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Genotypic Analysis of the Canine MultiDrug Resistance 1 (ABCB1) gene in Miniature American and Australian Shephards

Arieanna White, Undergraduate Researcher
Dr. Lee F. Rickords, Faculty Mentor & Miriam Laker, MS Lab Technician
Department of Animal, Dairy, and Veterinary Sciences, College of Agriculture

UtahState University UNDERGRADUATE RESEARCH

Introduction

Canine breeders who are breeding to improve genetics often utilize the ability to test breeding stock for deleterious alleles. A DNA sample, such as a cheek swab or blood sample, is sent to laboratory where they determine genotypes that the owner has requested to know. Once the breeder receives results, they are better able to plan crosses or decide if a dog should be castrated to avoid the inheritance of unwanted diseases. This is particularly important with purebred dogs due to the high ratio of homozygous alleles. I will be using canine DNA for the gene ABCB1 or Multidrug Resistance 1. Samples for this study were collected from the Miniature American Shepherd, Miniature Australian Shepherd, and Australian Shepherd breeds, all which are known to carry the ABCB1 gene. Paw Print Genetics (Spokane, WA) provided 6 DNA samples of known genotype for use as controls.

About ABCB1 (formerly MDR1): This gene mutation encodes P-glycoprotein. P-glycoprotein is a drug transport pump that is important in limiting the amount of drug that is absorbed and distributed to the brain, as well as aiding in the excretion of drugs **(Figure 1).** This specific gene mutation is more common in herding dog breeds.

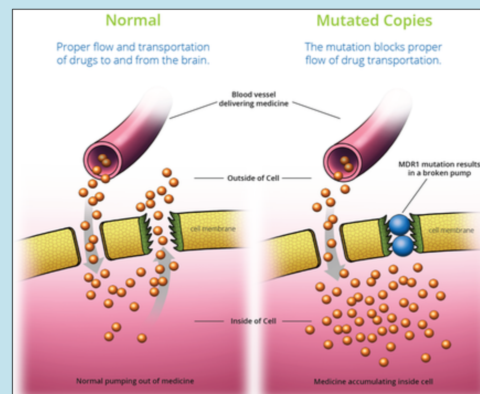

Figure 1

Objectives

- **Learn how to extract DNA from blood samples.**
- **Learn the technique of polymerase chain reaction (PCR).**
- **Learn genotyping methodology utilizing agarose gel electrophoresis to determine presence of mutation.**

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DNA Extraction

Blood samples were obtained from 7 individual dogs of the same breed and stored in EDTA tubes. DNA was extracted using the Qiagen DNeasy Kit following their protocol for purification of genomic DNA from animal blood. Proteinase K and RNase A were added to 100-200 µl of blood and PBS was added for a final volume of 224 µl and incubated at room temperature for 2 minutes. Cells were lysed during a ten minute incubation at 56°C **(Figure 2)** following the addition of Buffer AL **(Figure 3).** After incubation, ethanol was added and the samples were vortexed **(Figure 4)** and added to a spin column provided by Qiagen. Samples were centrifuged at maximum speed in a microcentrifuge **(Figure 5)** for one minute and the collection tube and flow through discarded.

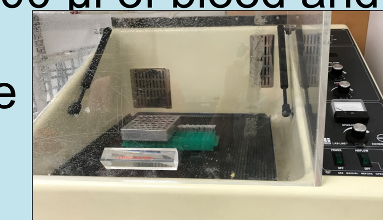
Figure 2

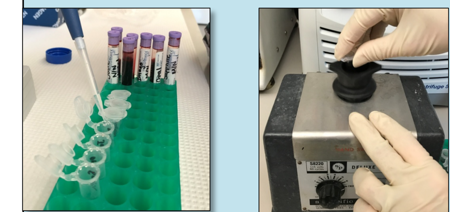
Figure 3 *Figure 4*

A wash buffer (Buffer AW1 – **Figure 6**) was added to the spin column and once again centrifuged at maximum speed for one minute. The collection tube and flow through were discarded. Another wash buffer (Buffer AW2) was added and samples were centrifuged at maximum speed for three minutes to ensure complete drying of the spin column. Spin columns were placed in microcentrifuge tubes and elution buffer (Buffer AE) was added and allowed to incubate at room temperature for one minute. One final centrifugation was performed to bring the buffer and DNA into the microcentrifuge tube. DNA concentrations were determined using the NanaDrop 2000. Results are given in **Table 1.**

Figure 5

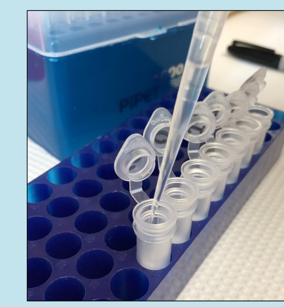
Figure 6

Table 1

OPAL	24.4 ng/µl	USU01	100 ng/µl
SAPPHIRE	20.8 ng/µl	USU02	100 ng/µl
LEGION	7.3 ng/µl	USU03	100 ng/µl
IMAGE	13.0 ng/µl	USU04	100 ng/µl
JANIE	3.3 ng/µl	USU05	100 ng/µl
RUGER	11.4 ng/µl	USU06	100 ng/µl
JOSIE	19.6 ng/µl		

Polymerase Chain Reaction

PCR was performed using Qiagen 2x Multiplex PCR Master Mix, 1 µM primers, and 20 ng DNA. The volume of DNA added to each tube was dependant upon each dog's DNA sample concentration. A thermocycler **(Figure 7)** was programmed to hold for 15 min at 95°C for denaturation of the DNA. A three step cycle of 95°C for 15 sec (denaturation), 60°C for 90 sec (annealing) and 72°C for 90 sec (extension) was repeated 40 times. A final extension at 72°C for 10 min was added to the end.

Primer sequences used (forward and reverse) credit to Paw Print Genetics:
ABCB1_SD_1_F:
GTTTCTTGAAATTCCTGCATTTGCAAAG
ABCB1_SD_1_R:
CATGATGCTGGTTTTTGGAAACATGAC

Figure 7

Gel Electrophoresis

We ran several gel types including **4% Agarose Gel (Figure 8), 3% Agarose Gel, and 8% Polyacrylamide Gel.** The most commonly used gel was made as follows:

8% Polyacrylamide Gel:
Ingredients were added in this order and mixed well: water, 5x TBE, 40% acrylamide:biacrylamide, 10% APS, and TEMED. Immediately poured gel in between glass plates gently using pipette. Allowed up to 1 hour to polymerize. Loaded 20 µl samples with 4 µl 6x loading buffer into each well. It was run at 40 volts for ~150 minutes (Completion - **Figure 9).**
After being run, all gel samples were stained to allow the bands to fluoresce under ultra-violet (UV) light **(Figure 10).**

Figure 8

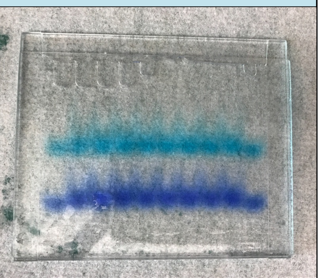
Figure 9

Base pair length of PCR products:
Wild type/Wild type: 61 base pair band
Wild type/Mutant: 57 and 61 base pairs (double band)
Mutant/Mutant: 57 base pair band

Expected Results:
The image depicted in **Figure 10** is an example of gel electrophoresis showing wild type (WT) and mutant (Mut) alleles (Paw Print Genetics). Both alleles are shown in wells 3 through 6. They ran PCR product of the ABCB1 gene. Well 3 and 6 depict WT/WT (61 bp). Well 4 depicts a double band, WT/Mut (61 & 57 bp). Well 5 depicts Mut/Mut (57 bp). The length of the base pairs is measured using a known DNA size ladder in lane M.

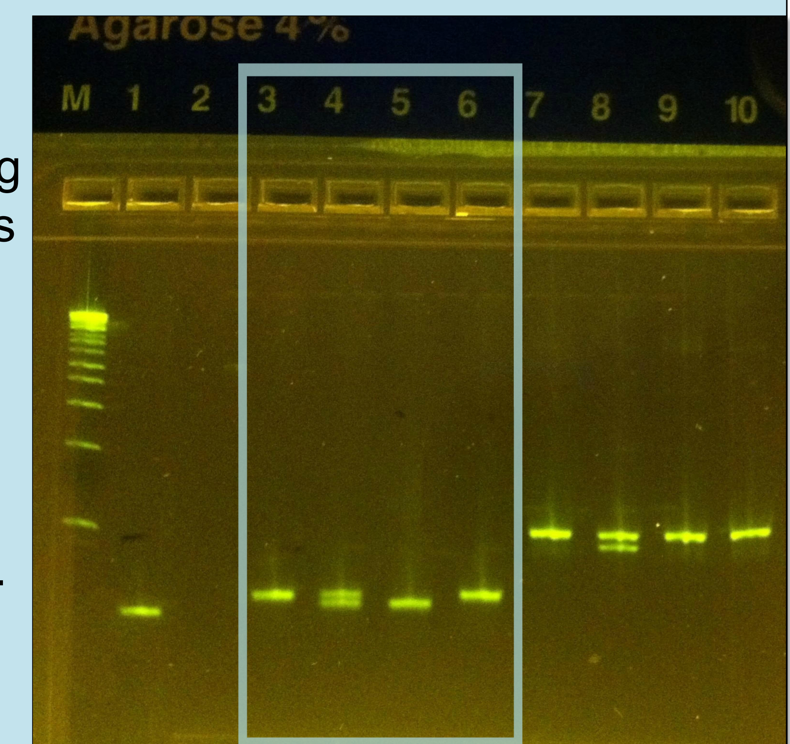

Figure 10

Conclusion

In conclusion, the objectives of the research were met. Experience and understanding of the processes involved in canine genotyping were accomplished. As it can happen, there were mishaps involved in the learning process causing less than ideal results with the gels. There may have been an issue with the polymerase chain reaction or gel electrophoresis. We are continuing the project to determine how this genotypic assay can be improved to increase reliability. Changes that can be made include lowering voltage when running the gel, modifying the buffer solution, adjusting the level of buffer above the gel, and altering the percent agarose of the gel.