Dog Genetics Lab

Oodles of Labradoodles™

miniPCR bio™ Dog Genetics Lab
Instructor's and Student's Guides

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Student's Guide Contents

Background information

Genotype and phenotype

For thousands of years, humans have been breeding animals and plants to carry traits that we find desirable. This is possible because we understand that many traits are passed down from parent to offspring. Domestic dogs are an excellent example of how selective breeding has changed organisms from their wild ancestors to be the domesticated pets we see today.

Figure 1. Physical diversity in dogs. Humans have selectively bred dogs to come in a wide array of shapes, colors, and sizes. Despite looking very different, all dogs are the same species: *Canis lupus familiaris.*

Humans breed dogs to select for traits that we want them to have, and many of these traits have to do with how dogs look. There are hundreds of recognized dog breeds that display extreme physical diversity, from the tiny Chihuahua to the massive Great Dane (Figure 1). Each dog breed has a characteristic appearance due to its genetics.

DNA contains the instructions for life, and it is a dog's DNA that determines the dog's appearance. More broadly, the information encoded in DNA determines how an organism develops and functions. Much of the information in DNA is organized in segments called *genes*. The word gene can be difficult to define exactly, but in this lab, we use it to mean a section of DNA that contains the instructions for making a specific protein. The instructions are written using the four building blocks of DNA, or *bases*: adenine (A), thymine (T), cytosine (C), and guanine (G). Because proteins carry out most of the functions in an organism, altering how proteins are made—by altering the sequence of bases in the corresponding genes—can change the organism.

We refer to an organism's genetic makeup as its *genotype*. We use the word *phenotype* to describe the organism's observable traits. A core principle in genetics is that an organism's genotype determines its phenotype, but phenotypes are often influenced by a combination of both genetics and the environment. For instance, a dog's overall size depends on several genes as well as environmental factors, like diet.

The dog phenotype you will study today is determined by a single gene. Scientists use the term *allele* to describe different versions of a gene. Different alleles for the same gene vary in their DNA sequence, which may affect how the corresponding protein is made. While alleles of the same gene can vary by as little as a single DNA base, alleles can also have substantially different sequences due to insertions or deletions of many DNA bases—this is the case for the gene you will examine today.

Furnishings in dogs

The phenotype you will investigate today has to do with the length of a dog's facial hair. Some dogs have bushier eyebrows and longer fur on their muzzles, making them look like they have a mustache. The term *furnishings* is used to describe this pattern of longer facial fur. Some dog breeds, like Poodles, have furnishings, while others, like Labrador Retrievers, do not (Figure 2).

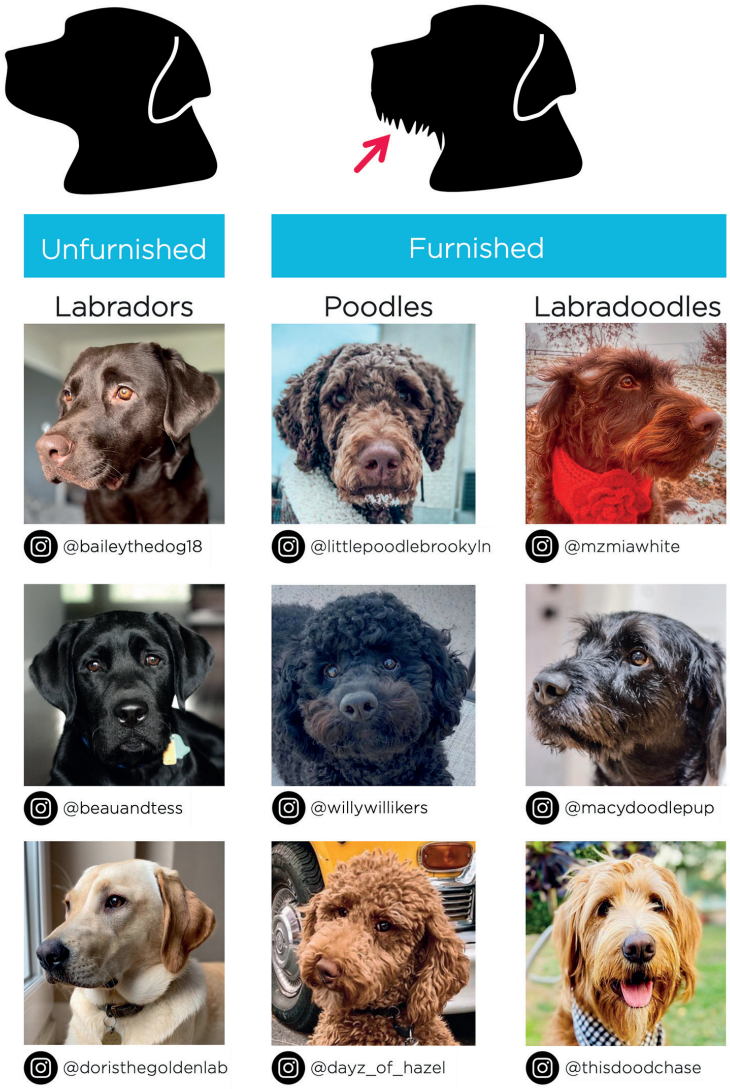


Figure 2. Furnishing in dogs
Furnishings—the presence of a mustache and bushy eyebrows—are found in many dog breeds. Labradors never have furnishings (left column), while Poodles always have furnishings (center column). You may not have seen a Poodle look like this since it is common to clip the hair on their face, but if you let their hair grow, Poodles do have furnishings. When you breed a Labrador and a Poodle, you get a Labradoodle with furnishings (right column).

Recently, scientists have discovered that a single gene controls whether or not a dog has furnishings (Cadieu *et al.*, 2009). The gene is called *RSPO2*, and it has two alleles (Figure 3): a dominant allele that results in furnishings and a recessive allele that results in a lack of furnishings. At the genetic level, the difference between the two *RSPO2* alleles is an insertion. The dominant allele contains an insertion of 167 base pairs (bp) near the end of the gene (Figure 3) (Cadieu *et al.*, 2009). Scientists sometimes use one-letter abbreviations for alleles—in this lab, we will use the letter "F" (for furnishings) and use an uppercase F for the dominant *RSPO2* allele and a lowercase f for the recessive *RSPO2* allele.

Figure 3. *RSPO2* alleles
The dominant *RSPO2* allele contains a 167 base pair (bp) insertion near the end of the *RSPO2* gene. The arrows on the recessive allele indicate the location of the insertion.

Like humans, dogs have two copies of each gene, one inherited from each of their parents. Only one copy of a dominant allele needs to be present to give a dominant phenotype. When both copies of an allele are recessive, the recessive phenotype will be expressed. That means that a dog that carries two copies of the dominant *RSPO2* allele (FF) or one copy of each *RSPO2* allele (Ff) will have furnishings. On the other hand, only dogs with two copies of the *RSPO2* recessive allele (ff) will be unfurnished (Figure 4).

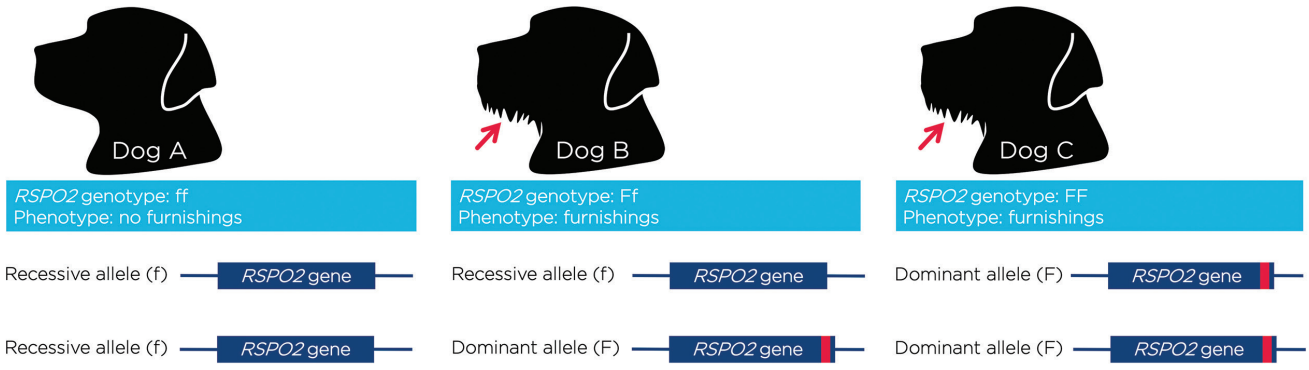


Figure 4. *RSPO2* genotype determines furnishings phenotype in dogs.
Like humans, dogs have two copies of each chromosome, one inherited from each parent. With two alleles for the *RSPO2* gene, there are three possible genotypes: two copies of the recessive allele (ff), two copies of the dominant allele (FF), or one copy of each allele (Ff). Having only recessive alleles causes an absence of furnishings phenotype (Dog A), while having one or two copies of the dominant allele causes the presence of furnishings (Dogs B and C).

Genotyping

For some traits, observing an organism's phenotype allows you to infer the underlying genotype. But often, you need to test the DNA to determine an organism's genotype with more certainty. *Genotyping* is a type of genetic test that reveals the allele(s) an organism carries in one or more regions of the genome.

Any type of genetic testing (Figure 5) starts with a biological sample like blood cells or a hair follicle. Scientists break open the collected cells and extract the DNA for analysis. Using a technique called *polymerase chain reaction* (PCR), scientists make many copies of the specific region of DNA that they want to study. Then an additional method is used to analyze the copied DNA. For genes like *RSPO2*, which have alleles that differ in length, a technique called *gel electrophoresis* can identify the different alleles. Gel electrophoresis allows scientists to separate DNA fragments based on length. For detailed explanations of PCR and gel electrophoresis, refer to https://www.minipcr.com/tutorials/.

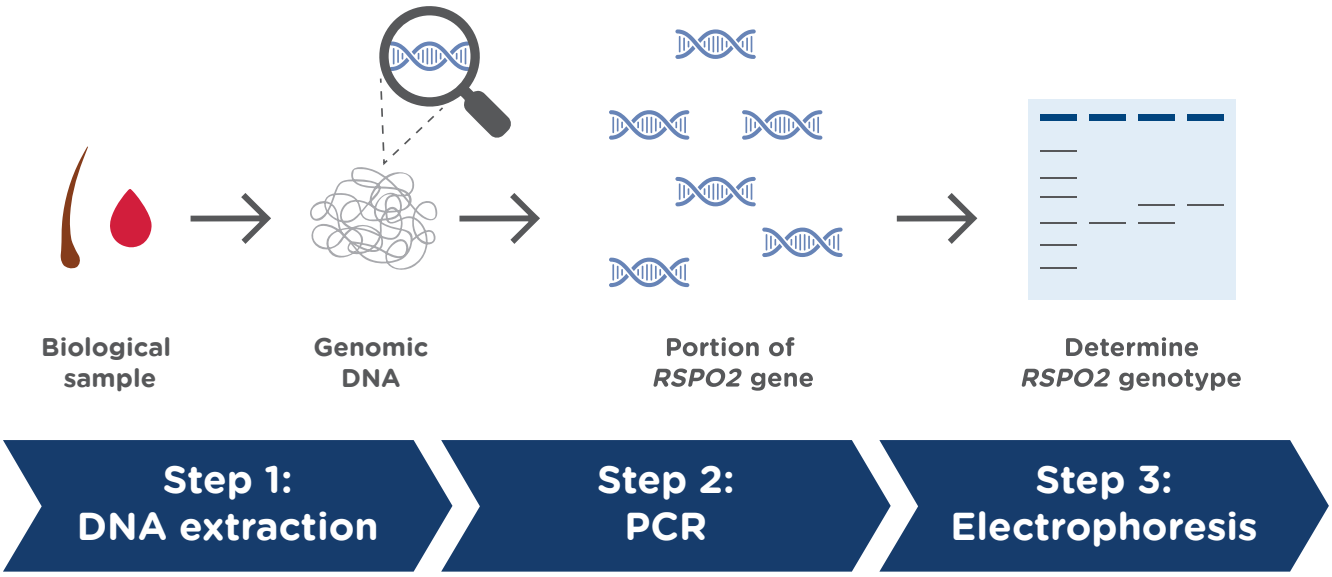


Figure 5. Genotyping dogs for *RSPO2*.
First, DNA is extracted from a biological sample, like blood or a hair follicle. Second, PCR is used to make many copies of just the locations in the genome that the scientists want to analyze. In this lab, that is a small portion of the *RSPO2* gene. Third, because the dominant *RSPO2* allele is 167 bp longer than the recessive allele, gel electrophoresis is used to identify which alleles were present in the dog's DNA. This information reveals the dog's *RSPO2* genotype.

In this experiment, PCR is used to copy just the end of the *RSPO2* gene because that is where the 167 bp insertion in the dominant allele is located. The recessive allele PCR product is approximately 250 bp and the dominant allele PCR product is approximately 400 bp (Figure 6). Then, gel electrophoresis can be used to separate the DNA by length and differentiate between the two alleles.

The gel shown in Figure 7 shows results examining both alleles of the *RSPO2* gene. Dog A only has the smaller band (~250 bp), which tells us that dog A only has the recessive allele and its *RSPO2* genotype is ff.
Dog B has two bands of different sizes (~400 bp and ~250 bp), which tells us that dog B has one copy of each *RSPO2* allele and has the Ff genotype.
Dog C only has the larger band (~400 bp), which tells us that dog C has two copies of the dominant allele and the FF genotype.

Because the *RSPO2* genotype directly determines the furnishing phenotype, we can use this information to infer whether each dog has furnishings or not. Having furnishings is a dominant trait, therefore having one copy of the dominant allele—either the Ff or FF genotype—will result in a dog with furnishings. We can conclude that both dog B (Ff) and dog C (FF) will have furnishings while dog A (ff) will not.

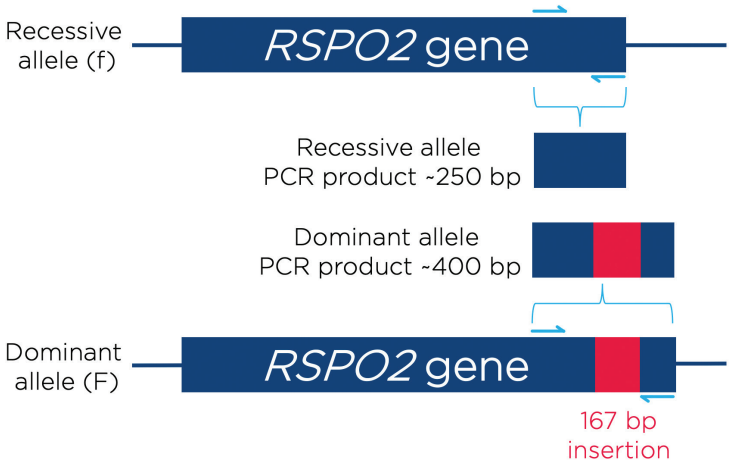


Figure 6. *RSPO2* genotyping PCR.
PCR is used to copy specific regions of DNA. In this experiment, the PCR is designed to copy the region of the *RSPO2* gene that differs between the recessive and dominant alleles (primers are shown in light blue). The reaction will generate a PCR product of approximately 250 bp for the recessive *RSPO2* allele (top), and a PCR product of approximately 400 bp for the dominant *RSPO2* allele (bottom).

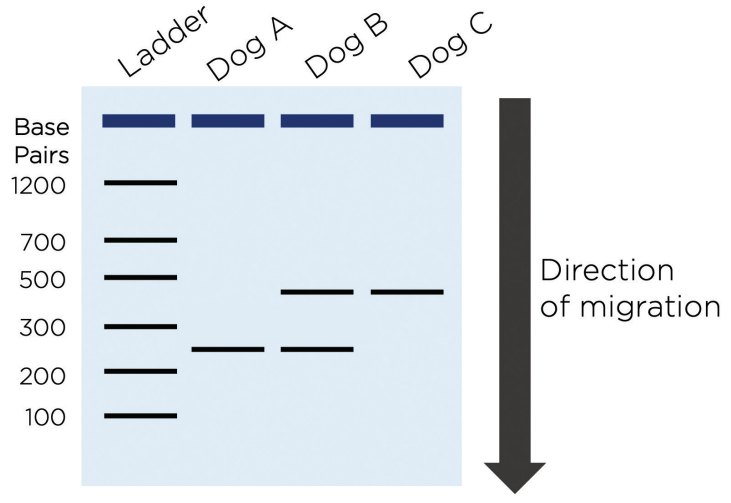


Figure 7. Gel electrophoresis.
After using PCR to copy a region of the *RSPO2* gene from dog DNA, gel electrophoresis can be used to determine the dog's *RSPO2* genotype. Because the dominant allele is 167 bp longer than the recessive allele, it will migrate more slowly on the gel. In this experiment, the PCR product for the dominant allele is around 400 bp (as seen in dogs B and C) and the recessive allele is around 250 bp (as seen in dogs A and B).

Today's lab

You have a Labradoodle named Molly. The term Labradoodle is used broadly to describe dogs that are part Poodle and part Labrador.

You are considering breeding Molly, and you take her to meet two potential mates: Zeus, a Poodle, and Otto, another Labradoodle. You decide to take some time to decide on a suitable match, but it turns out Molly had an opinion on the matter, and she surprises you with a litter of puppies!

Today you will use genetics to determine whether Zeus or Otto is the more likely father of Molly's puppies. While there are several different phenotypes that you could use to infer whether the father of Molly's puppies was a Poodle or a Labradoodle, we will focus on furnishings in today's lab. When puppies are young, you can't tell if they have furnishings or not because their fur is too short. If you want to determine whether Zeus or Otto is the father before the puppies' fur grows out, you can test their DNA to determine the puppies' *RSPO2* genotypes. Comparing a puppy's genotype with the potential fathers' genotype is a type of paternity testing, as it can reveal information on who the more likely father is.

Meet Molly's puppies!

@forevergreenlabradoodles

1. Astro
2. Buster
3. Chewy
4. Daisy
5. Elsa
6. Flora
7. Ginger
8. Hugo

Laboratory guide

Protective gloves and eyewear should be worn for the entirety of this experiment.

Gel electrophoresis - Pouring gels (before or during class period)

Important Gel Pouring Notes

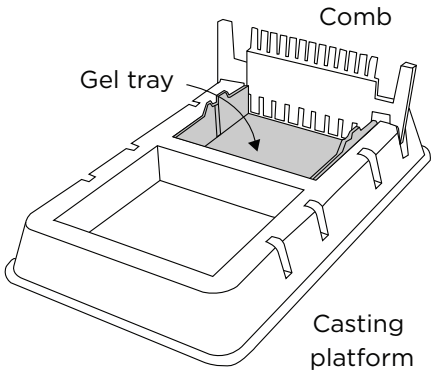
Gels can be prepared up to three days ahead of time and stored at ambient temperature, covered in air-tight plastic wrap and protected from light.

You will need 8 lanes plus one lane for ladder per group. It is possible for groups to share a gel by using two combs.

These instructions are designed for use with the blueGel™ electrophoresis system by miniPCR bio™. If using another electrophoresis system, these instructions may need to be adjusted according to the manufacturer's instructions.

1. **Prepare 1X TBE buffer (to be completed by teacher in advance)**
   - TBE buffer is often provided as liquid concentrate or powder.
   - Follow manufacturer's instructions to prepare 1X TBE buffer solution.

2. **Prepare a clean and dry casting platform with a gel tray and comb**
   - Place the clear gel tray in the white casting platform.
   - Place a well-forming comb at the top of the gel tray.



3. **Prepare a 2% agarose solution with a fluorescent DNA stain (e.g., SeeGreen™ or GelGreen®) using the method indicated by your instructor**

IMPORTANT NOTE: There are several ways to prepare agarose gels.

- Scan the QR code for detailed instructions on how to prepare agarose gels.
- Both written and video instructions are available.

www.minipcr.com/agarose-gel/

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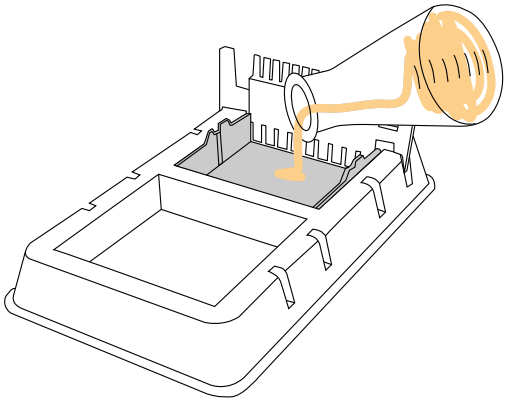
4. **Pour the agarose solution into the prepared casting platform with a gel tray and comb**
    - The agarose solution should cover the bottom of the gel tray and the bottom 3 mm of the comb (roughly the bottom 1/3 of the comb).

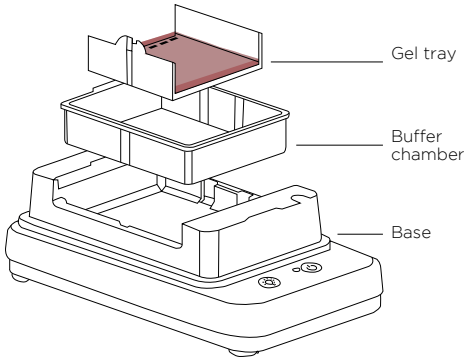
5. **Allow gel to solidify completely and remove the comb by pulling firmly upwards**
    - Gels will typically be ready in about 10 minutes.
    - Gel is ready when cool and firm to the touch.



Protective gloves and eyewear should be worn for the entirety of this experiment.

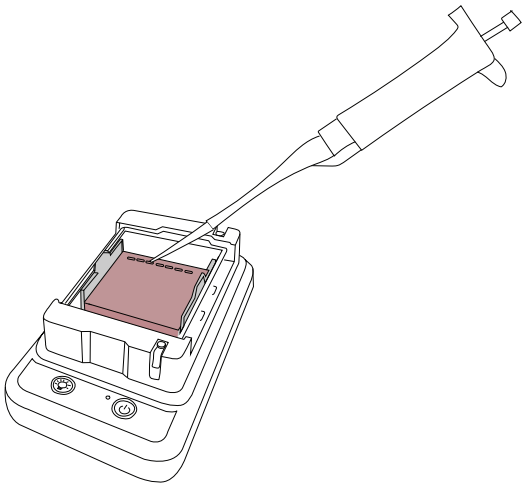
Gel electrophoresis - Running the gel

These instructions are designed for use with blueGel™ electrophoresis system by miniPCR bio™. If using another electrophoresis system, these instructions may need to be adjusted according to the manufacturer's instructions.



1. **Place the gel tray containing your gel in the buffer chamber**
   - Ensure that the clear buffer chamber is inside the blueGel™ electrophoresis system.
   - The wells of the gel should be on the same side as the negative electrode, away from the power button.

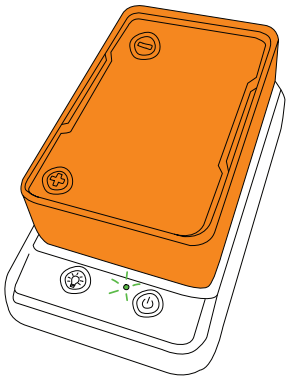
2. **Add 30 ml of 1X TBE electrophoresis buffer**
   - The buffer should just cover the gel and fill the wells.
   - Ensure that there are no air bubbles in the wells (shake the gel gently if bubbles need to be dislodged).



3. **Load samples onto the gel in the following sequence**
   - **Lane 1:** 10 µl Fast DNA Ladder 1
   - **Lane 2:** 10 µl Astro DNA
   - **Lane 3:** 10 µl Buster DNA
   - **Lane 4:** 10 µl Chewy DNA
   - **Lane 5:** 10 µl Daisy DNA
   - **Lane 6:** 10 µl Elsa DNA
   - **Lane 7:** 10 µl Flora DNA
   - **Lane 8:** 10 µl Ginger DNA
   - **Lane 9:** 10 µl Hugo DNA

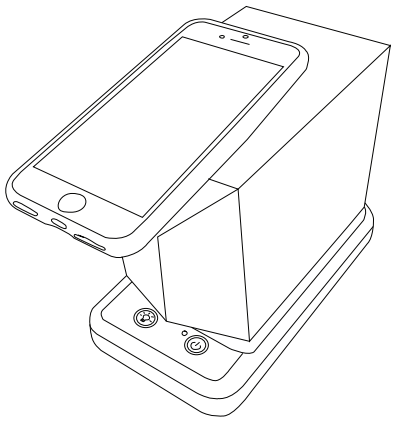
   Note: Change pipette tips between samples to prevent contamination.

4. **Place the orange cover on the blueGel™ electrophoresis system**
   - To prevent fogging, make sure that ClearView™ spray has been evenly applied to the inside of the orange cover.
   - Match the positive and negative electrode signs on the orange lid with the corresponding positive and negative signs on the blue base.
   - The electrodes of the lid should be aligned with the metal leads on the base.
   - The orange lid should sit flush with the blue base using little force.



5. **Press the "Run" button**
   - Check that the green light beside the power button remains illuminated.

6. **Conduct electrophoresis for 15-25 minutes**
   - Note: Check the progress of your samples every 10 minutes to monitor the migration of your DNA samples.
   - Longer electrophoresis times will result in better separation of similar sized DNA fragments. However, if run too long, small DNA fragments can run off the end of the gel or lose fluorescence.

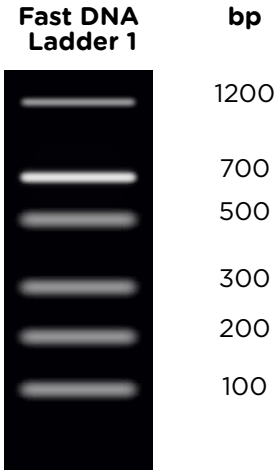


Gel electrophoresis – Visualizing results

1. **Press the "light bulb" button to turn on the blueGel™ transilluminator**
   - For best viewing, dim lights or use Fold-a-View™ photo documentation hood with a smartphone camera.
   - Gels may be viewed at the end of the run or periodically throughout the run.
   - If the image appears hazy, wipe off the inside of the orange cover and reapply ClearView™ spray.

2. **Ensure that there is sufficient DNA band resolution**
   - Run the gel longer if needed to increase resolution.

3. **Document your results**
   - Place Fold-a-View™ photo documentation hood on the blueGel™ electrophoresis system to take a picture with a smartphone or other digital camera.
   - Compare the bands from the DNA samples to the ladder to obtain size estimates.



Pre-lab study questions

Review

1. **Describe the relationship between genotype and phenotype.**

2. **What is an allele?**

3. **What does it mean for a dog to have furnishings?**

4. **What gene determines whether a dog has furnishings?**

5. **Fill in the table below using the following words and phrases: no furnishings, furnishings, FF, ff, Ff.**

Phenotype	Possible genotype(s)

6. **Explain why some phenotypes can be associated with more than one genotype, and other phenotypes can only be associated with one genotype.**

7. **Describe the genetic difference between the dominant allele (F) and the recessive allele (f) of the *RSPO2* gene.**

8. **Why is gel electrophoresis a good tool to differentiate between alleles of the *RSPO2* gene?**

9. **What size DNA fragments would you expect to see on a gel for dogs with the following *RSPO2* genotypes?**
   a. **FF**
   b. **ff**
   c. **Ff**

Critical thinking

1. **Poodles *always* have furnishings. Any time you breed two Poodles, all of their puppies will have furnishings.**
   a. **Based on this information, what do you expect a Poodle's *RSPO2* genotype to be? Use the symbols F and f to represent the alleles.**

   b. **Support your claim by filling out the Punnett square for a cross between two Poodles.**

Cross: _____ x _____
Poodle genotype | Poodle genotype

	Poodle	
Poodle		

2. **Labradors *never* have furnishings. Any time you breed two Labradors, none of their puppies will have furnishings.**
   a. **Based on this information, what do you expect a Labrador's *RSPO2* genotype to be? Use the symbols F and f to represent the alleles.**

   b. **Support your claim by filling out the Punnett square for a cross between two Labradors.**

Cross: _____ x _____
Labrador genotype | Labrador genotype

	Labrador	
Labrador		

3. **Dogs that are part Poodle and part Labrador are called Labradoodles.**
   ***First-generation Labradoodles* are generated by breeding a Poodle and a Labrador.**
   a. **Based on your answers to questions 1 and 2, fill in the Punnett square for a cross between a Poodle and a Labrador. Use the symbols F and f to represent the *RSPO2* alleles.**

Cross: _____ x _____
Poodle genotype | Labrador genotype

Labrador \ Poodle		

   b. **What are the expected genotypic ratios for a litter of "first-generation" Labradoodle puppies?**

   c. **What are the expected phenotypic ratios for a litter of "first-generation" Labradoodle puppies?**

4. **Based on your answers to the previous three questions, you can infer Molly, Zeus, and Otto's genotypes.**
   a. **Your dog Molly is a first-generation Labradoodle, meaning her parents were a Poodle and a Labrador. Knowing this, what is Molly's *RSPO2* genotype?**

b. Zeus is a Poodle. What is Zeus's *RSPO2* genotype?

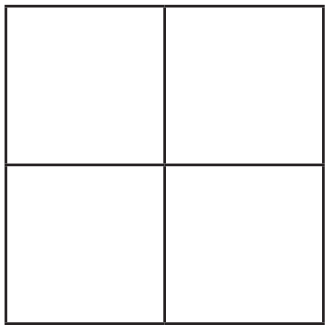
c. Otto, like Molly, is a first-generation Labradoodle. What is Otto's *RSPO2* genotype?

5. Assume Zeus is the father of Molly's puppies.
   a. Fill in the Punnett square for a cross between Molly and Zeus. Use the symbols F and f to represent the *RSPO2* alleles.

Cross: _____ x _____
Molly's genotype / Zeus's genotype

Molly
(1st generation Labradoodle)

Zeus
(Poodle)



   b. What are the expected <u>genotypic</u> ratios for a litter of puppies born to Molly and Zeus?

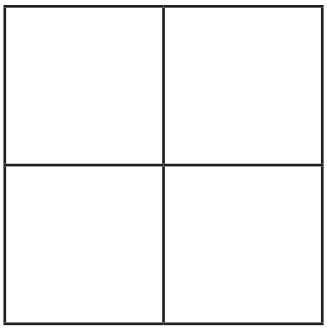
   c. What are the expected <u>phenotypic</u> ratios for a litter of puppies born to Molly and Zeus?

6. **Assume Otto is the father of Molly's puppies.**
   a. **Fill in the Punnett square for a cross between Molly and Otto. Use the symbols F and f to represent the *RSPO2* alleles.**

Cross: _____ x _____
Molly's genotype | Otto's genotype

Molly
(1st generation Labradoodle)

Otto
(1st generation Labradoodle)



   b. **What are the expected genotypic ratios for a litter of puppies born to Molly and Otto?**

   c. **What are the expected phenotypic ratios for a litter of puppies born to Molly and Otto?**

Post-lab study questions

Interpreting results

1. **Use the image on the right to illustrate your gel electrophoresis results. There are nine lanes on the gel: one for your ladder, and one for each puppy. Be sure to label each lane with the source of the sample.**

2. **Next to each band, write approximately how long (in base pairs) you think the DNA in that band is. Use the image of the ladder from page 18 to help you.**

3. **Fill in the table below with your genotyping results, then use that information to predict whether each puppy will have furnishings or not once their fur grows longer. If you need help, review Figure 7 on page 13.**

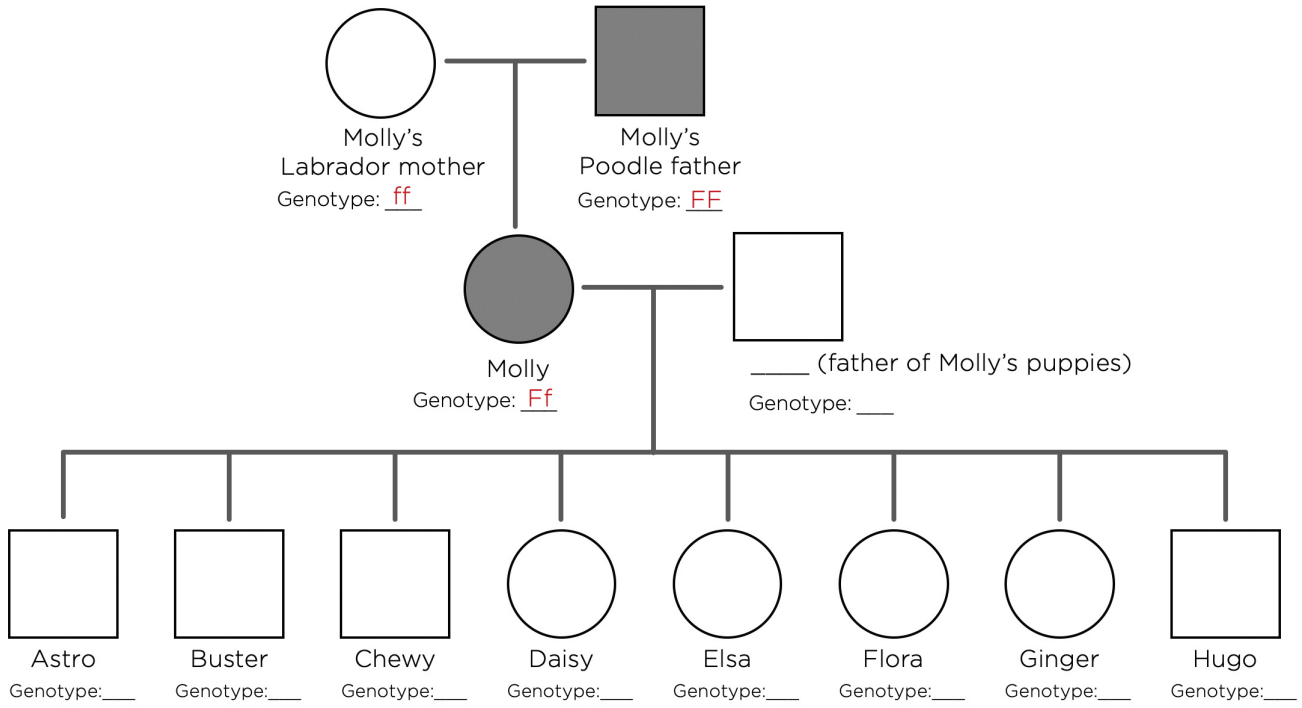
Puppy	Genotype	Predicted phenotype
Astro		
Buster		
Chewy		
Daisy		
Elsa		
Flora		
Ginger		
Hugo		

Critical thinking

1. **Who is the most likely father of Molly's puppies: Zeus or Otto? Explain your reasoning. If you need help, look back at your answers to *Critical thinking* pre-lab questions 5 and 6.**

Advanced questions

1. **Complete a pedigree for Molly's family that tracks the *RSPO2* gene by indicating each dog's genotype, and if appropriate, shading their shape in the pedigree to track the furnishings phenotype. Molly's mother, Molly's father, and Molly have already been filled in as an example. Use the symbols F and f to represent the *RSPO2* alleles.**



2. **Assume instead that you obtained the following experimental results. Is it possible for Molly to have a litter like this? If so, what could you say about the father of Molly's puppies? Explain your reasoning.**
   If you need help, look back at your answers to *Critical thinking* pre-lab questions 5 and 6.

Puppy	Genotype
Astro	FF
Buster	FF
Chewy	FF
Daisy	Ff
Elsa	FF
Flora	FF
Ginger	FF
Hugo	FF

CER Table

Fill in the table based on your results from the lab. Use the rubric on the next page to help your answers.

Question:

Based on your results, who is the most likely father of Molly's puppies?

Claim

Make a clear statement that answers the above question.

Evidence

Provide data from the lab that supports your claim.

Reasoning

Explain clearly why the data you presented supports your claim. Include the underlying scientific principles that link your evidence to your claim.

Score	4	3	2	1
CLAIM A statement that answers the original question/ problem.	Makes a clear, accurate, and complete claim.	Makes an accurate and complete claim.	Makes an accurate but incomplete or vague claim.	Makes a claim that is inaccurate.
EVIDENCE Data from the experiment that supports the claim. Data must be relevant and sufficient to support the claim.	All of the evidence presented is highly relevant and clearly sufficient to support the claim.	Provides evidence that is relevant and sufficient to support the claim.	Provides relevant but insufficient evidence to support the claim. May include some non-relevant evidence.	Only provides evidence that does not support claim.
REASONING Explain why your evidence supports your claim. This must include scientific principles/ knowledge that you have about the topic to show why the data counts as evidence.	Provides reasoning that clearly links the evidence to the claim. Relevant scientific principles are well integrated in the reasoning.	Provides reasoning that links the evidence to the claim. Relevant scientific principles are discussed.	Provides reasoning that links the evidence to the claim, but does not include relevant scientific principles or uses them incorrectly.	Provides reasoning that does not link the evidence to the claim. Does not include relevant scientific principles or uses them incorrectly.

Rubric score	3	4	5	6	7	8	9	10	11	12
Equivalent Grade	55	60	65	70	75	80	85	90	95	100

We recommend that teachers use the following scale when assessing this assignment using the rubric. Teachers should feel free to adjust this scale to their expectations.

Extension: Tracking the inheritance of multiple genes

Tracking the inheritance of multiple genes

In this lab, you genotyped Molly's puppies for a single gene that controls whether dogs have furnishings. But the presence or absence of a mustache is not the only coat phenotype observed in dogs. For example, dogs can have long or short fur, and their fur can be curly or straight. Two genes that influence dog coats are *FGF5* and *KRT71*. The *FGF5* gene specifies the length of the fur on the dog's body, with short fur being dominant to long fur. The *KRT71* gene specifies curliness of the fur, with curly fur being dominant to straight fur (Cadieu *et al*., 2009). These genes assort independently, which means they aren't linked.

Gene: *FGF5*	
Genotype	**Phenotype**
AA, Aa	Short fur
aa	Long fur

Gene: *KRT71*	
Genotype	**Phenotype**
BB, Bb	Curly fur
bb	Straight fur

Critical thinking

1. **Labradors *always* have short, straight fur. Any time you breed two Labradors, all of their puppies will have short, straight fur.**

   a. **Based on this information, what *FGF5* and *KRT71* genotypes do you expect in a Labrador? You may fill out the Punnett squares below if that helps you.**

Gene	Genotype	Phenotype
FGF5		Short fur
KRT71		Straight fur

Cross: _____ **x** _____
Labrador genotype **Labrador genotype**

Labrador

Labrador

Cross: _____ **x** _____
Labrador genotype **Labrador genotype**

Labrador

Labrador

   b. **Explain your reasoning.**

2. **Poodles *always* have long, curly fur. Any time you breed two Poodles, all of their puppies will have long, curly fur.**

   a. **Based on this information, what *FGF5* and *KRT71* genotypes do you expect in a Poodle? You may fill out the Punnett squares below if that helps you.**

Gene	Genotype	Phenotype
FGF5		Long fur
KRT71		Curly fur

Cross: _____ **x** _____
Poodle genotype **Poodle genotype**

Poodle

Poodle

Cross: _____ **x** _____
Poodle genotype **Poodle genotype**

Poodle

Poodle

   b. **Explain your reasoning.**

3. **Based on your answers to the previous two questions, what genotypes and phenotypes do you expect in a first-generation Labradoodle (a dog whose parents were a Poodle and a Labrador)? You may fill out the Punnett squares below if that helps you.**

Gene	Genotype	Phenotype
FGF5		
KRT71		

Cross: _____ (Poodle genotype) **x** _____ (Labrador genotype)

Poodle

Labrador

Cross: _____ (Poodle genotype) **x** _____ (Labrador genotype)

Poodle

Labrador

4. **Based on your answer to question 3, track *FGF5* and *KRT71* in a cross between two first-generation Labradoodles.**

   a. **Fill out the Punnett square below.**

Cross: _____ x _____
Labradoodle genotype | Labradoodle genotype

1st generation Labradoodle

1st generation Labradoodle

b. **What are the predicted phenotypic ratios from a cross between two first-generation Labradoodles? You might want to use a separate piece of paper to work out which genotypes correspond to which phenotypes.**

c. **Fur has to be a certain length in order to curl. So even if a dog's genotype for the *KRT71* gene specifies curly fur, if the dog's fur is short then it won't display the curly fur phenotype (Cadieu *et al.*, 2009). Knowing this, what are the actual predicted phenotypic ratios for the cross between two first-generation Labradoodles?**

5. **Poodle coat traits (long, curly fur) are generally considered favorable in Labradoodles. Imagine you want to breed Labradoodles and maximize the number of puppies with long, curly coats. What crosses would you set up? Explain your reasoning.**