THESIS

DEVELOPMENT OF A DNA FINGERPRINTING TECHNIQUE FOR PRONGHORN, *ANTILOCAPRA AMERICANA*

Submitted by

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WE HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER OUR SUPERVISION BY SCOTT T. BAUR ENTITLED "DEVELOPMENT OF A DNA FINGERPRINTING TECHNIQUE FOR PRONGHORN, *ANTILOCAPRA AMERICANA*" BE ACCEPTED AS FULFILLING PARTIAL REQUIREMENTS FOR THE THESIS IN ZOOLOGY.

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Abstract

DNA fingerprinting as a means of determining parentage can increase the accuracy and potential of reproductive studies. Such a study is currently being conducted at Weber State University: the investigation of male reproductive success in an island population of pronghorn (*Antilocapra americana*). To date, no published fingerprinting protocol specific for pronghorn exists. In this study, a DNA fingerprinting technique specific for pronghorn was developed and will subsequently be used to determine paternity.

Blood and ear-punch tissue samples were taken from each member of the pronghorn population. Results indicated that tissue samples are easier to obtain and digest, and yield substantially more DNA than the blood samples. Four restriction enzymes (*Hae* III, *Hinf* I, and *Hind* III/*Eco* RI) were screened to determine which produces an analyzable distribution of restricted DNA fragments. Gel electrophoresis was used to separate the variable-sized fragments, followed by Southern blotting onto a neutral nylon membrane, and hybridization with a chemiluminescent-labelled probe. Commercially available probes 33.15 and 33.6 were screened to determine the optimal restriction enzyme/probe combination.

Restriction enzyme *Hae* III and probe 33.6 yielded an analyzable pattern of bands with enough variability between

individuals to evaluate band sharing and thus assess genetic relatedness as a means of determining parentage.

Introduction

Animal behaviorists have traditionally relied primarily on field observations to support their hypotheses concerning reproductive success and genetic relatedness. Today, with the arrival of modern molecular techniques, many behavioral studies can be substantiated in the laboratory. A relatively new technique, known as DNA fingerprinting, provides a quantifiable laboratory method to determine parentage of a particular organism. Such a technique specific to pronghorn (*Antilocapra americana*) would be advantageous to a study of male reproductive success in this resource defending ungulate.

DNA fingerprinting was developed in 1985 by Alec Jeffreys and his colleagues (Jeffreys et al., 1985). In its short time as an established protocol, DNA fingerprinting has revolutionized criminalistics, as well as provided a modern tool for molecular biologists, systematists, evolutionary biologists, and behavioral ecologists. A DNA fingerprint is simply a genetic profile based on an organism's DNA. Although a large percentage of eukaryotic DNA is evolutionarily conserved, DNA fingerprinting is sufficiently sensitive to detect genetic variation between heterozygotic twins (Jeffreys et al., 1985).

Jeffreys' technique is based on long tandem repetitions of short nucleotide sequences known as 'minisatellites', or variable

number tandem repeats (VNTR), which share a common short core sequence (Jeffreys et al., 1985). Minisatellites are typically found in heterochromatin and are generally not transcribed. In contrast to the high degree of conservation found elsewhere in the genome, minisatellite sequences exhibit a distinct variation between individuals of a population, even siblings. The occurrence of minisatellite sequences is very high, as exemplified in some mammals where millions of minisatellite sequences constitute over 10% of the genome (Alberts et al., 1989).

Jeffreys et al. (1985) utilized a group of enzymes, known as restriction endonucleases, to detect minisatellite length variation by cleaving the DNA into a series of restriction fragments without cleaving the repeat unit. Restriction endonucleases were first isolated from bacteria in the 1970's by Daniel Nathans (Nathans, 1979). *In vivo*, the function of restriction endonucleases is to cleave foreign DNA molecules, thus protecting bacteria from invading viruses. *In vitro*, restriction endonucleases have become a powerful tool in molecular biology (Stryer, 1988).

Restriction sites commonly recognized by an endonuclease consist of 4 to 6 nucleotide pairs arranged in a palindromic sequence; a nucleotide-pair sequence that reads the same forward as backward. Different restriction endonucleases recognize different palindromic sequences. For example, *Hae* III, isolated

from the bacteria *Haemophilus aegyptius*, recognizes the nucleotide-pair sequence $\frac{GGCC}{CCGG}$ and catalyzes the hydrolysis of the phosphodiester bonds linking guanine and cytosine. On the other hand, *Hin*d III, isolated from *Hemophilus influenza*, recognizes the nucleotide-pair sequence $\frac{AAGCTT}{TTCGAA}$ and cleaves between the two adenine bases (Alberts et al., 1989).

Jeffreys et al. (1985) used gel electrophoresis to separate the restricted fragments of DNA based on size. An agarose matrix can effectively separate DNA fragments ranging in size from 100 nucleotides to more than 50,000 nucleotides, depending on the concentration used to make the gel (Micklos and Freyer, 1990). The resulting pattern of DNA bands on the gel was subsequently transferred by Jeffreys et al. (1985) to a nitrocellulose membrane, a process known as Southern blotting, and then immobilized on the membrane by baking.

Jeffreys et al. (1985) developed a series of probes to bind to the DNA minisatellites located on the membrane. He derived the probes from a 33-base pair repeat located in an intron (the noncoding sequence) of the human myoglobin gene. Jeffreys et al. found this sequence to be similar to the hypervariable minisatellites. The minisatellite variability between individuals could then be detected by labelling the probe with a radioactive isotope of phosphate (^{32}P) and exposing the probed nitrocellulose membrane to x-ray film. Jeffreys et al. termed the resulting pattern of bands a *DNA fingerprint*.

Through the years, Jeffreys et al's. technique has changed slightly with the advent of more modern technology. Probably the most notable difference in protocol is the use of a chemiluminescent-labelled probe instead of the traditional isotopic-labelled probe. Two chemiluminescent reactions have been most frequently used to replace isotopic labelling. The first reaction involves the oxidation of luminol in the presence of hydrogen peroxide. A catalyst such as horseradish peroxidase and other chemical enhancers, generally derivatives of phenol, are added to achieve the intensity and duration needed to expose the x-ray film. Light is produced in the second reaction from the decomposition of a stabilized, substituted dioxetane which undergoes rapid conversion to a luminescent derivative in the presence of calf intestinal alkaline phosphatase (Cunningham et al., 1994).

Since Jeffreys et al's. discovery in 1985, DNA fingerprinting has found a niche in several scientific applications. Human forensics, animal forensics, paternity analysis, and studies of genetic variation within a population are just a few of the many areas that utilize the power of DNA fingerprinting.

The first case testing the applicability of DNA fingerprinting was conducted by Jeffreys and his colleagues at the University of Leicester in England. Conventional methods based on human-leucocyte-associated antigens (HLAs) proved too

crude to be useful in determining whether a boy seeking to return to the United Kingdom was the son, and not the nephew, of a woman who resided there. DNA fingerprinting in this landmark case in 1985 set the stage for DNA evidence to be admitted in over 500 United States criminal cases (Hooper, 1992).

Although Jeffreys' work originated with humans, in 1987 he expanded DNA technology to animal forensics by applying DNA fingerprinting to dogs and cats (Jeffreys et al., 1987). This initiated the use of DNA fingerprinting as a tool in wildlife forensics. For example, Dr. Jerry Ruth, Senior Forensic Scientist at the National Fish and Wildlife Forensic (NFWF) Laboratory, routinely takes advantage of DNA fingerprinting techniques for three purposes - 1) to "match" two or more samples to the same animal; 2) to determine the minimum number of animals in a given sample; and 3) to determine parentage and/or "relatedness" (Ruth, 1993, personal communication).

Based on Jeffreys' original technique, Dr. Ruth and his colleagues developed protocols for several commonly illegally-hunted large North American mammals; such as gray wolf (*Canis lupus*), moose (*Alces alces*), white-tailed deer (*Odocoileus virginianus*), walrus (*Odobenus rosmarus divergens*), grizzly bear (*Ursus arctos horribilis*), black-tailed deer (*Odocoileus hemionus columbianus*), black bear (*Ursus americanus*), elk (*Cervus elaphus*), sea otter (*Enhydra lutris*), and mule deer (*Odocoileus hemionus hemionus*) (Ruth and Fain, 1993).

In addition to the multiple forensic applications, DNA fingerprinting provides a powerful molecular tool for research scientists. Probably the most popular applications include determining parentage and genetic variation among individuals in a population. A study conducted by Rabenold and Gortari (1991) applied DNA fingerprinting techniques to address gray wolf (*Canis lupus*) relocation plans in Yellowstone National Park. Scientists intend to monitor the genetic structure of the pack(s) which has now been introduced. To accomplish such a study, the relocated animals, which had known genotypes, would need to be distinguished from other unrelated and genetically different gray wolves that may potentially migrate into the park on their own (Linn, 1993). Such a distinction proved to be practically impossible phenotypically. Through DNA fingerprinting, the gray wolves of interest and other phenotypically similar wolves were able to be distinguished (Rabenold and Gortari, 1991).

Another relocation took place in January of 1993 when a small population of pronghorn, *Antilocapra americana*, was relocated to Antelope Island State Park, an island surrounded by the Great Salt Lake. Such a founding population provides numerous opportunities for research. A collaborative project by Dr. Sue Fairbanks and Dr. Amelia Ahern-Rindell is currently underway (1993-1998) to investigate the relative importance of territory quality and male phenotype to reproductive success in this ungulate (Fairbanks, 1994). To facilitate the study, an

accurate method of determining paternity will be needed. A similar study on red deer, *Cervus elaphus*, in Scotland, successfully utilized DNA fingerprinting to validate field observations to assign paternity to individual stags (Pemberton et al., 1991).

A quantifiable laboratory technique that can be used to determine paternity, such as DNA fingerprinting, is thus needed for the pronghorn study. *Antilocapra americana* is unique relative to other North American ungulates in that it is the only surviving species in the family Antilocapridae. However, some studies suggest that pronghorn may be closely related to the Bovidae and/or Cervidae families (O'Gara, 1978). Based on this similarity, fingerprinting techniques currently utilized for bovids or cervids could be slightly modified for use with pronghorn.

Methods

DNA Extraction From Blood and Ear-punches

At the time of reintroduction, blood and ear-punch samples were taken from each of the 26 pronghorn and stored at -70° C. We aliquoted 500 *u*l of each blood sample into 1.5 ml microcentrifuge tubes. We shaved the fur off each of the ear-punch samples to minimize unwanted protein. 500 ul of digestion buffer (100 mM Tris·HCl, 50 mM EDTA (ethylenediamine tetraacetic acid, disodium salt), 200 mM NaCl, 0.5% Triton X-100, 2% sodium dodecyl sulfate (SDS), proteinase K, and RNase A) was

added to each microcentrifuge tube. Tubes were allowed to rotate at 5 rpm overnight at 50° C. The DNA was then isolated through three extractions: 1) phenol (Tris-buffer saturated); 2) phenol:chloroform (1:1); and 3) chloroform:isoamyl alcohol (24:1).

Following the extractions, the DNA was precipitated using ethanol and centrifuged into a pellet. After removing any excess ethanol, the pellet was redissolved in 300 mM sodium acetate and purified by re-precipitation with ethanol. The final pellet was then dissolved in 10 mM Tris·HCl and 1 mM EDTA. The final DNA solution was then stored at -20° C. Concentrations were determined based on spectrophotometric absorbance readings at 260 nm (A_{260}) (**Table 1**).

Enzymatic Restriction of Genomic DNA

To obtain a final concentration of 1 *ug/ul* of DNA, the amount of 10 mM Tris·HCl, 1 mM EDTA was manipulated. Seven *ug* of DNA was then transferred to clean microcentrifuge tubes along with 5 to 7 units restriction enzyme/*ug* DNA. The solution was allowed to incubate at 37° C for three hours. Four restriction enzymes (*Hae* III, *Hin*d III/*Eco*RI, and *Hin*f I) were initially screened to determine which resulted in an optimal restriction of pronghorn DNA. Following restriction, the enzymes were inactivated by the addition of gel loading buffer (0.4% bromophenol blue, 0.4% xylene cyanol, and 25% Ficoll), which also

aided in the visualization of the samples during loading to the electrophoretic gel.

Agarose Gel Electrophoresis

The solution of restricted DNA and gel loading buffer was then heated briefly to 70° C to eliminate any secondary structure prior to loading onto the gel. After loading the samples into 15 *u*l wells, we covered the 1% agarose gel with 10 X TBE buffer (900 mM Tris·borate and 20 mM EDTA). The samples were electrophoresed at 34 V for 40 hours. We stained the gel using a 0.5 *u*g/ml solution of ethidium bromide for 30 min. The excess ethidium bromide was then eliminated with a 30 min water wash. The DNA was then visualized and photographed under ultraviolet light.

Southern Blotting

We prepared a 15 by 20 cm neutral nylon membrane (Magnagraph Nylon Transfer™, Fisher, St. Louis) by rinsing the membrane for 15 min with a 30% acetyl nitrile solution, followed by two 15 min water washes. The gel was then vacuum blotted onto the nylon membrane using high salt transfer at a pressure of 50 mbar:

1) 20 minutes with 0.25 N HCl (Depurination)
2) 20 minutes with 1.5 M NaCl/0.5 M NaOH (Denaturation)
3) 20 minutes with 1.5 M NaCl/1 M Tris·HCl (Neutralization)
4) 60 minutes with 10 X SSC (Transfer)

After blotting, the DNA was covalently bonded to the nylon membrane using a UV crosslinker (30 sec @ 1200 mJoules). The membrane was then rinsed with 10 X SSC (sodium chloride, sodium

citrate), twice with 1 X SSP (sodium chloride, sodium phosphate), and finally with 1 X SSP + 1% SDS.

Hybridization and Detection by Chemiluminescence Using Oligonucleotide Probes

All hybridization solutions were pre-heated to 50° C in a hybridization oven. The membrane was first placed in a pre-hybridization solution (5 X SSP, 1% SDS, and 1% casein hammarsten) and rotated at 5 rpm for 1 hr at 50° C. We then placed the membrane in a hybridization solution containing 8-10 *ul* Cellmark probe (Cellmark Diagnostics, Germantown) labelled with alkaline phosphatase and rotated the membrane for 30-60 min at 50° C. Following hybridization, the membrane was washed three times with hot (50° C) wash solutions (1 X SSP and 1% SDS) to remove any nonspecific binding of the probe. We then washed the membrane twice with room temperature washes (1 X SSC) to remove any residual probe. The membrane was then placed into a 'Ziplock' storage bag with the chemiluminescent substrate (Cellmark Lumi-Phos™). We added 5 ml of substrate and manipulated the membrane for 10 min to ensure thorough coverage. The membrane was then placed in a plastic development folder and inserted into a cassette along with Curix™ x-ray film (Fisher, St. Louis). We incubated the cassette at 30° C for 48-60 hr prior to developing.

Optimization for Pronghorn

In an attempt to optimize the protocol for pronghorn, we varied several parameters. We first considered the amount of DNA added to the restriction digest and subsequently loaded onto the electrophoretic gel. We conducted several series of reactions in which we added 3, 5, 7, and 9 *ug* of DNA. We then varied the concentration of restriction enzyme (*Hae* III) added to each reaction. In one of the above restriction series we added 5 U (1 unit of enzyme completely cleaves 1 *ug* lambda DNA in 1 hr at 37° C in a total volume of 50 *ul*) of enzyme/*ug* DNA. In another we added 7 U enzyme/*ug* DNA, and in another 9 U enzyme/*ug* DNA. We also varied the duration of the restrictions, allowing reactions to proceed for 3, 4, and 5 hrs.

After considering the parameters involved with enzymatic restriction of the DNA, we studied several variables specifically dealing with the hybridization of the membrane. We screened two probes by hybridizing a membrane containing several different individuals with probe 33.15, and then stripping the probe from the membrane. We then hybridized the same membrane with probe 33.6. Membranes were stripped by placing them in a 90° C solution of 0.1 X SSP and 0.5% SDS. The membranes were then shaken for 15 min until the stripping solution approached room temperature. We then placed the membranes in another 90° C stripping solution and repeated the procedure.

In addition to the type of probe used, we considered the effect of probe concentration and hybridization time on the ability of the probe to hybridize. We hybridized several membranes for 1 hr with probe concentrations of 7.5, 12, and 15 pmoles/ml hybridization solution. We also hybridized membranes for 30 min with a probe concentration of 30 pmoles/ml hybridization solution.

Results

We found that digestion of ear-punch tissue samples yielded substantially more DNA than blood samples (**Figure 1**). We also noted that the majority of DNA samples extracted from blood failed to restrict completely (data not shown). Among the four restriction enzymes screened, *Hae* III consistently yielded a more optimal digest than *Hind* III/*Eco*RI and *Hinf* I (**Figure 2**). In varying the concentration of *Hae* III, we occasionally noticed incomplete digestion when 5 U enzyme/*u*g DNA was used. We noted no significant difference between 7 and 9 U enzyme/*u*g DNA. We did, however, note an increase in relative band intensity with an increase in concentration of DNA added to the restriction digest; although 9 *u*g DNA reactions often failed to restrict completely. We noted no significant difference between 3, 4, and 5 hr restriction times (**Figure 3**).

In collaboration with Dr. Jerry Ruth from the NFWF, we found that hybridization with probe 33.15 yielded numerous intense

bands with little variation from one individual to another (**Figure 4**). On the other hand, probe 33.6 yielded a more simple pattern of bands with substantial variation between individuals (**Figure 5**). We also noted an increase in band intensity as we increased probe concentration from 7.5 to 12 pmoles/ml hybridization solution. We found little or no difference between 60 min hybridizations containing probe concentrations from 12 to 15 pmoles/ml and 30 min hybridizations containing 30 pmoles/ml.

Discussion

We concluded that DNA extracted from blood samples did not yield a sufficient amount of DNA to be useful for DNA fingerprinting. This is most likely due to the numerous anucleated red blood cells that fail to contribute any DNA. Other problems associated with blood samples included incomplete restriction due to degradative protein by-products, and difficulty in obtaining sufficient sample volumes from the newborn fawns due to their small vasculature. Ear-punch tissue samples, on the other hand, consistently restrict and are much easier to obtain from the fawns. In addition, collecting ear-punch samples from the fawns requires less handling, and therefore, less trauma to individuals during sampling.

We selected *Hae* III as the restriction enzyme of choice, due to the variation in size of the resulting fragments. *Hae* III is considered to be a frequent cutter because of its short recognition sequence. The shorter the recognition sequence, the

higher the probability that it will be contained in any given sequence of DNA, and therefore cleaved. Greater variability in the size of the restricted fragments will effectively increase the relative distance between bands after hybridization, thereby allowing for greater band variation to facilitate analysis.

In considering the other parameters involved in the enzymatic restriction of DNA, we concluded that 3 *ug* of DNA did not yield sufficiently intense bands. Either 5 or 7 *ug* of DNA provided an adequate band intensity for further analysis. We experienced difficulty in restriction of 9 *ug* samples, probably due to the increased amount of DNA.

We concluded that 5 U enzyme/*ug* DNA for 4 hrs, or 7 U enzyme/*ug* DNA for 3 hrs would consistently yield an adequate restriction. Five hr restrictions with any enzyme concentration proved to be unnecessary, as well as restrictions with 9 U enzyme/*ug* DNA.

We initially electrophoresed samples on a 0.8% agarose gel. We later switched to a 1% agarose gel for several reasons. First, the 1% gel was easier to handle and did not crack or stretch as easily as the 0.8% gel. Also, due to the increased density of the 1% gel over the 0.8% gel, the fragments migrated slower. The slower migration allowed us to electrophorese for a longer period of time, and thus achieve better band resolution in

the range from 25632 bp (base pairs) to 2390 bp (**Figures 4 and 5**). This region appears to contain the greatest variability between nonrelated individuals.

In screening the two probes, 33.15 and 33.6, we opted for 33.6 due to the simpler pattern of bands produced. Probe 33.15 failed to yield variation in the pattern of bands between individuals. The more simple pattern produced by 33.6 is explained upon comparison of the length of recognition sequences of the two enzymes. Probe 33.6 has a much longer recognition sequence than 33.15 (**Figures 4 and 5**). Similar to restriction enzymes, the longer the recognition sequence, the lower the probability that it is contained within a given segment of DNA, leading to fewer sites hybridized by the probe. Fewer sites hybridized by the probe leads to a simpler pattern of bands. Probe 33.15 contained a short recognition sequence that hybridized too frequently and produced too many bands for accurate analysis. The recognition sequence of probe 33.15, also probably hybridized to a common sequence found in most pronghorn, as demonstrated by the numerous bands shared by all individuals (**Figure 5**).

We concluded that a probe concentration of 7.5 pmoles/ml hybridization solution did not produce bands with sufficient intensity for analysis. Either a 60 min hybridization with a probe concentration from 12 to 15 pmoles/ml or a 30 min

hybridization with a probe concentration of 30 pmoles/ml will provide bands with sufficient intensity.

Following hybridization and the hot washes, we initially used two room temperature washes of 1 X SSP. We changed these two washes to 1 X SSC due to the potential of sodium phosphate interference with the alkaline phosphatase catalyzed reaction with Lumi-Phos™.

After the hybridized membrane was placed in a development folder and then inserted into a cassette with x-ray film, we tried allowing the chemiluminescent reaction to proceed at room temperature (25° C). We noted a drastic difference between autoradiograms exposed at room temperature and 30° C. Incubation of the cassette at 30° C was necessary for optimal alkaline phosphatase activity.

In conclusion, the *Hae* III/33.6 enzyme/probe combination yielded the optimal DNA fingerprint for subsequent analysis. Additional research will be required to optimize analysis procedures, as well as to develop statistical approaches to validate the determination of parentage. Each DNA fingerprint produced for the population by means of the above developed protocol can be analyzed using two published methods: 1) band sharing, and 2) genetic similarity. Band sharing (BS) is defined as the ratio of the number of shared bands between two individuals to the sum of all their bands (Pemberton et al., 1991; Rabenold and Gortari, 1991). Therefore, the greater the BS

value, the greater the chance of relatedness. Genetic similarity (GS) takes into account whether a band is heterozygous, homozygous, or null and may therefore be more accurate as a measure of relatedness (Mannen et al., 1993). To assist in evaluation, the autoradiograms may be scanned into a computer using a densitometer and analyzed with appropriate software (e.g. RFLP and Genetic Relatedness, Bio-Rad, Hercules).

Once DNA fingerprints are produced for each member of the population on Antelope Island, they can be analyzed to determine parentage, and more specifically, paternity. Parentage can be determined upon comparison of BS and/or GS. One would expect a much higher BS and GS between a parent and its offspring than between unrelated individuals. This can be directly attributed to the fact that all bands in the DNA fingerprint of the offspring must be accounted for by bands in either the fingerprint of the mother or of the father.

Following development of analysis and statistical procedures, DNA fingerprinting will provide a quantifiable laboratory method for determining paternity in a population of pronghorn. Such a highly accurate means of determining paternity will directly facilitate the aforementioned investigation of the relative importance of territory quality and male phenotype to male reproductive success in the pronghorn population reintroduced to Antelope Island.

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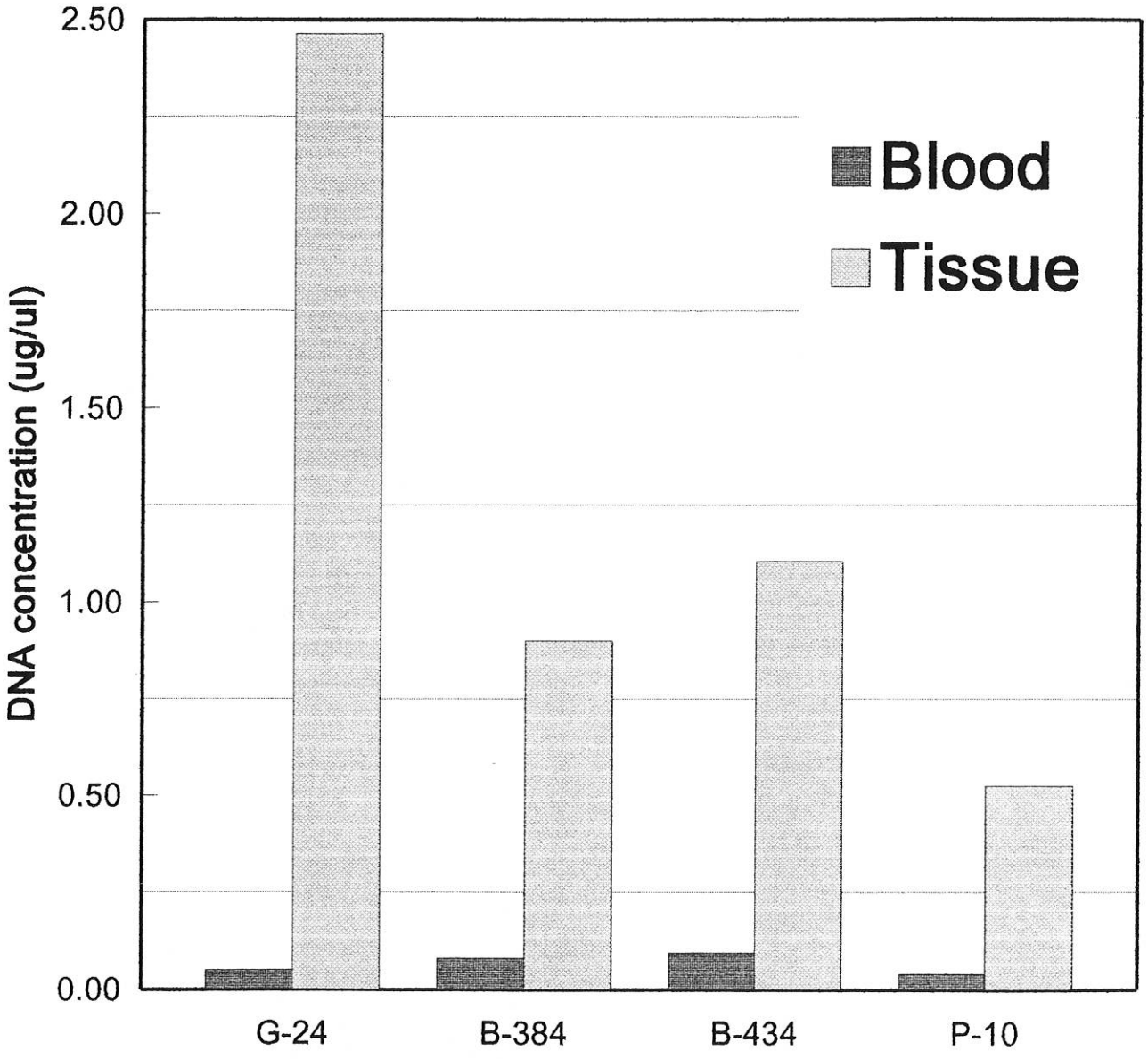
Table 1. *Absorbance and concentration of extracted DNA samples*[a]

Sample ID	A_{260}	*ug/ul* DNA	Sample ID	A_{260}	*ug/ul* DNA
B'	0.404	2.020	**O-20/G-4**	0.124	0.620
B''	0.350	1.750	**P-1/W-8**	0.148	0.740
B-373	0.206	1.030	**P-5**	0.223	1.115
B-384	0.180	0.900	**P-9**	0.277	1.385
B-384 (blood)	0.016	0.080	**P-10**	0.105	0.525
B-434	0.221	1.105	**P-10** (blood)	0.008	0.040
B-434 (blood)	0.019	0.095	**P-11**	0.282	1.410
B-556	0.228	1.140	**R-1**	0.163	0.815
B-5	0.123	0.615	**R-3**	0.315	1.575
B-6	0.150	0.750	**R-13**	0.229	1.145
B-7	0.137	0.685	**R-14**	0.206	1.030
B-8	0.368	1.840	**R-15**	0.238	1.190
G-1	0..309	1.545	**R-16**	0.096	0.480
G-3/W-9	0.144	0.720	**W-1**	0.264	1.320
G-21	0.338	1.690	**W-2**	0.139	0.695
G-22	0.213	1.065	**W-5**	0.547	2.735
G-23	0.400	2.000	**W-6**	0.216	1.080
G-24	0.488	2.463	**W-7**	0.187	0.935
G-24 (blood)	0.010	0.050	**Y-8**	0.976	4.880
G-25	0.283	1.415	**Y-9**	0.657	3.285
O-1	0.358	1.790	**Y-11**	0.478	2.390
O-3/P-2	0.178	0.890	**Y-25/B-10**	0.200	1.000
O-17	0.199	0.995	**Y-26**	0.114	0.570
O-18	0.176	0.880	**Y-27**	0.191	0.955
O-19A	0.288	1.440	**Y-28**	0.262	1.310
O-19B	0.466	2.330	**Y-29** (blood)	0.299	1.495

[a] **DNA (*u*g/ml) = A_{260} x dilution x 50 *u*g/OD_{260}**. Sample ID letters correspond to ear tag color.

Figure 1. *Concentration of DNA from blood vs tissue samples*[a]



[a] Sample concentrations were obtained from **Table 1**.

Figure 2. *Photograph of an electrophoretic gel screening 4 restriction enzymes* Samples were loaded onto a 1% agarose gel into wells on the right. They were electrophoresed for 40 hrs at 34 V. Anode on the right and cathode on the left.

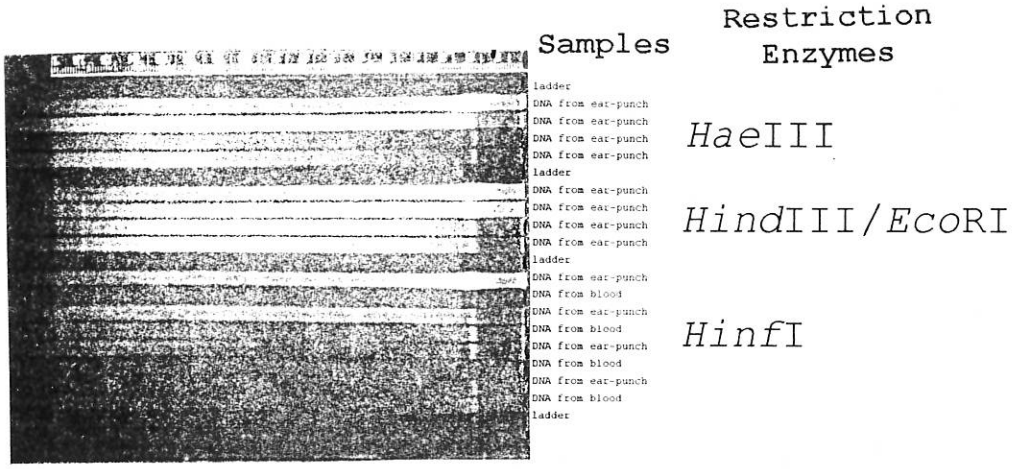


Figure 3. *Photograph of an electrophoretic gel restriction digest (*Hae *III)* All DNA was extracted from pronghorn O-19. Samples were loaded onto a 1% agarose gel into wells on the top. They were electrophoresed for 40 hrs at 34 V. Anode on the top and cathode on the bottom.

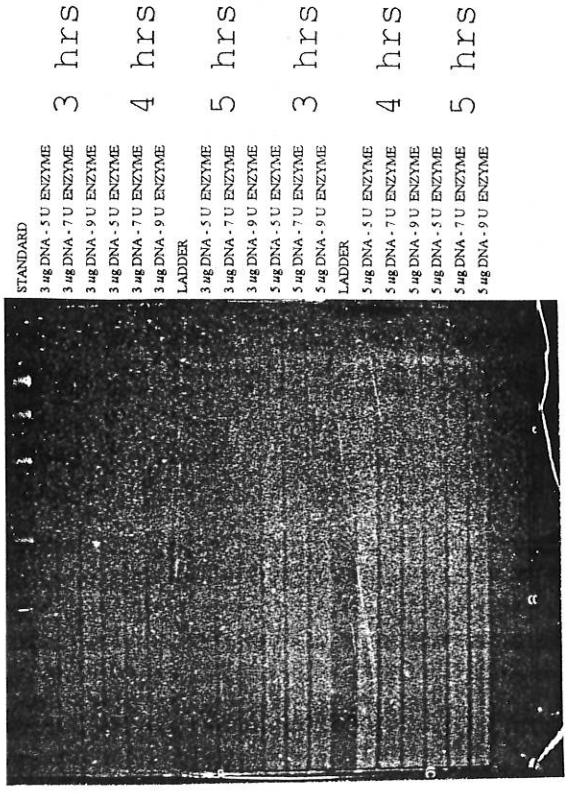
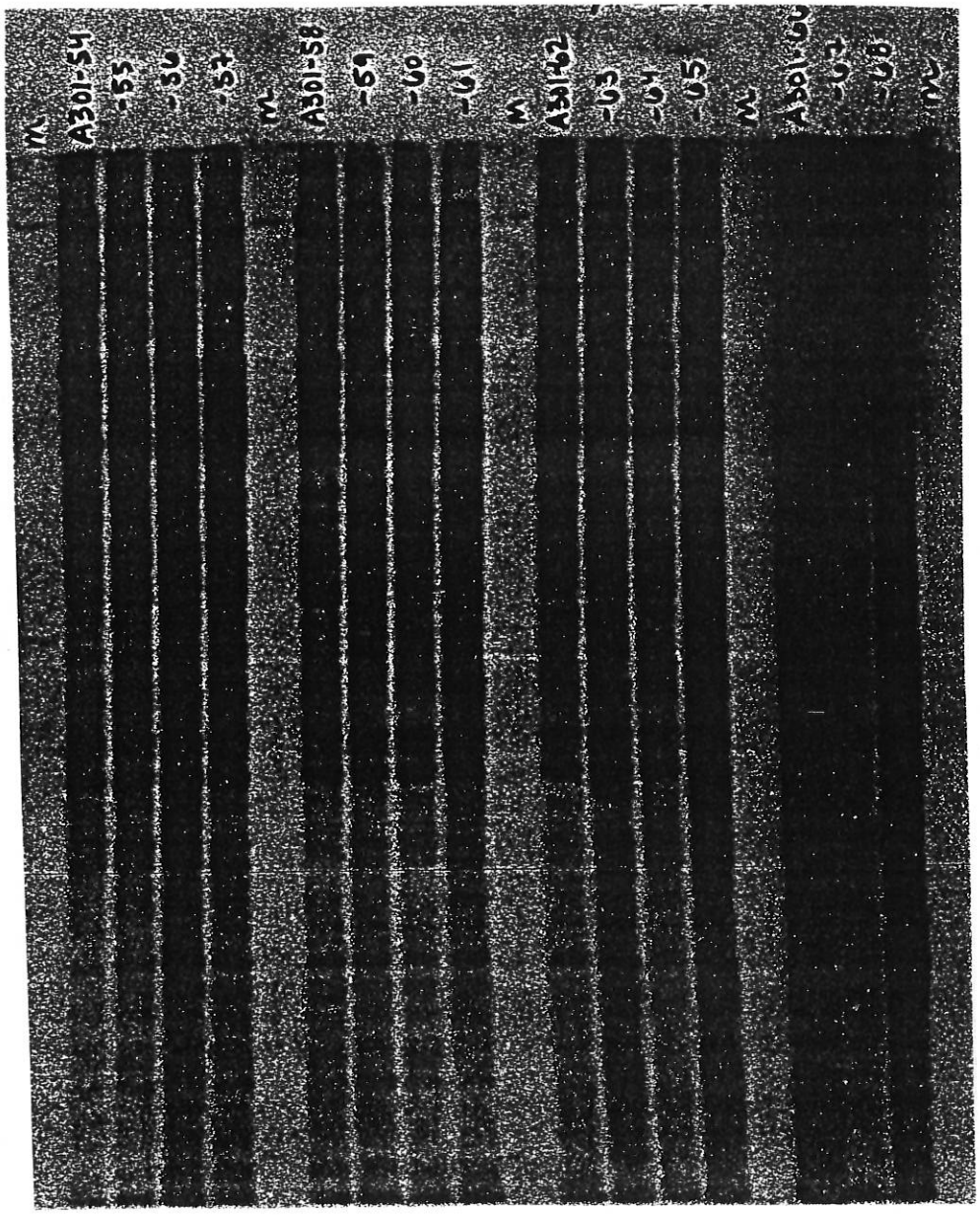
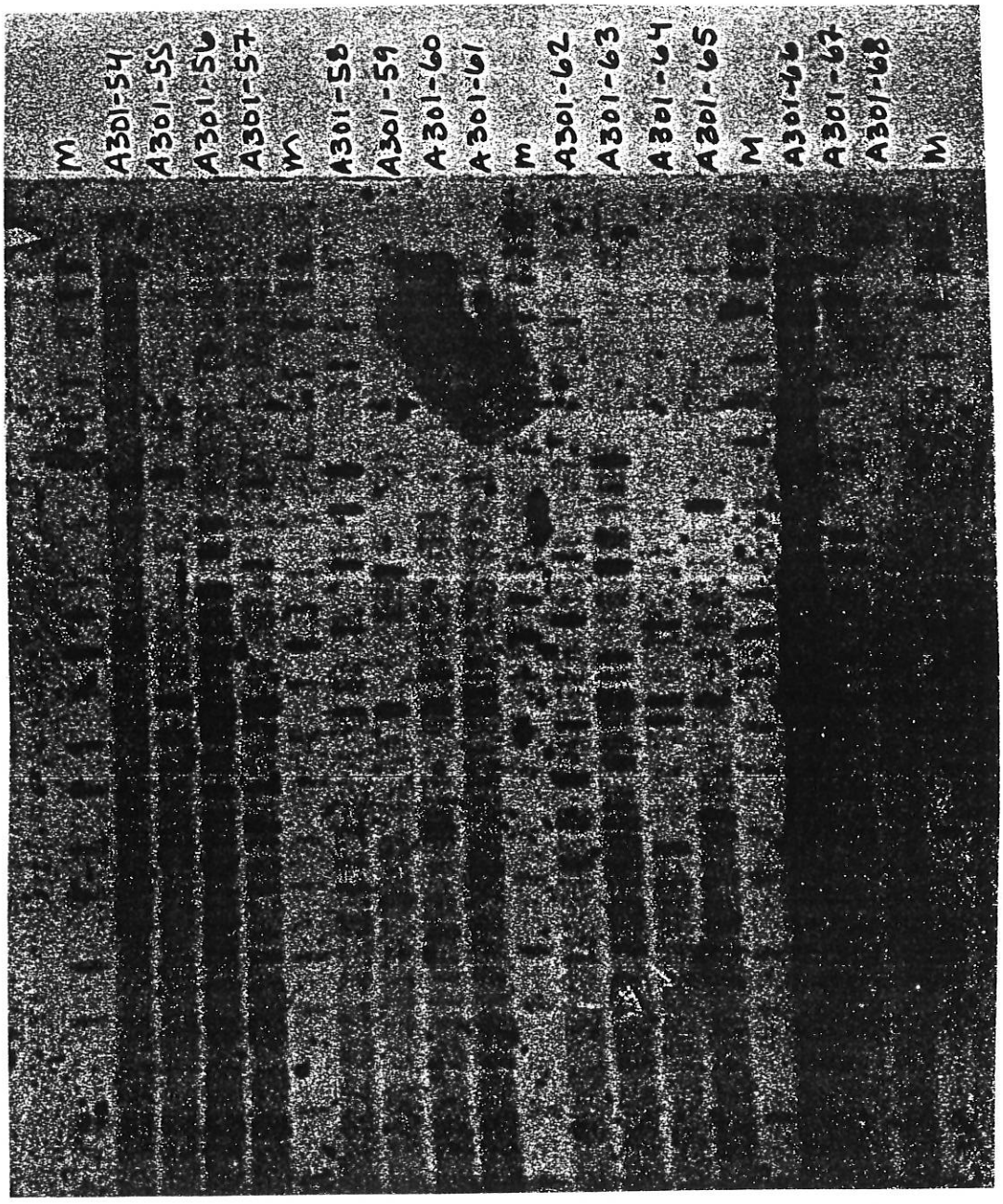


Figure 4. *Autoradiogram with probe 33.15*[a]



[a] The recognition sequence for probe 33.15 is AGAGGTGGGCAGGTG. This autoradiogram was produced at the NFWF Laboratory using *Hae* III as the restriction enzyme. Samples were electrophoresed on a 1% agarose gel for 40 hrs. M indicates a molecular weight standard and bp refers to base pairs.

Figure 5. *Autoradiogram with probe 33.6*[a]



[a] The recognition sequence for probe 33.6 is AGGGCTGGAGGAGGGCTGGAGGAGGGCTG-GAGG This autoradiogram was produced from the same membrane as **Figure 4** at the NFWF Laboratory using *Hae* III as the restriction enzyme. Samples were electrophoresed on a 1% agarose gel for 40 hrs. M indicates a molecular weight standard and bp refers to base pairs.