University of Nevada, Reno

# A Study of DNA Degradation for the Purpose of DNA Testing: Analysis of a Method to Improve DNA Profiling on Degraded DNA Samples

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Cell and Molecular Biology

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Abstract:

Concentration of higher molecular weight DNA allowed for a useable DNA profile to be obtained from a degraded DNA sample. DNA extraction methods were tested with tissue samples under three environmental conditions. The three tissue samples compared the time frame of in which DNA degradation occurred and the recovery of useable DNA profiles, to determine if the level of improvement was reproducible.

Short tandem repeats (STRs) and electrophoresis is used in forensic DNA analysis in order to determine a DNA profile. STRs are highly polymorphic regions with short repeated sequences. The Combined DNA Index System (CODIS) developed by the Federal Bureau of Investigation (FBI) consists of thirteen STR loci (D3S1358, THO1, D21S11, D18S51, D5S818, D13S317, D7S820, D16S539, CSF1PO, vWA, D8S1179, TPOX, and FGA). Multiple allelic variants are possible at each locus, which allow the profile obtained to discriminate between individuals.

DNA degradation can prevent an acceptable profile from being obtained. DNA degradation, an increased number of smaller DNA fragments, can be caused by heat, bacterial growth, ultraviolet light, time, and other environmental factors.

Known DNA profiles were established for the three tissue samples and the profiles were monitored for DNA degradation. The three tested environmental conditions were open air climate, stagnant water conditions and submerged soil

conditions. Sections of the tissue samples underwent DNA processing over defined time periods and the allelic profiles were recorded. Once DNA degradation was observed the samples were removed to prevent further degradation and the higher molecular weight DNA was concentrated and an improved DNA profile was obtained in two of the three samples.

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# INTRODUCTION

## GENERAL BACKGROUND

## History of DNA Typing

In 1985 Alec Jeffreys, an English geneticist, discovered that certain DNA regions contained repeated DNA sequences. These became known as variable number of tandem repeats (VNTRs). VNTRs differed between individuals which allowed for the development of DNA typing (1). Dr. Jeffreys developed a technique called restriction fragment length polymorphism (RLFP) which used a restriction enzyme to perform human identity tests. In 1986, England was the first to use DNA testing for forensic purposes and solved a double homicide (1).

DNA testing has achieved remarkable advancements over the years (1), with improved sensitivity, processing speed and power of discrimination. In the early years of DNA testing, large amounts of well preserved DNA were needed to obtain a successful profile. Such tests required 50 to 500ng of DNA. Today, only 0.1 to 1ng of DNA is needed to produce a DNA profile (1). The reduction in necessary DNA quantity has increased the opportunities to use DNA in forensic science. DNA testing originally took six to eight weeks and can now be conducted in hours. The power of discrimination had four possible outcomes, now has over three trillion (1). The development of multiplexed short tandem repeats (STR) combined both high power of discrimination and quick processing speed. STRs or microsatellites have repeating DNA regions that are 2 to 6 base pairs in length and are highly variable (1). These DNA markers are readily amplified using polymerase chain reaction (PCR), the enzymatic process which

replicates specific regions of DNA, without producing differential amplifications and functions with degraded DNA samples or low-quantity DNA templates.

In 1996 the Combined DNA Index System (CODIS) was developed by the Federal Bureau of Investigation (FBI). In 1997, the thirteen STR loci for the national DNA database were chosen (20).

TABLE 1: CODIS 13 STR LOCI

| Allele | Repeat Sequence | Allele Range | Chromosomal Location | Number of Alleles Seen |
|---|---|---|---|---|
| D3S1358 | [TCTG] [TCTA] | 8-21 | 3P21.31 | 24 |
| THO1 | TCAT | 3-14 | 11p15.5<br>Tyrosine hydroxylase, 1st intron | 20 |
| D21S11 | Complex [TCTA] [TCTG] | 7-39.2 | 21q21.1 | 82 |
| D18S51 | AGAA | 12-41.2 | 18q21.33 | 51 |
| D5S818 | AGAT | 7-18 | 5q23.2 | 15 |
| D13S317 | TATC | 5-16 | 13q31.1 | 17 |
| D7S820 | GATA | 5-16 | 7q21.11 | 30 |
| D16S539 | GATA | 5-16 | 16q24.1 | 19 |
| CSF1PO | TAGA | 5-16 | 5q33.1<br>c-fms proto-oncogene, 6th intron | 20 |
| vWA | [TCTG] [TCTA] | 10-25 | 12p13.31<br>Von Willebrand Factor, 40th intron | 28 |
| D8S1179 | [TCTA] [TCTG] | 7-20 | 8q24.13 | 17 |
| TPOX | GAAT | 4-16 | 2p25.3<br>Thyroid peroxidase, 10th intron | 15 |
| FGA | CTTT | 12.2-51.2 | 4q31.3<br>Alpha fibrinogen, 3rd intron | 80 |

The thirteen STR loci are the following: D3S1358, THO1, D21S11, D18S51, D5S818, D13S317, D7S820, D16S539, CSF1PO, vWA, D8S1179, TPOX, and FGA (3) (Table 1). The criteria for human identity allele selection includes high power of discrimination, low rates of mutations, separate chromosomal locations

(Table 1; Figure 1), smaller allele length (90 to 500 base pairs; for degraded DNA analysis), low stutter traits and reproducibility.

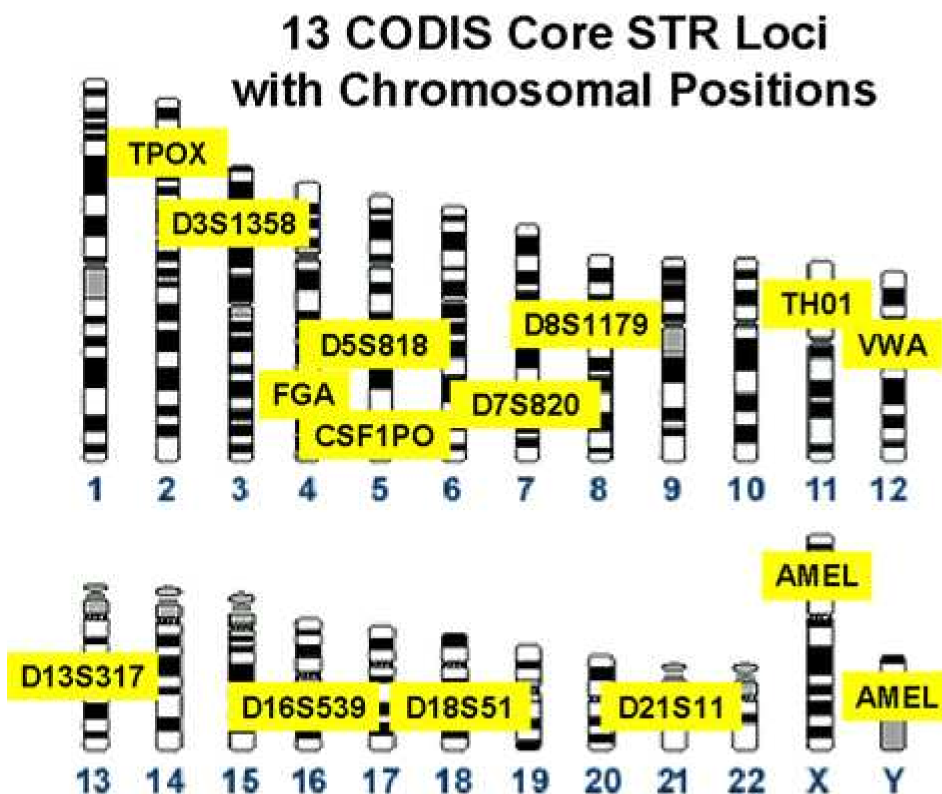


Figure 1: Chromosomal locations of the 13 CODIS STR loci.

Tetranucleotide, a sequence of four nucleotides repeated, STR loci have numerous characteristics that contribute to successful DNA typing. A narrow range of allele size allowed for multiplexing, reduced allelic drop out (the preferential amplification that represents the other allele (21)) from amplification of smaller alleles and reduced allelic stutter (a small peak that occurs around the true allele which results from halted polymerase activity).

DNA has two functions; create copies of information for cell division, and instructions for proteins. Nucleic acids are composed of a base (Adenine, Thymine, Cytosine and Guanine), a sugar and a phosphate. Nucleotides are complementary paired adenine and thymine linked with two hydrogen bonds or cytosine and guanine with three. The human genome is composed of three billion base pairs or twenty-three pairs of chromosomes (Figure 1), along with mitochondrial DNA. Mitochondrial DNA sequencing can be used for forensic DNA testing, sequencing the HV1 and HV2 regions (18). Since there are hundreds of copies of mtDNA per human cell mtDNA sequencing is used for the analysis of degraded samples. Unfortunately, mtDNA has low discrimination power because it is maternally inherited without recombination. The low discrimination power causes exclusions to be more meaningful than inclusions and explains why mtDNA sequencing is only conducted after the failure an autosomal profile.

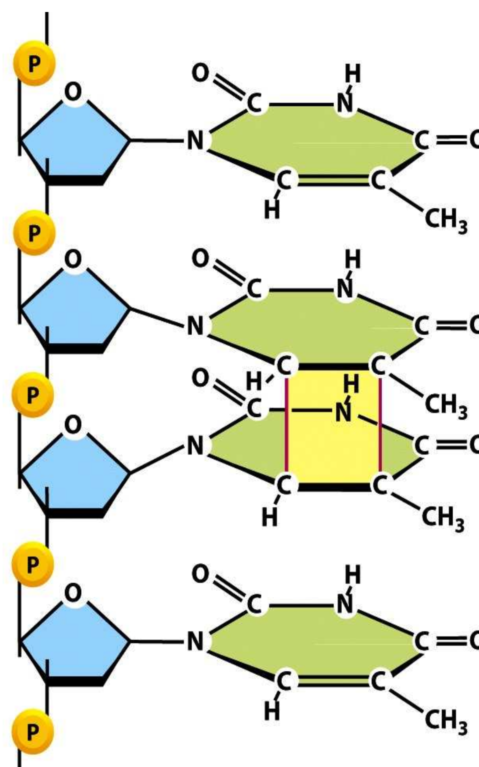


Figure 2 (2): A diagram of cross-linked adjacent thymine nucleotides commonly caused by UV irradiation.

Though current DNA methods have improved, DNA degradation is still a problem in forensic DNA testing. DNA degradation can be caused by chemical and enzymatic processes. DNA molecules are

exposed to cellular nucleases along with environmental conditions such as bacteria, fungus, insects and ultraviolet (UV) irradiation, which can cause hydrolytic cleavage, mainly at the glycosidic base sugar bond, and oxidative base damage (1). UV irradiation also prevents the passage of polymerase during PCR by causing the cross-linking of adjacent thymine nucleotides (2) (Figure 2). The PCR amplification is determined by the number of DNA molecules within the biological samples that break in the regions where the primer anneals or between the forward and reverse primers.

The purpose of this study was to determine if a useable DNA profile could be obtained from a degraded sample by the concentration of higher molecular weight DNA. DNA extraction methods were tested with tissue samples under three environmental DNA degradation conditions, open air climate conditions, stagnant water conditions and submerged soil conditions. Known DNA profiles were established and DNA degradation of human muscle tissue was monitored. Sections of the tissue samples underwent DNA processing over defined time periods and the allelic profiles were recorded. These environmental conditions compared the time frame of DNA degradation and the recovery of useable DNA profiles, to determine if the level of improvement was reproducible. The concentration of higher molecular weight DNA or chromosomal DNA was conducted and an improved DNA profile was obtained in two of the three samples.

# EXPERIMENTAL PROCEDURES

# AND PROTOCOLS

Gentra Puregene Protocols:

The Gentra Puregene system (6) was one of the DNA extraction methods used for the isolation of DNA on tested tissue samples. This method uses SDS lysis solution, sodium acetate and alcohol for DNA isolation and purification.

The tested biological tissue sample was placed in a fresh tube along with the Puregene lysis solution, proteinase K and dithiothreitol (See Gentra Puregene Handbook for ratios; Please note that if an insufficient amount of lysis solution is used the histones may not properly unwind (19) and reduced DNA concentration) and heated at 55 ℃ until digested. Protein precipi tation solution, which was used to separate the protein from the solution, was added, vortexed and centrifuged at maximum speed (approximately 14,000xg rpm) for five minutes. The supernatant was transferred to a fresh tube and an equal volume of iso-propanol was added. The solutions were gently mixed and centrifuged at maximum speed for twenty minutes. The supernatant was discarded and the DNA pellet washed with 70% ethanol. The sample was centrifuged ten minutes at maximum speed and supernatant discarded. The DNA pellet was allowed to dry and re-suspended in TE (Tris Buffer and Ethylenediaminetetraacetic acid solution) or elution solution.

1% Agarose Gel:

The agarose gel (9) was used to separate the degraded DNA fragments from the chromosomal DNA (17). A one percent agarose gel was created using agarose and 1xTris-acetate (TAE). The mixture was heated to a boil, allowed to cool while

swirled, ethidium bromide (EtBr) was added, swirled and then the gel was poured and allowed to solidify. Loading dye was added to the DNA sample, mixed and loaded into the gel.

Qiagen QIAquick Gel Extraction Kit Protocol:

Qiagen QIAquick Gel Extraction Kit Protocol (10) was used to dissolve the gel. The chromosomal DNA fragment band was cut from the gel and placed in a clean tube. Three volumes of buffer QG was added to 1 volume of gel (300 µl of buffer QG to 100 mg of gel) and incubated for 10 minutes at 50℃ until the gel was completely dissolved. The tube was vortexed every 2 to 3 minutes to assist dissolving during incubation. Once the gel was completely dissolved two volumes of DNA IQ lysis solution was added. DNA extraction was conducted using DNA IQ Protocols.

DNA IQ Protocols:

DNA IQ System (11, 12) is a DNA extraction method which uses magnetic beads for DNA isolation. The tested sample was combined with an appropriate amount of DNA IQ lysis solution and 7 µl of resin, magnetic beads. The solution was mixed and incubated at room temperature for 5 minutes. The tube was placed in the magnetic stand and the solution discarded without disturbing the resin. Lysis solution was added and the sample was removed from the magnetic stand and mixed. The sample was returned to the magnetic stand and the lysis solution discarded. 100 µl of 1x wash buffer was added and the sample was removed

from the magnetic stand and mixed. The tube was returned to the magnetic stand and the wash buffer discarded. The wash buffer steps were repeated twice more. The sample remained in the magnetic stand and allowed to dry for 5 minutes. 30 µl of elution buffer was added and incubated at 65 ℃ for 5 minutes, which caused the resin to separate from the DNA. The sample was vortexed and placed on the magnetic stand. Elution buffer was removed and placed in a clean tube.

PowerPlex 16 Hot Start (PP16HS) PCR Amplification Protocols:

The PowerPlex® 16 HS System (4, 14) (Table 2) allowed the co-amplification and three-color detection of sixteen loci (fifteen STR loci; D3S1358, TH01, D21S11, D18S51, Penta E, D5S818, D13S317, D7S820, D16S539, CSF1PO, Penta D, vWA, D8S1179, TPOX, FGA and Amelogenin). One primer for each of the D3S1358, TH01, D21S11, D18S51, and Penta E loci was labeled with fluorescein (FL); one primer for each of the D5S818, D13S317, D7S820, D16S539, CSF1PO, and Penta D loci was labeled with 6-carboxy-4´,5´-dichloro-2´,7´-dimethoxy-fluorescein (JOE); and one primer for each of the vWA, D8S1179, TPOX, FGA and Amelogenin loci was labeled with carboxy-tetramethylrhodamine (TMR).

For the PP16HS system 3.75 µl water, 2.5 µl PP16HS master mix, 1.25 µl primer, 1 µl to 5 µl of extracted DNA, depending on DNA concentration, were combined. The PCR thermal cycler run conditions were the following: heat to

95℃ for 2 minutes followed by 10 cycles of 94℃ for 30 seconds, 60℃ for 30 seconds, 70℃ for 45 seconds, 22 cycles of 90℃ for 30 seconds, 60℃ for 30 seconds, 70℃ for 45 seconds and 30 minutes at 60℃ with 4℃ soak.

**Table 2: PowerPlex 16 HS Loci Information**

| STR Locus | Chromosome Location | Repeat Sequence 5' → 3' | Dye Label | Repeat Numbers of Allelic Ladder | Size Range of Allelic Ladder (Bases) |
|---|---|---|---|---|---|
| D3S1358 | 3p | TCTA Complex | FL | 12-20, | 115-147 |
| THO1 | 11p15.5 | AATG (24) | FL | 4-9, 9.3, 10, 11, 13.3 | 156-195 |
| D21S11 | 21q11-21q21 | TCTA Complex (24) | FL | 24, 24.2, 25, 25.2, 26-28, 28.2, 29, 29.2, 30, 30.2, 31, 31.2, 32, 32.2, 33, 33.2, 34, 34.2, 35, 35.2, 36-38 | 203-259 |
| D18S51 | 18q21.3 | AGAA (24) | FL | 8-10, 10.2, 11-13, 13.2, 14-27 | 290-366 |
| Penta E | 15q | AAAGA | FL | 5-24, | 379-474 |
| D5S818 | 5q23.3-32 | AGAT | JOE | 7-16, | 119-155 |
| D13S317 | 13q22-q31 | TATC | JOE | 7-15, | 176-208 |
| D7S820 | 7q11.21-22 | GATA | JOE | 6-14, | 215-247 |
| D16S539 | 16q24-qter | GATA | JOE | 5, 8-15 | 264-304 |
| CSF1PO | 5q33.3-34 | AGAT | JOE | 6-15, | 321-357 |
| Penta D | 21q | AAAGA | JOE | 2.2, 3.2, 5, 7-17 | 376-449 |
| vWA | 12p12-pter | TCTA | TMR | 10-22, | 123-171 |
| D8S1179 | 8q | TCTA Complex (24) | TMR | 7-18, | 203-247 |
| TPOX | 2p24-2pter | AATG | TMR | 6-13, | 262-290 |
| FGA | 4q28 | TTTC | TMR | 16-18, 18.2, 19, 19.2, 20, 20.2, 21, 21.2, 22, 22.2, 23, 23.2, 24, 24.2, 25, 25.2, 26-30, 31.2, 43.2, 44.2, 45.2, 46.2 | 322-444 |
| Amelogenin | Xp22.1-22.3 and Y | N/A[a] | TMR | X, Y | 106, 112 |

AmpFℓSTR® Identifiler® Plus PCR Amplification Protocols:

The AmpFℓSTR® Identifiler® Plus PCR Amplification Kit (5, 13), from Applied Biosystems, used a short tandem repeat (STR) multiplex assay which amplifies 15 tetranucleotide repeat loci, which included D8S1179, D21S11, D7S820, CSF1PO, D3S1358, THO1, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818, FGA, and the Amelogenin gender-determining marker in a single PCR.

For the Identifiler system 5.0 μl of Identifiler master mix, 2.50 μl primer, 1 μl to 5 μl of extracted DNA, depending on DNA concentration, were combined. The PCR thermal cycler run conditions were the following: heat to 95℃ for 11 minutes followed by 28 cycles of 94℃ for 20 seconds, 59℃ for 3 minutes, and the final extension is 60℃ for 10 minutes with 4℃ hold.

ABI 3100 Genetic Analyzer Protocols:

A mixture of 16.5 μl of formamide and ILS 600 size standard was added with the appropriate 0.6 μl of PCR product in a 96 well septum plate. The samples were denatured by heating the plate at 95℃ for five min utes. The samples were cooled for 30 seconds with a frozen metal chiller block. The plate was placed the 3100 plate holder, the retainer was snapped into place and the entire unit was loaded into the 3100. The 3100 used the PP16HS run conditions with polymer 3100 pop 4 and filter set Z or Identifiler run conditions with polymer 3100 pop 4 and filter set G5.

Data Analyzed using GeneMapper ID version 3.2.1

The 3100 project file was selected to analyze and add to list. PP16HS settings along with ILS 600 were used for analysis. The samples were labeled appropriately (i.e. allelic ladder, positive control, negative control, and sample) and the plate was analyzed giving the allele calls for the appropriate allelic profile.

# RESULTS

## PART 1:

## TISSUE SAMPLE #1

## OPEN AIR CLIMATE CONDITIONS

Tissue Samples #1: Open Air Climate Conditions

Figure 3: Tissue Sample #1 (a section of female human muscle tissue) photo progression of monitored tissue decay/degradation over the time period of 304 days.

On May 18, 2010 a sample of female human muscle tissue collected from the upper thigh (Length 2.5 in, Width 1.0 in, Depth 1.0 in, Mass 82.1 grams) was exposed to typical weather conditions of Reno, Nevada 89503 (Figure 3) and allowed to decay/degrade over the time period of 304 days. Muscle tissue typically contains 50-500ng/mg of DNA. Weather conditions were monitored and recorded by both temperature and type (7, 8) (Figures 4, 5). On May 18, 2010 a section of tissue was collected and processed using Puregene protocol to establish the known profile of the sample before DNA degradation took place (Table 3, 7; Figure 7).

Sections of the tissue sample were collected at known time points and DNA profiles were established using Puregene and DNA IQ protocols (Table 3, 7). DNA degradation was first noticed on February 7, 2011 with an allelic drop out of the one of the longer loci, Penta D (Table 3). On March 9, 2011, a section of tissue sample #1 was cut and processed using Puregene protocols and the profile obtained from the March 09, 2011 sample produced ten useable loci. Three loci produced the correct profile but were below laboratory threshold (15, 16) (D18S51, CSF1PO, TPOX), one locus produced an allelic drop-in (Amelogenin) and two loci failed to produces allele calls (Penta E, Penta D) (Table 3; Figure 8).

On March 17, 2011 tissue sample #1 was removed from the sample area and placed in the freezer to prevent further degradation. A section of tissue was collected and processed using Puregene protocols. The extracted DNA was run

on a 1% agarose gel (Figure 6). The chromosomal DNA bands were extracted and the gel was dissolved using Qiagen QIAquick Gel Extraction Kit Protocol. Once the gel was dissolved the DNA was extracted using DNA IQ, amplified using PP16HS and continued using the 3100 capillary electrophoresis. After this process all a profile for all sixteen loci were obtained (Table 3, 7; Figure 9).

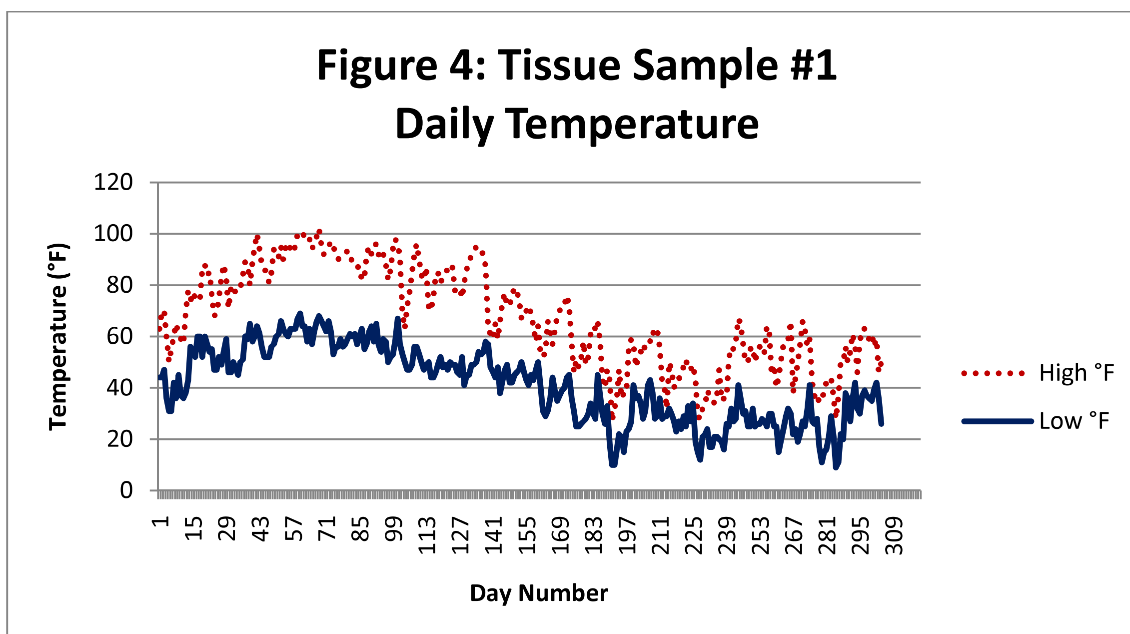


Figure 4: Shows the high (red dots) and low (blue line) temperatures (°F) for Tissue Sample #1 over the time period of 304 days (7, 8).

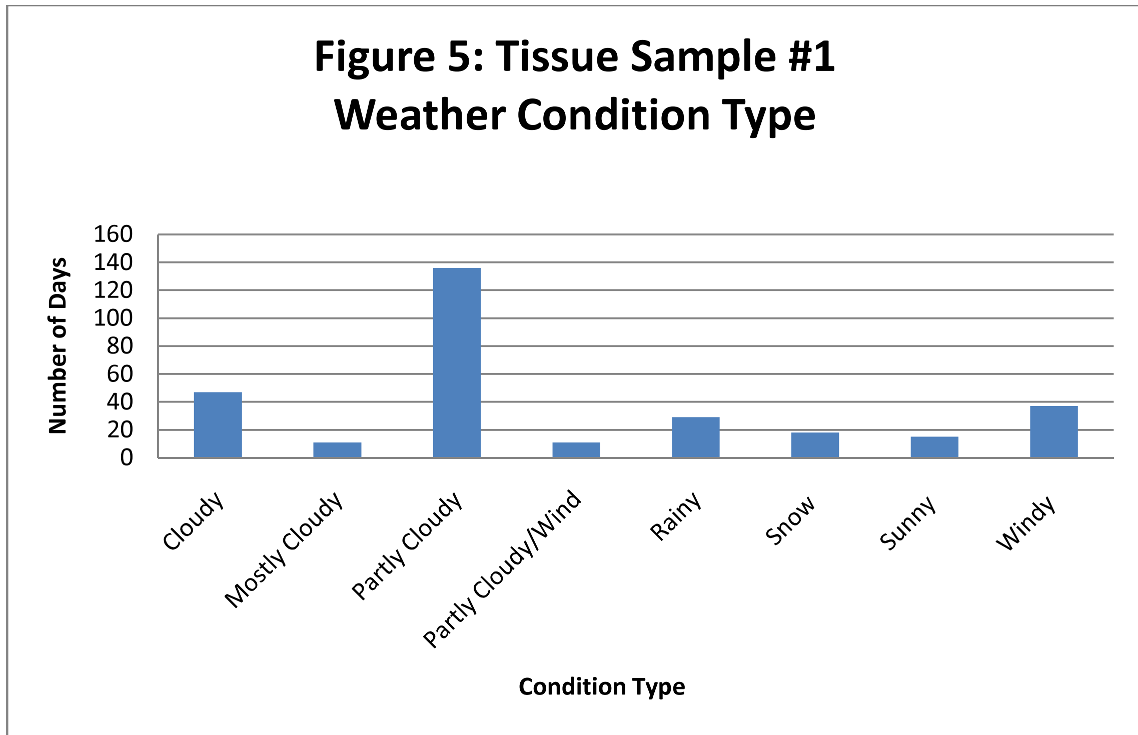


Figure 5: The number of days in the 304 day time period that Tissue Sample #1 experienced the different weather condition types (7, 8).

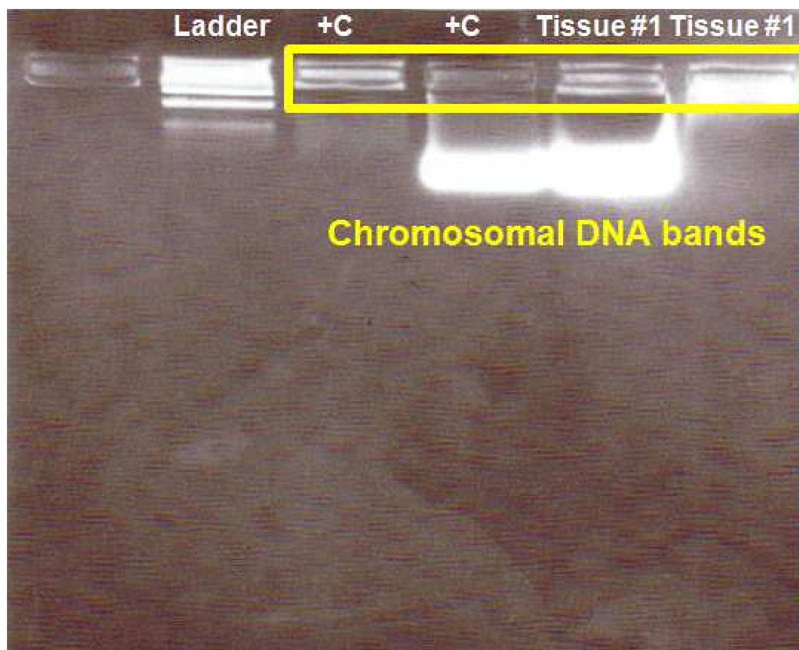


Figure 6: Tissue Sample #1 gel image of Chromosomal DNA bands (box in yellow) present after DNA degradation and extraction.

**Table 3: Tissue Sample #1 DNA profiles**

| Alleles | Sample 1 05/18/2010 | Sample 1 03/09/2011 | Sample 1 03/23/2011 |
|---|---|---|---|
| D3S1358 | 15 | 15 | 15 |
| THO1 | 7, 8 | 7, 8 | 7, 8 |
| D21S11 | 29 | 29 | 29 |
| D18S51 | 15, 17 | 15,* 17* | 15, 17 |
| Penta E | 9, 14 | <, < | 9, 14 |
| D5S818 | 12, 13 | 12, 13 | 12, 13 |
| D13S317 | 12, 13 | 12, 13 | 12, 13 |
| D7S820 | 8, 12 | 8, 12 | 8, 12 |
| D16S539 | 12, 13 | 12, 13 | 12, 13 |
| CSF1PO | 10, 11 | 10*, 11* | 10, 11 |
| Penta D | 9, 10 | <, < | 9, 10 |
| vWA | 11, 17 | 11, 17 | 11, 17 |
| D8S1179 | 12, 15 | 12, 15 | 12, 15 |
| TPOX | 8, 9 | 8*, 9* | 8, 9 |
| FGA | 22, 24 | 22, 24 | 22, 24 |
| Amelogenin | X | X, Y^ | X |

*Below Laboratory Threshold (Peak heights below 150 rfu)

^Allelic Drop-in (False Peak)

<No Allele Present

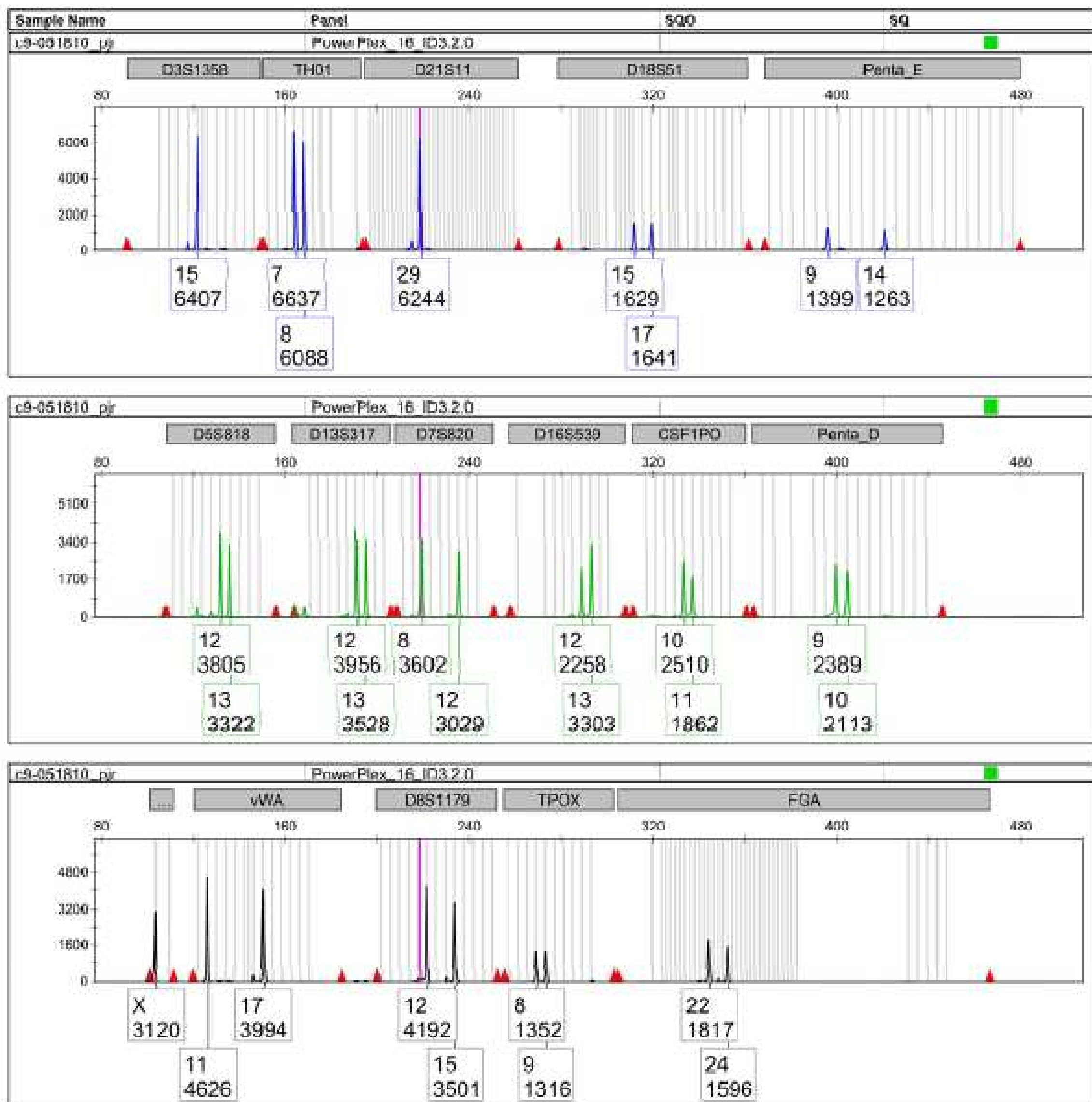


Figure 7: Tissue Sample #1 3100 Profile. Established known profile for Tissue Sample #1 on May 18, 2010 before DNA degradation affect. All sixteen loci produced alleles with sufficient peak heights.

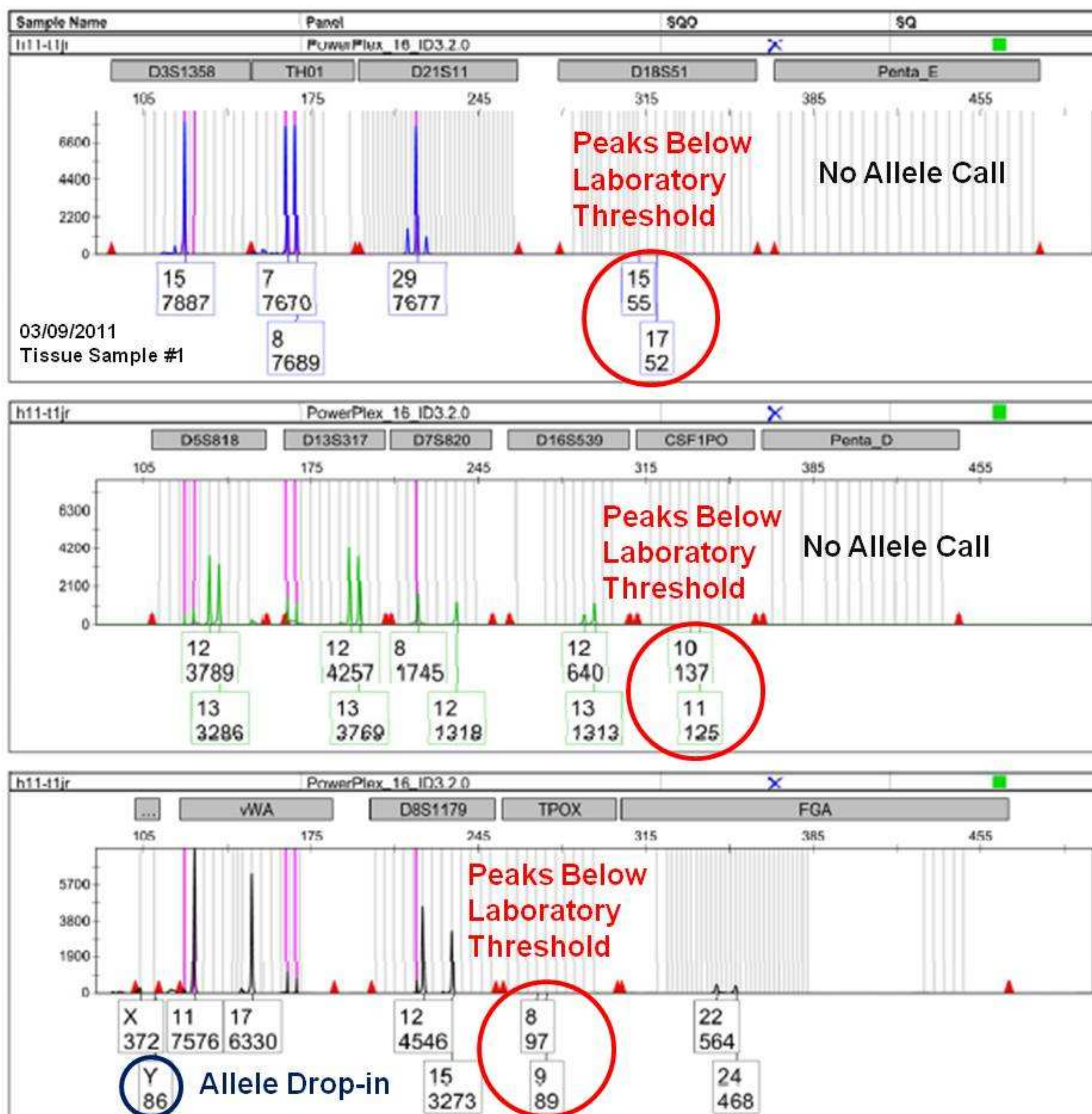


Figure 8: Tissue Sample #1 degraded 3100 profile. March 09, 2011 DNA degraded profile of Tissue Sample #1. Ten loci produced acceptable alleles. Three loci, (D15S51, CSF1PO, and TPOX) produced the correct profile but were below laboratory threshold (red circles). One locus produced an allelic drop-in (Amelogenin) and two loci failed to produce allele calls (Penta E and Penta D).

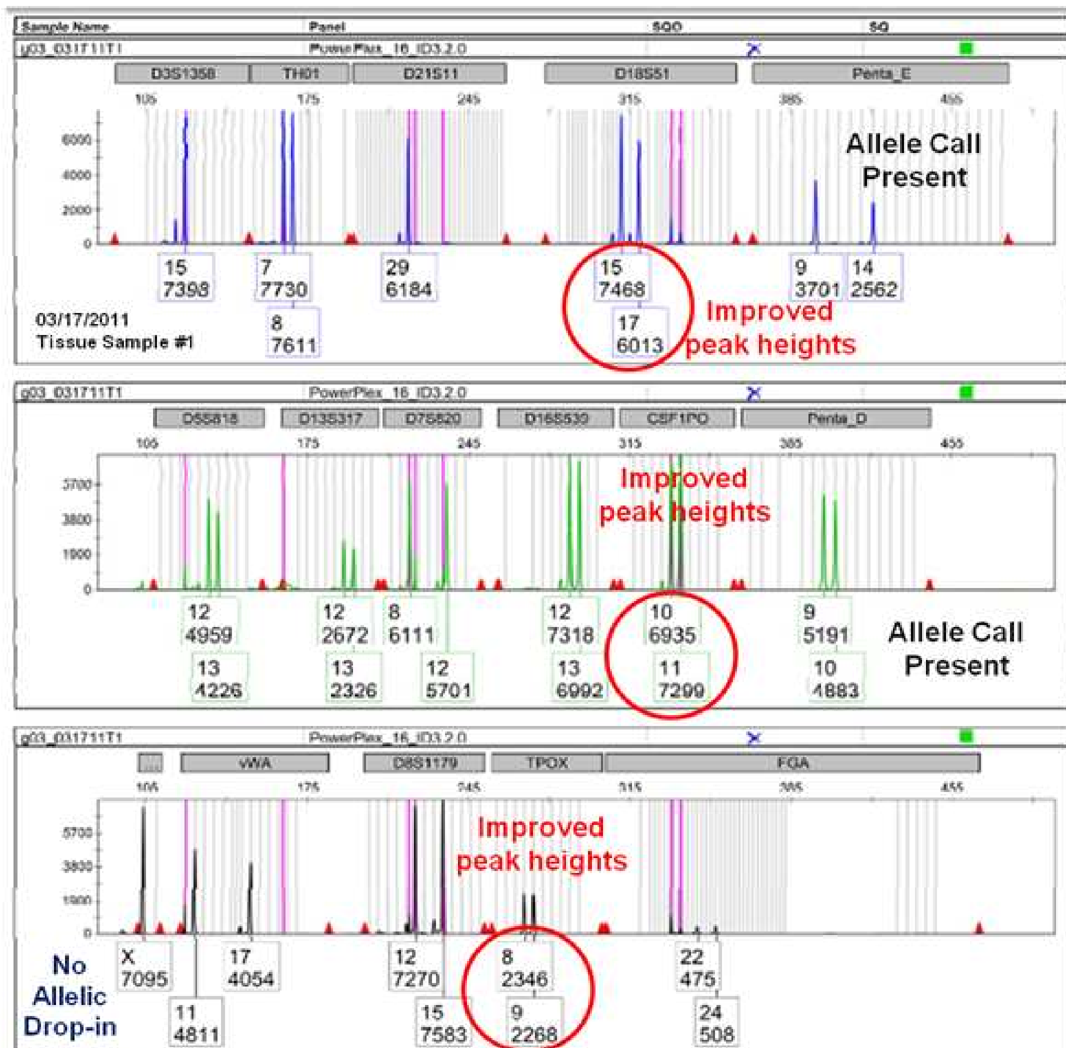


Figure 9: Improved 3100 profile for Tissue Sample #1. The March 17, 2011 DNA profile for Tissue Sample #1 produced a complete profile with acceptable peak heights. The three loci, (D15S51, CSF1PO, and TPOX) that produced the correct profile but were below laboratory threshold showed improved peak heights (red circles). The locus that produced an allelic drop-in (Amelogenin) was no longer present. The two loci that failed to produce allele calls (Penta E and Penta D) now showed allele calls with acceptable peak heights.

RESULTS

PART 2:

TISSUE SAMPLE #2

STAGNANT WATER CONDITIONS

Sample #2: Stagnant Water Conditions

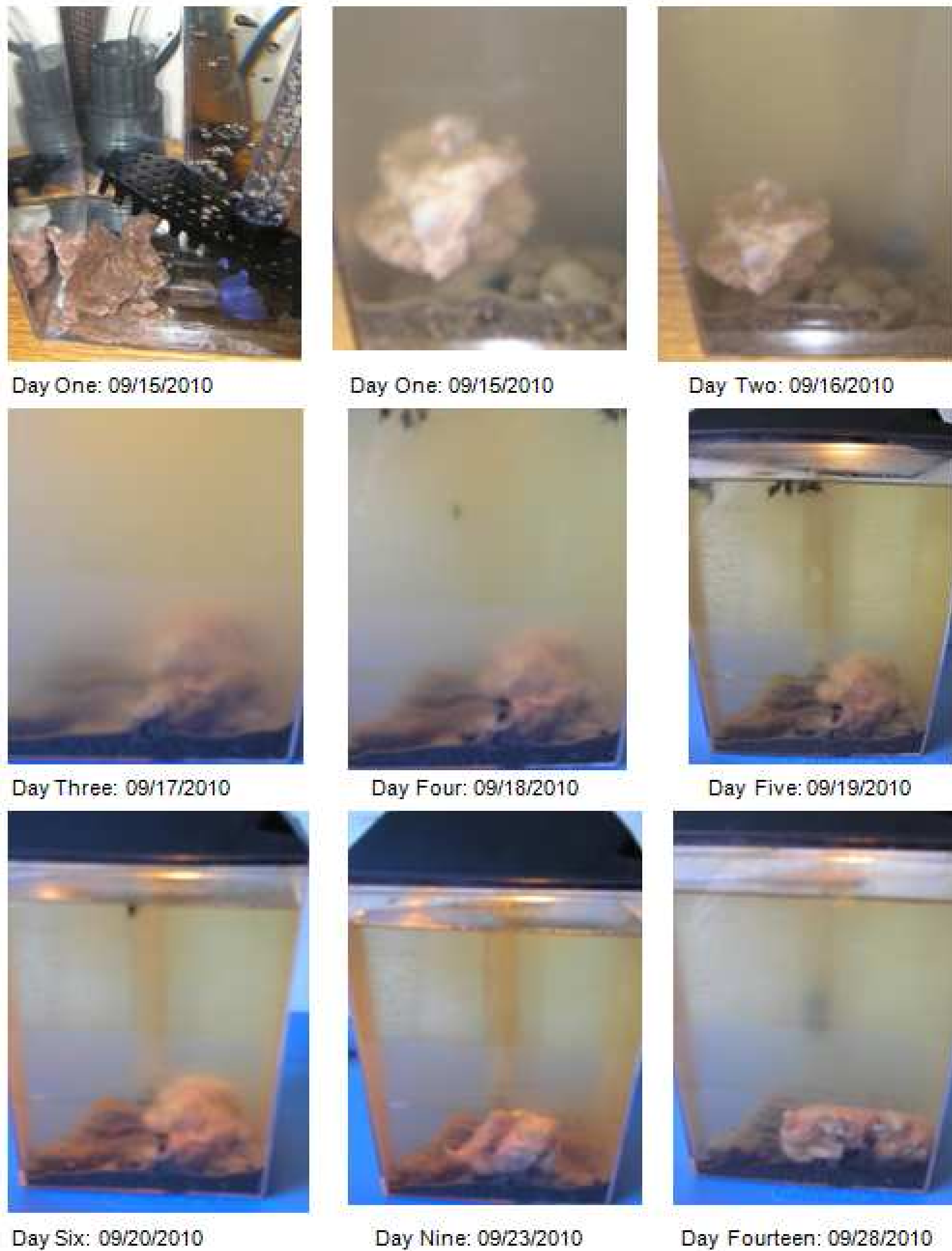


Figure 10: Tissue Sample #2 (a section of male human muscle tissue) monitored progression of tissue decay/degradation over the time period of fourteen days.

On September 15, 2010 a section of male human muscle tissue (Length 1.5 in, Width 1.0 in, Depth 1.0 in) was exposed to stagnant water conditions with bottom soil and water from the Truckee River and allowed to decay (Figure 10). A sample of the section of tissue was collected at known time points and processed using Puregene and DNA IQ protocols (Table 4). DNA degradation was noticed immediately. The sample was allowed to degrade for fourteen days and was then removed and placed in a freezer to prevent further degradation. Tissue samples were collected and processed using Puregene protocols. The extraction DNA was run on a 1% agarose gel. The faint chromosomal DNA bands (Figure 17) were extracted and the gel was dissolved using Qiagen QIAquick Gel Extraction Kit Protocol. Once the gel was dissolved the DNA was extracted using DNA IQ, amplified using PP16HS and continued using the 3100 capillary electrophoresis. No useable profile was recovered from the degraded sample.

Table 4: Tissue Sample #2 DNA profiles

| **Alleles** | **Sample 2 Known Profile** | **Sample 2 09/15/2010** | **Sample 2 09/20/2010** | **Sample 2 09/23/2010** |
|---|---|---|---|---|
| D3S1358 | 15, 17 | 15, 17 | 14^, 15, 17 | 15, 17 |
| THO1 | 7, 9.3 | 7, 9.3 | 6^, 7, 9.3 | 6^, 7, 9.3, 10^ |
| D21S11 | 31, 32.2 | 31, 32.2 | <, < | <, < |
| D18S51 | 12, 14 | 12, < | <, < | <, < |
| Penta E | 5, 11 | <, < | <, < | <, < |
| D5S818 | 11, 12 | 11, 12 | 11, 12 | 11, 16^ |
| D13S317 | 12, 15 | 12, 15 | 12, < | 12, < |
| D7S820 | 9, 12 | 9, 12 | <, < | <, 12 |
| D16S539 | 13 | 13 | 12^ | < |
| CSF1PO | 11, 12 | 11, < | <, < | <, < |
| Penta D | 11 | 11 | 12^ | < |
| vWA | 16, 20 | 16, 20 | 16, < | 10^, 11^, 12^, 13^, 14^, 15^, 16, 17^, 18^, 19^, 20, 21^, 22 |
| D8S1179 | 12, 15 | 12, 15 | <, < | 15, 18^ |

| TPOX | 8 | 8 | < | < |
|---|---|---|---|---|
| FGA | 21, 23 | 21, 23 | <, < | 23, 27^ |
| Amelogenin | X, Y | X, Y | X, Y | X, < |

*Below Laboratory Threshold (Peak heights below 150 rfu)
^Allelic Drop-in (False Peak)
<No Allele Present

# RESULTS

## PART 3:

## TISSUE SAMPLE #3

## SUBMERGED SOIL CONDITIONS

Tissue Sample #3: SUBMERGED SOIL SAMPLE

Figure 11: Tissue Sample #3 (a section of female human muscle tissue) photo progression monitoring tissue decay/degradation over the time period of thirty days.

On March 08, 2011 a section of female human muscle tissue (Length 2.0 in, Width 1.0 in, Depth 1.0 in, 75.3 grams) was buried in soil collected from the depth of five feet and exposed to typical Reno, Nevada weather conditions (7, 8) and was allowed to decay/degrade for thirty days (Figure 11). On March 08, 2011 a section of tissue was collected and processed using Puregene protocols to establish the known profile for tissue sample #3. A sample of the section of tissue was collected at known time points and processed using Puregene and DNA IQ

protocols (Figure 14) to determine the start of when DNA degradation begins to affect the profile (Table 5).

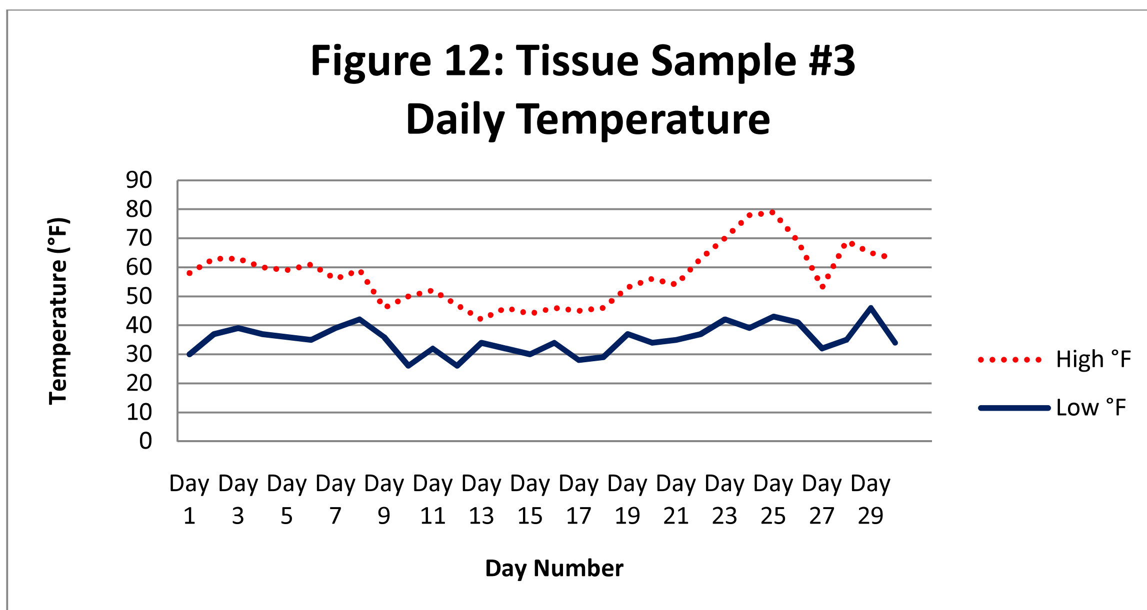


Figure 12: Tissue Sample #3 Daily temperatures (7, 8) range, the high (red dots) and low (blue line) temperatures (°F) over the time period of 30 days.

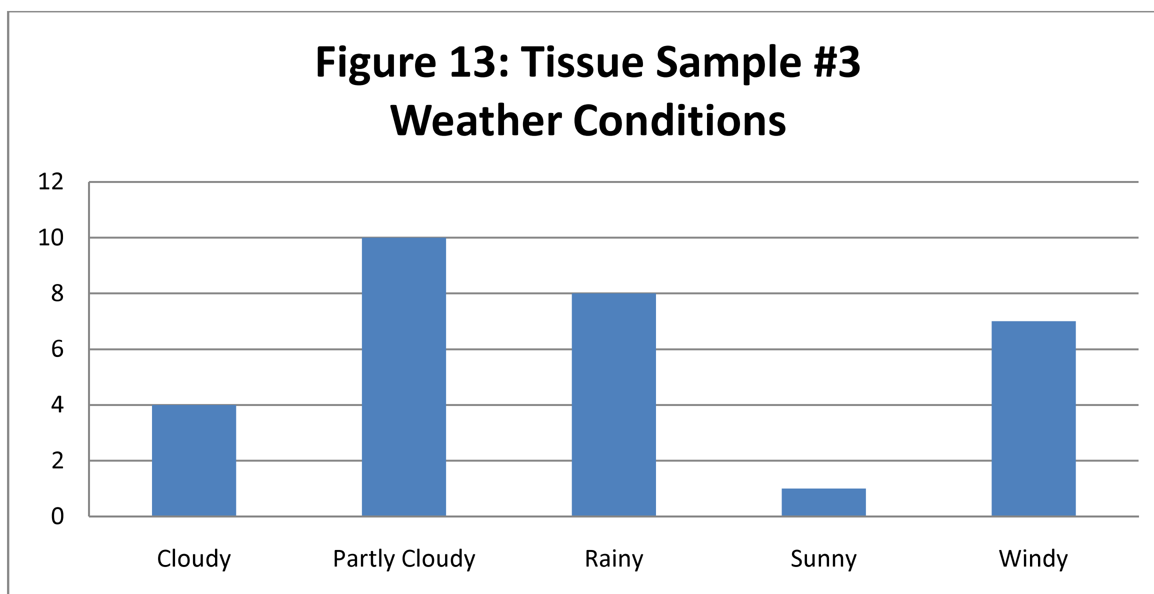


Figure 13: Tissue Sample #3 weather conditions (7, 8) for the 30 day time period that Tissue Sample #3 experienced the different weather condition types.

On April 01, 2010, DNA degradation was noticed affecting eleven of the loci (Figure 15). On April 06, 2011 tissue sample #3 was removed from the sample area and placed in the freezer to prevent further degradation. A section of tissue was collected and processed using Puregene protocols. The extraction DNA was run on a 1% agarose gel. The chromosomal DNA bands were present and cut from the gel for DNA extraction. The gel was dissolved using Qiagen QIAquick Gel Extraction Kit Protocol. Once the gel was dissolved, the DNA was extracted using DNA IQ, amplified using PP16HS and continued using the 3100 capillary electrophoresis. The PCR reactions were run using 3 µl, 5 µl and 8.75 µl of DNA for Tissue Sample #3. The profile that obtained the most allele calls with the optimal peak height used 3 µl of DNA. After this process fifteen of the sixteen loci obtained useable allelic calls (Figure 16). The only locus unusable was FGA the correct allelic profile was present but peak heights were below laboratory threshold.

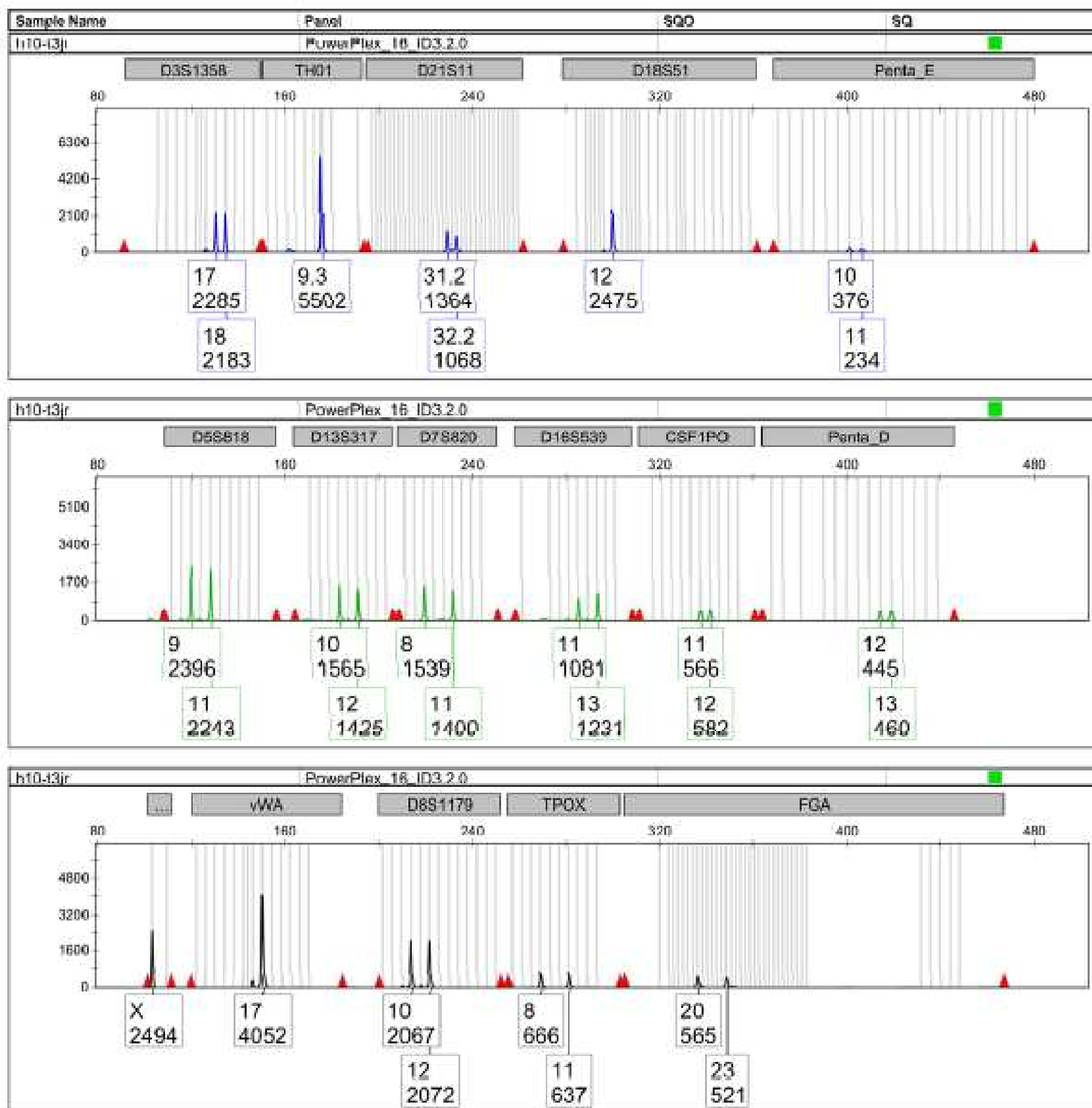


Figure 14: Tissue Sample #3 3100 Profile. Established known profile for Tissue Sample #3 on March 8, 2011 before DNA degradation affect. All sixteen loci are present with sufficient peak heights.

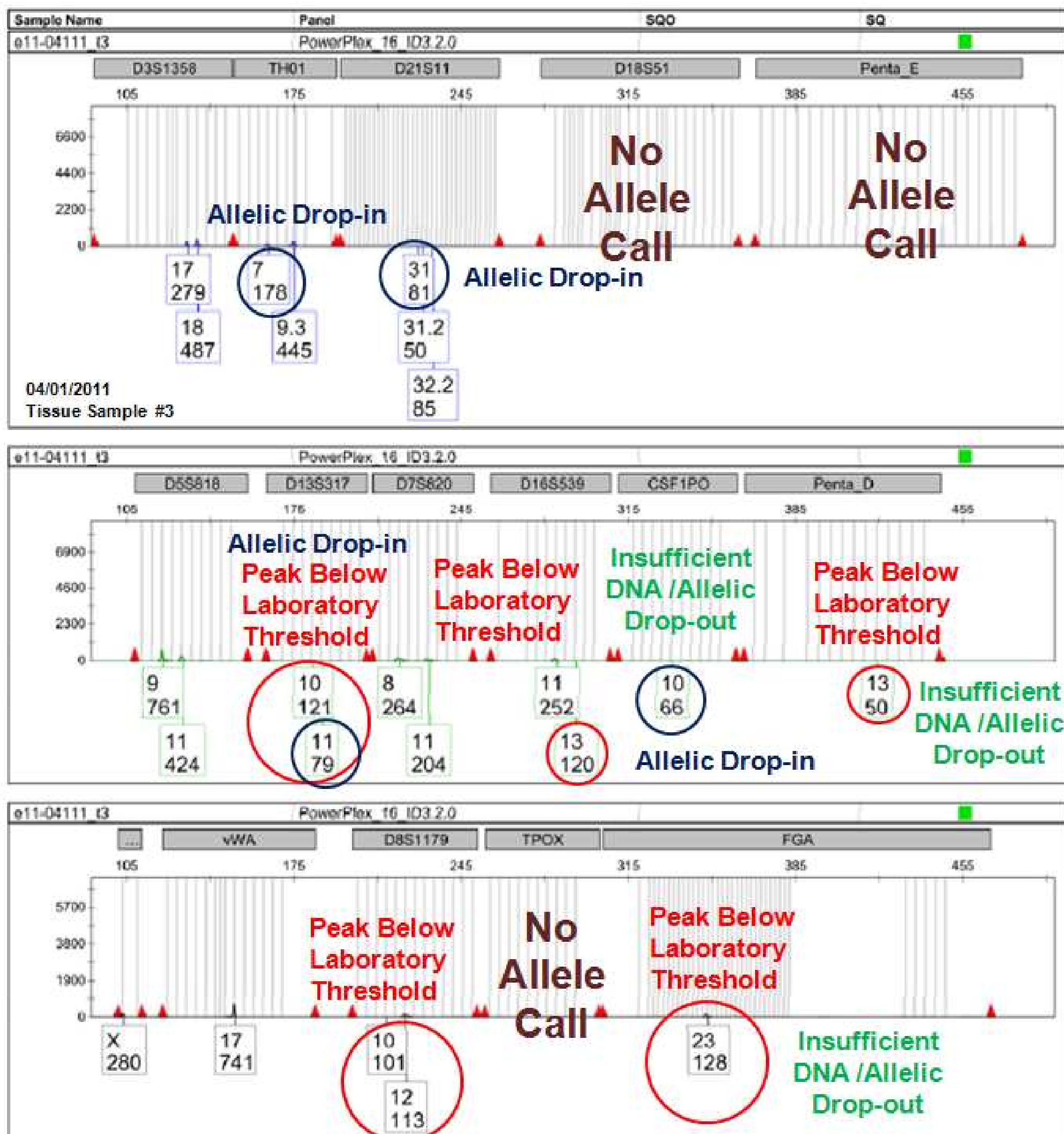


Figure 15: Tissue Sample #3 degraded 3100 profile. April 01, 2011 DNA degraded profile of Tissue Sample #3. Five loci were acceptable. Five loci, (D13S317, D16S539, Penta D, D8S1179 and FGA) produced the correct profile but were below laboratory threshold (red circles) or partial alleles. Three loci (THO1, D21S11, and CSF1PO) produced an allelic drop-in (blue circles) and three loci failed to produce allele calls (D18S51, Penta E, and TPOX).

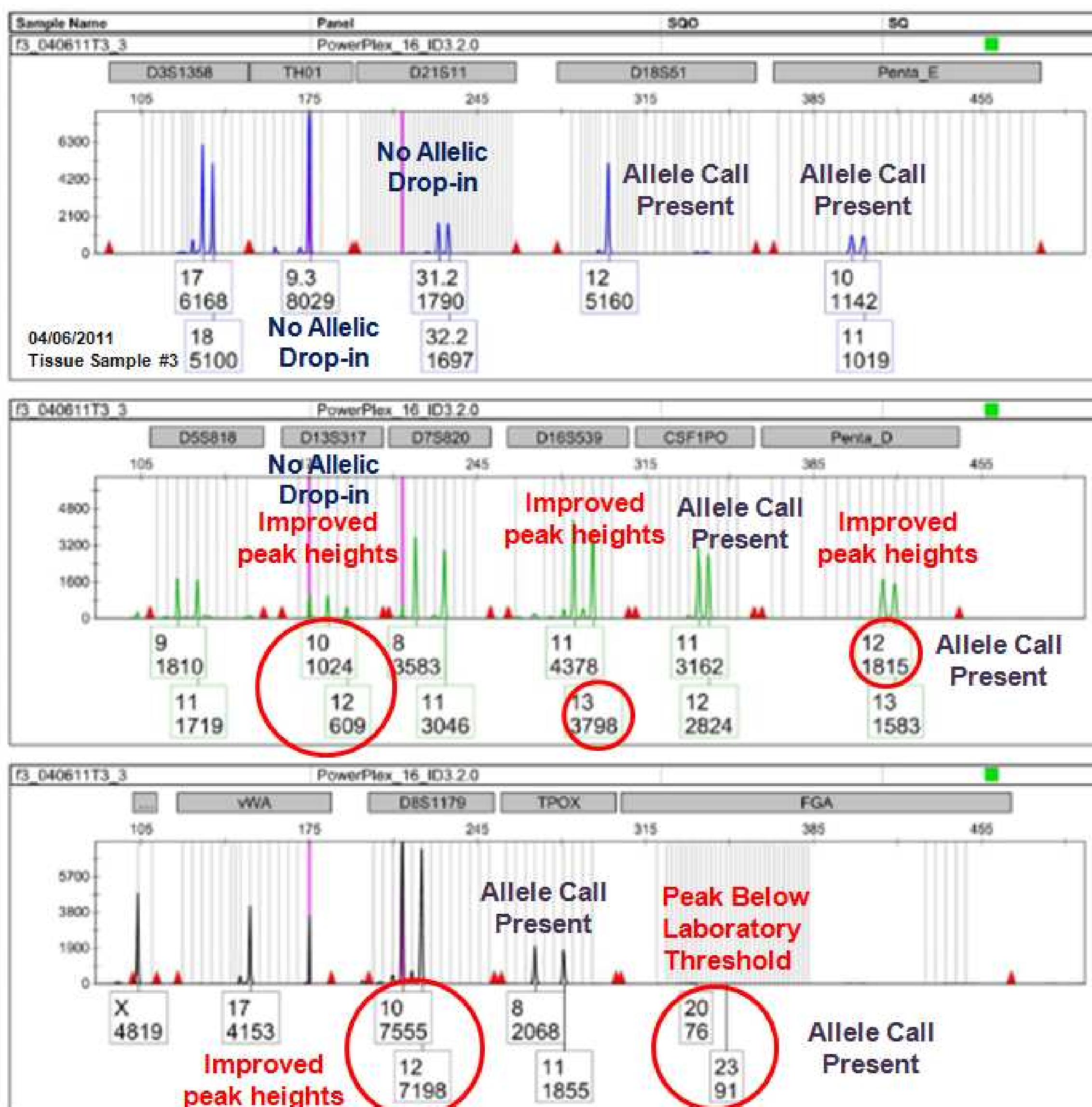


Figure 16: Improved 3100 profile for Tissue Sample #3. The April 6, 2011 DNA profile for Tissue Sample #3 produced a allele calls for 15 loci with acceptable peak heights. Four of the five loci, (D13S317, D16S539, Penta D, D8S1179 and FGA) that produced peaks below laboratory threshold (red circles) or partial alleles have improved and acceptable peak heights. FGA produced the correct allele calls with peaks below laboratory threshold. The three loci (THO1, D21S11, and CSF1PO) that produced peaks due to allelic drop-in (blue circles) were no longer present and three loci failed to produce allele calls (D18S51, Penta E, and TPOX) produced the correct allele calls.

**Table 5: Tissue Sample #3 DNA profiles**

| Alleles | Sample 3 03/08/2011 | Sample 3 04/01/2011 | Sample 3 04/06/2011 |
|---|---|---|---|
| D3S1358 | 17, 18 | 17, 18 | 17, 18 |
| THO1 | 9.3 | 7^, 9.3 | 9.3 |
| D21S11 | 31.2, 32.2 | 31^, 31.2, 32.2 | 31.2, 32.2 |
| D18S51 | 12 | < | 12 |
| Penta E | 10, 11 | <, < | 10, 11 |
| D5S818 | 9, 11 | 9, 11 | 9, 11 |
| D13S317 | 10, 12 | 10*, 11*^ | 10, 12 |
| D7S820 | 8, 11 | 8, 11 | 8, 11 |
| D16S539 | 11, 13 | 11, 13* | 11, 13 |
| CSF1PO | 11, 12 | 10^, <, < | 11, 12 |
| Penta D | 12, 13 | <, 13* | 12, 13 |
| vWA | 17 | 17 | 17 |
| D8S1179 | 10, 12 | 10*, 12* | 10, 12 |
| TPOX | 8, 11 | <, < | 8, 11 |
| FGA | 20, 23 | <, 23* | 20*, 23* |
| Amelogenin | X | X | X |

*Below Laboratory Threshold (Peak heights below 150 rfu)

^Allelic Drop-in (False Peak)

<No Allele Present

# CONCLUSIONS

Tissue Sample #1

Based on the small amount of DNA degradation experienced by tissue sample #1 it can be concluded that tissue left in dry open air climate will retain a function profile for lengthily time period. Once DNA degradation occurs that the longer loci degrade first, which is typical of most degradation patterns, but with the use of gel electrophoresis to separate the intact chromosomal DNA a complete profile can be regained.

Tissue Sample #2:

Based on the serious amount of DNA degradation experienced by tissue sample #2, the speed in which the DNA degradation occurred and that no useable profile was able to be recovered it suggests that the tissue was of poor quality at the start of testing and/or immersion in water causes rapid degradation. This leads to the belief that the amount of intact chromosomal DNA was not of sufficient quantity to regain a useable profile.

Tissue Sample #3

Based on the data obtained from tissue sample #3, including amount of DNA degradation, time frame and recovery, it shows that the use of gel electrophoresis to separate the intact chromosomal DNA allows for a more complete profile to be obtained. The effect of increased DNA quantity to the PCR reaction suggests that inhibitors can remain present in the DNA and reduced the

PCR reaction yield. It may be possible to digest a larger section of tissue and repeat this process to gain more chromosomal DNA and balance out the inhibition.

Overall Conclusions:

The data determines that using gel electrophoresis to separate the intact chromosomal DNA from the degraded fragments allows a useable DNA profile to be obtained for a sample with DNA degradation as long as intact chromosomal DNA is still present. Once the sample has degraded to the point where no useable loci can be obtained for the initial extraction attempt, obtaining a useable profile using gel electrophoresis as a filter is unlikely. Improvement in the DNA profile was noticed in samples which a partial profile was obtained in the initial attempt which is extremely helpful for DNA comparison and human identification.

SUPPLEMENTAL DATA

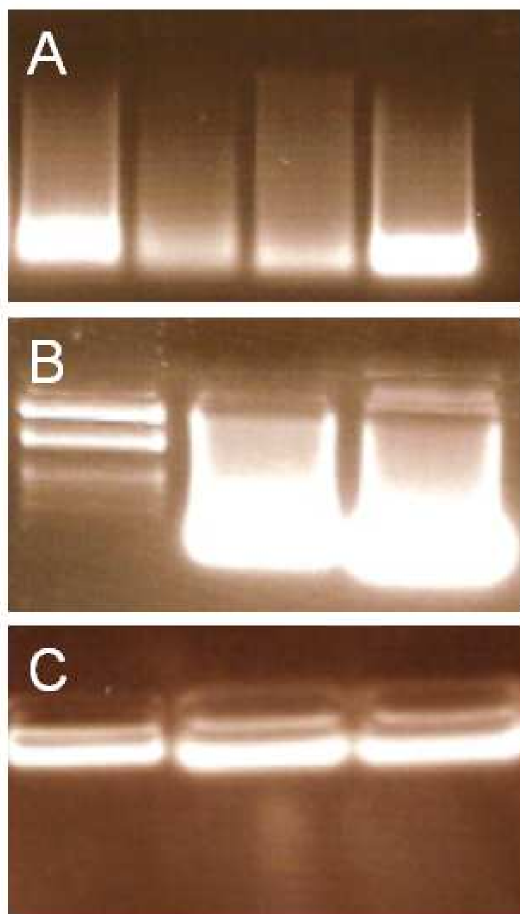


Figure 17: Tissue Sample #2 gel images.

A) DNA extracted from tissue sample using Puregene protocols. No chromosomal DNA present.

B) Wells loaded as followed: Ladder, Positive Control, and Tissue Samples 2. Tissue sample 2 produced a faint band.

C) Wells loaded as followed: Positive Control, Tissue Sample 2 and Tissue Sample 2. Tissue sample 2 produced faint bands.

| Table #6: Weather Conditions Temperature: Tissue Sample #1 | | | | |
|---|---|---|---|---|
| Day # | Date | High °F | Low °F | Condition |
| Day 1 | 18-May-10 | 63 | 44 | Mostly Cloudy |
| Day 2 | 19-May-10 | 70 | 44 | Light Rain/Wind |
| Day 3 | 20-May-10 | 70 | 47 | Partly Cloudy |
| Day 4 | 21-May-10 | 60 | 36 | Partly Cloudy/Wind |
| Day 5 | 22-May-10 | 50 | 31 | Cloudy |
| Day 6 | 23-May-10 | 55 | 31 | Cloudy/Sun |
| Day 7 | 24-May-10 | 60 | 42 | Partly Cloudy |
| Day 8 | 25-May-10 | 65 | 36 | Mostly Cloudy |
| Day 9 | 26-May-10 | 61 | 45 | Light Rain |
| Day 10 | 27-May-10 | 59 | 37 | Mostly Cloudy |
| Day 11 | 28-May-10 | 57 | 36 | Partly Cloudy/Wind |
| Day 12 | 29-May-10 | 69 | 38 | Partly Cloudy |
| Day 13 | 30-May-10 | 78 | 43 | Clouds |

| | | | | |
|---|---|---|---|---|
| Day 14 | 31-May-10 | 75 | 56 | Mostly Cloudy |
| Day 15 | 1-Jun-10 | 73 | 54 | Mostly Cloudy |
| Day 16 | 2-Jun-10 | 77 | 52 | Cloudy |
| Day 17 | 3-Jun-10 | 76 | 60 | Mostly Cloudy |
| Day 18 | 4-Jun-10 | 75 | 60 | Windy |
| Day 19 | 5-Jun-10 | 86 | 52 | Partly Cloudy |
| Day 20 | 6-Jun-10 | 88 | 60 | Partly Cloudy |
| Day 21 | 7-Jun-10 | 84 | 57 | Partly Cloudy |
| Day 22 | 8-Jun-10 | 85 | 54 | Cloudy/Windy |
| Day 23 | 9-Jun-10 | 76 | 55 | Partly Cloudy/Wind |
| Day 24 | 10-Jun-10 | 68 | 47 | Partly Cloudy |
| Day 25 | 11-Jun-10 | 69 | 47 | Partly Cloudy |
| Day 26 | 12-Jun-10 | 75 | 52 | Partly Cloudy |
| Day 27 | 13-Jun-10 | 83 | 49 | Partly Cloudy |
| Day 28 | 14-Jun-10 | 88 | 54 | Windy/Partly Cloudy |
| Day 29 | 15-Jun-10 | 83 | 59 | Windy/Partly Cloudy |
| Day 30 | 16-Jun-10 | 71 | 46 | Partly Cloudy |
| Day 31 | 17-Jun-10 | 79 | 46 | Partly Cloudy |
| Day 32 | 18-Jun-10 | 80 | 50 | Partly Cloudy |
| Day 33 | 19-Jun-10 | 77 | 48 | Partly Cloudy |
| Day 34 | 20-Jun-10 | 78 | 45 | Partly Cloudy |
| Day 35 | 21-Jun-10 | 79 | 50 | Partly Cloudy |
| Day 36 | 22-Jun-10 | 84 | 51 | Partly Cloudy |
| Day 37 | 23-Jun-10 | 89 | 60 | Partly Cloudy |
| Day 38 | 24-Jun-10 | 88 | 59 | Mostly Cloudy |
| Day 39 | 25-Jun-10 | 80 | 65 | Mostly Cloudy |
| Day 40 | 26-Jun-10 | 89 | 58 | Partly Cloudy |
| Day 41 | 27-Jun-10 | 95 | 60 | Partly Cloudy |
| Day 42 | 28-Jun-10 | 100 | 64 | Windy/Partly Cloudy |
| Day 43 | 29-Jun-10 | 94 | 61 | Windy/Partly Cloudy |
| Day 44 | 30-Jun-10 | 88 | 56 | Windy/Partly Cloudy |
| Day 45 | 1-Jul-10 | 87 | 52 | Windy/Partly Cloudy |
| Day 46 | 2-Jul-10 | 84 | 52 | Windy/Partly Cloudy |
| Day 47 | 3-Jul-10 | 81 | 52 | Partly Cloudy |
| Day 48 | 4-Jul-10 | 85 | 56 | Partly Cloudy |
| Day 49 | 5-Jul-10 | 94 | 57 | Partly Cloudy |
| Day 50 | 6-Jul-10 | 91 | 60 | Partly Cloudy |
| Day 51 | 7-Jul-10 | 91 | 61 | Mostly Cloudy |
| Day 52 | 8-Jul-10 | 95 | 66 | Partly Cloudy |
| Day 53 | 9-Jul-10 | 90 | 63 | Partly Cloudy |

| | | | | |
|---|---|---|---|---|
| Day 54 | 10-Jul-10 | 95 | 61 | Partly Cloudy |
| Day 55 | 11-Jul-10 | 93 | 60 | Partly Cloudy |
| Day 56 | 12-Jul-10 | 95 | 63 | Windy |
| Day 57 | 13-Jul-10 | 93 | 63 | Partly Cloudy |
| Day 58 | 14-Jul-10 | 95 | 63 | Sunny |
| Day 59 | 15-Jul-10 | 100 | 67 | Partly Cloudy |
| Day 60 | 16-Jul-10 | 99 | 69 | Rainy |
| Day 61 | 17-Jul-10 | 100 | 64 | Partly Cloudy |
| Day 62 | 18-Jul-10 | 99 | 64 | Sunny |
| Day 63 | 19-Jul-10 | 97 | 58 | Sunny |
| Day 64 | 20-Jul-10 | 98 | 63 | Partly Cloudy |
| Day 65 | 21-Jul-10 | 94 | 57 | Sunny |
| Day 66 | 22-Jul-10 | 96 | 62 | Sunny |
| Day 67 | 23-Jul-10 | 99 | 66 | Partly Cloudy |
| Day 68 | 24-Jul-10 | 102 | 68 | Rainy |
| Day 69 | 25-Jul-10 | 95 | 66 | Rainy |
| Day 70 | 26-Jul-10 | 92 | 64 | Rainy |
| Day 71 | 27-Jul-10 | 94 | 62 | Partly Cloudy |
| Day 72 | 28-Jul-10 | 95 | 66 | Partly Cloudy |
| Day 73 | 29-Jul-10 | 97 | 62 | Partly Cloudy |
| Day 74 | 30-Jul-10 | 95 | 53 | Partly Cloudy |
| Day 75 | 31-Jul-10 | 92 | 56 | Sunny |
| Day 76 | 1-Aug-10 | 90 | 56 | Partly Cloudy |
| Day 77 | 2-Aug-10 | 91 | 59 | Windy |
| Day 78 | 3-Aug-10 | 92 | 56 | Partly Cloudy |
| Day 79 | 4-Aug-10 | 93 | 57 | Partly Cloudy |
| Day 80 | 5-Aug-10 | 93 | 59 | Partly Cloudy |
| Day 81 | 6-Aug-10 | 93 | 61 | Partly Cloudy |
| Day 82 | 7-Aug-10 | 88 | 60 | Windy |
| Day 83 | 8-Aug-10 | 87 | 61 | Rainy |
| Day 84 | 9-Aug-10 | 87 | 57 | Partly Cloudy |
| Day 85 | 10-Aug-10 | 87 | 60 | Windy |
| Day 86 | 11-Aug-10 | 82 | 63 | Windy |
| Day 87 | 12-Aug-10 | 83 | 55 | Partly Cloudy |
| Day 88 | 13-Aug-10 | 92 | 57 | Partly Cloudy |
| Day 89 | 14-Aug-10 | 95 | 62 | Partly Cloudy |
| Day 90 | 15-Aug-10 | 92 | 64 | Rainy |
| Day 91 | 16-Aug-10 | 95 | 58 | Sunny |
| Day 92 | 17-Aug-10 | 96 | 65 | Windy |
| Day 93 | 18-Aug-10 | 92 | 57 | Windy |

| | | | | |
|---|---|---|---|---|
| Day 94 | 19-Aug-10 | 90 | 54 | Partly Cloudy |
| Day 95 | 20-Aug-10 | 92 | 59 | Windy |
| Day 96 | 21-Aug-10 | 88 | 58 | Windy |
| Day 97 | 22-Aug-10 | 82 | 50 | Partly Cloudy |
| Day 98 | 23-Aug-10 | 86 | 52 | Partly Cloudy |
| Day 99 | 24-Aug-10 | 94 | 53 | Sunny |
| Day 100 | 25-Aug-10 | 98 | 58 | Partly Cloudy |
| Day 101 | 26-Aug-10 | 96 | 67 | Windy |
| Day 102 | 27-Aug-10 | 91 | 57 | Windy |
| Day 103 | 28-Aug-10 | 69 | 53 | Windy |
| Day 104 | 29-Aug-10 | 63 | 50 | Cloudy |
| Day 105 | 30-Aug-10 | 72 | 47 | Partly Cloudy |
| Day 106 | 31-Aug-10 | 77 | 47 | Partly Cloudy |
| Day 107 | 1-Sep-10 | 85 | 49 | Partly Cloudy |
| Day 108 | 2-Sep-10 | 92 | 56 | Partly Cloudy |
| Day 109 | 3-Sep-10 | 96 | 56 | Windy |
| Day 110 | 4-Sep-10 | 90 | 53 | Sunny |
| Day 111 | 5-Sep-10 | 84 | 50 | Windy |
| Day 112 | 6-Sep-10 | 82 | 47 | Partly Cloudy |
| Day 113 | 7-Sep-10 | 87 | 49 | Partly Cloudy |
| Day 114 | 8-Sep-10 | 71 | 50 | Windy |
| Day 115 | 9-Sep-10 | 70 | 44 | Partly Cloudy |
| Day 116 | 10-Sep-10 | 76 | 44 | Partly Cloudy |
| Day 117 | 11-Sep-10 | 84 | 46 | Partly Cloudy |
| Day 118 | 12-Sep-10 | 85 | 49 | Partly Cloudy |
| Day 119 | 13-Sep-10 | 81 | 52 | Partly Cloudy |
| Day 120 | 14-Sep-10 | 83 | 48 | Partly Cloudy |
| Day 121 | 15-Sep-10 | 85 | 49 | Partly Cloudy |
| Day 122 | 16-Sep-10 | 86 | 47 | Partly Cloudy |
| Day 123 | 17-Sep-10 | 85 | 50 | Windy |
| Day 124 | 18-Sep-10 | 87 | 49 | Windy |
| Day 125 | 19-Sep-10 | 78 | 49 | Windy |
| Day 126 | 20-Sep-10 | 78 | 46 | Partly Cloudy |
| Day 127 | 21-Sep-10 | 79 | 45 | Windy |
| Day 128 | 22-Sep-10 | 75 | 52 | Windy |
| Day 129 | 23-Sep-10 | 81 | 41 | Partly Cloudy |
| Day 130 | 24-Sep-10 | 86 | 45 | Partly Cloudy |
| Day 131 | 25-Sep-10 | 90 | 45 | Partly Cloudy |
| Day 132 | 26-Sep-10 | 91 | 49 | Partly Cloudy |
| Day 133 | 27-Sep-10 | 93 | 49 | Partly Cloudy |

| Day 134 | 28-Sep-10 | 95 | 50 | Partly Cloudy |
|---|---|---|---|---|
| Day 135 | 29-Sep-10 | 93 | 54 | Partly Cloudy |
| Day 136 | 30-Sep-10 | 93 | 53 | Partly Cloudy |
| Day 137 | 1-Oct-10 | 92 | 54 | Sunny |
| Day 138 | 2-Oct-10 | 87 | 58 | Mostly Cloudy |
| Day 139 | 3-Oct-10 | 63 | 57 | Windy |
| Day 140 | 4-Oct-10 | 61 | 48 | Mostly Cloudy |
| Day 141 | 5-Oct-10 | 59 | 46 | Windy |
| Day 142 | 6-Oct-10 | 65 | 44 | Sunny |
| Day 143 | 7-Oct-10 | 59 | 48 | Cloudy |
| Day 144 | 8-Oct-10 | 66 | 38 | Cloudy |
| Day 145 | 9-Oct-10 | 74 | 43 | Partly Cloudy |
| Day 146 | 10-Oct-10 | 77 | 47 | Mostly Sunny |
| Day 147 | 11-Oct-10 | 72 | 49 | Partly Cloudy |
| Day 148 | 12-Oct-10 | 72 | 42 | Partly Cloudy |
| Day 149 | 13-Oct-10 | 76 | 42 | Partly Cloudy |
| Day 150 | 14-Oct-10 | 79 | 45 | Partly Cloudy |
| Day 151 | 15-Oct-10 | 78 | 46 | Partly Cloudy |
| Day 152 | 16-Oct-10 | 74 | 47 | Cloudy |
| Day 153 | 17-Oct-10 | 67 | 50 | Rainy |
| Day 154 | 18-Oct-10 | 67 | 46 | Windy |
| Day 155 | 19-Oct-10 | 70 | 43 | Partly Cloudy |
| Day 156 | 20-Oct-10 | 72 | 41 | Partly Cloudy |
| Day 157 | 21-Oct-10 | 69 | 45 | Windy |
| Day 158 | 22-Oct-10 | 64 | 43 | Windy |
| Day 159 | 23-Oct-10 | 58 | 47 | Cloudy |
| Day 160 | 24-Oct-10 | 64 | 50 | Cloudy |
| Day 161 | 25-Oct-10 | 53 | 40 | Windy |
| Day 162 | 26-Oct-10 | 52 | 31 | Windy |
| Day 163 | 27-Oct-10 | 54 | 29 | Windy |
| Day 164 | 28-Oct-10 | 66 | 31 | Partly Cloudy |
| Day 165 | 29-Oct-10 | 62 | 36 | Partly Cloudy |
| Day 166 | 30-Oct-10 | 57 | 44 | Rainy |
| Day 167 | 31-Oct-10 | 62 | 39 | Sunny |
| Day 168 | 1-Nov-10 | 68 | 35 | Partly Cloudy |
| Day 169 | 2-Nov-10 | 70 | 37 | Sunny |
| Day 170 | 3-Nov-10 | 72 | 39 | Partly Cloudy |
| Day 171 | 4-Nov-10 | 72 | 40 | Partly Cloudy |
| Day 172 | 5-Nov-10 | 76 | 44 | Cloudy |
| Day 173 | 6-Nov-10 | 72 | 45 | Cloudy |

| | | | | |
|---|---|---|---|---|
| Day 174 | 7-Nov-10 | 57 | 36 | Rainy |
| Day 175 | 8-Nov-10 | 46 | 31 | Rainy |
| Day 176 | 9-Nov-10 | 51 | 25 | Partly Cloudy |
| Day 177 | 10-Nov-10 | 48 | 25 | Snow |
| Day 178 | 11-Nov-10 | 51 | 26 | Partly Cloudy |
| Day 179 | 12-Nov-10 | 58 | 27 | Partly Cloudy |
| Day 180 | 13-Nov-10 | 49 | 28 | Partly Cloudy |
| Day 181 | 14-Nov-10 | 52 | 30 | Cloudy |
| Day 182 | 15-Nov-10 | 61 | 34 | Partly Cloudy |
| Day 183 | 16-Nov-10 | 64 | 30 | Partly Cloudy |
| Day 184 | 17-Nov-10 | 63 | 28 | Cloudy |
| Day 185 | 18-Nov-10 | 66 | 45 | Cloudy |
| Day 186 | 19-Nov-10 | 58 | 37 | Snow |
| Day 187 | 20-Nov-10 | 43 | 30 | Snow |
| Day 188 | 21-Nov-10 | 40 | 26 | Snow |
| Day 189 | 22-Nov-10 | 47 | 33 | Partly Cloudy |
| Day 190 | 23-Nov-10 | 43 | 18 | Snow |
| Day 191 | 24-Nov-10 | 27 | 10 | Snow |
| Day 192 | 25-Nov-10 | 32 | 10 | Partly Cloudy |
| Day 193 | 26-Nov-10 | 36 | 16 | Partly Cloudy |
| Day 194 | 27-Nov-10 | 47 | 22 | Snow |
| Day 195 | 28-Nov-10 | 36 | 21 | Partly Cloudy |
| Day 196 | 29-Nov-10 | 38 | 15 | Partly Cloudy |
| Day 197 | 30-Nov-10 | 38 | 23 | Cloudy |
| Day 198 | 1-Dec-10 | 55 | 24 | Partly Cloudy |
| Day 199 | 2-Dec-10 | 59 | 27 | Rainy |
| Day 200 | 3-Dec-10 | 57 | 41 | Cloudy |
| Day 201 | 4-Dec-10 | 48 | 36 | Rainy |
| Day 202 | 5-Dec-10 | 52 | 37 | Rainy |
| Day 203 | 6-Dec-10 | 54 | 34 | Rainy |
| Day 204 | 7-Dec-10 | 57 | 28 | Partly Cloudy |
| Day 205 | 8-Dec-10 | 55 | 31 | Cloudy |
| Day 206 | 9-Dec-10 | 57 | 41 | Cloudy |
| Day 207 | 10-Dec-10 | 58 | 43 | Rainy |
| Day 208 | 11-Dec-10 | 62 | 38 | Cloudy |
| Day 209 | 12-Dec-10 | 60 | 28 | Sunny |
| Day 210 | 13-Dec-10 | 62 | 29 | Cloudy |
| Day 211 | 14-Dec-10 | 56 | 36 | Snow |
| Day 212 | 15-Dec-10 | 42 | 28 | Partly Cloudy |
| Day 213 | 16-Dec-10 | 40 | 29 | Cloudy |

| | | | | |
|---|---|---|---|---|
| Day 214 | 17-Dec-10 | 32 | 29 | Snow |
| Day 215 | 18-Dec-10 | 50 | 32 | Rainy |
| Day 216 | 19-Dec-10 | 47 | 30 | Snow |
| Day 217 | 20-Dec-10 | 40 | 27 | Cloudy |
| Day 218 | 21-Dec-10 | 43 | 23 | Cloudy |
| Day 219 | 22-Dec-10 | 43 | 27 | Snow |
| Day 220 | 23-Dec-10 | 47 | 24 | Cloudy |
| Day 221 | 24-Dec-10 | 48 | 29 | Cloudy |
| Day 222 | 25-Dec-10 | 51 | 25 | Cloudy |
| Day 223 | 26-Dec-10 | 47 | 33 | Snow |
| Day 224 | 27-Dec-10 | 47 | 27 | Partly Cloudy |
| Day 225 | 28-Dec-10 | 49 | 34 | Snow |
| Day 226 | 29-Dec-10 | 43 | 19 | Snow |
| Day 227 | 30-Dec-10 | 29 | 15 | Cloudy |
| Day 228 | 31-Dec-10 | 28 | 12 | Cloudy |
| Day 229 | 1-Jan-11 | 32 | 21 | Cloudy |
| Day 230 | 2-Jan-11 | 33 | 22 | Cloudy |
| Day 231 | 3-Jan-11 | 39 | 24 | Cloudy |
| Day 232 | 4-Jan-11 | 37 | 17 | Partly Cloudy |
| Day 233 | 5-Jan-11 | 36 | 17 | Rainy |
| Day 234 | 6-Jan-11 | 34 | 21 | Snow |
| Day 235 | 7-Jan-11 | 37 | 21 | Snow |
| Day 236 | 8-Jan-11 | 47 | 20 | Partly Cloudy |
| Day 237 | 9-Jan-11 | 35 | 19 | Cloudy |
| Day 238 | 10-Jan-11 | 36 | 16 | Sunny |
| Day 239 | 11-Jan-11 | 36 | 26 | Cloudy |
| Day 240 | 12-Jan-11 | 51 | 25 | Cloudy |
| Day 241 | 13-Jan-11 | 56 | 32 | Rainy |
| Day 242 | 14-Jan-11 | 55 | 27 | Partly Cloudy |
| Day 243 | 15-Jan-11 | 58 | 28 | Cloudy |
| Day 244 | 16-Jan-11 | 67 | 41 | Windy |
| Day 245 | 17-Jan-11 | 65 | 36 | Partly Cloudy |
| Day 246 | 18-Jan-11 | 57 | 30 | Cloudy |
| Day 247 | 19-Jan-11 | 50 | 31 | Partly Cloudy |
| Day 248 | 20-Jan-11 | 50 | 25 | Partly Cloudy |
| Day 249 | 21-Jan-11 | 55 | 25 | Partly Cloudy |
| Day 250 | 22-Jan-11 | 56 | 32 | Partly Cloudy |
| Day 251 | 23-Jan-11 | 50 | 25 | Partly Cloudy |
| Day 252 | 24-Jan-11 | 54 | 26 | Partly Cloudy |
| Day 253 | 25-Jan-11 | 56 | 26 | Partly Cloudy |

| | | | | |
|---|---|---|---|---|
| Day 254 | 26-Jan-11 | 53 | 28 | Cloudy |
| Day 255 | 27-Jan-11 | 54 | 27 | Partly Cloudy |
| Day 256 | 28-Jan-11 | 64 | 25 | Partly Cloudy |
| Day 257 | 29-Jan-11 | 61 | 30 | Windy |
| Day 258 | 30-Jan-11 | 45 | 30 | Rainy |
| Day 259 | 31-Jan-11 | 49 | 25 | Partly Cloudy |
| Day 260 | 1-Feb-11 | 40 | 25 | Partly Cloudy |
| Day 261 | 2-Feb-11 | 44 | 15 | Partly Cloudy |
| Day 262 | 3-Feb-11 | 51 | 20 | Partly Cloudy |
| Day 263 | 4-Feb-11 | 57 | 24 | Partly Cloudy |
| Day 264 | 5-Feb-11 | 59 | 29 | Cloudy |
| Day 265 | 6-Feb-11 | 59 | 32 | Partly Cloudy |
| Day 266 | 7-Feb-11 | 66 | 30 | Windy |
| Day 267 | 8-Feb-11 | 38 | 22 | Cloudy |
| Day 268 | 9-Feb-11 | 44 | 24 | Partly Cloudy |
| Day 269 | 10-Feb-11 | 50 | 19 | Partly Cloudy |
| Day 270 | 11-Feb-11 | 57 | 22 | Partly Cloudy |
| Day 271 | 12-Feb-11 | 66 | 27 | Windy |
| Day 272 | 13-Feb-11 | 63 | 25 | Partly Cloudy |
| Day 273 | 14-Feb-11 | 56 | 32 | Windy |
| Day 274 | 15-Feb-11 | 59 | 41 | Windy |
| Day 275 | 16-Feb-11 | 42 | 27 | Cloudy |
| Day 276 | 17-Feb-11 | 37 | 26 | Cloudy |
| Day 277 | 18-Feb-11 | 34 | 28 | Snow |
| Day 278 | 19-Feb-11 | 36 | 17 | Partly Cloudy |
| Day 279 | 20-Feb-11 | 35 | 11 | Partly Cloudy |
| Day 280 | 21-Feb-11 | 39 | 15 | Rainy |
| Day 281 | 22-Feb-11 | 42 | 16 | Partly Cloudy |
| Day 282 | 23-Feb-11 | 43 | 21 | Partly Cloudy |
| Day 283 | 24-Feb-11 | 44 | 29 | Windy |
| Day 284 | 25-Feb-11 | 36 | 21 | Cloudy |
| Day 285 | 26-Feb-11 | 28 | 9 | Snow |
| Day 286 | 27-Feb-11 | 37 | 11 | Partly Cloudy |
| Day 287 | 28-Feb-11 | 50 | 22 | Partly Cloudy |
| Day 288 | 1-Mar-11 | 50 | 20 | Partly Cloudy |
| Day 289 | 2-Mar-11 | 56 | 38 | Rainy |
| Day 290 | 3-Mar-11 | 50 | 36 | Partly Cloudy |
| Day 291 | 4-Mar-11 | 54 | 27 | Cloudy |
| Day 292 | 5-Mar-11 | 61 | 36 | Cloudy |
| Day 293 | 6-Mar-11 | 56 | 42 | Rainy |

| | | | | |
|---|---|---|---|---|
| Day 294 | 7-Mar-11 | 46 | 32 | Rainy |
| Day 295 | 8-Mar-11 | 58 | 30 | Cloudy |
| Day 296 | 9-Mar-11 | 63 | 37 | Cloudy |
| Day 297 | 10-Mar-11 | 63 | 39 | Rainy |
| Day 298 | 11-Mar-11 | 60 | 37 | Partly Cloudy |
| Day 299 | 12-Mar-11 | 59 | 36 | Cloudy |
| Day 300 | 13-Mar-11 | 61 | 35 | Windy |
| Day 301 | 14-Mar-11 | 56 | 39 | Rainy |
| Day 302 | 15-Mar-11 | 59 | 42 | Rainy |
| Day 303 | 16-Mar-11 | 46 | 36 | Rainy |
| Day 304 | 17-Mar-11 | 50 | 26 | Partly Cloudy |

**Table 7: Tissue #1 DNA profiles**

| **Alleles** | **Sample 1 05/18/2010** | **Sample 1 05/20/2010** | **Sample 1 05/22/2010** | **Sample 1 05/24/2010** | **Sample 1 05/26/2010** |
|---|---|---|---|---|---|
| D3S1358 | 15 | 15 | 15 | 15 | 15 |
| THO1 | 7, 8 | 7, 8 | 7, 8 | 7, 8 | 7, 8 |
| D21S11 | 29 | 29 | 29 | 29 | 29 |
| D18S51 | 15, 17 | 15, 17 | 15, 17 | 15, 17 | 15, 17 |
| Penta E | 9, 14 | 9, 14 | 9, 14 | 9, 14 | 9, 14 |
| D5S818 | 12, 13 | 12, 13 | 12, 13 | 12, 13 | 12, 13 |
| D13S317 | 12, 13 | 12, 13 | 12, 13 | 12, 13 | 12, 13 |
| D7S820 | 8, 12 | 8, 12 | 8, 12 | 8, 12 | 8, 12 |
| D16S539 | 12, 13 | 12, 13 | 12, 13 | 12, 13 | 12, 13 |
| CSF1PO | 10, 11 | 10, 11 | 10, 11 | 10, 11 | 10, 11 |
| Penta D | 9, 10 | 9, 10 | 9, 10 | 9, 10 | 9, 10 |
| vWA | 11, 17 | 11, 17 | 11, 17 | 11, 17 | 11, 17 |
| D8S1179 | 12, 15 | 12, 15 | 12, 15 | 12, 15 | 12, 15 |
| TPOX | 8, 9 | 8, 9 | 8, 9 | 8, 9 | 8, 9 |
| FGA | 22, 24 | 22, 24 | 22, 24 | 22, 24 | 22, 24 |
| Amelogenin | X | X | X | X | X |
| **Alleles** | **Sample 1 05/28/2010** | **Sample 1 05/30/2010** | **Sample 1 06/01/2010** | **Sample 1 06/03/2010** | **Sample 1 06/05/2010** |
| D3S1358 | 15 | 15 | 15 | 15 | 15 |
| THO1 | 7, 8 | 7, 8 | 7, 8 | 7, 8 | 7, 8 |
| D21S11 | 29 | 29 | 29 | 29 | 29 |
| D18S51 | 15, 17 | 15, 17 | 15, 17 | 15, 17 | 15, 17 |
| Penta E | 9, 14 | 9, 14 | 9, 14 | 9, 14 | 9, 14 |
| D5S818 | 12, 13 | 12, 13 | 12, 13 | 12, 13 | 12, 13 |

| D13S317 | 12, 13 | 12, 13 | 12, 13 | 12, 13 | 12, 13 |
|---|---|---|---|---|---|
| D7S820 | 8, 12 | 8, 12 | 8, 12 | 8, 12 | 8, 12 |
| D16S539 | 12, 13 | 12, 13 | 12, 13 | 12, 13 | 12, 13 |
| CSF1PO | 10, 11 | 10, 11 | 10, 11 | 10, 11 | 10, 11 |
| Penta D | 9, 10 | 9, 10 | 9, 10 | 9, 10 | 9, 10 |
| vWA | 11, 17 | 11, 17 | 11, 17 | 11, 17 | 11, 17 |
| D8S1179 | 12, 15 | 12, 15 | 12, 15 | 12, 15 | 12, 15 |
| TPOX | 8, 9 | 8, 9 | 8, 9 | 8, 9 | 8, 9 |
| FGA | 22, 24 | 22, 24 | 22, 24 | 22, 24 | 22, 24 |
| Amelogenin | X | X | X | X | X |
| **Alleles** | **Sample 1 06/07/2010** | **Sample 1 06/09/2010** | **Sample 1 06/11/2010** | **Sample 1 06/13/2010** | **Sample 1 06/16/2010** |
| D3S1358 | 15 | 15 | 15 | 15 | 15 |
| THO1 | 7, 8 | 7, 8 | 7, 8 | 7, 8 | 7, 8 |
| D21S11 | 29 | 29 | 29 | 29 | 29 |
| D18S51 | 15, 17 | 15, 17 | 15, 17 | 15, 17 | 15, 17 |
| Penta E | 9, 14 | 9, 14 | 9, 14 | 9, 14 | 9, 14 |
| D5S818 | 12, 13 | 12, 13 | 12, 13 | 12, 13 | 12, 13 |
| D13S317 | 12, 13 | 12, 13 | 12, 13 | 12, 13 | 12, 13 |
| D7S820 | 8, 12 | 8, 12 | 8, 12 | 8, 12 | 8, 12 |
| D16S539 | 12, 13 | 12, 13 | 12, 13 | 12, 13 | 12, 13 |
| CSF1PO | 10, 11 | 10, 11 | 10, 11 | 10, 11 | 10, 11 |
| Penta D | 9, 10 | 9, 10 | 9, 10 | 9, 10 | 9, 10 |
| vWA | 11, 17 | 11, 17 | 11, 17 | 11, 17 | 11, 17 |
| D8S1179 | 12, 15 | 12, 15 | 12, 15 | 12, 15 | 12, 15 |
| TPOX | 8, 9 | 8, 9 | 8, 9 | 8, 9 | 8, 9 |
| FGA | 22, 24 | 22, 24 | 22, 24 | 22, 24 | 22, 24 |
| Amelogenin | X | X | X | X | X |
| **Alleles** | **Sample 1 06/21/2010** | **Sample 1 07/06/2010** | **Sample 1 07/28/2010** | **Sample 1 09/02/2010** | **Sample 1 02/07/2011** |
| D3S1358 | 15 | 15 | 15 | 15 | 15 |
| THO1 | 7, 8 | 7, 8 | 7, 8 | 7, 8 | 7, 8 |
| D21S11 | 29 | 29 | 29 | 29 | 29 |
| D18S51 | 15, 17 | 15, 17 | 15, 17 | 15, 17 | 15, 17 |
| Penta E | Identifiler | 9, 14 | 9, 14 | 9, 14 | 9, 14 |
| D5S818 | 12, 13 | 12, 13 | 12, 13 | 12, 13 | 12, 13 |
| D13S317 | 12, 13 | 12, 13 | 12, 13 | 12, 13 | 12, 13 |
| D7S820 | 8, 12 | 8, 12 | 8, 12 | 8, 12 | 8, 12 |
| D16S539 | 12, 13 | 12, 13 | 12, 13 | 12, 13 | 12, 13 |
| CSF1PO | 10, 11 | 10, 11 | 10, 11 | 10, 11 | 10, 11 |
| Penta D | Identifiler | 9, 10 | 9, 10 | 9, 10 | <, < |

| | | | | | |
|---|---|---|---|---|---|
| vWA | 11, 17 | 11, 17 | 11, 17 | 11, 17 | 11, 17 |
| D8S1179 | 12, 15 | 12, 15 | 12, 15 | 12, 15 | 12, 15 |
| TPOX | 8, 9 | 8, 9 | 8, 9 | 8, 9 | 8, 9 |
| FGA | 22, 24 | 22, 24 | 22, 24 | 22, 24 | 22, 24 |
| Amelogenin | X | X | X | X | X |

| **Alleles** | **Sample 1 03/09/2011** | **Sample 1 03/23/2011** |
|---|---|---|
| D3S1358 | 15 | 15 |
| THO1 | 7, 8 | 7, 8 |
| D21S11 | 29 | 29 |
| D18S51 | 15,* 17* | 15, 17 |
| Penta E | <, < | 9, 14 |
| D5S818 | 12, 13 | 12, 13 |
| D13S317 | 12, 13 | 12, 13 |
| D7S820 | 8, 12 | 8, 12 |
| D16S539 | 12, 13 | 12, 13 |
| CSF1PO | 10*, 11* | 10, 11 |
| Penta D | <, < | 9, 10 |
| vWA | 11, 17 | 11, 17 |
| D8S1179 | 12, 15 | 12, 15 |
| TPOX | 8*, 9* | 8, 9 |
| FGA | 22, 24 | 22, 24 |
| Amelogenin | X, Y^ | X |

Table 8: Tissue Sample #3: Weather Conditions

| Day # | Date | High °F | Low °F | Condition |
|---|---|---|---|---|
| Day 1 | 8-Mar-11 | 58 | 30 | Cloudy |
| Day 2 | 9-Mar-11 | 63 | 37 | Cloudy |
| Day 3 | 10-Mar-11 | 63 | 39 | Rainy |
| Day 4 | 11-Mar-11 | 60 | 37 | Partly Cloudy |
| Day 5 | 12-Mar-11 | 59 | 36 | Cloudy |
| Day 6 | 13-Mar-11 | 61 | 35 | Windy |
| Day 7 | 14-Mar-11 | 56 | 39 | Rainy |
| Day 8 | 15-Mar-11 | 59 | 42 | Rainy |
| Day 9 | 16-Mar-11 | 46 | 36 | Rainy |
| Day 10 | 17-Mar-11 | 50 | 26 | Partly Cloudy |
| Day 11 | 18-Mar-11 | 52 | 32 | Windy |
| Day 12 | 19-Mar-11 | 47 | 26 | Rainy |

| Day 13 | 20-Mar-11 | 42 | 34 | Rainy |
|---|---|---|---|---|
| Day 14 | 21-Mar-11 | 46 | 32 | Partly Cloudy |
| Day 15 | 22-Mar-11 | 44 | 30 | Partly Cloudy |
| Day 16 | 23-Mar-11 | 46 | 34 | Rainy |
| Day 17 | 24-Mar-11 | 45 | 28 | Rainy |
| Day 18 | 25-Mar-11 | 46 | 29 | Partly Cloudy |
| Day 19 | 26-Mar-11 | 53 | 37 | Windy |
| Day 20 | 27-Mar-11 | 56 | 34 | Windy |
| Day 21 | 28-Mar-11 | 54 | 35 | Sunny |
| Day 22 | 29-Mar-11 | 63 | 37 | Cloudy |
| Day 23 | 30-Mar-11 | 70 | 42 | Partly Cloudy |
| Day 24 | 31-Mar-11 | 78 | 39 | Partly Cloudy |
| Day 25 | 1-Apr-11 | 79 | 43 | Partly Cloudy |
| Day 26 | 2-Apr-11 | 69 | 41 | Windy |
| Day 27 | 3-Apr-11 | 53 | 32 | Partly Cloudy |
| Day 28 | 4-Apr-11 | 69 | 35 | Partly Cloudy |
| Day 29 | 5-Apr-11 | 65 | 46 | Windy |
| Day 30 | 6-Apr-11 | 63 | 34 | Windy |

**Table 9: Tissue Sample #3 DNA profile**

| **Alleles** | **Sample 3 03/08/2011** | **Sample 3 03/15/2011** | **Sample 3 04/01/2011** | **Sample 3 04/06/2011** |
|---|---|---|---|---|
| D3S1358 | 17, 18 | 17, 18 | 17, 18 | 17, 18 |
| THO1 | 9.3 | 9.3 | 7^, 9.3 | 9.3 |
| D21S11 | 31.2, 32.2 | 31.2, 32.2 | 31^, 31.2, 32.2 | 31.2, 32.2 |
| D18S51 | 12 | 12 | < | 12 |
| Penta E | 10, 11 | 10, 11 | <, < | 10, 11 |
| D5S818 | 9, 11 | 9, 11 | 9, 11 | 9, 11 |
| D13S317 | 10, 12 | 10, 12 | 10*, 11* | 10, 12 |
| D7S820 | 8, 11 | 8, 11 | 8, 11 | 8, 11 |
| D16S539 | 11, 13 | 11, 13 | 11, 13* | 11, 13 |
| CSF1PO | 11, 12 | 11, 12 | 10^, <, < | 11, 12 |
| Penta D | 12, 13 | 12, 13 | <, 13* | 12, 13 |
| vWA | 17 | 17 | 17 | 17 |
| D8S1179 | 10, 12 | 10, 12 | 10*, 12* | 10, 12 |
| TPOX | 8, 11 | 8, 11 | <, < | 8, 11 |
| FGA | 20, 23 | 20, 23 | <, 23* | 20*, 23* |
| Amelogenin | X | X | X | X |

*Below Laboratory Threshold (Peak heights below 150 rfu)
^Allele Drop-in (False Peak)
<No Allele Present

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