Analysis of Microsatellite Markers in American Pit Bull Terriers
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PROJECT SUMMARY

Breed assignment in animal shelters occurs based on canine appearance and shelter staff knowledge. Assigning a dog to the breed American Pit Bull Terrier (APBT) can have a negative effect on the adoptability of a dog in an animal shelter. The canine genome has many markers that are used to identify a canine’s breed profile using DNA. Little is known about combinations of different types of markers to identify one specific breed. Short tandem repeats and single nucleotide polymorphisms are the types of markers used in this study to test for any patterns in APBT genetics for possible identification. Successful DNA extraction methods from cotton swabs have been designed. SNP primers are highly sensitive during PCR and more work is needed to design an efficient protocol for amplification.

INTRODUCTION

- Animal shelter agents assign breeds to incoming canines based on subjective opinion that is highly dependent on personal knowledge of various breeds.
- The breed assigned to these canines has a profound impact on the adoptability of the canine since potential adopters may only be looking for certain breeds or a homeowner’s insurance may not allow certain breeds.
- Studies have examined the reliability of shelter agents and veterinarians to correctly identify breeds against a DNA analysis for confirmation. More specifically, one study concluded that breed assignment of American Pit Bull Terriers (APBT) lacks consistency and visual identification is unreliable.
- The reality is that shelters are dominantly populated by APBTs. In order to keep breed variability in a shelter, usually an APBT is the next canine to be euthanized to make space for incoming surrenders and strays.
- The trouble with breed misidentification is that there are more pressing matters occurring on a daily basis in a shelter that cause acceptance of the current standards for breed assignment. Developing an identification method for APBTs can decrease the amount of misidentified canines coming into animal shelters.
- Microsatellite markers in the canine genome are used in canine breed profiling arrays and can be useful in combination to determine patterns consistent with specific breeds such as the APBT. Combining results from amplification of short tandem repeats (STR) and single nucleotide polymorphisms (SNP) using buccal DNA from canines, a population pattern may show APBTs have a certain pattern that differs from other breeds.

OBJECTIVES

1) Design a DNA extraction method from canine buccal swabs taken from the York County Shelter for the Prevention of Cruelty to Animals.
2) Obtain amplicons from PCR of desired SNP and STR markers.
3) Genotype markers and obtain frequency data for population analysis.

PILOT STUDY

Purpose: Determine whether the wooden end or the cotton end of a sterile swab is best for further experimentation.

- The microsatellite markers were amplified via PCR using the DNA samples extracted from the cotton end.
- PCR amplification of the STR primers for PEZ1 and FDX1 using the DNA samples extracted from the cotton end showed more diversity in the across-breed comparison (Brouillette and Venta 2002).
- The microsatellite markers in the canine genome are used in canine breed profiling arrays and can be useful in combination to determine patterns consistent with specific breeds such as the APBT. Combining results from amplification of short tandem repeats (STR) and single nucleotide polymorphisms (SNP) using buccal DNA from canines, a population pattern may show APBTs have a certain pattern that differs from other breeds.

EXPECTED RESULTS

- Cotton end of the sterile swab was found to be the best sample collection method.
- The microsatellite markers were amplified via PCR using the DNA samples extracted from the cotton end.
- PCR amplification of the STR primers for PEZ1 and FDX1 and the SNP primers for SCGB and TMY5 showed a discrepancy in banding for TMY5 but was successful for the other markers.
- Restriction digestion to genotype SNPs failed because sample volumes were insufficient.

PROPOSED METHODS

- Use developed Chelex Extraction method
- Quantify DNA using Nanodrop
- Low Yield: no further use for study
- High Yield: efficient method for sampling canines
- Buccal swab canine with wooden end
- Buccal swab canine with cotton end
- Polyacrylamide Gel Electrophoresis and Capillary Gel Electrophoresis
- Genotypes Identified
- Data Analysis of Genotypes

LITERATURE CITED


ACKNOWLEDGEMENTS

I would like to thank Dr. Ronald Barrier for being my mentor. I would also like to thank Melissa Smith, Executive Director at the York County Shelter for the Prevention of Cruelty to Animals, for her support of this study and allowing the opportunity for sample collection.

Table 1. Concentrations (ng/µL) from DNA extractions via the cotton end and wooden end of a sterile swab quantified by a NanoDrop 1000 spectrophotometer.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Primer sequence 1</th>
<th>Primer sequence 2</th>
<th>Restriction enzyme 1</th>
<th>Restriction enzyme 2</th>
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</tr>
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</table>

Figure 1. Agarose gel image of 18s RNA PCR to verify eukaryotic DNA isolation from swab extractions A) 100 bp ladder B) 18s template C) Great Dane wooden D) Great Dane cotton E) Boxer wooden F) Boxer cotton.

Table 2. Primer sequences and restriction enzymes used to assay SNP loci from Brouillette and Venta’s 2002 study.

Table 3. Theoretical statistical analysis of genotype frequencies for SNPs of 30 collected samples from American Pit Bull Terriers in York, PA.

Table 4. Expected and observed genotypes for American Pit Bull Terriers in York, PA.