

**SINGAPORE POLYTECHNIC**  
Chemical Process and Biotechnology Department

**DIPLOMA IN BIOTECHNOLOGY**

Final Year Project 1998/1999

DBT 9813

**DEVELOPMENT OF RAPID STRAIN  
TYPING OF *AEDES* MOSQUITO BY  
RANDOM AMPLIFIED POLYMORPHIC  
TYPING ADULT DNA (RAPD)**

**Supervisors:** Mrs. Koh Siok Im  
Dr. Eric Yap

**Project Team:** Chia Yeong Chit (9656640)  
Ling Han Tong Maurice (9640308)  
Wong Chong Kum Edwin (9640225)

## CONTENTS

Pages	
Acknowledgement .....	1
Abbreviations.....	2
Abstract.....	4
Introduction.....	5
<b>Literature Review</b>	
Dengue in Singapore .....	6
DNA Extraction Methods.....	9
Randomly Amplified Polymorphic DNA.....	11
Artificial Intelligence in Data Analysis.....	15
<b>Materials and Methods</b>	
DNA Extraction Methods .....	16
Quantification of DNA .....	18
Primer Preparation.....	18
Randomly Amplified Polymorphic DNA.....	19
Data Analysis .....	21
<b>Results and Discussion</b>	
DNA Extraction from Individual Mosquito Larva.....	23
Quantification of DNA .....	26
RAPD: PCR Optimization.....	29
RAPD: Fingerprinting .....	33

Conclusion ..... 43

References ..... 44

## Appendices

Appendix I: Spectrometrical Analysis of Primers .....	48
Appendix II: Spectrometrical Analysis of Extraction Methods.....	49
Appendix III: Spectrometrical Analysis of Single Extraction .....	50
Appendix IV: Agarose Gel Photographs of Extraction Methods .....	52
Appendix V: Agarose Gel Photographs of PCR Optimization.....	54
Appendix VI: RAPD: Agarose Gel Photographs and Tabulations .....	57
Appendix VII: Similarity Index and Percent Match Matrixes.....	129
Appendix VIII: Optimization of Neural Network in STRAIN .....	164
Appendix IX: STRAIN: Source Code .....	165
Appendix X: STRAIN: User Guide .....	184

## ACKNOWLEDGEMENT

We would like to express our heartfelt appreciation to our project supervisor, Mrs. Koh Siock Im of Singapore Polytechnic and our co-supervisor, Dr. Eric P.H. Yap of Defense Medical Research Institute (DMRI) for their valuable guidance and concern throughout the entire project.

We would also like to extend our gratitude to Mdm. Shen Jie, Technical Officer of the Molecular Biology Laboratory, Singapore Polytechnic for her timely assistance, and the Ministry of Environment Vector Control Unit for their precious mosquito larvae samples.

Some special thanks were given to the group of friends around us who never failed to shower upon us their encouragement and enlivens the atmosphere in the laboratory. We would glad to show our appreciation to a special person, Eric Wong, who took great efforts and considerable time in assisting us debugging the computer program, STRAIN.

This entire work was financially sponsored by the Singapore Polytechnic and Singapore Totalisation Board through the project 11-27801-45-2442.

PCR

polymerase chain reaction

RPM

revolutions per minute

SDS

sodium dodecyl sulphate

ul

microlitres

## ABBREVIATIONS

°C	degrees Centigrade
MΩ·cm	megaohms centimetres
/	per
%	percent
DNA	deoxyribonucleic acid
ddH <sub>2</sub> O	distilled, deionized water
dNTPs	deoxynucleotide triphosphate
dsDNA	double stranded DNA
DTT	dithiothreitol
EDTA	ethylenediaminetetraacetic acid
g	gravities
l	litres
nm	nanometres
M	molar
ml	millilitres
mg	milligrams
OD	optical density
PCR	polymerase chain reaction
rpm	revolutions per minute
SDS	sodium dodecyl sulphate
µl	microlitres

## Abbreviations

μg micrograms

μM micromolar

UV ultraviolet (ability individual myxospores of *Acanthocephala* to transform DNA to double strand break)

V discrete (discrete as vector for Deegue virus using RAPD fingerprinting)

w/v weight per volume (methodology of preparing DNA samples used)

selected based on efficiency and efficiency of RAPD analysis satisfied both criteria.

Nine 16-base primers were evaluated and only five of them, A3, B1, C3, A4 and A5, were found potentially suitable for RAPD fingerprinting. All fingerprint profiles were analyzed by a in-house designed artificial intelligence software but only those of A3, B1 and C3 were analyzed using Similarity Index and Percent Matching. Out of these three methods, only the results from the software were highly promising. The best result was obtained from the profiles by primer C3 with 98% accuracy and a standard deviation of 1%. Thus, we concluded that RAPD fingerprinting of genomic DNA could be employed to discretely characterize individual myxospores of *Acanthocephala* in terms of their ability to perform as a vector for Deegue virus.

## ABSTRACT

In this study, we were able identify individual mosquito larva of *Aedes aegypti* in terms of their discrete ability to function as vector for Dengue virus using RAPD fingerprinting of genomic DNA. Four different methodologies of genomic DNA isolation were evaluated based on economy and efficiency and 24 hour SDS lysis satisfied both criterias. Nine 10-base primers were evaluated and only five of them, A2, B1, C13, AA1 and AA3, were found potentially suitable for RAPD fingerprinting. All fingerprinting profiles were analyzed by a in-house designed artificial intelligence software but only those of A1 and B1 were analyzed using Similarity Index and Percent Matching. Out of these three methods, only the results from the software were highly promising. The best result was obtained from the profiles by primer C13 with 98% accuracy and a standard deviation of 3%. Thus, we concluded that RAPD fingerprinting of genomic DNA could be employed to discretely characterize individual mosquito of *Aedes aegypti* in terms of their ability to perform as a vector for Dengue virus.

Fingerprinting method, *Aedes aegypti*, Rambdi® Amplified Length Polymorphism (RAPD), Primers, Economy and efficient method of Isolation of genomic DNA, Individual mosquito larva and the primers used in this study will also be investigated.

## INTRODUCTION

*Dengue in Singapore*  
Since the turn of the decade, incidences of Dengue Hemorrhagic Fever (DHF) and Dengue Fever (DF) in Singapore is on the uptrend. Of these two, DHF cases have appealed to public attention due to its potentially lethal consequences.

Despite the concreteness of Dengue virus being the causative agent of both DHF and DF, Dengue infection remains a three façade issue. Firstly, the virulence and epidemiological occurrence of Dengue virus. Secondly, the immunological susceptibility of individuals to the virus. Thirdly, the competency of mosquito, namely, *Aedes aegypti*, to function as a vector and its geographical distribution.

This project aimed at identifying individual mosquito in terms of capacity to act as vector for Dengue virus at the genomic level. In simple terms, it is an attempt to classify individual mosquito based on their ability to transmit Dengue virus using genomic fingerprinting methods, in this project, Randomly Amplified DNA Polymorphism (RAPD). Prior to this, an economic and efficient method of isolation of genomic DNA from individual mosquito larva and the primers used for strain typing will also be investigated.

*There are altogether 4 dengue virus serotypes. Dengue 1 virus predominated in 1993, 1996 and 1997. Dengue 2 virus is predominating in 1998, 1999, 2000 and 2001 in 1992 and 1994 (Goh, 1998).*

## LITERATURE REVIEW

### **Dengue in Singapore**

Dengue fever had always been of public awareness since the first epidemic of the disease in 1901 in Singapore. The haemorrhagic form of the disease, dengue haemorrhagic fever was documented in 1960. At that time, there were 70 hospitalised cases. By 1966, the number of cases for dengue haemorrhagic fever had increased to 630 hospitalised cases with 24 deaths. It was very alarming to the public and in June 1966, the Vector Control Unit was set-up (Goh, 1998). Extensive research was carried out to study the two dengue vectors, *Aedes aegypti* and *Aedes albopictus*.

*Aedes aegypti* is an indoor breeder while *Aedes albopictus* is an outdoor breeder. Their breeding habitats are similar where virtually anything that is able to hold even a small portion of water. These includes drains, roof-gutters, flower pots, plastic bags, containers and etc. According to a survey, it was shown that 99% of those identified breeding habitats were artificial habitats like containers plastic bags (Reiter, 1998). *Aedes albopictus* is found to be closely related with rainfall (Tan et. al., 1998).

There are all together 4 dengue virus serotypes. Dengue 1 virus predominates 1995, 1996 and 1997. Dengue 2 virus is predominating in 1990, 1991, 1993 and Dengue 3 in 1992 and 1994 (Goh, 1998).

Dengue fever and dengue hemorrhagic fever affect all age group of human population with age group between 15-24 years old being the highest. There is a slight male dominance over female with a ratio of 1.6:1 and Chinese are more affected compared to Indians and Malays (Goh, 1998). As construction sites are the majors breeding places of mosquitoes, foreign workers are more easily affected. Last of all, compound houses had higher morbidity rate of 5.6 times than high-rise building flats and 2.4 times than private condominiums (Tan *et. al.*, 1998).

The cases of dengue fever and dengue hemorrhagic fever had been on the rise from 1986 to 1997 with a slight decrease in 1993. In 1986 (354 cases with 1 death); 1987 (436 cases with 2 deaths); 1989 (944 cases with 2 deaths); 1990 (1733 cases with 3 deaths); 1991 (2179 cases with 6 deaths); 1992 (2878 cases with 6 deaths); 1993 (946 cases with no death); 1994 (1239 cases with 1 death); 1995 (2008 cases with 1 death); 1996 (3128 cases with 3 deaths) and 1997 (4300 cases with 1 death) (Goh, 1998).

One reason that dengue fever and dengue hemorrhagic fever cases are increasing is that the immunity level of the population to the virus is dropping from 45.6% in 1982-1984 to 39.6% in 1993. It was also observed that the residents in the affected areas have higher level of immunity against dengue virus (Goh, 1998). Other reasons include population growth, urbanization and tourism. Rapid population growth give rise to the population, this will enable the *Aedes* mosquitoes to have a larger target. When a place undergo urbanization, more construction sites will be set up and they are the majors breeding habitats for *Aedes* mosquitoes. Every year, 5%-8% of the dengue fever cases are

imported dengue fever. With at least 5 cases out of 100 infected cases were imported, more attention should be focus on tourism.

As there is yet a dengue virus vaccine available for large-scale immunization, community-based eradication of *Aedes* mosquitoes is still the main approach for the control of dengue. This includes public education, law enforcement, search and destroy operations and finding innovative methods to eradicate breeding sites of the *Aedes* mosquitoes' larvae.

In 1966, a pilot study was conducted to show the effect of health education and source reduction had on the control of dengue. The results showed that the House Index (HI) was brought down from 16% to 2%. The goal of the search and destroy operations was to maintain the HI at 1% to 2%. Active and continuous participation is required as the mosquitoes' breeding habitats are formed as fast as they are destroyed (Goh, 1998).

In October 1968, a one-month long "Keep Singapore Clean" campaign was set to change the attitude of Singaporeans so as to keep Singapore clean. Pamphlets, stickers, posters were given out and there was also a major exhibition at the Singapore Conference Exhibition Concourse. Several other mobile exhibitions were held at six community centers. Following by the enforcement of law to keep Singapore clean, the effect was very good and the streets were observed to be cleaner with less litter. The law that was enforced was known as the Destruction of Disease-Bearing Insects Act (Reiter, 1998).

There are several other measures that are taken to control dengue. These includes the American ovitrap, mosquito-proof roof-gutters, Reslin-50E changed to Actelin 50EC when the mosquitoes are resistant to it. There was also a suggestion to heat the drain to 95°C for 1 hour to eliminate the larvae. It was very effective but factors like cost and different conditions of the drain had to be taken into consideration. As the cost is too high and the conditions are hard to control, the idea cannot be implemented.

Another method of control is through the use of insecticides such as Chlordip 100WP with Fenpropane K and EC (Hector et al., 1993). Many people have feared the idea of having their house fogged to eliminate *Aedes* mosquitoes, as it will leave their furniture oily and house to be smelly. This is because the primitive carrier used in fogging is kerosene. Actelin 50EC is now used as a carrier and fogging is not sticky and smelly.

Another method of control is through re-designing of public structures. It has been suggested over many other DNA isolation protocols is that the Public structures like drains and roof-gutters can be re-designed to become mosquito-proof. Other simpler methods like using Bactimos granules or insecticides paint, PC-cide on flowerpots or roof-gutters to aid in the control of dengue.

### DNA Extraction Methods

The first step in DNA extraction is to break down the cell wall of the sample. This can be done by using detergents and protease inhibitors. The idea of DNA extraction is to remove the proteins and nucleic acids from the sample. Various methodologies had been previously employed in attempt to isolate genomic DNA from entomological samples and other samples of minute quantities, such as, forensic samples. Many different combinations of homemade lysis buffer with SDS as the prime lysing ingredient were used in insect studies. These includes, 1% SDS with Proteinase K buffered at pH 8.0 (Corley et. al., 1993); 0.1% SDS at pH 8.0 (Severson et.

*al.*, 1993); 0.5% SDS with 0.2M sucrose at pH 9.1 (Mutebi *et. al.*, 1997); and 0.1% SDS with spermine, spermidine and Proteinase K at pH 8.0 (Apostol *et. al.*, 1993 and Ballinger-Crabtree *et. al.*, 1992). Despite the common use of SDS in the above mentioned buffers, the amount of genomic DNA isolated varies greatly, ranging from 0.5 $\mu$ g (Severson *et. al.*, 1993) to 10 $\mu$ g (Ballinger-Crabtree *et. al.*, 1992). For forensic studies, the main protocol used is Chelex® 100 extraction. Similarly, many variations are in existence, such as, Chelex® 100 resin with Proteinase K and DTT (Herber *et. al.*, 1997). Chelex® 100 resin is a chelating exchange resin, which will prevent degradation of DNA by chelation of metal ions in solutions of low ionic strength (Walsh *et. al.*, 1991).

In 1997, a new form of DNA isolation using a patented product, DNAZOL, was introduced. Its major advantage over many other DNA isolation protocols is that the entire process can be completed within 30 minutes and it is suitable for nearly all forms of DNA isolation, from small fragments to genomic DNA. At the same time, the DNA extracted using this method can directly be used for other molecular biology applications (Chomczynski *et. at.*, 1997).

DNAZOL is in fact a mixture of detergent and guanidine thiocyanate. The idea of DNA isolation using detergent dated in the primordial period of molecular biology. SDS, itself, is a detergent. On the other hand, guanidine-containing compounds had been used in DNA isolation at least a decade before the use of DNAZOL (Verma, 1998).

### ***Randomly Amplified Polymorphic DNA (RAPD)***

The year 1983 marked an innovation of impact in the field of DNA technology with the invention of polymerase chain reaction (PCR) by Kary Mullis. Ten years later, Kary Mullis was conferred Nobel Prize in Chemistry for this invention (Karp, 1999).

However, before PCR techniques were established, for years, DNA fingerprinting technology had relied on restriction fragment length polymorphism (RFLP) analysis. Although RFLP is a very efficient technique, it is costly and labourious, thus, not suitable for high throughput.

A novel PCR-based strategy involving the use of arbitrary primers to amplify genomic DNA has been developed. In a relatively short time, random amplification methodology has became very popular due to its ability to generate high quality fingerprints using very small amounts of starting DNA, normally less than 10ng. This ability is unsurpassed even using RFLP together with radioisotopic probes.

Arbitrarily primed PCR (AP-PCR) reactions involved the use of a single oligonucleotide of arbitrary sequence, which primes the amplification of several discrete DNA segments. Each of these anonymous but reproducible fragments is derived from a region of the genome that contains, on opposite DNA strands, two primer-binding sites located within an amplifiable distance of each other. Any difference between the pattern of amplified fragments reveals a polymorphism in the original DNA template, hence, the name, RAPD.

All classes of mutations can be potentially detected by means of random amplification fingerprinting, even though most of the identified polymorphisms are supposed to be caused by substitutions. Single base substitution within the primer-binding region may prevent amplification by introducing a mismatch; this is supported by the observation that most single base changes in a primer sequence results in a complete change in the amplification pattern. Substitutions outside the primer-binding site can be detected if they result in stable secondary structures, which inhibits elongation by *Taq* polymerase. Other classes of mutations can be isolated if they remove a primer-binding site or change the distance between two primer-binding sites.

The amplification strategy is common in AP-PCR, RAPD-PCR and DNA amplification fingerprinting (DAF). They differ in the length of primers used, amplification conditions, separation and visualization of the amplified fragments; consequently generate markedly different fingerprints.

Amplifications performed using two arbitrary primers generate reproducible patterns that are different from those obtained with each single primer. Pairwise combination of primers provides an advantage over single primers: it allows for generation of a much higher number of bands due to combinatorial binding of primers to the template DNA. Amplification products generated by 2 different primers can be directly analyzed by cycle sequencing.

By improving either primer design or amplification strategy, researchers are able to tailor fingerprints in such a way that peculiar requirements of specific areas of application can be met, such as, those pertaining to the complexity of amplification pattern, level of polymorphisms detected and nature of amplified products. Strategies based on improvement of primer design involves the use of mini-hairpin primers with hairpin structure at their 5' end, or primers with degenerate bases. Primers that are biased towards particular sequence motifs, promoter consensus sequences or gene families have also been used.

The improvement of amplification strategy is the basis of both ASAP analysis and tecMAAP: the former is a dual-step amplification strategy in which fingerprints are generated by re-amplification of a previous fingerprinting profile, while the latter involves endonuclease cleavage of the template prior to its amplification with arbitrary primers. More recently, further alternative strategies like AFLP, RAHM, RAMPO and DS-PCR have been proposed.

AFLP is a technique, which produces highly complex profiles by arbitrary amplification of subsets of restriction fragments ligated to adaptor cassettes.

Randomly amplified hybridization microsatellites (RAHM) and random amplified microsatellite polymorphisms (RAMPO) are methods which combines random DNA amplification with hybridization to microsatellite-complementary oligonucleotide probes.

This approach can be usefully applied in genetic analysis of species where little or no intraspecific variations is detected by random amplification alone.

Double stringency PCR (DS-PCR) is a technique combining microsatellite-specific priming with arbitrary priming. In this approach, random amplification is targetted to highly polymorphic genomic regions.

Randomly amplification technology is an efficient tool to quickly and easily screen a very large number of loci for possible DNA polymorphism (RAPD). These polymorphisms have proved to be able to discriminate between closely related individuals. In addition, easy identification makes RAPD potentially useful in many areas of genetic research, such as, gene mapping, marker-assisted selection on breeding programs and individual or strain identification.

However, in RAPD, the presence of band does not allow discrimination between homozygosity and heterozygosity at a particular locus.

RAPD, AP-PCR, DAF and other PCR-based fingerprinting approaches are rather sensitive to template DNA quality, reaction conditions and machine type. Quality and quantity of template DNA have emerged as the main factors affecting reproducibility. Therefore, the preparation of the template DNA is crucial. (Micheli and Bova, 1996)

### **Artificial Intelligence in Data Analysis**

There is a long history in the utilization of information technology and artificial intelligence (AI) in the field of biology despite its relatively low profile. In the mid-1970s, an expert system, MYCIN, was developed at Stanford University to assist doctors in the diagnosis of bacteremia and meningitis (Shortliffe, 1976). This event marks the intersection of biology and AI. From then onwards, MYCIN has been mentioned in numerous AI-based literatures as elucidation (Durkin, 1994). In the last decade, many instances of AI-based analytical programs were used in molecular biology, for example, prediction of protein folding by genetic algorithm (Dandekar and Argos, 1997) and artificial neural network (ANN) (Qian and Sejnowski, 1988). ANN is inspired by the human brain, which is a biological neural network. Upon training the ANN with a known data set, it is able to abstractly characterize and adapt itself to the data given. This property of ANN situates it in an advantageous position to analyze large amounts of data, such as, from RFLP (Carson *et. al.*, 1995) or hyperspectral fingerprinting (Goodacre *et. al.*, 1998), in a short time, which is otherwise near impossible using conventional statistical means.

### **Protocol 2: Using CellCounter**

In this protocol, we will use CellCounter to count the number of cells in a sample. The sample must be a single cell suspension. Cells can be obtained from a tissue sample, a blood sample, or a culture. Numerous grants provide a description on how to obtain a single cell suspension. A brief description of the process is provided below.

## MATERIALS AND METHODS

The larva was crushed with 1ml of absolute ethanol and dried overnight at -20°C. The pellet was resuspended in 200µl of absolute ethanol and air-dried overnight at -20°C.

### DNA Extractions Methods

#### Protocol 1: By SDS Lysis

200µl of lysis buffer (2% (w/v) SDS; 0.2M NaCl; 25mM EDTA; 10mM Tris-HCl, pH 8.0) was added to the pulverized mosquito larva and incubated at room temperature. For optimization purposes, the samples were incubated at four different lengths of time, 15 hours, 17 hours, 20 hours and 24 hours. After incubation, equal volume of phenol-chloroform (1:1) was added and mixed by gentle inversion. The sample was then spun at 14000 rpm (Eppendorf Centrifuge 5415C) for 10 minutes at room temperature. The aqueous layer was transferred into a new tube with 5µl of 10mg/ml RNase A and incubated for 30 minutes, after which another step of phenol-chloroform extraction was carried out as described above. To the aqueous layer, 0.1 volume of 3M sodium acetate and 2.5 volume of absolute ethanol was added and mixed. The mixture was incubated overnight at -20°C and spun at 14000 rpm for 30 minutes. The pellet was air-dried and solubilized in 20µl of T-10 buffer (10mM Tris-HCl, pH 8.5).

#### Protocol 2: Using QIAamp DNA Mini Kit

To the crushed larva samples, 1ml of QIAamp DNA Mini Kit solution (Qiagen, Germany) was added. Numerous gentle pipetting was essential to ensure sufficient lysis of cells. 500µl of absolute ethanol was mixed into the mixture and incubated for 3 minutes at

room temperature. After incubation, the mixture was spun at 4000g for 2 minutes and the supernatant was discarded. The pellet was washed with 1ml of absolute ethanol, air-dried and reconstituted in 20 $\mu$ l of T-10 buffer.

**Protocol 3: Using QIAamp Tissue Kit (QIAGEN)** 100 mg of larva was added to a tube containing 180 $\mu$ l of Buffer ATL was added to each sample of powdered larva, followed by the addition of 20 $\mu$ l of Proteinase K. The mixture was left for incubation for 2 hours at 55°C. To this mixture, 200 $\mu$ l of Buffer AL was added and incubated for 10 minutes at 70°C. After incubation, 210 $\mu$ l of absolute ethanol was added and mixed. The mixture was then applied to a QIAamp spin column, which was placed in a collection tube, and centrifuged at 8000 rpm for 1 minute. The filtrate collected was discarded. 500 $\mu$ l of Buffer AW was placed into the spin column and spun for 1 minute. The filtrate was discarded. This step of washing with Buffer AW was repeated with a centrifugation force of 14000 rpm for 3 minutes. The DNA bound to the column was eluted twice with 200 $\mu$ l of Buffer AE preheated to 70°C and centrifuged at 8000 rpm for 1 minute. All buffer, enzymes and columns used in this protocol were provided for in the kit, with the exception of ethanol.

**Protocol 4: Using Chelex 100 ® Resin**

200 $\mu$ l of 5% (w/v) Chelex 100® resin (Bio-Rad), 4 $\mu$ l of 17.8mg/ml Proteinase K and 6 $\mu$ l of 1M DTT was added to each grounded sample. The resulting mixture was incubated overnight at 56°C. The mixture was then boiled for 10 minutes and centrifuged at 14000 rpm for 10 minutes to remove the Chelex 100® resin.

**Quantification of DNA**

All the DNA extracted were subjected to quantification using spectrophotometer (Shimadzu UV-Visible Recording Spectrophotometer UV-160A) with the exception of those extracted using QIAamp Tissue Kit and DNA<sub>ZOL</sub> solution. 5μl of reconstituted DNA was diluted with 995μl of ddH<sub>2</sub>O ( $\geq 18.1\text{M}\Omega\text{-cm}$ ) to give a 1 in 200 dilution. The diluted sample was then measured at OD<sub>260</sub> and OD<sub>280</sub>. The ratio of OD<sub>260</sub> and OD<sub>280</sub> was obtained. All results were tabulated in the appendices. The concentration of DNA was calculated based on the relation that OD<sub>260</sub> of 1 is equivalent to 50μg/ml of dsDNA.

**Primer Preparation**

The primers used in this project were 1.5mer, 2.5mer and 3.5mer. All the primers used in this project were commercially synthesized by Gibco, Life Technologies, SPD. The entire amount of lyophilized primer was re-hydrated in 300μl of autoclaved ddH<sub>2</sub>O. The concentration of the stock primer solution was spectrophotometrically determined based on the following formula,

$$[\text{primer}] = \frac{\text{OD}_{260} \times 30}{5 \times Nmers \times 300} \times 10^6 \mu\text{M}$$

where Nmers is the number of bases on the primer. The primers were then diluted into the working concentration of 10μM and aliquoted into 100μl aliquots.

**Randomly Amplified Polymorphic DNA (RAPD)****Section 1: PCR Optimization**

Optimization of PCR conditions was carried out prior to fingerprinting using nine commercially synthesized 10-base random primers [A2 (5' TGCCGAGCTG 3'); B1 (5' GTTCGCTCC 3'); B3 (5' CATCCCCCTG 3'); C9 (5' CTCACCGTCC 3'); C13 (5' AAGCCTCGTC 3'); AA1 (5' GTTGCATCC 3'); AA2 (5' TTTGCCCGGA 3'); AA3 (5' CCACAGCAGT 3'); AA4 (5' GGACCCTTAC 3')] (Gibco, Life Technologies, SPD) as previously described (Ballinger-Crabtree *et. al.*, 1992 and Mutebi *et. al.*, 1997). For each of the primer, the optimal concentration of magnesium ions (Promega) was determined by a series of titration. The concentrations titrated were 1.5mM, 2.0mM and 2.5mM per reaction mixture. The economically optimal concentrations of dNTPs (Perkin Elmer) and AmpliTaq® polymerase (Perkin Elmer) were similarly confirmed by titrations. The concentrations of dNTPs titrated were 0.2mM, 0.4mM, 0.6mM, 0.8mM and 1.0mM per reaction mixture, whereas for AmpliTaq polymerase, the concentrations were 1.0 unit, 1.5 units and 2.5 units per reaction mixture. Machine optimization was also carried out for six of the nine primers, namely, C9, C13, AA1, AA2, AA3 and AA4. The PCR machines were Perkin Elmer Cetus and Perkin Elmer GeneAmp System 2400. The optimal conditions determined for each primer were used in the actual DNA fingerprinting by RAPD. All optimization reactions and fingerprinting were carried out in 50 $\mu$ l reaction volume using the program as described in the next section.

We currently perform an RAPD with the following dye suggested to avoid smearing of the gel bands before the gel was exposed to UV radiation and the image was captured on Polaroid film (Polaroid).

## Section 2: RAPD Fingerprinting

Eighty-four mosquito larvae of two different strains, resistant to Dengue virus and susceptible to Dengue virus, were obtained from the Ministry of Environment, Vector Control Unit. The genomic DNA from these larvae was individually isolated using SDS lysis. All sample genomic DNA isolated from individual mosquito larva were diluted 1:50 times with autoclaved ddH<sub>2</sub>O. 1μl of diluted template DNA was added to 2X PCR buffer (Perkin Elmer) to a total volume of 10μl before subjected to heat-denaturation at 98°C for eight minutes. If Perkin Elmer Cetus was to be used as the thermal-cycler, one to two drops of autoclaved mineral oil (Sigma) was added to the sample prior to heat-denaturation. Upon completion, the samples were cooled to 4°C where 3μl of 10X PCR buffer, 1.5 units of AmpliTaq polymerase, 4μl of 10mM dNTPs, magnesium ions, 2μl of 10μM primer and autoclaved ddH<sub>2</sub>O were added to each reaction to provide a total volume of 50μl. PCR was carried out using the temperature profile: 94°C, 2 minutes; 40 cycles of 94°C, 1 minute, 35°C, 2 minutes, 72°C, 3 minutes; 72°C, 4 minutes. 10μl of PCR product was mixed with 2μl of 0.6X [10 times dilution of 6X dye with glycerol (Merck)] DNA loading dye (Promega) before loading into a pre-cast 2.5% (w/v) ethidium bromide incorporated agarose (Sigma) gel in a submarine unit (Hoefer Max Submarine Unit HE99X, and Bio-Rad DNA Sub Cell™) for analysis, with pGEM digested with restriction endonucleases HinfI, RsaI and SmaI as marker (Promega) and 1X TAE (4.84g/l Tris; 1.142ml/l glacial acetic acid; 0.05M EDTA) as the running buffer. The samples were electrophoresed at 100V until the blue tracking dye migrated to about one-thirds of the gel length before the gel was exposed to UV radiation and the image was captured on Polaroid film (Fuji).

### Data Analysis

#### Protocol 1: Computerized Calculation of Similarity Index and Percent Match

Each visually distinguishable band on the photograph was individually scored for its molecular weight ( $MW_{(bp)j}$ ) using the marker lane as standard and the following formula:

$$MW_{(bp)j} = 10^{\left[ \frac{N \sum X_i Y_i - \sum X_i \sum Y_i}{N \sum Y_i^2 - (\sum Y_i)^2} Z_j + \frac{N \sum X_i Y_i - \sum X_i \sum Y_i}{N \sum X_i^2 - (\sum X_i)^2} \right]}$$

Where N is the number of bands of the marker used as standard; X is the migration distance of band  $i$  of the marker; Y is the logarithmic value of the molecular weight in base pairs of band  $i$  of the marker; and Z is the migration distance of band  $j$  of the sample.

The molecular weight of the largest and smallest observed fragments was identified for each primer and its logarithmic scale of the difference was divided into 20 categories. All the scored bands for each individual sample was placed into the 20 defined categories. All the data were entered into an in-house designed computer program, STRAIN. (Refer to Appendix X for the user's guide for STRAIN.) Calculations of Similarity Index (S.I.) and Percent Match (%M) between each individual mosquito larva were done electronically. The S.I. between two individuals was calculated using the following formula,

$$S.I. = \frac{2N_{AB}}{N_A + N_B}$$

where  $N_{AB}$  was the number of matching existent category between the two samples,  $N_A$  and  $N_B$  were the number of scored categories, representing the presence of bands, in Sample A and Sample B respectively. The %M was calculated as follows,

$$\%M = \frac{N_{AB}}{N_T}$$

where  $N_{AB}$  was the sum of the number of existent and non-existent groups between the samples, whereas  $N_T$  was the number of scored categories.

### Protocol 2: Analysis Using Artificial Neural Network Model

An extremely simplified form of neural network was presented in STRAIN (refer to Appendix X for assistance on the operation of the program) for the purpose of rapid identification of strain type, namely, resistant strain and susceptible strain, from RAPD fingerprinting. The fingerprints were categorized as described above and stored in a computer database through STRAIN. The neural network architecture consisted of a multilayer feedforward network with twenty input nodes, five hidden nodes and one output node (with two states). The network was trained with 15 known data samples of each strain using supervised learning for a number of repetitive cycles. For optimization purposes, a range between 1000 to 10000 cycles with 10 intervals were used. Each interval was graded for its subsequent accuracy when tested with known data, and its reproducibility (triplicates). In all, RAPD fingerprints obtained using five primers, namely, C13, B1, A2, AA1 and AA3, were evaluated.

## RESULTS AND DISCUSSIONS

### **DNA Extraction from Individual Mosquito Larva**

In order to evaluate the usability of the four DNA isolation protocols in extracting genomic DNA from mosquito larvae, the isolated DNA samples were subjected to visual analysis for intactness using 1% (v/v) agarose gel electrophoresis. Isolated DNA samples using SDS lysis and Chelex® 100 resin were spectrophotometrically analyzed. Based on experience, spectrophotometrical analysis of small quantities of DNA isolated using ion exchange chromatography, such as, those in QIAamp Tissue Kit, will give rise to unrealistic values. For the purpose of visual evaluation, the amount of DNA isolated must be significant. To ensure this, five mosquito larvae were used per isolation and for the results to be statistically significant, every variation of isolation methods were carried out in quadruplets.

Comparing the presence and intactness of DNA isolated by the four protocols using agarose gel electrophoresis, SDS lysis method is consistent in terms of quality and gave the best yield, even though very smearable bands were observed (see Appendix IV). In terms of optimal lysis time, 24 hour lysis gave the best quality results as compared with other durations of lysis (see Appendix IV, Figure 1 and 2). According to spectrophotometric analysis, SDS lysis method yields an average of  $2.42\mu\text{g}$  of DNA per mosquito larva. Although the average yield of DNA were slightly different for each duration of lysis [see Appendix II (T1A – T1D represents 24 hour lysis, T2A – T2D

represents 20 hour lysis, T3A – T3D represents 17 hour lysis, T4A – T4D represents 15 hour lysis)], the difference were insignificant ( $F = 0.177$ ,  $p > 0.25$ ). The ratio of  $OD_{260}$  to  $OD_{280}$  on average was around 1.7, indicating considerably pure DNA. SDS lysis method was considerably economical, about S\$0.20 per isolation, when compared with the other three methods, making it especially useful for screening. However, this method was not without any disadvantage. The major deterrent was that this method was very labour intensive. Every complete isolation of DNA required approximately 48 hours.

DNA isolation using QIAamp Tissue Kit gave the most consistent and reproducible results on agarose gel, even though the bands were faint and smearable (see Appendix IV, Figure 3). Moreover, the yield of DNA was considerably lower than that of SDS lysis. In the aspects of economy, it was so far the most expensive method, costing S\$4.00 per isolation. The sheer expense of this method might not render it very attractive despite the quality of DNA isolated. This was especially true when it was used in routine screening. However, the use of QIAamp Tissue Kit was faster and easier than both SDS lysis method and using Chelex® 100 resin, with an average processing time of 4 hours per isolation.

Comparing all four methods, including using DNAZOL and Chelex® 100 resin had a consistency in the quantity of DNA, however, the quality of DNA were rather consistent (see Appendix IV, Figure 4). These inconsistencies might be due inherently to the procedure of cell lysis. Cells were lysed by numerous pipetting in the solution, which required considerable experience to ensure complete lysis. The quality and quantity of

DNA, as visually analyzed, were very much similar to that isolated using QIAamp Tissue Kit. Compared with QIAamp Tissue Kit, DNA isolation with DNAZOL solution was much more economical, S\$0.90 per isolation, and faster, every extraction only required 30 minutes. If experience was available and time was crucial, DNA isolation using DNAZOL solution would prove to be very useful.

Analysis by electrophoresis of the DNA samples isolated using Chelex® 100 resin yield neither observable bands nor smears (see Appendix IV, Figure 5). However, spectrophotometry showed that the average yield of DNA per larva was  $2.12\mu\text{g}$ . The average ratio of  $\text{OD}_{260}$  to  $\text{OD}_{280}$  was 0.98 [see Appendix II (Sample C1 – C4)], indicating the presence of high amounts of contaminants. These contaminants might be protein contaminants associated with the genomic DNA. These proteins might be wrapping around or tightly bounded to the genomic DNA, as *in vivo*, and rendered it relatively impermeable to ethidium bromide, therefore, not visualized on agarose gel. This method is very easy to perform, even to amateurs, however, it required about 16 hours to complete an isolation.

There are several methods that one could use to determine the quantity of DNA. We considered four methods, isolations using DNAZOL and Chelex® 100 resin had a much higher put-through than the other two methods due to the level of tediousness.

A summary of the evaluation is given in Table 1.

**Table 1: Evaluation of DNA Isolation Protocols**

	<b>DNAzol</b>	<b>QIAamp TK</b>	<b>Chelex® 100</b>	<b>SDS lysis</b>
<b>Processing Time</b>	30 minutes	4 hours	16 hours	48 hours
<b>Skill Needed<sup>a</sup></b>	++	+++	+	++++
<b>Quantity of DNA<sup>b</sup></b>	++	+++	+++	++++
<b>Cost per isolation</b>	S\$0.90	S\$4.00	S\$0.60	S\$0.20
<b>Consistency<sup>c</sup></b>	++	++++	++	+++

<sup>a</sup> more pluses indicates higher skill level

<sup>b</sup> more pluses indicates greater quantity

<sup>c</sup> more pluses indicates greater consistency

After careful evaluation, it was decided that 24 hours of SDS lysis would be the choice of genomic DNA isolation for use in this project.

### **Quantification of DNA**

Quantification of DNA is necessary as it let the operator knows how much of DNA was extracted from the sample. In our project, we had successfully extracted DNA from a single larva of *Aedes* mosquito. The amount of DNA extracted is of concern because if the DNA is present in very low quantities, it may hinder the processes of other procedures after DNA extraction, such as, restriction digestion and PCR.

For the purity of the DNA sample, the ratio of absorbance at wavelengths 260nm over 280nm is used. There are several methods that one could use to determine the quantity of DNA. We choose to use the spectrophotometric assay. Two wavelengths were used to assay the quantity of extracted DNA, 260nm and 280nm. The absorbance readings from 260nm were used to find the concentration of DNA by applying the formula:

$$1 \text{ OD}_{260} = 50 \mu\text{g/ml}$$

This problem can be overcome by the addition of proteinase to digest large protein molecules into smaller fragments. If the ratio is more than 2.0, this indicated gross degradation of the sample DNA. This is mainly

There are several factors affecting the determination of the quantity of DNA extracted using the spectrophotometric assay. First of all, the operator must be aware of the molecular structure of the DNA sample in question. The formula stated above is applied to only double-stranded DNA, which is the type extracted from mosquito larva. If the DNA that is to be quantified is single-stranded, then the formula used should be:

$$1 \text{ OD}_{260} = 40 \mu\text{g/ml}$$

This is because the bases of single-stranded DNA are more exposed to the environment compared to the bases of double-stranded DNA. Increase in the number of bases exposed to the environment will result in increase in absorbance readings. As the bases of double-stranded DNA are less exposed, it will require more amount of double-stranded DNA to give the same absorbance reading at wavelength of 260nm as that of single-stranded DNA. The other factor to be considered is the conformation of the DNA. If the DNA is highly folded and compact, more amount of DNA is required to give the same absorbance reading at 260nm compared to that that is less folded.

For the purity of the DNA sample, the ratio of absorbance at wavelengths 260nm over 280nm is used. For pure double-stranded DNA, the ratio should be between 1.8 to 2.0. if the ratio is less than 1.8, this shows that the sample solution has protein contamination. This problem might have arose as a result of insufficient or incorrect performance of phenol-chloroform extraction. However, in some cases where the protein molecule is too big, it cannot effectively enter into the organic layer. This problem can be overcome by the addition of proteinase to digest large protein molecules into smaller fragments. If the ratio is more than 2.0, this indicated gross degradation of the sample DNA. This is mainly

due to improper handling of DNA during the extraction stage, such as, vigorous shaking and pipetting. In some cases, the DNA might be contaminated due to inappropriate storage and was broken down by external DNases.

With reference to Appendix III, the purity range of the extracted DNA accepted was between 1.4 to 2.2. We found that even at the ratio of 1.4, the DNA provides good results for subsequent procedures. It was generally observed that there was some protein contamination in most of the isolated DNA samples and the protein was believed to be mostly histones, which normally wrapped around eukaryotic genomic DNA.

The average amount of DNA extracted per larva is  $16.42\mu\text{g}$  with a standard deviation of  $14.11\mu\text{g}$ . The amount of DNA isolated was very high compared to those reported in literatures ( $10\mu\text{g}$  per larva). However, the standard deviation was also very large, meaning that the quantity of DNA fluctuates over a large range, with the lowest being  $0.8\mu\text{g}$  per larva to the highest being  $117.8\mu\text{g}$  per larva. This fluctuation might be due to the different sizes of larva in the sample. Apart from the difference in sizes, the larvae were also present in different stages with some in the larvae stage and some in the pupa stage of their life cycle. There is no way that we can think of to obtain all larvae in the same stage and growth. However, it might be possible to minimize the difference by weighing the larvae. A range of acceptable weights can then be determined so that the standard deviation will be reduced to a much lower value.

**RAPD: PCR Optimization**

All genomic DNA isolated from pooled samples during the phase of optimization of DNA isolation, namely, by SDS lysis and QIAamp Tissue Kit, were used for PCR optimization. To eliminate the possibility of poor quality DNA, the DNA samples were analyzed by both 1% (w/v) agarose (Sigma) gel and spectrophotometrically. The results proved to be satisfactory [see Appendix II, Appendix III and the above discussion (DNA Extraction from Individual Mosquito Larva)].

To avoid unnecessary complications, the entire scheme of PCR optimization was carried out in four stages. The objective of the first stage is to achieve PCR amplification of the template, namely, genomic DNA from mosquito larva. In this stage, two different PCR systems were evaluated, namely, Taq polymerase from Promega and AmpliTaq® polymerase from Perkin Elmer. It was found that only AmpliTaq® polymerase from Perkin Elmer ensured PCR amplification. It was also at this stage where it was made known that neat DNA samples straight from the isolation process using SDS lysis were not suitable for PCR, however a 2% (v/v) neat sample gave satisfactory results as 1% (v/v) neat sample. In the case of those extracted by QIAamp Tissue Kit, neat samples gave relatively high level of amplification. The need for dilution may be due to large amounts of inhibitors of PCR present in the neat sample that were removed when the DNA was isolated using QIAamp Tissue Kit. These inhibitors might be residual proteins, SDS or even phenol. By performing a 1:50 or 1:100 dilution using nanopure water, the inhibitors might be diluted to below inhibitory concentrations. It was also in a later part of the project that it was realized that the source of dNTPs also played an important role

in PCR amplification in this instance. It was brought to notice that using dNTPs from Promega, together with AmpliTaq® polymerase, will not result in amplification despite all other conditions were unvaried.

Stemming from the cost of dNTPs and AmpliTaq® polymerase, the second stage of PCR optimization aimed at determining the economical concentrations of dNTPs and AmpliTaq® polymerase per reaction. Through a series of titrations, it was concluded that the economical concentrations of dNTPs and AmpliTaq® polymerase were 0.8mM and 1.5U per reaction respectively (see Appendix V, Figure 1 and 2). These economical concentrations might be result of the inherent limitation of the PCR cycling conditions. As a consequence of the number of fixed cycles, which is 40 cycles, in the PCR program, any higher amounts of dNTPs than 0.8mM will result in excess and eventually wasted. This might also be true for AmpliTaq® polymerase, assuming no changes in other parameters. In short, 0.8mM dNTPs and 1.5U AmpliTaq® polymerase per reaction were non-limiting factors in terms of the amount of PCR product. The most likely limiting factor was the number of amplification cycles.

After the amount of dNTPs and polymerase were ascertained, magnesium titrations were carried out for each individual primer. For six of the nine primers (C9, C13, AA1, AA2, AA3 and AA4), magnesium titration was simultaneously carried out with machine evaluation (Perkin Elmer Cetus and Perkin Elmer GeneAmp System 2400). Machine evaluation was attempted with two purposes in mind. Firstly, to maximize use of available equipments. Perkin Elmer Cetus had a capacity of 48 reactions per run,

whereas, Perkin Elmer GeneAmp System 2400 had 24. However, due to the lack of a heated cover, Perkin Elmer Cetus required the addition of mineral oil into the reaction mixture. This proved to be a hinderance when the PCR products were to be analyzed on agarose gels. Secondly, to check if the PCR products were machine dependent. In theory, due to the difference in the rate of heating and cooling, different machines might yield slightly different compositions of PCR products and it is vital to PCR-based fingerprinting.

The PCR products were analyzed on 2.5% (w/v) agarose gel and the optimum magnesium ions concentration and machine were judged visually, based on clarity of bands, resolution of bands and number of bands (more number of bands were preferred).

A very interesting phenomenon was discovered when the resolved PCR products were judged for machine dependency, different primers seemed to work better for different machines, occasionally, irregardless of magnesium concentration. A possible reason for this occurrence might be the preferential annealing of the primer to the template at the characteristic rate of cooling of the particular thermal-cycler.

In terms of optimal concentration of magnesium ions and PCR thermal-cycler usage, A2 primer required 1.5mM with Perkin Elmer GeneAmp System 2400; B1, 1.5mM with Perkin Elmer GeneAmp System 2400 (Appendix V, Figure 3); B3, 2.5mM with Perkin Elmer GeneAmp System 2400 (Appendix V, Figure 4); C9, 2.5mM with either Perkin Elmer GeneAmp System 2400 or Perkin Elmer Cetus (Appendix V, Figure 5); C13,

2.5mM with Perkin Elmer Cetus (Appendix V, Figure 6); AA1, 2.5mM with Perkin Elmer Cetus (Appendix V, Figure 7); AA2, 2mM with Perkin Elmer Cetus (Appendix V, Figure 8); AA3, 2mM with Perkin Elmer Cetus (Appendix V, Figure 9); and AA4, 2.5mM 5mM with either Perkin Elmer GeneAmp System 2400 or Perkin Elmer Cetus (Appendix V, Figure 10).

The optimum PCR conditions for each primer were summarized in Table 2 below.



It was observed visually that RAPC fingerprinting with some primers gave better results than others in a number of aspects. The quality of the products of fingerprinting of mosquito genomic DNA were primarily visually evaluated based on the gel images as shown in Fig. 10-14. The various RAPC condition were, during the analysis, compared at 10000 dpi resolution on scanner gel and the quality of amplified bands

**Table 2: Summary of PCR Optimized Conditions**

Primer	Magnesium Ions Concentration <sup>a</sup>	Machine Usage <sup>b</sup>	DNTPs Concentration <sup>a</sup>	Amount of AmpliTaq® polymerase <sup>a</sup>
A2	1.5mM	2400	0.8mM	1.5U
B1	1.5mM	2400	0.8mM	1.5U
B3	2.5mM	2400	0.8mM	1.5U
C9	2.5mM	2400 / Cetus	0.8mM	1.5U
C13	2.5mM	Cetus	0.8mM	1.5U
AA1	2.5mM	Cetus	0.8mM	1.5U
AA2	2mM	Cetus	0.8mM	1.5U
AA3	2mM	2400 / Cetus	0.8mM	1.5U
AA4	2.5mM	Cetus	0.8mM	1.5U

<sup>a</sup> per reaction<sup>b</sup> 2400 denotes Perkin Elmer GeneAmp System 2400, Cetus denotes Perkin Elmer Cetus**RAPD: Fingerprinting**

Genomic DNA isolated from individual mosquito larva using 24 hour SDS lysis were diluted 1:50 using nanopure water before carrying out RAPD fingerprinting using each of the nine stated primers at optimized conditions mentioned in Table 2 above. The resultant PCR products were analyzed by 2.5% (w/v) agarose gel and the gel images were captured on Polaroid film and shown in Appendix VI.

It was observed visually that RAPD fingerprinting with some primers gave better results than others in a number of aspects. The usability of the primers in fingerprinting of mosquito genomic DNA were primarily, visually evaluated based on the gel images as captured on film. The criteria for evaluation were, ability to amplify fragments of mosquito genomic DNA, resolution on agarose gel and the visibility of amplified bands.

The ability to amplify mosquito genomic DNA was scored for each of the nine primers by calculating the percentage of the samples amplified. The presence of at least a visually observable band signifies amplification whereas the absence of band would mean no amplification. Based on this, the amplification percentage of primers A2, B1, B3, C9, C13, AA1, AA2, AA3 and AA4 were 95.2%, 98.8%, 41.7%, 73.8%, 92.8%, 96.4%, 64.3%, 100% and 60.7% respectively.

Besides amplification frequency, the PCR products differed in terms of resolution and observability of bands when resolved in 2.5% (w/v) agarose gel. The PCR products yield by primer A2 were relatively visible, however, the bands appeared to be slightly fuzzy with a background smear (see Appendix VI, Figures 1 to 8), whereas the bands of PCR products amplified using primer B1, as shown in Appendix VI, Figures 9 to 16, were better resolved with less background smear. Moreover, the individual bands appeared to be brighter than those of A2. Most of the PCR products amplified using primer B3 were smears instead of bands. Even when bands were present, they were mostly overcast by the background smear (see Appendix VI, Figures 17 to 24). The gel images of primer C9 showed relatively strong background smear, which affected the resolution, and individual bands were mostly weakly visible (see Appendix VI, Figures 25 to 32). The resolution of PCR products yielded by primer C13 were quite good but the visibility of distinct bands were not extremely magnificent (see Appendix VI, Figures 33 to 40). The products of primer AA1 as shown in Appendix VI, Figures 41 to 48, individual bands were barely visible although the resolution appeared to be acceptable in a few images. As with those of primer AA2 (see Appendix VI, Figures 49 to 56), both the visibility and resolution of

distinct bands were relatively poor. Appendix VI, Figures 57 to 64 showed the agarose gel images of the PCR products by primer AA3. Most of the bands as observed from each sample in these images were rather bright and distinct. PCR products by primer AA4 did not resolve into very distinctive bands. There was also a degree of background smear and to make things worse, the bands were not very bright for visual distinction (see Appendix VI, Figures 65 to 72).

Each figure in Appendix VI was accompanied by a table illustrating the primer used to fingerprint the samples, strain type and the molecular weights of each visually distinct band of each sample in base-pairs.

The visual evaluation of each primers based on the RAPD fingerprints captured on agarose gel images were summarized in Table 3 below.

**Table 3: Summary of Visual Evaluation of Primers**

Primer	Amplification Percentage	Visibility of Bands <sup>a</sup>	Resolution of Images <sup>a</sup>
A2	95.2 %	++++	++
B1	98.8 %	+++++	++++
B3	41.7 %	+	-
C9	73.8 %	++	-- ++
C13	92.8 %	++	+++++
AA1	96.4 %	+	++
AA2	64.3 %	+	-
AA3	100 %	+++++	++++
AA4	60.7 %	++	-

<sup>a</sup> more pluses denotes better visibility or resolution.

Based on the above evaluation, five primer were short-listed to be usable for future applications of RAPD fingerprinting of mosquito genomic DNA out of the nine primers used in this study. They were primers A2, B1, C13, AA1 and AA3.

The main consideration when short-listing the primers was the amplification percentage of the primer. In another words, the proportion of the total samples that can be amplified by the primer in question. This was followed by the overall resolution of bands and lastly, visibility of bands. The amplification percentage must be kept in mind simply because it played a critical role if the primer were to be used for routine screening or any similar applications. If a primer with low amplification percentage was used routinely, it would directly increase the cost and severely compromised the results than if a primer with high amplification percentage was used. It should be noted that the amplification percentage was independent of the template DNA since primer, such as, AA3, had ensured amplification in this study. In order to maintain economy and quality of fingerprinting results, amplification percentage of 90% was used as cut-off. In another words, only primers with amplification percentage of more than 90% were acceptable.

As with regard to amplification percentage, a very strange observation was noted in the gel images of primers C9 and AA2 (see Appendix VI, Figures 25 to 32 and 49 to 56). The amplification percentages were high for the strains that were resistant to dengue virus but low for the strains that were susceptible to dengue virus. In fact, the difference was so great that it lowered the overall percentages of both primers to below 80%. It was unlikely that this difference was resultant from either the operator or the reagents as it

was not seen across the board, for example, in the case of primer B1, the amplification percentage for resistant strains was lower than that for susceptible strains. Should this be a reproducible event, meaning, not due to chance or other external factors, it could be developed into a differential assay, which would group the larvae into resistant or susceptible strains, and this was exactly the eventual aim of this study.

Both visibility of bands and resolution of images were also important factors, which would affect the final result of the fingerprinting profile. If individual bands were very weak, it might be possible to neglect the presence of some bands in preference for visually distinct bands. This could be made much worse if the resolution was poor either due to fuzziness of bands or presence of strong background smears. Even with distinct bands, the presence of strong background smears would adversely affect visual differentiation of the bands and it was very possible to miss a few weak bands due to overcast. Poor resolution of bands was also a problem faced when tabulating the results based on gel images. Resolution meant the visual ability to distinguish two separate bands as distinct. In poorly resolved images, it was very likely to mistake two close bands as one band. Therefore, it was very clear that the visibility of bands and the resolution of images could severely affect the final results in many different aspects.

However, it was possible to improve the resolution by analyzing the fingerprinting products on sequencing gels instead of agarose gel. Sequencing gel enhances resolution by two ways, firstly, due to the length of the gel, close bands could be better separated. Secondly, the porosity of sequencing gel was much more uniformed and smaller than that

of agarose gel. Another alternative was to incorporate Nusieve agarose into normal agarose gels and use it for electrophoretic analysis of RAPD fingerprinting products. The incorporation of Nusieve agarose would result in sharper bands. The main objective of using Nusieve agarose was to decrease the fuzziness of individual bands, however, the drawbacks of it was that Nusieve agarose gel were very soft even at high concentrations and Nusieve agarose was very costly.

A survey of molecular weights of individual bands in each sample profile of the five selected primers were not very promising. No individual bands could be used directly to differentiate the samples into either resistant or susceptible strains. Therefore, in order to differentiate the samples, the fingerprinting profile must be processed and analyzed. The first step of analysis involved the grouping of molecular weight of each band of each sample into 20 categories for each primer. For each primer, a visual survey was made to identify the range of molecular weights for the amplified bands. The range of molecular weight were divided into 20 intervals using logarithmic scale.

The range of molecular weights of amplified bands for each primer were as follows; A2, 303 to 2505; B1, 196 to 2004; C13, 144 to 5158; AA1, 244 to 2791; AA3, 169 to 2701. All the molecular weights were classified primer-wise into the 20 intervals. The intervals were then scored for discrete presence of bands. Therefore, these 20 intervals represented the processed fingerprint of each sample of each primer and they were used for the calculation of Similarity Index, Percent Matching and analysis using a in-house designed artificial intelligence software.

Due to sheer sample size, only the fingerprinting data of two primers, A2 and B1, were used for calculating Similarity Index and Percent Matching and the results were displayed in Appendix VII. Also due to sample size, a comprehensive dendogram could not be drawn, however, based on the numerals shown, several observations could be made. Judging by the numerical distribution in the matrices, relatedness measure using Similarity Index seemed to be better at clustering subspecies of *Aedes aegypti* than Percent Matching. Both matrices of Similarity Index seemed to reveal that there were more than one subspecies that were resistant to dengue virus, the same was also true for susceptible strains. However, despite its wide-spread use in analysis of fingerprinting data, both Similarity Index and Percent Matching had limited use in this study.

Similarity Index and Percent Matching were useful in identifying and proving the presence of subspecies. However, they were more geared towards academic studies and was impractical to use in routine screenings due to the nature of the methods.

Since RAPD fingerprinting could be considered complex data, use of information technology in its analysis seemed feasible. Manual analysis could be tedious and unrewarding especially if done routinely. A survey of literatures revealed instances of artificial intelligence being employed in analyzing fingerprinting data (see Literature Review, Artificial Intelligence in Data Analysis). There were numerous paradigms of artificial intelligence used in analysis of complex, incomplete or noisy data. These paradigms included expert systems, genetic algorithms and artificial neural networks. Expert systems required a pre-defined set of rules as basis for analysis, which was not

readily obtained in this case. In fact, expert systems were usually used to analyze incomplete data, like in the case of MYCIN. Therefore, the use of expert system in our analysis was infeasible. Between genetic algorithm and artificial neural networks, artificial neural network was preferred for two reasons. Firstly, coding procedures and logic of artificial neural networks were much easier than genetic algorithms. Secondly, the presentation procedures of artificial neural network was easier than that of genetic algorithm. Based on this short evaluation, artificial neural network was deemed to be applicable in our analysis and a small computer program, STRAIN, was the result (see Appendix IX for the source code for STRAIN and Appendix X for STRAIN's User Guide).

Processed fingerprints of all the five primers were entered into STRAIN and the first 15 profiles of each strain of each primer were used as training data. The main purpose of training was to allow the neural network to adapt to the given data. In complicated softwares, there were many parameters governing the training process, however, for the ease of use, only one parameter could be user-defined, the rest were coded into the system. This single parameter represented the most important scope in training neural networks, it was the number of times the training data was fed into the system.

An attempt to optimize the number of times the training data was fed into the system, in short, the number of cycles, for each primer was carried out. A range from 1000 cycles to 10000 cycles with an interval of 1000 cycles was used in this process. (Please refer to Appendix X, STRAIN: User's Guide for specific details on the operation of the

program.) After training, a different set of 20 profiles, comprising of 10 susceptible strains and 10 resistant strains, was used to test the accuracy of the neural network. This was done in triplicates and the best results for each primer (see Appendix VIII for detailed results of testing) were, for A2, 68% accuracy,  $s = 3\%$  at 6000 cycles; for B1, 78% accuracy,  $s = 3\%$  at 6000 cycles; for C13, 98% accuracy,  $s = 3\%$  at 3000 cycles; for AA1, 77% accuracy,  $s = 3\%$  at 6000 cycles; for AA3, 52% accuracy,  $s = 3\%$  at 10000 cycles.

It was observed that primer C13 gave promising results whereas primer AA3 did not appear to be very useful in differentiating strains in our testing. In fact, due to the nature of neural networks, there was no reason as to why high levels of accuracy could not be achieved, however, in our case, the reasons could be plentiful. Firstly, the optimal number of cycles to train the current neural network might not lie within our range of cycles. This might just be the reason why primer AA3 did not perform up to expectations. A scan across the profiles obtained using primer AA3 would show that the distinction between the two was even fuzzier than that of primer C13. Therefore, even at 10000 cycles, the network might not able to accustom itself to a level, which would render it useful. It was very possible that if the training for primer AA3 were to extend to higher number of cycles like 50000 cycles, the results would have been much better. However, it was important to realize that the process of training was very time consuming.

Secondly, the inefficiency of the primer could be due to the nature of the network. This could explain why primers, such as, A2 and AA1, did not perform as well as primer C13.

It was mentioned before that a large number of parameters were coded into the system instead of being user-defined. This, in itself, was an inherent barrier to better performance. A number of more important factors that were fixed were number of hidden nodes, number of hidden layers and the learning rate. In STRAIN, the number of hidden nodes was fixed at five and the number of hidden layers was one. The learning rate in our case was re-defined into absolute numerals. A combination of these three factors would somewhat define the complexity of the neural network to a certain degree. In the analysis of very complicated data, such as shares analysis and defense applications, the number of hidden nodes required might be as large as a few hundred and there might be five to ten hidden layers with varied numbers of hidden nodes per layer. With the use of a more advanced neural network, it would not be surprising that better accuracy could be achieved.

However, despite all, we were able to conclude two things from the above results. Firstly, in this study, primer C13 could accurately differentiate between strains that were resistant to dengue virus and strains that were susceptible to dengue virus. Most importantly, it was also concluded that the ability of individual mosquito of *Aedes aegypti* to act as vector for dengue virus could be identified using RAPD fingerprinting of its genomic DNA.

## CONCLUSION

Four established protocols were investigated for their efficiency of isolating genomic DNA from individual mosquito larva and 24 hour SDS lysis had emerged to be both economical and efficient. Nine primers were evaluated for their potential application in strain typing of *Aedes aegypti*, however, only five of the nine, A2, B1, C13, AA1 and AA3, were found satisfactory. Two sets of fingerprinting profiles were analyzed using Similarity Index and Percent Matching, and all five sets were analyzed using an in-house designed computer software employing simple artificial intelligence. Out of these three methods, in terms of strain typing, the in-house software gave highly satisfactory results. Using the profile obtained by primer C13, it had correctly classified the test samples with 98% accuracy and a standard deviation of 3%. In short, the ability of individual mosquito of *Aedes aegypti* to function as a vector for Dengue virus could be identified using RAPD fingerprinting of its genomic DNA.

**REFERENCES**

Apostol, B.L., Black IV, W.C., Miller, B.R., Reiter, P., Beaty, B.J. 1993. **Estimation of the number of full sibling families at an oviposition site using RAPD-PCR markers: applications to the mosquito *Aedes aegypti*.** *Theor. Appl. Genet.* 86:991 - 1000.

Ballinger-Crabtree, Mary E., Black IV, William C., Miller, Barry R. 1992. **Use of Genetic Polymorphisms Detected by the Random-Amplified Polymorphism DNA Polymerase Chain Reaction (RAPD-PCR) for Differentiation and Identification of *Aedes aegypti* Subspecies and Populations.** *Am. J. Trop. Med. Hyg.* 47(6):893 - 901.

Carson, C. Andrew, Keller, James M., McAdoo, Kelly K., Wang Dayou, Higgins, Barbara, Bailey, Craig W., Thorne, James G., Hahn, Allen W. 1995. ***Escherichia coli* O157:H7 Restriction Pattern Recognition by Artificial Neural Network.** *J. Clin. Microbiol.* 33(11):2894 – 2898.

Chomczynski P, Mackey K, Drews R, Wilfinger W. 1997. **DNAzol: a reagent for the rapid isolation of genomic DNA.** *Biotechniques* 22(3):550 - 3.

Corley, J., Rabinovich, M., Seigelchifer, M., Zorzopoulos, J., Corley, E. 1993. **Sperm Utilization in Honeybees as Detected by M13 DNA Fingerprints.** In *DNA Fingerprinting: State of the Science*. Pena, S.D.J., Chakraborty, R., Epplen, J.T., Jeffrey, A.J. (ed), pp. 355 - 362.

- Dandekar, Thomas, Argos, Patrick. 1997. **Applying Experimental Data to Protein Fold Prediction with the Genetic Algorithm.** *Protein Engineering* 10(8):877 – 893.
- Durkin, John. 1994. **MYCIN.** In *Expert System: Design and Development*. Prentice Hall International, Inc., United States of America.
- Goh, K.T. 1998. **Dengue - A Re-emerging Infectious Disease in Singapore.** In *Dengue in Singapore*. Goh, K.T. (ed.) Institute of Environmental Epidemiology, Ministry of Environment, Singapore.
- Goodacre, Rpyston, Timmins, Eadaoin M., Burton, Rebecca, Kaderbhai, Naheed, Woodward, Andrew M., Kell, Douglas B., Rooney, Paul J. 1998. **Rapid Identification of Urinary Tract Infection Bacteria Using Hyperspectral Whole-Organism Fingerprinting and Artificial Neural Networks.** *Microbiology* 144:1157 – 1170.
- Herber, Birgit, Herold, Kurt. 1997. **DNA Typing of Human Dandruff.** *J. Forensic Sci.* 43(3):648 - 656,
- Karp, Gerald. 1999. **Techniques in Cell and Molecular Biology.** In *Cell and Molecular Biology, 2nd edition*. John Wiley & Sons, Inc., Canada.

Micheli, M.R., Bova, Rodolfo. 1996. **Fingerprinting Methods Based on Arbitrarily Primer PCR.** Springer-Verlag, Berlin. pp. 3 – 9.

Mutebi, J.P., Black IV, W.C., Bosio, C.F., Sweeney Jr, W.P., Craig Jr, G.B. 1997. **Linkage Map for the Asian Tiger Mosquito [*Aedes (Stegomyia) albopictus*] Based on SSCP Analysis of RAPD Markers.** *J. Hered.* 88:489 - 494.

Qian Ning, Sejnowski, Terrence J. 1988. **Predicting the Secondary Structure of Globular Proteins Using Neural Network Models.** *J. Mol. Biol.* 202:865 – 884.

Reiter, P. 1998. **Dengue Control in Singapore.** In *Dengue in Singapore.* Goh, K.T. (ed.) Institute of Environmental Epidemiology, Ministry of Environment, Singapore.

Severson, D.W., Mori, A., Zhang, Y., Christensen, B.M. 1993. **Linkage Map for *Aedes aegypti* Using Restriction Fragment Length Polymorphisms.** *J. Hered.* 84:241 - 247.

Shortliffe, E.H. 1976. **Computer-Based Medical Consultation, MYCIN.** American Elsevier, United States of America.

Tan, B.T., Lawther, J.F., Lam-Phua, S.G., Lee, K.M. 1998. **Research in *Aedes* Control.** In *Dengue in Singapore.* Goh, K.T. (ed.) Institute of Environmental Epidemiology, Ministry of Environment, Singapore.

Verma M. 1998. **High molecular weight DNA isolation by guanidine hydrochloride or guanidinium isothiocyanate treatment.** *Biotechniques* 6(9):848, 850, 853.

Walsh, P. Sean, Metzger, David A., Higuchi, Russell. 1991. **Chelex ® 100 as a Medium for Simple Extraction of DNA for PCR-Based Typing from Forensic Material.** *BioTechniques* 10(4):506 - 513.

**APPENDIX I:****SPECTROMETRICAL ANALYSIS OF PRIMERS**

All the lysophilized primers were hydrated and analyzed spectrophotometrically in accordance to the procedure stated in the section, Materials and Methods, under the heading, Primer Preparation. All absorbance readings obtained were tabulated as follows:

Primer	Wavelength in nanometers										
	200	210	220	230	240	250	260	270	280	290	300
A2	0.324	0.182	0.104	0.078	0.051	0.090	0.087	0.074	0.051	0.018	-0.003
B1	-0.001	-0.006	0.008	0.013	0.014	0.022	0.030	0.036	0.027	0.016	0.006
B3	0.113	0.043	0.060	0.057	0.045	0.044	0.058	0.067	0.055	0.027	0.007
C13	0.395	0.260	0.141	0.084	0.072	0.078	0.087	0.085	0.064	0.037	0.012
C9	0.275	0.178	0.093	0.047	0.031	0.031	0.035	0.040	0.031	0.016	0.004
AA1	0.268	0.179	0.088	0.045	0.039	0.045	0.048	0.047	0.035	0.020	0.008
AA2	0.265	0.176	0.086	0.044	0.038	0.045	0.049	0.048	0.035	0.018	0.005
AA3	0.334	0.211	0.109	0.064	0.056	0.060	0.065	0.061	0.041	0.016	0.003
AA4	0.400	0.264	0.145	0.089	0.077	0.083	0.093	0.092	0.071	0.037	-0.015

**APPENDIX II:****SPECTROPHOTOMETRICAL ANALYSIS OF EXTRACTION METHODS**

5 mosquito larvae were used for each extraction.

Sample	OD <sub>260</sub>	OD <sub>280</sub>	OD <sub>260</sub> /OD <sub>280</sub>	Concentration of DNA (μg/μl)	Total Amount of DNA Extracted (μg)
T1A	0.075	0.047	1.6	0.75	37.50
T1B	0.037	0.021	1.8	0.37	18.50
T1C	0.062	0.037	1.7	0.62	31.00
T1D	0.068	0.042	1.6	0.68	34.00
T2A	0.058	0.035	1.7	0.58	29.00
T2B	0.044	0.026	1.7	0.44	22.00
T2C	0.083	0.049	1.7	0.83	41.50
T2D	0.056	0.031	1.8	0.56	28.00
T3A	0.039	0.026	1.5	0.39	19.50
T3B	0.094	0.056	1.7	0.94	47.00
T3C	0.051	0.035	1.5	0.51	25.50
T3D	0.030	0.015	2.0	0.30	15.00
T4A	0.065	0.040	1.6	0.65	32.50
T4B	0.029	0.017	1.7	0.29	14.50
T4C	0.063	0.037	1.7	0.63	31.50
T4D	0.053	0.035	1.5	0.53	26.50
C1	0.047	0.047	1.0	0.47	23.50
C2	0.071	0.072	1.0	0.71	35.50
C3	0.042	0.045	0.9	0.42	21.00
C4	0.052	0.052	1.0	0.52	26.00

**APPENDIX III:****SPECTROPHOTOMETRICAL ANALYSIS OF SINGLE EXTRactions**

Sample	OD <sub>260</sub>	OD <sub>280</sub>	OD <sub>260</sub> /OD <sub>280</sub>	Concentration of DNA (μg/μl)	Total Amount of DNA Extracted (μg)
R1	0.101	0.067	1.5	1.01	20.20
R2	0.068	0.041	1.7	0.68	13.60
R3	0.053	0.029	1.8	0.53	10.60
R4	0.037	0.019	1.9	0.37	7.40
R5	0.033	0.022	1.5	0.33	6.60
R7	0.037	0.021	1.8	0.37	7.40
R15	0.041	0.026	1.6	0.41	8.20
R16	0.042	0.021	2.0	0.42	8.40
R17	0.032	0.018	1.8	0.32	6.40
R18	0.066	0.040	1.7	0.66	13.20
R20	0.047	0.026	1.8	0.47	9.40
R22	0.022	0.015	1.5	0.22	4.40
R23	0.048	0.032	1.5	0.48	9.60
R25	0.038	0.022	1.7	0.38	7.60
R27	0.037	0.021	1.8	0.37	7.40
R28	0.051	0.026	2.0	0.51	10.20
R50	0.004	0.002	2.0	0.04	0.80
R53	0.589	0.404	1.5	5.89	117.80
R55	0.046	0.022	2.1	0.46	9.20
R56	0.070	0.041	1.7	0.70	14.00
R57	0.108	0.072	1.5	1.08	21.60
R62	0.114	0.075	1.5	1.14	22.80
R63	0.083	0.056	1.5	0.83	16.60
R65	0.070	0.047	1.5	0.70	14.00
R66	0.032	0.019	1.7	0.32	6.40
R71	0.039	0.023	1.7	0.39	7.80
R74	0.100	0.068	1.5	1.00	20.00
R76	0.095	0.065	1.5	0.95	19.00
R77	0.050	0.025	2.0	0.50	10.00
R78	0.151	0.104	1.5	1.51	30.20
R79	0.054	0.035	1.5	0.54	10.80
R83	0.063	0.037	1.7	0.63	12.60
R84	0.077	0.051	1.5	0.77	15.40
R85	0.073	0.048	1.5	0.73	14.60
R91	0.033	0.018	1.8	0.33	6.60
R93	0.073	0.050	1.5	0.73	14.60
R94	0.106	0.071	1.5	1.06	21.20
R95	0.077	0.051	1.5	0.77	15.40
R98	0.073	0.047	1.6	0.73	14.60
R99	0.049	0.024	2.0	0.49	9.80

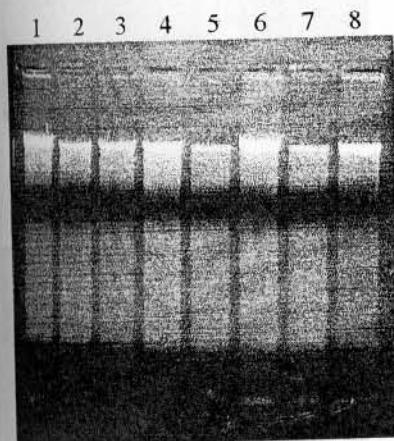
Appendix III: Spectrophotometrical Analysis of Single Extractions

---

R103	0.036	0.020	1.8	0.36	7.20
R105	0.018	0.010	1.8	0.18	3.60
S1	0.117	0.082	1.4	1.17	23.40
S2	0.085	0.058	1.5	0.85	17.00
S5	0.111	0.079	1.4	1.11	22.20
S6	0.080	0.055	1.5	0.80	16.00
S7	0.116	0.082	1.4	1.16	23.20
S8	0.107	0.073	1.5	1.07	21.40
S9	0.134	0.099	1.4	1.34	26.80
S10	0.137	0.099	1.4	1.37	27.40
S11	0.136	0.099	1.4	1.36	27.20
S12	0.065	0.041	1.6	0.65	13.00
S13	0.090	0.063	1.4	0.90	18.00
S14	0.114	0.081	1.4	1.14	22.80
S15	0.083	0.057	1.5	0.83	16.60
S16	0.095	0.067	1.4	0.95	19.00
S17	0.098	0.065	1.5	0.98	19.60
S20	0.056	0.036	1.6	0.56	11.20
S21	0.139	0.098	1.4	1.39	27.80
S23	0.115	0.084	1.4	1.15	23.00
S24	0.107	0.072	1.5	1.07	21.40
S25	0.139	0.097	1.4	1.39	27.80
S27	0.092	0.062	1.5	0.92	18.40
S28	0.148	0.105	1.4	1.48	29.60
S29	0.225	0.163	1.4	2.25	45.00
S30	0.086	0.050	1.7	0.86	17.20
S31	0.120	0.084	1.4	1.20	24.00
S32	0.149	0.105	1.4	1.49	29.80
S33	0.133	0.093	1.4	1.33	26.60
S34	0.163	0.119	1.4	1.63	32.60
S35	0.140	0.098	1.4	1.40	28.00
S36	0.137	0.096	1.4	1.37	27.40
S37	0.044	0.032	1.4	0.44	8.80
S38	0.032	0.021	1.5	0.32	6.40
S39	0.021	0.010	2.1	0.21	4.20
S40	0.025	0.014	1.7	0.25	5.00
S44	0.017	0.009	2.0	0.17	3.40
S45	0.036	0.024	1.5	0.36	7.20
S48	0.021	0.010	2.2	0.21	4.20
S49	0.032	0.023	1.4	0.32	6.40
S50	0.036	0.025	1.4	0.36	7.20
S51	0.019	0.011	1.7	0.19	3.80
S52	0.039	0.024	1.6	0.39	7.80
S54	0.021	0.009	2.3	0.21	4.20

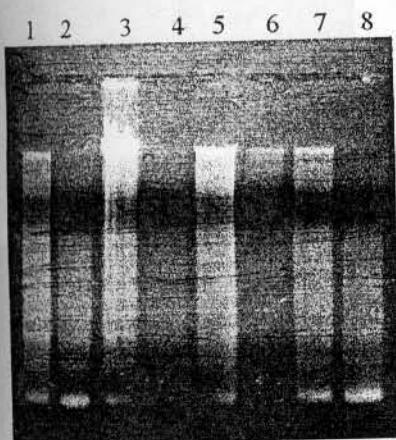
## APPENDIX IV:

### AGAROSE GEL PHOTOGRAPHS OF EXTRACTION METHODS



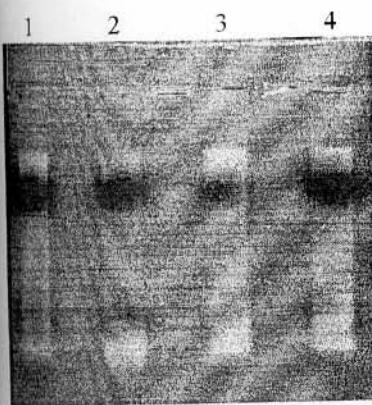
**Figure 1: Agarose Gel Analysis of DNA Isolated Using SDS Lysis Method, Part 1.**

Lane 1 to 4: Samples were incubated for 24 hours.  
Lane 5 to 8: Samples were incubated for 20 hours.



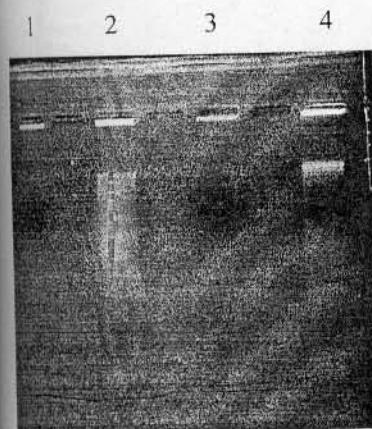
**Figure 2: Agarose Gel Analysis of DNA Isolated Using SDS Lysis Method, Part 2.**

Lane 1 to 4: Samples were incubated for 17 hours.  
Lane 5 to 8: Samples were incubated for 15 hours.



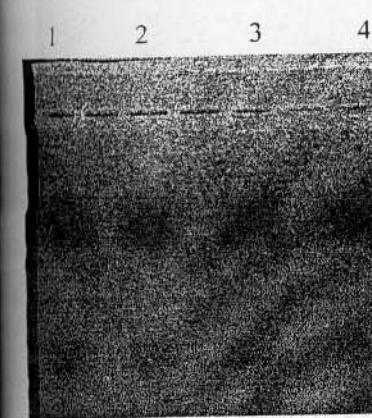
**Figure 3: Agarose Gel Analysis of DNA Isolated Using QIAamp Tissue Kit.**

Lane 1 to 4: Four repeats of isolation.



**Figure 4: Agarose Gel Analysis of DNA Isolated Using DNAZOL Solution.**

Lane 1 to 4: Four repeats of isolation.

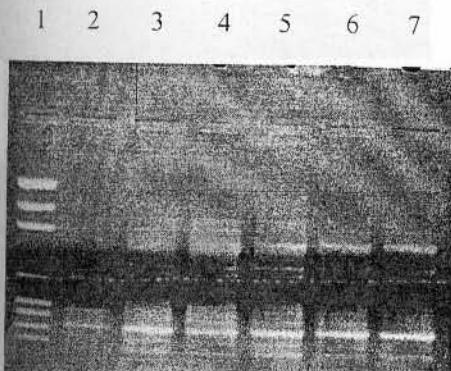


**Figure 5: Agarose Gel Analysis of DNA Isolated Using Chelex® 100 resin.**

Lane 1 to 4: Four repeats of isolation.

## APPENDIX V:

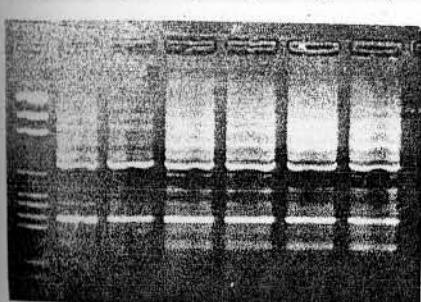
### AGAROSE GEL PHOTOGRAPHS OF PCR OPTIMIZATION



**Figure 1: Optimization of Concentration of dNTPs.**

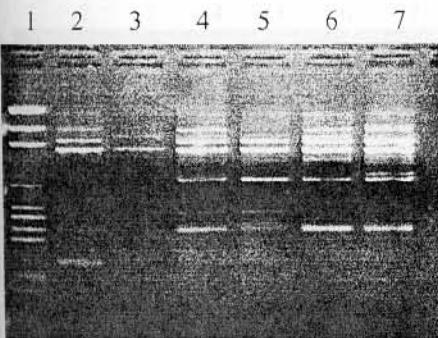


**Figure 2: Optimization of Concentration of AmpliTaq® Polymerase.**



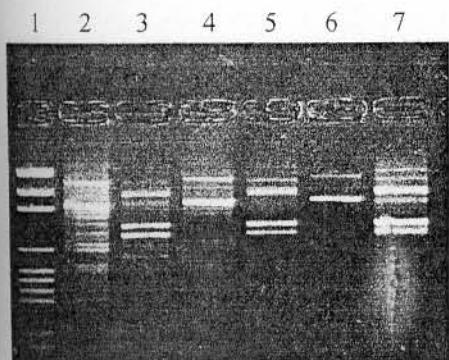
**Figure 3: Magnesium Ions Titration for Primer B1.**

Lane 1: pGEM-digested marker.  
Lane 2 and 3: 1.5mM magnesium ions.  
Lane 4 and 5: 2.0mM magnesium ions.  
Lane 6 and 7: 2.5mM magnesium ions.



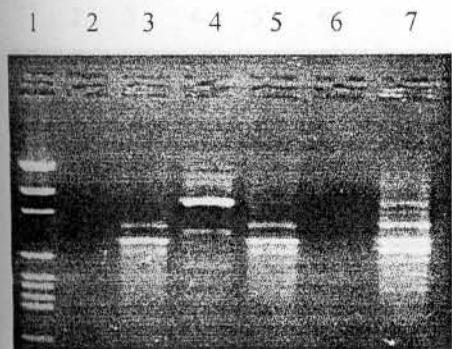
**Figure 4: Magnesium Ions Titration for Primer B3.**

Lane 1: pGEM-digested marker.  
Lane 2 and 3: 1.5mM magnesium ions.  
Lane 4 and 5: 2.0mM magnesium ions  
Lane 6 and 7: 2.5mM magnesium ions



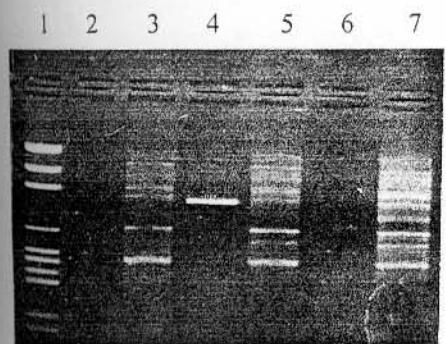
**Figure 5: Magnesium Ions Titration for Primer C9.**

Lane 1: pGEM-digested marker. Lane 2 and 7:  
2.5mM magnesium ions using 2400 and Cetus  
respectively. Lane 3 and 6: 1.5mM magnesium ions  
using Cetus and 2400 respectively. Lane 4 and 5:  
2.0mM using 2400 and Cetus respectively.



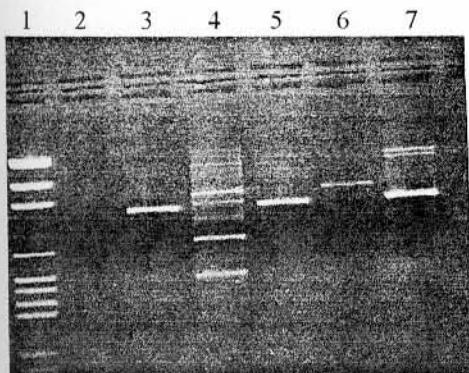
**Figure 6: Magnesium Ions Titration for Primer C13.**

Lane 1: pGEM-digested marker. Lane 2 and 3:  
1.5mM magnesium ions using 2400 and Cetus  
respectively. Lane 4 and 5: 2.0mM magnesium  
ions using 2400 and Cetus respectively. Lane 6 and  
7: 2.5mM magnesium ions using 2400 and Cetus  
respectively.



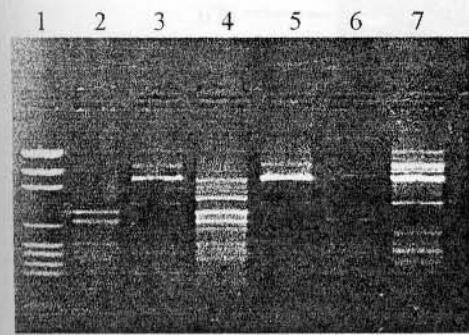
**Figure 7: Magnesium Ions Titration for Primer AA1.**

Lane 1: pGEM-digested marker. Lane 2 and 3:  
1.5mM magnesium ions using 2400 and Cetus  
respectively. Lane 4 and 5: 2.0mM magnesium ions  
using 2400 and Cetus respectively. Lane 6 and 7:  
2.5mM magnesium ions using 2400 and Cetus  
respectively.



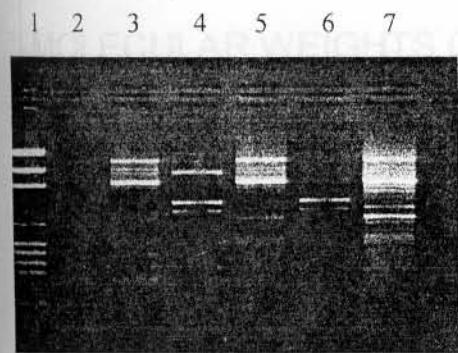
**Figure 8: Magnesium Ions Titration for Primer AA2.**

Lane 1: pGEM-digested marker. Lane 2 and 3: 1.5mM magnesium ions using 2400 and Cetus respectively. Lane 4 and 5: 2.0mM magnesium ions using 2400 and Cetus respectively. Lane 6 and 7: 2.5mM magnesium ions using 2400 and Cetus respectively.



**Figure 9: Magnesium Ions Titration for Primer AA3.**

Lane 1: pGEM-digested marker. Lane 2 and 3: 1.5mM magnesium ions using 2400 and Cetus respectively. Lane 4 and 5: 2.0mM magnesium ions using 2400 and Cetus respectively. Lane 6 and 7: 2.5mM magnesium ions using 2400 and Cetus respectively.



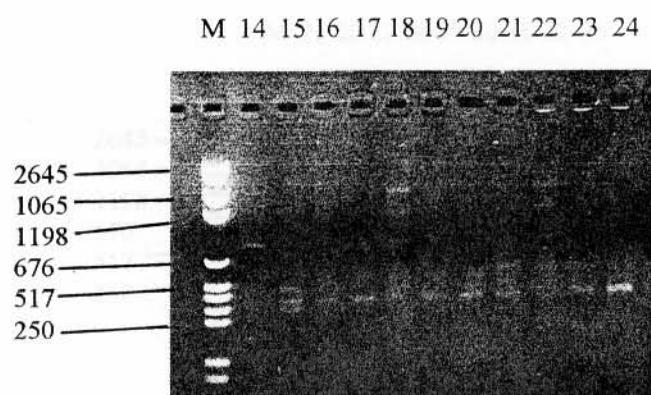
**Figure 10: Magnesium Ions Titration for Primer AA4.**

Lane 1: pGEM-digested marker. Lane 2 and 3: 1.5mM magnesium ions using 2400 and Cetus respectively. Lane 4 and 5: 2.0mM magnesium ions using 2400 and Cetus respectively. Lane 6 and 7: 2.5mM magnesium ions using 2400 and Cetus respectively.

Note: Cetus denotes Perkin Elmer Cetus and 2400 denotes Perkin Elmer GeneAmp PCR System 2400.

**APPENDIX VI:****RAPD: AGAROSE GEL PHOTOGRAPHS AND TABULATIONS****Figure 1: RAPD-DNA Fingerprinting Using Primer A2 (Part 1 of 8)**

PRIMER NAME: A2												
STRAIN TYPE: RESISTANT												
LANE M: pGEM-digested marker												
MOLECULAR WEIGHTS OF BANDS												
1	2	3	4	5	6	7	8	9	10	11	12	13
1923	2276	2034	1298	1923	2276	1923	1536	1923	2034	1923	1452	
1719	783	1373	1160	1536	2034	1298	1227	1719	1923	1298	927	
1452	559	1097	927	1160	1097	1037	1097	1452	1818	927	599	
1298	473	953	500	1037	953	927	927	1335	1625	599	399	
927	399	829	399	559	559	599	626	1097	1452	399		
741		559		529	399	399	559	981	1335			
644		399		399			399	741	1067			
559								399	953			
500									829			
399									741			
									662			
									399			

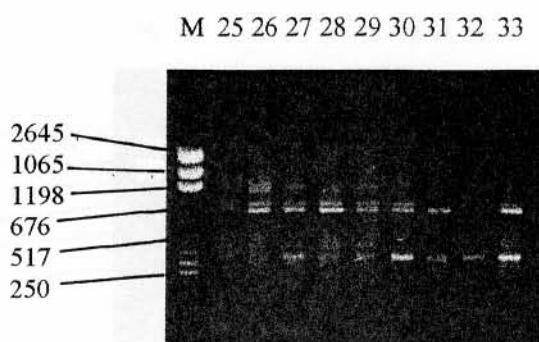


**Figure 2: RAPD-DNA Fingerprinting Using Primer A2 (Part 2 of 8)**

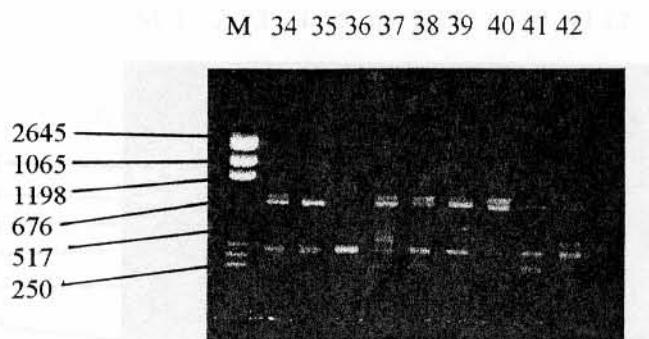
PRIMER NAME: A2
STRAIN TYPE: RESISTANT
LANE M: pGEM-digested marker
MOLECULAR WEIGHTS OF BANDS
14    15    16    17    18    19    20    21    22    23    24
1871    1215    856    856    1726    856    856    856    1680    856    953
979    979    460    513    1248    381    381    587    1354    381    879
879    587    381    381    856            473    879            381
448            381                 381    381                 381
381                                 381                         381
342                                 342                         342

#### MOLECULAR WEIGHTS OF BANDS

14	15	16	17	18	19	20	21	22	23	24
1871	1215	856	856	1726	856	856	856	1680	856	953
979	979	460	513	1248	381	381	587	1354	381	879
879	587	381	381	856			473	879		381
	448			381			381	381		
	381									
	342									

**Figure 3: RAPD-DNA Fingerprinting Using Primer A2 (Part 3 of 8)**

PRIMER NAME: A2								
STRAIN TYPE: RESISTANT								
LANE M: pGEM-digested marker								
MOLECULAR WEIGHTS OF BANDS								
<b>25</b>	<b>26</b>	<b>27</b>	<b>28</b>	<b>29</b>	<b>30</b>	<b>31</b>	<b>32</b>	<b>33</b>
1783	2505	1908	908	1908	1908	2042	1035	1454
1358	1908	1665	1369	1665	1454	1454	967	1035
1185	1556	1464	1035	1358	1269	1269	688	967
1035	1454	1269	903	1035	1035	967	561	688
934	1313	1035	816	903	903	903	490	601
788	1035	934	643	816	737	428	399	524
643	903	601	561	737	524			399
524	561	524	490	643	399			
399	490	399	413	524				
304	399	284		458				
				399				



**Figure 4: RAPD-DNA Fingerprinting Using Primer A2 (Part 4 of 8)**

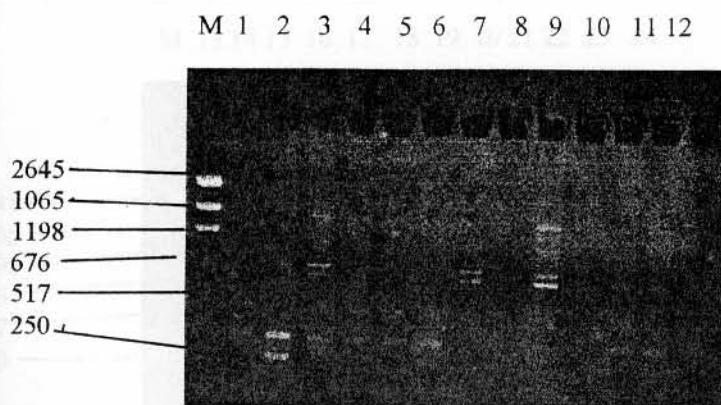
**PRIMER NAME: A2**

**STRAIN TYPE: RESISTANT**

**LANE M: pGEM-digested marker**

**MOLECULAR WEIGHTS OF BANDS**

34	35	36	37	38	39	40	41	42
1827	1827	1097	1714	965	2075	1827	906	797
1328	1328	965	1246	850	1947	1509	658	618
1029	1029	618	1029	544	1714	1328	561	479
906	906	510	906	479	618	1029	421	395
618	544	449	748	395	510	906	306	306
544	395	395	618	306	395	544		223
395						479		
						421		



**Figure 5: RAPD-DNA Fingerprinting Using Primer A2 (Part 5 of 8)**

PRIMER NAME: A2											
STRAIN TYPE: SUSCEPTIBLE											
LANE M: pGEM-digested marker											
MOLECULAR WEIGHTS OF BANDS											
1	2	3	4	5	6	7	8	9	10	11	12
919	1097	1636	1636	1636	919	1636		1565		1636	1636
841	919	919	919	1147	841	1147		1432		1565	1565
564	841	841	841	919	395	919		1199		841	841
395	539	564	564	841	303	804		1147		395	589
303	395	395	395	564				919		303	395
	303	331	331	395				804			
				303							

M 13 14 15 16 17 18 19 20 21 22 23 24

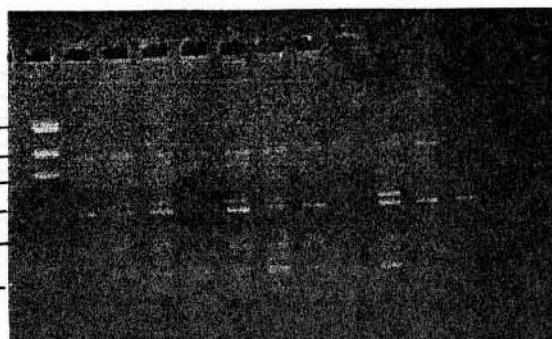


Figure 6: **RAPD-DNA Fingerprinting Using Primer A2 (Part 6 of 8)**

**PRIMER NAME:** A2

**STRAIN TYPE: SUSCEPTIBLE**

**LANE M: pGEM-digested marker**

#### MOLECULAR WEIGHTS OF BANDS

13	14	15	16	17	18	19	20	21	22	23	24
1587	1587	1814	1587	1587	1587	1587		1587	1660	1660	1660
1162	1215	1587	930	930	1452	1452		1215	1215	851	1328
930	930	1389	851	814	1328	1389		1063	973	652	1112
851	814	930	456	545	1215	1271		930	851	596	930
545	814		382	499	930	930		851	399	522	814
382	545				814	851		652		399	681
	382				652	545		477		365	
					545	382		399			
					456	349		334			
					382	306					
					292						

Appendix VI: RAPD: Agarose Gel Photographs and Tabulations

M 25 26 27 28 29 30 31 32 33

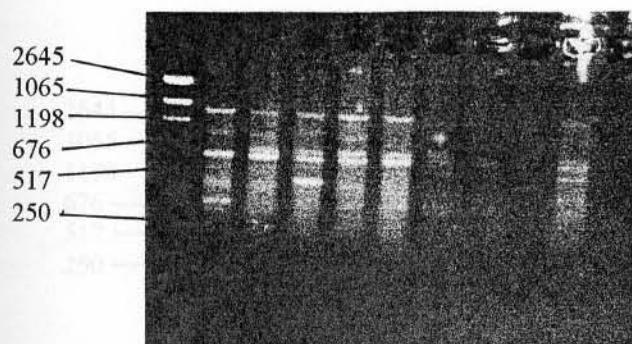


Figure 7: **RAPD-DNA Fingerprinting Using Primer A2 (Part 7 of 8)**

PRIMER NAME: A2

STRAIN TYPE: SUSCEPTIBLE

LANE M: pGEM-digested marker

MOLECULAR WEIGHTS OF BANDS

25	26	27	28	29	30	31	32	33
1601	1512	1512	1512	1512	955	955	759	1696
1071	1071	1134	1201	851	851	851	603	1512
759	851	902	851	759	716	381	5349	902
508	759	759	759	508	381			851
381	508	569	508	403				716
340			381				603	
							508	
							403	

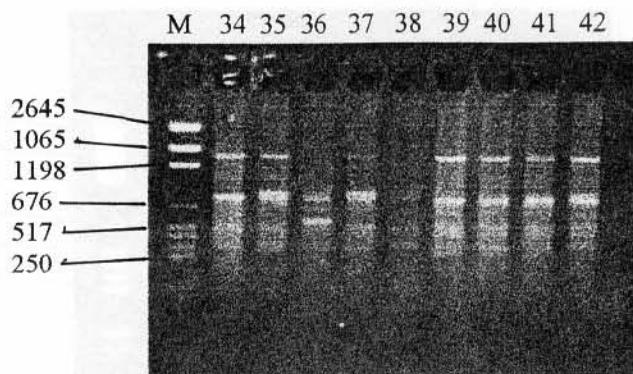


Figure 8: **RAPD-DNA Fingerprinting Using Primer A2 (Part 8 of 8)**

PRIMER NAME: A2

STRAIN TYPE: SUSCEPTIBLE

LANE M: pGEM-digested marker

MOLECULAR WEIGHTS OF BANDS

34	35	36	37	38	39	40	41	42
1488	1488	880	1578	880	1488	1488	1488	1488
1049	1049	830	830	783	1112	1112	1112	1112
783	830	657	521	491	783	783	783	783
491	739	585	367	389	697	697	697	697
367	463				491	491	491	491
					367	367	367	367

Appendix VI: RAPD: Agarose Gel Photographs and Tabulations

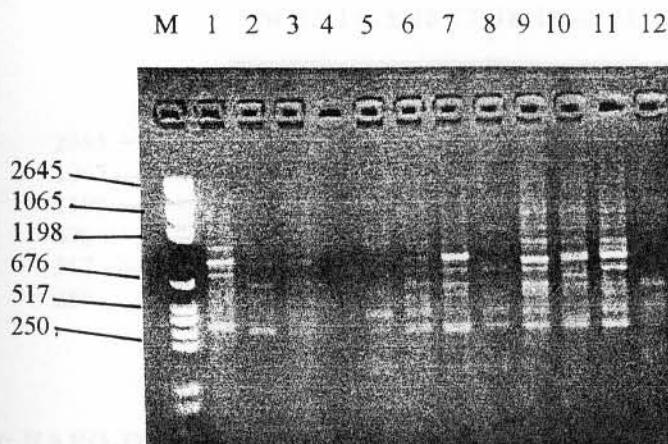


Figure 9: RAPD-DNA Fingerprinting Using Primer B1 (Part 1 of 8)

PRIMER NAME: B1											
STRAIN TYPE: RESISTANT											
LANE M: pGEM-digested marker											
MOLECULAR WEIGHTS OF BANDS											
1	2	3	4	5	6	7	8	9	10	11	12
1616	1050	2873		1866	1958	954	1334	2261	1696	1541	1866
1272	867	1779		1541	909	826	826	1958	1400	1156	1400
1102	716	1616		1400	788	682	620	1541	1156	1050	1050
954	591	1272		1156	650	620	650	1272	954	954	650
788	465	1050		954	537	591	488	1102	826	788	537
650	403	9090		788	433	488	403	867	751	650	274
591		716		591	366	403	226	788	620	591	
403		537		465	238	226		650	488	488	
		488		226				563	403	403	
		422						465			
		384						384			

Appendix VI: RAPD: Agarose Gel Photographs and Tabulations

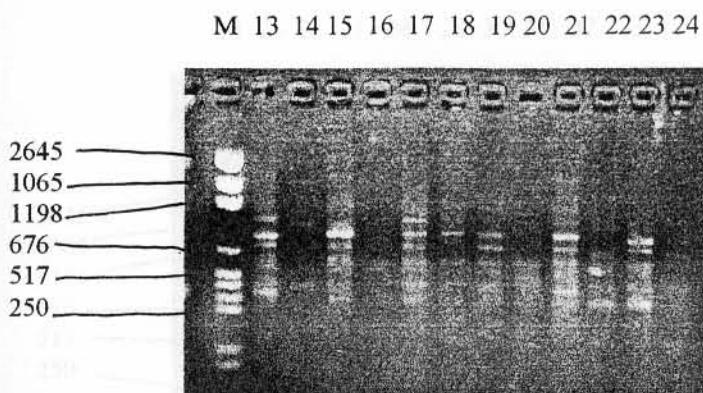


Figure 10: **RAPD-DNA Fingerprinting Using Primer B1 (Part 2 of 8)**

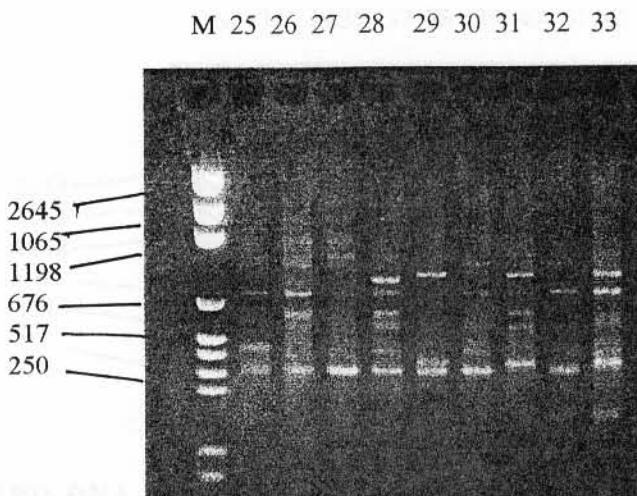
PRIMER NAME: B1

STRAIN TYPE: RESISTANT

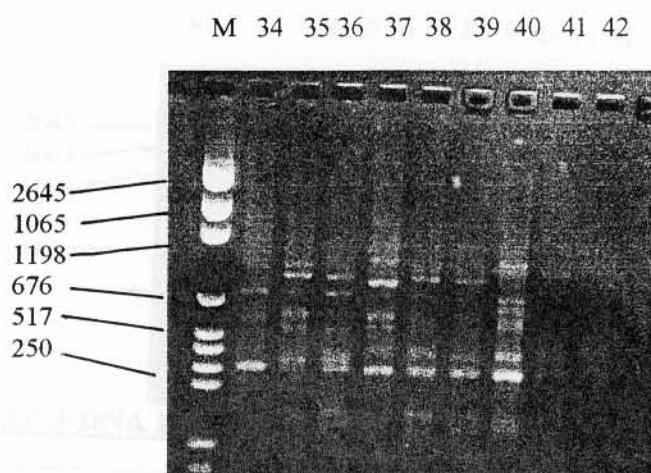
LANE M: pGEM-digested marker

**MOLECULAR WEIGHTS OF BANDS**

13	14	15	16	17	18	19	20	21	22	23	24
1855	1765	1855	1521	1129	1247	1247	1521	1310	1521	838	1377
1447	1022	1377	1310	926	1022	926	1377	1247	1247	759	1129
1187	564	1247	564	759	881	759	1247	1074	1129	536	838
1022	440	838		654	622	622	654	926	973	440	687
838		759		564	440	564	564	759	838	361	462
722		622		462	379	462	398	622	722		379
440		564		379	231	379	255	564	592		231
379		418				255		462	462		
		379						379	379		



**Figure 11: RAPD-DNA Fingerprinting Using Primer B1 (Part 3 of 8)**

Figure 12: **RAPD-DNA Fingerprinting Using Primer B1 (Part 4 of 8)**

<b>PRIMER NAME:</b> B1									
<b>STRAIN TYPE: RESISTANT</b>									
<b>LANE M: pGEM-digested marker</b>									
<b>MOLECULAR WEIGHTS OF BANDS</b>									
<b>34</b>	<b>35</b>	<b>36</b>	<b>37</b>	<b>38</b>	<b>39</b>	<b>40</b>	<b>41</b>	<b>42</b>	
1898	1644	1644	1704	927	1234	1149	1069	648	
1530	1477	1149	1425	803	1069	1069	504	603	
1149	1279	996	1191	648	927	803	392	486	
996	1149	803	960	582	803	721	264	392	
696	1032	696	863	469	648	648	237		
603	927	561	696	392	561	603			
522	803	522	625	255	452	452			
421	648	469	504		378	378			
378	561	452	421			221			
421		392							
		255							
		237							

Appendix VI: RAPD: Agarose Gel Photographs and Tabulations



**Figure 13: RAPD-DNA Fingerprinting Using Primer B1 (Part 5 of 8)**

PRIMER NAME: B1

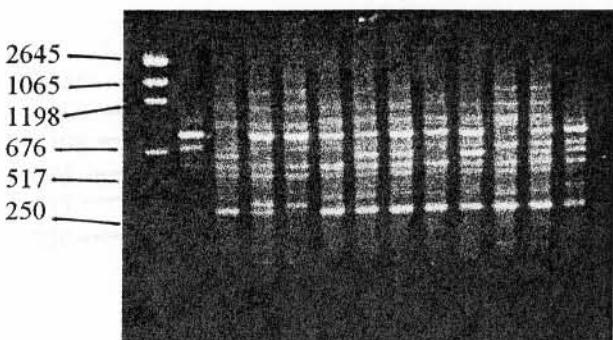
STRAIN TYPE: SUSCEPTIBLE

LANE M: pGEM-digested marker

**MOLECULAR WEIGHTS OF BANDS**

1	2	3	4	5	6	7	8	9	10	11	12
1061	890	890	1510	1061	1016	1445	1445	1445	1510	1383	1016
762	683	683	1211	890	746	1159	1211	1211	1323	1266	890
612	625	625	1061	714	598	1061	890	1016	1016	1061	779
548	385	368	930	368	548	890	683	890	890	930	625
368	368		890		368	746	612	683	797	815	572
199	199		746			612	368	625	625	653	439
			625			572		572	572	598	368
			572			368		368	368	420	
			368								

M 13 14 15 16 17 18 19 20 21 22 23 24

Figure 14: **RAPD-DNA Fingerprinting Using Primer B1 (Part 6 of 8)**

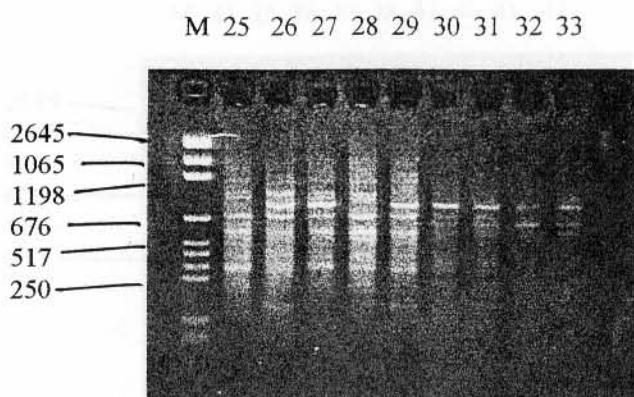
PRIMER NAME: B1

STRAIN TYPE: SUSCEPTIBLE

LANE M: pGEM-digested marker

## MOLECULAR WEIGHTS OF BANDS

13	14	15	16	17	18	19	20	21	22	23	24
1297	1699	1484	1777	1859	1484	1484	1859	1484	1859	1484	990
1083	1484	1297	1484	1484	1297	1297	1484	1297	1484	1297	905
905	1297	1036	1297	1297	1083	1083	1297	1083	1297	1083	756
756	990	905	1036	1083	905	990	1083	1013	1083	990	691
603	773	631	905	990	691	905	905	905	1013	905	631
368	691	577	756	905	631	791	756	756	905	773	577
	631	421	631	631	577	691	631	691	756	691	421
	577	368	577	577	440	631	577	631	631	631	368
	440	196	461	421	368	577	440	577	577	577	196
	368		402	368	196	440	368	440	440	440	
	196		196	235		368	235	368	368	368	
					196		235	235	235	235	
						196					

Figure 15: RAPD-DNA Fingerprinting Using Primer B1 (Part 7 of 8)

PRIMER NAME: B1									
STRAIN TYPE: SUSCEPTIBLE									
LANE M: pGEM-digested marker									
MOLECULAR WEIGHTS OF BANDS									
25	26	27	28	29	30	31	32	33	
1035	1093	1288	1093	1603	878	878	878	928	
980	928	1093	1007	1398	787	787	787	878	
787	787	1007	787	1288	706	706	706	706	
599	632	878	632	1093	632	632	632	632	
567	567	787	567	980	356				
356	356	632	431	878					
	212	599	356	787					
		356	212	632					
				356					
				212					

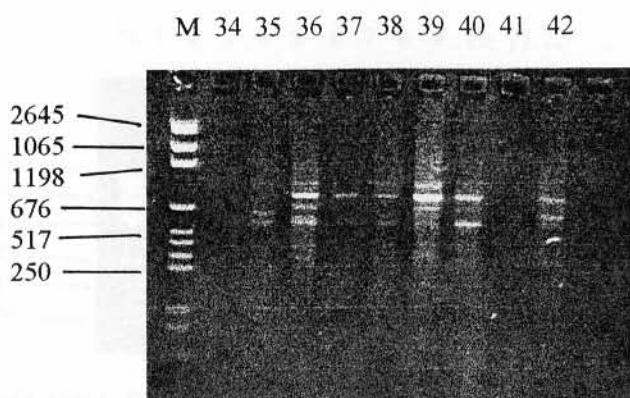
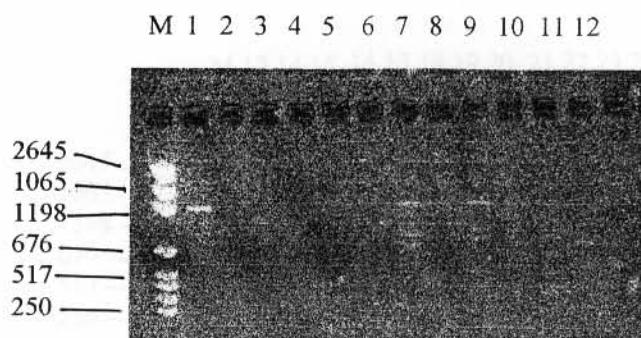


Figure 16: **RAPD-DNA Fingerprinting Using Primer B1 (Part 8 of 8)**

PRIMER NAME:	B1																																																															
STRAIN TYPE:	SUSCEPTIBLE																																																															
LANE M:	pGEM-digested marker																																																															
MOLECULAR WEIGHTS OF BANDS																																																																
<table><thead><tr><th>34</th><th>35</th><th>36</th><th>37</th><th>38</th><th>39</th><th>40</th><th>41</th><th>42</th></tr></thead><tbody><tr><td>625</td><td>695</td><td>1066</td><td>908</td><td>1066</td><td>1066</td><td>958</td><td></td><td>958</td></tr><tr><td></td><td>625</td><td>1011</td><td>625</td><td>984</td><td>908</td><td>908</td><td></td><td>774</td></tr><tr><td></td><td></td><td>908</td><td></td><td>908</td><td>774</td><td>774</td><td></td><td>695</td></tr><tr><td></td><td></td><td>774</td><td></td><td>695</td><td></td><td>625</td><td></td><td></td></tr><tr><td></td><td></td><td>642</td><td></td><td>625</td><td></td><td></td><td></td><td></td></tr><tr><td></td><td></td><td>625</td><td></td><td></td><td></td><td></td><td></td><td></td></tr></tbody></table>		34	35	36	37	38	39	40	41	42	625	695	1066	908	1066	1066	958		958		625	1011	625	984	908	908		774			908		908	774	774		695			774		695		625					642		625							625						
34	35	36	37	38	39	40	41	42																																																								
625	695	1066	908	1066	1066	958		958																																																								
	625	1011	625	984	908	908		774																																																								
		908		908	774	774		695																																																								
		774		695		625																																																										
		642		625																																																												
		625																																																														



**Figure 17: RAPD-DNA Fingerprinting Using Primer B3 (Part 1 of 8)**

**PRIMER: B3**

**STRAIN TYPE: RESISTANT**

**LANE M: pGEM-digested markers**

**MOLECULAR WEIGHTS OF BANDS**

1	2	3	4	5	6	7	8	9	10	11	12
1761						1761		1336	1576	1336	727
1576						1576		907	1336	1070	651
1336						1336		812	1131	812	
1131						858		494	907	651	
907						727			768	374	
727						651			651		
						583			522		



Figure 18: RAPD-DNA Fingerprinting Using Primer B3 (Part 2 of 8)

PRIMER: B3												
STRAIN TYPE: RESISTANT												
LANE M: pGEM-digested markers												
MOLECULAR WEIGHTS OF BANDS												
13	14	15	16	17	18	19	20	21	22	23	24	
1664		2072		2072	1411		1015	1197	1015	1197		
1411		1664		1336	910		910	910	861	1073		
1265		1411		961			772	815	772	961		
961		1197		772				772		861		
655		961		498						772		
321		861		400								
		692										
		620										

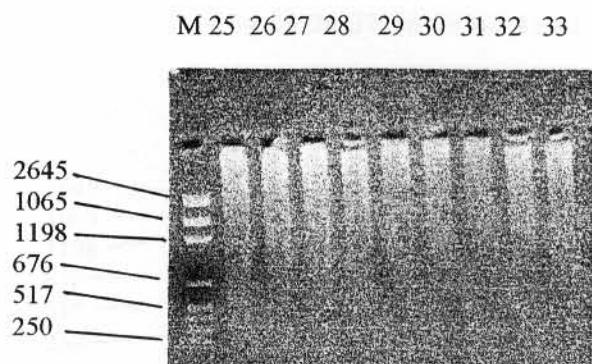


Figure 19: RAPD-DNA Fingerprinting Using Primer B3 (Part 3 of 8)

<b>PRIMER: B3</b>
<b>STRAIN TYPE: RESISTANT</b>
<b>LANE M: pGEM-digested markers</b>
<b>MOLECULAR WEIGHTS OF BANDS</b>
25    26    27    28    29    30    31    32    33

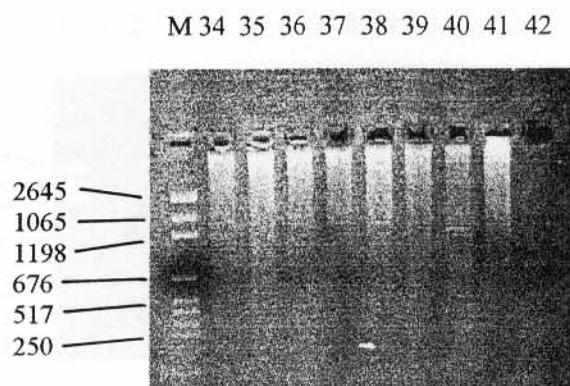


Figure 20: **RAPD-DNA Fingerprinting Using Primer B3 (Part 4 of 8)**

PRIMER: B3									
STRAIN TYPE: RESISTANT									
LANE M: pGEM-digested markers									
MOLECULAR WEIGHTS OF BANDS									
34	35	36	37	38	39	40	41	42	
					1494				
					1341				
					825				
					782				

M 1 2 3 4 5 6 7 8 9 10 11 12

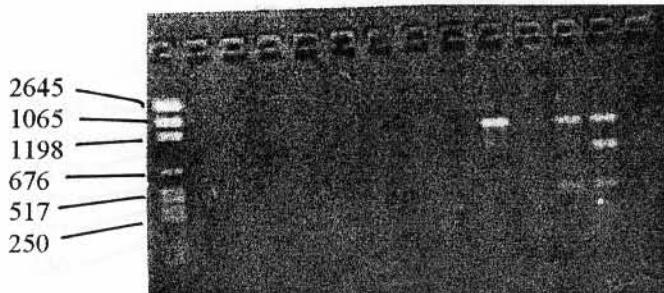


Figure 21: **RAPD-DNA Fingerprinting Using Primer B3 (Part 5 of 8)**

**PRIMER: B3**

**STRAIN TYPE: SUSCEPTIBLE**

**LANE M: pGEM-digested markers**

**MOLECULAR WEIGHTS OF BANDS**

1	2	3	4	5	6	7	8	9	10	11	12
								1237		1237	
								885		885	
								484		396	
								370			

M 13 14 15 16 17 18 19 20 21 22 23 24

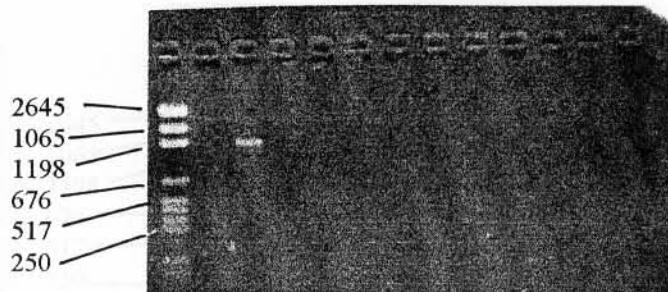


Figure 22: RAPD-DNA Fingerprinting Using Primer B3 (Part 6 of 8)

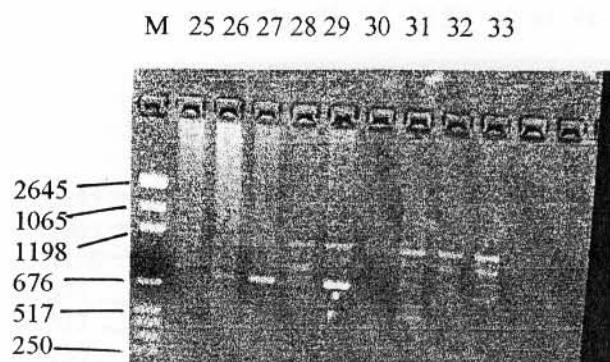
**PRIMER: B3**

**STRAIN TYPE: SUSCEPTIBLE**

**LANE M: pGEM-digested markers**

**MOLECULAR WEIGHTS OF BANDS**

13	14	15	16	17	18	19	20	21	22	23	24
1166											



**Figure 23: RAPD-DNA Fingerprinting Using Primer B3 (Part 7 of 8)**

PRIMER: B3								
STRAIN TYPE: SUSCEPTIBLE								
LANE M: pGEM-digested markers								
MOLECULAR WEIGHTS OF BANDS								
25	26	27	28	29	30	31	32	33
1220	784	1220	1220	1167	1220	1167	1455	1455
		784	1022	784		1022	1220	1167
			895			857	1022	978
			784			750	354	718
			687			527		354
			504			339		
			339					

M 34 35 36 37 38 39 40 41 42



**Figure 24: RAPD-DNA Fingerprinting Using Primer B3 (Part 8 of 8)**

**PRIMER: B3**

**STRAIN TYPE: SUSCEPTIBLE**

**LANE M: pGEM-digested markers**

**MOLECULAR WEIGHTS OF BANDS**

34	35	36	37	38	39	40	41	42
2319	1802	2678		2877	1802	1304	1561	1561
2158	1258	1506		2583	1401	5498	1258	1304
1802	1129	1258		1304	1258	5498		398
1506	817	817		1213	788			
1252	760			943				
1089	427			847				
817				682				
635				427				
476				398				
384								

Appendix VI: RAPD: Agarose Gel Photographs and Tabulations

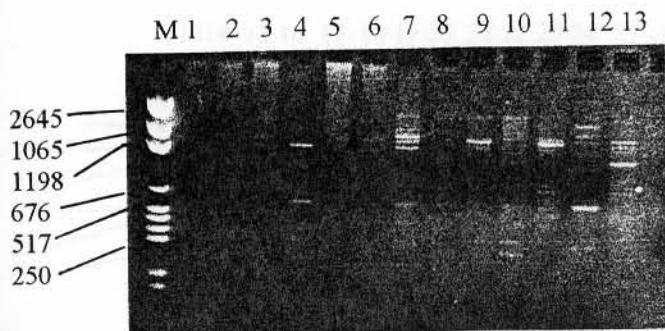
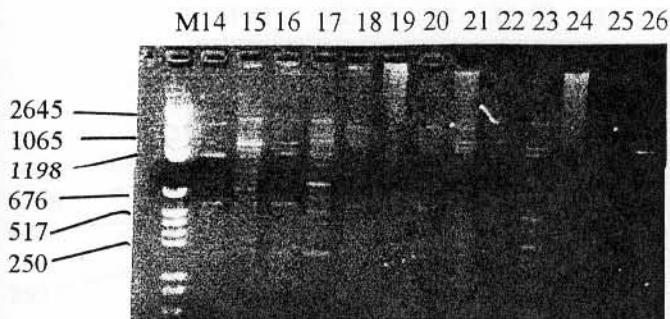


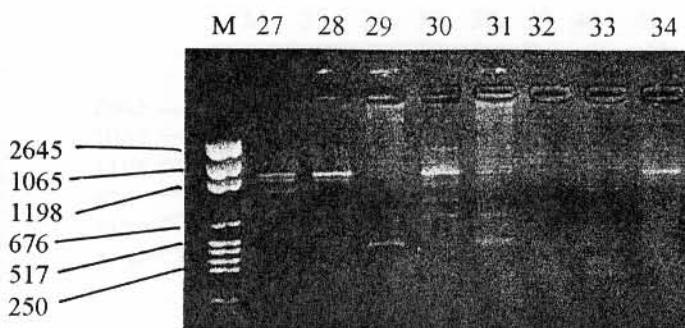
Figure 25: RAPD-DNA Fingerprinting Using Primer C9 (Part 1 of 8)

PRIMER NAME: C9													
STRAIN TYPE: RESISTANT													
LANE M: pGEM-digested marker													
MOLECULAR WEIGHTS OF BANDS													
1	2	3	4	5	6	7	8	9	10	11	12	13	
773	2221	2221	3431	1628	1680	2595	2676	2676	2595	1844	3431	2676	
	1733	1733	1962	1194	1394	2364	2364	1787	2438	1733	2848	1844	
1054	174	1530			603	2088	2088	1628	1962	1577	2676	1680	
773		1122			470	1844	1157	961	1787	1484	2364	1577	
603		603			324	1680	286	903	1628	875	1962	1270	
						1530		822	1484	797	1787	991	
						875		532	1231	603	1438	797	
						797			991	532	1270		
						470			848		1122		
						345			683		603		
									603		324		
									532				
									334				
									286				



**Figure 26: RAPD-DNA Fingerprinting Using Primer C9 (Part 2 of 8)**

PRIMER NAME: C9													
STRAIN TYPE: RESISTANT													
LANE M: pGEM-digested marker													
MOLECULAR WEIGHTS OF BANDS													
14	15	16	17	18	19	20	21	22	23	24	25	26	
2349	2233	2601	2233	2069	1343	2069	2017	1917	2233	2412	1042	2233	
1732	2017	2349	2069	1917	1213	1732	1688	1732	2017	1450	694	1917	
1413	1822	2069	1966	1732		990	1605	1487	1776			1605	
1378	1646	1822	1869	1605		850	1525	1413	1605			1450	
712	1564	1605	1688	1069		694	1413	1343	1487			1124	
623	1487	1487	1413	694		596	1124	1096	1213			1069	
	1042	1413	1213			417	1042	870	1153			990	
	895	1124	1153			358	941	712	965			941	
	850	694	918				870	417	895			829	
	694	324	850				768	368	870			730	
	429		768				694		694			538	
	358		694				417		596				
			659				368		511				
			377						358				
									264				



**Figure 27: RAPD-DNA Fingerprinting Using Primer C9 (Part 3 of 8)**

PRIMER NAME:	C9						
STRAIN TYPE:	RESISTANT						
LANE M:	pGEM-digested marker						
<b>MOLECULAR WEIGHTS OF BANDS</b>							
<b>27    28    29    30    31    32    33    34</b>							
2393	2393	2393	2393	2393	2393	2545	2545
1655	1605	1605	1707	1707	2183	2321	2251
1464	1464	1464	1605	1605	1815	1815	1815
1217	484	484	1044	792	1707	1557	1707
531	296	296	792	515	1377	1255	1557
351					1145	1012	1464
					1012	923	1294
					842	658	1145
					744	619	895
					679	515	744
					582	455	403
					531	403	
					484	335	
					428	262	
					356		

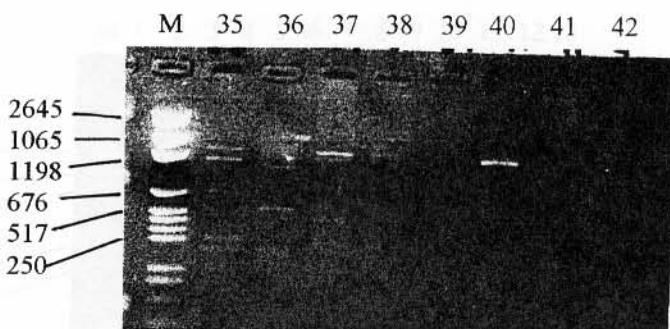


Figure 28: RAPD-DNA Fingerprinting Using Primer C9 (Part 4 of 8)

PRIMER NAME: C9							
STRAIN TYPE: RESISTANT							
LANE M: pGEM-digested marker							
MOLECULAR WEIGHTS OF BANDS							
35	36	37	38	39	40	41	42
2782	2511	2598	2598	2266	610	2266	2266
2116	2266	1724	2266	1910	405	1610	1724
1910	2116	1556	1977	1453	1312	1405	1405
1610	1910	1357	1610	1225	709	1069	1225
1312	1846	1225	1405	841	619	813	998
1069	1666	1069	662	759	540	662	871
965	1556	813	578	578		578	786
759	1226	662	504	471		504	578
540	871	504	411	371		440	471
471	709	411	383	292		383	335
411	578	358	323	222		312	255
323	504	323	273			273	
273	383	292	169				
	335						
	273						



Figure 29: **RAPD-DNA Fingerprinting Using Primer C9 (Part 5 of 8)**

PRIMER NAME: C9

STRAIN TYPE: SUSCEPTIBLE

LANE M: pGEM-digested marker

MOLECULAR WEIGHTS OF BANDS

1	2	3	4	5	6	7	8	9	10	11	12	13
2009				1411				1411		1411		1411
1457				1201								766
1240												
1056												
929												
766												

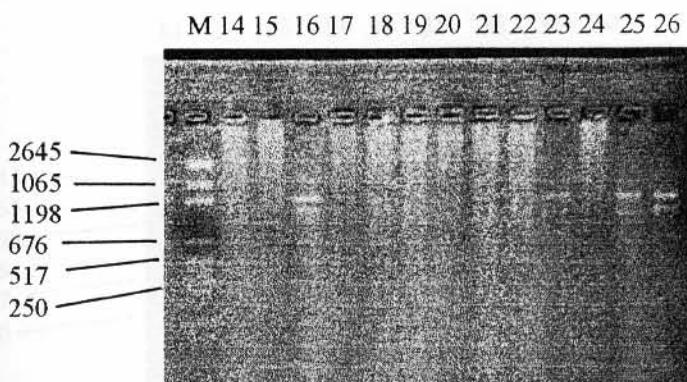


Figure 30: **RAPD-DNA Fingerprinting Using Primer C9 (Part 6 of 8)**

PRIMER NAME: C9

STRAIN TYPE: SUSCEPTIBLE

LANE M: pGEM-digested marker

MOLECULAR WEIGHTS OF BANDS

14	15	16	17	18	19	20	21	22	23	24	25	26
		1419							1336		1336	1185
		1185							1051		1051	1051
		1019							755		755	755

Appendix VI: RAPD: Agarose Gel Photographs and Tabulations

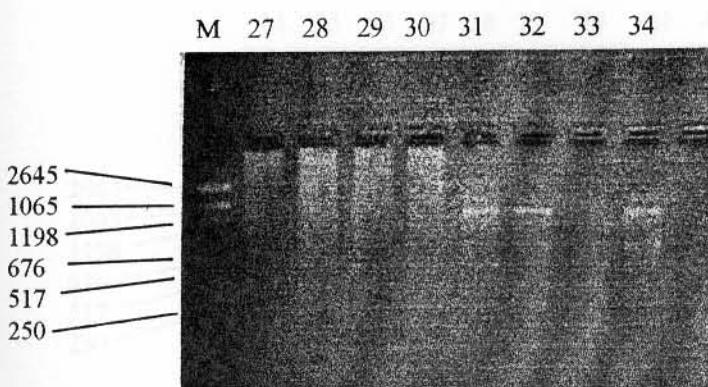
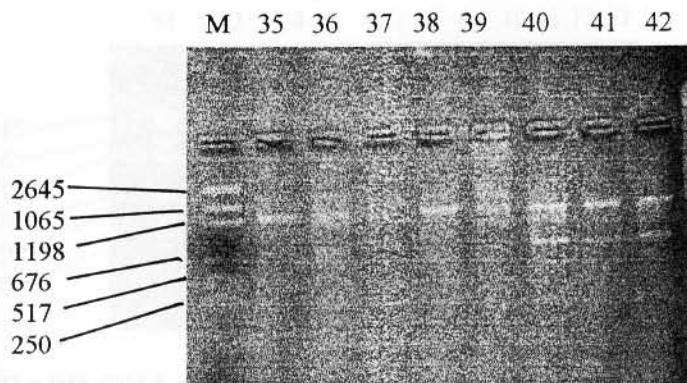
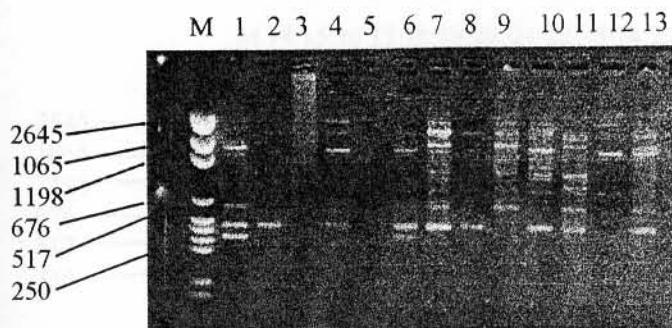


Figure 31: **RAPD-DNA Fingerprinting Using Primer C9 (Part 7 of 8)**

PRIMER NAME:	C9																																																
STRAIN TYPE:	SUSCEPTIBLE																																																
LANE M:	pGEM-digested marker																																																
MOLECULAR WEIGHTS OF BANDS																																																	
<table><thead><tr><th>27</th><th>28</th><th>29</th><th>30</th><th>31</th><th>32</th><th>33</th><th>34</th></tr></thead><tbody><tr><td></td><td></td><td></td><td></td><td>2067</td><td>1388</td><td></td><td>2067</td></tr><tr><td></td><td></td><td></td><td></td><td>1810</td><td>1064</td><td></td><td>1694</td></tr><tr><td></td><td></td><td></td><td></td><td>1388</td><td>714</td><td></td><td>1388</td></tr><tr><td></td><td></td><td></td><td></td><td>1064</td><td></td><td></td><td>714</td></tr><tr><td></td><td></td><td></td><td></td><td>714</td><td></td><td></td><td></td></tr></tbody></table>		27	28	29	30	31	32	33	34					2067	1388		2067					1810	1064		1694					1388	714		1388					1064			714					714			
27	28	29	30	31	32	33	34																																										
				2067	1388		2067																																										
				1810	1064		1694																																										
				1388	714		1388																																										
				1064			714																																										
				714																																													

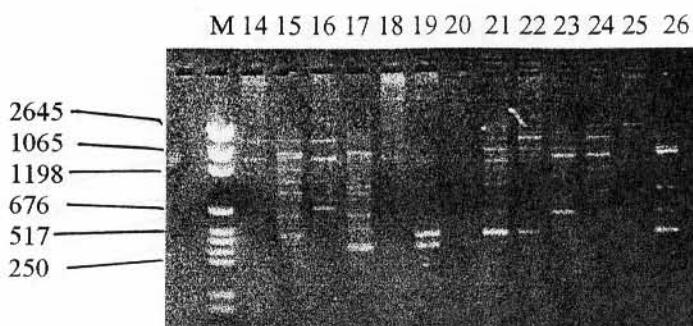
Figure 32: RAPD-DNA Fingerprinting Using Primer C9 (Part 8 of 8)

PRIMER NAME: C9																																																
STRAIN TYPE: SUSCEPTIBLE																																																
LANE M: pGEM-digested marker																																																
MOLECULAR WEIGHTS OF BANDS																																																
<table border="1"> <thead> <tr> <th>35</th><th>36</th><th>37</th><th>38</th><th>39</th><th>40</th><th>41</th><th>42</th></tr> </thead> <tbody> <tr> <td>1484</td><td>2063</td><td>767</td><td>1300</td><td>1808</td><td>1300</td><td>1300</td><td>1300</td></tr> <tr> <td>1140</td><td>1484</td><td></td><td>767</td><td>1389</td><td>1103</td><td>767</td><td>767</td></tr> <tr> <td>767</td><td>767</td><td></td><td>651</td><td>767</td><td>767</td><td>552</td><td>589</td></tr> <tr> <td></td><td></td><td></td><td></td><td></td><td>517</td><td>267</td><td>267</td></tr> <tr> <td></td><td></td><td></td><td></td><td></td><td>267</td><td></td><td></td></tr> </tbody> </table>	35	36	37	38	39	40	41	42	1484	2063	767	1300	1808	1300	1300	1300	1140	1484		767	1389	1103	767	767	767	767		651	767	767	552	589						517	267	267						267		
35	36	37	38	39	40	41	42																																									
1484	2063	767	1300	1808	1300	1300	1300																																									
1140	1484		767	1389	1103	767	767																																									
767	767		651	767	767	552	589																																									
					517	267	267																																									
					267																																											

Figure 33: RAPD-DNA Fingerprinting Using Primer C13 (Part 1 of 8)

PRIMER NAME: C13													
STRAIN TYPE: RESISTANT													
LANE M: pGEM-digested marker													
MOLECULAR WEIGHTS OF BANDS													
1	2	3	4	5	6	7	8	9	10	11	12	13	
1835	2199	2199	2716		2336	2635	2336	2635	2635	2199	3158	2336	
1676	1949		2199		1727	2336	1727	2199	2336	2070	2716	2199	
1486	1727		1626		1578	2199	890	1835	2199	1835	1531	1780	
658	517		517		1278	1835	517	1626	1727	1167	890	1626	
487					890	1727		1441	1531	945		678	
406					699	1531		1132	1441	890		487	
					517	1203		658	1317	699			
					431	1066			1167	517			
						890			1099				
						699			890				
						517			699				
									517				

Appendix VI: RAPD: Agarose Gel Photographs and Tabulations



**Figure 34: RAPD-DNA Fingerprinting Using Primer C13 (Part 2 of 8)**

PRIMER NAME: C13
STRAIN TYPE: RESISTANT
LANE M: pGEM-digested marker

**MOLECULAR WEIGHTS OF BANDS**

14	15	16	17	18	19	20	21	22	23	24	25	26
2690	1966	2541	2690	1705	1911		2400	2400	2142	2400	2333	1911
2333	1657	2023	2541	1611	1437		2268	1966	1911	1966	2142	1857
1911	1522	1522	2400	768	1358		1705	1657	1657	1705	1522	1611
1479	1282	813	2023	647	964		1397	911	1522	1522		1522
685	1081	725	1805	410	611		1211	813	1081	1246		885
911			1705		460		1021	725	992	1112		647
647			1522		388		937	487	836	911		487
487			1021				861		647	685		309
			911				685		487	577		
			647				515			487		
			577									
			546									
			410									

Appendix VI: RAPD: Agarose Gel Photographs and Tabulations

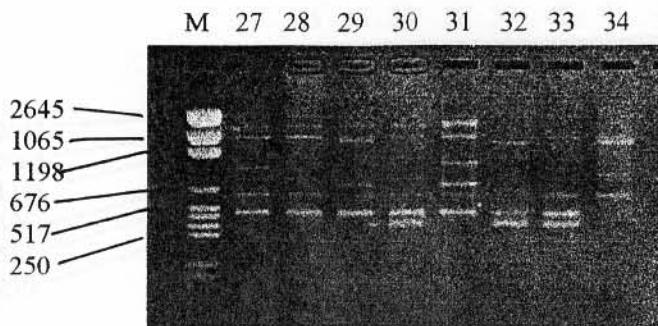
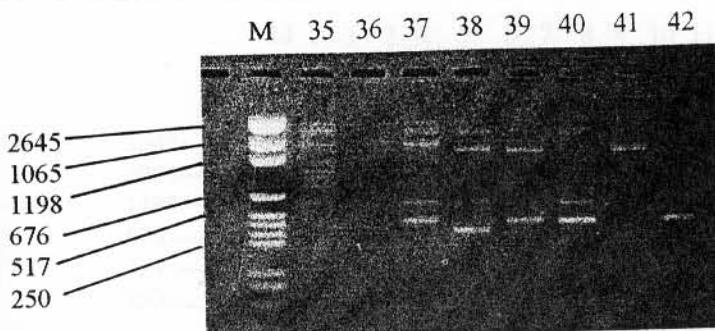


Figure 35: RAPD-DNA Fingerprinting Using Primer C13 (Part 3 of 8)

PRIMER NAME: C13							
STRAIN TYPE: RESISTANT							
LANE M: pGEM-digested marker							
MOLECULAR WEIGHTS OF BANDS							
27	28	29	30	31	32	33	34

2213	1951	1951	2078	2078	1517	1832	2357
2078	1615	1517	1720	1615	712	1667	2213
1775	1073	759	860	1073	488	1142	1667
1073	648	628	669	759	404	1007	1565
860	458	458	554	628		860	916
735			404	458		669	648
690						488	
520						404	



**Figure 36: RAPD-DNA Fingerprinting Using Primer C13 (Part 4 of 8)**

PRIMER NAME: C13							
STRAIN TYPE: RESISTANT							
LANE M: pGEM-digested marker							
MOLECULAR WEIGHTS OF BANDS							
35	36	37	38	39	40	41	42
2499		2499	2499	2499	2346	3657	1603
2346		2346	2133	2202	2066	3023	850
2273		2202	1603	1820	1820	2346	480
1763		1820	1167	1603	1603	1603	
1096		1167	850	512	1096		
965		660	660		850		
850		480	397		660		
749					480		
163							
144							

Appendix VI: RAPD: Agarose Gel Photographs and Tabulations



**Figure 37: RAPD-DNA Fingerprinting Using Primer C13 (Part 5 of 8)**

PRIMER NAME: C13

STRAIN TYPE: SUSCEPTIBLE

LANE M: pGEM-digested marker

MOLECULAR WEIGHTS OF BANDS

1	2	3	4	5	6	7	8	9	10	11	12	13
1520	1022	1813	1735	1813	1813	1813	2362	1813	1735	1588	1980	2162
978		1520	1520	1520	1588	1520	2163	1520	1520	1068	1735	1816
576		751	1274	895	1022	1022	1895		1274		1588	1526
442		629	1022	602	751	629	1588		462		1331	1176
		442	576		576						1116	906
												729
												493

Appendix VI: RAPD: Agarose Gel Photographs and Tabulations

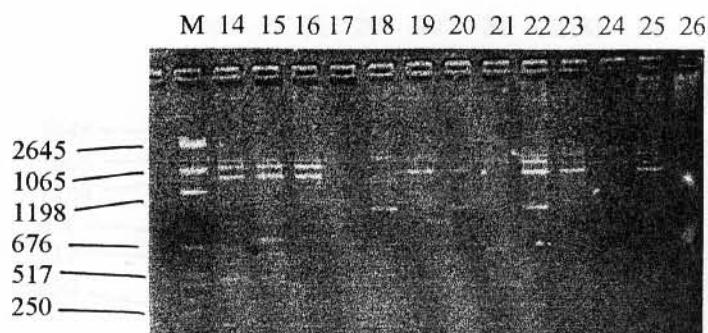


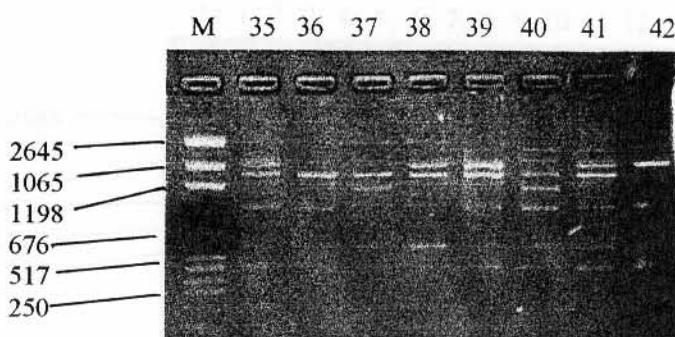
Figure 38: **RAPD-DNA Fingerprinting Using Primer C13 (Part 6 of 8)**

PRIMER NAME: C13													
STRAIN TYPE: SUSCEPTIBLE													
LANE M: pGEM-digested marker													
MOLECULAR WEIGHTS OF BANDS													
14	15	16	17	18	19	20	21	22	23	24	25	26	
1739	1739	5158	1816	1816	1665	1739	1816	1816		1816		1504	
1594	1526	5158	1032	1594	1399	729	1739	1526		1526		991	
761	761		830		1078	640	1526						
640							1032						
493													



Figure 39: RAPD-DNA Fingerprinting Using Primer C13 (Part 7 of 8)

PRIMER NAME:	C13																																																																
STRAIN TYPE:	SUSCEPTIBLE																																																																
LANE M:	pGEM-digested marker																																																																
MOLECULAR WEIGHTS OF BANDS																																																																	
<table border="1"><thead><tr><th>27</th><th>28</th><th>29</th><th>30</th><th>31</th><th>32</th><th>33</th><th>34</th></tr></thead><tbody><tr><td>2081</td><td>1729</td><td>1729</td><td>1729</td><td>2081</td><td>1729</td><td>1729</td><td>1740</td></tr><tr><td>1811</td><td>1504</td><td>1504</td><td>1504</td><td>1729</td><td>1504</td><td>1576</td><td>1657</td></tr><tr><td>1504</td><td>991</td><td>991</td><td>991</td><td>1504</td><td>623</td><td>1038</td><td>881</td></tr><tr><td>823</td><td></td><td>684</td><td>684</td><td>1309</td><td>472</td><td></td><td>597</td></tr><tr><td>472</td><td></td><td></td><td>595</td><td>991</td><td></td><td></td><td>469</td></tr><tr><td></td><td></td><td></td><td></td><td>623</td><td></td><td></td><td></td></tr><tr><td></td><td></td><td></td><td></td><td>472</td><td></td><td></td><td></td></tr></tbody></table>		27	28	29	30	31	32	33	34	2081	1729	1729	1729	2081	1729	1729	1740	1811	1504	1504	1504	1729	1504	1576	1657	1504	991	991	991	1504	623	1038	881	823		684	684	1309	472		597	472			595	991			469					623								472			
27	28	29	30	31	32	33	34																																																										
2081	1729	1729	1729	2081	1729	1729	1740																																																										
1811	1504	1504	1504	1729	1504	1576	1657																																																										
1504	991	991	991	1504	623	1038	881																																																										
823		684	684	1309	472		597																																																										
472			595	991			469																																																										
				623																																																													
				472																																																													



**Figure 40: RAPD-DNA Fingerprinting Using Primer C13 (Part 8 of 8)**

PRIMER NAME:	C13						
STRAIN TYPE:	SUSCEPTIBLE						
LANE M:	pGEM-digested marker						
MOLECULAR WEIGHTS OF BANDS							
35	36	37	38	39	40	41	42
1504	1827	1657	2013	2013	2013	2113	2130
1020	1504	1504	1657	1740	1657	1657	1657
469	1238	597	1433	1433	1365	971	597
	881		971	1238	925	597	496
	425		469	925	569		
				597	425		
				446			

Appendix VI: RAPD: Agarose Gel Photographs and Tabulations



**Figure 41: RAPD-DNA Fingerprinting Using Primer AA1 (Part 1 of 8)**

PRIMER NAME: AA1

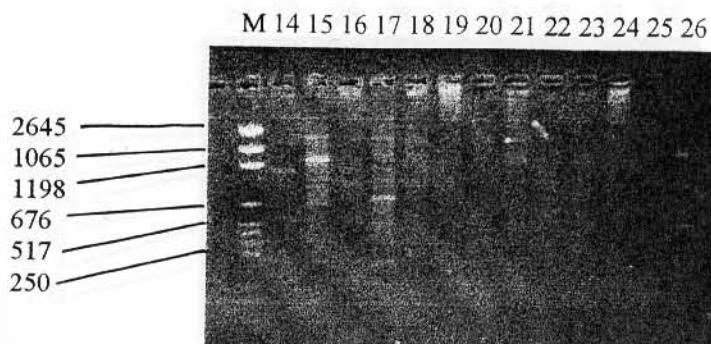
STRAIN TYPE: RESISTANT

LANE M: pGEM-digested marker

MOLECULAR WEIGHTS OF BANDS

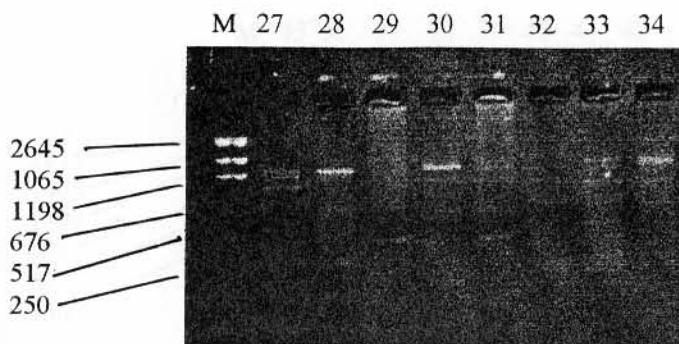
1	2	3	4	5	6	7	8	9	10	11	12	13
1578	1578	1488	1324	2113	2113	2113	2113	2113	2113	2113	1880	2113
696	933		1018	1404	1578	1673	1673	1445	1488	1404	1488	1488
	696		585	1048	1363	1488	1111	783	1111	760	989	1111
					738	1404	568	696	738	717	585	830
					620	1286	274	491	585		317	
						738			326			
						551			290			
						412						
						326						

Appendix VI: RAPD: Agarose Gel Photographs and Tabulations



**Figure 42: RAPD-DNA Fingerprinting Using Primer AA1 (Part 2 of 8)**

PRIMER NAME: AA1														
STRAIN TYPE: RESISTANT														
LANE M: pGEM-digested marker														
MOLECULAR WEIGHTS OF BANDS														
14	15	16	17	18	19	20	21	22	23	24	25	26		
1988	1988	1988	1988	1867	1988	1867	1867	2553	2116	569	1205	1548		
1548	1500	1454	1754	1754	1754	1548	1548	1548	1548			938	1063	
1205	1609	1205	1548	1366	1548	1205	1454	1454	1366				938	
569	1205	938	1205	881	1205	644	1283	1283	828			443		
502	881	828	1063	730	1063	569		1132	777					
391	686	644	938	502	938			938	730					
268	589	504	730		750			730	569					
	304		686		686			569	502					
			605		605				324					
			534		534									
			471		471									
			252		252									



**Figure 43: RAPD-DNA Fingerprinting Using Primer AA1 (Part 3 of 8)**

PRIMER NAME: AA1							
STRAIN TYPE: RESISTANT							
LANE M: pGEM-digested marker							
MOLECULAR WEIGHTS OF BANDS							
27	28	29	30	31	32	33	34
1513	1419	1370	2223	1613	1955	1613	1513
1331	1331	1834	1513	1513	1834	1331	370
1099	1099	1513	1171	1171	906	1099	286
850	906	1171	850	748	850	850	
702	797	906	748	448	448	748	
448	478	478			325	543	
305	305	347			286	448	
		286			325		
						286	

Appendix VI: RAPD: Agarose Gel Photographs and Tabulations

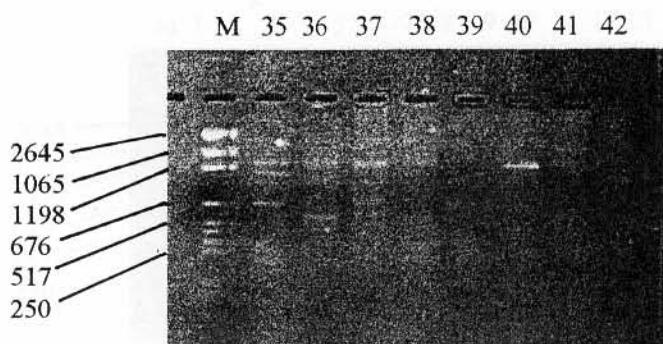


Figure 44: **RAPD-DNA Fingerprinting Using Primer AA1 (Part 4 of 8)**

PRIMER NAME:	AA1						
STRAIN TYPE:	RESISTANT						
LANE M:	pGEM-digested marker						
MOLECULAR WEIGHTS OF BANDS							
35	36	37	38	39	40	41	42
1491	2264	1491	2264	2264	2112	2791	1491
1210	1970	743	1970	1714	1491	1970	1129
982	1599	562	1599	1211	1210	1491	982
693	1129	280	1491	1129	1053	797	797
301	743		562	693	797	562	562
244	562		489	426	693	322	456
	244			301	562		301
				261	456		

Appendix VI: RAPD: Agarose Gel Photographs and Tabulations



Figure 45: RAPD-DNA Fingerprinting Using Primer AA1 (Part 5 of 8)

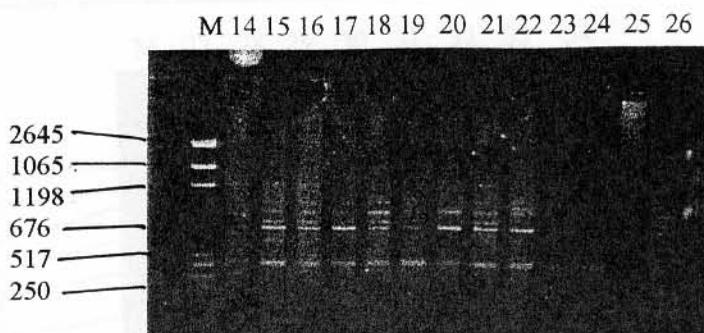
PRIMER NAME: AA1

STRAIN TYPE: SUSCEPTIBLE

LANE M: pGEM-digested marker

MOLECULAR WEIGHTS OF BANDS

1	2	3	4	5	6	7	8	9	10	11	12	13
1973	2076	2547	1875	2420	2680	2076	1693	1781	2185	1781	1973	1781
1609	1693	2185	1609	2185	2185	1781	1528	1311	1973	1528	1781	1452
1311	1528	1781	1528	1781	1781	1609	872	872	1781	1452	1452	872
787	828	1528	917	1528	1609	1452			1380	872	1311	
		1380		872	1452	1311			872		872	
		872			917	872						



**Figure 46: RAPD-DNA Fingerprinting Using Primer AA1 (Part 6 of 8)**

**PRIMER NAME:** AA1

**STRAIN TYPE: SUSCEPTIBLE**

**LANE M: pGEM-digested marker**

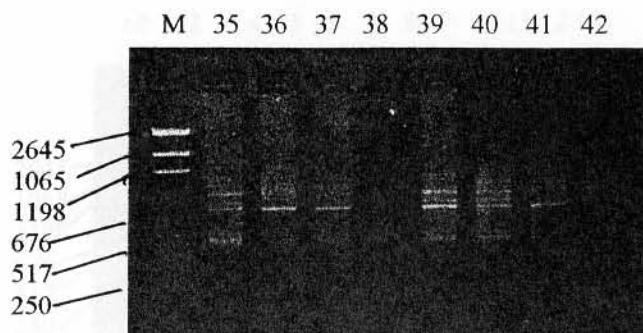
**MOLECULAR WEIGHTS OF BANDS**

14	15	16	17	18	19	20	21	22	23	24	25	26
1317	1387	1387	1071	1127	785	1017	1317	1017	965	965	965	870
1187	965	1071	917	1017	708	785	1187	785	785	785	745	785
870	826	965	785	870	468	493	1071	468	745	745	468	708
785	785	826	468	785			965		468	468		638
468	638	745		493			870					468
	493	493					638					
							468					



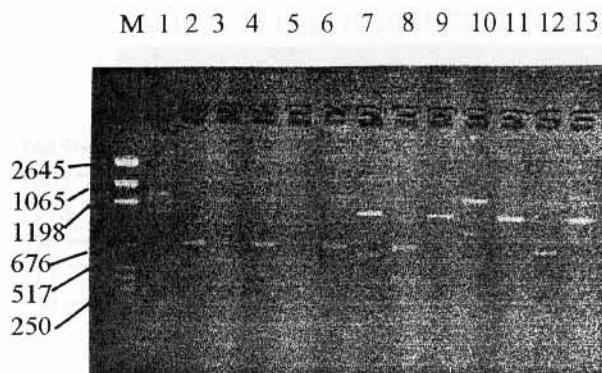
Figure 47: RAPD-DNA Fingerprinting Using Primer AA1 (Part 7 of 8)

PRIMER NAME:	AA1						
STRAIN TYPE:	SUSCEPTIBLE						
LANE M:	pGEM-digested marker						
MOLECULAR WEIGHTS OF BANDS							
27	28	29	30	31	32	33	34
1113	1053	1113	1243			1243	893
997	893	997	997			1113	756
893	716	893	893			943	460
716	460	716	716			716	
		435	435			460	



**Figure 48: RAPD-DNA Fingerprinting Using Primer AA1 (Part 8 of 8)**

PRIMER NAME:	AA1						
STRAIN TYPE:	SUSCEPTIBLE						
LANE M:	pGEM-digested marker						
MOLECULAR WEIGHTS OF BANDS							
35	36	37	38	39	40	41	42
1429	695	908	487	1206	1429	1513	1276
1276	1351	766		961	1206	1351	1077
1077	961	545		858	1139	1139	908
961	724	487		766	961	1017	724
858	460			487	858	811	487
766					766	487	
545							
487							



**Figure 49: RAPD-DNA Fingerprinting Using Primer AA2 (Part 1 of 8)**

**PRIMER: AA2**

**STRAIN TYPE: RESISTANT**

**LANE M: pGEM-digested markers**

**MOLECULAR WEIGHTS OF BANDS**

1	2	3	4	5	6	7	8	9	10	11	12	13
1973	1581	1416	730	1268	1538	1200	1135	1135	1496	1581	1496	1973
1496	1268	730	469	910	910	653	730		1135	1200	1135	1617
1200	730	585	356		730		469		910	910	730	1496
	469	469			469						585	1135
												910

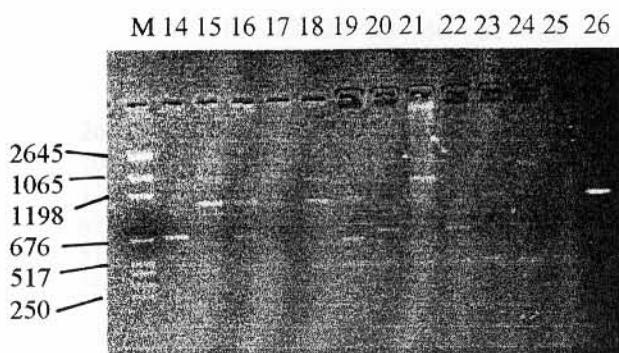
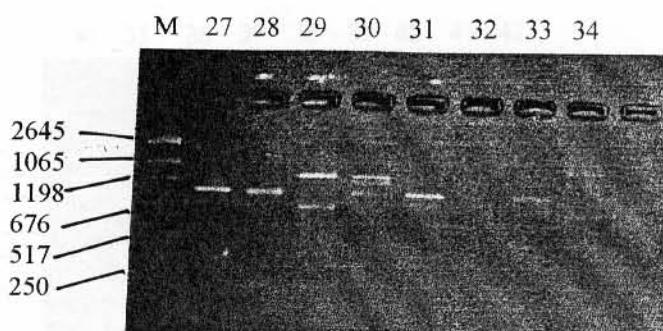


Figure 50: RAPD-DNA Fingerprinting Using Primer AA2 (Part 2 of 8)

PRIMER: AA2													
STRAIN TYPE: RESISTANT													
LANE M: pGEM-digested markers													
MOLECULAR WEIGHTS OF BANDS													
14	15	16	17	18	19	20	21	22	23	24	25	26	
2327	1992	1620	1620	1538	1187	1460	1460	1992	1187	1706	468	1538	
1460	1620	1187	1071	1127	965	965	1187	1706	917	1250	309	1250	
1250	1127	726	745	708	638	870	1017	1538	606	1127	251	1071	
917	965	468		493	468	708	480	1250	493	468			
708	708				422	546	400	546					
546	638					468		493					
	546												



**Figure 51: RAPD-DNA Fingerprinting Using Primer AA2 (Part 3 of 8)**

PRIMER: AA2							
STRAIN TYPE: RESISTANT							
LANE M: pGEM-digested markers							
MOLECULAR WEIGHTS OF BANDS							
27	28	29	30	31	32	33	34
1992	1049	1879	1404	2051		1112	2174
1112		1324	1286	1324		880	1992
783		1049	1080	1144		697	1879
		830					1773
		437					1404
							1178
							1080
							1049
							989

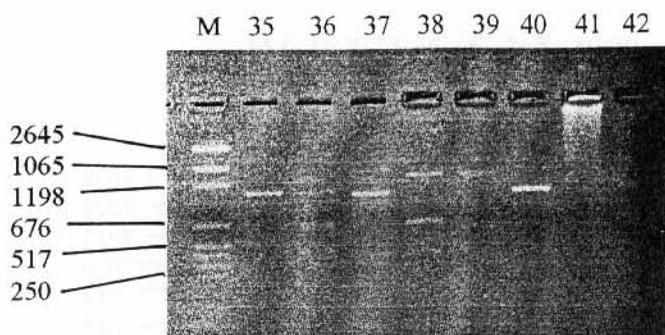


Figure 52: **RAPD-DNA Fingerprinting Using Primer AA2 (Part 4 of 8)**

<b>PRIMER: AA2</b>							
<b>STRAIN TYPE: RESISTANT</b>							
<b>LANE M: pGEM-digested markers</b>							
<b>MOLECULAR WEIGHTS OF BANDS</b>							
35	36	37	38	39	40	41	42
1158	1379	1643	1550	1462	1158		1158
	1093	1093	770	647	917		917
	865	917	543	456	456		727
	685	456	456				543
	456						456

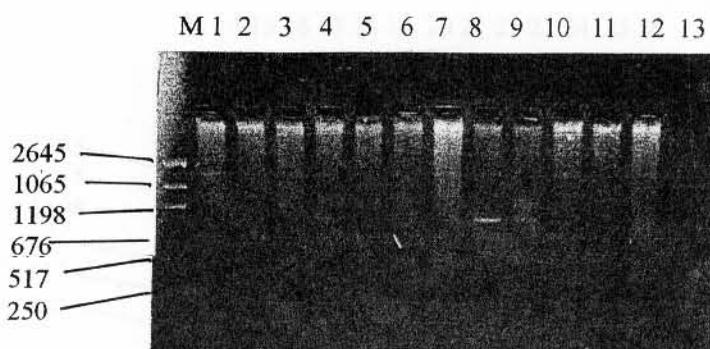


Figure 53: **RAPD-DNA Fingerprinting Using Primer AA2 (Part 5 of 8)**

**PRIMER: AA2**

**STRAIN TYPE: SUSCEPTIBLE**

**LANE M: pGEM-digested markers**

**MOLECULAR WEIGHTS OF BANDS**

1	2	3	4	5	6	7	8	9	10	11	12	13
			1529				1163	1461				
			1396					1396				
			1163					1163				

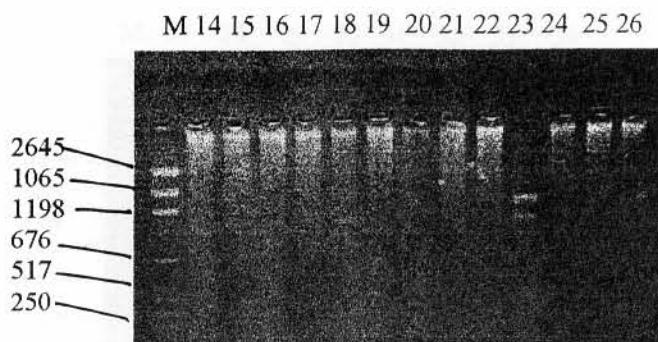


Figure 54: RAPD-DNA Fingerprinting Using Primer AA2 (Part 6 of 8)

**PRIMER: AA2**

**STRAIN TYPE: SUSCEPTIBLE**

**LANE M: pGEM-digested markers**

**MOLECULAR WEIGHTS OF BANDS**

14	15	16	17	18	19	20	21	22	23	24	25	26
				1142					1560			
									1452			
									1199			

Appendix VI: RAPD: Agarose Gel Photographs and Tabulations

---

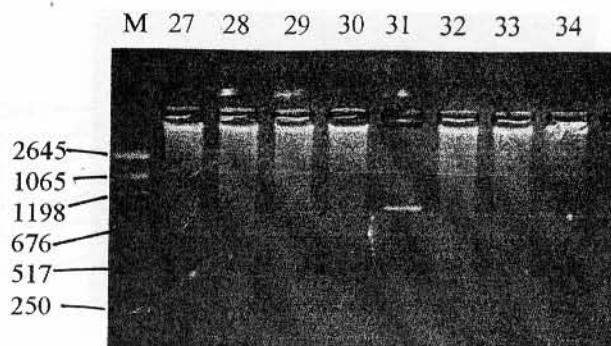


Figure 55: RAPD-DNA Fingerprinting Using Primer AA2 (Part 7 of 8)

PRIMER: AA2							
STRAIN TYPE: SUSCEPTIBLE							
LANE M: pGEM-digested markers							
MOLECULAR WEIGHTS OF BANDS							
27	28	29	30	31	32	33	34

1176

Appendix VI: RAPD: Agarose Gel Photographs and Tabulations

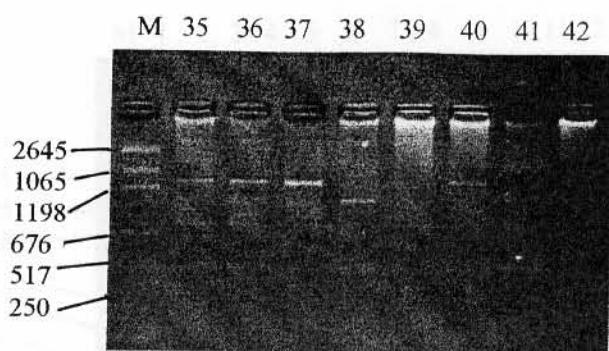


Figure 56: **RAPD-DNA Fingerprinting Using Primer AA2 (Part 8 of 8)**

<b>PRIMER: AA2</b>							
<b>STRAIN TYPE: SUSCEPTIBLE</b>							
<b>LANE M: pGEM-digested markers</b>							
<b>MOLECULAR WEIGHTS OF BANDS</b>							
35	36	37	38	39	40	41	42
1398	1398	1398	1132		1398	1398	
1132	1132	439	439			1132	
635	871					439	
	439						

Appendix VI: RAPD: Agarose Gel Photographs and Tabulations

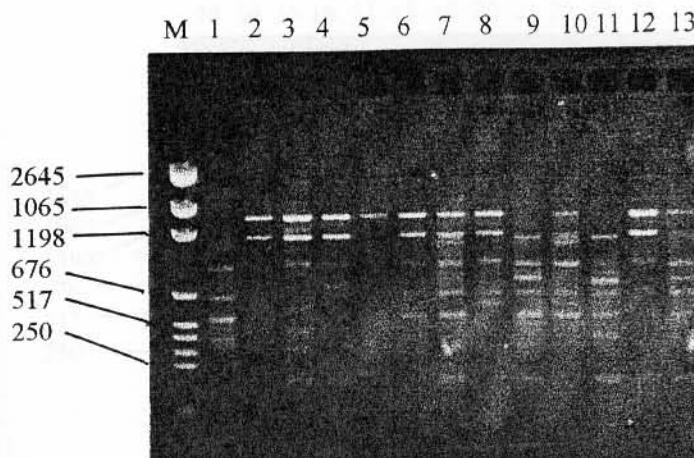
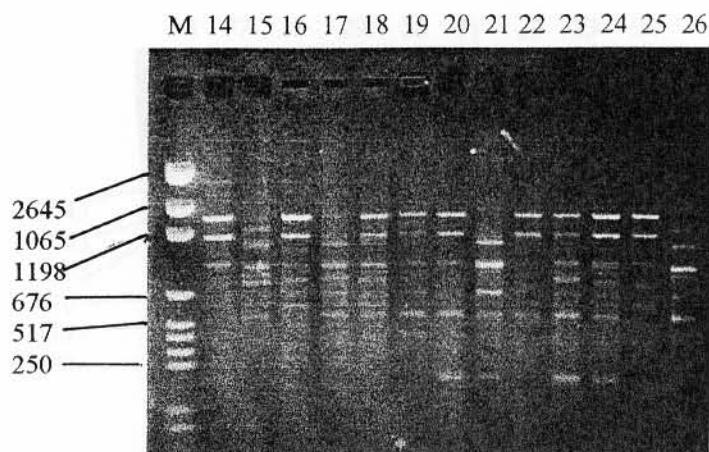


Figure 57: RAPD-DNA Fingerprinting Using Primer AA3 (Part 1 of 8)

PRIMER: AA3													
STRAIN TYPE: RESISTANT													
LANE M: pGEM-digested markers													
MOLECULAR WEIGHTS OF BANDS													
1	2	3	4	5	6	7	8	9	10	11	12	13	
1210	1549	2701	2291	1517	1549	1549	1549	1549	1549	1235	1249	1549	
1137	1261	2387	1581	1235	1261	1261	1397	1397	1261	888	1235	1369	
888	964	1581	1235	1185	926	1185	1261	1261	1210	818	926	1235	
818	613	1397	926	926	666	1005	926	1210	1137	785	694	1161	
785		1287	269	613	520	926	666	945	926	666	520	907	
639		1137		520	406	785	613	870	666	542	269	769	
509		964		441	344	666	432	835	520	423		666	
406		852		374	269	531		680		169		520	
		588		317		269		553				269	
		459		248				269					
		269											

Figure 58: RAPD-DNA Fingerprinting Using Primer AA3 (Part 2 of 8)

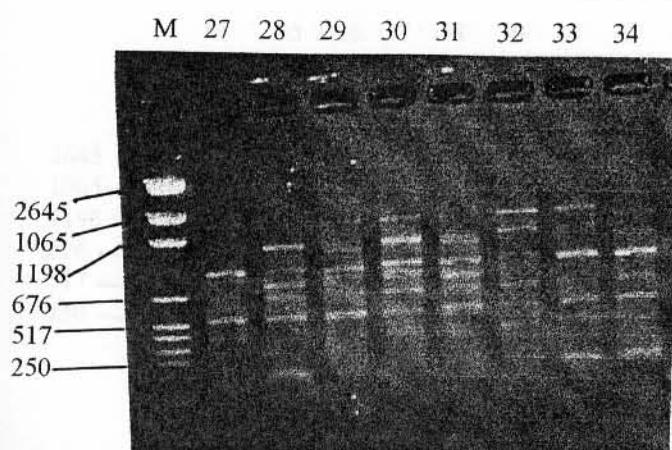
PRIMER: AA3

STRAIN TYPE: RESISTANT

LANE M: pGEM-digested markers

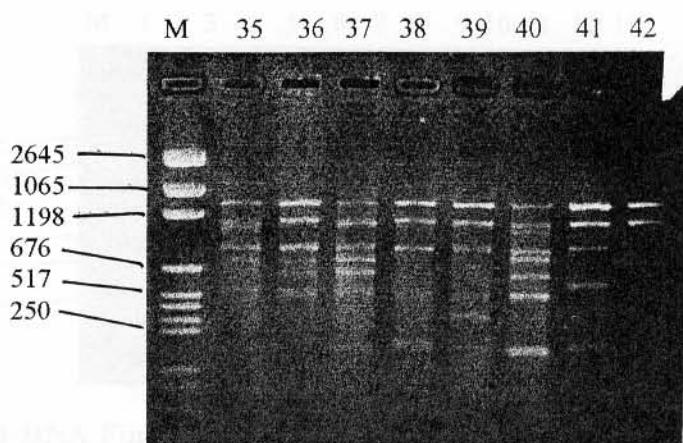
## MOLECULAR WEIGHTS OF BANDS

14	15	16	17	18	19	20	21	22	23	24	25	26
2121	1503	1534	1534	1534	1331	1534	1227	1534	1534	1540	1534	1331
1917	1358	1227	1253	1253	1253	1386	1179	1253	1253	1253	1253	1087
1503	1203	1087	1155	924	924	1253	924	963	1087	924	924	852
1227	1132	906	1087	852	818	963	852	852	924	755	786	802
924	906	786	906	786	668	852	835	725	818	668	592	642
755	816	580	786	696	546	725	696	546	786	592		503
616	770	379	668	616	428	546	546	274	668	546		
274	535	336	535	546		274	274		546	274		
				428					274			



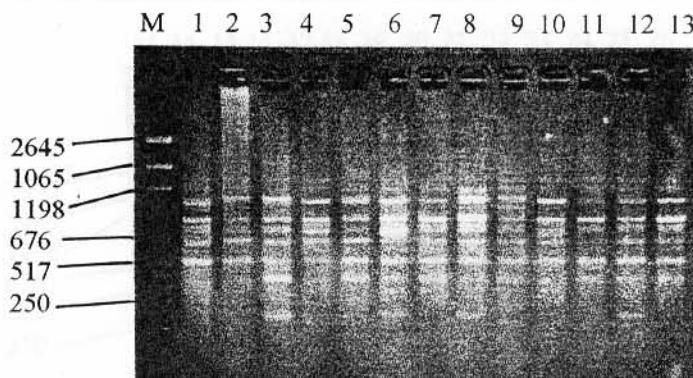
**Figure 59: RAPD-DNA Fingerprinting Using Primer AA3 (Part 3 of 8)**

PRIMER: AA3							
STRAIN TYPE: RESISTANT							
LANE M: pGEM-digested markers							
MOLECULAR WEIGHTS OF BANDS							
27	28	29	30	31	32	33	34
1403	1096	1341	1341	1755	1403	1403	1282
1254	819	1048	1048	1403	1121	1282	1048
1025	701	959	819	1121	819	1121	819
819	613	801	733	1072	749	819	626
749	479	701	599	819	599	749	479
479	245	573	438	585	479	626	245
383		479	245	599	383	479	
				479		383	
						245	



**Figure 60: RAPD-DNA Fingerprinting Using Primer AA3 (Part 4 of 8)**

PRIMER: AA3							
STRAIN TYPE: RESISTANT							
LANE M: pGEM-digested markers							
MOLECULAR WEIGHTS OF BANDS							
35	36	37	38	39	40	41	42
1802	1440	1440	1440	1440	1440	1440	1440
1440	1150	1150	1203	1203	1203	1203	1203
1150	840	840	840	840	1052	879	879
1075	374	734	642	614	840	561	768
840	614	512	490	490	768	274	490
734	490	392	392	392	614		
642		262	262	262	490		
490				392			
392				262			

Figure 61: **RAPD-DNA Fingerprinting Using Primer AA3 (Part 5 of 8)**

PRIMER: AA3

STRAIN TYPE: SUSCEPTIBLE

LANE M: pGEM-digested markers

MOLECULAR WEIGHTS OF BANDS

1	2	3	4	5	6	7	8	9	10	11	12	13
1069	1484	1790	1484	1876	1630	1416	2061	1555	1120	1416	1416	1289
1020	1174	1484	1416	1555	1416	1289	1790	1416	886	1289	1289	1120
845	886	1120	1120	1289	1289	1120	1484	1289	734	1069	1069	886
770	807	886	929	1174	1120	886	1289	1174	638	886	886	807
701	701	807	807	1069	1069	807	1120	1020	529	770	807	669
638	529	669	701	886	770	669	929	886	399	669	669	529
505	418	554	638	770	669	529	807	807		529	529	418
399		438	554	669	609	418	669	669		482	418	
		262	418	529	529	381	529	529		418	262	
			262	418	438	262	418	418				
					363		381	363				
					274		262	301				

Appendix VI: RAPD: Agarose Gel Photographs and Tabulations



Figure 62: **RAPD-DNA Fingerprinting Using Primer AA3 (Part 6 of 8)**

**PRIMER: AA3**

**STRAIN TYPE: SUSCEPTIBLE**

**LANE M: pGEM-digested markers**

**MOLECULAR WEIGHTS OF BANDS**

14	15	16	17	18	19	20	21	22	23	24	25	26
1789	1250	1250	1495	1367	1307	1250	1495	1367	1307	1143	1045	1789
1495	1093	1045	1250	1093	1195	1045	1250	1143	1143	913	835	1495
1250	873	873	1093	873	1045	873	1143	913	873	764	764	1195
1093	799	764	1045	764	873	764	873	799	730	638	638	1045
835	668	668	873	668	799	668	764	730	668	534	584	955
764	558	534	799	610	698	638	668	558	510	273	510	835
610	427		668	510	558	534	534	446			390	764
534			534	408		427	408	273			273	638
668			427	373								510
273			273	273								261

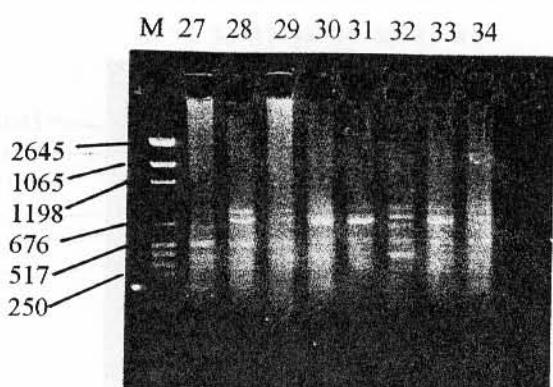


Figure 63: RAPD-DNA Fingerprinting Using Primer AA3 (Part 7 of 8)

PRIMER: AA3							
STRAIN TYPE: SUSCEPTIBLE							
LANE M: pGEM-digested markers							
MOLECULAR WEIGHTS OF BANDS							
27	28	29	30	31	32	33	34
1439	818	818	818	818	865	818	818
1285	730	730	690	690	730	690	730
1148	520	520	520	520	652	520	616
520		415			520		520
415					415		
370							
313							
264							

Appendix VI: RAPD: Agarose Gel Photographs and Tabulations

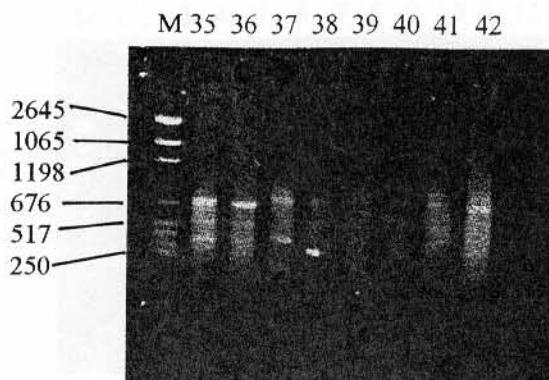
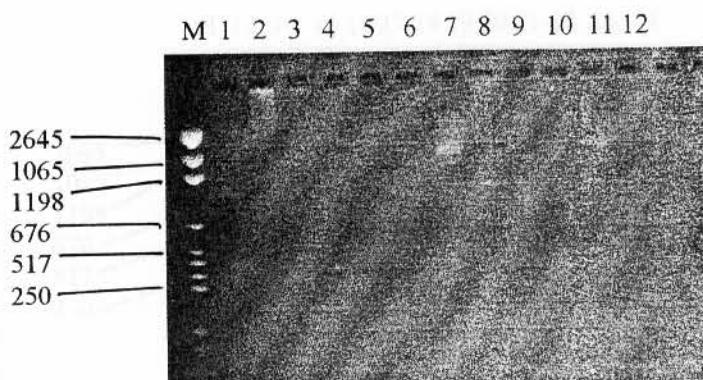


Figure 64: RAPD-DNA Fingerprinting Using Primer AA3 (Part 8 of 8)

<b>PRIMER: AA3</b>							
<b>STRAIN TYPE: SUSCEPTIBLE</b>							
<b>LANE M: pGEM-digested markers</b>							
<b>MOLECULAR WEIGHTS OF BANDS</b>							
35	36	37	38	39	40	41	42
738	872	825	2512	825	698	1288	1288
660	738	738	2126	738	558	1089	1030
472	500	528	500	624	500	872	825
378		400	400	472		738	660
338		256				590	472
						528	
						423	



**Figure 65: RAPD-DNA Fingerprinting Using Primer AA4 (Part 1 of 8)**

**PRIMER: AA4**

**STRAIN TYPE: RESISTANT**

**LANE M: pGEM-digested markers**

**MOLECULAR WEIGHTS OF BANDS**

1	2	3	4	5	6	7	8	9	10	11	12
1435						2142		1667	1843	1667	
1298						1753		1508	1435	1298	
1062						1586		1298	1234	1062	
914						1435		1174	1117	961	
806						1298		1010	961	786	
						1117		827	869		
						1010			748		

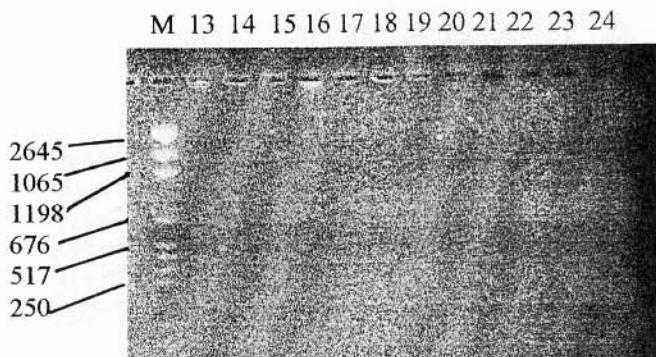


Figure 66: RAPD-DNA Fingerprinting Using Primer AA4 (Part 2 of 8)

PRIMER: AA4												
STRAIN TYPE: RESISTANT												
LANE M: pGEM-digested markers												
MOLECULAR WEIGHTS OF BANDS												
13	14	15	16	17	18	19	20	21	22	23	24	
		1690										
		1533										
		1143										
		1037										

Appendix VI: RAPD: Agarose Gel Photographs and Tabulations

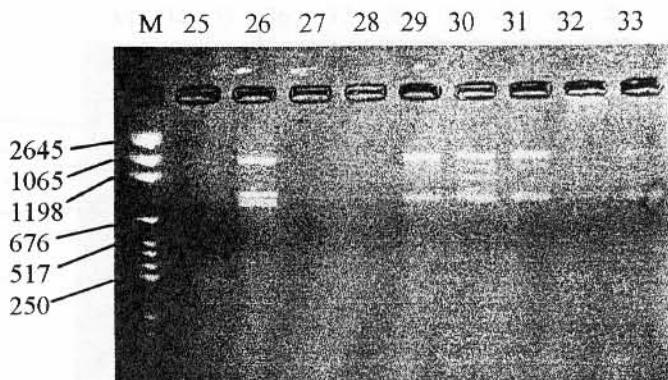


Figure 67: RAPD-DNA Fingerprinting Using Primer AA4 (Part 3 of 8)

PRIMER: AA4								
STRAIN TYPE: RESISTANT								
LANE M: pGEM-digested markers								
MOLECULAR WEIGHTS OF BANDS								
25	26	27	28	29	30	31	32	33
1518	1603			1603	1603	1603		1603
1361	1361			1399	1361	1361		1221
1037	1221			1037	1157	1221		1037
930	1037			881	982	1157		881
	982				881	982		
	881					881		

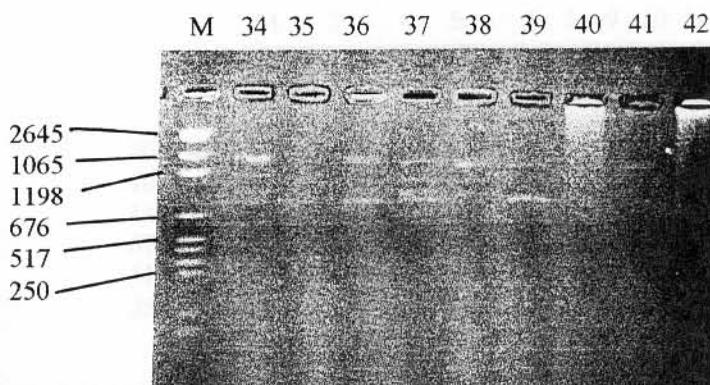


Figure 68: **RAPD-DNA Fingerprinting Using Primer AA4 (Part 4 of 8)**

**PRIMER: AA4**

**STRAIN TYPE: RESISTANT**

**LANE M: pGEM-digested markers**

**MOLECULAR WEIGHTS OF BANDS**

34	35	36	37	38	39	40	41	42
1947		1661	1575	1661			1575	
1661		1575	1417	1087				
1344		1031	1274					
1031		879	1087					
879			978					
791			791					

Appendix VI: RAPD: Agarose Gel Photographs and Tabulations

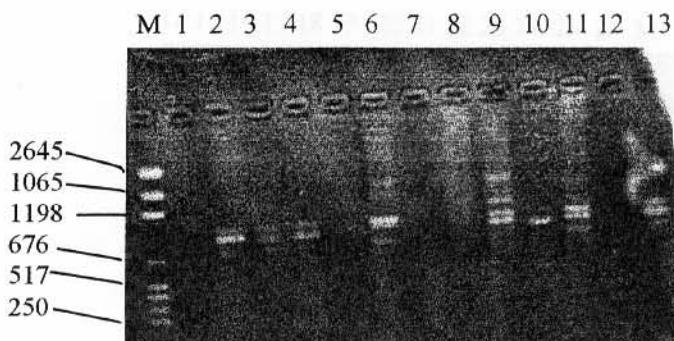


Figure 69: RAPD-DNA Fingerprinting Using Primer AA4 (Part 5 of 8)

**PRIMER: AA4**

**STRAIN TYPE: SUSCEPTIBLE**

**LANE M: pGEM-digested markers**

**MOLECULAR WEIGHTS OF BANDS**

1	2	3	4	5	6	7	8	9	10	11	12	13
1402	1039	1712	1549	1628				1712	989	940		989
1039	895	1474		1402				1148	851	851		895
940	770			989				1039		733		770
770				770				895				

Appendix VI: RAPD: Agarose Gel Photographs and Tabulations



Figure 70: RAPD-DNA Fingerprinting Using Primer AA4 (Part 6 of 8)

**PRIMER: AA4**

**STRAIN TYPE: SUSCEPTIBLE**

**LANE M: pGEM-digested markers**

**MOLECULAR WEIGHTS OF BANDS**

14	15	16	17	18	19	20	21	22	23	24	25	26
1674	1504	1504	1031	1031	1031				977	1031	1674	1587
1504	1279	1279	926	926	926						1425	1212
1279	1089	1089		832	832						1031	977
1089	832	788									926	788
926												
788												

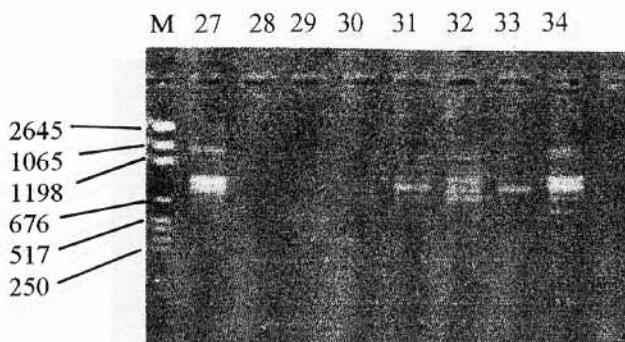


Figure 71: RAPD-DNA Fingerprinting Using Primer AA4 (Part 7 of 8)

PRIMER: AA4							
STRAIN TYPE: SUSCEPTIBLE							
LANE M: pGEM-digested markers							
MOLECULAR WEIGHTS OF BANDS							
27	28	29	30	31	32	33	34
2186			822	874	1515	822	1425
1611			727		987		929
1341					929		822
929					822		684
822					727		570
309							

Appendix VI: RAPD: Agarose Gel Photographs and Tabulations

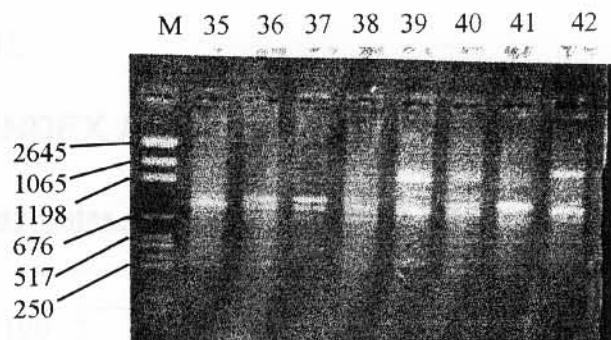


Figure 72: RAPD-DNA Fingerprinting Using Primer AA4 (Part 8 of 8)

PRIMER: AA4							
STRAIN TYPE: SUSCEPTIBLE							
LANE M: pGEM-digested markers							
MOLECULAR WEIGHTS OF BANDS							
35	36	37	38	39	40	41	42
1126	994	994	1708	1608	1608	1608	1514
1164	881	881	1426	1342	1426	1426	1342
1121	781	781	1264	1121	1121	936	936
994			1055	936	994	781	881
830			881	830	830	544	735
			781	379	379	336	
				317			

**APPENDIX VII:****SIMILARITY INDEX AND PERCENT MATCH MATRIXES****Table 1: Similarity Index of Samples Based on Primer A2**

R1	100																
R2	53	100															
R3	47	33	100														
R4	53	40	50	100													
R5	38	36	46	36	100												
R7	38	55	77	55	50	100											
R15	50	18	31	55	50	33	100										
R16	47	33	57	67	62	62	62	100									
R17	71	33	29	33	46	15	62	29	100								
R18	73	24	53	35	44	33	56	42	74	100							
R20	53	20	33	60	36	36	91	67	50	47							
R22	43	22	55	44	20	40	60	55	36	38							
R25	31	0	20	25	44	22	67	20	40	40							
R27	25	18	15	36	33	17	67	46	46	33							
R28	15	25	40	25	22	22	22	20	20	27							
R50	31	50	40	50	22	22	22	20	20	27							
R53	43	22	36	44	20	20	40	36	55	50							
R55	17	29	44	29	25	25	25	22	22	29							
R56	17	29	44	29	25	25	25	22	22	29							
R57	53	29	38	57	40	27	53	50	38	67							
R62	57	22	55	44	20	32	40	36	55	50							
R63	17	29	44	29	25	35	25	22	22	29							
R65	33	29	44	57	25	34	50	44	22	29							
R66	60	27	71	40	50	40	38	47	47	64							
R71	70	53	47	53	63	25	63	59	59	64							
R74	74	29	50	43	53	50	80	63	75	67							
R76	74	43	63	43	53	50	53	38	50	67							
R77	76	38	56	25	47	50	47	33	67	78							
R78	78	46	53	46	57	40	71	53	80	70							
R79	40	0	50	40	0	40	36	33	33	47							
R83	63	55	46	55	50	35	50	46	31	44							
R84	59	33	57	33	46	43	62	57	43	53							
R85	59	33	43	50	62	36	92	71	57	53							
R91	63	36	46	55	67	50	83	62	62	56							
R93	38	36	46	73	33	46	50	62	15	22							
R94	50	18	31	55	17	46	50	62	46	44							
R95	50	55	62	55	33	50	33	46	15	33							
	<b>R1</b>	<b>R2</b>	<b>R3</b>	<b>R4</b>	<b>R5</b>	<b>R7</b>	<b>R15</b>	<b>R16</b>	<b>R17</b>	<b>R18</b>							

Appendix VII: Similarity Index and Percent Match Matrixes

---

<b>R98</b>	50	36	31	36	33	50	50	31	46	44
<b>R99</b>	56	31	27	46	57	33	57	53	40	50
<b>R103</b>	40	20	33	20	18	50	18	33	0	24
<b>R105</b>	27	40	33	40	18	33	36	33	17	24
<b>S1</b>	40	40	67	40	36	29	36	50	17	35
<b>S2</b>	38	36	77	55	50	36	33	62	15	33
<b>S5</b>	38	36	62	36	50	18	33	62	15	44
<b>S6</b>	14	22	36	22	40	55	20	36	18	38
<b>S7</b>	38	36	62	36	50	67	33	62	15	44
<b>S8</b>	29	22	55	44	20	50	40	36	18	38
<b>S9</b>	14	0	55	44	40	20	20	55	0	38
<b>S11</b>	63	29	63	43	40	50	40	63	38	67
<b>S13</b>	38	0	62	55	33	40	33	62	31	56
<b>S14</b>	14	22	36	22	40	40	40	55	18	38
<b>S15</b>	14	0	55	44	40	33	20	55	0	38
<b>S16</b>	35	33	71	50	62	20	31	71	14	42
<b>S17</b>	50	36	62	55	50	40	50	77	31	56
<b>S20</b>	59	33	71	33	46	62	31	57	43	63
<b>S21</b>	40	20	67	40	18	50	36	33	33	47
<b>S23</b>	40	40	50	40	36	46	18	50	0	35
<b>S24</b>	53	29	63	43	40	36	40	63	38	57
<b>S27</b>	29	44	36	44	20	36	40	36	18	25
<b>S28</b>	27	0	17	20	18	40	36	17	50	47
<b>S29</b>	38	18	31	0	17	0	17	31	15	33
<b>S30</b>	38	0	46	55	17	17	50	62	31	44
<b>S31</b>	38	55	15	36	50	33	33	31	46	44
<b>S32</b>	27	40	17	20	36	17	18	17	33	47
<b>S33</b>	40	20	33	40	36	0	18	50	17	47
<b>S34</b>	40	40	33	60	36	36	36	50	33	47
<b>S35</b>	29	44	36	44	40	18	20	36	18	38
<b>S36</b>	43	44	55	44	20	20	40	36	36	50
<b>S37</b>	31	25	60	50	22	44	44	40	20	40
<b>S38</b>	33	29	22	0	25	25	0	22	0	14
<b>S39</b>	56	46	40	46	29	29	43	53	40	60
<b>S40</b>	27	40	0	20	36	0	18	17	33	35
<b>S44</b>	25	36	15	18	33	0	17	15	31	44
<b>S45</b>	29	0	36	22	0	20	40	36	0	38
<b>S48</b>	14	22	36	0	40	20	0	36	0	25
<b>S49</b>	43	44	55	67	20	40	40	36	18	38
<b>S50</b>	38	36	15	36	33	17	0	31	15	33
<b>S51</b>	38	36	15	36	33	17	0	31	15	33
<b>S52</b>	38	36	15	36	33	17	0	31	15	33
<b>S54</b>	38	36	15	36	33	17	0	31	15	33
<b>R1</b>	<b>R2</b>	<b>R3</b>	<b>R4</b>	<b>R5</b>	<b>R7</b>	<b>R15</b>	<b>R16</b>	<b>R17</b>	<b>R18</b>	

Appendix VII: Similarity Index and Percent Match Matrixes

R20	100										
R22	67	100									
R25	50	29	100								
R27	55	40	22	100							
R28	25	29	0	44	100						
R50	25	29	0	22	67	100					
R53	44	25	0	40	57	57	100				
R55	29	33	0	25	80	80	67	100			
R56	29	33	0	25	80	80	67	100	100		
R57	43	31	33	40	33	50	46	36	36	100	
R62	44	75	29	20	29	29	50	33	33	31	
R63	29	33	0	25	80	80	67	100	100	36	
R65	57	67	40	25	40	40	33	50	50	36	
R66	27	43	31	25	31	31	43	33	33	63	
R71	53	43	46	38	15	31	29	17	17	63	
R74	71	62	50	53	17	17	46	18	18	44	
R76	43	46	50	27	33	50	31	36	36	67	
R77	38	40	43	35	43	29	40	31	31	50	
R78	62	50	55	43	18	18	33	20	20	47	
R79	40	44	25	36	25	0	22	0	0	29	
R83	36	40	44	33	22	44	20	25	25	67	
R84	50	73	40	46	20	20	18	22	22	50	
R85	83	55	60	62	20	20	36	22	22	50	
R91	73	40	67	50	22	22	40	25	25	53	
R93	55	60	22	50	44	44	20	25	25	40	
R94	55	40	22	33	0	0	40	0	0	27	
R95	36	40	22	17	44	67	40	50	50	67	
R98	55	40	22	33	22	44	40	25	25	27	
R99	46	17	55	43	18	18	17	0	0	59	
R103	20	22	25	18	25	0	0	0	0	43	
R105	40	44	0	36	50	75	44	57	57	57	
S1	40	44	25	18	50	50	44	57	57	57	
S2	36	40	22	17	44	44	40	50	50	53	
S5	36	40	22	17	44	44	40	50	50	67	
S6	22	25	0	20	57	57	50	67	67	62	
S7	36	40	22	17	44	44	40	50	50	67	
S8	44	50	29	20	57	57	50	67	67	62	
S9	22	25	29	0	29	29	25	33	33	46	
S11	43	46	17	40	50	33	46	36	36	67	
S13	36	40	22	17	22	22	40	25	25	53	
S14	44	50	0	40	57	57	50	67	67	46	
S15	22	25	29	0	29	29	25	33	33	46	
S16	33	36	20	15	40	40	36	44	44	63	
S17	55	40	22	33	44	44	60	50	50	67	
<b>R20</b>		<b>R22</b>	<b>R25</b>	<b>R27</b>	<b>R28</b>	<b>R50</b>	<b>R53</b>	<b>R55</b>	<b>R56</b>	<b>R57</b>	

Appendix VII: Similarity Index and Percent Match Matrixes

---

<b>S20</b>	33	55	20	15	40	40	55	44	44	50
<b>S21</b>	40	67	25	36	75	50	44	57	57	43
<b>S23</b>	20	22	25	0	25	50	22	29	29	57
<b>S24</b>	43	46	17	40	33	33	46	36	36	67
<b>S27</b>	44	50	0	40	57	86	50	67	67	46
<b>S28</b>	20	0	25	55	25	25	67	29	29	43
<b>S29</b>	18	20	0	33	22	22	40	25	25	27
<b>S30</b>	55	40	22	33	22	22	60	25	25	40
<b>S31</b>	18	20	22	50	22	44	20	25	25	53
<b>S32</b>	0	0	25	18	25	50	22	29	29	57
<b>S33</b>	20	22	25	0	0	0	0	0	0	43
<b>S34</b>	40	22	0	36	50	75	67	57	57	71
<b>S35</b>	22	25	0	20	57	86	50	67	67	62
<b>S36</b>	44	50	29	20	57	57	50	67	67	46
<b>S37</b>	50	57	33	22	67	67	57	80	80	50
<b>S38</b>	0	0	0	0	0	0	0	0	0	18
<b>S39</b>	46	50	18	29	36	55	50	40	40	59
<b>S40</b>	0	0	25	36	0	25	0	0	0	43
<b>S44</b>	0	0	22	33	22	44	20	25	25	53
<b>S45</b>	44	50	29	20	29	29	25	33	33	46
<b>S48</b>	0	0	0	20	29	29	25	33	33	31
<b>S49</b>	44	50	29	20	57	86	50	67	67	62
<b>S50</b>	0	0	0	17	0	22	0	0	0	40
<b>S51</b>	0	0	0	17	0	22	0	0	0	40
<b>S52</b>	0	0	0	17	0	22	0	0	0	40
<b>S54</b>	0	0	0	17	0	22	0	0	0	40
<b>R20</b>	<b>R22</b>	<b>R25</b>	<b>R27</b>	<b>R28</b>	<b>R50</b>	<b>R53</b>	<b>R55</b>	<b>R56</b>	<b>R57</b>	
<b>R62</b>	100									
<b>R63</b>	33	100								
<b>R65</b>	67	50	100							
<b>R66</b>	57	33	33	100						
<b>R71</b>	43	17	33	50	100					
<b>R74</b>	62	18	36	63	74	100				
<b>R76</b>	46	36	36	74	74	67	100			
<b>R77</b>	53	31	31	76	57	70	80	100		
<b>R78</b>	50	20	40	56	78	82	71	74	100	
<b>R79</b>	44	0	29	27	40	43	29	38	46	100
<b>R83</b>	40	25	50	63	63	53	80	59	57	18
<b>R84</b>	55	22	44	71	59	75	75	67	67	33
<b>R85</b>	36	22	44	47	71	88	63	56	80	33
<b>R91</b>	40	25	50	50	75	80	67	59	86	36
<b>R93</b>	40	25	50	38	38	40	40	35	29	36
<b>R94</b>	40	0	25	38	25	53	13	35	43	36
<b>R95</b>	40	50	50	63	50	40	67	47	43	18
<b>R62</b>	<b>R63</b>	<b>R65</b>	<b>R66</b>	<b>R71</b>	<b>R74</b>	<b>R76</b>	<b>R77</b>	<b>R78</b>	<b>R79</b>	

Appendix VII: Similarity Index and Percent Match Matrixes

<b>R98</b>	40	25	25	25	38	53	40	35	29	18
<b>R99</b>	17	0	20	33	78	59	59	53	63	46
<b>R103</b>	22	0	29	53	27	29	43	50	31	40
<b>R105</b>	22	57	29	40	27	29	43	25	15	0
<b>S1</b>	44	57	57	67	40	43	57	50	46	20
<b>S2</b>	40	50	50	75	38	40	53	47	43	18
<b>S5</b>	40	50	50	63	50	40	53	47	43	18
<b>S6</b>	25	67	33	43	29	15	31	27	17	0
<b>S7</b>	40	50	50	63	50	40	53	47	43	18
<b>S8</b>	50	67	67	57	29	31	46	40	33	22
<b>S9</b>	25	33	33	43	29	15	31	27	17	22
<b>S11</b>	46	36	36	63	63	56	67	70	59	57
<b>S13</b>	40	25	25	50	50	40	40	35	43	55
<b>S14</b>	25	67	33	29	29	31	31	27	17	0
<b>S15</b>	25	33	33	43	29	15	31	27	17	22
<b>S16</b>	36	44	44	71	47	38	50	44	40	17
<b>S17</b>	40	50	50	50	63	53	53	47	57	36
<b>S20</b>	73	44	44	71	59	63	63	67	53	33
<b>S21</b>	67	57	57	53	40	43	57	63	46	60
<b>S23</b>	22	29	29	40	53	29	57	38	31	20
<b>S24</b>	46	36	36	63	63	56	56	50	59	43
<b>S27</b>	25	67	33	29	29	31	46	27	17	0
<b>S28</b>	22	29	0	40	27	43	29	38	31	20
<b>S29</b>	20	25	0	50	13	40	40	47	14	0
<b>S30</b>	40	25	25	50	25	53	27	35	29	36
<b>S31</b>	20	25	25	25	50	27	40	35	43	0
<b>S32</b>	0	29	0	27	40	14	43	38	31	0
<b>S33</b>	22	0	29	40	27	14	29	38	31	20
<b>S34</b>	22	57	29	27	53	29	43	25	31	20
<b>S35</b>	25	67	33	29	43	15	46	27	17	0
<b>S36</b>	50	67	67	43	29	31	46	53	50	22
<b>S37</b>	57	80	80	46	31	33	50	43	36	25
<b>S38</b>	0	0	0	33	17	18	36	31	20	0
<b>S39</b>	50	40	40	44	44	47	47	53	38	15
<b>S40</b>	0	0	0	13	40	14	29	25	31	0
<b>S44</b>	0	25	0	25	38	13	40	35	29	0
<b>S45</b>	25	33	33	43	14	31	46	40	17	22
<b>S48</b>	0	33	0	29	29	15	31	27	17	0
<b>S49</b>	50	67	67	43	43	31	62	40	33	22
<b>S50</b>	0	0	0	25	25	0	27	24	14	0
<b>S51</b>	0	0	0	25	25	0	27	24	14	0
<b>S52</b>	0	0	0	25	25	0	27	24	14	0
<b>S54</b>	0	0	0	25	25	0	27	24	14	0
<b>R62</b>	<b>R63</b>	<b>R65</b>	<b>R66</b>	<b>R71</b>	<b>R74</b>	<b>R76</b>	<b>R77</b>	<b>R78</b>	<b>R79</b>	

Appendix VII: Similarity Index and Percent Match Matrixes

---

<b>R83</b>	100									
<b>R84</b>	77	100								
<b>R85</b>	62	71	100							
<b>R91</b>	67	62	92	100						
<b>R93</b>	50	46	46	33	100					
<b>R94</b>	17	31	46	33	50	100				
<b>R95</b>	67	46	46	50	50	17	100			
<b>R98</b>	33	31	46	33	50	33	33	100		
<b>R99</b>	57	40	67	71	43	29	43	29	100	
<b>R103</b>	55	50	33	36	36	18	55	0	46	100
<b>R105</b>	36	33	33	18	55	18	73	55	15	20
<b>S1</b>	55	50	50	55	36	18	91	18	31	60
<b>S2</b>	50	46	46	50	50	33	83	17	29	55
<b>S5</b>	50	46	46	50	33	17	83	17	43	55
<b>S6</b>	20	18	18	20	20	0	60	20	17	22
<b>S7</b>	50	46	46	50	33	17	83	17	43	55
<b>S8</b>	40	36	36	40	40	20	80	20	17	44
<b>S9</b>	20	18	18	20	40	40	40	0	33	22
<b>S11</b>	53	63	50	53	40	27	53	13	59	57
<b>S13</b>	17	31	31	33	33	50	33	0	43	18
<b>S14</b>	20	36	36	20	40	20	40	40	17	0
<b>S15</b>	20	18	18	20	40	40	40	0	33	22
<b>S16</b>	46	43	43	46	46	31	77	15	40	50
<b>S17</b>	50	46	62	67	33	33	67	17	57	36
<b>S20</b>	46	57	43	46	31	31	62	31	40	33
<b>S21</b>	36	50	33	36	55	18	55	18	31	40
<b>S23</b>	55	33	33	36	36	18	73	18	62	40
<b>S24</b>	40	50	50	53	27	27	67	13	47	43
<b>S27</b>	40	36	36	20	60	20	60	60	17	0
<b>S28</b>	18	17	33	36	0	36	18	18	31	0
<b>S29</b>	33	46	31	17	17	33	33	33	14	36
<b>S30</b>	17	31	46	33	50	83	33	33	29	18
<b>S31</b>	50	31	31	33	33	17	33	33	43	0
<b>S32</b>	36	17	17	18	18	18	36	18	46	0
<b>S33</b>	36	33	17	18	36	55	18	0	31	40
<b>S34</b>	36	17	33	36	36	18	55	36	46	0
<b>S35</b>	40	18	18	20	40	0	60	40	33	0
<b>S36</b>	40	36	36	40	40	40	60	20	17	22
<b>S37</b>	44	40	40	44	44	22	67	22	18	25
<b>S38</b>	50	44	22	25	0	0	25	0	20	57
<b>S39</b>	43	40	40	29	57	57	57	57	38	15
<b>S40</b>	36	17	17	18	18	18	18	18	46	0
<b>S44</b>	33	15	15	17	17	17	33	17	43	0
<b>S45</b>	40	55	36	20	40	40	40	20	17	44
<b>R83</b>	<b>R84</b>	<b>R85</b>	<b>R91</b>	<b>R93</b>	<b>R94</b>	<b>R95</b>	<b>R98</b>	<b>R99</b>	<b>R103</b>	

Appendix VII: Similarity Index and Percent Match Matrixes

<b>S48</b>	20	18	18	20	0	0	40	0	33	22
<b>S49</b>	60	36	36	40	60	20	80	40	33	22
<b>S50</b>	33	15	0	0	33	33	17	17	29	18
<b>S51</b>	33	15	0	0	33	33	17	17	29	18
<b>S52</b>	33	15	0	0	33	33	17	17	29	18
<b>S54</b>	33	15	0	0	33	33	17	17	29	18
<b>R83</b>	<b>R84</b>	<b>R85</b>	<b>R91</b>	<b>R93</b>	<b>R94</b>	<b>R95</b>	<b>R98</b>	<b>R99</b>	<b>R103</b>	
<b>R105</b>	100									
<b>S1</b>	60	100								
<b>S2</b>	55	91	100							
<b>S5</b>	55	91	83	100						
<b>S6</b>	67	67	60	80	100					
<b>S7</b>	55	91	83	100	80	100				
<b>S8</b>	67	89	80	80	75	80	100			
<b>S9</b>	22	44	60	60	50	60	50	100		
<b>S11</b>	29	57	53	67	46	67	46	46	100	
<b>S13</b>	18	36	50	50	40	50	40	80	57	100
<b>S14</b>	67	44	40	60	75	60	50	50	46	40
<b>S15</b>	22	44	60	60	50	60	50	100	46	80
<b>S16</b>	50	83	92	92	73	92	73	73	63	62
<b>S17</b>	36	73	67	83	60	83	60	60	80	67
<b>S20</b>	33	67	62	77	55	77	55	55	75	62
<b>S21</b>	40	60	55	55	44	55	67	44	71	55
<b>S23</b>	40	60	55	73	44	73	44	67	57	55
<b>S24</b>	43	71	67	80	62	80	62	46	78	67
<b>S27</b>	89	44	40	40	50	40	50	25	31	20
<b>S28</b>	20	20	18	18	22	18	22	22	29	36
<b>S29</b>	36	36	33	33	20	33	20	20	40	17
<b>S30</b>	36	36	50	33	20	33	40	60	40	67
<b>S31</b>	36	18	17	33	40	33	20	20	27	17
<b>S32</b>	40	20	18	36	44	36	22	44	29	36
<b>S33</b>	0	20	36	36	22	36	22	67	43	55
<b>S34</b>	60	40	36	55	67	55	44	44	57	55
<b>S35</b>	67	44	40	60	75	60	50	50	46	40
<b>S36</b>	44	67	60	60	50	60	75	50	46	40
<b>S37</b>	50	75	67	67	57	67	86	57	50	44
<b>S38</b>	0	29	25	25	0	25	0	0	36	0
<b>S39</b>	62	46	43	57	50	57	50	50	47	43
<b>S40</b>	20	0	0	18	22	18	0	22	14	18
<b>S44</b>	36	18	17	33	40	33	20	40	27	33
<b>S45</b>	44	44	40	40	25	40	50	50	46	40
<b>S48</b>	22	44	40	60	50	60	25	50	46	40
<b>S49</b>	67	67	60	60	50	60	75	50	46	40
<b>S50</b>	18	0	17	17	20	17	0	40	27	33
<b>R105</b>	<b>S1</b>	<b>S2</b>	<b>S5</b>	<b>S6</b>	<b>S7</b>	<b>S8</b>	<b>S9</b>	<b>S11</b>	<b>S13</b>	

Appendix VII: Similarity Index and Percent Match Matrixes

S51	18	0	17	17	20	17	0	40	27	33
S52	18	0	17	17	20	17	0	40	27	33
S54	18	0	17	17	20	17	0	40	27	33
R105	S1	S2	S5	S6	S7	S8	S9	S11	S13	
S14	100									
S15	50	100								
S16	55	73	100							
S17	60	60	77	100						
S20	55	55	71	77	100					
S21	44	44	50	55	67	100				
S23	44	67	67	73	67	40	100			
S24	46	46	75	80	75	57	57	100		
S27	75	25	36	40	36	44	44	31	100	
S28	22	22	17	36	33	20	20	43	22	100
S29	40	20	31	33	46	18	36	40	40	55
S30	40	60	46	50	46	36	36	40	40	55
S31	40	20	31	33	31	18	36	40	40	36
S32	44	44	33	36	33	20	60	29	44	40
S33	22	67	50	36	33	20	40	29	0	0
S34	67	44	50	73	50	40	60	57	67	40
S35	75	50	55	60	55	44	67	46	75	22
S36	50	50	55	60	55	67	44	46	50	22
S37	57	57	60	67	60	75	50	50	57	25
S38	0	0	22	25	22	0	29	18	0	0
S39	67	50	53	57	67	46	62	47	67	31
S40	22	22	17	18	17	0	40	29	22	40
S44	40	40	31	33	31	18	55	40	40	55
S45	50	50	36	40	36	44	44	31	50	22
S48	50	50	55	60	55	22	67	62	25	44
S49	50	50	55	60	55	67	67	46	75	22
S50	20	40	31	17	15	0	36	27	20	18
S51	20	40	31	17	15	0	36	27	20	18
S52	20	40	31	17	15	0	36	27	20	18
S54	20	40	31	17	15	0	36	27	20	18
S14	S15	S16	S17	S20	S21	S23	S24	S27	S28	
S29	100									
S30	50	100								
S31	17	0	100							
S32	18	18	73	100						
S33	18	36	36	40	100					
S34	18	36	55	60	20	100				
S35	20	20	60	67	22	89	100			
S36	20	40	40	44	44	44	50	100		
S37	22	44	22	25	25	50	57	86	100	
S29	S30	S31	S32	S33	S34	S35	S36	S37		

Appendix VII: Similarity Index and Percent Match Matrixes

S38	50	0	0	0	29	0	0	0	0	100
S39	43	57	57	62	46	62	67	67	55	0
S40	18	0	91	80	40	40	44	22	0	0
S44	33	17	83	91	36	55	60	40	22	0
S45	60	60	0	22	44	22	25	50	57	33
S48	60	20	40	44	22	44	50	25	29	33
S49	20	40	40	44	22	67	75	75	86	0
S50	33	17	67	55	73	36	40	20	0	25
S51	33	17	67	55	73	36	40	20	0	25
S52	33	17	67	55	73	36	40	20	0	25
S54	33	17	67	55	73	36	40	20	0	25
	<b>S29</b>	<b>S30</b>	<b>S31</b>	<b>S32</b>	<b>S33</b>	<b>S34</b>	<b>S35</b>	<b>S36</b>	<b>S37</b>	<b>S38</b>
S39	100									
S40	46	100								
S44	57	91	100							
S45	50	0	20	100						
S48	33	44	60	25	100					
S49	67	22	40	50	25	100				
S50	43	73	67	20	40	20	100			
S51	43	73	67	20	40	20	100	100		
S52	43	73	67	20	40	20	100	100	100	
S54	43	73	67	20	40	20	100	100	100	100
	<b>S39</b>	<b>S40</b>	<b>S44</b>	<b>S45</b>	<b>S48</b>	<b>S49</b>	<b>S50</b>	<b>S51</b>	<b>S52</b>	<b>S54</b>

**Table 2: Percent Matching of Samples Based on Primer A2**

R1	100									
R2	65	100								
R3	55	60	100							
R4	65	70	70	100						
R5	50	65	65	65	100					
R7	50	75	85	75	70	100				
R15	60	55	55	75	70	60	100			
R16	55	60	70	80	75	75	75	100		
R17	75	60	50	60	65	45	75	50	100	
R18	70	35	55	45	50	40	60	45	75	100
R20	65	60	60	80	65	65	95	80	70	55
R22	60	65	75	75	60	70	80	75	65	50
R25	55	60	60	70	75	65	85	60	70	55
R27	40	55	45	65	60	50	80	65	65	40
R28	45	70	70	70	65	65	65	60	60	45
R50	55	80	70	80	65	65	65	60	60	45
R53	60	65	65	75	60	60	70	65	75	60
R55	50	75	75	75	70	70	70	65	65	50
	<b>R1</b>	<b>R2</b>	<b>R3</b>	<b>R4</b>	<b>R5</b>	<b>R7</b>	<b>R15</b>	<b>R16</b>	<b>R17</b>	<b>R18</b>

**Appendix VII: Similarity Index and Percent Match Matrixes**

<b>R56</b>	50	75	75	75	70	70	70	65	65	50
<b>R57</b>	55	50	50	70	55	45	65	60	50	65
<b>R62</b>	70	65	75	75	60	70	70	65	75	60
<b>R63</b>	50	75	75	75	70	70	70	65	65	50
<b>R65</b>	60	75	75	85	70	80	80	75	65	50
<b>R66</b>	60	45	75	55	60	60	50	55	55	60
<b>R71</b>	70	65	55	65	70	60	70	65	65	60
<b>R74</b>	75	50	60	60	65	55	85	70	80	65
<b>R76</b>	75	60	70	60	65	55	65	50	60	65
<b>R77</b>	75	50	60	40	55	45	55	40	70	75
<b>R78</b>	80	65	65	65	70	60	80	65	85	70
<b>R79</b>	55	50	70	70	45	65	65	60	60	55
<b>R83</b>	70	75	65	75	70	70	70	65	55	50
<b>R84</b>	65	60	70	60	65	65	75	70	60	55
<b>R85</b>	65	60	60	70	75	65	95	80	70	55
<b>R91</b>	70	65	65	75	80	70	90	75	75	60
<b>R93</b>	50	65	65	85	60	70	70	75	45	30
<b>R94</b>	60	55	55	75	50	60	70	75	65	50
<b>R95</b>	60	75	75	75	60	70	60	65	45	40
<b>R98</b>	60	65	55	65	60	60	70	55	65	50
<b>R99</b>	60	55	45	65	70	50	70	65	55	50
<b>R103</b>	55	60	60	60	55	65	55	60	40	35
<b>R105</b>	45	70	60	70	55	55	65	60	50	35
<b>S1</b>	55	70	80	70	65	75	65	70	50	45
<b>S2</b>	50	65	85	75	70	80	60	75	45	40
<b>S5</b>	50	65	75	65	70	70	60	75	45	50
<b>S6</b>	40	65	65	65	70	60	60	65	55	50
<b>S7</b>	50	65	75	65	70	70	60	75	45	50
<b>S8</b>	50	65	75	75	60	70	70	65	55	50
<b>S9</b>	40	55	75	75	70	70	60	75	45	50
<b>S11</b>	65	50	70	60	55	55	55	70	50	65
<b>S13</b>	50	45	75	75	60	60	60	75	55	60
<b>S14</b>	40	65	65	65	70	60	70	75	55	50
<b>S15</b>	40	55	75	75	70	70	60	75	45	50
<b>S16</b>	45	60	80	70	75	75	55	80	40	45
<b>S17</b>	60	65	75	75	70	70	70	85	55	60
<b>S20</b>	65	60	80	60	65	65	55	70	60	65
<b>S21</b>	55	60	80	70	55	65	65	60	60	55
<b>S23</b>	55	70	70	70	65	65	55	70	40	45
<b>S24</b>	55	50	70	60	55	55	55	70	50	55
<b>S27</b>	50	75	65	75	60	60	70	65	55	40
<b>S28</b>	45	50	50	60	55	45	65	50	70	55
<b>S29</b>	50	55	55	45	50	50	50	55	45	40
<b>S30</b>	50	45	65	75	50	60	70	75	55	50
<b>R1</b>	<b>R2</b>	<b>R3</b>	<b>R4</b>	<b>R5</b>	<b>R7</b>	<b>R15</b>	<b>R16</b>	<b>R17</b>	<b>R18</b>	

Appendix VII: Similarity Index and Percent Match Matrixes

<b>S31</b>	50	75	45	65	70	50	60	55	65	50
<b>S32</b>	45	70	50	60	65	45	55	50	60	55
<b>S33</b>	55	60	60	70	65	65	55	70	50	55
<b>S34</b>	55	70	60	80	65	55	65	70	60	55
<b>S35</b>	50	75	65	75	70	60	60	65	55	50
<b>S36</b>	60	75	75	75	60	70	70	65	65	60
<b>S37</b>	55	70	80	80	65	75	75	70	60	55
<b>S38</b>	60	75	65	65	70	70	60	65	55	40
<b>S39</b>	60	65	55	65	50	50	60	65	55	60
<b>S40</b>	45	70	40	60	65	45	55	50	60	45
<b>S44</b>	40	65	45	55	60	40	50	45	55	50
<b>S45</b>	50	55	65	65	50	60	70	65	45	50
<b>S48</b>	40	65	65	55	70	60	50	65	45	40
<b>S49</b>	60	75	75	85	60	70	70	65	55	50
<b>S50</b>	50	65	45	65	60	50	40	55	45	40
<b>S51</b>	50	65	45	65	60	50	40	55	45	40
<b>S52</b>	50	65	45	65	60	50	40	55	45	40
<b>S54</b>	50	65	45	65	60	50	40	55	45	40
	<b>R1</b>	<b>R2</b>	<b>R3</b>	<b>R4</b>	<b>R5</b>	<b>R7</b>	<b>R15</b>	<b>R16</b>	<b>R17</b>	<b>R18</b>
<b>R20</b>	100									
<b>R22</b>	85	100								
<b>R25</b>	80	75	100							
<b>R27</b>	75	70	65	100						
<b>R28</b>	70	75	70	75	100					
<b>R50</b>	70	75	70	65	90	100				
<b>R53</b>	75	70	65	70	85	85	100			
<b>R55</b>	75	80	75	70	95	95	90	100		
<b>R56</b>	75	80	75	70	95	95	90	100	100	
<b>R57</b>	60	55	60	55	60	70	65	65	65	100
<b>R62</b>	75	90	75	60	75	75	80	80	80	55
<b>R63</b>	75	80	75	70	95	95	90	100	100	65
<b>R65</b>	85	90	85	70	85	85	80	90	90	65
<b>R66</b>	45	60	55	40	55	55	60	60	60	65
<b>R71</b>	65	60	65	50	45	55	50	50	50	65
<b>R74</b>	80	75	70	65	50	50	65	55	55	50
<b>R76</b>	60	65	70	45	60	70	55	65	65	70
<b>R77</b>	50	55	60	45	60	50	55	55	55	50
<b>R78</b>	75	70	75	60	55	55	60	60	60	55
<b>R79</b>	70	75	70	65	70	60	65	65	65	50
<b>R83</b>	65	70	75	60	65	75	60	70	70	75
<b>R84</b>	70	85	70	65	60	60	55	65	65	60
<b>R85</b>	90	75	80	75	60	60	65	65	65	60
<b>R91</b>	85	70	85	70	65	65	70	70	70	65
<b>R93</b>	75	80	65	70	75	75	60	70	70	55
	<b>R20</b>	<b>R22</b>	<b>R25</b>	<b>R27</b>	<b>R28</b>	<b>R50</b>	<b>R53</b>	<b>R55</b>	<b>R56</b>	<b>R57</b>

Appendix VII: Similarity Index and Percent Match Matrixes

---

<b>R94</b>	75	70	65	60	55	55	70	60	60	45
<b>R95</b>	65	70	65	50	75	85	70	80	80	75
<b>R98</b>	75	70	65	60	65	75	70	70	70	45
<b>R99</b>	65	50	75	60	55	55	50	50	50	65
<b>R103</b>	60	65	70	55	70	60	55	65	65	60
<b>R105</b>	70	75	60	65	80	90	75	85	85	70
<b>S1</b>	70	75	70	55	80	80	75	85	85	70
<b>S2</b>	65	70	65	50	75	75	70	80	80	65
<b>S5</b>	65	70	65	50	75	75	70	80	80	75
<b>S6</b>	65	70	65	60	85	85	80	90	90	75
<b>S7</b>	65	70	65	50	75	75	70	80	80	75
<b>S8</b>	75	80	75	60	85	85	80	90	90	75
<b>S9</b>	65	70	75	50	75	75	70	80	80	65
<b>S11</b>	60	65	50	55	70	60	65	65	65	70
<b>S13</b>	65	70	65	50	65	65	70	70	70	65
<b>S14</b>	75	80	65	70	85	85	80	90	90	65
<b>S15</b>	65	70	75	50	75	75	70	80	80	65
<b>S16</b>	60	65	60	45	70	70	65	75	75	70
<b>S17</b>	75	70	65	60	75	75	80	80	80	75
<b>S20</b>	60	75	60	45	70	70	75	75	75	60
<b>S21</b>	70	85	70	65	90	80	75	85	85	60
<b>S23</b>	60	65	70	45	70	80	65	75	75	70
<b>S24</b>	60	65	50	55	60	60	65	65	65	70
<b>S27</b>	75	80	65	70	85	95	80	90	90	65
<b>S28</b>	60	55	70	75	70	70	85	75	75	60
<b>S29</b>	55	60	55	60	65	65	70	70	70	45
<b>S30</b>	75	70	65	60	65	65	80	70	70	55
<b>S31</b>	55	60	65	70	65	75	60	70	70	65
<b>S32</b>	50	55	70	55	70	80	65	75	75	70
<b>S33</b>	60	65	70	45	60	60	55	65	65	60
<b>S34</b>	70	65	60	65	80	90	85	85	85	80
<b>S35</b>	65	70	65	60	85	95	80	90	90	75
<b>S36</b>	75	80	75	60	85	85	80	90	90	65
<b>S37</b>	80	85	80	65	90	90	85	95	95	70
<b>S38</b>	65	70	75	60	75	75	70	80	80	55
<b>S39</b>	65	70	55	50	65	75	70	70	70	65
<b>S40</b>	50	55	70	65	60	70	55	65	65	60
<b>S44</b>	45	50	65	60	65	75	60	70	70	65
<b>S45</b>	75	80	75	60	75	75	70	80	80	65
<b>S48</b>	55	60	65	60	75	75	70	80	80	55
<b>S49</b>	75	80	75	60	85	95	80	90	90	75
<b>S50</b>	45	50	55	50	55	65	50	60	60	55
<b>S51</b>	45	50	55	50	55	65	50	60	60	55
<b>S52</b>	45	50	55	50	55	65	50	60	60	55
	<b>R20</b>	<b>R22</b>	<b>R25</b>	<b>R27</b>	<b>R28</b>	<b>R50</b>	<b>R53</b>	<b>R55</b>	<b>R56</b>	<b>R57</b>

Appendix VII: Similarity Index and Percent Match Matrixes

<b>S54</b>	45	50	55	50	55	65	50	60	60	55
	<b>R20</b>	<b>R22</b>	<b>R25</b>	<b>R27</b>	<b>R28</b>	<b>R50</b>	<b>R53</b>	<b>R55</b>	<b>R56</b>	<b>R57</b>
<b>R62</b>	100									
<b>R63</b>	80	100								
<b>R65</b>	90	90	100							
<b>R66</b>	70	60	60	100						
<b>R71</b>	60	50	60	50	100					
<b>R74</b>	75	55	65	65	75	100				
<b>R76</b>	65	65	65	75	75	70	100			
<b>R77</b>	65	55	55	75	55	70	80	100		
<b>R78</b>	70	60	70	60	80	85	75	75	100	
<b>R79</b>	75	65	75	45	55	60	50	50	65	100
<b>R83</b>	70	70	80	70	70	65	85	65	70	55
<b>R84</b>	75	65	75	75	65	80	80	70	75	60
<b>R85</b>	65	65	75	55	75	90	70	60	85	60
<b>R91</b>	70	70	80	60	80	85	75	65	90	65
<b>R93</b>	70	70	80	50	50	55	55	45	50	65
<b>R94</b>	70	60	70	50	40	65	35	45	60	65
<b>R95</b>	70	80	80	70	60	55	75	55	60	55
<b>R98</b>	70	70	70	40	50	65	55	45	50	55
<b>R99</b>	50	50	60	40	80	65	65	55	70	65
<b>R103</b>	65	65	75	65	45	50	60	60	55	70
<b>R105</b>	65	85	75	55	45	50	60	40	45	50
<b>S1</b>	75	85	85	75	55	60	70	60	65	60
<b>S2</b>	70	80	80	80	50	55	65	55	60	55
<b>S5</b>	70	80	80	70	60	55	65	55	60	55
<b>S6</b>	70	90	80	60	50	45	55	45	50	55
<b>S7</b>	70	80	80	70	60	55	65	55	60	55
<b>S8</b>	80	90	90	70	50	55	65	55	60	65
<b>S9</b>	70	80	80	60	50	45	55	45	50	65
<b>S11</b>	65	65	65	65	65	60	70	70	65	70
<b>S13</b>	70	70	70	60	60	55	55	45	60	75
<b>S14</b>	70	90	80	50	50	55	55	45	50	55
<b>S15</b>	70	80	80	60	50	45	55	45	50	65
<b>S16</b>	65	75	75	75	55	50	60	50	55	50
<b>S17</b>	70	80	80	60	70	65	65	55	70	65
<b>S20</b>	85	75	75	75	65	70	70	70	65	60
<b>S21</b>	85	85	85	65	55	60	70	70	65	80
<b>S23</b>	65	75	75	55	65	50	70	50	55	60
<b>S24</b>	65	65	65	65	65	60	60	50	65	60
<b>S27</b>	70	90	80	50	50	55	65	45	50	55
<b>S28</b>	65	75	65	55	45	60	50	50	55	60
<b>S29</b>	60	70	60	60	30	55	55	55	40	45
<b>S30</b>	70	70	70	60	40	65	45	45	50	65
	<b>R62</b>	<b>R63</b>	<b>R65</b>	<b>R66</b>	<b>R71</b>	<b>R74</b>	<b>R76</b>	<b>R77</b>	<b>R78</b>	<b>R79</b>

Appendix VII: Similarity Index and Percent Match Matrixes

---

<b>S31</b>	60	70	70	40	60	45	55	45	60	45
<b>S32</b>	55	75	65	45	55	40	60	50	55	50
<b>S33</b>	65	65	75	55	45	40	50	50	55	60
<b>S34</b>	65	85	75	45	65	50	60	40	55	60
<b>S35</b>	70	90	80	50	60	45	65	45	50	55
<b>S36</b>	80	90	90	60	50	55	65	65	70	65
<b>S37</b>	85	95	95	65	55	60	70	60	65	70
<b>S38</b>	70	80	80	60	50	55	65	55	60	65
<b>S39</b>	70	70	70	50	50	55	55	55	50	45
<b>S40</b>	55	65	65	35	55	40	50	40	55	50
<b>S44</b>	50	70	60	40	50	35	55	45	50	45
<b>S45</b>	70	80	80	60	40	55	65	55	50	65
<b>S48</b>	60	80	70	50	50	45	55	45	50	55
<b>S49</b>	80	90	90	60	60	55	75	55	60	65
<b>S50</b>	50	60	60	40	40	25	45	35	40	45
<b>S51</b>	50	60	60	40	40	25	45	35	40	45
<b>S52</b>	50	60	60	40	40	25	45	35	40	45
<b>S54</b>	50	60	60	40	40	25	45	35	40	45
	<b>R62</b>	<b>R63</b>	<b>R65</b>	<b>R66</b>	<b>R71</b>	<b>R74</b>	<b>R76</b>	<b>R77</b>	<b>R78</b>	<b>R79</b>
<b>R83</b>	100									
<b>R84</b>	85	100								
<b>R85</b>	75	80	100							
<b>R91</b>	80	75	60	100						
<b>R93</b>	70	65	75	60	100					
<b>R94</b>	50	55	80	60	70	100				
<b>R95</b>	80	65	100	70	70	50	100			
<b>R98</b>	60	55	95	60	70	60	60	100		
<b>R99</b>	70	55	65	80	60	50	60	50	100	
<b>R103</b>	75	70	65	65	65	55	75	45	65	100
<b>R105</b>	65	60	65	55	75	55	85	75	45	60
<b>S1</b>	75	70	65	75	65	55	95	55	55	80
<b>S2</b>	70	65	75	70	70	60	90	50	50	75
<b>S5</b>	70	65	60	70	60	50	90	50	60	75
<b>S6</b>	60	55	60	60	60	50	80	60	50	65
<b>S7</b>	70	65	70	70	60	50	90	50	60	75
<b>S8</b>	70	65	65	70	70	60	90	60	50	75
<b>S9</b>	60	55	65	60	70	70	70	50	60	65
<b>S11</b>	65	70	55	65	55	45	65	35	65	70
<b>S13</b>	50	55	65	60	60	70	60	40	60	55
<b>S14</b>	60	65	65	60	70	60	70	70	50	55
<b>S15</b>	60	55	55	60	70	70	70	50	60	65
<b>S16</b>	65	60	60	65	65	55	85	45	55	70
<b>S17</b>	70	65	55	80	60	60	80	50	70	65
<b>S20</b>	65	70	65	65	55	55	75	55	55	60
	<b>R83</b>	<b>R84</b>	<b>R85</b>	<b>R91</b>	<b>R93</b>	<b>R94</b>	<b>R95</b>	<b>R98</b>	<b>R99</b>	<b>R103</b>

Appendix VII: Similarity Index and Percent Match Matrixes

<b>S21</b>	65	70	55	65	75	55	75	55	55	70
<b>S23</b>	75	60	60	65	65	55	85	55	75	70
<b>S24</b>	55	60	75	65	45	45	75	35	55	60
<b>S27</b>	70	65	60	60	80	60	80	80	50	55
<b>S28</b>	55	50	60	65	45	65	55	55	55	50
<b>S29</b>	60	65	60	50	50	60	60	60	40	65
<b>S30</b>	50	55	60	60	70	90	60	60	50	55
<b>S31</b>	70	55	65	60	60	50	60	60	60	45
<b>S32</b>	65	50	60	55	55	55	65	55	65	50
<b>S33</b>	65	60	55	55	65	75	55	45	55	70
<b>S34</b>	65	50	65	65	65	55	75	65	65	50
<b>S35</b>	70	55	55	60	70	50	80	70	60	55
<b>S36</b>	70	65	50	70	70	70	80	60	50	65
<b>S37</b>	75	70	50	75	75	65	85	65	55	70
<b>S38</b>	80	75	60	70	60	60	70	60	60	85
<b>S39</b>	60	55	55	50	70	70	70	70	50	45
<b>S40</b>	65	50	65	55	55	55	55	55	65	50
<b>S44</b>	60	45	70	50	50	50	60	50	60	45
<b>S45</b>	70	75	65	60	70	70	70	60	50	75
<b>S48</b>	60	55	55	60	50	50	70	50	60	65
<b>S49</b>	80	65	50	70	80	60	90	70	60	65
<b>S50</b>	60	45	35	40	60	60	50	50	50	55
<b>S51</b>	60	45	35	40	60	60	50	50	50	55
<b>S52</b>	60	45	35	40	60	60	50	50	50	55
<b>S54</b>	60	45	35	40	60	60	50	50	50	55
<b>R83</b>	<b>R84</b>	<b>R85</b>	<b>R91</b>	<b>R93</b>	<b>R94</b>	<b>R95</b>	<b>R98</b>	<b>R99</b>	<b>R103</b>	
<b>R105</b>	100									
<b>S1</b>	80	100								
<b>S2</b>	75	95	100							
<b>S5</b>	75	95	90	100						
<b>S6</b>	85	85	80	90	100					
<b>S7</b>	75	95	90	100	90	100				
<b>S8</b>	85	95	90	90	90	90	100			
<b>S9</b>	65	75	80	80	80	80	80	100		
<b>S11</b>	50	70	65	75	65	75	65	65	100	
<b>S13</b>	55	65	70	70	70	70	70	90	75	100
<b>S14</b>	85	75	70	80	90	80	80	80	65	70
<b>S15</b>	65	75	80	80	80	80	80	100	65	90
<b>S16</b>	70	90	95	95	85	95	85	85	70	75
<b>S17</b>	65	85	80	90	80	90	80	80	85	80
<b>S20</b>	60	80	75	85	75	85	75	75	80	75
<b>S21</b>	70	80	75	75	75	75	85	75	80	75
<b>S23</b>	70	80	75	85	75	85	75	85	70	75
<b>S24</b>	60	80	75	85	75	85	75	65	80	75
<b>R105</b>	<b>S1</b>	<b>S2</b>	<b>S5</b>	<b>S6</b>	<b>S7</b>	<b>S8</b>	<b>S9</b>	<b>S11</b>	<b>S13</b>	

Appendix VII: Similarity Index and Percent Match Matrixes

<b>S27</b>	95	75	70	70	80	70	80	70	55	60
<b>S28</b>	60	60	55	55	65	55	65	65	50	65
<b>S29</b>	65	65	60	60	60	60	60	60	55	50
<b>S30</b>	65	65	70	60	60	60	70	80	55	80
<b>S31</b>	65	55	50	60	70	60	60	60	45	50
<b>S32</b>	70	60	55	65	75	65	65	75	50	65
<b>S33</b>	50	60	65	65	65	65	65	85	60	75
<b>S34</b>	80	70	65	75	85	75	75	75	70	75
<b>S35</b>	85	75	70	80	90	80	80	80	65	70
<b>S36</b>	75	85	80	80	80	80	90	80	65	70
<b>S37</b>	80	90	85	85	85	85	95	85	70	75
<b>S38</b>	65	75	70	70	70	70	70	70	65	60
<b>S39</b>	75	65	60	70	70	70	70	70	55	60
<b>S40</b>	60	50	45	55	65	55	55	65	40	55
<b>S44</b>	65	55	50	60	70	60	60	70	45	60
<b>S45</b>	75	75	70	70	70	70	80	80	65	70
<b>S48</b>	65	75	70	80	80	80	70	80	65	70
<b>S49</b>	85	85	80	80	80	80	90	80	65	70
<b>S50</b>	55	45	50	50	60	50	50	70	45	60
<b>S51</b>	55	45	50	50	60	50	50	70	45	60
<b>S52</b>	55	45	50	50	60	50	50	70	45	60
<b>S54</b>	55	45	50	50	60	50	50	70	45	60
<b>R105</b>	<b>S1</b>	<b>S2</b>	<b>S5</b>	<b>S6</b>	<b>S7</b>	<b>S8</b>	<b>S9</b>	<b>S11</b>	<b>S13</b>	
<b>S14</b>	100									
<b>S15</b>	80	100								
<b>S16</b>	75	85	100							
<b>S17</b>	80	80	85	100						
<b>S20</b>	75	75	80	85	100					
<b>S21</b>	75	75	70	75	80	100				
<b>S23</b>	75	85	80	85	80	70	100			
<b>S24</b>	65	65	80	85	80	70	70	100		
<b>S27</b>	90	70	65	70	65	75	75	55	100	
<b>S28</b>	65	65	50	65	60	60	60	60	65	100
<b>S29</b>	70	60	55	60	65	55	65	55	70	75
<b>S30</b>	70	80	65	70	65	65	65	55	70	75
<b>S31</b>	70	60	55	60	55	55	65	55	70	65
<b>S32</b>	75	75	60	65	60	60	80	50	75	70
<b>S33</b>	65	85	70	65	60	60	70	50	55	50
<b>S34</b>	85	75	70	85	70	70	80	70	85	70
<b>S35</b>	90	80	75	80	75	75	85	65	90	65
<b>S36</b>	80	80	75	80	75	85	75	65	80	65
<b>S37</b>	85	85	80	85	80	90	80	70	85	70
<b>S38</b>	70	70	65	70	65	65	75	55	70	65
<b>S39</b>	80	70	65	70	75	65	75	55	80	55
<b>S14</b>	<b>S15</b>	<b>S16</b>	<b>S17</b>	<b>S20</b>	<b>S21</b>	<b>S23</b>	<b>S24</b>	<b>S27</b>	<b>S28</b>	

Appendix VII: Similarity Index and Percent Match Matrixes

---

<b>S40</b>	65	65	50	55	50	50	70	50	65	70
<b>S44</b>	70	70	55	60	55	55	75	55	70	75
<b>S45</b>	80	80	65	70	65	75	75	55	80	65
<b>S48</b>	80	80	75	80	75	65	85	75	70	75
<b>S49</b>	80	80	75	80	75	85	85	65	90	65
<b>S50</b>	60	70	55	50	45	45	65	45	60	55
<b>S51</b>	60	70	55	50	45	45	65	45	60	55
<b>S52</b>	60	70	55	50	45	45	65	45	60	55
<b>S54</b>	60	70	55	50	45	45	65	45	60	55
	<b>S14</b>	<b>S15</b>	<b>S16</b>	<b>S17</b>	<b>S20</b>	<b>S21</b>	<b>S23</b>	<b>S24</b>	<b>S27</b>	<b>S28</b>
<b>S29</b>	100									
<b>S30</b>	70	100								
<b>S31</b>	50	40	100							
<b>S32</b>	55	55	85	100						
<b>S33</b>	55	65	65	70	100					
<b>S34</b>	55	65	75	80	60	100				
<b>S35</b>	60	60	80	85	65	95	100			
<b>S36</b>	60	70	70	75	75	75	80	100		
<b>S37</b>	65	75	65	70	70	80	85	95	100	
<b>S38</b>	80	60	60	65	75	65	70	70	75	100
<b>S39</b>	60	70	70	75	65	75	80	80	75	50
<b>S40</b>	55	45	95	90	70	70	75	65	60	65
<b>S44</b>	60	50	90	95	65	75	80	70	65	60
<b>S45</b>	80	80	50	65	75	65	70	80	85	80
<b>S48</b>	80	60	70	75	65	75	80	70	75	80
<b>S49</b>	60	70	70	75	65	85	90	90	95	70
<b>S50</b>	60	50	80	75	85	65	70	60	55	70
<b>S51</b>	60	50	80	75	85	65	70	60	55	70
<b>S52</b>	60	50	80	75	85	65	70	60	55	70
<b>S54</b>	60	50	80	75	85	65	70	60	55	70
	<b>S29</b>	<b>S30</b>	<b>S31</b>	<b>S32</b>	<b>S33</b>	<b>S34</b>	<b>S35</b>	<b>S36</b>	<b>S37</b>	<b>S38</b>
<b>S39</b>	100									
<b>S40</b>	65	100								
<b>S44</b>	70	95	100							
<b>S45</b>	70	55	60	100						
<b>S48</b>	60	75	80	70	100					
<b>S49</b>	80	65	70	80	70	100				
<b>S50</b>	60	85	80	60	70	60	100			
<b>S51</b>	60	85	80	60	70	60	100	100		
<b>S52</b>	60	85	80	60	70	60	100	100	100	
<b>S54</b>	60	85	80	60	70	60	100	100	100	100
	<b>S39</b>	<b>S40</b>	<b>S44</b>	<b>S45</b>	<b>S48</b>	<b>S49</b>	<b>S50</b>	<b>S51</b>	<b>S52</b>	<b>S54</b>

**Table 3: Similarity Index of Samples Based on Primer B1**

<b>R1</b>	100										
<b>R2</b>	57	100									
<b>R3</b>	67	50	100								
<b>R5</b>	47	40	53	100							
<b>R7</b>	40	15	59	50	100						
<b>R15</b>	53	62	35	50	43	100					
<b>R16</b>	53	62	35	50	29	86	100				
<b>R17</b>	56	63	60	63	47	47	59	100			
<b>R18</b>	71	67	63	67	25	63	63	53	100		
<b>R20</b>	71	67	53	67	38	63	50	63	67	100	
<b>R22</b>	43	17	50	27	46	15	31	50	13	27	
<b>R23</b>	38	57	56	47	40	27	27	67	47	59	
<b>R25</b>	67	60	43	15	0	36	36	29	46	46	
<b>R27</b>	50	57	56	59	40	40	53	67	71	47	
<b>R28</b>	36	22	15	50	0	20	40	46	33	33	
<b>R50</b>	53	46	47	63	57	57	43	59	63	75	
<b>R53</b>	40	62	35	38	29	57	57	47	50	50	
<b>R55</b>	40	46	47	63	43	43	29	47	63	63	
<b>R56</b>	53	31	24	50	14	43	57	47	50	63	
<b>R57</b>	63	57	67	71	40	40	40	67	71	71	
<b>R62</b>	38	57	44	71	40	53	40	67	71	71	
<b>R63</b>	31	55	53	14	50	33	33	40	43	29	
<b>R65</b>	27	31	35	50	43	57	71	59	50	38	
<b>R66</b>	63	47	57	50	44	33	33	67	50	70	
<b>R71</b>	67	53	70	64	40	60	60	78	73	73	
<b>R74</b>	67	38	70	32	47	47	47	50	63	53	
<b>R76</b>	67	46	47	50	57	57	43	47	63	75	
<b>R77</b>	50	43	67	24	40	40	53	56	35	47	
<b>R78</b>	75	57	56	47	40	40	53	67	59	71	
<b>R79</b>	53	62	71	50	43	43	29	47	50	75	
<b>R83</b>	60	44	73	38	53	32	42	64	57	48	
<b>R84</b>	56	63	50	42	24	35	47	60	53	53	
<b>R85</b>	47	27	53	56	63	50	38	53	44	67	
<b>R91</b>	78	50	50	53	24	59	59	60	74	74	
<b>R93</b>	42	35	48	50	56	67	56	48	60	50	
<b>R94</b>	59	40	42	44	38	63	50	42	67	67	
<b>R95</b>	40	46	35	38	43	71	57	59	50	50	
<b>R98</b>	50	57	44	47	40	67	53	67	59	71	
<b>R99</b>	47	67	42	56	50	63	63	74	56	67	
<b>R103</b>	15	18	40	14	50	17	17	27	0	14	
<b>R105</b>	33	40	29	31	36	55	55	57	31	46	
<b>S1</b>	43	50	50	27	46	15	15	50	27	40	
<b>R1</b>	<b>R2</b>	<b>R3</b>	<b>R5</b>	<b>R7</b>	<b>R15</b>	<b>R16</b>	<b>R17</b>	<b>R18</b>	<b>R20</b>		

Appendix VII: Similarity Index and Percent Match Matrixes

---

<b>S2</b>	46	18	27	29	50	50	33	40	29	43
<b>S5</b>	50	20	29	31	55	55	36	43	31	46
<b>S6</b>	53	46	47	63	43	29	14	59	50	75
<b>S7</b>	50	40	57	31	55	18	0	43	31	46
<b>S8</b>	46	55	53	29	50	17	17	53	29	43
<b>S9</b>	53	46	47	63	43	29	14	59	50	75
<b>S10</b>	43	17	25	53	46	46	31	50	40	67
<b>S11</b>	63	43	44	59	53	40	27	67	47	82
<b>S12</b>	53	46	47	50	29	43	43	71	50	50
<b>S13</b>	80	62	47	38	29	71	71	59	63	63
<b>S14</b>	71	67	63	40	46	46	31	50	53	67
<b>S15</b>	71	50	63	53	46	31	31	63	53	53
<b>S16</b>	78	38	60	53	47	47	47	60	63	63
<b>S17</b>	71	40	53	44	38	50	50	63	44	67
<b>S20</b>	84	59	67	60	33	56	56	67	70	80
<b>S21</b>	67	38	60	63	59	59	59	70	42	63
<b>S23</b>	71	40	53	44	38	50	50	63	44	67
<b>S24</b>	67	50	50	42	35	59	59	70	53	63
<b>S25</b>	74	47	67	70	67	56	56	76	50	70
<b>S27</b>	74	47	57	60	56	56	56	67	50	70
<b>S28</b>	74	47	67	70	67	56	56	76	50	70
<b>S29</b>	78	50	60	63	59	59	59	70	53	74
<b>S30</b>	67	46	47	38	57	57	43	47	50	63
<b>S31</b>	62	55	53	43	50	33	17	53	43	57
<b>S32</b>	67	46	47	38	57	43	29	59	38	63
<b>S33</b>	67	62	47	38	43	43	57	82	50	50
<b>S34</b>	67	62	47	25	43	43	43	59	38	63
<b>S35</b>	71	40	63	33	50	38	38	63	56	44
<b>S36</b>	33	40	29	15	55	36	36	57	31	31
<b>S37</b>	36	44	15	17	40	40	40	46	33	33
<b>S38</b>	36	44	15	17	40	40	40	46	33	33
<b>S39</b>	50	40	29	31	55	55	36	43	46	46
<b>S40</b>	22	29	0	20	0	25	25	18	20	20
<b>S44</b>	40	25	0	18	22	44	44	33	18	36
<b>S45</b>	77	55	40	43	50	50	33	53	43	71
<b>S48</b>	40	25	17	36	22	44	22	17	36	36
<b>S49</b>	67	40	29	31	36	55	36	43	31	62
<b>S50</b>	55	44	46	33	40	20	0	31	33	50
<b>S51</b>	55	44	31	50	40	40	20	31	50	50
<b>S54</b>	55	22	31	33	60	40	20	31	50	50
<b>R1</b>	<b>R2</b>	<b>R3</b>	<b>R5</b>	<b>R7</b>	<b>R15</b>	<b>R16</b>	<b>R17</b>	<b>R18</b>	<b>R20</b>	
<b>R22</b>	100									
<b>R23</b>	29	100								
<b>R25</b>	20	33	100							
	<b>R22</b>	<b>R23</b>	<b>R25</b>							

Appendix VII: Similarity Index and Percent Match Matrixes

<b>R27</b>	29	75	33	100						
<b>R28</b>	22	18	29	36	100					
<b>R50</b>	15	40	18	53	20	100				
<b>R53</b>	15	67	55	67	20	43	100			
<b>R55</b>	15	40	18	53	20	86	43	100		
<b>R56</b>	46	40	36	53	60	43	43	43	100	
<b>R57</b>	29	50	33	63	36	80	53	80	40	100
<b>R62</b>	0	63	17	63	36	80	53	80	40	75
<b>R63</b>	18	62	22	62	0	33	50	33	17	31
<b>R65</b>	31	40	0	53	20	57	57	43	43	53
<b>R66</b>	35	74	53	63	29	56	56	44	56	53
<b>R71</b>	42	67	47	67	38	60	60	50	60	67
<b>R74</b>	50	56	43	56	15	47	59	35	47	56
<b>R76</b>	15	53	36	67	20	86	57	71	57	67
<b>R77</b>	57	38	33	38	18	40	40	27	40	50
<b>R78</b>	43	63	50	75	36	67	67	53	67	75
<b>R79</b>	31	53	36	27	20	43	29	43	29	53
<b>R83</b>	56	70	38	70	13	42	53	32	42	50
<b>R84</b>	38	56	43	56	46	24	47	24	47	44
<b>R85</b>	40	59	31	59	33	63	50	50	63	47
<b>R91</b>	38	56	57	56	46	47	59	35	71	56
<b>R93</b>	35	32	27	42	14	67	56	67	44	53
<b>R94</b>	27	47	46	47	33	50	50	38	63	35
<b>R95</b>	31	27	18	40	20	71	43	71	43	53
<b>R98</b>	29	50	33	50	18	80	67	67	40	75
<b>R99</b>	27	59	31	59	17	75	75	63	38	71
<b>R103</b>	55	31	22	15	0	17	50	33	17	31
<b>R105</b>	20	17	25	33	29	73	36	55	36	50
<b>S1</b>	33	43	40	43	22	46	46	46	15	57
<b>S2</b>	18	15	22	31	25	67	33	50	33	46
<b>S5</b>	20	17	25	33	29	73	36	55	36	50
<b>S6</b>	15	67	36	53	40	71	57	71	43	80
<b>S7</b>	20	50	25	33	0	55	36	55	0	67
<b>S8</b>	36	46	44	46	25	50	50	50	17	62
<b>S9</b>	15	67	36	53	40	71	57	71	43	80
<b>S10</b>	17	43	20	43	44	77	46	62	62	57
<b>S11</b>	29	63	33	50	36	80	53	67	53	75
<b>S12</b>	31	53	36	53	60	43	57	43	43	67
<b>S13</b>	46	40	55	53	40	43	57	29	57	53
<b>S14</b>	17	57	60	57	22	62	62	62	31	71
<b>S15</b>	33	43	40	57	44	62	46	62	31	86
<b>S16</b>	25	44	43	56	46	59	35	47	59	56
<b>S17</b>	40	47	46	47	50	50	50	38	63	59
<b>S20</b>	35	42	53	42	43	56	33	44	56	63
	<b>R22</b>	<b>R23</b>	<b>R25</b>	<b>R27</b>	<b>R28</b>	<b>R50</b>	<b>R53</b>	<b>R55</b>	<b>R56</b>	<b>R57</b>

Appendix VII: Similarity Index and Percent Match Matrixes

<b>S21</b>	50	56	43	56	46	47	59	35	59	56
<b>S23</b>	40	47	46	47	50	50	50	38	63	59
<b>S24</b>	38	56	43	56	46	47	59	35	59	56
<b>S25</b>	47	63	40	63	43	56	56	44	56	63
<b>S27</b>	35	53	40	53	43	56	56	44	56	63
<b>S28</b>	47	63	40	63	43	56	56	44	56	63
<b>S29</b>	38	56	43	56	46	59	59	47	59	67
<b>S30</b>	15	40	36	53	20	71	43	57	43	53
<b>S31</b>	18	46	44	46	25	67	50	67	17	77
<b>S32</b>	31	40	36	40	20	71	43	57	29	67
<b>S33</b>	46	53	36	67	40	57	57	43	43	67
<b>S34</b>	31	53	55	53	20	57	57	43	43	53
<b>S35</b>	40	47	31	47	17	50	38	38	25	59
<b>S36</b>	20	50	0	50	0	55	36	36	18	33
<b>S37</b>	22	36	0	36	0	40	20	20	20	18
<b>S38</b>	22	36	0	36	0	40	20	20	20	18
<b>S39</b>	20	33	0	33	0	55	18	36	18	33
<b>S40</b>	0	0	40	22	50	25	25	25	25	22
<b>S44</b>	25	0	33	20	40	44	22	22	44	20
<b>S45</b>	36	31	44	31	25	67	33	50	33	62
<b>S48</b>	0	0	33	20	40	44	22	44	22	40
<b>S49</b>	40	17	50	17	29	55	36	36	36	50
<b>S50</b>	22	36	29	18	0	40	20	40	0	55
<b>S51</b>	0	18	29	36	33	60	20	60	20	55
<b>S54</b>	22	18	0	18	0	60	0	40	20	36
	<b>R22</b>	<b>R23</b>	<b>R25</b>	<b>R27</b>	<b>R28</b>	<b>R50</b>	<b>R53</b>	<b>R55</b>	<b>R56</b>	<b>R57</b>
<b>R62</b>	100									
<b>R63</b>	46	100								
<b>R65</b>	53	33	100							
<b>R66</b>	53	38	33	100						
<b>R71</b>	67	33	60	75	100					
<b>R74</b>	44	53	59	57	78	100				
<b>R76</b>	67	50	43	67	60	59	100			
<b>R77</b>	25	46	53	53	57	67	40	100		
<b>R78</b>	50	46	53	74	67	67	80	63	100	
<b>R79</b>	53	50	14	44	50	47	43	53	40	100
<b>R83</b>	40	59	53	70	72	82	53	60	70	42
<b>R84</b>	44	53	24	57	52	50	35	56	56	59
<b>R85</b>	59	43	38	70	73	63	75	47	59	50
<b>R91</b>	56	27	47	67	87	80	59	44	67	47
<b>R93</b>	63	38	67	45	67	67	56	42	42	33
<b>R94</b>	59	43	38	60	73	74	63	35	47	50
<b>R95</b>	67	33	57	33	60	47	57	40	40	29
<b>R98</b>	75	31	67	53	76	67	67	50	63	40
	<b>R62</b>	<b>R63</b>	<b>R65</b>	<b>R66</b>	<b>R71</b>	<b>R74</b>	<b>R76</b>	<b>R77</b>	<b>R78</b>	<b>R79</b>

Appendix VII: Similarity Index and Percent Match Matrixes

---

<b>R99</b>	71	43	75	60	64	53	63	47	71	38
<b>R103</b>	15	40	33	25	22	40	17	46	31	33
<b>R105</b>	50	22	55	40	47	29	55	50	50	18
<b>S1</b>	43	55	15	47	32	38	46	43	57	46
<b>S2</b>	46	20	33	38	44	40	67	31	46	17
<b>S5</b>	50	22	36	40	47	43	73	33	50	18
<b>S6</b>	80	33	29	67	60	47	71	27	67	57
<b>S7</b>	50	44	18	40	35	43	55	33	50	55
<b>S8</b>	46	60	17	50	33	40	50	46	62	50
<b>S9</b>	80	33	29	67	60	47	71	27	67	57
<b>S10</b>	71	18	46	59	63	50	77	29	57	31
<b>S11</b>	75	31	40	74	67	56	80	38	75	53
<b>S12</b>	67	33	43	44	70	59	43	40	53	43
<b>S13</b>	40	33	43	44	70	71	57	53	67	43
<b>S14</b>	57	55	15	59	53	50	77	43	71	62
<b>S15</b>	57	36	31	47	53	50	62	43	71	46
<b>S16</b>	56	40	35	67	70	60	71	44	67	47
<b>S17</b>	47	29	38	60	73	63	63	59	71	50
<b>S20</b>	53	25	33	64	75	57	56	53	63	67
<b>S21</b>	44	27	47	67	78	60	59	56	67	47
<b>S23</b>	47	29	38	60	73	63	63	59	71	50
<b>S24</b>	56	40	47	57	78	70	59	56	67	47
<b>S25</b>	53	38	44	73	75	57	67	53	74	56
<b>S27</b>	53	38	44	64	67	57	67	53	74	56
<b>S28</b>	53	38	44	73	75	57	67	53	74	56
<b>S29</b>	56	40	47	67	70	60	71	56	78	59
<b>S30</b>	53	50	29	56	50	47	86	40	67	43
<b>S31</b>	62	40	17	50	44	40	67	31	62	50
<b>S32</b>	53	33	29	56	50	47	71	40	67	43
<b>S33</b>	53	50	57	56	60	59	57	53	80	29
<b>S34</b>	40	50	29	67	50	47	71	53	80	43
<b>S35</b>	47	43	50	50	64	74	50	47	59	38
<b>S36</b>	50	67	55	40	35	43	55	33	50	18
<b>S37</b>	36	50	40	29	25	31	40	18	36	20
<b>S38</b>	36	50	40	29	25	31	40	18	36	20
<b>S39</b>	50	44	36	27	35	43	55	17	33	36
<b>S40</b>	22	0	0	17	14	0	25	0	22	0
<b>S44</b>	20	0	22	31	27	17	44	20	40	0
<b>S45</b>	46	20	17	50	44	40	67	31	62	50
<b>S48</b>	40	0	0	15	27	17	44	0	20	22
<b>S49</b>	33	0	18	40	47	43	55	33	50	36
<b>S50</b>	36	25	0	29	25	31	40	18	36	60
<b>S51</b>	55	25	0	29	25	15	60	0	36	40
<b>S54</b>	36	25	20	29	25	31	60	18	36	40
	<b>R62</b>	<b>R63</b>	<b>R65</b>	<b>R66</b>	<b>R71</b>	<b>R74</b>	<b>R76</b>	<b>R77</b>	<b>R78</b>	<b>R79</b>

Appendix VII: Similarity Index and Percent Match Matrixes

---

<b>R83</b>	100									
<b>R84</b>	55	100								
<b>R85</b>	57	42	100							
<b>R91</b>	64	60	63	100						
<b>R93</b>	52	29	60	57	100					
<b>R94</b>	57	53	78	84	70	100				
<b>R95</b>	32	24	50	47	78	50	100			
<b>R98</b>	50	33	59	67	74	59	80	100		
<b>R99</b>	57	42	44	53	70	44	63	82	100	
<b>R103</b>	35	27	29	13	50	14	33	31	43	100
<b>R105</b>	25	14	46	29	53	31	73	67	62	22
<b>S1</b>	44	63	40	25	35	27	31	43	53	55
<b>S2</b>	24	27	57	40	50	43	67	62	43	20
<b>S5</b>	25	14	62	43	53	46	73	67	46	22
<b>S6</b>	42	47	63	59	44	50	43	67	63	33
<b>S7</b>	38	29	31	29	27	15	36	50	46	44
<b>S8</b>	47	53	43	27	38	29	33	46	57	60
<b>S9</b>	42	47	63	59	44	50	43	67	63	33
<b>S10</b>	33	25	80	63	59	67	62	71	53	18
<b>S11</b>	50	44	71	67	53	59	53	75	71	31
<b>S12</b>	42	59	50	71	44	50	57	67	50	33
<b>S13</b>	53	59	50	82	44	63	57	67	50	17
<b>S14</b>	44	50	53	50	35	40	46	57	53	36
<b>S15</b>	44	50	40	50	35	27	46	57	53	36
<b>S16</b>	55	60	63	70	48	63	47	44	42	13
<b>S17</b>	48	63	67	74	40	56	50	59	44	29
<b>S20</b>	52	67	50	76	45	60	44	53	50	13
<b>S21</b>	55	50	74	70	48	53	47	56	53	40
<b>S23</b>	48	63	67	74	40	56	50	59	44	29
<b>S24</b>	55	70	63	80	48	63	59	67	53	27
<b>S25</b>	61	57	70	67	45	50	44	53	60	38
<b>S27</b>	52	67	60	67	45	50	44	53	60	38
<b>S28</b>	61	57	70	67	45	50	44	53	60	38
<b>S29</b>	55	60	63	70	48	53	47	56	63	40
<b>S30</b>	42	47	63	47	44	50	57	53	50	17
<b>S31</b>	35	40	43	40	38	29	50	62	57	40
<b>S32</b>	42	47	50	47	44	38	57	67	63	33
<b>S33</b>	63	59	38	59	44	38	57	67	75	33
<b>S34</b>	53	59	50	47	33	38	43	53	63	33
<b>S35</b>	67	53	33	63	50	44	50	59	56	29
<b>S36</b>	50	29	31	29	40	31	55	50	62	22
<b>S37</b>	40	31	17	31	29	33	40	36	50	0
<b>S38</b>	40	31	17	31	29	33	40	36	50	0
<b>S39</b>	38	29	31	43	40	46	55	50	46	0
	<b>R83</b>	<b>R84</b>	<b>R85</b>	<b>R91</b>	<b>R93</b>	<b>R94</b>	<b>R95</b>	<b>R98</b>	<b>R99</b>	<b>R103</b>

Appendix VII: Similarity Index and Percent Match Matrixes

<b>S40</b>	0	18	20	18	17	20	25	22	20	0
<b>S44</b>	14	17	36	33	31	36	44	40	36	0
<b>S45</b>	35	40	43	53	38	43	50	62	57	20
<b>S48</b>	0	17	36	33	31	36	44	40	18	0
<b>S49</b>	25	29	46	57	40	46	55	67	46	22
<b>S50</b>	27	31	17	31	14	17	20	36	33	25
<b>S51</b>	13	31	33	31	29	33	40	36	33	0
<b>S54</b>	27	15	33	31	29	33	40	36	33	0
	<b>R83</b>	<b>R84</b>	<b>R85</b>	<b>R91</b>	<b>R93</b>	<b>R94</b>	<b>R95</b>	<b>R98</b>	<b>R99</b>	<b>R103</b>
<b>R105</b>	100									
<b>S1</b>	40	100								
<b>S2</b>	67	55	100							
<b>S5</b>	75	40	89	100						
<b>S6</b>	36	62	50	55	100					
<b>S7</b>	25	60	44	50	73	100				
<b>S8</b>	44	91	40	44	67	67	100			
<b>S9</b>	36	62	50	55	100	73	67	100		
<b>S10</b>	60	33	73	80	77	40	36	77	100	
<b>S11</b>	50	57	62	67	93	67	62	93	86	100
<b>S12</b>	36	46	50	55	71	55	50	71	62	67
<b>S13</b>	36	31	50	55	43	36	33	43	46	53
<b>S14</b>	40	67	55	60	77	80	73	77	50	71
<b>S15</b>	40	67	55	60	77	80	73	77	50	71
<b>S16</b>	43	50	67	57	59	43	40	59	63	67
<b>S17</b>	46	53	71	62	63	46	43	63	67	71
<b>S20</b>	40	47	50	40	56	40	38	56	47	63
<b>S21</b>	43	38	53	57	59	43	40	59	63	67
<b>S23</b>	46	53	71	62	63	46	43	63	67	71
<b>S24</b>	43	50	67	57	59	43	40	59	63	67
<b>S25</b>	40	47	50	53	67	53	50	67	59	74
<b>S27</b>	40	59	63	53	67	53	50	67	59	74
<b>S28</b>	40	47	50	53	67	53	50	67	59	74
<b>S29</b>	43	50	53	57	71	57	53	71	63	78
<b>S30</b>	55	62	83	73	57	55	50	57	62	67
<b>S31</b>	44	73	60	67	83	89	80	83	55	77
<b>S32</b>	55	77	83	73	71	73	67	71	62	80
<b>S33</b>	55	62	50	55	57	55	67	57	46	67
<b>S34</b>	55	77	67	55	57	55	67	57	46	67
<b>S35</b>	31	53	57	46	50	62	43	50	40	59
<b>S36</b>	50	40	44	50	36	50	44	36	40	50
<b>S37</b>	29	22	25	29	20	29	25	20	22	36
<b>S38</b>	29	22	25	29	20	29	25	20	22	36
<b>S39</b>	25	20	44	50	36	50	22	36	40	50
<b>S40</b>	40	29	33	40	25	0	33	25	29	22
	<b>R105</b>	<b>S1</b>	<b>S2</b>	<b>S5</b>	<b>S6</b>	<b>S7</b>	<b>S8</b>	<b>S9</b>	<b>S10</b>	<b>S11</b>

Appendix VII: Similarity Index and Percent Match Matrixes

---

<b>S44</b>	67	25	57	67	22	0	29	22	50	40
<b>S45</b>	44	55	60	67	67	67	60	67	55	77
<b>S48</b>	33	25	57	67	44	33	29	44	50	40
<b>S49</b>	50	40	67	75	55	50	44	55	60	67
<b>S50</b>	0	44	25	29	60	86	50	60	22	55
<b>S51</b>	29	44	50	57	60	57	50	60	44	55
<b>S54</b>	29	22	50	57	40	57	25	40	44	55
	<b>R105</b>	<b>S1</b>	<b>S2</b>	<b>S5</b>	<b>S6</b>	<b>S7</b>	<b>S8</b>	<b>S9</b>	<b>S10</b>	<b>S11</b>
<b>S12</b>	100									
<b>S13</b>	71	100								
<b>S14</b>	62	62	100							
<b>S15</b>	77	62	83	100						
<b>S16</b>	59	59	63	63	100					
<b>S17</b>	75	75	67	67	84	100				
<b>S20</b>	56	67	59	59	86	80	100			
<b>S21</b>	71	71	63	63	70	84	67	100		
<b>S23</b>	75	75	67	67	84	100	80	84	100	
<b>S24</b>	82	82	63	63	80	95	76	80	95	100
<b>S25</b>	67	67	71	71	76	80	73	95	80	76
<b>S27</b>	67	67	71	71	86	90	82	86	90	86
<b>S28</b>	67	67	71	71	76	80	73	95	80	76
<b>S29</b>	71	71	75	75	80	84	76	90	84	80
<b>S30</b>	43	57	77	62	82	75	67	59	75	71
<b>S31</b>	67	50	91	91	53	57	50	53	57	53
<b>S32</b>	57	57	77	77	71	75	67	59	75	71
<b>S33</b>	71	71	62	77	59	63	56	59	63	71
<b>S34</b>	43	57	77	62	71	75	67	59	75	71
<b>S35</b>	63	63	53	67	74	67	70	53	67	74
<b>S36</b>	36	36	40	40	43	31	27	29	31	43
<b>S37</b>	20	40	22	22	31	17	29	15	17	31
<b>S38</b>	20	40	22	22	31	17	29	15	17	31
<b>S39</b>	36	55	40	40	43	31	40	29	31	43
<b>S40</b>	25	25	29	29	18	20	17	18	20	18
<b>S44</b>	22	44	25	25	33	36	31	33	36	33
<b>S45</b>	50	67	73	73	53	57	63	53	57	53
<b>S48</b>	44	44	50	50	33	36	31	33	36	33
<b>S49</b>	55	73	60	60	43	62	53	57	62	57
<b>S50</b>	40	40	67	67	31	33	43	31	33	31
<b>S51</b>	40	40	67	67	46	33	43	31	33	31
<b>S54</b>	20	40	44	44	46	33	43	31	33	31
	<b>S12</b>	<b>S13</b>	<b>S14</b>	<b>S15</b>	<b>S16</b>	<b>S17</b>	<b>S20</b>	<b>S21</b>	<b>S23</b>	<b>S24</b>
<b>S25</b>	100									
<b>S27</b>	91	100								
<b>S28</b>	100	91	100							
	<b>S25</b>	<b>S27</b>	<b>S28</b>							

Appendix VII: Similarity Index and Percent Match Matrixes

---

S29	95	95	95	100						
S30	67	78	67	71	100					
S31	63	63	63	67	67	100				
S32	67	78	67	71	86	83	100			
S33	67	67	67	71	57	67	71	100		
S34	67	78	67	71	86	67	86	71	100	
S35	60	70	60	63	63	57	75	75	63	100
S36	40	40	40	43	55	44	55	73	55	62
S37	29	29	29	31	40	25	40	60	40	50
S38	29	29	29	31	40	25	40	60	40	50
S39	40	40	40	43	55	44	55	55	36	62
S40	17	17	17	18	25	33	25	25	25	0
S44	31	31	31	33	44	29	44	44	44	18
S45	63	63	63	67	67	80	83	67	67	57
S48	31	31	31	33	44	57	44	22	22	18
S49	53	53	53	57	55	67	73	55	55	46
S50	43	43	43	46	40	75	60	40	40	50
S51	43	43	43	46	60	75	60	40	40	33
S54	43	43	43	46	60	50	60	40	40	50
	S25	S27	S28	S29	S30	S31	S32	S33	S34	S35
S36	100									
S37	86	100								
S38	86	100	100							
S39	75	86	86	100						
S40	0	0	0	0	100					
S44	33	40	40	33	67	100				
S45	44	50	50	67	33	57	100			
S48	0	0	0	33	67	50	57	100		
S49	25	29	29	50	40	67	89	67	100	
S50	29	33	33	57	0	0	75	40	57	100
S51	29	33	33	57	50	40	75	80	57	67
S54	57	67	67	86	0	40	75	40	57	67
	S36	S37	S38	S39	S40	S44	S45	S48	S49	S50
S51	100									
S54	67	100								
	S51	S54								

**Table 4: Percent Matching of Samples Based on Primer B1**

R1	100									
R2	70	100								
R3	70	60	100							
R5	55	55	55	100						
R7	55	45	65	60	100					
	<b>R1</b>	<b>R2</b>	<b>R3</b>	<b>R5</b>	<b>R7</b>					

Appendix VII: Similarity Index and Percent Match Matrixes

---

<b>R15</b>	65	75	45	60	60	100				
<b>R16</b>	65	75	45	60	50	90	100			
<b>R17</b>	60	70	60	65	55	55	65	100		
<b>R18</b>	75	75	65	70	40	70	70	55	100	
<b>R20</b>	75	75	55	70	50	70	60	65	70	100
<b>R22</b>	60	50	60	45	65	45	55	60	35	45
<b>R23</b>	50	70	60	55	55	45	45	70	55	65
<b>R25</b>	80	80	60	45	45	65	65	50	65	65
<b>R27</b>	60	70	60	65	55	55	65	70	75	55
<b>R28</b>	65	65	45	70	50	60	70	65	60	60
<b>R50</b>	65	65	55	70	70	70	60	65	70	80
<b>R53</b>	55	75	45	50	50	70	70	55	60	60
<b>R55</b>	55	65	55	70	60	60	50	55	70	70
<b>R56</b>	65	55	35	60	40	60	70	55	60	70
<b>R57</b>	70	70	70	75	55	55	55	70	75	75
<b>R62</b>	50	70	50	75	55	65	55	70	75	75
<b>R63</b>	55	75	65	40	70	60	60	55	60	50
<b>R65</b>	45	55	45	60	60	70	80	65	60	50
<b>R66</b>	65	55	55	50	50	40	40	65	50	70
<b>R71</b>	65	55	65	60	40	60	60	75	70	70
<b>R74</b>	70	50	70	35	55	55	55	50	65	55
<b>R76</b>	75	65	55	60	70	70	60	55	70	80
<b>R77</b>	60	60	70	35	55	55	65	60	45	55
<b>R78</b>	80	70	60	55	55	55	65	70	65	75
<b>R79</b>	65	75	75	60	60	60	50	55	60	80
<b>R83</b>	60	50	70	35	55	35	45	60	55	45
<b>R84</b>	60	70	50	45	35	45	55	60	55	55
<b>R85</b>	55	45	55	60	70	60	50	55	50	70
<b>R91</b>	80	60	50	55	35	65	65	60	75	75
<b>R93</b>	45	45	45	50	60	70	60	45	60	50
<b>R94</b>	65	55	45	50	50	70	60	45	70	70
<b>R95</b>	55	65	45	50	60	80	70	65	60	60
<b>R98</b>	60	70	50	55	55	75	65	70	65	75
<b>R99</b>	55	75	45	60	60	70	70	75	60	70
<b>R103</b>	45	55	55	40	70	50	50	45	30	40
<b>R105</b>	60	70	50	55	65	75	75	70	55	65
<b>S1</b>	60	70	60	45	65	45	45	60	45	55
<b>S2</b>	65	55	45	50	70	70	60	55	50	60
<b>S5</b>	70	60	50	55	75	75	65	60	55	65
<b>S6</b>	65	65	55	70	60	50	40	65	60	80
<b>S7</b>	70	70	70	55	75	55	45	60	55	65
<b>S8</b>	65	75	65	50	70	50	50	65	50	60
<b>S9</b>	65	65	55	70	60	50	40	65	60	80
<b>S10</b>	60	50	40	65	65	65	55	60	55	75
<b>R1</b>	<b>R2</b>	<b>R3</b>	<b>R5</b>	<b>R7</b>	<b>R15</b>	<b>R16</b>	<b>R17</b>	<b>R18</b>	<b>R20</b>	

Appendix VII: Similarity Index and Percent Match Matrixes

---

<b>S11</b>	70	60	50	65	65	55	45	70	55	85
<b>S12</b>	65	65	55	60	50	60	60	75	60	60
<b>S13</b>	85	75	55	50	50	80	80	65	70	70
<b>S14</b>	80	80	70	55	65	65	55	60	65	75
<b>S15</b>	80	70	70	65	65	55	55	70	65	65
<b>S16</b>	80	50	60	55	55	55	55	60	65	65
<b>S17</b>	75	55	55	50	50	60	60	65	50	70
<b>S20</b>	85	65	65	60	40	60	60	65	70	80
<b>S21</b>	70	50	60	65	65	65	65	70	45	65
<b>S23</b>	75	55	55	50	50	60	60	65	50	70
<b>S24</b>	70	60	50	45	45	65	65	70	55	65
<b>S25</b>	75	55	65	70	70	60	60	75	50	70
<b>S27</b>	75	55	55	60	60	60	60	65	50	70
<b>S28</b>	75	55	65	70	70	60	60	75	50	70
<b>S29</b>	80	60	60	65	65	65	65	70	55	75
<b>S30</b>	75	65	55	50	70	70	60	55	60	70
<b>S31</b>	75	75	65	60	70	60	50	65	60	70
<b>S32</b>	75	65	55	50	70	60	50	65	50	70
<b>S33</b>	75	75	55	50	60	60	70	85	60	60
<b>S34</b>	75	75	55	40	60	60	60	65	50	70
<b>S35</b>	75	55	65	40	60	50	50	65	60	50
<b>S36</b>	60	70	50	45	75	65	65	70	55	55
<b>S37</b>	65	75	45	50	70	70	70	65	60	60
<b>S38</b>	65	75	45	50	70	70	70	65	60	60
<b>S39</b>	70	70	50	55	75	75	65	60	65	65
<b>S40</b>	65	75	45	60	60	70	70	55	60	60
<b>S44</b>	70	70	40	55	65	75	75	60	55	65
<b>S45</b>	85	75	55	60	70	70	60	65	60	80
<b>S48</b>	70	70	50	65	65	75	65	50	65	65
<b>S49</b>	80	70	50	55	65	75	65	60	55	75
<b>S50</b>	75	75	65	60	70	60	50	55	70	70
<b>S51</b>	75	75	55	70	70	70	60	55	60	70
<b>S54</b>	75	65	55	60	80	70	60	55	60	70
	<b>R1</b>	<b>R2</b>	<b>R3</b>	<b>R5</b>	<b>R7</b>	<b>R15</b>	<b>R16</b>	<b>R17</b>	<b>R18</b>	<b>R20</b>
<b>R22</b>	100									
<b>R23</b>	50	100								
<b>R25</b>	60	60	100							
<b>R27</b>	50	80	60	100						
<b>R28</b>	65	55	75	65	100					
<b>R50</b>	45	55	55	65	60	100				
<b>R53</b>	45	75	75	75	60	60	100			
<b>R55</b>	45	55	55	65	60	90	60	100		
<b>R56</b>	65	55	65	65	80	60	60	60	100	
<b>R57</b>	50	60	60	70	65	85	65	85	55	100
	<b>R22</b>	<b>R23</b>	<b>R25</b>	<b>R27</b>	<b>R28</b>	<b>R50</b>	<b>R53</b>	<b>R55</b>	<b>R56</b>	<b>R57</b>

Appendix VII: Similarity Index and Percent Match Matrixes

---

<b>R62</b>	30	70	50	70	65	85	65	85	55	80
<b>R63</b>	55	75	65	75	60	60	70	60	50	55
<b>R65</b>	55	55	45	65	60	70	70	60	60	65
<b>R66</b>	45	75	65	65	50	60	60	50	60	55
<b>R71</b>	45	65	55	65	50	60	60	50	60	65
<b>R74</b>	60	60	60	60	45	55	65	45	55	60
<b>R76</b>	45	65	65	75	60	90	70	80	70	75
<b>R77</b>	70	50	60	50	55	55	55	45	55	60
<b>R78</b>	60	70	70	80	65	75	75	65	75	80
<b>R79</b>	55	65	65	45	60	60	50	60	50	65
<b>R83</b>	60	70	50	70	35	45	55	35	45	50
<b>R84</b>	50	60	60	60	65	35	55	35	55	50
<b>R85</b>	55	65	55	65	60	70	60	60	70	55
<b>R91</b>	50	60	70	60	65	55	65	45	75	60
<b>R93</b>	45	35	45	45	40	70	60	70	50	55
<b>R94</b>	45	55	65	55	60	60	60	50	70	45
<b>R95</b>	55	45	55	55	60	80	60	80	60	65
<b>R98</b>	50	60	60	60	55	85	75	75	55	80
<b>R99</b>	45	65	55	65	50	80	80	70	50	75
<b>R103</b>	75	55	65	45	60	50	70	60	50	55
<b>R105</b>	60	50	70	60	75	85	65	75	65	70
<b>S1</b>	60	60	70	60	65	65	65	65	45	70
<b>S2</b>	55	45	65	55	70	80	60	70	60	65
<b>S5</b>	60	50	70	60	75	85	65	75	65	70
<b>S6</b>	45	75	65	65	70	80	70	80	60	85
<b>S7</b>	60	70	70	60	65	75	65	75	45	80
<b>S8</b>	65	65	75	65	70	70	70	70	50	75
<b>S9</b>	45	75	65	65	70	80	70	80	60	85
<b>S10</b>	50	60	60	60	75	85	65	75	75	70
<b>S11</b>	50	70	60	60	65	85	65	75	65	80
<b>S12</b>	55	65	65	65	80	60	70	60	60	75
<b>S13</b>	65	55	75	65	70	60	70	50	70	65
<b>S14</b>	50	70	80	70	65	75	75	75	55	80
<b>S15</b>	60	60	70	70	75	75	65	75	55	90
<b>S16</b>	40	50	60	60	65	65	45	55	65	60
<b>S17</b>	55	55	65	55	70	60	60	50	70	65
<b>S20</b>	45	45	65	45	60	60	40	50	60	65
<b>S21</b>	60	60	60	60	65	55	65	45	65	60
<b>S23</b>	55	55	65	55	70	60	60	50	70	65
<b>S24</b>	50	60	60	60	65	55	65	45	65	60
<b>S25</b>	55	65	55	65	60	60	60	50	60	65
<b>S27</b>	45	55	55	55	60	60	60	50	60	65
<b>S28</b>	55	65	55	65	60	60	60	50	60	65
<b>S29</b>	50	60	60	60	65	65	65	55	65	70
	<b>R22</b>	<b>R23</b>	<b>R25</b>	<b>R27</b>	<b>R28</b>	<b>R50</b>	<b>R53</b>	<b>R55</b>	<b>R56</b>	<b>R57</b>

Appendix VII: Similarity Index and Percent Match Matrixes

---

<b>S30</b>	45	55	65	65	60	80	60	70	60	65
<b>S31</b>	55	65	75	65	70	80	70	80	50	85
<b>S32</b>	55	55	65	55	60	80	60	70	50	75
<b>S33</b>	65	65	65	75	70	70	70	60	60	75
<b>S34</b>	55	65	75	65	60	70	70	60	60	65
<b>S35</b>	55	55	55	55	50	60	50	50	40	65
<b>S36</b>	60	70	60	70	65	75	65	65	55	60
<b>S37</b>	65	65	65	65	70	70	60	60	60	55
<b>S38</b>	65	65	65	65	70	70	60	60	60	55
<b>S39</b>	60	60	60	60	65	75	55	65	55	60
<b>S40</b>	65	55	85	65	90	70	70	70	70	65
<b>S44</b>	70	50	80	60	85	75	65	65	75	60
<b>S45</b>	65	55	75	55	70	80	60	70	60	75
<b>S48</b>	60	50	80	60	85	75	65	75	65	70
<b>S49</b>	70	50	80	50	75	75	65	65	65	70
<b>S50</b>	65	65	75	55	70	70	60	70	50	75
<b>S51</b>	55	55	75	65	80	80	60	80	60	75
<b>S54</b>	65	55	65	55	70	80	50	70	60	65
	<b>R22</b>	<b>R23</b>	<b>R25</b>	<b>R27</b>	<b>R28</b>	<b>R50</b>	<b>R53</b>	<b>R55</b>	<b>R56</b>	<b>R57</b>
<b>R62</b>	100									
<b>R63</b>	65	100								
<b>R65</b>	65	60	100							
<b>R66</b>	55	50	40	100						
<b>R71</b>	65	40	60	70	100					
<b>R74</b>	50	65	65	55	75	100				
<b>R76</b>	75	70	60	70	60	65	100			
<b>R77</b>	40	65	65	55	55	70	55	100		
<b>R78</b>	60	65	65	75	65	70	85	70	100	
<b>R79</b>	65	70	40	50	50	55	60	65	55	100
<b>R83</b>	40	65	55	65	65	80	55	60	70	45
<b>R84</b>	50	65	35	55	45	50	45	60	60	65
<b>R85</b>	65	60	50	70	70	65	80	55	65	60
<b>R91</b>	60	45	55	65	85	80	65	50	70	55
<b>R93</b>	65	50	70	40	60	65	60	45	45	40
<b>R94</b>	65	60	50	60	70	75	70	45	55	60
<b>R95</b>	75	60	70	40	60	55	70	55	55	50
<b>R98</b>	80	55	75	55	75	70	75	60	70	55
<b>R99</b>	75	60	80	60	60	55	70	55	75	50
<b>R103</b>	45	70	60	40	30	55	50	65	55	60
<b>R105</b>	70	65	75	55	55	50	75	70	70	55
<b>S1</b>	60	75	45	55	35	50	65	60	70	65
<b>S2</b>	65	60	60	50	50	55	80	55	65	50
<b>S5</b>	70	65	65	55	55	60	85	60	70	55
<b>S6</b>	85	60	50	70	60	55	80	45	75	70
	<b>R62</b>	<b>R63</b>	<b>R65</b>	<b>R66</b>	<b>R71</b>	<b>R74</b>	<b>R76</b>	<b>R77</b>	<b>R78</b>	<b>R79</b>

Appendix VII: Similarity Index and Percent Match Matrixes

---

<b>S7</b>	70	75	55	55	45	60	75	60	70	75
<b>S8</b>	65	80	50	60	40	55	70	65	75	70
<b>S9</b>	85	60	50	70	60	55	80	45	75	70
<b>S10</b>	80	55	65	65	65	60	85	50	70	55
<b>S11</b>	80	55	55	75	65	60	85	50	80	65
<b>S12</b>	75	60	60	50	70	65	60	55	65	60
<b>S13</b>	55	60	60	50	70	75	70	65	75	60
<b>S14</b>	70	75	45	65	55	60	85	60	80	75
<b>S15</b>	70	65	55	55	55	60	75	60	80	65
<b>S16</b>	60	55	45	65	65	60	75	50	70	55
<b>S17</b>	55	50	50	60	70	65	70	65	75	60
<b>S20</b>	55	40	40	60	70	55	60	55	65	70
<b>S21</b>	50	45	55	65	75	60	65	60	70	55
<b>S23</b>	55	50	50	60	70	65	70	65	75	60
<b>S24</b>	60	55	55	55	75	70	65	60	70	55
<b>S25</b>	55	50	50	70	70	55	70	55	75	60
<b>S27</b>	55	50	50	60	60	55	70	55	75	60
<b>S28</b>	55	50	50	70	70	55	70	55	75	60
<b>S29</b>	60	55	55	65	65	60	75	60	80	65
<b>S30</b>	65	70	50	60	50	55	90	55	75	60
<b>S31</b>	75	70	50	60	50	55	80	55	75	70
<b>S32</b>	65	60	50	60	50	55	80	55	75	60
<b>S33</b>	65	70	70	60	60	65	70	65	85	50
<b>S34</b>	55	70	50	70	50	55	80	65	85	60
<b>S35</b>	55	60	60	50	60	75	60	55	65	50
<b>S36</b>	70	85	75	55	45	60	75	60	70	55
<b>S37</b>	65	80	70	50	40	55	70	55	65	60
<b>S38</b>	65	80	70	50	40	55	70	55	65	60
<b>S39</b>	70	75	65	45	45	60	75	50	60	65
<b>S40</b>	65	70	60	50	40	45	70	55	65	60
<b>S44</b>	60	65	65	55	45	50	75	60	70	55
<b>S45</b>	65	60	50	60	50	55	80	55	75	70
<b>S48</b>	70	65	55	45	45	50	75	50	60	65
<b>S49</b>	60	55	55	55	55	60	75	60	70	65
<b>S50</b>	65	70	50	50	40	55	70	55	65	80
<b>S51</b>	75	70	50	50	40	45	80	45	65	70
<b>S54</b>	65	70	60	50	40	55	80	55	65	70
	<b>R62</b>	<b>R63</b>	<b>R65</b>	<b>R66</b>	<b>R71</b>	<b>R74</b>	<b>R76</b>	<b>R77</b>	<b>R78</b>	<b>R79</b>
<b>R83</b>	100									
<b>R84</b>	50	100								
<b>R85</b>	55	45	100							
<b>R91</b>	60	60	65	100						
<b>R93</b>	45	25	60	55	100					
<b>R94</b>	55	55	80	85	70	100				
	<b>R83</b>	<b>R84</b>	<b>R85</b>	<b>R91</b>	<b>R93</b>	<b>R94</b>				

Appendix VII: Similarity Index and Percent Match Matrixes

---

<b>R95</b>	35	35	60	55	80	60	100			
<b>R98</b>	50	40	65	70	75	65	85	100		
<b>R99</b>	55	45	50	55	70	50	70	85	100	
<b>R103</b>	45	45	50	35	60	40	60	55	60	100
<b>R105</b>	40	40	65	50	65	55	85	80	75	65
<b>S1</b>	50	70	55	40	45	45	55	60	65	75
<b>S2</b>	35	45	70	55	60	60	80	75	60	60
<b>S5</b>	40	40	75	60	65	65	85	80	65	65
<b>S6</b>	45	55	70	65	50	60	60	75	70	60
<b>S7</b>	50	50	55	50	45	45	65	70	65	75
<b>S8</b>	55	65	60	45	50	50	60	65	70	80
<b>S9</b>	45	55	70	65	50	60	60	75	70	60
<b>S10</b>	40	40	85	70	65	75	75	80	65	55
<b>S11</b>	50	50	75	70	55	65	65	80	75	55
<b>S12</b>	45	65	60	75	50	60	70	75	60	60
<b>S13</b>	55	65	60	85	50	70	70	75	60	50
<b>S14</b>	50	60	65	60	45	55	65	70	65	65
<b>S15</b>	50	60	55	60	45	45	65	70	65	65
<b>S16</b>	50	60	65	70	45	65	55	50	45	35
<b>S17</b>	45	65	70	75	40	60	60	65	50	50
<b>S20</b>	45	65	50	75	40	60	50	55	50	30
<b>S21</b>	50	50	75	70	45	55	55	60	55	55
<b>S23</b>	45	65	70	75	40	60	60	65	50	50
<b>S24</b>	50	70	65	80	45	65	65	70	55	45
<b>S25</b>	55	55	70	65	40	50	50	55	60	50
<b>S27</b>	45	65	60	65	40	50	50	55	60	50
<b>S28</b>	55	55	70	65	40	50	50	55	60	50
<b>S29</b>	50	60	65	70	45	55	55	60	65	55
<b>S30</b>	45	55	70	55	50	60	70	65	60	50
<b>S31</b>	45	55	60	55	50	50	70	75	70	70
<b>S32</b>	45	55	60	55	50	50	70	75	70	60
<b>S33</b>	65	65	50	65	50	50	70	75	80	60
<b>S34</b>	55	65	60	55	40	50	60	65	70	60
<b>S35</b>	65	55	40	65	50	50	60	65	60	50
<b>S36</b>	60	50	55	50	55	55	75	70	75	65
<b>S37</b>	55	55	50	55	50	60	70	65	70	60
<b>S38</b>	55	55	50	55	50	60	70	65	70	60
<b>S39</b>	50	50	55	60	55	65	75	70	65	55
<b>S40</b>	35	55	60	55	50	60	70	65	60	70
<b>S44</b>	40	50	65	60	55	65	75	70	65	65
<b>S45</b>	45	55	60	65	50	60	70	75	70	60
<b>S48</b>	30	50	65	60	55	65	75	70	55	65
<b>S49</b>	40	50	65	70	55	65	75	80	65	65
<b>S50</b>	45	55	50	55	40	50	60	65	60	70
	<b>R83</b>	<b>R84</b>	<b>R85</b>	<b>R91</b>	<b>R93</b>	<b>R94</b>	<b>R95</b>	<b>R98</b>	<b>R99</b>	<b>R103</b>

Appendix VII: Similarity Index and Percent Match Matrixes

---

<b>S51</b>	35	55	60	55	50	60	70	65	60	60
<b>S54</b>	45	45	60	55	50	60	70	65	60	60
	<b>R83</b>	<b>R84</b>	<b>R85</b>	<b>R91</b>	<b>R93</b>	<b>R94</b>	<b>R95</b>	<b>R98</b>	<b>R99</b>	<b>R103</b>
<b>R105</b>	100									
<b>S1</b>	70	100								
<b>S2</b>	85	75	100							
<b>S5</b>	90	70	95	100						
<b>S6</b>	65	75	70	75	100					
<b>S7</b>	70	80	75	80	85	100				
<b>S8</b>	75	95	70	75	80	85	100			
<b>S9</b>	65	75	70	75	100	85	80	100		
<b>S10</b>	80	60	85	90	85	70	65	85	100	
<b>S11</b>	70	70	75	80	95	80	75	95	90	100
<b>S12</b>	65	65	70	75	80	75	70	80	75	75
<b>S13</b>	65	55	70	75	60	65	60	60	65	65
<b>S14</b>	70	80	75	80	85	90	85	85	70	80
<b>S15</b>	70	80	75	80	85	90	85	85	70	80
<b>S16</b>	60	60	75	70	65	60	55	65	70	70
<b>S17</b>	65	65	80	75	70	65	60	70	75	75
<b>S20</b>	55	55	60	55	60	55	50	60	55	65
<b>S21</b>	60	50	65	70	65	60	55	65	70	70
<b>S23</b>	65	65	80	75	70	65	60	70	75	75
<b>S24</b>	60	60	75	70	65	60	55	65	70	70
<b>S25</b>	55	55	60	65	70	65	60	70	65	75
<b>S27</b>	55	65	70	65	70	65	60	70	65	75
<b>S28</b>	55	55	60	65	70	65	60	70	65	75
<b>S29</b>	60	60	65	70	75	70	65	75	70	80
<b>S30</b>	75	75	90	85	70	75	70	70	75	75
<b>S31</b>	75	85	80	85	90	95	90	90	75	85
<b>S32</b>	75	85	90	85	80	85	80	80	75	85
<b>S33</b>	75	75	70	75	70	75	80	70	65	75
<b>S34</b>	75	85	80	75	70	75	80	70	65	75
<b>S35</b>	55	65	70	65	60	75	60	60	55	65
<b>S36</b>	80	70	75	80	65	80	75	65	70	70
<b>S37</b>	75	65	70	75	60	75	70	60	65	65
<b>S38</b>	75	65	70	75	60	75	70	60	65	65
<b>S39</b>	70	60	75	80	65	80	65	65	70	70
<b>S40</b>	85	75	80	85	70	75	80	70	75	65
<b>S44</b>	90	70	85	90	65	70	75	65	80	70
<b>S45</b>	75	75	80	85	80	85	80	80	75	85
<b>S48</b>	80	70	85	90	75	80	75	75	80	70
<b>S49</b>	80	70	85	90	75	80	75	75	80	80
<b>S50</b>	65	75	70	75	80	95	80	80	65	75
<b>S51</b>	75	75	80	85	80	85	80	80	75	75
	<b>R105</b>	<b>S1</b>	<b>S2</b>	<b>S5</b>	<b>S6</b>	<b>S7</b>	<b>S8</b>	<b>S9</b>	<b>S10</b>	<b>S11</b>

Appendix VII: Similarity Index and Percent Match Matrixes

---

<b>S54</b>	75	65	80	85	70	85	70	70	75	75
	<b>R105</b>	<b>S1</b>	<b>S2</b>	<b>S5</b>	<b>S6</b>	<b>S7</b>	<b>S8</b>	<b>S9</b>	<b>S10</b>	<b>S11</b>
<b>S12</b>	100									
<b>S13</b>	80	100								
<b>S14</b>	75	75	100							
<b>S15</b>	85	75	90	100						
<b>S16</b>	65	65	70	70	100					
<b>S17</b>	80	80	75	75	85	100				
<b>S20</b>	60	70	65	65	85	80	100			
<b>S21</b>	75	75	70	70	70	85	65	100		
<b>S23</b>	80	80	75	75	85	100	80	85	100	
<b>S24</b>	85	85	70	70	80	95	75	80	95	100
<b>S25</b>	70	70	75	75	75	80	70	95	80	75
<b>S27</b>	70	70	75	75	85	90	80	85	90	85
<b>S28</b>	70	70	75	75	75	80	70	95	80	75
<b>S29</b>	75	75	80	80	80	85	75	90	85	80
<b>S30</b>	60	70	85	75	85	80	70	65	80	75
<b>S31</b>	80	70	95	95	65	70	60	65	70	65
<b>S32</b>	70	70	85	85	75	80	70	65	80	75
<b>S33</b>	80	80	75	85	65	70	60	65	70	75
<b>S34</b>	60	70	85	75	75	80	70	65	80	75
<b>S35</b>	70	70	65	75	75	70	70	55	70	75
<b>S36</b>	65	65	70	70	60	55	45	50	55	60
<b>S37</b>	60	70	65	65	55	50	50	45	50	55
<b>S38</b>	60	70	65	65	55	50	50	45	50	55
<b>S39</b>	65	75	70	70	60	55	55	50	55	60
<b>S40</b>	70	70	75	75	55	60	50	55	60	55
<b>S44</b>	65	75	70	70	60	65	55	60	65	60
<b>S45</b>	70	80	85	85	65	70	70	65	70	65
<b>S48</b>	75	75	80	80	60	65	55	60	65	60
<b>S49</b>	75	85	80	80	60	75	65	70	75	70
<b>S50</b>	70	70	85	85	55	60	60	55	60	55
<b>S51</b>	70	70	85	85	65	60	60	55	60	55
<b>S54</b>	60	70	75	75	65	60	60	55	60	55
	<b>S12</b>	<b>S13</b>	<b>S14</b>	<b>S15</b>	<b>S16</b>	<b>S17</b>	<b>S20</b>	<b>S21</b>	<b>S23</b>	<b>S24</b>
<b>S25</b>	100									
<b>S27</b>	90	100								
<b>S28</b>	100	90	100							
<b>S29</b>	95	95	95	100						
<b>S30</b>	70	80	70	75	100					
<b>S31</b>	70	70	70	75	80	100				
<b>S32</b>	70	80	70	75	90	90	100			
<b>S33</b>	70	70	70	75	70	80	80	100		
<b>S34</b>	70	80	70	75	90	80	90	80	100	
	<b>S25</b>	<b>S27</b>	<b>S28</b>	<b>S29</b>	<b>S30</b>	<b>S31</b>	<b>S32</b>	<b>S33</b>	<b>S34</b>	<b>S35</b>

**Appendix VII: Similarity Index and Percent Match Matrixes**

---

<b>S35</b>	60	70	60	65	70	70	80	80	70	100
<b>S36</b>	55	55	55	60	75	75	75	85	75	75
<b>S37</b>	50	50	50	55	70	70	70	80	70	70
<b>S38</b>	50	50	50	55	70	70	70	80	70	70
<b>S39</b>	55	55	55	60	75	75	75	75	65	75
<b>S40</b>	50	50	50	55	70	80	70	70	70	50
<b>S44</b>	55	55	55	60	75	75	75	75	75	55
<b>S45</b>	70	70	70	75	80	90	90	80	80	70
<b>S48</b>	55	55	55	60	75	85	75	65	65	55
<b>S49</b>	65	65	65	70	75	85	85	75	75	65
<b>S50</b>	60	60	60	65	70	90	80	70	70	70
<b>S51</b>	60	60	60	65	80	90	80	70	70	60
<b>S54</b>	60	60	60	65	80	80	80	70	70	70
	<b>S25</b>	<b>S27</b>	<b>S28</b>	<b>S29</b>	<b>S30</b>	<b>S31</b>	<b>S32</b>	<b>S33</b>	<b>S34</b>	<b>S35</b>
<b>S36</b>	100									
<b>S37</b>	95	100								
<b>S38</b>	95	100	100							
<b>S39</b>	90	95	95	100						
<b>S40</b>	75	80	80	75	100					
<b>S44</b>	80	85	85	80	95	100				
<b>S45</b>	75	80	80	85	80	85	100			
<b>S48</b>	70	75	75	80	95	90	85	100		
<b>S49</b>	70	75	75	80	85	90	95	90	100	
<b>S50</b>	75	80	80	85	80	75	90	85	85	100
<b>S51</b>	75	80	80	85	90	85	90	95	85	90
<b>S54</b>	85	90	90	95	80	85	90	85	85	90
	<b>S36</b>	<b>S37</b>	<b>S38</b>	<b>S39</b>	<b>S40</b>	<b>S44</b>	<b>S45</b>	<b>S48</b>	<b>S49</b>	<b>S50</b>
<b>S51</b>	100									
<b>S54</b>	90	100								
	<b>S51</b>	<b>S54</b>								

Note: All numeric figures above are in terms of percentages.

**APPENDIX VIII:****OPTIMIZATION OF NEURAL NETWORK IN STRAIN**

Two-strain differential based on artificial neural network encoded in STRAIN was tested for each of the five primers, A2, B1, C13, AA1 and AA3 using known data ( $N = 30$ ) from 1000 cycles to 10000 cycles. Each of the tests was carried out in triplicates and the results in percent accuracy, together with standard deviation were tabulated as follows:

Primer	Cycles									
	1000	2000	3000	4000	5000	6000	7000	8000	9000	10000
A2	60	75	65	55	60	65	55	50	55	60
	60	45	55	75	60	70	75	65	80	60
	60	65	65	60	80	70	70	70	75	75
	60	62	62	63	67	68	67	62	70	65
	s	0	15	6	10	12	3	10	13	9
B1	80	75	75	70	80	80	75	75	85	70
	70	60	70	60	60	80	70	80	80	75
	80	65	70	70	80	75	65	60	70	70
	77	67	72	67	73	78	70	72	78	72
	s	6	8	3	6	12	3	5	10	8
C13	85	90	100	90	85	80	75	90	75	80
	85	90	100	95	90	85	90	75	85	80
	90	95	95	95	95	90	85	90	85	75
	87	92	98	93	90	85	83	85	82	78
	s	3	3	3	3	5	5	8	9	6
AA1	70	75	70	65	70	75	70	75	70	65
	65	70	60	70	60	75	70	70	70	75
	85	70	70	65	55	80	80	65	70	85
	73	72	67	67	62	77	73	70	70	75
	s	10	3	6	3	8	3	3	5	0
AA3	30	40	50	55	55	45	35	50	45	50
	40	35	45	50	55	45	40	55	50	50
	45	40	65	40	40	60	55	45	40	55
	38	38	52	48	50	50	43	50	45	52
	s	8	3	8	8	9	9	10	5	3

## APPENDIX IX:

### STRAIN: SOURCE CODE

STRAIN is written in Microsoft® Visual Basic version 5.0.

#### File: Main\_Form.frm

VERSION 5.00

```
Object = "{BDC217C8-ED16-11CD-956C-0000C04E4C0A}#1.1#0";
"TABCTL32.OCX"
```

```
Object = "{6B7E6392-850A-101B-AFC0-4210102A8DA7}#1.1#0";
"COMCTL32.OCX"
```

```
Begin VB.Form Main_Form
    BorderStyle = 1 Fixed Single
    Caption = "Strain"
    ClientHeight = 4365
    ClientLeft = 45
    ClientTop = 330
    ClientWidth = 4770
    Icon = "Main_Form.frx":0000
    LinkTopic = "Form1"
    MaxButton = 0 False
    ScaleHeight = 4365
    ScaleWidth = 4770
    StartUpPosition = 3 'Windows Default
    Begin TabDlg.SSTab SSTab1
        Height = 4275
        Left = 30
        TabIndex = 0
        Top = 30
        Width = 4695
        _ExtentX = 8281
        _ExtentY = 7541
        _Version = 327680
        Tabs = 5
        TabsPerRow = 5
        TabHeight = 520
        TabCaption(0) = "Input"
        TabPicture(0) = "Main_Form.frx":030A
        Tab(0).ControlCount= 8
        Tab(0).ControlEnabled= -1 True
        Tab(0).Control(0)= "Label1"
        Tab(0).Control(0).Enabled= 0 False
```

```
Tab(0).Control(1)= "Label2"
Tab(0).Control(1).Enabled= 0 'False
Tab(0).Control(2)= "Label3"
Tab(0).Control(2).Enabled= 0 'False
Tab(0).Control(3)= "Primer_Name"
Tab(0).Control(3).Enabled= 0 'False
Tab(0).Control(4)= "Sample_Name"
Tab(0).Control(4).Enabled= 0 'False
Tab(0).Control(5)= "Strain"
Tab(0).Control(5).Enabled= 0 'False
Tab(0).Control(6)= "Band"
Tab(0).Control(6).Enabled= 0 'False
Tab(0).Control(7)= "Add"
Tab(0).Control(7).Enabled= 0 'False
TabCaption(1) = "Remove"
TabPicture(1) = "Main_Form.frx":0326
Tab(1).ControlCount= 7
Tab(1).ControlEnabled= 0 False
Tab(1).Control(0)= "List_All"
Tab(1).Control(0).Enabled= -1 True
Tab(1).Control(1)= "Strain_r"
Tab(1).Control(1).Enabled= -1 True
Tab(1).Control(2)= "Band_r"
Tab(1).Control(2).Enabled= -1 True
Tab(1).Control(3)= "Frame2"
Tab(1).Control(3).Enabled= 0 False
Tab(1).Control(4)= "Frame1"
Tab(1).Control(4).Enabled= 0 False
Tab(1).Control(5)= "Remove"
Tab(1).Control(5).Enabled= -1 True
Tab(1).Control(6)= "Label4"
Tab(1).Control(6).Enabled= 0 False
TabCaption(2) = "Process"
TabPicture(2) = "Main_Form.frx":0342
Tab(2).ControlCount= 1
Tab(2).ControlEnabled= 0 False
Tab(2).Control(0)= "Process"
Tab(2).Control(0).Enabled= -1 True
TabCaption(3) = "Training"
TabPicture(3) = "Main_Form.frx":035E
Tab(3).ControlCount= 5
Tab(3).ControlEnabled= 0 False
Tab(3).Control(0)= "ProgressBar1"
Tab(3).Control(0).Enabled= 0 False
Tab(3).Control(1)= "List"
Tab(3).Control(1).Enabled= -1 True
```

```
Tab(3).Control(2)= "Frame3"
Tab(3).Control(2).Enabled= 0 'False
Tab(3).Control(3)= "Loops"
Tab(3).Control(3).Enabled= -1 'True
Tab(3).Control(4)= "Label5"
Tab(3).Control(4).Enabled= 0 'False
TabCaption(4) = "Test"
TabPicture(4) = "Main_Form.frx":037A
Tab(4).ControlCount= 3
Tab(4).ControlEnabled= 0 'False
Tab(4).Control(0)= "Label6"
Tab(4).Control(0).Enabled= 0 False
Tab(4).Control(1)= "Test_Band"
Tab(4).Control(1).Enabled= 0 False
Tab(4).Control(2)= "Test"
Tab(4).Control(2).Enabled= 0 False
Begin ComctlLib.ProgressBar ProgressBar1
    Height      = 225
    Left         = -74910
    TabIndex     = 27
    Top          = 2520
    Width        = 4395
    _ExtentX     = 7752
    _ExtentY     = 397
    _Version     = 327680
    BorderStyle  = 1
    Appearance   = 0
    MouseIcon    = "Main_Form.frx":0396
End
Begin VB.CommandButton Test
    Caption      = "Test"
    Height       = 405
    Left         = -71760
    TabIndex     = 17
    Top          = 780
    Width        = 825
End
Begin VB.TextBox Test_Band
    Height       = 285
    Left         = -74850
    MaxLength    = 20
    TabIndex     = 16
    Top          = 870
    Width        = 2955
End
Begin VB.CommandButton List
End
```

```
Caption      = "List"
Height       = 225
Left         = -74880
TabIndex     = 13
Top          = 660
Width        = 2415
End
Begin VB.Frame Frame3
Caption      = "Primer"
Height       = 1935
Left         = -72330
TabIndex     = 24
Top          = 510
Width        = 1815
Begin VB.ListBox Pri_4_Train
Height       = 1620
Left         = 210
TabIndex     = 15
Top          = 240
Width        = 1455
End
End
Begin VB.TextBox Loops
Height       = 285
Left         = -74820
MaxLength    = 9
TabIndex     = 14
Top          = 1200
Width        = 2295
End
End
Begin VB.CommandButton Process
Caption      = "Process SI and Percent Match"
Height       = 405
Left         = -74760
TabIndex     = 12
Top          = 1410
Width        = 4155
End
Begin VB.CommandButton List_All
Caption      = "List"
Height       = 285
Left         = -74760
TabIndex     = 6
Top          = 420
Width        = 3375
End
```

```
Begin VB.CheckBox Strain_r
    Caption      = "Strain"
    Height       = 255
    Left         = -71370
    TabIndex     = 9
    Top          = 870
    Width        = 735
End
Begin VB.TextBox Band_r
    Height       = 285
    Left         = -74400
    MaxLength    = 20
    TabIndex     = 10
    Top          = 2220
    Width        = 3915
End
Begin VB.Frame Frame2
    Caption      = "Sample"
    Height       = 1335
    Left         = -73140
    TabIndex     = 22
    Top          = 780
    Width        = 1725
End
Begin VB.ListBox Sample_List
    Height       = 840
    Left         = 180
    TabIndex     = 8
    Top          = 360
    Width        = 1335
End
Begin VB.Frame Frame1
    Caption      = "Primer"
    Height       = 1335
    Left         = -74850
    TabIndex     = 21
    Top          = 780
    Width        = 1725
End
Begin VB.ListBox Primer_List
    Height       = 840
    Left         = 180
    TabIndex     = 7
    Top          = 360
    Width        = 1335
End
End
```

```
Begin VB.CommandButton Remove
    Caption      = "Remove"
    Height       = 315
    Left         = -74850
    TabIndex     = 11
    Top          = 2580
    Width        = 4365
End
Begin VB.CommandButton Add
    Caption      = "Add"
    Height       = 315
    Left         = 720
    TabIndex     = 5
    Top          = 2040
    Width        = 1005
End
Begin VB.TextBox Band
    Height       = 285
    Left         = 690
    MaxLength    = 20
    TabIndex     = 4
    Top          = 1710
    Width        = 2955
End
Begin VB.CheckBox Strain
    Caption      = "Strain"
    Height       = 255
    Left         = 2250
    TabIndex     = 3
    Top          = 1140
    Width        = 735
End
Begin VB.TextBox Sample_Name
    Height       = 285
    Left         = 1470
    MaxLength    = 6
    TabIndex     = 2
    Top          = 1140
    Width        = 735
End
Begin VB.TextBox Primer_Name
    Height       = 285
    Left         = 690
    MaxLength    = 6
    TabIndex     = 1
    Top          = 1110
```

```
Width      = 735
End
Begin VB.Label Label6
Caption    = "Band"
Height     = 225
Left       = -74850
TabIndex   = 26
Top        = 630
Width      = 555
End
Begin VB.Label Label5
Caption    = "Times To Loop"
Height     = 315
Left       = -74850
TabIndex   = 25
Top        = 960
Width      = 1125
End
Begin VB.Label Label4
Caption    = "Band"
Height     = 225
Left       = -74850
TabIndex   = 23
Top        = 2220
Width      = 555
End
Begin VB.Label Label3
Caption    = "Band"
Height     = 225
Left       = 690
TabIndex   = 20
Top        = 1470
Width      = 555
End
Begin VB.Label Label2
Caption    = "Sample Name"
Height     = 405
Left       = 1560
TabIndex   = 19
Top        = 660
Width      = 555
End
Begin VB.Label Label1
Caption    = "Primer Name"
Height     = 405
Left       = 840
```

```

TabIndex      = 18
Top          = 660
Width        = 555
End
End
End
Attribute VB_Name = "Main_Form"
Attribute VB_GlobalNameSpace = False
Attribute VB_Creatable = False
Attribute VB_PredeclaredId = True
Attribute VB_Exposed = False
Private Sub Add_Click()
    If Verify_Input = True Then
        Dim rs As Recordset
        Dim SQL_STATEMENT As String
        SQL_STATEMENT = "SELECT Rap.Primer, Rap.Sample, Rap.Strain, Rap.Band
    " & " From Rap WHERE (((Rap.Primer)=""" + Primer_Name + """) AND
    ((Rap.Sample)=""" + Sample_Name + """));"
        Set rs = db.OpenRecordset(SQL_STATEMENT, dbOpenDynaset)
        If rs.RecordCount = 0 Then
            MsgBox "Adding...", vbOKOnly, "Add"
            rs.AddNew
            rs![Primer] = Primer_Name
            rs![Sample] = Sample_Name
            rs![Strain] = Strain
            rs![Band] = Band
        Else
            MsgBox "Updating...", vbOKOnly, "Update"
            rs.Requery
            rs.Edit
            rs![Primer] = Primer_Name
            rs![Sample] = Sample_Name
            rs![Strain] = Strain
            rs![Band] = Band
        End If
        rs.Update
        rs.Close
    Else
        MsgBox "Incorrect Input Detected. Pls Verify Before Adding record.", vbCritical,
    "Incorrect Input!"
    End If
End Sub

Private Sub Form_Load()
    Call Main
End Sub

```

```

Private Sub List_All_Click()
    Dim rs As Recordset
    Dim SQL_STATEMENT As String
    SQL_STATEMENT = "SELECT Rap.Primer" &_
        " From Rap GROUP BY Rap.Primer;" 
    Set rs = db.OpenRecordset(SQL_STATEMENT, dbOpenDynaset)
    Primer_List.Clear
    Sample_List.Clear
    If rs.RecordCount = 0 Then
        MsgBox "No record found", vbCritical, "No Record Found"
    Else
        rs.MoveFirst
        Do Until rs.EOF
            Primer_List.AddItem rs![Primer]
            rs.MoveNext
        Loop
    End If
    rs.Close
End Sub

```

```

Private Sub List_Click()
    Dim rs_r As Recordset
    Dim SQL_STATEMENT As String
    SQL_STATEMENT = "SELECT * FROM Primer_For_Train;" 
    Set rs_r = db.OpenRecordset(SQL_STATEMENT, dbOpenDynaset)
    If rs_r.RecordCount = 0 Then
        MsgBox "No record Found", vbCritical, "No Record"
        Exit Sub
    Else
        Pri_4_Train.Clear
        rs_r.MoveFirst
        Do Until rs_r.EOF
            Pri_4_Train.AddItem rs_r![Primer]
            rs_r.MoveNext
        Loop
    End If
End Sub

```

```

Private Sub Loops_Change()
    If Not (IsNumeric(Loops)) Then
        Loops = "1"
    Else
        If CLng(Loops) < 1 Then
            Loops = "1"

```

```

End If
End If
End Sub

Private Sub Pri_4_Train_DblClick()
Dim cum As Double
Dim temp As Integer
Dim i As Integer
Dim j As Integer
Dim k As Long
Dim Primer0 As Recordset
Dim Primer1 As Recordset
Dim SQL_STATEMENT As String
SQL_STATEMENT = "SELECT Rap.Primer, Rap.Strain, Rap.Band From Rap
WHERE (((Rap.Primer)=""" + Pri_4_Train + """) AND ((Rap.Strain)=True));"
Set Primer1 = db.OpenRecordset(SQL_STATEMENT, dbOpenDynaset)
SQL_STATEMENT = "SELECT Rap.Primer, Rap.Strain, Rap.Band From Rap
WHERE (((Rap.Primer)=""" + Pri_4_Train + """) AND ((Rap.Strain)=FALSE));"
Set Primer0 = db.OpenRecordset(SQL_STATEMENT, dbOpenDynaset)
For i = 0 To 4
    Randomize
    'w(i) = CDec(((0.05 - 0 + 0.05) * Rnd + 0))
    w(i) = Rnd * 0.05
    MsgBox CStr(Format(w(i), "0.00000"))
Next i

'MsgBox "w0" + Format(w(0), "0.0000")
'MsgBox "w1" + Format(w(1), "0.0000")
'MsgBox "w2" + Format(w(2), "0.0000")
'MsgBox "w3" + Format(w(3), "0.0000")
'MsgBox "w4" + Format(w(4), "0.0000")

If IsNumeric(Loops) Then
    If CLng(Loops) < 1 Then
        MsgBox "Invalid Quantity", vbCritical, "Error"
        Exit Sub
    End If
    ProgressBar1.Max = CLng(Loops)
    For k = 1 To CLng(Loops)
        ProgressBar1.Value = k
        Primer0.MoveFirst
        Primer1.MoveFirst
        For j = 1 To 15
            'for 0
            cum = 0
            For i = 1 To 20

```

```

temp = CInt(Mid(Primer0![Band], i, 1))
cum = cum + (temp * w(0))
cum = cum + (temp * w(1))
cum = cum + (temp * w(2))
cum = cum + (temp * w(3))
cum = cum + (temp * w(4))

Next i
cum = cum / 5
If cum < 0.5 Then
  If Primer0![Strain] = True Then
    w(0) = w(0) + 0.0001
    w(1) = w(1) + 0.0001
    w(2) = w(2) + 0.0001
    w(3) = w(3) + 0.0001
    w(4) = w(4) + 0.0001
  End If
  'cum = 0
Else
  If Primer0![Strain] = False Then
    w(0) = w(0) - 0.0001
    w(1) = w(1) - 0.0001
    w(2) = w(2) - 0.0001
    w(3) = w(3) - 0.0001
    w(4) = w(4) - 0.0001
  End If
  'cum = 1
End If
'for 1
cum = 0
For i = 1 To 20
  temp = CInt(Mid(Primer1![Band], i, 1))
  cum = cum + (temp * w(0))
  cum = cum + (temp * w(1))
  cum = cum + (temp * w(2))
  cum = cum + (temp * w(3))
  cum = cum + (temp * w(4))

Next i
cum = cum / 5
If cum < 0.5 Then
  If Primer1![Strain] = True Then
    w(0) = w(0) + 0.0001
    w(1) = w(1) + 0.0001
    w(2) = w(2) + 0.0001
    w(3) = w(3) + 0.0001
    w(4) = w(4) + 0.0001
  End If

```

```

'cum = 0
Else
  If Primer1![Strain] = False Then
    w(0) = w(0) - 0.0001
    w(1) = w(1) - 0.0001
    w(2) = w(2) - 0.0001
    w(3) = w(3) - 0.0001
    w(4) = w(4) - 0.0001
  End If
  'cum = 1
End If
Primer0.MoveNext
Primer1.MoveNext
Next j
Next k
Else
  MsgBox "Invalid Quantity", vbCritical, "Error"
End If
' MsgBox "w0" + Format(w(0), "0.0000")
' MsgBox "w1" + Format(w(1), "0.0000")
' MsgBox "w2" + Format(w(2), "0.0000")
' MsgBox "w3" + Format(w(3), "0.0000")
' MsgBox "w4" + Format(w(4), "0.0000")
End Sub

Private Sub Primer_List_Click()
  Dim rs As Recordset
  Dim SQL_STATEMENT As String
  SQL_STATEMENT = "SELECT Rap.Primer, Rap.Sample From Rap WHERE
((Rap.Primer)=""" + Primer_List + """));"
  Set rs = db.OpenRecordset(SQL_STATEMENT, dbOpenDynaset)
  Primer_List.Clear
  Sample_List.Clear
  If rs.RecordCount = 0 Then
    MsgBox "No record found", vbCritical, "No Record Found"
  Else
    rs.MoveFirst
    Do Until rs.EOF
      Sample_List.AddItem rs![Sample]
      rs.MoveNext
    Loop
  End If
  rs.Close
End Sub

Private Sub Process_Click()

```

```

Dim rs_r As Recordset
Dim rs_s As Recordset
Dim rs_s2 As Recordset
Dim rs_out As Recordset

Dim pmatch_count As Integer
Dim si_count As Integer
Dim si_band As Integer

Dim pmatch_index As Double
Dim si_index As Double

Dim a As String
Dim b As String

Dim i As Integer
Dim SQL_STATEMENT As String

SQL_STATEMENT = "SELECT pmatch.* FROM pmatch;"
Set rs_out = db_out.OpenRecordset(SQL_STATEMENT, dbOpenDynaset)
If rs_out.RecordCount > 0 Then
    rs_out.MoveFirst
    Do Until rs_out.EOF
        rs_out.Delete
        rs_out.MoveNext
    Loop
End If

SQL_STATEMENT = "SELECT Primer.* FROM Primer;"
Set rs_r = db.OpenRecordset(SQL_STATEMENT, dbOpenDynaset)

If rs_r.RecordCount = 0 Then
    MsgBox "No record Found", vbCritical, "No Record"
    Exit Sub
Else
    rs_r.MoveFirst
    'loops the primer
    Do Until rs_r.EOF
        SQL_STATEMENT = "SELECT Rap.Primer, Rap.Sample, Rap.Band From Rap
WHERE (((Rap.Primer)=""" + rs_r![Primer] + """));"
        Set rs_s = db.OpenRecordset(SQL_STATEMENT, dbOpenDynaset)
        If rs_s.RecordCount = 0 Then
            MsgBox "No record Found", vbCritical, "No record"
            Exit Sub
        Else
            'loops the sample
        End If
    Loop
End If

```

```

rs_s.MoveFirst
Do Until rs_s.EOF
    SQL_STATEMENT = "SELECT Rap.Primer, Rap.Sample, Rap.Band From
Rap WHERE (((Rap.Primer)="" + rs_r![Primer] + ""));"
    Set rs_s2 = db.OpenRecordset(SQL_STATEMENT, dbOpenDynaset)
    If rs_s2.RecordCount = 0 Then
        MsgBox "No record Found", vbCritical, "No Record"
        Exit Sub
    Else
        rs_s2.MoveFirst
        Do Until rs_s2.EOF
            'perform operation here..
            pmatch_count = 0
            si_count = 0
            si_band = 0

            pmatch_index = 0
            si_index = 0

            For i = 1 To 20
                a = Mid(CStr(rs_s![Band]), i, 1)
                b = Mid(CStr(rs_s2![Band]), i, 1)
                If a = b Then
                    pmatch_count = pmatch_count + 1
                End If
                If a = 1 And b = 1 Then
                    si_count = si_count + 1
                    si_band = si_band + 2
                Else
                    If a = 1 Then
                        si_band = si_band + 1
                    End If
                    If b = 1 Then
                        si_band = si_band + 1
                    End If
                End If
            Next i
            pmatch_index = (pmatch_count / 20) * 100
            si_index = 2 * si_count / si_band * 100
            'pumps into DB
            SQL_STATEMENT = "SELECT pmatch.* FROM pmatch;"
            Set rs_out = db_out.OpenRecordset(SQL_STATEMENT,
dbOpenDynaset)
            rs_out.AddNew
            rs_out![Primer] = rs_r![Primer]
            rs_out![Sample1] = rs_s![Sample]

```

```

        rs_out![Sample2] = rs_s2![Sample]
        rs_out![%Match] = pmatch_index
        rs_out![SI] = si_index
        rs_out.Update
        rs_s2.MoveNext
    Loop
End If
rs_s.MoveNext
If rs_s.RecordCount = 1 Then
    '100 percent
Else
    'End If
    Loop
End If
rs_r.MoveNext
Loop
End If
MsgBox "Done!", vbOKOnly, "Done"
End Sub

Private Sub Remove_Click()
    Dim rs As Recordset
    Dim SQL_STATEMENT As String
    SQL_STATEMENT = "SELECT Rap.Primer, Rap.Sample, Rap.Strain, Rap.Band " _
        & " From Rap WHERE (((Rap.Primer)=""" + Primer_List + """) AND " _
        ((Rap.Sample)=""" + Sample_List + """));"

    Set rs = db.OpenRecordset(SQL_STATEMENT, dbOpenDynaset)
    If rs.RecordCount = 0 Then
        MsgBox "No record found", vbCritical, "No Record Found"
    Else
        rs.Requery
        rs.Delete
    End If
    rs.Close
    Primer_List.Clear
    Sample_List.Clear
    Strain_r.Value = 0
    Band_r = ""
End Sub

Private Sub Sample_List_Click()
    Dim rs As Recordset
    Dim SQL_STATEMENT As String
    SQL_STATEMENT = "SELECT Rap.Primer, Rap.Sample, Rap.Strain, Rap.Band " _

```

---

```

& " From Rap WHERE (((Rap.Primer)=""" + Primer_List + """) AND
((Rap.Sample)=""" + Sample_List + """));"

Set rs = db.OpenRecordset(SQL_STATEMENT, dbOpenDynaset)
'Primer_List.Clear
'Sample_List.Clear
If rs.RecordCount = 0 Then
    MsgBox "No record found", vbOKOnly, "No Record Found"
Else
    rs.Requery

    If rs![Strain] = True Then
        Strain_r.Value = 1
    Else
        Strain_r.Value = 0
    End If
    Band_r = rs![Band]
End If
rs.Close

End Sub

Private Sub Test_Click()
    Dim cum As Double
    If Verify_Input1 = True Then
        cum = 0
        For i = 1 To 20
            temp = CInt(Mid(Test_Band, i, 1))
            cum = cum + (temp * w(0))
            cum = cum + (temp * w(1))
            cum = cum + (temp * w(2))
            cum = cum + (temp * w(3))
            cum = cum + (temp * w(4))
        Next i
        cum = cum / 5
        If cum < 0.5 Then
            MsgBox "It is a SUSCEPTIBLE strain!", vbOKOnly, "RESULTS"
        Else
            MsgBox "It is a RESISTANT strain!", vbOKOnly, "RESULTS"
        End If
    Else
        MsgBox "Incorrect Input Detected.", vbCritical, "Error"
    End If

End Sub

```

**File: Strain.bas**

Attribute VB\_Name = "Module1"

Option Explicit

```

Public db As Database
Public db_out As Database
Public w(5) As Double
Sub Main()
    Set db = OpenDatabase(App.Path & "\Rapdata.mdb")
    Set db_out = OpenDatabase(App.Path & "\Rapdata_Out.mdb")
    Dim i As Integer
    For i = 0 To 4
        Randomize
        'w(i) = CDec(((0.05 - 0 + 0.05) * Rnd + 0))
        w(i) = Rnd * 0.05
        'MsgBox CStr(Format(w(i), "0.00000"))
    Next i

End Sub
Function Verify_Input() As Boolean
    Dim i As Integer
    If (Len(Main_Form.Primer_Name) < 2) Or (Len(Main_Form.Sample_Name) < 2) Or
    (Len(Main_Form.Band) < 20) Then
        Verify_Input = False
    Else
        For i = 1 To 20
            If (IsNumeric(Mid(Main_Form.Band, i, 1))) And
            (IsNumeric(Mid(Main_Form.Band, i, 1))) Then
                If (CInt(Mid(Main_Form.Band, i, 1)) = 0) Or (CInt(Mid(Main_Form.Band, i,
                1)) = 1) Then
                    Verify_Input = True
                Else
                    Verify_Input = False
                    Exit For
                End If
            Else
                Verify_Input = False
                Exit For
            End If
        Next i
    End If
End Function

```

```

End If
Next i

End If
End Function
Function Verify_Input1() As Boolean
    Dim i As Integer
    If (Len(Main_Form.Test_Band) < 20) Then
        Verify_Input1 = False
    Else
        For i = 1 To 20
            If (IsNumeric(Mid(Main_Form.Test_Band, i, 1))) And
                (IsNumeric(Mid(Main_Form.Test_Band, i, 1))) Then
                If (CInt(Mid(Main_Form.Test_Band, i, 1)) = 0) Or
                    (CInt(Mid(Main_Form.Test_Band, i, 1)) = 1) Then
                    Verify_Input1 = True
                Else
                    Verify_Input1 = False
                    Exit For
                End If
            Else
                Verify_Input1 = False
                Exit For
            End If
        Next i
    End If
End Function

```

**File: Strain.vbp**

Type=Exe

```

Reference=*\G{00020430-0000-0000-C000-
00000000046}#2.0#0#..\SYSTEM\STDOLE2.TLB#OLE Automation
Object={BDC217C8-ED16-11CD-956C-0000C04E4C0A}#1.1#0; TABCTL32.OCX
Object={5E9E78A0-531B-11CF-91F6-C2863C385E30}#1.0#0; MSFLXGRD.OCX
Object={FAEEE763-117E-101B-8933-08002B2F4F5A}#1.1#0; DBLIST32.OCX
Object={00028C01-0000-0000-0000-00000000046}#1.0#0; DBGRID32.OCX
Object={F9043C88-F6F2-101A-A3C9-08002B2F49FB}#1.1#0; COMDLG32.OCX
Object={6B7E6392-850A-101B-AFC0-4210102A8DA7}#1.1#0; COMCTL32.OCX
Object={FE0065C0-1B7B-11CF-9D53-00AA003C9CB6}#1.0#0; COMCT232.OCX
Object={48E59290-9880-11CF-9754-00AA00C00908}#1.0#0; MSINET.OCX
Object={3B7C8863-D78F-101B-B9B5-04021C009402}#1.1#0; RICHTX32.OCX
Object={02B5E320-7292-11CF-93D5-0020AF99504A}#1.0#0; MSCHART.OCX
Object={248DD890-BB45-11CF-9ABC-0080C7E7B78D}#1.0#0; MSWINSCK.OCX
Object={20C62CAE-15DA-101B-B9A8-444553540000}#1.1#0; MSMAPI32.OCX

```

```

Object={C1A8AF28-1257-101B-8FB0-0020AF039CA3}#1.1#0; MCI32.OCX
Object={27395F88-0C0C-101B-A3C9-08002B2F49FB}#1.1#0; PICCLP32.OCX
Object={6FBA474E-43AC-11CE-9A0E-00AA0062BB4C}#1.0#0; SYSINFO.OCX
Object={648A5603-2C6E-101B-82B6-000000000014}#1.1#0; MSCOMM32.OCX
Object={C932BA88-4374-101B-A56C-00AA003668DC}#1.1#0; MSMASK32.OCX
Reference=*\\G{00025E01-0000-0000-C000-00000000046}#4.0#0#..\..\PROGRAM
FILES\COMMON FILES\MICROSOFT SHARED\DAO\DAO350.DLL#Microsoft
DAO 3.5 Object Library
Form=Main_Form.frm
Module=Module1; strain.bas
IconForm="Main_Form"
Startup="Main_Form"
ExeName32="Strain.exe"
Command32=""
Name="Project1"
HelpContextID="0"
CompatibleMode="0"
MajorVer=1
MinorVer=0
RevisionVer=0
AutoIncrementVer=0
ServerSupportFiles=0
VersionCompanyName="Company"
CompilationType=0
OptimizationType=0
FavorPentiumPro(tm)=0
CodeViewDebugInfo=0
NoAliasing=0
BoundsCheck=0
OverflowCheck=0
FlPointCheck=0
FDIVCheck=0
UnroundedFP=0
StartMode=0
Unattended=0
ThreadPerObject=0
MaxNumberOfThreads=1

```

## APPENDIX X:

### STRAIN: USER'S GUIDE

STRAIN is a computer program specifically designed for basic analysis and allows for two-strain differential of a species using DNA fingerprinting data. This program enables the calculation of Similarity Index (SI) and Percent Matching (%M) whereas, the two-strain differential is accomplished by an Artificial Neural Network in-built into the program. This guide is intended to assist the end-user in the operation of this program. Programmers should refer to Appendix IX for the source code for this program for better understanding of the algorithms used.

STRAIN requires a minimum computer system, which meets the following requirements:

- A Pentium or equivalent system
- Windows 95 operating system
- VB5 runtime library (can be downloaded from [www.microsoft.com](http://www.microsoft.com))
- MS Access 95 (if calculation of SI and %M are required)
- Winzip 6 (can be downloaded from [www.winzip.com](http://www.winzip.com))

To setup the program, run **Setup.exe** by double clicking on the icon. Type the path of the desired directory (e.g. c:\Strain) into the text box and click on the button, **Unzip**. Click **Close** to end setup.

To run the program, go to the directory in which the program is installed and double click on the icon **Strain.exe**. It should respond by bringing up a window with five tabs.

If everything goes well, the window should consist of five tabs labeled **Input**, **Remove**, **Process**, **Training** and **Test**. The **Input** tab is for the user to enter fingerprinting data, which will be used in either the calculation of SI, %M, or to train the neural network or both.

The **Input** tab consists of four input fields, Primer name, Sample name, Strain and Band. Primer name field is for the user to enter the primer name. It can take up to six alpha numeric characters. Sample name field is for the entry of sample name, it can take up to six alphanumeric characters. Strain field is a tick box (a tick represents that it is a resistant strain), which is subsequently used for two-strain differential in the training of the neural network. Band field allows for the entry of fingerprinting data. The fingerprinting data must be entered in only '1's or '0's and it is mandatory to enter 20 characters for this field. This set of data will then be used for the calculation of SI, %M and neural network training. After entering the information in all four fields, click **Add** button to register data in the database. Should there be any errors in the fingerprints but not in the primer name and sample name, to make amendments, simply re-enter the fingerprinting data. It will replace the original data without warning.

However, if the error is not in the fingerprinting data, it is then necessary to delete the data from the database from the **Remove** tab. This tab consists of two list boxes, two buttons and a text box. The steps for removal of samples from the database is as follows:

Firstly, click on the **List** button. This will list out all the available primers in the database.

Secondly, click on the name of the primer of which the sample that is to be deleted belongs in the list box. This will list out all the recorded samples belonging to the desired primer in the database.

Thirdly, click on the name of the sample that is to be deleted. It will response by showing the fingerprinting information in the Band text box.

Finally, click on the **Remove** button to delete the particular sample from the database.

---

The **Process** tab is used when the user had entered all the data and wants to calculate the Similarity Index and Percent Matching between any two individuals. To begin the calculation, simply click on the sole button on this tab.

NOTE: all previous calculations of SI and %M will be deleted.

A message box will appear indicating the completion of this process. The results can only be viewed through MS Access. In MS Access, open the database with the file name, **rapdata\_out.mdb**, located in the directory where STRAIN is. After opening the database, open the table, **pmatch**, located on the **Table Tab**.

The fourth and fifth tabs are used in the two-strain differential using artificial intelligence neural network. The fourth tab, **Training**, consists of the bare necessities to train the network. It is importance of training a neural network cannot be over-emphasized since the integrity of it depends entirely on its training process. In order to train the network, there must be a minimum of 15 fingerprints of each strain in the database. These 30 fingerprints are then used to train the network. The number of training cycles is critical to the integrity of the network. Too low a number of cycles will result in poor resolution, whereas too high a number causes noise intolerance.

The steps to train the network are as follows:

Firstly, enter the number of training cycles in the text box.

Secondly, click on the **List** button to list all available primers in the database.

Finally, double click on the primer name to initiate training. The progress bar will indicate the commencement of training.

After training, the user can then proceed to either testing the system with known data or using the system to identify the strain. Either way, the steps taken are the same. To do this, go to the **Test** tab. Enter the fingerprint into the test box and click on the **Test** button. A message box will appear indicating whether the strain is resistant or susceptible.

Ease of use is the greatest strength of this program even though its functions are relatively limited. With future improvements, this program may be of great assistance in

analyzing complex fingerprinting data, which are currently out of the capabilities of this program.