Dog Genetics Lab

Oodles of Labradoodles™

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

Version: 1.2
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Instructor's Guide Contents

At a glance

Molly the Labradoodle has surprised you with a litter of puppies! Use genetics to examine the link between genotype and phenotype in Molly's puppies, and in the process determine the most likely father.

This lab offers students an introduction to Mendelian genetics. Students will use gel electrophoresis to analyze DNA samples from the surprise litter of puppies to track the inheritance of a single trait, and will then use that information to determine the puppies' father.

TECHNIQUES

Micropipetting
Gel electrophoresis

TOPICS

Mendelian inheritance
Genotype to phenotype
Biotechnology

LEVEL

Middle school
General high school
Advanced high school

WHAT YOU NEED

Micropipettes
Gel electrophoresis and visualization system

AP CONNECTION

Biology units
5.3, 6.1, 6.7, 6.8, 7.3

Skills and Practices
1.A-C, 2.A, 2.C-D, 3.D, 5.A, 6.A-E

Planning your time

SINGLE CLASS PERIOD: 45 MINUTES* if gels are prepared in advance

Gel electrophoresis → **Interpret results**

* Allow an additional 25 minutes if students will be preparing the gels in class.

Additional Supports

Help your students build proficiency in pipetting and gel electrophoresis with additional instructional videos, worksheets, and activities available at: https://www.minipcr.com/tutorials/.

Taking it further—extension activities, page 30.
Tracking the inheritance of multiple genes: give your students practice with dihybrid crosses using dog genetics.

For answers to the lab study questions and extensions, email answers@minipcr.com. Please include the name of the lab, as well as your name, school, and title in the body of the email.

Materials needed

Supplied in Kit (KT-1506-01)

Reagents and supplies	Amount provided in kit	Amount needed per lab group	Storage	Teacher's checklist
DNA Samples • Astro DNA • Buster DNA • Chewy DNA • Daisy DNA • Elsa DNA • Flora DNA • Ginger DNA • Hugo DNA	**150 µl each**	**15 µl each**	Freezer	
Fast DNA Ladder 1	**150 µl**	**15 µl**	Freezer	

Sold Separately in Learning Lab Companion Kit (KT-1510-01)

This lab requires reagents for running and visualizing DNA samples on a 2% agarose gel with a fluorescent DNA stain (e.g., SeeGreen™ or GelGreen®). The Learning Lab Companion Kit provides enough electrophoresis reagents for 8 groups when using the blueGel™ electrophoresis system. Gels can also be prepared with agarose tabs or agarose powder. Refer to https://www.minipcr.com/agarose-gel/ for detailed instructions.

Reagents and supplies	Amount provided in kit	Amount needed per lab group	Storage	Teacher's checklist
All-in-one agarose tabs	**8**	**One tab per agarose gel** (2% agarose gel)	Room temp., protected from light	
TBE electrophoresis buffer • 1X working solution	**Supplied as liquid concentrate or powder** Sufficient to prepare 600 ml of 1X working solution	**30 ml of 1X solution** per blueGel™ system	Room temp.	
Plastic tubes	**50 1.7 ml microtubes** **100 0.2 ml microtubes**	**9 tubes** 1.7 ml or 0.2 ml tubes can be used		

Materials needed (cont.)

Supplied by teacher

Available at miniPCR.com

Reagents and supplies	Amount needed per lab group	Teacher's checklist
Horizontal gel electrophoresis apparatus: *e.g.,* blueGel™ electrophoresis system	**1** (can be shared between groups if you use two combs per gel)	
Blue light transilluminator *Note: A blue light transilluminator is integrated in the blueGel™ electrophoresis system.		
Micropipettes • 2-20 µl adjustable or 10 µl fixed volume	**1**	
Disposable micropipette tips	**At least 9 per group**	
Distilled water for making agarose gels and diluting TBE buffer	**50 ml per gel**	
Flask or beaker to dissolve agarose		
Microwave or hot plate to dissolve agarose		
Other supplies: • Disposable laboratory gloves • Protective eyewear • Permanent marker • Cup to dispose of tips		

Lab setup

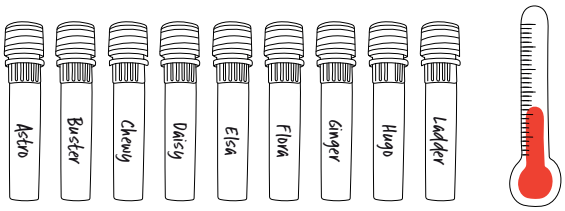
The following activities can be carried out by the instructor ahead of class. Reagents are sufficient to be used with eight student groups. Reagents are stable at room temperature for 24 hours, but should remain cold for short-term storage and frozen for long-term storage.

A. Dispense reagents

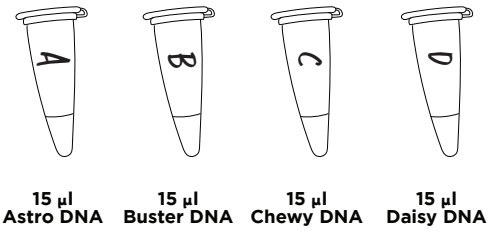
- Thaw tubes containing the DNA samples by placing them on a rack or water bath at room temperature.
- For each lab group, dispense the following reagents into nine labeled microtubes (you can use either 1.7 ml or 0.2 ml tubes).
  - Puppy DNA samples 15 µl each
    - Astro DNA
    - Buster DNA
    - Chewy DNA
    - Daisy DNA
    - Elsa DNA
    - Flora DNA
    - Ginger DNA
    - Hugo DNA

    Note: samples already contain loading dye
  - Fast DNA Ladder 1 15 µl

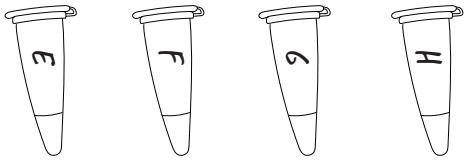
Defrost tubes



Aliquot reagents



15 µl Astro DNA | **15 µl Buster DNA** | **15 µl Chewy DNA** | **15 µl Daisy DNA**



15 µl Elsa DNA | **15 µl Flora DNA** | **15 µl Ginger DNA** | **15 µl Hugo DNA**

15 µl ladder

✖ **number of groups**

up to 8

B. Distribute supplies and reagents to lab groups

Check	At the start of this experiment, every lab group should have:	Amount
	DNA samples for analysis • Astro DNA • Buster DNA • Chewy DNA • Daisy DNA • Elsa DNA • Flora DNA • Ginger DNA • Hugo DNA	15 µl of each sample
	Fast DNA ladder 1	15 µl
	2-20 µl micropipette or 10 µl fixed volume micropipette	1
	Micropipette tips	9
	9 wells in an electrophoresis gel	

C. Prepare for gel electrophoresis

- Prepare 1X TBE buffer.
  - TBE buffer is often provided as liquid concentrate or powder.
  - Follow manufacturer's instructions to prepare 1X TBE buffer solution.
  - Volume to prepare depends on the method used to prepare gels; see "Important Note" below.
- Gels can be poured in advance of the class.
  - This lab requires running and visualizing DNA samples on a 2% agarose gel with a fluorescent DNA stain (e.g., SeeGreen™ or GelGreen®).
  - Pre-poured gels can be stored at ambient temperature, in a sealed container or wrapped in plastic wrap, and protected from light for up to three days.
- Have the banding pattern of the Fast DNA Ladder 1 handy (page 18) to help interpret the electrophoresis results.

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/

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).

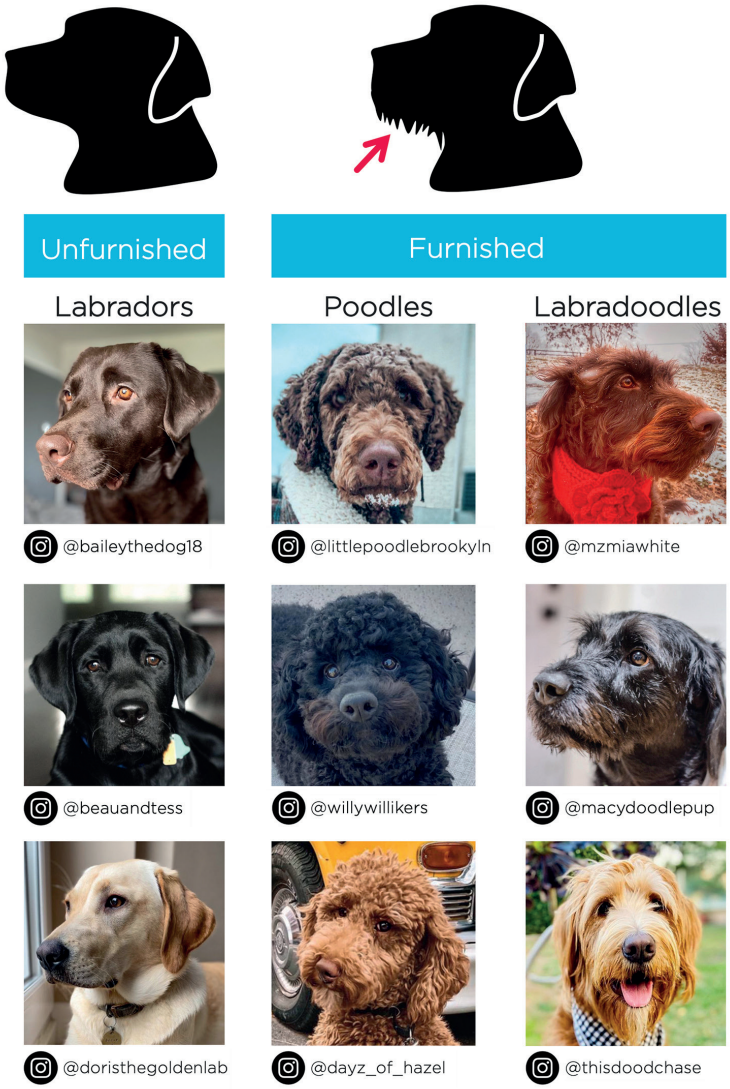


Figure 2. Furnishing in dogs
Furnishings—the presence of a mustache and bushy eyebrows—are found in many dog breeds. Labradors never have furnishings (left column), while Poodles always have furnishings (center column). You may not have seen a Poodle look like this since it is common to clip the hair 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).

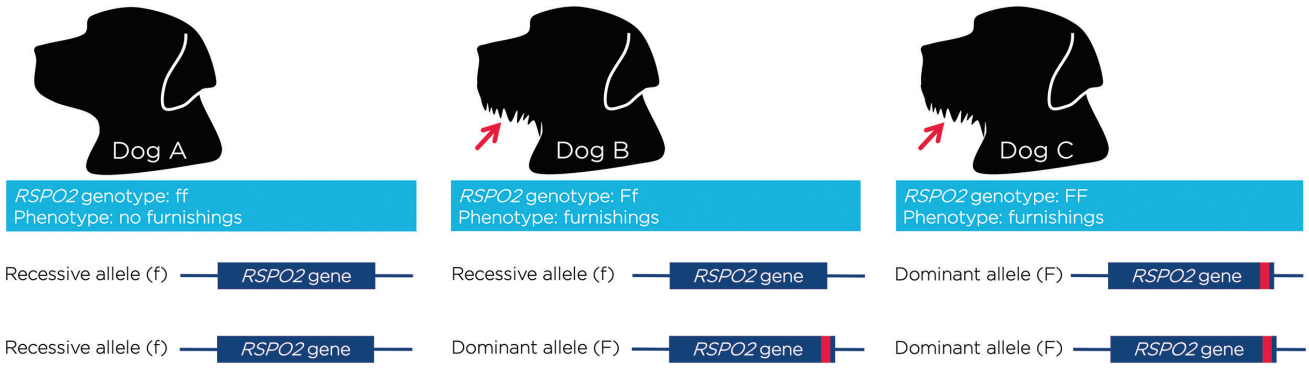


Figure 4. *RSPO2* genotype determines furnishings phenotype in dogs.
Like humans, dogs have two copies of each chromosome, one inherited from each parent. With two alleles for the *RSPO2* gene, there are three possible genotypes: two copies of the recessive allele (ff), two copies of the dominant allele (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/.

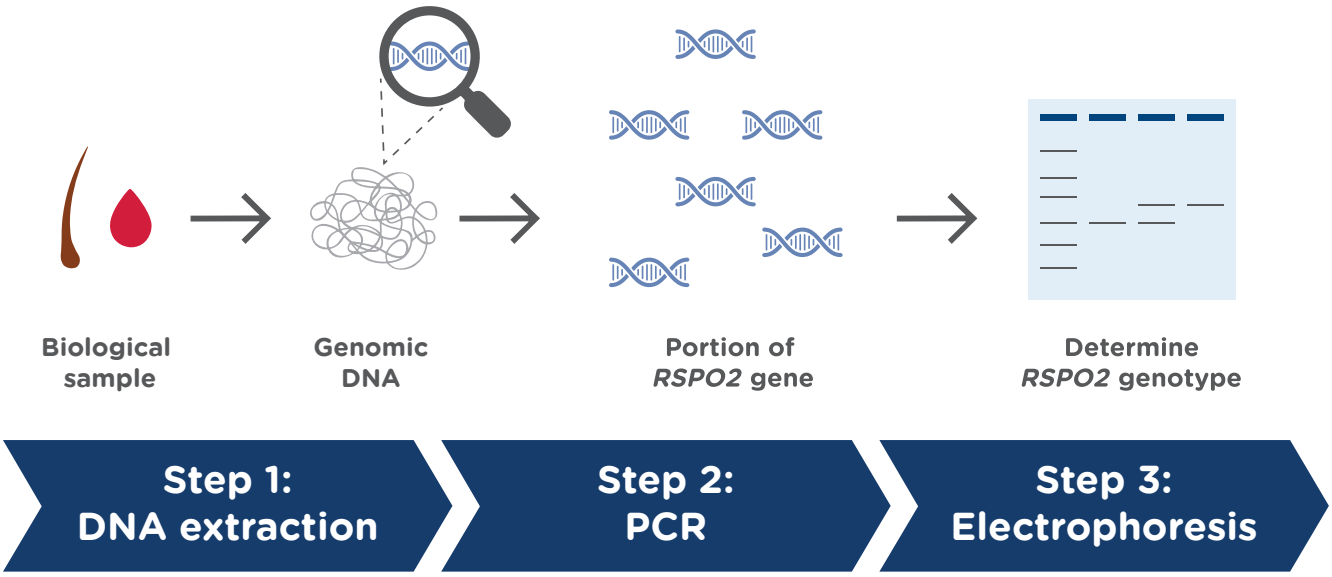


Figure 5. Genotyping dogs for *RSPO2*.
First, DNA is extracted from a biological sample, like blood or a hair follicle. Second, PCR is used to make many copies of just the locations in the genome that the scientists want to analyze. In this lab, that is a small portion of the *RSPO2* gene. Third, because 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.

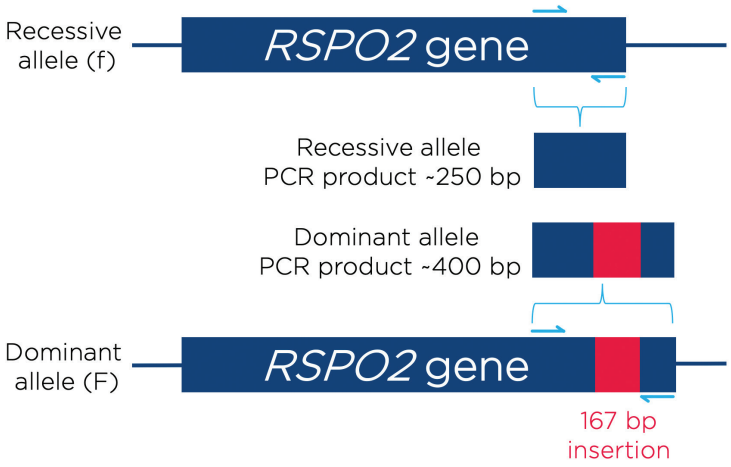


Figure 6. *RSPO2* genotyping PCR.
PCR is used to copy specific regions of DNA. In this experiment, the PCR is designed to copy the region of the *RSPO2* gene that differs between the recessive and dominant alleles (primers are shown in light blue). The reaction will generate a PCR product of approximately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

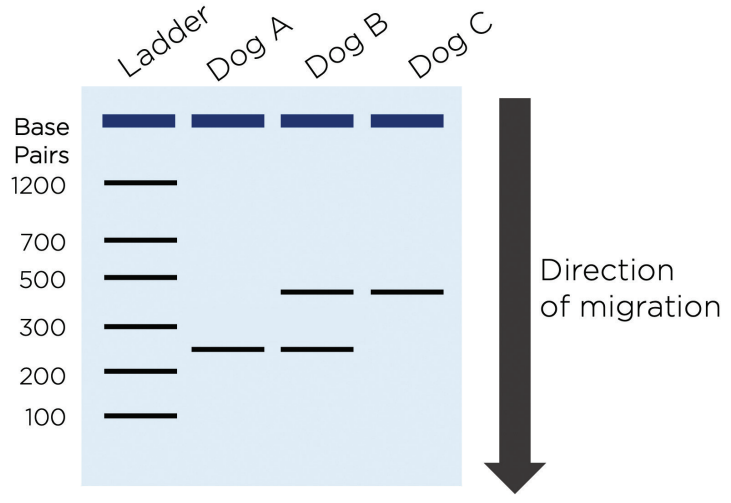


Figure 7. Gel electrophoresis.
After using PCR to copy a region of the *RSPO2* gene from dog DNA, gel electrophoresis can be used to determine the dog's *RSPO2* genotype. Because the dominant allele is 167 bp longer than the recessive allele, it will migrate more slowly on the gel. In this experiment, 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

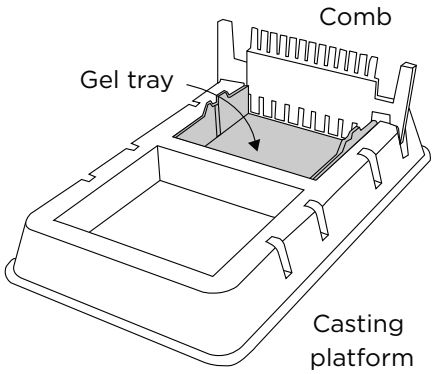
Gels can be prepared up to three days ahead of time and stored at ambient temperature, covered in air-tight plastic wrap and protected from light.

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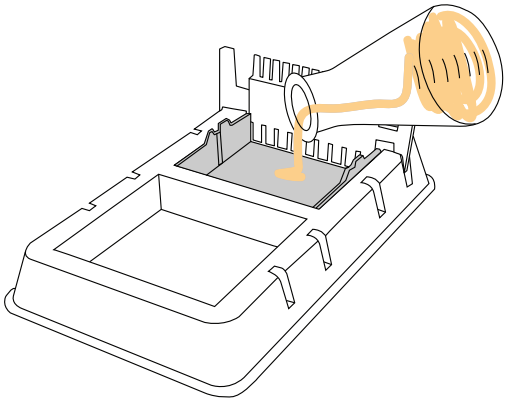
4. **Pour the agarose solution into the prepared casting platform with a gel tray and comb**
    - The agarose solution should cover the bottom of the gel tray and the bottom 3 mm of the comb (roughly the bottom 1/3 of the comb).

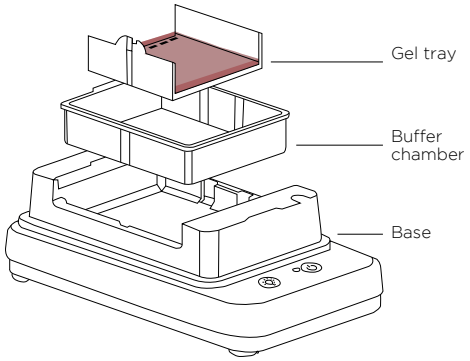
5. **Allow gel to solidify completely and remove the comb by pulling firmly upwards**
    - Gels will typically be ready in about 10 minutes.
    - Gel is ready when cool and firm to the touch.



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

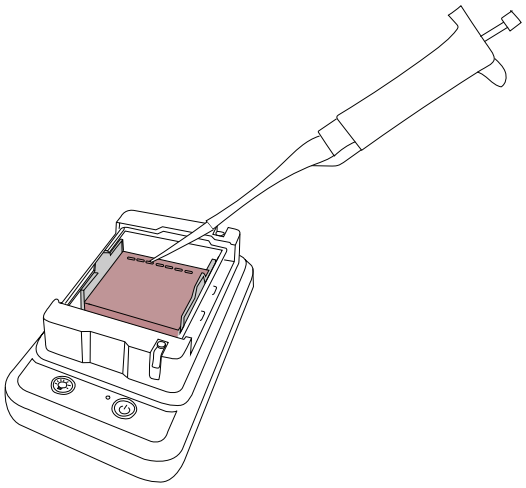
Gel electrophoresis - Running the gel

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.

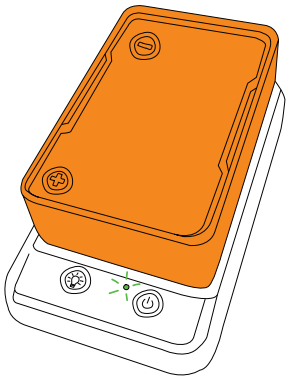
2. **Add 30 ml of 1X TBE electrophoresis buffer**
   - The buffer should just cover the gel and fill the wells.
   - Ensure that there are no air bubbles in the wells (shake the gel gently if bubbles need to be dislodged).



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

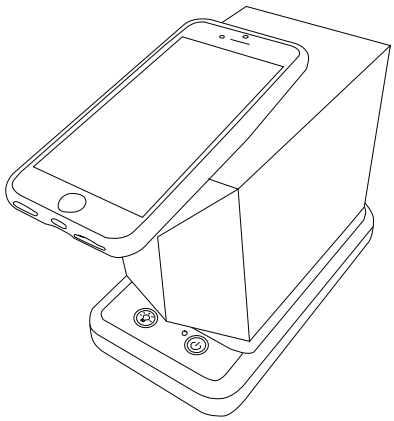
   Note: Change pipette tips between samples to prevent contamination.

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.

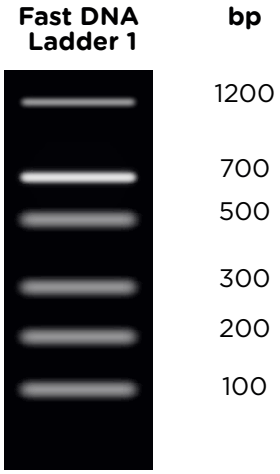


Gel electrophoresis – Visualizing results

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?

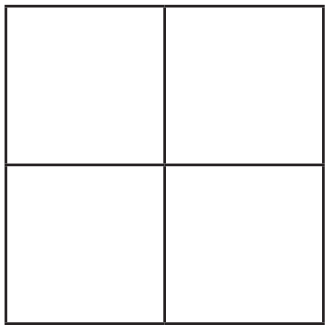
c. Otto, like Molly, is a first-generation Labradoodle. What is Otto's *RSPO2* genotype?

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?

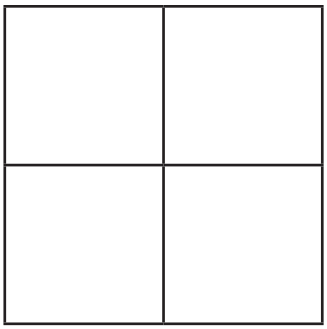
   c. What are the expected <u>phenotypic</u> ratios for a litter of puppies born to Molly and Zeus?

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.**

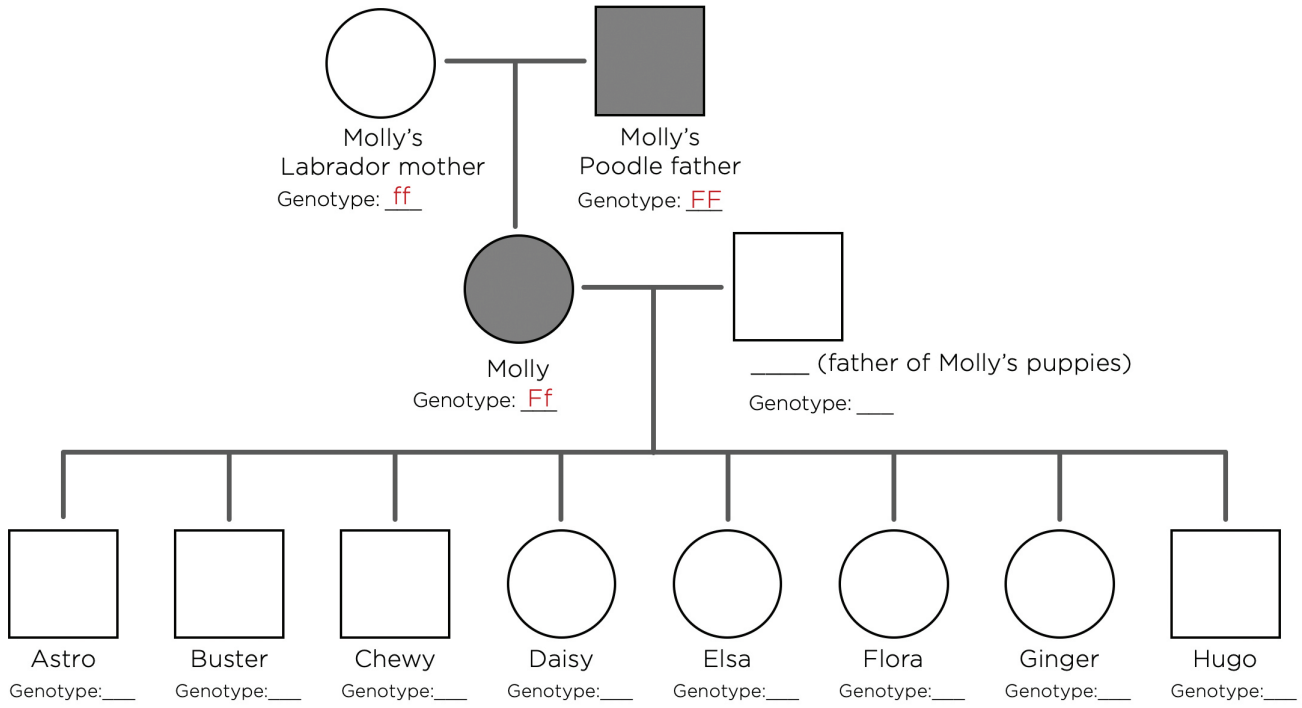
Puppy	Genotype	Predicted phenotype
Astro		
Buster		
Chewy		
Daisy		
Elsa		
Flora		
Ginger		
Hugo		

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.**

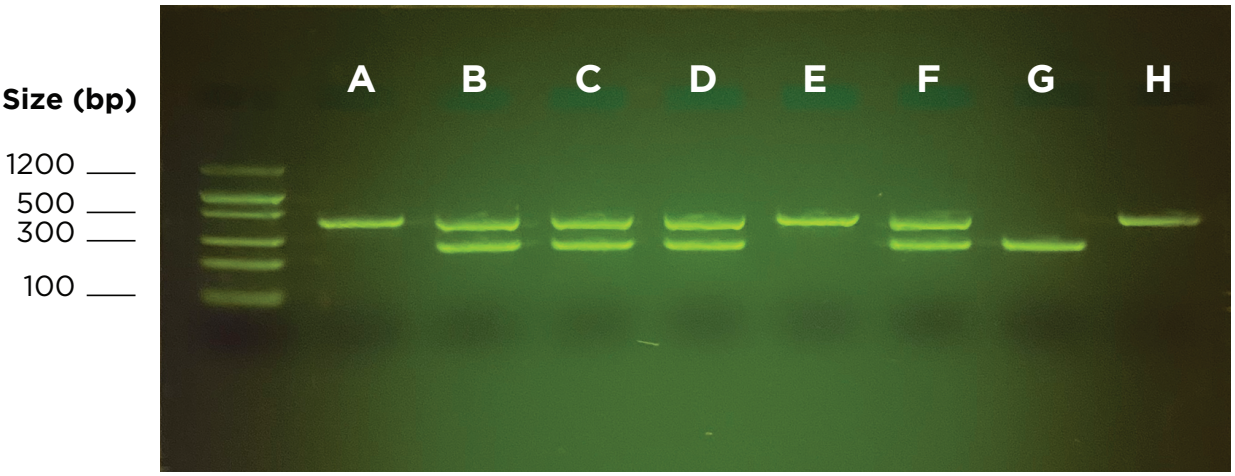
c. **Fur has to be a certain length in order to curl. So even if a dog's genotype for the *KRT71* gene specifies curly fur, if the dog's fur is short then it won't display the curly fur phenotype (Cadieu *et al.*, 2009). Knowing this, what are the actual predicted phenotypic ratios for the cross between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

Instructor's Guide

Expected results

Gel electrophoresis results are expected to resemble the gel image below.



This image represents results obtained after a 15 minute run using a blueGel™

- Astro DNA: ~400 bp band
- Buster DNA: ~400 bp + ~250 bp bands
- Chewy DNA: ~400 bp + ~250 bp bands
- Daisy DNA: ~400 bp + ~250 bp bands
- Elsa DNA: ~400 bp band
- Flora DNA: ~400 bp + ~250 bp bands
- Ginger DNA: ~250 bp band
- Hugo: ~400 bp band

Interpretation

The larger ~400 bp band represents the dominant allele of the *RSPO2* gene and the smaller ~250 bp band represents the recessive allele of the *RSPO2* gene. The puppies' genotypes and predicted phenotypes are shown in the table to the right.

For answers to the lab study questions and extension, email answers@minipcr.com. Please include the name of the lab, as well as your name, school, and title in the body of the email.

Puppy	Genotype	Phenotype
Astro	FF	Furnishings
Buster	Ff	Furnishings
Chewy	Ff	Furnishings
Daisy	Ff	Furnishings
Elsa	FF	Furnishings
Flora	Ff	Furnishings
Ginger	ff	No furnishings
Hugo	FF	Furnishings

Notes on lab design

This lab serves as an introduction to the relationship between genotype and phenotype. We believe our approach provides the right balance between intellectual engagement, inquiry, and accessibility. The design of this lab has simplified certain elements to achieve these goals.

- This lab uses prepared DNA to simulate the results of PCR amplification of a section of the *RSPO2* gene from dog genomic DNA.
- Paternity testing typically analyzes several genetic loci to determine parentage. We simplified the analysis to focus on a single gene with two alleles associated with a clear physical phenotype. While there are a few approaches to using DNA to determine parentage, most paternity testing in humans and dogs mirrors forensic science. It examines regions of the genome that contain variable number short tandem repeats. For more information on this topic, refer to the DNAdots article on DNA fingerprinting (https://dnadots.minipcr.com/dnadots/dna-fingerprinting).
- For simplicity, we omitted a discussion of the specific nature of the genetic difference between the *RSPO2* alleles. However, depending on the level of your class, this information may be appropriate to discuss. The 167 bp insertion in the dominant allele of the *RSPO2* gene is not located in the protein-coding region, but in the 3' untranslated region. Scientists believe that the dominant allele is associated with increased expression of RSPO2 protein (Cadieu *et al.*, 2009).
- We also omitted a discussion of RSPO2 protein function, although depending on your class's level, this information may be appropriate to discuss. The RSPO2 protein is involved in a signaling pathway involved in hair follicle development (Andl *et al*., 2002).
- The dominant allele of the *RSPO2* gene is associated with furnishings, but can also influence a dog's overall coat. In dogs that don't have long fur, having one or two copies of the dominant *RSPO2* allele causes a "wire" coat, which is slightly longer and has a coarser texture (Cadieu *et al*., 2009). Comparing a wire Dachshund with a smooth Dachshund demonstrates this trait. While a dog's *RSPO2* genotype is associated with whether they have wire fur, we chose to omit a discussion of the wire hair phenotype because wire hair does not always accompany furnishings (see next bullet).

Smooth Dachshund

Gene	Genotype
RSPO2	ff
FGF5	AA

Wire Dachshund

Gene	Genotype
RSPO2	FF
FGF5	AA

Note: While dogs with an *RSPO2* genotype of FF or Ff will display furnishings, purebread dogs tend to be homozygous to ensure the inheritance of desired traits.

- As described in the previous bullet, the dominant *RSPO2* allele gives a dog furnishings and wire hair. Dogs with long fur and furnishings are an exception. In dogs with the dominant *RSPO2* allele and two copies of the recessive *FGF5*, furnishings are present, but the fur is long and smooth instead of being wiry (Cadieu *et al*., 2009). This phenotype can be seen in Bearded Collies. This example shows how the relationship between genotype and phenotype can be more complicated than a single gene that always specifies a single trait.

Bearded Collie

Gene	Genotype
RSPO2	FF
FGF5	aa

Note: While dogs with an *RSPO2* genotype of FF or Ff will display furnishings, purebread dogs tend to be homozygous to ensure the inheritance of desired traits.

Before carrying out this lab, students should have basic competence using a micropipette, and should understand the concept of gel electrophoresis. See the Additional Student Supports section of this lab (page 41) for ways to scaffold this assignment for students who may be less comfortable with the aforementioned skills.

Differentiation

This lab serves as an introduction to the relationship between genotype and phenotype. With simple modifications, this activity can be used effectively in classes ranging from middle school through advanced high school.

Introductory classes: Focus on the relationship between *RSPO2* genotype and the furnishings phenotype, and how to genotype using gel electrophoresis. The standard introduction and review questions for this lab take this approach.

Advanced classes: Use the optional advanced questions and extension activity to challenge students to consider the inheritance of more than one gene that affects a dog's coat phenotype.

Additional student supports

At miniPCR bio™, we are committed to preparing students to be successful in the laboratory through high quality curriculum and training. We have created an extensive set of resources to help your students succeed in molecular biology techniques, all of which are available for free download at the miniPCR bio™ tutorials page of our website.

https://www.minipcr.com/tutorials/.

Those activities most relevant to this lab are listed below.

Micropipetting: Video and activity resources to train students in the basic use of a micropipette.

Gel electrophoresis: Video and worksheet activity instructing students on the fundamentals and practice of agarose gel electrophoresis.

PCR: While students do not perform PCR in this lab, the samples they analyze represent PCR products. If you want to discuss PCR in more detail with your students, we have a video and worksheet activity instructing students on the fundamentals and practice of PCR.

Extension activities

The following optional extension activities are provided for students to explore topics more deeply.

Tracking the inheritance of multiple genes (page 30): If your class has covered dihybrid crosses, this activity allows students to practice with a dog genetics example. Explore the inheritance of two genes that both affect a dog's coat phenotype (long vs. short fur and straight vs. curly fur).

Suggested reading: Advanced students can explore the scientific research that this lab is based upon:

Cadieu, E., Neff, M.W., Quignon, P., Walsh, K., Chase, K., Parker, H.G., VonHoldt, B.M., Rhue, A., Boyko, A., Byers, A., et al. (2009). Coat variation in the domestic dog is governed by variants in three genes. Science 326, 150–153.

Placement in unit

Genetics

This lab can be used to discuss Mendelian genetics and gives a real-world example of the relationship between genotype and phenotype.

- While the lab focuses on dog genetics, it can be used as a jumping-off point to discuss other examples of the relationship between genotype and phenotype in humans. The miniPCR® Sickle Cell Genetics Lab (https://www.minipcr.com/product/sickle-cell-genetics-lab/) is a gel electrophoresis activity that allows students to dive deeper into the connection between mutations and protein structure in the context of human disease.
- Once students have mastered their molecular biology skills with this lab, you can continue with the miniPCR® Plant Genetics Lab (https://www.minipcr.com/product/plant-genetics-lab-brassica-rapa/), where students test plants using PCR and gel electrophoresis to correlate genotype and phenotype. You may also continue with the miniPCR® PTC Taster Lab (https://www.minipcr.com/product/minipcr-genotype-to-phenotype-ptc-taster-lab/), where students use PCR-RFLP to analyze their own DNA to correlate their own genotype with their ability to taste a bitter chemical.

Biotechnology

This lab can be used as a culminating activity to demonstrate mastery of micropipetting and gel electrophoresis. For this approach, we recommend spending more time on some of the activities discussed in the Additional student supports section of this lab (page 41) before beginning the experiment.

Learning goals and skills developed

Student Learning Goals:

- Correlate genotype and phenotype
- Predict genotype and phenotype using Punnett squares
- Apply basic probability rules to genetic analysis
- Solve real-world problems using genetic analysis

Scientific Inquiry Skills:

- Students will use experimental results to make conclusions and solve a real-world problem
- Students will follow detailed experimental protocols
- Students will make a claim based in scientific evidence
- Students will use reasoning to justify a scientific claim
- Students will follow laboratory safety protocols

Molecular Biology Skills:

- Micropipetting
- Preparation of agarose gels
- Agarose gel DNA electrophoresis
- Staining, visualization, and molecular weight analysis of DNA fragments

Standards alignment

Next Generation Science Standards

Students who demonstrate understanding can:

HS-LS3-1.	Ask questions to clarify relationships about the role of DNA and chromosomes in coding the instructions for characteristic traits passed from parents to offspring.
HS-LS3-3.	Apply concepts of statistics and probability to explain the variation and distribution of expressed traits in a population.

Science and Engineering Practice	Disciplinary Core Ideas	Crosscutting Concepts
• Asking Questions and Defining Problems • Developing and Using Models • Planning and Carrying Out Investigations • Analyzing and Interpreting Data • Using Mathematics and Computational Thinking • Constructing Explanations and Designing Solutions • Engaging in Argument from Evidence • Obtaining, Evaluating, and Communicating Information	LS1.A: From Molecules to Organisms: Structures and Processes LS3.A: Inheritance of Traits	• Patterns • Cause and Effect • Systems and System Models • Interdependence of Science, Engineering, and Technology

Common Core ELA/Literacy Standards

RST.9-10.1	Cite specific textual evidence to support analysis of science and technical texts, attending to the precise details of explanations or descriptions.
RST.9-10.3	Follow precisely a complex multistep procedure when carrying out experiments, taking measurements, or performing technical tasks, attending to special cases or exceptions defined in the text.
RST.9-10.4	Determine the meaning of symbols, key terms, and other domain-specific words and phrases as they are used in a specific scientific or technical context relevant to grades 9-10 texts and topics.
RST.9-10.5	Analyze the structure of the relationships among concepts in a text, including relationships among key terms (e.g., force, friction, reaction force, energy).
RST.9-10.9	Compare and contrast findings presented in a text to those from other sources (including their own experiments), noting when the findings support or contradict previous explanations or accounts.
WHST.9-10.1	Write arguments focused on discipline-specific content.
WHST.9-10.2	Write informative/explanatory texts, including the narration of historical events, scientific procedures/ experiments, or technical processes.
WHST.9-10.9	Draw evidence from informational texts to support analysis, reflection, and research.

* For simplicity, this activity has been aligned to high school NGSS and grades 9-10 Common Core standards.

Ordering information

To order miniPCR® Dog Genetics Lab kits, you can:

Call (781)-990-8PCR

email us at orders@minipcr.com

visit https://www.minipcr.com

miniPCR® Dog Genetics Lab (catalog no. KT-1506-01) contains the following reagents:

- 8 puppy DNA samples (Astro, Buster, Chewy, Daisy, Elsa, Flora, Ginger, Hugo)
- Fast DNA Ladder 1

Materials are sufficient for 8 lab groups, or 32 students

All components should be kept frozen at -20°C for long-term storage

Reagents must be used within 12 months of shipment

Other reagents needed

- Agarose (electrophoresis grade)
- Fluorescent DNA stain (e.g., SeeGreen™ or GelGreen®)
- Gel electrophoresis buffer (e.g., 1X TBE)
- Distilled or deionized water (to dilute TBE buffer concentrate)

Note: Agarose, DNA stain, and TBE buffer are available at minipcr.com as part of the Learning Lab Companion Kit (KT-1510-01)

About miniPCR bio Learning Labs™

This Learning Lab was developed by the miniPCR bio™ team in an effort to help more students understand concepts in molecular biology and to gain hands-on experience in real biology and biotechnology experimentation.

We believe, based on our direct involvement working in educational settings, that it is possible for these experiences to have a real impact in students' lives. Our goal is to increase everyone's love of DNA science, scientific inquiry, and STEM. We develop Learning Labs™ to help achieve these goals, working closely with educators, students, academic researchers, and others committed to science education.

The guiding premise for this lab is that a ~45-minute electrophoresis-based experiment can give students hands-on experience with genetics and provide the right balance between intellectual engagement, inquiry, and discussion.

Starting on a modest scale working with Massachusetts public schools, miniPCR bio Learning Labs™ have been well received, and their use is growing rapidly through academic and outreach collaborations across the world.