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**Evolution Characterization of *Escherichia coli*  
Using RFLP DNA Fingerprinting**

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## **Abstract**

*Escherichia coli* are commonly found in intestine of human and any adaptation or evolution may affect the human body. The relationship between *E. coli* and food additives is less studied as compared to antibiotics. *E. coli* within our human gut are consistently interacting with the food additives; thus, it is important to investigate this relationship. In this study, we observed the evolution of *E. coli* cultured in different concentration of food additives (sodium chloride, benzoic acid and monosodium glutamate), singly or in combination, over 70 passages. Adaptability over time was estimated by generation time and cell density at stationary phase. Polymerase Chain Reaction (PCR) / Restriction Fragments Length Polymorphism (RFLP) using 3 primers and restriction endonucleases each was used to characterize adaptation/evolution at genomic level. The amplification and digestion profiles were tabulated and analyzed by Nei-Li Dissimilarity Index. Our results demonstrated that *E. coli* in every treatment had adapted over 465 generations. The types of stress were discovered to be different even though different concentrations of same additives were used. Genomic analysis by RFLP shows that the stress response in *E. coli* is similar. In addition, monosodium glutamate may be a nutrient source and support acid resistance in *E. coli*.

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## **Abbreviations**

BSA	Bovine Serum Albumin
BHI	Brain Heart Infusion
CC	Correlation Coefficient
DI	Dissimilarity Index
DNA	Deoxyribonucleic Acid
dNTPs	Deoxyribonucleotide Triphosphates
EDTA	Ethylenedinitrilotetraacetic Acid
H BA	High Concentration of Benzoic acid
H MSG	High Concentration of Monosodium Glutamate Treatment
H SALT	High Concentration of Salt Treatment
H COMB	Low Concentration of Combination Treatment
LB	Luria Bertani broth
L BA	Low Concentration of Benzoic acid Treatment
L MSG	Low Concentration of Monosodium Glutamate Treatment
L SALT	Low Concentration of Salt Treatment
L COMB	Low Concentration of Combination Treatment
NB	Nutrient Broth
SNP	Single Nucleotide Polymorphisms
STE	Sodium Chloride–Tris–EDTA

## **1. Introduction**

The Gram negative bacterium, *Escherichia coli*, is commonly found in intestine of human. As a normal flora of the gut, *E. coli* can benefit the host by producing Vitamin K or preventing pathogenic bacteria growth in the intestine. However, these bacteria may adapt or evolve which may affect the human body.

*E. coli* evolution to antibiotics tolerance and resistance are widely studied but the mechanisms to non-antibiotic agents, such as preservatives are less understood. Food additives are commonly used in limiting microbial growth and flavoring in various types of food products. Since *E. coli* within our human gut are consistently interacting with the food additives, it is important to investigate their relationship.

This project aimed to observe the adaptation of *E. coli* cultured in different concentration of food additives, namely sodium chloride, benzoic acid and monosodium glutamate. *E. coli* cells are cultured in 8 different media over 70 passages and swapped at intervals among the treatments. Adaptability over time is estimated by generation time and cell density of 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**

Evolution is the result of genetic changes within a population from one generation to the other. These changes refer to the modification to the DNA sequence resulting in genetic variation. The genetic traits in the individual are inherited down from one generation to the other. As these individual genetic changes accumulate over time, a population can be formed through the process of genetic divergence (Lenski et al., 1991). These traits may vary within population and show heritable difference of the organisms. Genetic changes originated in any generation are usually small and the difference accumulated in each successive generations can cause substantial changes in the population. Eventually, new species may be emerged from the ancestor (a speciation event).

### ***2.1 Sources and Effects of Genetic Variation***

There are three sources of genetic variation: mutation, gene flow and sexual reproduction. A mutation is defined as a permanent change in the DNA sequence of the gene, ranging from a single nucleotide base to a large portion of a gene sequence. The accumulations of many mutations result in evolutionary changes of the population. There are two ways in which gene mutation can occur. The first way is by inheriting the mutation from a parent; the process is known as hereditary mutation. This means that the gene mutation is passed from the parent to the offspring and the next successive generation will contain the genetic variation. The second way is that mutation can be acquired during the lifespan of the organisms. Due to environmental, chemical or physical stress, acquired mutation may occur to improve the survivability and adaptability of the organism (Travisano, 1997).

Gene flow is defined as the transfer of allele of genes from one population to the other (Morjan and Rieseberg, 2004). Migration of gene may result in new genetic variants being introduced to the gene pool of the particular population. For example, species of grass grow on both sides of the road are likely to transport pollen grains from one side to the other. When the pollen grains from one side are able to fertilize the grass on the other side and produce viable offspring, the allele will be successfully transported from one population to the other. The transferring of genes within or across the population has different effects on

evolution. Within the population, gene flow can introduce or reintroduce genetic variants to the population, increase the genetic variation of the organisms. If the gene flow is across the population, it can make distant population genetically similar to one another (Buczkowski et al., 2004) which help to reduce the chance of speciation.

Sexual reproduction is the production of offspring with the combination of genetic material from parental gene which introduces new gene combination into the population, resulting in diversity. Sexual reproduction is important as it can introduce new combination of gene to every successive generation which increases the ability of a species to evolve (Colegrave and Collins, 2008). This implies that advantageous traits from the parental gene may be combined together and transferred to the offspring. However, there is also a possibility that good combination of genes may be removed.

The two main mechanisms responsible for evolution are natural selection and genetic drift. The process whereby the heritable traits are passed on over successive generation to improve the survivability of organism is known as natural selection (Hurst, 2009). For natural selection to occur, there are two essential requirements to be met. Heritable variation for the particular traits must be present and able to exist within the population. In addition, there must be differential survival and reproduction associated with the possession of that trait. Through natural selection, the advantageous traits are passed on to the next generation and more offspring will be able to survive and adapt better. On the other hand, the trait that does not confer an advantage is unlikely to be passed over to the next generation.

An example (Saccheri et al., 2008) will be peppered moths (*Biston betularia*) in England. The original peppered moths were light gray which blended in with the tree trunk. However, during the industrial revolution, many industrial released huge amount of air pollutant. With change in environment, the camouflage of the original moth loses its function because the tree trunks are covered with air pollutant and turned darker. The dark gray peppered moths which once at disadvantage and eaten by predators, now survived and bred while their lighter counterparts were eaten up. Through natural selection and adaption

over a period of time, the peppered moths eventually change from light gray to dark gray to match the colour of the tree trunk.

The central concept of natural selection is the evolutionary fitness of an organism (Orr, 2009). The fitness refers to the proportion of subsequent generation that contains the genes. This concept measures the organism survivability and reproducibility, determining the size of its genetic contributed to the next generation. For example, if an allele of one gene confers better fitness over the other allele in the population, this allele will be selected and passed over to the next generation (Lenz et al., 2009). Subsequently, this particular allele will become more common within the population after each successive generation.

The second mechanism for evolution is genetic drift (Mank et al., 2009). Genetic drift is the change in the frequency of a gene variant occurring in a population due to random sampling. As compared to natural selection which determines the genetic variant due to successive generation, genetic drift randomly determines the variant for the next generation and is not affected by physical, chemical or environmental stress. The variant randomly selected may be beneficial (Mank et al., 2009), neutral (Bloom et al., 2007) or even detrimental (Munguia-Vega et al., 2007) to the next generation.

Genetic drift can have several important effects on evolution. The drift will stop eventually when an allele disappeared from the population or replaced the other alleles entirely. This mean that the genetic variation in the populations is reduced and the population's ability to evolve in response to new stress may be lowered. Another issue is that the effect of the genetic drift is larger in small population and smaller in large population (Otto and Whitlock, 1997). Genetic drift occurs faster and has more drastic impact in smaller population. Thus, rare and endangered species which exist in a smaller population will be affected most by the drift. Genetic drift can also contribute to the process of speciation (Devaux and Lande, 2008). Through the process of genetic drift, there is possibility that a small isolated population will be diverged from the larger population.

Evolution affects the behavior of the organisms and influences every aspect of them. The outcomes of evolution can lead to adaptation, extinction and speciation. Adaptation is the process whereby an organism change in behavior, physiology and structure to become more

suitability in an environment. For example in bacteria, *Escherichia coli* evolved the ability to utilize citric acid as a nutrient source (Blount et al., 2008) and *Flavobacterium sp.* able to grow on the by-product of nylon manufacture through the two newly evolved enzymes (Okada et al., 1983). Speciation is the process whereby a species diverges into two or more descendant species. The competition among same species of organism may result in the divergence of different species. However, only the stronger species will be able to survive and reproduce. Although evolution can lead to beneficial advantages in organism, a certain proportion of species are able to survive and reproduce by out-competing the other proportion, which may cause extinction of a species. (Kutschera and Niklas, 2004).

## 2.2 *Experimental Advantages of Escherichia coli*

Although evolution occurs in every species, it is not feasible to study evolution in an experimental setting with many species due to long generation time. On the other hand, there are several advantages of utilizing bacteria, especially *Escherichia coli* to perform evolution experiments. Firstly, *E. coli* are able to grow rapidly in simple chemically-defined environment (Lenski et al., 1991), allowing easy condition manipulation and generation monitoring. For example, concoction of chemicals can be added to the culture medium or growing them in different temperatures (Cooper et al., 2001). At the same time, rapid generation time allows more chance for mutation (Travisano and Lenski, 1996; Travisano, 1997). In addition, being asexual implies that all the factors affecting the mutation will be solely based on the environment and not due to genetic segregation.

Secondly, *E. coli* can be preserved in ultra-low freezer (-80°C) for an extended amount of time and resurrected to compare with the newly derived cells, without being concerned about the instability and having to rely on test kits that may change over the years (Acha et al., 2005). Preserved cells can be resurrected to restart the experiment should the need arise.

Lastly, *E. coli* is a model organism in genetics, genomics, molecular biology, biochemistry and cell physiology. It is well studied and a number of strains have been fully sequenced, including both pathogenic (*E. coli* O157:H7) and non-pathogenic strain (*E. coli* ATCC 8739 and *E. coli* S88). This not only provides information about *E. coli* that helps interpret

results from studies but also enable researchers to determine the identity of the gene mutated, throughout the course of experiments.

### **2.3 Examples of Evolution Experiments with Bacteria**

Considering the advantages of bacterial cells in the study of evolution, there had been a number of studies using various strains of bacteria, such as *E. coli K12 MG1655* (Fong et al., 2005), *E. coli W ATCC 9637* (Daumy et al., 1985), *Caulobacter crescentus* (Ackermann et al., 2007), *Thermus thermophilus* (Akanuma et al., 1998), *Pseudomonas aeruginosa* (Prijambada et al., 1995) and *Proteus rettgeri* (Daumy et al., 1985).

A study (Daumy et al., 1985) demonstrating 2 different isoforms of Penicillin G acylase from *E. coli* and *Proteus rettgeri* with distinctly physical (isoelectric point and molecular weight) and biochemical (regulation and chemical repressibility) activities can be made to evolve to similar physical and biochemical characteristics using selective media. Apart from altering biochemical properties of a protein, thermal stability has also been improved. In another study (Akanuma et al., 1998), the thermostability of 3-Isopropylmalate dehydrogenase, an enzyme involved in leucine biosynthesis, from *Bacillus subtilis* was improved from 37°C to 70°C in *Thermus thermophiles* through sequential increase in culturing temperature. A sequence analysis of the mutant enzyme showed 3 amino acid substitutions as compared to the original enzyme in *Bacillus subtilis*.

### **2.4 Long-Term Experimental Evolution in *Escherichia coli***

*E. coli* is an organism for a well known long-term experimental evolution studies spanning more than 2 decades (Lenski, 1988, Blount et al., 2008). A series of studies had been carried out (Travisano, 1997, Travisano and Lenski, 1996, Cooper, 2002, Rozen and Lenski, 2000) to observe the pattern of adaptation by looking at the mean fitness in a constant selective environment. Arabinose-using ( $\text{Ara}^+$ ) and non arabinose-using ( $\text{Ara}^-$ ) clones of *E. coli* B (Lenski, 1988) were inoculated into 12 tubes (6 replicates each of  $\text{Ara}^+$  and  $\text{Ara}^-$  clones) of minimal salt media. Fitness was measured by the ratio of  $\text{Ara}^+$  to  $\text{Ara}^-$  colonies. Unique genetic markers have since evolved to allow identification of each strain. The mean rate of increase in fitness relative to the ancestor was  $1.9 \times 10^{-4}$  per generation over 2000 generation (Lenski et al., 1991). In the first 1000 generations, the rate of increase in fitness

relative to the ancestor was  $2.7 \times 10^{-4}$  per generation. In the last 1000 generations, the rate of fitness increment relative to the ancestor continued to increase by at a lower rate of  $1.1 \times 10^{-4}$  per generation. This suggests that all population adapted to glucose (Lenski et al., 1991). An important adaptation that occurred in one of the twelve populations: the bacteria evolved the ability to utilise citrate as a source of energy (Blount et al., 2008). The researchers noticed a dramatically expanded population-size in one of the samples. They found that there were clones in this population that can metabolize the citrate included in the growth medium to permit iron acquisition. Analysis of samples of the population frozen earlier discovered that a citrate-using variant had evolved in the population at some point between 31000 and 31500 generations (Blount et al., 2008). They used a number of genetic markers unique to this population to exclude the possibility that the citrate-using *E. coli* were contaminants.

Another adaptation that occurred in the *E. coli* was an increase in cell size (Philippe et al., 2009). This change was partly the result of a mutation that changed the expression of a gene for a penicillin binding protein, which allowed the mutant bacteria to out-compete ancestral bacteria under the conditions in the long-term evolution experiment (rich LB broth). Although this mutation increased fitness under these conditions, it also increased the bacteria's sensitivity to osmotic stress and decreased their ability to survive long periods in stationary phase cultures; hence, the phenotype of this adaptation depends on the environment of the cells (Philippe et al., 2009).

Temperature is one of the physical factors that affect the growth of living things by altering the rate of metabolism (Acha et al., 2005). At higher temperatures, proteins in the cells can start to denature and loses its functional structure. In a study (Cooper et al., 2001), *E. coli* was cultured for 20,000 generations in low ( $20^{\circ}\text{C}$ ) and high ( $41\text{--}42^{\circ}\text{C}$ ) temperatures. Most populations were lost during the early phase of rapid adaptation to the temperature but the surviving populations were believed to undergo antagonistic pleiotropy, as the population that mutates at high rates due to DNA repair, does not die out to the adapting population with low mutation rates. Under the concept of antagonistic pleiotropy, adaptation to the selective environment and loss of function in other environments are caused by the same mutations, which mediate a trade-off between performances across environments (Cooper

et al., 2001). The population adapted fastest during the initially exposure and decreases over time. The same trend will apply to the rate of functional loss process, which will be fastest at the early phase and decreases with time. This is different from mutation accumulation which the rate of functional loss is constant.

Another study by Sezonov et al. (2007) suggested that as *E. coli* grow by constant replication, the resulting increase in cell density causes each bacteria cell to compress the other bacteria cells for more spaces to replicate and this may change the *E. coli* morphology. The change in morphology affects the metabolic activity of the bacteria and compressing the bacteria cells give rise to smaller cells. Decreased cell size may have lower metabolism requirement compared to its original. In addition, Sezonov et al. (2007) suggested that the limits of proportionality between cell count to OD600 spectrophotometric reading is 0.3 and the cell size beyond OD600 of 0.3 reduces non-linearly to only about 30% of the size at OD600 2.0. This suggests that follow up experiments measuring cell density using optical density may results in problems of inaccuracy in cell density estimation. Increasing population implies higher demands for carbon source; however, most nutrients has already depleted after the log phase. Therefore, bacteria cells will suffer overcrowding and compete for nutrient if subculture is not perform regularly.

## 2.5 Effects of Chemicals on Bacteria

In terms of the effects of chemical treatments, bacterial resistance and tolerance to antibiotics are well established and the mechanisms widely studied (Cardonha et al., 2005, Karami et al., 2007, Langsrud et al., 2004, Odahara et al., 2006, Pezzotti et al., 2003). In contrast, mechanisms of insusceptibility to non-antibiotic agents, such as preservatives and antiseptics, are less understood. Food additives are important means of limiting microbial growth (Salmond et al., 1984) and adding flavors to various types of food products. For example, citric acid inhibits the growth of proteolytic strains of *Clostridium botulinum* (Russell, 1991), because of its chelating properties which decrease the pH in the medium. Sodium chloride can inhibit the growth of many bacteria such as *Listeria monocytogenes* (Garner et al., 2006), *Ochrobactrum anthropi* (Kesseru et al., 2002), and *Latobacillus plantarum* (Glaasker et al., 1998). However, some bacteria are able to grow well in high salt conditions (Kobayashi et al., 2000, Kushwaha and Kates, 1979, Chan et al., 1979).

Fatty acids (Sheu and Freese, 1972, Carson and Daneo-Moore, 1980, Saito and Tomioka, 1988, Speert et al., 1979, Miller et al., 1977), such as formic (Dashper and Reynolds, 2000), acetic (Roe et al., 2002), propionic acid (Maruyama and Kitamura, 1985, Salmond et al., 1984), is also capable of inhibiting bacteria growth.

## **2.6 Fitness Definition**

Generation time trend was employed to determine the adaptability of cells to different stress level because in an adaption study (Helling et al., 1987), *E. coli* was cultured in a constant environment for 765 generations. Mutations occur mostly during the first half of the culture, indicating that adaption took place during the early half of the experiment. More variants were seen in subsequent passages suggesting that variants had a faster generation time. Therefore, a decrease in generation time would be indicative of variants.

## **2.7 Aims and Hypothesis of Project**

The aim of the project is to observe the evolution of *E. coli* growing in different concentration of food additives, namely sodium chloride, benzoic acid and monosodium glutamate. High concentration of food additives is expected to induce more chemical stress as compared to lower concentration which is likely to be expected in shorter generation time for *E. coli* cultured in low concentration additives. After several passages, *E. coli* is expected to adapt to their individual treatment. Low concentration treated cells are swapped to high concentration media and vice versa at regular intervals. We expected that low concentration cells cannot grow well (longer generation time) in high concentration media and it needs to undergo mutations to adapt. On the other hand, high concentration treated cells are able to grow well (shorter generation time) in low concentration media as the chemical stress is lower. As the cells adapt to different media, mutations resulting in differences in PCR/RFLP profile are likely to occur.

### **3. Materials and Methods**

#### ***3.1 Extended Viability in Different Media***

*Escherichia coli* (ATCC 8739, Microbiologics Incorporated) were inoculated into 3 different media (Nutrient Broth, Brain Heart Infusion and Luria-Bertani Broth) and cultured for 6 weeks at 37°C. Viable cell counts were estimated weekly by serial dilution (ranging from 10<sup>-2</sup> to 10<sup>-9</sup>) and spread plates. OD600 readings were recorded before viable cell count was performed.

#### ***3.2 Main Culture Experiment***

Lysophilised *Escherichia coli* ATCC 8739 (Reference Passage 4 from ATCC) were revived on nutrient agar plate and incubated at 37°C before inoculating into 8 different treatment supplementation in Nutrient Broth [0.025% (w/v) as high monosodium glutamate (H MSG); 0.0025% (w/v) as low monosodium glutamate (L MSG); 0.025% (w/v) as high benzoic acid (H BA); 0.0025% (w/v) as low benzoic acid (L BA); 1% (w/v) NaCl as high salt (H SALT), Nutrient Broth as low salt (L SALT); 0.025% (w/v) monosodium glutamate, 0.025% (w/v) benzoic acid and 1% (w/v) NaCl as high combination (H COMB); 0.0025% (w/v) monosodium glutamate and 0.0025% (w/v) benzoic acid as low combination (L COMB)]. Subculture was performed using 1% of the previous culture on every Monday, Wednesday and Friday (Figure 3.1, Experiment B). OD600 readings were taken before the next subculture to estimate the number of generation within the current passage. In addition, OD600 readings were taken on day 5 and 7 of each Wednesday's and Friday's culture and generation time was measured on every 3<sup>rd</sup> passage (Figure 3.1, Experiment A). Glycerol stocks for each treatment were made from 1% of the culture for every 12<sup>th</sup> passage after culturing on MacConkey agar. This was maintained for 70 passages.

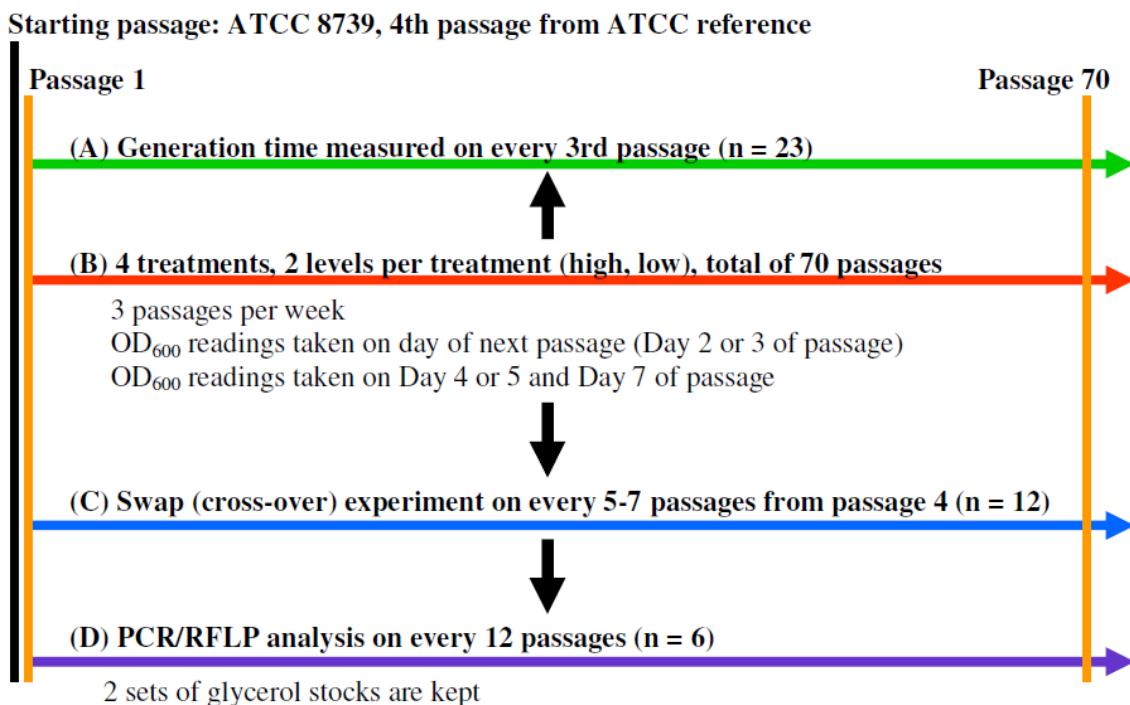


Figure 3.1: Complete Experimental Design

### 3.3 *Treatment Swapping Experiment*

*Escherichia coli* cultured in the different treatments were swapped to other treatments fortnightly (every 5-7 passages interval) to observe the generation time (Figure 3.1, Experiment C). Four types of swaps were carried out where cells were inoculated 1 in 100 dilutions to the new treatment medium. The first swap set involves the inoculation of the basal medium (L SALT) treated cells into 6 other non-salt media. For example, cells grown in L SALT were inoculated into H MSG treatment. For the second set, cells cultured in high and low concentrations of each treatment were swapped. For example, cells cultured in H BA were inoculated into L BA and vice versa. In the third set, cells from single high concentration treatments (H MSG, H BA and H SALT) were inoculated into H Comb. In the last set, cells from the single low concentration treatments (L MSG, L BA and L SALT) were inoculated into L COMB respectively. OD600 readings were recorded for each swap at intervals and generation times were calculated for each interval.

### **3.4 Polymerase Chain Reaction / Restriction Fragments Length Polymorphism**

**Genomic DNA Extraction.** Genomic DNA was extracted from the treatment cultures at every 12 passages for Polymerase Chain Reaction and Restriction Fragment Length Polymorphism. The DNA extraction protocol is based on (Cheng and Jiang, 2006) proposed method for DNA extraction of Gram-negative bacteria. Cells were harvested by centrifugation at 4000rpm for 15 minutes. The pellet was washed twice with 400 $\mu$ l STE (100 mM NaCl, 10 mM Tris/HCl, 1 mM EDTA, pH 8.0). The suspension was centrifuged at 13000rpm for 2 minutes and the pellets were resuspended in 200 $\mu$ l Tris/HCl buffer before 200 $\mu$ l of phenol/chloroform/isoamyl alcohol (25:24:1) was added to the cell suspension and vortex-mixed for 60 seconds to lyse the cells and obtain a white suspension. The aqueous phase was separated from the organic phase by centrifugation at 13000rpm for 5 minutes. The white interphase was removed using a sterile toothpick and the cell lysate (aqueous phase) was further purified by phenol/chloroform treatment until the white interphase was no longer seen by repeating this procedure two to three times. The aqueous phase was extracted with 200 $\mu$ l of chloroform and centrifuged at 13000rpm for 5 minutes to remove traces of phenol from the aqueous phase. Equal volume of isopropanol was added to the aqueous phase and incubated at -20°C for at least 30 minutes to precipitate the DNA. The precipitate was centrifuged at 13000rpm for 20 minutes and the isopropanol was decanted, followed by air-drying of the precipitate. The precipitate was dissolved in 100 $\mu$ l of Tris/HCl, pH 8.0 and stored at -20°C.

**Polymerase Chain Reaction.** A total volume of 50 $\mu$ l in each reaction was prepared using 2 $\mu$ l of DNA template in 10pmoles of dNTPs, 50pmoles of primer, 1 unit of Taq polymerase and 1X standard buffer (with 1.5mM of MgCl<sub>2</sub>) provided by the supplier (New England Biolabs, Inc.). A total of 3 primers were used separately: Primer 5, CgCgCTggC; Primer 6, gCTggCggC; and Primer 7, CAggCggCg. The PCR reaction was carried out (Hybaid Limited, PCR Express) with the cycling condition of initial denaturation at 95°C for 10 minutes; 35 cycles of amplification at 95°C for 1 minute, 27°C for 1 minute, 72°C for 3 minutes; followed by a final extension at 72°C for 10 minutes before gel electrophoresis in 2% (w/v) agarose gel with 1X GelRed.

**Restriction Fragments Length Polymorphism.** 11 $\mu$ l of PCR product was digested with 1 unit of restriction endonuclease (TaqI, Hinfl or MspI) in a total of 20 $\mu$ l with 1X restriction digestion buffer and 100ng/ $\mu$ l acetylated BSA provided by the supplier. Each reaction was incubated at 37°C (65°C for TaqI) for 16 hours before analysis on 2% (w/v) agarose gel with 1X GelRed.

### 3.5 Data Analysis

**Five and Seven Day Cell Density.** Cell density was estimated from the corresponding OD600 readings using cell size correction suggested by Sezonov et al. (2007). Briefly, cell size remain constant up to OD600 0.3, being equivalent to  $5 \times 10^7$  cells per milliliter. After OD600 0.3, cell size decreases and relationship between the OD600 and the cell density changes. The correction graph shows a standard curve of Cell density =  $52137400 * \ln(\text{OD600 reading}) + 118718650$ .

Cell density for five and seven day cultures were compared using a quotient of cell density at day seven and day five. A value of 100% would indicate no increase in cell density from the fifth day to the seventh whereas a decrease from this value means a higher cell density on the fifth day than on the seventh day and vice versa for an increase in the value.

**Generation Time.** OD600 readings taken at intervals of up to 435 minutes after inoculation into fresh media was used to analyze the generation time of each treatment at every 3 passages (Experiment A on Figure 3.1) and the swap experiment on every 5-7 passages (Experiment C on Figure 3.1). Readings for the first interval were not used for the generation time calculations due to the inclusion of the lag phase of growth and the geometric mean of the subsequent intervals, using cell density after cell size correction, was obtained to represent the average generation time for the treatment. In experiment A, the mean generation times were compared within the same treatment across passages, and between treatments within the same passage.

**Swap Treatment.** In the first swap set (low salt concentration treatment into 6 other non-salt treatments) which also serves as a control, generation times of each treatment swap

across passages were compared in order to determine the effects of Nutrient Broth (L SALT) on the adaptations of the cells growing in it. No change in the general linear trend (gradient of zero) of generation time across passages indicates that Nutrient Broth is unlikely to cause any adaptations that may assist the cells to grow better on other treatments. An increase or decrease may suggest otherwise. The gradients of the generation times across passages were tested using t-test for regression coefficient (Gopal, 2006) to determine if they were statistically different from zero using the formula,

$$t - \text{statistic} = \frac{bS_x}{S_{y.x}}(n-1)^{-\frac{1}{2}},$$

where

$$S_x^2 = \frac{\sum (x_i - \bar{x})^2}{n-1}$$

$$b = \frac{\sum x_i y_i - \frac{1}{n} \sum x_i \sum y_i}{\sum x_i^2 - \frac{1}{n} (\sum x_i)^2}$$

$$S_{y.x}^2 = \frac{\sum (y_i - \bar{y} - b(x_i - \bar{x}))^2}{n-2}$$

As for the second experiment (high treatment cells into low treatment media and vice versa), the difference in stress level on cells between high and low treatments, and the adaptability of *E. coli* growing in high and low treatments can be determined. Generation time of each treatment swap was compared across passages to analyse the effects of different treatment concentrations.

Adaptability of cells growing in low treatment was analyzed through comparing generation times of the four swaps from low treatment to high treatment across passages. The adaptability of cells, growing in low treatment, increases if a trend of increasing generation time is observed, likely due to the reduction in effects of high concentration treatments on the low treatment cells as adaptability improves.

Similarly, adaptability of cells cultured in high treatment was analyzed by comparison of generation times of the remaining four swaps from high to low treatment across passages. Adaptability of cells to high concentration treatment increases if a trend of increasing generation times is observed. This may a result of adaptation to a high concentration treatment and thus the effects of low concentration treatment is minimized. Another probable observation is that, the generation time patterns of both high treatment cells into low treatment and vice versa, is similar to that of swapping low salt concentration treatment cells into either high or low concentration combination treatment. The basis of this observation is that high and low treatments each causes a different type of stress on the cells, therefore by swapping either ways, it would be equivalent to inoculating the cells into a new kind of stress and this is exactly what is occurring when low salt treatment cells are inoculated into high or low concentration treatment.

Swap set three and four (high single treatment to high combination treatment, low single treatment to low combination) was used to analyze the similarities of the effects of different types of single treatment on the cells. An observation of similar growth patterns among the treatments may indicate the same type of stress on the cells.

**Polymerase Chain Reaction / Restriction Fragment Length Polymorphism.** Migration distance of bands for the PCR and RFLP gels between different treatments within the same passage were tabulated and a Nei-Li dissimilarity index (DI) (Nei and Li, 1979) was obtained for each pair-wise comparison (28 in total) between all treatments per passage. Nei-Li DI is a measure of how different two organisms are, according to the presence and absence of common bands after a restriction endonuclease digest. The banding patterns of the two organisms are compared and the DI is obtained by using the following equation:

$$1 - \frac{2 \times \text{number of regions where both species are present}}{\left[ 2 \times (\text{number of regions where both species are present}) + \right.} \left. \frac{\text{number of regions where only one species is present}}{} \right]$$

A maximum value of 1 is obtained when there are no common bands between the two comparing samples while a minimum value of 0 is obtained when the two samples have the

same exact bands. The geometric mean for the DI of each pair-wise comparison was then calculated for each passage and the average DI per passage, regardless whether it is from a PCR or RFLP gel, is obtained. The comparisons are first analyzed by contrasting the DI with the passages in a graph. Then, analysis through mathematically derived equations shows the effects of each comparison. The effects of MSG, BA, and SALT are given the term MSG, BA and S respectively and the baseline effects from NB is termed NB.

All the single treatments effects would be the addition of effects from NB and the additive(s) which is shown as,

$$H\ MSG = NB + 10\ MSG$$

$$L\ MSG = NB + MSG$$

$$H\ BA = NB + 10\ BA$$

$$L\ BA = NB + BA$$

$$H\ SALT = NB + S$$

$$L\ SALT = NB$$

$$H\ COMB = NB + 10\ MSG + 10\ BA + S$$

$$L\ COMB = NB + MSG + BA$$

The multiplication of 10 to the effects in high treatments is to factor in the 10 times difference between the high and low treatments concentrations with the exception of salt since no additional salt was added and the salt content in L SALT was not derived from NB.

The effects of comparisons between treatments would therefore be,

$$H\ COMB = NB + 10\ MSG + 10\ BA + S$$

$$L\ COMB = NB + MSG + BA$$

Thus,  $H\ COMB / L\ COMB = 9\ MSG + 9\ BA + S$ , suggesting that the difference between the genome of cells from the treatments are due to the differing constituents between the treatments. The effects of all comparisons are shown in Table 3.1

Comparisons	Factors in Each Comparison						
	MSG	BA	Salt	9MSG	9BA	10MSG	10BA
LMSG / LSALT	■						
LBA / LCOMB	■						
LMSG / LCOMB		■					
LBA / LSALT		■					
HMSG / LSALT					■		
HBA / LSALT							■
LMSG / HSALT	■		■				
LBA / H SALT		■	■				
LMSG / LBA	■	■					
LSALT / LCOMB	■	■					
HSALT / LCOMB	■	■	■				
HMSG / HSALT			■		■		
HBA / HCOMB			■		■		
HMSG / HCOMB			■				■
HBA / HSALT			■			■	
HMSG / HBA			■			■	
HSALT / HCOMB			■			■	
LSALT / HCOMB			■			■	
HMSG / L BA		■	■			■	
LMSG / HBA	■	■					■
HMSG / LCOMB		■	■	■			
HBA / LCOMB	■	■			■		
LMSG / HCOMB			■	■			■
LBA / HCOMB			■	■	■		
HMSG / LMSG				■	■		
HBA / LBA				■	■		
HSALT / LSALT			■				
HCOMB / LCOMB			■	■	■		

Table 3.1: Effects of the 28 pair-wise comparisons among the 8 treatments. The shaded areas represent the individual effects of the comparisons.

Contrasting the two comparisons, L MSG / L SALT and L MSG / L COMB, the resulting difference was from MSG. The DI of the two comparisons over the passages was plotted against each other and the correlation coefficient (CC) value obtained. The CC value was then statistically tested against the CC value of 0.95 (~1) using the Z-test for two correlation coefficients (Gopal, 2006). A P-value of more than 0.05 would indicate that the null hypothesis (CC is equal to 0.95) is not rejected and the test is not statistically significant, whereas a P-value of lesser than 0.05 would indicate that the calculated CC is

not equal to 0.95. The CC values were obtained and tested from comparisons having resulting effects of MSG, BA, BA + MSG, 10MSG + S, 10BA + S and 10MSG + 10BA.

## 4. Results

### 4.1 Different Media

At the start of the experiment, *Escherichia coli* in Brain Heart Infusion (BHI) had the highest OD600 reading of 0.157 (refer to appendix A for full data). *E. coli* in Nutrient broth (NB) and Luria-Bertani broth (LB) started with similar OD600 readings of 0.088 and 0.098 respectively. After the 1st 5 days in culture, the OD600 readings of *E. coli* in NB increased by 13.5 times which is the highest as compared to the LB (13 times) and BHI (11.1 times). Although the OD 600 readings of *E. coli* in the 3 different media were increasing after 5 days, the differences in OD600 readings between 5 and 41 days in culture is considerably lower (ranging from 1.39 times to 1.53 times). The linear regression gradient (Figure 4.1) of *E. coli* in BHI was the steepest followed by that of *E. coli* in LB and then *E. coli* in NB.

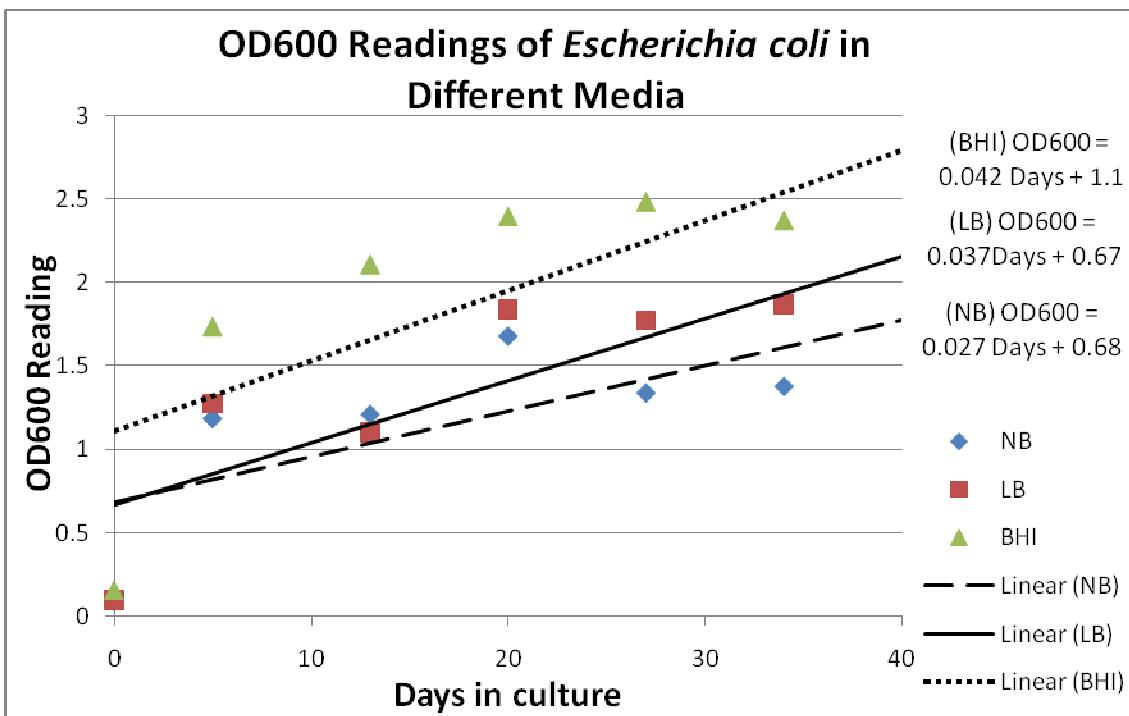


Figure 4.1: OD600 readings of *Escherichia coli* in Nutrient broth (NB), Luria-Bertani broth (LB) and Brain Heart Infusion (BHI) cultured over 41 days.

The viable count of *E. coli* in LB is the highest ( $>3 \times 10^{10}$  CFU/ml) during day 5 in culture as compared to viable plate count of NB ( $1.1 \times 10^7$  CFU/ml) and BHI ( $3.8 \times 10^9$  CFU/ml). However, the viable count of *E. coli* in LB decreased over the 41 days in culture (Figure 4.2). The viable count of *E. coli* in NB increased greatly to its peak between Day 20 and 25 before decreasing over the remaining day in culture. As for the viable count of *E. coli* in BHI, it increased to its highest point (more than  $3.0 \times 10^{10}$  CFU/mL) between Day 10 and 15 and decreased from Day 15 onwards.

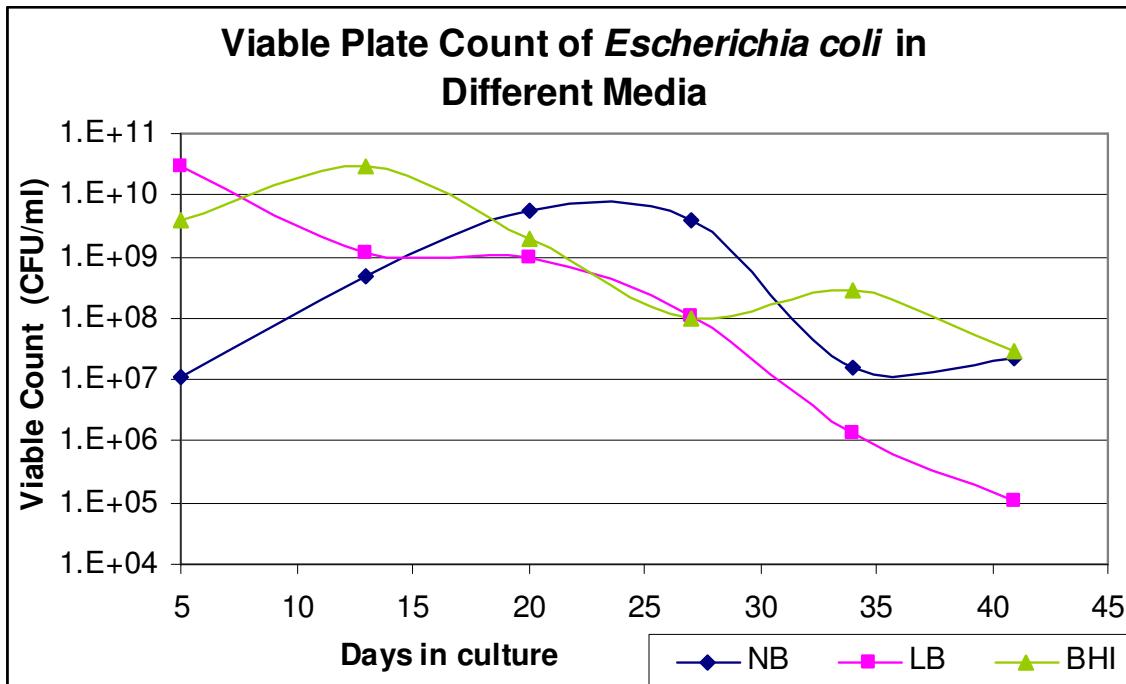
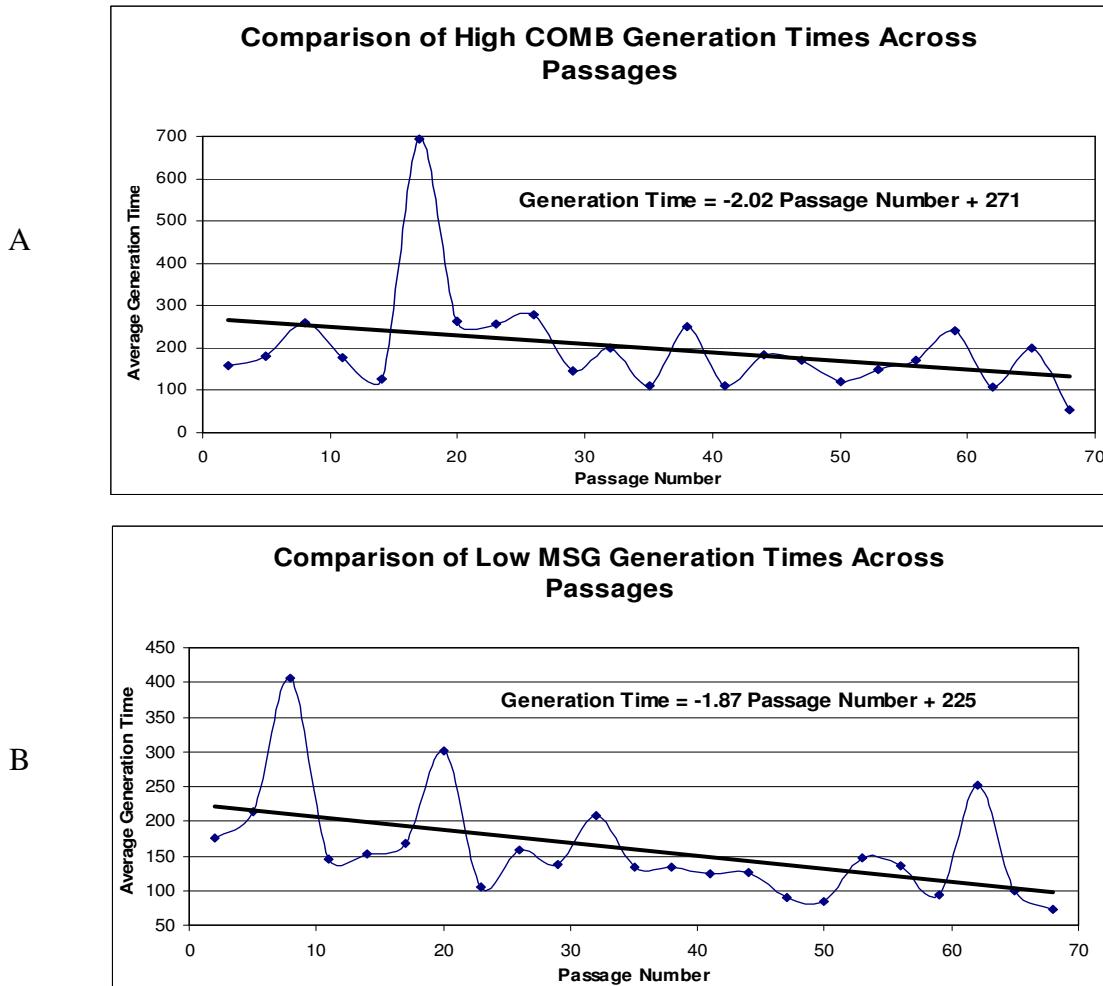
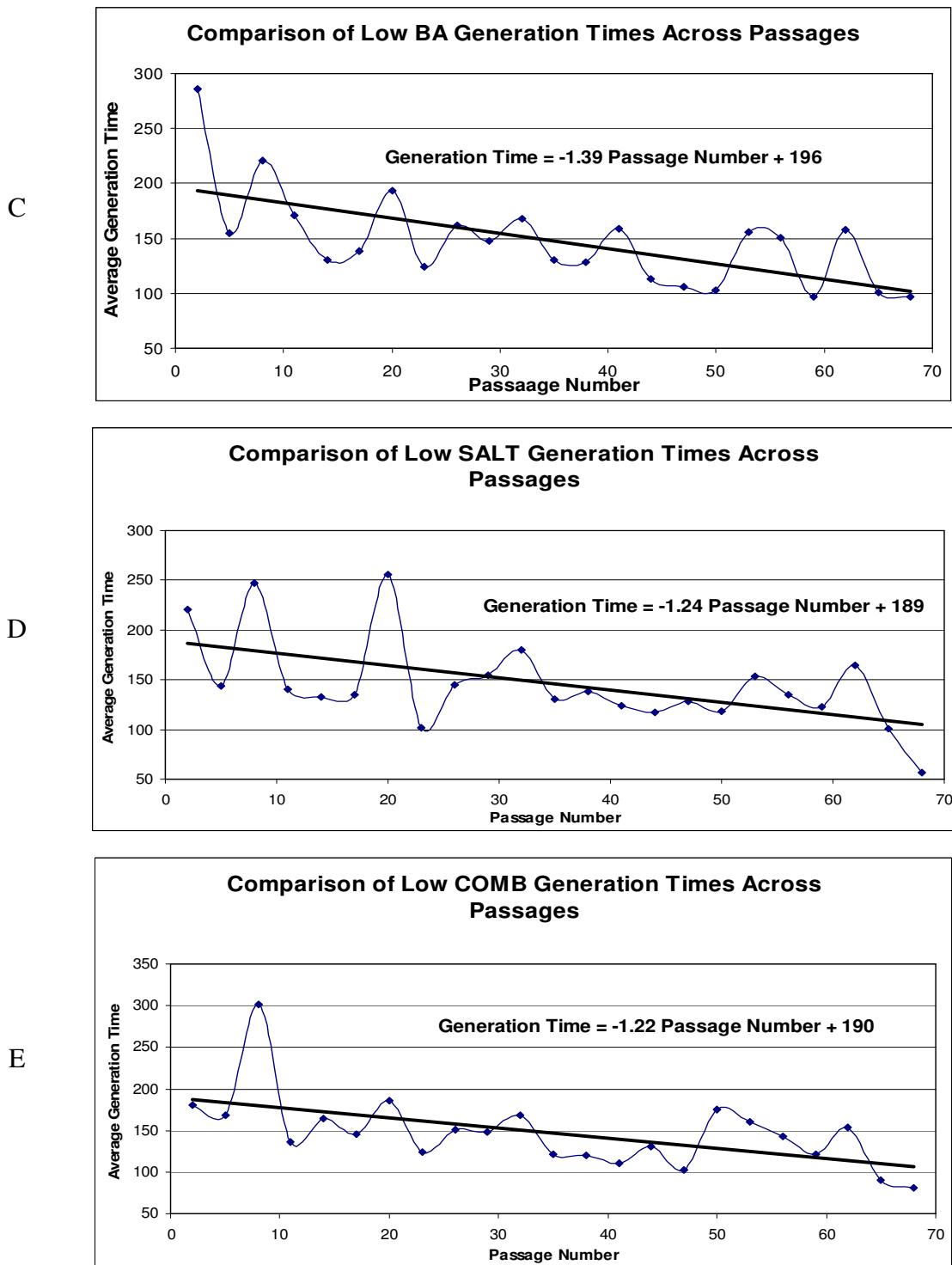


Figure 4.2: Viable plate count of *Escherichia coli* in Nutrient broth (NB), Luria-Bertani broth (LB) and Brain Heart Infusion (BHI) cultured over 41 days. When the number of colonies exceed 300 at  $10^8$  dilutions, it is plotted as  $3 \times 10^{10}$  CFU/ml.

## 4.2 Generation Time

Analysis of the generation times all eight treatments over the passages had shown different rates of decreasing generation times (Figure 4.3). The steepest decline in generation time occurs in H COMB treatment (Figure 4.3A) where approximately 2.02 minutes reduction in generation time per passage over 70 passages was seen, followed by L MSG (1.87 minutes), L BA (1.39 minutes), L SALT (1.24 minutes), L COMB (1.22 minutes), H BA (1.15 minutes), H SALT (1.12 minutes) and finally H MSG (0.906 minutes). The intercept on the generation time axis (Figure 4.3) may be used to estimate the generation time of the cells in each treatment media for the first passage which is indicative of the level of initial stress on the cells. On this basis, the treatment exerting the highest level of stress on the cells would be H COMB, followed by L MSG, H BA, L BA, L COMB, L SALT, H SALT and H MSG.





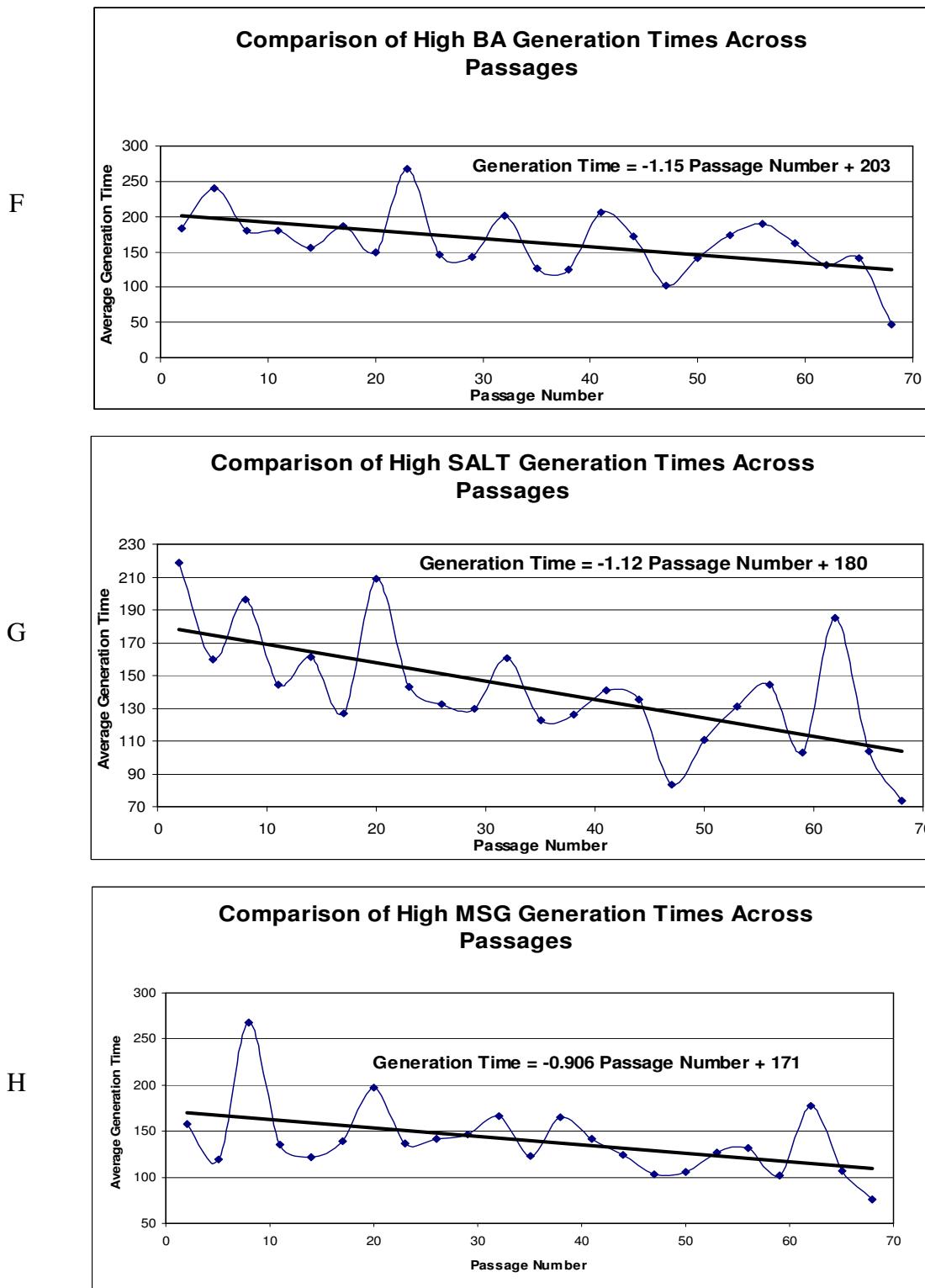


Figure 4.3: Generation times of the eight treatments: High MSG (A), Low MSG (B), High BA (C), Low BA (D), High Salt (E), Low Salt (F), High COMB (G) and Low COMB (H) across 70 passages arranged according to the stress level from highest to lowest based on the gradient of the linear regression line.

### 4.3 Day 5 and Day 7 Cell Density

At the start of the experiment, all treatments show a random trend. After 28 passages the coefficient of variation of all treatments starts to decline, except L SALT, H COMB and L COMB, as shown in Table 4.1. The coefficient of variation of L SALT, H COMB and L COMB started to declined after passage 43.

Passage	H MSG	L MSG	H BA	L BA	H SALT	L SALT	H COMB	L COMB
1-13	3.16%	4.75%	3.12%	7.69%	7.19%	4.61%	5.88%	5.56%
14-26	6.02%	5.56%	2.04%	3.88%	1.43%	1.88%	5.25%	1.74%
28-43	1.66%	2.84%	2.08%	1.75%	1.53%	6.96%	8.17%	10.13%
44-56	1.17%	1.97%	1.10%	1.48%	2.56%	3.89%	3.15%	4.20%
58-70	1.39%	1.51%	1.12%	1.88%	3.70%	3.84%	1.67%	1.54%

Table 4.1: Tabulation of Coefficient of Variation of all treatments for 70 passages.

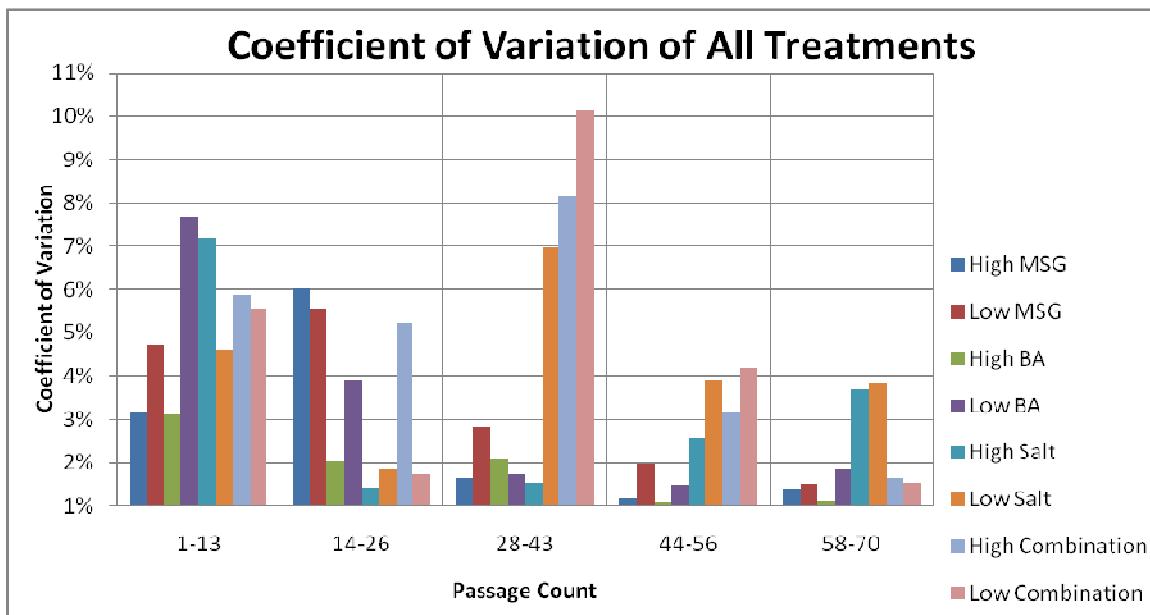


Figure 4.4: Coefficent of Variation of all treatments for 70 passages.

The ratio of Day 7 to Day 5 cell density for MSG treatments (Figure 4.5) between passages 1 and 10 varies between 100% and 105%. However, the maximum variation of the ratio increase from 5% to 15% (H MSG – 100% to 115%, L MSG – 85% to 100%), from passage 11 to 28. After passage 28, both ratios started to stabilize at 100% till the end of the experiment (Passage 70).

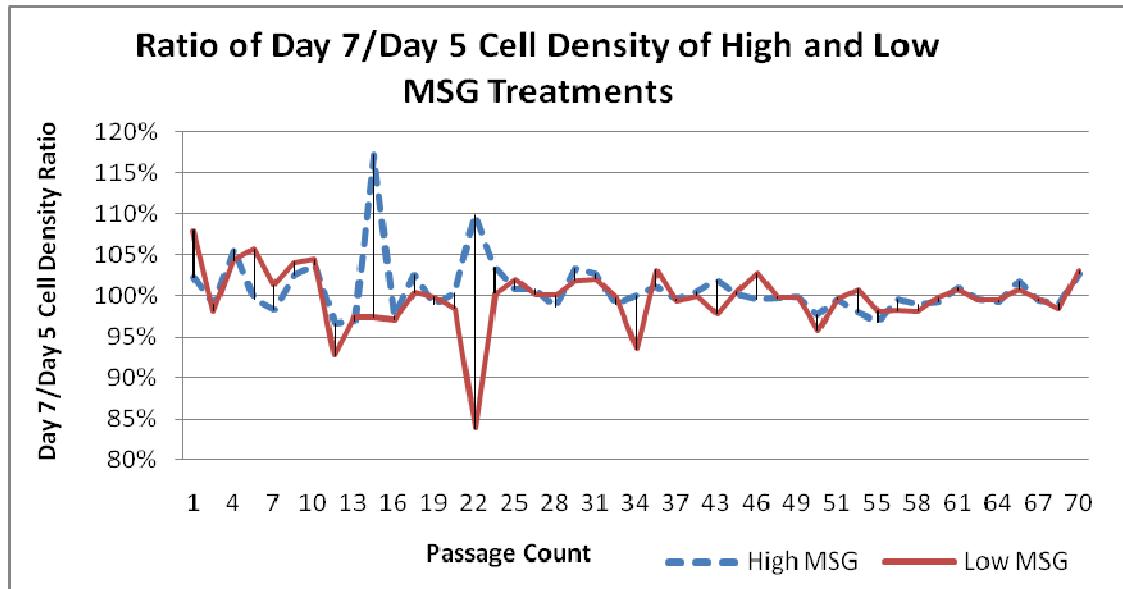


Figure 4.5: Ratio of Day 7 to Day 5 cell density of High and Low MSG treatments over 70 passages.

From Figure 4.6, the ratio for H BA treatment was relatively constant with 5% variation between passages 1 and 10. On the other hand, the maximum variation of the ratio for L BA treatment was up to 20%. The fluctuation starts to reduce in both BA treatments from passage 13 to 28. H BA treatment remains constant within 5% variation and the ratio variation for L BA treatments reduced from 20% to 10%. After passage 29, both ratios started to stabilize at 100% till the end of the experiment (Passage 70).

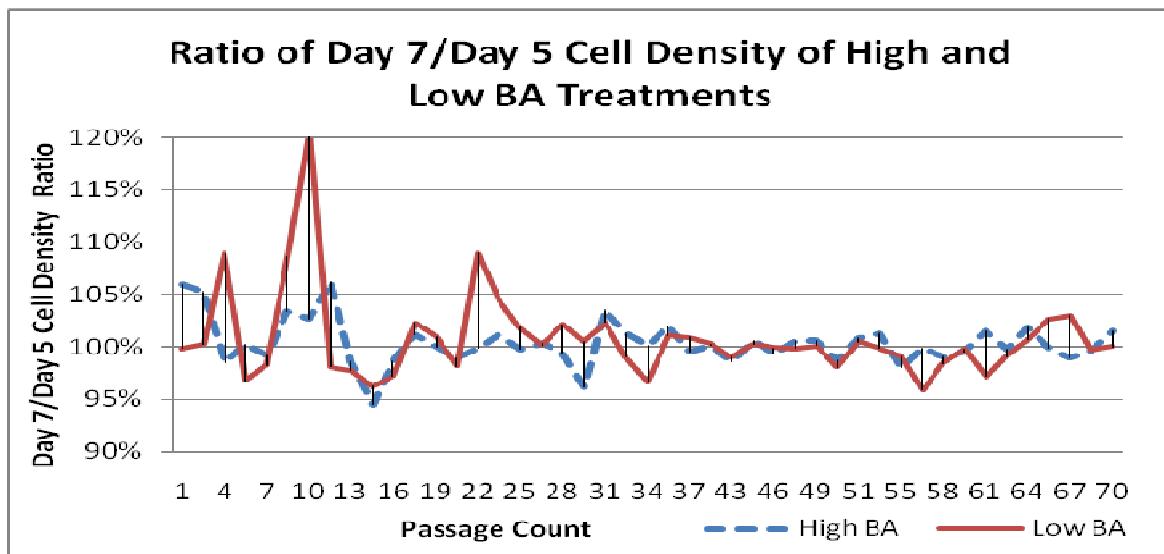


Figure 4.6: Ratio of Day 7 to Day 5 cell density of High and Low BA treatments over 70 passages.

The ratio of Day 7 to Day 5 cell density (Figure 4.7) for both salt treatments showed high fluctuation (H SALT – 20%, LOW SALT – 10%). After passage 13, H SALT treatment remained constant with variation of 5% till passage 67 and rises to 108% at passage 70. L SALT treatment was constant from passage 13 to 37 within 5% variation. At passage 43, L SALT treatment rose to 120% and the following passages fluctuate at about 10% till passage 70.

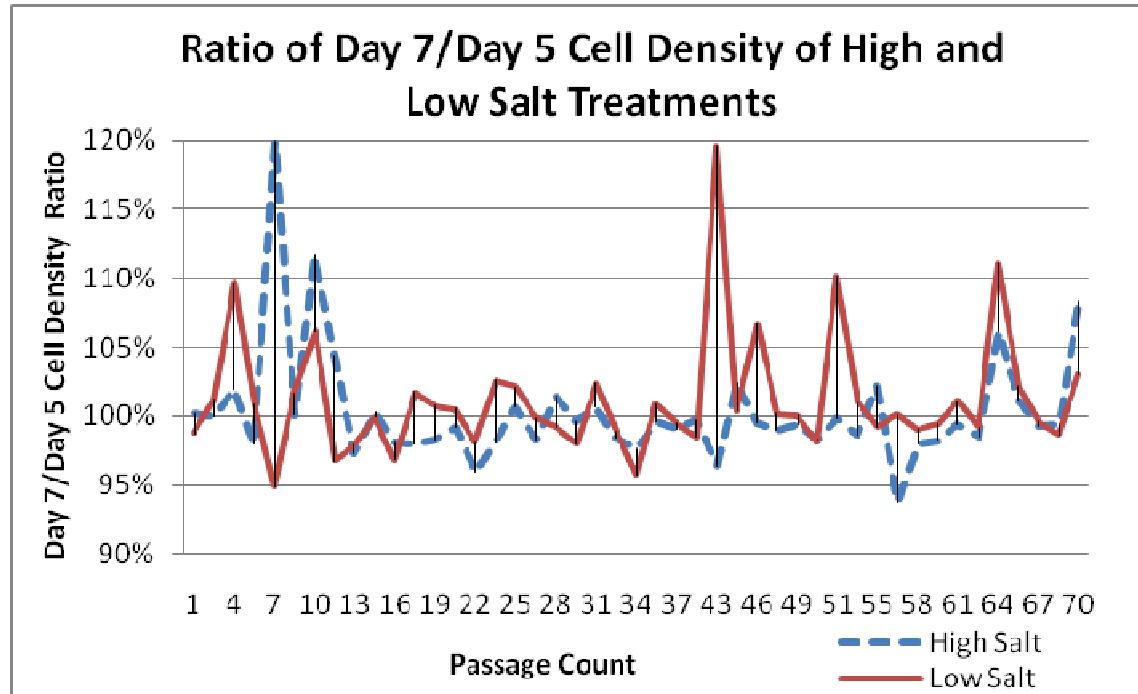


Figure 4.7: Ratio of Day 7 to Day 5 cell density of High and Low Salt treatments over 70 passages.

The ratio of cell density for combination treatment (Figure 4.8) began with a rise followed by a decline after passage 13. From Passage 14 to 28, both treatments were relatively stable around 100% but H COMB treatment increased by 15% at passage 22. From passage 29 to 46, both treatments showed a maximum variation of 20% (H COMB – 80% to 100%, L COMB – 100% to 120%). Passage 43 onwards, H COMB treatment then started to stabilise with maximum variation of 8% from passage 43 onwards, while on the contrary, L COMB treatment had a slightly higher maximum fluctuation of 12% until passage 58 when it gradually became constant around 100% till passage 70.

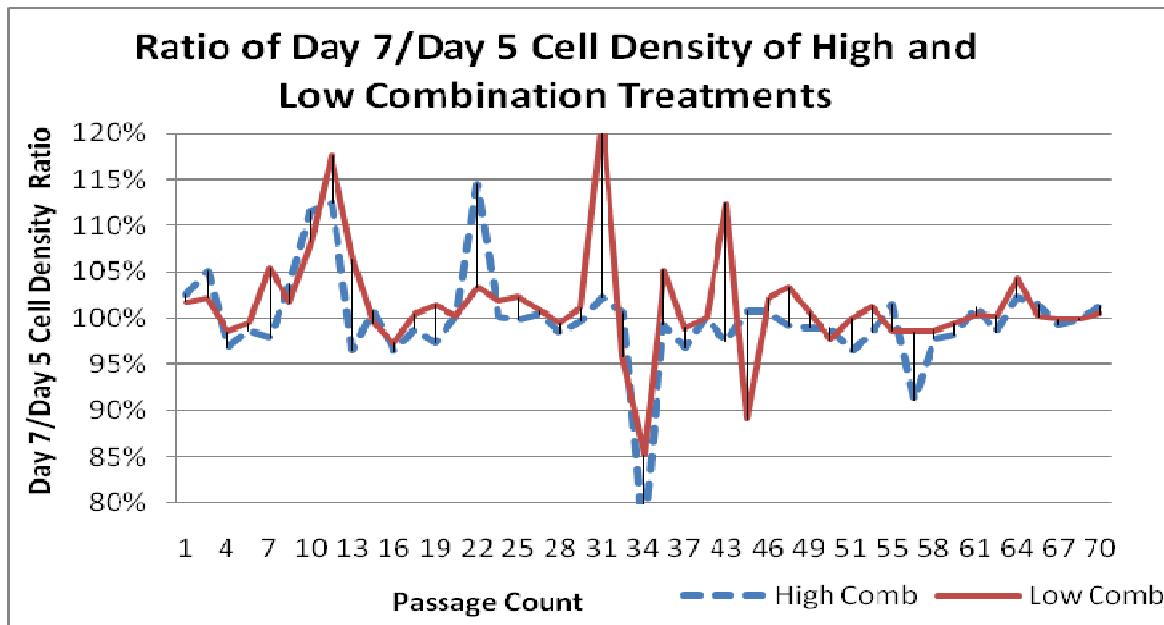


Figure 4.8: Ratio of Day 7 to Day 5 cell density of High and Low Combination treatments over 70 passages.

#### 4.4 Swap Experiment

From Figure 4.9, it shows the generation time trend of low salt treatment cells inoculated into 6 other non-salt treatment media over 12 swaps. The linear regression gradients of the equations are not equal to zero which indicates that the generation times are not constant for the 6 swaps. Although there are changes in the general trend of generation time across the passages, the p-value calculated for the 6 swaps is more than 0.05 which is not significant: L SALT cells to H MSG media, 0.475509; L SALT to L MSG media is 0.421721; L SALT cells to H BA media is 0.250415, L SALT cells to L BA media is 0.4660235; L SALT cells to H COMB media is 0.484.887; L SALT cells to L COMB is 0.443381.

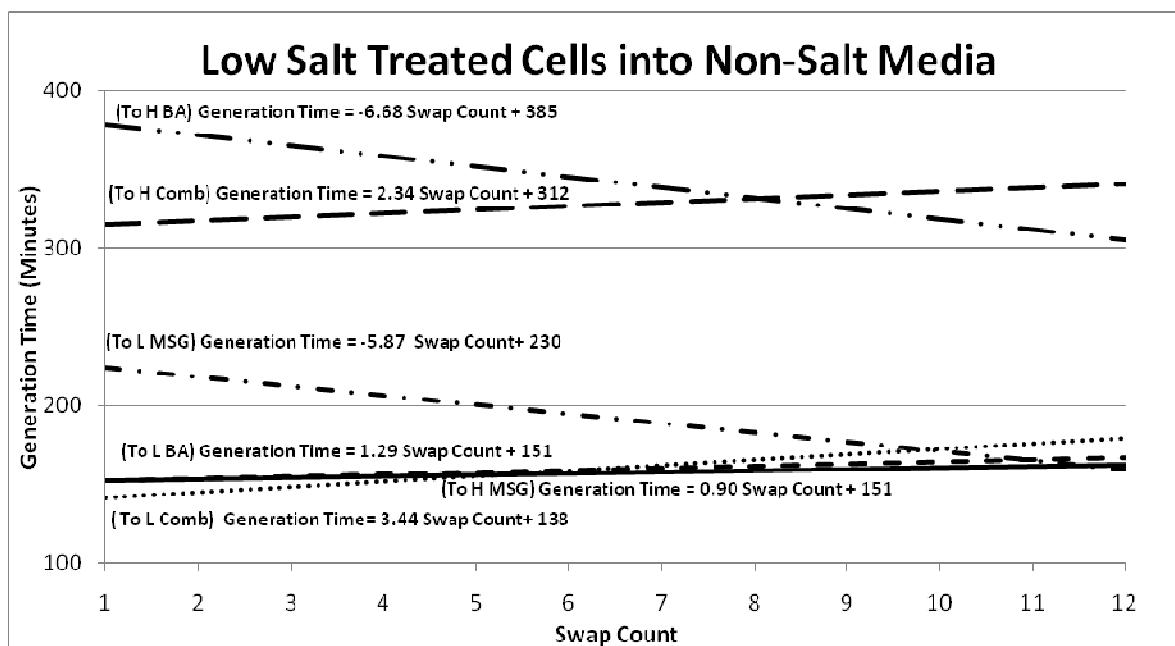


Figure 4.9: Generation Time Trend of Low Salt Treated Cells into Non-Salt Treated Media (High MSG, Low MSG, High BA, Low BA, High Combination and Low Combination) over 12 Swaps.

From Figure 4.10, the generation times trend of the 4 swaps (MSG, BA, Salt and Combination) from low treatment to high treatment over 12 swaps change in a decreasing manner. With reference to the regression equations, the linear regression gradient of low treatment cells into high treatment media for combination treatment is the steepest followed by that for BA, MSG and salt treatments. At swap count between 8 and 9, the generation time is almost the same for MSG, salt and combination but the generation time for BA is still distantly higher.

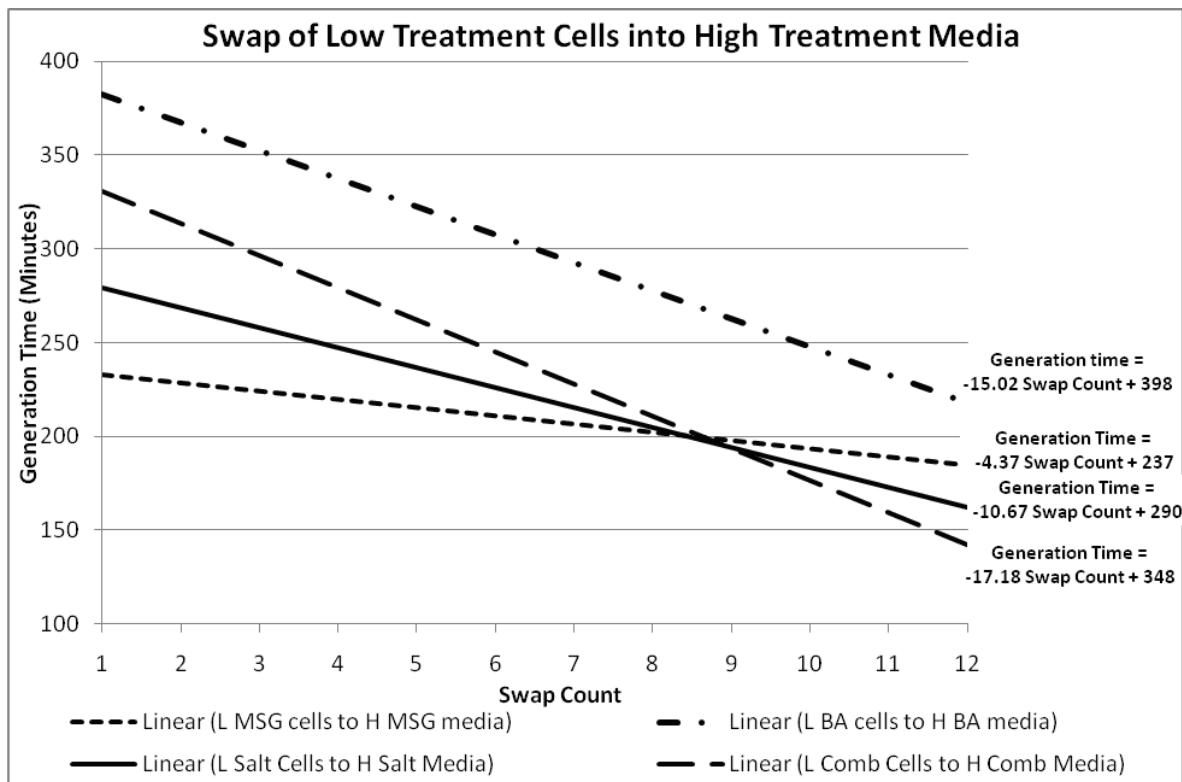


Figure 4.10: Generation Time Trend of Low Treatment Cells into High Treatment Media over 12 Swaps.

From Figure 4.11, the generation time trend of the 4 swaps (MSG, BA, salt and combination) from high treatment to low treatment over 12 swaps change in a decreasing manner. With reference to the regression equations, the linear regression gradient of high treatment cells into low treatment media for MSG treatment is the steepest followed by that for BA, combination and salt treatment. The generation time of high treatment cells into low treatment media for MSG treatment is almost the same as for salt treatment between swap count 4 and 5, and as for combination treatment between swap count 8 and 9. The generation time for BA is consistently lowest among the treatments.

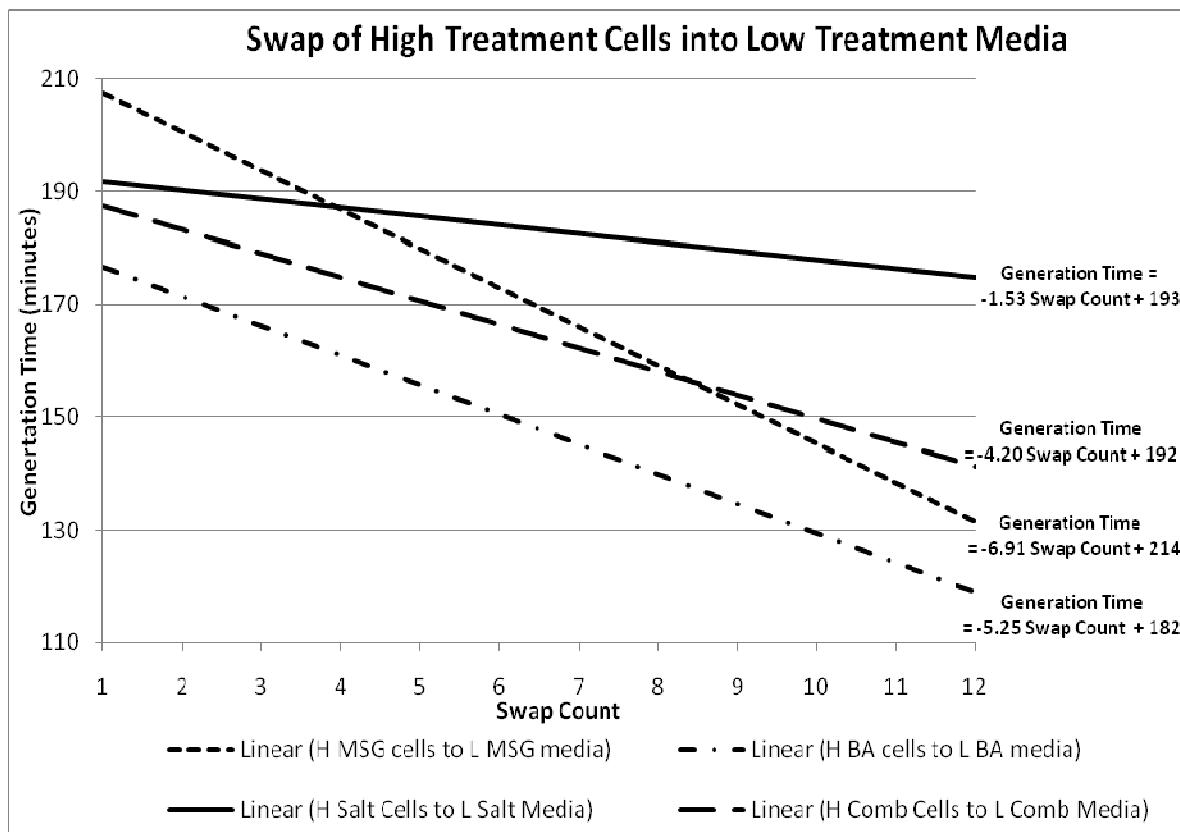


Figure 4.11: Generation Time Trend of High Treatment Cells into Low Treatment Media over 12 Swaps.

The generation time of swapping high concentration treated cells in to high combination medium showed similar trends (Figure 4.12). At the 8<sup>th</sup> swap, H BA suddenly increases in generation time, due to the retarding growth. The OD600 reading ranges from 0.069 to 0.084 over a period of 3 days. After the 10<sup>th</sup> swap, generation time for all treatments remain constant at 200 minutes till the 12<sup>th</sup> swap.

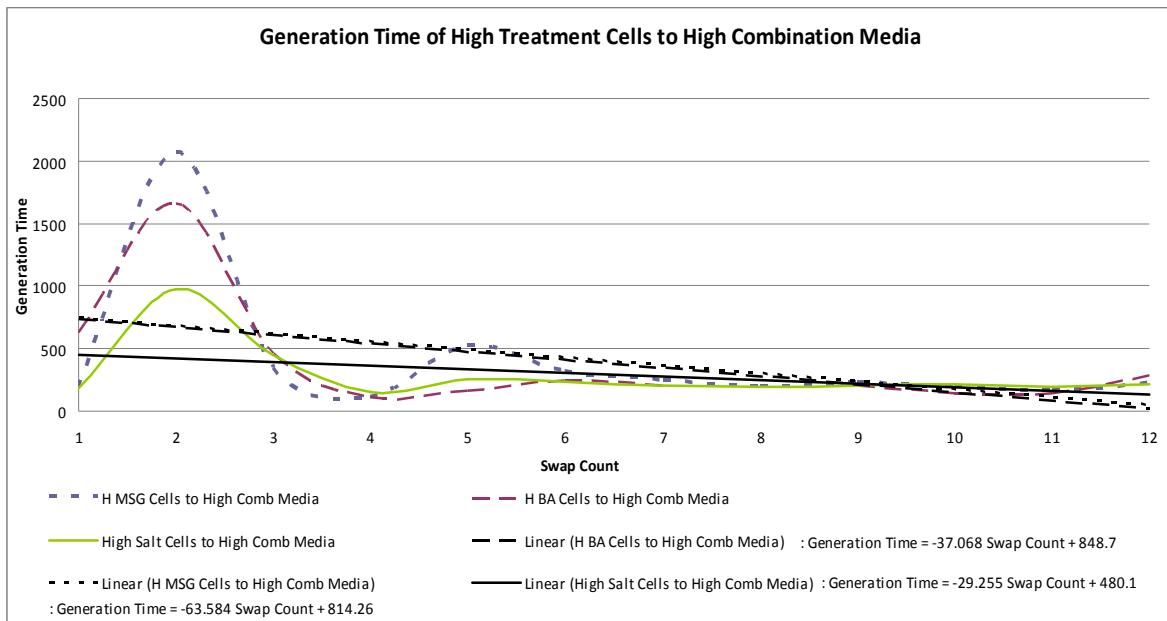


Figure 4.12: Generation time of High Treatment Cells to High Combination Media for 12 swaps.

The generation time of swapping low concentration treated cells in to low combination medium showed similar trends (Figure 4.13). At the 2<sup>nd</sup> swap, L Salt suddenly increases in generation time, due to the slow growth. The OD600 reading ranges from 0.077 to 0.545 over a period of 3 days. After the 4<sup>th</sup> swap, generation time for all treatment follows a similar trend till the 12<sup>th</sup> swap.

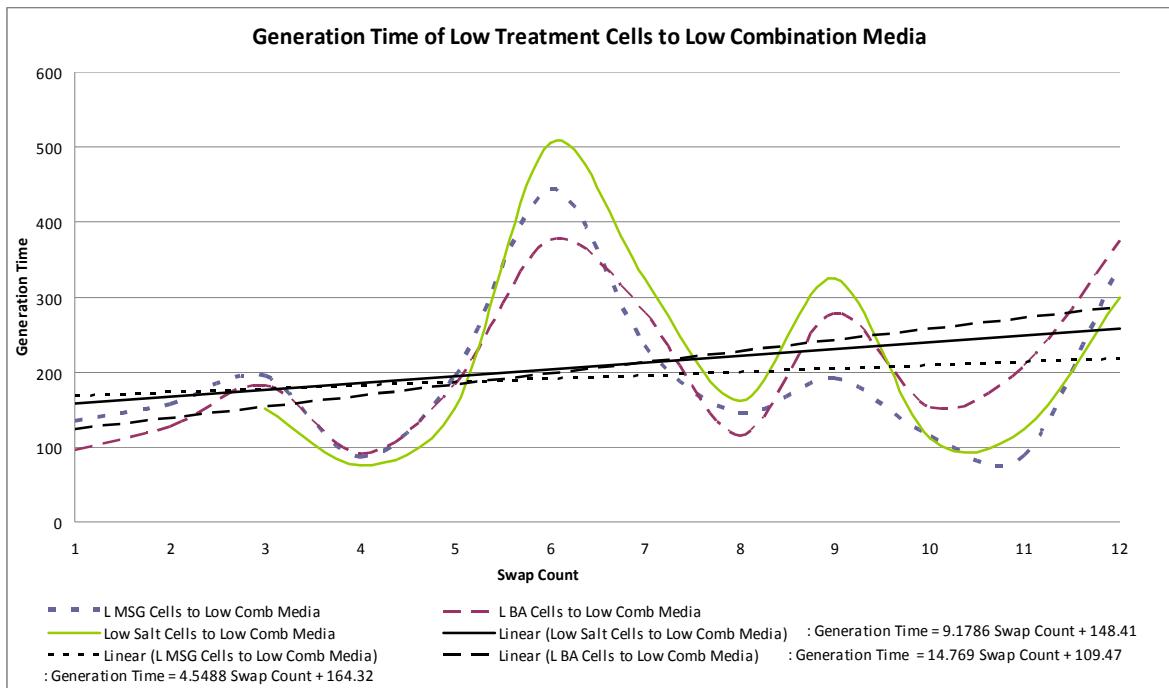


Figure 4.13: Generation time of Low Treatment Cells to Low Combination Media for 12 swaps.

## 4.5 Polymerase Chain Reaction / Restriction Fragment Length Polymorphism

Electrophoresis agarose gels of the PCR and RFLP products for the 8 treatments were used to study the differences between the genome of the *E. coli* cells of the treatments across the passages. Nei-Li Dissimilarity Index (DI) was utilised to mathematically calculate the dissimilarity between pair-wise comparisons of the treatments.

The dissimilarity index of the 28 comparisons showed a trend of convergence from PCR/RFLP #4 onwards (Figure 4.14).

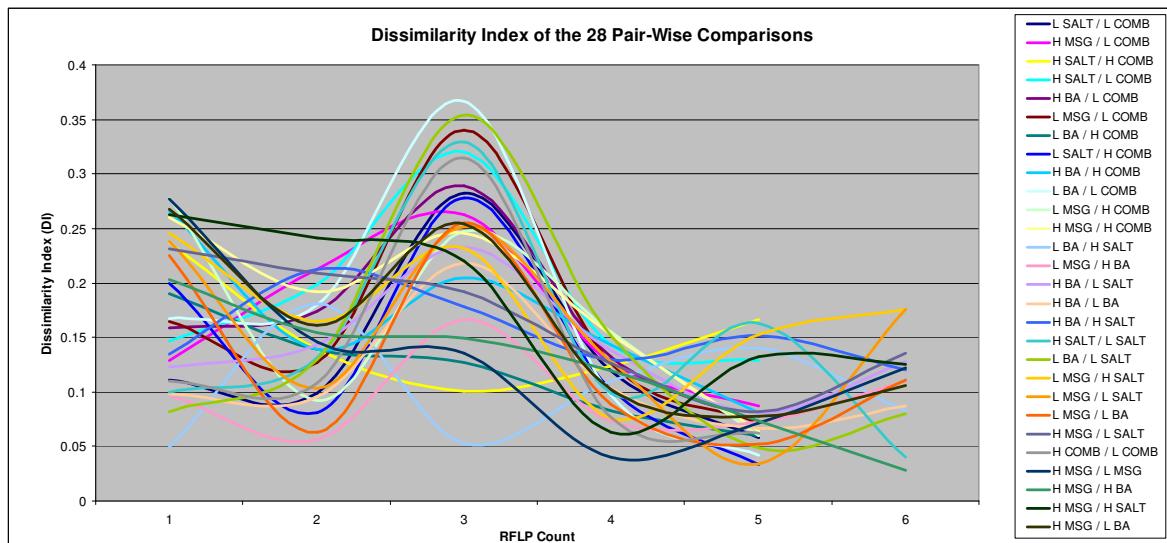


Figure 4.14: Dissimilarity index of the 28 pair-wise comparisons for the 6 PCR/RFLP. The data points of comparisons with H COMB or L COMB of PCR/RFLP #6 were excluded from this and subsequent analysis due to unusually high dissimilarity index which is caused by an error in the PCR of H COMB and L COMB of PCR/RFLP #6 (Appendice G: Gel 9, 15, 21, 27, 33, 39, 45, 51, 57, 63, 69) resulting in almost blank lanes on subsequent RFLP.

This trend is further elaborated with the estimation of the maximum and minimum mean values (Figure 4.15) which shows converging linear regression line across the 6 PCR/RFLP.

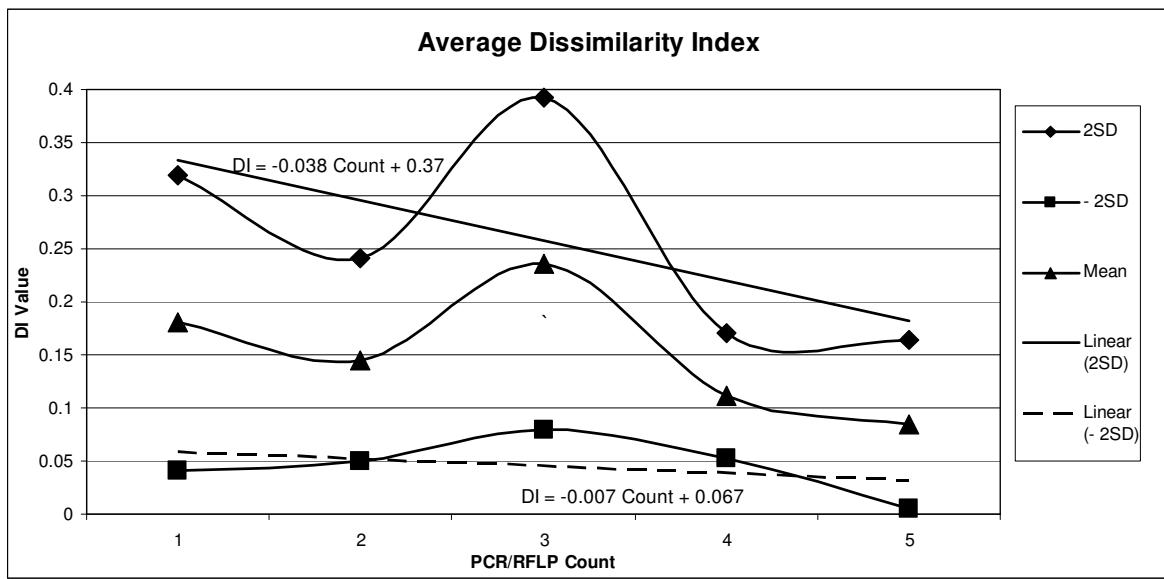


Figure 4.15: Estimation of the average maximum and minimum mean values of the DI for each PCR/RFLP count.

Six resulting effects (Table 4.17) obtained from the comparisons were analysed. The similarity among the six resulting effects is that each type of effects has 2 originating comparisons. Therefore by plotting the 2 comparisons against each other and testing for significance, we can deduce whether the genomic differences in each of the 2 comparisons are actually a consequent effect from the resulting effects.

All resulting effects are not statistically significant except for 10BA + S. This suggests that the PCR/RFLP-inferred genetic distance between H MSG and H COMB, and H BA and H SALT vary independently (not correlated).

PCR-RFLP Comparison	Resulting Effects	Correlation Coefficient	Z Statistic	P-value	Significant
LMSG/LS, LBA/LC	MSG	0.786	-0.944	0.173	No
LMSG/LC, LBA/LS	BA	0.934	-0.175	0.431	No
LMSG/LBA, LS/LC	BA + MSG	0.764	-1.012	0.156	No
HMSG/HS, HBA/HC	10MSG + S	0.631	-1.333	0.091	No
HMSG/HC, HBA/HS	10BA + S	0.142	-2.068	0.019	Yes
HMSG/H BA, HS/HC	10MSG + 10BA	0.437	-0.167	0.434	No

Table 4.2: Tabulation of P-value for the resulting effects.

## 5. Discussion

### 5.1 Nutrient Broth Sustains Continuous Growth Up to 24 Days

*Escherichia coli* (ATCC 8739) was cultured in 3 different media (Nutrient broth, Luria-Bertani broth, Brain Heart Infusion) to determine the most suitable medium for subsequent adaptation experiments. The 3 different media vary in constituents: Nutrient broth (Atlas, 2004) consists of peptone, yeast extract, beef extract, sodium chloride and water; Luria-Bertani broth (Sezonov et al., 2007); consists of tryptone, yeast extract, sodium chloride and water; while Brain Heart Infusion (Atlas, 2004) consists of beef heart extract, calf brain extract, disodium hydrogen phosphate, glucose, peptone, sodium chloride and water. Graph of OD600 readings and viable plate count of *E. coli* in different media was recorded (Appendix A) and plotted (Figure 4.1 and 4.2).

The OD600 readings of *E. coli* measured are related to the cell density (Sezonov et al., 2007). With reference to the OD600 readings result (Figure 4.1), *E. coli* cultured in Brain Heart Infusion was the highest initially after inoculation (Day 0). However, after 5 day in culture, the cell density of *E. coli* in Nutrient broth demonstrated the highest increase in turbidity (13.5 times) as compared with *E. coli* in Luria-Bertani broth and Brain Heart Infusion.

As cell density includes both dead and live cells, viable plate count for *E. coli* in 3 different media was performed to determine which media able to produce highest amount of live cells and cells density. *E. coli* in Luria-Bertani broth has the highest viable cell count at Day 5 but the viable cell count decreases over the remaining days in culture. This suggests that even though Luria-Bertani broth has high concentration of catabolizable amino acids, there is a lack of recoverable sugars (Sezonov et al., 2007), resulting in a decrease of growth rate and cell mass. *E. coli* cultured in Brain Heart Infusion shows to have increased in viable plate count till Day 10. The presence of peptone and infusion (calf brain and beef heart) serves as sources of carbon, nitrogen, essential growth factors, amino acids and vitamins. However, after culturing over 41 days, the viable plate count in the graph shows fluctuations which may indicate unreliability in the amount of live cells and cells density.

As for *E. coli* in nutrient broth, the viable plate count shows relatively constant increase till day 24. In contrast to Luria-Bertani broth, sources of carbon in nutrient broth are recoverable sugars (Sezonov et al., 2007) which support growth rate. Since subsequent adaptation experiments required the *E. coli* to be cultured in different treatments for a week, it is important that the cells viability are sustained within this time frame and our results shows that NB supports consistent growth up to 24 days. Hence, nutrient broth shown to be the ideal media for *E. coli* with highest cell density and live cells provided for DNA extraction (Kubitschek, 1974).

## **5.2 Nutrient Broth Does Not Prime Cells for Growth in Other Treatments**

Since *E. coli* cells were grown in NB with the various supplementations of treatments, it was important that adaptations to the cells are a result of the treatments rather than from the NB. The generation time trend for *E. coli* cells from L SALT (NB) inoculated into 6 different media (Figure 4.9) showed that nutrient broth may not have a serious impact on adaptability as none of the regression gradients are statistically different from a gradient of zero. Although there are changes in the general trend of generation time, the increase or decrease in the generation time for 6 non-salt media are statistically insignificant. Since the general generation time trend remained almost the same, nutrient broth (L SALT media) is unlikely cause any adaptations that may assist the cells in growing better in our treatments tested (MSG, BA and combination).

## **5.3 Cells Adapt to Their Individual Treatments**

The low concentration treated cells were observed to be adapting to their environment as seen from the decreasing generation time (Figure 4.3). Low treatment cells inoculated into high treatment media also showed decrease in generation time across the swaps. This suggests that the low treatment cells had gradually adapted to its own individual treatment before the swap, causing it to be less stressed when swapped into high treatment media.

The generation times of cells from low single treatments to low combination treatment was observed to increase gradually across passages. This suggests that the individual low concentration treatments are not stressful enough to induce significant adaptations such that when they are placed into the low combination treatment, the cells could not cope with the

stress induced by the combination treatment. Furthermore, the increasing trend may also suggest that the individual low concentration treatments are inducing changes to the cells causing them to be less adaptable to the low combination treatment across the passages.

The effects of accumulating L MSG, L BA and L SALT do not increase the adaptability of cells growing in L COMB but decreases it as seen from the generation time analysis (Figure 4.3). It had been suggested that the presence of MSG counteracts the effects of drop in pH caused by BA (Moreau, 2007, Bjornsdottir et al., 2006, Bhagwat et al., 2005, Wilks and Slonczewski, 2007). This is achieved by increasing the resistance of *E. coli* cells against the lowered pH, which will otherwise kill the cells. This suggests that the effects of L MSG and L BA cancel out each other, leaving only L SALT which is further supported by the similarity between the adaptability of L SALT (-1.24 minutes per generation) and L COMB (-1.22 minutes per generation).

Our results indicated that *E. coli* cultured in H COMB treatment have the greatest decline in the generation time over 70 passages. Since higher stress level may force the cells to adapt quickly in order to survive, this suggests that the *E. coli* cells in H COMB treatment are experiencing the highest level of stress among the eight treatments due to the three different stresses in addition to the possible effects of the interacting treatments (MSG with BA, MSG with SALT, BA with SALT) as compared to the other treatments except L COMB. The accumulation of stresses is evident from the slower decrease in generation times of H MSG, H BA and H SALT when contrasted with H COMB.

In contrast with H COMB, our results illustrated that H MSG induced the lowest decrease in generation time as compared to the rest of the treatments, indicating that the presence of MSG is aiding the growth of cells. It is likely that glutamate serves as an additional source of nutrient for the *E. coli* cells. Thus, H MSG may be causing the least amount of stress to the cells since the treatment actually helps the cells to grow better and a low level of stress may lead to a lower rate of adaptation, which explains the lowest decrease in generation time. This may also suggests that H MSG is a more favorable environment for *E. coli* to grow compared to the basal NB (L SALT). However, this does not apply for L MSG which induces the second most stressful conditions to cells (Figure 4.3). A possible explanation

could be that low amounts of MSG (~0.0025% w/v) causes a significant amount of stress to *E. coli* cells. Keeping in mind that L MSG is more stressful than H MSG, L MSG treated cells when swapped into H MSG media would be a swap from a higher level of stress to a lower level of stress. The resulting effects is shown in Figure 4.10 where the initial generation time is the lowest (237 min), suggesting that the cells experience very little stress when swapping to H MSG media.

Our results shows that high concentration treated cells swapped into low treatment media showed declining generation time (Figure 4.11). This suggests that high concentration treated cells are likely to have adapted to its own individual treatments as the decreasing generation time suggests the presence of lesser stress. Although both high and low concentrations appear to result in some adaptations as measured by generation time, the rate of adaptation differs (Figure 4.10 and 4.11). The general decline in generation times of low treatment cells to high treatment media is steeper than that of the reverse.

Theoretically, low treatment cells inoculated into high treatment media should experience more stress than high treatment cells inoculated into low treatment media (Doudoroff, 1940), provided that the type of stress is similar. However, our results suggested otherwise. High concentration treated cells inoculated into low concentration treatment media appeared more stressed as a possible reason is that the type of stress may be different, even though low and high treatment contains same type of additives and differing only in concentration.

Focusing on the swap from high individual concentration treatment to combined high concentration treatment, the similar trend of adaption in high combination media suggested that the stress level of each high treatment is similar. This is supported by our results from generation time analysis (Figure 4.3) where the three high individual concentration treatments ranked last which is proportionate to the stress level.

Similarly, the stress levels of three individual low treatments are almost the same since the increase in generation time of the three swaps, from low individual treatment to low combination treatment, do not differ much. This is supported by generation time analysis

(Figure 4.3) where the three low individual treatments were grouped together suggesting that their stress levels may be similar.

#### **5.4 Cells Adapt After 25 Passages**

In the early phase (before Passage 25) of the experiment, the coefficient of variation of the Day 7 and Day 5 ratio for all treatment fluctuates randomly for at least 10 passages. Another reason could be the deceleration phase (Day 5) is too long before it reaches the stationary phase (Day 7), resulting in higher cell density in the Day 7 causing the ratio to be above 100%. Some *E. coli* may not survive in treatments in stationary phase (Day 5) for long period of time and cause the death phase (Day 7) to be brought forward, resulting in lower cell density in Day 7 culture and the ratio dropped below 100%. This is supported by generation time trend (Figure 4.3) where the fluctuations after Passage 10-20 were lesser as compared to before the 10<sup>th</sup> passage. This suggests that *E. coli* is trying to adapt to the different treatments but the varying peaks and valleys suggests that the growth is still not stable for each passage.

After passage 25, the variation of the Day 7 and Day 5 ratio for most treatments (all except L SALT and L COMB) reduced and stabilised at 100%. This suggests that *E. coli* in these treatments had gradually adapted to the environment which is supported by the generation time analysis where the fluctuations in generation times stabilized approximately after passage 25. This also suggests that the maximum cell density potential of each treatment was reached within 25 passages, leaving cells in stationary phase from Day 5 to Day 7. A possible reason for the fluctuations in L SALT is that the maximum potential of the cells growing in L SALT has not been reached since from the experiment on extended viability of *E. coli* cells in NB, it was shown that cells continue to divide up until 25 days in culture which is representative of the maximum growth potential.

Evidence of adaptation can be seen from the generation times of the eight treatments that were estimated on every third passage by using the cell size correction formulae to determine the cell density at intervals where it showed a general trend of different rates of decreasing generation across passages. The shortening in generation time trend indicates

that the *E. coli* cells are adapting to their respective environment such that they can grow better.

### **5.5 Cells from Different Treatment Becomes Genetically Similar**

Our results from PCR/RFLP showed a converging trend in DI indicating that the *E. coli* from all treatments are getting similar (Figure 4.15) suggesting that they mutate in a similar manner. This suggests that they may evolve the same type of stress mechanism and DNA repair.

*E. coli* exposed to stresses would respond to counteract the effects. Tucker et al. (2006) had shown that *E. coli* in nitric oxide (NO) will reduce the NO to nitrous oxide under anaerobic conditions which is harmless to the cell. In another study, *E. coli* produces methylglyoxal to counteract toxic electrophiles (Ferguson, 1999).

The cells from all treatments may have experienced different types and levels of stresses, however, it is likely the cells might have adapted and activated similar stress-handling mechanism whether or not the type of stress is similar as a study (Zhang et al., 2006) found a group of 66 genes that was activated in both oxidative and heat stress in *Desulfovibrio vulgaris* suggesting the presence of a general stress response. In addition, Cuny et al. (2007) suggested the presence of global stress response in *E. coli*. This is despite the cascades that regulate gene expression in *E. coli* in response to stresses such as heat, pH and salt are unknown (Ahmad, 1999).

However, the similar response and mutations to the *E. coli* of the treatments could not be determined for the whole genome. Primers 5, 6, and 7 amplified 0.37% of the whole genome. Hence, this study can only imply that areas of the genome that were amplified and analysis were approaching genetic similarity. The genes responsible for stress-handling mechanism may also not be present in the amplified regions of the genome. It is unlikely that the genetic distance of *E. coli* in the eight treatments reaches zero as the declining trend is likely to taper off. In addition, spontaneous mutation and the selective environment may prohibit them from being identical.

Statistical analysis of the selected comparisons indicates that the effects of all the treatments are non-significant except 10BA + S (Table 4.17). Statistical tests for MSG and BA effect suggested that different gels provided constant and reliable results; thus, providing reliability in our study.

Statistical tests suggest that MSG and BA, and MSG and salt are likely to interact with each other. However, 10BA + S do not appear to have an interacting effect. This suggests that high combination media contains 10MSG + 10BA + S and the interacting effects of MSG and BA, and MSG and salt.

Results from swap analysis indicated that low salt cells when swapped to high combination media showed an increase in generation time. This suggests that the high stress in High combination media results in difficulties for the cells from L SALT to grow and caused an increase in doubling time. This might be due to the additional combined stress produced by MSG interacting with BA and S. The presence of the additional interacting stress of the combination treatment can also be seen from the analysis of generation time where the stress level of H COMB is much higher than the three individual high concentration treatments.

However, some interacting effects may not be hindering the growth of the bacteria. BA kills bacteria by lowering the pH of the media whereas MSG has the effects of pH resistance on the cells. Hence, presence of MSG may aid the growth of the *E. coli* living in low pH environment (Bhagwat et al., 2005) caused by the presence of BA. On the other hand, combined effects from MSG + S could be harmful to the cells as salt may increase the high sodium content provided by MSG in media. The high sodium environment actually changes the environment to be even more selective.

## **6. Recommendations**

There are several areas for improvement in this study. Firstly, an effective method of contamination check or contamination prevention would be needed. A suggestion would be to use crystal violet concurrently in the treatments media to prevent the growth of Gram positive bacteria during the experiments as it had been shown that Gram positive bacteria could be inhibited at the concentration of 1:100 000 (Packer, 1943). However, this has to be investigated as to whether crystal violet will affect the growth of *E. coli*.

Secondly, the concentrations of the low treatments can also be assessed to determine the lowest concentration where the treatments pose a significant stress on the cells. This is necessary since we do not know whether or not the low concentrations we used in this study are significant.

Lastly, improvement can be made to the coverage of the genome of *Escherichia coli* ATCC 8739 during the PCR and RFLP experiment. This study only covered a limited portion of 0.374% of the whole genome. Thus, in order to accurately study the changes of the genome throughout the passages, more coverage would be needed. Alternatively, SNP microarray study can be employed to efficiently detect the changes to the genome.

## **7. Conclusion**

In conclusion, our results have showed that *E. coli* cells from the eight treatments have evolved and adapted to their individual environments over the course of this study without any significant effects from the Nutrient Broth. Even though the same additive was used at two different concentrations, the types of stress were not the same despite the level of stress experienced by the cells of either high or low concentration treatment media were the same. In addition, MSG may provide as an additional source of nutrient and also counteracts the effects of BA. The genomic analysis by RFLP found that the cells from the various treatments may trigger a global stress response as the cells are getting genetically similar.

## 8. References

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## Appendix A – Extended Bacterial Culture in Different Medium

*Escherichia coli* were inoculated into 3 different media (Nutrient Broth, Brain-Heart Infusion broth and Luria-Bertani broth) and cultured for 6 weeks at 37°C. Viable cell counts were estimated weekly by serial dilution ( $10^{-2}$  to  $10^{-9}$ ) and spread plates. OD600 readings were recorded before viable cell count was performed. Tabulation of the full results are listed below.

Media	Days in Culture	OD 600 Reading	Viable Count by Spread Plate (CFU/ml)
Nutrient Broth (NB)	0 (Initial)	0.088	Not enumerated
	5	1.184	1.1e7
	13	1.209	4.9e8
	20	1.678	5.7e9
	27	1.338	3.8e9
	34	1.377	1.6e7
	41	1.744	2.3e7
Luria Bertani Broth (LB)	0 (Initial)	0.098	Not enumerated
	5	1.273	>3.0e10**
	13	1.103	1.1e9
	20	1.834	9.3e8
	27	1.771	1.1e8
	34	1.868	1.3e6
	41	1.945	1.1e5
Brain Heart Infusion (BHI)	0 (Initial)	0.157	Not enumerated
	5	1.735	3.8e9
	13	2.107	>3.0e10**
	20	2.395	1.9e9
	27	2.482	1.0e8
	34	2.370	2.9e8
	41	2.408	3.0e7

\*\* more than 300 CFU/ml at 10e8 dilution

## Appendix B – Number of Generations

This is the tabulation of number of log phase generation of the main culture experiment for the 8 different treatments. OD600 readings were taken on every passage to estimate the number of log phase generations within the passage. In total, the number of log phase generation across 70 passages is estimated.

Passage Number	High Monosodium Glutamate (MSG)		Low Monosodium Glutamate (MSG)	
	OD 600 at Start of next Passage	Logarithmic Phase Generations	OD 600 at Start of next Passage	Logarithmic Phase Generations
0	0.898	6.49	0.898	6.58
1	0.717	6.65	0.813	6.45
2	0.721	6.57	0.624	6.50
3	0.656	6.78	0.523	6.67
4	0.789	6.94	0.536	7.03
5	1.250	6.46	0.894	6.52
6	0.931	6.79	0.749	6.79
7	1.168	6.78	0.919	6.60
8	1.489	6.23	0.863	6.07
9	0.768	6.95	0.428	7.45
10	1.240	6.61	1.237	6.34
11	1.175	6.29	0.771	6.24
12	0.689	6.38	0.469	6.80
13	0.500	6.35	0.555	7.00
14	0.375	6.98	0.887	6.87
15	0.526	6.67	1.281	6.10
16	0.540	7.17	0.583	7.21
17	1.123	6.06	1.339	6.36
18	0.507	7.06	0.849	6.85
19	0.867	6.91	1.182	6.70
20	1.346	6.54	1.301	6.63
21	1.120	6.48	1.264	6.22
22	0.872	6.84	0.668	7.04
23	1.190	6.30	1.199	6.43
24	0.706	6.93	0.849	6.91
25	1.084	6.71	1.313	6.68
26	1.210	6.56	1.411	6.12
27	1.048	6.17	0.635	6.62
28	0.547	7.23	0.616	7.12
29	1.257	6.34	1.235	6.53
30	0.779	6.85	1.016	6.63
31	1.071	6.71	0.988	6.85
32	1.193	6.51	1.398	6.49

33	0.965	6.17	1.079	6.38
34	0.517	7.30	0.725	7.07
35	1.315	6.65	1.432	6.12
36	1.319	6.68	0.644	7.10
37	1.405	6.70	1.273	6.73
38	1.565	6.56	1.476	6.58
39	1.343	6.61	1.310	6.64
40	1.262	6.73	1.307	6.70
41	1.467	6.16	1.454	6.15
42	0.686	6.60	0.670	6.66
43	0.647	7.18	0.681	7.15
44	1.478	6.39	1.510	6.52
45	0.958	6.72	1.210	6.48
46	1.074	6.78	0.928	6.85
47	1.344	6.62	1.306	6.27
48	1.296	6.66	0.726	7.04
49	1.330	6.69	1.342	6.68
50	1.450	6.56	1.438	6.60
51	1.249	6.58	1.320	6.55
52	1.120	6.58	1.117	6.59
53	1.010	6.64	1.030	6.50
54	1.009	6.52	0.831	6.78
55	0.837	7.00	1.018	6.86
56	1.510	6.53	1.478	6.56
57	1.233	6.65	1.269	6.65
58	1.241	6.75	1.281	6.72
59	1.497	6.38	1.470	6.16
60	0.951	6.91	0.687	7.11
61	1.490	6.63	1.423	6.65
62	1.441	6.61	1.426	6.59
63	1.352	6.61	1.298	6.49
64	1.279	6.71	0.997	6.59
65	1.432	6.58	0.915	6.84
66	1.281	6.23	1.249	6.27
67	0.686	6.97	0.703	7.04
68	1.116	6.69	1.295	6.37
69	1.214	6.65	0.841	6.74
70	1.218	6.49	0.975	6.58
Cumulative Generations		465.26		465.12

Passage Number	High Benzoic Acid (BA)		Low Benzoic Acid (BA)	
	OD 600 at Start of next Passage	Logarithmic Phase Generations	OD 600 at Start of next Passage	Logarithmic Phase Generations
0	0.898	6.44	0.898	6.67
1	0.678	6.75	0.939	6.45

2	0.780	6.84	0.714	6.52
3	1.044	6.27	0.609	6.62
4	0.617	7.09	0.595	7.12
5	1.177	6.19	1.179	6.09
6	0.612	7.03	0.540	7.13
7	1.066	6.63	1.059	6.32
8	1.041	5.80	0.659	6.89
9	0.374	6.94	0.927	6.00
10	0.501	7.13	0.419	7.15
11	0.940	6.10	0.760	6.44
12	0.467	6.78	0.585	6.75
13	0.545	6.78	0.667	6.87
14	0.639	7.06	0.913	6.86
15	1.170	6.06	1.300	5.80
16	0.519	7.27	0.421	7.31
17	1.242	6.53	0.959	6.16
18	1.030	6.69	0.510	7.28
19	1.117	6.70	1.233	6.24
20	1.223	6.32	0.674	6.66
21	0.748	6.51	0.691	6.64
22	0.630	7.03	0.684	6.96
23	1.100	6.41	1.083	6.56
24	0.775	6.64	0.947	6.77
25	0.768	6.98	1.162	6.73
26	1.300	6.63	1.342	6.57
27	1.268	6.62	1.172	6.61
28	1.220	6.55	1.100	6.78
29	1.045	6.60	1.389	6.43
30	0.981	6.69	0.972	6.22
31	1.057	5.76	0.548	7.26
32	0.362	7.60	1.349	6.15
33	1.183	6.24	0.640	6.71
34	0.652	7.14	0.698	7.09
35	1.384	6.61	1.391	6.63
36	1.294	6.69	1.354	6.59
37	1.402	6.65	1.233	6.71
38	1.406	6.59	1.397	6.63
39	1.271	6.64	1.359	6.61
40	1.266	6.65	1.288	6.70
41	1.274	6.25	1.437	6.26
42	0.695	6.58	0.777	6.60
43	0.636	7.18	0.733	7.09
44	1.435	6.59	1.494	6.55
45	1.314	6.47	1.266	6.56
46	0.981	6.83	1.099	6.78
47	1.332	6.24	1.386	6.66

48	0.713	7.03	1.418	6.52
49	1.296	6.70	1.139	6.75
50	1.431	6.56	1.375	6.63
51	1.239	6.33	1.338	6.54
52	0.759	6.99	1.115	6.71
53	1.314	6.40	1.244	6.46
54	0.882	6.80	0.920	6.71
55	1.134	6.79	1.017	6.76
56	1.476	6.56	1.232	6.68
57	1.273	6.65	1.313	6.66
58	1.296	6.71	1.356	6.69
59	1.465	6.47	1.468	6.15
60	1.078	6.81	0.683	6.64
61	1.424	6.64	0.680	7.12
62	1.421	6.44	1.425	6.62
63	1.013	6.79	1.369	6.46
64	1.284	6.63	1.007	6.84
65	1.247	6.60	1.411	6.13
66	1.162	6.30	0.642	7.06
67	0.699	7.06	1.193	6.72
68	1.329	6.56	1.361	6.55
69	1.144	6.61	1.163	6.71
70	1.075	6.44	1.306	6.67
Cumulative Generations		465.18		465.30

Passage Number	High Salt		Low Salt	
	OD 600 at Start of next Passage	Logarithmic Phase Generations	OD 600 at Start of next Passage	Logarithmic Phase Generations
0	0.898	6.56	0.898	6.30
1	0.797	6.81	0.565	6.80
2	1.016	6.72	0.689	6.90
3	1.156	6.61	1.000	6.12
4	1.094	6.75	0.501	7.20
5	1.319	6.42	1.060	6.64
6	0.911	6.28	1.055	6.62
7	0.558	7.18	1.009	6.81
8	1.193	6.62	1.341	6.45
9	1.145	6.43	0.969	6.50
10	0.822	6.75	0.788	6.68
11	0.968	6.48	0.833	6.15
12	0.757	6.76	0.452	7.08
13	0.898	6.45	0.766	6.87
14	0.682	7.04	1.086	6.70
15	1.247	5.87	1.189	5.78
16	0.441	7.43	0.396	7.48

17	1.270	6.57	1.135	6.28
18	1.129	6.56	0.665	6.96
19	0.983	6.85	1.058	6.81
20	1.389	6.63	1.398	6.50
21	1.345	6.61	1.100	6.57
22	1.268	6.59	0.974	6.81
23	1.165	6.33	1.280	6.36
24	0.728	6.89	0.820	6.89
25	1.055	6.47	1.216	6.67
26	0.808	6.89	1.281	6.56
27	1.191	6.56	1.106	6.72
28	1.042	6.78	1.262	6.32
29	1.309	6.39	0.763	6.79
30	0.862	6.69	0.945	6.79
31	0.926	6.86	1.197	6.69
32	1.319	6.42	1.289	6.54
33	0.915	6.62	1.078	6.31
34	0.887	6.90	0.667	7.12
35	1.348	6.62	1.385	6.63
36	1.300	6.63	1.355	6.51
37	1.276	6.63	1.070	6.73
38	1.248	6.66	1.233	6.69
39	1.281	6.54	1.331	6.60
40	1.067	6.78	1.234	6.32
41	1.356	6.37	0.745	6.78
42	0.872	6.48	0.913	6.35
43	0.695	7.09	0.614	7.21
44	1.399	6.49	1.446	6.51
45	1.067	6.38	1.141	6.84
46	0.719	7.04	1.623	6.54
47	1.327	6.64	1.350	6.66
48	1.309	6.67	1.385	6.62
49	1.372	6.65	1.327	6.70
50	1.380	6.60	1.462	6.58
51	1.271	6.15	1.293	6.30
52	0.611	7.05	0.754	6.96
53	1.092	6.65	1.220	6.45
54	1.103	6.76	0.900	6.83
55	1.344	6.62	1.221	6.71
56	1.289	6.63	1.378	6.60
57	1.259	6.67	1.279	6.66
58	1.319	6.66	1.324	6.70
59	1.369	6.37	1.476	6.11
60	0.878	6.50	0.651	7.12
61	0.720	6.96	1.344	6.68
62	1.162	6.54	1.425	6.60

63	0.989	6.78	1.319	6.19
64	1.243	6.45	0.666	7.11
65	0.906	6.70	1.348	6.63
66	0.989	6.44	1.310	6.25
67	0.732	6.98	0.715	7.07
68	1.235	6.38	1.386	6.55
69	0.811	6.83	1.167	6.67
70	1.074	6.56	1.214	6.30
Cumulative Generations		465.18		465.62

Passage Number	High Combination		Low Combination	
	OD 600 at Start of next Passage	Logarithmic Phase Generations	OD 600 at Start of next Passage	Logarithmic Phase Generations
0	0.898	6.54	0.898	6.49
1	0.771	6.77	0.719	6.83
2	0.926	6.71	0.940	6.76
3	1.030	6.61	1.139	6.54
4	0.978	6.85	0.961	6.68
5	1.372	6.02	1.020	6.69
6	0.549	7.06	1.098	6.53
7	0.961	6.38	0.911	6.89
8	0.657	0.00	1.364	6.40
9	0.168	8.80	0.909	6.67
10	1.117	6.67	0.953	6.60
11	1.167	6.29	0.888	6.55
12	0.685	6.88	0.779	6.34
13	0.953	6.90	0.530	7.20
14	1.470	6.59	1.154	6.65
15	1.343	6.09	1.163	6.01
16	0.589	7.20	0.489	7.35
17	1.350	6.29	1.311	6.08
18	0.766	6.81	0.574	6.47
19	0.979	6.85	0.472	7.40
20	1.375	6.50	1.351	6.51
21	1.068	6.53	1.082	6.29
22	0.889	6.83	0.651	7.06
23	1.201	5.85	1.212	6.48
24	0.426	7.40	0.930	6.84
25	1.136	6.67	1.280	6.62
26	1.190	6.35	1.227	6.13
27	0.761	6.94	0.583	7.09
28	1.208	6.71	1.102	6.74
29	1.348	6.34	1.292	6.57
30	0.823	6.72	1.135	6.16

31	0.918	6.84	0.573	6.94
32	1.260	6.34	0.849	6.64
33	0.785	6.66	0.845	6.52
34	0.803	6.95	0.707	6.94
35	1.305	6.65	1.101	6.75
36	1.324	6.39	1.324	6.60
37	0.870	6.94	1.219	6.75
38	1.421	6.54	1.462	6.59
39	1.185	6.64	1.328	6.09
40	1.185	6.34	0.590	7.20
41	0.742	6.79	1.352	6.33
42	0.914	6.42	0.821	6.45
43	0.664	7.09	0.630	7.18
44	1.315	6.60	1.433	6.52
45	1.210	6.45	1.159	6.57
46	0.892	6.90	1.021	6.81
47	1.365	6.20	1.361	6.57
48	0.686	7.07	1.193	6.70
49	1.318	6.69	1.321	6.69
50	1.437	6.49	1.446	6.34
51	1.103	6.52	0.869	6.36
52	0.903	6.84	0.595	6.76
53	1.240	6.63	0.687	6.59
54	1.201	6.69	0.639	7.09
55	1.312	6.66	1.230	6.71
56	1.356	6.58	1.378	6.60
57	1.218	6.55	1.266	6.63
58	1.043	6.84	1.239	6.39
59	1.452	6.11	0.830	6.53
60	0.643	7.11	0.711	6.57
61	1.291	6.70	0.646	7.16
62	1.421	6.07	1.432	6.14
63	0.597	7.18	0.662	7.07
64	1.323	6.62	1.266	6.68
65	1.261	6.15	1.348	6.61
66	0.608	6.79	1.260	6.22
67	0.734	7.02	0.666	7.12
68	1.326	6.30	1.373	6.53
69	0.772	6.87	1.135	6.68
70	1.096	6.54	1.208	6.49
Cumulative Generations		460.34		465.25

## Appendix C – Generation Time Estimation

This is the tabulation of the log phase generation time of main culture experiment in 8 different treatments. On every 3<sup>rd</sup> passage, OD600 readings were taken regularly after subculture to monitor the log phase. This measures the time taken for one log phase generation to take place which is used to interpret the adaptability of the cells.

Passage	Generation Time in Minutes (Geometric Mean) of each Treatment							
	High MSG	Low MSG	High BA	Low BA	High Salt	Low Salt	High Comb	Low Comb
2	157.50	175.92	183.10	285.58	218.68	221.00	159.66	180.10
5	118.93	213.81	240.28	154.48	159.53	143.33	181.75	168.96
8	268.22	406.53	180.46	220.26	195.97	247.68	260.08	301.26
11	135.37	145.11	180.33	171.41	144.12	140.09	176.18	135.43
14	121.91	153.80	154.96	130.11	161.02	132.79	128.19	164.30
17	139.67	168.32	186.65	138.67	126.49	135.01	693.27	145.96
20	197.05	302.31	149.71	193.07	208.89	256.01	261.34	186.44
23	136.02	105.86	267.69	124.30	143.30	102.17	255.17	123.80
26	141.48	159.41	145.34	161.68	132.24	144.73	278.83	150.28
29	146.24	137.74	142.50	147.64	129.32	154.65	145.44	148.29
32	165.86	207.62	200.95	167.66	160.50	180.14	200.84	168.53
35	123.13	133.96	126.02	130.68	122.78	130.15	111.65	121.48
38	164.99	134.74	124.99	128.51	126.12	138.05	249.49	120.44
41	142.19	125.14	206.62	158.90	140.62	123.59	111.14	110.00
44	124.01	126.79	171.25	112.70	135.31	117.44	184.12	130.06
47	103.17	90.84	102.58	106.33	83.51	128.64	171.98	102.81
50	106.00	84.21	141.78	102.84	110.90	118.15	121.47	175.54
53	126.55	147.77	172.74	155.76	130.81	153.62	149.12	159.87
56	132.03	135.85	190.47	150.94	144.27	135.10	170.35	142.79
59	102.31	92.87	162.60	97.04	103.14	122.77	239.66	121.31
62	177.55	252.73	131.95	157.45	185.29	164.64	106.36	153.58
65	106.95	106.95	106.95	106.95	106.95	106.95	106.95	106.95
68	76.28	76.28	76.28	76.28	76.28	76.28	76.28	76.28

## Appendix D – Cell Density at Stationary Phase

This is the tabulation of cell density every 5 and 7 days of every passage. The cell density is estimated from the OD600 reading taken on 5 and 7 days of the passage. This could estimate the growth rate which is used to estimate the adaptability of the *E. coli* during stationary phase.

**Ratio of Day 7/ Day 5 Cell Density of High MSG Treatment**

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
1	0.876	0.919	1.12E+08	1.14E+08	102.23%
2	1.177	1.146	1.27E+08	1.26E+08	98.91%
4	1.090	1.249	1.23E+08	1.30E+08	105.76%
5	1.159	1.146	1.26E+08	1.26E+08	99.53%
7	1.202	1.153	1.28E+08	1.26E+08	98.31%
8	1.356	1.447	1.35E+08	1.38E+08	102.52%
10	1.109	1.212	1.24E+08	1.29E+08	103.73%
11	1.280	1.175	1.32E+08	1.27E+08	96.61%
13	1.343	1.244	1.34E+08	1.30E+08	97.02%
14	0.913	1.329	1.14E+08	1.34E+08	117.17%
16	1.305	1.225	1.33E+08	1.29E+08	97.51%
17	1.106	1.174	1.24E+08	1.27E+08	102.51%
19	1.375	1.335	1.35E+08	1.34E+08	98.86%
20	1.274	1.284	1.31E+08	1.32E+08	100.31%
22	0.857	1.056	1.11E+08	1.22E+08	109.84%
23	1.100	1.193	1.24E+08	1.28E+08	103.42%
25	1.318	1.343	1.33E+08	1.34E+08	100.74%
26	1.333	1.363	1.34E+08	1.35E+08	100.87%
28	1.271	1.227	1.31E+08	1.29E+08	98.60%
29	1.129	1.225	1.25E+08	1.29E+08	103.40%
31	1.203	1.284	1.28E+08	1.32E+08	102.65%
32	1.323	1.284	1.33E+08	1.32E+08	98.83%
34	1.328	1.327	1.34E+08	1.33E+08	99.97%
35	1.262	1.297	1.31E+08	1.32E+08	101.09%
37	1.371	1.354	1.35E+08	1.35E+08	99.52%
38	1.535	1.553	1.41E+08	1.42E+08	100.43%
43	1.053	1.099	1.21E+08	1.24E+08	101.84%
44	1.377	1.382	1.35E+08	1.36E+08	100.14%
46	1.327	1.309	1.33E+08	1.33E+08	99.47%
47	1.297	1.289	1.32E+08	1.32E+08	99.76%
49	1.327	1.322	1.33E+08	1.33E+08	99.85%
50	1.401	1.319	1.36E+08	1.33E+08	97.69%

51	1.260	1.247	1.31E+08	1.30E+08	99.59%
53	1.218	1.157	1.29E+08	1.26E+08	97.92%
55	1.425	1.313	1.37E+08	1.33E+08	96.89%
56	1.384	1.368	1.36E+08	1.35E+08	99.55%
58	1.346	1.307	1.34E+08	1.33E+08	98.86%
59	1.418	1.389	1.37E+08	1.36E+08	99.21%
61	1.357	1.393	1.35E+08	1.36E+08	101.01%
62	1.452	1.432	1.38E+08	1.37E+08	99.48%
64	1.266	1.242	1.31E+08	1.30E+08	99.24%
65	1.373	1.434	1.35E+08	1.38E+08	101.68%
67	1.441	1.416	1.38E+08	1.37E+08	99.34%
68	1.273	1.244	1.31E+08	1.30E+08	99.08%
70	1.269	1.360	1.31E+08	1.35E+08	102.75%

#### Ratio of Day 7/ Day 5 Cell Density of Low MSG Treatment

Passage	OD 600 at Day 5	OD 600 at Day 7	Cell Density at Day	Cell Density Day 7	Ratio of Day 7, Day 5 Cell Density
1	0.722	0.842	1.02E+08	1.10E+08	107.88%
2	1.001	0.958	1.19E+08	1.16E+08	98.07%
4	1.179	1.311	1.27E+08	1.33E+08	104.35%
5	0.945	1.073	1.16E+08	1.22E+08	105.72%
7	0.833	0.856	1.09E+08	1.11E+08	101.30%
8	0.833	0.907	1.09E+08	1.14E+08	104.06%
10	1.109	1.234	1.24E+08	1.30E+08	104.49%
11	0.903	0.771	1.13E+08	1.05E+08	92.73%
13	1.274	1.193	1.31E+08	1.28E+08	97.39%
14	1.155	1.083	1.26E+08	1.23E+08	97.34%
16	1.255	1.167	1.31E+08	1.27E+08	97.10%
17	1.237	1.249	1.30E+08	1.30E+08	100.39%
19	1.157	1.152	1.26E+08	1.26E+08	99.82%
20	1.312	1.260	1.33E+08	1.31E+08	98.41%
22	1.680	1.069	1.46E+08	1.22E+08	83.83%
23	1.080	1.088	1.23E+08	1.23E+08	100.31%
25	1.220	1.280	1.29E+08	1.32E+08	101.94%
26	1.407	1.410	1.37E+08	1.37E+08	100.08%
28	1.149	1.151	1.26E+08	1.26E+08	100.07%
29	1.180	1.235	1.27E+08	1.30E+08	101.87%
31	1.293	1.361	1.32E+08	1.35E+08	102.02%
32	1.377	1.368	1.35E+08	1.35E+08	99.75%
34	1.406	1.187	1.36E+08	1.28E+08	93.53%
35	1.273	1.377	1.31E+08	1.35E+08	103.12%
37	1.363	1.339	1.35E+08	1.34E+08	99.31%
38	1.459	1.458	1.38E+08	1.38E+08	99.97%
43	1.420	1.337	1.37E+08	1.34E+08	97.71%

44	1.411	1.431	1.37E+08	1.37E+08	100.54%
46	1.106	1.181	1.24E+08	1.27E+08	102.76%
47	1.270	1.265	1.31E+08	1.31E+08	99.84%
49	1.355	1.348	1.35E+08	1.34E+08	99.80%
50	1.411	1.266	1.37E+08	1.31E+08	95.86%
51	1.285	1.273	1.32E+08	1.31E+08	99.63%
53	0.999	1.016	1.19E+08	1.20E+08	100.74%
55	1.384	1.315	1.36E+08	1.33E+08	98.03%
56	1.406	1.338	1.36E+08	1.34E+08	98.11%
58	1.315	1.251	1.33E+08	1.30E+08	98.04%
59	1.401	1.391	1.36E+08	1.36E+08	99.73%
61	1.348	1.372	1.34E+08	1.35E+08	100.69%
62	1.416	1.398	1.37E+08	1.36E+08	99.51%
64	1.287	1.268	1.32E+08	1.31E+08	99.41%
65	1.266	1.289	1.31E+08	1.32E+08	100.72%
67	1.414	1.397	1.37E+08	1.36E+08	99.54%
68	1.261	1.207	1.31E+08	1.29E+08	98.26%
70	1.004	1.077	1.19E+08	1.23E+08	103.08%

#### Ratio of Day 7/ Day 5 Cell Density of High BA Treatment

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
1	0.754	0.849	1.04E+08	1.10E+08	105.95%
2	1.015	1.146	1.19E+08	1.26E+08	105.30%
4	1.255	1.208	1.31E+08	1.29E+08	98.48%
5	1.145	1.153	1.26E+08	1.26E+08	100.29%
7	1.155	1.134	1.26E+08	1.25E+08	99.24%
8	1.074	1.164	1.22E+08	1.27E+08	103.43%
10	1.088	1.159	1.23E+08	1.26E+08	102.68%
11	0.825	0.940	1.09E+08	1.15E+08	106.26%
13	1.297	1.250	1.32E+08	1.30E+08	98.55%
14	1.168	1.021	1.27E+08	1.20E+08	94.47%
16	1.266	1.224	1.31E+08	1.29E+08	98.66%
17	1.202	1.237	1.28E+08	1.30E+08	101.17%
19	1.256	1.257	1.31E+08	1.31E+08	100.03%
20	1.233	1.203	1.30E+08	1.28E+08	99.01%
22	1.001	0.998	1.19E+08	1.19E+08	99.87%
23	1.001	1.027	1.19E+08	1.20E+08	101.13%
25	1.363	1.355	1.35E+08	1.35E+08	99.77%
26	1.300	1.313	1.32E+08	1.33E+08	100.39%
28	1.299	1.280	1.32E+08	1.32E+08	99.42%
29	1.077	0.985	1.23E+08	1.18E+08	96.20%
31	1.220	1.330	1.29E+08	1.34E+08	103.49%
32	1.356	1.401	1.35E+08	1.36E+08	101.26%
34	1.314	1.318	1.33E+08	1.33E+08	100.12%

35	1.278	1.344	1.32E+08	1.34E+08	102.00%
37	1.357	1.339	1.35E+08	1.34E+08	99.48%
38	1.345	1.349	1.34E+08	1.34E+08	100.12%
43	1.271	1.227	1.31E+08	1.29E+08	98.60%
44	1.355	1.376	1.35E+08	1.35E+08	100.60%
46	1.235	1.213	1.30E+08	1.29E+08	99.28%
47	1.281	1.302	1.32E+08	1.32E+08	100.64%
49	1.314	1.335	1.33E+08	1.34E+08	100.62%
50	1.372	1.328	1.35E+08	1.34E+08	98.74%
51	1.128	1.150	1.25E+08	1.26E+08	100.81%
53	1.243	1.285	1.30E+08	1.32E+08	101.33%
55	1.399	1.328	1.36E+08	1.34E+08	98.01%
56	1.363	1.361	1.35E+08	1.35E+08	99.94%
58	1.310	1.281	1.33E+08	1.32E+08	99.12%
59	1.377	1.361	1.35E+08	1.35E+08	99.55%
61	1.328	1.380	1.34E+08	1.36E+08	101.50%
62	1.435	1.425	1.38E+08	1.37E+08	99.73%
64	1.170	1.225	1.27E+08	1.29E+08	101.89%
65	1.264	1.260	1.31E+08	1.31E+08	99.87%
67	1.405	1.369	1.36E+08	1.35E+08	99.01%
68	1.266	1.254	1.31E+08	1.31E+08	99.62%
70	0.999	1.035	1.19E+08	1.21E+08	101.56%

#### Ratio of Day 7/ Day 5 Cell Density of Low BA Treatment

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
1	0.979	0.976	1.18E+08	1.17E+08	99.86%
2	1.035	1.041	1.21E+08	1.21E+08	100.25%
4	1.086	1.341	1.23E+08	1.34E+08	108.94%
5	1.261	1.163	1.31E+08	1.27E+08	96.78%
7	1.166	1.119	1.27E+08	1.25E+08	98.31%
8	0.993	1.208	1.18E+08	1.29E+08	108.63%
10	0.664	0.975	9.74E+07	1.17E+08	120.57%
11	0.790	0.760	1.06E+08	1.04E+08	98.10%
13	1.368	1.291	1.35E+08	1.32E+08	97.76%
14	1.167	1.065	1.27E+08	1.22E+08	96.24%
16	1.190	1.112	1.28E+08	1.24E+08	97.23%
17	1.031	1.088	1.20E+08	1.23E+08	102.33%
19	1.209	1.238	1.29E+08	1.30E+08	100.96%
20	1.246	1.191	1.30E+08	1.28E+08	98.19%
22	0.812	0.978	1.08E+08	1.18E+08	108.99%
23	0.950	1.048	1.16E+08	1.21E+08	104.41%
25	1.260	1.319	1.31E+08	1.33E+08	101.82%
26	1.346	1.351	1.34E+08	1.34E+08	100.14%

28	1.142	1.203	1.26E+08	1.28E+08	102.16%
29	1.313	1.331	1.33E+08	1.34E+08	100.53%
31	1.239	1.313	1.30E+08	1.33E+08	102.33%
32	1.312	1.281	1.33E+08	1.32E+08	99.06%
34	1.328	1.220	1.34E+08	1.29E+08	96.69%
35	1.285	1.320	1.32E+08	1.33E+08	101.06%
37	1.244	1.273	1.30E+08	1.31E+08	100.92%
38	1.390	1.399	1.36E+08	1.36E+08	100.25%
43	1.434	1.399	1.38E+08	1.36E+08	99.06%
44	1.434	1.444	1.38E+08	1.38E+08	100.26%
46	1.295	1.292	1.32E+08	1.32E+08	99.91%
47	1.351	1.346	1.34E+08	1.34E+08	99.86%
49	1.397	1.401	1.36E+08	1.36E+08	100.11%
50	1.310	1.246	1.33E+08	1.30E+08	98.03%
51	1.241	1.256	1.30E+08	1.31E+08	100.48%
53	1.201	1.197	1.28E+08	1.28E+08	99.86%
55	1.440	1.407	1.38E+08	1.37E+08	99.12%
56	1.213	1.096	1.29E+08	1.23E+08	95.89%
58	1.402	1.345	1.36E+08	1.34E+08	98.41%
59	1.391	1.384	1.36E+08	1.36E+08	99.81%
61	1.410	1.309	1.37E+08	1.33E+08	97.16%
62	1.473	1.438	1.39E+08	1.38E+08	99.10%
64	1.260	1.284	1.31E+08	1.32E+08	100.75%
65	1.319	1.411	1.33E+08	1.37E+08	102.64%
67	1.255	1.354	1.31E+08	1.35E+08	103.03%
68	1.346	1.335	1.34E+08	1.34E+08	99.68%
70	1.256	1.257	1.31E+08	1.31E+08	100.03%

#### Ratio of Day 7/ Day 5 Cell Density of High Salt Treatment

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
1	1.038	1.042	1.21E+08	1.21E+08	100.17%
2	0.826	0.826	1.09E+08	1.09E+08	100.00%
4	1.221	1.284	1.29E+08	1.32E+08	102.03%
5	1.223	1.162	1.29E+08	1.27E+08	97.94%
7	0.788	1.184	1.06E+08	1.28E+08	119.97%
8	1.190	1.193	1.28E+08	1.28E+08	100.10%
10	0.833	1.063	1.09E+08	1.22E+08	111.64%
11	0.880	0.968	1.12E+08	1.17E+08	104.43%
13	1.208	1.130	1.29E+08	1.25E+08	97.29%
14	1.265	1.276	1.31E+08	1.31E+08	100.34%
16	1.141	1.090	1.26E+08	1.23E+08	98.10%
17	1.214	1.156	1.29E+08	1.26E+08	98.02%
19	1.163	1.115	1.27E+08	1.24E+08	98.26%

20	1.264	1.240	1.31E+08	1.30E+08	99.24%
22	1.198	1.084	1.28E+08	1.23E+08	95.93%
23	1.012	0.968	1.19E+08	1.17E+08	98.06%
25	1.224	1.244	1.29E+08	1.30E+08	100.65%
26	1.301	1.245	1.32E+08	1.30E+08	98.27%
28	1.119	1.158	1.25E+08	1.26E+08	101.43%
29	1.231	1.219	1.30E+08	1.29E+08	99.61%
31	1.202	1.220	1.28E+08	1.29E+08	100.60%
32	1.345	1.291	1.34E+08	1.32E+08	98.41%
34	1.246	1.176	1.30E+08	1.27E+08	97.68%
35	1.248	1.238	1.30E+08	1.30E+08	99.68%
37	1.171	1.145	1.27E+08	1.26E+08	99.08%
38	1.274	1.266	1.31E+08	1.31E+08	99.75%
43	1.367	1.246	1.35E+08	1.30E+08	96.42%
44	1.257	1.335	1.31E+08	1.34E+08	102.40%
46	1.278	1.262	1.32E+08	1.31E+08	99.50%
47	1.268	1.237	1.31E+08	1.30E+08	99.02%
49	1.338	1.317	1.34E+08	1.33E+08	99.38%
50	1.355	1.295	1.35E+08	1.32E+08	98.25%
51	1.197	1.193	1.28E+08	1.28E+08	99.86%
53	1.210	1.169	1.29E+08	1.27E+08	98.60%
55	1.323	1.400	1.33E+08	1.36E+08	102.21%
56	1.296	1.104	1.32E+08	1.24E+08	93.68%
58	1.305	1.239	1.33E+08	1.30E+08	97.96%
59	1.324	1.264	1.33E+08	1.31E+08	98.19%
61	1.322	1.301	1.33E+08	1.32E+08	99.37%
62	1.216	1.173	1.29E+08	1.27E+08	98.54%
64	1.031	1.187	1.20E+08	1.28E+08	106.11%
65	1.167	1.200	1.27E+08	1.28E+08	101.15%
67	1.318	1.295	1.33E+08	1.32E+08	99.31%
68	1.168	1.153	1.27E+08	1.26E+08	99.47%
70	0.825	0.982	1.09E+08	1.18E+08	108.36%

#### Ratio of Day 7/ Day 5 Cell Density of Low Salt Treatment

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
1	1.151	1.117	1.26E+08	1.24E+08	98.76%
2	0.933	0.958	1.15E+08	1.16E+08	101.20%
4	0.985	1.227	1.18E+08	1.29E+08	109.71%
5	1.074	1.096	1.22E+08	1.23E+08	100.86%
7	1.150	1.016	1.26E+08	1.20E+08	94.87%
8	1.198	1.249	1.28E+08	1.30E+08	101.70%
10	0.809	0.919	1.08E+08	1.14E+08	106.17%
11	0.893	0.833	1.13E+08	1.09E+08	96.79%
13	1.259	1.195	1.31E+08	1.28E+08	97.92%

14	1.177	1.176	1.27E+08	1.27E+08	99.97%
16	1.275	1.177	1.31E+08	1.27E+08	96.83%
17	1.035	1.078	1.21E+08	1.23E+08	101.76%
19	1.167	1.188	1.27E+08	1.28E+08	100.73%
20	1.313	1.330	1.33E+08	1.34E+08	100.50%
22	1.025	0.981	1.20E+08	1.18E+08	98.09%
23	1.112	1.184	1.24E+08	1.28E+08	102.63%
25	1.145	1.205	1.26E+08	1.28E+08	102.12%
26	1.293	1.289	1.32E+08	1.32E+08	99.88%
28	1.220	1.196	1.29E+08	1.28E+08	99.20%
29	1.311	1.245	1.33E+08	1.30E+08	97.97%
31	1.228	1.304	1.29E+08	1.33E+08	102.42%
32	1.381	1.341	1.36E+08	1.34E+08	98.87%
34	1.329	1.192	1.34E+08	1.28E+08	95.75%
35	1.289	1.318	1.32E+08	1.33E+08	100.88%
37	1.317	1.301	1.33E+08	1.32E+08	99.52%
38	1.339	1.288	1.34E+08	1.32E+08	98.49%
43	0.834	1.257	1.09E+08	1.31E+08	119.58%
44	1.381	1.395	1.36E+08	1.36E+08	100.39%
46	1.135	1.335	1.25E+08	1.34E+08	106.75%
47	1.339	1.345	1.34E+08	1.34E+08	100.17%
49	1.346	1.348	1.34E+08	1.34E+08	100.06%
50	1.428	1.360	1.37E+08	1.35E+08	98.15%
51	0.970	1.219	1.17E+08	1.29E+08	110.17%
53	1.239	1.271	1.30E+08	1.31E+08	101.02%
55	1.387	1.360	1.36E+08	1.35E+08	99.25%
56	1.269	1.273	1.31E+08	1.31E+08	100.13%
58	1.311	1.280	1.33E+08	1.32E+08	99.06%
59	1.397	1.380	1.36E+08	1.36E+08	99.53%
61	1.300	1.337	1.32E+08	1.34E+08	101.11%
62	1.462	1.432	1.39E+08	1.37E+08	99.22%
64	0.987	1.269	1.18E+08	1.31E+08	111.10%
65	1.335	1.411	1.34E+08	1.37E+08	102.16%
67	1.420	1.406	1.37E+08	1.36E+08	99.62%
68	1.352	1.306	1.34E+08	1.33E+08	98.66%
70	1.096	1.177	1.23E+08	1.27E+08	103.01%

#### Ratio of Day 7/ Day 5 Cell Density of High Combination Treatment

Passage	OD 600 Day 5	OD 600 Day 7	Cell Density at Day 5	Cell Density at Day 7	Ratio of Day 7, Day 5 Cell Density
1	1.067	1.131	1.22E+08	1.25E+08	102.49%
2	1.082	1.220	1.23E+08	1.29E+08	105.10%
4	1.315	1.214	1.33E+08	1.29E+08	96.87%
5	1.229	1.189	1.29E+08	1.28E+08	98.67%
7	1.238	1.177	1.30E+08	1.27E+08	97.97%

8	1.214	1.333	1.29E+08	1.34E+08	103.78%
10	0.788	0.998	1.06E+08	1.19E+08	111.59%
11	0.891	1.167	1.13E+08	1.27E+08	112.48%
13	1.322	1.210	1.33E+08	1.29E+08	96.54%
14	1.343	1.372	1.34E+08	1.35E+08	100.83%
16	1.166	1.070	1.27E+08	1.22E+08	96.47%
17	1.235	1.198	1.30E+08	1.28E+08	98.78%
19	1.318	1.230	1.33E+08	1.30E+08	97.29%
20	1.247	1.259	1.30E+08	1.31E+08	100.38%
22	0.777	1.043	1.06E+08	1.21E+08	114.54%
23	1.032	1.037	1.20E+08	1.21E+08	100.21%
25	1.113	1.111	1.24E+08	1.24E+08	99.92%
26	1.147	1.161	1.26E+08	1.27E+08	100.50%
28	1.094	1.054	1.23E+08	1.21E+08	98.43%
29	1.240	1.229	1.30E+08	1.29E+08	99.64%
31	1.153	1.219	1.26E+08	1.29E+08	102.30%
32	1.269	1.288	1.31E+08	1.32E+08	100.59%
34	1.311	0.714	1.33E+08	1.01E+08	76.15%
35	1.188	1.166	1.28E+08	1.27E+08	99.24%
37	1.160	1.076	1.26E+08	1.23E+08	96.90%
38	1.344	1.349	1.34E+08	1.34E+08	100.14%
43	1.270	1.190	1.31E+08	1.28E+08	97.41%
44	1.188	1.210	1.28E+08	1.29E+08	100.75%
46	1.046	1.063	1.21E+08	1.22E+08	100.69%
47	1.269	1.239	1.31E+08	1.30E+08	99.05%
49	1.226	1.193	1.29E+08	1.28E+08	98.90%
50	1.344	1.307	1.34E+08	1.33E+08	98.91%
51	1.189	1.090	1.28E+08	1.23E+08	96.45%
53	1.211	1.166	1.29E+08	1.27E+08	98.47%
55	1.312	1.361	1.33E+08	1.35E+08	101.44%
56	1.409	1.118	1.37E+08	1.25E+08	91.17%
58	1.345	1.269	1.34E+08	1.31E+08	97.74%
59	1.352	1.290	1.34E+08	1.32E+08	98.18%
61	1.261	1.300	1.31E+08	1.32E+08	101.21%
62	1.453	1.395	1.38E+08	1.36E+08	98.46%
64	1.235	1.308	1.30E+08	1.33E+08	102.31%
65	1.284	1.335	1.32E+08	1.34E+08	101.54%
67	1.332	1.309	1.34E+08	1.33E+08	99.32%
68	1.244	1.240	1.30E+08	1.30E+08	99.87%
70	0.906	0.934	1.14E+08	1.15E+08	101.40%

**Ratio of Day 7/ Day 5 Cell Density of Low Combination Treatment**

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
1	1.090	1.137	1.23E+08	1.25E+08	101.79%
2	1.031	1.086	1.20E+08	1.23E+08	102.25%
4	1.260	1.215	1.31E+08	1.29E+08	98.55%
5	1.201	1.185	1.28E+08	1.28E+08	99.45%
7	0.972	1.099	1.17E+08	1.24E+08	105.46%
8	1.242	1.296	1.30E+08	1.32E+08	101.71%
10	0.817	0.969	1.08E+08	1.17E+08	108.22%
11	0.643	0.888	9.57E+07	1.13E+08	117.59%
13	1.142	1.327	1.26E+08	1.33E+08	106.23%
14	1.291	1.273	1.32E+08	1.31E+08	99.45%
16	1.267	1.184	1.31E+08	1.28E+08	97.30%
17	1.209	1.224	1.29E+08	1.29E+08	100.50%
19	1.242	1.289	1.30E+08	1.32E+08	101.49%
20	1.279	1.288	1.32E+08	1.32E+08	100.28%
22	0.840	0.901	1.10E+08	1.13E+08	103.33%
23	1.075	1.124	1.22E+08	1.25E+08	101.90%
25	1.155	1.220	1.26E+08	1.29E+08	102.26%
26	1.271	1.297	1.31E+08	1.32E+08	100.80%
28	1.189	1.173	1.28E+08	1.27E+08	99.45%
29	1.256	1.292	1.31E+08	1.32E+08	101.13%
31	0.790	1.236	1.06E+08	1.30E+08	121.93%
32	1.086	0.984	1.23E+08	1.18E+08	95.82%
34	1.336	0.913	1.34E+08	1.14E+08	85.17%
35	1.032	1.164	1.20E+08	1.27E+08	105.21%
37	1.257	1.223	1.31E+08	1.29E+08	98.91%
38	1.423	1.427	1.37E+08	1.37E+08	100.11%
43	0.938	1.235	1.15E+08	1.30E+08	112.43%
44	1.362	1.027	1.35E+08	1.20E+08	89.08%
46	1.257	1.327	1.31E+08	1.33E+08	102.16%
47	1.209	1.312	1.29E+08	1.33E+08	103.31%
49	1.329	1.351	1.34E+08	1.34E+08	100.64%
50	1.420	1.335	1.37E+08	1.34E+08	97.65%
51	1.252	1.252	1.30E+08	1.30E+08	100.00%
53	1.151	1.189	1.26E+08	1.28E+08	101.34%
55	1.279	1.235	1.32E+08	1.30E+08	98.61%
56	1.292	1.246	1.32E+08	1.30E+08	98.57%
58	1.326	1.281	1.33E+08	1.32E+08	98.65%
59	1.350	1.331	1.34E+08	1.34E+08	99.45%
61	1.380	1.391	1.36E+08	1.36E+08	100.31%
62	1.464	1.474	1.39E+08	1.39E+08	100.26%
64	1.104	1.220	1.24E+08	1.29E+08	104.21%
65	1.279	1.285	1.32E+08	1.32E+08	100.19%
67	1.414	1.409	1.37E+08	1.37E+08	99.86%

68	1.323	1.323	1.33E+08	1.33E+08	100.00%
70	1.055	1.068	1.22E+08	1.22E+08	100.53%

## Appendix E – Swap Treatments

This is the tabulation of the generation time of swap experiment. On every 5<sup>th</sup> to 7<sup>th</sup> passage, OD600 readings were taken regularly after subculture to monitor the log phase. This measures the time taken for 1 generation to take place which is used to estimate the adaptability of the cells in the media. Below are the geometric means of the generation time for every swap experiment.

### Experiment 1: Low Salt to Different treatments

Swap Count	Generation Time in Minutes (Geometric Mean) of each Treatment					
	H MSG	L MSG	H BA	L BA	H COMB	L COMB
1 (26/6)	102.98	124.25	411.52	101.23	108.14	93.28
2 (10/7)	150.75	138.55	131.21	130.40	130.36	118.87
3 (24/7)	183.81	353.12	651.05	209.49	782.63	166.17
4 (7/8)	100.98	215.71	128.41	106.39	139.49	115.33
5 (21/8)	148.07	146.37	626.92	156.47	728.81	179.42
6 (4/9)	266.11	408.51	274.49	269.80	284.70	277.51
7 (18/9)	167.63	173.28	268.40	182.04	249.60	167.84
8 (2/10)	167.49	121.93	176.25	157.35	170.89	176.08
9 (16/10)	225.55	213.46	570.36	208.08	347.23	218.94
10 (30/10)	119.07	111.94	308.40	102.74	185.48	106.20
11 (13/11)	77.68	129.80	385.78	105.37	507.24	111.23
12 (27/11)	174.50	161.34	170.06	184.74	301.63	190.99

### Experiment 2: High to Low, Low to High

Swap Count	Generation Time in Minutes (Geometric Mean) of each Treatment							
	H MSG Cells to L MSG	L MSG Cells to H MSG	H BA Cells to L BA	L BA Cells to H BA	H Salt Cells to L Salt	L Salt Cells to H Salt	H Comb Cells to L Comb	L Comb Cells to H Comb
1 (26/6)	101.17	124.50	87.87	589.45	94.57	103.99	131.69	385.69
2 (10/7)	400.54	273.56	285.02	322.78	223.56	275.54	371.90	256.03
3 (24/7)	148.34	179.55	171.64	399.06	279.39	651.05	122.73	412.93
4 (7/8)	90.74	115.23	92.41	168.82	90.09	91.04	101.43	143.92
5 (21/8)	158.67	192.13	150.34	291.40	155.98	132.83	142.67	307.08
6 (4/9)	241.23	461.63	266.17	237.19	211.53	274.50	262.25	232.69
7 (18/9)	237.33	399.49	103.60	239.73	360.03	268.40	69.97	113.81
8 (2/10)	112.58	140.99	177.27	232.75	146.77	163.79	147.00	310.65
9 (16/10)	164.52	259.89	77.49	219.35	254.46	203.38	170.53	179.16
10(30/10)	104.86	105.31	103.54	351.34	88.72	141.24	125.16	169.70
11(13/11)	93.64	82.80	90.74	351.22	114.03	146.63	89.55	156.38
12 (27/11)	179.77	168.06	167.85	198.90	180.04	197.73	236.86	169.14

### Experiment 3: High Single Treatments to High Combination Treatment

Swap Count	Generation Time in Minutes (Geometric Mean) of each Treatment		
	H MSG Cells to High Comb Media	H BA Cells to High Comb Media	High Salt Cells to High Comb Media
1 (26/6)	184.93	625.80	184.27
2 (10/7)	2070.26	1661.56	974.75
3 (24/7)	345.18	464.10	450.52
4 (7/8)	113.65	115.18	155.71
5 (21/8)	518.56	166.63	255.41
6 (4/9)	314.69	246.25	232.72
7 (18/9)	250.32	202.15	207.87
8 (2/10)	195.35	3029.77	195.60
9 (16/10)	230.07	202.15	207.87
10 (30/10)	196.64	145.29	212.31
11 (13/11)	168.97	144.07	190.73
12 (27/11)	222.97	290.11	211.56

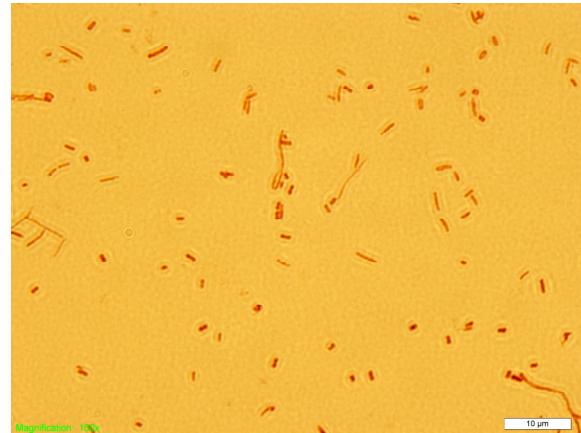
### Experiment 4: Low Single Treatments to Low Combination Treatment

Swap Count	Generation Time in Minutes (Geometric Mean) of each Treatment		
	L MSG Cells to Low Comb Media	L BA Cells to Low Comb Media	L Salt Cells to Low Comb Media
1 (26/6)	133.78	96.52	101.13
2 (10/7)	156.55	127.39	2754.07
3 (24/7)	194.40	182.16	150.71
4 (7/8)	85.79	91.87	74.92
5 (21/8)	194.40	182.16	150.71
6 (4/9)	444.13	375.79	505.05
7 (18/9)	235.86	278.94	325.44
8 (2/10)	144.97	115.60	162.14
9 (16/10)	190.81	278.94	325.44
10 (30/10)	113.02	153.55	111.50
11 (13/11)	90.06	207.67	123.90
12 (27/11)	342.92	374.97	299.14

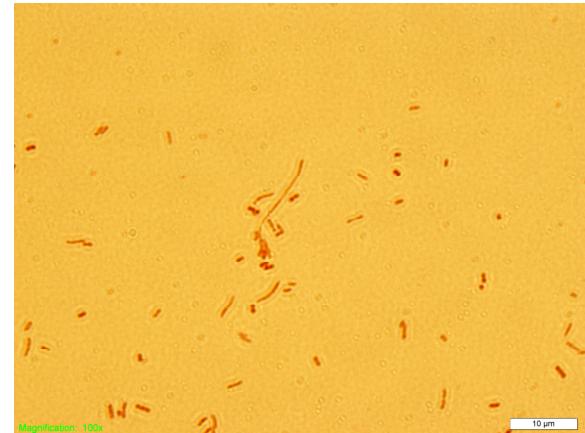
## Appendix F – Gram Staining Pictures

Contamination check is conducted by Gram staining ensure only Gram negative bacteria are growing in the media. Below is the Gram staining pictures on passage 69.

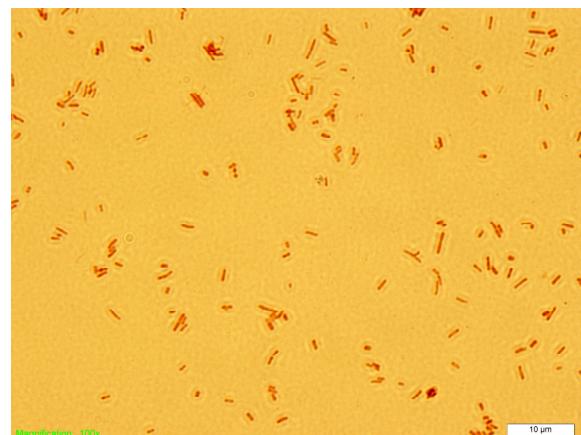
### Passage 69



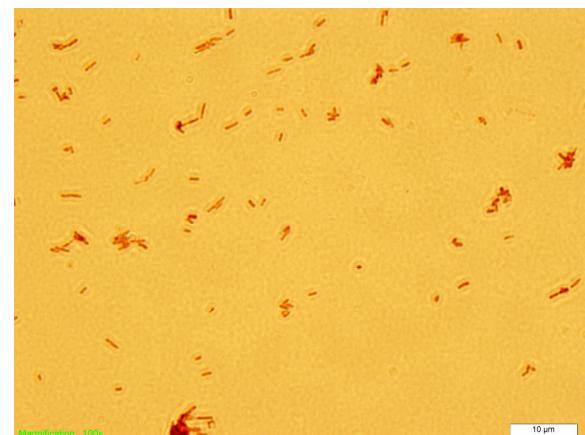
High MSG



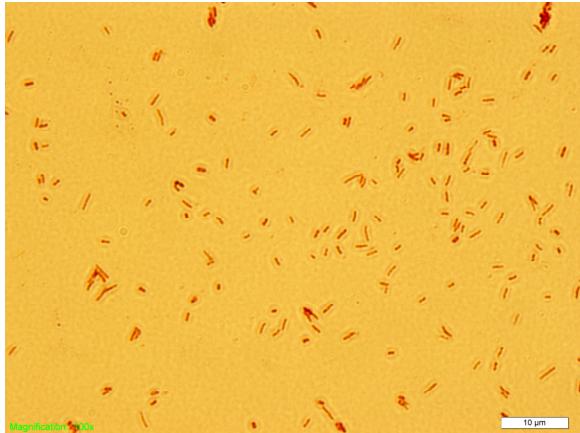
Low MSG



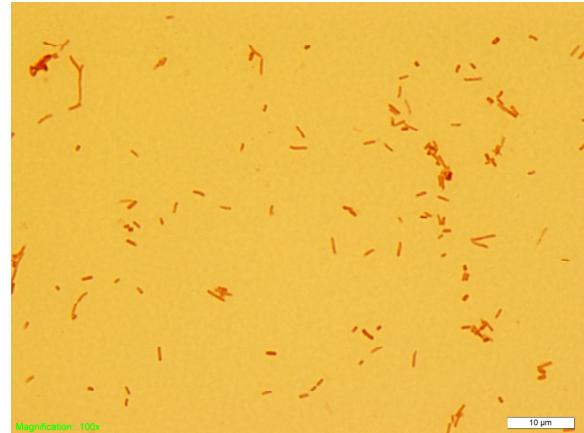
High BA



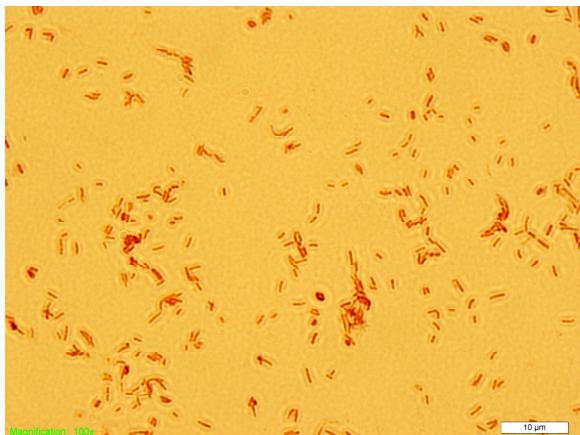
Low BA



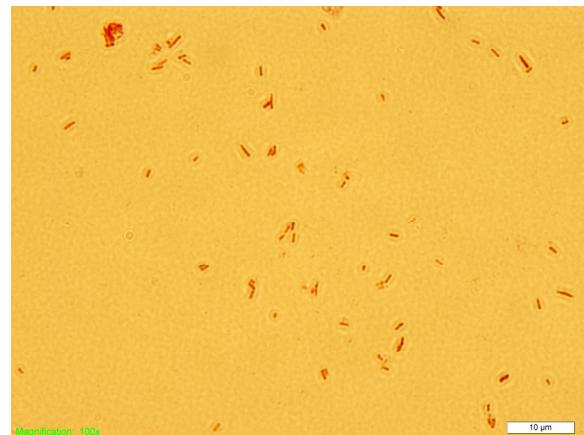
High Salt



Low Salt



High Combination



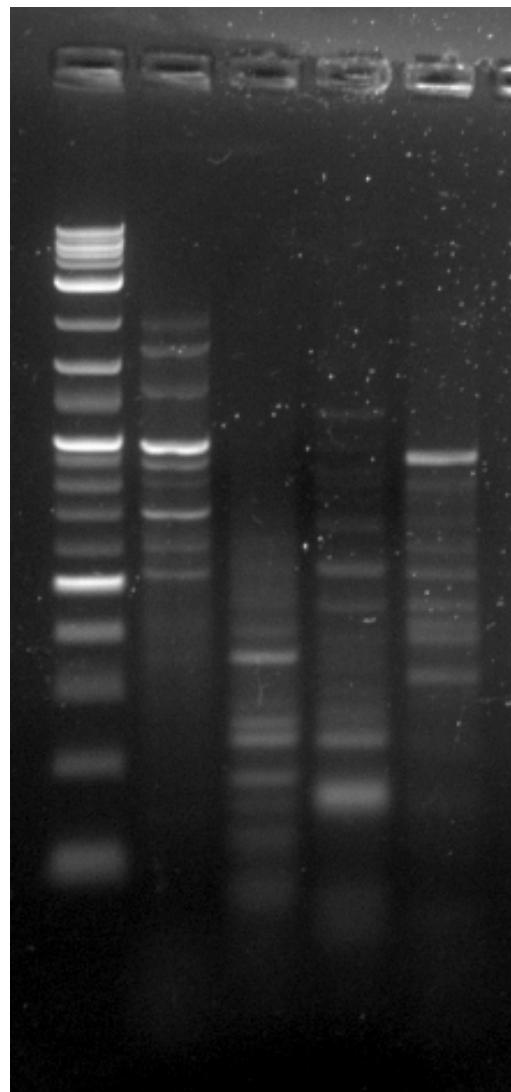
Low Combination

## **Appendix G – Agarose Gel Electrophoresis of PCR-RFLP**

Genetic studies on main culture *E. coli* was conducted by PCR-RFLP on every 12<sup>th</sup> passage. Below are the gel pictures of the *E. coli* on every 12<sup>th</sup> passage.

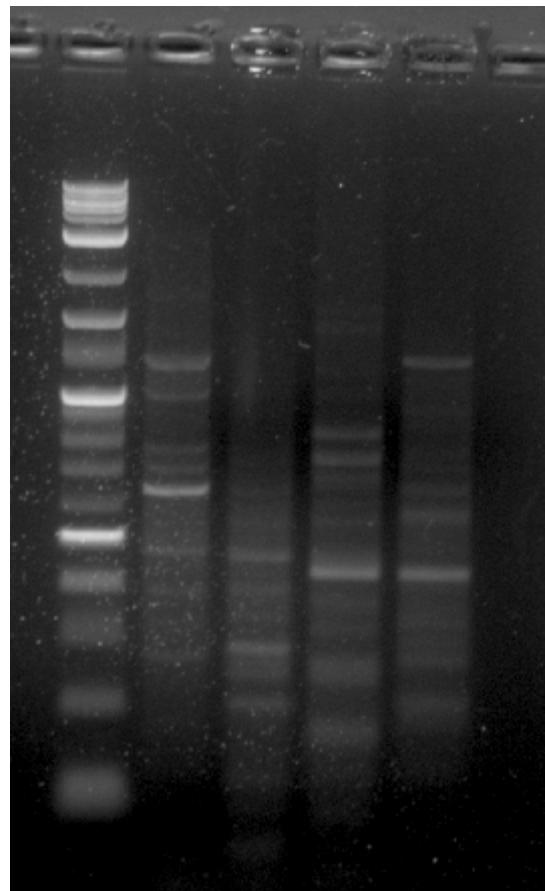
G.1 – ATCC 8739, PCR; MspI; TaqI; HinfI (sorted by primers)

Agarose Gel 1: ATCC 8739 PCR, Primer 5, digested by MspI, TaqI and HinfI



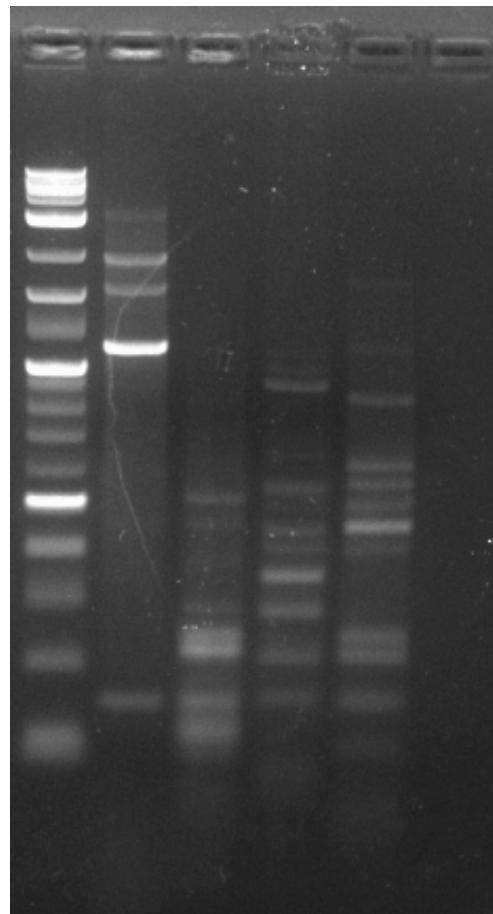
Log 2 Marker		Sample Lane			
Distance	Molecular Weight	PCR	MspI	TaqI	HinfI
2.2	3000	3.2			
3.3	2000	3.5			
3.8	1500	4.0		4.0	
4.7	1000	4.7			4.7
4.9	900	4.9			
5.2	800	5.1			5.1
6.5	500	5.5		5.5	
		5.9		5.9	5.9
		6.2			6.2
			7.3	7.3	7.3
			7.8		
			8.1		
			8.3	8.3	
			8.8	8.8	

Agarose Gel 2: ATCC 8739 PCR, Primer 6, digested by MspI, TaqI and HinfI



Log 2 Marker		Sample Lane			
Distance	Molecular Weight	PCR	MspI	TaqI	HinfI
2.0	3000	4.1			4.1
3.1	2000	4.4			
3.6	1500			4.9	4.9
4.6	1000			5.2	
4.8	900	5.5		5.5	5.5
5.1	800	5.9		5.9	5.9
6.4	500			6.1	
		6.7	6.7	6.7	
			7.7		

Agarose Gel 3: ATCC 8739 PCR, Primer 7, digested by MspI, TaqI and HinfI

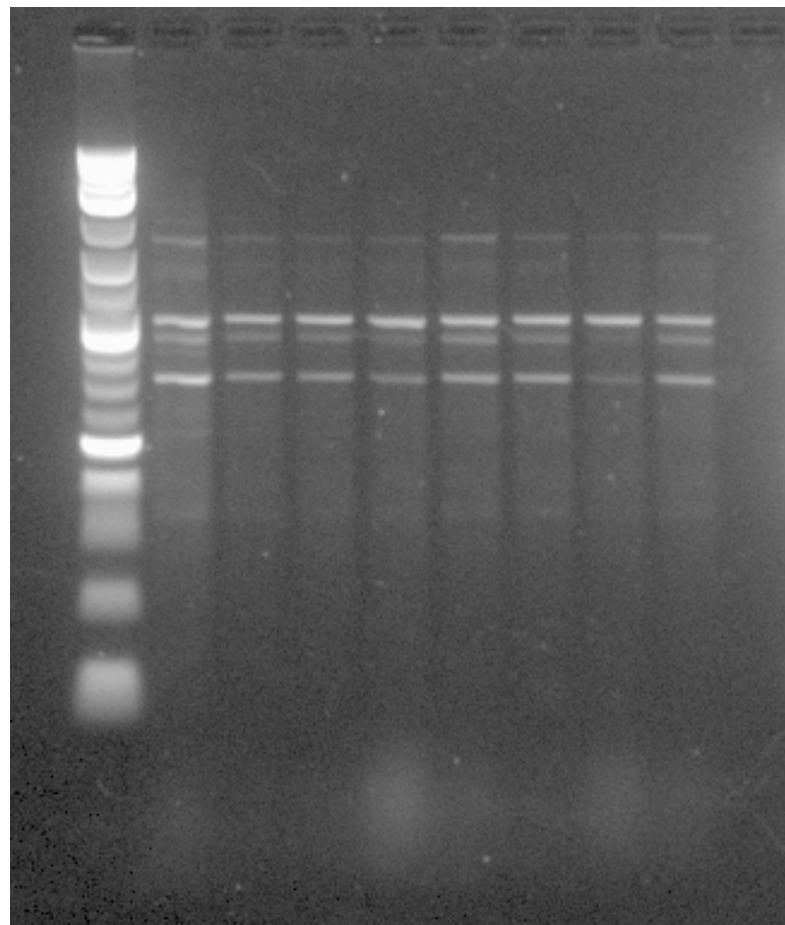


Log 2 Marker		Sample Lane			
Distance	Molecular Weight	PCR	MspI	TaqI	HinfI
2.3	3000	2.8			
2.7	2000	3.2			3.2
3.3	1500	4.0			4.0
4.3	1000			4.4	
4.4	900				5.4
4.7	800			5.6	
6.0	500		6.0		6.0
			6.3	6.3	6.3
				6.5	6.5
				6.9	
			7.4	7.4	
			8.0	8.0	8.0

## G.2 – PCR Gels

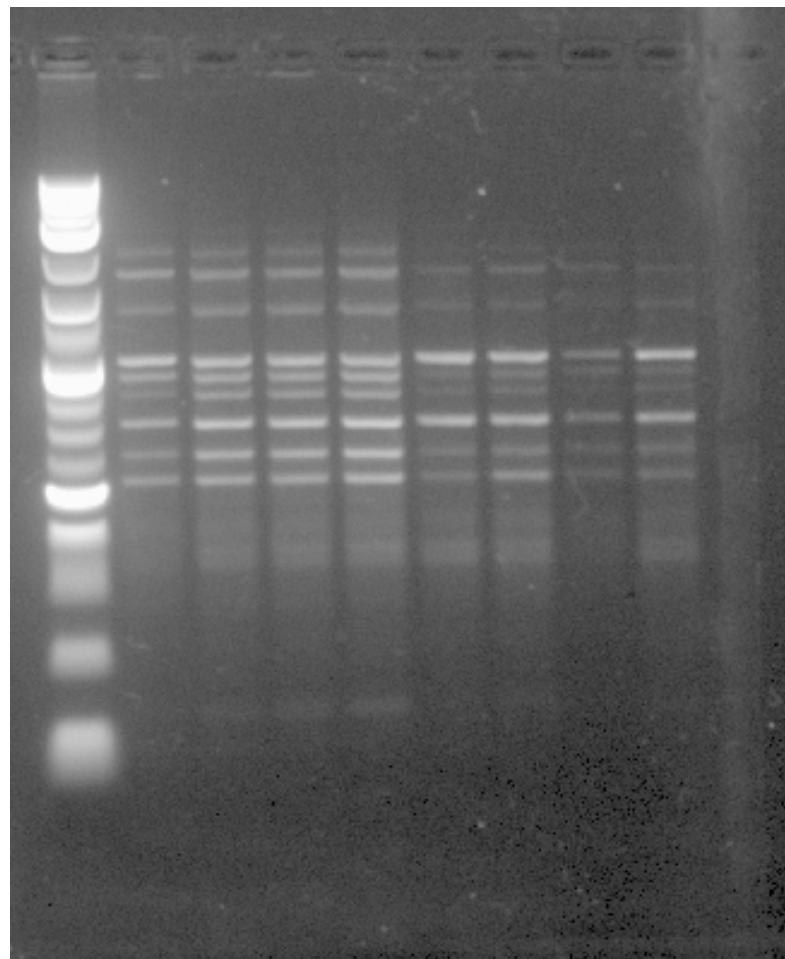
### G.2.a – Primer 5

Agarose Gel 4: 8/7 culture, PCR Primer 5



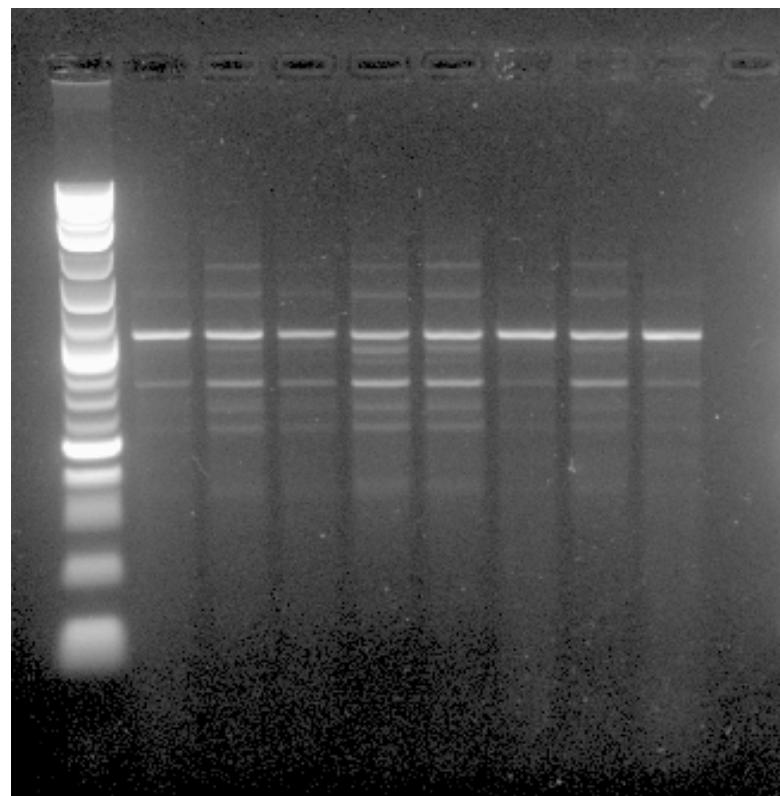
Log 2 Marker		Treatments							
Distance	Molecular Weight	H MSG	L MSG	H BA	L BA	H SALT	L SALT	H COMB	L COMB
2.0	3000	2.3	2.3	2.3	2.3	2.3	2.3	2.3	2.3
2.8	1500	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2
3.5	1000	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4
4.7	500	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8
5.1	400								
5.7	300								
6.4	200								

Agarose Gel 5: 5/8 Culture, PCR Primer 5



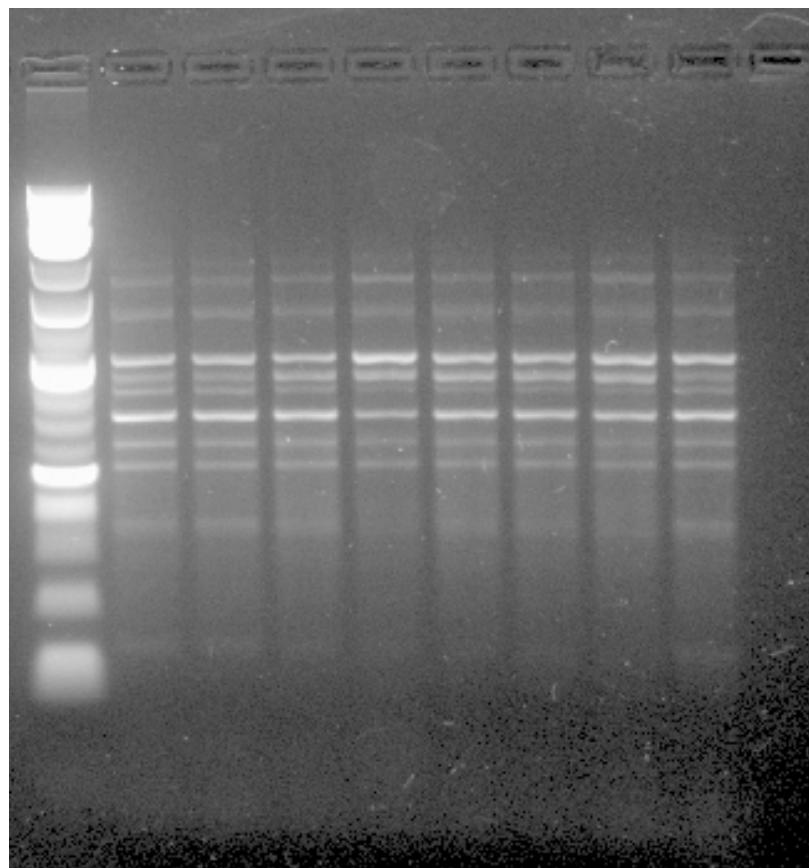
Log 2 Marker		Treatments							
Distance	Molecular Weight	H MSG	L MSG	H BA	L BA	H SALT	L SALT	H COMB	L COMB
2.1	3000	2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.2
2.5	2000	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4
2.9	1500	2.9	2.9	2.9	2.9	2.9	2.9	2.9	2.9
3.7	1000	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4
4.0	900	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6
4.3	800	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8
5.0	500	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1
		4.4	4.4	4.4	4.4	4.4	4.4	4.4	4.4
		4.7	4.7	4.7	4.7	4.7	4.7	4.7	4.7

Agarose Gel 6: 2/9 Culture, PCR Primer 5



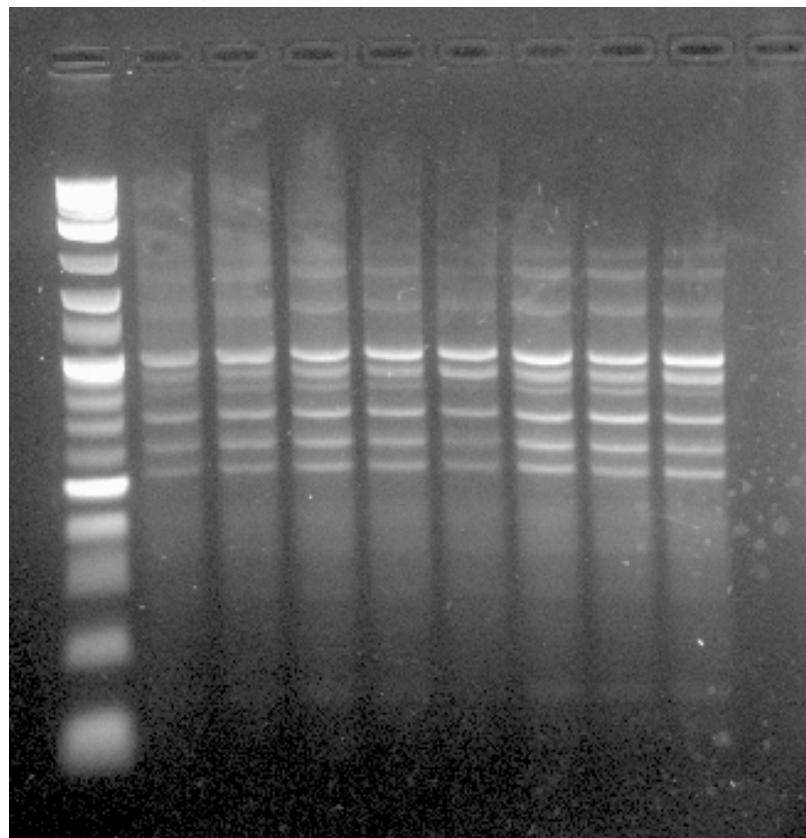
Log 2 Marker		Treatments							
Distance	Molecular Weight	H MSG	L MSG	H BA	L BA	H SALT	L SALT	H COMB	L COMB
2.1	3000	2.4	2.4	2.4	2.4	2.4	2.4	2.4	
2.4	2000	2.7	2.7	2.7	2.7	2.7	2.7	2.7	
2.8	1500	3.15	3.15	3.15	3.15	3.15	3.15	3.15	3.15
3.5	1000		3.3	3.3	3.3	3.3		3.3	
3.7	900		3.4		3.4	3.4			
3.9	800	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7
4.5	500	4.0	4.0	4.0	4.0	4.0		4.0	
		4.2	4.2	4.2	4.2	4.2		4.2	

Agarose gel 7: 30/9 Culture, PCR Primer 5



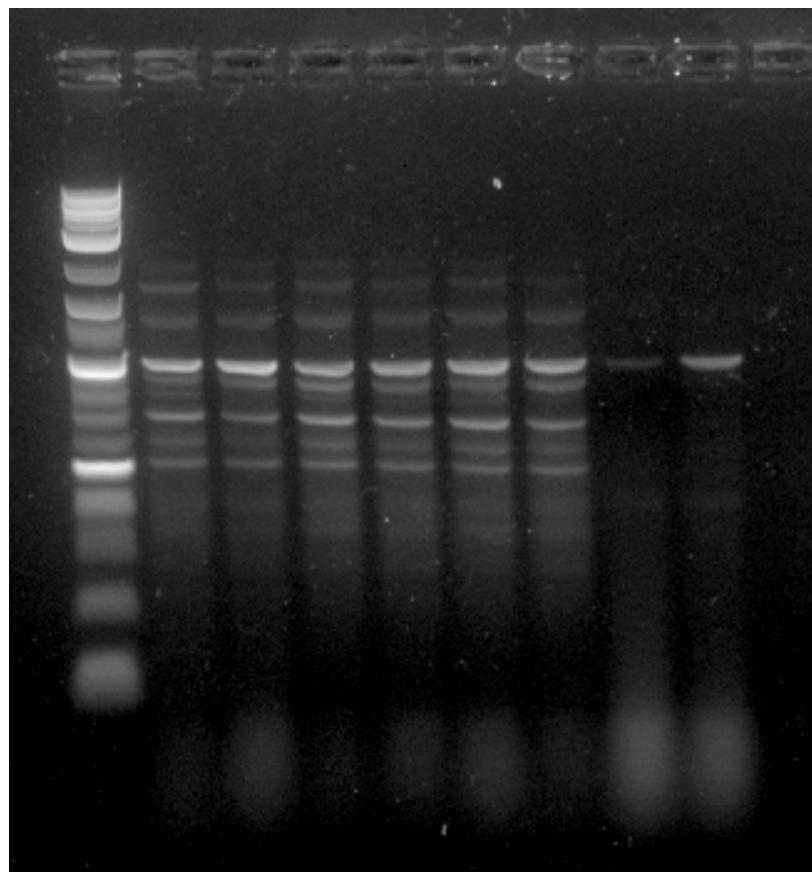
Log 2 Marker		Treatments							
Distance	Molecular Weight	H MSG	L MSG	H BA	L BA	H SALT	L SALT	H COMB	L COMB
2.0	3000	2.15	2.15	2.15	2.15		2.15	2.15	2.15
2.4	2000	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4
2.8	1500	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7
3.45	1000	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2
3.7	900	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4
3.9	800	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5
4.5	500	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8
		4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1
		4.3	4.3	4.3	4.3	4.3	4.3	4.3	4.3
		5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0

Agarose gel 8: 28/10 Culture, PCR Primer 5



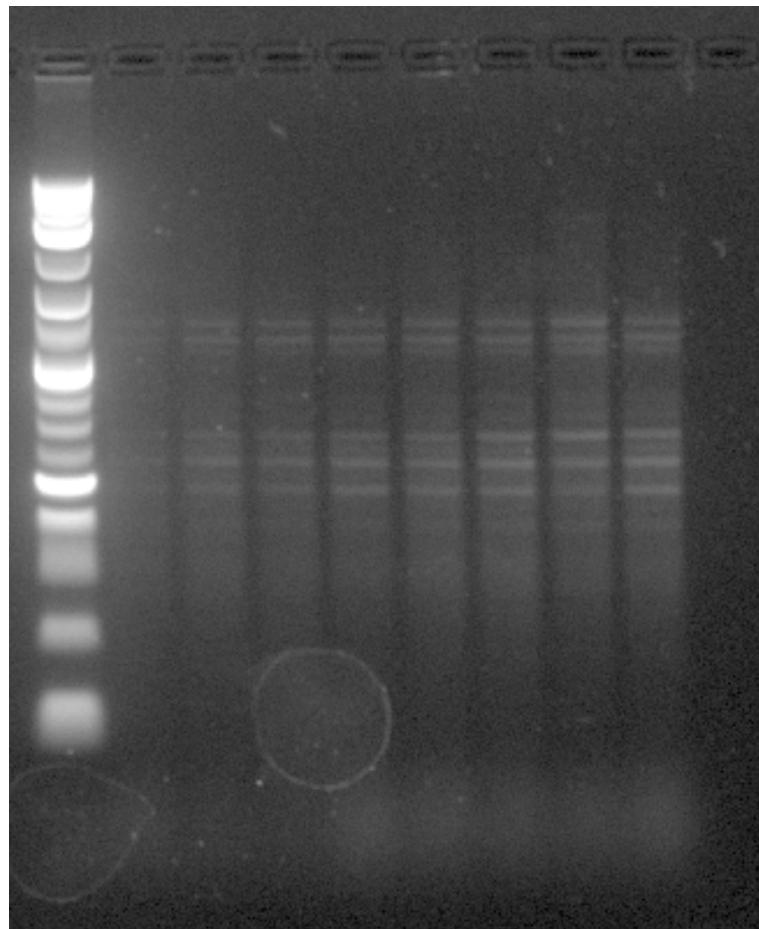
Log 2 Marker		Treatments							
Distance	Molecular Weight	H MSG	L MSG	H BA	L BA	H SALT	L SALT	H COMB	L COMB
2.0	3000						2.3	2.3	2.3
2.4	2000	2.6	2.6	2.6	2.6		2.6	2.6	2.6
2.8	1500	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
3.6	1000	3.55	3.55	3.55	3.55	3.55	3.55	3.55	3.55
4.0	900	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7
4.25	800	3.9	3.9	3.9	3.9		3.9	3.9	3.9
5.0	500	4.2	4.2	4.2	4.2	4.2	4.2	4.2	4.2
		4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5
		4.8	4.8	4.8	4.8	4.8	4.8	4.8	4.8

Agarose gel 9: 25/11 Culture, PCR Primer 5



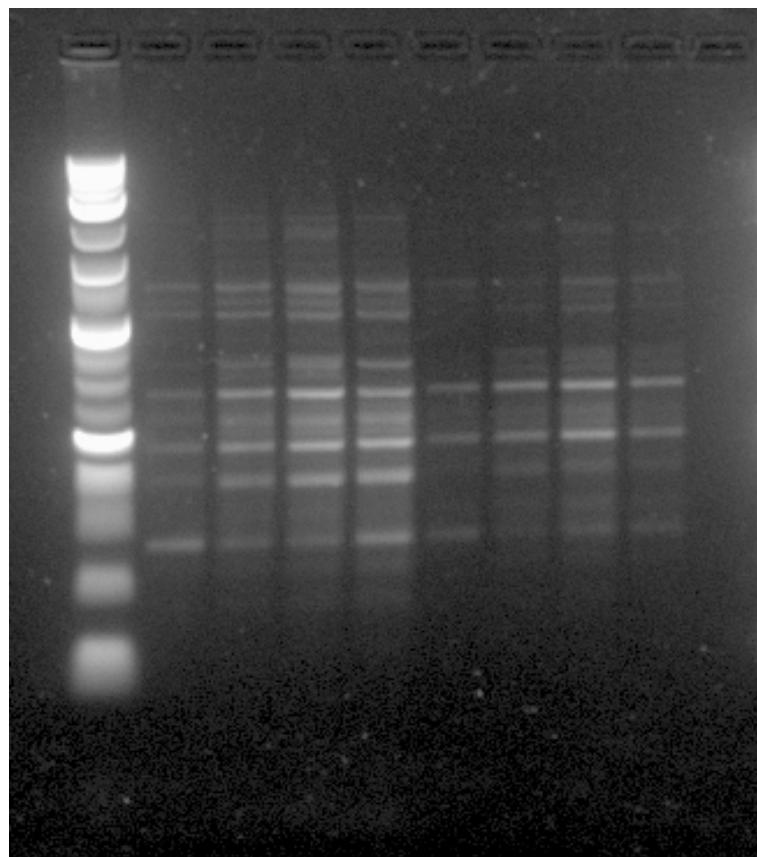
Log 2 Marker		Treatments							
Distance	Molecular Weight	H MSG	L MSG	H BA	L BA	H SALT	L SALT	H COMB	L COMB
2.2	3000	2.5	2.5	2.5	2.5	2.5			
2.6	2000	2.7	2.7	2.7	2.7	2.7	2.7		
3.0	1500	3.1	3.1	3.1	3.1	3.1	3.1		
3.7	1000	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6
4.0	900	3.8	3.8	3.8	3.8	3.8	3.8		
4.3	800	3.9		3.9		3.9			
4.9	500	4.2	4.2	4.2	4.2	4.2	4.2		
		4.5	4.5	4.5	4.5	4.5	4.5		
		4.8	4.8	4.8	4.8	4.8	4.8		
			5.3	5.3	5.3	5.3	5.3	5.3	5.3
			5.6	5.6	5.6	5.6	5.6		

G.2.b – Primer 6  
 Agarose Gel 10: 8/7 Culture, PCR Primer 6



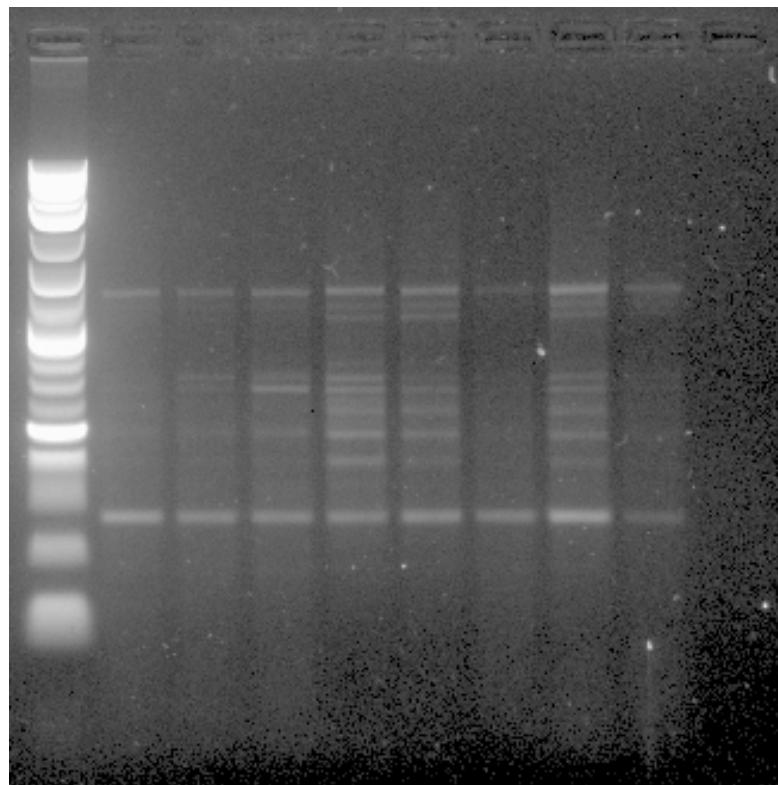
Log 2 Marker		Treatments							
Distance	Molecular Weight	H MSG	L MSG	H BA	L BA	H SALT	L SALT	H COMB	L COMB
2.1	3000		3.1	3.1	3.1	3.1	3.1	3.1	3.1
2.9	1500		3.3	3.3	3.3	3.3	3.3	3.3	3.3
3.7	1000			3.4		3.4	3.4	3.4	3.4
4.9	500		4.4	4.4	4.4	4.4	4.4	4.4	4.4
5.3	400		4.7	4.7	4.7	4.7	4.7	4.7	4.7
5.9	300		5.0	5.0	5.0	5.0	5.0	5.0	5.0
6.7	200								

Agarose Gel 11: 5/8 Culture, PCR Primer 6



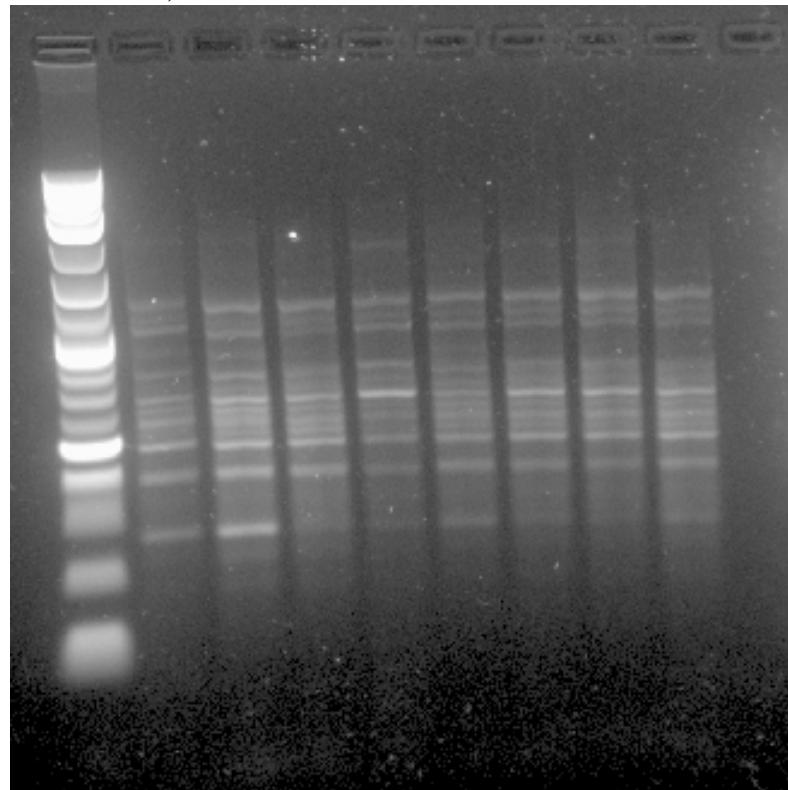
Log 2 Marker		Treatments							
Distance	Molecular Weight	H MSG	L MSG	H BA	L BA	H SALT	L SALT	H COMB	L COMB
2.1	3000		2.0	2.0	2.0				
2.4	2000		2.3	2.3			2.3	2.3	2.3
2.8	1500	2.9	2.9	2.9	2.9	2.9	2.9	2.9	2.9
3.6	1000			3.0					
3.8	900	3.2	3.2	3.2	3.2		3.2	3.2	3.2
4.1	800		3.8	3.8	3.8		3.8	3.8	3.8
4.8	500	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1
		4.4	4.4	4.4	4.4				
		4.7	4.7	4.7	4.7	4.7	4.7	4.7	4.7
		5.1	5.1	5.1	5.1	5.1	5.1	5.1	5.1
		5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8

Agarose gel 12: 2/9 Culture, PCR Primer 6



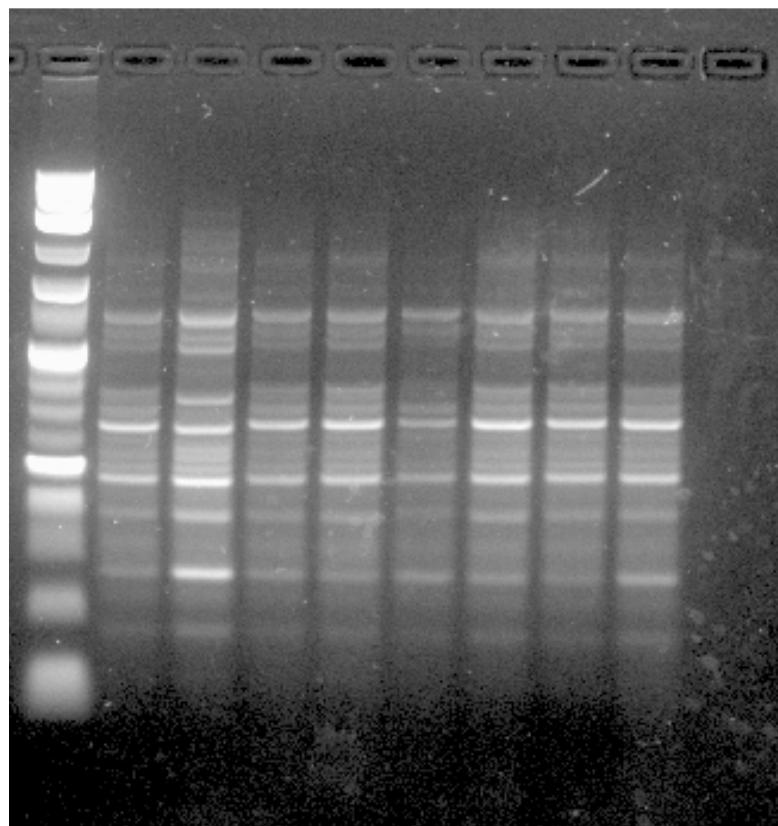
Log 2 Marker		Treatments							
Distance	Molecular Weight	H MSG	L MSG	H BA	L BA	H SALT	L SALT	H COMB	L COMB
2.05	3000	2.8	2.8	2.8	2.8	2.8	2.8	2.8	2.8
2.4	2000				3.0	3.0		3.0	
2.8	1500				3.1	3.1		3.1	
3.45	1000		3.8	3.8	3.8	3.8		3.8	3.8
3.65	900			3.9	3.9	3.9		3.9	3.9
3.9	800				4.15	4.15		4.15	
4.4	500			4.4	4.4	4.4		4.4	
					4.7	4.7		4.7	
		5.3	5.3	5.3	5.3	5.3	5.3	5.3	5.3

Agarose gel 13: 30/9 Culture, PCR Primer 6



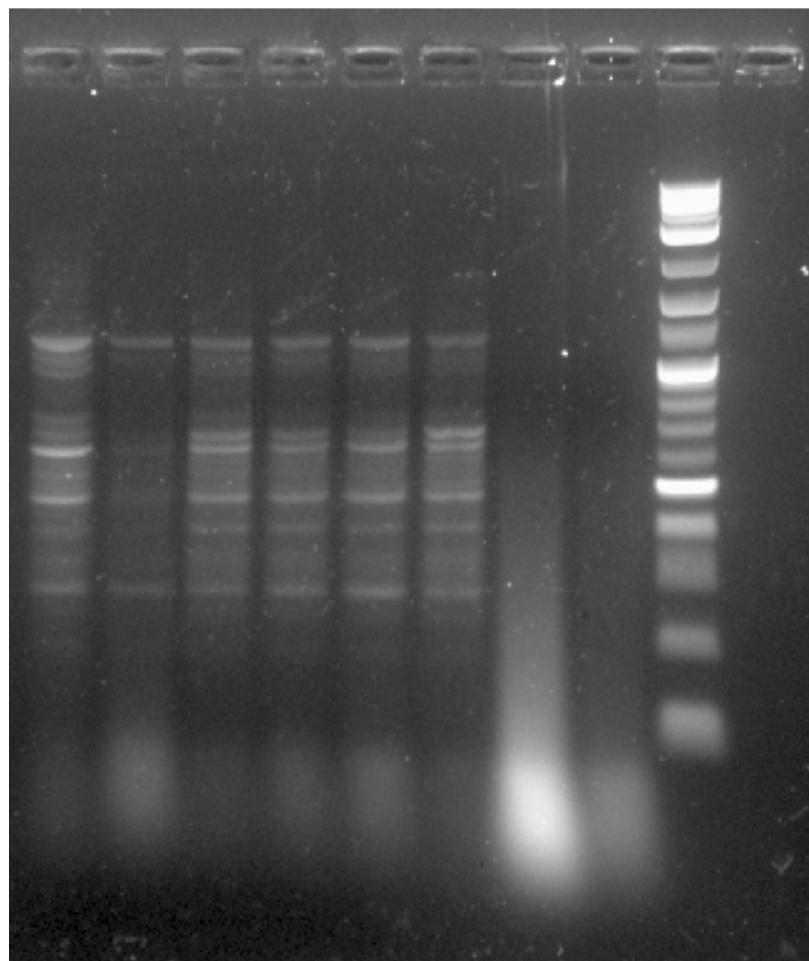
Log 2 Marker		Treatments							
Distance	Molecular Weight	H MSG	L MSG	H BA	L BA	H SALT	L SALT	H COMB	L COMB
2.15	3000	2.8	2.8	2.8	2.8	2.8	2.8	2.8	2.8
2.5	2000	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
2.9	1500	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1
3.5	1000	3.55	3.55	3.55	3.55	3.55	3.55	3.55	3.55
3.7	900	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8
4.0	800	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
4.5	500	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1
		4.35	4.35	4.35	4.35	4.35	4.35	4.35	4.35
		4.7	4.7	4.7	4.7	4.7	4.7	4.7	4.7
		5.4	5.4	5.4	5.4	5.4	5.4	5.4	5.4

Agarose gel 14: 28/10 Culture, PCR Primer 6



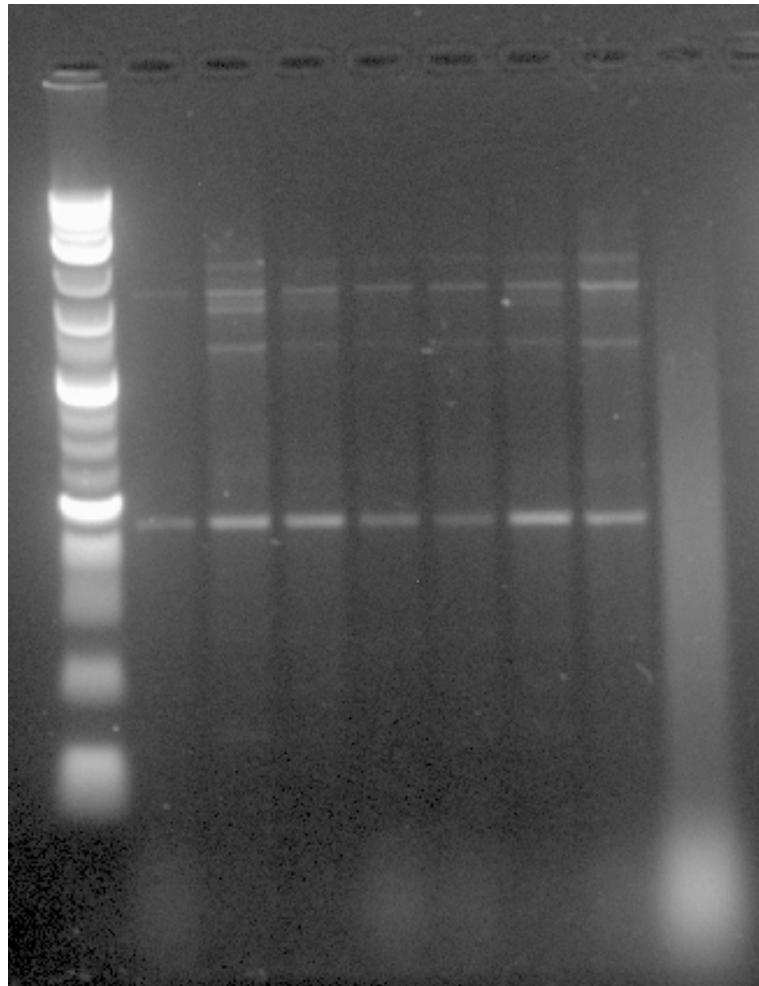
Log 2 Marker		Treatments							
Distance	Molecular Weight	H MSG	L MSG	H BA	L BA	H SALT	L SALT	H COMB	L COMB
2.1	3000	2.6	2.6	2.6	2.6		2.6	2.6	2.6
2.5	2000		2.9	2.9	2.9		2.9	2.9	
2.9	1500	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2
3.8	1000	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4
4.1	900	3.5	3.5	3.5	3.5		3.5	3.5	3.5
4.4	800	4.1	4.1	4.1	4.1		4.1	4.1	4.1
5.0	500	4.2	4.2	4.2	4.2	4.2	4.2	4.2	4.2
		4.3	4.3	4.3	4.3	4.3	4.3	4.3	4.3
		4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5
		4.9	4.9	4.9	4.9	4.9	4.9	4.9	4.9
		5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
		5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.6
		6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0
		6.3	6.3	6.3	6.3	6.3	6.3	6.3	6.3
		7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0

Agarose gel 15: 25/11 Culture, PCR Primer 6



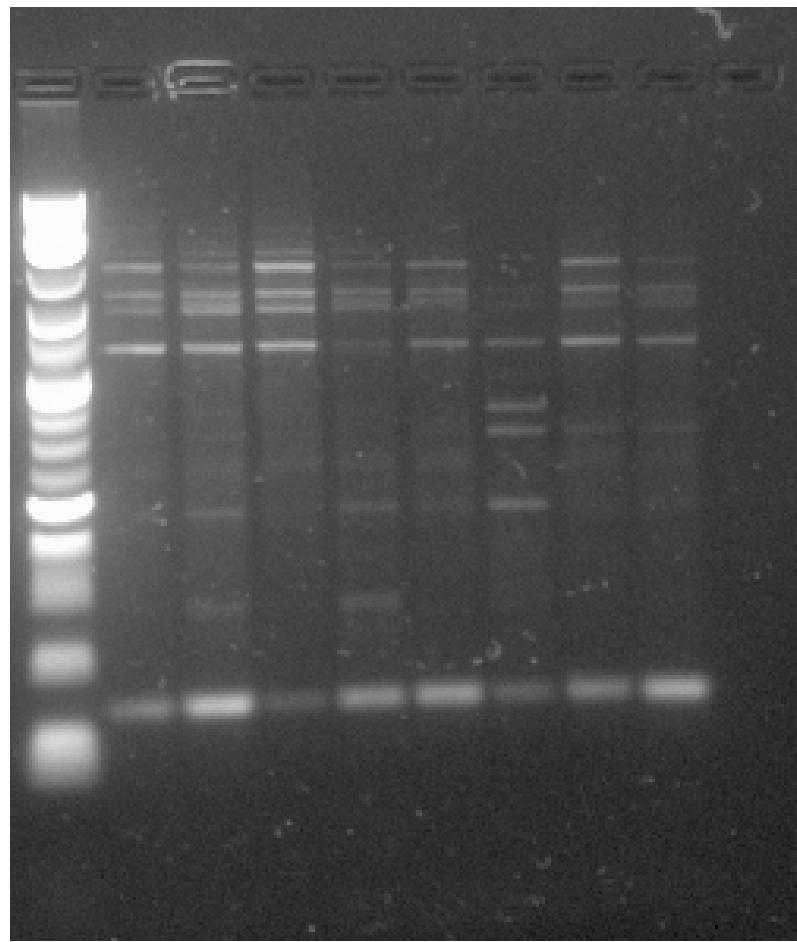
Log 2 Marker		Treatments							
Distance	Molecular Weight	H MSG	L MSG	H BA	L BA	H SALT	L SALT	H COMB	L COMB
2.1	3000	3.2	3.2	3.2	3.2	3.2	3.2		
2.4	2000	3.4	3.4	3.4	3.4	3.4	3.4		
2.8	1500	3.5		3.5		3.5	3.5		
3.6	1000	4.1		4.1					
3.9	900	4.2		4.2	4.2	4.2	4.2		
4.15	800	4.3	4.3	4.3	4.3	4.3	4.3		
4.8	500	4.9	4.9	4.9	4.9	4.9	4.9		
		5.2	5.2	5.2	5.2	5.2	5.2		
		5.9	5.9	5.9	5.9	5.9	5.9		

G.2.c – Primer 7  
 Agarose Gel 16: 8/7 culture, PCR Primer 7



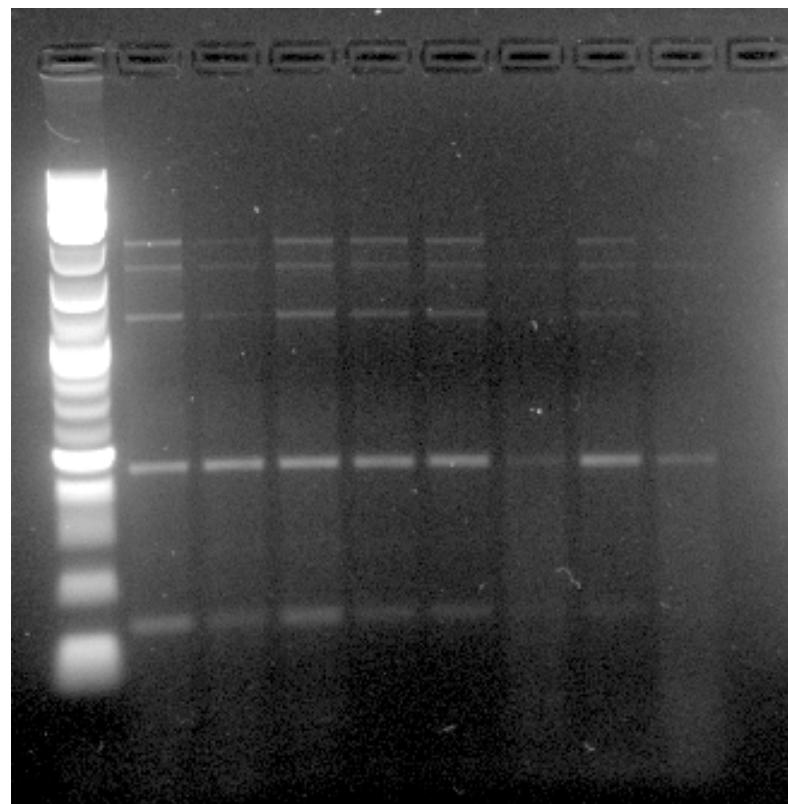
Log 2 Marker		Treatments							
Distance	Molecular Weight	H MSG	L MSG	H BA	L BA	H SALT	L SALT	H COMB	L COMB
2.2	3000		2.4	2.4	2.4	2.4	2.4	2.4	2.4
3.0	1500	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6
3.8	1000		2.8						
5.2	500	5.3	5.3	5.3	5.3	5.3	5.3	5.3	5.3
5.7	400		3.3	3.3	3.3	3.3	3.3	3.3	3.3
6.3	300								
7.2	200								

Agarose Gel 17: 5/8 Culture, PCR Primer 7



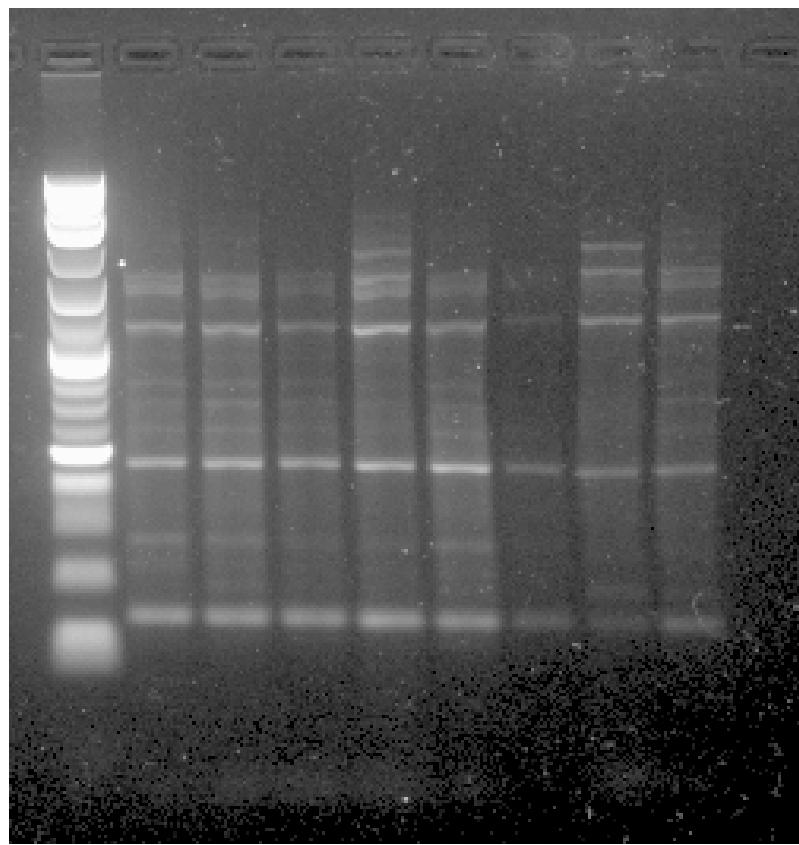
Log 2 Marker		Treatments							
Distance	Molecular Weight	H MSG	L MSG	H BA	L BA	H SALT	L SALT	H COMB	L COMB
1.6	3000	1.5	1.5	1.5	1.5				
1.8	2000	1.6	1.6	1.6	1.6	1.6		1.6	1.6
2.2	1500	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8
2.8	1000	2.0	2.0	2.0	2.0		2.0	2.0	2.0
3.0	900	2.3	2.3	2.3	2.3	2.3	2.3	2.3	2.3
3.2	800						2.8		
3.7	500					3.1	3.1	3.1	3.1
			3.3	3.3	3.3	3.3	3.3	3.3	3.3
			3.7	3.7	3.7	3.7	3.7		3.7
			4.5		4.5	4.5			
		5.3	5.3	5.3	5.3	5.3	5.3	5.3	5.3

Agarose gel 18: 2/9 Culture, PCR Primer 7



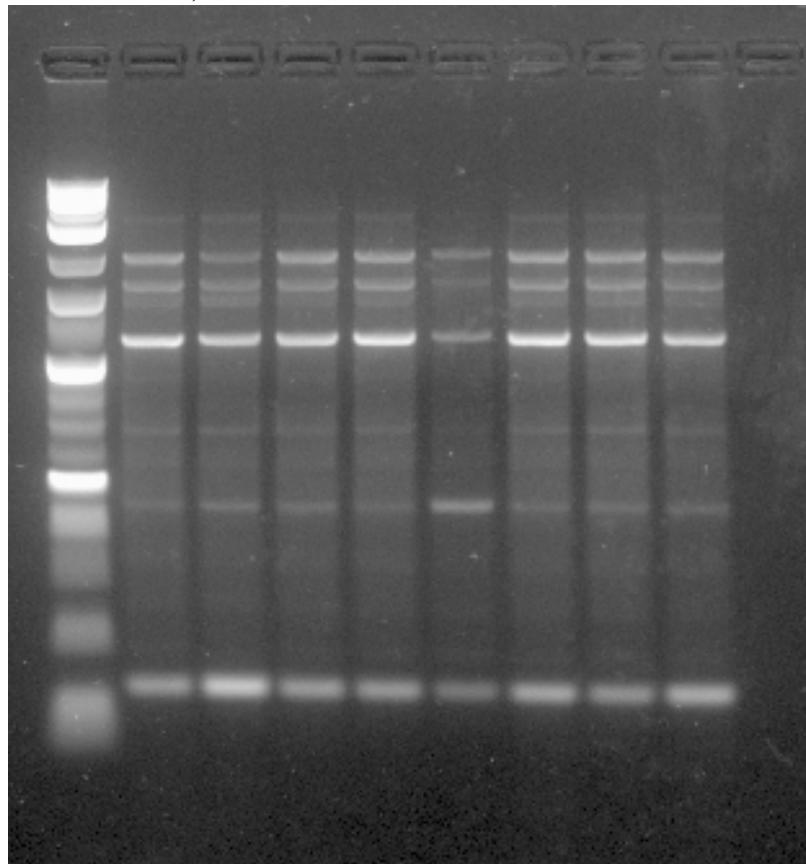
Log 2 Marker		Treatments							
Distance	Molecular Weight	H MSG	L MSG	H BA	L BA	H SALT	L SALT	H COMB	L COMB
2.0	3000	2.0	2.0	2.0	2.0	2.0		2.0	
2.2	2000	2.3	2.3	2.3	2.3	2.3	2.3	2.3	
2.7	1500	2.8	2.8	2.8	2.8	2.8		2.8	
3.4	1000	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5
3.6	900	6.2	6.2	6.2	6.2	6.2		6.2	
3.9	800								
4.4	500								

Agarose gel 19: 30/9 Culture, PCR Primer 7



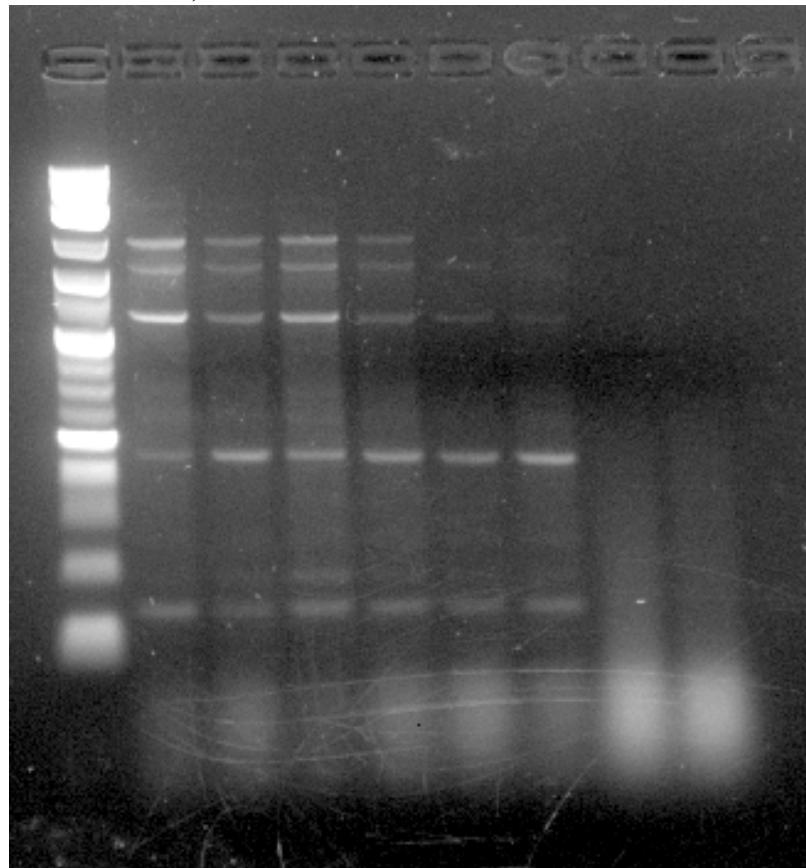
Log 2 Marker		Treatments							
Distance	Molecular Weight	H MSG	L MSG	H BA	L BA	H SALT	L SALT	H COMB	L COMB
1.5	3000				1.7			1.7	
1.9	2000	1.9	1.9	1.9	1.9	1.9		1.9	1.9
2.2	1500	2.1	2.1	2.1	2.1	2.1			2.1
2.7	1000	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4
2.9	900	2.9	2.9			2.9			
3.25	800	3.0	3.0			3.0			
3.5	500	3.2	3.2			3.2			
		3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5
		4.2	4.2		4.2	4.2			
								4.65	4.65
		4.8	4.8	4.8	4.8	4.8	4.8	4.8	4.8

Agarose gel 20: 28/10 Culture, PCR Primer 7



Log 2 Marker		Treatments							
Distance	Molecular Weight	H MSG	L MSG	H BA	L BA	H SALT	L SALT	H COMB	L COMB
2.1	3000	1.85	1.85	1.85	1.85		1.85	1.85	1.85
2.4	2000	2.3	2.3	2.3	2.3	2.3	2.3	2.3	2.3
2.9	1500	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6
3.6	1000	2.8	2.8	2.8	2.8		2.8	2.8	2.8
4.0	900	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3
4.25	800	4.3	4.3	4.3	4.3		4.3	4.3	4.3
4.9	500	5.1	5.1	5.1	5.1	5.1	5.1	5.1	5.1
		7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3

Agarose gel 21: 25/11 Culture, PCR Primer 7

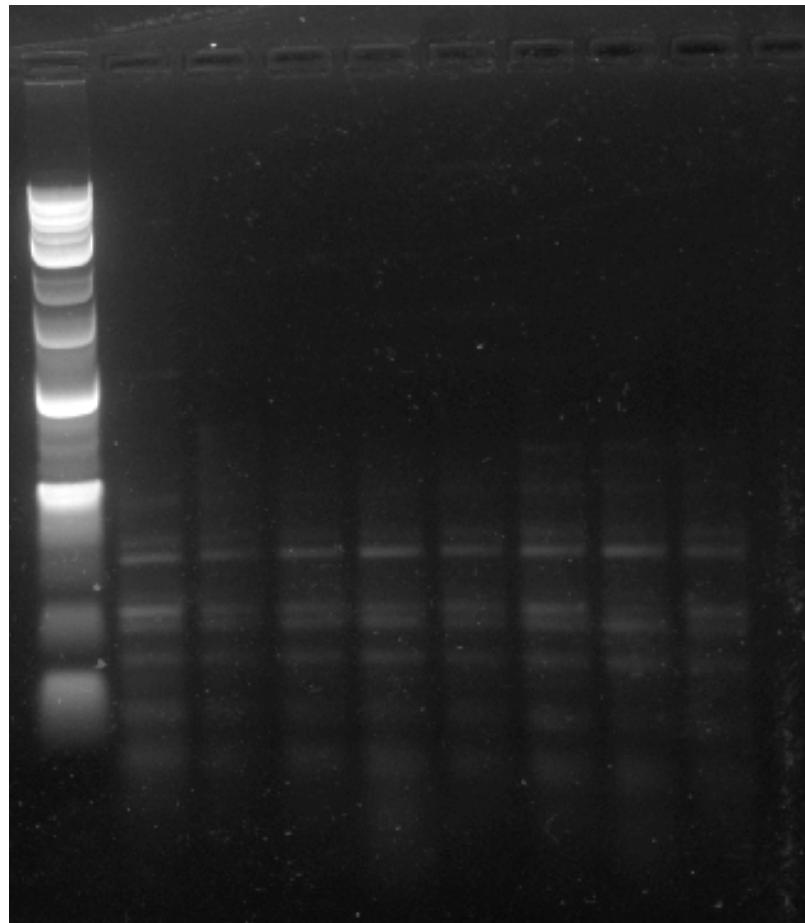


Log 2 Marker		Treatments							
Distance	Molecular Weight	H MSG	L MSG	H BA	L BA	H SALT	L SALT	H COMB	L COMB
2.1	3000	2.3	2.3	2.3	2.3				
2.4	2000	2.6	2.6	2.6	2.6	2.6			
2.85	1500	3.2	3.2	3.2	3.2	3.2	3.2		
3.6	1000	4.9	4.9	4.9	4.9	4.9	4.9		
3.9	900	6.45	6.45	6.45					
4.1	800	6.8	6.8	6.8	6.8	6.8	6.8		
4.8	500								

### G.3 – Gel Photographs of Restriction Digestion of PCR Products

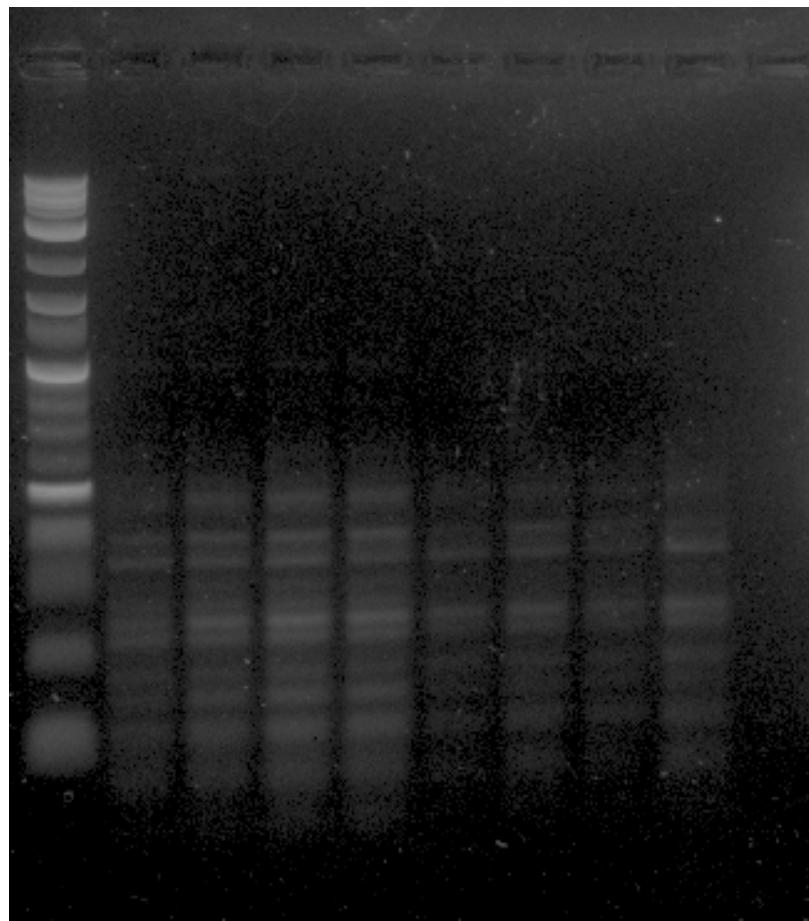
#### G.3.a – MspI, P5

Agarose gel 22: 8/7 Culture PCR products Primer 5, Digested by MspI



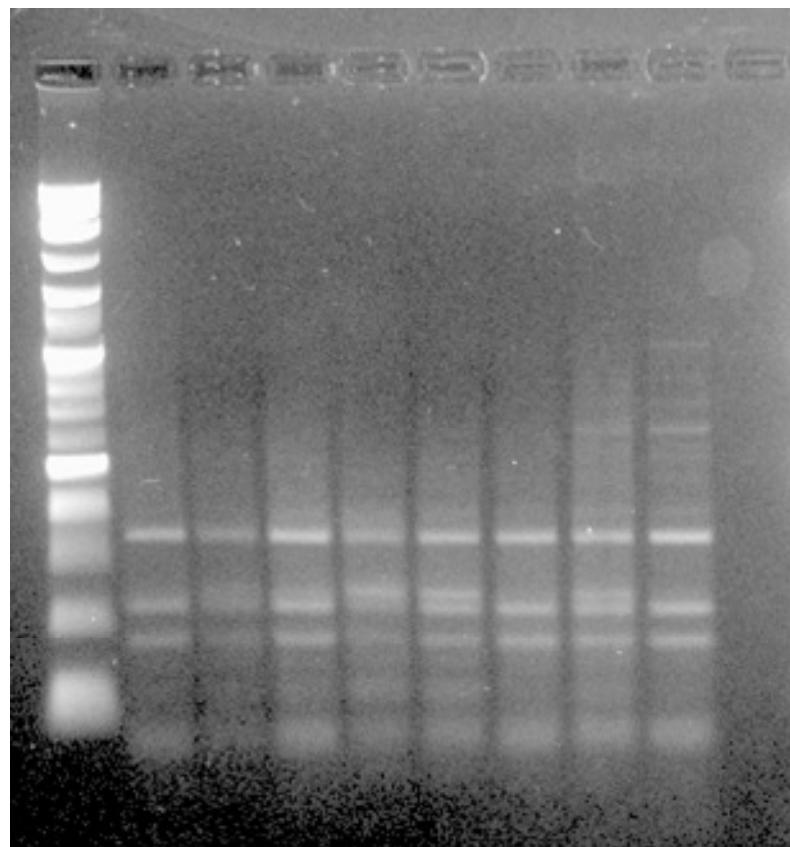
Log 2 Marker		Treatments							
Distance	Molecular Weight	H MSG	L MSG	H BA	L BA	H SALT	L SALT	H COMB	L COMB
2.3	3000						4.6	4.6	4.6
2.8	2000						5.0	5.0	5.0
3.4	1500	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7
4.1	1000	6.3	6.3	6.3	6.3	6.3	6.3	6.3	6.3
4.3	900	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6
4.5	800	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0
5.2	500	7.4	7.4	7.4	7.4		7.4	7.4	7.4
			8.0	8.0	8.0				
		8.1					8.1	8.1	8.1

Agarose gel 23: 5/8 Culture PCR products Primer 5, Digested by MspI



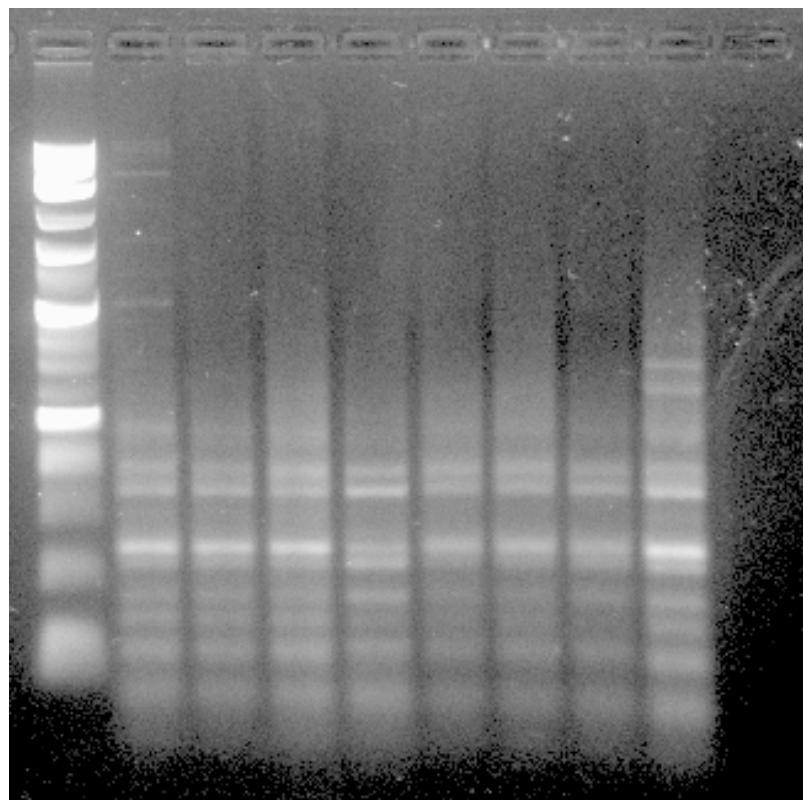
Log 2 Marker		Treatments							
Distance	Molecular Weight	H MSG	L MSG	H BA	L BA	H SALT	L SALT	H COMB	L COMB
2.2	3000	5.4	5.4	5.4	5.4	5.4	5.4	5.4	5.4
2.6	2000	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0
3.2	1500	6.3	6.3	6.3	6.3	6.3	6.3	6.3	6.3
4.0	1000	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5
4.3	900	6.8	6.8	6.8	6.8	6.8	6.8	6.8	6.8
5.0	800	7.0	7.0	7.0	7.0		7.0	7.0	7.0
5.4	500	7.3	7.3	7.3	7.3		7.3		7.3
		7.7	7.7	7.7	7.7		7.7		7.7
		8.0	8.0	8.0	8.0				8.0
		8.4	8.4	8.4	8.4			8.4	8.4
		9.0	9.0	9.0	9.0				9.0

Agarose gel 24: 2/9 Culture PCR products Primer 5, Digested by MspI



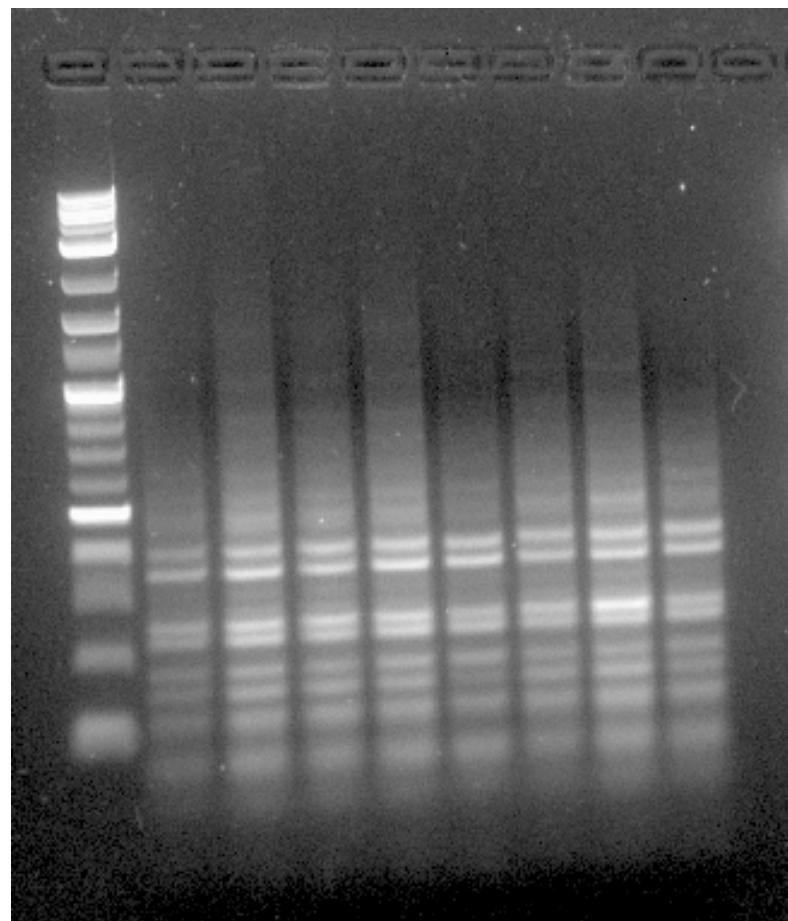
Log 2 Marker		Treatments							
Distance	Molecular Weight	H MSG	L MSG	H BA	L BA	H SALT	L SALT	H COMB	L COMB
2.2	3000								3.1
2.6	2000								3.5
2.9	1500							4.0	4.0
3.4	1000	5.3	5.3	5.3	5.3	5.3	5.3	5.3	5.3
3.6	900	6.2	6.2	6.2	6.2	6.2	6.2	6.2	6.2
3.9	800	6.5		6.5	6.5	6.5	6.5	6.5	6.5
4.6	500			7.2	7.2	7.2		7.2	
		7.7		7.7	7.7	7.7	7.7		7.7

Agarose gel 25: 30/9 Culture PCR products Primer 5, Digested by MspI



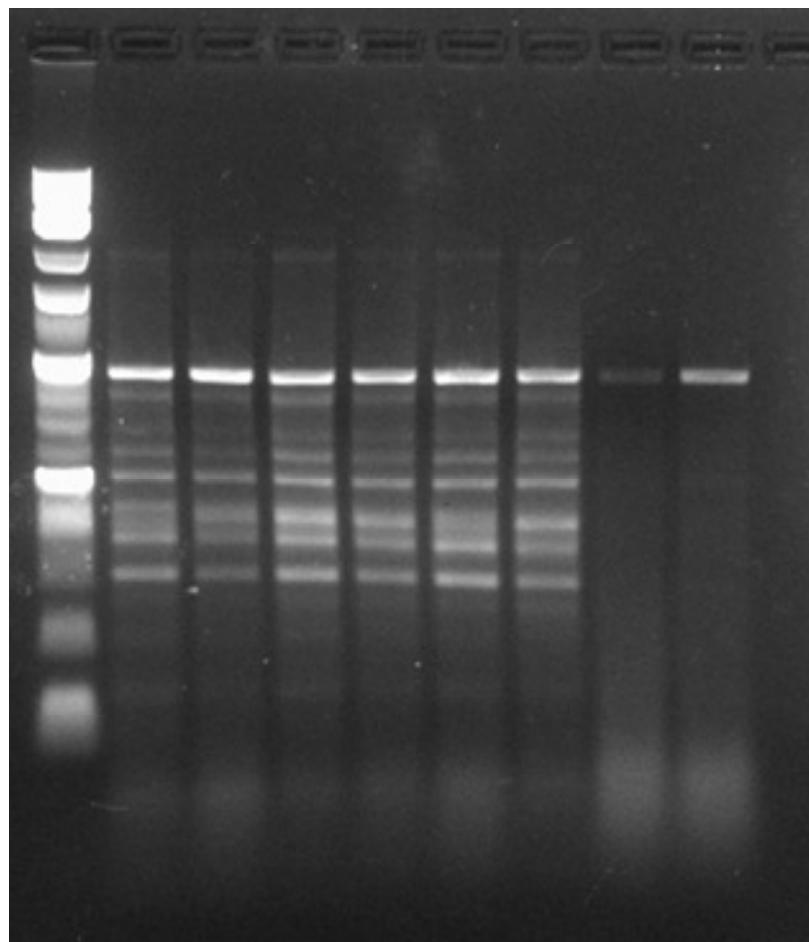
Log 2 Marker		Treatments							
Distance	Molecular Weight	H MSG	L MSG	H BA	L BA	H SALT	L SALT	H COMB	L COMB
2.1	3000	1.5							
2.5	2000	2.2							
2.7	1500	3.0							
3.2	1000								3.7
3.5	900								4.0
3.8	800	4.9	4.9	4.9	4.9	4.9	4.9	4.9	4.9
4.7	500	5.2	5.2	5.2	5.2	5.2	5.2	5.2	5.2
		5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8
		6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0
		6.4	6.4	6.4	6.4	6.4	6.4	6.4	6.4
		6.7	6.7	6.7	6.7	6.7	6.7	6.7	6.7
		7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.2
		7.7	7.7	7.7	7.7	7.7	7.7	7.7	7.7

Agarose gel 26: 28/10 Culture PCR products Primer 5, Digested by MspI



Log 2 Marker		Treatments							
Distance	Molecular Weight	H MSG	L MSG	H BA	L BA	H SALT	L SALT	H COMB	L COMB
2.0	3000		4.9	4.9	4.9		4.9		4.9
2.4	2000		5.3	5.3	5.3		5.3	5.3	5.3
2.9	1500	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5
3.2	1000	6.1	6.1	6.1	6.1	6.1	6.1	6.1	6.1
3.4	900	6.3	6.3	6.3	6.3	6.3	6.3	6.3	6.3
4.1	800	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6
5.0	500	6.9	6.9	6.9	6.9	6.9	6.9	6.9	6.9
		7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.2
		7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5
			8.0	8.0	8.0	8.0	8.0	8.0	8.0

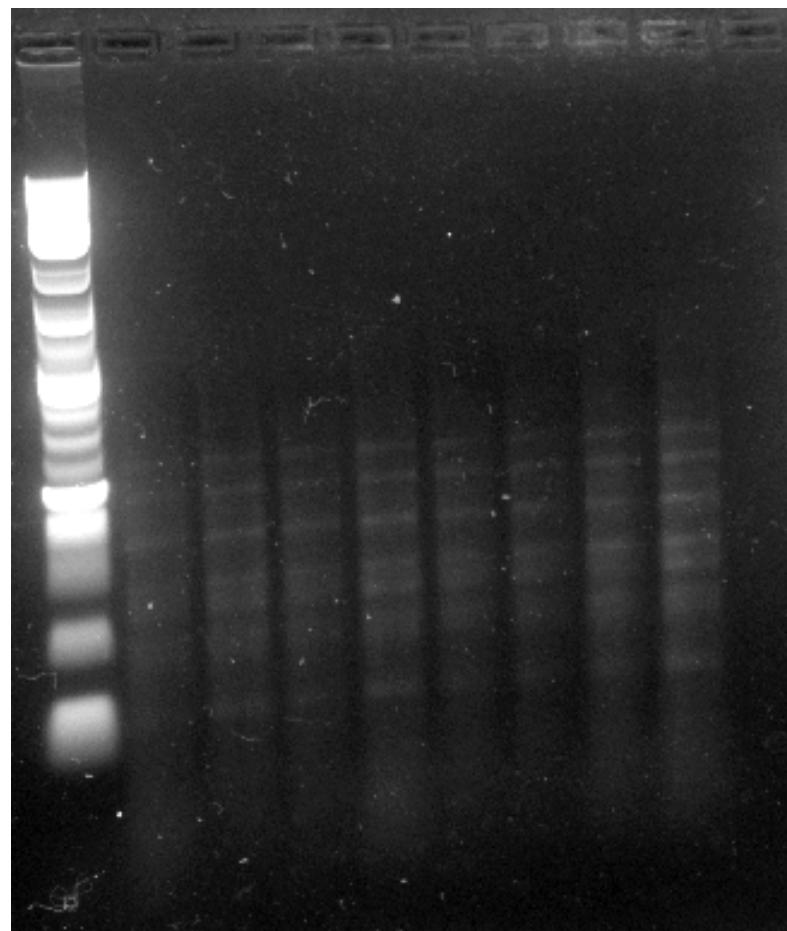
Agarose gel 27: 25/11 Culture PCR products Primer 5, Digested by MspI



Log 2 Marker		Treatments							
Distance	Molecular Weight	H MSG	L MSG	H BA	L BA	H SALT	L SALT	H COMB	L COMB
2.4	3000	2.7		2.7		2.7	2.7		
2.9	2000	4.4	4.4	4.4	4.4	4.4	4.4	4.4	4.4
3.4	1500	4.7	4.7	4.7	4.7	4.7	4.7		
4.4	1000	5.1	5.1	5.1	5.1	5.1	5.1		
4.7	900	5.5	5.5	5.5	5.5	5.5	5.5		
5.0	800	5.8	5.8	5.8	5.8	5.8	5.8		
5.9	500	6.2	6.2	6.2	6.2	6.2	6.2		
		6.6	6.6	6.6	6.6	6.6	6.6		
		7.0	7.0	7.0	7.0	7.0	7.0		

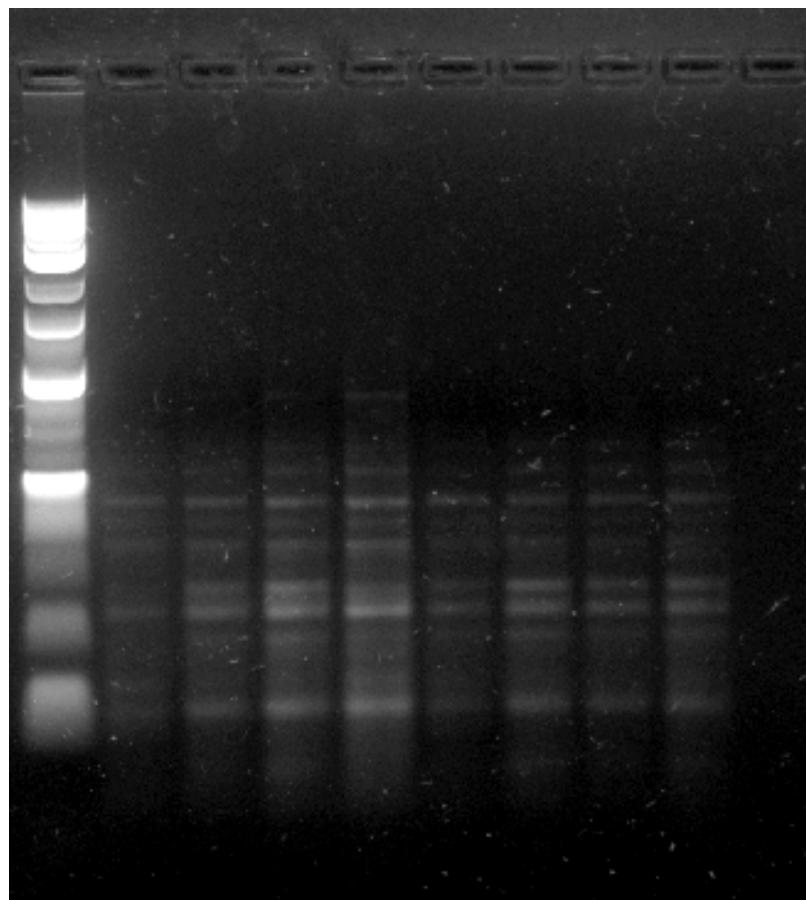
G.3.b – MspI, P6

Agarose gel 28: 8/7 Culture PCR products Primer 6, Digested by MspI



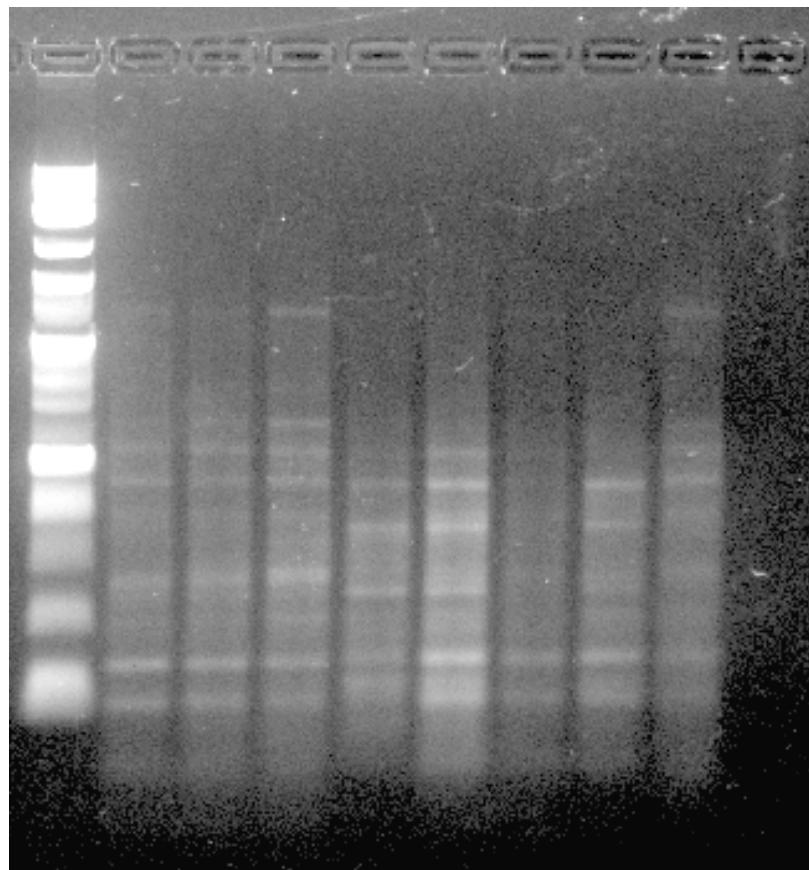
Log 2 Marker		Treatments							
Distance	Molecular Weight	H MSG	L MSG	H BA	L BA	H SALT	L SALT	H COMB	L COMB
2.6	3000	5.2	5.2	5.2	5.2	5.2	5.2	5.2	5.2
3.0	2000	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7
3.6	1500	6.2	6.2	6.2	6.2	6.2	6.2	6.2	6.2
4.5	1000	6.8	6.8	6.8	6.8	6.8	6.8	6.8	6.8
4.7	900	7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.2
5.0	800	7.8	7.8	7.8	7.8	7.8	7.8	7.8	
5.7	500	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5

Agarose gel 29: 5/8 Culture PCR products Primer 6, Digested by MspI



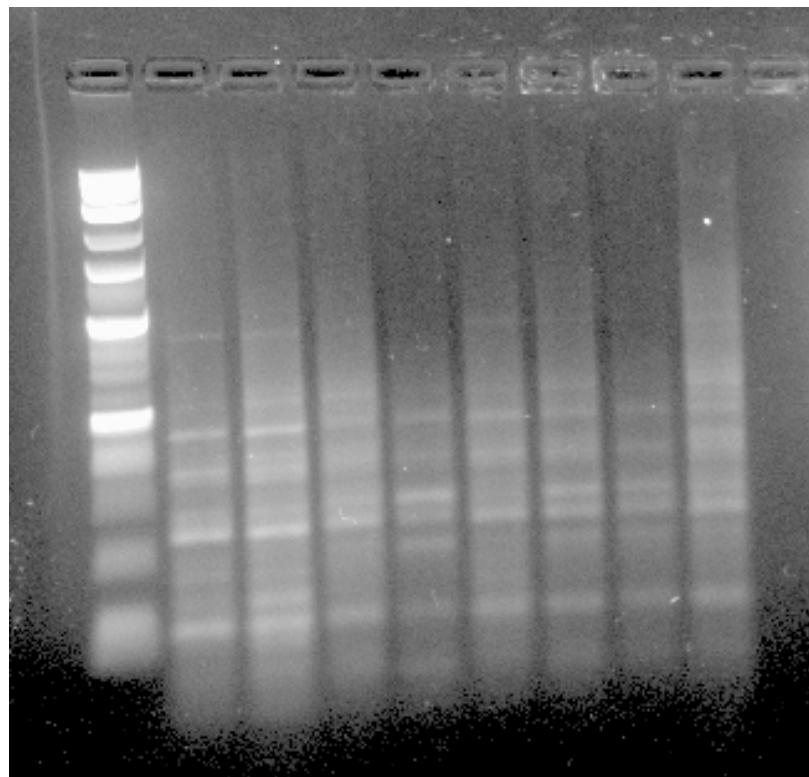
Log 2 Marker		Treatments							
Distance	Molecular Weight	H MSG	L MSG	H BA	L BA	H SALT	L SALT	H COMB	L COMB
2.2	3000				3.7				
2.5	2000			4.5	4.5	4.5		4.5	4.5
3.0	1500	4.9	4.9	4.9	4.9	4.9	4.9	4.9	4.9
3.6	1000	5.2	5.2	5.2	5.2	5.2	5.2	5.2	5.2
3.8	900	5.4	5.4	5.4	5.4	5.4	5.4	5.4	5.4
4.1	800	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9
4.7	500	6.2	6.2	6.2	6.2	6.2	6.2	6.2	6.2
		7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3

Agarose gel 30: 2/9 Culture PCR products Primer 6, Digested by MspI



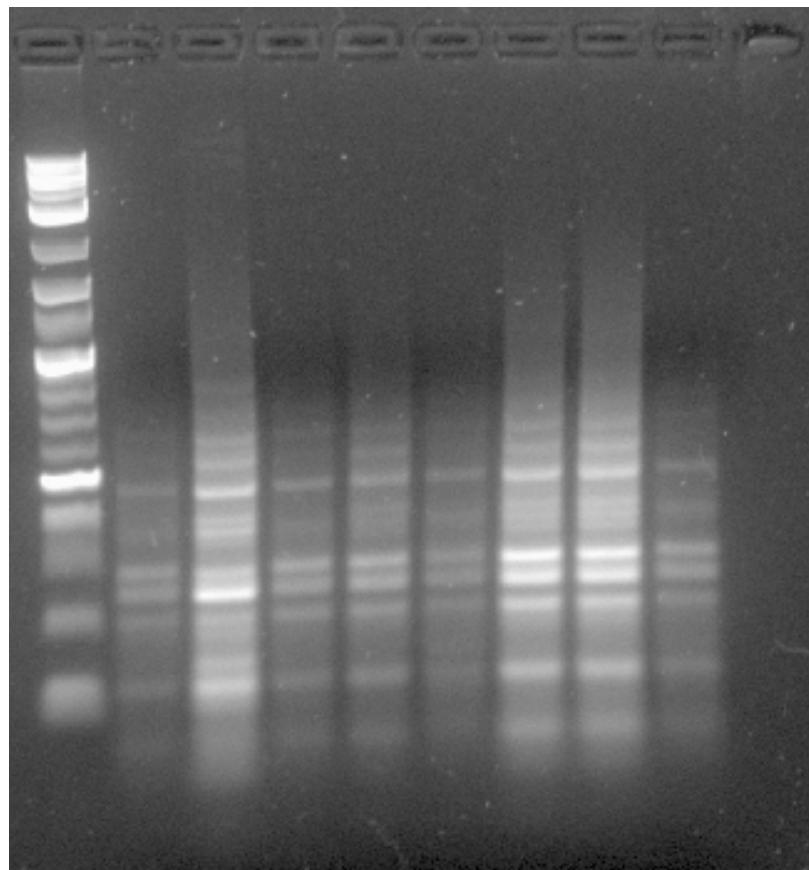
Log 2 Marker		Treatments							
Distance	Molecular Weight	H MSG	L MSG	H BA	L BA	H SALT	L SALT	H COMB	L COMB
2.2	3000	2.8	2.8	2.8			2.8	2.8	2.8
2.7	2000	4.1	4.1	4.1					4.1
3.0	1500	4.5	4.5	4.5	4.5	4.5		4.5	4.5
3.3	1000	4.9	4.9	4.9	4.9	4.9		4.9	
3.8	900	5.3	5.3	5.3	5.3	5.3		5.3	5.3
4.0	800	6.0	6.0	6.0	6.0	6.0		6.0	6.0
4.8	500	6.5		6.5		6.5		6.5	6.5
		7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0
		7.5	7.5	7.5	7.5	7.5	7.5	7.5	

Agarose gel 31: 30/9 Culture PCR products Primer 6, Digested by MspI



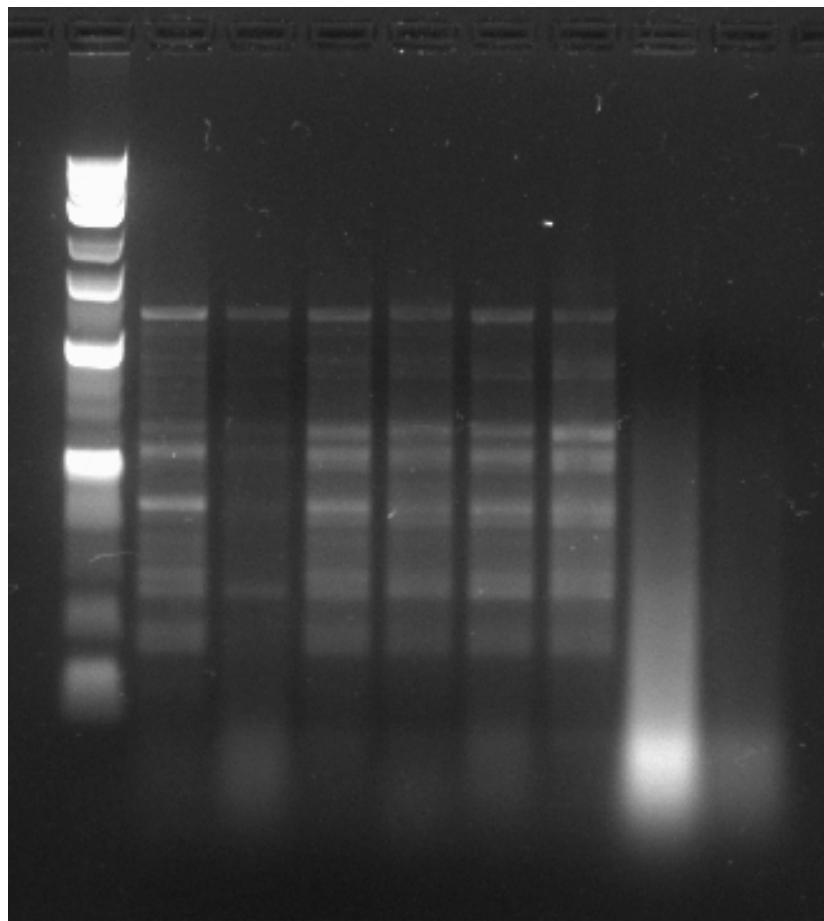
Log 2 Marker		Treatments							
Distance	Molecular Weight	H MSG	L MSG	H BA	L BA	H SALT	L SALT	H COMB	L COMB
2.2	3000	3.3	3.3	3.3		3.3	3.3		
2.6	2000	4.1	4.1	4.1		4.1	4.1		
2.8	1500	4.5	4.5	4.5	4.5	4.5	4.5	4.5	
3.3	1000	5.0	5.0	5.0	5.0	5.0	5.0	5.0	
3.6	900	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7
3.9	800	5.9	5.9		5.9	5.9	5.9	5.9	5.9
4.6	500	6.4	6.4	6.4	6.4			6.4	6.4
		6.7	6.7		6.7		6.7		6.7
		7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.2

Agarose gel 32: 28/10 Culture PCR products Primer 6, Digested by MspI



Log 2 Marker		Treatments							
Distance	Molecular Weight	H MSG	L MSG	H BA	L BA	H SALT	L SALT	H COMB	L COMB
2.0	3000		4.5				4.5	4.5	
2.4	2000		4.8		4.8		4.8	4.8	
2.9	1500	5.2	5.2	5.2	5.2	5.2	5.2	5.2	5.2
3.2	1000		5.4		5.4		5.4	5.4	5.4
3.4	900	5.5	5.5		5.5		5.5	5.5	
4.1	800	6.1	6.1	6.1	6.1	6.1	6.1	6.1	6.1
5.0	500	6.4	6.4	6.4	6.4	6.4	6.4	6.4	6.4
		6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6
		7.2	7.2			7.2	7.2	7.2	7.2
		7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5
							7.9	7.9	

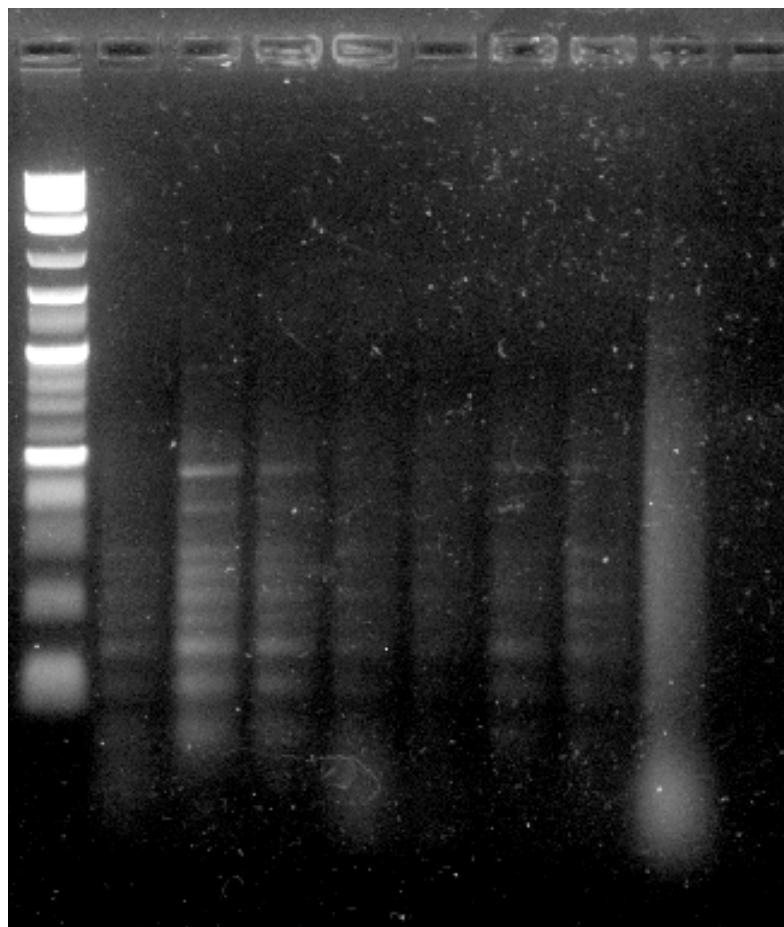
Agarose gel 33: 25/11 Culture PCR products Primer 6, Digested by MspI



Log 2 Marker		Treatments							
Distance	Molecular Weight	H MSG	L MSG	H BA	L BA	H SALT	L SALT	H COMB	L COMB
2.4	3000	3.7	3.7	3.7	3.7	3.7	3.7		
2.9	2000	4.2		4.2	4.2				
3.4	1500	4.4				4.4	4.4		
4.2	1000	5.1	5.1	5.1	5.1	5.1	5.1		
4.5	900	5.4	5.4	5.4	5.4	5.4	5.4		
5.0	800	6.1	6.1	6.1	6.1	6.1	6.1		
5.6	500	7.0		7.0		7.0	7.0		
		7.3	7.3	7.3	7.3	7.3	7.3		
		8.0		8.0	8.0	8.0	8.0		

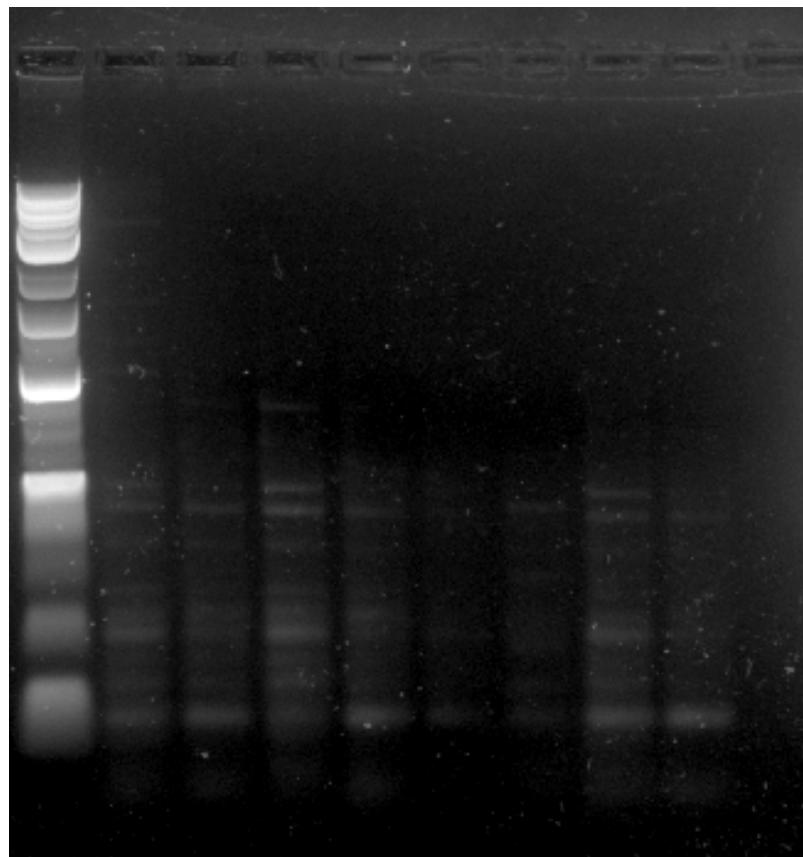
G.3.c – MspI, P7

Agarose gel 34: 8/7 Culture PCR products Primer 7, Digested by MspI



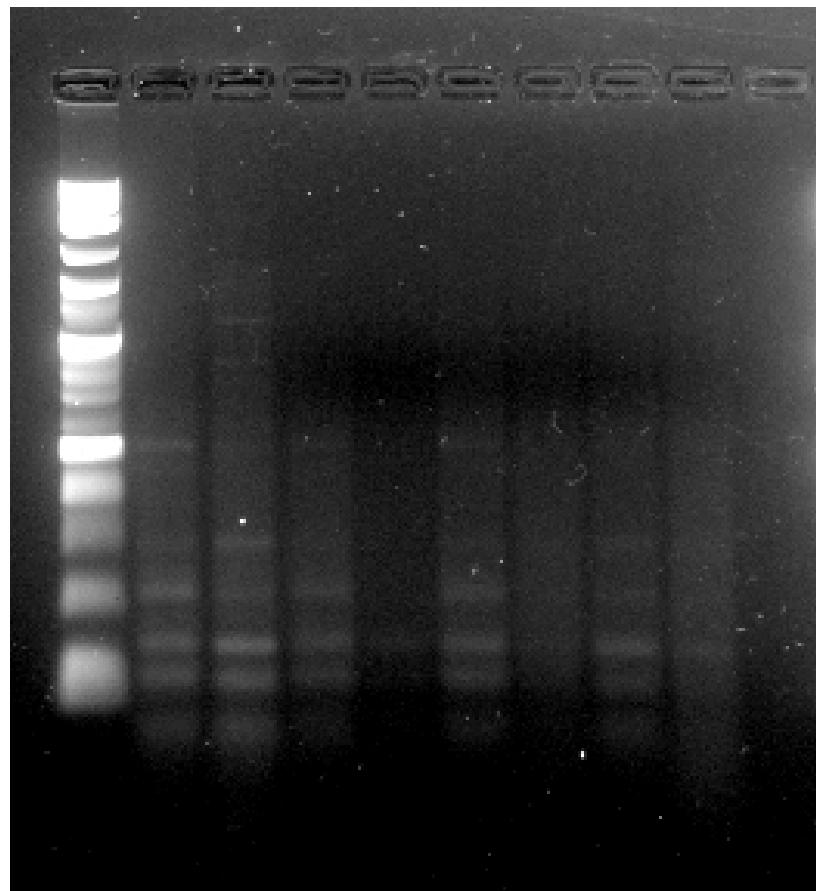
Log 2 Marker		Treatments							
Distance	Molecular Weight	H MSG	L MSG	H BA	L BA	H SALT	L SALT	H COMB	L COMB
2.0	3000		4.8						
2.4	2000						5.0	5.0	
2.8	1500	5.3	5.3	5.3					
3.6	1000	5.7	5.8	5.7					
3.7	900		6.2	6.2				6.2	
3.8	800	6.5	6.4	6.5	6.5	6.5	6.5	6.5	
4.7	500	6.8	7.0	6.8	6.8	6.8	6.8	6.8	
		7.4	7.5	7.4					
								7.8	
			8.0	8.0			8.0		

Agarose gel 35: 5/8 Culture PCR products Primer 7, Digested by MspI



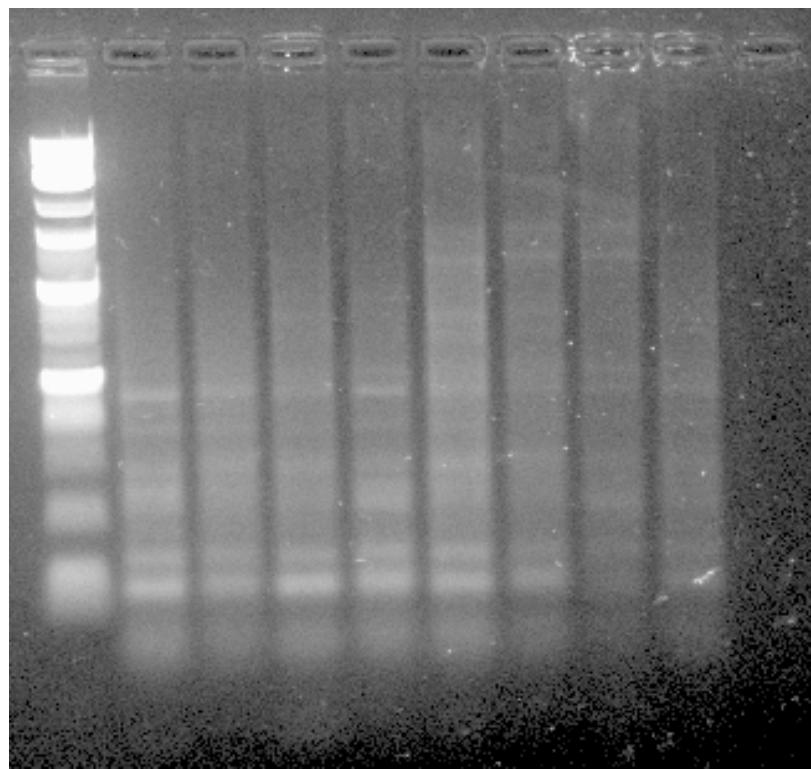
Log 2 Marker		Treatments							
Distance	Molecular Weight	H MSG	L MSG	H BA	L BA	H SALT	L SALT	H COMB	L COMB
2.3	3000		4.0	4.0					
2.7	2000	5.0	5.0	5.0				5.0	5.0
3.3	1500	5.3	5.3	5.3	5.3		5.3	5.3	5.3
4.0	1000	5.7	5.7	5.7	5.7				
4.2	900	6.2		6.2					
4.4	800	6.5	6.5	6.5	6.5				
5.1	500	6.8	6.8	6.8	6.8		6.8		
		7.4	7.4	7.4	7.4		7.4	7.4	7.4
		7.8	7.8	7.8	7.8	7.8	7.8	7.8	7.8

Agarose gel 36: 2/9 Culture PCR products Primer 7, Digested by MspI



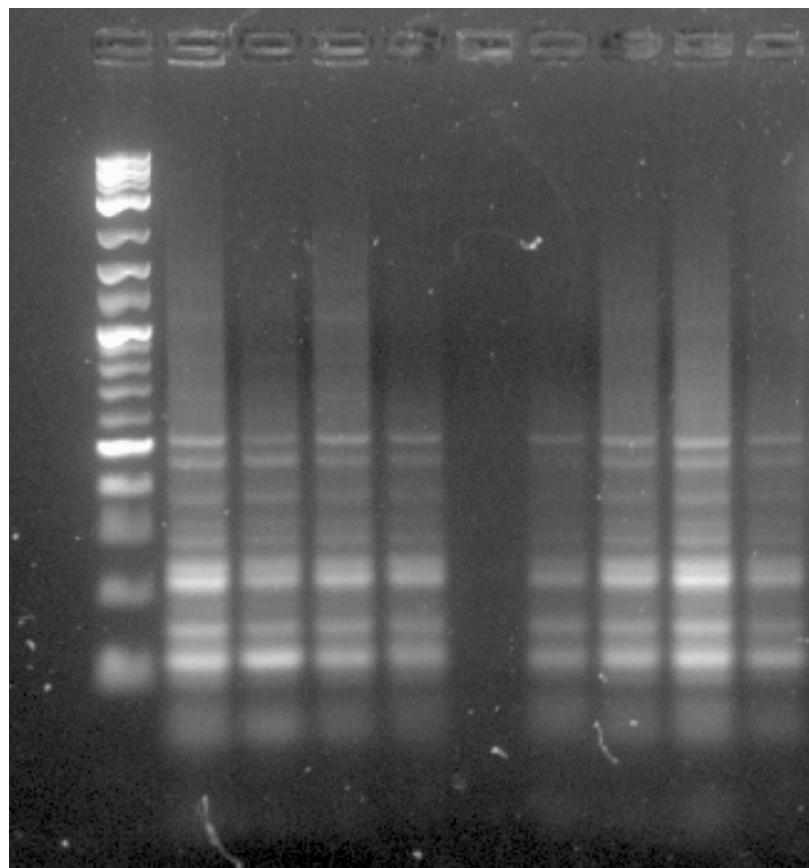
Log 2 Marker		Treatments							
Distance	Molecular Weight	H MSG	L MSG	H BA	L BA	H SALT	L SALT	H COMB	L COMB
1.9	3000	4.0							
2.3	2000	5.1	5.1						
2.6	1500	5.6	5.6	5.6	5.6	5.6			
3.0	1000	6.4	6.4	6.4	6.4	6.4			
3.3	900	6.7	6.7	6.7	6.7	6.7	6.7	6.7	
3.5	800	7.4	7.4	7.4		7.4	7.4	7.4	
4.1	500								

Agarose gel 37: 30/9 Culture PCR products Primer 7, Digested by MspI



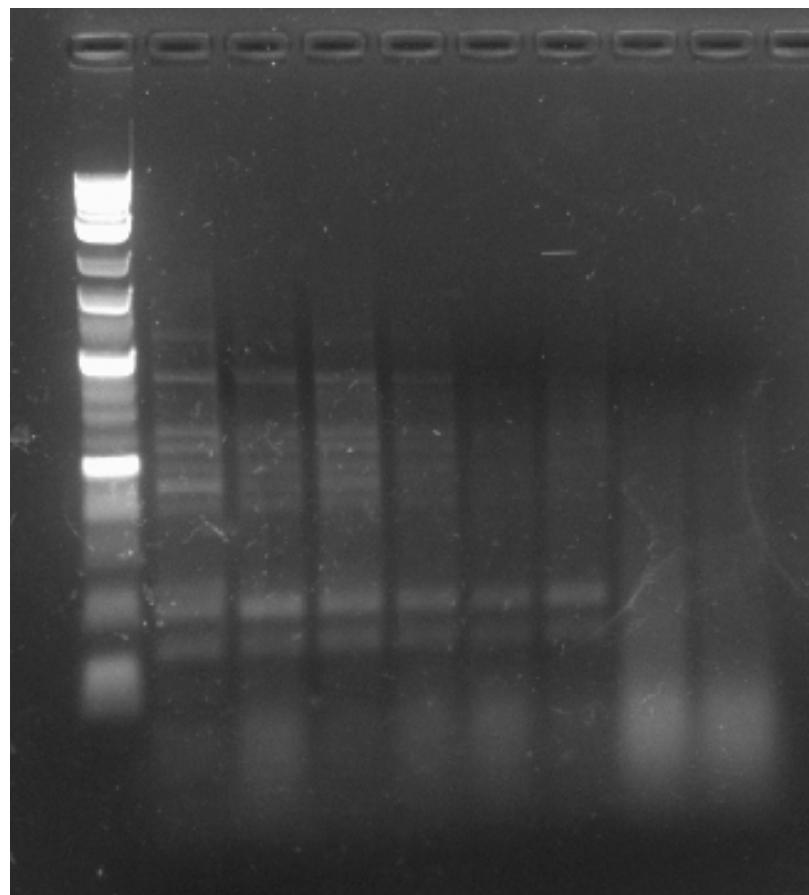
Log 2 Marker		Treatments							
Distance	Molecular Weight	H MSG	L MSG	H BA	L BA	H SALT	L SALT	H COMB	L COMB
2.1	3000					2.1	2.1	2.1	
2.4	2000					2.6	2.6	2.6	
2.7	1500	4.0				4.0	4.0	4.0	4.0
3.2	1000	4.4				4.4	4.4	4.4	4.4
3.4	900	5.2	5.2	5.2	5.2	5.2	5.2	5.2	5.2
3.7	800	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8
4.3	500	6.4	6.4	6.4	6.4	6.4	6.4	6.4	6.4
		6.9	6.9	6.9	6.9	6.9	6.9	6.9	6.9
		7.7			7.7	7.7			

Agarose gel 38: 28/10 Culture PCR products Primer 7, Digested by MspI



Log 2 Marker		Treatments							
Distance	Molecular Weight	H MSG	L MSG	H BA	L BA	H SALT	L SALT	H COMB	L COMB
2.0	3000	4.6	4.6	4.6	4.6	4.6	4.6	4.6	4.6
2.4	2000	4.9	4.9	4.9	4.9		4.9	4.9	4.9
2.9	1500	5.3	5.3	5.3	5.3		5.3	5.3	5.3
3.8	1000	5.6	5.6	5.6			5.6	5.6	
4.2	900	5.8	5.8	5.8			5.8	5.8	
4.4	800	6.1	6.1	6.1	6.1	6.1	6.1	6.1	6.1
5.0	500	6.3	6.3	6.3	6.3	6.3	6.3	6.3	6.3
		6.8	6.8	6.8	6.8	6.8	6.8	6.8	6.8
		7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.2

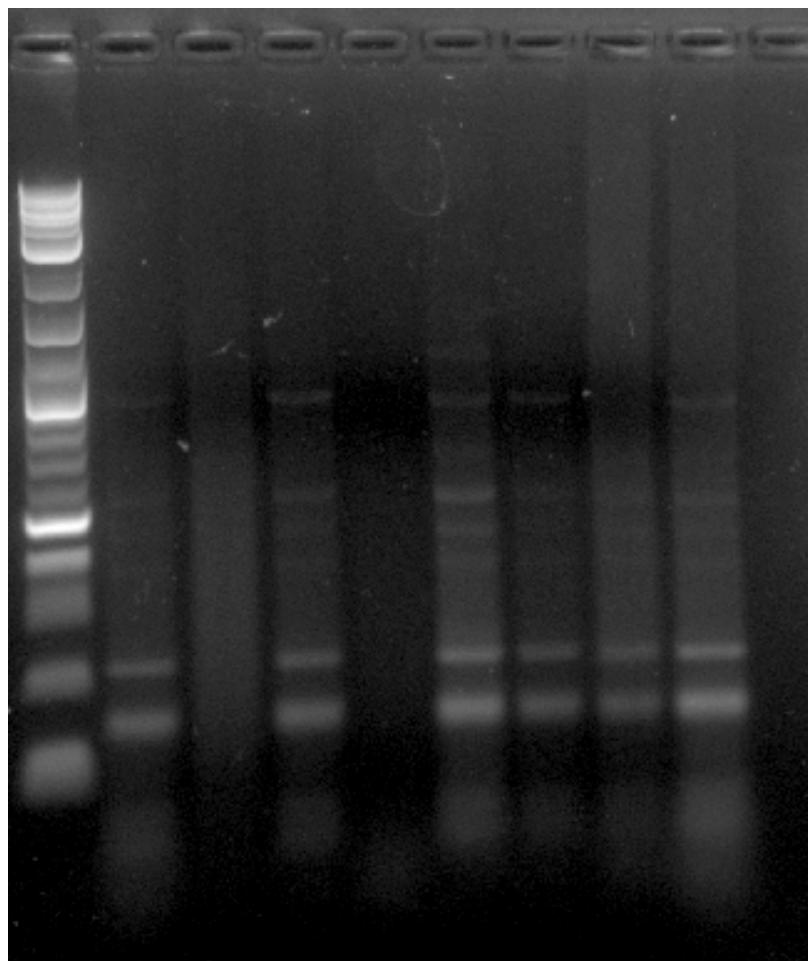
Agarose gel 39: 25/11 Culture PCR products Primer 7, Digested by MspI



Log 2 Marker		Treatments							
Distance	Molecular Weight	H MSG	L MSG	H BA	L BA	H SALT	L SALT	H COMB	L COMB
2.9	3000	3.8							
3.4	2000	4.3	4.3	4.3	4.3				
3.8	1500	4.9	4.9	4.9	4.9				
4.2	1000	5.2	5.2	5.2	5.2		5.2		
4.5	900	5.5	5.5	5.5					
4.8	800	5.7	5.7	5.7					
5.6	500	6.0	6.0	6.0					
		7.4	7.4	7.4	7.4	7.4	7.4		
		7.9	7.9	7.9	7.9	7.9	7.9		

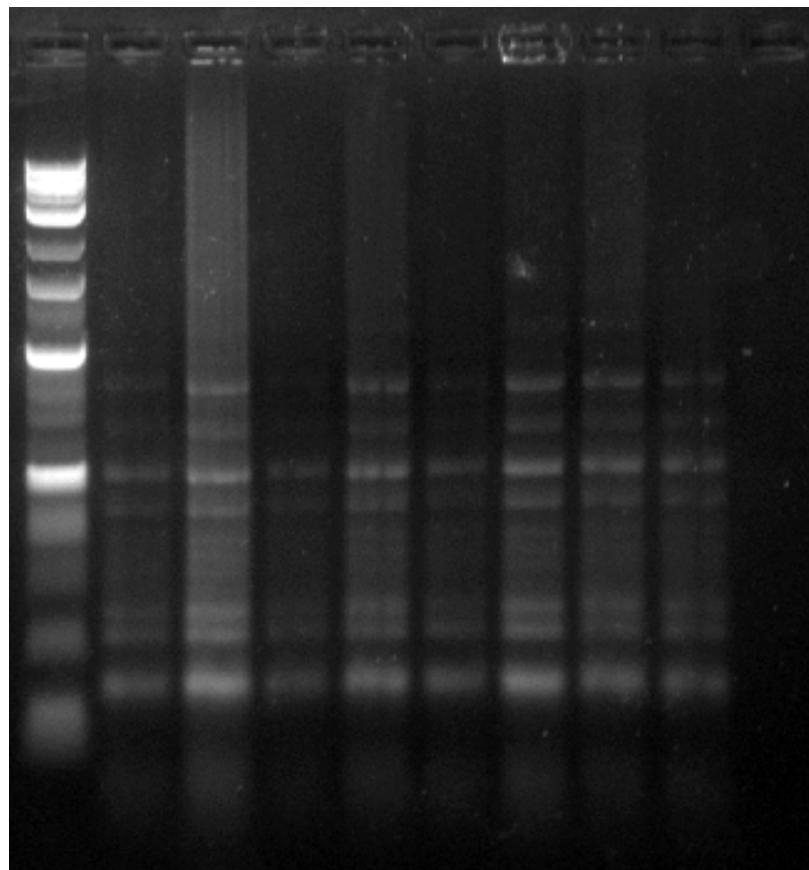
G.3.d – TaqI, Primer 5

Agarose gel 40: 8/7 Culture PCR products Primer 5, Digested by TaqI



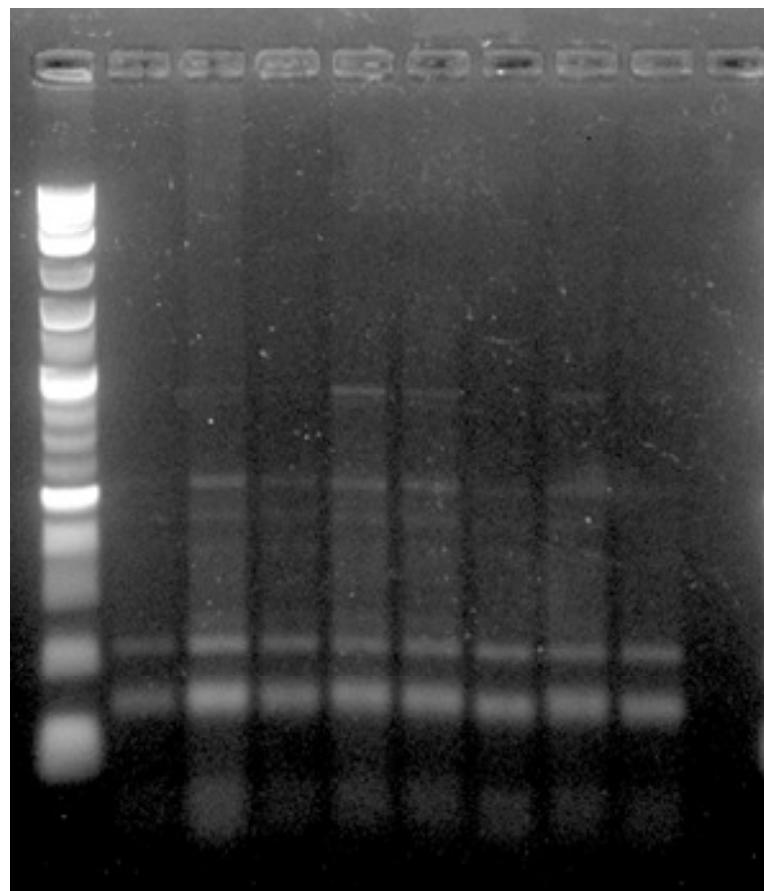
Log 2 Marker		Treatments							
Distance	Molecular Weight	H MSG	L MSG	H BA	L BA	H SALT	L SALT	H COMB	L COMB
2.4	3000					3.5			
2.8	2000	4.0		4.0		4.0	4.0		4.0
3.2	1500					4.6		4.6	4.6
4.1	1000			5.1		5.1	5.1	5.1	5.1
4.4	900			5.5		5.5		5.5	5.5
4.75	800	6.8		6.8		6.8	6.8	6.8	6.8
5.3	500	7.5		7.5		7.5	7.5	7.5	7.5

Agarose gel 41: 5/8 Culture PCR products Primer 5, Digested by TaqI



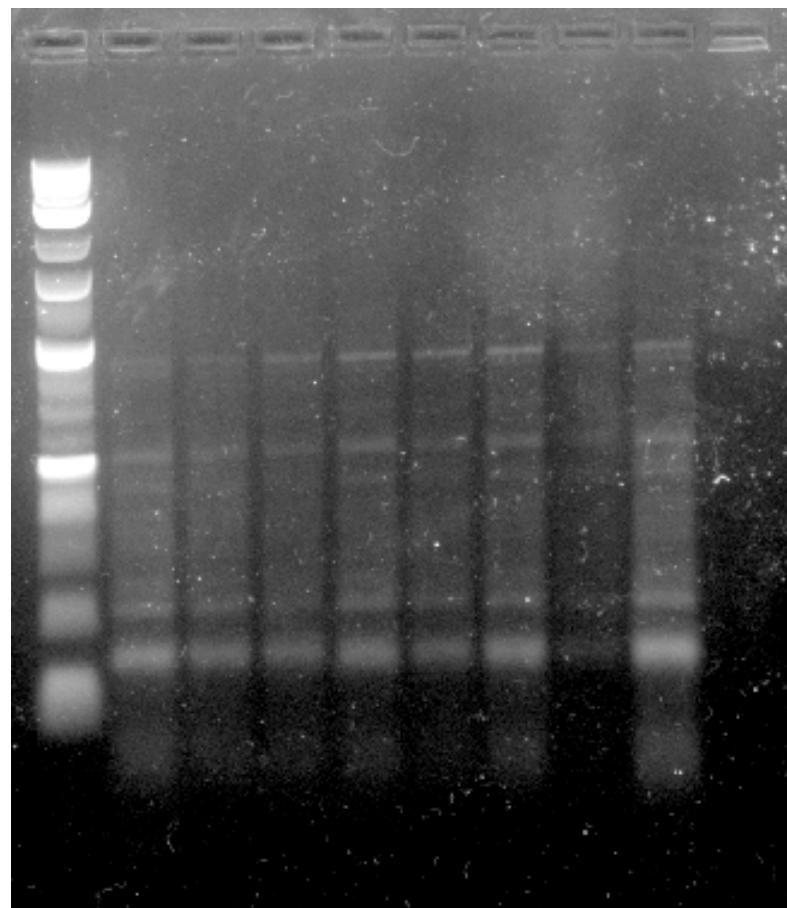
Log 2 Marker		Treatments							
Distance	Molecular Weight	H MSG	L MSG	H BA	L BA	H SALT	L SALT	H COMB	L COMB
2.1	3000	4.2	4.2		4.2	4.2	4.2	4.2	4.2
2.6	2000	4.7	4.7	4.7	4.7	4.7	4.7	4.7	4.7
3.0	1500	5.2	5.2	5.2	5.2	5.2	5.2	5.2	5.2
3.8	1000	5.6	5.6		5.6	5.6	5.6	5.6	5.6
4.2	900	6.0	6.0		6.0	6.0	6.0	6.0	6.0
4.6	800	6.4	6.4	6.4	6.4	6.4	6.4	6.4	6.4
5.2	500	6.9	6.9	6.9	6.9	6.9	6.9	6.9	6.9
		7.8	7.8	7.8	7.8	7.8	7.8	7.8	7.8

Agarose gel 42: 2/9 Culture PCR products Primer 5, Digested by TaqI



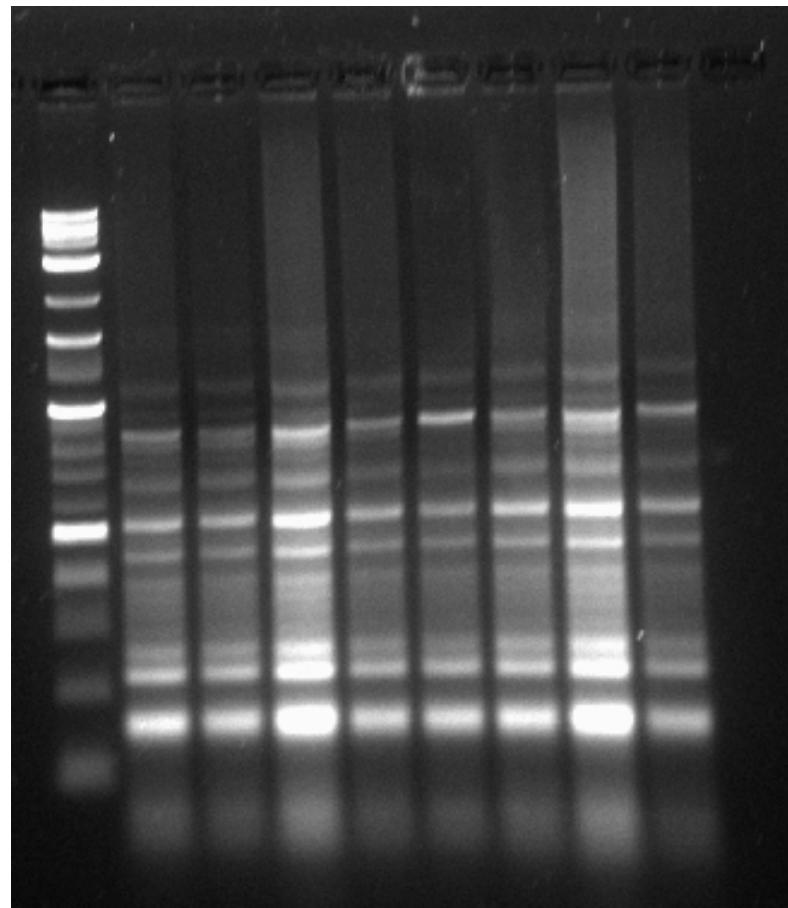
Log 2 Marker		Treatments							
Distance	Molecular Weight	H MSG	L MSG	H BA	L BA	H SALT	L SALT	H COMB	L COMB
2.3	3000		3.9		3.9	3.9		3.9	
2.7	2000		5.1	5.1	5.1	5.1	5.1	5.1	
3.2	1500	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1
4.0	1000	7.8	7.8	7.8	7.8	7.8	7.8	7.8	7.8
4.35	900								
4.65	800								
5.4	500								

Agarose gel 43: 30/9 Culture PCR products Primer 5, Digested by TaqI



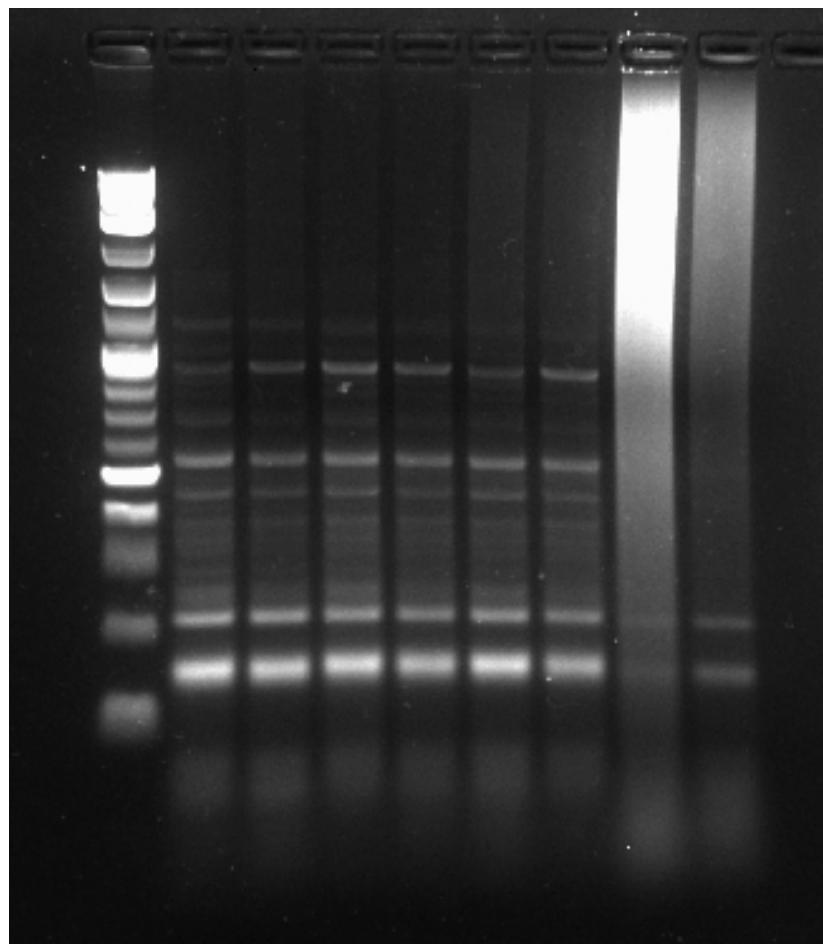
Log 2 Marker		Treatments							
Distance	Molecular Weight	H MSG	L MSG	H BA	L BA	H SALT	L SALT	H COMB	L COMB
2.4	3000	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
2.8	2000	5.2	5.2	5.2	5.2	5.2	5.2	5.2	5.2
3.3	1500	7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.2
4.2	1000	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0
4.5	900								
5.0	800								
5.5	500								

Agarose gel 44: 28/10 Culture PCR products Primer 5, Digested by TaqI



Log 2 Marker		Treatments							
Distance	Molecular Weight	H MSG	L MSG	H BA	L BA	H SALT	L SALT	H COMB	L COMB
2.1	3000	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6
2.5	2000	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1
3.0	1500	4.2		4.2				4.2	
3.8	1000	4.6	4.6	4.6	4.6	4.6	4.6	4.6	4.6
4.0	900	5.1	5.1	5.1	5.1	5.1	5.1	5.1	5.1
4.2	800	5.4	5.4	5.4	5.4	5.4	5.4	5.4	5.4
5.2	500	5.7	5.7	5.7	5.7	5.7	5.7	5.7	
				5.9			5.9		
		6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6
		6.8	6.8	6.8	6.8	6.8	6.8	6.8	6.8
		7.45	7.45	7.45	7.45	7.45	7.45	7.45	7.45

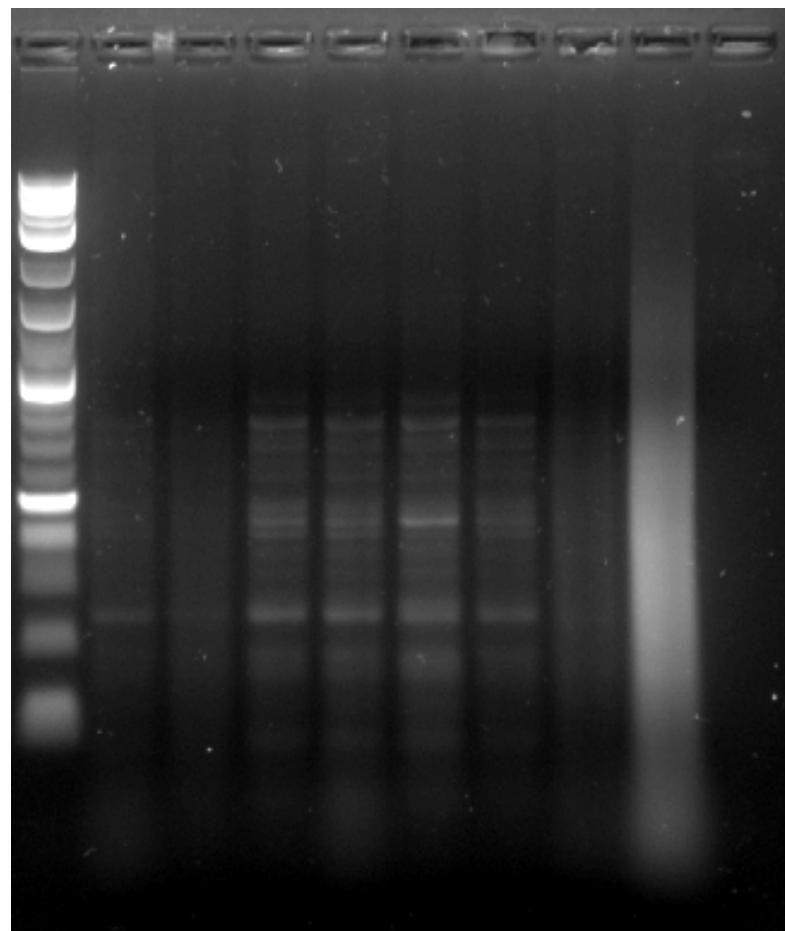
Agarose gel 45: 25/11 Culture PCR products Primer 5, Digested by TaqI



Log 2 Marker		Treatments							
Distance	Molecular Weight	H MSG	L MSG	H BA	L BA	H SALT	L SALT	H COMB	L COMB
2.5	3000	3.7	3.7	3.7	3.7	3.7	3.7		
2.9	2000	4.3	4.3	4.3	4.3	4.3	4.3		
3.4	1500	5.0		5.0	5.0	5.0	5.0		
4.4	1000	5.6	5.6	5.6	5.6	5.6	5.6		
4.7	900	6.0	6.0	6.0	6.0	6.0	6.0		
5.0	800	6.4	6.4	6.4	6.4	6.4	6.4		
5.3	500	6.6	6.6	6.6	6.6	6.6	6.6		
		6.8	6.8	6.8	6.8	6.8	6.8		
		7.0	7.0	7.0	7.0	7.0	7.0		
		7.3	7.3	7.3	7.3	7.3	7.3		
		7.7	7.7	7.7	7.7	7.7	7.7	7.7	7.7
		8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5

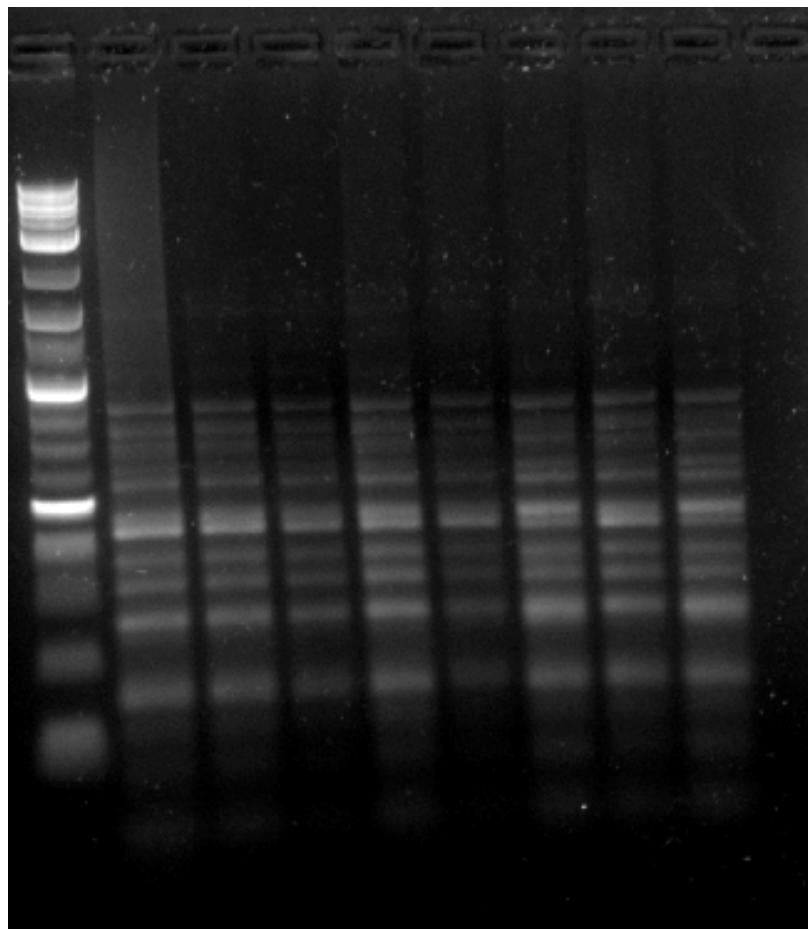
G.3.e – TaqI, P6

Agarose gel 46: 8/7 Culture PCR products Primer 6, Digested by TaqI



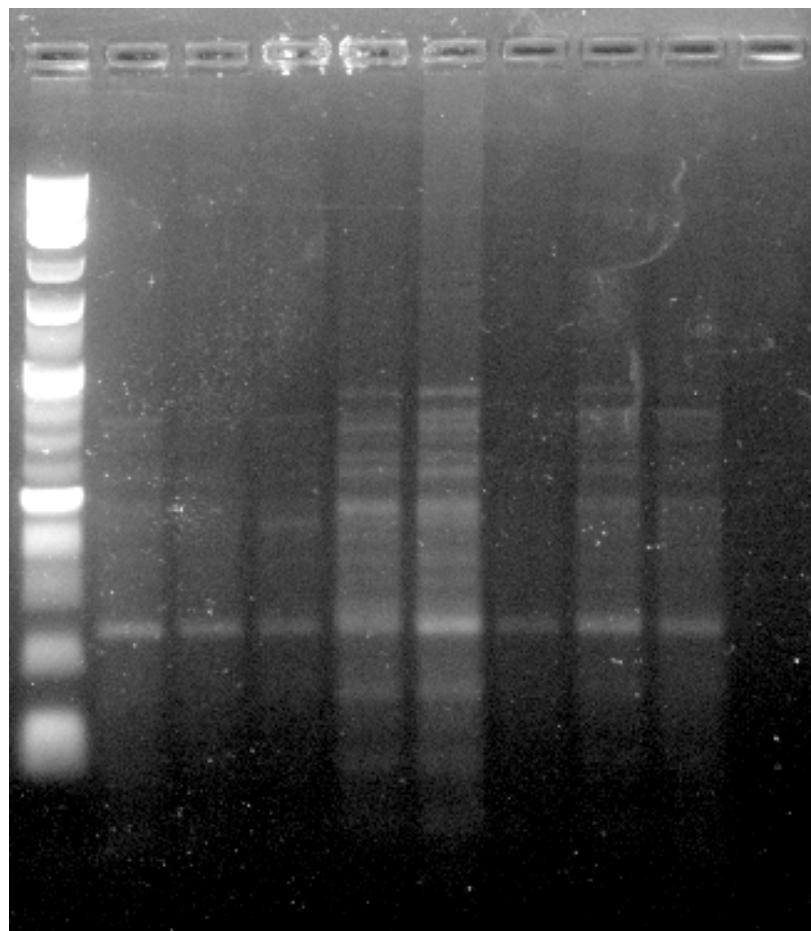
Log 2 Marker		Treatments							
Distance	Molecular Weight	H MSG	L MSG	H BA	L BA	H SALT	L SALT	H COMB	L COMB
2.1	3000	4.1		4.1	4.1	4.1	4.1	4.1	
2.5	2000	4.35		4.35	4.35	4.35	4.35	4.35	
3.0	1500			4.6	4.6	4.6	4.6		
3.75	1000	4.7		4.7	4.7	4.7	4.7	4.7	
4.1	900	5.0	5.0	5.0	5.0	5.0	5.0	5.0	
4.4	800	5.2	5.2	5.2	5.2	5.2	5.2	5.2	
5.0	500	5.4	5.4	5.4	5.4	5.4	5.4	5.4	
			6.2	6.2	6.2	6.2	6.2	6.2	
				6.9	6.9	6.9	6.9	6.9	

Agarose gel 47: 5/8 Culture PCR products Primer 6, Digested by TaqI



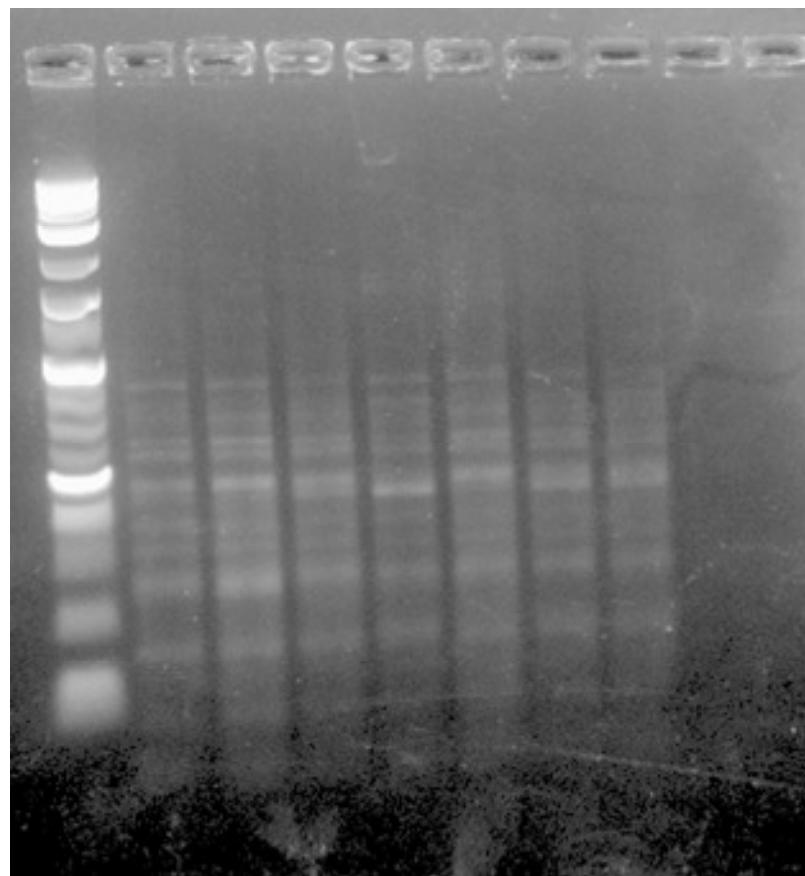
Log 2 Marker		Treatments							
Distance	Molecular Weight	H MSG	L MSG	H BA	L BA	H SALT	L SALT	H COMB	L COMB
2.1	3000	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.9
2.6	2000	4.2	4.2	4.2	4.2	4.2	4.2	4.2	4.2
3.0	1500	4.4	4.4	4.4	4.4	4.4	4.4	4.4	4.4
3.2	1000	4.6	4.6	4.6	4.6	4.6	4.6	4.6	4.6
4.1	900	4.7	4.7	4.7	4.7	4.7	4.7	4.7	4.7
4.4	800	5.1	5.1	5.1	5.1	5.1	5.1	5.1	5.1
5.0	500	5.2	5.2	5.2	5.2	5.2	5.2	5.2	5.2
		5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.6
		5.85	5.85	5.85	5.85	5.85	5.85	5.85	5.85
		6.3	6.3	6.3	6.3	6.3	6.3	6.3	6.3
		7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1
		7.9	7.9	7.9	7.9	7.9	7.9	7.9	7.9

Agarose gel 48: 2/9 Culture PCR products Primer 6, Digested by TaqI



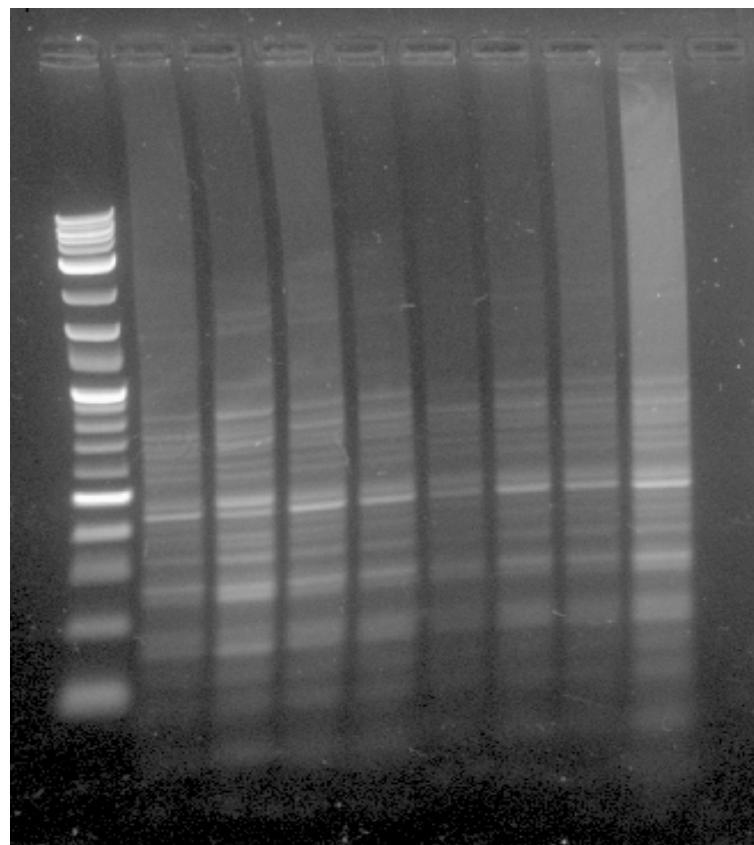
Log 2 Marker		Treatments							
Distance	Molecular Weight	H MSG	L MSG	H BA	L BA	H SALT	L SALT	H COMB	L COMB
2.25	3000				4.0	4.0		4.0	
2.7	2000					4.3			4.3
3.15	1500				4.8	4.8			
4.0	1000				5.0	5.0			
4.3	900				5.4	5.4		5.4	5.4
4.7	800			5.6					
5.4	500	6.8	6.8	6.8	6.8	6.8	6.8	6.8	6.8

Agarose gel 49: 30/9 Culture PCR products Primer 6, Digested by TaqI



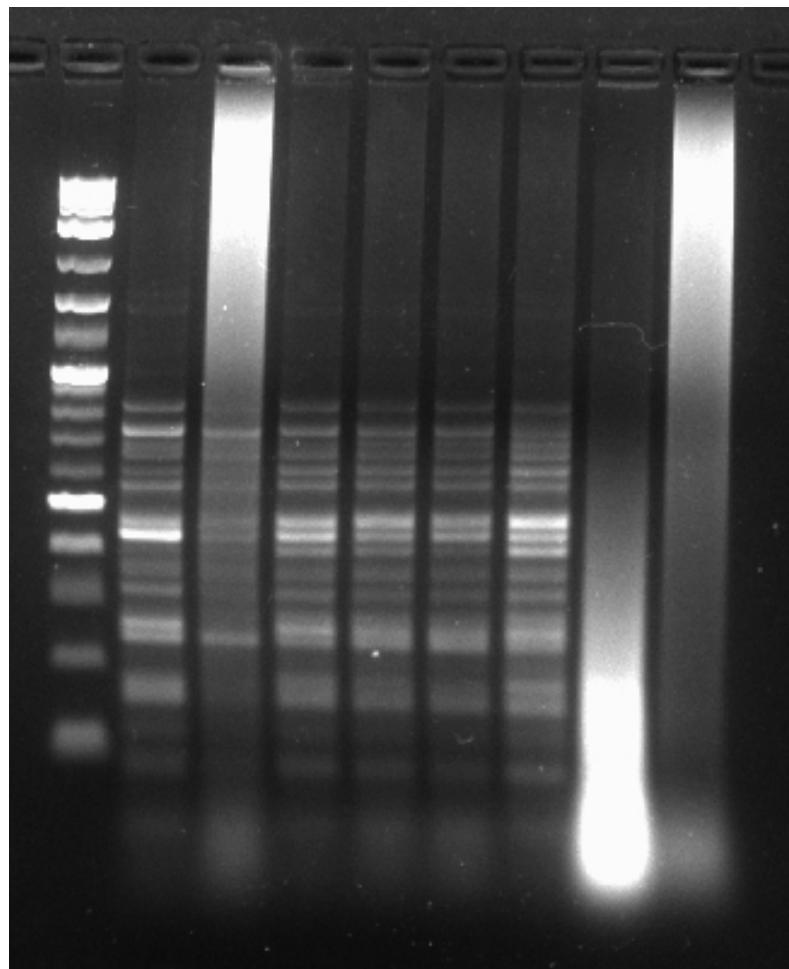
Log 2 Marker		Treatments							
Distance	Molecular Weight	H MSG	L MSG	H BA	L BA	H SALT	L SALT	H COMB	L COMB
2.2	3000	4.0	4.0	4.0	4.0	4.0	4.0	4.0	
2.6	2000	4.4	4.4	4.4		4.4			
3.1	1500	4.85	4.85	4.85	4.85	4.85			
3.9	1000	4.95	4.95	4.95	4.95	4.95	4.95	4.95	
4.3	900	5.3	5.3		5.3	5.3		5.3	
4.6	800		5.5	5.5					
5.3	500	5.75	5.75						
		6.1	6.1	6.1	6.1			6.1	
		6.6	6.6	6.6	6.6	6.6	6.6	6.6	
		7.3	7.3	7.3	7.3	7.3	7.3	7.3	

Agarose gel 50: 28/10 Culture PCR products Primer 6, Digested by TaqI



Log 2 Marker		Treatments							
Distance	Molecular Weight	H MSG	L MSG	H BA	L BA	H SALT	L SALT	H COMB	L COMB
2.9	3000		3.5						
3.3	2000		3.7	3.7	3.7	3.7	3.7		
3.7	1500	4.4	4.4	4.4					
4.55	1000	4.75	4.75	4.75	4.75	4.75	4.75	4.75	4.75
4.7	900	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
4.95	800	5.1	5.1	5.1	5.1	5.1	5.1	5.1	5.1
5.9	500	5.25	5.25	5.25	5.25		5.25	5.25	5.25
		5.4	5.4	5.4	5.4	5.4	5.4	5.4	5.4
		5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.6
		5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9
		6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0
		6.25	6.25	6.25	6.25	6.25	6.25	6.25	6.25
			6.3				6.3	6.3	6.3
			6.4	6.4	6.4	6.4			6.4
		6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6
		7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0
			7.1	7.1	7.1		7.1		7.1
		7.8	7.8	7.8	7.8	7.8	7.8	7.8	7.8

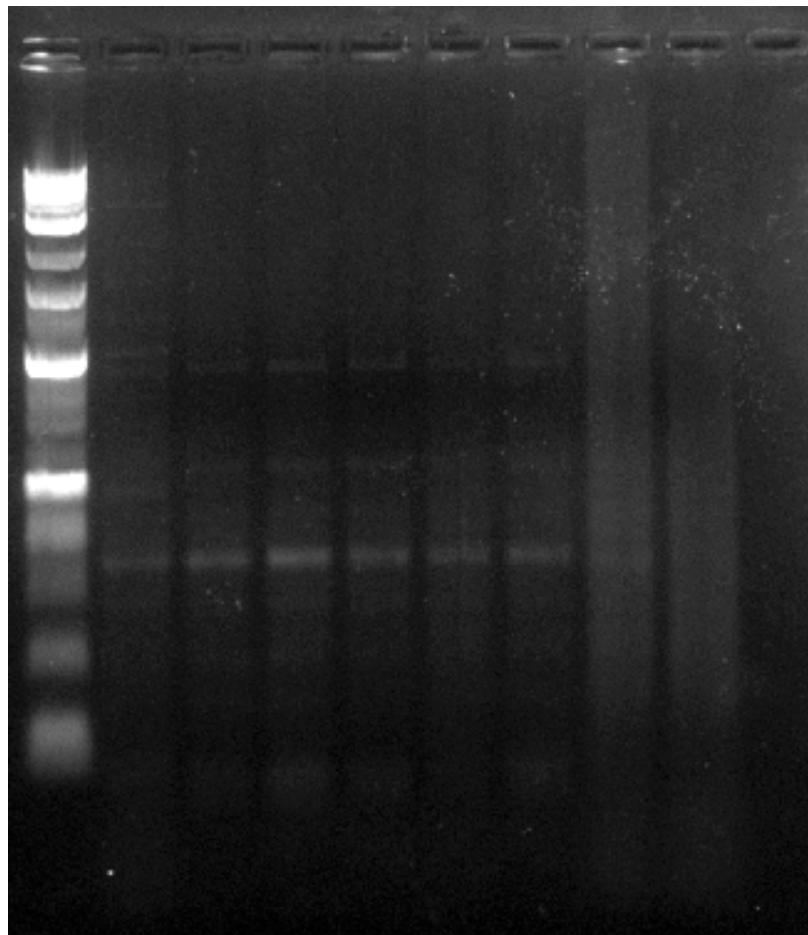
Agarose gel 51: 25/11 Culture PCR products Primer 6, Digested by TaqI



Log 2 Marker		Treatments							
Distance	Molecular Weight	H MSG	L MSG	H BA	L BA	H SALT	L SALT	H COMB	L COMB
2.1	3000	4.5	4.5	4.5	4.5	4.5	4.5		
2.5	2000	4.8	4.8	4.8	4.8	4.8	4.8		
2.9	1500	5.1	5.1	5.1	5.1	5.1	5.1		
4.1	1000	5.3	5.3	5.3	5.3	5.3	5.3		
4.2	900	5.5	5.5	5.5	5.5	5.5	5.5		
4.6	800	5.95	5.95	5.95	5.95	5.95	5.95		
5.7	500	6.15	6.15	6.15	6.15	6.15	6.15		
				6.3	6.3	6.3	6.3		
		6.7		6.7	6.7	6.7	6.7		
		6.9		6.9	6.9	6.9	6.9		
		7.4		7.4	7.4	7.4	7.4		
		7.6	7.6	7.6	7.6	7.6	7.6		
		8.0		8.0	8.0	8.0	8.0		
		8.3		8.3	8.3	8.3	8.3		

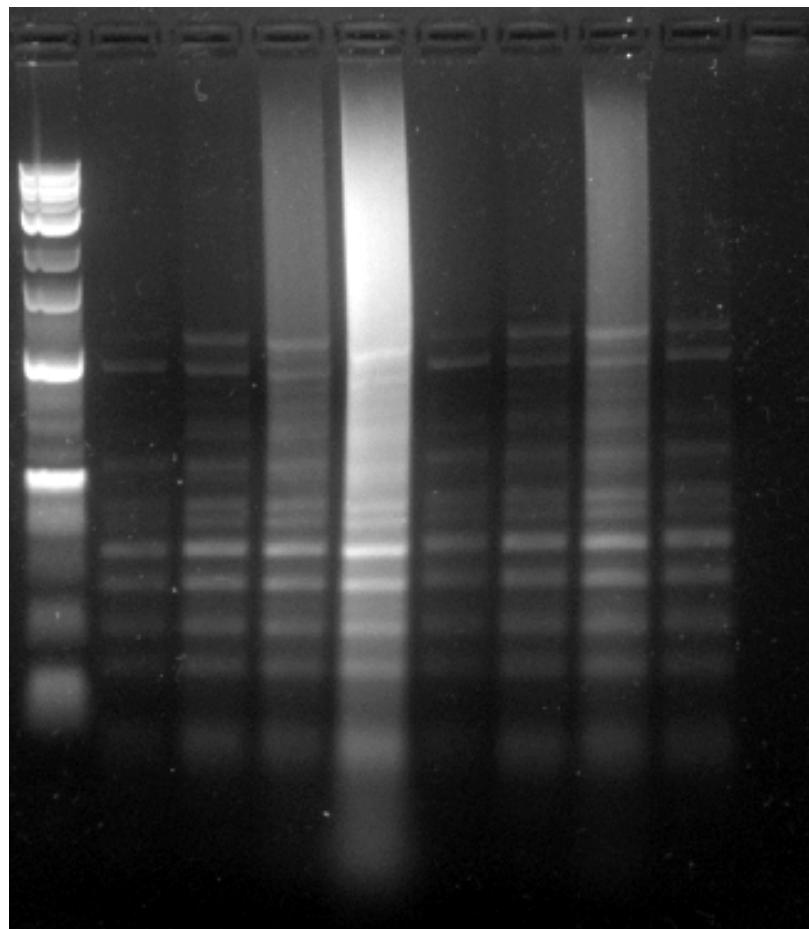
G.3.f – TaqI, P7

Agarose gel 52: 8/7 Culture PCR products Primer 7, Digested by TaqI



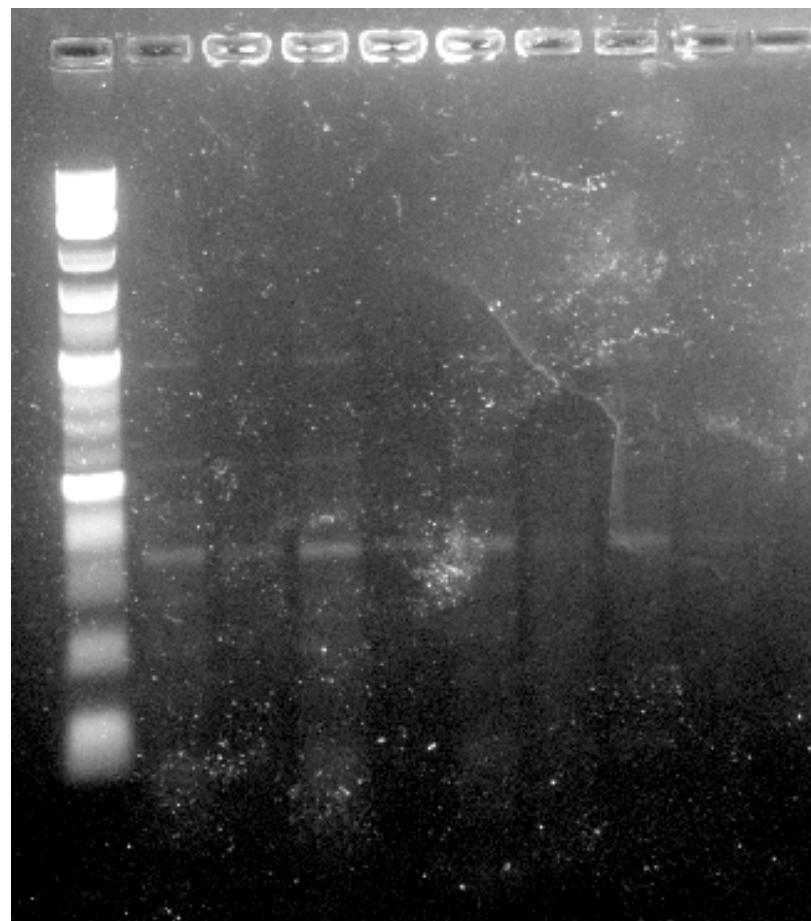
Log 2 Marker		Treatments							
Distance	Molecular Weight	H MSG	L MSG	H BA	L BA	H SALT	L SALT	H COMB	L COMB
2.0	3000		3.5	3.5	3.5	3.5	3.5		
2.4	2000	4.7	4.7	4.7					
2.85	1500		5.2	5.2					
3.0	1000	5.7	5.7	5.7	5.7	5.7	5.7		
4.0	900								
4.25	800								
4.9	500								

Agarose gel 53: 5/8 Culture PCR products Primer 7, Digested by TaqI



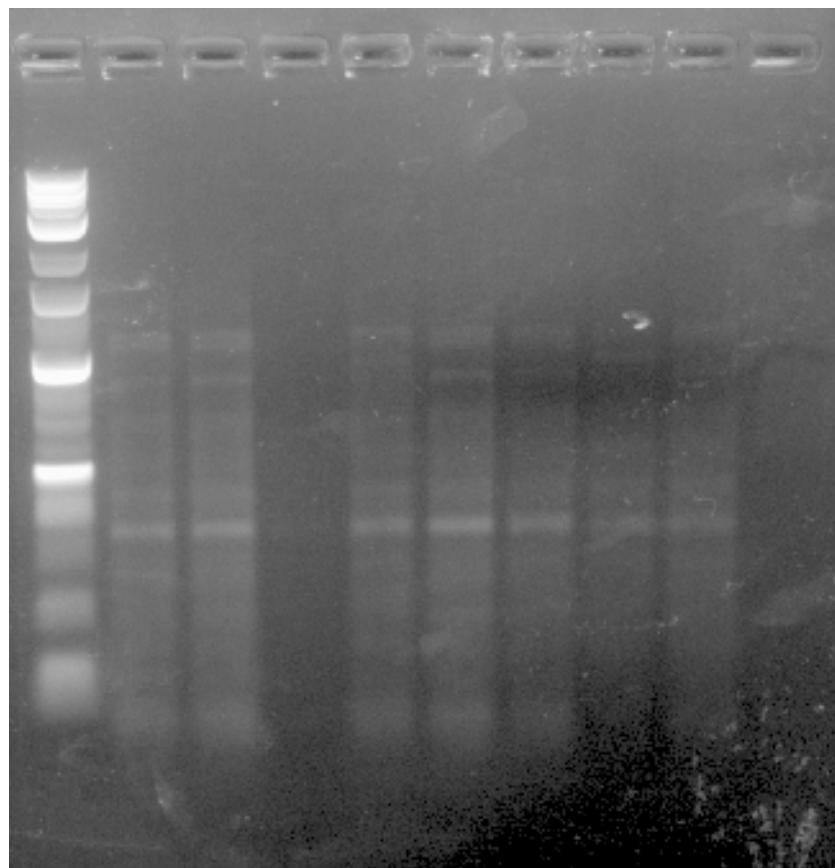
Log 2 Marker		Treatments							
Distance	Molecular Weight	H MSG	L MSG	H BA	L BA	H SALT	L SALT	H COMB	L COMB
2.1	3000	3.3	3.3	3.3		3.3	3.3	3.3	3.3
2.5	2000			3.5	3.5				
2.95	1500	3.6	3.6	3.6		3.6	3.6	3.6	3.6
3.2	1000		4.3	4.3		4.3	4.3	4.3	4.3
4.0	900	4.8	4.8	4.8		4.8	4.8	4.8	4.8
4.3	800	5.1	5.1	5.1	5.1	5.1	5.1	5.1	5.1
4.9	500	5.3	5.3	5.3	5.3	5.3	5.3	5.3	5.3
		5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.6
		5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9
		6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5
		6.9	6.9	6.9	6.9	6.9	6.9	6.9	6.9
			7.8	7.8	7.8		7.8	7.8	7.8

Agarose gel 54: 2/9 Culture PCR products Primer 7, Digested by TaqI



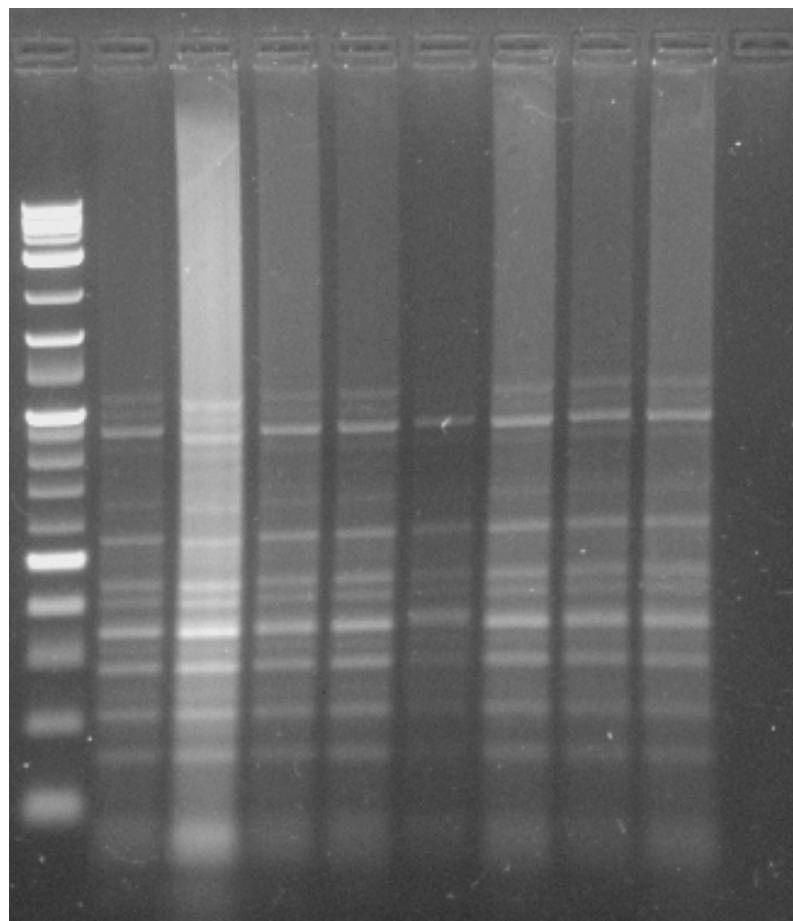
Log 2 Marker		Treatments							
Distance	Molecular Weight	H MSG	L MSG	H BA	L BA	H SALT	L SALT	H COMB	L COMB
2.4	3000	4.2	4.2	4.2					
2.8	2000			6.2					
3.35	1500	6.6	6.6	6.6	6.6	6.6		6.6	
4.2	1000								
4.6	900								
5.0	800								
5.75	500								

Agarose gel 55: 30/9 Culture PCR products Primer 7, Digested by TaqI



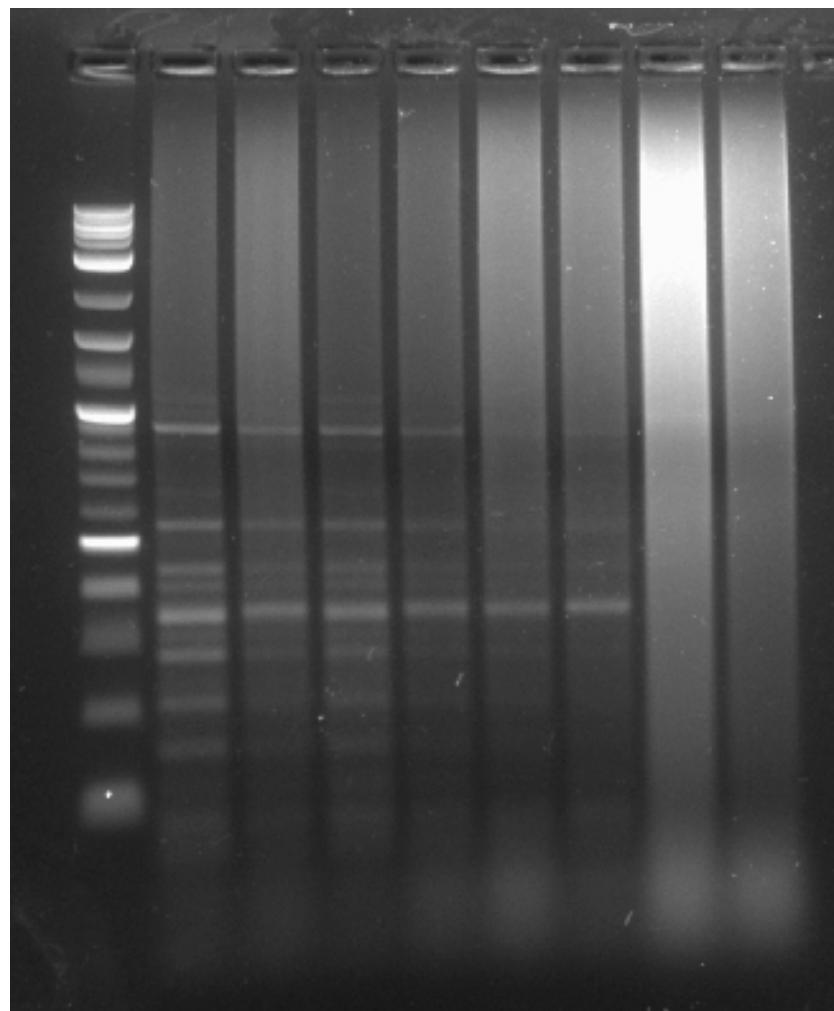
Log 2 Marker		Treatments							
Distance	Molecular Weight	H MSG	L MSG	H BA	L BA	H SALT	L SALT	H COMB	L COMB
2.3	3000	3.6	3.6		3.6	3.6	3.6	3.6	3.6
2.7	2000	4.1	4.1			4.1	4.1		
3.2	1500	4.6	4.6				4.6		
4.0	1000	5.1	5.1		5.1	5.1	5.1	5.1	5.1
4.3	900	5.45	5.45		5.45	5.45			
4.6	800	5.95	5.95		5.95	5.95	5.95	5.95	5.95
5.2	500								

Agarose gel 56: 28/10 Culture PCR products Primer 7, Digested by TaqI



Log 2 Marker		Treatments							
Distance	Molecular Weight	H MSG	L MSG	H BA	L BA	H SALT	L SALT	H COMB	L COMB
2.4	3000	3.8		3.8	3.8		3.8	3.8	3.8
2.8	2000	3.95	3.95		3.95				
3.2	1500	4.0	4.0					4.0	
4.1	1000	4.2	4.2	4.2	4.2	4.2	4.2	4.2	4.2
4.3	900	4.4			4.4		4.4	4.4	
4.6	800	5.0	5.0	5.0	5.0		5.0	5.0	5.0
5.65	500	5.35	5.35	5.35	5.35	5.35	5.35	5.35	5.35
		5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9
		6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0
		6.35	6.35	6.35	6.35	6.35	6.35	6.35	6.35
		6.5		6.5	6.5			6.5	6.5
		6.75	6.75	6.75	6.75	6.75	6.75	6.75	6.75
		7.15	7.15	7.15			7.15		
		7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3
		7.7	7.7	7.7	7.7	7.7	7.7	7.7	7.7

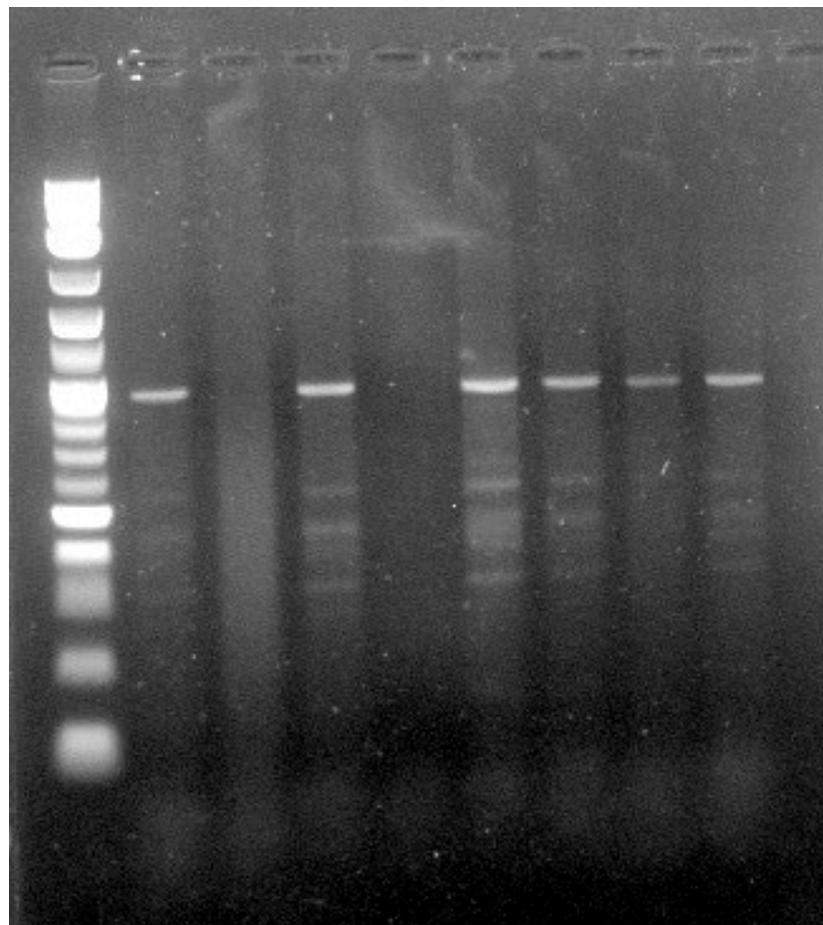
Agarose gel 57: 25/11 Culture PCR products Primer 7, Digested by TaqI



Log 2 Marker		Treatments							
Distance	Molecular Weight	H MSG	L MSG	H BA	L BA	H SALT	L SALT	H COMB	L COMB
2.6	3000			4.4					
3.0	2000	4.6	4.6	4.6	4.6	4.6	4.6		
3.6	1500	5.5		5.5					
4.5	1000	5.9	5.9	5.9	5.9	5.9	5.9		
4.7	900	6.5	6.5	6.5	6.5	6.5	6.5		
5.0	800	6.7	6.7	6.7	6.7	6.7	6.7		
6.2	500	7.1	7.1	7.1					
		7.4	7.4	7.4	7.4	7.4	7.4		
		8.2	8.2	8.2					
		8.8		8.8					

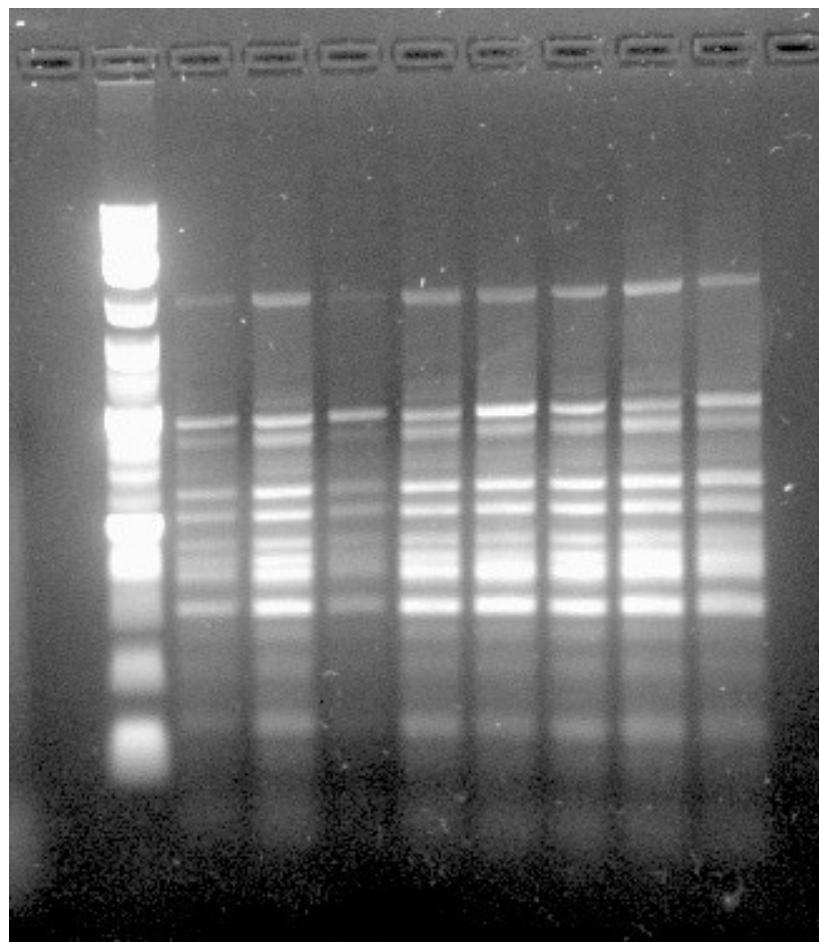
G.3.g – Hinfl, P5

Agarose gel 58: 8/7 Culture PCR products Primer 5, Digested by Hinfl



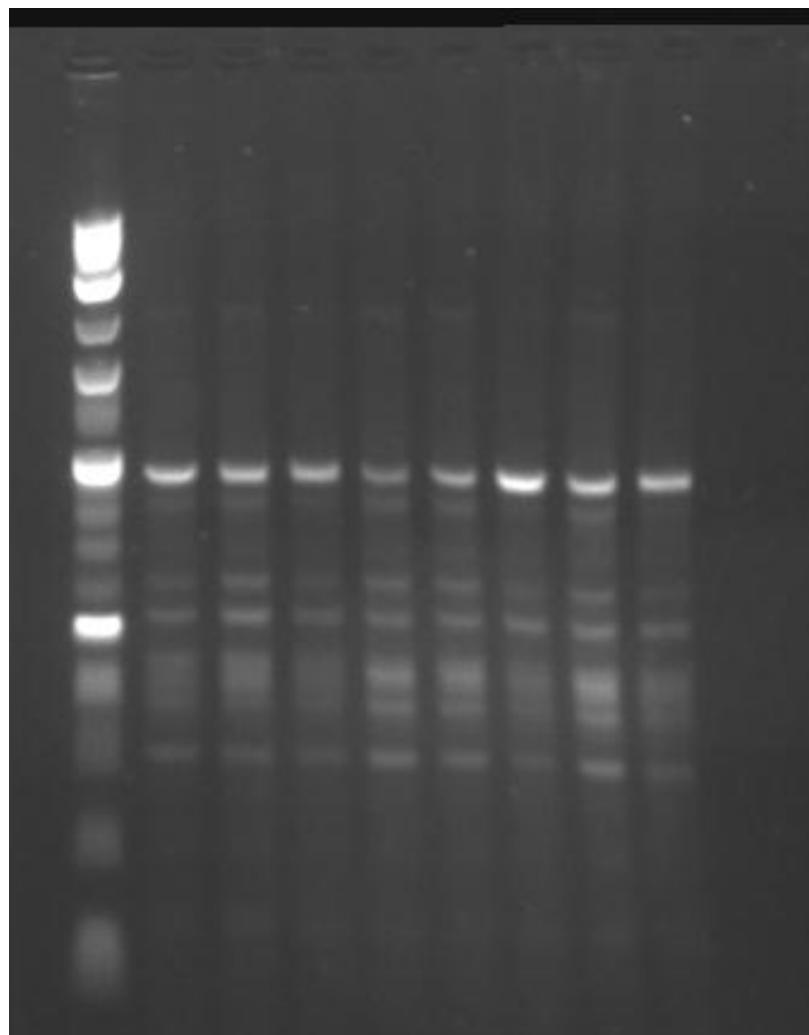
Log 2 Marker		Treatments							
Distance	Molecular Weight	H MSG	L MSG	H BA	L BA	H SALT	L SALT	H COMB	L COMB
2.4	3000	4.2		4.2		4.2	4.2	4.2	4.2
2.9	2000					4.8			4.8
3.4	1500					5.1			
4.3	1000	5.4		5.4		5.4	5.4		5.4
4.7	900	5.8		5.8		5.8	5.8		5.8
5.0	800	6.1		6.1		6.1	6.1		6.1
5.7	500	6.6		6.6		6.6	6.6		6.6

Agarose gel 59: 5/8 Culture PCR products Primer 5, Digested by Hinfl



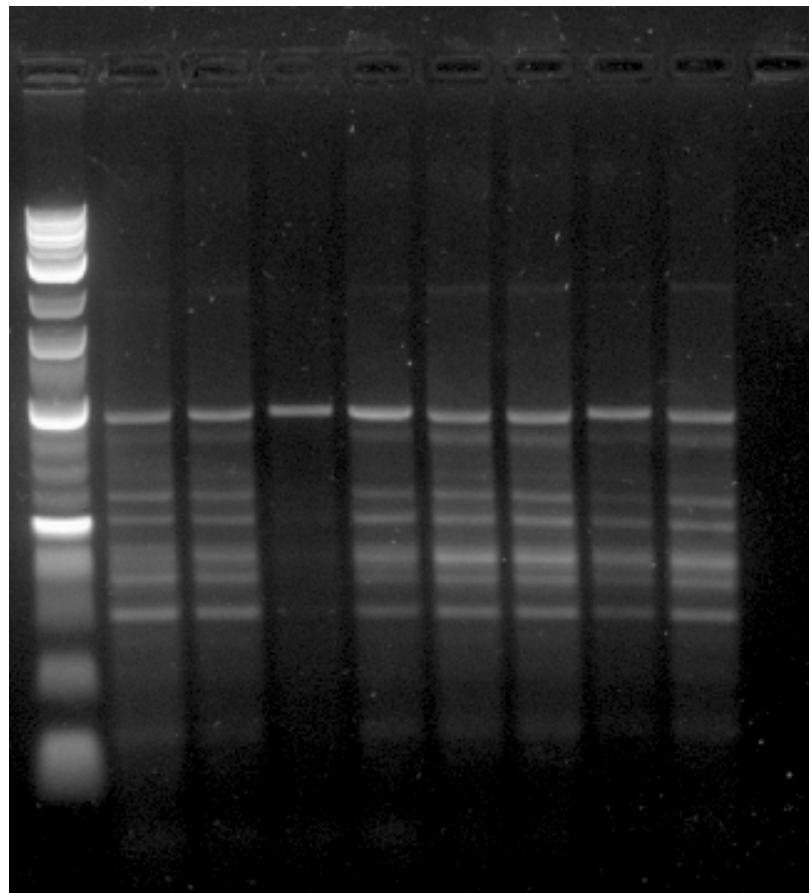
Log 2 Marker		Treatments							
Distance	Molecular Weight	H MSG	L MSG	H BA	L BA	H SALT	L SALT	H COMB	L COMB
2.6	3000	2.8	2.8	2.8	2.8	2.8	2.8	2.8	2.8
3.0	2000	4.2	4.2	4.2	4.2	4.2	4.2	4.2	4.2
3.5	1500	4.4	4.4	4.4	4.4	4.4	4.4	4.4	4.4
4.3	1000		4.75					4.75	4.75
4.55	900	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
4.8	800	5.3	5.3	5.3	5.3	5.3	5.3	5.3	5.3
5.4	500	5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.6
		5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8
		6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0
			6.4	6.4	6.4	6.4	6.4	6.4	6.4
			6.6		6.6	6.6	6.6	6.6	6.6
			7.0		7.0	7.0	7.0	7.0	7.0
			7.2		7.2	7.2	7.2	7.2	7.2

Agarose gel 60: 2/9 Culture PCR products Primer 5, Digested by Hinfl



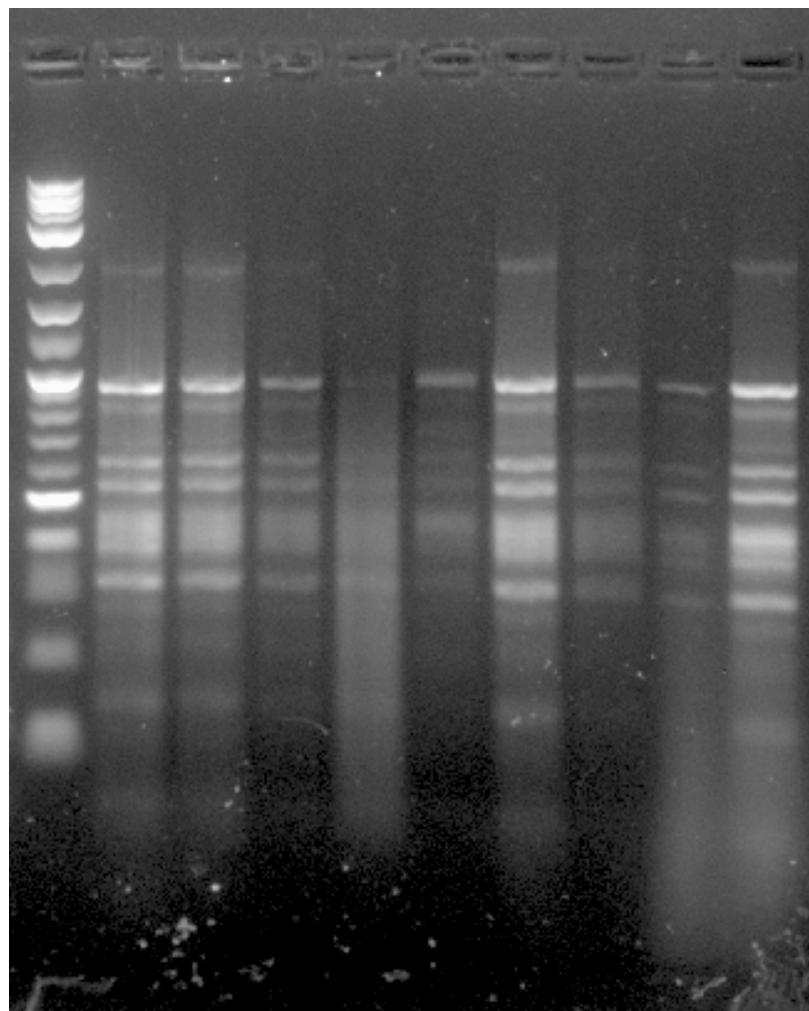
Log 2 Marker		Treatments							
Distance	Molecular Weight	H MSG	L MSG	H BA	L BA	H SALT	L SALT	H COMB	L COMB
2.9	3000	3.1	3.1		3.1	3.1		3.1	
3.4	2000	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
4.0	1500	5.4	5.4	5.4	5.4	5.4	5.4	5.4	5.4
5.0	1000	5.9	5.9	5.9		5.9	5.9	5.9	5.9
5.45	900	6.2	6.2	6.2	6.2	6.2	6.2	6.2	6.2
5.9	800	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6
6.8	500	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1
		8.2	8.2	8.2	8.2	8.2	8.2	8.2	8.2

Agarose gel 61: 30/9 Culture PCR products Primer 5, Digested by HinfI



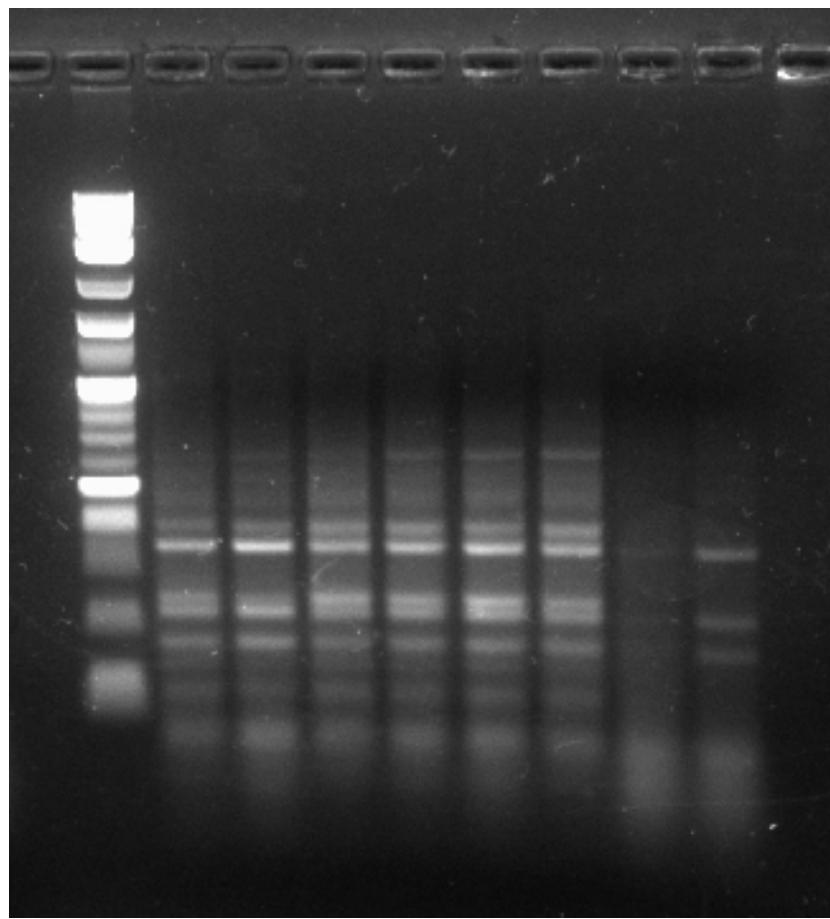
Log 2 Marker		Treatments							
Distance	Molecular Weight	H MSG	L MSG	H BA	L BA	H SALT	L SALT	H COMB	L COMB
2.4	3000	2.7	2.7		2.7	2.7	2.7	2.7	2.7
2.85	2000	4.2	4.2	4.2	4.2	4.2	4.2	4.2	4.2
3.3	1500	4.4	4.4		4.4	4.4	4.4	4.4	4.4
4.2	1000	4.9	4.9			4.9	4.9		4.9
4.6	900	5.15	5.15	5.15	5.15	5.15	5.15	5.15	5.15
4.85	800	5.4	5.4	5.4	5.4	5.4	5.4	5.4	5.4
5.5	500	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9
		6.2	6.2		6.2	6.2	6.2	6.2	6.2
		6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6

Agarose gel 62: 28/10 Culture PCR products Primer 5, Digested by HinfI



Log 2 Marker		Treatments							
Distance	Molecular Weight	H MSG	L MSG	H BA	L BA	H SALT	L SALT	H COMB	L COMB
2.1	3000	1.9	1.9	1.9	1.9	1.9	1.9	1.9	1.9
2.5	2000	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7
2.95	1500	4.0	4.0		4.0	4.0	4.0	4.0	4.0
3.7	1000	4.4		4.4	4.4	4.4		4.4	4.4
3.85	900	4.6	4.6	4.6	4.6	4.6	4.6	4.6	4.6
4.1	800	4.9	4.9	4.9	4.9	4.9	4.9	4.9	4.9
5.1	500	5.4	5.4	5.4	5.4	5.4	5.4	5.4	5.4
		5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.6
		6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0

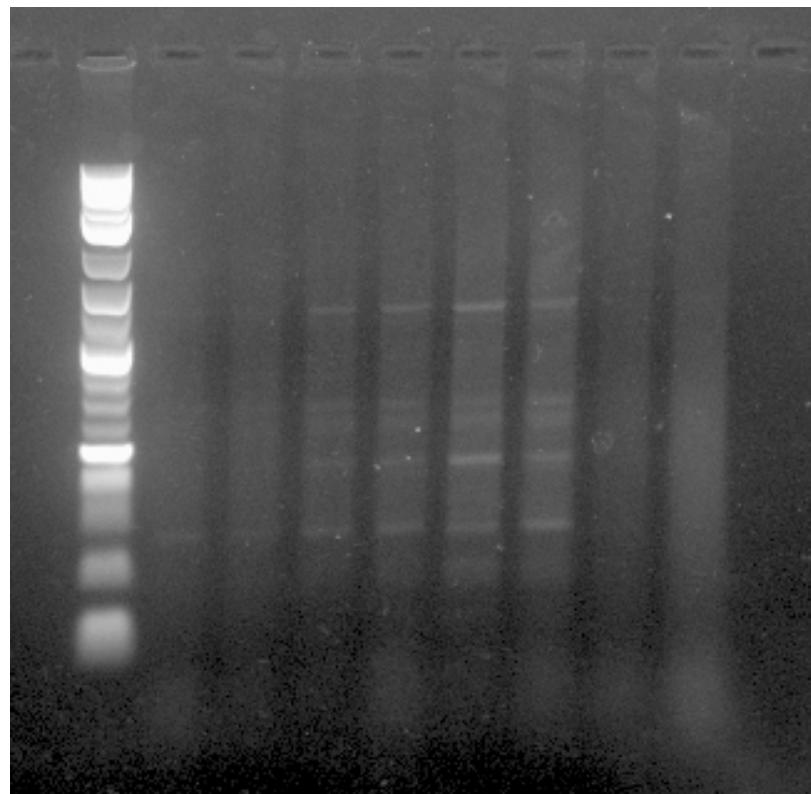
Agarose gel 63: 25/11 Culture PCR products Primer 5, Digested by HinfI



Log 2 Marker		Treatments							
Distance	Molecular Weight	H MSG	L MSG	H BA	L BA	H SALT	L SALT	H COMB	L COMB
2.4	3000	5.2	5.2	5.2	5.2	5.2	5.2		
2.9	2000	5.5	5.5	5.5	5.5	5.5	5.5		
3.4	1500	5.8	5.8	5.8	5.8	5.8	5.8		
4.2	1000	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0
4.4	900	6.7	6.7	6.7	6.7	6.7	6.7		
4.7	800	6.9	6.9	6.9	6.9	6.9	6.9	6.9	6.9
5.4	500	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3
		8.0	8.0	8.0	8.0	8.0	8.0		

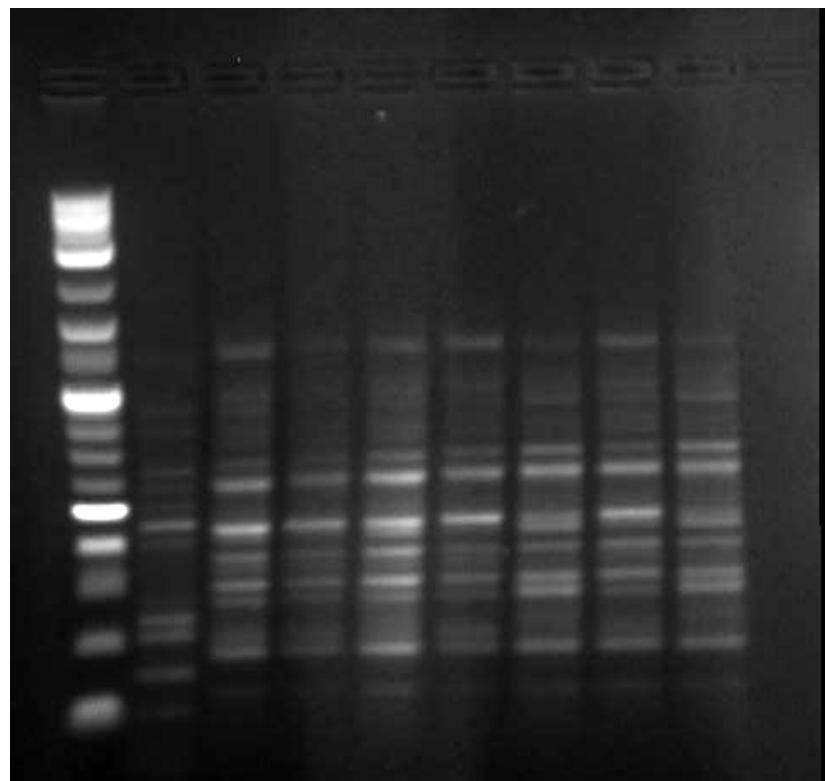
G.3.h – Hinfl, P6

Agarose gel 64: 8/7 Culture PCR products Primer 6, Digested by Hinfl



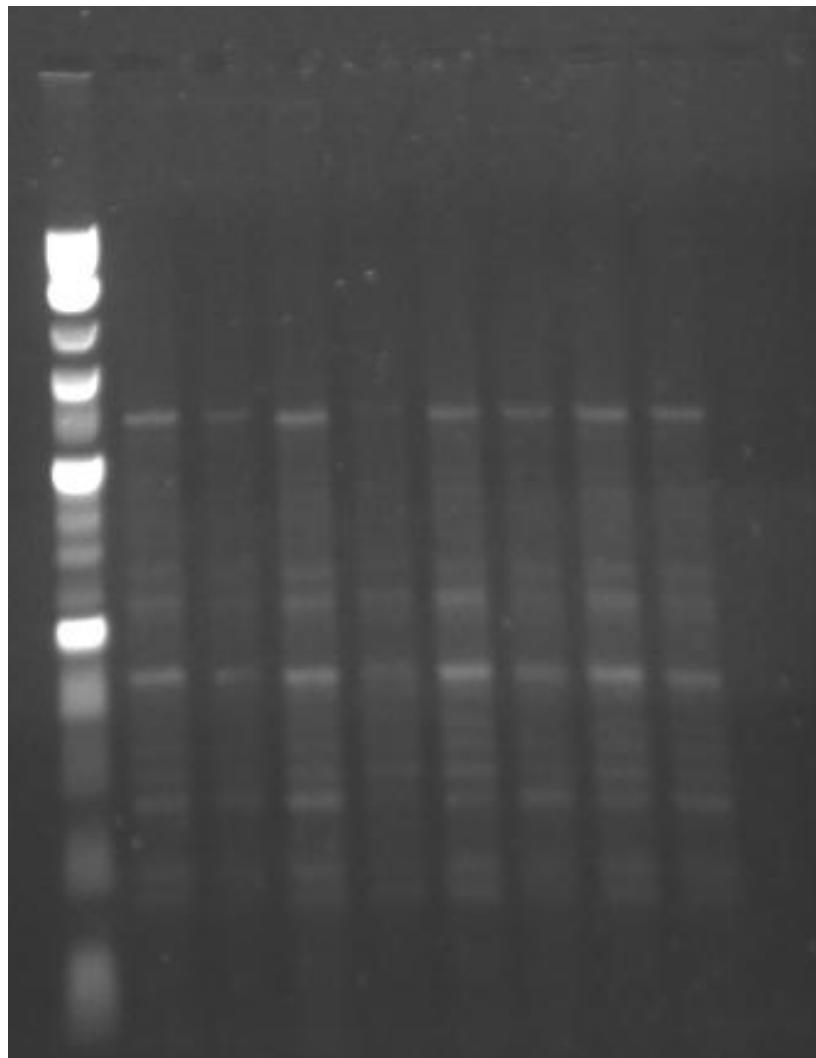
Log 2 Marker		Treatments							
Distance	Molecular Weight	H MSG	L MSG	H BA	L BA	H SALT	L SALT	H COMB	L COMB
2.4	3000			3.4	3.4	3.4	3.4		
2.9	2000			3.7	3.7				
3.3	1500		4.2	4.2	4.2				
4.1	1000		4.6	4.6	4.6	4.6	4.6		
4.3	900			5.85	5.85	5.85	5.85		
4.6	800	6.2	6.2	6.2	6.2	6.2	6.2	6.2	6.2
5.2	500								

Agarose gel 65: 5/8 Culture PCR products Primer 6, Digested by Hinfl



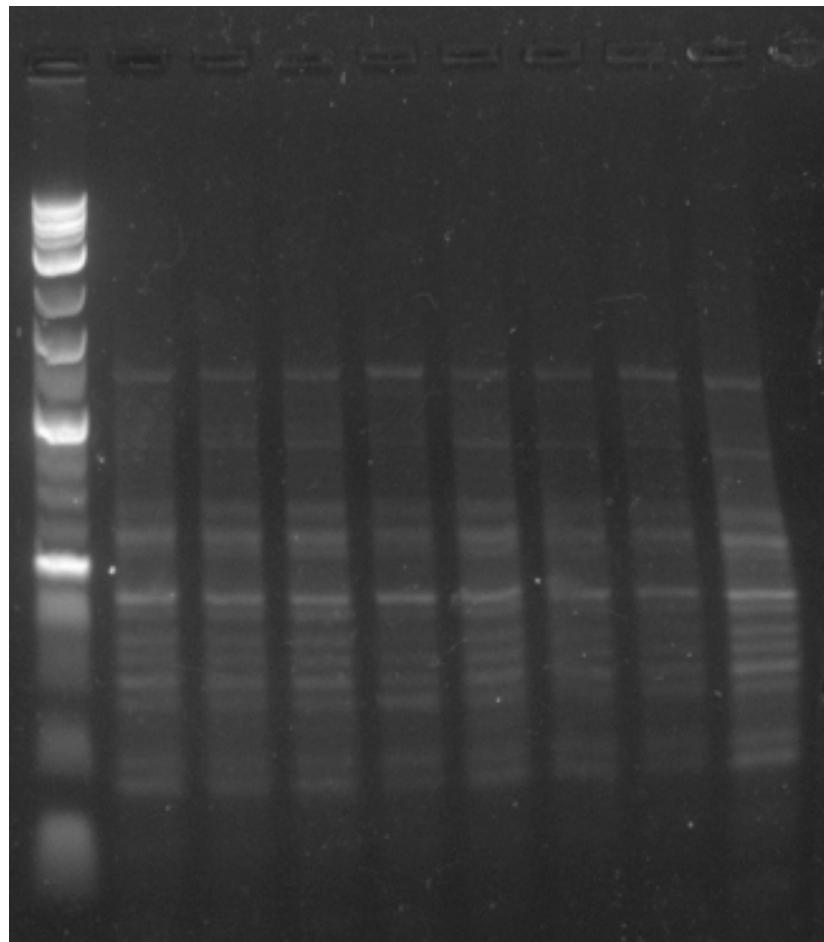
Log 2 Marker		Treatments							
Distance	Molecular Weight	H MSG	L MSG	H BA	L BA	H SALT	L SALT	H COMB	L COMB
2.15	3000		3.3	3.3	3.3	3.3	3.3	3.3	3.3
2.4	2000	4.0					4.0	4.0	4.0
2.6	1500	4.3	4.3	4.3	4.3	4.3	4.3	4.3	4.3
3.9	1000	4.55	4.55	4.55	4.55	4.55	4.55	4.55	4.55
4.25	900	4.8	4.8	4.8	4.8	4.8	4.8	4.8	4.8
4.5	800	5.3	5.3	5.3	5.3	5.3		5.3	
5.2	500						5.4		5.4
		5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7
			6.1	6.1	6.1	6.1	6.1	6.1	6.1
			6.25	6.25	6.25	6.25	6.25	6.25	6.25
		6.45							
		6.7	6.7			6.7	6.7	6.7	6.7
			6.85	6.85	6.85	6.85	6.85	6.85	6.85
		7.2			7.2				
					7.4	7.4	7.4	7.4	7.4

Agarose gel 66: 2/9 Culture PCR products Primer 6, Digested by Hinfl



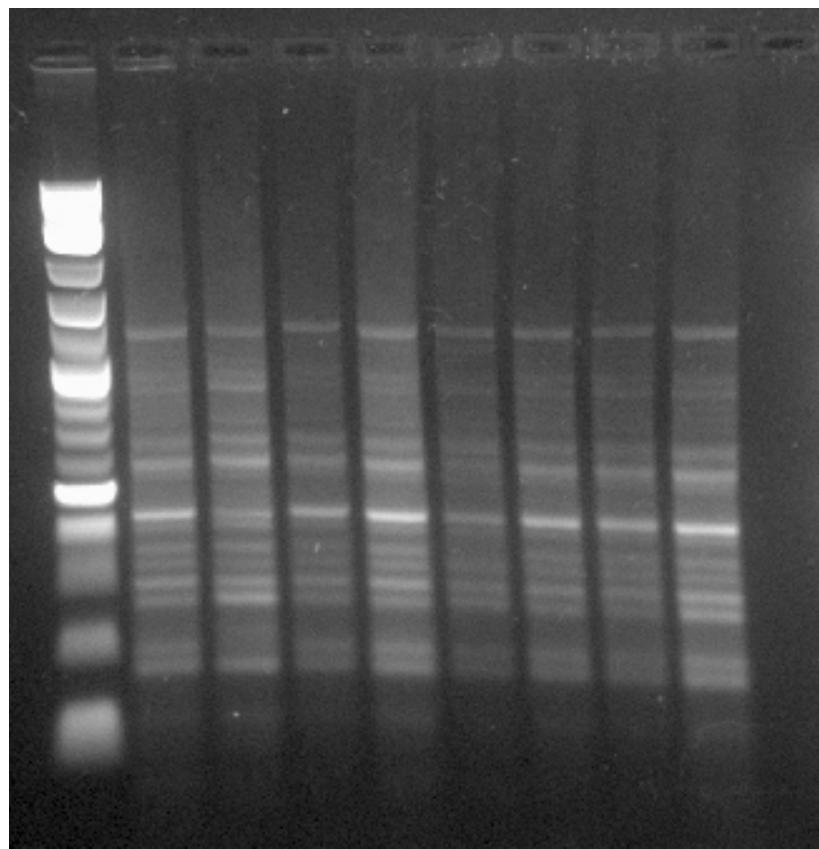
Log 2 Marker		Treatments							
Distance	Molecular Weight	H MSG	L MSG	H BA	L BA	H SALT	L SALT	H COMB	L COMB
2.3	3000	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1
3.25	2000	4.8			4.8	4.8		4.8	4.8
4.3	1500	5.0			5.0	5.0	5.0	5.0	5.0
4.9	1000	5.2			5.2	5.2		5.2	5.2
5.3	900	5.6			5.6	5.6			
5.7	800	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9
6.7	500	6.3	6.3	6.3	6.3	6.3	6.3	6.3	6.3
		7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1
		7.3		7.3	7.3	7.3	7.3		
		7.6		7.6	7.6	7.6	7.6	7.6	
		7.8		7.8	7.8	7.8	7.8	7.8	7.8
		8.2		8.2	8.2	8.2	8.2	8.2	8.2
		8.5	8.5	8.5		8.5	8.5	8.5	8.5
		9.3		9.3		9.3		9.3	
						9.6			

Agarose gel 67: 30/9 Culture PCR products Primer 6, Digested by HinfI



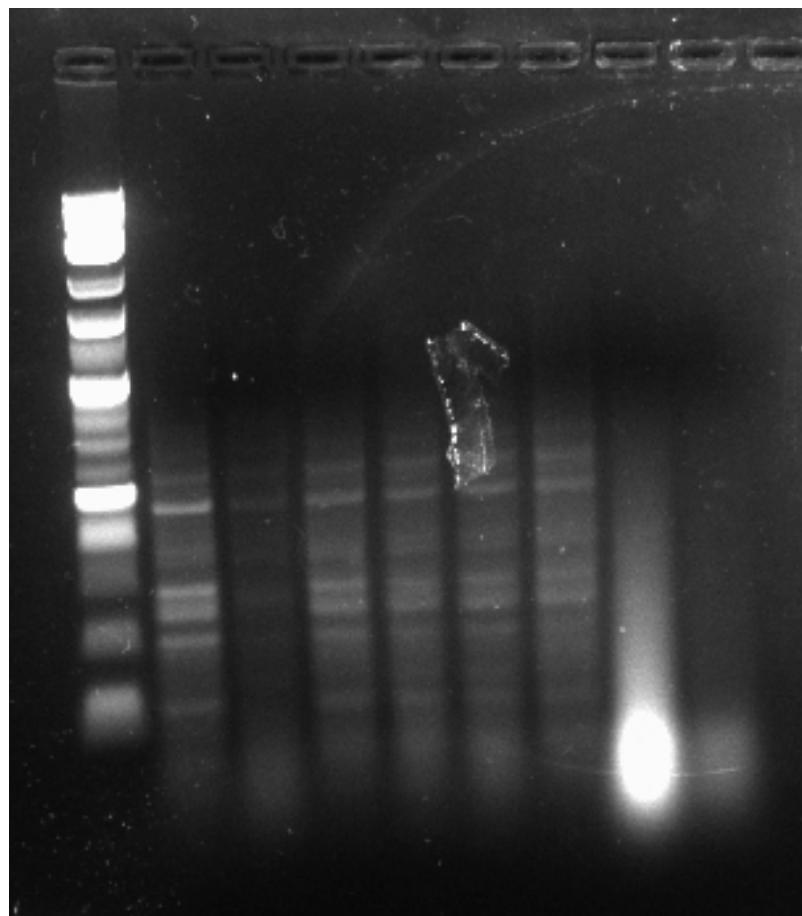
Log 2 Marker		Treatments							
Distance	Molecular Weight	H MSG	L MSG	H BA	L BA	H SALT	L SALT	H COMB	L COMB
2.5	3000	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7
2.9	2000	4.3	4.3	4.3	4.3	4.3			
3.4	1500	4.6	4.6	4.6	4.6	4.6	4.6	4.6	4.6
4.4	1000	5.3	5.3	5.3	5.3	5.3	5.3	5.3	5.3
4.8	900	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5
5.0	800	5.65	5.65	5.65	5.65				5.65
5.9	500	6.25	6.25	6.25	6.25	6.25	6.25	6.25	6.25
		6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5
		6.8	6.8	6.8	6.8	6.8	6.8	6.8	6.8
		7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0
		7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3
		7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5
		8.2	8.2	8.2	8.2	8.2	8.2	8.2	8.2
		8.45	8.45	8.45	8.45	8.45	8.45	8.45	8.45

Agarose gel 68: 28/10 Culture PCR products Primer 6, Digested by HinfI



Log 2 Marker		Treatments							
Distance	Molecular Weight	H MSG	L MSG	H BA	L BA	H SALT	L SALT	H COMB	L COMB
2.4	3000	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4
2.8	2000	3.9	3.9		3.9	3.9	3.9	3.9	3.9
3.8	1500	4.1	4.1		4.1	4.1	4.1	4.1	4.1
4.1	1000	4.45	4.45	4.45	4.45		4.45	4.45	4.45
4.4	900	4.75	4.75	4.75	4.75	4.75	4.75	4.75	4.75
4.7	800	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
5.4	500	5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.6
		5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7
		6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0
		6.15	6.15	6.15	6.15	6.15	6.15	6.15	6.15
		6.4	6.4	6.4	6.4	6.4	6.4	6.4	6.4
		6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6

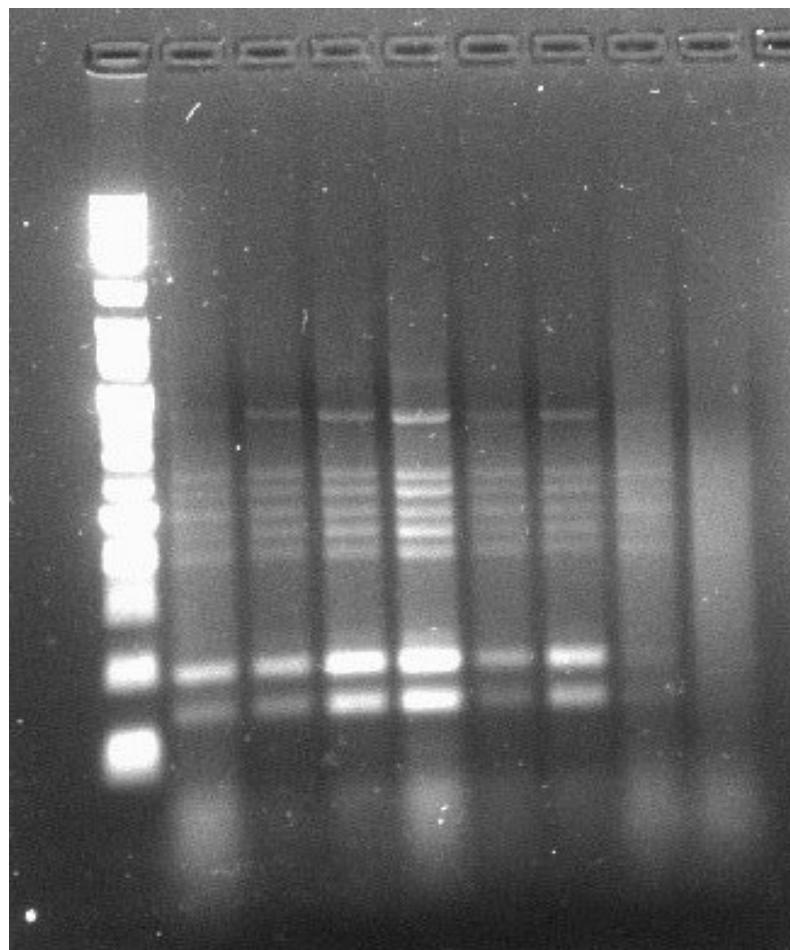
Agarose gel 69: 25/11 Culture PCR products Primer 6, Digested by HinfI



Log 2 Marker		Treatments							
Distance	Molecular Weight	H MSG	L MSG	H BA	L BA	H SALT	L SALT	H COMB	L COMB
2.6	3000	5.2		5.2	5.2	5.2	5.2		
3.0	2000	5.5		5.5	5.5	5.5	5.5		
3.5	1500	5.8	5.8	5.8	5.8	5.8	5.8		
4.4	1000	6.5		6.5	6.5	6.5	6.5		
4.7	900	6.9	6.9	6.9	6.9	6.9	6.9		
5.0	800	7.2	7.2	7.2	7.2	7.2	7.2		
5.7	500	7.5	7.5	7.5	7.5	7.5	7.5		
		7.8	7.8	7.8	7.8	7.8	7.8		
		8.0	8.0	8.0	8.0	8.0	8.0		
		8.4	8.4	8.4	8.4	8.4	8.4		

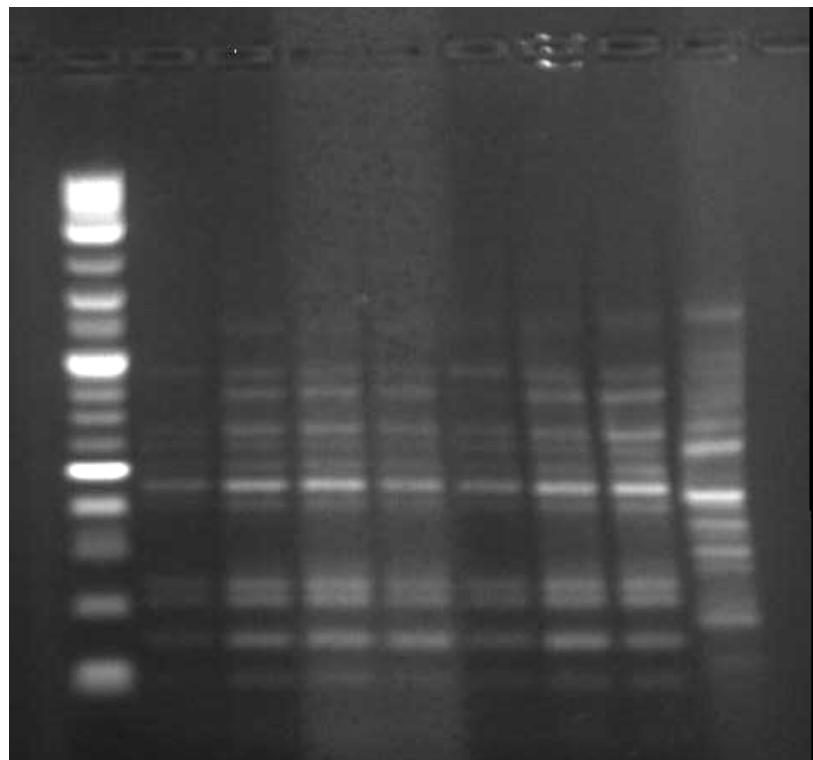
G.3.i – HinfI, P7

Agarose gel 70: 8/7 Culture PCR products Primer 7, Digested by HinfI



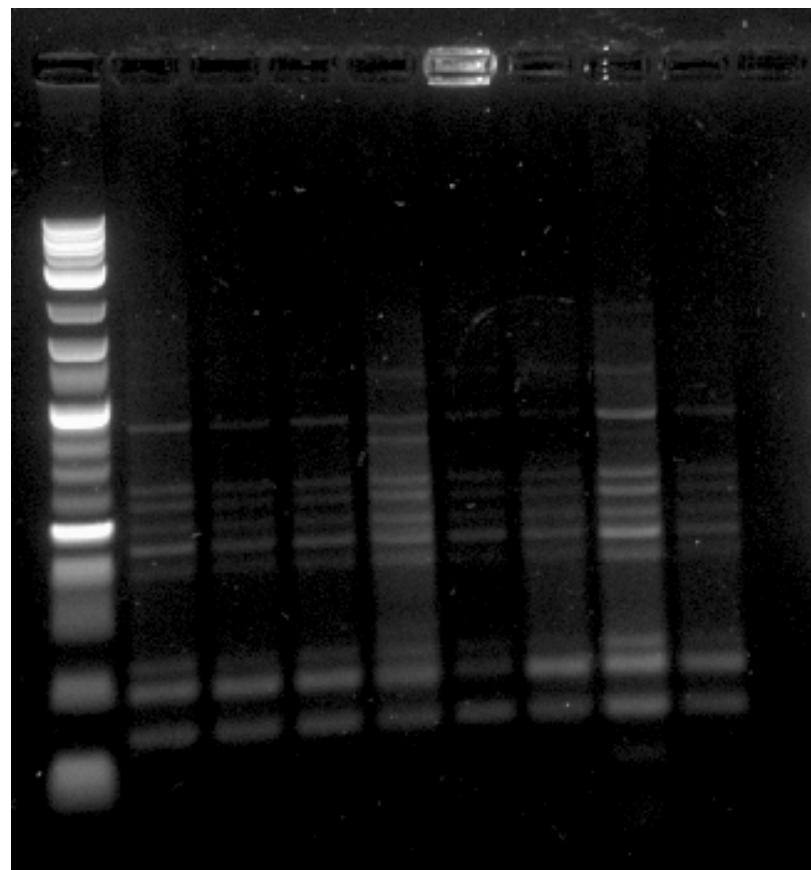
Log 2 Marker		Treatments							
Distance	Molecular Weight	H MSG	L MSG	H BA	L BA	H SALT	L SALT	H COMB	L COMB
2.9	3000		4.85	4.85	4.85	4.85	4.85	4.85	
3.2	2000	5.6	5.6	5.6	5.6	5.6	5.6	5.6	
3.8	1500	5.9	5.9	5.9	5.9	5.9	5.9	5.9	
4.8	1000	6.1	6.1	6.1	6.1	6.1	6.1	6.1	
5.2	900	6.4	6.4	6.4	6.4	6.4	6.4	6.4	
5.4	800	6.7	6.7	6.7	6.7	6.7	6.7	6.7	
6.3	500	8.2	8.2	8.2	8.2	8.2	8.2	8.2	
		8.6	8.6	8.6	8.6	8.6	8.6	8.6	

Agarose gel 71: 5/8 Culture PCR products Primer 7, Digested by Hinfl



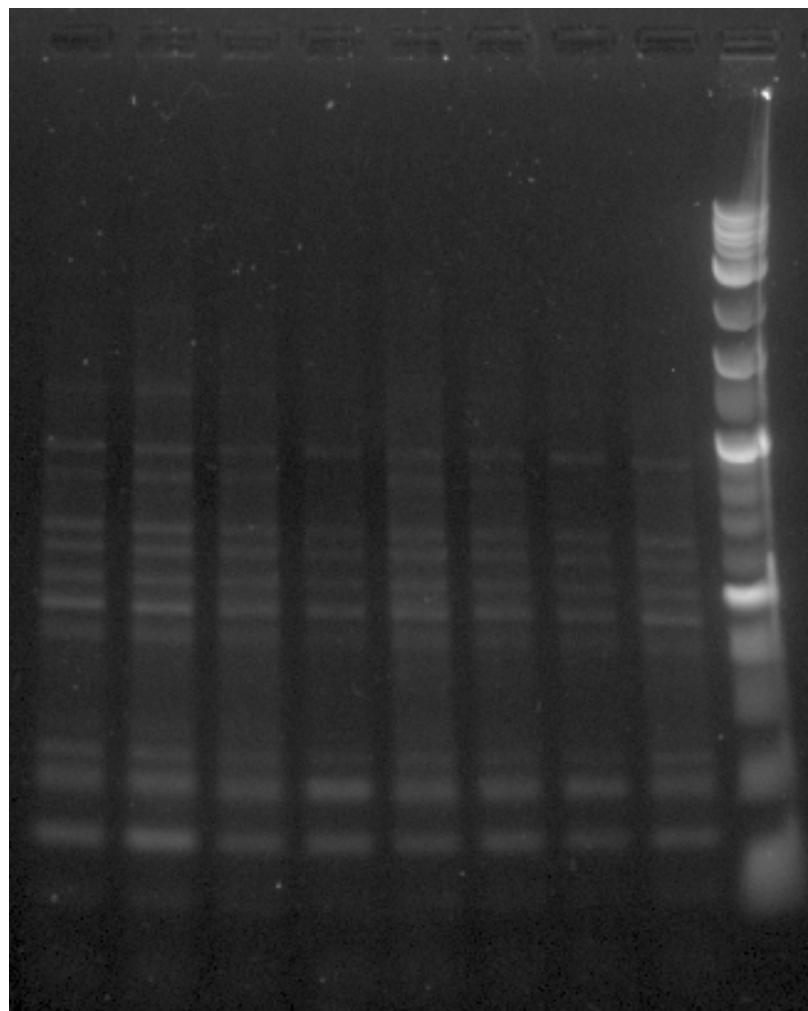
Log 2 Marker		Treatments							
Distance	Molecular Weight	H MSG	L MSG	H BA	L BA	H SALT	L SALT	H COMB	L COMB
2.4	3000		3.45	3.45			3.45	3.45	3.45
2.8	2000	4.1	4.1	4.1	4.1	4.1	4.1	4.1	
3.2	1500		4.4	4.4	4.4	4.4	4.4	4.4	
4.0	1000	4.8	4.8	4.8	4.8	4.8	4.8	4.8	4.8
4.25	900		5.05	5.05	5.05	5.05	5.05	5.05	5.05
4.55	800		5.2	5.2	5.2	5.2	5.2	5.2	
5.2	500	5.45	5.45	5.45	5.45	5.45	5.45	5.45	
									5.6
			5.7	5.7	5.7	5.7	5.7	5.7	
									5.9
									6.2
			6.6	6.6	6.6	6.6	6.6	6.6	
			6.8	6.8	6.8	6.8	6.8	6.8	
									7.1
		7.4	7.4	7.4	7.4	7.4	7.4	7.4	

Agarose gel 72: 2/9 Culture PCR products Primer 7, Digested by Hinfl



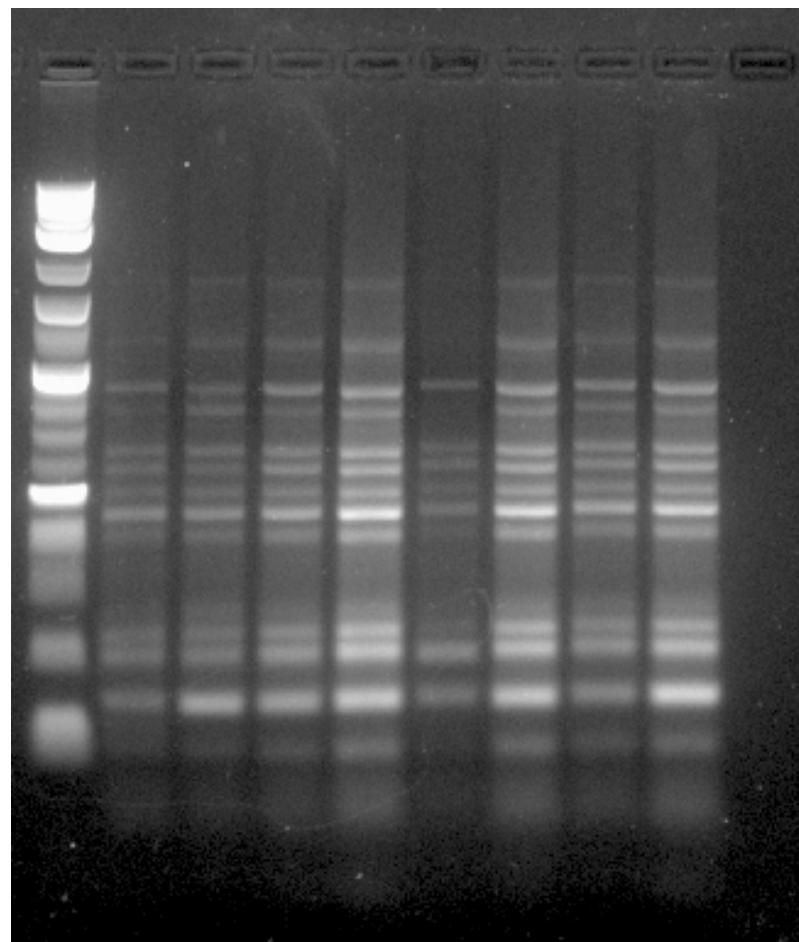
Log 2 Marker		Treatments							
Distance	Molecular Weight	H MSG	L MSG	H BA	L BA	H SALT	L SALT	H COMB	L COMB
3.0	3000							3.4	
3.4	2000				4.2		4.2	4.2	
4.0	1500	4.7	4.7	4.7	4.7	4.7	4.7	4.7	4.7
4.9	1000				5.1			5.1	
5.2	900	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5
5.5	800	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8
6.9	500	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0
		6.4	6.4	6.4	6.4	6.4	6.4	6.4	6.4
		6.7	6.7	6.7	6.7	6.7	6.7	6.7	6.7
					7.2				
		7.9	7.9	7.9	7.9	7.9	7.9	7.9	7.9
		8.2	8.2	8.2	8.2	8.2	8.2	8.2	8.2
		8.8	8.8	8.8	8.8	8.8	8.8	8.8	8.8

Agarose gel 73: 30/9 Culture PCR products Primer 7, Digested by HinfI



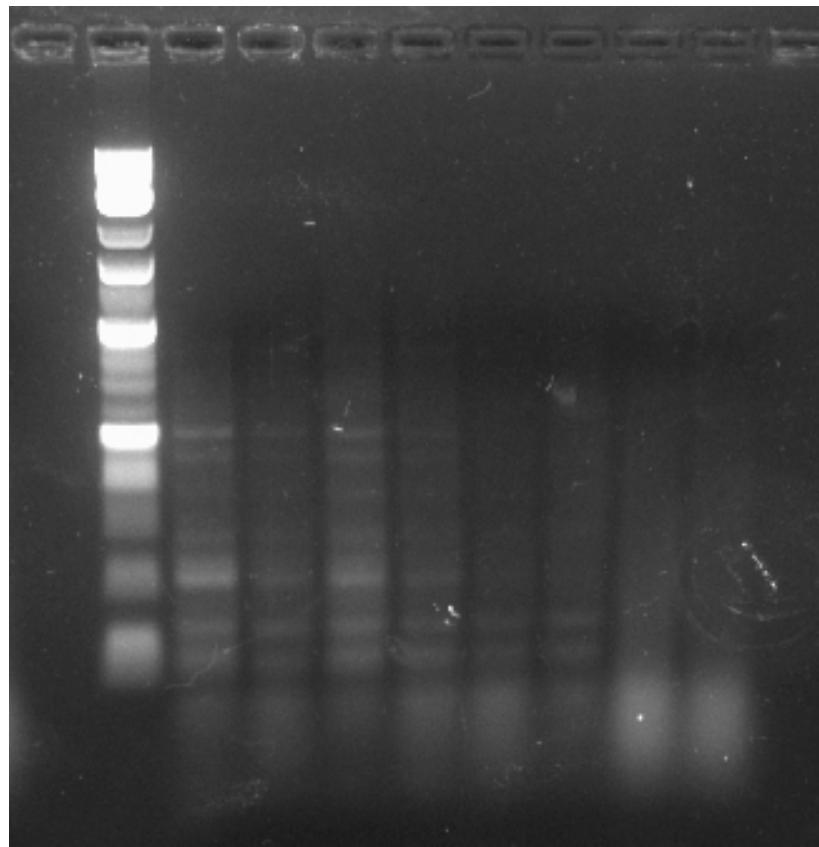
Log 2 Marker		Treatments							
Distance	Molecular Weight	H MSG	L MSG	H BA	L BA	H SALT	L SALT	H COMB	L COMB
2.6	3000	3.8	3.8	3.8					
3.1	2000	4.4	4.4	4.4	4.4	4.4	4.4	4.4	4.4
3.6	1500	4.7	4.7	4.7		4.7	4.7		
4.0	1000	5.2	5.2	5.2	5.2	5.2	5.2	5.2	5.2
5.0	900	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5
5.3	800	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8
6.05	500	6.1	6.1	6.1	6.1	6.1	6.1	6.1	6.1
		6.4	6.4	6.4	6.4	6.4	6.4	6.4	6.4
		7.4	7.4	7.4		7.4	7.4		7.4
		7.7	7.7	7.7	7.7	7.7	7.7	7.7	7.7
		8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0
		8.6	8.6	8.6	8.6	8.6	8.6	8.6	8.6

Agarose gel 74: 28/10 Culture PCR products Primer 7, Digested by HinfI



Log 2 Marker		Treatments							
Distance	Molecular Weight	H MSG	L MSG	H BA	L BA	H SALT	L SALT	H COMB	L COMB
2.4	3000		2.9	2.9	2.9		2.9	2.9	2.9
2.8	2000		3.6	3.6	3.6		3.6	3.6	3.6
3.3	1500	4.2	4.2	4.2	4.2	4.2	4.2	4.2	4.2
4.1	1000	4.45	4.45	4.45	4.45	4.45	4.45	4.45	4.45
4.5	900	4.95	4.95	4.95	4.95	4.95	4.95	4.95	4.95
4.8	800	5.2	5.2	5.2	5.2	5.2	5.2	5.2	5.2
5.6	500	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5
		5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7
		6.0	6.0	6.0	6.0		6.0	6.0	6.0
					6.9		6.9	6.9	6.9
		7.2	7.2	7.2	7.2		7.2	7.2	7.2
		7.45	7.45	7.45	7.45	7.45	7.45	7.45	7.45
		8.1	8.1	8.1	8.1	8.1	8.1	8.1	8.1

Agarose gel 75: 25/11 Culture PCR products Primer 7, Digested by HinfI



Log 2 Marker		Treatments							
Distance	Molecular Weight	H MSG	L MSG	H BA	L BA	H SALT	L SALT	H COMB	L COMB
2.6	3000	5.2		5.2					
3.0	2000	5.5		5.5					
3.6	1500	5.9		5.9					
4.5	1000	6.4		6.4					
4.7	900	6.6		6.6					
5.0	800	7.15	7.15	7.15	7.15				
6.2	500	7.8	7.8	7.8	7.8	7.8	7.8		
		8.3	8.3	8.3	8.3	8.3	8.3		

## Appendix H – Dissimilarity Matrix

The Dissimilarity Index obtained using Nei-Li DI is tabulated into the following matrixes according to PCR/RFLP count.

### PCR/RFLP #1 (Passage 10)

	H MSG	L MSG	H BA	L BA	H SALT	L SALT	H COMB	L COMB
H MSG	0.0							
L MSG	0.277	0.0						
H BA	0.203	0.0972	0.0					
L BA	0.268	0.225	0.0980	0.0				
H SALT	0.263	0.246	0.135	0.0491	0.0			
L SALT	0.231	0.238	0.123	0.0819	0.0997	0.0		
H COMB	0.260	0.278	0.266	0.190	0.237	0.200	0.0	
L COMB	0.129	0.165	0.159	0.167	0.147	0.111	0.110	0.0

### PCR/RFLP #2 (Passage 22)

	H MSG	L MSG	H BA	L BA	H SALT	L SALT	H COMB	L COMB
H MSG	0.0							
L MSG	0.146	0.0						
H BA	0.154	0.0561	0.0					
L BA	0.161	0.0630	0.0956	0.0				
H SALT	0.241	0.165	0.212	0.181	0.0			
L SALT	0.209	0.103	0.143	0.130	0.131	0.0		
H COMB	0.192	0.0921	0.139	0.139	0.139	0.0811	0.0	
L COMB	0.212	0.127	0.174	0.180	0.199	0.0993	0.108	0.0

### PCR/RFLP #3 (Passage 34)

	H MSG	L MSG	H BA	L BA	H SALT	L SALT	H COMB	L COMB
H MSG	0.0							
L MSG	0.136	0.0						
H BA	0.149	0.166	0.0					
L BA	0.254	0.255	0.218	0.0				
H SALT	0.220	0.232	0.178	0.0526	0.0			
L SALT	0.192	0.252	0.233	0.354	0.329	0.0		
H COMB	0.246	0.247	0.205	0.127	0.101	0.278	0.0	
L COMB	0.263	0.340	0.289	0.367	0.320	0.282	0.315	0.0

**PCR/RFLP #4 (Passage 46)**

	H MSG	L MSG	H BA	L BA	H SALT	L SALT	H COMB	L COMB
H MSG	0.0							
L MSG	0.136	0.0						
H BA	0.149	0.166	0.0					
L BA	0.254	0.255	0.218	0.0				
H SALT	0.220	0.232	0.178	0.0526	0.0			
L SALT	0.192	0.252	0.233	0.354	0.329	0.0		
H COMB	0.246	0.247	0.205	0.127	0.101	0.278	0.0	
L COMB	0.263	0.340	0.289	0.367	0.320	0.282	0.315	0.0

**PCR/RFLP #5 (Passage 58)**

	H MSG	L MSG	H BA	L BA	H SALT	L SALT	H COMB	L COMB
H MSG	0.0							
L MSG	0.0718	0.0						
H BA	0.0734	0.0729	0.0					
L BA	0.0778	0.0521	0.0665	0.0				
H SALT	0.132	0.153	0.152	0.140	0.0			
L SALT	0.0823	0.0337	0.0808	0.0489	0.163	0.0		
H COMB	0.0639	0.0599	0.0807	0.0595	0.166	0.0336	0.0	
L COMB	0.0868	0.0717	0.0727	0.0418	0.129	0.0580	0.0622	0.0

**PCR/RFLP #6 (Passage 70)**

	H MSG	L MSG	H BA	L BA	H SALT	L SALT	H COMB	L COMB
H MSG	0.0							
L MSG	0.122	0.0						
H BA	0.0279	0.110	0.0					
L BA	0.106	0.111	0.0866	0.0				
H SALT	0.125	0.176	0.120	0.0830	0.0			
L SALT	0.136	0.176	0.128	0.0798	0.0403	0.0		
H COMB	0.697	0.658	0.665	0.653	0.665	0.651	0.0	
L COMB	0.697	0.648	0.665	0.653	0.665	0.651	0.0	0.0

## Appendix I – Bactome: A module for analysing bacterial omics

Bactome is a collection of functions, implemented in Python programming language, to find primers, determine restriction digestion profile, analyse the migrated distance of the bands in gel electrophoresis, calculate generation time and number of generations.

Primer design was performed to determine the suitable primer for PCR. The primer sequence obtained is used as forward and reverse primer in each reaction. Genome of the bacteria was scanned and cut into equal fragments length. The longest common substring (LCS), which is the longest sequence between two fragments, was determined and compared. All LCS were first determined according to the size of amplicon and minimum length of primers, given that the forward and reverse primer sequence is equal. LCS was generated with the start fragment position, end fragment position and primer sequence. For easier analysis of the primer sequence, Inversion of the LCS list was performed to classify into primer sequence with start fragment position, end fragment position and amplicon size. In addition, primer design can be used in microarray, sequencing and selection for specific bases.

```
def fasta_seq(fasta='ATCC8739.fasta'):
    f = SeqIO.parse(open(fasta, 'rU'), 'fasta').next()
    return f.seq

def generate_fragments(seq, tablefile, fragment_size=15):
    genome_fragment = anydbm.open(tablefile, 'c')
    last_start = len(seq) - fragment_size
    count = 0
    for start in range(0, last_start, fragment_size):
        genome_fragment[str(count)] = seq[start:15+start].data
        count = count + 1
    if (count % 1000) == 0:
        print str(count) + ' fragments inserted into ' + tablefile
    print str(count) + ' fragments inserted into ' + tablefile
    genome_fragment.close()

def LCS(seq, test):
    m = len(seq)
    n = len(test)
    L = [[0] * (n+1) for i in xrange(m+1)]
    LCS = set()
    longest = 0
    for i in xrange(m):
        for j in xrange(n):
            if seq[i] == test[j]:
                L[i+1][j+1] = L[i][j] + 1
                if L[i+1][j+1] > longest:
                    longest = L[i+1][j+1]
                    LCS.add((i+1, j+1))
            else:
                L[i+1][j+1] = 0
    return LCS, longest
```

```

        v = L[i][j] + 1
        L[i+1][j+1] = v
        if v > longest:
            longest = v
            LCS = set()
        if v == longest:
            LCS.add(seq[i-v+1:i+1])
    return LCS

def generate_all_LCS(fragfile, LCSfile,
                     min_primer=7, max_interval=198, min_interval=6):
    genome_fragment = anydbm.open(fragfile, 'c')
    num_of_frag = len(genome_fragment)
    num_analyzed = 0
    result = {}
    fragment_size = len(genome_fragment['0'])
    for size in range(max_interval, min_interval, -1):
        com_seq = anydbm.open(LCSfile, 'c')
        print 'Analysing for ' + str(fragment_size * size) + 'bp
amplicons'
        num_com = 0
        for frag in range(0, num_of_frag - size):
            p1 = genome_fragment[str(frag)]
            p2 = genome_fragment[str(frag+size)]
            p2 = Seq(p2, generic_dna).reverse_complement().data
            try: common_seq = LCS(p1, p2).pop()
            except : common_seq = ''
            num_analyzed = num_analyzed + 1
            if len(common_seq) > min_primer:
                com_seq['|'.join([str(frag), str(frag+size),
                                  str(size)])] = common_seq
                num_com = num_com + 1
            if (num_analyzed % 10000) == 0:
                print str(num_analyzed) + ' pairs analyzed'
                print '      ' + str(num_com) + ' LCS more than ' + \
                      str(min_primer) + 'bp for ' + \
                      str(15 * size) + 'bp amplicons'
        com_seq.close()
        result[size] = str(num_com)
    print str(num_analyzed) + ' pairs analyzed'
    print '      ' + str(num_com) + ' LCS more than ' + str(min_primer)+\
          'bp for ' + str(15 * size) + 'bp amplicons'
    genome_fragment.close()
    return result

def get_LCS_by_amplicon(LCSfile, amplicon_size):
    com_seq = anydbm.open(LCSfile, 'c')
    keys = com_seq.keys()
    return ([com_seq[x]
            for x in keys
            if x.split('|')[2] == str(amplicon_size)],
           [x
            for x in keys
            if x.split('|')[2] == str(amplicon_size)])

def LCS_uniqueness(LCSfile, amplicon_size):
    LCS = get_LCS_by_amplicon(LCSfile, amplicon_size)[0]
    unique_LCS = [x

```

```

        for x in LCS
            if LCS.count(x) == 1]
non_unique_LCS = list(set([(LCS.count(x), x)
                           for x in LCS
                           if LCS.count(x) > 1]))
return (unique_LCS, non_unique_LCS)

def LCS_tabulate(LCSfile, max_interval=198, min_interval=6,
                 fragment_size=15, p='yes'):
    result = {}
    for amplicon_size in range(max_interval, min_interval, -1):
        (unique_LCS, non_unique_LCS) = LCS_uniqueness(LCSfile,
                                                       amplicon_size)
        if p == 'yes':
            print 'Longest Common Substring (primer) for amplicon \
size of ' + str(amplicon_size*fragment_size)
            print 'Unique Primers (only one amplicon). N = ' + \
                  str(len(unique_LCS))
            print ' '.join(unique_LCS)
            print
            print 'Non-Unique Primers (more than one amplicon)'
            print '\t'.join(['# amplicons', 'Primer sequence'])
            for lcs in non_unique_LCS:
                print '\t\t'.join([str(lcs[0]), lcs[1]])
            print
            print
        result[str(amplicon_size)] = (unique_LCS, non_unique_LCS)
    return result

def inverse_LCS(LCSfile, inverseLCSfile, max_interval=198, min_interval=6,
               fragment_size=15):
    inverseLCSfile = marshaldbm(inverseLCSfile, 'c')
    count = 0
    for amplicon_size in range(max_interval, min_interval, -1):
        seq, pos = get_LCS_by_amplicon(LCSfile, amplicon_size)
        print 'Processing for amplicon size of ' + \
              str(amplicon_size*fragment_size)
        for index in range(len(seq)):
            count = count + 1
            try:
                temp = inverseLCSfile[seq[index]]
                inverseLCSfile[seq[index]] = temp + [pos[index]]
            except KeyError:
                inverseLCSfile[seq[index]] = [pos[index]]
            if (count % 1000) == 0:
                print str(count) + ' LCS processed'
    print str(count) + ' LCS processed'
    inverseLCSfile.close()

def look_for_primers(inverseLCSfile, num_of_amplicons, min_tm, p='yes'):
    f = marshaldbm(inverseLCSfile, 'c')
    keys = f.keys()
    primers = []
    for k in keys:
        temperature = 4*(k.count('G')+k.count('C')) + \
                      2*(k.count('A')+k.count('T'))
        if (len(f[k])) == num_of_amplicons and \
           temperature > min_tm-1 and \

```

```

        not k.endswith('A') and not k.endswith('T'):
            primers.append(k)
        if p == 'yes':
            print k
            print f[k]
            print
    return primers

def generate_primers_from_fasta(fasta='ATCC8739.fasta',
                                 genome_fragment='genome_fragment.table',
                                 max_primer_length=15,
                                 min_primer_length=7,
                                 max_amplicon_length=3100,
                                 min_amplicon_length=300,
                                 primer_file='primer.table',
                                 inverse_primer_file='invprimer.table'):

    seq = fasta_seq(fasta)
    generate_fragments(seq, genome_fragment,
fragment_size=max_primer_length)
    generate_all_LCS(genome_fragment, primer_file,
                      min_primer_length,
                      int(max_amplicon_length/max_primer_length),
                      int(min_amplicon_length/max_primer_length))
    inverse_LCS(primer_file, inverse_primer_file,
                int(max_amplicon_length/max_primer_length),
                int(min_amplicon_length/max_primer_length),
                max_primer_length)

```

Restriction enzyme cuts a specific region in the genome which can indicate the presence of certain sequence in the genome (a string of DNA letters). If there is mutation at the cutting region, the same restriction enzyme cannot cut the same site anymore as the sequence is changed resulting the decrease in number of fragment. In exchange, the restriction enzyme may be able to cut other regions in the genome results in more fragments generation and also the mutated cutting site can now be cut by another restriction site. This tool enables us to digest the genome virtually which allows us to see the expected fragments and also tell us the restriction enzyme that is able to cut the genome then can be used to select restriction enzyme to purchase for following laboratory experiments. This tool uses the library in Biopython for the restriction enzyme cutting site then it scans the cutting site in the genome. If it is able to find the cutting site in the genome this indicates the restriction enzyme is able to be used for RFLP. It returns the location and number of cutting site for the restriction enzyme and the length of fragment generated by restriction enzyme. This tool can also be used for microsatellite and single nucleotide polymorphisms detection and gene mapping.

```

def restriction_digest(seq, enzyme, max_band=23130, min_band=2000,
                      linear=False, p='yes'):
    digest = enzyme.search(seq, linear=linear)

```

```

digest.sort()
fragment = [digest[x+1] - digest[x]
            for x in range(len(digest) - 1)]
fragment.sort()
ogel = [x for x in fragment if x > max_band]
gel = [x for x in fragment if x <= max_band and x >= min_band]
ugel = [x for x in fragment if x < min_band]
ogel.sort()
gel.sort()
ugel.sort()
if p == 'yes':
    print 'Enzyme: ' + str(enzyme)
    print 'Restriction site: ' + enzyme.site
    print 'Number of fragments: ' + str(len(fragment))
    print 'Number of fragments (x > ' + str(max_band) + '): ' + \
          str(len(ogel))
    print 'Number of fragments (' + str(max_band) + ' < x < ' + \
          str(min_band) + '): ' + str(len(gel))
    print 'Number of fragments (x < ' + str(min_band) + '): ' + \
          str(len(ugel))
    print
return (len(fragment), ogel, gel, ugel)

def restriction_supplier(seq, max_band=23130, min_band=2000,
                        suppliers='ACEGFIHKJMONQPSRUVX',
                        linear=False, p='yes'):
    from Bio.Restriction import RestrictionBatch
    count = 0
    result = {}
    for enzyme in RestrictionBatch(first=[],
                                     suppliers=[x.upper()
                                                 for x in suppliers]):
        try:
            digest = restriction_digest(seq, enzyme, max_band, min_band,
                                         linear, p)
        except MemoryError:
            print 'Memory Error during ' + str(enzyme) + ' digestion'
            result[str(enzyme)] = (digest[0], len(digest[1]),
                                    len(digest[2]), len(digest[3]))
        count = count + 1
    if p != 'yes':
        if count % 10 == 0:
            print str(count) + ' restriction endonuclease processed'
    return result

```

Generation time is a measure of time taken for one generation to take place which indicates the fitness of the bacteria at that point of time. This function calculates the generation time of bacteria cells (the length of time for the number of cells to multiply to twice the number) using the turbidity of the culture media measured as optical density at 600nm wavelength (OD600). Sezonov et al. (2007) suggested that the limits of proportionality between cell count to OD600 spectrophotometric reading is 0.3 and the cell size beyond OD600 of 0.3 reduces non-linearly to only about 30% of the size at OD600 2.0. Limit of proportionality,

correction gradient and intercept are reverse engineered from the graph of Sezonov Sezonov et al. (2007). Before OD600 0.3, every 0.3 OD600 reading is represent 50 million cells. However, after OD600 0.3 the cell density follows the relationship, Cell density = 52137400 \* ln (OD600 reading) + 118718650. These 2 relationships only apply to *Escherichia coli*. Using the Information, we use the generation time calculator to determine the cell density at each time. The number of generation is obtained by subtracting the log of cell density at Time 2 with the log of cell density at Time 1 then divided by log 2. In order to measure the time taken for one generation, time difference (Time 2 – Time 1) is divided by the number of generation. This function will return the number of generation and time taken for one generation. Apart using this tool to measure the logarithm phase, this tool can be used to measure the stationary phase of the bacteria growth.

```
def generation(A2, A1, min2, min1,
              conv=50000000/0.3,
              limit=0.301,
              gradient=52137400,
              intercept=118718650):
    if A1 < limit:
        y = A1 * conv
    else:
        y = gradient * math.log(A1, math.e) + intercept
    if A2 < limit:
        x = A2 * conv
    else:
        x = gradient * math.log(A2, math.e) + intercept
    gen = (math.log10(x) - math.log10(y))/math.log10(2)
    time_diff = min2 - min1
    return (gen, time_diff/gen, x, y)
```

Total generation is a measure number of generation has taken place during our entire experiment. This function calculates the initial cell density (Day it is subculture) and divided by the dilution to obtain the cell density that is subcultured. It then calculates the final cell density (Day to subculture). Generation taken place per passage is obtained by subtract cell density subcultured from final cell density. Total generation is the additional of all generation taken place per passage.

```
def totalgen(ODs, p='yes', dilution=100,
            conv=50000000/0.3, limit=0.301,
            gradient=52137400, intercept=118718650):
    ODs = [float(x) for x in ODs if x != '']
    gen_list = [0] * (len(ODs) - 1)
    for passage in range(len(ODs)-1):
```

```

if ODs[passage] < limit:
    initial_count = ODs[passage] * conv
else:
    initial_count = gradient * math.log(ODs[passage], math.e) + \
                    intercept
if ODs[passage+1] < limit:
    final_count = ODs[passage+1] * limit
else:
    final_count = gradient * math.log(ODs[passage+1], math.e) + \
                    intercept
initial_count = initial_count / float(dilution)
gen = (math.log10(final_count) - \
       math.log10(initial_count)) / math.log10(2)
if gen < 0: gen = 0.0
gen_list[passage] = gen
if p == 'yes':
    print 'Number of cells inoculate from Passage ' + \
          str(passage) + ' = ' +str(initial_count)
    print 'Number of cells at subculture from Passage ' + \
          str(passage+1) + ' = ' +str(final_count)
    print 'Between Passage ' + str(passage) + ' and Passage ' + \
          str(passage+1) + ', Number of generations = ' + \
          str(gen_list[passage])
    print 'Total number of generations since Passage 0 = ' + \
          str(sum(gen_list))
    print
return gen_list

```

This tool provides the function analysing the migrated distance of the bands in gel electrophoresis using the method to calculate dissimilarity developed by Nei and Li (1979). Dissimilarity index =  $1 - [2 \times (\text{number of regions where both species are present}) / [(2 \times (\text{number of regions where both species are present})) + (\text{number of regions where only one species is present})]]$ . There is 2 type of comparison: set and list. Set comparison is used for processing set-based (unordered or nominal) distance of categorical data. List comparison is used for processing list-based (ordered or ordinal) distance of categorical data. For our experiment, list comparison is preferred. It compare the band migrated distance for one sample (original) to another sample (test). If the bands were similar it returns zero dissimilarity index. On the hand, if the band at that certain distance only appears on of the sample, it will return the dissimilarity index. This tool can be also used for SDS-PAGE gel electrophoresis.

```

def setCompare(original, test, absent):
    original_only = float(len([x for x in original if x not in test]))
    test_only = float(len([x for x in test if x not in original]))
    both = float(len([x for x in original if x in test]))
    return (original_only, test_only, both)

```

```

def listCompare(original, test, absent):
    original = list(original)
    test = list(test)
    original_only = 0.0
    test_only = 0.0
    both = 0.0
    for i in range(len(original)):
        if original[i] == absent and test[i] == absent: pass
        elif original[i] == test[i]: both = both + 1
        elif original[i] <> absent and test[i] == absent:
            original_only = original_only + 1
        elif original[i] == absent and test[i] <> absent:
            test_only = test_only + 1
        else: pass
    return (original_only, test_only, both)

def Nei_Li(original, test, absent=0, type='Set'):
    if type == 'Set':
        (original_only, test_only, both) = setCompare(original,
                                                       test, absent)
    else:
        (original_only, test_only, both) = listCompare(original,
                                                       test, absent)
    return 1-((2*both)/((2*both)+original_only+test_only))

def randomization(statfunction, fulldata,testdata, iteration=1000):
    from copads.Operations import sample_wr
    test_statistic = statfunction(testdata)
    randomized_statistic = [statfunction(sample_wr(fulldata,
len(testdata)))
                             for i in range(iteration)]
    #print randomized_statistic, test_statistic
    more = [1 for x in randomized_statistic
            if x >= test_statistic]
    return sum(more) / float(len(randomized_statistic))

```

The distance matrices of the various gels, tabulated into an excel file where one gel occupies one sheet, will be calculated for the 28 DI for each gel using the following tool. To remove inaccuracy due to empty lanes, the tool removes empty lanes in all the distance matrices and then calculates the DI values. The average DI for each comparison across the 6 passages was then calculated and returned.

```

from xlrd import open_workbook
from bactome import Nei_Li

Gelfile = open_workbook('(NEW) Tabulations for gels.xls')

def check_gel(gel):
    lanes = []
    for lane in range(len(gel)):
        if len([band
                for band in gel[lane][2:]])

```

```

        if type(band) == type(0.0)]) > 0:
            lanes.append(lane)
    return lanes

def process_gel(gelfile, gelnumber):
    gel = gelfile.sheet_by_index(gelnumber-1)
    gel_data = []
    result = {}
    for col in range(gel.ncols):
        column = [cell.value for cell in gel.col(col)]
        gel_data.append(column)
    lanes = check_gel(gel_data)
    if len(lanes) != 8: print gelnumber, lanes
    for test in lanes:
        for original in lanes:
            try: result[(test,original)] = Nei_Li(gel_data[test][2:],
                                         gel_data[original][2:],
                                         '', 'List')
            except:
                if test != original: print gelnumber, test, original
    return result

complete = {}
for gelnumber in range(1, 73):
    try:
        complete[gelnumber] = process_gel(Gelfile, gelnumber)
        print str(gelnumber) + ' completed'
    except ZeroDivisionError: pass

def calc_avg_dist_per_passage(rflp, complete):
    average_dist = {}
    for row in range(8):
        for col in range(8):
            dist = []
            for gel in range(rflp, 72, 6):
                try: dist.append(complete[gel][(row, col)])
                except KeyError: pass
                try: average_dist[(row+1, col+1)] =
sum(dist)/float(len(dist))
                except: print rflp-1, row+1, col+1, dist
    return average_dist

def calc_dist(complete):
    dist = {}
    for rflp in range(1, 7):
        dist[rflp] = calc_avg_dist_per_passage(rflp, complete)
    return dist

dist = calc_dist(complete)

for row in range(1,9):
    for col in range(1,9):
        for passage in range(1,7):
            print passage, row, col, dist[passage][(row, col)]
print

```