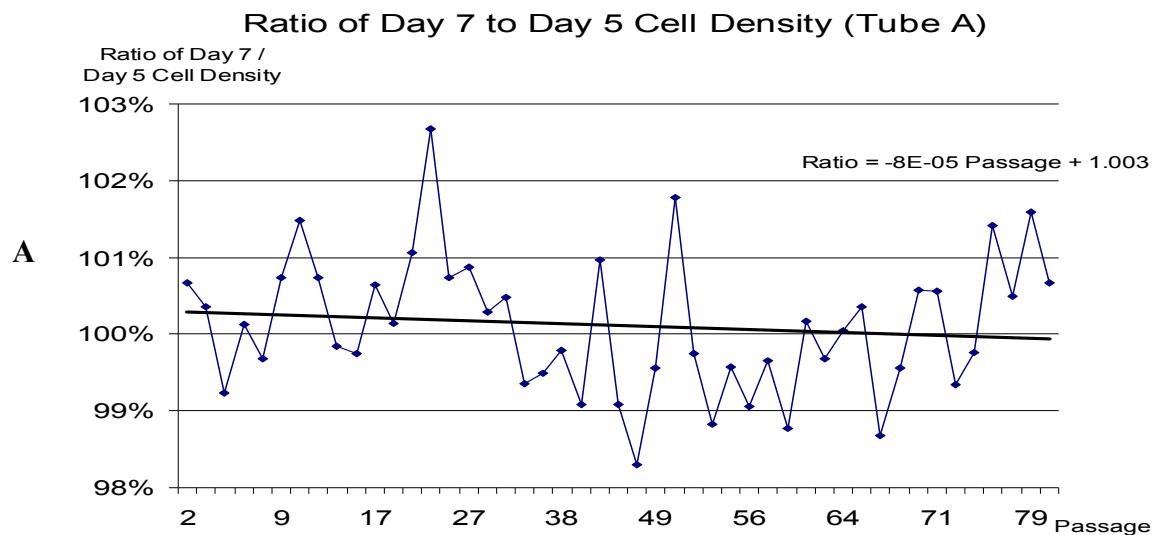
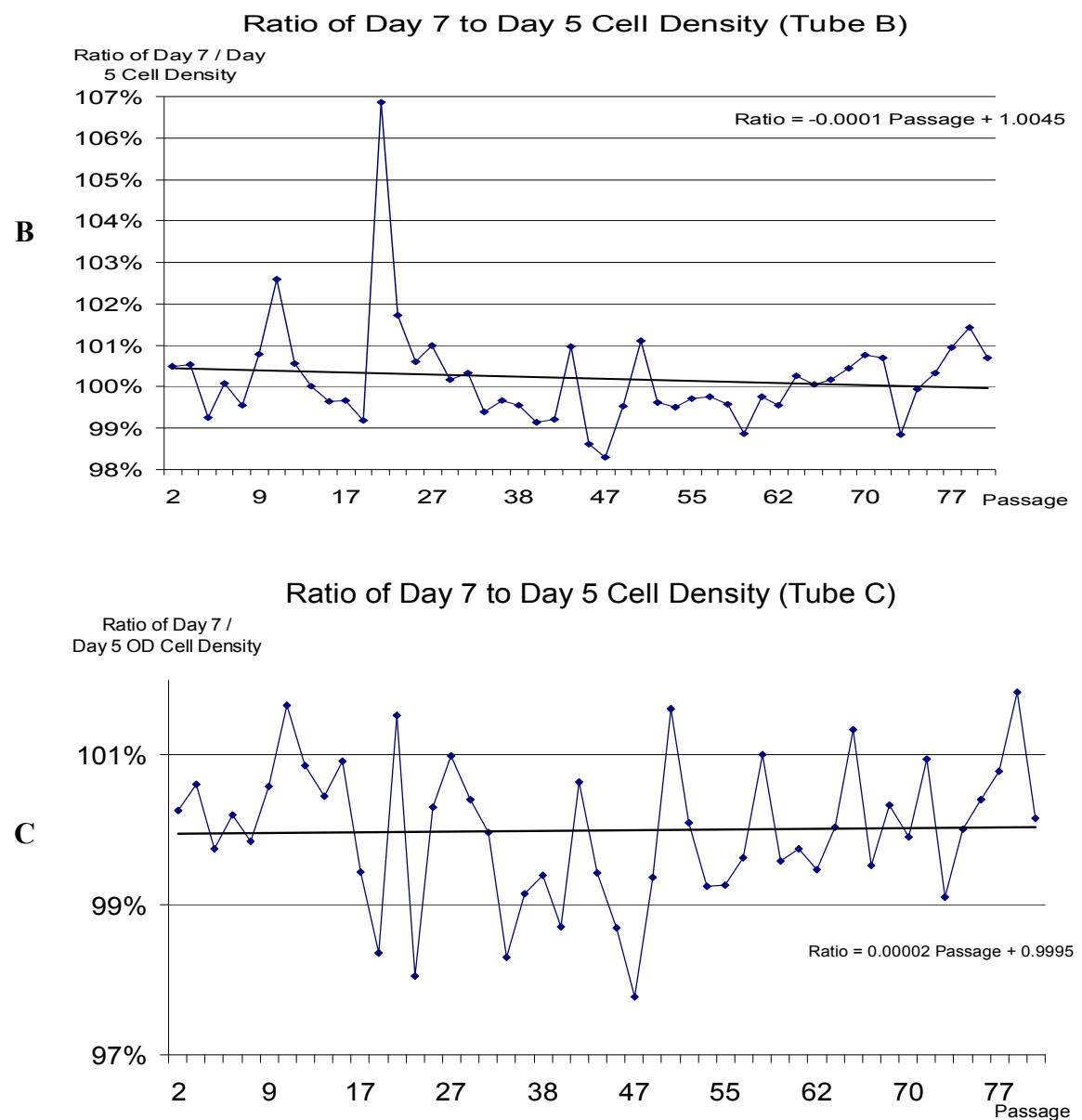


Figure 4.5: Coefficient of variation for all four tubes over 80 passages.

Generally the ratio of Day 7 to Day 5 cell density has decreased over 80 passages, except for Tube C, which increased (Figure 4.6). However, the rate of decrease and increase for all four tubes are very gradual. The tubes with the fastest rate of decline are Tube B and Tube D at a decrease of 0.0001% every passage. Tube C is the only tube with an increase of ratio. The ratio of Tube C has increased over a rate of 0.00002% every passage. The ratio of all four tubes has maintained at around 99% to 101% even though the amount of NaCl is increased from 3% to 8% over the course of 80 passages.





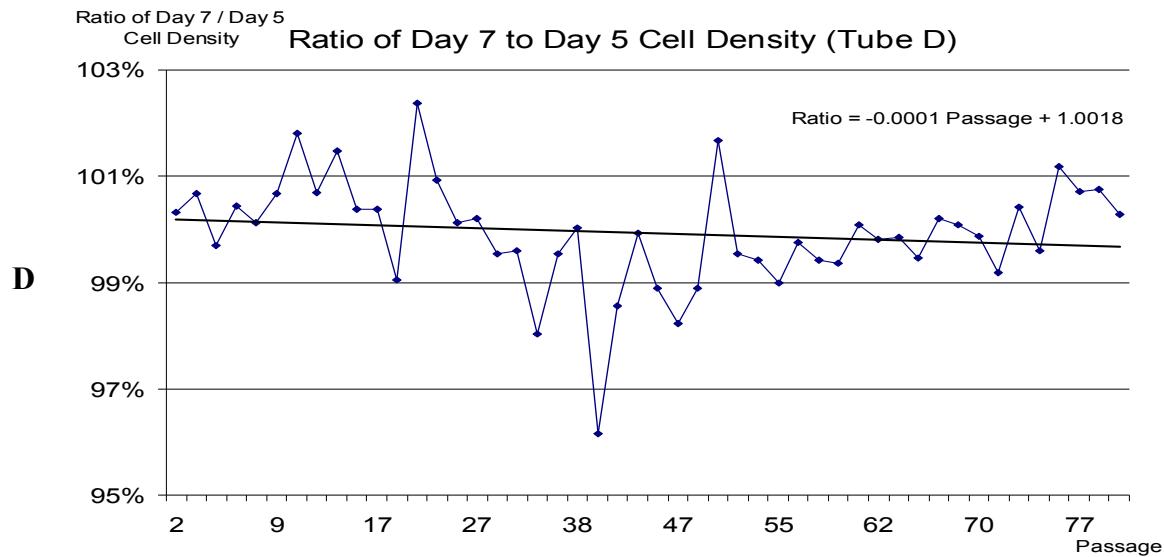


Figure 4.6: Ratio of day 7 to day 5 cell density of Tubes A, B, C and D over 80 Passages.
Tube A (A), Tube B (B), Tube C (C), Tube (D).

4.4 Minimum Inhibitory Concentration (MIC) – R² Values

Table 4.3 shows the polynomial fittings of the MIC data. The polynomials range from x^1 to x^6 . Values below 0.950 are only found in the fittings of x^3 and lower polynomials. There are no r^2 values lower than 0.95 in the fittings of x^4 and the higher polynomials.

Power	Time (hour)	Tube A	Tube B	Tube C	Tube D
x^1	17	0.945	0.920	0.868	0.901
	19	0.956	0.926	0.852	0.891
	21	0.959	0.938	0.888	0.910
	23	0.969	0.947	0.928	0.941
x^2	17	0.942	0.921	0.877	0.902
	19	0.956	0.927	0.875	0.904
	21	0.959	0.941	0.895	0.917
	23	0.969	0.952	0.929	0.942
x^3	17	0.988	0.971	0.920	0.985
	19	0.981	0.969	0.910	0.983
	21	0.989	0.972	0.912	0.985
	23	0.992	0.980	0.944	0.988
x^4	17	0.991	0.979	0.956	0.985
	19	0.994	0.979	0.961	0.984
	21	0.995	0.983	0.972	0.986
	23	0.998	0.987	0.983	0.991
x^5	17	0.991	0.996	0.972	0.995
	19	0.997	0.995	0.971	0.997
	21	0.995	0.996	0.988	0.994

	23	0.998	0.994	0.989	0.996
x^6	17	1	1	1	1
	19	1	1	1	1
	21	1	1	1	1
	23	1	1	1	1

Table 4.3: Fitting of the MIC graphs using different polynomial equations. Values shown are the r^2 values between the original OD600 readings and fitted equations. r^2 values that are below 0.950 are shaded.

As shown on the Figure 4.7 below, the x^6 fitting of the OD600 readings of Tube A at 19 hours passes through every data point of the original data. This gives rise to many gaps in between the original data points. Although the r^2 values of the x^6 fittings as shown in the table are equal to 1, the gaps from the graph indicate that the fitting of the data is forced in order to achieve the r^2 values of 1. The presence of such gaps in the x^4 fitting is much lesser as compared to that of x^6 .

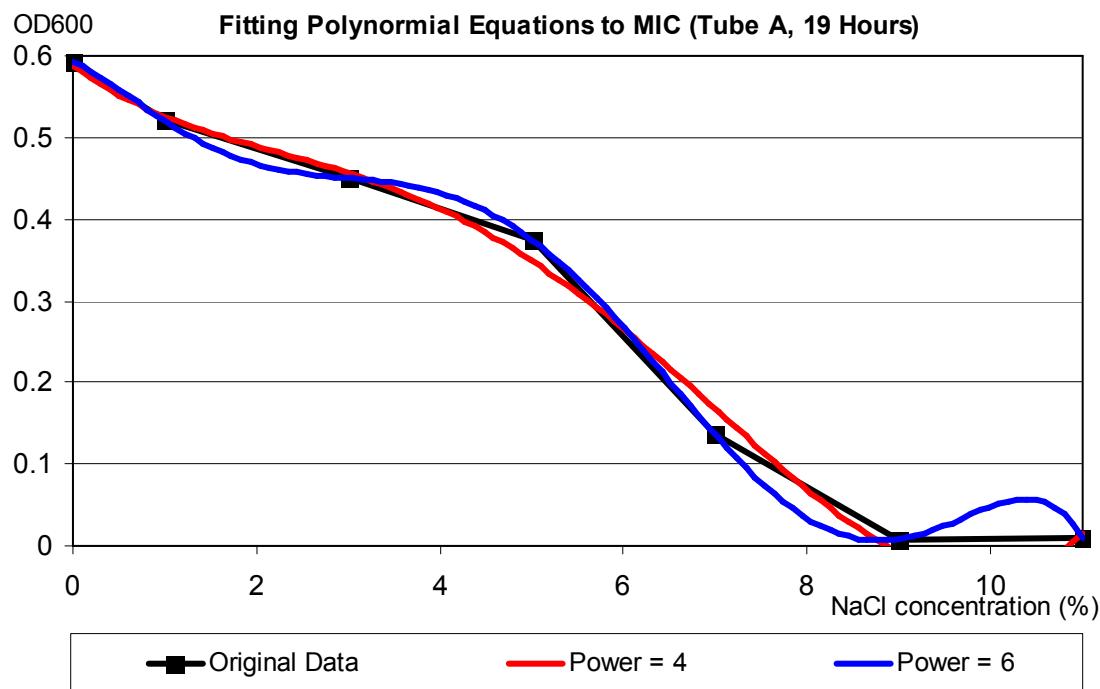


Figure 4.7: Fitting of Polynomial Equations to MIC (Tube A, 19 hours).

From Figure 4.8 below, the x^4 fitted OD600 readings at 21 hours is almost the same as the x^4 fitted OD600 readings at 23 hours. The x^4 fitted OD600 readings of 17 and 19 hours do not intersect the other lines.

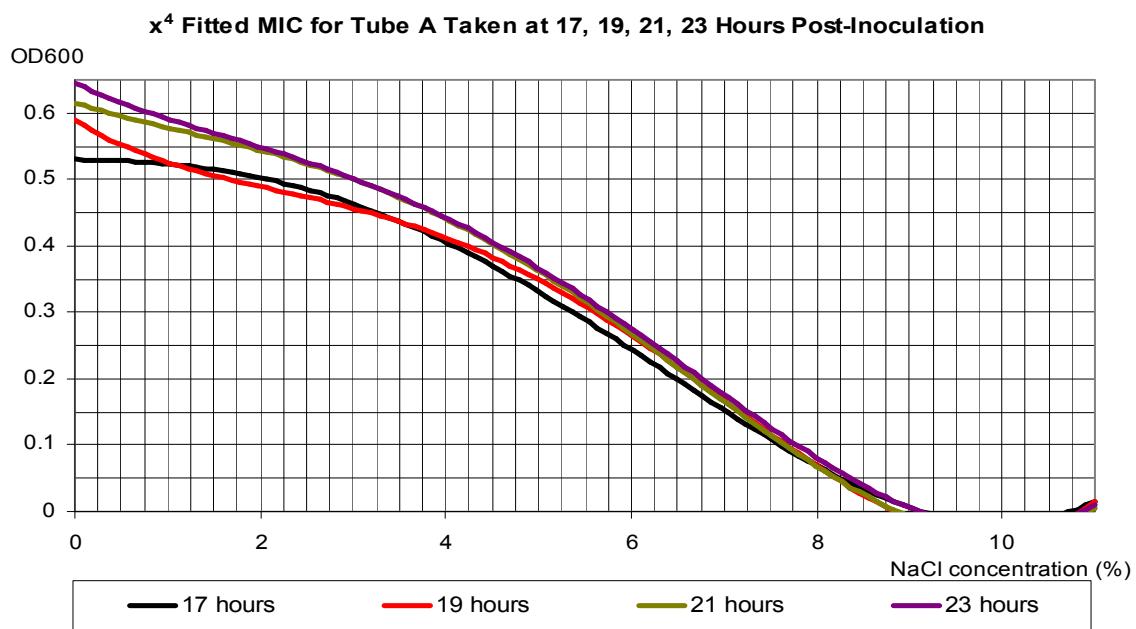


Figure 4.8: x^4 fitted MIC for Tube A taken at 17, 19, 21 and 23 hours post-inoculation.

4.5 Minimum Inhibitory Concentration

The concentration of NaCl whereby the OD is at the maximum as shown in Figure 4.9 has decreased over 80 passages, except for Tube B. The fastest rate of decrease is Tube D, at a rate of 0.1269% every passage. The tube with the slowest rate of decrease is Tube A, which decreased at 0.0308% every passage. The concentration of NaCl whereby the OD is at the maximum for Tube D has decreased by about 1% (from 2.0 to 0.4) over the 80 passages.

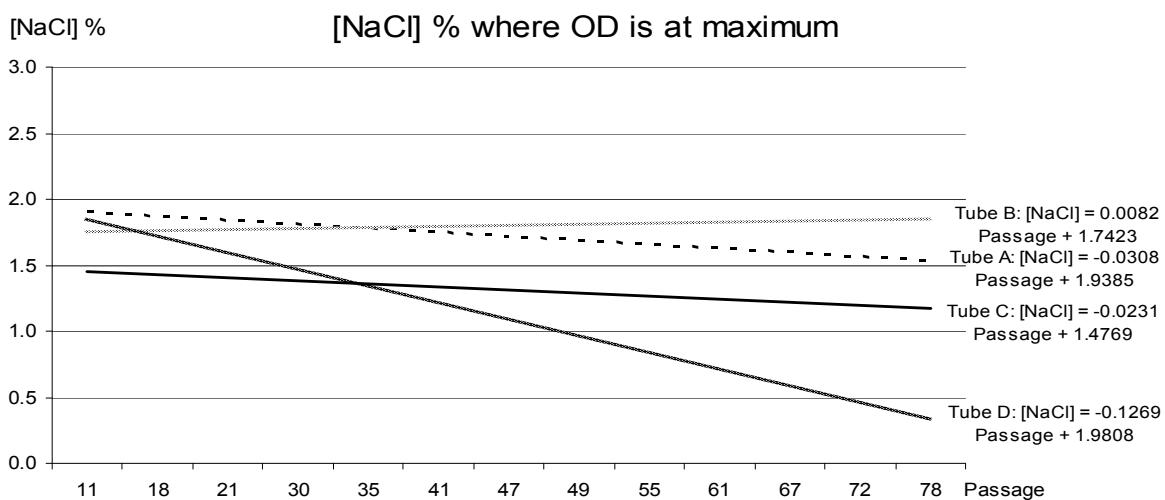


Figure 4.9: Concentration of NaCl (%) whereby the OD is at maximum.

Generally, it is observed that the concentration of NaCl whereby the OD is at half of maximum has increased over the course of 80 passages, except for Tube C (Figure 4.10). The concentration of NaCl whereby the OD is at half for Tube C has decreased at a rate of -0.0679% every passage from more than 5.8% to around 4.8%, approximately a decrement of 1% over the 80 passages. The concentration of NaCl whereby the OD is at half for the rest of the tubes have increased gradually, with the fastest increase in Tube A at a rate of 0.0275% every passage and the slowest increase in Tube D at a rate of 0.0013% every passage. In overall, the concentrations of the tubes except for Tube C have maintained at around 5.5 to 5.8%.

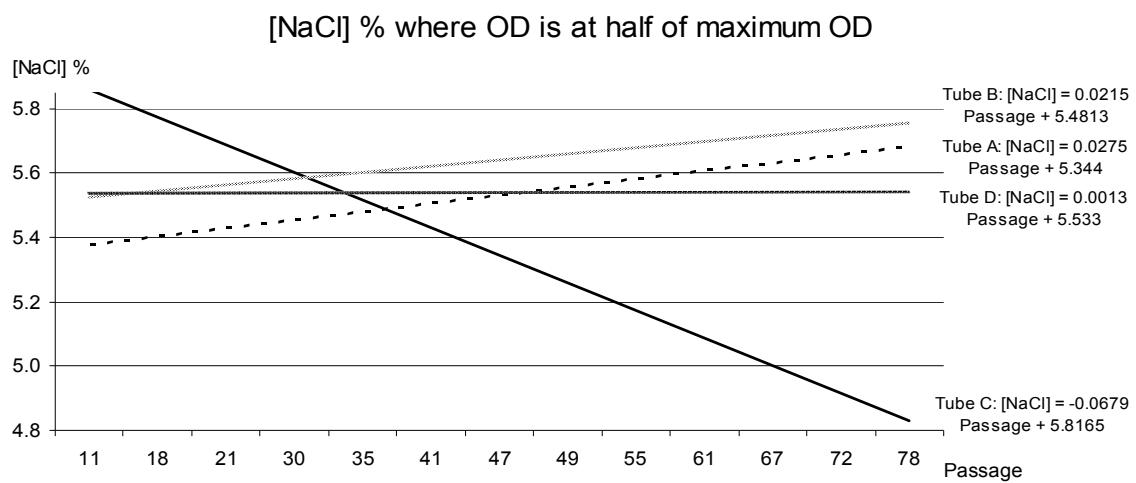


Figure 4.10: Concentration of NaCl (%) whereby the OD is at half of maximum.

The area of the curve (AUC) whereby the concentration of NaCl is more than 7.5% has increased for all four tubes over 80 passages (Figure 4.11). The fastest rate of increase is Tube B, at 0.0144% AUC for every passage. The slowest rate of increase is Tube A, at a rate of 0.003 every passage. The AUC for Tube B is the highest, at more than 0.16.

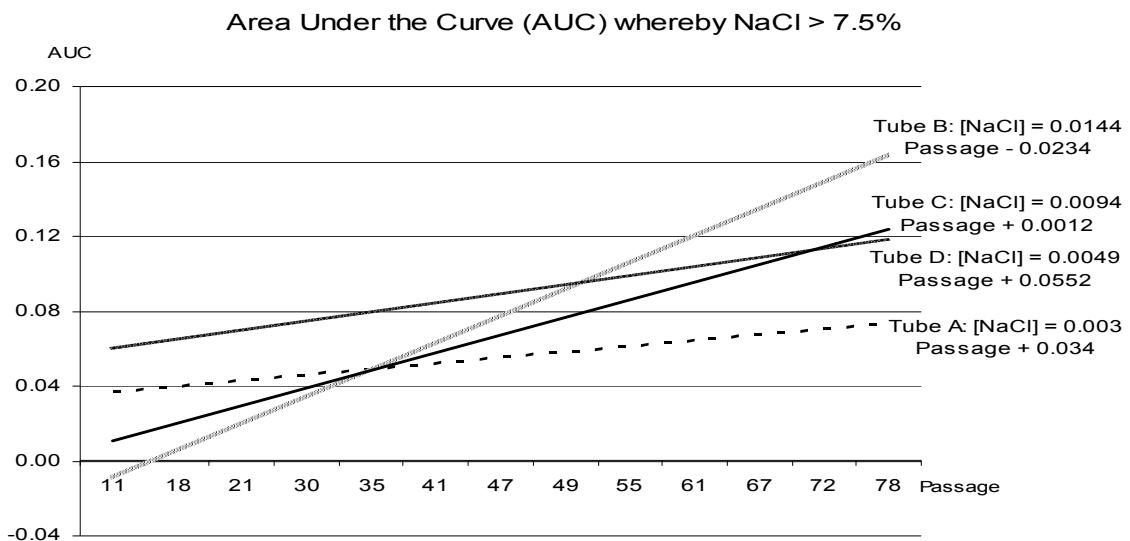


Figure 4.11: Area under the curve whereby the concentration of NaCl is more than 7.5%.

The *E. coli* are able to grow at 10% NaCl as shown by the MIC data for 11% NaCl for the four tubes (Figure 4.12). The growth has generally remained stable throughout, with a rapid increase at passage 64 onwards. The OD reading of nearly 0 at Passage 64 has increased to around 0.15 to 0.25 for the four tubes. The least amount of increase is Tube C, followed by Tube B and lastly Tube D. Tube A has the highest amount of increase in OD.

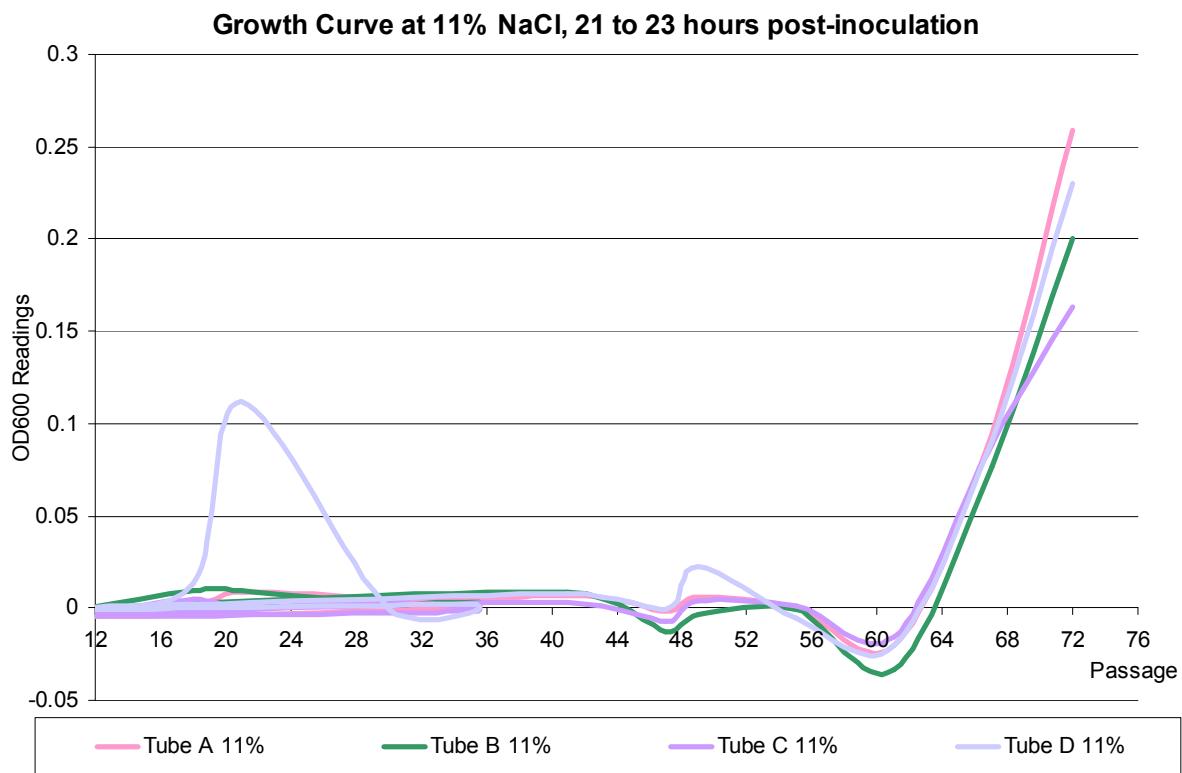


Figure 4.12: Growth Curve at 11% NaCl, 21 to 23 hours post-inoculation.

4.6 Colony MIC

Table 4.4 shows the tabulations of means and standard deviations of colony MICs performed on Passage 44, 53 and 72. The mean ODmax across all three colony MICs has decreased generally, with an increase in Passage 53. However, the standard deviation of Passage 53 is also the highest, which means that there is much variability in the ODmax of the colonies of which MIC was performed on. The concentration of NaCl whereby the OD is at half of maximum has maintained at around 4% to 6% across all passages, with an increase in Passage 53 and a subsequent decrease in Passage 72. Lastly, the %AUC has increased generally across all passages, with little variability except for Passage 72, tube D.

Passage [NaCl]%	Tube	ODmax		1/2ODMax		%AUC[NaCl>7.5%]	
		Mean	SD	Mean	SD	Mean	SD
44 (5%)	A	1.060	1.007	5.360	0.842	0.026	0.048
	B	0.160	0.347	5.570	0.525	0.041	0.044
	C	0.520	0.755	4.890	0.669	0.070	0.085
	D	1.856	0.810	5.456	0.609	0.096	0.129
53 (6%)	A	2.290	1.613	6.430	0.736	0.122	0.150
	B	1.760	1.883	6.550	0.443	0.091	0.123
	C	1.450	1.701	6.400	0.445	0.079	0.126
	D	0.556	1.245	6.533	0.827	0.094	0.122
72 (7%)	A	0.960	1.171	4.190	1.574	0.091	0.139
	B	0.130	0.236	3.560	1.767	0.157	0.148
	C	0.790	0.972	5.800	1.327	0.080	0.075
	D	0.378	0.474	4.689	0.971	0.205	1.756

Table 4.4: Table of Means and Standard Deviations (SD) of Colony MIC for Passage 44, 53 and 72.

4.7 Polymerase Chain Reaction / Restriction Fragment Length

Polymorphism

The electrophoresis agarose gels of the PCR and RFLP products for all four treatments were used to study the differences between the genomes of the four treatments. The Nei-Li

Dissimilarity Index (DI) was utilized to mathematically calculate the dissimilarity of the genomes between the pair-wise combinations of treatments. The DI values are then entered into a dissimilarity matrix for each passage. The mean DI and the standard deviation of each passage are calculated as the four treatments are essentially duplicates; the results are then plotted in Figure 4.12.

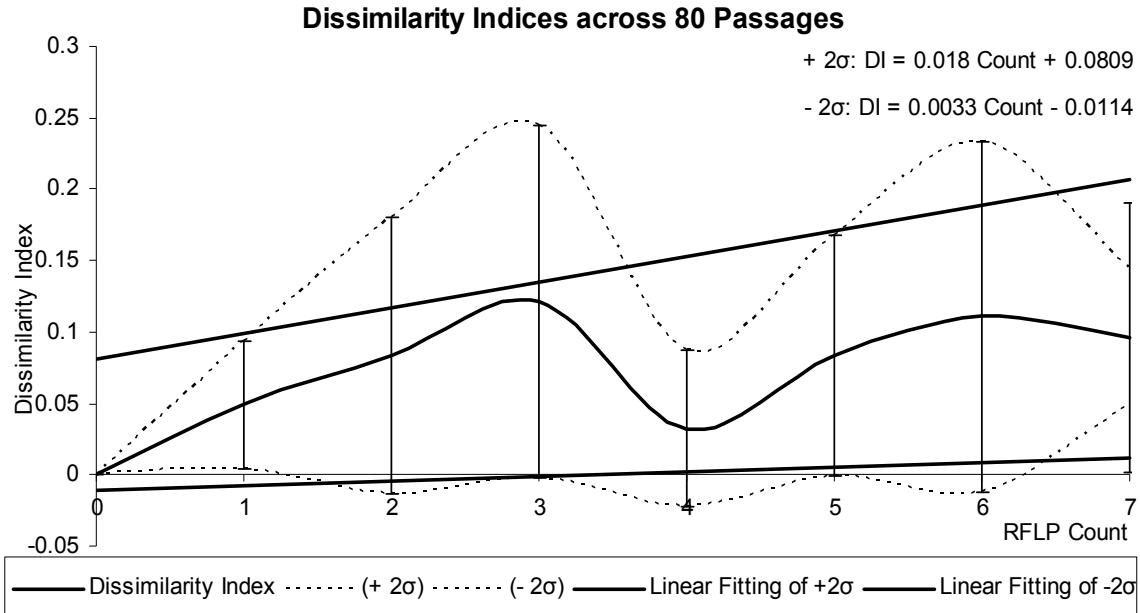


Figure 4.13: Dissimilarity Indices across 80 passages, error bars are $\pm 2\sigma$. RFLP count: 1 (P11), 2 (P21), 3 (P30), 4 (P42), 5 (P55), 6 (P66) and 7 (P76).

The mean DI has increased gradually, but dipped from RFLP count 3 onwards. This dip is followed by another increase. The increase of DI between counts 1 – 3 is faster as compared to between counts 4 – 6. The linear fittings of the two error bars have been steadily increasing, with the $+2\sigma$ linear fitting increasing at a rate of 0.018 for every RFLP count and the -2σ linear fitting increasing at a rate of 0.0033 for every RFLP count. The bounded area between the two linear fittings has also increased, gradually forming a fan-shaped area. The rate of increase of the $+2\sigma$ linear fitting is also higher than that of the -2σ linear fitting.

5. Discussion

5.1 Increase NaCl Concentration Increases Generation Time

The generation times of cells was observed to increase gradually across passages (Figure 4.3). This demonstrated that the increase in salt (NaCl) concentration increases the generation times of *E. coli* as the treatments are inducing changes to the cells affecting their ability to adapt and divide. This is supported by study showing that bacteria that are exposed to NaCl typically exhibits a prolonged lag phase and decreasing growth rate in increasing NaCl concentration (Carlucci & Pramer, 1960).

Our results also showed that only treatment is statistically significant (*p*-value = 0.01) but not the replicates (*p*-value = 0.559) nor the interaction between the treatments and replicates (*p*-value = 0.539). Tubes B and D showed fastest decline in the ratio of Day 7 to Day 5 cell density (Figure 4.6) which correlates to the highest increase in generation time among the 4 tubes. This suggests that the cells in Tubes B and D adapts slower as compared to Tube A and C throughout the passages. The drop in cell density of the Day 7 to Day 5 readings may be linked to the increased in generation time of cells throughout the passages. On the other hand, our results suggest that the number of generation increases over passage (an average of 6.69 generations of all 4 Tubes in passage 1 as compared to 7.46 generations in passage 70). This suggests that cells take a period of time to adapt and divide in higher salt concentration as generation time is measured up to 360 minutes which may be insufficient for cells to adapt and grow. This is supported in Liu et al., 2005 study which demonstrated that bacteria cells need time to adapt to the change in environmental conditions. However, Tube C shows an increase in cell density across passages, the generation time of tube C increases. Due to environmental stresses, acquired mutation may occur to improve the survivability and adaptability of *E. coli* in Tube C (Travisano, 1997).

5.2 Decrease in Generation Time in Same NaCl Concentration until Next Concentration Increase

According to our hypothesis, when *E. coli* adapts to the salt concentration over passages, the generation time is expected to decrease showing an improved fitness until the next increase in

salt concentration where *E. coli* slowly adapts. However, our results suggested otherwise as the generation time from all tubes vary differently from one another in terms of generation time (Figure 4.4). Despite the difference in adaptation among cells in different tubes, they are able to reach and adapt to 10% NaCl. As our cells are cultured along the same treatment across passages, theoretically it should exhibit the same stress mechanism as the level of stress is similar for all tubes. However, based on the RFLP results, it showed an increase in mean DI and linear fittings which have suggested that *E. coli* in all tubes mutated differently in terms of stress mechanisms to adapt to the treatment. This is different from a study (Zhang et al., 2006) which demonstrated that the identification of 66 genes in *Desulfovibrio vulgaris* showed a similar stress response to both environmental perturbations when activated in both oxidative and heat stress.

Although Zhang et al (2006) used 2 stress, this study uses increasing stress which may be viewed as multiple stresses even though the chemical is the same. Collectively, this may suggest genetic robustness in adapting to different stress environments. However, this may lead to ecological specialization over extended culture in high salt environment resulting in the decline of robustness to adapt to new environmental stress (Devictor et al., 2010). Further studies are needed to ascertain the extend of genetic robustness in *E. coli*. Nevertheless, our results suggest that when *E. coli* is exposed to a single type of stress over time is likely to result in different stress mechanisms in order to adapt.

5.3 Comparing Generation Time with Lee et al. (2010)

Passage 1 of *E. coli* ATCC 8739 is obtained from (Lee et al., 2010) passage 70 cultured in high salt (1% NaCl) media. Generation time of passage 68 (Lee et al., 2010) was done and Student-t test was done to compare if the generation time taken from passage 68 was statistically ‘significant’ to our passage 1. Based on the results, it suggests that the generation time calculated for both passage 68 is the same as passage 1.

5.4 Stationary Phase of *Escherichia coli*

The ratio of Day 7 to Day 5 cell density of the four different tube cultures of *E. coli* was shown to decrease across the 80 passages, suggesting a general rightward shift of the bacteria

growth curve, placing the 5 day OD reading taken to be past the start of the stationary phase, and the 7 day OD reading to be taken around the death phase. This rightward shift suggests a shortened duration of the log phase, which implies that the bacteria have acclimatized to the salt concentration in the nutrient broth. An increase in the Day 7 to Day 5 cell density ratio on inoculation into a higher salt concentration would indicate an increase in the lag time for cells to adapt to the increased stress. The bacteria cells would first have to adapt to the increased stress by forming essential coenzymes required to process the essential nutrients and substrates in the nutrient media.

In the previous study on *Listeria monocytogenes* (Muñoz-Cuevas et al., 2010), *L. monocytogenes* at the exponential growth phase, when subjected to a change in environment, in order to adapt to the new conditions, would undergo a lag phase which lasted till the bacterial cells could overcome the new conditions. Several scenarios such as changes in temperature or water activity confirmed the predictions (Muñoz-Cuevas et al., 2010). This would correlate with the increase in generation time and decrease in number of generations at every new increase of salt concentration for the *E. coli* cells, as the increase in salt concentration would reduce the water activity in the nutrient broth for the bacteria. This results in the *E. coli* cells having to undergo an extended lag phase to overcome and adapt to the new conditions. Bacteria that are exposed to NaCl typically exhibit a prolonged lag phase and the growth rate of the cells decreases in increasing NaCl concentration (Carlucci & Pramer, 1960). This is because the bacterial cells need time to adapt to the change in environmental conditions (Liu et al., 2005).

It was also further noticed that when *L. monocytogenes* was in the process of adapting to new conditions but was again subjected to environmental fluctuations, to overcome the new lag phase, the sum of the work needed to be done, was the work required to overcome the new lag phase as well as the work required to be done to overcome the previously uncompleted lag phase. In short, the bacteria cells have to overcome the sum of both conditions in order to fully adapt and overcome these external stresses (Muñoz-Cuevas et al., 2010).

5.5 Generations of *Escherichia coli*

Our results demonstrated that the number of generations per passage increases in each subsequent passage with the maximum seen in Tube C, indicating that the number of generations in the tube has adapted relatively better than the other tube cultures. Despite the increase in salt concentration across the passages, *E. coli* bacteria have successfully managed to replicate and sustain itself, evident from the increase in number of generations across passages. This fulfilled the objectives of our experiment to fully adapt the *E. coli* cells to 10% NaCl.

This is also supported by Figure 4.3, where the trend line for generation time is lowest for tube culture C. This suggests that the acclimatization of *E. coli* to unfavourable environments and conditions by subjecting them to small doses of the unfavourable condition which was the growing salt concentration was effective in allowing them to resist it once they have acclimatized to it over time (Doudoroff, 1940).

From Figure 4.1, a visible spike in the number of generations can be seen over passage 19. The salt concentration at passage 19 was 4%. The spike in number of generations indicate an increased number of generations and complete acclimatization to salt at 4% concentration. In a previous study (Hrenovic and Ivankovic, 2009), *E. coli* was shown to have an absence of bacterial decay at 3.5% salt concentration, which was primarily attributed to the enriched nutrients available in the media. This could suggest that X1 nutrient broth media used for the halophilization of *E. coli*, was sufficient enough for *E. coli* to fully utilize and delay bacterial decay.

5.6 Fourth Power Polynomial Fitting of the Data Taken at 21 to 23

Hours Post-inoculation is Accurate for Analysis of MIC Data

Our results demonstrate low correlation ($r^2 < 0.95$) for the MIC data values with the polynomial fitting curves of x^1 , x^2 and x^3 . This suggests that the first three polynomial fittings are unsuitable for the analysis of the MIC data. On the other hand, polynomial fittings of x^4 , x^5 and x^6 showed high correlation ($r^2 > 0.95$) particularly the r^2 values of all of x^6 which are equal to 1. This would suggest that the fitting of x^6 should be the best. However, the x^6 fitting is shown to pass through every single data point, with the presence of large gaps in between

the data points. This indicates that although the r^2 values of x^6 are equal to 1, the gaps in between the data points suggest overfitting. This may result in inaccuracies in the calculations of the data. Our results suggest that overfitting is occurring at x^5 , as evident by the considerable size of the gaps between the fittings of x^4 and x^6 . The fitting of x^4 is then chosen for the analysis of the MIC data.

The x^4 fitted polynomial curve at 21 hours (Figure 4.5) is shown to be almost the same as the x^4 fitted polynomial curve at 23 hours. The similarity of both lines indicates that the growth of the *E. coli* is generally the same at the time frame between 21 to 23 hours. This is in contrast to the difference between the x^4 fitted OD600 readings at 17 and 19 hours. Thus, the OD600 readings are taken at 21 to 23 hours post-inoculation because this is where the growth of the *E. coli* is generally the same in this timeframe.

5.7 *E. coli* Cells Adapt to 1% Increase in NaCl in about One Month

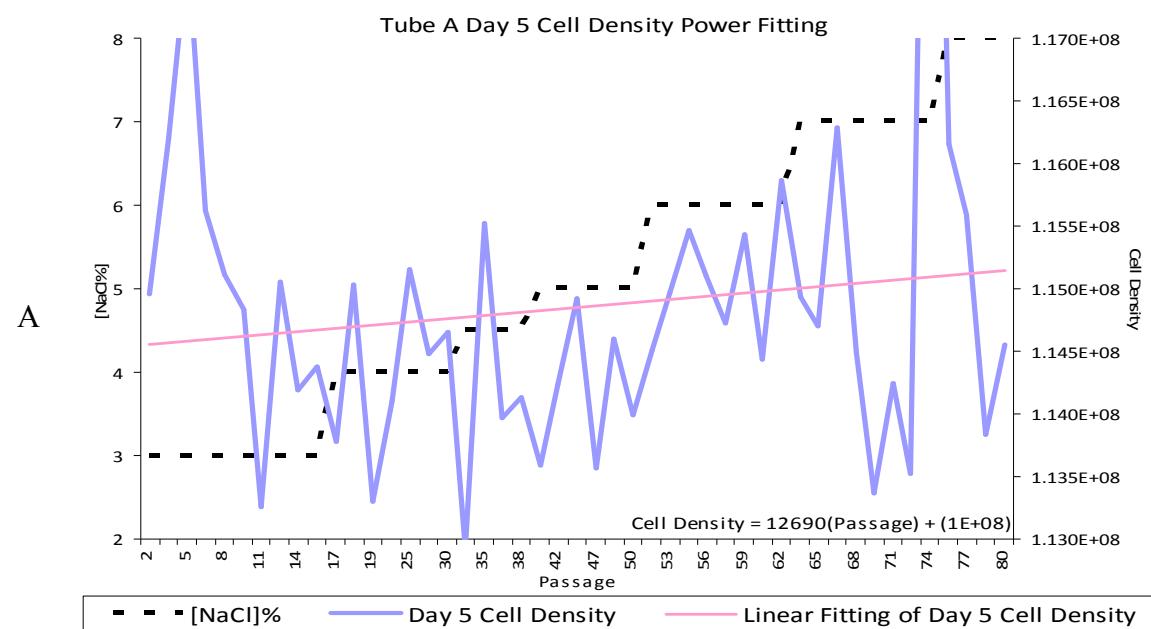
The MIC experiments are conducted every two weeks to check the adaptability of the cells and used to plan for the increase in [NaCl]. The concentration of NaCl for 1/2ODMax has increased slightly for all tubes, except for Tube C. This suggests that the cells can already tolerate the concentration of NaCl of which they were inoculated into as the number of passages increased. Although the 1/2ODMax illustrates the concentration of the salt whereby 50% of the cells are surviving, this suggests that the cells are already able to grow at that specific concentration of NaCl as the incubation period for the MIC experiment is only 21 to 23 hours post-inoculation. The concentration of NaCl at 1/2ODMax could be caused by the fast growers, which proliferate faster than the slower growers within the time span of 21 to 23 hours. If the experiment is left to run for more than 23 hours, the whole population would have expanded large enough to show that the *E. coli* can successfully adapt to that specific concentration of NaCl. This result is expected as the increase in resistance can be improved by repeated exposures to increasing concentrations of antibiotics (Adam et al., 2008). The repetitive exposures to increasing concentrations of NaCl could result in the same effect. The speed of adaptation of *E. coli* to various antibiotics varies according to the rate of selection; a steep increase in the concentration of antibiotics reduces the chance of evolving resistance to the antibiotics (Perron et al., 2008). A rate 1% increment per month could make it easier for the *E. coli* to adapt to the concentration of NaCl.

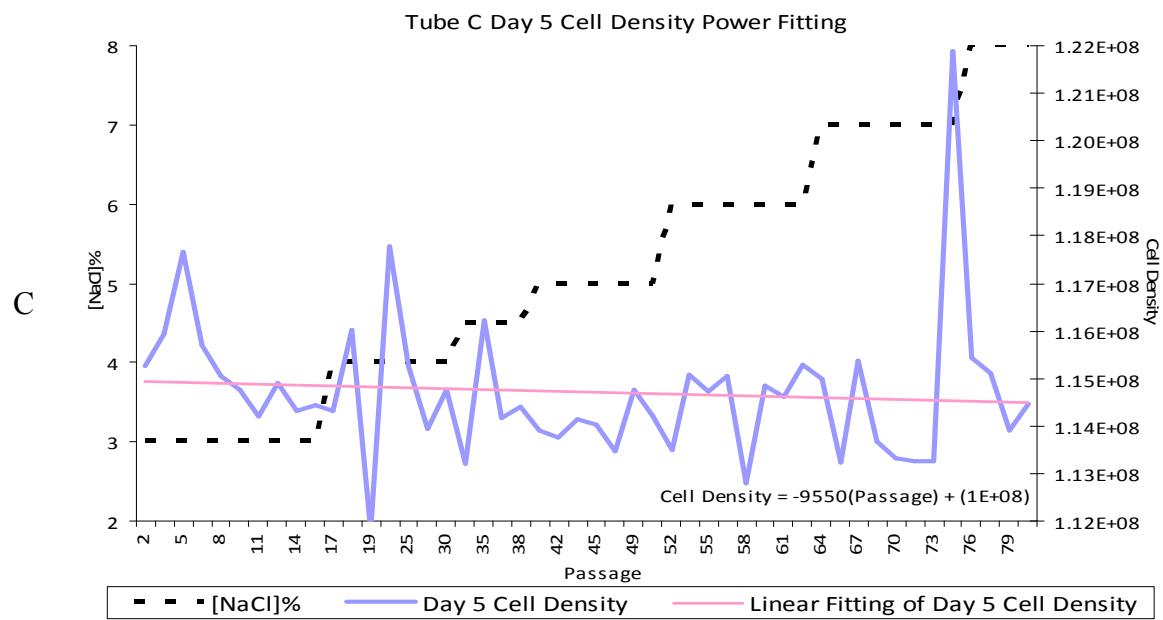
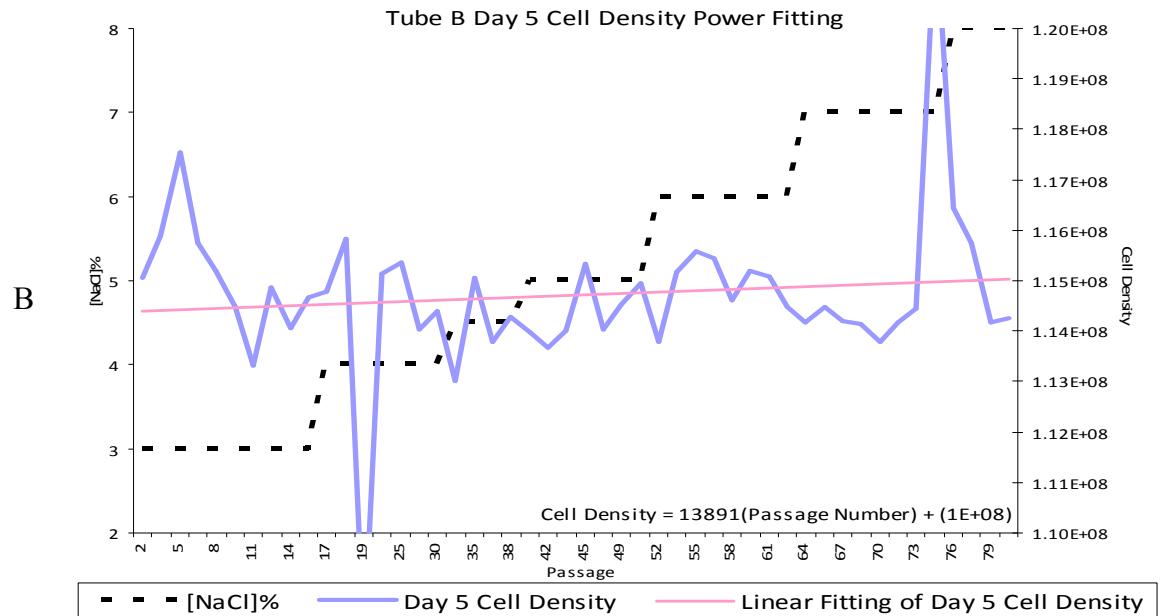
The rapid increase of the Area under the Curve (AUC) for NaCl that is more than 7.5% (Figure 4.9) suggests the proportion of the cell population which is able to grow at $[NaCl] > 7.5\%$ increases as the number of passages increase. 7.5% NaCl is set as a benchmark of adaptability to NaCl by the *E. coli*. The benchmark is set at 7.5% NaCl as the *E. coli* is found to be unable to proliferate significantly in 7% NaCl, after 72 hours of incubation (Hrenovic and Ivankovic, 2009). The proportion of cells which have passed this benchmark can be said to have the highest adaptability within the whole population. Over time, this proportion continues to increase as the cells with the highest adaptability proliferate. The increase in AUC is also present even though the concentration of NaCl is increased (1% increment every time) during the course of the 80 passages. This indicates that the cells were able to handle the additional stress caused by the higher concentration of NaCl. However, the 1/2ODMax of Tube C does not tally with the AUC of Tube C. In the case of Tube C, the 1/2ODMax has decreased over passages, whereas the AUC of Tube C still continues to increase over the passages. A reason for this observation could be that the cells in Tube C were able to grow in a wider range of NaCl concentrations. The speed of commensal *E. coli* adapting to nalidixic acid, ciprofloxacin and levofloxacin was found to be 7 days, as suggested by Fantin et al. (2009). The study was done on healthy individuals and a regimen of varying concentrations of oral ciprofloxacin was administered over a period of 14 days. This suggests that the speed of *E. coli* adapting to NaCl could also be quite similar. However, the difference in microbicidal effects of NaCl and antibiotics, and the conditions of the experiment make the estimation of the real speed of *E. coli* adapting to NaCl difficult.

The colony MIC experiment is used to check and reinforce the accuracy of the MIC experiments by acting as a sampling method for analyzing the adaptation of the general population to the NaCl. The data shown in Figure 4.4 suggests that generally the data of the colony MIC corroborates with the data of the MIC experiment (Figures 4.7 – 4.9), with the exception of Figure 4.8. The reason for this exception could be that the number of colonies taken is not representative of the whole population. The 10 colonies could be insufficient to demonstrate that the whole population is already adapted to the concentration of NaCl of which they are grown in. However, the variations between the colonies of the colony MIC data show that dominance by the clones of a particular cell is not well established. This suggests that although the whole population is adapting well to the NaCl, there is insufficient evidence of an establishment of dominance by clones of a particular cell.

The Day 7/Day 5 ratios in Table 4.2 and Figure 4.3 show that the Coefficients of Variation (COV) at 4% NaCl and at 5% NaCl are the highest. The COV of the four tubes is 0.83% to 2.55% at 4% NaCl and 1.1% to 1.69% at 5% NaCl, suggesting that the growth of the cells is the most unstable during 4% NaCl and 5% NaCl. The COV has decreased from 5% NaCl onwards to 8% NaCl (from > 1% to < 1%), which indicates an increase in the stability of the growth of the *E. coli*. The adaptability of the *E. coli* to the NaCl can be known and predicted by looking at the COV. A decrease in COV would mean that the growth of the *E. coli* is getting more stable.

Figure 5.1 shows the linear fitting of the Day 5 cell density of the four tubes to the passage. The increasing gradients of the linear fittings of Tube A, B and D suggest that the *E. coli* has sufficiently adapted to the increasing NaCl across passages (dotted line). The fluctuations of the cell density are likely to be caused by the measurement errors made by the spectrophotometer. The decrease of the gradient of Tube C is found to be non-significant (*p*-value = 0.999). Therefore, the *E. coli* cells in Tube C can be also said to be adapting well to the monthly increase of [NaCl] by 1%.





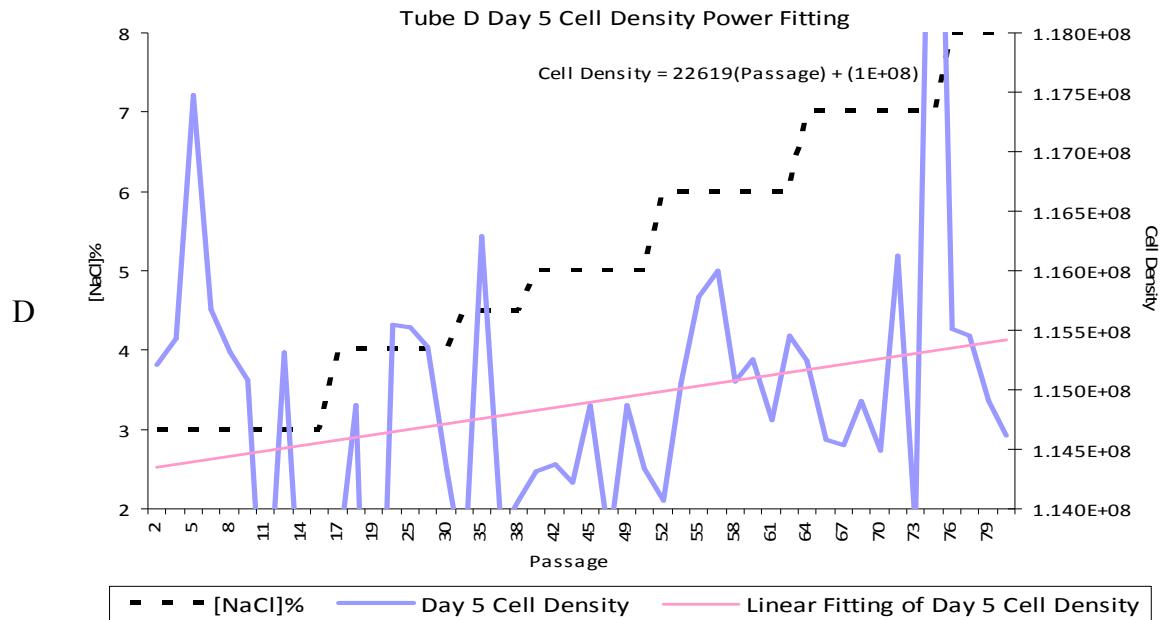


Figure 5.1: Linear fittings of Day 5 Cell Density; A (Tube A), B (Tube B), C (Tube C) and D (Tube D).

5.8 Cells from Later Passages are More Genetically Different from the Original Cells

Our PCR/RFLP results showed that the mean DI has increased over the course of 80 passages. This suggests that the mean difference between the four treatments of later passages is higher than the mean difference of the four treatments of earlier passages. The increasing DI is expected as the NaCl is known to cause stress on the cells. An increasing concentration of NaCl should exert more stress, which in turn cause more mutations in the genomes of the *E. coli*. The increasing bounded area of the linear fittings of $\pm 2\sigma$ suggests that the differences of the genomes of the *E. coli* cells within the four treatments are increasing. The increase in mean DI could be the reason behind the increasing spectrum of NaCl of which the *E. coli* can grow in, as shown by the MIC data (Figures 4.9 to 4.11). The increase in $1/2OD_{max}$ and the AUC, together with the decrease in OD_{max} suggests that the range of NaCl of which *E. coli* can grow in has expanded. However, the mean DI has dipped from RFLP count 3 to count 4, with a reduction in the variations as indicated by the $\pm 2\sigma$ linear fittings. This could be that the genomes of the *E. coli* have reached a convergence. This convergence is temporary as the mean DI has continued to increase over the passages that are cultured in 5% to 8% NaCl.

The convergence between RFLP count 3 and count 4 (P32, 4% NaCl and P42, 5% NaCl) could have a correlation to the COV (Figure 4.5). The COV of 4% to 5% NaCl is increasing and at the same time the similarity in the genomes of the *E. coli* is also increasing. The reason behind this correlation could be that the slow growers within the population are dying off, producing a critical ‘weeding point’ at 4% NaCl. This correlates to the study done by Hrenovic and Ivankovic (2009). Their results suggest that the *E. coli* is already experiencing stress at 5% NaCl and no significant growth could be observed at 7% NaCl, even after an incubation period of 72 hours. The convergence would then arise due to competition between the surviving fast growers within the population, these fast growers have adapted to the NaCl and survived.

The results of the number of generations also support the above mentioned correlation. The large spike observed in the data showed that the number of generations has increased rapidly from P16 (4% NaCl) and this large number of generations decreased at around P21 (4% NaCl). The rapid increase in the number of generations suggests that it could be caused by the fast growers, which has survived the 4% NaCl and managed to proliferate and compete. The COV and the number of generations have both dipped to low levels when the concentration of NaCl is more than 4% and at the same time the mean DI of the four treatments starts to increase steadily again. This suggests that the competition of the fast growers could have caused more genetic differences to arise and result in the stabilization of cell growth. The increase in the mean DI and linear fittings suggest that the genomes of the *E. coli* have mutated differently and developed different mechanisms to adapt to the increased NaCl. This could correlate to a study on the resistance of *E. coli* to ampicillin, where the *E. coli* developed different mechanisms to resist the anti-microbial effects of various antibiotics (Sáenz et al., 2004). The genomes of the *E. coli* were found to be different and they had different resistances to other antibiotics, although all of them were resistant to ampicillin.

6. Recommendations

There are several areas for improvement in this study. Firstly, MacConkey broth could be substituted as a nutrient medium for the growth of Escherichia coli. This will prevent the growth of gram positive bacteria during the experiment, as well as prevent fluctuations in the OD600 readings upon decontamination due to residue red dyes from MacConkey broth/agar.

Secondly, the adapted E. coli bacteria could be tested with other forms of salt such as benzoic acid, and potassium chloride. This is to evaluate the adaptability of *E. coli* and to find out if the evolution of the bacteria in sodium chloride, aids in its survival in other forms of salt.

Thirdly, we could increase the number of viable colonies taken from colony MIC, to broaden the amount of data obtained, giving a better gauge to the overall mutation and evolution of *E. coli* under salt stress.

Lastly, SNP microarray study can be employed to efficiently detect the changes in the *E. coli* ATCC 8739 genome instead of using PCR/RFLP. This is because the PCR/RFLP method only covers 0.374% of the total genome. By using SNP microarray, it would allow a better insight to the study of the changes in the genome.

7. Conclusion

Our results showed that *E. coli* cell cultures from the four different tubes have evolved and adapted to 10% NaCl concentration in their environment over the course of 80 passages. The generation time increased with the increase in salt stress, while the adaptation to the stress was seen with the increase in number of generations based on cell density at stationary phase. The analysis of the RFLP data and the Day7/Day 5 data showed that an increase in genetic similarities is correlated with a decrease in COV.

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Appendix A – Generation Time

The following series of tables show the OD readings of the Generation Time experiment. Readings are taken at intervals of up to 360 minutes.

Passage	Treatment	Tube	Timings (Minutes)				
			0	120	240	300	360
3	3% NaCl	A	0.050	0.048	0.072	0.131	0.250
		B	0.026	0.016	0.059	0.133	0.222
		C	0.019	0.009	0.050	0.120	0.203
		D	0.026	0.070	0.050	0.116	0.218
6	3% NaCl	A	-0.003	0.010	0.097	0.141	0.231
		B	-0.002	0.008	0.092	0.134	0.222
		C	0.000	0.012	0.096	0.142	0.229
		D	0.016	0.075	0.199	0.246	0.296
			0	120	195	255	315
12	3% NaCl	A	0.080	0.141	0.163	0.208	0.254
		B	0.099	0.135	0.165	0.217	0.282
		C	0.072	0.130	0.172	0.236	0.280
		D	0.101	0.136	0.168	0.205	0.282
			0	120	180	240	300
15	3% NaCl	A	0.009	0.013	0.036	0.114	0.173
		B	0.022	0.016	0.016	0.015	0.013
		C	0.008	0.009	0.009	0.084	0.149
		D	0.014	0.020	0.020	0.116	0.177
			0	120	180	240	
18	4% NaCl	A	-0.055	0.104	0.011	0.023	
		B	-0.058	0.023	0.071	0.132	
		C	-0.014	0.009	0.163	0.185	
		D	-0.011	-	-	-	
			0	120	180	240	300
22	4% NaCl	A	-0.015	0.005	0.011	0.022	0.042
		B	-0.017	0.002	0.007	0.019	0.038
		C	-0.007	0.016	0.033	0.055	0.098
		D	-0.018	0.001	0.005	0.016	0.034
25	4% NaCl	A	0.003	0.001	0.008	0.016	0.030
		B	0.000	0.001	0.009	0.020	0.043
		C	0.005	0.008	0.012	0.022	0.054
		D	0.008	0.011	0.020	0.030	0.054
28	4% NaCl	A	0.004	0.010	0.021	0.041	0.075
		B	0.007	0.012	0.019	0.036	0.068
		C	0.002	0.014	0.023	0.030	0.078
		D	0.006	0.019	0.032	0.068	0.123
31	4.5% NaCl	A	0.006	0.011	0.016	0.062	0.133
		B	0.009	0.013	0.013	0.046	0.109
		C	0.004	0.014	0.012	0.053	0.118

		D	0.010	0.014	0.023	0.058	0.133
31	5% NaCl	A	0.006	0.007	0.010	0.015	0.014
		B	0.008	0.006	0.006	0.006	0.004
		C	-0.001	-0.002	-0.002	0.011	0.011
		D	0.008	0.008	0.008	0.009	0.010
34	4.25% NaCl	A	0.013	0.020	0.025	0.045	0.065
		B	0.017	0.051	0.166	0.229	0.232
		C	0.017	0.016	0.022	0.036	0.054
		D	0.039	0.039	0.045	0.057	0.078
34	4.5% NaCl	A	0.013	0.023	0.028	0.040	0.057
		B	0.023	0.031	0.034	0.044	0.058
		C	0.002	0.007	0.008	0.017	0.030
		D	0.014	0.015	0.020	0.029	0.045
37	4.5% NaCl	A	-0.002	0.011	0.047	0.069	0.100
		B	0.003	0.005	0.022	0.039	0.065
		C	-0.004	0.005	0.006	0.022	0.046
		D	0.004	0.012	0.031	0.044	0.067
39	4.5% NaCl	A	0.004	0.025	0.030	0.036	0.055
		B	0.006	0.020	0.025	0.026	0.054
		C	-0.002	0.017	0.024	0.025	0.051
		D	0.022	0.046	0.046	0.081	0.128
42	4.5% NaCl	A	0.038	0.047	0.053	0.064	0.095
		B	0.010	0.015	0.020	0.028	0.060
		C	0.008	0.015	0.018	0.025	0.047
		D	0.010	0.024	0.029	0.051	0.086
42	5% NaCl	A	0.005	0.008	0.010	0.015	0.300
		B	0.002	0.010	0.013	0.017	0.034
		C	0.004	0.001	0.011	0.015	0.029
		D	0.004	0.006	0.009	0.013	0.027
45	5% NaCl	A	0.000	-0.009	-0.004	0.008	
		B	0.002	-0.009	0.003	0.007	
		C	0.001	-0.015	-0.003	0.003	
		D	-0.001	-0.007	0.004	0.009	
48	5% NaCl	A	0.009	0.005	0.000	0.001	0.002
		B	0.001	0.006	0.002	0.008	0.013
		C	0.011	0.009	0.004	0.008	0.015
		D	0.004	0.005	0.002	0.004	0.011
50	5% NaCl	A	0.085	0.117	0.253	0.309	
		B	-0.001	0.011	0.024	0.054	
		C	0.050	0.013	0.025	0.054	
		D	-0.003	0.011	0.022	0.053	
53	5% NaCl		0	120		225	300
		A	-0.007	-0.012		-0.005	0.043
		B	0.001	-0.010		0.000	0.047
		C	-0.011	-0.008		0.003	0.045
53	5.5% NaCl	D	-0.012	-0.016		0.003	0.026
		A	0.005	-0.005		0.005	0.028
		B	0.007	-0.003		0.016	0.029

		C	0.020	0.012		0.015	0.038
		D	0.008	0.013		0.011	0.034
53	6% NaCl	A	0.004	-0.015		-0.002	0.018
		B	0.006	-0.011		-0.004	0.018
		C	0.003	-0.016		0.006	0.015
		D	0.002	-0.016		-0.001	0.016
			0	120	195	240	300
56	6% NaCl	A	0.013	0.009	0.02	0.010	0.022
		B	0.007	0.023	0.032	0.024	0.032
		C	0.014	0.024	0.027	0.030	0.037
		D	0.018	0.021	0.03	0.029	0.036
			0	120	180	240	300
59	6% NaCl	A	0.002	0.011	0.014	0.024	0.031
		B	0.025	0.004	0.004	0.008	0.011
		C	0.007	0.007	0.009	0.013	0.017
		D	0.020	0.000	0	0.003	0.007
62	6% NaCl	A	0.000	-0.015	-0.01		0.013
		B	-0.005	-0.017	-0.014		0.011
		C	0.005	-0.020	-0.025		-0.002
		D	0.003	-0.014	-0.01		0.015
			0	120	210	240	300
68	7% NaCl	A	0.013	0.016	0.015	0.021	0.023
		B	0.017	0.017	0.016	0.015	0.014
		C	0.016	0.021	0.127	0.2	0.241
		D	0.013	0.021	0.015	0.019	0.023
71	7% NaCl	A	0.005	0.010		-0.001	0.001
		B	0.008	0.006		0.004	0.002
		C	0.003	0.003		0.001	0.004
		D	0.008	0.004		0.01	0.011
74 (MacConkey)	7% NaCl	A	2.173	2.107	2.128	2.114	2.088
		B	2.173	2.045	2.088	2.088	2.088
		C	1.888	1.860	1.817	1.864	1.884
		D	2.011	1.945	1.936	1.964	1.936
77	8% NaCl	A	0.036	0.008	0.011	0.011	0.013
		B	0.039	0.048	0.064	0.04	0.046
		C	0.017	0.014	0.023	0.036	0.042
		D	0.023	0.016	0.013	0.089	0.066
80	8% NaCl	A	0.010	0.006	0.01	0.012	0.018
		B	0.004	0.011	0.016	0.01	0.016
		C	0.007	0.005	0.006	0.015	0.021
		D	0.008	0.014	0.018	0.02	0.020

The following table shows the geometric mean of each treatment from each tube. The geometric mean is calculated using turbidity measurement of cell culture (OD600 readings) between two time points taken from generation time in minutes except the first reading which comprises of the lag phase.

Passage, [NaCl]%/ Treatment	Generation Time in Minutes (Geometric Mean) of each Treatment			
	A	B	C	D
3, 3% NaCl	97.16	64.21	56.70	57.08
6, 3% NaCl	69.99	67.70	71.78	155.47
12, 3% NaCl	233.50	184.13	181.09	188.56
15, 3% NaCl	52.76		36.76	48.25
18, 4% NaCl	56.38	49.74		68.68
18, 3% NaCl				
18, 0% NaCl	70.06	51.42	34.78	53.53
22, 4% NaCl	58.82	43.61	69.57	37.08
25, 4% NaCl	42.98	37.69	68.82	79.63
28, 4% NaCl	62.14	72.76	60.38	67.60
31, 4.5% NaCl	57.05	39.83	38.14	57.36
31, 5% NaCl	109.36			373.33
34, 4.25% NaCl	114.2	244.16	104.20	189.26
34, 4.5% NaCl	142.52	221.95	107.96	115.27
37, 4.5% NaCl	70.32	54.96	74.39	72.14
39, 4.5% NaCl	172.20	224.04	192.81	81.74
42, 4.5% NaCl	200.31	99.16	123.91	108.82
42, 5% NaCl	64.26	113.82	52.74	87.07
45, 5% NaCl		49.08		51.29
48, 5% NaCl	60.00	50.69	63.00	49.67
50, 5% NaCl	85.06	52.29	58.61	53.27
53, 5% NaCl			19.20	24.07
53, 5.5% NaCl	30.18	87.41	135.06	46.07
53, 6% NaCl			56.74	
56, 6% NaCl	58.60	150.85	295.91	167.43
59, 6% NaCl	129.31	88.52	142.63	49.08
62, 6% NaCl				
68, 7% NaCl	168.09		70.74	138.38
71, 7% NaCl			30.00	436.35
74(MacConkey), 7% NaCl	12694.81	6001.47	7295.46	8529.16
77, 8% NaCl	180.31	207.41	128.02	21.62
80, 8% NaCl	123.96	99.10	108.57	255.58

Appendix B – 2 Day Generations and Cell Density

This following table is the tabulation of the number of generations that are calculated from the 2-Day OD readings. OD600 readings were taken of the 2-Day subcultures from all four tube cultures, A, B, C, and D, and subsequently calculated to produce the number of generations. These readings were taken to monitor the stationary phase of *E. coli*. These results can be analysed to estimate the ability of *E. coli* acclimatizing to increasing concentrations of salt.

Number of Generations

Comparison of Number of Generations across Different Tubes				
Passage	Tube A	Tube B	Tube C	Tube D
1	6.73	6.66	6.50	6.86
2	6.50	6.51	6.60	6.42
3	6.80	6.88	6.88	6.89
4	6.60	6.57	6.48	6.44
5	6.93	6.96	6.95	7.05
7	6.55	6.60	6.58	6.64
8	6.69	6.61	6.66	6.68
9	6.51	6.53	6.56	6.55
10	6.64	6.66	6.80	6.56
11	6.49	6.49	6.27	6.57
13	6.68	6.70	6.68	6.57
14	6.68	6.68	6.67	6.49
16	6.61	6.42	6.47	6.85
17	6.84	7.63	7.15	6.80
19	7.63	12.11	9.89	8.72
20	6.71	6.76	7.24	6.75
21	6.68	6.71	6.10	7.45
24	6.70	7.40	6.72	6.43
26	6.58	6.69	6.73	6.98
27	6.67	6.59	6.56	6.22
29	6.76	6.71	6.71	6.66
32	6.79	6.78	6.74	6.71
33	6.34	6.37	6.38	6.39
34	6.96	6.67	6.67	6.75
35	6.57	6.85	6.84	6.76
36	6.86	6.80	6.83	6.79
37	6.74	6.80	6.76	6.83
38	6.65	6.66	6.70	6.64
40	7.03	6.91	6.89	6.90
41	6.61	6.84	6.82	6.89

43	6.68	6.63	6.66	6.43
44	6.84	6.95	6.92	6.98
46	6.80	6.80	7.00	6.89
47	7.57	7.65	7.55	7.61
49	6.22	6.09	6.03	6.15
52	7.85	7.97	7.80	7.78
55	7.30	7.70	7.73	7.66
58	6.82	7.67	7.44	7.08
60	6.37	6.38	6.36	6.74
61	7.07	6.76	7.03	7.12
67	7.54	7.38	7.52	7.19
70	7.32	7.37	7.39	7.26
73	8.49	8.46	8.56	8.62
76	7.55	7.57	7.57	7.14

The following tables are the tabulations of the OD600 of respective treatments, and their number of cells upon subculture and the number cells inoculated in the passage. The 2-Day cell density / Inoculated density ratio is subsequently calculated and tabulated as follows.

Treatment A 2-Day Cell Density/Inoculated Density Ratio

Passage	Treatment A	No. of Cells on Subculture	No. of Cells Inoculated	2-Day Cell Density/ Inoculated Density
1	0.473	84322960.48	796854.70	105.82
2	0.517	76503988.00	843229.60	90.73
3	0.445	85222765.37	765039.88	111.40
4	0.526	82475378.97	852227.65	96.78
5	0.499	100862095.77	824753.79	122.29
6	0.710	81526441.56	1008620.96	80.83
7	0.490	76621019.25	815264.42	93.98
8	0.446	79242686.70	766210.19	103.42
9	0.469	72233042.22	792426.87	91.15
10	0.410	72233042.22	722330.42	100.00
11	0.410	64723203.95	722330.42	89.60
12	0.355	66739818.87	647232.04	103.12
13	0.369	68545022.36	667398.19	102.70
14	0.382	70289808.60	685450.22	102.55
15	0.395	64723203.95	702898.09	92.08
16	0.355	63082120.26	647232.04	97.46
17	0.344	72360051.78	630821.20	114.71
18	0.411	0.06	723600.52	0.00

19	0.209	69223021.51	348333.33	198.73
20	0.387	72360051.78	692230.22	104.53
21	0.411	74351220.73	723600.52	102.75
22	0.427	73983622.97	743512.21	99.51
23	0.424	75796229.62	739836.23	102.45
24	0.439	78683866.77	757962.30	103.81
25	0.464	79464548.31	786838.67	100.99
26	0.471	76269134.45	794645.48	95.98
27	0.443	77547930.77	762691.34	101.68
28	0.454	73983622.97	775479.31	95.40
29	0.424	80015106.75	739836.23	108.15
30	0.476			
31				
32	0.405	79242686.70	715933.11	110.68
33	0.469	64132405.56	792426.87	80.93
34	0.351	79905459.18	641324.06	124.59
35	0.475	76033218.21	799054.59	95.15
36	0.441	88301882.88	760332.18	116.14
37	0.558	94463543.59	883018.83	106.98
38	0.628	94712014.60	944635.44	100.26
39	0.631	68408358.06	947120.15	72.23
40	0.381	89684918.88	684083.58	131.10
41	0.573	87643692.36	896849.19	97.72
42	0.551	72105722.49	876436.92	82.27
43	0.409	73860512.17	721057.22	102.43
44	0.423	84724792.14	738605.12	114.71
45	0.521	75677330.14	847247.92	89.32
46	0.438	84524263.44	756773.30	111.69
47	0.519	160789475.24	845242.63	190.23
48	2.241	92776101.81	1607894.75	57.70
49	0.608	69088125.18	927761.02	74.47
50	0.386	0.07	690881.25	0.00
51	0.234	0.07	390000.00	0.00
52	0.236	90943468.96	393333.33	231.21
53	0.587	0.08	909434.69	0.00
54	0.269	57824128.90	448333.33	128.98
55	0.311	90854573.11	578241.29	157.12
56	0.586	75914858.56	908545.73	83.56
57	0.440	74228975.89	759148.59	97.78

58	0.426	83918007.77	742289.76	113.05
59	0.513	76269134.45	839180.08	90.89
60	0.443	63082120.26	762691.34	82.71
61	0.344	84724792.14	630821.20	134.31
62	0.521	0.07	847247.92	0.00
63	0.246	0.05	410000.00	0.00
64	0.158	59959186.90	263333.33	227.69
65	0.324	0.08	599591.87	0.00
66	0.250	0.08	416666.67	0.00
67	0.262	81526441.56	436666.67	186.70
68	0.490	0.07	815264.42	0.00
69	0.249	0.08	415000.00	0.00
70	0.258	68545022.36	430000.00	159.41
71	0.382	0.09	685450.22	0.00
72	0.291	0.08	485000.00	0.00
73	0.262	157102551.64	436666.67	359.78
74	2.088	0.09	1571025.52	0.00
75	0.295	0.08	491666.67	0.00
76	0.274	85716027.32	456666.67	187.70
77	0.531	0.09	857160.27	0.00
78	0.298	0.09	496666.67	0.00
79	0.290	0.09	483333.33	0.00
80	0.287			

Treatment B 2-Day Cell Density/Inoculated Density Ratio

Passage	Treatment B	No. of Cells on Subculture	No. of Cells Inoculated	2-Day Cell Density/Inoculated Density
1	0.501	83816276.22	826839.29	101.37
2	0.512	76151309.76	838162.76	90.86
3	0.442	89593849.17	761513.10	117.65
4	0.572	84924552.54	895938.49	94.79
5	0.523	105697635.02	849245.53	124.46
6	0.779	82787891.80	1056976.35	78.33
7	0.502	80124524.21	827878.92	96.78
8	0.477	78458650.87	801245.24	97.92
9	0.462	72486752.69	784586.51	92.39
10	0.412	73489425.90	724867.53	101.38
11	0.420	66171558.20	734894.26	90.04

12	0.365	67301952.61	661715.58	101.71
13	0.373	70157647.82	673019.53	104.24
14	0.394	71850146.49	701576.48	102.41
15	0.407	67301952.61	718501.46	93.67
16	0.373	57656214.42	673019.53	85.67
17	0.310	114201058.64	576562.14	198.07
18	0.917	0.00	1142010.59	0.00
19	0.009	66456462.73	15000.00	4430.43
20	0.367	71978091.10	664564.63	108.31
21	0.408	75558158.88	719780.91	104.97
22	0.437	70815126.89	755581.59	93.72
23	0.399	0.08	708151.27	0.00
24	0.255	71593311.49	425000.00	168.45
25	0.405	75677330.14	715933.11	105.70
26	0.438	78345677.04	756773.30	103.53
27	0.461	75318996.08	783456.77	96.14
28	0.435	72739234.54	753189.96	96.57
29	0.414	76269134.45	727392.35	104.85
30	0.443			
31				
32	0.413	79575126.08	726131.46	109.59
33	0.472	65885088.25	795751.26	82.80
34	0.363	66880921.52	658850.88	101.51
35	0.370	76970545.03	668809.22	115.09
36	0.449	86009759.93	769705.45	111.74
37	0.534	95856141.38	860097.60	111.45
38	0.645	97054755.09	958561.41	101.25
39	0.660	63082120.26	970547.55	65.00
40	0.344	75677330.14	630821.20	119.97
41	0.438	86977086.38	756773.30	114.93
42	0.544	72865018.44	869770.86	83.77
43	0.415	71978091.10	728650.18	98.78
44	0.408	89044064.81	719780.91	123.71
45	0.566	72233042.22	890440.65	81.12
46	0.410	80451405.53	722330.42	111.38
47	0.480	161459838.99	804514.06	200.69
48	2.270	95531801.92	1614598.39	59.17
49	0.641	64869863.40	955318.02	67.90
50	0.356	0.05	648698.63	0.00
51	0.179	0.06	298333.33	0.00
52	0.208	86881157.37	346666.67	250.62

53	0.543	0.07	868811.57	0.00
54	0.222	0.08	370000.00	0.00
55	0.255	88581440.66	425000.00	208.43
56	0.561	73983622.97	885814.41	83.52
57	0.424	0.07	739836.23	0.00
58	0.245	83201682.86	408333.33	203.76
59	0.506	78458650.87	832016.83	94.30
60	0.462	65452410.25	784586.51	83.42
61	0.360	70815126.89	654524.10	108.19
62	0.399	0.07	708151.27	0.00
63	0.216	0.03	360000.00	0.00
64	0.104	0.09	173333.33	0.00
65	0.289	0.08	481666.67	0.00
66	0.262	0.08	436666.67	0.00
67	0.259	71721887.14	431666.67	166.15
68	0.406	0.07	717218.87	0.00
69	0.216	0.07	360000.00	0.00
70	0.224	61855301.31	373333.33	165.68
71	0.336	0.08	618553.01	0.00
72	0.265	0.08	441666.67	0.00
73	0.267	156170377.11	445000.00	350.94
74	2.051	69759143.46	1561703.77	44.67
75	0.391	0.08	697591.43	0.00
76	0.263	83098542.57	438333.33	189.58
77	0.505	0.08	830985.43	0.00
78	0.254	0.09	423333.33	0.00
79	0.292	0.09	486666.67	0.00
80	0.294			

Treatment C 2-Day Cell Density/Inoculated Density Ratio

Passage	Treatment C	No. of Cells on Subculture	No. of Cells Inoculated	2-Day Cell Density/Inoculated Density
1	0.585	82265991.54	907655.25	90.64
2	0.497	79795580.53	822659.92	97.00
3	0.474	94130396.13	797955.81	117.96
4	0.624	83816276.22	941303.96	89.04
5	0.512	103858502.73	838162.76	123.91

6	0.752	80668193.37	1038585.03	77.67
7	0.482	77202267.18	806681.93	95.70
8	0.451	77891317.74	772022.67	100.89
9	0.457	73489425.90	778913.18	94.35
10	0.420	81738814.15	734894.26	111.23
11	0.492	63233462.61	817388.14	77.36
12	0.345	70025151.18	632334.63	110.74
13	0.393	71978091.10	700251.51	102.79
14	0.408	73365141.22	719780.91	101.93
15	0.419	70553129.36	733651.41	96.17
16	0.397	62625439.39	705531.29	88.76
17	0.341	88674294.47	626254.39	141.59
18	0.562	0.01	886742.94	0.00
19	0.046	72739234.54	76666.67	948.77
20	0.414	109690349.13	727392.35	150.80
21	0.841	75199002.01	1096903.49	68.56
22	0.434	72360051.78	751990.02	96.22
23	0.411	75796229.62	723600.52	104.75
24	0.439	80015106.75	757962.30	105.57
25	0.476	74351220.73	800151.07	92.92
26	0.427	78908114.00	743512.21	106.13
27	0.466	74594853.88	789081.14	94.53
28	0.429	73365141.22	745948.54	98.35
29	0.419	76621019.25	733651.41	104.44
30	0.446			
31				
32	0.428	79575126.08	744731.80	106.85
33	0.472	66456462.73	795751.26	83.51
34	0.367	67441544.13	664564.63	101.48
35	0.374	77086534.84	674415.44	114.30
36	0.450	87548983.16	770865.35	113.57
37	0.550	94877006.51	875489.83	108.37
38	0.633	98840723.01	948770.07	104.18
39	0.683	63684871.49	988407.23	64.43
40	0.348	75318996.08	636848.71	118.27
41	0.435	84924552.54	753189.96	112.75

42	0.523	67719610.92	849245.53	79.74
43	0.376	68681329.36	677196.11	101.42
44	0.383	82891647.85	686813.29	120.69
45	0.503	67161986.36	828916.48	81.02
46	0.372	86009759.93	671619.86	128.06
47	0.534	161689014.74	860097.60	187.99
48	2.280	93963020.71	1616890.15	58.11
49	0.622	61387698.17	939630.21	65.33
50	0.333	0.05	613876.98	0.00
51	0.169	0.07	281666.67	0.00
52	0.229	85222765.37	381666.67	223.29
53	0.526	0.07	852227.65	0.00
54	0.229	0.07	381666.67	0.00
55	0.246	86881157.37	410000.00	211.91
56	0.543	0.08	868811.57	0.00
57	0.280	0.09	466666.67	0.00
58	0.292	84322960.48	486666.67	173.27
59	0.517	80233712.52	843229.60	95.15
60	0.478	65741260.85	802337.13	81.94
61	0.362	85912032.82	657412.61	130.68
62	0.533	0.07	859120.33	0.00
63	0.221	0.03	368333.33	0.00
64	0.106	0.06	176666.67	0.00
65	0.213	0.08	355000.00	0.00
66	0.267	0.07	445000.00	0.00
67	0.237	72739234.54	395000.00	184.15
68	0.414	0.07	727392.35	0.00
69	0.217	0.07	361666.67	0.00
70	0.227	63534835.70	378333.33	167.93
71	0.347	0.07	635348.36	0.00
72	0.241	0.07	401666.67	0.00
73	0.241	151631519.14	401666.67	377.51
74	1.880	68817280.94	1516315.19	45.38
75	0.384	0.07	688172.81	0.00
76	0.223	70553129.36	371666.67	189.83
77	0.397	0.07	705531.29	0.00

78	0.236	0.08	393333.33	0.00
79	0.271	0.09	451666.67	0.00
80	0.299			

Treatment D 2-Day Cell Density/Inoculated Density Ratio

Passage	Treatment D	No. of Cells on Subculture	No. of Cells Inoculated	2-Day Cell Density/Inoculated Density
1	0.428	86785051.54	744731.80	116.53
2	0.542	74351220.73	867850.52	85.67
3	0.427	88114674.78	743512.21	118.51
4	0.556	76621019.25	881146.75	86.96
5	0.446	101227973.76	766210.19	132.12
6	0.715	79464548.31	1012279.74	78.50
7	0.471	79019876.96	794645.48	99.44
8	0.467	80884083.53	790198.77	102.36
9	0.484	76033218.21	808840.84	94.00
10	0.441	71593311.49	760332.18	94.16
11	0.405	67858090.15	715933.11	94.78
12	0.377	75677330.14	678580.90	111.52
13	0.438	71721887.14	756773.30	94.77
14	0.406	64428641.59	717218.87	89.83
15	0.353	62010241.69	644286.42	96.25
16	0.337	71593311.49	620102.42	115.45
17	0.405	79905459.18	715933.11	111.61
18	0.475	0.03	799054.59	0.00
19	0.094	66171558.20	156666.67	422.37
20	0.365	71205671.04	661715.58	107.61
21	0.402	124253566.59	712056.71	174.50
22	1.112	72613146.45	1242535.67	58.44
23	0.413	89136099.08	726131.46	122.75
24	0.567	76737788.39	891360.99	86.09
25	0.447	76151309.76	767377.88	99.24
26	0.442	96017557.61	761513.10	126.09
27	0.647	71721887.14	960175.58	74.70
28	0.406	74837353.85	717218.87	104.34
29	0.431	75914858.56	748373.54	101.44
30	0.440			
31				
32	0.427	77891317.74	743512.21	104.76
33	0.457	65452410.25	778913.18	84.03
34	0.360	70553129.36	654524.10	107.79
35	0.397	76503988.00	705531.29	108.43
36	0.445	84924552.54	765039.88	111.01
37	0.523	96498829.34	849245.53	113.63
38	0.653	96178475.64	964988.29	99.67

39	0.649	62625439.39	961784.76	65.11
40	0.341	74594853.88	626254.39	119.11
41	0.429	88581440.66	745948.54	118.75
42	0.561	79019876.96	885814.41	89.21
43	0.467	67996202.55	790198.77	86.05
44	0.378	86107304.21	679962.03	126.64
45	0.535	71464417.98	861073.04	82.99
46	0.404	84724792.14	714644.18	118.56
47	0.521	165777731.93	847247.92	195.67
48	2.466	95368872.42	1657777.32	57.53
49	0.639	67580762.90	953688.72	70.86
50	0.375	0.05	675807.63	0.00
51	0.176	0.07	293333.33	0.00
52	0.230	84524263.44	383333.33	220.50
53	0.519	0.07	845242.63	0.00
54	0.249	0.08	415000.00	0.00
55	0.276	92947324.95	460000.00	202.06
56	0.610	63834476.75	929473.25	68.68
57	0.349	63233462.61	638344.77	99.06
58	0.345	85321791.80	632334.63	134.93
59	0.527	60598917.62	853217.92	71.02
60	0.328	64576130.80	605989.18	106.56
61	0.354	89866582.48	645761.31	139.16
62	0.575	0.08	898665.82	0.00
63	0.282	0.04	470000.00	0.00
64	0.128	0.09	213333.33	0.00
65	0.299	0.09	498333.33	0.00
66	0.298	56808433.14	496666.67	114.38
67	0.305	82891647.85	568084.33	145.91
68	0.503	0.09	828916.48	0.00
69	0.283	0.09	471666.67	0.00
70	0.293	74716244.86	488333.33	153.00
71	0.430	56120140.63	747162.45	75.11
72	0.301	0.07	561201.41	0.00
73	0.234	153027040.60	390000.00	392.38
74	1.931	73737109.98	1530270.41	48.19
75	0.422	58984627.68	737371.10	79.99
76	0.318	83098542.57	589846.28	140.88
77	0.505	0.08	830985.43	0.00
78	0.252	57487757.41	420000.00	136.88
79	0.309	59959186.90	574877.57	104.30
80	0.324			

Appendix C – Cell Density at Stationary Phase

The following four tables show the OD600 of the passages 5 days and 7 days after subculture. The OD600 readings are used to estimate the cell density at the stationary phase of the *E. coli*. The changes in the stationary phase can in turn be used to estimate the adaptability of the *E. coli* to increasing NaCl concentration from Passage 2 (3% NaCl) to Passage 80 (10% NaCl).

Ratio of Day 7 / Day 5 Cell Density – Tube A

Passage	OD 600 at Day 5	OD 600 at Day 7	Cell Density at Day 5	Cell Density at Day 7	Ratio of Day 7, Day 5 Cell Density
2	0.486	0.563	1.15E+08	1.16E+08	100.67%
3	0.617	0.669	1.16E+08	1.17E+08	100.36%
5	0.824	0.692	1.18E+08	1.17E+08	99.23%
6	0.552	0.567	1.16E+08	1.16E+08	100.12%
8	0.501	0.467	1.15E+08	1.15E+08	99.68%
9	0.475	0.559	1.15E+08	1.16E+08	100.74%
11	0.351	0.484	1.13E+08	1.15E+08	101.48%
12	0.495	0.582	1.15E+08	1.16E+08	100.73%
14	0.419	0.405	1.14E+08	1.14E+08	99.84%
15	0.435	0.412	1.14E+08	1.14E+08	99.75%
17	0.388	0.446	1.14E+08	1.15E+08	100.64%
18	0.493	0.509	1.15E+08	1.15E+08	100.14%
19	0.354	0.446	1.13E+08	1.15E+08	101.06%
21	0.412	0.739	1.14E+08	1.17E+08	102.67%
25	0.505	0.594	1.15E+08	1.16E+08	100.73%
27	0.443	0.537	1.14E+08	1.15E+08	100.88%
30	0.459	0.489	1.15E+08	1.15E+08	100.29%
33	0.329	0.365	1.13E+08	1.13E+08	100.48%
35	0.542	0.470	1.16E+08	1.15E+08	99.36%
36	0.402	0.360	1.14E+08	1.13E+08	99.50%
38	0.415	0.396	1.14E+08	1.14E+08	99.79%
41	0.374	0.306	1.14E+08	1.13E+08	99.08%
42	0.423	0.523	1.14E+08	1.15E+08	100.97%
45	0.483	0.395	1.15E+08	1.14E+08	99.09%
47	0.372	0.257	1.14E+08	1.12E+08	98.30%
49	0.454	0.412	1.15E+08	1.14E+08	99.56%
50	0.404	0.597	1.14E+08	1.16E+08	101.79%
52	0.446	0.422	1.15E+08	1.14E+08	99.75%
53	0.487	0.376	1.15E+08	1.14E+08	98.83%
55	0.536	0.487	1.15E+08	1.15E+08	99.57%
56	0.500	0.406	1.15E+08	1.14E+08	99.06%
58	0.465	0.431	1.15E+08	1.14E+08	99.65%

59	0.532	0.405	1.15E+08	1.14E+08	98.77%
61	0.440	0.456	1.14E+08	1.15E+08	100.16%
62	0.579	0.539	1.16E+08	1.15E+08	99.68%
64	0.484	0.489	1.15E+08	1.15E+08	100.05%
65	0.463	0.501	1.15E+08	1.15E+08	100.36%
67	0.627	0.467	1.16E+08	1.15E+08	98.68%
68	0.444	0.403	1.14E+08	1.14E+08	99.56%
70	0.358	0.406	1.13E+08	1.14E+08	100.58%
71	0.424	0.479	1.14E+08	1.15E+08	100.56%
73	0.369	0.320	1.14E+08	1.13E+08	99.35%
74	2.101	1.984	1.23E+08	1.22E+08	99.76%
76	0.611	0.837	1.16E+08	1.18E+08	101.41%
77	0.549	0.612	1.16E+08	1.16E+08	100.49%
79	0.392	0.555	1.14E+08	1.16E+08	101.59%
80	0.450	0.522	1.15E+08	1.15E+08	100.68%

Ratio of Day 7 / Day 5 Cell Density – Tube B

Passage	OD 600 at Day 5	OD 600 at Day 7	Cell Density at Day 5	Cell Density at Day 7	Ratio of Day 7, Day 5 Cell Density
2	0.495	0.551	1.15E+08	1.16E+08	100.49%
3	0.581	0.653	1.16E+08	1.16E+08	100.53%
5	0.796	0.674	1.18E+08	1.17E+08	99.26%
6	0.565	0.574	1.16E+08	1.16E+08	100.07%
8	0.508	0.460	1.15E+08	1.15E+08	99.55%
9	0.442	0.526	1.14E+08	1.15E+08	100.79%
11	0.355	0.623	1.13E+08	1.16E+08	102.59%
12	0.478	0.541	1.15E+08	1.16E+08	100.56%
14	0.409	0.410	1.14E+08	1.14E+08	100.01%
15	0.459	0.424	1.15E+08	1.14E+08	99.64%
17	0.470	0.437	1.15E+08	1.14E+08	99.67%
18	0.574	0.479	1.16E+08	1.15E+08	99.19%
19	0.123	0.508	1.08E+08	1.15E+08	106.86%
21	0.504	0.739	1.15E+08	1.17E+08	101.73%
25	0.526	0.600	1.15E+08	1.16E+08	100.59%
27	0.408	0.508	1.14E+08	1.15E+08	101.00%
30	0.436	0.452	1.14E+08	1.15E+08	100.16%
33	0.334	0.358	1.13E+08	1.13E+08	100.32%
35	0.495	0.432	1.15E+08	1.14E+08	99.38%
36	0.389	0.361	1.14E+08	1.13E+08	99.66%
38	0.427	0.388	1.14E+08	1.14E+08	99.56%
41	0.402	0.333	1.14E+08	1.13E+08	99.14%
42	0.379	0.319	1.14E+08	1.13E+08	99.21%
44	0.404	0.500	1.14E+08	1.15E+08	100.98%
45	0.522	0.384	1.15E+08	1.14E+08	98.61%
47	0.407	0.280	1.14E+08	1.12E+08	98.29%

49	0.447	0.404	1.15E+08	1.14E+08	99.54%
50	0.484	0.617	1.15E+08	1.16E+08	101.10%
52	0.388	0.358	1.14E+08	1.13E+08	99.63%
53	0.505	0.454	1.15E+08	1.15E+08	99.52%
55	0.548	0.514	1.16E+08	1.15E+08	99.71%
56	0.534	0.506	1.15E+08	1.15E+08	99.76%
58	0.456	0.415	1.15E+08	1.14E+08	99.57%
59	0.508	0.396	1.15E+08	1.14E+08	98.87%
61	0.499	0.473	1.15E+08	1.15E+08	99.76%
62	0.443	0.402	1.14E+08	1.14E+08	99.56%
64	0.417	0.442	1.14E+08	1.14E+08	100.27%
65	0.444	0.450	1.14E+08	1.15E+08	100.06%
67	0.420	0.436	1.14E+08	1.14E+08	100.17%
68	0.415	0.457	1.14E+08	1.15E+08	100.44%
70	0.388	0.458	1.14E+08	1.15E+08	100.76%
71	0.417	0.486	1.14E+08	1.15E+08	100.70%
73	0.441	0.342	1.14E+08	1.13E+08	98.84%
74	1.900	1.872	1.22E+08	1.22E+08	99.94%
76	0.646	0.697	1.16E+08	1.17E+08	100.34%
77	0.564	0.695	1.16E+08	1.17E+08	100.94%
79	0.418	0.570	1.14E+08	1.16E+08	101.42%
80	0.424	0.494	1.14E+08	1.15E+08	100.70%

Ratio of Day 7 / Day 5 Cell Density – Tube C

Passage	OD 600 at Day 5	OD 600 at Day 7	Cell Density at Day 5	Cell Density at Day 7	Ratio of Day 7, Day 5 Cell Density
2	0.515	0.545	1.15E+08	1.16E+08	100.26%
3	0.585	0.670	1.16E+08	1.17E+08	100.61%
5	0.817	0.772	1.18E+08	1.17E+08	99.75%
6	0.559	0.584	1.16E+08	1.16E+08	100.20%
8	0.495	0.479	1.15E+08	1.15E+08	99.85%
9	0.468	0.532	1.15E+08	1.15E+08	100.58%
11	0.420	0.605	1.14E+08	1.16E+08	101.67%
12	0.481	0.581	1.15E+08	1.16E+08	100.86%
14	0.429	0.474	1.14E+08	1.15E+08	100.45%
15	0.439	0.537	1.14E+08	1.15E+08	100.92%
17	0.430	0.381	1.14E+08	1.14E+08	99.45%
18	0.594	0.413	1.16E+08	1.14E+08	98.37%
19	0.265	0.368	1.12E+08	1.14E+08	101.53%
21	0.837	0.539	1.18E+08	1.15E+08	98.05%
25	0.516	0.551	1.15E+08	1.16E+08	100.30%
27	0.400	0.497	1.14E+08	1.15E+08	100.99%
30	0.468	0.512	1.15E+08	1.15E+08	100.41%
33	0.348	0.346	1.13E+08	1.13E+08	99.97%
35	0.618	0.423	1.16E+08	1.14E+08	98.30%

36	0.418	0.347	1.14E+08	1.13E+08	99.15%
38	0.437	0.383	1.14E+08	1.14E+08	99.40%
41	0.398	0.300	1.14E+08	1.12E+08	98.71%
42	0.386	0.444	1.14E+08	1.14E+08	100.64%
44	0.416	0.367	1.14E+08	1.13E+08	99.43%
45	0.407	0.306	1.14E+08	1.13E+08	98.70%
47	0.365	0.225	1.13E+08	1.11E+08	97.78%
49	0.467	0.407	1.15E+08	1.14E+08	99.38%
50	0.420	0.599	1.14E+08	1.16E+08	101.62%
52	0.367	0.375	1.13E+08	1.14E+08	100.10%
53	0.498	0.422	1.15E+08	1.14E+08	99.25%
55	0.466	0.397	1.15E+08	1.14E+08	99.27%
56	0.494	0.455	1.15E+08	1.15E+08	99.63%
58	0.321	0.399	1.13E+08	1.14E+08	101.01%
59	0.475	0.434	1.15E+08	1.14E+08	99.59%
61	0.454	0.430	1.15E+08	1.14E+08	99.75%
62	0.518	0.461	1.15E+08	1.15E+08	99.47%
64	0.490	0.494	1.15E+08	1.15E+08	100.04%
65	0.350	0.468	1.13E+08	1.15E+08	101.34%
67	0.526	0.474	1.15E+08	1.15E+08	99.53%
68	0.380	0.409	1.14E+08	1.14E+08	100.34%
70	0.355	0.348	1.13E+08	1.13E+08	99.91%
71	0.351	0.431	1.13E+08	1.14E+08	100.95%
73	0.352	0.290	1.13E+08	1.12E+08	99.11%
74	1.830	1.838	1.22E+08	1.22E+08	100.02%
76	0.532	0.583	1.15E+08	1.16E+08	100.41%
77	0.500	0.595	1.15E+08	1.16E+08	100.79%
79	0.398	0.595	1.14E+08	1.16E+08	101.84%
80	0.442	0.458	1.14E+08	1.15E+08	100.16%

Ratio of Day 7 / Day 5 Cell Density – Tube D

Passage	OD 600 at Day 5	OD 600 at Day 7	Cell Density at Day 5	Cell Density at Day 7	Ratio of Day 7, Day 5 Cell Density
2	0.510	0.547	1.15E+08	1.16E+08	100.32%
3	0.532	0.618	1.15E+08	1.16E+08	100.68%
5	0.788	0.734	1.17E+08	1.17E+08	99.68%
6	0.558	0.614	1.16E+08	1.16E+08	100.43%
8	0.520	0.534	1.15E+08	1.15E+08	100.12%
9	0.498	0.577	1.15E+08	1.16E+08	100.67%
11	0.314	0.464	1.13E+08	1.15E+08	101.81%
12	0.520	0.605	1.15E+08	1.16E+08	100.68%
14	0.331	0.455	1.13E+08	1.15E+08	101.47%
15	0.379	0.412	1.14E+08	1.14E+08	100.38%
17	0.375	0.408	1.14E+08	1.14E+08	100.39%
18	0.478	0.388	1.15E+08	1.14E+08	99.05%

19	0.205	0.339	1.10E+08	1.13E+08	102.37%
21	0.544	0.669	1.16E+08	1.17E+08	100.93%
25	0.542	0.557	1.16E+08	1.16E+08	100.12%
27	0.525	0.548	1.15E+08	1.16E+08	100.19%
30	0.431	0.389	1.14E+08	1.14E+08	99.53%
33	0.365	0.335	1.13E+08	1.13E+08	99.61%
35	0.627	0.404	1.16E+08	1.14E+08	98.03%
36	0.390	0.353	1.14E+08	1.13E+08	99.54%
38	0.410	0.413	1.14E+08	1.14E+08	100.03%
41	0.430	0.185	1.14E+08	1.10E+08	96.15%
42	0.434	0.316	1.14E+08	1.13E+08	98.55%
44	0.422	0.416	1.14E+08	1.14E+08	99.93%
45	0.478	0.375	1.15E+08	1.14E+08	98.90%
47	0.383	0.260	1.14E+08	1.12E+08	98.22%
49	0.478	0.375	1.15E+08	1.14E+08	98.90%
50	0.432	0.623	1.14E+08	1.16E+08	101.67%
52	0.410	0.371	1.14E+08	1.14E+08	99.54%
53	0.496	0.437	1.15E+08	1.14E+08	99.43%
55	0.569	0.454	1.16E+08	1.15E+08	98.98%
56	0.594	0.563	1.16E+08	1.16E+08	99.76%
58	0.497	0.437	1.15E+08	1.14E+08	99.42%
59	0.515	0.447	1.15E+08	1.15E+08	99.36%
61	0.467	0.475	1.15E+08	1.15E+08	100.08%
62	0.535	0.513	1.15E+08	1.15E+08	99.81%
64	0.513	0.496	1.15E+08	1.15E+08	99.85%
65	0.452	0.402	1.15E+08	1.14E+08	99.47%
67	0.448	0.469	1.15E+08	1.15E+08	100.21%
68	0.481	0.491	1.15E+08	1.15E+08	100.09%
70	0.444	0.432	1.14E+08	1.14E+08	99.88%
71	0.608	0.508	1.16E+08	1.15E+08	99.19%
73	0.392	0.429	1.14E+08	1.14E+08	100.41%
74	1.849	1.683	1.22E+08	1.21E+08	99.60%
76	0.541	0.702	1.16E+08	1.17E+08	101.18%
77	0.534	0.624	1.15E+08	1.16E+08	100.70%
79	0.481	0.567	1.15E+08	1.16E+08	100.75%
80	0.455	0.484	1.15E+08	1.15E+08	100.28%

Appendix D – Minimum Inhibitory Concentration (MIC)

The following series of tables show the OD readings of the MIC experiment. The first table is Tube A, followed by Tube B, Tube C and lastly Tube D. All OD readings are taken at a wavelength of 600nm and are taken 21 to 23 hours post-inoculation.

MIC OD Readings – Tube A

MIC OD	[NaCl]%						
	0%	1%	3%	5%	7%	9%	11%
11	0.684	0.672	0.866	0.464	0.118	0.006	-0.004
18	0.788	0.692	0.821	0.357	0.106	0.010	0.001
21	0.632	0.542	0.752	0.398	0.143	0.006	0.009
30	0.644	0.703	0.793	0.362	0.139	0.006	0.005
35	0.511	0.710	0.344	0.240	0.090	0.014	0.000
11	0.684	0.672	0.866	0.464	0.118	0.006	-0.004
41	0.554	0.536	0.530	0.455	0.008	0.002	0.007
47	0.783	0.773	1.248	0.844	0.169	0.002	-0.002
49	0.705	0.680	0.840	0.696	0.104	0.012	0.006
55	0.722	0.762	0.968	0.678	0.148	0.037	0.000
61	0.471	0.515	0.771	0.433	0.049	-0.022	-0.020
67	0.565	0.637	0.560	0.453	0.127	0.049	0.094
72	0.549	0.746	0.610	0.401	0.418	0.289	0.259
78	0.731	0.832	0.388	0.332	0.459	0.157	0.073

MIC OD Readings – Tube B

MIC OD	[NaCl]%						
	0%	1%	3%	5%	7%	9%	11%
11	0.616	0.613	0.863	0.355	0.075	0.006	-0.001
18	0.817	0.675	0.783	0.327	0.150	0.007	0.010
21	0.589	0.585	0.741	0.364	0.125	0.004	0.010
30	0.678	0.658	0.744	0.381	0.085	0.021	0.002
35	0.507	0.849	0.488	0.634	0.092	0.021	0.003
11	0.616	0.613	0.863	0.355	0.075	0.006	-0.001
41	0.534	0.385	0.59	0.406	0.008	0.007	0.009
47	0.708	0.796	0.718	0.666	0.144	-0.001	-0.013
49	0.761	0.720	0.927	0.757	0.123	0.030	-0.003
55	0.628	0.676	0.933	0.755	0.135	0.011	-0.001
61	0.432	0.446	0.732	0.461	0.078	-0.018	-0.033
67	0.508	0.879	0.798	0.317	0.308	0.019	0.077
72	0.804	0.742	0.791	0.501	0.301	0.288	0.200
78	0.788	1.062	0.594	0.28	0.386	0.151	0.036

MIC OD Readings – Tube C

MIC OD	[NaCl]%						
	0%	1%	3%	5%	7%	9%	11%
Passage	0%	1%	3%	5%	7%	9%	11%
11	0.637	0.657	0.706	0.376	0.089	-0.003	-0.004
18	0.883	0.778	0.627	0.450	0.224	0.005	0.005
21	0.599	0.562	0.757	0.396	0.085	0.002	0.000
30	0.659	0.627	0.757	0.425	0.005	0.010	0.003
35	0.556	0.574	0.704	0.636	0.090	0.004	-0.002
11	0.637	0.657	0.706	0.376	0.089	-0.003	-0.004
41	0.67	0.642	0.494	0.453	0.002	0.008	0.003
47	0.919	0.705	1.354	0.792	0.158	0.064	-0.007
49	0.787	0.913	0.736	0.688	0.191		0.004
55	0.779	0.701	0.732	0.612	0.141	0.01	0.001
61	0.54	0.561	0.628	0.444	0.085	-0.021	-0.016
67	0.58	0.797	0.664	0.307	0.238	0.17	0.089
72	0.55	1.013	0.984	0.407	0.309	0.241	0.163
78	0.734	0.962	0.224	0.307	0.176	0.115	0.017

MIC OD Readings – Tube D

MIC OD	[NaCl]%						
	0%	1%	3%	5%	7%	9%	11%
Passage	0%	1%	3%	5%	7%	9%	11%
11	0.686	0.719	0.870	0.370	0.090	0.008	-0.001
18	0.757	0.830	0.777	0.317	0.165	0.003	0.013
21	0.606	0.623	0.712	0.374	0.252	0.159	0.112
30	0.670	0.639	0.784	0.363	0.035	0.007	-0.001
35	0.568	0.433		0.549	0.090	0.012	0.002
11	0.686	0.719	0.870	0.370	0.090	0.008	-0.001
41	0.577	0.613	0.715	0.565	0.004	0.007	0.008
47	0.680	0.958	1.235	0.961	0.142	0.010	-0.001
49	0.726	0.767	1.060	0.582	0.115	0.002	0.023
55	0.783	0.813	0.759	0.621	0.106	0.012	-0.005
61	0.985	0.569	0.760	0.431	0.124	-0.009	-0.020
67	0.814	0.702	0.675	0.544	0.255	0.106	0.091
72	0.873	0.790	0.689	0.387	0.340	0.286	0.230
78	0.795	1.062	0.359	0.314	0.284	0.040	0.061

The following series of tables are the coefficients of equations which are made by plotting the graphs using the OD readings and then fitted onto the following polynomial: $OD = M_4(\%NaCl)^4 + M_3(\%NaCl)^3 + M_2(\%NaCl)^2 + M_1(\%NaCl) + M_0$.

MIC Coefficients – Tube A

Passage	Coefficients				
	M ₄	M ₃	M ₂	M ₁	M ₀
11	-0.00007	0.0051	-0.0732	0.2179	0.6338
18	-0.0001	0.0058	-0.066	0.1366	0.7353
21	0.0001	0.00008	-0.0296	0.1035	0.5816
30	-0.0003	0.01	-0.101	0.2539	0.613
35	-0.0005	0.0112	-0.0847	0.1283	0.5554
41	-0.00005	0.0061	-0.0967	0.3599	0.3862
47	0.0004	-0.0018	-0.0575	0.3144	0.6987
49	0.0004	-0.0051	-0.0076	0.1059	0.665
55	0.0001	0.0022	-0.0638	0.2443	0.6757
61	-0.00006	0.0053	-0.0808	0.2814	0.42
67	0.00005	0.0013	-0.0332	0.0932	0.5667
72	-0.0005	0.0124	-0.0974	0.2135	0.5721
78	-0.0002	0.0027	-0.0048	-0.1078	0.7988

MIC Coefficients – Tube B

Passage	Coefficients				
	M ₄	M ₃	M ₂	M ₁	M ₀
11	-0.0003	0.0104	-0.11	0.3001	0.5537
18	-0.00003	0.0029	-0.0395	0.0558	0.7685
21	-0.0001	0.0053	-0.0673	0.1895	0.5489
30	-0.0002	0.0066	-0.0729	0.1726	0.6387
35	-0.0001	0.0054	-0.0687	0.2004	0.5661
41	0.0003	-0.0047	0.0065	0.0233	0.4779
47	0.0003	-0.0027	-0.0161	0.0859	0.7114
49	0.0004	-0.0053	-0.0101	0.1219	0.7117
55	0.0004	-0.0032	-0.0324	0.2181	0.5832
61	0.0001	0.0011	-0.0501	0.02235	0.3812
67	-0.0008	0.0211	-0.1838	0.4675	0.5326
72	-0.0002	0.0063	-0.057	0.1037	0.7682
78	-0.0009	0.0208	-0.1422	0.2062	0.8501

MIC Coefficients – Tube C

Passage	Coefficients				
	M ₄	M ₃	M ₂	M ₁	M ₀
11	-0.0002	0.0063	-0.0714	0.1753	0.6089
18	0.0003	-0.0054	0.0285	-0.1304	0.8837
21	-0.00003	0.0038	-0.0596	0.1801	0.5489
30	-0.0001	0.0057	-0.0724	0.1918	0.6087
35	0.0004	-0.0052	-0.0041	0.1054	0.5284
41	0.0002	-0.0016	-0.0055	-0.0157	0.665
47	0.0004	-0.0023	-0.0474	0.2575	0.7866
49	0.0005	-0.0082	0.0169	0.012	0.8177
55	0.0004	-0.007	0.0203	-0.0214	0.7549
61	0.0001	0.000009	-0.0311	0.1173	0.5185
67	-0.0008	0.0202	-0.1571	0.3351	0.5874
72	-0.0014	0.0359	-0.2905	0.7201	0.552
78	-0.0006	0.0115	-0.0639	-0.0242	0.827

MIC Coefficients – Tube D

Passage	Coefficients				
	M ₄	M ₃	M ₂	M ₁	M ₀
11	-0.0004	0.012	-0.1193	0.2993	0.6375
18	-0.0004	0.0123	-0.1093	0.2206	0.7433
21	-0.0003	0.008	-0.0753	0.1799	0.5775
30	-0.0002	0.0083	-0.0888	0.2184	0.6174
35	0.0007	-0.0133	0.0705	-0.1225	0.5429
41	0.0001	0.0011	-0.0478	0.1822	0.5426
47	-0.00002	0.0068	-0.1292	0.5174	0.6398
49	-0.0001	0.0073	-0.1049	0.3422	0.6624
55	0.0002	-0.00053	-0.0288	0.089	0.7709
61	0.0007	-0.0132	0.0804	-0.2417	0.9097
67	0.0004	-0.0064	0.0302	-0.0896	0.7987
72	-0.0003	0.0067	-0.0458	0.0111	0.8571
78	-0.0005	0.0101	-0.0635	-0.0016	0.8959

The following series of tables are the tabulations of the MIC experiment. The coefficients are used to calculate the three values: Concentration of NaCl whereby OD is at maximum and whereby OD is at half of maximum and the percentage of AUC whereby the NaCl is more than 7.5%.

MIC Calculations – Tube A

Passage	[NaCl%]ODmax	[NaCl%]1/2ODmax	%AUC[NaCl>7.5%]
11	1.8	5.5	0.005
18	1.2	5.2	0.175
21	1.8	5.7	0.000
30	1.6	5.2	0.050
35	0.9	4.0	0.023
41	2.4	5.7	0.003
47	2.7	6.4	0.136
49	2.5	6.2	0.011
55	2.2	6.0	0.002
61	2.2	5.5	0.000
67	1.6	5.7	0.002
72	1.5	6.6	0.236
78	0.0	4.2	0.076

MIC Calculations – Tube B

Passage	[NaCl%]ODmax	[NaCl%]1/2ODmax	%AUC[NaCl>7.5%]
11	1.8	5.2	0.004
18	0.8	5.1	0.013
21	1.8	5.5	0.032
30	1.5	5.1	0.000
35	1.8	5.8	0.090
41	2.1	5.8	0.000
47	2	6.4	0.156
49	2.5	6.2	0.011
55	2.8	6.7	0.170
61	2.5	5.8	0.000
67	1.8	4.9	0.009
72	1.1	6.5	0.224
78	0.9	4.3	0.259

MIC Calculations – Tube C

Passage	[NaCl%]ODmax	[NaCl%]1/2ODmax	%AUC[NaCl>7.5%]
11	1.5	5.0	0.003
18	0.0	5.2	0.058
21	1.8	5.5	0.003
30	1.6	5.3	0.006
35	2.7	6.4	0.021
41	0.0	5.5	0.234
47	2.5	6.5	0.145
49	1.9	5.8	0.000
55	0.0	6.0	0.000
61	1.9	5.6	0.000
67	1.4	4.7	0.247
72	1.8	4.8	0.250
78	0.0	3.2	0.000

MIC Calculations – Tube D

Passage	[NaCl%]ODmax	[NaCl%]1/2ODmax	%AUC[NaCl>7.5%]
11	1.6	4.9	0.001
18	1.3	4.8	0.180
21	1.5	5.5	0.017
30	1.5	5.2	0.142
35	0.0	7.0	0.098
41	2.1	5.6	0.000
47	2.5	5.8	0.000
49	2.1	5.6	0.005
55	1.5	6.0	0.147
61	0.0	5.6	0.203
67	0.0	7.4	0.268
72	0.1	4.9	0.101
78	0.0	3.7	0.000

Appendix E – Colony MIC

The following series of tables show the OD values of the colonies which were measured using a spectrophotometer at 600nm. . The colonies are labeled with their treatment (A – D) in front, followed by A – J, which denotes the colony taken from the treatment. All OD readings were taken at 21 – 23 hours post-inoculation.

Colony MIC OD Readings – P44

Colony MIC OD	[NaCl]%						
Treatment/Colony	0%	1%	3%	5%	7%	9%	11%
AA	0.474	0.412	0.249	0.280	0.024	0.005	0.014
AB	0.463	0.441	0.317	0.466	0.042	0.006	0.010
AC	0.233	0.321	0.408	0.299	0.024	0.008	0.011
AD	0.644	0.621	0.417	0.250	0.090	0.012	0.016
AE	0.393	0.404	0.294	0.518	0.020	0.012	0.023
AF	0.592	0.596	0.480	0.270	0.027	0.007	0.010
AG	0.373	0.378	0.355	0.305	0.036	0.009	0.020
AH	0.365	0.350	0.367	0.305	0.039	0.015	0.011
AI	0.228	0.330	0.343	0.306	0.025	0.005	0.029
AJ	0.265	0.325	0.352	0.252	0.030	0.009	0.020
BA	0.382	0.383	0.230	0.309	0.021	0.006	0.017
BB	0.422	0.410	0.331	0.258	0.034	0.012	0.018
BC	0.443	0.414	0.307	0.417	0.041	0.016	0.023
BD	0.450	0.430	0.347	0.473	0.056	0.014	0.031
BE	0.475	0.465	0.341	0.288	0.029	0.030	0.020
BF	0.457	0.392	0.302	0.357	0.031	0.014	0.014
BG	0.437	0.478	0.305	0.354	0.027	0.019	0.023
BH	0.464	0.415	0.339	0.344	0.029	0.005	0.028
BI	0.460	0.466	0.354	0.330	0.039	0.026	0.023
BJ	0.430	0.477	0.350	0.344	0.040	0.036	0.023
CA	0.745	0.682	0.386	0.264	0.071	0.041	0.023
CB	0.459	0.434	0.306	0.300	0.047	0.044	0.034
CC	0.538	0.473	0.338	0.309	0.026	0.013	0.025
CD	0.447	0.475	0.295	0.330	0.038	0.008	0.016
CE	0.436	0.435	0.329	0.375	0.031	0.019	0.012
CF	0.409	0.398	0.307	0.269	0.037	0.020	0.046
CG	0.397	0.429	0.381	0.438	0.023	0.023	0.017
CH	0.766	0.784	0.696	0.377	0.119	0.038	0.038
CI	0.746	0.787	0.649		0.022	0.031	0.040
CJ	0.488	0.501	0.419	0.234	0.022	0.035	0.029
DA	0.547	0.595	0.694	0.099	0.041	0.019	0.028
DB	0.244	0.676	0.640	0.469	0.051	0.028	0.052
DC	0.495	0.562	0.700	0.213	0.046	0.013	0.024
DD	0.391	0.378	0.273	0.308	0.024	0.036	0.014

DE	0.486	0.558	0.538	0.412	0.032	0.018	0.025
DF	0.411	0.605	0.468	0.505	0.038	0.017	0.023
DG	0.390	0.531	0.499	0.416	0.027	0.032	0.029
DH	0.219	0.453	0.480	0.538	0.038	0.038	0.032
DI	0.475	0.431	0.453	0.499	0.042	0.017	0.018
DJ	0.496	0.506	0.575	0.314	0.028	0.026	0.036

Colony MIC OD Readings – P53

Colony MIC OD	[NaCl]%						
Treatment/Colony	0%	1%	3%	5%	7%	9%	11%
AA	0.561	0.402	0.368	0.321	0.026	0.006	0.012
AB	0.558	0.429	0.339	0.405	0.030	0.005	0.003
AC	0.320	0.280	0.317	0.353	0.024	0.006	0.015
AD	0.309	0.307	0.280	0.414	0.033	0.001	0.015
AE	0.353	0.322	0.315	0.394	0.016	0.045	0.018
AF	0.281	0.264	0.261	0.348	0.057	0.003	0.008
AG	0.354	0.338	0.382	0.441	0.020	0.006	0.003
AH	0.385	0.323	0.280	0.414	0.043	0.006	0.006
AI	0.352	0.320	0.380	0.421	0.022	-0.005	0.014
AJ	0.350	0.321	0.352	0.395	0.020	0.008	0.005
BA	0.330	0.302	0.319	0.447	0.021	-0.001	0.021
BB	0.322	0.299	0.314	0.456	0.060	-0.004	0.009
BC	0.342	0.255	0.221	0.374	0.069	-0.004	0.008
BD	0.365	0.314	0.288	0.417	0.085	0.008	0.023
BE	0.299	0.267	0.246	0.406	0.036	0.010	0.007
BF	0.339	0.394	0.321	0.395	0.086	-0.006	0.012
BG	0.325	0.288	0.292	0.360	0.018	0.007	0.001
BH	0.385	0.320	0.260	0.337	0.025	-0.009	0.001
BI	0.313	0.323	0.211	0.378	0.021	0.008	0.011
BJ	0.327	0.347	0.221	0.308	0.049	0.015	0.002
CA	0.384	0.366	0.284	0.406	0.022	0.005	0.010
CB	0.360	0.368	0.321	0.369	0.125	0.009	0.013
CC	0.340	0.383	0.322	0.390	0.053	0.007	0.010
CD	0.386	0.423	0.283	0.388	0.091	0.011	0.018
CE	0.306	0.372	0.301	0.349	0.083	0.033	0.002
CF	0.352	0.390	0.383	0.279	0.064	0.016	0.008
CG	0.402	0.368	0.366	0.347	0.136	0.015	0.003
CH	0.348	0.344	0.318	0.437	0.039	0.022	0.006
CI	0.377	0.380	0.323	0.414	0.089	0.016	0.015
CJ	0.282	0.280	0.231	0.466	0.048	0.024	0.005
DA	0.321	0.345	0.275	0.383	0.020	0.007	0.002
DB	0.329	0.288	0.362	0.290	0.039	0.005	0.001
DC	0.380	0.307	0.291	0.319	0.030	0.003	0.013
DD	0.325	0.307	0.286	0.335	0.047	0.019	0.013
DE	0.445	0.324	0.306	0.318	0.249	0.011	0.001
DF	0.559	0.353	0.276	0.434	0.059	-0.001	-0.001

DG	0.324	0.265	0.240	0.345	0.082	0.030	-0.001
DH	0.321	0.330	0.254	0.315	0.025	0.011	-0.004
DI	0.349	0.357	0.315	0.356	0.073	0.002	0.005
DJ	0.392	0.371	0.286	0.313	0.041	0.020	0.025

Colony MIC OD Readings – P72

Colony MIC OD	[NaCl]%						
Treatment/Colony	0%	1%	3%	5%	7%	9%	11%
AA	0.547	0.659	0.160	0.279	0.082	0.099	0.137
AB	0.593	0.675	0.238	0.174	0.194	0.166	0.104
AC	0.538	0.562	0.095	0.312	0.040	0.095	0.129
AD	0.239	0.278	0.240	0.217	0.164	0.172	0.105
AE	0.358	0.553	0.247	0.231	0.168	0.097	0.158
AF	0.318	0.320	0.246	0.207	0.016	0.011	0.005
AG	0.565	0.566	0.163	0.186	0.172	0.132	0.069
AH	0.137	0.162	0.118	0.201	0.042	0.060	0.121
AI	0.574	0.615	0.214	0.136	0.102	0.093	0.091
AJ	0.167	0.180	0.195	0.132	0.010	0.000	0.009
BA	0.445	0.499	0.192	0.225	0.117	0.103	0.111
BB	0.416	0.574	0.163	0.236	0.082	0.051	0.090
BC	0.409	0.465	0.286	0.195	0.035	0.008	0.052
BD	0.473	0.587	0.192	0.131	0.040	0.054	0.123
BE	0.628	0.529	0.145	0.176	0.075	0.088	0.071
BF	0.495	0.616	0.253	0.238	0.132	0.099	0.036
BG	0.187	0.148	0.052	0.120	0.136	0.071	0.139
BH	0.530	0.534	0.136	0.155	0.022	0.073	0.066
BI	0.164	0.132	0.076	0.193	0.072	0.064	0.101
BJ	0.593	0.600	0.150	0.150	0.031	0.075	0.068
CA	0.274	0.290	0.266	0.240	0.129	0.137	0.093
CB	0.502	0.598	0.189	0.198	0.112	0.132	0.086
CC	0.334	0.293	0.264	0.255	0.090	0.110	0.118
CD	0.374	0.350	0.332	0.282	0.110	0.061	0.072
CE	0.309	0.304	0.282	0.294	0.087	0.150	0.075
CF	0.391	0.308	0.248	0.308	0.057	0.112	0.088
CG	0.292	0.327	0.210	0.262	0.124	0.067	0.065
CH	0.250	0.290	0.215	0.241	0.069	0.092	0.094
CI	0.384	0.317	0.377	0.058	0.096	0.167	0.103
CJ	0.072	0.107	0.167	0.116	0.051	0.027	0.110
DA	0.494	0.535	0.250	0.185	0.150	0.165	0.111
DB	0.341	0.441	0.241	0.188	0.127	0.147	0.111
DC	0.510	0.617	0.396	0.254	0.101	0.124	0.113
DD	0.401	0.352	0.393	0.175	0.065	0.054	0.069
DE	0.334	0.289	0.177	0.229	0.125	0.152	0.086
DF	0.442	0.490	0.281	0.281	0.082	0.070	0.103
DG	0.413	0.451	0.298	0.234	0.081	0.061	0.126
DH	0.574	0.554	0.261	0.237	0.075	0.154	0.105

DI	0.537	0.536	0.257	0.189	0.116	0.143	0.054
DJ	0.334	0.274	0.176	0.248	0.087	0.115	0.101

The following series of tables are the coefficients of equations which are made by plotting the graphs using the OD readings and then fitted onto the following polynomial: $OD = M_4(\%NaCl)^4 + M_3(\%NaCl)^3 + M_2(\%NaCl)^2 + M_1(\%NaCl) + M_0$.

Colony MIC Coefficients – P44

Treatment/ Colony	Coefficients				
	M ₄	M ₃	M ₂	M ₁	M ₀
AA	0.0002	-0.0047	0.0293	-0.1137	0.4802
AB	0.0005	-0.0093	0.0500	-0.1043	0.4720
AC	-0.00005	0.0035	-0.0510	0.1826	0.2170
AD	-0.00009	0.0029	-0.0235	-0.0250	0.6509
AE	0.0005	-0.0105	0.0538	-0.0848	0.4043
AF	-0.0002	0.0057	-0.0526	0.0753	0.5836
AG	0.0001	-0.0011	-0.0085	0.0322	0.3658
AH	0.0001	-0.0015	-0.0060	0.0318	0.3512
AI	0.00002	0.0018	-0.0363	0.1389	0.2255
AJ	-0.00004	0.0028	-0.0392	0.1244	0.2553
BA	0.0002	-0.0041	0.0206	-0.0634	0.3942
BB	0.00005	0.0002	-0.0113	0.0064	0.4184
BC	0.0004	-0.0077	0.0408	-0.0897	0.4481
BD	0.0005	-0.0092	0.0465	-0.0838	0.4545
BE	0.00002	0.0007	-0.0135	-0.0014	0.4745
BF	0.0003	-0.0066	0.0367	-0.0976	0.4550
BG	0.0001	-0.0014	-0.0023	-0.0085	0.4507
BH	0.0003	-0.0050	0.0224	-0.0608	0.4599
BI	0.00009	-0.0006	-0.0078	0.0028	0.4615
BJ	0.00002	0.0009	-0.0194	0.0378	0.4362
CA	-0.00009	0.0023	-0.0119	-0.0926	0.7562
CB	0.0001	-0.0015	0.0040	-0.0409	0.4611
CC	0.0002	-0.0031	0.0147	-0.0777	0.0536
CD	0.0001	-0.0018	0.0028	-0.0286	0.4619
CE	0.0002	-0.0036	0.0118	-0.0285	0.4391
CF	0.0001	-0.0011	-0.0023	-0.0120	0.4088
CG	0.0002	-0.0034	0.0029	0.0281	0.3949
CH	-0.0002	0.0077	-0.0727	0.1278	0.7521
CI	-0.0005	0.0137	-0.1122	0.1902	0.7301
CJ	-0.0002	0.0063	-0.0556	0.0935	0.4775
DA	-0.0008	0.0197	-0.1594	0.3408	0.5023
DB	-0.0006	0.0183	-0.1777	0.5338	0.2600
DC	-0.0005	0.0152	-0.1352	0.3292	0.4523
DD	0.0001	-0.0022	0.0071	-0.0306	0.3924
DE	-0.00004	0.0036	-0.0522	0.1500	0.4747
DF	-0.000007	0.0030	-0.0515	0.1723	0.4292

DG	-0.0001	0.0056	-0.0684	0.2067	0.3866
DH	0.00003	0.0029	-0.0609	0.2655	0.2233
DI	0.0005	-0.0079	0.0316	-0.0298	0.4596
DJ	-0.0002	0.0060	-0.0660	0.1692	0.4646

Colony MIC Coefficients – P53

Treatment/ Colony	Coefficients				
	M ₄	M ₃	M ₂	M ₁	M ₀
AA	0.0004	-0.0073	0.0436	-0.1363	0.5406
AB	0.0005	-0.0101	0.0617	-0.1668	0.5504
AC	0.0004	-0.0064	0.0273	-0.0259	0.3065
AD	0.0005	-0.0086	0.0416	-0.051	0.3105
AE	0.0003	-0.0055	0.0224	-0.0247	0.3423
AF	0.0004	-0.0077	0.0385	-0.0519	0.2798
AG	0.0004	-0.0068	0.0237	0.0012	0.3399
AH	0.0005	-0.0106	0.0606	-0.1184	0.3835
AI	0.0004	-0.0075	0.029	-0.0115	0.3358
AJ	0.0004	-0.0062	0.0232	-0.0122	0.3364
BA	0.0005	-0.01	0.0482	-0.0556	0.3229
BB	0.0006	-0.0112	0.0574	-0.0728	0.3197
BC	0.0006	-0.0131	0.0816	-0.169	0.3433
BD	0.0006	-0.0114	0.065	-0.1183	0.3664
BE	0.0005	-0.0102	0.0555	-0.0884	0.2988
BF	0.0003	-0.0053	0.0188	-0.0063	0.3515
BG	0.0004	-0.0067	0.0312	-0.0418	0.3159
BH	0.0004	-0.0082	0.047	-0.1064	0.3831
BI	0.0004	-0.0075	0.0398	-0.0734	0.3253
BJ	0.0002	-0.0039	0.0187	-0.0449	0.3402
CA	0.0004	-0.0077	0.0391	-0.0724	0.3876
CB	0.0003	-0.0065	0.0314	-0.0469	0.3677
CC	0.0003	-0.0042	0.0114	0.0074	0.3461
CD	0.0003	-0.0058	0.0279	-0.0546	0.4051
CE	0.0001	-0.0016	-0.0034	0.0341	0.3154
CF	-0.00001	0.0019	-0.0301	0.086	0.345
CG	0.0003	-0.006	0.0286	-0.0489	0.3986
CH	0.0004	-0.0074	0.0332	-0.0358	0.3462
CI	0.0004	-0.0073	0.0351	-0.0524	0.3829
CJ	0.0006	-0.0113	0.0626	-0.0927	0.2899
DA	0.0003	-0.0053	0.0205	-0.0184	0.3261
DB	0.0002	-0.0029	0.0029	0.0222	0.308
DC	0.0004	-0.0069	0.0367	-0.079	0.3703
DD	0.0003	-0.0052	0.0229	-0.0316	0.3214
DE	0.0006	-0.0128	0.0869	-0.2086	0.4496
DF	0.0008	-0.0174	0.1172	-0.2951	0.5521
DG	0.0004	-0.0088	0.0529	-0.1069	0.3228
DH	0.0002	-0.0035	0.0127	-0.0198	0.3246

DI	0.0003	-0.0052	0.021	-0.0237	0.3519
DJ	0.0002	-0.0038	0.0166	-0.0448	0.3932

Colony MIC Coefficients – P72

Treatment/ Colony	Coefficients				
	M ₄	M ₃	M ₂	M ₁	M ₀
AA	-0.0002	0.0034	-0.0161	-0.0725	0.6059
AB	-0.0016	0.0296	-0.1551	0.1396	0.6131
AC	0.0001	-0.0029	0.0303	-0.1775	0.5884
AD	-0.0002	0.0035	-0.0262	0.0545	0.2408
AE	-0.0003	0.0072	-0.0548	0.0882	0.4059
AF	0.00002	0.0005	-0.0114	0.0118	0.317
AG	-0.0002	0.0027	0.00004	-0.1362	0.6064
AH	0.0002	-0.0031	0.0137	-0.0132	0.1439
AI	-0.0004	0.0075	-0.0395	-0.0452	0.6106
AJ	-0.000003	0.0011	-0.0172	0.0516	0.1595
BA	-0.0001	0.0024	-0.0119	-0.0494	0.479
BB	-0.0002	0.0047	-0.0317	0.0023	0.4712
BC	-0.0001	0.0041	-0.0359	0.0421	0.4225
BD	-0.0004	0.0088	-0.0573	0.0276	0.5143
BE	0.00001	-0.0014	0.0286	-0.2178	0.6551
BF	-0.0003	0.0077	-0.0491	0.0264	0.5364
BG	0.0002	-0.0059	0.046	-0.1316	0.2034
BH	-0.0002	0.0039	-0.0147	-0.0965	0.5629
BI	0.0003	-0.0064	0.0436	-0.0995	0.1719
BJ	-0.0003	0.0051	-0.0201	-0.1043	0.6308
CA	-0.00006	0.0018	-0.0173	0.0383	0.2711
CB	-0.0004	0.0079	-0.0446	-0.01	0.5452
CC	0.0001	-0.0018	0.0074	-0.0277	0.3268
CD	0.0001	-0.0022	0.0052	-0.0116	0.3684
CE	-0.00003	0.0011	-0.0139	0.0308	0.2998
CF	0.0002	-0.0045	0.0286	-0.0881	0.3825
CG	0.0001	-0.0022	0.0104	-0.0303	0.3086
CH	0.000003	0.0007	-0.012	0.0285	0.2555
CI	-0.0005	0.0112	-0.0768	0.1203	0.3477
CJ	0.00006	-0.00008	-0.0117	0.0637	0.0668
DA	-0.0004	0.0075	-0.0427	-0.0008	0.5177
DB	-0.0004	0.0082	-0.0566	0.082	0.3612
DC	-0.0004	0.0107	-0.0803	0.1279	0.5245
DD	-0.0001	0.0036	-0.0351	0.0626	0.3765
DE	0.00004	-0.0014	0.0151	-0.0774	0.3384
DF	-0.00002	0.0013	-0.0146	-0.0061	0.4614
DG	-0.00005	0.0022	-0.0223	0.0186	0.4245
DH	-0.0002	0.0048	-0.0245	-0.0529	0.5886
DI	-0.0003	0.0066	-0.0349	-0.0292	0.5539
DJ	0.0002	-0.004	0.0294	-0.0992	0.3364

The following three tables are the tabulations of the colony MIC experiment. The colonies are labeled with their treatment (A – D) in front, followed by A – J, which denotes the colony taken from the treatment. These values are obtained by calculating using the coefficient values that are shown above. The three values shown in the following tables are: Concentrations of NaCl where OD is at maximum, where OD is at half of maximum and the percentage AUC where concentration of NaCl is 7.5% and higher is calculated. The first table is P44 (5% NaCl), followed by P53 (6% NaCl) and lastly P72 (7% NaCl).

Colony MIC Calculations – P44

Treatment/Colony	[Nacl%]ODmax	[Nacl%]1/2ODmax	%AUC[NaCl>7.5%]
AA	0.0	4.1	0.012
AB	0.0	6.7	0.152
AC	2.3	5.7	0.004
AD	0.0	4.2	0.067
AE	0.0	6.1	0.002
AF	0.8	4.5	0.003
AG	1.5	5.5	0.000
AH	1.7	5.5	0.006
AI	2.3	5.8	0.009
AJ	2.0	5.5	0.007
BA	0.0	5.2	0.000
BB	0.3	5.3	0.084
BC	0.0	6.2	0.015
BD	0.0	6.7	0.101
BE	0.0	5.0	0.035
BF	0.0	5.3	0.003
BG	0.0	5.2	0.000
BH	0.0	5.8	0.120
BI	0.2	5.5	0.037
BJ	1.1	5.5	0.017
CA	0.0	3.5	0.057
CB	0.0	5.1	0.014
CC	0.0	4.8	0.147
CD	0.0	5.0	0.002
CE	0.0	5.6	0.000
CF	0.0	5.2	0.007
CG	2.2	5.8	0.001
CH	1.0	5.1	0.244
CI	1.0	4.2	0.076
CJ	1.0	4.6	0.151
DA	1.4	4.1	0.312
DB	2.1	5.2	0.093
DC	1.6	4.8	0.263
DD	0.0	5.2	0.005

DE	1.7	5.4	0.006
DF	2.0	5.7	0.007
DG	2.0	5.6	0.173
DH	2.7	6.1	0.020
DI	3.0	6.1	0.288
DJ	1.6	5.0	0.007

Colony MIC Calculations – P53

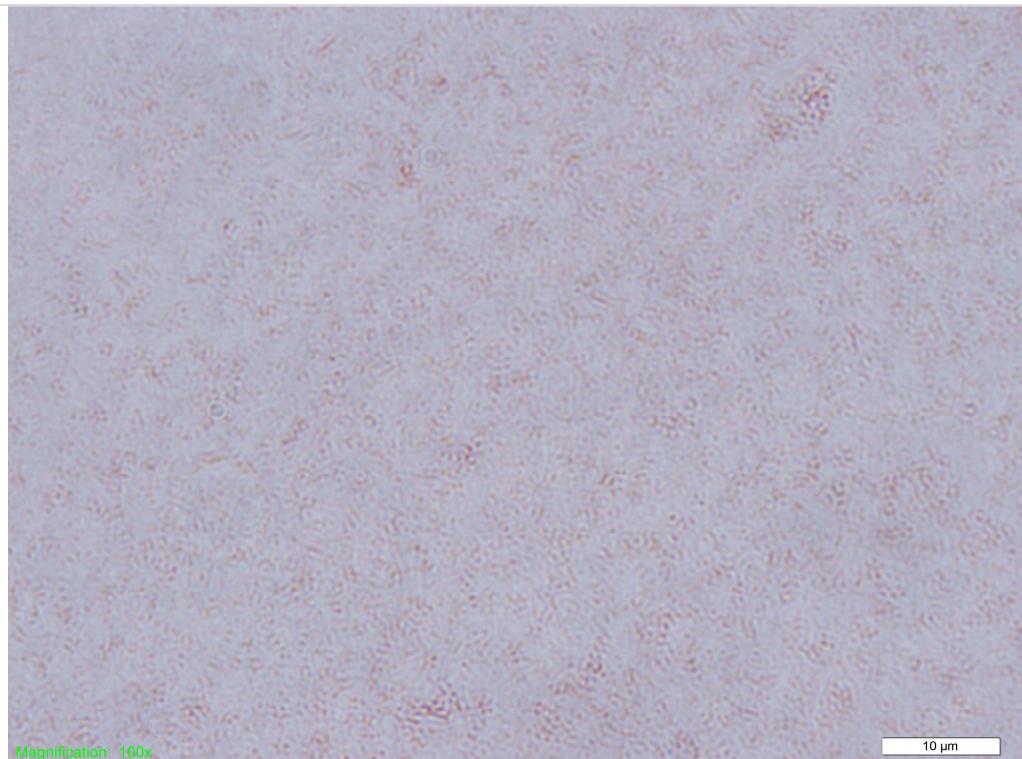
Treatment/Colony	[NaCl%]ODmax	[NaCl%]1/2ODmax	%AUC[NaCl>7.5%]
AA	0.0	5.4	0.228
AB	0.0	5.6	0.017
AC	3.4	6.5	0.334
AD	3.9	6.7	0.321
AE	2.7	6.3	0.004
AF	3.6	6.8	0.014
AG	3.1	6.5	0.011
AH	0.0	6.2	0.000
AI	3.1	6.2	0.000
AJ	3.1	8.1	0.293
BA	3.5	6.3	0.000
BB	4.1	7.4	0.171
BC	0.0	6.5	0.002
BD	0.0	6.9	0.278
BE	3.8	6.6	0.002
BF	2.8	6.5	0.010
BG	3.4	6.5	0.308
BH	0.0	6.0	0.002
BI	0.0	6.9	0.130
BJ	0.0	5.9	0.005
CA	0.0	6.3	0.007
CB	0.0	6.2	0.000
CC	2.7	6.5	0.304
CD	0.0	6.2	0.008
CE	2.2	6.1	0.000
CF	1.7	5.6	0.018
CG	0.0	6.3	0.005
CH	3.3	6.7	0.015
CI	0.0	7.1	0.115
CJ	4.6	7.0	0.313
DA	2.7	6.4	0.007
DB	2.3	6.1	0.006
DC	0	6.1	0.327
DD	0.0	7.0	0.149
DE	0.0	8.6	0.270
DF	0.0	6.1	0.024
DG	0.0	6.5	0.005

DH	0.0	6.1	0.007
DI	2.7	6.7	0.062
DJ	0.0	5.6	0.000

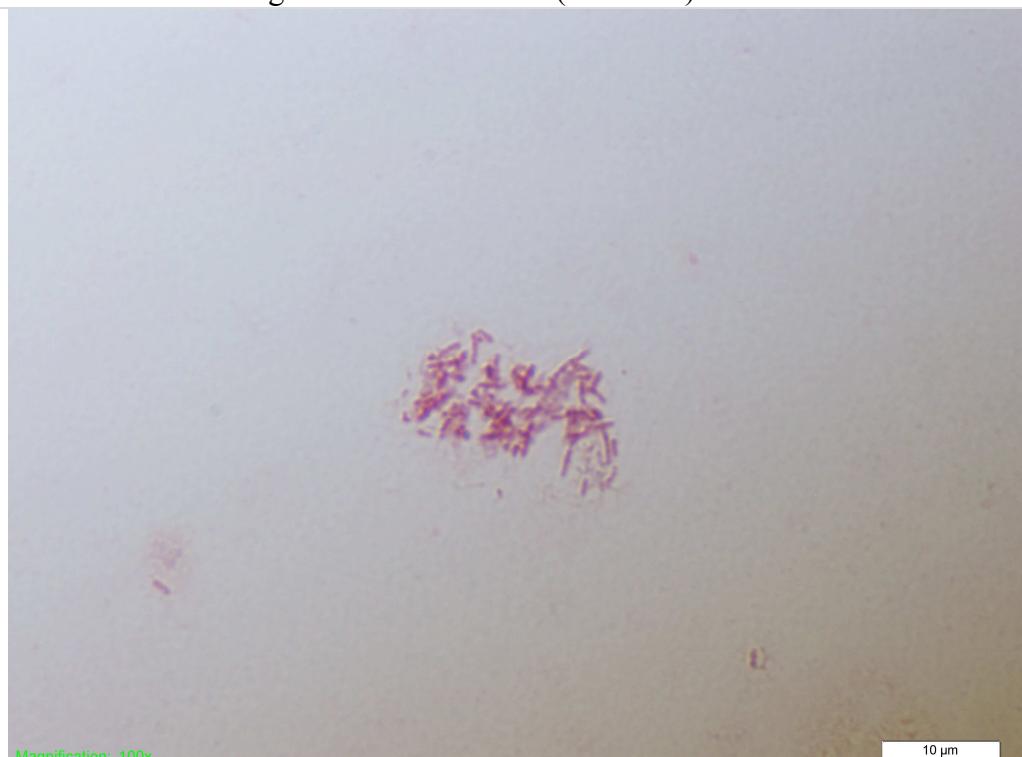
Colony MIC Calculations – P72

Treatment/Colony	[NaCl%]ODmax	[NaCl%]1/2ODmax	%AUC[NaCl>7.5%]
AA	0.0	3.2	0.005
AB	0.5	2.9	0.258
AC	0.0	2.5	0.017
AD	1.4	5.7	0.000
AE	1.9	4.6	0.049
AF	0.5	5.2	0.013
AG	0.0	2.5	0.058
AH	3.5	6.8	0.421
AI	0.0	2.9	0.016
AJ	1.8	5.6	0.077
BA	0.0	3.7	0.238
BB	0.0	3.9	0.055
BC	0.7	4.5	0.321
BD	0.3	3.3	0.028
BE	0.0	2.0	0.087
BF	0.3	4.1	0.403
BG	0.0	1.3	0.023
BH	0.0	2.6	0.054
BI	0.0	7.7	0.323
BJ	0.0	2.5	0.039
CA	1.4	7.9	0.203
CB	0.0	3.3	0.008
CC	0.0	6.1	0.048
CD	0.0	5.6	0.002
CE	1.3	6.6	0.083
CF	0.0	5.7	0.013
CG	0.0	6.1	0.011
CH	1.3	6.4	0.145
CI	1.0	3.9	0.127
CJ	2.9	6.4	0.163
DA	0.0	3.5	0.000
DB	0.9	4.2	0.001
DC	1.0	4.5	0.326
DD	1.1	5.0	0.126
DE	0.0	5.9	0.177
DF	0.0	4.8	0.074
DG	0.4	4.9	0.115
DH	0.0	3.4	0.340
DI	0.0	3.4	0.334
DJ	0.0	6.1	0.350

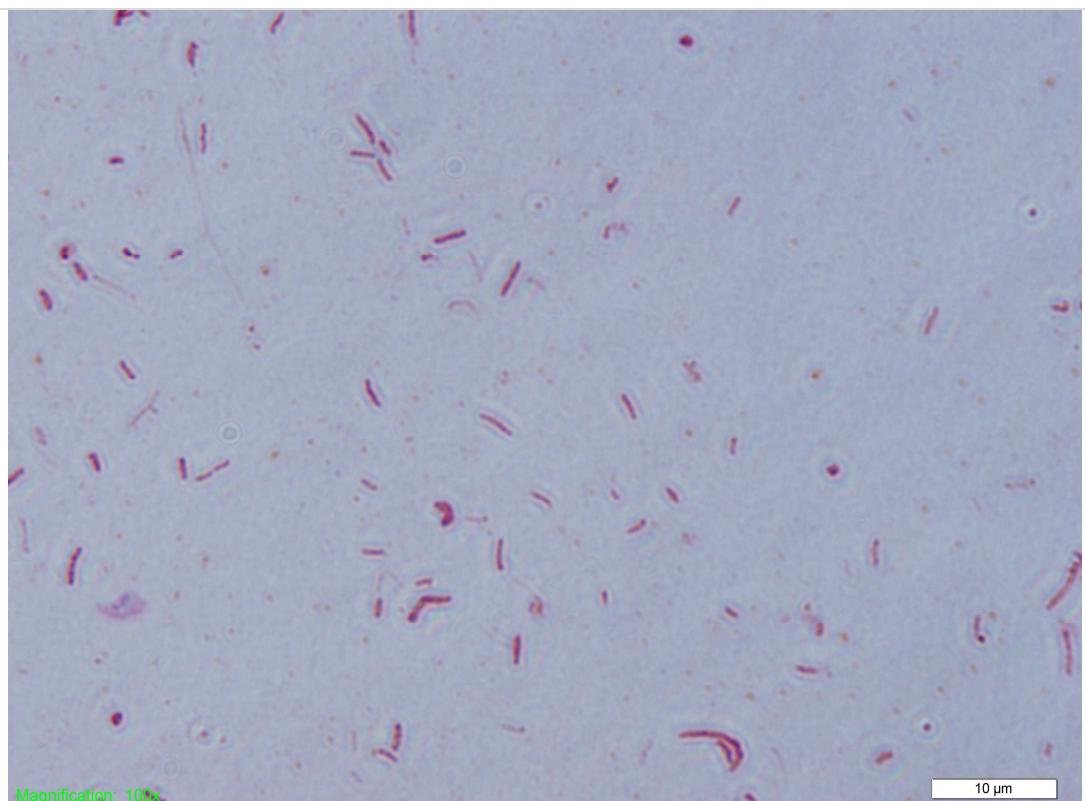
Appendix F – Gram Staining Photos



Gram Stain of Passage 46 Tube Culture A (5% NaCl)

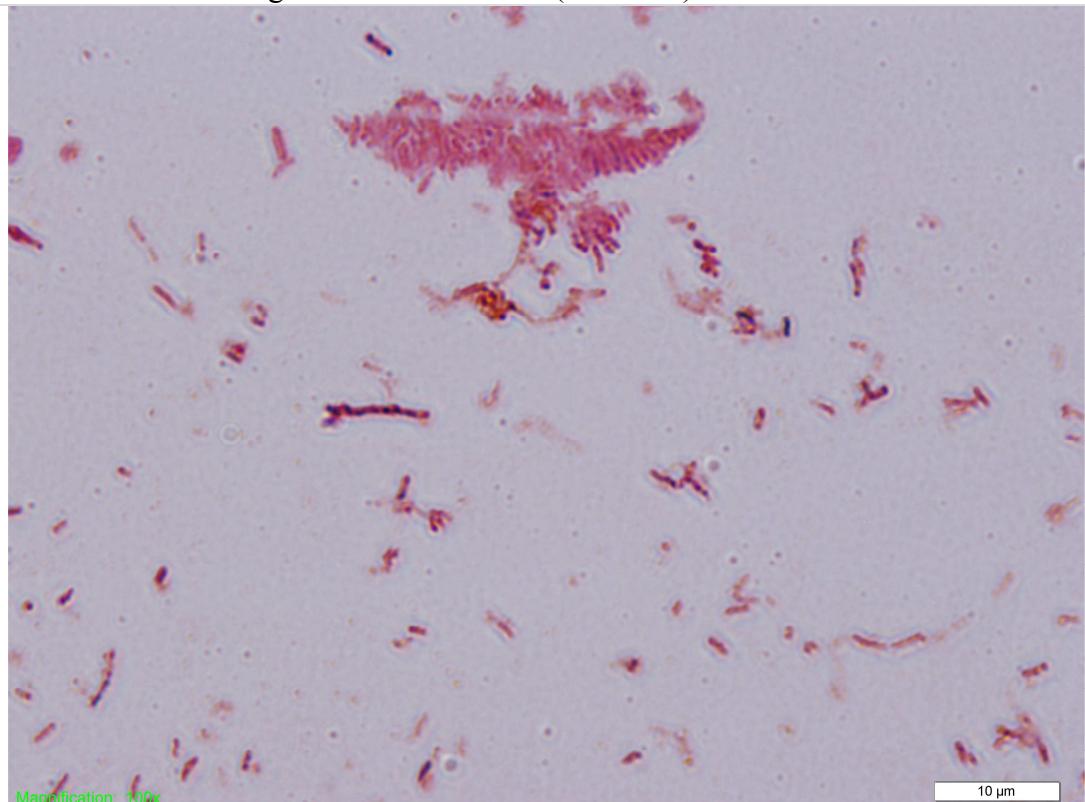


Gram Stain of Passage 46 Tube Culture B (5% NaCl)



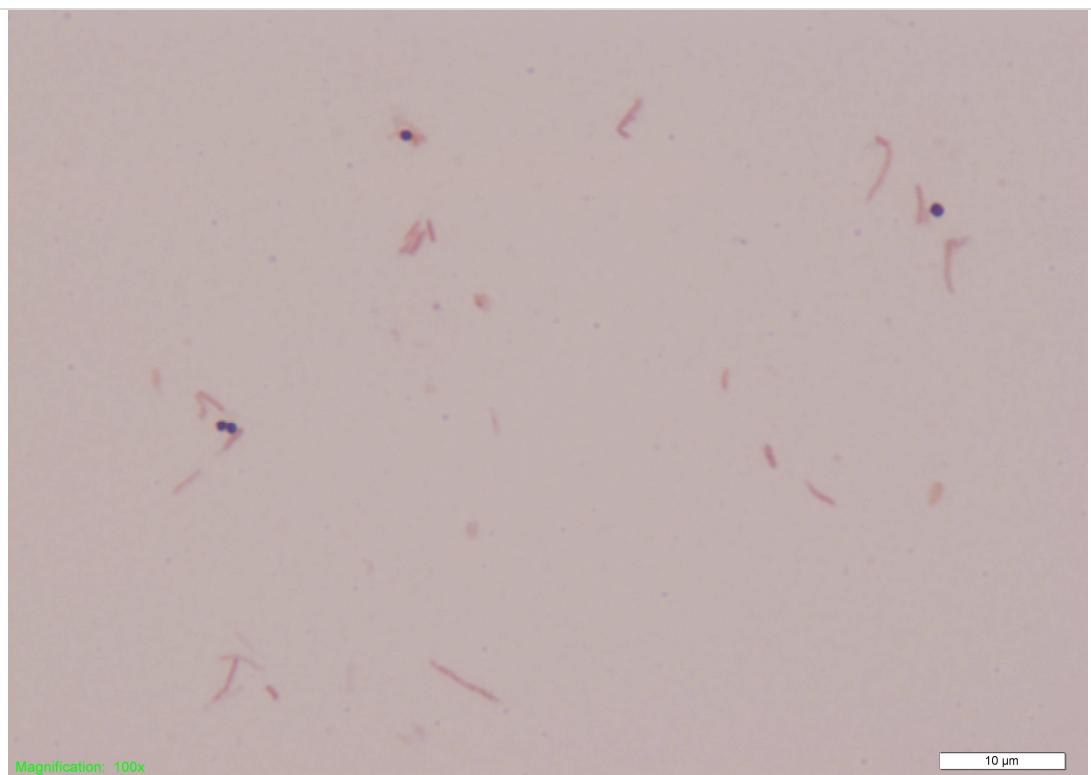
Magnification: 100x

Gram Stain of Passage 46 Tube Culture C (5% NaCl)

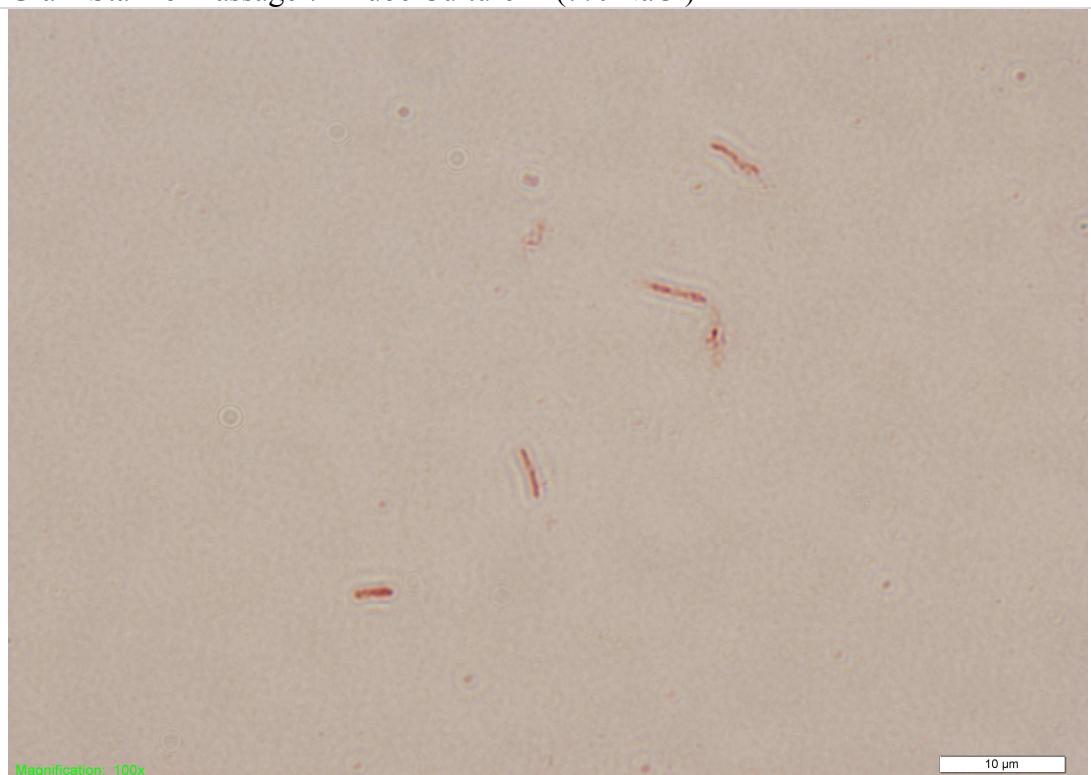


Magnification: 100x

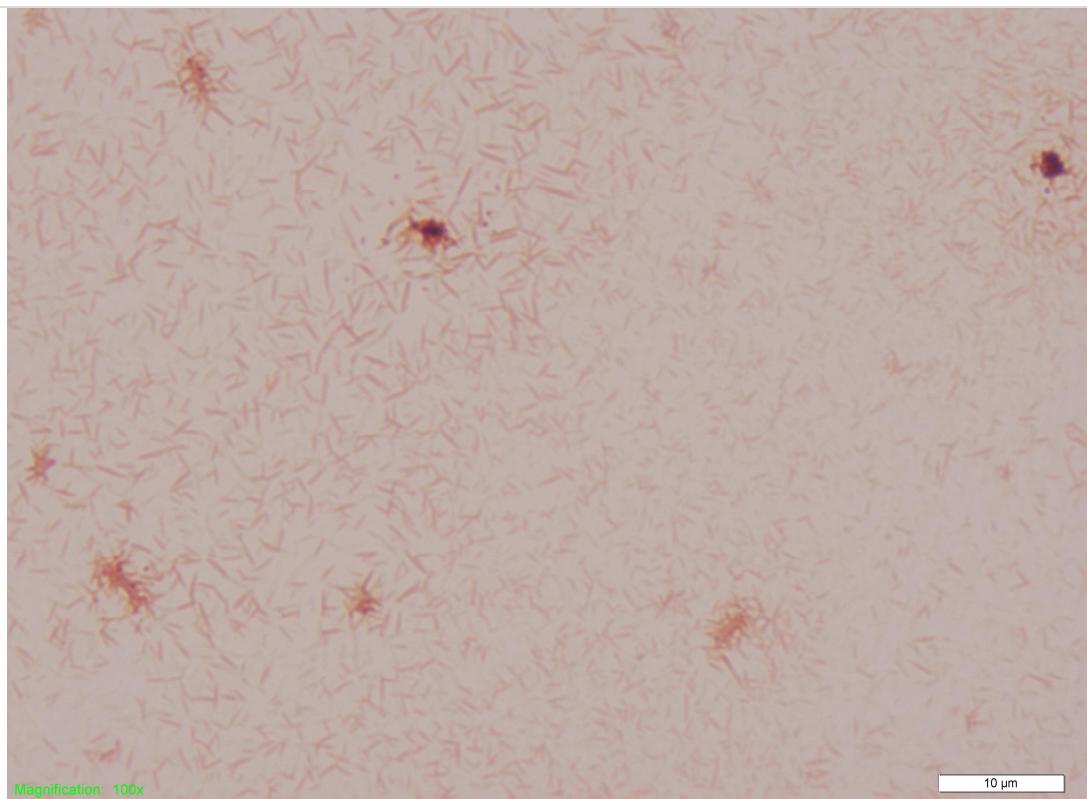
Gram Stain of Passage 46 Tube Culture D (5% NaCl)



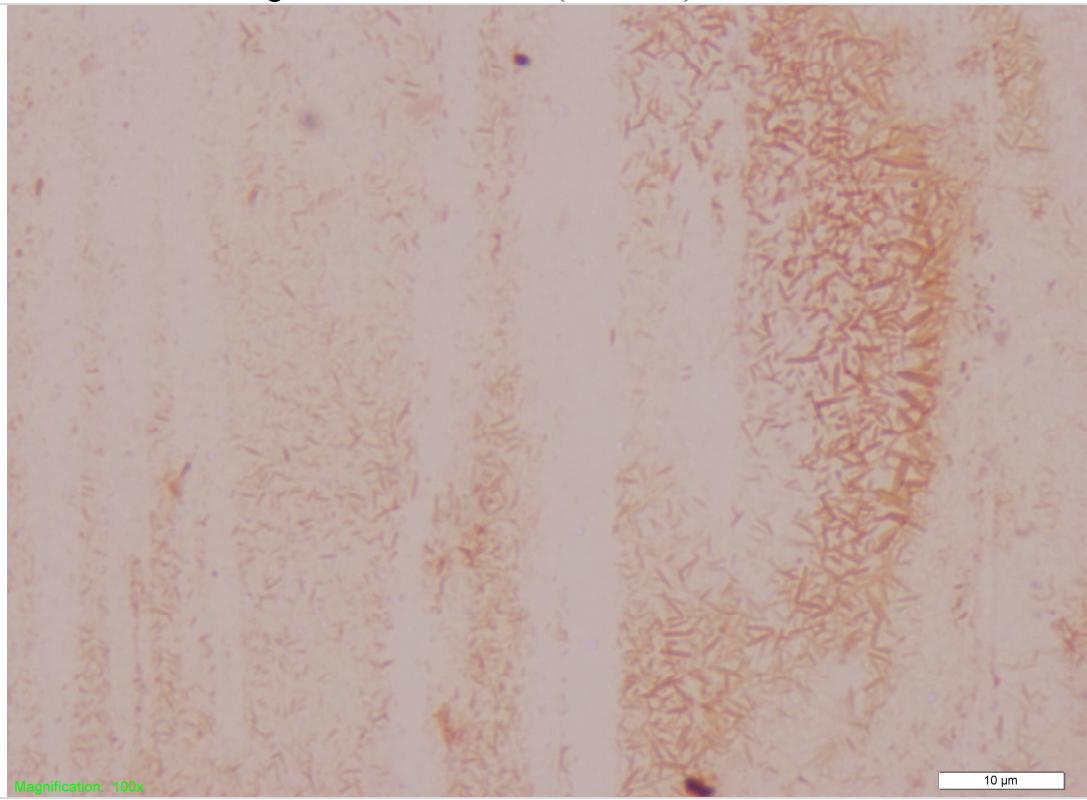
Magnification: 100x
Gram Stain of Passage 72 Tube Culture A (7% NaCl)



Magnification: 100x
Gram Stain of Passage 72 Tube Culture B (7% NaCl)



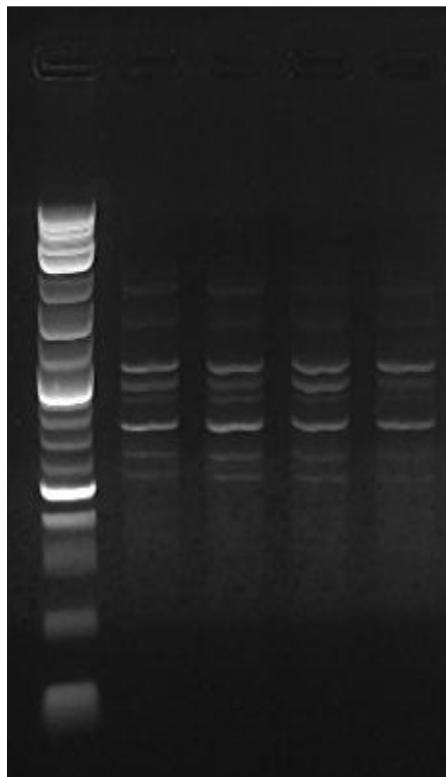
Gram Stain of Passage 72 Tube Culture A (7% NaCl)



Gram Stain of Passage 72 Tube Culture A (7% NaCl)

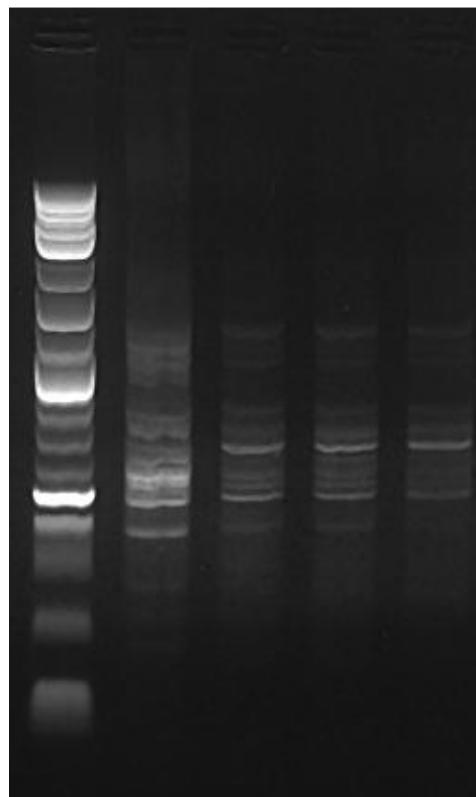
Appendix G – PCR/RFLP Agarose Gel Photos

G.1. PCR of Passage 11 with Primer 5



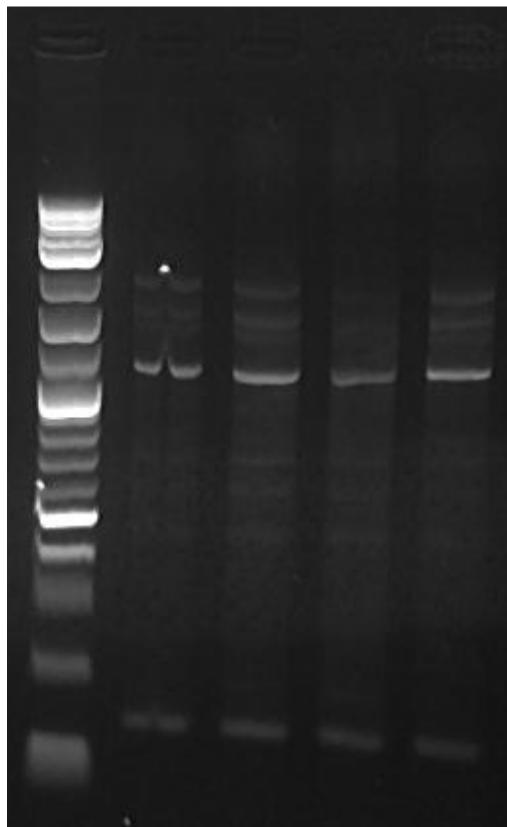
Log 2 Marker		Treatments			
Distance	Molecular Weight	Primer 5			
36	0.8kb	A	B	C	D
38	0.7kb	25	25	25	25
41	0.6kb	27	27	27	27
43	0.5kb	32	32	32	32
46	0.4kb	33	33	33	33
49	0.3kb	36	36	36	36
56	0.2kb	39	39	39	39
64	0.1kb	41	41	41	41
		42	42	42	42

G.2. PCR of Passage 11 with Primer 6



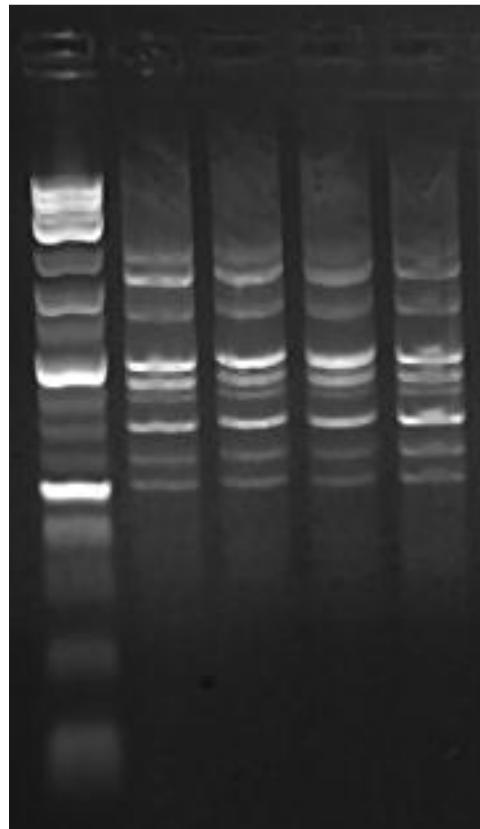
Log 2 Marker		Treatments			
Distance	Molecular Weight	Primer 6			
		A	B	C	D
37	0.8kb				
43	0.7kb		30	30	30
47	0.6kb	32			
49	0.5kb	40	40	40	40
52	0.4kb	42	42	42	42
57	0.3kb	45	45	45	45
63	0.2kb	50	50	50	50
70	0.1kb	53	53	53	53

G.3. PCR of Passage 11 with Primer 7



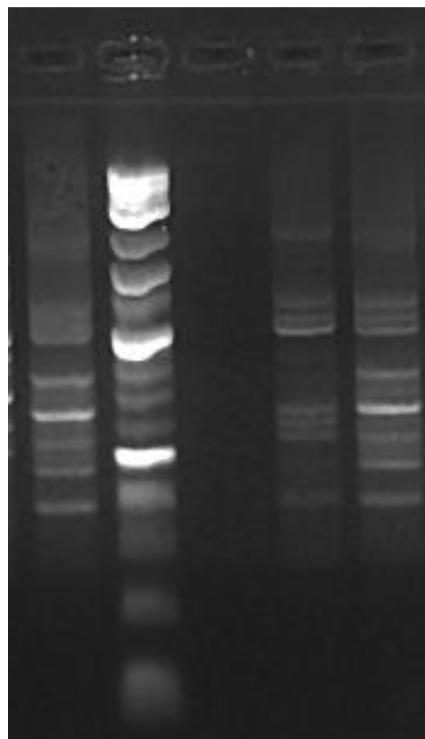
Log 2 Marker		Treatments			
Distance	Molecular Weight	Primer 7			
		A	B	C	D
41	0.8kb				
43	0.7kb	25	25	25	25
46	0.6kb	27	27	27	27
48	0.5kb	34			34
53	0.4kb		36	36	
56	0.3kb	79	79		
64	0.2kb			81	81
72	0.1kb				

G.4. PCR of Passage 21 with Primer 5



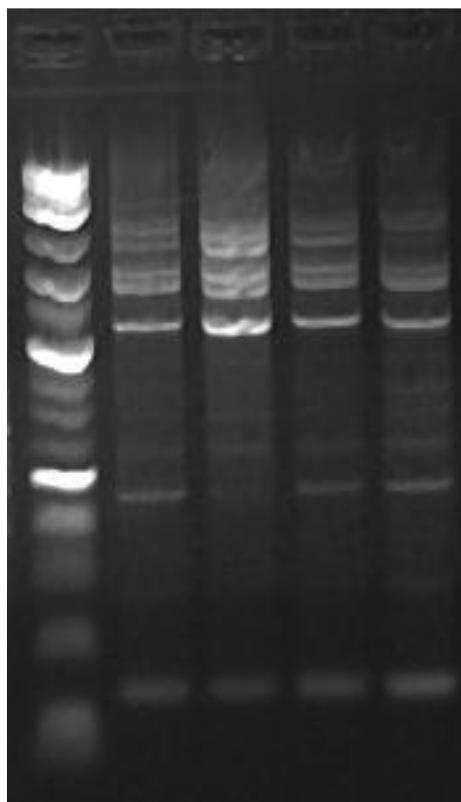
Log 2 Marker		Treatments			
Distance	Molecular Weight	Primer 5			
35	0.8kb	A	B	C	D
37	0.7kb	21	21	21	21
41	0.6kb	23	23	23	23
43	0.5kb	27	27	27	27
47	0.4kb	32	32	32	32
52	0.3kb	34	34	34	34
59	0.2kb	38	38	38	38
67	0.1kb	41	41	41	41
		44	44	44	44

G.5. PCR of Passage 21 with Primer 6



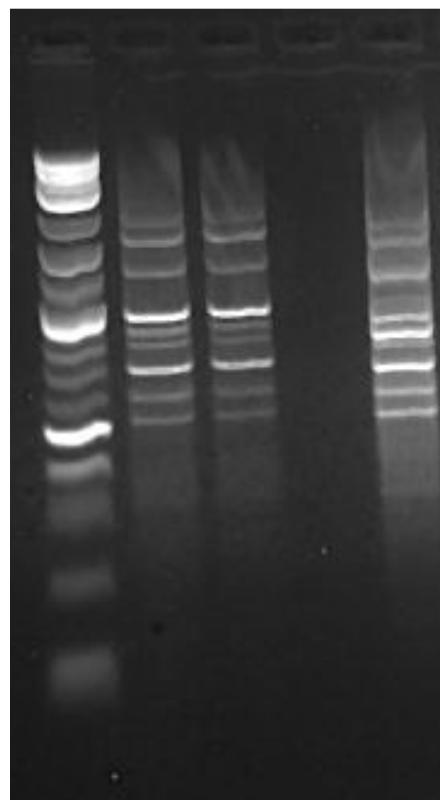
Log 2 Marker		Treatments			
Distance	Molecular Weight	Primer 6			
		A	B	C	D
35	0.8kb				
37	0.7kb			21	21
41	0.6kb			27	27
43	0.5kb	31		31	31
47	0.4kb	35			35
52	0.3kb	39		39	39
59	0.2kb	42			42
67	0.1kb	45			
		49		49	49

G.6. PCR of Passage 21 with Primer 7



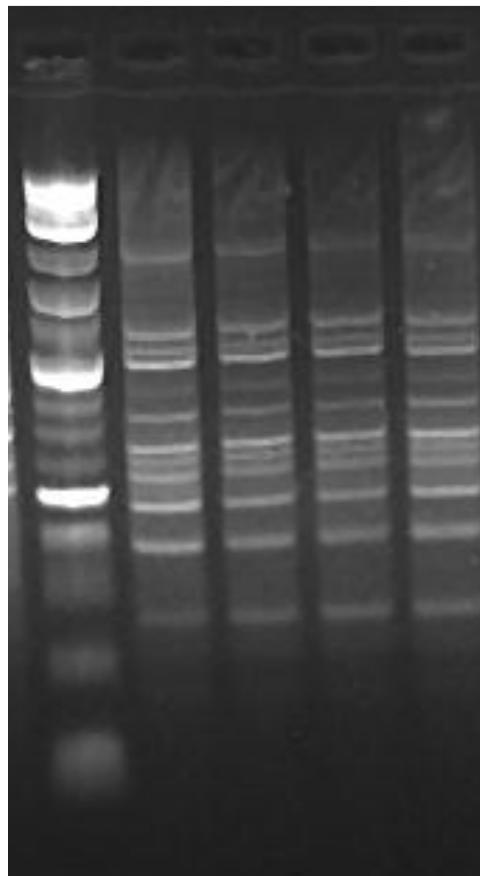
Log 2 Marker		Treatments			
Distance	Molecular Weight	Primer 7			
35	0.8kb	A	B	C	D
37	0.7kb	19	19	19	19
41	0.6kb	22	22	22	22
43	0.5kb	24	24	24	24
47	0.4kb	27	27	27	27
52	0.3kb	30	30	30	30
59	0.2kb	46		46	46
67	0.1kb	66	66	66	66

G.7. PCR of Passage 30 with Primer 5



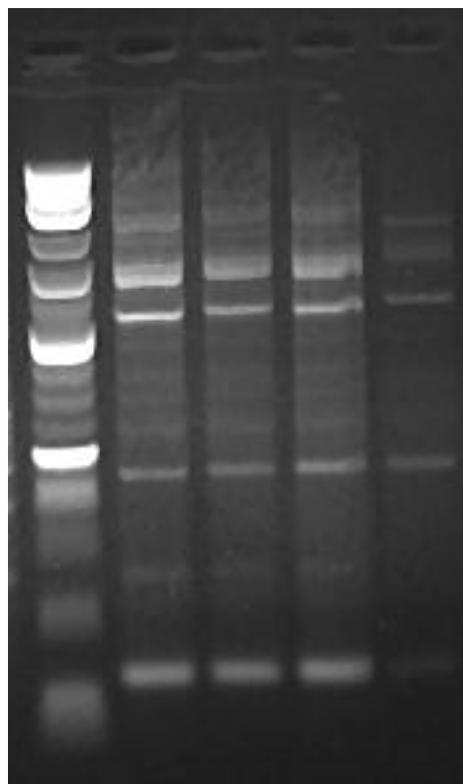
Log 2 Marker		Treatments			
Distance	Molecular Weight	Primer 5			
33	0.8kb	A	B	C	D
36	0.7kb	21	21		21
39	0.6kb	23	23		23
42	0.5kb	27	27		27
46	0.4kb	32	32		32
49	0.3kb	33	33		33
57	0.2kb	35	35		35
63	0.1kb	38	38		38
		40	40		40
		43	43		43

G.8. PCR of Passage 30 with Primer 6



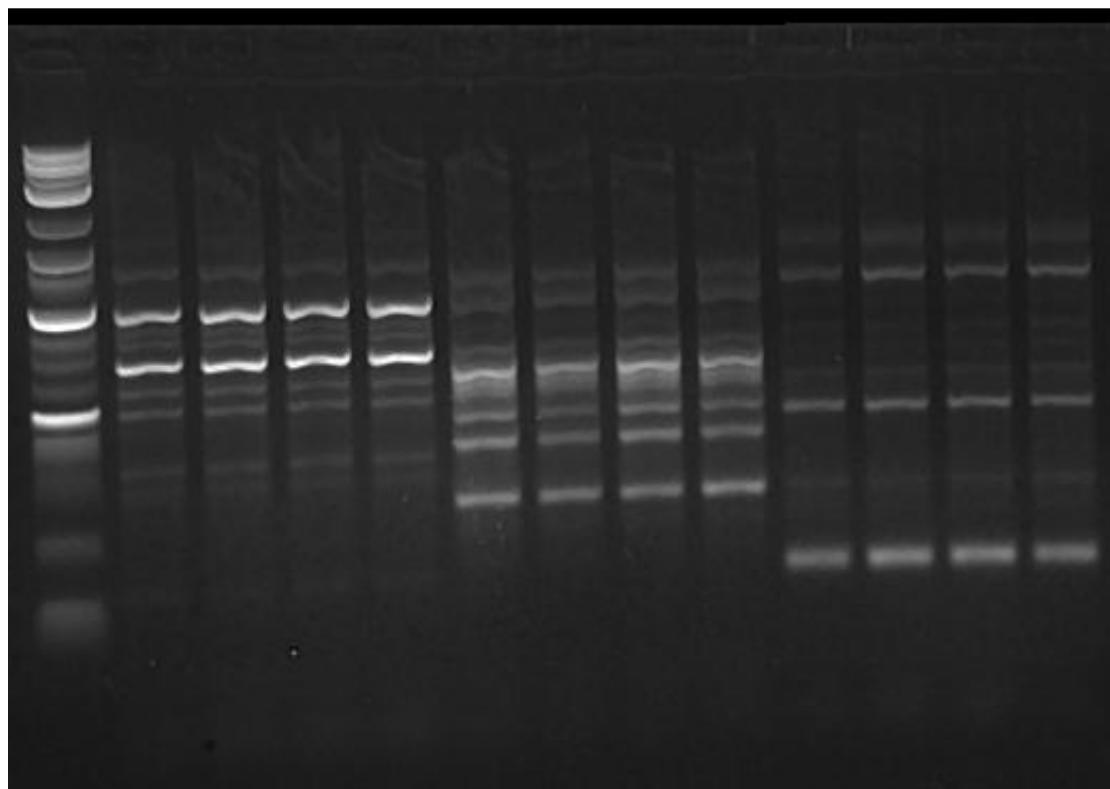
Log 2 Marker		Treatments			
Distance	Molecular Weight	Primer 6			
		A	B	C	D
33	0.8kb				
36	0.7kb	22	22	22	22
39	0.6kb	28	28	28	28
42	0.5kb	31	31	31	31
46	0.4kb	36	36	36	36
49	0.3kb	38	38	38	38
57	0.2kb	42	42	42	42
63	0.1kb	44	44	44	44
		47	47	47	47
		54	54	54	54

G.9. PCR of Passage 30 with Primer 7



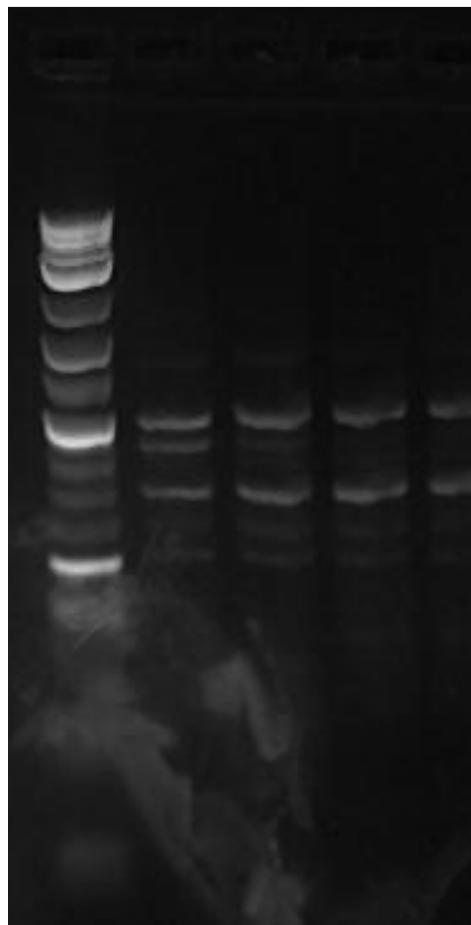
Log 2 Marker		Treatments			
Distance	Molecular Weight	Primer 7			
		A	B	C	D
33	0.8kb				
36	0.7kb	20	20	20	20
39	0.6kb	25	25	25	25
42	0.5kb	28	28	28	28
46	0.4kb	44	44	44	44
49	0.3kb	65	65	65	65
57	0.2kb				
63	0.1kb				

G.10. PCR of Passage 42 with Primers 5, 6 and 7



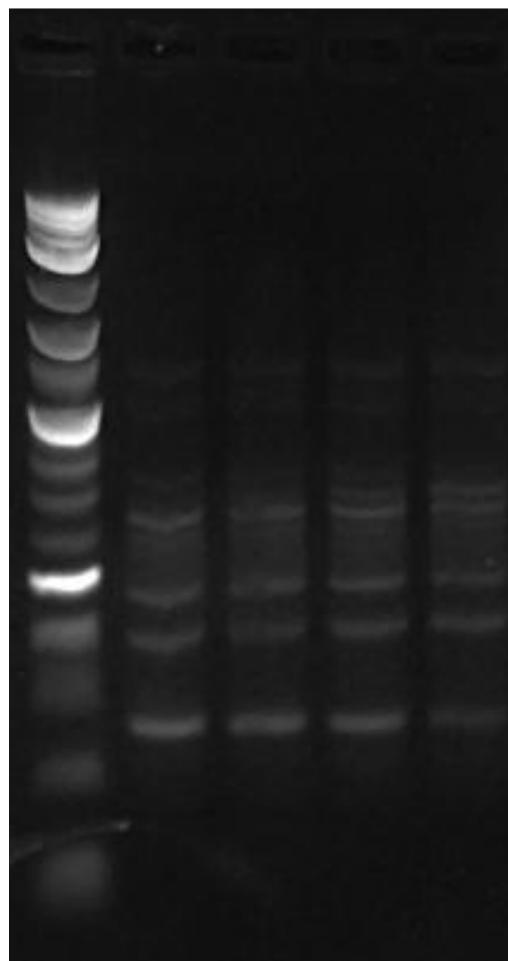
Log 2 Marker		Treatments											
Distance	Molecular Weight	Primer 5				Primer 6				Primer 7			
		A	B	C	D	A	B	C	D	A	B	C	D
31	0.8kb	A	B	C	D	A	B	C	D	A	B	C	D
34	0.7kb	24	24	24	24	23	23	23	23	18	18	18	18
37	0.6kb	28	28	28	28	26	26	26	26	22	22	22	22
40	0.5kb	30	30	30	30	31	31	31	31	26	26	26	26
43	0.4kb	31	31	31	31	34	34	34	34	30	30	30	30
47	0.3kb	34	34	34	34	37	37	37	37	33	33	33	33
55	0.2kb	37	37	37	37	38	38	38	38	37	37	37	37
63	0.1kb	39	39	39	39	42	42	42	42	46	46	46	46
		46	46	46	46	47	47	47	47	55	55	55	55
		49	49	49	49								

G.11. PCR of Passage 55 with Primer 5



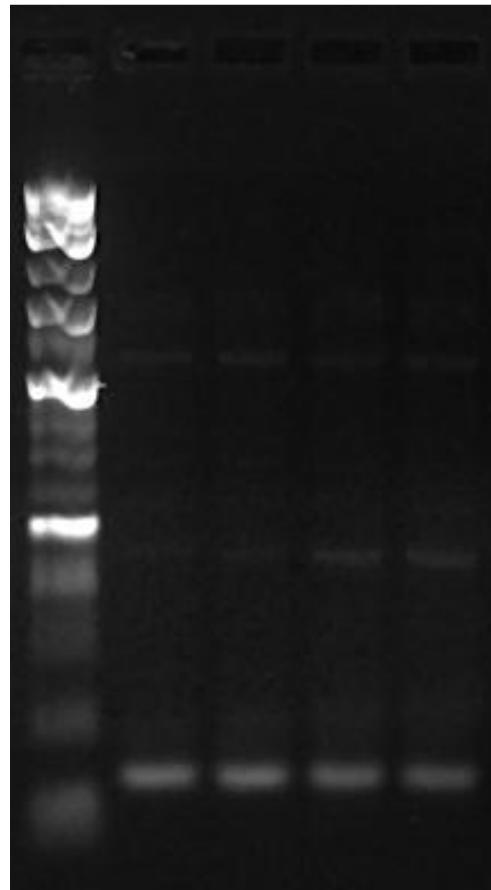
Log 2 Marker		Treatments			
Distance	Molecular Weight	Primer 5			
40	0.8kb	A	B	C	D
42	0.7kb	39	39	39	39
46	0.6kb	42			
50	0.5kb	46	46	46	46
53	0.4kb	47	47	47	47
60	0.3kb	53	53	53	53
67	0.2kb				
72	0.1kb				

G.12. PCR of Passage 55 with Primer 6



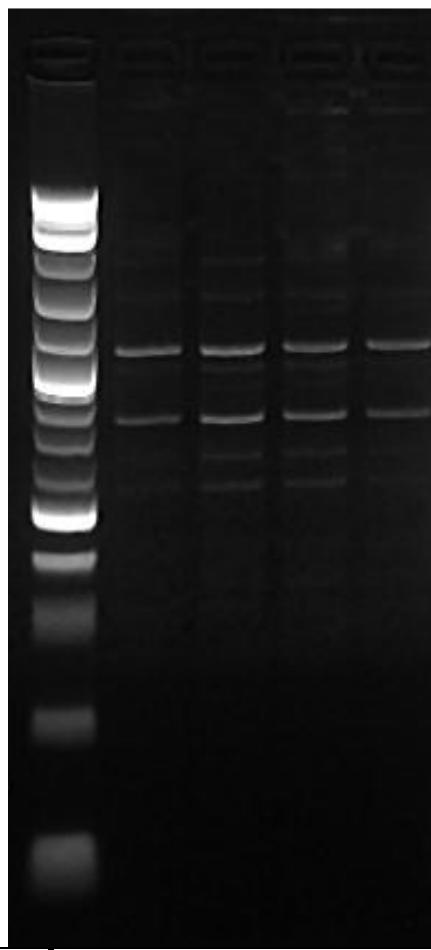
Log 2 Marker		Treatments			
Distance	Molecular Weight	Primer 6			
44	0.8kb	A	B	C	D
47	0.7kb	34	34	34	34
52	0.6kb	37	37	37	37
55	0.5kb	47	47	47	47
60	0.4kb	50	50	50	50
65	0.3kb	57	57	57	57
75	0.2kb	62	62	62	62
85	0.1kb	70	70	70	70

G.13. PCR of Passage 55 with Primer 7



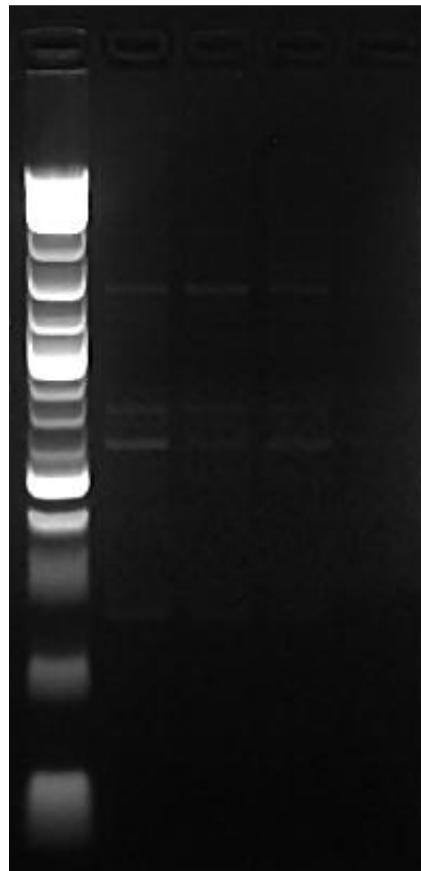
Log 2 Marker		Treatments			
Distance	Molecular Weight	Primer 7			
40	0.8kb	A	B	C	D
43	0.7kb	27	27	27	27
47	0.6kb	33	33	33	33
50	0.5kb	53	53	53	53
54	0.4kb	77	77	77	77
60	0.3kb				
69	0.2kb				
78	0.1kb				

G.14. PCR of Passage 66 with Primer 5



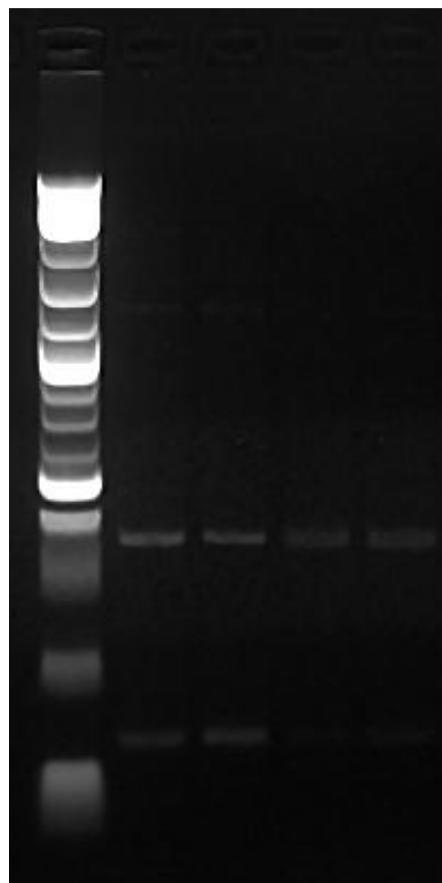
Log 2 Marker		Treatments			
Distance	Molecular Weight	Primer 5			
40	0.8kb	A	B	C	D
43	0.7kb	35	35	35	35
47	0.6kb	43	43	43	43
50	0.5kb	47	47	47	47
56	0.4kb	49	49	49	49
60	0.3kb				
74	0.2kb				
88	0.1kb				

G.15. PCR of Passage with of Primer 6



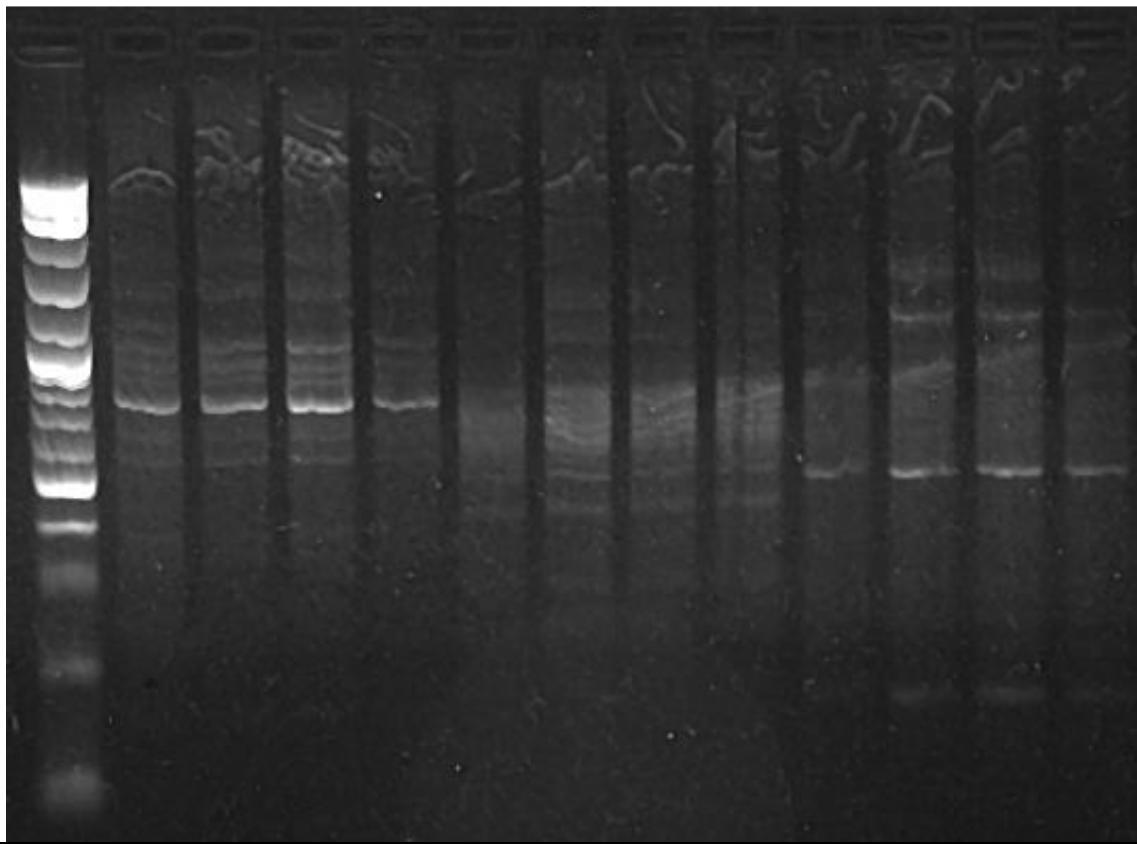
Log 2 Marker		Treatments			
Distance	Molecular Weight	Primer 6			
40	0.8kb	A	B	C	D
43	0.7kb	29	29	29	
45	0.6kb	42	42	42	
49	0.5kb	47	47	47	
54	0.4kb	65	65	65	
57	0.3kb				
70	0.2kb				
84	0.1kb				

G.16. PCR of Passage 66 with Primer 7



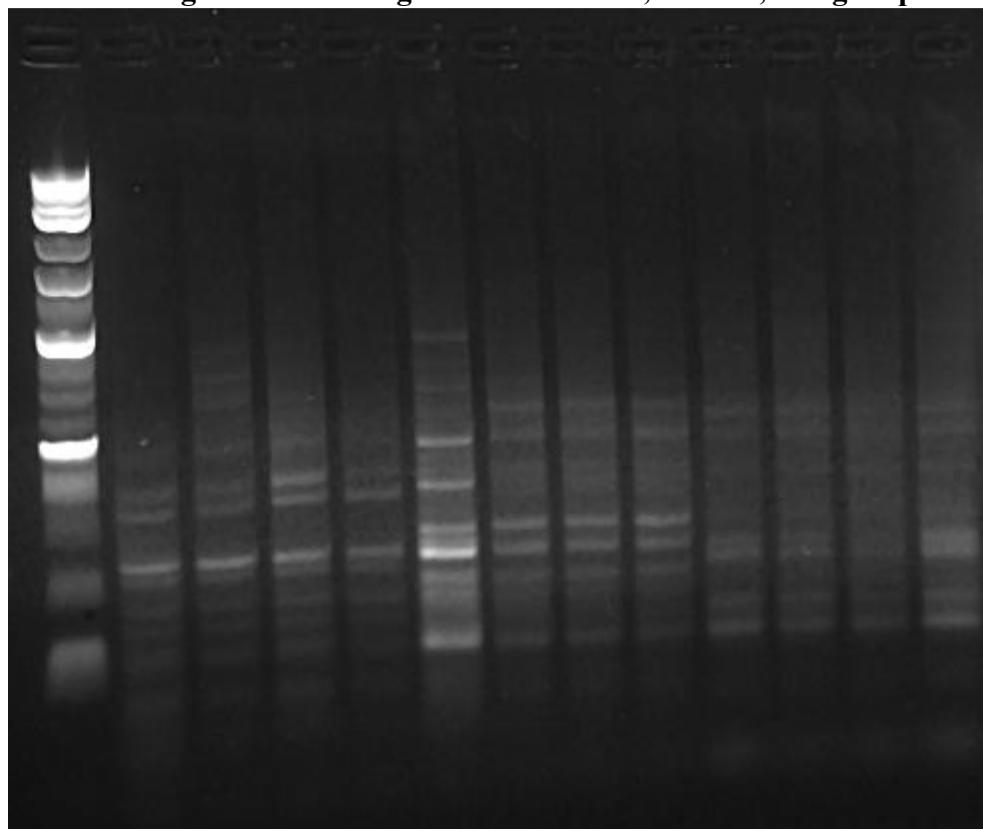
Log 2 Marker		Treatments			
Distance	Molecular Weight	Primer 7			
41	0.8kb	A	B	C	D
43	0.7kb	32	32		
47	0.6kb	58	58	58	58
50	0.5kb	80	80		
54	0.4kb				
57	0.3kb				
70	0.2kb				
84	0.1kb				

G.17. PCR of Passage 76 with Primers 5, 6 and 7



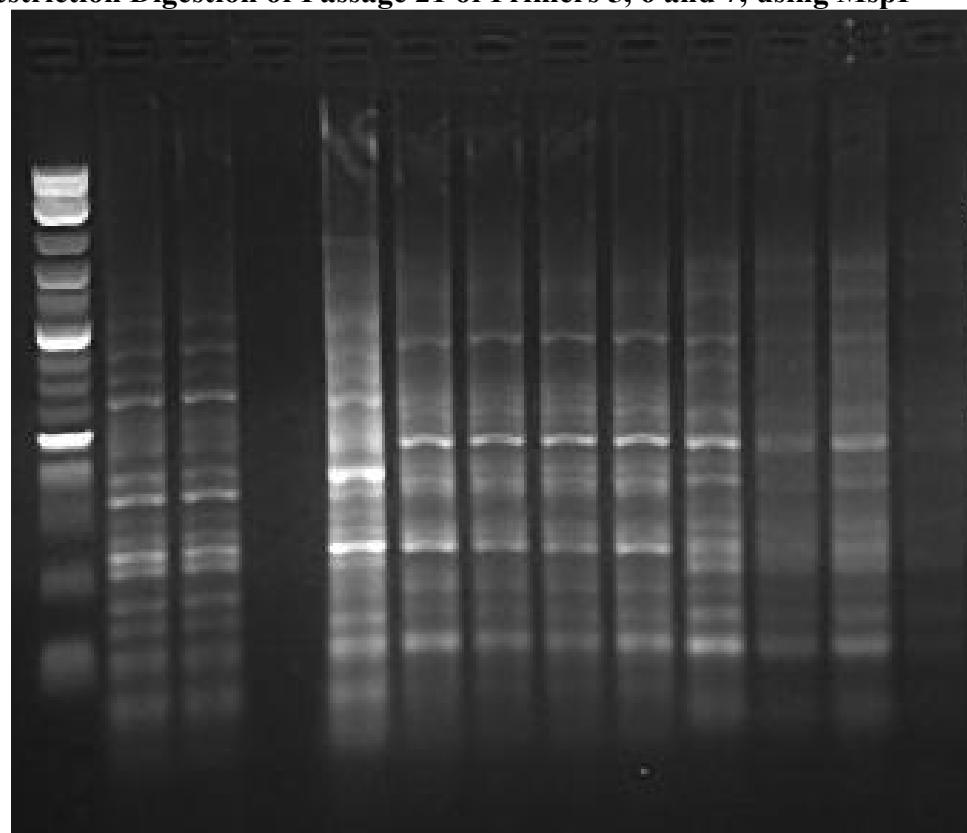
Log 2 Marker		Treatments											
Distance	Molecular Weight	Primer 5				Primer 6				Primer 7			
37	0.8kb	A	B	C	D	A	B	C	D	A	B	C	D
41	0.7kb	14	14	14	14		29				26	26	
44	0.6kb	23	23	23	23		33	33			30	30	30
49	0.5kb	32	32	32	32	37	37	37	39	39	39	39	39
53	0.4kb	34	34	34	34	41	41	41	41	49	49	49	49
60	0.3kb	36	36	36	36	43	43	43	43	74	74	74	74
69	0.2kb	40	40	40	40	47	47	47	47				
83	0.1kb	43	43	43	43	52	52	52	52				
		46	46	46	46	60	60	60	60				

G.18. Restriction Digestion of Passage 11 of Primers 5, 6 and 7, using MspI



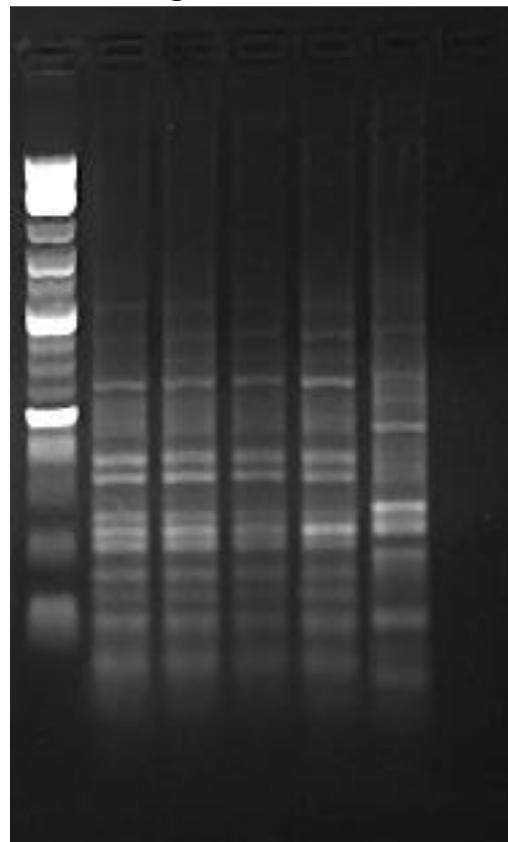
Log 2 Marker		Treatments											
Distance	Molecular Weight	Primer 5				Primer 6				Primer 7			
		A	B	C	D	A	B	C	D	A	B	C	D
43	0.8kb												
47	0.7kb	62	62	62	62	40				50	50	50	50
51	0.6kb	66	66	66	66		50	50	50	65	65	65	65
54	0.5kb	72	72	72	72	55	55	55	55	75	75	75	75
57	0.4kb					60	60	60	60	80	80	80	80
63	0.3kb					65	65	65	65				
71	0.2kb					68	68	68	68				
82	0.1kb					81	81	81	81				

G.19. Restriction Digestion of Passage 21 of Primers 5, 6 and 7, using MspI



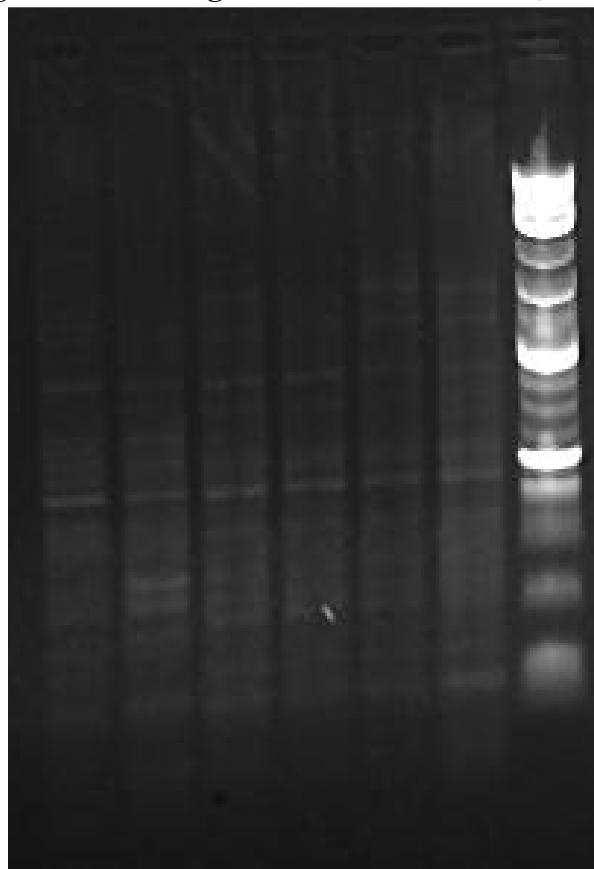
Log 2 Marker		Treatments											
Distance	Molecular Weight	Primer 5				Primer 6				Primer 7			
		A	B	C	D	A	B	C	D	A	B	C	D
28	0.8kb	A	B	C	D	A	B	C	D	A	B	C	D
30	0.7kb	21	21			21	27	27	27	27	27	27	
33	0.6kb	30	30			30	37	37	37	37	37	37	
35	0.5kb	35	35			35	41	41	41	41	41	41	
37	0.4kb	40	40			40	47	47	47	47	47	47	
42	0.3kb	43	43			43	56	56	56	56	50	50	
48	0.2kb	47	47			47					53	53	
53	0.1kb	52	52			52					57	57	
		54	54			54							
		57	57			57							
		62	62			62							

G.20. Restriction Digestion of Passage 30 of Primers 5 and 6, using MspI



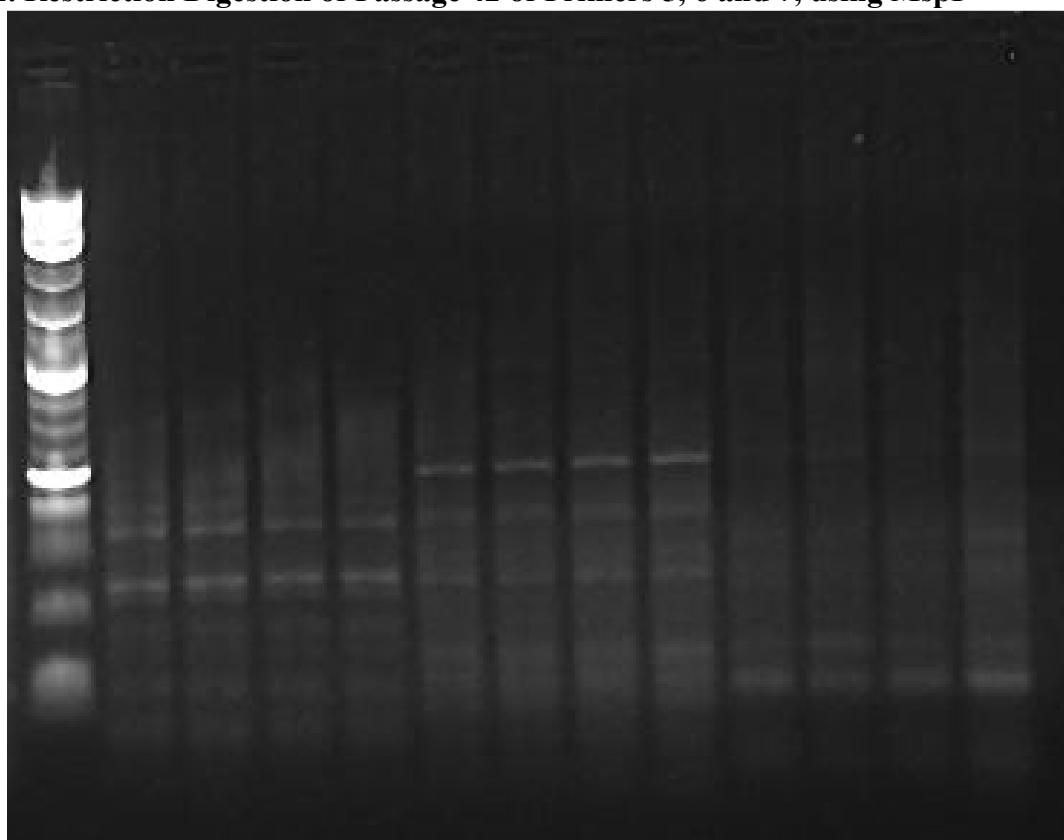
Log 2 Marker		Treatments					
Distance	Molecular Weight	Primer 5				Primer 6	
		A	B	C	D	A	B
28	0.8kb	A	B	C	D	A	B
30	0.7kb	34	34	34	34	34	
33	0.6kb	40	40	40	40	37	
35	0.5kb	41	41	41	41	46	
37	0.4kb	47	47	47	47	50	
42	0.3kb	52	52	52	52	51	
48	0.2kb	57	57	57	57	58	
53	0.1kb	60	60	60	60		

G.21. Restriction Digestion of Passage 30 of Primers 6 and 7, using MspI



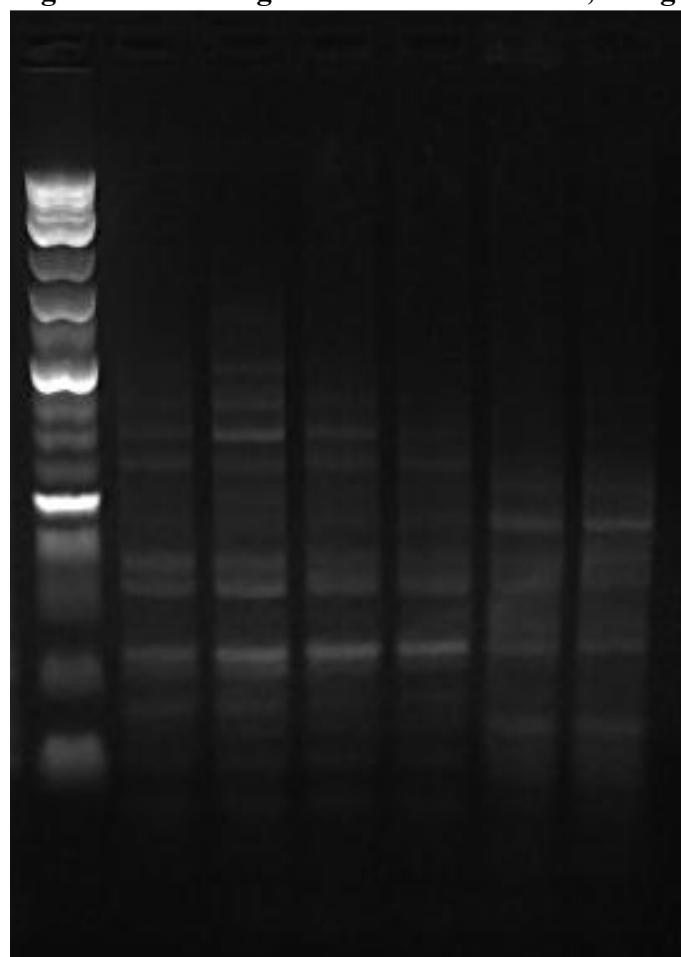
Log 2 Marker		Treatments					
Distance	Molecular Weight	Primer 6			Primer 7		
C	D	A	B	C	D		
31	0.8kb						
33	0.7kb	20		23		23	23
36	0.6kb		31			25	25
37	0.5kb	32				30	30
41	0.4kb		36	31	31		
44	0.3kb		41	40	40	40	40
49	0.2kb	42					58
56	0.1kb		47	59	59	59	
			59				
		61					

G.22. Restriction Digestion of Passage 42 of Primers 5, 6 and 7, using MspI



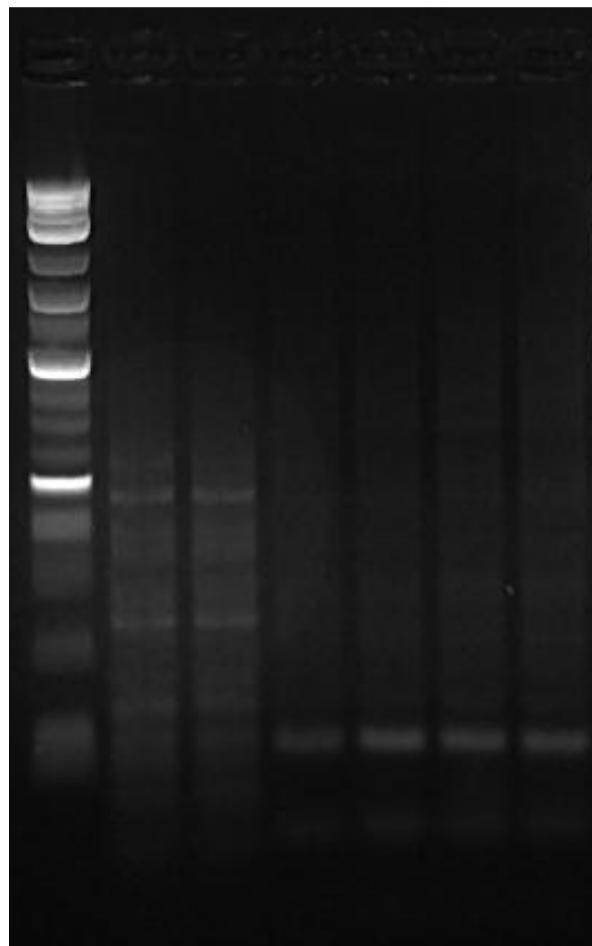
Log 2 Marker		Treatments											
Distance	Molecular Weight	Primer 5				Primer 6				Primer 7			
		A	B	C	D	A	B	C	D	A	B	C	D
31	0.8kb	A	B	C	D	A	B	C	D	A	B	C	D
33	0.7kb	40	40	40	40	40	40	40	40	39	39	39	39
36	0.6kb	44	44	44	44	44	44	44	44				47
37	0.5kb	49	49	49	49	50	50	50	50	57			
41	0.4kb	52	52	52	52	56	56	56	56		58	58	58
44	0.3kb	57	57	57	57	57	57	57	57	60			
49	0.2kb										69	69	69
56	0.1kb												

G.23. Restriction Digestion of Passage 55 of Primers 5 and 6, using MspI



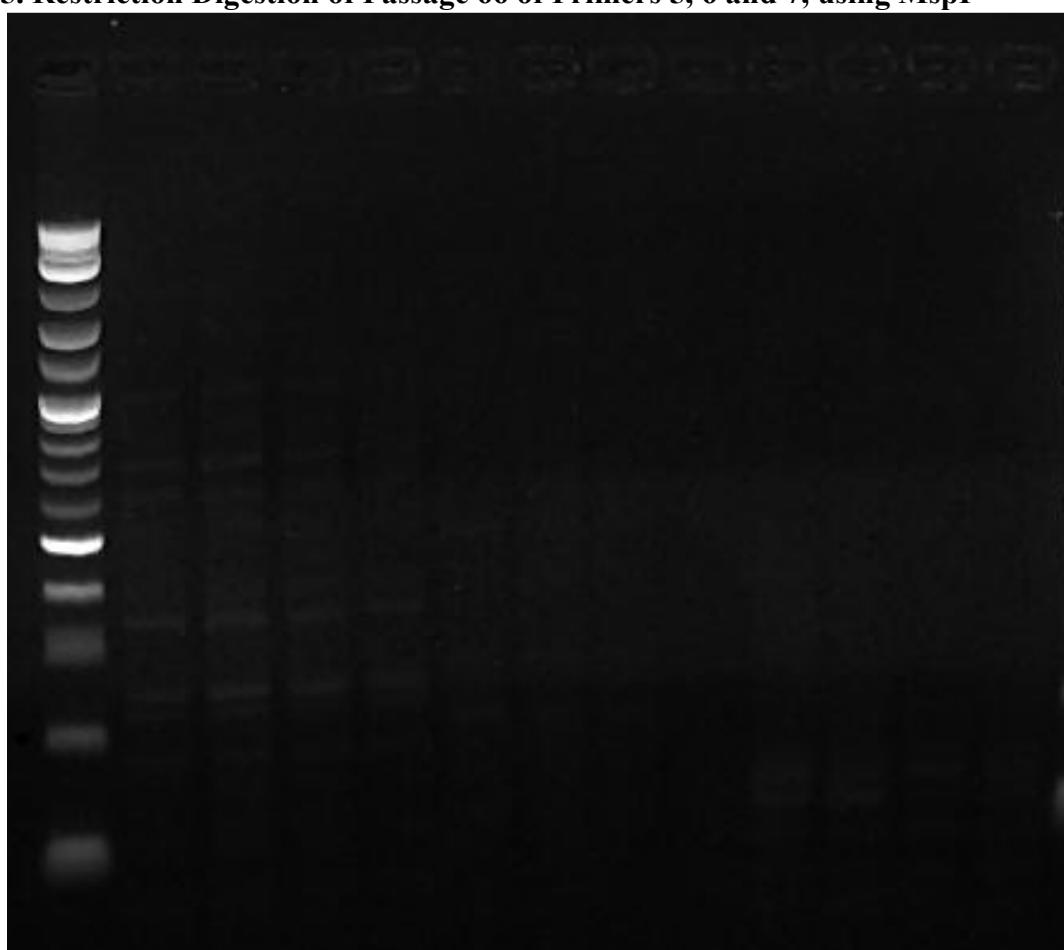
Log 2 Marker		Treatments					
Distance	Molecular Weight	Primer 5				Primer 6	
A	B	C	D	A	B		
40	0.8kb						
43	0.7kb	41	41	41	41	53	53
46	0.6kb	44	44	44	44	65	65
49	0.5kb	57	57	57	57	73	73
54	0.4kb	60	60	60	60		
57	0.3kb	67	67	67	67		
67	0.2kb	73	73				
75	0.1kb						

G.24. Restriction Digestion of Passage 55 of Primers 6 and 7, using MspI



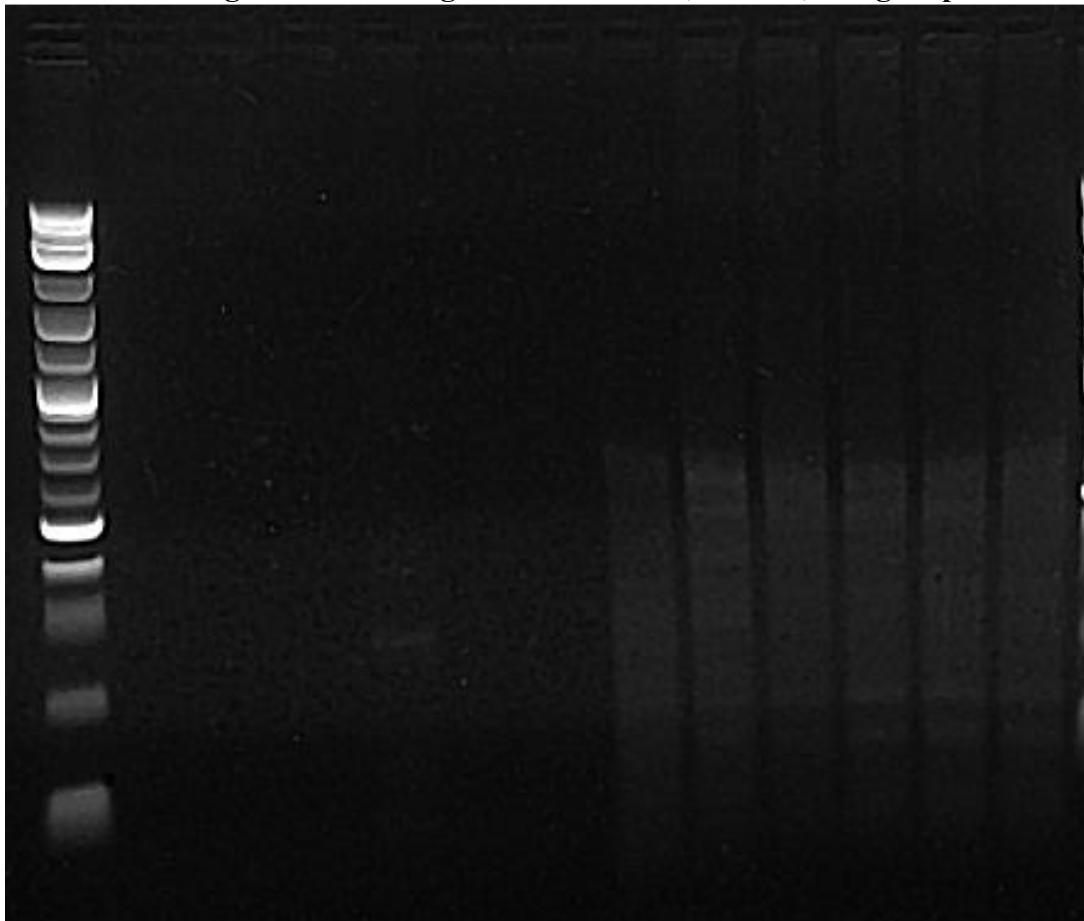
Log 2 Marker		Treatments					
Distance	Molecular Weight	Primer 6		Primer 7			
		C	D	A	B	C	D
37	0.8kb	C	D	A	B	C	D
46	0.7kb	50	50	65	65	65	65
49	0.6kb	53	53	75	75	75	75
53	0.5kb	67	67	85	85	85	85
57	0.4kb	79	79	92	92	92	92
63	0.3kb	82	82				
73	0.2kb						
83	0.1kb						

G. 25. Restriction Digestion of Passage 66 of Primers 5, 6 and 7, using MspI



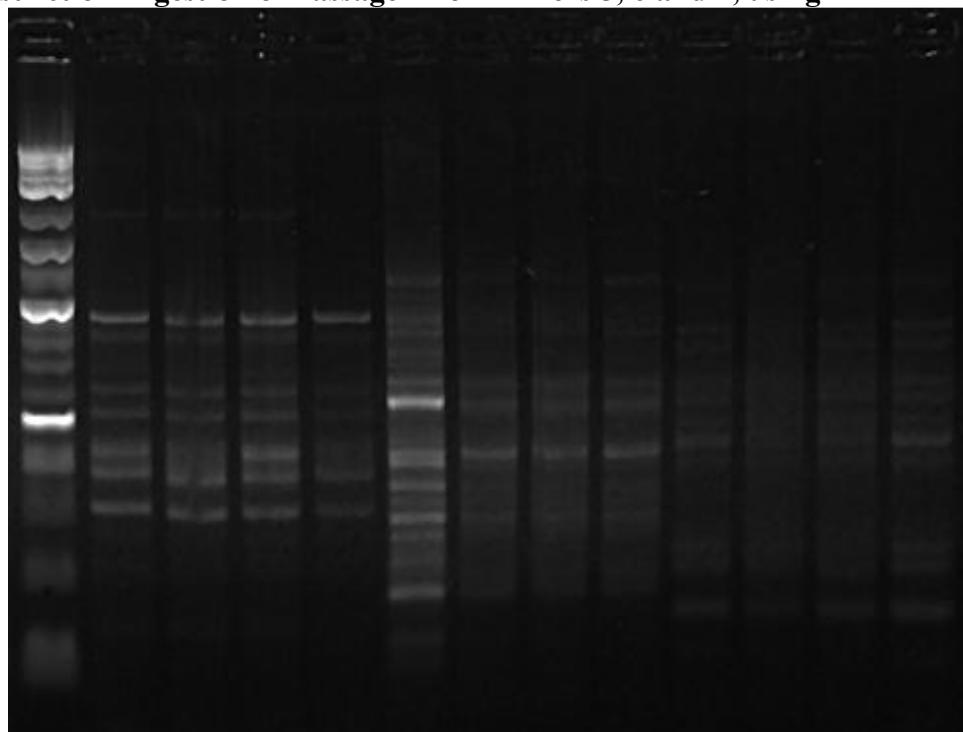
Log 2 Marker		Treatments											
Distance	Molecular Weight	Primer 5				Primer 6				Primer 7			
		A	B	C	D	A	B	C	D	A	B	C	D
30	0.8kb	A	B	C	D	A	B	C	D	A	B	C	D
31	0.7kb	25	25	25		35	35	35		58	58	58	58
34	0.6kb	31	31	31		38	38	38		60	60		
37	0.5kb	43	43	43	43	49	49	49					
40	0.4kb	49	49	49	49	51	51	51					
44	0.3kb	50	50	50	50								
51	0.2kb	54	54	54	54								
60	0.1kb												

G.26. Restriction Digestion of Passage 76 of Primers 5, 6 and 7, using MspI



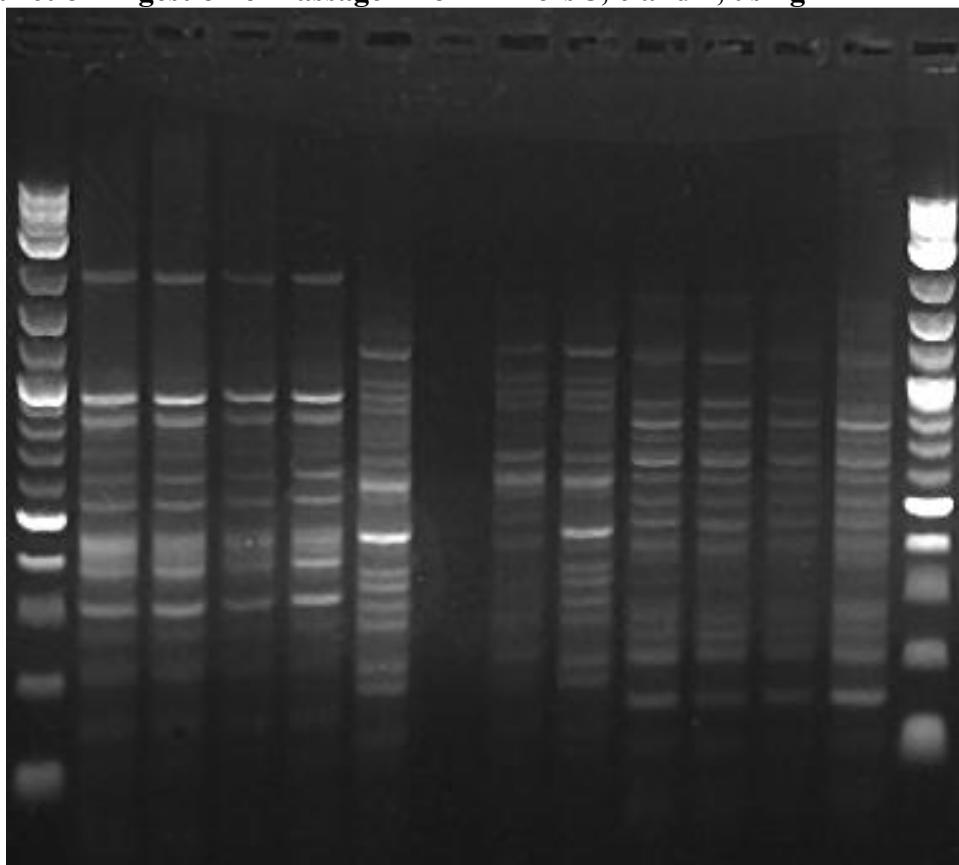
Log 2 Marker		Treatments											
Distance	Molecular Weight	Primer 5				Primer 6				Primer 7			
		A	B	C	D	A	B	C	D	A	B	C	D
45	0.8kb												
49	0.7kb					73				52	52	52	52
53	0.6kb					88				55	55	55	55
57	0.5kb					91				61	61	61	61
62	0.4kb									66	66	66	66
67	0.3kb									76	76	76	76
77	0.2kb									78	78	78	78
90	0.1kb												

G.27. Restriction Digestion of Passage 11 of Primers 5, 6 and 7, using *HinfI*



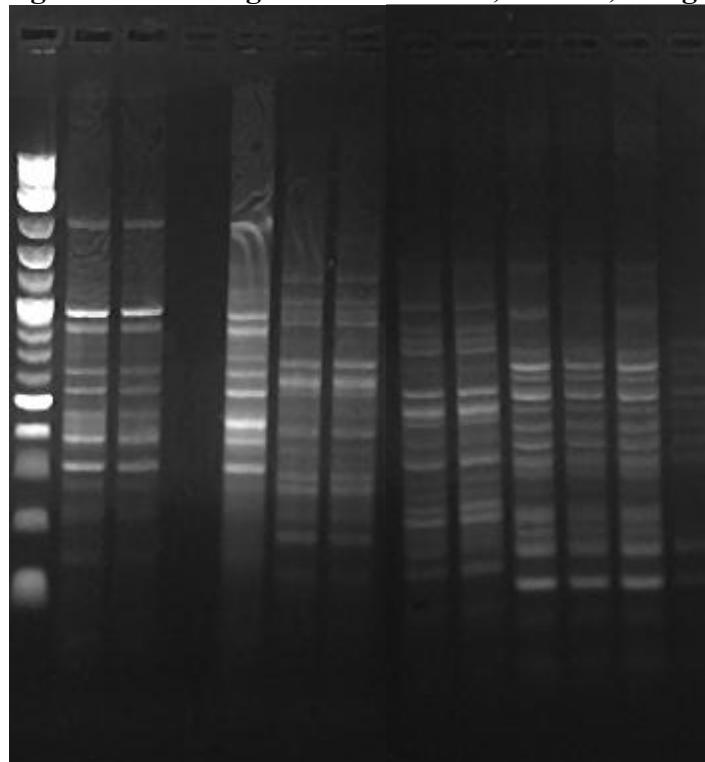
Log 2 Marker		Treatments											
Distance	Molecular Weight	P11 – Primer 5				P11 – Primer 6				P11 – Primer 7			
		A	B	C	D	A	B	C	D	A	B	C	D
20	3kb	23	23	23		32	32	32	32	32	32	32	32
28	1.5kb	37	37	37	37	37	37	37	37	38	38	38	38
32	1.2kb	39	39	39	39	45	45	45	45	41	41	41	41
36	1.0kb	47	47	47	47	48	48	48	48	45	45	45	45
50	0.5kb	50	50	50	50	55	55	55	55	48	48	48	48
55	0.4kb	55	55	55	55	59	59	59	59	50	50	50	50
		58	58	58	58	63	63	63	63	53	53	53	53
		62	62	62	62	73				67	67	67	67
										70	70	70	70
										75	75	75	75

G.28. Restriction Digestion of Passage 21 of Primers 5, 6 and 7, using *HinfI*



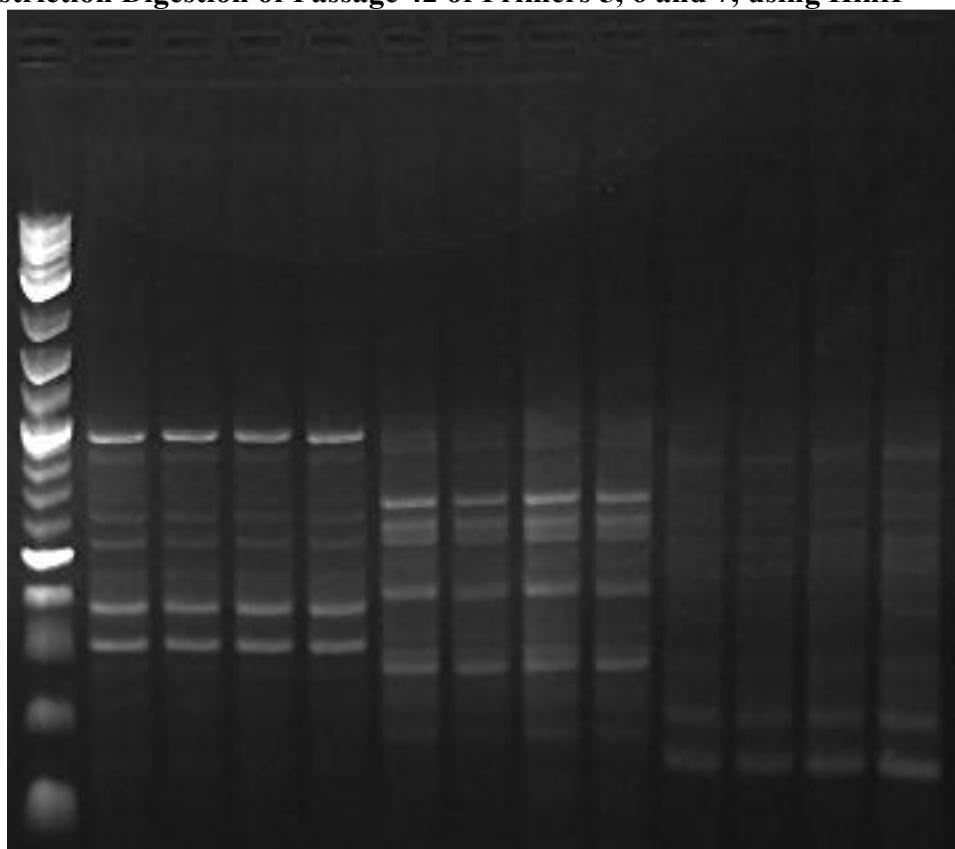
Log 2 Marker		Treatments											
Distance	Molecular Weight	P21 – Primer 5				P21 – Primer 6				P21 – Primer 7			
		A	B	C	D	A	B	C	D	A	B	C	D
23	3kb	26	26	26	26	34		34	34	27	27		27
30	1.5kb	39	39	39	39	37		37	37	34	34	34	34
35	1.2kb	41	41	41	41	38		38	38	39	39	39	39
38	1.0kb	43	43	43	43	40		40	40	41	41	41	41
52	0.5kb	45	45	45	45	45		45	45	42	42	42	42
56	0.4kb	47	47	47	47	47		47	47	44	44	44	44
		50	50	50	50	52		52	52	46	46	46	46
		55	55	55	55	56			56	48	48	48	48
		61	61	61	61	58		58	58	51	51	51	51
		64	64	64	64	60			60	54	54	54	54
		68	68	68	68	66		66	66	63	63	63	63
		74	74	74	74	68			68	70	70	70	70

G.29. Restriction Digestion of Passage 30 of Primers 5, 6 and 7, using *HinfI*



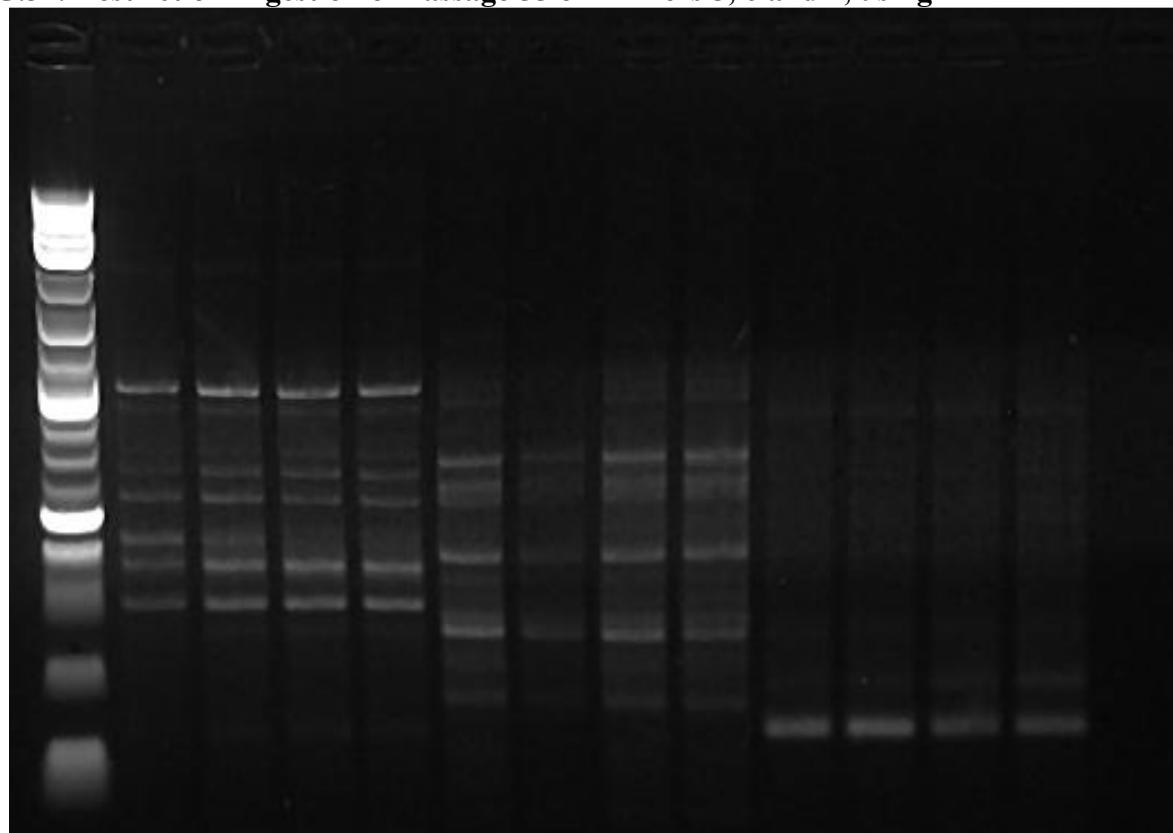
Log 2 Marker		Treatments											
Distance	Molecular Weight	P30 – Primer 5				P30 – Primer 6				P30 – Primer 7			
		A	B	C	D	A	B	C	D	A	B	C	D
23	3kb	25	25			25	29	29	29	29	29	29	
30	1.5kb	37	37			37	35	35	35	35	35	35	
33	1.2kb	39	39			39	38	38	38	38	40	40	40
38	1.0kb	45	45			45	45	45	45	45	42	42	42
49	0.5kb	47	47			47	47	47	47	47	44	44	44
52	0.4kb	52	52			52	52	52	52	52	46	46	46
		54	54			54	55	55	55	55	48	48	48
		58	58			58	59	59	59	59	50	50	50
		64	64			64	60	60	60	60	52	52	52
		69	69			69	68	68	68	68	55	55	55
						70	70	70	70	70	59	59	59
										70	70	70	70

G.30. Restriction Digestion of Passage 42 of Primers 5, 6 and 7, using *HinfI*



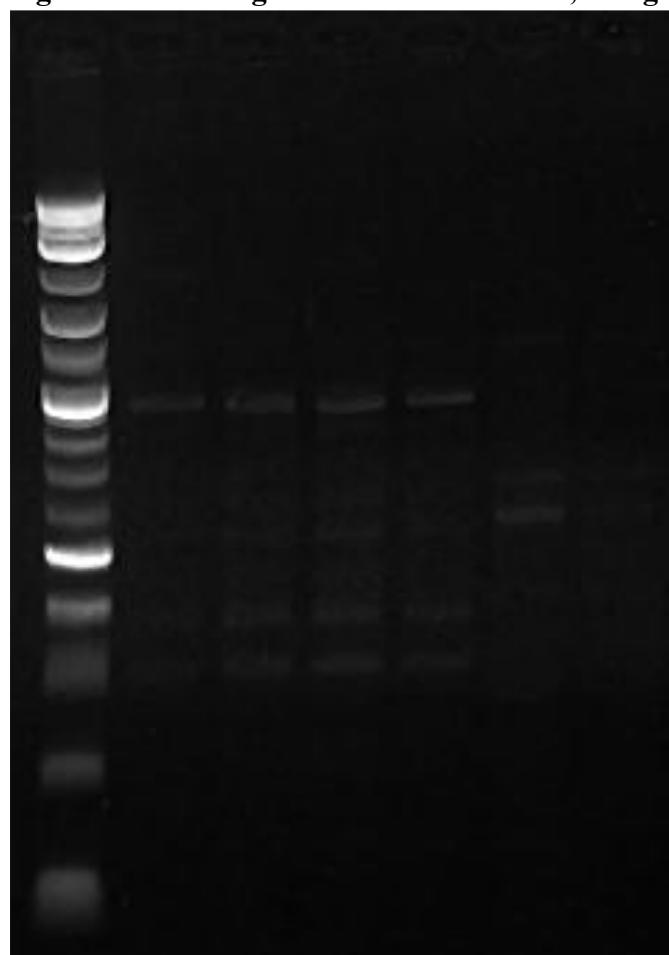
Log 2 Marker		Treatments											
Distance	Molecular Weight	P30 – Primer 5				P30 – Primer 6				P30 – Primer 7			
		A	B	C	D	A	B	C	D	A	B	C	D
24	3kb	39	39	39	39	40	40	40	40	42	42	42	42
32	1.5kb	41	41	41	41	45	45	45	45	46	46	46	46
35	1.2kb	44	44	44	44	47	47	47	47	50	50	50	50
39	1.0kb	47	47	47	47	49	49	49	49	53	53	53	53
50	0.5kb	49	49	49	49	60	60	60	60	57	57	57	57
54	0.4kb	52	52	52	52	62	62	62	62	62	62	62	62
		55	55	55	55	65	65	65	65	68	68	68	68
		59	59	59	59	68	68	68	68	72	72	72	72

G.31. Restriction Digestion of Passage 55 of Primers 5, 6 and 7, using *HinfI*



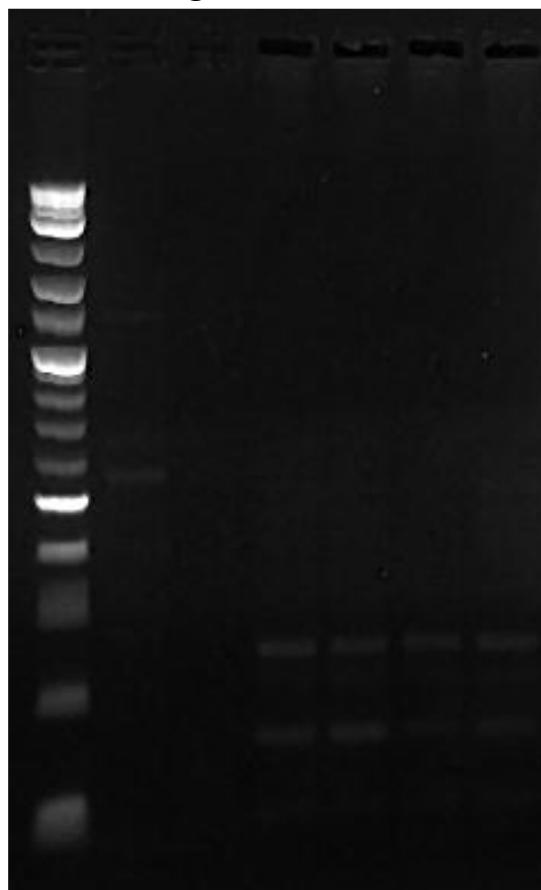
Log 2 Marker		Treatments											
Distance	Molecular Weight	P55 – Primer 5				P55 – Primer 6				P55 – Primer 7			
		A	B	C	D	A	B	C	D	A	B	C	D
25	3kb	25	25	25	25	34		34	34	43	43	43	43
34	1.5kb	40	40	40	40	39	39	39	39	50	50	50	50
37	1.2kb	43	43	43	43	49	49	49	49	56	56	56	56
43	1kb	48	48	48	48	53	53	53	53	60	60	60	60
56	0.5kb	50	50	50	50	60	60	60	60	68	68	68	68
50	0.4kb	53	53	53	53	68	68	68	68	75	75	75	75
		58	58			70	70	70	70	80	80	80	80
		61	61	61	61	73	73	73	73				
		66	66	66	66	77	77	77	77				
		80	80	80	80								

G.32. Restriction Digestion of Passage 66 of Primer 5 and 6, using *HinfI*



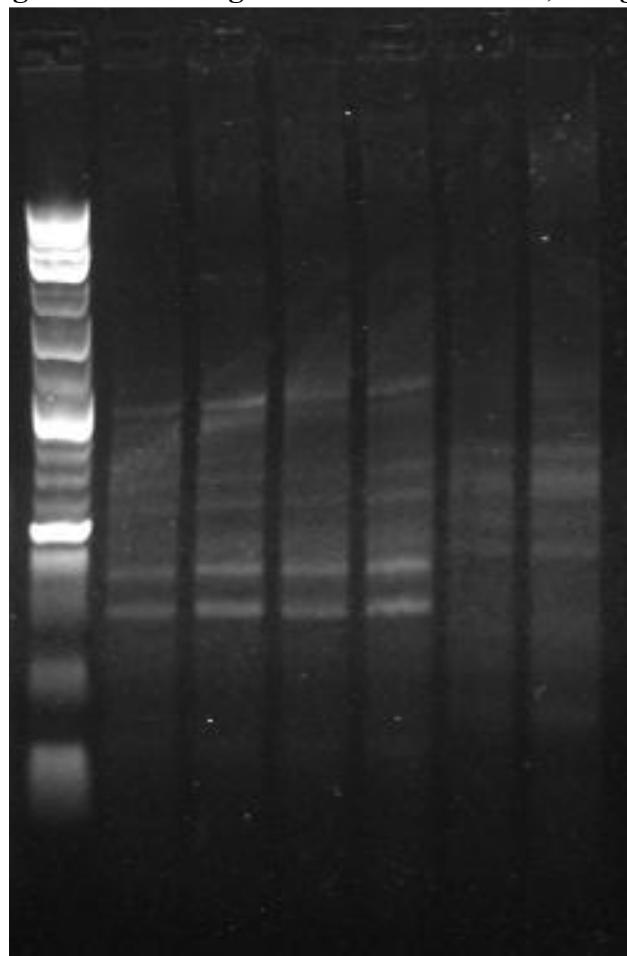
Log 2 Marker		Treatments					
Distance	Molecular Weight	Primer 5				Primer 6	
		A	B	C	D	A	B
24	0.8kb						
27	0.7kb	23	23	23	23	20	20
29	0.6kb	26	26	26	26	26	26
32	0.5kb	27	27	27	27	29	29
35	0.4kb	30	30	30	30	35	
39	0.3kb	36	36	36	36	41	
46	0.2kb	40	40	40	40		
53	0.1kb						

G.33. Restriction Digestion of Passage 66 of Primer 6 and 7, using *HinfI*



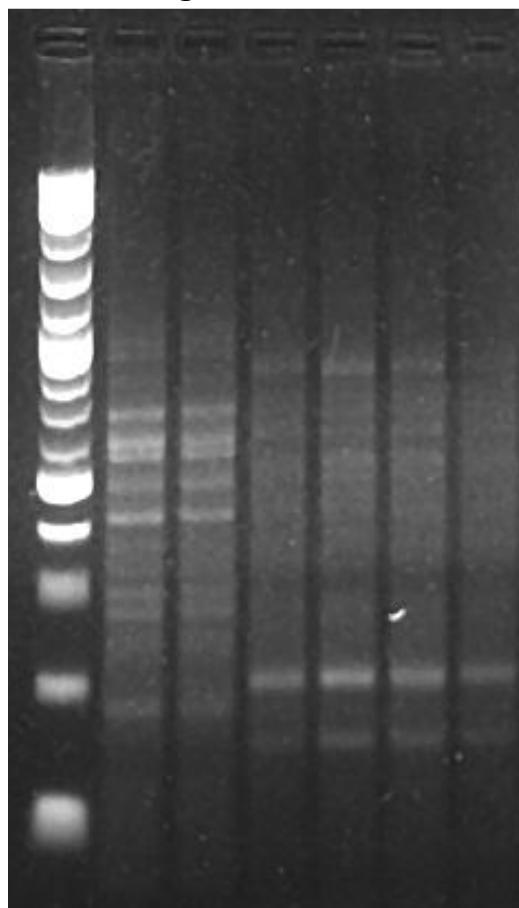
Log 2 Marker		Treatments					
Distance	Molecular Weight	Primer 6		Primer 7			
27	0.8kb	C	D	A	B	C	D
29	0.7kb	23		45	45	45	45
33	0.6kb	32		47	47	47	47
35	0.5kb			53	53	53	53
39	0.4kb			58	58	58	58
43	0.3kb						
50	0.2kb						
60	0.1kb						

G.34. Restriction Digestion of Passage 76 of Primer 5 and 6, using *HinfI*



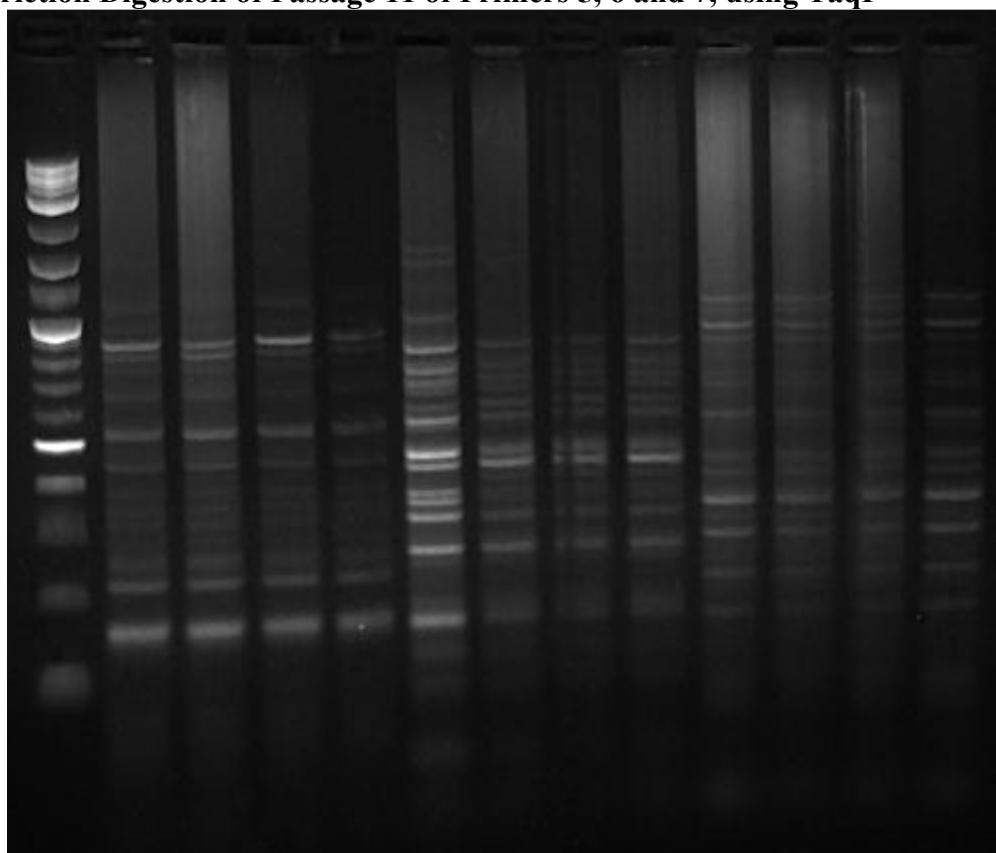
Log 2 Marker		Treatments					
Distance	Molecular Weight	Primer 5				Primer 6	
		A	B	C	D	A	B
53	0.8kb						
59	0.7kb	50	50	50	50	54	54
63	0.6kb	57	57	57	57	57	57
68	0.5kb	60	60	60	60	63	63
72	0.4kb	63	63	63	63	77	77
78	0.3kb	70	70	70	70		
88	0.2kb	76	76	76	76		
100	0.1kb						

G.35. Restriction Digestion of Passage 76 of Primers 6 and 7, using *HinfI*



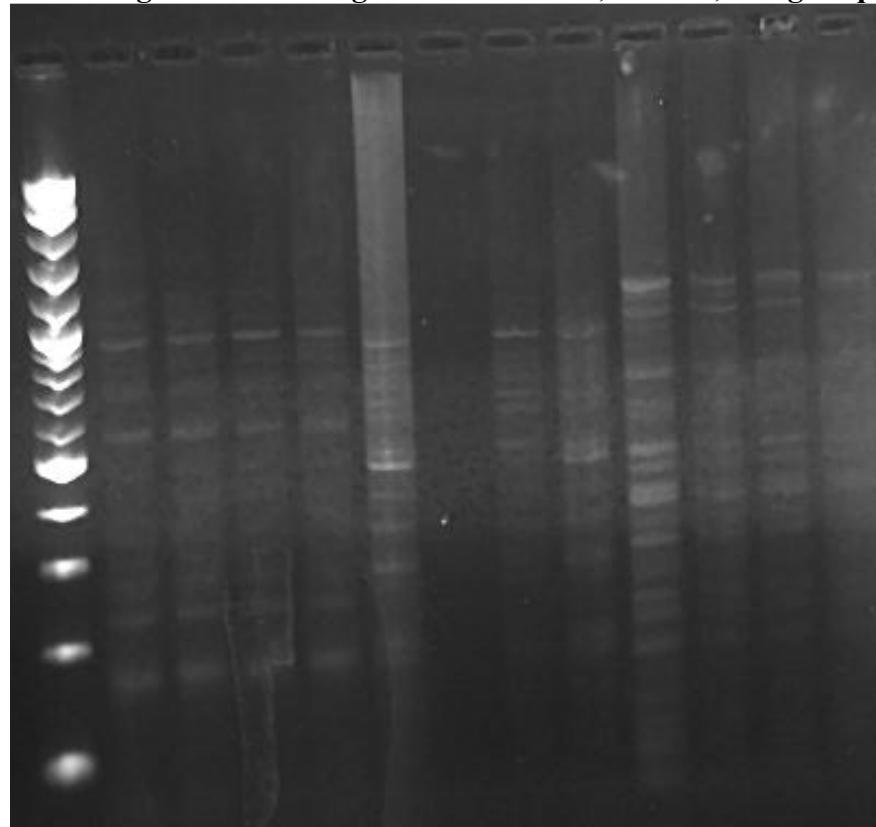
Log 2 Marker		Treatments					
Distance	Molecular Weight	Primer 6		Primer 7			
35	0.8kb	C	D	A	B	C	D
37	0.7kb	31	31	31	31	31	31
39	0.6kb	37	37	40	40	40	40
44	0.5kb	39	39		50	50	
47	0.4kb	41	41	62	62	62	62
55	0.3kb	45	45	69	69	69	69
64	0.2kb	47	47				
77	0.1kb	53	53				
		55	55				
		65	65				
		69	69				

G.36. Restriction Digestion of Passage 11 of Primers 5, 6 and 7, using TaqI



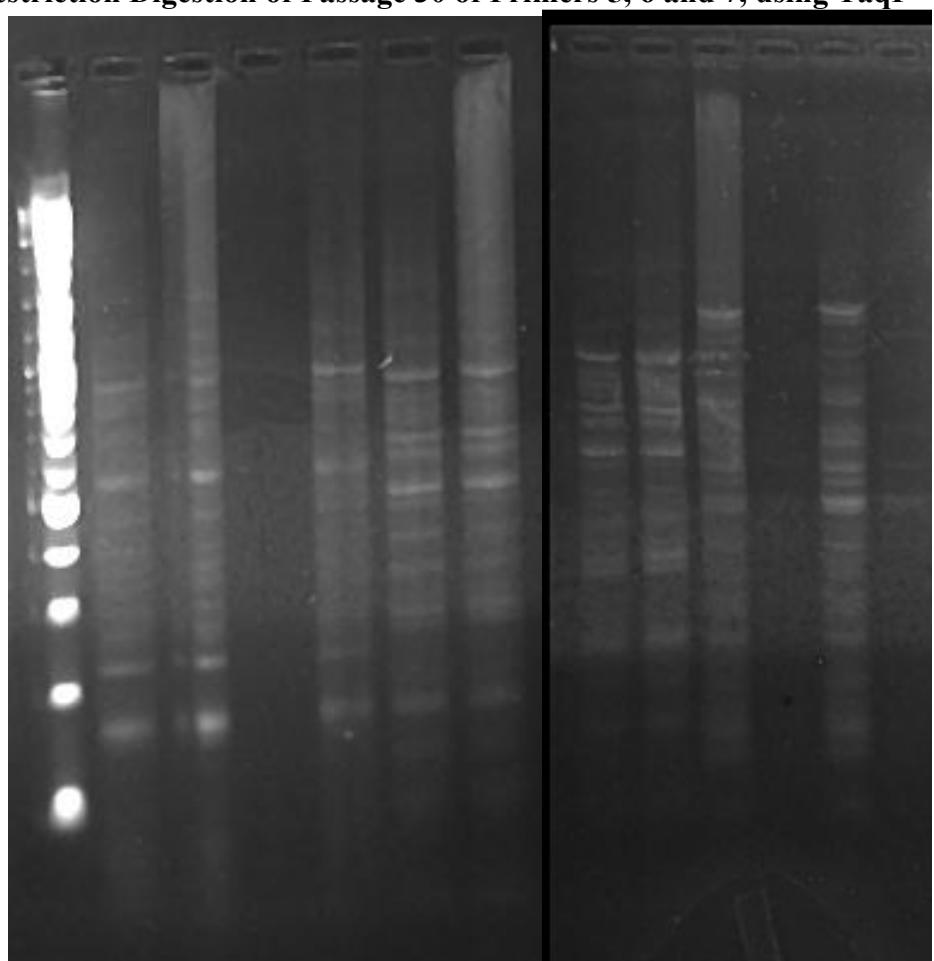
Log 2 Marker		Treatments											
Distance	Molecular Weight	P11 – Primer 5				P11 – Primer 6				P11 – Primer 7			
		A	B	C	D	A	B	C	D	A	B	C	D
43	0.8kB	36	36	36	36	27.5				35	35	35	35
46.5	0.7kB	38.5	38.5	38.5	38.5	29.5	29.5	29.5	29.5	38	38	38	38
50	0.6kB	41	41	41	41	32	32	32	32	40	40	40	40
54	0.5kB	43	43	43	43	41	41	41	41	46	46	46	46
59	0.4kB	44	44	44	44	44	44	44	44	50	50	50	50
64	0.3kB	47.5	47.5	47.5	47.5	46	46	46	46	55	55	55	55
74	0.2kB	53	53	53	53	49	49	49	49	57.5	57.5	57.5	57.5
		70	70	70	70	51	51	51	51	61	61	61	61
		73	73	73	73	55	55	55	55	65	65	65	65
		79	79	79	79	57	57	57	57	71	71	71	71
						61	61	61	61				
						62.5	62.5	62.5	62.5				

G.37. Restriction Digestion of Passage 21 of Primers 5, 6 and 7, using TaqI



Log 2 Marker		Treatments											
Distance	Molecular Weight	P21 – Primer 5				P21 – Primer 6				P21 – Primer 7			
		A	B	C	D	A	B	C	D	A	B	C	D
39	0.8kB	31	31	31	31	37		37	37	31	31	31	31
42	0.7kB	42	42	42	52.5	52.5		43	52	35	35	35	35
46	0.6kB	47	47	47	55	55		45	63	42	42	42	42
51	0.5kB	52	52	52	59	59		50		46	46	46	46
56	0.4kB	66	66	66	64.5	64.5				51	51	51	51
63	0.3kB	69	69	69						53	53	53	53
73	0.2kB	72	72	72						56			
										60			
										62			
										69			
										72			

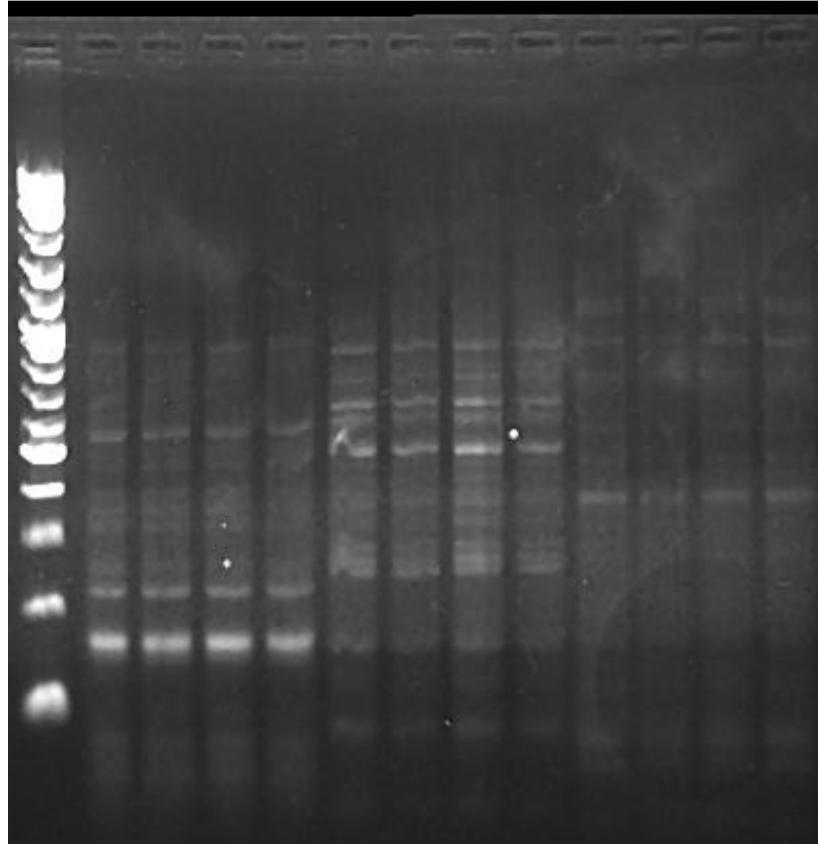
G.38. Restriction Digestion of Passage 30 of Primers 5, 6 and 7, using TaqI



Log 2 Marker		Treatments											
Distance	Molecular Weight	P30 – Primer 5				P30 – Primer 6				P30 – Primer 7			
		A	B	C	D	A	B	C	D	A	B	C	D
38	0.8kB	34	34			34	35	35	36	36	35		35
41	0.7kB	41	41			41	41.5	41.5	41	41	38		
45	0.6kB	45	45			45	42.5	42.5	42.5	42.5	40		40
48	0.5kB	50	50			50	47.5	47.5	46.5	46.5	47		47
53	0.4kB	66	66			66	52.5	52.5	53	53	51		51
59	0.3kB	73	73			73	56	56	59	59	55		55
69	0.2kB					62	62	62	62	57			57
						71	71	68	68	60			60
								78	78	65			65

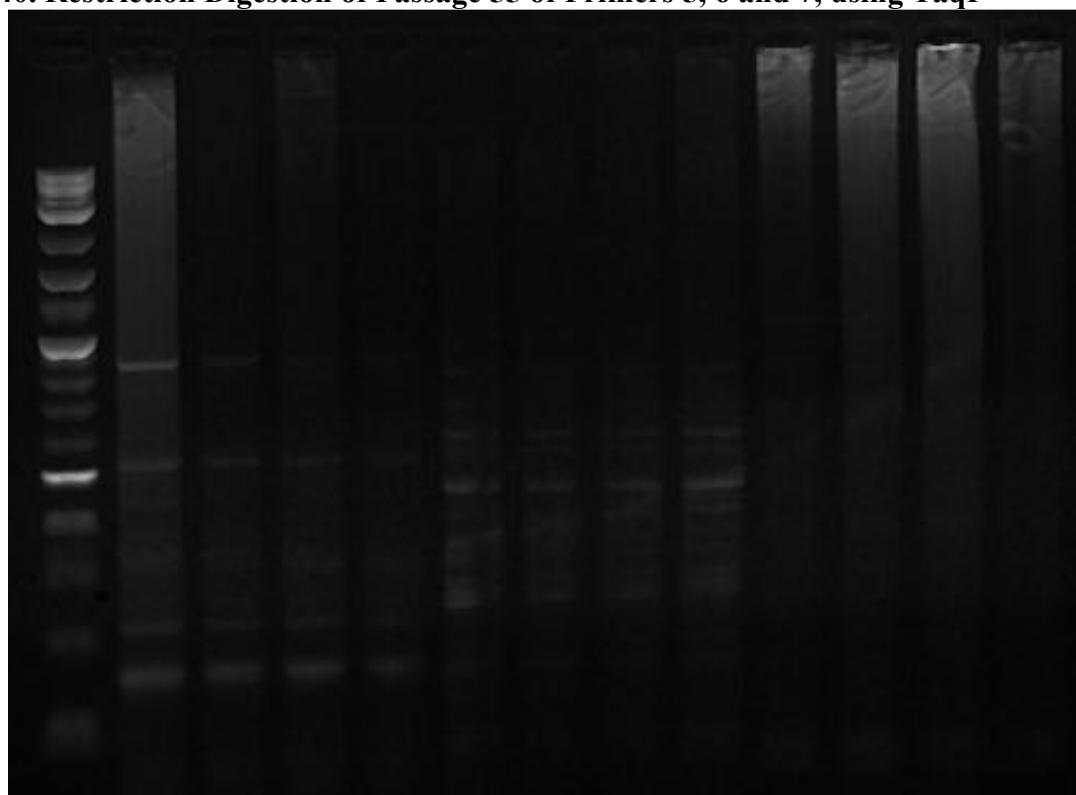
*P30 – Primer 6 Treatment A and B are located on the same gel as P20, whereas P30 – Primer 6 Treatment C onwards are located on the same gel as P42, thus these other samples share the ladder on P42's gel.

G.39. Restriction Digestion of Passage 42 of Primers 5, 6 and 7, using TaqI



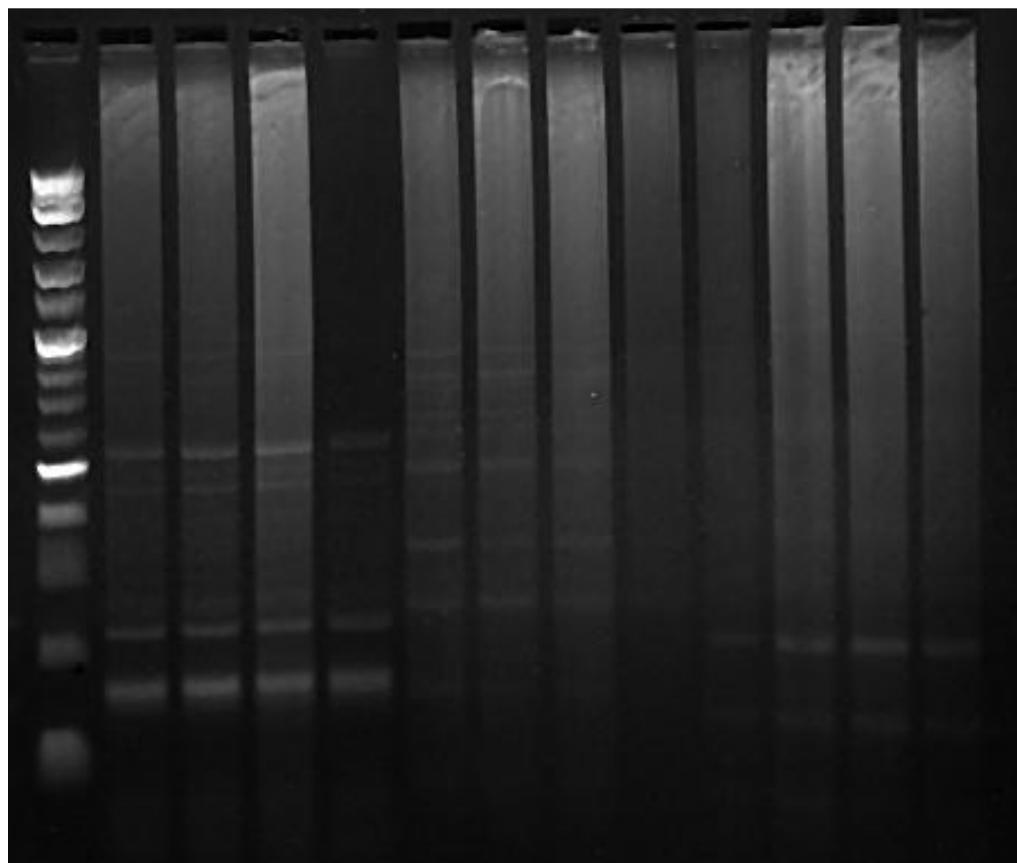
Log 2 Marker		Treatments											
Distance	Molecular Weight	P42 – Primer 5				P42 – Primer 6				P42 – Primer 7			
		A	B	C	D	A	B	C	D	A	B	C	D
42.5	0.8kB	39	39	39	39	40	40	40	40	33	33	33	33
46	0.7kB	50	50	50	50	41.5	41.5	41.5	41.5	37	37	37	37
49.5	0.6kB	54	54	54	54	43.5	43.5	43.5	43.5	42	42	42	42
52	0.5kB	60	60	60	60	52.5	52.5	52.5	52.5	50	50	50	50
57	0.4kB	70	70	70	70	59	59	59	59	53	53	53	53
63	0.3kB	72	72	72	72	66	66	66	66	56.5	56.5	56.5	56.5
72	0.2kB												

G.40. Restriction Digestion of Passage 55 of Primers 5, 6 and 7, using TaqI



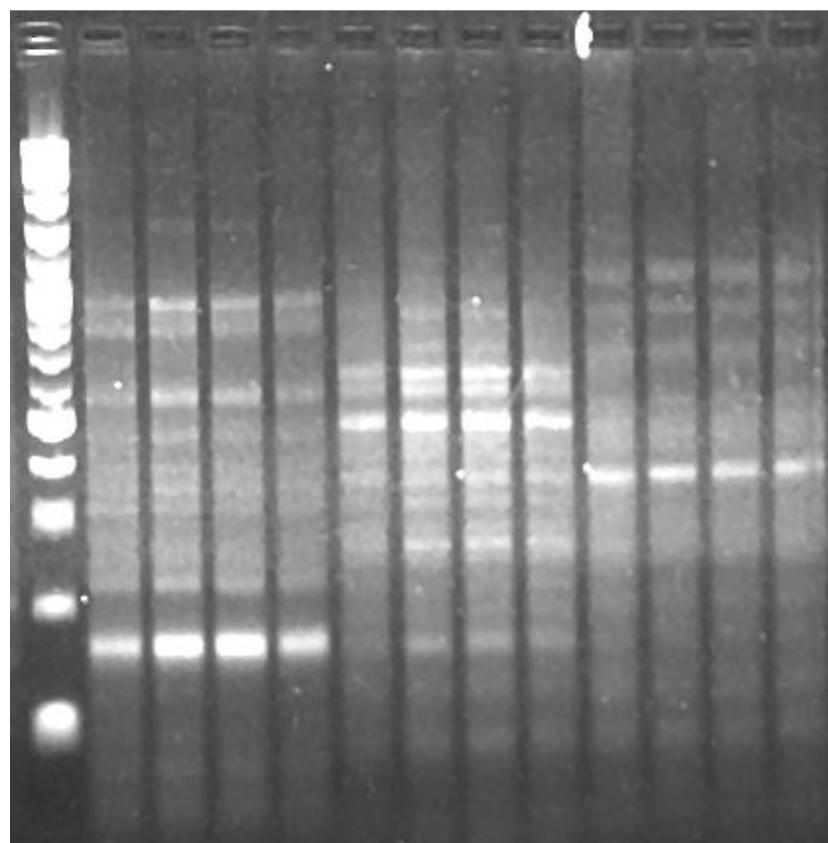
Log 2 Marker		Treatments											
Distance	Molecular Weight	P55 – Primer 5				P55 – Primer 6				P55 – Primer 7			
		A	B	C	D	A	B	C	D	A	B	C	D
42	0.8kB	40	40	40	40	41	41	41	41	59	59	59	59
46	0.7kB	51.5	51.5	51.5	51.5	45							
50	0.6kB	56	56	56	56	49	49	49	49				
54	0.5kB	64	64	64	64	51	51	51	51				
60	0.4kB	72	72	72	72	55	55	55	55				
66	0.3kB	78	78	78	78	62	62	62	62				
74	0.2kB					69	69	69	69				
						77	77	77	77				

G.41. Restriction Digestion of Passage 66 of Primers 5, 6 and 7, using TaqI



Log 2 Marker		Treatments											
Distance	Molecular Weight	Primer 5				Primer 6				Primer 7			
		A	B	C	D	A	B	C	D	A	B	C	D
29	0.8kb	A	B	C	D	A	B	C	D	A	B	C	D
31	0.7kb	25	25	25		26	26	26		42	42	42	42
34	0.6kb	33	33	33	33	28	28	28		51	51	51	51
36	0.5kb	48	48	48	48	35	35	35		58	58	58	58
40	0.4kb	52	52	52	52	42	42	42					
43	0.3kb					47	47	47					
50	0.2kb												
60	0.1kb												

G.42. Restriction Digestion of Passage 76 of Primers 5, 6 and 7, using TaqI



Log 2 Marker		Treatments											
Distance	Molecular Weight	Primer 5				Primer 6				Primer 7			
33	0.8kb	A	B	C	D	A	B	C	D	A	B	C	D
36	0.7kb	15	15	15	15	30	30	30	30	26	26	26	26
39	0.6kb	29	29	29	29	36	36	36	36	29	29	29	29
42	0.5kb	32	32	32	32	38	38	38	38	34	34	34	34
47	0.4kb	40	40	40	40	42	42	42	42	47	47	47	47
52	0.3kb	43	43	43	43	48	48	48	48				
61	0.2kb	59	59	59	59	51	51	51	51				
73	0.1kb	66	66	66	66	55	55	55	55				
						66	66	66	66				

Appendix H – Dissimilarity Matrix

PCR/RFLP #1 (Passage 11)

	A	B	C	D
A	0.0			
B	0.057790	0.0		
C	0.078623	0.020833	0.0	
D	0.063345	0.047222	0.026389	0.0
Mean	0.049034	Standard Dev	0.022219	

PCR/RFLP #2 (Passage 21)

	A	B	C	D
A	0.0			
B	0.041227	0.0		
C	0.115303	0.015050	0.0	
D	0.138626	0.072115	0.118634	0.0
Mean	0.083493	Standard Dev	0.048758	

PCR/RFLP #3 (Passage 30)

	A	B	C	D
A	0.0			
B	0.014286	0.0		
C	0.176906	0.100000	0.0	
D	0.167860	0.104091	0.161364	0.0
Mean	0.120751	Standard Dev	0.061750	

PCR/RFLP #4 (Passage 42)

	A	B	C	D
A	0.0			
B	0.055556	0.0		
C	0.055556	0.000000	0.0	
D	0.059524	0.011905	0.011905	0.0
Mean	0.032408	Standard Dev	0.027195	

PCR/RFLP #5 (Passage 55)

	A	B	C	D
A	0.0			
B	0.079717	0.0		
C	0.11011	0.100197	0.0	
D	0.11011	0.100197	0.000000	0.0
Mean	0.083389	Standard Dev	0.042333	

PCR/RFLP #6 (Passage 66)

	A	B	C	D
A	0.0			
B	0.020833	0.0		
C	0.152778	0.152778	0.0	
D	0.147024	0.147024	0.042857	0.0
Mean	0.110549	Standard Dev	0.061414	

PCR/RFLP #7 (Passage 76)

	A	B	C	D
A	0.0			
B	0.050397	0.0		
C	0.143803	0.106667	0.0	
D	0.114286	0.136508	0.027195	0.0
Mean	0.096476	Standard Dev	0.047300	

Appendix I – MIC Analysis Script

The following Python script is used to process the MIC data and calculate the concentration of NaCl whereby the OD is at maximum, the concentration of NaCl whereby OD is at half of maximum and the AUC (area under the curve) of which NaCl is more than 7.5%.

```
def half_ODmax(m4, m3, m2, m1, m0):
    conc = 0.0
    conc_max = 9.0
    ODmax = 0.0
    while conc < conc_max:
        OD = (m4 * conc ** 4) + (m3 * conc ** 3) + \
              (m2 * conc ** 2) + (m1 * conc) + m0
        if OD > ODmax:
            ODmax = OD
            conc_ODmax = conc
        #print str(conc), str(OD)
        conc = conc + 0.1
    print 'Highest OD is ' + str(ODmax) + ' at [NaCl] = ' +
str(conc_ODmax)
    ODhalf = ODmax / 2
    print '1/2 ODmax = ' + str(ODhalf)
    conc = conc_ODmax
    OD = 0.0
    conc_ODhalf = 0.0
    while conc < conc_max:
        OD = (m4 * conc ** 4) + (m3 * conc ** 3) + \
              (m2 * conc ** 2) + (m1 * conc) + m0
        # print str(conc), str(OD)
        if OD < ODhalf:
            #c_OD = OD
            conc_ODhalf = conc
            break
        conc = conc + 0.1
    print '[NaCl] at 1/2 ODmax = ' + str(conc_ODhalf) + '%'
    return (ODmax, conc_ODmax, conc_ODhalf)

def ODroot(m4, m3, m2, m1, m0):
    conc = 0.0
    while conc < 10.9:
        OD = (m4 * conc ** 4) + (m3 * conc ** 3) + \
              (m2 * conc ** 2) + (m1 * conc) + m0
        # print '\t'.join([str(conc), str(OD)])
        if OD < 0.0:
            return conc + 0.1
        conc = conc + 0.1
    return conc
```

```

def AUC(x, m4, m3, m2, m1, m0):
    return m4/5 * x ** 5 + m3/4 * x ** 4 + m2/3 * x ** 3 + 
m1/2 * x **2 + m0 * x

def AUCratio(x, AUChalf, AUC):
    return(AUChalf / AUC)

m4=0.0
m3=0.0
m2=0.0
m1=0.0
m0=0.0

x = half_ODmax(m4, m3, m2, m1, m0)
print x
auc1 = AUC(7.5, m4, m3, m2, m1, m0)
auc = AUC(ODroot(m4, m3, m2, m1, m0), m4, m3, m2, m1, m0)

print str(auc), str(auc1), str(auc1/auc), '| %AUC>[NaCl7.5%] =
' + str (1-(auc1/auc))

```