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# DNA Fingerprinting: Identification of Organisms Using the Polymerase Chain Reaction and Various Primers

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# DNA Fingerprinting: Identification of Organisms Using the Polymerase Chain Reaction and Various Primers

By

Vera Santoleri

Submitted to the Department of Biological Sciences of the State University of New York College at Brockport in fulfillment of a Thesis requirement as part of a Master of Science degree

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## Abstract

The study undertaken involved small scale DNA isolation from eight different fruits using a modified technique written for leaf material. Genetic analysis of this extracted DNA was performed by PCR. Four primers known to target specific DNA sequences were utilized: Analu, Bactoribo, HHF1, and Mitocox. PCR with the Analu, HHF1, and Mitocox primers resulted in a unique pattern of bands that enabled each fruit to be differentiated. Since one major band was observed with the Bactoribo primers and the size of that amplified DNA fragment was either the same or very similar for each fruit, they could not be distinguished based on this primer. Furthermore, the amplification products yielded by the fruits were different from the positive control, thus allowing them to be distinguished also. In most cases, 10 µl of fruit DNA extract in the PCR resulted in the best banding pattern, although informative bands were detected with 1 and 5 µl of DNA also. Interestingly, 5 µl of fruit DNA extract in the PCR reaction yielded variable results whereby in some cases, such as with the analu primers, either fewer bands were seen compared to 1 and 10 µl of DNA, or no bands were visible at all, thus providing less meaningful data. Like RFLP and RAPD analysis, this study demonstrated that the entire genome does not have to be sequenced to detect DNA polymorphisms between different organisms.

## Introduction

DNA fingerprinting, a technology based upon slight differences that exist in DNA sequences found in the genome, has become a powerful tool in the identification of individuals. This variability in DNA sequences between individuals is known as DNA polymorphism (many forms). The concept of DNA fingerprinting was discovered in 1985 when the English geneticist Alec J. Jeffreys, from the University of Leicester, noticed the presence of specific base sequences between chromosomal genes. Such sequences were neither transcribed nor translated, thus having no known function in the cell. It was further observed that these sequences were repeated tandemly many times. Moreover, the number of times the base sequences repeated themselves varied from one individual to the next, hence their name variable number of tandem repeats, or VNTRs (Alcamo 1996). The emphasis of DNA fingerprinting, then, is on utilizing the concept that DNA differs to a certain extent within individuals to retrieve genetic information that is unique to each individual, just as the traditional ink fingerprint is unique and enables identification.

The discovery of DNA fingerprinting has had a profound impact in the area of criminal forensics, whereby DNA from hair, semen, blood, and other tissue samples at a crime scene or on a victim can be analyzed, and a suspect identified with a high degree of certainty. Moreover, paternity testing is facilitated because analysis of DNA samples taken from the child, mother, and father in question can be performed to determine, with much accuracy, the likelihood of the man actually being the biological father (Alcamo 1996).

Currently, there are two particular techniques utilized in deriving a DNA fingerprint. These include the Restriction Fragment Length Polymorphism analysis (RFLP) and the Random Amplified Polymorphic DNA analysis (RAPD). The idea behind the RFLP analysis is that the number of times a restriction site (a palindromic base sequence) recognized by a restriction endonuclease is present in the genome is different from one individual to another. This procedure involves cutting genomic DNA into several fragments with a restriction endonuclease. To prevent repeating sequences from being internally cut by a restriction endonuclease, one is chosen that cuts around them. Some of the most common restriction enzymes used are *Hae III, Taq I, Hinf I,*

and *Pst I* (Easteal et al. 1991). The fragments produced from digestion with the restriction enzyme are then separated by size through gel electrophoresis. The distance migrated by the DNA fragments is inversely proportional to the log of the base pairs comprising the fragment. Therefore, small fragments, which contain fewer repeating sequences, migrate further than the large fragments consisting of a greater number of repeating sequences. These fragments are then denatured into single strands and transferred from the gel onto a nylon or nitrocellulose membrane through the process of Southern blotting, where they are immobilized. Hybridization of a radioactively or fluorescently labeled mini- or microsatellite probe (derived from human DNA, M13 bacteriophage DNA, or synthetically made) to complementary DNA fragments on the membrane yields a banding pattern that is unique for each individual, since these RFLPs are inherited in a Mendelian fashion. Differences in RFLP patterns can also occur due to point mutations whereby a single base is substituted by another, thus either eliminating a restriction site or forming an additional one. Moreover, a deletion or insertion of a base sequence into the DNA results in the shortening or lengthening of the fragments, respectively. Point mutations, deletions, and insertions will alter the banding pattern due to changes in the size and/or number of resulting restriction fragments (Heldt 1997).

Ryskov et al. (1988) conducted the very first plant DNA fingerprinting study by performing an RFLP analysis. This was done by digesting the DNA of two barley cultivars with a restriction endonuclease, performing a Southern blot, and hybridizing a radioactive minisatellite DNA probe that came from the M13 bacteriophage to complementary sequences in the restriction fragments. These barley cultivars were able to be differentiated based on the bands appearing after autoradiography (Nybom 1994).

DNA polymorphisms detected after hybridization of the probe to specific DNA sequences depend more so on the type of probe used than the restriction enzyme chosen. Vosman et al. (1992) conducted a study on 15 tomato cultivars using two different microsatellite probes: $(GATA)_4$ and $(GACA)_4$. All 15 cultivars were distinguishable with both probes. However, the number of bands depicting DNA polymorphisms, as well as the total number of bands seen, was

lower with the $(GACA)_4$ probe. Interestingly, many of the bands detected with the $(GACA)_4$ probe were the same as those detected with the $(GATA)_4$ probe. It was concluded based on these observations that the GACA repeating sequence was located on the same restriction fragment as the GATA repeating sequence, and that these two repeats were, therefore, in close proximity to each other.

The random amplified polymorphic DNA (RAPD) technique, also known as DNA amplification fingerprinting (DAF), or arbitrarily primed polymerase chain reaction (AP-PCR), was developed in 1990 by John Williams and his colleagues, and it has become the preferred procedure over RFLP analysis (Kaemmer et al. 1992). The RAPD method involves the use of PCR to amplify sequences of DNA. Rather than using two primers that flank a specific area of interest by binding to opposite strands of the DNA molecule, one oligonucleotide primer is utilized, and it is typically composed of 10 arbitrary nucleotides that are now commercially available. Williams et al. (1990) demonstrated that the primer must be comprised of at least 9 nucleotides for meaningful results to be achieved. The RAPD technique yields several bands that provide the means for differentiating individuals. As in the RFLP analysis, DNA polymorphisms arise because DNA sequences are unique to the individual. Also, point mutations that create or destroy primer binding sites, as well as deletions and insertions that change the size of the amplified sequence effect the pattern of bands seen on the gel (Heldt 1997).

Depending on the sequence of the single arbitrary primer used, different levels of DNA polymorphisms can be seen. For instance, Kaemmer et al. (1992) set out to distinguish 15 Banana cultivars from each other. To do so, they performed PCR with two single 10-mer primers whose sequences were 5' CGACCGCAGT 3' and 5' CCCTCTGCGG 3'. It was found that, individually, the former primer yielded mostly a smear of bands with the exception of four cultivars that had distinct bands. With the latter primer, on the other hand, the cultivars that produced a smear gave rise to distinguishable bands. As a way of achieving further distinctive band patterns of the PCR products, two short primers can be utilized together. Kaemmer et al. (1992) demonstrated this by performing PCR with the two single primers mentioned above,

simultaneously. They discovered that for most of the 15 banana cultivars the band patterns were unique and different from the patterns observed with the single primers.

On a similar note, the study conducted by Williams et al. (1990) showed that by simply changing one nucleotide in a single primer, different banding patterns could be visualized. While testing two species of soybean, *Glycine max* and *Glycine soja*, one 10-mer primer as well as 10 others that differed from it by the substitution of one base at each consecutive position, were used in PCR. Most of the base substitutions resulted in a pattern of bands different than that observed with the original primer, with some presenting new polymorphisms. Thus, just one base can effect whether or not a DNA sequence will be amplified. RAPD analysis has become the technique of choice because unlike the RFLP analysis, it does not require Southern blotting or the hybridizations and the washings associated with it, it is rapid, simple, and requires very small amounts of DNA which need not even be purified (Heldt 1997). Moreover, since the nucleotide sequence of the primer used is arbitrary, there is no need to have prior genetic knowledge of the DNA being analyzed.

## Purpose

Like RAPD analysis, this research project utilized the PCR procedure for amplifying sequences of DNA from fruit genomes. However, rather than specifying the sequence to be copied with an arbitrary single 10-mer primer, two primers complementary to opposite strands of the template DNA were used. These primers flank specific DNA sequences in specific organisms. Because these primers have not been used in fruit genome analysis, it was unknown from the start whether these primers would yield PCR products, and if they did, whether the resulting PCR products would be similar to the positive control. Based on the success of this study, the procedure for isolating fruit DNA as well as the use of defined primers to amplify DNA sequences, can be incorporated into future genetics labs, whereby students extract DNA from a particular fruit, perform PCR, electrophorese the PCR products, and compare the pattern of bands to those observed in this study.

## Materials and Methods

### DNA preparation

This project entailed the use of DNA from eight various fruits chosen by the researcher. These included a golden delicious apple purchased at a local apple orchard, as well as a banana, red seedless grape, navel orange, plum tomato, mango, kiwi, and cantaloupe, which were purchased at Wegmans Food Market. The fruit DNA was isolated according to a protocol meant for extracting plant DNA from leaf material. The procedure was slightly modified such that the tissue from which the DNA was isolated was the fleshy part of the fruit instead of the leaf. Whether this procedure would be a success was unknown, but it was indeed successful on the first attempt. To begin, between 0.1 and 0.2 grams of the inside of the fruit was weighed out and transferred to a microcentrifuge tube. The tissue was homogenized by repeatedly mashing it with a wooden pestle. To the tube was added 400 µl of a DNA extraction buffer containing 0.2 M Tris-HCl pH 8.0, 0.25 M NaCl, 0.025 M EDTA, and 0.5% SDS. The contents were vortexed for five seconds and centrifuged at top speed for 1 min. Three hundred µl of the supernatant was transferred to a fresh microcentrifuge tube and an equal volume of 2-propanol was added to precipitate the DNA. Following this, the tubes were spun at top speed for 5 min, the supernatant was aspirated, and the pellet washed with 300 µl of 70% ethanol. After centrifuging for another 5 min, the supernatant was again aspirated and the pellet dried in a vacuum dessicator. Finally, the DNA was dissolved in 100 µl of TE (Tris EDTA) buffer (Clapp 1996). It should be noted that the DNAs were extracted on two different days. During the isolation of Apple, Grape, and Orange DNA, warm 2-propanol was used. This did not appear to hinder the precipitation of the DNA. Moreover, the DNA was dissolved in 400 µl of TE. In the case of the Kiwi, Mango, Cantaloupe, Banana, and Tomato, which were isolated on another day, cold 2-propanol was utilized and the DNA was dissolved in the 100 µl of TE called for in the protocol.

DNAs used as a positive control for the primers involved in this study included purified *E. coli* DNA and chelex treated *Ratticus norvecigus* liver DNA which were isolated in Dr. Kline's laboratory by Jeffrey Kiggins, a former graduate student; purified *Saccharomyces cerevisiae* DNA

purchased from Sigma Corporation; and chelex treated *Homo sapiens* DNA isolated from the researcher according to a noninvasive DNA isolation method (Bloom et al. 1996).

**PCR Primers**

Four primers, which target specific DNA sequences, were used in the PCR reactions. These primers, purchased from GIBCO BRL, include: Analu, Bactoribo, HHF1, and Mitocox primers. Each of the primers are composed of a pair of oligonucleotide sequences that recognize complementary nucleotides on opposite strands of the template DNA. The Analu, or animal Alu, primers are composed of two identical sequences 26 nucleotides long. Like the Alu primers, which target repeating suquences found specifically in humans, these oligonucleotides flank a tandemly repeating sequence in animals that are dispersed throughout their genome. The Bactoribo primers contain a 20- and 21-mer nucleotide sequence. As its name implies, it is a bacterial primer specific for the 16S ribosomal RNA gene. The HHF1 primers consist of a 19- and 20-mer nucleotide sequence. Together, they flank the histone 4 gene in *S. cerevisiae*, commonly known as baker's yeast. Histones, which are unique to eukaryotes, are proteins that function in the packaging of DNA into chromosomes (Griffiths et al. 1996). Lastly, the Mitocox primers, consisting of a 25- and 26-mer nucleotide sequence, are specific for the mitochondrial gene coding for the cytochrome c oxidase I subunit. This enzyme is utilized in the electron transport chain during respiration. Refer to Table 1 of Appendix A for the sequences of nucleotides comprising these primers.

**Polymerase Chain Reaction (PCR)**

PCR, a powerful tool used for amplifying DNA, was developed by Kary Mullis in 1985 (Mullis 1990). Though the discovery of this *in vitro* DNA synthesis procedure was at first met with skepticism, it quickly gained popularity and presently has widespread applications in the areas of forensic pathology, diagnostic medicine, genetic testing, cloning, and sequencing, to name a few (Erlich 1992). The wide use of PCR can be attributed to its simplicity, low cost, and time saving qualities. One of the benefits of using PCR is that it requires only a few reagents: genomic DNA (target DNA), primers that flank the DNA region of interest, deoxyribonucleotide

triphosphates (dNTPs), Taq Polymerase, $Mg^{+2}$ ions, and Taq polymerase buffer. The genomic DNA does not have to be purified, thus making PCR quite a forgiving tool. The primers in the reaction bind to complementary nucleotides in the template DNA, and they provide a free 3' OH for the incorporation of the first dNTP into the newly synthesizing strand. Taq polymerase is a heat resistant DNA polymerase originally isolated from the thermophilic bacterium *Thermus aquaticus*. It is now produced in large quantities by the bacterium *E. coli* through recombinant methods. Until the discovery of this enzyme, the klenow fragment of DNA Polymerase I was utilized, but since it was sensitive to high temperatures of PCR, fresh enzyme had to be added during each cycle. $Mg^{+2}$ is a cofactor utilized by Taq polymerase to incorporate dNTPs into the growing DNA strand. Taq polymerase buffer optimizes conditions for DNA synthesis by providing Tris-HCl, KCl, and additional $Mg^{+2}$ ions.

Table 2 of Appendix A illustrates the concentrations and amounts of the reagents used in the PCR reactions of this study. To simplify the process of preparing for PCR, a master mix was made that contained all the reagents except for the template DNA and Taq polymerase. The appropriate volume was then transferred to individual PCR tubes, at which point the DNA and Taq polymerase were then added. The total volume of the PCR reagents in the tubes was 50 μl. Prior to putting the tubes in the thermal cycler, 50 μl of oil was added on top of the contents to prevent possible evaporation due to the high temperature involved with PCR. Note that three different amounts of fruit DNA extract, 1, 5, and 10 μl were tested to determine the effect of DNA concentration on the resulting PCR products. Furthermore, the human and rat chelex DNAs were added to the reaction tubes without dilution, but the *E. coli* and *S. cerevisiae* DNAs were diluted to 0.1 ng prior to their addition to the tubes.

The amplification of DNA during PCR is accomplished in three steps that occur in a thermal cycler: DNA denaturation, primer annealing, and primer extension. During the first step, the temperature is increased to a temperature of 94° or 95° C so that the hydrogen bonds holding the double stranded DNA molecule together are broken, and the DNA is thus melted, or separated, into two individual strands. Following this, the temperature is dropped to enable the

binding of the primers to their complementary region on the opposite strands. The primers flank the region of interest, and therefore, specify the DNA sequence to be amplified. The annealing temperature ranges from 35°C to 65°C depending on the specificity desired. Low annealing temperatures allow mismatched base pairing between the primer and the template strand, thus resulting in the indiscriminate amplification of many DNA sequences. Increasing the annealing temperature ensures perfect base pairing of the primer to the template strand. As a result, fewer DNA sequences are amplified. Too high a temperature may prohibit the primers from binding to the DNA at all, however. The final step in DNA amplification is the extension of the primers through the incorporation of deoxynucleotide triphosphates. This polymerization reaction occurs at 72°C and is catalyzed by the heat stable Taq polymerase. Since 72°C is the optimum temperature for the activity of Taq polymerase, the primer annealing temperature can be increased to increase the specificity of the reaction, without hindering the polymerization activity of the polymerase. [The Klenow enzyme obtained optimum activity at 37°C. This low temperature allowed mismatched base pairing to occur between the primer and template DNA strand, therefore resulting in nonspecific amplification of DNA sequences (Erlich 1992).]

After one PCR cycle a complementary strand is synthesized for each single stranded DNA template. With each cycle the number of DNA molecules is doubled, their ends specified by the primer. For the first two cycles, though, synthesis continues past the region of interest resulting in fragments of variable length. By the third cycle, the primers have defined both ends of the synthesized DNA fragment and each subsequent amplification yields an exponential doubling of this fragment. Typically, the number of cycles ranges from 30 to 35, therefore resulting in millions of copies of the sequence of interest. The temperature and time allotted for each of the three steps are found in Table 3 of Appendix A. These PCR conditions, which vary slightly according to the primer pair used, had been previously found in the literature and were effective in obtaining informative data in a similar study conducted by Jeffrey Kiggins (Altuschul et al. 1990, Loughney et al. 1982, Lunt et al. 1996, Kiggins 1999, and Smith et al. 1983).

### Detection of PCR Products

After the completion of PCR, 5 μl of 10X loading buffer was added to the PCR tubes. This buffer consists of sucrose, which changes the density of the DNA solution and thus enables it to sink to the bottom of the well, in addition to bromophenol blue and xylene cyanol dye, which migrate toward the anode like the DNA and allows indirect visualization of the migrating DNA across the gel. Ten μl of the PCR samples were loaded onto a 2% agarose mini-gel containing 8 wells. In addition to fruit DNA, each gel contained a positive control and a negative control (no DNA) as well as a 100 bp DNA Ladder (0.05 μg /μl) containing 11 DNA fragments of known size: 1500, 1000, 900, 800, 700, 600, 500, 400, 300, 200, and 100 bp. Once the samples were loaded, the gel was run for approximately one hour at 100 Volts. Following this, the gel was stained with ethidium bromide for roughly 5 min, rinsed in distilled water for several seconds, and then visualized under medium range UV light. Ethidium bromide is an agent that intercalates between double stranded DNA and fluoresces when exposed to UV light (Griffiths 1996). A Polaroid photograph was taken of the gel before discarding it.

## Results

### Analu Primers

Amplification products resulting from PCR with 1 μl of fruit DNA extract can be seen in Figures 1 and 2. It is evident that the Analu primers yielded multiple bands for each fruit. In most cases, only the calculated fragment size of the major PCR products will be mentioned. The fragment size of the minor products will be presented in tables together with the major products. Tomato yielded 11 bands, including 8 minor and 3 major bands. The major amplification products were calculated to be 980, 800, and 540 bp in length. The minor products are displayed in Table 1 of Appendix B. Cantaloupe gave 6 bands. The 4 major products were 1640, 680, 420, and 230 bp. Rat DNA, which served as a positive control, resulted in a smear (many unresolvable bands) with two bands visible within it. The brighter of the two bands was 410 bp long while the second band, which was more difficult to see because it blended in with the smear, was 320 bp. Human

DNA isolated from the researcher was thought to be a negative control because it contains Alu repeats different from animal Alu repeats. However, like the rat DNA, it resulted in a long smear of indistinguishable bands. Eleven bands were apparent with Mango. The major amplification product was 350 bp. Apple yielded 7 bands, two of which were major bands. These major bands represented a 610 and 390 bp amplification product. Turning to figure 2, Banana yielded 10 bands, with 2 major bands. The brighter of the two was 640 bp, while the other was 500 bp. For Kiwi, 9 bands were detected with certainty. One major band was present, and it was 720 bp. Rat gave a smear with two bright bands measuring 390 and 300 bp. Human DNA resulted in a smear of bands, also. Only five distinguishable bands were found with Grape. The major amplification product was 420 bp. Orange produced 6 measurable bands, with a major product of 360 bp.

Figure 1

Figure 2

Figures 1and 2: PCR products from 1 μl fruit DNA + Analu primers.
Figure 1. Lanes 1-8: Tomato, Cantaloupe, No DNA, Ladder, Rat, Human, Mango, Apple
Figure 2. Lanes 1-8: Banana, Kiwi, No DNA, Ladder, Rat, Human, Grape, Orange

Figures 3 and 4 represent amplification products obtained from the use of 5 µl of fruit DNA extract in the PCR reaction. In addition, *E. coli* was utilized as a negative control in place of human DNA. From figure 3 it can be seen that Apple, Kiwi, and Banana, did not yield any PCR products, while Tomato produced very light bands. Two were readily detected at 330 and 250 bp, but there was also a fuzzy region between 600 and 400 bp that was difficult to measure. Rat gave two major bands representing a 420 and 310 bp fragment. Although it was thought that *E. coli* would not yield amplification products, PCR with the Analu primers resulted in a slew of bands. Seventeen bands were produced, and from these, there were four major bands measuring at 1600, 1320, 1090, and 310 bp. The minor bands varied in intensity, with the bands greater than 1500 bp being very light. Turning to figure 4, Orange gave one bright band at 380 bp. Mango did not yield any amplification products. Rat yielded two major amplification products measuring 420 and 310 bp in length. *E. coli* produced 17 bands with the most intense being 1590, 1310, 1130, and 310 bp. Cantaloupe yielded 3 bands. The major PCR product was a 420 bp fragment. Grape gave two very faint bands at 440 and 380 bp.

Figure 3

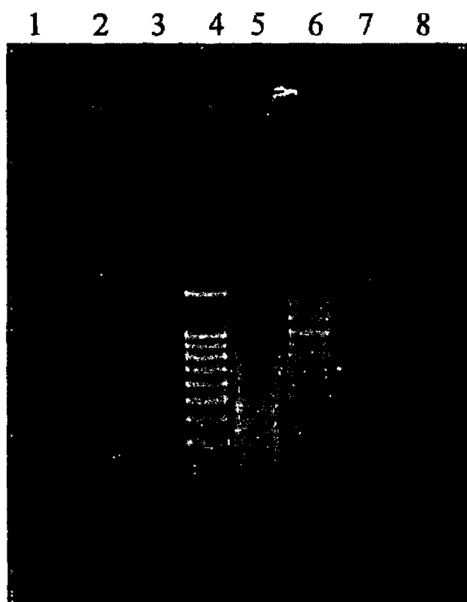



Figure 4

Figures 3 and 4: PCR products from 5 µl of fruit DNA + Analu primers.
Figure 3. Lanes 1-8: Apple, Kiwi, No DNA, Ladder, Rat, E. coli, Banana, Tomato
Figure 4. Lanes 1-8: Orange, Mango, No DNA, Ladder, Rat, E. coli, Cantaloupe, Grape

Figures 5 and 6 represent amplification products from PCR with 10 μl of fruit DNA extract. Looking at figure 5, Kiwi yielded only one visible band at 720 bp. Two bands were detected with Apple. The major band represented a 410 bp fragment. With rat, only one band at 430 bp was detectable. *E. coli* displayed 17 bands with the major bands measuring 1590, 1380, 1090, 830, and 310 bp. Banana gave no PCR products. Tomato yielded 12 bands, four of which represented major products measuring at 1150, 950, 450, and 340 bp. Looking at figure 6, four bands were observed for Orange. The major amplification product was 440 bp. Grape gave six amplification products with two major bands representing 520 and 250 bp fragments. Rat resulted in one band measured to be 430 bp. *E. coli* yielded 18 bands with the four major bands representing a 1560, 1320, 1120, and 830 bp fragment. Five distinguishable bands were detected with Cantaloupe. The four major products present were 1630, 670, 390, and 220 bp. The most intense band was produced by the 670 bp fragment. Mango yielded 10 bands. The 4 major bands represented an 800, 380, 320, and 250 bp fragment. There were also faint bands in the high base pair region that were difficult to measure.

Figure 5

1 2 3 4 5 6 7 8

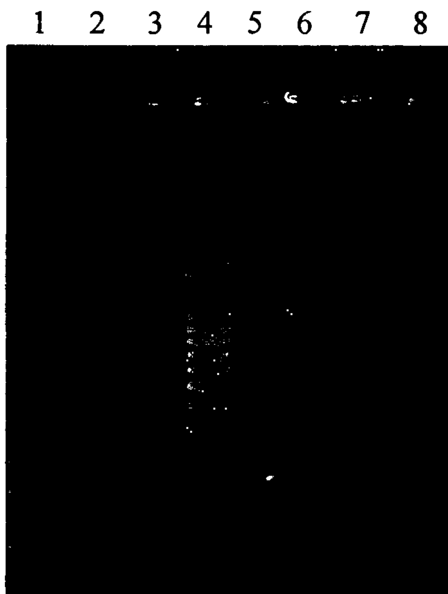
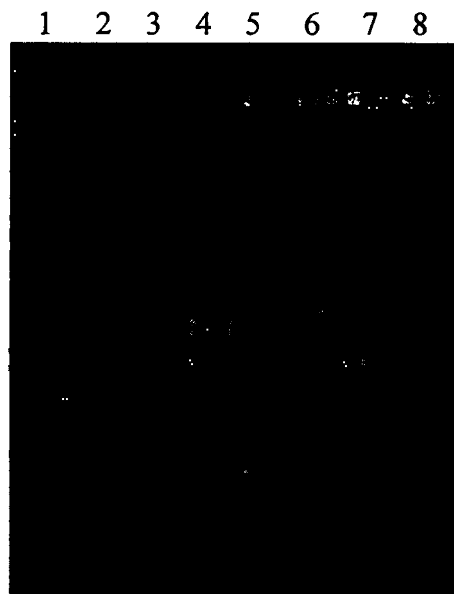


Figure 6

1 2 3 4 5 6 7 8



Figures 5 and 6: PCR products from 10 μl of fruit DNA + Analu primers.
Figure 5. Lanes 1-8: Kiwi, Apple, No DNA, Ladder, Rat, E. coli, Banana, Tomato
Figure 6. Lanes 1-8: Orange, Grape, No DNA, Ladder, Rat, E. coli, Cantaloupe, Mango

**Bactoribo Primers**

The PCR products resulting from the use of 1 μl of fruit DNA extract are indicated in Figures 7 and 8. Minor amplification products are displayed in Table 2 of Appendix B. Observing figure 7, Cantaloupe and Kiwi both gave a major amplification product of 680 bp as well as three and four minor products, respectively. The positive control, *E. coli*, yielded a 780 bp fragment different from the fruits and slightly greater in size. No PCR product was visible for Grape. Banana yielded a major amplification product of 680 bp, in addition to 2 minor products. Looking at Figure 8, Tomato and Mango both gave a 670 bp fragment. In addition to the major band, Mango also displayed 2 minor bands. *E. coli* yielded a 780 bp fragment. Meanwhile, Orange and Apple yielded a 740 bp fragment.

Figure 7

1 2 3 4 5 6 7 8

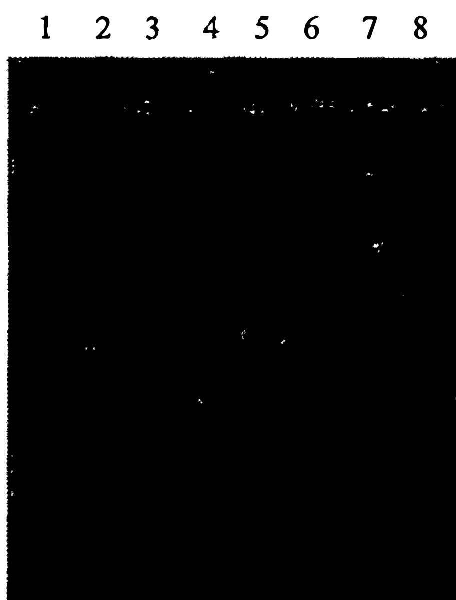


Figure 8

1 2 3 4 5 6 7 8

Figures 7 and 8. PCR products from 1 μl of fruit DNA + Bactoribo primers
Figure 7. Lanes 1-8: Cantaloupe, Kiwi, No DNA, Ladder, E. coli, Grape, Banana, Blank
Figure 8. Lanes 1-8: Tomato, Mango, No DNA, Ladder, E. coli, Apple, Orange, Blank

Figures 9 and 10 represent PCR products resulting from the use of 5 μl of fruit DNA extract. In several instances the bands appeared brighter and thicker, thus indicating an abundance of PCR product. In figure 9, five bands were detected for Orange. The major product was 700 bp. Apple yielded two amplification products with a major product of 730 bp. *E. coli* yielded a 790 bp fragment. Grape gave one band representing a 730 bp fragment. Cantaloupe produced four bands, with the major amplification product being a 700 bp fragment. Tomato yielded a major amplification product of 670 bp as well as two minor bands in the high base pair region. According to figure 10, Mango and Kiwi both yielded a 710 bp fragment, in addition to three and two minor bands, respectively. *E. coli* gave a 740 bp amplification product. Banana migrated further than the rest, producing a 650 bp fragment.

Figure 9

1 2 3 4 5 6 7 8

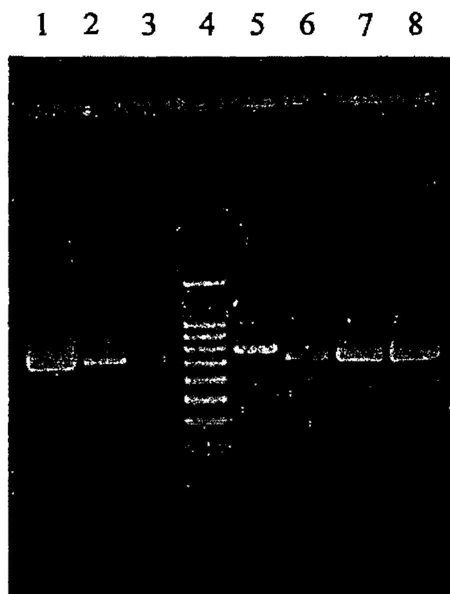
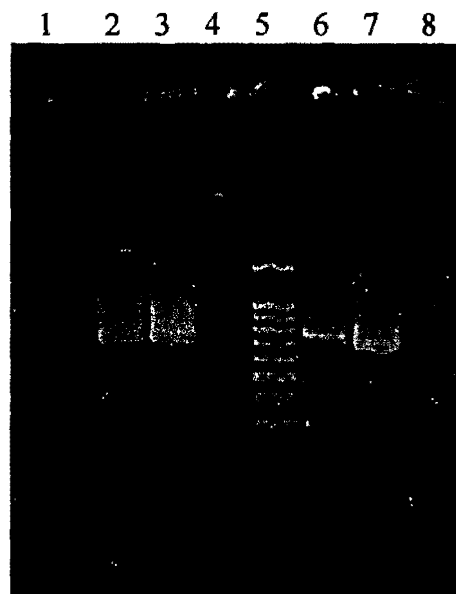


Figure 10

1 2 3 4 5 6 7 8



Figures 9 and 10. PCR products from 5 μl of fruit DNA + Bactoribo primers.
Figure 9. Lanes 1-8: Orange, Apple, No DNA, Ladder, E. coli, Grape, Cantaloupe, Tomato
Figure 10. Lanes 1-8: Blank, Mango, Kiwi, No DNA, Ladder, E. coli, Banana, Blank

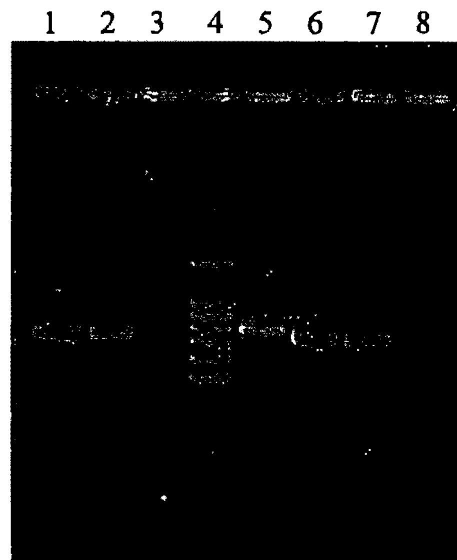
PCR products resulting from the use of 10 μl of fruit DNA extract can be seen in Figures 11 and 12. Observing figure 11, Mango, Grape, Kiwi, and Banana each gave a PCR product of 710 bp. In addition, three and two minor bands were visible with Mango and Kiwi, respectively. *E. coli* yielded a 780 bp fragment. Turning to figure 12, Orange and Apple each yielded a 720 bp fragment, while Cantaloupe and Tomato both gave a major product 690 bp in length and 3 minor products. Three minor bands were also visible for Orange. *E. coli* yielded a 790 bp fragment.

Figure 11

1 2 3 4 5 6 7 8

Figure 12

1 2 3 4 5 6 7 8



Figures 11 and 12. PCR products from 10 μl fruit DNA + Bactoribo primers.
Figure 11. Lanes 1-8: Mango, Grape, No DNA, Ladder, E. coli, Kiwi, Banana, Blank
Figure 12. Lanes 1-8: Orange, Apple, No DNA, Ladder, E. coli, Cantaloupe, Tomato, Blank

**HHF1 Primers**

Figures 13 and 14 represent PCR products resulting from the use of 1 μl of fruit DNA extract. Minor bands are presented in Table 3 of Appendix B. In figure 13, Grape yielded very light bands. The five distinguishable amplification products were 640, 530, 440, 370, and 240 bp in length. A fuzzy region was also present between 800 and 1500 bp. Kiwi yielded 14 bands.

The two major bands represented a 1010 and 330 bp fragment. The positive control, *S. cerevisiae*, gave 11 detectable bands. The major amplification product was 330 bp. *E. coli*, which was believed to be a negative control, yielded one band at 1610 bp. Six bands were observed for Cantaloupe. There were two major bands, and the brighter of the two was 580 bp while the other was 330 bp. Extremely faint bands were present for Apple. The two measurable bands indicated a 380 and 320 bp fragment. Looking at figure 14, Mango gave 7 bands that were fairly light. The most intense band was produced by a 430 bp fragment. Three faint bands representing a 870, 450, and 410 bp fragment were observed for Orange. Ten bands were detected for *S. cerevisiae*. The major amplification product was 340 bp in length. *E. coli* gave no detectable amplification product. Banana yielded one major band measuring 620 bp. Tomato resulted in 11 bands. The three major products were 620, 430, and 290 bp long.

Figure 13

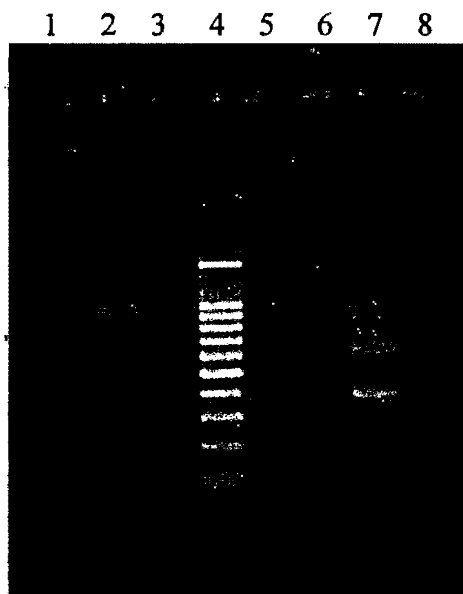
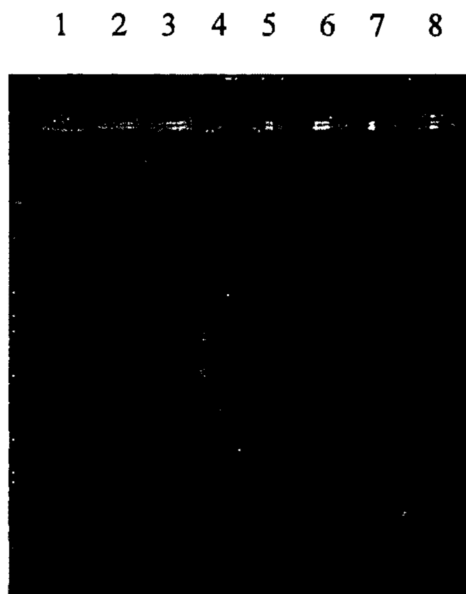



Figure 14




Figures 13 and 14. PCR products from 1 μl of fruit DNA + HHF1 primers.
Figure 13. Lanes 1-8: Grape, Kiwi, No DNA, Ladder, S. cerevisiae, E. coli, Cantaloupe, Apple
Figure 14. Lanes 1-8: Mango, Orange, No DNA, Ladder, S. cerevisiae, E. coli, Banana, Tomato

Figures 15 and 16 depict the PCR products resulting from the use of 5 μl of fruit DNA extract. In figure 15, Orange gave one amplification product of 440 bp. Kiwi yielded four discernible bands with the two major products measuring 610 and 170 bp. *S. cerevisiae* yielded 8 bands, the major product being 350 bp. *E. coli* gave an amplification product of 1600 bp. Nine bands were detected for Cantaloupe. There were two major bands, with the brighter band measuring 350 bp and the lighter of the two, 610 bp. Apple gave two light bands with the brighter of the two representing a 380 bp fragment and the other a 320 bp fragment. Looking at figure 16, twelve bands were detected with Tomato. The two major amplification products included a 620 and 300 bp fragment. Grape yielded a 440 bp amplification product. *S. cerevisiae* yielded 9 bands, the major band representing a 350 bp fragment. *E. coli* resulted in one band measuring 1710 bp. Eighteen bands were visible for Banana. The major PCR product was 650 bp. Mango yielded seven observable bands. The three major products were 960, 540, and 420 bp, while the minor bands were extremely faint.

Figure 15

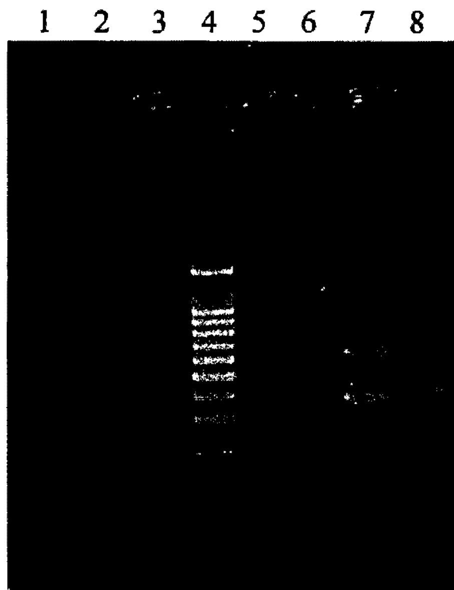



Figure 16

Figures 15 and 16. PCR products from 5 μl of fruit DNA + HHF1 primers.
Figure 15. Lanes 1-8: Orange, Kiwi, No DNA, Ladder, S. cerevisiae, E. coli, Cantaloupe, Apple
Figure 16. Lanes 1-8: Tomato, Grape, No DNA, Ladder, S. cerevisae, E. coli, Banana, Mango

Figures 17 and 18 indicate the PCR products resulting from the use of 10 μl of fruit DNA extract. According to figure 17, 14 bands were observed for Tomato. The two major bands were indicative of a 460 and 340 bp fragment. Banana yielded 21 bands. The major product was 680 bp long. *S. cerevisiae* yielded five faint bands and one major band at 320 bp. No PCR product was observed for *E. coli*. Mango resulted in 13 bands. The six major amplification products included a 1720, 1380, 970, 890, 850, and 420 bp fragment. Twelve bands were visible with Kiwi. Three major bands were visible, with the most intense measuring 1010 bp in length, while the other two were 440 and 290 bp. Turning to figure 18, three bands were observed for Orange. The major band represented a 940 bp fragment. Grape yielded 15 bands. The four major bands were 1160, 1060, 640, and 540 bp. *S. cerevisiae* produced six observable bands, with a major amplification product of 340 bp. *E. coli* again yielded no bands. Apple gave nine bands. The two major amplification products were 2610 and 1700 bp long. The remaining minor bands were all very faint. Ten bands were detected for Cantaloupe with the three major bands representing a 720, 610, and 350 bp fragment. The 350 bp band was the brightest.

Figure 17

1 2 3 4 5 6 7 8

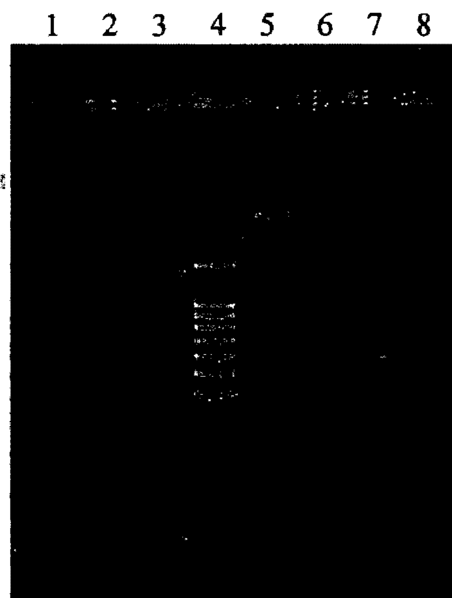
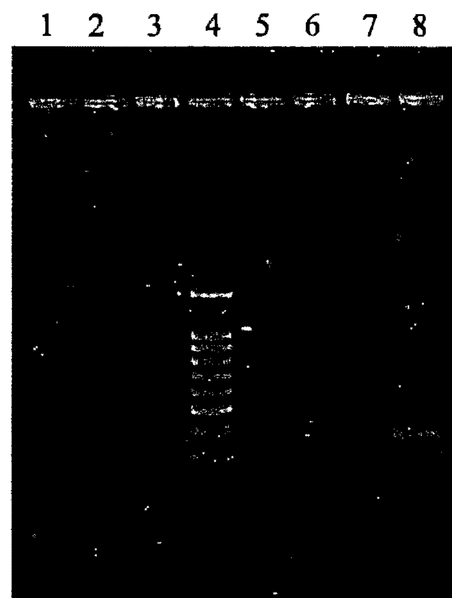


Figure 18

1 2 3 4 5 6 7 8



Figures 17 and 18. PCR products from 10 μl of fruit DNA + HHF1 primers.
Figure 17. Lanes 1-8: Tomato, Banana, No DNA, Ladder, S. cerevisiae, E. coli, Mango, Kiwi
Figure 18. Lanes 1-8: Orange, Grape, No DNA, Ladder, S. cerevisiae, E. coli, Apple, Cantaloupe

**Mitocox Primers**

Figures 19 and 20 represent the PCR products obtained from the use of 1 μl of fruit DNA extract. Minor bands are depicted in Table 4 of Appendix B. Looking at figure 19, Mango resulted in six light bands that measured 1110, 720, 600, 410, 280, and 180 bp. A fuzzy region around 400 bp was detectable for Apple, but could not be measured. The positive control, Human DNA, yielded 6 bands with the major band representing a 690 bp fragment. *E. coli* gave a very light band at 410 bp. Nine bands were detected for Cantaloupe, and the major amplification product was 540 bp in length. Orange yielded 2 bands similar in intensity representing a 320 and 210 bp fragment. In figure 20, nine bands were observed for Tomato. The two major PCR products were 430 and 190 bp long. Grape yielded two light bands measuring 400 and 330 bp. Human DNA gave 7 bands with the major band being 690 bp. *E. coli* yielded a faint band at 400 bp. Kiwi resulted in 5 bands. The major amplification product was 500 bp. One light band was detectable for Banana at 340 bp.

Figure 19

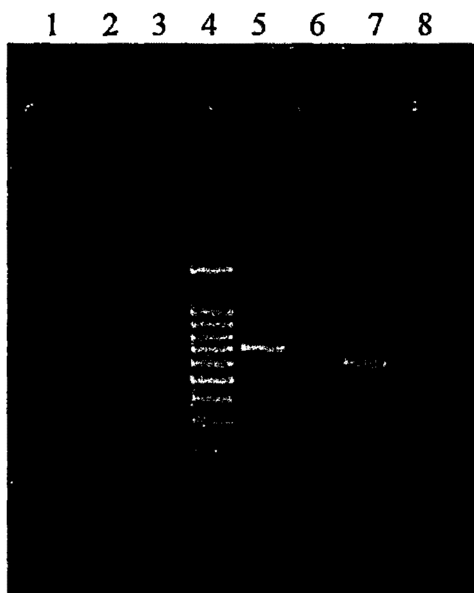
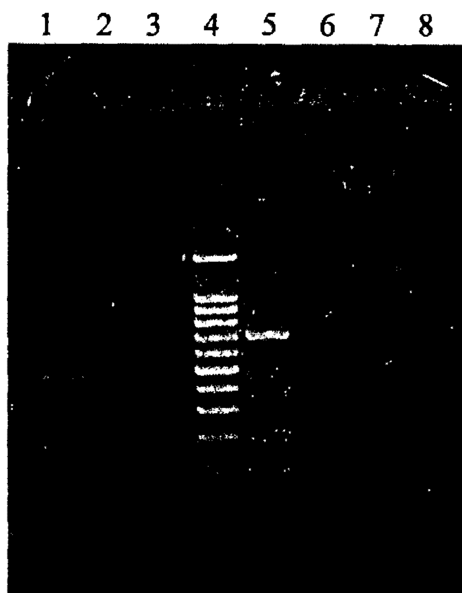



Figure 20




Figures 19 and 20. PCR products from 1 μl of fruit DNA + Mitocox primers.
Figure 19. Lanes 1-8: Mango, Apple, No DNA, Ladder, Human, E. coli, Cantaloupe, Orange
Figure 20. Lanes 1-8: Tomato, Grape, No DNA, Ladder, Human, E. coli, Kiwi, Banana

PCR products resulting from the use of 5 μl of fruit DNA extract are shown in figures 21 and 22. In figure 21, five bands were observed for Tomato, and the major amplification product was 180 bp. No bands were detectable for Grape. Human DNA gave one PCR product measuring 690 bp. *E. coli* yielded two bands representing a 820 and 340 bp fragment. Kiwi yielded 2 bands. The major band was indicative of a 460 bp fragment. Banana failed to yield a PCR product. Looking at figure 22, Mango resulted in 5 discernible bands. The major band was 440 bp. Two light bands indicative of a 530 and 300 bp fragment were observed for Apple. Seven bands were detected for Human DNA, including a major product of 690 bp. *E. coli* yielded 2 bands measuring at 830 and 380 bp. Cantaloupe gave 6 bands with the major product being 580 bp. Orange yielded 4 bands all of which were similar in intensity. They represented a 910, 830, 330, and 210 bp fragment.

Figure 21

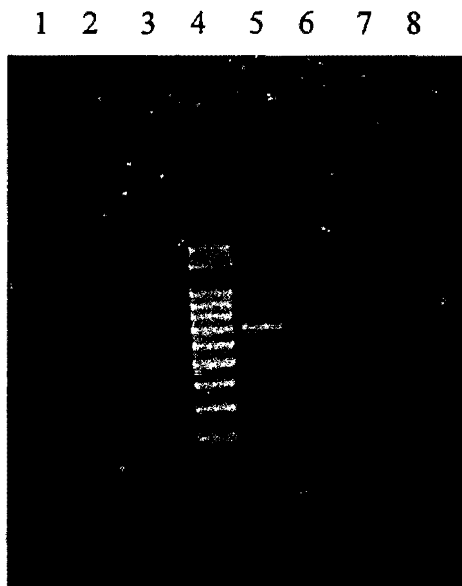
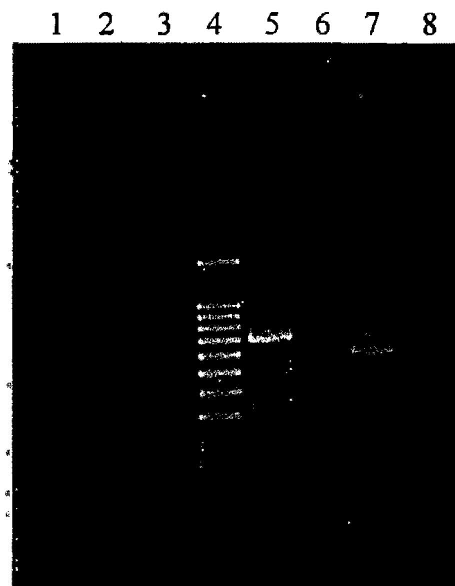



Figure 22




Figures 21 and 22. PCR products from 5 μl of fruit DNA + Mitocox primers.
Figure 21. Lanes 1-8: Tomato, Grape, No DNA, Ladder, Human, E. coli, Kiwi, Banana
Figure 22. Lanes 1-8: Mango, Apple, No DNA, Ladder, Human, E. coli, Cantaloupe, Orange

Figures 23 and 24 depict the PCR products resulting from the use of 10 μl of fruit DNA. Looking at figure 23, Mango yielded 8 bands. The two major amplification products were 1050 and 490 bp in length. Five bands were detected for Banana. The four major bands represented a 1000, 820, 270, and 170 bp fragment. Human DNA gave 4 visible bands with the major product being 680 bp in length. *E. coli* gave no detectable bands. Orange resulted in 3 bands with the two major products measuring at 330 and 220 bp. Cantaloupe yielded 7 bands, and the major band represented a 540 bp fragment. According to figure 24, six bands were observed for Apple. The major band represented a 680 bp amplification product. Tomato gave 11 amplification products. The 5 major products were 1060 bp, with the greatest intensity, as well as 440, 250, 190, and 150 bp fragments. Human DNA yielded 6 bands with the major product being 680 bp in length. *E. coli* yielded two very faint bands indicative of a 1170 and a 440 bp fragment. Kiwi resulted in 9 bands. The 2 major products were 920 and 460 bp in length. Grape yielded 14 discernible bands. The 5 major bands represented a 2550, 620, 490, 380, and 310 bp fragment.

Figure 23

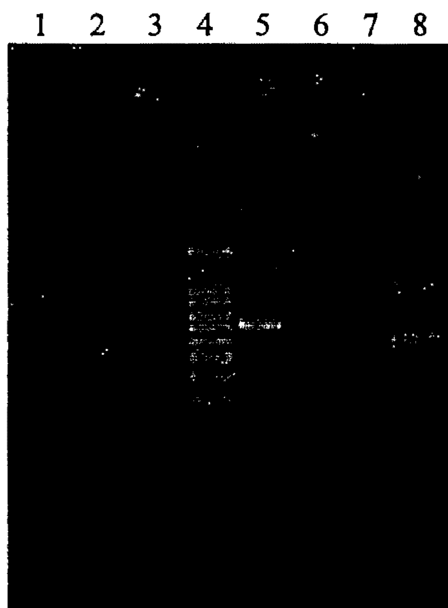
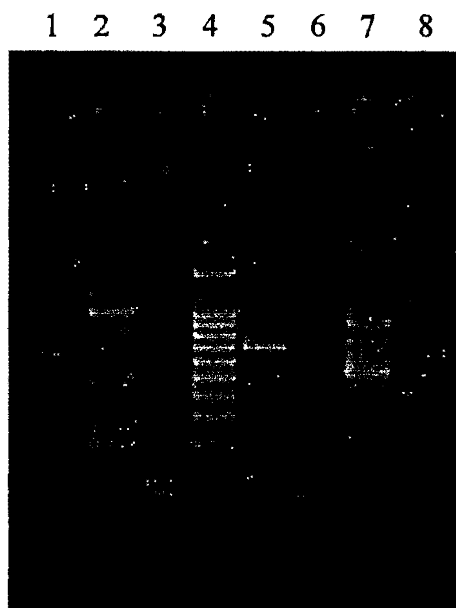



Figure 24




Figures 23 and 24. PCR products from 10 μl of fruit DNA + Mitocox primers.
Figure 23. Lanes 1-8: Mango, Banana, No DNA, Ladder, Human, E. coli, Orange, Cantaloupe
Figure 24. Lanes 1-8: Apple, Tomato, No DNA, Ladder, Human, E. coli, Kiwi, Grape

## Discussion

### Analu Primers

The Analu primers yielded multiple bands in many cases, especially with the 1 and 10 μl DNA reactions. For an unknown reason, Tomato, Orange, Cantaloupe, and Grape gave fewer PCR products with 5 μl of DNA than observed for either 1 or 10 μl. The remaining fruits, Kiwi, Mango, Apple, and Banana yielded no amplification products at all with 5 μl of DNA. Furthermore, Banana resulted in no PCR products in the 10 μl reaction either. It is possible that the concentration of Banana DNA was too high, and thus hindered the PCR reaction. When too much DNA is present, all the primers bind to their complementary sequences on the template DNA, and there is not an ample amount of the primers left to sustain further amplification. For Kiwi, Mango, and Apple, on the other hand, amplification products were detected with 10 μl of DNA. Inexplicably, when PCR was repeated using 5 μl of fruit DNA, the same banding patterns were observed.

The numerous bands displayed by the fruits are most likely due to the low primer annealing temperature in the PCR. An advantage of these multiple bands is their revelation of DNA polymorphisms present among the fruit genomes. Since each fruit yielded a pattern of bands that differed from one fruit to the next, it was possible to distinguish the fruits from each other with the Analu primers.

Like human Alu repeats, animal Alu repeats are short DNA sequences that are interspersed throughout the genome and occur frequently. In humans, for example, more than 1 million copies of this repetitive sequence can be found, accounting for 5% of the entire genome. They are normally located in spacer DNA that exists between genes but can also be found within the introns of genes. These repeating sequences have no known function since they are not transcribed (Kirby 1990). The abundance of this Alu family of sequences is the reason why Rat DNA yielded so many bands that they appeared as a smear, with the exception of the two bands that were detected within the smear. The brightness of those two bands indicates that those particular DNA sequences are found most often. Human DNA was used at first as a negative

control because human and animal Alu sequences differ in nucleotide composition, but like Rat, a smear was present. A combination of low stringency conditions and the complementarity of template DNA sequences to the primers probably resulted in the long smear of bands. *E. coli* DNA was utilized to replace Human DNA in the remaining reactions, and surprisingly, the result was the presence of numerous distinguishable bands. No amplification products were expected to be seen since 99% of the *E. coli* genome consists of single copy sequences, but it is evident that there are several DNA sequences that the Analu primers bind to under low stingency conditions (Kirby 1990).

**Bactoribo Primers**

Each fruit yielded one major amplification product of similar size with the bactoribo primers. The only exception where a PCR product was not detected was with 1 µl of Grape DNA extract in the PCR reaction. The initial dilution of the isolated DNA in 400 µl of TE buffer may have contributed to this poor amplification. Apple and Orange DNA, which were dissolved in 400 µl of TE buffer as well, produced a lighter band than the remaining five fruits. Due to the small quantity of DNA isolated from the eight fruits, the DNA samples were not quantitated. Thus, the relative amount of DNA in the 1 µl pipetted for PCR may have been different enough to give varying quantities of PCR product. In many cases, minor products were observed in addition to the major product, thus providing further evidence for the mismatched base pairing occurring between template DNA and primer at the low primer annealing temperature used in this reaction.

The fruit DNA major amplification product was smaller in size than that for *E. coli*, thus enabling the differentiation of a prokaryote such as *E. coli* from eukaryotic fruit. Recall that prokaryotic and eukaryotic ribosomes differ in size. The 70S prokaryotic ribosome contains a 50S and 30S subunit whereas the 80S eukaryotic ribosome consists of a 60S and 40S subunit. Within these subunits reside rRNA. The prokaryotic 30S subunit contains a 16S rRNA, while the equivalent in the eukaryotic 40S subunit is the 18S rRNA (Griffiths et al. 1996). The universal bactoribo primers target a region of the 16S rRNA gene that is conserved among bacteria. The

18S rRNA gene found in the fruit DNAs may also contain conserved regions whose sequence is similar enough to the 16S sequence to bind with the primers in this low specificity reaction.

It was evident from the gel that the fruit PCR products did not all migrate the same distance. This can be due to two factors. First, the thickness of the bands varied in some instances, thereby resulting in different migration distances. Second, the slight differences in size of the fragments can also be due to variation in the number of nucleotides resulting from an insertion or deletion of bases.

**HHF1 Primers**

PCR with the HHF1 primers resulted in multiple bands just as the Analu primers did. Therefore, all the fruits were identifiable based on their distinctive banding patterns. The best results were observed with the use of 10 μl of fruit DNA extract in the PCR reaction, because bands were detectable for each one of the fruits. Of all the primers, HHF1 resulted in the greatest number of bands, thus suggesting that many DNA sequences are present in the fruit genome that are complementary enough to the HHF1 primers to enable binding under low stringency conditions. Because plant genomes contain a lot of repetitive sequences, it is possible that the HHF1 primers bound to these sequences and thus produced a slew of bands. *S. cerevisiae*, which was utilized as the positive control and was known to yield a 371 bp amplification product, resulted in several minor products in addition to a major product that ranged from 320 to 350 bp in the six gels that were run. Although the fragment appeared to be closer to 371 bp on the gel, calculations using the line of best fit based on the migration distance and the size of the Ladder DNA fragments gave a different estimate. The statistical $R^2$ value for all the standard curve graphs was approximately 0.98. This indicates that there is not a perfect one-to-one relationship between migration distance of the Ladder DNA fragments and their size, which explains why the calculation of the size of the amplification products is not truly accurate.

The HHF1 primers were designed to flank the two histone 4 genes present in the *S. cerevisiae* genome. Histones, which are proteins unique to eukaryotes, assist in the packaging

of DNA into tightly coiled chromosomes (Griffiths et al. 1996). Since histones do not exist in prokaryotes, *E. coli* was utilized as a negative control. Two different concentrations of *E. coli* were used in the PCR procedure. When 5 µl of DNA was used, a band greater than 1500 bp was detected, but with 1 µl of DNA, no amplification product was visible. Because there was only one band observed, and it was detected with the greater DNA concentration, it was most likely due to the unspecific binding of the primers to the template DNA.

**Mitocox Primers**

Like the Analu and HHF1 primers, the Mitocox Primers resulted in many amplification products. Again, the banding patterns were unique to the individual fruits, and therefore enabled them to be distinguished from each other. The best results were observed with 10 µl of DNA extract. Both Banana and Grape failed to yield measurable bands with 5 µl of DNA; only one and two bands were seen, respectively, with 1 µl of DNA but more were detected with 10 µl of DNA. In the case of Apple and Orange, the number of amplification products increased with an increase in DNA concentration.

Mitocox primers target a 710 bp region of the mitochondrial gene coding for the cytochrome c oxidase I subunit (COI) (Folmer et al. 1994). The COI gene has become the focus of many "taxonomic, population and evolutionary" studies in animals, because it contains regions that are highly conserved (Lunt et al. 1996). Human DNA, the positive control, yielded several minor PCR products in addition to a major product that ranged from 680 to 690 bp, which is very close to the 710 bp fragment expected. There was one instance, however, where only one amplification product was present, and this resulted when 5 µl of Human DNA extract was used in the PCR instead of 1 µl. *E. coli* yielded one and sometimes two amplification products. These were faint and difficult to measure in some cases. Since *E. coli* does not contain mitochondrial DNA, the bands observed were most likely due to unspecific primer-template DNA binding during PCR.

In conclusion, this study first demonstrated that DNA can be isolated from the fleshy part of a fruit using a protocol for leaf tissue. Moreover, the eight different fruits tested could be distinguished from each other by performing a genetic analysis using the PCR technique together with various primers that target specific DNA sequences. Like the RAPD method, the result is a pattern of bands that is unique for each individual, and this enables identification. Furthermore, the results of this study indicate that using different concentrations of fruit DNA extract in the PCR procedure can lead to the revelation of new bands and/or the disappearance of bands. Moreover, the major amplification product that is observed may change with the different DNA concentrations added to the PCR mix. That is, what is seen to be the major PCR product with a certain concentration of DNA in the PCR may be the minor product when another DNA concentration is used. This particular study showed that in most cases, 10 µl of fruit DNA extract in the PCR displayed DNA polymorphisms the best and allowed the greatest opportunity for differentiating the fruits tested. Though the complete banding pattern produced by each fruit was informative, it was also found that, with the exception of the Bactoribo reactions, the fruits could be distinguished from each other based solely on their major band(s) alone. This comparison of major bands is illustrated in Table 5 of Appendix B, and for the sake of simplicity, it includes just the major band(s) resulting from the 10 µl DNA reactions for each of the four primers. Finally, DNA polymorphisms were observed between the fruits and the positive controls; of the amplification products detected with the fruits, very few were the same size as the positive control.

**Suggestions for Future Work**

This study was essentially a survey that sought to determine whether the use of unconventional primers in PCR would enable DNA differences to be seen among eight various fruits whose DNA was obtained by a small-scale isolation method. Due to the fact that all of the PCR reactions were not repeated to demonstrate the reproducibility of the results observed for each fruit, it cannot be stated with certainty that a particular fruit will exhibit a banding pattern

specific for the primer pair used, everytime a PCR reaction is run. Performing additional reactions would enable such results to be achieved. Furthermore, if each fruit DNA had been isolated on a large-scale basis, the DNA could have been quantitated so that the exact concentration of the DNA in the PCR reaction was known. In this case, the amount of DNA in the reactions would be consistent for each fruit, and it could be stated with increased confidence that the differences seen in banding patterns are due to the genetic makeup of the individual fruits and not the variability in the starting amount of template DNA. Moreover, the reproducibility of the results can be easily demonstrated if the experiments are repeated using the same amount of DNA each time.

Although the combination of primers and PCR conditions used in this study allowed DNA polymorphisms to be observed in the fruits, future studies can focus on determining what the effects of altering the primer annealing temperature are on the amplification products detected. Furthermore, single oligonucleotide primers of arbitrary sequence can be utilized individually or together in PCR to discover additional DNA polymorphisms in fruit.

# Appendix A

**Table 1. Primer sequences and target sites**

| Primer Name | Target sequence | Primer Sequence | # Nucleotides |
|---|---|---|---|
| Analu | Animal Alu sequence | (F) 5' GTGGATCACCTGAGGTCAGGAGTTTC 3' | 26 mer |
| | | (R) 5' GTGGATCACCTGAGGTCAGGAGTTTC 3' | 26 mer |
| Bactoribo | Bacterial rRNA gene | (F) 5' GATCCTGGCTCAGGATGAAC 3' | 20 mer |
| | | (R) 5' GGACTACCAGGGTATCTAATC 3' | 21 mer |
| HHF1 | *S. cerevisiae* | (F) 5' AACAAAAACAAGCAACAAA 3' | 19 mer |
| | histone 4 gene | (R) 5' ACCGTTTTCTTAGAATTAGC 3' | 20 mer |
| Mitocox | Cytochrome C oxidase | (F) 5' GGTCAACAAATCATAAAGATATTGG 3' | 25 mer |
| | subunit I gene | (R) 5' TAAACTTCAGGGTGACCAAAAAATCA 3' | 26 mer |

(F) represents forward primer
(R) represents reverse primer

**Table 2. PCR Reagents needed for 50 ul reaction**

| Reagents | Analu Primers |
|---|---|
| 10X Taq Buffer with $Mg^{+2}$ * | 5 µl |
| $Mg^{+2}$ (25 mM) | 5 µl |
| Primer Mix (5 pmol/µl each) | 5 µl |
| dNTPs (5 mM each) | 2 µl |
| Distilled Water ** | 31.5 µl |
| Target DNA | 1 µl |
| Taq Polymerase (5 units/µl) | 0.5 µl |

| Reagents | Bactoribo Primers |
|---|---|
| 10X Taq Buffer with $Mg^{+2}$ * | 5 µl |
| $Mg^{+2}$ (25 mM) | 5 µl |
| Primer Mix (1.4 pmol/µl each) | 5 µl |
| dNTPs (5 mM each) | 2 µl |
| Distilled Water ** | 31.5 µl |
| Target DNA | 1 µl |
| Taq Polymerase (5 units/µl) | 0.5 µl |

| Reagents | HHF1 Primers |
|---|---|
| 10X Taq Buffer with $Mg^{+2}$ * | |
| $Mg^{+2}$ (25 mM) | 5 µl |
| Primer Mix (2.5 pmol/µl each) | 5 µl |
| dNTPs (5 mM each) | 2 µl |
| Distilled Water ** | 31.5 µl |
| Target DNA | 1 µl |
| Taq Polymerase (5 units/µl) | 0.5 µl |

| Reagents | Mitocox Primers |
|---|---|
| 10X Taq Buffer with $Mg^{+2}$ * | 5 µl |
| $Mg^{+2}$ (25 mM) | 5 µl |
| Primer Mix (5 pmol/µl each) | 5 µl |
| dNTPs (5 mM each) | 2 µl |
| Distilled Water ** | 31.5 µl |
| Target DNA | 1 µl |
| Taq Polymerase (5 units/µl) | 0.5 µl |

* Taq DNA polymerase 10X buffer:

| Promega | Sigma |
|---|---|
| 100 mM Tris-HCl pH 9.0 | 100 mM Tris-HCl pH 8.3 |
| 500 mM KCl | 500 mM KCl |
| 15 mM $MgCl_2$ | 15 mM $MgCl_2$ |
| 1% Triton X-100 | 0.01% gelatin |

** For 5 and 10 µl of DNA, 27.5 and 22.5 µl of distilled water was used.

Table 3. PCR Cycles

| Thermal Cycler Steps | Primers<br>Analu | Bactoribo | HHF1 | Mitocox |
|---|---|---|---|---|
| 1. DNA Denaturation | 95° C / 1 min | 94° C / 1 min | 94° C / 1 min | 95° C / 1 min |
| 2. Primer Annealing | 40° C / 1 min | 40° C / 2 min | 37° C / 2 min | 40° C / 1 min |
| 3. Primer Extension | 72° C / 1.5 min | 72° C / 2 min | 72° C / 2 min | 72° C / 1.5 min |
| # Cycles * | 35 | 30 | 30 | 35 |

* After cycles were completed, there was additional primer extension at 72° C for 10 min.

## Appendix B

**Table 1. PCR products resulting from the use of 1, 5, and 10 ul of fruit DNA + Analu primers**

| Organism | DNA Concentration 1 ul | 5 ul | 10 ul |
|---|---|---|---|
| Banana | **640, 500,** 2020, 1340, 590, 460, 410, 360, 270, 250 | Nothing | Nothing |
| Kiwi | **720,** 1450, 890, 610, 540, 420, 360, 300, 250 | Nothing | **720** |
| Grape | **420,** 1180, 1090, 690, 590 | 440, 380 | **520, 250,** 900, 800, 440, 330 |
| Tomato | **980, 800, 540,** 1100, 900, 660, 610, 460, 420, 350, 220 | 330, 250 | **1150, 950, 450, 340,** 1830, 1670, 790, 650, 590, 520, 410, 240 |
| Orange | **360,** 1130, 960, 720, 610, 290 | **380** | **440,** 1210, 700, 320 |
| Cantaloupe | **1640, 680, 420, 230,** 900, 390 | **420,** 660, 230 | **1630, 670, 390, 220,** 500 |
| Apple | **610, 390,** 1570, 1240, 980, 740, 350 | Nothing | **410,** 590 |
| Mango | **350,** 1450, 1290, 1140, 1020, 900, 800, 710, 500, 390, 240 | Nothing | **800, 380, 320, 250,** 1770, 1430, 1160, 900, 670, 480 |

| Organism | 1 ul | 5 ul | 10 ul |
|---|---|---|---|
| Rat | **410, 320** | **420, 310** | **430** |
| | **390, 300** | **420, 310** | **430** |
| E. coli | | **1600, 1320, 1090, 310,** 4370, 3440, 2840, 2460, 1840, 940, 860, 780, 640, 560, 480, 440, 250 | **1590, 1380, 1090, 830, 310,** 4080, 3380, 2930, 2430, 1830, 950, 750, 650, 540, 470, 430, 240 |
| | | **1590, 1310, 1130, 310,** 4260, 3330, 2870, 2360, 1850, 970, 840, 800, 660, 570, 490, 440, 250 | **1560, 1320, 1120, 830,** 3760, 3050, 2690, 2280, 1770, 940, 770, 700, 650, 550, 480, 440, 320, 250 |
| Human | Smear | | |
| | Smear | | |

**Bold Face indicates major bands**

Table 2. PCR products resulting from the use of 1, 5, and 10 ul of fruit DNA + Bactoribo primers

| Organism | DNA Concentration 1 ul | 5 ul | 10 ul |
|---|---|---|---|
| Banana | **680,** 860, 750 | **650** | **710** |
| Kiwi | **680,** 2300, 1740, 350, 280 | **710,** 2150, 1720 | **710,** 2240, 1740 |
| Grape | Nothing | **730** | **710** |
| Tomato | **670** | **670,** 2340, 1680 | **690,** 2390, 2010, 1700 |
| Orange | **740** | **700,** 2440, 1980, 1680, 1420 | **720,** 2390, 2010, 1700 |
| Cantaloupe | **680,** 2530, 2090, 1740 | **700,** 2340, 1980, 1680 | **690,** 2390, 2010, 1700 |
| Apple | **740** | **730,** 1420 | **720** |
| Mango | **670,** 1440, 900 | **710,** 2450, 2060, 1720 | **710,** 2240, 1890, 1600 |

| Organism | 1 ul | 5 ul | 10 ul |
|---|---|---|---|
| E. coli | **780** | **790** | **790** |
| | **780** | **740** | **780** |

**Bold Face indicates major bands**

Table 3. PCR products resulting from the use of 1, 5, and 10 ul of fruit DNA + HHF1 primers

| Organism | DNA Concentration: 1 ul | 5-ul | 10 ul |
|---|---|---|---|
| Banana | **620** | **650,** 2900, 2640, 2280, 1970, 1550, 1410, 1280, 1110, 1000, 910, 750, 560, 420, 380, 330, 210, 150 | **680,** 2930, 2680, 2240, 1970 1800, 1720, 1440, 1260, 1160, 1060, 1010, 930, 780, 600, 520, 460, 400, 340, 290, 150 |
| Kiwi | **1010, 330,** 1930, 1760, 1610, 1400, 1270, 840, 670, 610, 530, 440, 270, 180 | **610, 170,** 320, 280 | **1010, 440, 290,** 2560, 2350, 1880, 1720, 1380, 1260, 850, 620, 160 |
| Grape | 640, 530, 440, 370, 240 | 440 | **1160, 1060, 640, 540,** 2110, 1850, 1630,1500, 820, 720, 430, 380, 320, 270, 240 |
| Tomato | **620, 430, 290,** 1390, 1150, 1100, 870, 720, 520, 200, 160 | **620, 300,**1410, 1110, 870, 790, 510, 420, 360, 240, 200, 160 | **460, 340,** 2150, 1970, 1580, 1440, 1210, 1160, 810, 680, 570, 420, 230, 180 |
| Orange | 870, 450, 410 | 440 | **940,** 490, 430 |
| Cantaloupe | **580, 330,** 920, 700, 200, 150 | **610, 350,** 960, 730, 500, 420, 290, 200, 140 | **720, 610, 350,** 1020, 940, 510, 420, 300, 200, 150 |
| Apple | 380, 320 | 380, 320 | **2610, 1700,** 1560, 980, 860, 790, 400, 320, 260 |
| Mango | 1760, 1460, 1210, 830, 650, 430, 180 | **960, 540, 420,** 1160, 360, 260, 180 | **1720, 1380, 970, 890, 850, 420,** 2450, 2240, 1970, 1110, 650, 340, 170 |

| Organism | 1 ul | 5-ul | 10 ul |
|---|---|---|---|
| S.cerevisiae | **340,** 2820, 2330, 2120, 1680, 1530, 1390, 870, 720, 650 | **350,** 2650, 2210, 2110, 1460, 1270, 1160, 880 | **340,** 2200, 2020, 1630, 1430, 1320 |
| | **330,** 2550, 2120, 1930, 1680, 1530, 1400, 1270, 1160, 840, 730 | **350,** 2900, 2170, 1710, 1550, 1410, 1160, 830, 720 | **320,** 1380, 1260, 1160, 850, 650 |
| E. coli | Nothing | 1600 | Nothing |
| | 1610 | 1710 | Nothing |

**Bold Face indicates major bands**

Table 4. PCR products resulting from the use of 1, 5, and 10 ul of fruit DNA + Mitocox primers

| Organism | DNA Concentration 1 ul | 5 ul | 10 ul |
|---|---|---|---|
| Banana | 340 | Nothing | **1000, 820, 270, 170,** 710 |
| Kiwi | **500,** 380, 260, 220, 190 | **460,** 210 | **920, 460,** 790, 720, 620, 400, 310, 250, 210, 180 |
| Grape | 400, 330 | Nothing | **2550, 620, 490, 380, 310,** 2200, 1420, 1290, 1170, 790, 230, 200, 180, 120 |
| Tomato | **430, 190,** 960, 800, 690, 500, 380, 330, 270 | **180,** 630, 410, 260, 150 | **1060, 440, 250, 190, 150,** 2810, 2310, 1900, 1420, 870, 720 |
| Orange | 320, 210 | 910, 830, 330, 210 | **330, 220,** 620 |
| Cantaloupe | **540,**1060, 920, 660, 450, 390, 290, 230,140 | **580,** 1100, 910, 420, 240, 160 | **540,** 1050, 910, 650, 380, 230, 150 |
| Apple | Nothing | 530, 300 | **680,** 870, 760, 460, 400, 220 |
| Mango | 1110, 720, 600, 410, 280, 180 | **440,** 1100, 580, 250, 190 | **1050, 490,** 2250, 2050, 620, 380, 290, 170 |
| | | | |
| Human | **690,** 500, 430, 300, 260, 200, 130 | **690** | **680,** 510, 440, 300, 190, 120 |
| | **690,** 520, 450, 290, 200, 130 | **690,** 500, 440, 300, 250, 190, 130 | **680,** 320, 200, 130 |
| E. coli | 400 | 820, 340 | 1170, 440 |
| | 410 | 830, 380 | Nothing |

**Bold Face indicates major bands**

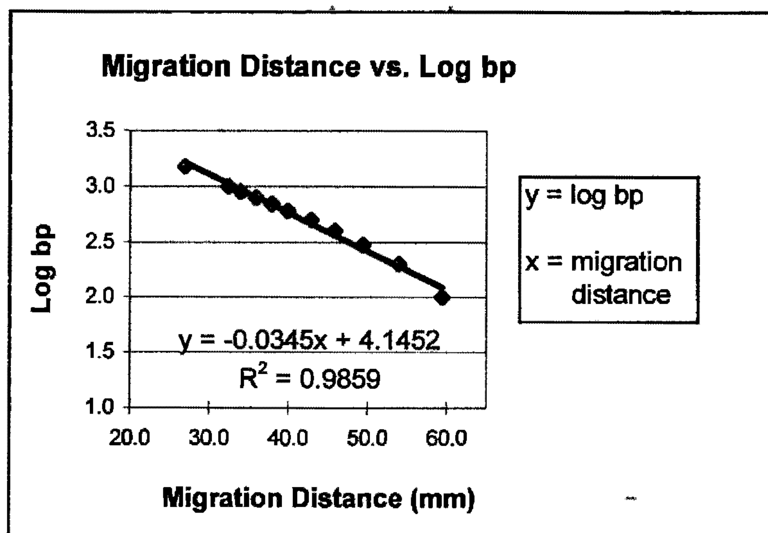
Table 5. Summary of major amplification products for Analu, Bactoribo, HHF1, and Mitocox primers

| Organism | Primers: Analu | Bactoribo | HHF1 | Mitocox |
|---|---|---|---|---|
| Banana | Nothing | 710 | 680 | 1000, 820, 270, 170 |
| Kiwi | 720 | 710 | 1010, 440, 290 | 920, 460 |
| Grape | 520, 250 | 710 | 1160, 1060, 640, 540 | 2550, 620, 490, 380, 310 |
| Tomato | 1150, 950, 450, 340 | 690 | 460, 340 | 1060, 440, 250, 190, 150 |
| Orange | 440 | 720 | 940 | 330, 220 |
| Cantaloupe | 1630, 670, 390, 220 | 690 | 720, 610, 350 | 540 |
| Apple | 410 | 720 | 2610, 1700 | 680 |
| Mango | 800, 380, 320, 250 | 710 | 1720, 1380, 970, 890, 850, 420 | 1050, 490 |

## Appendix C. Calculation of size of PCR Products

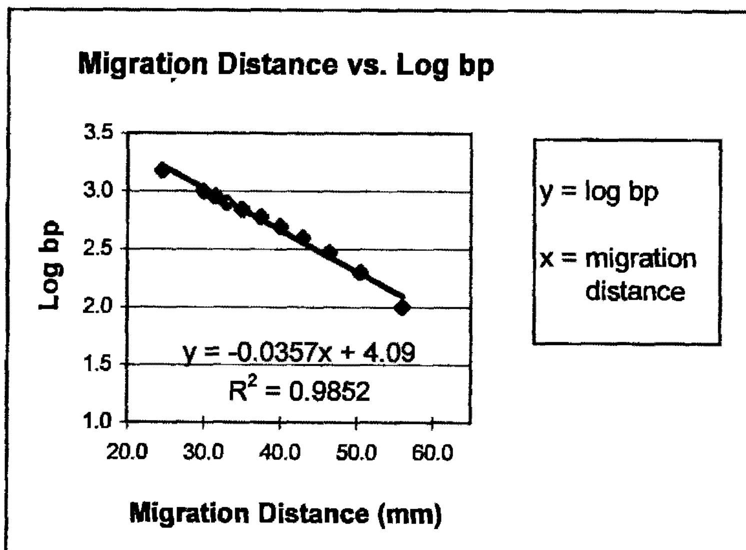
**Analu primers: 1 ul of fruit DNA**

| Ladder (bp) | Dist. Mig. (mm) | Log (bp) | Organism | PCR Frag. (mm) | Calc. log bp | Frag. (bp) |
|---|---|---|---|---|---|---|
| 1500 | 27.0 | 3.1761 | Rat | 44.5 | 2.6100 | 410 |
| 1000 | 32.5 | 3.0000 | | 47.5 | 2.5065 | 320 |
| 900 | 34.0 | 2.9542 | Tomato | 32.0 | 3.0412 | 1,100 |
| 800 | 36.0 | 2.9031 | | 33.5 | 2.9895 | 980 |
| 700 | 38.0 | 2.8451 | | 34.5 | 2.9550 | 900 |
| 600 | 40.0 | 2.7782 | | 36.0 | 2.9032 | 800 |
| 500 | 43.0 | 2.6990 | | 38.5 | 2.8170 | 660 |
| 400 | 46.0 | 2.6021 | | 39.5 | 2.7825 | 610 |
| 300 | 49.5 | 2.4771 | | 41.0 | 2.7307 | 540 |
| 200 | 54.0 | 2.3010 | | 43.0 | 2.6617 | 460 |
| 100 | 59.5 | 2.0000 | | 44.0 | 2.6272 | 420 |
| | | | | 46.5 | 2.5410 | 350 |
| | | | | 52.0 | 2.3512 | 220 |
| | | | Cantaloupe | 27.0 | 3.2137 | 1,640 |
| | | | | 34.5 | 2.9550 | 900 |
| | | | | 38.0 | 2.8342 | 680 |
| | | | | 44.0 | 2.6272 | 420 |
| | | | | 45.0 | 2.5927 | 390 |
| | | | | 51.5 | 2.3685 | 230 |
| | | | Mango | 28.5 | 3.1620 | 1,450 |
| | | | | 30.0 | 3.1102 | 1,290 |
| | | | | 31.5 | 3.0585 | 1,140 |
| | | | | 33.0 | 3.0067 | 1,020 |
| | | | | 34.5 | 2.9550 | 900 |
| | | | | 36.0 | 2.9032 | 800 |
| | | | | 37.5 | 2.8515 | 710 |
| | | | | 42.0 | 2.6962 | 500 |
| | | | | 45.0 | 2.5927 | 390 |
| | | | | 46.5 | 2.5410 | 350 |
| | | | | 51.0 | 2.3857 | 240 |
| | | | Apple | 27.5 | 3.1965 | 1,570 |
| | | | | 30.5 | 3.0930 | 1,240 |
| | | | | 33.5 | 2.9895 | 980 |
| | | | | 37.0 | 2.8687 | 740 |
| | | | | 39.5 | 2.7825 | 610 |
| | | | | 45.0 | 2.5927 | 390 |
| | | | | 46.5 | 2.5410 | 350 |



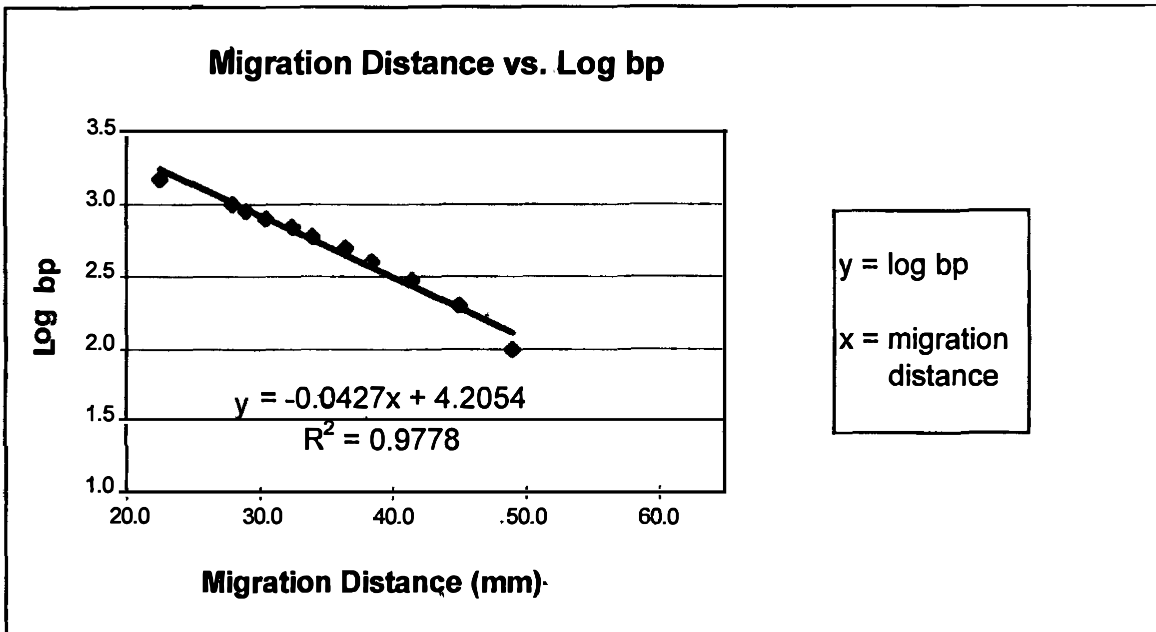
**Analu primers: 1 ul of fruit DNA**

| Ladder (bp) | Dist. Mig. (mm) | Log (bp) | Organism. | PCR Frag. (mm) | Calc. log bp | Frag. (bp) |
|---|---|---|---|---|---|---|
| 1500 | 24.5 | 3.1761 | Rat | 42.0 | 2.5906 | 390 |
| 1000 | 30.0 | 3.0000 | | 45.0 | 2.4835 | 300 |
| 900 | 31.5 | 2.9542 | Banana | 22.0 | 3.3046 | 2,020 |
| 800 | 33.0 | 2.9031 | | 27.0 | 3.1261 | 1,340 |
| 700 | 35.0 | 2.8451 | | 36.0 | 2.8048 | 640 |
| 600 | 37.5 | 2.7782 | | 37.0 | 2.7691 | 590 |
| 500 | 40.0 | 2.6990 | | 39.0 | 2.6977 | 500 |
| 400 | 43.0 | 2.6021 | | 40.0 | 2.6620 | 460 |
| 300 | 46.5 | 2.4771 | | 41.5 | 2.6085 | 410 |
| 200 | 50.5 | 2.3010 | | 43.0 | 2.5549 | 360 |
| 100 | 56.0 | 2.0000 | | 46.5 | 2.4300 | 270 |
| | | | | 47.5 | 2.3943 | 250 |
| | | | Kiwi | 26.0 | 3.1618 | 1,450 |
| | | | | 32.0 | 2.9476 | 890 |
| | | | | 34.5 | 2.8584 | 720 |
| | | | | 36.5 | 2.7870 | 610 |
| | | | | 38.0 | 2.7334 | 540 |
| | | | | 41.0 | 2.6263 | 420 |
| | | | | 43.0 | 2.5549 | 360 |
| | | | | 45.0 | 2.4835 | 300 |
| | | | | 47.5 | 2.3943 | 250 |
| | | | Grape | 28.5 | 3.0726 | 1,180 |
| | | | | 29.5 | 3.0369 | 1,090 |
| | | | | 35.0 | 2.8405 | 690 |
| | | | | 37.0 | 2.7691 | 590 |
| | | | | 41.0 | 2.6263 | 420 |
| | | | Orange | 29.0 | 3.0547 | 1,130 |
| | | | | 31.0 | 2.9833 | 960 |
| | | | | 34.5 | 2.8584 | 720 |
| | | | | 36.5 | 2.7870 | 610 |
| | | | | 43.0 | 2.5549 | 360 |
| | | | | 45.5 | 2.4657 | 290 |



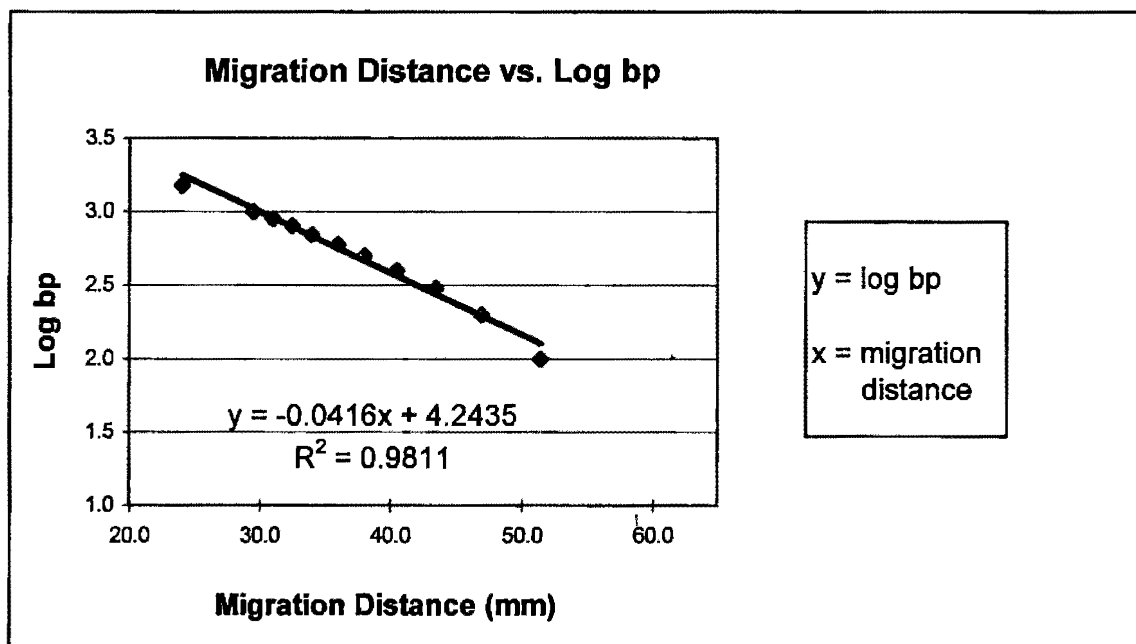
**Analu primers: 5 ul of fruit DNA**

| Ladder (bp) | Dist. Mig. (mm) | Log (bp) | Organism | PCR Frag. (mm) | Calc. log bp | Frag. (bp) |
|---|---|---|---|---|---|---|
| 1500 | 22.5 | 3.1761 | Rat | 37.0 | 2.6255 | 420 |
| 1000 | 28.0 | 3.0000 | | 40.0 | 2.4974 | 310 |
| 900 | 29.0 | 2.9542 | E. coli | 13.5 | 3.6290 | 4,260 |
| 800 | 30.5 | 2.9031 | | 16.0 | 3.5222 | 3,330 |
| 700 | 32.5 | 2.8451 | | 17.5 | 3.4582 | 2,870 |
| 600 | 34.0 | 2.7782 | | 19.5 | 3.3728 | 2,360 |
| 500 | 36.5 | 2.6990 | | 22.0 | 3.2660 | 1,850 |
| 400 | 38.5 | 2.6021 | | 23.5 | 3.2020 | 1,590 |
| 300 | 41.5 | 2.4771 | | 25.5 | 3.1166 | 1,310 |
| 200 | 45.0 | 2.3010 | | 27.0 | 3.0525 | 1,130 |
| 100 | 49.0 | 2.0000 | | 28.5 | 2.9885 | 970 |
| | | | | 30.0 | 2.9244 | 840 |
| | | | | 30.5 | 2.9031 | 800 |
| | | | | 32.5 | 2.8177 | 660 |
| | | | | 34.0 | 2.7536 | 570 |
| | | | | 35.5 | 2.6896 | 490 |
| | | | | 36.5 | 2.6469 | 440 |
| | | | | 40.0 | 2.4974 | 310 |
| | | | | 42.5 | 2.3907 | 250 |
| | | | Orange | 38.0 | 2.5828 | 380 |
| | | | Cantaloupe | 32.5 | 2.8177 | 660 |
| | | | | 37.0 | 2.6255 | 420 |
| | | | | 43.0 | 2.3693 | 230 |
| | | | Grape | 36.5 | 2.6469 | 440 |
| | | | | 38.0 | 2.5828 | 380 |



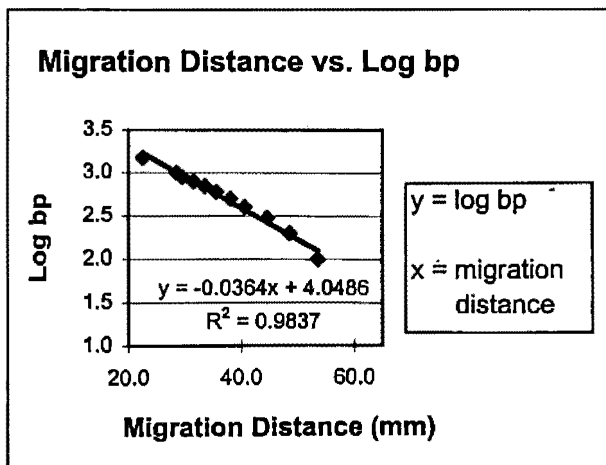
**Analu primers: 5 ul of fruit DNA**

| Ladder (bp) | Dist. Mig. (mm) | Log (bp) | Organism | PCR Frag. (mm) | Calc. log bp | Frag. (bp) |
|---|---|---|---|---|---|---|
| 1500 | 24.0 | 3.1761 | Rat | 39.0 | 2.6211 | 420 |
| 1000 | 29.5 | 3.0000 | | 42.0 | 2.4963 | 310 |
| 900 | 31.0 | 2.9542 | E. coli | 14.5 | 3.6403 | 4,370 |
| 800 | 32.5 | 2.9031 | | 17.0 | 3.5363 | 3,440 |
| 700 | 34.0 | 2.8451 | | 19.0 | 3.4531 | 2,840 |
| 600 | 36.0 | 2.7782 | | 20.5 | 3.3907 | 2,460 |
| 500 | 38.0 | 2.6990 | | 23.5 | 3.2659 | 1,840 |
| 400 | 40.5 | 2.6021 | | 25.0 | 3.2035 | 1,600 |
| 300 | 43.5 | 2.4771 | | 27.0 | 3.1203 | 1,320 |
| 200 | 47.0 | 2.3010 | | 29.0 | 3.0371 | 1,090 |
| 100 | 51.5 | 2.0000 | | 30.5 | 2.9747 | 940 |
| | | | | 31.5 | 2.9331 | 860 |
| | | | | 32.5 | 2.8915 | 780 |
| | | | | 34.5 | 2.8083 | 640 |
| | | | | 36.0 | 2.7459 | 560 |
| | | | | 37.5 | 2.6835 | 480 |
| | | | | 38.5 | 2.6419 | 440 |
| | | | | 42.0 | 2.4963 | 310 |
| | | | | 44.5 | 2.3923 | 250 |
| | | | Tomato | 41.5 | 2.5171 | 330 |
| | | | | 44.5 | 2.3923 | 250 |



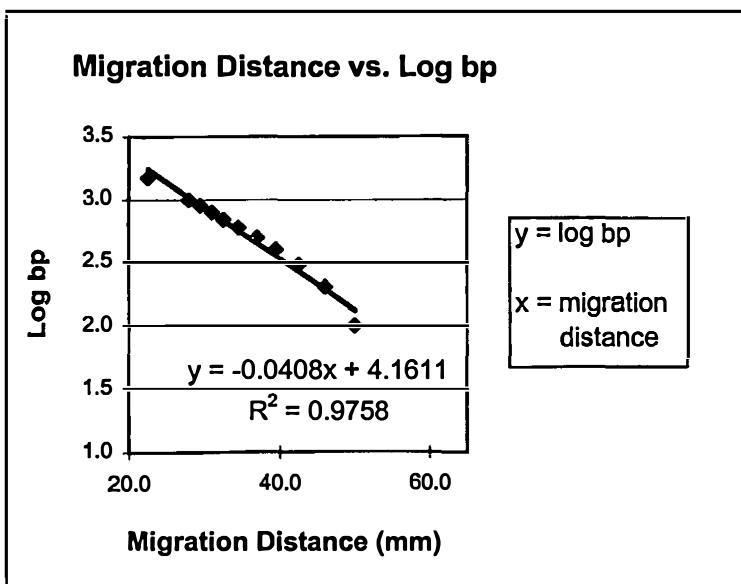
**Analu primers: 10 ul of fruit DNA**

| Ladder (bp) | Dist. Mig. (mm) | Log (bp) | Organism | PCR Frag. (mm) | Calc. log bp | Frag. (bp) |
|---|---|---|---|---|---|---|
| 1500 | 22.5 | 3.1761 | Rat | 39.0 | 2.6290 | 430 |
| 1000 | 28.5 | 3.0000 | E.coli | 13.0 | 3.5754 | 3,760 |
| 900 | 29.5 | 2.9542 | | 15.5 | 3.4844 | 3,050 |
| 800 | 31.5 | 2.9031 | | 17.0 | 3.4298 | 2,690 |
| 700 | 33.5 | 2.8451 | | 19.0 | 3.3570 | 2,280 |
| 600 | 35.5 | 2.7782 | | 22.0 | 3.2478 | 1,770 |
| 500 | 38.0 | 2.6990 | | 23.5 | 3.1932 | 1,560 |
| 400 | 40.5 | 2.6021 | | 25.5 | 3.1204 | 1,320 |
| 300 | 44.5 | 2.4771 | | 27.5 | 3.0476 | 1,120 |
| 200 | 48.5 | 2.3010 | | 29.5 | 2.9748 | 940 |
| 100 | 53.5 | 2.0000 | | 31.0 | 2.9202 | 830 |
| | | | | 32.0 | 2.8838 | 770 |
| | | | | 33.0 | 2.8474 | 700 |
| | | | | 34.0 | 2.8110 | 650 |
| | | | | 36.0 | 2.7382 | 550 |
| | | | | 37.5 | 2.6836 | 480 |
| | | | | 38.5 | 2.6472 | 440 |
| | | | | 42.5 | 2.5016 | 320 |
| | | | | 45.5 | 2.3924 | 250 |
| | | | Orange | 26.5 | 3.0840 | 1,210 |
| | | | | 33.0 | 2.8474 | 700 |
| | | | | 38.5 | 2.6472 | 440 |
| | | | | 42.5 | 2.5016 | 320 |
| | | | Grape | 30.0 | 2.9566 | 900 |
| | | | | 31.5 | 2.9020 | 800 |
| | | | | 36.5 | 2.7200 | 520 |
| | | | | 38.5 | 2.6472 | 440 |
| | | | | 42.0 | 2.5198 | 330 |
| | | | | 45.5 | 2.3924 | 250 |
| | | | Cantaloupe | 23.0 | 3.2114 | 1,630 |
| | | | | 33.5 | 2.8292 | 670 |
| | | | | 37.0 | 2.7018 | 500 |
| | | | | 40.0 | 2.5926 | 390 |
| | | | | 47.0 | 2.3378 | 220 |
| | | | Mango | 22.0 | 3.2478 | 1,770 |
| | | | | 24.5 | 3.1568 | 1,430 |
| | | | | 27 | 3.0658 | 1,160 |
| | | | | 30 | 2.9566 | 900 |
| | | | | 31.5 | 2.9020 | 800 |
| | | | | 33.5 | 2.8292 | 670 |
| | | | | 37.5 | 2.6836 | 480 |
| | | | | 40.5 | 2.5744 | 380 |
| | | | | 42.5 | 2.5016 | 320 |
| | | | | 45.5 | 2.3924 | 250 |



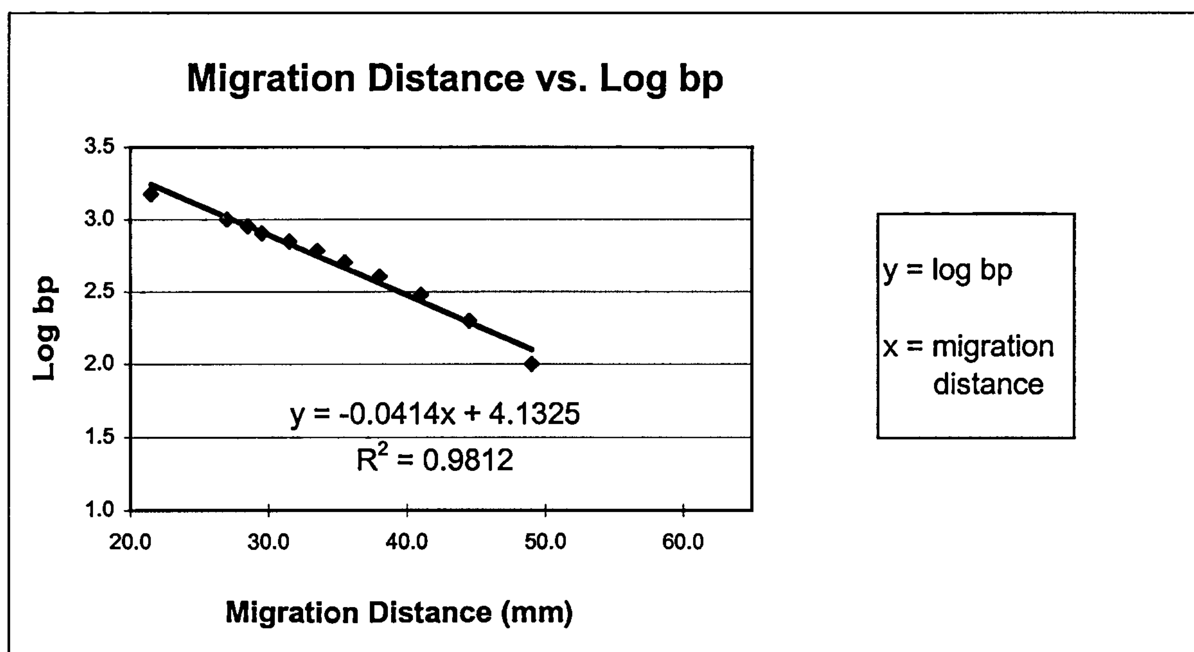
**Analu primers: 10 ul of fruit DNA**

| Ladder (bp) | Dist. Mig. (mm) | Log (bp) | Organism | PCR Frag. (mm) | Calc. log bp | Frag. (bp) |
|---|---|---|---|---|---|---|
| 1500 | 22.5 | 3.1761 | Rat | 37.5 | 2.6311 | 430 |
| 1000 | 28.0 | 3.0000 | E.coli | 13.5 | 3.6103 | 4,080 |
| 900 | 29.5 | 2.9542 | | 15.5 | 3.5287 | 3,380 |
| 800 | 31.0 | 2.9031 | | 17.0 | 3.4675 | 2,930 |
| 700 | 32.5 | 2.8451 | | 19.0 | 3.3859 | 2,430 |
| 600 | 34.5 | 2.7782 | | 22.0 | 3.2635 | 1,830 |
| 500 | 37.0 | 2.6990 | | 23.5 | 3.2023 | 1,590 |
| 400 | 39.5 | 2.6021 | | 25.0 | 3.1411 | 1,380 |
| 300 | 42.5 | 2.4771 | | 27.5 | 3.0391 | 1,090 |
| 200 | 46.0 | 2.3010 | | 29.0 | 2.9779 | 950 |
| 100 | 50.0 | 2.0000 | | 30.5 | 2.9167 | 830 |
| | | | | 31.5 | 2.8759 | 750 |
| | | | | 33.0 | 2.8147 | 650 |
| | | | | 35.0 | 2.7331 | 540 |
| | | | | 36.5 | 2.6719 | 470 |
| | | | | 37.5 | 2.6311 | 430 |
| | | | | 41.0 | 2.4883 | 310 |
| | | | | 43.5 | 2.3863 | 240 |
| | | | Kiwi | 32.0 | 2.8555 | 720 |
| | | | Apple | 34.0 | 2.7739 | 590 |
| | | | | 38.0 | 2.6107 | 410 |
| | | | Tomato | 22.0 | 3.2635 | 1,830 |
| | | | | 23.0 | 3.2227 | 1,670 |
| | | | | 27.0 | 3.0595 | 1,150 |
| | | | | 29.0 | 2.9779 | 950 |
| | | | | 31.0 | 2.8963 | 790 |
| | | | | 33.0 | 2.8147 | 650 |
| | | | | 34.0 | 2.7739 | 590 |
| | | | | 35.5 | 2.7127 | 520 |
| | | | | 37.0 | 2.6515 | 450 |
| | | | | 38.0 | 2.6107 | 410 |
| | | | | 40.0 | 2.5291 | 340 |
| | | | | 43.5 | 2.3863 | 240 |



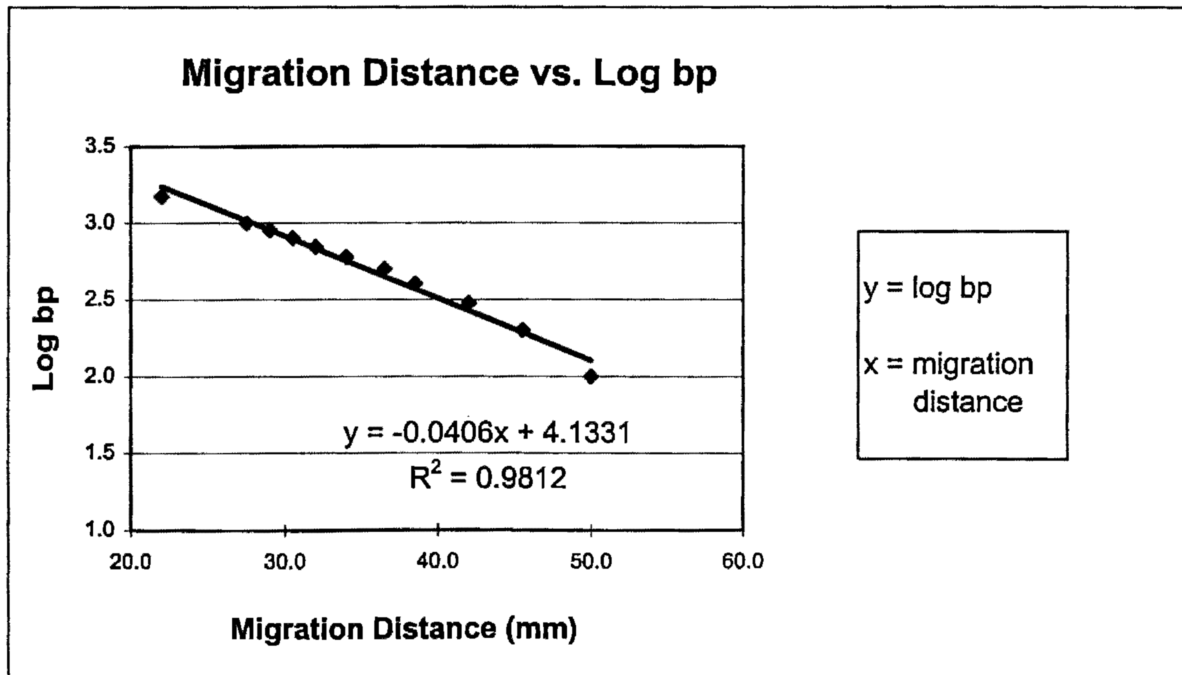
**Bactoribo primers: 1 ul of fruit DNA**

| Ladder (bp) | Dist. Mig. (mm) | Log (bp) | Organism | PCR Frag. (mm) | Calc. log bp | Frag. (bp) |
|---|---|---|---|---|---|---|
| 1500 | 21.5 | 3.1761 | E. coli | 30.0 | 2.8905 | 780 |
| 1000 | 27.0 | 3.0000 | Tomato | 31.5 | 2.8284 | 670 |
| 900 | 28.5 | 2.9542 | Mango | 23.5 | 3.1596 | 1,440 |
| 800 | 29.5 | 2.9031 | | 28.5 | 2.9526 | 900 |
| 700 | 31.5 | 2.8451 | | 31.5 | 2.8284 | 670 |
| 600 | 33.5 | 2.7782 | Apple | 30.5 | 2.8698 | 740 |
| 500 | 35.5 | 2.6990 | Orange | 30.5 | 2.8698 | 740 |
| 400 | 38.0 | 2.6021 | | | | |
| 300 | 41.0 | 2.4771 | | | | |
| 200 | 44.5 | 2.3010 | | | | |
| 100 | 49.0 | 2.0000 | | | | |



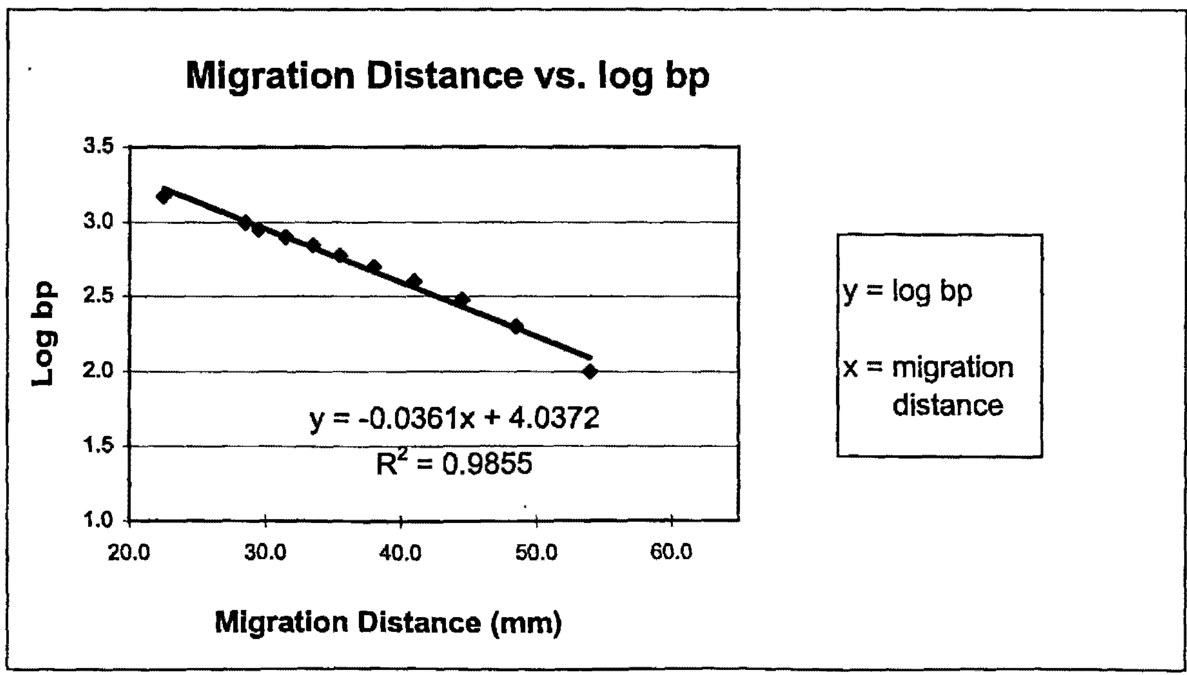
**Bactoribo primers: 1 ul of fruit DNA**

| Ladder (bp) | Dist. Mig. (mm) | Log (bp) | Organism | PCR Frag. (mm) | Calc. log bp | Frag. (bp) |
|---|---|---|---|---|---|---|
| 1500 | 22.0 | 3.1761 | E. coli | 30.5 | 2.8948 | 780 |
| 1000 | 27.5 | 3.0000 | Cantaloupe | 18.0 | 3.4023 | 2,530 |
| 900 | 29.0 | 2.9542 | | 20.0 | 3.3211 | 2,090 |
| 800 | 30.5 | 2.9031 | | 22.0 | 3.2399 | 1,740 |
| 700 | 32.0 | 2.8451 | | 32.0 | 2.8339 | 680 |
| 600 | 34.0 | 2.7782 | Kiwi | 19.0 | 3.3617 | 2,300 |
| 500 | 36.5 | 2.6990 | | 22.0 | 3.2399 | 1,740 |
| 400 | 38.5 | 2.6021 | | 32.0 | 2.8339 | 680 |
| 300 | 42.0 | 2.4771 | | 39.0 | 2.5497 | 350 |
| 200 | 45.5 | 2.3010 | | 41.5 | 2.4482 | 280 |
| 100 | 50.0 | 2.0000 | Banana | 29.5 | 2.9354 | 860 |
| | | | | 31.0 | 2.8745 | 750 |
| | | | | 32.0 | 2.8339 | 680 |



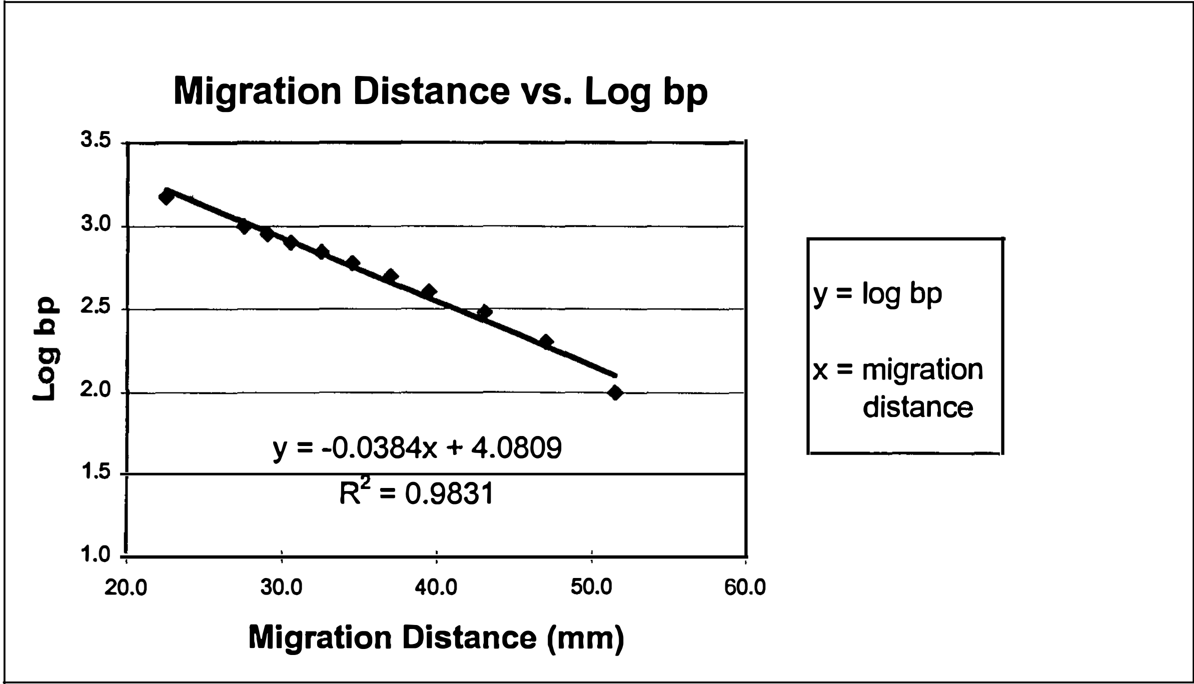
**Bactoribo primers: 5 ul of fruit DNA**

| Ladder (bp) | Dist. Mig. (mm) | Log (bp) | Organism | PCR Frag. (mm) | Calc. log bp | Frag. (bp) |
|---|---|---|---|---|---|---|
| 1500 | 22.5 | 3.1761 | E. coli | 31.5 | 2.9001 | 790 |
| 1000 | 28.5 | 3.0000 | Orange | 18.0 | 3.3874 | 2,440 |
| 900 | 29.5 | 2.9542 | | 20.5 | 3.2972 | 1,980 |
| 800 | 31.5 | 2.9031 | | 22.5 | 3.2250 | 1,680 |
| 700 | 33.5 | 2.8451 | | 24.5 | 3.1528 | 1,420 |
| 600 | 35.5 | 2.7782 | | 33.0 | 2.8459 | 700 |
| 500 | 38.0 | 2.6990 | Apple | 24.5 | 3.1528 | 1,420 |
| 400 | 41.0 | 2.6021 | | 32.5 | 2.8640 | 730 |
| 300 | 44.5 | 2.4771 | Grape | 32.5 | 2.8640 | 730 |
| 200 | 48.5 | 2.3010 | Cantaloupe | 18.5 | 3.3694 | 2,340 |
| 100 | 54.0 | 2.0000 | | 20.5 | 3.2972 | 1,980 |
| | | | | 22.5 | 3.2250 | 1,680 |
| | | | | 33.0 | 2.8459 | 700 |
| | | | Tomato | 18.5 | 3.3694 | 2,340 |
| | | | | 22.5 | 3.2250 | 1,680 |
| | | | | 33.5 | 2.8279 | 670 |



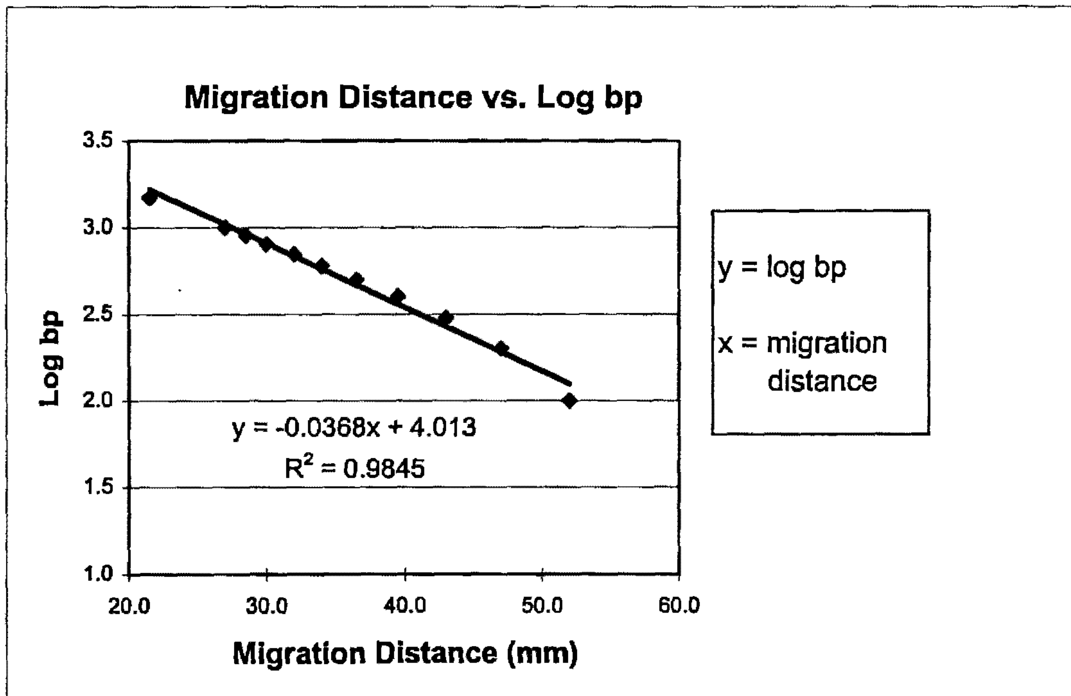
**Bactoribo primers: 5 ul of fruit DNA**

| Ladder (bp) | Dist. Mig. (mm) | Log (bp) | Organism | PCR Frag. (mm) | Calc. log bp | Frag. (bp) |
|---|---|---|---|---|---|---|
| 1500 | 22.5 | 3.1761 | E. coli | 31.5 | 2.8713 | 740 |
| 1000 | 27.5 | 3.0000 | Mango | 18.0 | 3.3897 | 2,450 |
| 900 | 29.0 | 2.9542 | | 20.0 | 3.3129 | 2,060 |
| 800 | 30.5 | 2.9031 | | 22.0 | 3.2361 | 1,720 |
| 700 | 32.5 | 2.8451 | | 32.0 | 2.8521 | 710 |
| 600 | 34.5 | 2.7782 | Kiwi | 19.5 | 3.3321 | 2,150 |
| 500 | 37.0 | 2.6990 | | 22.0 | 3.2361 | 1,720 |
| 400 | 39.5 | 2.6021 | | 32.0 | 2.8521 | 710 |
| 300 | 43.0 | 2.4771 | Banana | 33.0 | 2.8137 | 650 |
| 200 | 47.0 | 2.3010 | | | | |
| 100 | 51.5 | 2.0000 | | | | |



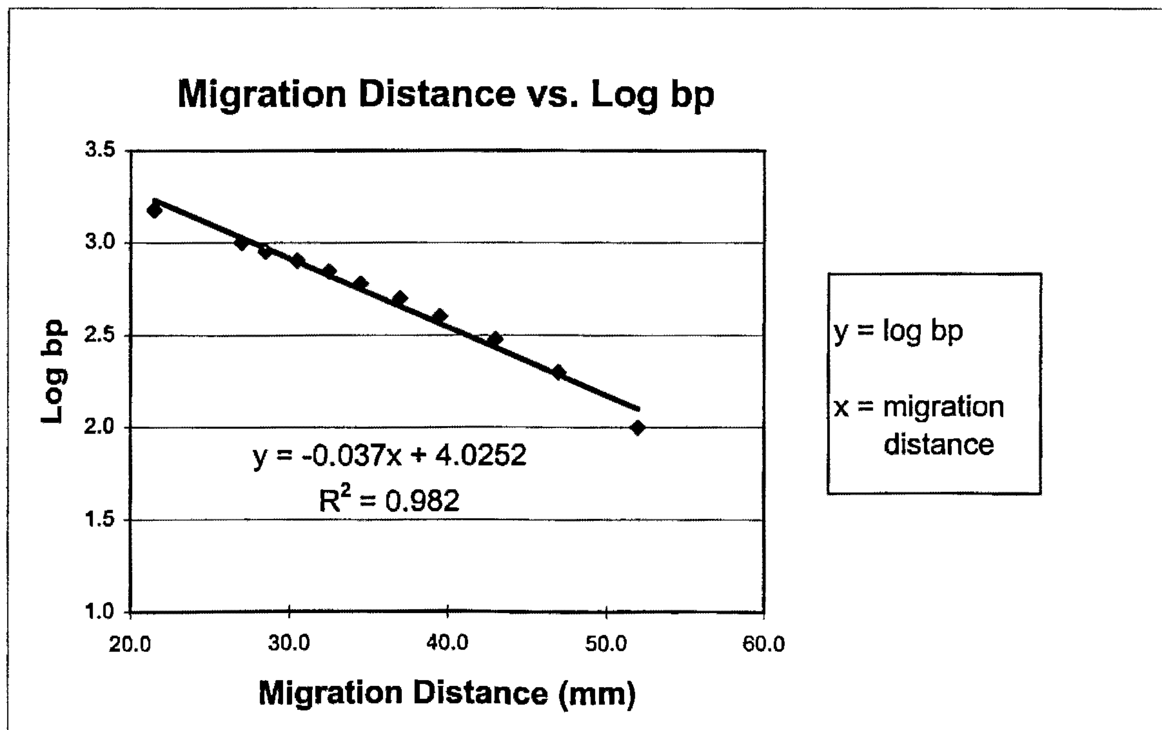
**Bactoribo primers: 10 ul of fruit DNA**

| Ladder (bp) | Dist. Mig. (mm) | Log (bp) | Organism | PCR Frag. (mm) | Calc. log bp | Frag..(bp) |
|---|---|---|---|---|---|---|
| 1500 | 21.5 | 3.1761 | E. coli | 30.5 | 2.8906 | 780 |
| 1000 | 27.0 | 3.0000 | Mango | 18.0 | 3.3506 | 2,240 |
| 900 | 28.5 | 2.9542 | | 20.0 | 3.2770 | 1,890 |
| 800 | 30.0 | 2.9031 | | 22.0 | 3.2034 | 1,600 |
| 700 | 32.0 | 2.8451 | | 31.5 | 2.8538 | 710 |
| 600 | 34.0 | 2.7782 | Grape | 31.5 | 2.8538 | 710 |
| 500 | 36.5 | 2.6990 | Kiwi | 18.0 | 3.3506 | 2,240 |
| 400 | 39.5 | 2.6021 | | 21.0 | 3.2402 | 1,740 |
| 300 | 43.0 | 2.4771 | | 31.5 | 2.8538 | 710 |
| 200 | 47.0 | 2.3010 | Banana | 31.5 | 2.8538 | 710 |
| 100 | 52.0 | 2.0000 | | | | |



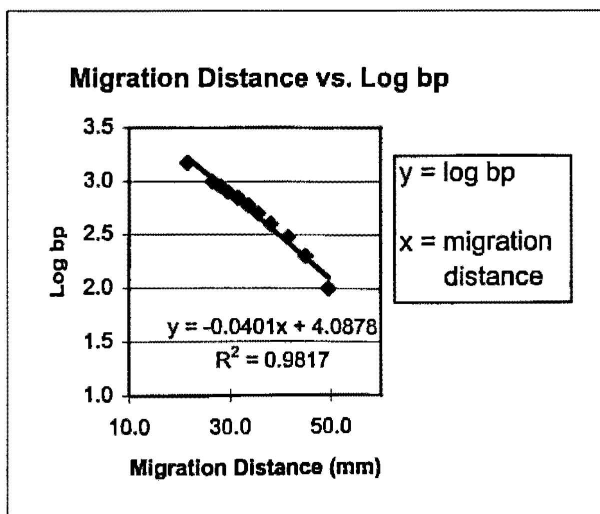
**Bactoribo primers: 10 ul of fruit DNA**

| Ladder (bp) | Dist. Mig. (mm) | Log (bp) | Organism | PCR Frag. (mm) | Calc. log bp | Frag. (bp) |
|---|---|---|---|---|---|---|
| 1500 | 21.5 | 3.1761 | E. coli | 30.5 | 2.8967 | 790 |
| 1000 | 27.0 | 3.0000 | Orange | 17.5 | 3.3777 | 2,390 |
| 900 | 28.5 | 2.9542 | | 19.5 | 3.3037 | 2,010 |
| 800 | 30.5 | 2.9031 | | 21.5 | 3.2297 | 1,700 |
| 700 | 32.5 | 2.8451 | | 31.5 | 2.8597 | 720 |
| 600 | 34.5 | 2.7782 | Apple | 31.5 | 2.8597 | 720 |
| 500 | 37.0 | 2.6990 | Cantaloupe | 17.5 | 3.3777 | 2,390 |
| 400 | 39.5 | 2.6021 | | 19.5 | 3.3037 | 2,010 |
| 300 | 43.0 | 2.4771 | | 21.5 | 3.2297 | 1,700 |
| 200 | 47.0 | 2.3010 | | 32.0 | 2.8412 | 690 |
| 100 | 52.0 | 2.0000 | Tomato | 17.5 | 3.3777 | 2,390 |
| | | | | 19.5 | 3.3037 | 2,010 |
| | | | | 21.5 | 3.2297 | 1,700 |
| | | | | 32.0 | 2.8412 | 690 |



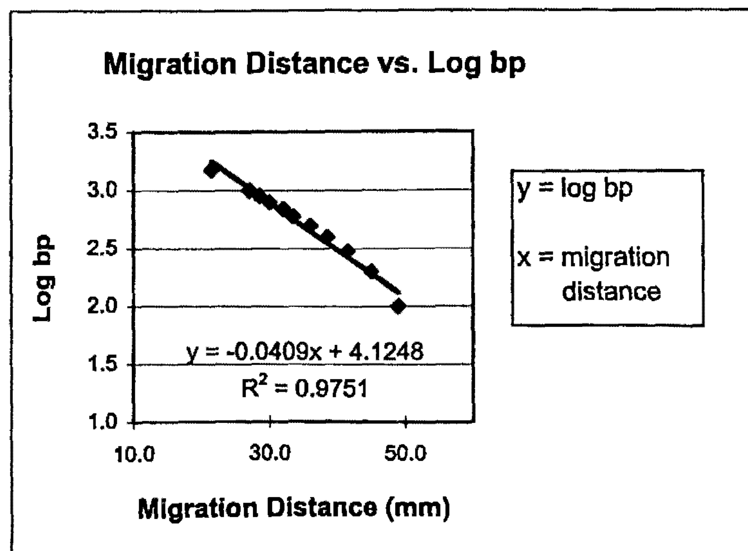
**HHF1 primers: 1 ul of fruit DNA**

| Ladder (bp) | Dist. Mig. (mm) | Log (bp) | Organism | PCR Frag. (mm) | Calc. log bp | Frag. (bp) |
|---|---|---|---|---|---|---|
| 1500 | 21.5 | 3.1761 | S. cerevisiae | 17.0 | 3.4061 | 2,550 |
| 1000 | 26.5 | 3.0000 | | 19.0 | 3.3259 | 2,120 |
| 900 | 28.0 | 2.9542 | | 20.0 | 3.2858 | 1,930 |
| 800 | 29.5 | 2.9031 | | 21.5 | 3.2257 | 1,680 |
| 700 | 31.5 | 2.8451 | | 22.5 | 3.1856 | 1,530 |
| 600 | 33.5 | 2.7782 | | 23.5 | 3.1455 | 1,400 |
| 500 | 35.5 | 2.6990 | | 24.5 | 3.1054 | 1,270 |
| 400 | 38.0 | 2.6021 | | 25.5 | 3.0653 | 1,160 |
| 300 | 41.5 | 2.4771 | | 29.0 | 2.9249 | 840 |
| 200 | 45.0 | 2.3010 | | 30.5 | 2.8648 | 730 |
| 100 | 49.5 | 2.0000 | | 39.0 | 2.5239 | 330 |
| | | | E. coli | 22.0 | 3.2056 | 1,610 |
| | | | Grape | 32.0 | 2.8046 | 640 |
| | | | | 34.0 | 2.7244 | 530 |
| | | | | 36.0 | 2.6442 | 440 |
| | | | | 38.0 | 2.5640 | 370 |
| | | | | 42.5 | 2.3836 | 240 |
| | | | Kiwi | 20.0 | 3.2858 | 1,930 |
| | | | | 21.0 | 3.2457 | 1,760 |
| | | | | 22.0 | 3.2056 | 1,610 |
| | | | | 23.5 | 3.1455 | 1,400 |
| | | | | 24.5 | 3.1054 | 1,270 |
| | | | | 27.0 | 3.0051 | 1,010 |
| | | | | 29.0 | 2.9249 | 840 |
| | | | | 31.5 | 2.8247 | 670 |
| | | | | 32.5 | 2.7846 | 610 |
| | | | | 34.0 | 2.7244 | 530 |
| | | | | 36.0 | 2.6442 | 440 |
| | | | | 39.0 | 2.5239 | 330 |
| | | | | 41.5 | 2.4237 | 270 |
| | | | | 46.0 | 2.2432 | 180 |
| | | | Cantaloupe | 28.0 | 2.9650 | 920 |
| | | | | 31.0 | 2.8447 | 700 |
| | | | | 33.0 | 2.7645 | 580 |
| | | | | 39.0 | 2.5239 | 330 |
| | | | | 44.5 | 2.3034 | 200 |
| | | | | 48 | 2.1630 | 150 |
| | | | Apple | 37.5 | 2.5841 | 380 |
| | | | | 39.5 | 2.5039 | 320 |



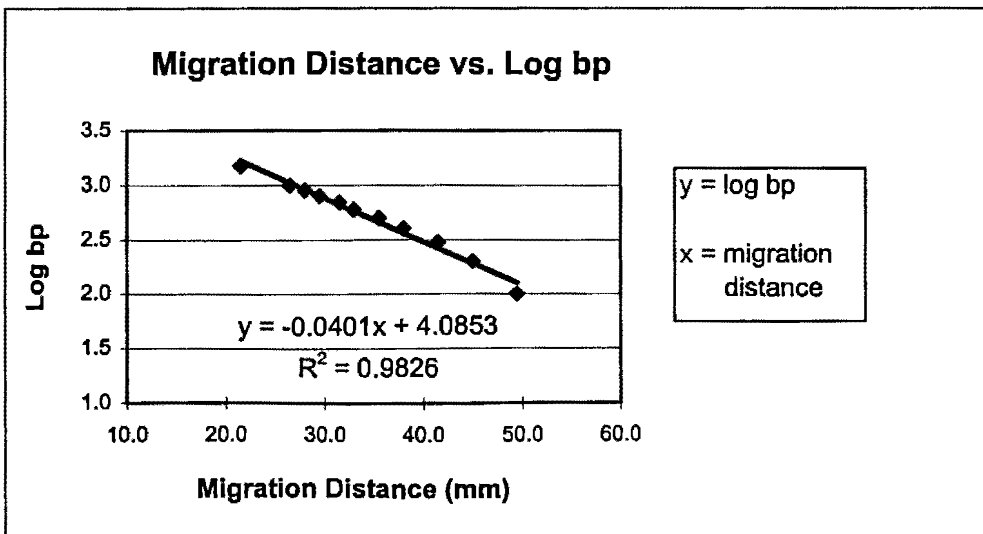
**HHF1 primers: 1 ul of fruit DNA**

| Ladder (bp) | Dist. Mig. (mm) | Log (bp) | Organism | PCR Frag. (mm) | Calc. log bp | Frag. (bp) |
|---|---|---|---|---|---|---|
| 1500 | 21.5 | 3.1761 | S. cerevisiae | 16.5 | 3.4500 | 2,820 |
| 1000 | 27.0 | 3.0000 | | 18.5 | 3.3682 | 2,330 |
| 900 | 28.5 | 2.9542 | | 19.5 | 3.3273 | 2,120 |
| 800 | 30.0 | 2.9031 | | 22.0 | 3.2250 | 1,680 |
| 700 | 32.0 | 2.8451 | | 23.0 | 3.1841 | 1,530 |
| 600 | 33.5 | 2.7782 | | 24.0 | 3.1432 | 1,390 |
| 500 | 36.0 | 2.6990 | | 29.0 | 2.9387 | 870 |
| 400 | 38.5 | 2.6021 | | 31.0 | 2.8569 | 720 |
| 300 | 41.5 | 2.4771 | | 32.0 | 2.8160 | 650 |
| 200 | 45.0 | 2.3010 | | 39.0 | 2.5297 | 340 |
| 100 | 49.0 | 2.0000 | Mango | 21.5 | 3.2455 | 1,760 |
| | | | | 23.5 | 3.1637 | 1,460 |
| | | | | 25.5 | 3.0819 | 1,210 |
| | | | | 29.5 | 2.9183 | 830 |
| | | | | 32.0 | 2.8160 | 650 |
| | | | | 36.5 | 2.6320 | 430 |
| | | | | 45.5 | 2.2639 | 180 |
| | | | Orange | 29.0 | 2.9387 | 870 |
| | | | | 36.0 | 2.6524 | 450 |
| | | | | 37.0 | 2.6115 | 410 |
| | | | Banana | 32.5 | 2.7956 | 620 |
| | | | Tomato | 24.0 | 3.1432 | 1,390 |
| | | | | 26.0 | 3.0614 | 1,150 |
| | | | | 26.5 | 3.0410 | 1,100 |
| | | | | 29.0 | 2.9387 | 870 |
| | | | | 31.0 | 2.8569 | 720 |
| | | | | 32.5 | 2.7956 | 620 |
| | | | | 34.5 | 2.7138 | 520 |
| | | | | 36.5 | 2.6320 | 430 |
| | | | | 40.5 | 2.4684 | 290 |
| | | | | 44.5 | 2.3048 | 200 |
| | | | | 47.0 | 2.2025 | 160 |



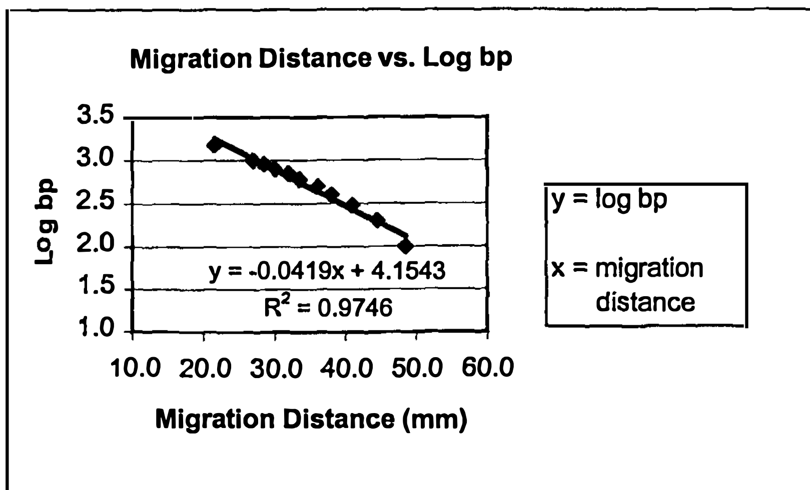
**HHF1 primers: 5 ul of fruit DNA**

| Ladder (bp) | Dist. Mig. (mm) | Log (bp) | Organism | PCR frag. | Calc. Log bp | Frag. (bp) |
|---|---|---|---|---|---|---|
| 1500 | 21.5 | 3.1761 | S. cerevisiae | 16.5 | 3.4237 | 2,650 |
| 1000 | 26.5 | 3.0000 | | 18.5 | 3.3435 | 2,210 |
| 900 | 28.0 | 2.9542 | | 19.0 | 3.3234 | 2,110 |
| 800 | 29.5 | 2.9031 | | 23.0 | 3.1630 | 1,460 |
| 700 | 31.5 | 2.8451 | | 24.5 | 3.1029 | 1,270 |
| 600 | 33.0 | 2.7782 | | 25.5 | 3.0628 | 1,160 |
| 500 | 35.5 | 2.6990 | | 28.5 | 2.9425 | 880 |
| 400 | 38.0 | 2.6021 | | 38.5 | 2.5415 | 350 |
| 300 | 41.5 | 2.4771 | E. coli | 22.0 | 3.2031 | 1,600 |
| 200 | 45.0 | 2.3010 | Orange | 36.0 | 2.6417 | 440 |
| 100 | 49.5 | 2.0000 | Kiwi | 32.5 | 2.7821 | 610 |
| | | | | 39.5 | 2.5014 | 320 |
| | | | | 41.0 | 2.4412 | 280 |
| | | | | 46.0 | 2.2407 | 170 |
| | | | Cantaloupe | 27.5 | 2.9826 | 960 |
| | | | | 30.5 | 2.8623 | 730 |
| | | | | 32.5 | 2.7821 | 610 |
| | | | | 34.5 | 2.7019 | 500 |
| | | | | 36.5 | 2.6217 | 420 |
| | | | | 38.5 | 2.5415 | 350 |
| | | | | 40.5 | 2.4613 | 290 |
| | | | | 44.5 | 2.3009 | 200 |
| | | | | 48.0 | 2.1605 | 140 |
| | | | Apple | 37.5 | 2.5816 | 380 |
| | | | | 39.5 | 2.5014 | 320 |



HHF1 primers: 5 ul of fruit DNA

| Ladder (bp) | Dist. Mig. (mm) | Log (bp) | Organism | PCR frag. | Calc. Log bp | Frag. (bp) |
|---|---|---|---|---|---|---|
| 1500 | 21.5 | 3.1761 | S. cerevisiae | 16.5 | 3.4630 | 2,900 |
| 1000 | 27.0 | 3.0000 | | 19.5 | 3.3373 | 2,170 |
| 900 | 28.5 | 2.9542 | | 22.0 | 3.2325 | 1,710 |
| 800 | 30.0 | 2.9031 | | 23.0 | 3.1906 | 1,550 |
| 700 | 32.0 | 2.8451 | | 24.0 | 3.1487 | 1,410 |
| 600 | 33.5 | 2.7782 | | 26.0 | 3.0649 | 1,160 |
| 500 | 36.0 | 2.6990 | | 29.5 | 2.9183 | 830 |
| 400 | 38.0 | 2.6021 | | 31.0 | 2.8554 | 720 |
| 300 | 41.0 | 2.4771 | | 38.5 | 2.5412 | 350 |
| 200 | 44.5 | 2.3010 | E. coli | 22.0 | 3.2325 | 1,710 |
| 100 | 48.5 | 2.0000 | Tomato | 24.0 | 3.1487 | 1,410 |
| | | | | 26.5 | 3.0440 | 1,110 |
| | | | | 29.0 | 2.9392 | 870 |
| | | | | 30.0 | 2.8973 | 790 |
| | | | | 32.5 | 2.7926 | 620 |
| | | | | 34.5 | 2.7088 | 510 |
| | | | | 36.5 | 2.6250 | 420 |
| | | | | 38.0 | 2.5621 | 360 |
| | | | | 40.0 | 2.4783 | 300 |
| | | | | 42.5 | 2.3736 | 240 |
| | | | | 44.0 | 2.3107 | 200 |
| | | | | 46.5 | 2.2060 | 160 |
| | | | Grape | 36.0 | 2.6459 | 440 |
| | | | Banana | 16.5 | 3.4630 | 2,900 |
| | | | | 17.5 | 3.4211 | 2,640 |
| | | | | 19.0 | 3.3582 | 2,280 |
| | | | | 20.5 | 3.2954 | 1,970 |
| | | | | 23.0 | 3.1906 | 1,550 |
| | | | | 24.0 | 3.1487 | 1,410 |
| | | | | 25.0 | 3.1068 | 1,280 |
| | | | | 26.5 | 3.0440 | 1,110 |
| | | | | 27.5 | 3.0021 | 1,000 |
| | | | | 28.5 | 2.9602 | 910 |
| | | | | 30.5 | 2.8764 | 750 |
| | | | | 32.0 | 2.8135 | 650 |
| | | | | 33.5 | 2.7507 | 560 |
| | | | | 36.5 | 2.6250 | 420 |
| | | | | 37.5 | 2.5831 | 380 |
| | | | | 39.0 | 2.5202 | 330 |
| | | | | 43.5 | 2.3317 | 210 |
| | | | | 47.0 | 2.1850 | 150 |
| | | | Mango | 26.0 | 3.0649 | 1,160 |
| | | | | 28.0 | 2.9811 | 960 |
| | | | | 34.0 | 2.7297 | 540 |
| | | | | 36.5 | 2.6250 | 420 |
| | | | | 38.0 | 2.5621 | 360 |
| | | | | 41.5 | 2.4155 | 260 |
| | | | | 45.5 | 2.2479 | 180 |

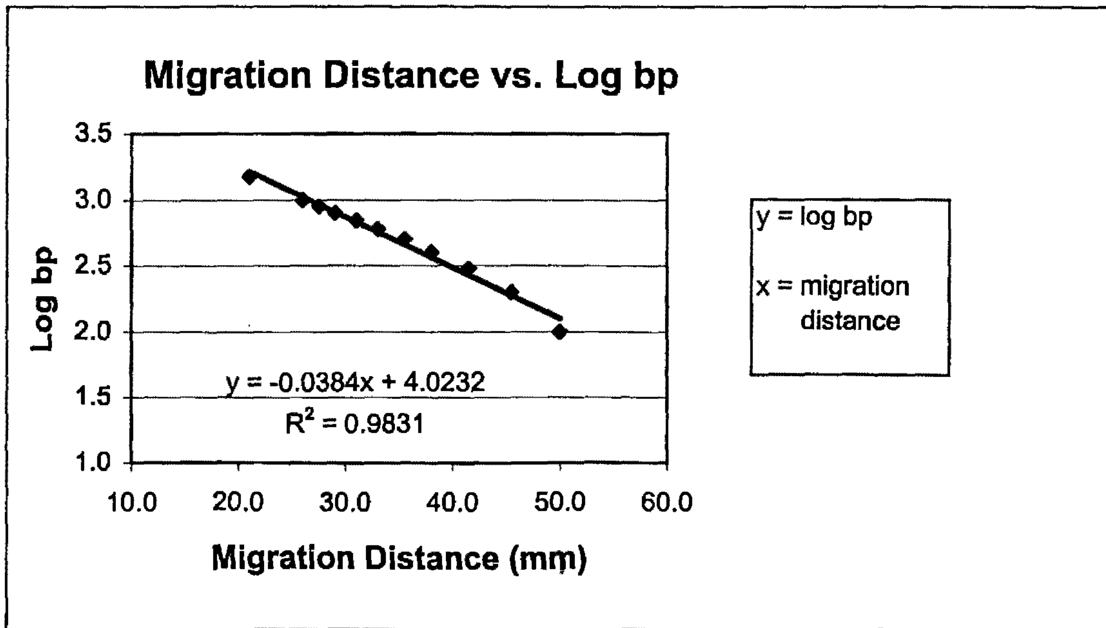


**HHF1 primers: 10 ul of fruit DNA**

| Ladder (bp) | Dist. Mig. (mm) | Log (bp) | Organism | PCR frag. | Calc. Log bp | Frag. (bp) |
|---|---|---|---|---|---|---|
| 1500 | 21.0 | 3.1761 | S. cerevisiae | 23.0 | 3.1400 | 1,380 |
| 1000 | 26.0 | 3.0000 | | 24.0 | 3.1016 | 1,260 |
| 900 | 27.5 | 2.9542 | | 25.0 | 3.0632 | 1,160 |
| 800 | 29.0 | 2.9031 | | 28.5 | 2.9288 | 850 |
| 700 | 31.0 | 2.8451 | | 31.5 | 2.8136 | 650 |
| 600 | 33.0 | 2.7782 | | 39.5 | 2.5064 | 320 |
| 500 | 35.5 | 2.6990 | Tomato | 18.0 | 3.3320 | 2,150 |
| 400 | 38.0 | 2.6021 | | 19.0 | 3.2936 | 1,970 |
| 300 | 41.5 | 2.4771 | | 21.5 | 3.1976 | 1,580 |
| 200 | 45.5 | 2.3010 | | 22.5 | 3.1592 | 1,440 |
| 100 | 50.0 | 2.0000 | | 24.5 | 3.0824 | 1,210 |
| | | | | 25.0 | 3.0632 | 1,160 |
| | | | | 29.0 | 2.9096 | 810 |
| | | | | 31.0 | 2.8328 | 680 |
| | | | | 33.0 | 2.7560 | 570 |
| | | | | 35.5 | 2.6600 | 460 |
| | | | | 36.5 | 2.6216 | 420 |
| | | | | 39.0 | 2.5256 | 340 |
| | | | | 43.5 | 2.3528 | 230 |
| | | | | 46.0 | 2.2568 | 180 |
| | | | Banana | 14.5 | 3.4664 | 2,930 |
| | | | | 15.5 | 3.4280 | 2,680 |
| | | | | 17.5 | 3.3512 | 2,240 |
| | | | | 19.0 | 3.2936 | 1,970 |
| | | | | 20.0 | 3.2552 | 1,800 |
| | | | | 20.5 | 3.2360 | 1,720 |
| | | | | 22.5 | 3.1592 | 1,440 |
| | | | | 24.0 | 3.1016 | 1,260 |
| | | | | 25.0 | 3.0632 | 1,160 |
| | | | | 26.0 | 3.0248 | 1,060 |
| | | | | 26.5 | 3.0056 | 1,010 |
| | | | | 27.5 | 2.9672 | 930 |
| | | | | 29.5 | 2.8904 | 780 |
| | | | | 31.0 | 2.8328 | 680 |
| | | | | 32.5 | 2.7752 | 600 |
| | | | | 34.0 | 2.7176 | 520 |
| | | | | 35.5 | 2.6600 | 460 |
| | | | | 37.0 | 2.6024 | 400 |
| | | | | 39.0 | 2.5256 | 340 |
| | | | | 40.5 | 2.4680 | 290 |
| | | | | 48.0 | 2.1800 | 150 |
| | | | Mango | 16.5 | 3.3896 | 2,450 |
| | | | | 17.5 | 3.3512 | 2,240 |
| | | | | 19.0 | 3.2936 | 1,970 |
| | | | | 20.5 | 3.2360 | 1,720 |
| | | | | 23.0 | 3.1400 | 1,380 |

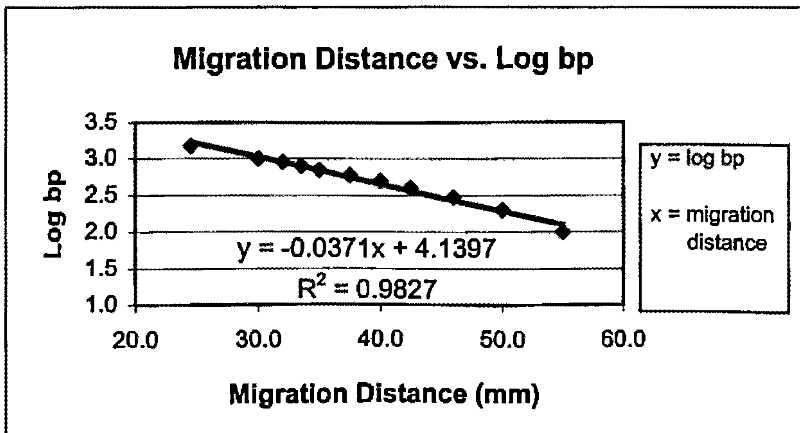
**HHF1 primers: 10 ul of fruit DNA**

| Organism | PCR frag. | Calc. Log bp | Frag. (bp) |
|---|---|---|---|
| Mango | 25.5 | 3.0440 | 1,110 |
| | 27.0 | 2.9864 | 970 |
| | 28.0 | 2.9480 | 890 |
| | 28.5 | 2.9288 | 850 |
| | 31.5 | 2.8136 | 650 |
| | 36.5 | 2.6216 | 420 |
| | 39 | 2.5256 | 340 |
| | 47 | 2.2184 | 170 |
| Kiwi | 16 | 3.4088 | 2,560 |
| | 17 | 3.3704 | 2,350 |
| | 19.5 | 3.2744 | 1,880 |
| | 20.5 | 3.2360 | 1,720 |
| | 23.0 | 3.1400 | 1,380 |
| | 24.0 | 3.1016 | 1,260 |
| | 26.5 | 3.0056 | 1,010 |
| | 28.5 | 2.9288 | 850 |
| | 32.0 | 2.7944 | 620 |
| | 36.0 | 2.6408 | 440 |
| | 40.5 | 2.4680 | 290 |
| | 47.5 | 2.1992 | 160 |



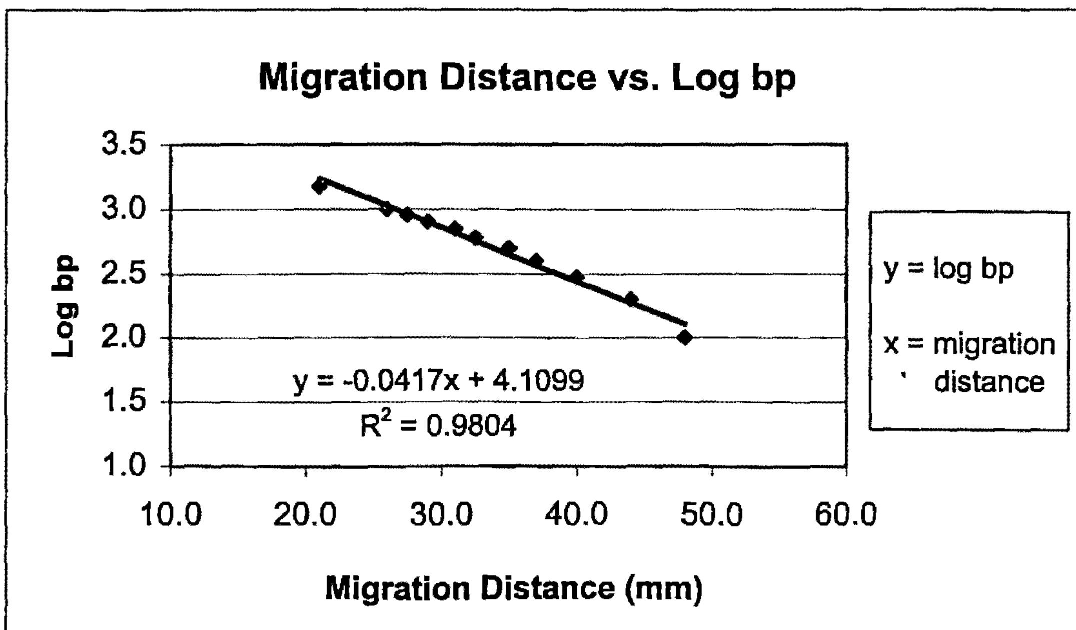
HHF1 primers: 10 ul of fruit DNA

| Ladder (bp) | Dist. Mig. (mm) | Log (bp) | Organism | PCR frag. | Calc. Log bp | Frag. (bp) |
|---|---|---|---|---|---|---|
| 1500 | 24.5 | 3.1761 | S. cerevisiae | 21.5 | 3.3421 | 2,200 |
| 1000 | 30.0 | 3.0000 | | 22.5 | 3.3050 | 2,020 |
| 900 | 32.0 | 2.9542 | | 25.0 | 3.2122 | 1,630 |
| 800 | 33.5 | 2.9031 | | 26.5 | 3.1566 | 1,430 |
| 700 | 35.0 | 2.8451 | | 27.5 | 3.1195 | 1,320 |
| 600 | 37.5 | 2.7782 | | 43.5 | 2.5259 | 340 |
| 500 | 40.0 | 2.6990 | Orange | 31.5 | 2.9711 | 940 |
| 400 | 42.5 | 2.6021 | | 39.0 | 2.6928 | 490 |
| 300 | 46.0 | 2.4771 | | 40.5 | 2.6372 | 430 |
| 200 | 50.0 | 2.3010 | Grape | 22.0 | 3.3235 | 2,110 |
| 100 | 55.0 | 2.0000 | | 23.5 | 3.2679 | 1,850 |
| | | | | 25.0 | 3.2122 | 1,630 |
| | | | | 26.0 | 3.1751 | 1,500 |
| | | | | 29.0 | 3.0638 | 1,160 |
| | | | | 30.0 | 3.0267 | 1,060 |
| | | | | 33.0 | 2.9154 | 820 |
| | | | | 34.5 | 2.8598 | 720 |
| | | | | 36.0 | 2.8041 | 640 |
| | | | | 38.0 | 2.7299 | 540 |
| | | | | 40.5 | 2.6372 | 430 |
| | | | | 42.0 | 2.5815 | 380 |
| | | | | 44.0 | 2.5073 | 320 |
| | | | | 46.0 | 2.4331 | 270 |
| | | | | 47.5 | 2.3775 | 240 |
| | | | Apple | 19.5 | 3.4163 | 2,610 |
| | | | | 24.5 | 3.2308 | 1,700 |
| | | | | 25.5 | 3.1937 | 1,560 |
| | | | | 31.0 | 2.9896 | 980 |
| | | | | 32.5 | 2.9340 | 860 |
| | | | | 33.5 | 2.8969 | 790 |
| | | | | 41.5 | 2.6001 | 400 |
| | | | | 44.0 | 2.5073 | 320 |
| | | | | 46.5 | 2.4146 | 260 |
| | | | Cantaloupe | 30.5 | 3.0082 | 1,020 |
| | | | | 31.5 | 2.9711 | 940 |
| | | | | 34.5 | 2.8598 | 720 |
| | | | | 36.5 | 2.7856 | 610 |
| | | | | 38.5 | 2.7114 | 510 |
| | | | | 41.0 | 2.6186 | 420 |
| | | | | 43.0 | 2.5444 | 350 |
| | | | | 45.0 | 2.4702 | 300 |
| | | | | 49.5 | 2.3033 | 200 |
| | | | | 53.0 | 2.1734 | 150 |



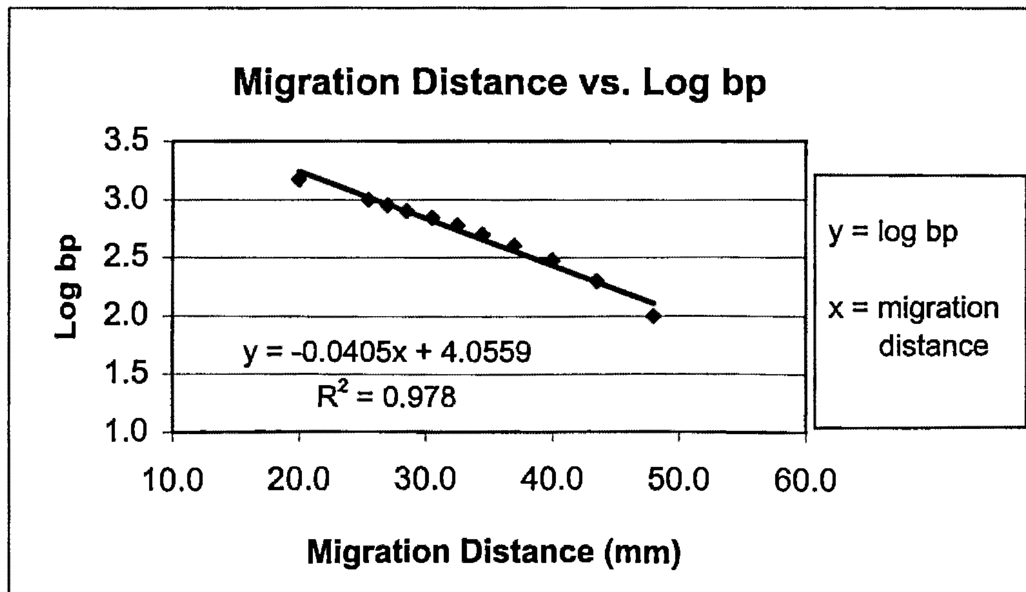
**Mitocox primers: 1 ul of fruit DNA**

| Ladder (bp) | Dist. Mig. (mm) | Log (bp) | Organism | PCR frag. | Calc. Log bp | Frag. (bp) |
|---|---|---|---|---|---|---|
| 1500 | 21.0 | 3.1761 | Human | 30.5 | 2.8381 | 690 |
| 1000 | 26.0 | 3.0000 | | 33.5 | 2.7130 | 520 |
| 900 | 27.5 | 2.9542 | | 35.0 | 2.6504 | 450 |
| 800 | 29.0 | 2.9031 | | 39.5 | 2.4628 | 290 |
| 700 | 31.0 | 2.8451 | | 43.5 | 2.2960 | 200 |
| 600 | 32.5 | 2.7782 | | 48.0 | 2.1083 | 130 |
| 500 | 35.0 | 2.6990 | E.coli | 36.0 | 2.6087 | 410 |
| 400 | 37.0 | 2.6021 | Mango | 25.5 | 3.0466 | 1,110 |
| 300 | 40.0 | 2.4771 | | 30.0 | 2.8589 | 720 |
| 200 | 44.0 | 2.3010 | | 32.0 | 2.7755 | 600 |
| 100 | 48.0 | 2.0000 | | 36.0 | 2.6087 | 410 |
| | | | | 40.0 | 2.4419 | 280 |
| | | | | 44.5 | 2.2543 | 180 |
| | | | Cantaloupe | 26.0 | 3.0257 | 1,060 |
| | | | | 27.5 | 2.9632 | 920 |
| | | | | 31.0 | 2.8172 | 660 |
| | | | | 33.0 | 2.7338 | 540 |
| | | | | 35.0 | 2.6504 | 450 |
| | | | | 36.5 | 2.5879 | 390 |
| | | | | 39.5 | 2.4628 | 290 |
| | | | | 42.0 | 2.3585 | 230 |
| | | | | 47.0 | 2.1500 | 140 |
| | | | Orange | 38.5 | 2.5045 | 320 |
| | | | | 43 | 2.3168 | 210 |



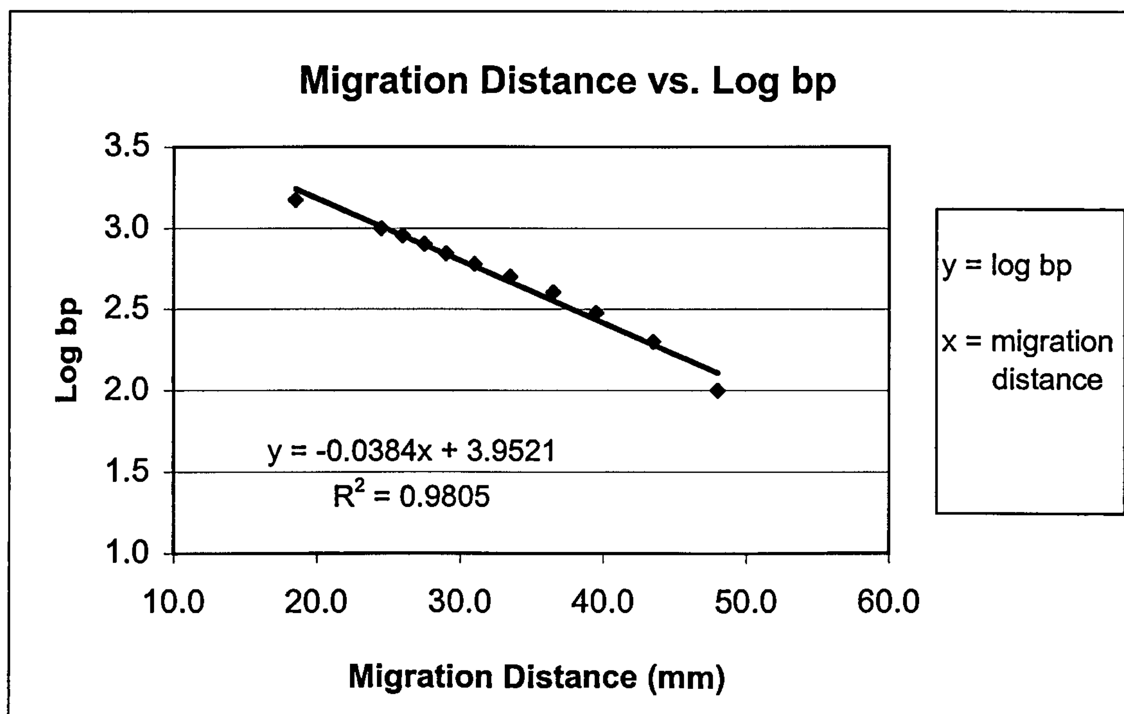
**Mitocox primers: 1 ul of fruit DNA**

| Ladder (bp) | Dist. Mig. (mm) | Log (bp) | Organism | PCR frag. | Calc. Log bp | Frag. (bp) |
|---|---|---|---|---|---|---|
| 1500 | 20.0 | 3.1761 | Human | 30.0 | 2.8409 | 690 |
| 1000 | 25.5 | 3.0000 | | 33.5 | 2.6992 | 500 |
| 900 | 27.0 | 2.9542 | | 35.0 | 2.6384 | 430 |
| 800 | 28.5 | 2.9031 | | 39.0 | 2.4764 | 300 |
| 700 | 30.5 | 2.8451 | | 40.5 | 2.4157 | 260 |
| 600 | 32.5 | 2.7782 | | 43.5 | 2.2942 | 200 |
| 500 | 34.5 | 2.6990 | | 48.0 | 2.1119 | 130 |
| 400 | 37.0 | 2.6021 | E.coli | 36.0 | 2.5979 | 400 |
| 300 | 40.0 | 2.4771 | Tomato | 26.5 | 2.9827 | 960 |
| 200 | 43.5 | 2.3010 | | 28.5 | 2.9017 | 800 |
| 100 | 48.0 | 2.0000 | | 30.0 | 2.8409 | 690 |
| | | | | 33.5 | 2.6992 | 500 |
| | | | | 35.0 | 2.6384 | 430 |
| | | | | 36.5 | 2.5777 | 380 |
| | | | | 38.0 | 2.5169 | 330 |
| | | | | 40.0 | 2.4359 | 270 |
| | | | | 44.0 | 2.2739 | 190 |
| | | | Grape | 36.0 | 2.5979 | 400 |
| | | | | 38.0 | 2.5169 | 330 |
| | | | Kiwi | 33.5 | 2.6992 | 500 |
| | | | | 36.5 | 2.5777 | 380 |
| | | | | 40.5 | 2.4157 | 260 |
| | | | | 42.5 | 2.3347 | 220 |
| | | | | 44 | 2.2739 | 190 |
| | | | Banana | 37.5 | 2.5372 | 340 |



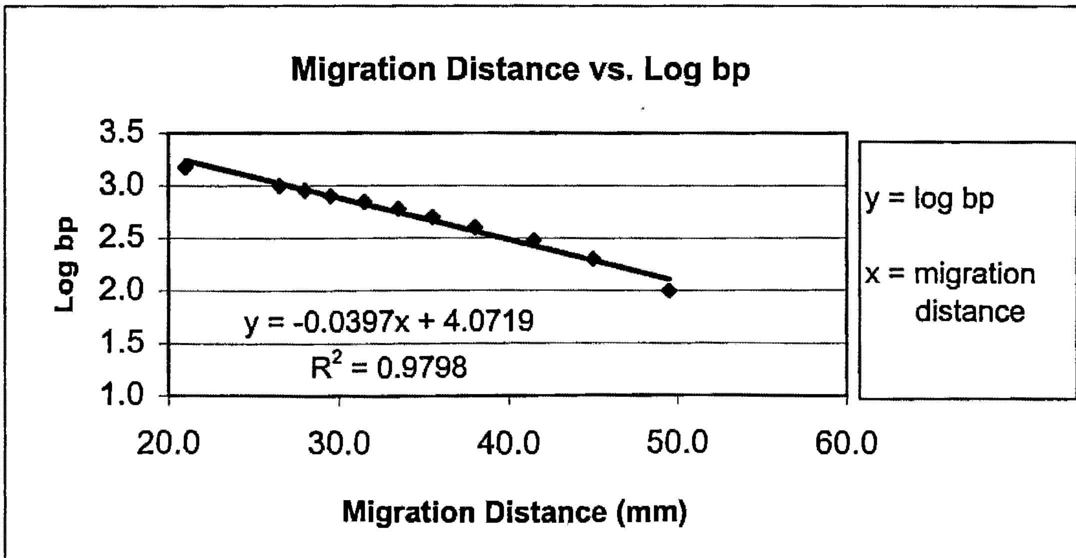
**Mitocox primers: 5 ul of fruit DNA**

| Ladder (bp) | Dist. Mig. (mm) | Log (bp) | Organism | PCR frag. | Calc. Log bp | Frag. (bp) |
|---|---|---|---|---|---|---|
| 1500 | 18.5 | 3.1761 | Human | 29.0 | 2.8385 | 690 |
| 1000 | 24.5 | 3.0000 | Tomato | 30.0 | 2.8001 | 630 |
| 900 | 26.0 | 2.9542 | | 35.0 | 2.6081 | 410 |
| 800 | 27.5 | 2.9031 | | 40.0 | 2.4161 | 260 |
| 700 | 29.0 | 2.8451 | | 44.5 | 2.2433 | 180 |
| 600 | 31.0 | 2.7782 | | 46.5 | 2.1665 | 150 |
| 500 | 33.5 | 2.6990 | Kiwi | 33.5 | 2.6657 | 460 |
| 400 | 36.5 | 2.6021 | | 42.5 | 2.3201 | 210 |
| 300 | 39.5 | 2.4771 | E.coli | 27.0 | 2.9153 | 820 |
| 200 | 43.5 | 2.3010 | | 37.0 | 2.5313 | 340 |
| 100 | 48.0 | 2.0000 | | | | |



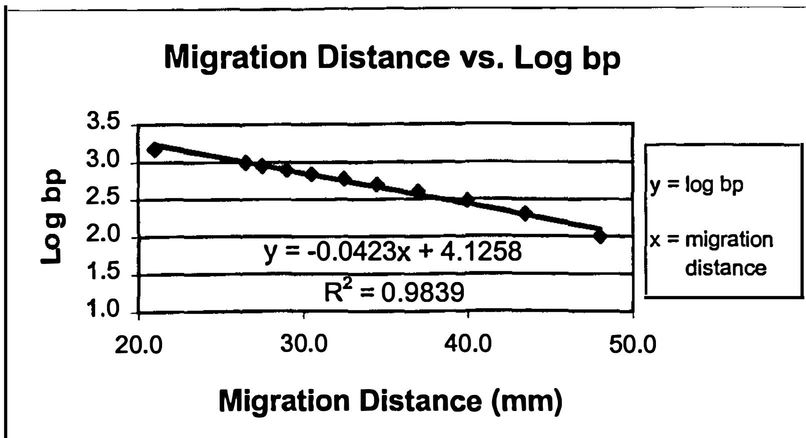
**Mitocox primers: 5 ul of fruit DNA**

| Ladder (bp) | Dist. Mig. (mm) | Log (bp) | Organism | PCR frag. | Calc. Log bp | Frag. (bp) |
|---|---|---|---|---|---|---|
| 1500 | 21.0 | 3.1761 | Human | 31.0 | 2.8412 | 690 |
| 1000 | 26.5 | 3.0000 | | 34.5 | 2.7023 | 500 |
| 900 | 28.0 | 2.9542 | | 36.0 | 2.6427 | 440 |
| 800 | 29.5 | 2.9031 | | 40.0 | 2.4839 | 300 |
| 700 | 31.5 | 2.8451 | | 42.0 | 2.4045 | 250 |
| 600 | 33.5 | 2.7782 | | 45.0 | 2.2854 | 190 |
| 500 | 35.5 | 2.6990 | | 49.5 | 2.1068 | 130 |
| 400 | 38.0 | 2.6021 | E.coli | 29.0 | 2.9206 | 830 |
| 300 | 41.5 | 2.4771 | | 37.5 | 2.5832 | 380 |
| 200 | 45.0 | 2.3010 | Mango | 26.0 | 3.0397 | 1,100 |
| 100 | 49.5 | 2.0000 | | 33.0 | 2.7618 | 580 |
| | | | | 36.0 | 2.6427 | 440 |
| | | | | 42.0 | 2.4045 | 250 |
| | | | | 45.0 | 2.2854 | 190 |
| | | | Apple | 34.0 | 2.7221 | 530 |
| | | | | 40.0 | 2.4839 | 300 |
| | | | Cantaloupe | 26.0 | 3.0397 | 1,100 |
| | | | | 28.0 | 2.9603 | 910 |
| | | | | 33.0 | 2.7618 | 580 |
| | | | | 36.5 | 2.6229 | 420 |
| | | | | 42.5 | 2.3847 | 240 |
| | | | | 47.0 | 2.2060 | 160 |
| | | | Orange | 28.0 | 2.9603 | 910 |
| | | | | 29.0 | 2.9206 | 830 |
| | | | | 39.0 | 2.5236 | 330 |
| | | | | 44.0 | 2.3251 | 210 |



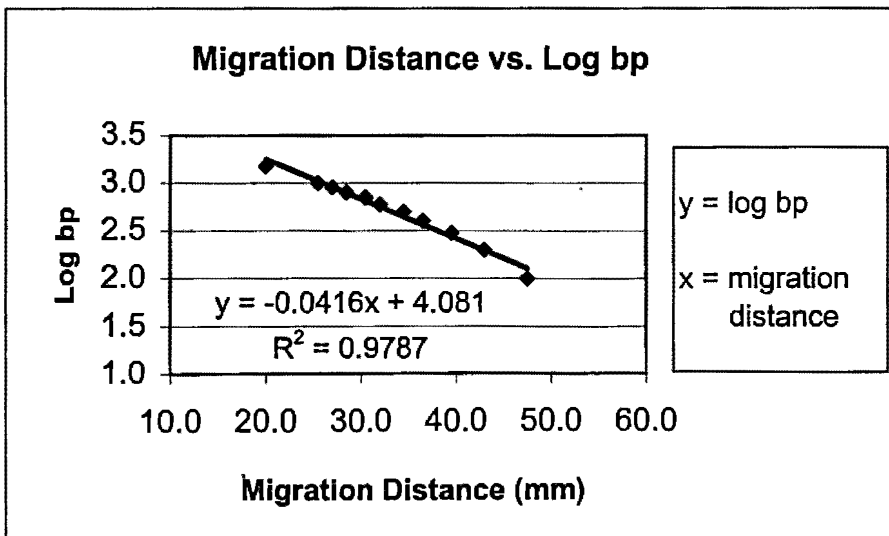
**Mitocox primers: 10 ul of fruit DNA**

| Ladder (bp) | Dist. Mig. (mm) | Log (bp) | Organism | PCR frag. | Calc. Log bp | Frag. (bp) |
|---|---|---|---|---|---|---|
| 1500 | 21.0 | 3.1761 | Human | 30.5 | 2.8357 | 680 |
| 1000 | 26.5 | 3.0000 | | 33.5 | 2.7088 | 510 |
| 900 | 27.5 | 2.9542 | | 35.0 | 2.6453 | 440 |
| 800 | 29.0 | 2.9031 | | 39.0 | 2.4761 | 300 |
| 700 | 30.5 | 2.8451 | | 43.5 | 2.2858 | 190 |
| 600 | 32.5 | 2.7782 | | 48.0 | 2.0954 | 120 |
| 500 | 34.5 | 2.6990 | E.coli | 25.0 | 3.0683 | 1,170 |
| 400 | 37.0 | 2.6021 | | 35.0 | 2.6453 | 440 |
| 300 | 40.0 | 2.4771 | Apple | 28.0 | 2.9414 | 870 |
| 200 | 43.5 | 2.3010 | | 29.5 | 2.8780 | 760 |
| 100 | 48.0 | 2.0000 | | 30.5 | 2.8357 | 680 |
| | | | | 34.5 | 2.6665 | 460 |
| | | | | 36.0 | 2.6030 | 400 |
| | | | | 42.0 | 2.3492 | 220 |
| | | | Tomato | 16.0 | 3.4490 | 2,810 |
| | | | | 18.0 | 3.3644 | 2,310 |
| | | | | 20.0 | 3.2798 | 1,900 |
| | | | | 23.0 | 3.1529 | 1,420 |
| | | | | 26.0 | 3.0260 | 1,060 |
| | | | | 28.0 | 2.9414 | 870 |
| | | | | 30.0 | 2.8568 | 720 |
| | | | | 35.0 | 2.6453 | 440 |
| | | | | 41.0 | 2.3915 | 250 |
| | | | | 43.5 | 2.2858 | 190 |
| | | | | 46.0 | 2.1800 | 150 |
| | | | Kiwi | 27.5 | 2.9626 | 920 |
| | | | | 29.0 | 2.8991 | 790 |
| | | | | 30.0 | 2.8568 | 720 |
| | | | | 31.5 | 2.7934 | 620 |
| | | | | 34.5 | 2.6665 | 460 |
| | | | | 36.0 | 2.6030 | 400 |
| | | | | 38.5 | 2.4973 | 310 |
| | | | | 41.0 | 2.3915 | 250 |
| | | | | 42.5 | 2.3281 | 210 |
| | | | | 44.5 | 2.2435 | 180 |
| | | | Grape | 17.0 | 3.4067 | 2,550 |
| | | | | 18.5 | 3.3433 | 2,200 |
| | | | | 23.0 | 3.1529 | 1,420 |
| | | | | 24.0 | 3.1106 | 1,290 |
| | | | | 25.0 | 3.0683 | 1,170 |
| | | | | 29.0 | 2.8991 | 790 |
| | | | | 31.5 | 2.7934 | 620 |
| | | | | 34.0 | 2.6876 | 490 |
| | | | | 36.5 | 2.5819 | 380 |
| | | | | 38.5 | 2.4973 | 310 |
| | | | | 41.5 | 2.3704 | 230 |
| | | | | 43.0 | 2.3069 | 200 |
| | | | | 44.0 | 2.2646 | 180 |
| | | | | 48.0 | 2.0954 | 120 |



**Mitocox primers: 10 ul of fruit DNA**

| Ladder (bp) | Dist. Mig. (mm) | Log (bp) | Organism | PCR frag. | Calc. Log bp | Frag. (bp) |
|---|---|---|---|---|---|---|
| 1500 | 20.0 | 3.1761 | Human | 30.0 | 2.8330 | 680 |
| 1000 | 25.5 | 3.0000 | | 38.0 | 2.5002 | 320 |
| 900 | 27.0 | 2.9542 | | 43.0 | 2.2922 | 200 |
| 800 | 28.5 | 2.9031 | | 47.0 | 2.1258 | 130 |
| 700 | 30.5 | 2.8451 | Mango | 17.5 | 3.3530 | 2,250 |
| 600 | 32.0 | 2.7782 | | 18.5 | 3.3114 | 2,050 |
| 500 | 34.5 | 2.6990 | | 25.5 | 3.0202 | 1,050 |
| 400 | 36.5 | 2.6021 | | 31.0 | 2.7914 | 620 |
| 300 | 39.5 | 2.4771 | | 33.5 | 2.6874 | 490 |
| 200 | 43.0 | 2.3010 | | 36.0 | 2.5834 | 380 |
| 100 | 47.5 | 2.0000 | | 39.0 | 2.4586 | 290 |
| | | | | 44.5 | 2.2298 | 170 |
| | | | Banana | 26.0 | 2.9994 | 1,000 |
| | | | | 28.0 | 2.9162 | 820 |
| | | | | 29.5 | 2.8538 | 710 |
| | | | | 39.5 | 2.4378 | 270 |
| | | | | 44.5 | 2.2298 | 170 |
| | | | Orange | 31.0 | 2.7914 | 620 |
| | | | | 37.5 | 2.5210 | 330 |
| | | | | 42.0 | 2.3338 | 220 |
| | | | Cantaloupe | 25.5 | 3.0202 | 1,050 |
| | | | | 27.0 | 2.9578 | 910 |
| | | | | 30.5 | 2.8122 | 650 |
| | | | | 32.5 | 2.7290 | 540 |
| | | | | 36.0 | 2.5834 | 380 |
| | | | | 41.5 | 2.3546 | 230 |
| | | | | 45.5 | 2.1882 | 150 |



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