Dog Genetics Lab

Oodles of Labradoodles™

Dog Genetics Lab:
Oodles of Labradoodles™
KT-1506-01

Version: 1.3
Release: April 2025

Contents

At a glance

Lab overview

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

General high school
Advanced high school

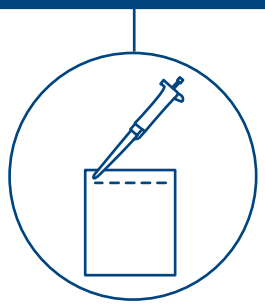
Planning your time

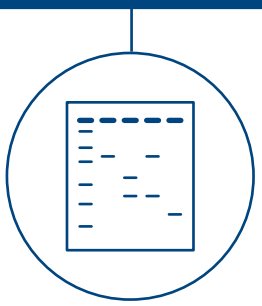
SINGLE CLASS: 45 min.

See the next page for detailed class time requirements

Teacher prep (30 min.) → **Gel electrophoresis** → **Interpret results**

See page 7 for details. We recommend that the teacher prepare the gels before class.





Technical support

If you have any questions about implementing this activity, contact support@minipcr.com.

Class time requirements

This activity can be completed in a 45-minute class period if the gels have been prepared in advance.

Steps		Time required
Prep	Make gels	We recommend the teacher prepare the gels outside of class (see page 7). Allot 30 minutes of class time if you opt to have students prepare the gels.
1	Load gel	10 minutes
2	Run gel	15-25 minutes The gel does not need to be actively monitored during this time.
3	Interpret results	5 minutes

Materials needed

Supplied in kit (KT-1506-01)

- Kit contains DNA samples for eight lab groups.
- If kept in the freezer, reagents can be stored for 12 months after receipt. If kept in the refrigerator, reagents can be stored for 6 months after receipt.
- Reagents for preparing gels, plastic tubes for distributing samples to individual groups, and pipette tips are sold separately. Refer to the section below for details.

Contents	Provided	Required per group	Storage
Simulated dog 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

Electrophoresis reagents and plastics sold separately

- This lab requires 2% agarose gels with a fluorescent DNA stain (*e.g.,* SeeGreen™ or GelGreen®) and plastic tubes to distribute reagents to individual groups.
- The Learning Lab Companion Kit (KT-1510-01) provides sufficient reagents to prepare and run eight gels when using the blueGel or Bandit electrophoresis systems, as well as plastic tubes to distribute samples to student groups.
- Alternatively, bulk electrophoresis reagents and plastics (tubes, pipette tips) are available for purchase from miniPCR bio.
- Gel electrophoresis reagents and plastics can also be purchased from other suppliers.

Required equipment

- This lab is compatible with any horizontal gel electrophoresis system in combination with:
  - A fluorescent DNA stain (*e.g.,* SeeGreen™ or GelGreen®).
  - A transilluminator that is compatible with the DNA stain used. Fluorescent DNA stains typically require blue light (~470 nm) or UV (~260 nm) illumination.
- The table below outlines gel electrophoresis equipment from miniPCR bio that meets these requirements.

AVAILABLE AT MINIPCR.COM

Item	Recommended quantity
Gel electrophoresis and visualization system Option 1: blueGel™ OR GELATO™ electrophoresis systems with integrated blue light transilluminator	1 blueGel per group 1 GELATO can be shared by two groups
Option 2: Bandit™ STEM electrophoresis kit paired with the Viewit™ Illumination Kit	1 Bandit + 1 Viewit per group
Option 3: Bandit™ STEM electrophoresis kit paired with a blueBox™ blue light transilluminator	1 Bandit per group + 1 blueBox for the class to share
Micropipettes and tips 2-20 µl adjustable or 10 µl fixed volume	1 pipette per group

Other materials supplied by user

- Distilled water
- Microwave or hot plate
- Heat-resistant flask or beaker
- Disposable laboratory gloves
- Protective eyewear
- Fine-tipped permanent marker

Teacher prep

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

Overview

The table below provides an overview of the teacher prep, and the subsequent pages provide detailed instructions.

Prep	Time required	Timeline
Dispense reagents	10 minutes	Can be completed up to one week before before use.
Prepare electrophoresis buffer and agarose gels	20 minutes	Varies - If using gel reagents from miniPCR, gels can be prepared up to five days before use.

Dispense reagents

- DNA samples can be dispensed up to one week in advance and stored in the refrigerator until use.
- This kit provides sufficient reagents for eight lab groups.

Materials needed

From the lab kit (stored in the freezer):

- Dog DNA samples
  - Astro DNA
  - Buster DNA
  - Chewy DNA
  - Daisy DNA
  - Elsa DNA
  - Flora DNA
  - Ginger DNA
  - Hugo DNA
- Fast DNA Ladder 1

Supplied by user:

- Plastic tubes for dispensing reagents. 1.5 ml or 0.2 ml tubes can be used
- 2-20 µl micropipette and tips
- Fine-tipped permanent marker

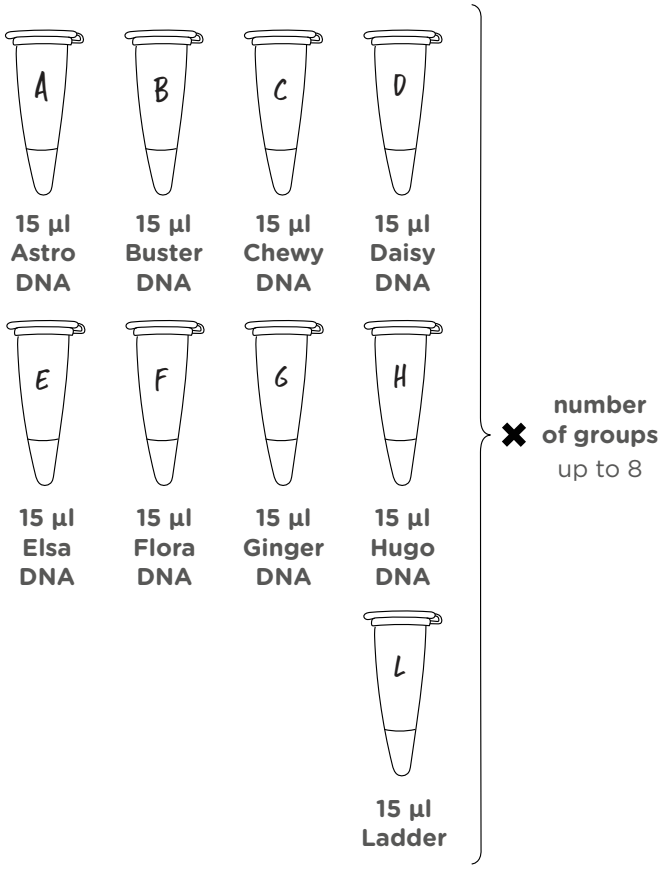
1. Thaw reagents by placing tubes at room temperature.

2. Collect the liquid at the bottom of each tube. Either spin briefly in a microcentrifuge or shake the liquid down with a flick of the wrist.

3. When you open each tube, check for liquid stuck inside the cap. If necessary, put the cap back on and repeat step 2.

4. For each lab group, dispense the following reagents into labeled plastic tubes. 1.5 ml or 0.2 ml plastic tubes can be used.

- Astro DNA (label tube as “A”)	15 µl
- Buster DNA (tube B)	15 µl
- Chewy DNA (tube C)	15 µl
- Daisy DNA (tube D)	15 µl
- Elsa DNA (tube E)	15 µl
- Flora DNA (tube F)	15 µl
- Ginger DNA (tube G)	15 µl
- Hugo DNA (tube H)	15 µl
- Fast DNA Ladder 1 (tube L)	15 µl



5. If you are preparing the DNA samples more than 24 hours before class, store the tubes in the refrigerator until use. Dispensed DNA samples can be stored in the refrigerator for up to one week before use.

Prepare gel electrophoresis buffer and agarose gels

1. Prepare electrophoresis buffer.
   - Follow the manufacturer's instructions to prepare buffer solution.
   - The volume of buffer needed varies based on the gel electrophoresis system you are using.
     - For the blueGel and Bandit electrophoresis systems, 600 ml of TBE buffer is sufficient for at least eight gel runs.
     - For other systems, refer to the manufacturer's instructions for:
       (1) The buffer volume needed to prepare agarose gels.
       (2) The buffer volume needed for use as running buffer.

2. Prepare 2% agarose gels with fluorescent DNA stain.
   - Each group will need eight lanes for samples, plus one lane for the ladder.
   - This lab kit is compatible with any molecular grade agarose and fluorescent DNA stain (*e.g.,* SeeGreen™ or GelGreen®).
   - The volume of gel needed varies based on the gel electrophoresis system you are using. Refer to the manufacturer's instructions.
   - If using gel electrophoresis reagents from miniPCR bio, gels can be prepared up to five days in advance. Store prepared gels at room temperature in an airtight container protected from light. Do NOT soak the gels in buffer or wrap them in paper towels.

Detailed instructions for preparing buffer and gels for miniPCR electrophoresis systems

blueGel
https://links.minipcr.com/gelpouring

Bandit
https://links.minipcr.com/BanditDNAgel

Student workstation setup

At the start of this experiment, every lab group should have:	
Dog DNA samples: • Astro DNA • Buster DNA • Chewy DNA • Daisy DNA • Elsa DNA • Flora DNA • Ginger DNA • Hugo DNA	15 µl each
Fast DNA Ladder 1	15 µl
2-20 µl micropipette or 10 µl fixed volume micropipette	1
Micropipette tips	At least 9
Electrophoresis buffer * Volume depends on your electrophoresis system	30 ml TBE if using a blueGel™ or Bandit™
9 wells in a 2% agarose gel with fluorescent DNA stain	

Student guide

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.

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.

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.*

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).

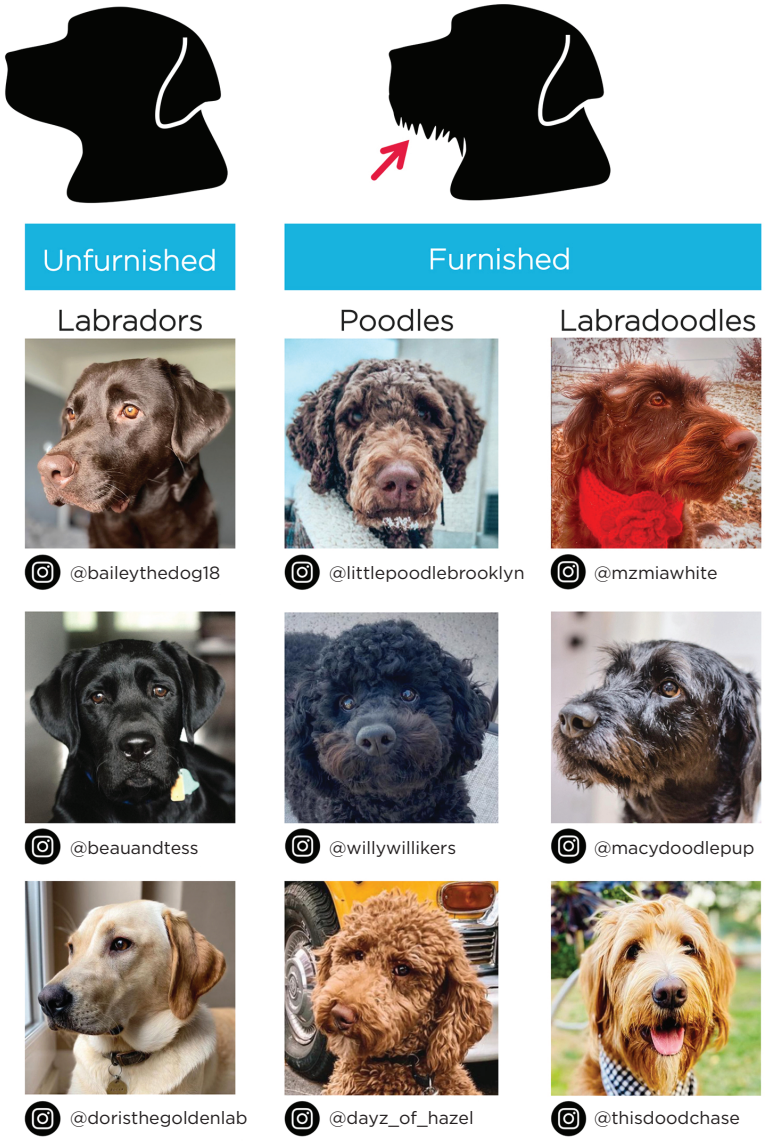


Figure 2. Furnishing in dogs
Furnishings—the presence of a mustache and bushy eyebrows—are found in many dog breeds. Labradors never have furnishings (left column), while Poodles always have furnishings (center column). You may not have seen a Poodle look like this since it is common to clip the hair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

RSPO2 gene

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.

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.

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.

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).

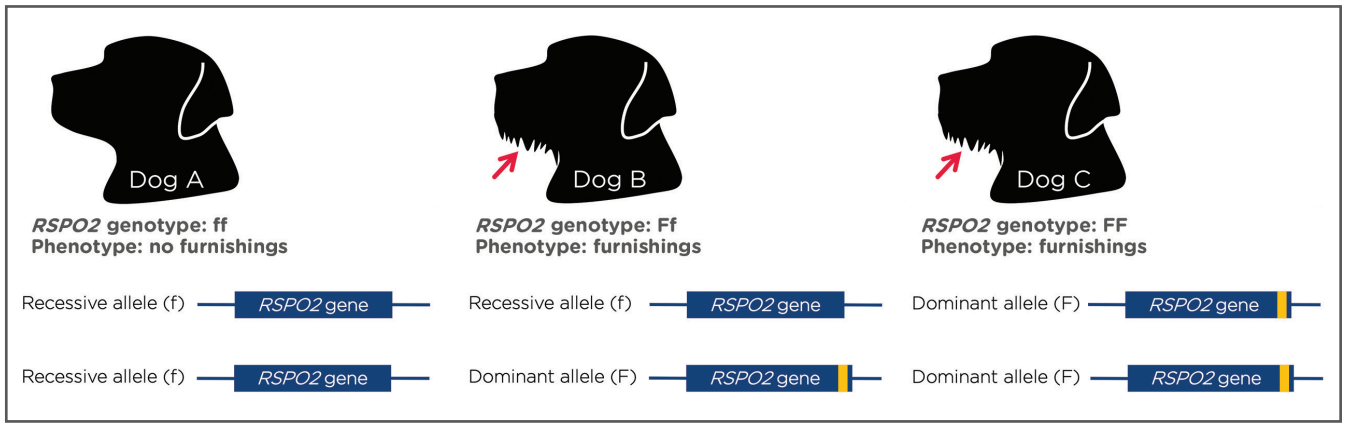


Figure 4. *RSPO2* genotype determines furnishings phenotype in dogs.
Like humans, dogs have two copies of each chromosome, one inherited from each parent. With two alleles for the *RSPO2* gene, there are three possible genotypes: two copies of the recessive allele (ff), two copies of the dominant allele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

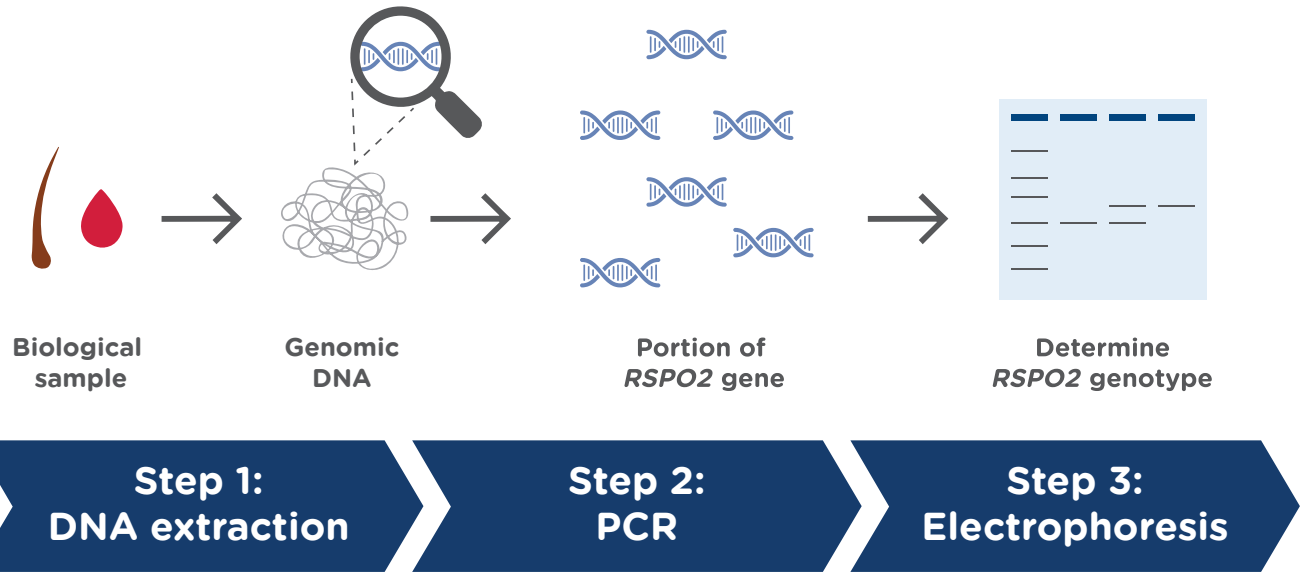


Figure 5. Genotyping dogs for *RSPO2*.
First, DNA is extracted from a biological sample, like blood or a hair follicle. Second, PCR is used to make many copies of just the locations in the genome that the scientists want to analyze. In this lab, that is a small portion of the *RSPO2* gene. Third, because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

Interpreting gel electrophoresis results

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.

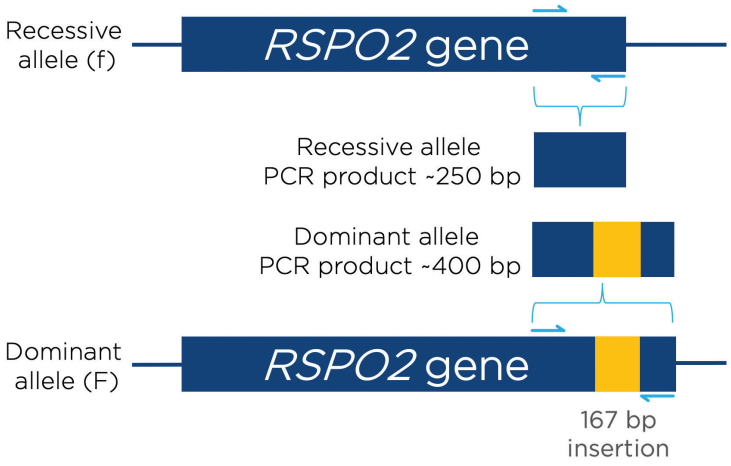


Figure 6. *RSPO2* genotyping PCR.
PCR is used to copy specific regions of DNA. In this experiment, the PCR is designed to copy the region of the *RSPO2* gene that differs between the recessive and dominant alleles (primers are shown in light blue). The reaction will generate a PCR product of approximately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

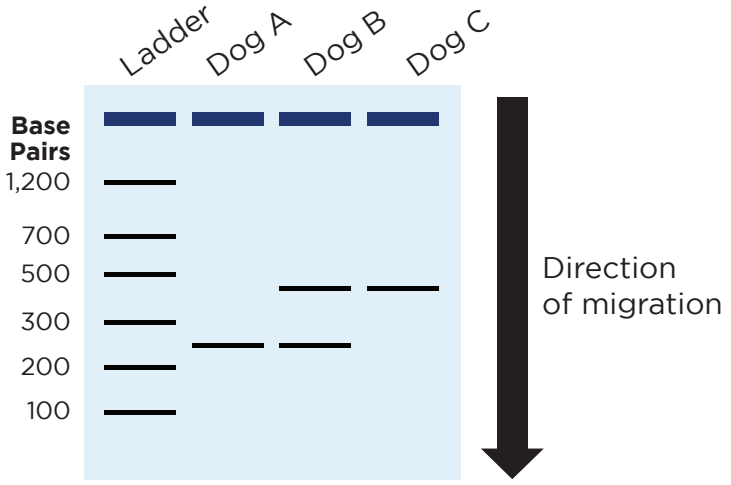


Figure 7. Gel electrophoresis.
After using PCR to copy a region of the *RSPO2* gene from dog DNA, gel electrophoresis can be used to determine the dog's *RSPO2* genotype. Because the dominant allele is 167 bp longer than the recessive allele, it will migrate more slowly on the gel. In this experiment,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

Student lab protocol

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

1. Place the prepared gel into the electrophoresis chamber.

2. Add enough electrophoresis buffer to fill the chamber and just cover the gel.
   - You will need 30 ml of TBE buffer for a blueGel™ or Bandit™ electrophoresis system. Do not overfill the chamber.
   - If using another electrophoresis system, refer to the manufacturer's instructions for the recommended buffer type and volume.

3. Use a micropipette to load samples in the following order. To prevent contamination, use a new tip for each sample.
   - Well 1: 10 µl Fast DNA Ladder 1
   - Well 2: 10 µl Astro DNA
   - Well 3: 10 µl Buster DNA
   - Well 4: 10 µl Chewy DNA
   - Well 5: 10 µl Daisy DNA
   - Well 6: 10 µl Elsa DNA
   - Well 7: 10 µl Flora DNA
   - Well 8: 10 µl Ginger DNA
   - Well 9: 10 µl Hugo DNA

Detailed operating instructions for miniPCR electrophoresis systems

blueGel
https://links.minipcr.com/blueGelRun

Bandit
https://links.minipcr.com/BanditViewit

4. Run the gel for 15-25 minutes.
   - The blueGel™ and Bandit™ electrophoresis systems run at a fixed voltage.
   - If using another gel electrophoresis system, set the voltage in the 70-90 V range.

5. To visualize the DNA samples, turn on the blue light in your electrophoresis system, or move the gel to a transilluminator.

6. If needed, continue to run the gel until there is sufficient separation between the 200-500 bp bands in the ladder to interpret the results.

7. If desired, take a photo to document the results.

8. Compare the bands from the DNA samples to the Fast DNA Ladder 1 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

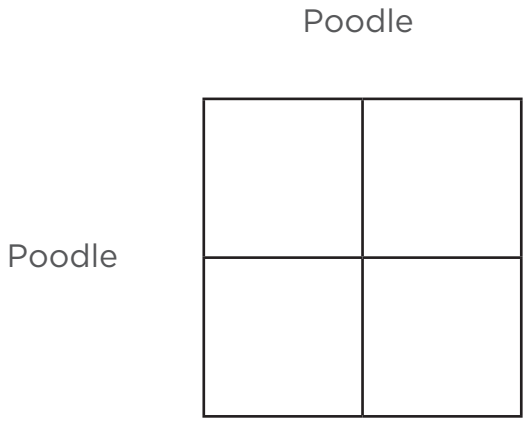
   c. Ff

Critical thinking

10. 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.

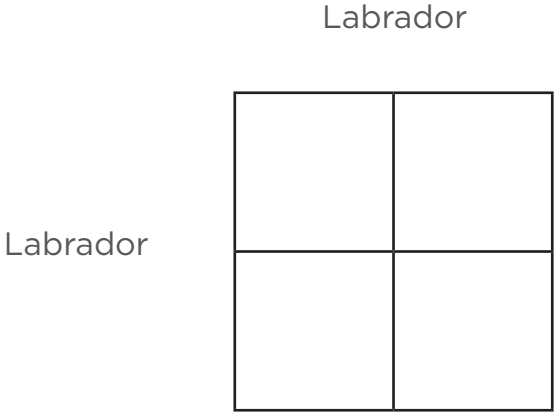
Cross: _____ (Poodle genotype) x _____ (Poodle genotype)



11. 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.

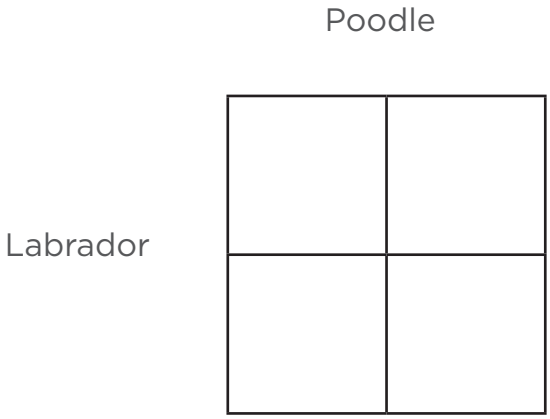
    b. Support your claim by filling out the Punnett square for a cross between two Labradors.

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



12. 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



    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?

13. 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?

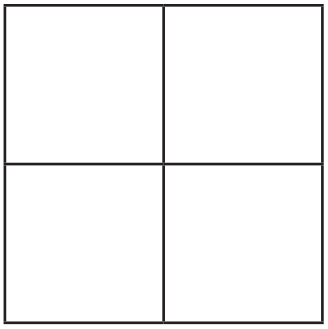
c. Otto, like Molly, is a first-generation Labradoodle. What is Otto's *RSPO2* genotype?

14. 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?

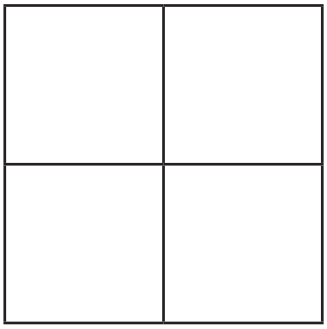
c. What are the expected <u>phenotypic</u> ratios for a litter of puppies born to Molly and Zeus?

15. 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?

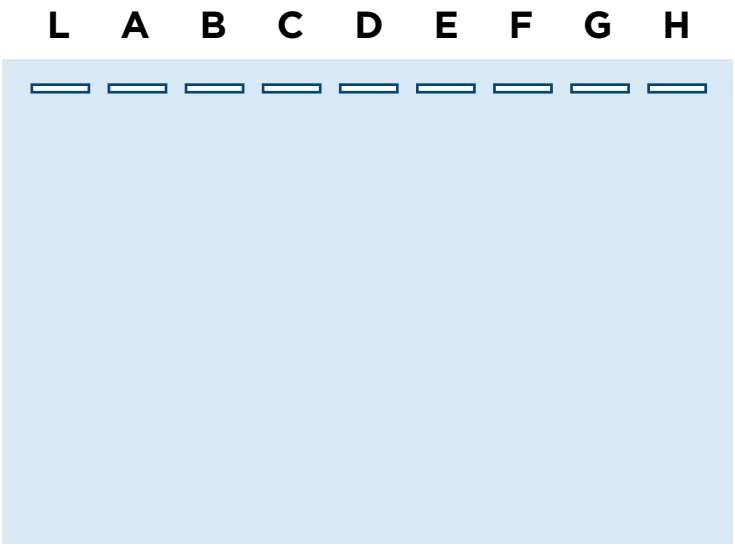
    c. What are the expected phenotypic ratios for a litter of puppies born to Molly and Otto?

Post-lab study questions

Interpreting results

1. Use the image on the right to draw what your gel looks like. For each sample, draw the bands that you see on your actual gel.

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



3. Use your gel electrophoresis results to complete the table below.
   a. Use checkmarks to record the gel electrophoresis results in the first two rows of the table.
   b. Use the results to determine each puppy's genotype and predicted phenotype, and record that in the third and fourth rows of the table.

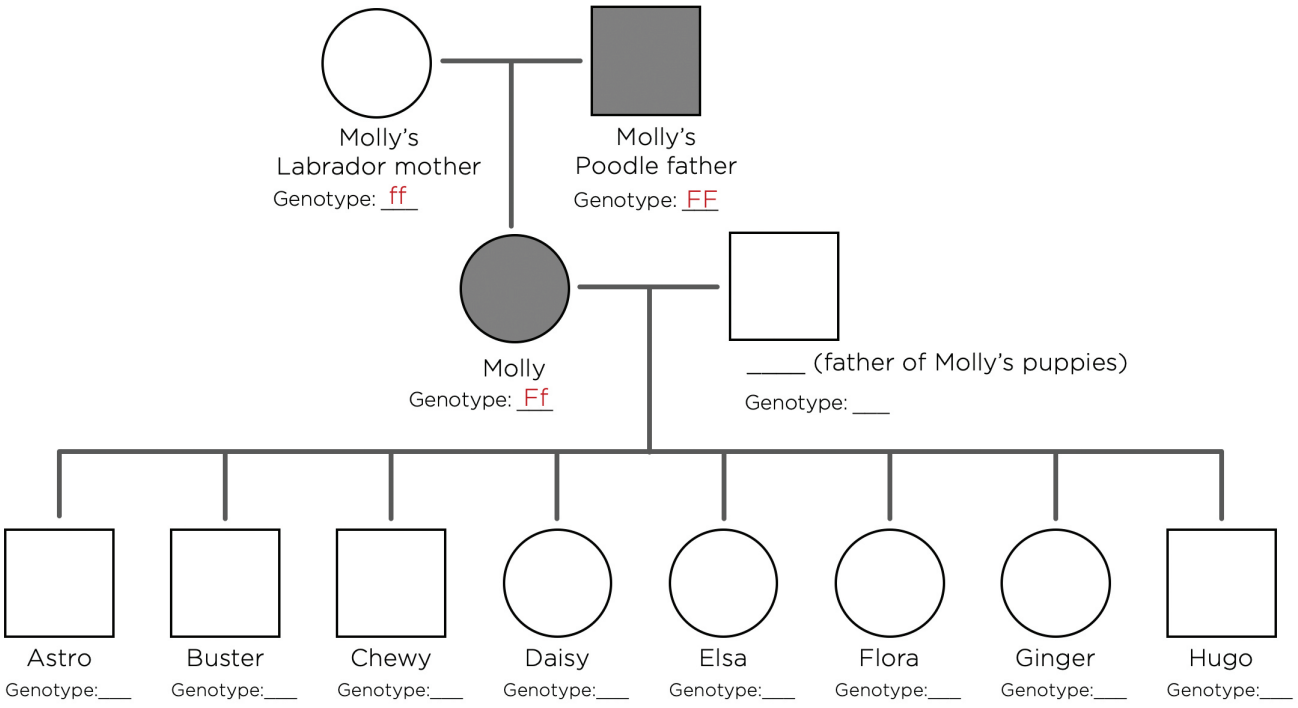
	Astro	Buster	Chewy	Daisy	Elsa	Flora	Ginger	Hugo
Dominant F allele (400 bp)								
Recessive f allele (250 bp)								
RSPO2 genotype (FF/Ff/ff)								
Predicted phenotype (Furnishings/ no furnishings)								

Critical thinking

4. 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 14 and 15.

Advanced questions

5. 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.



6. 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.

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.

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

Extension: 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: _____ (Labradoodle genotype) x _____ (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.

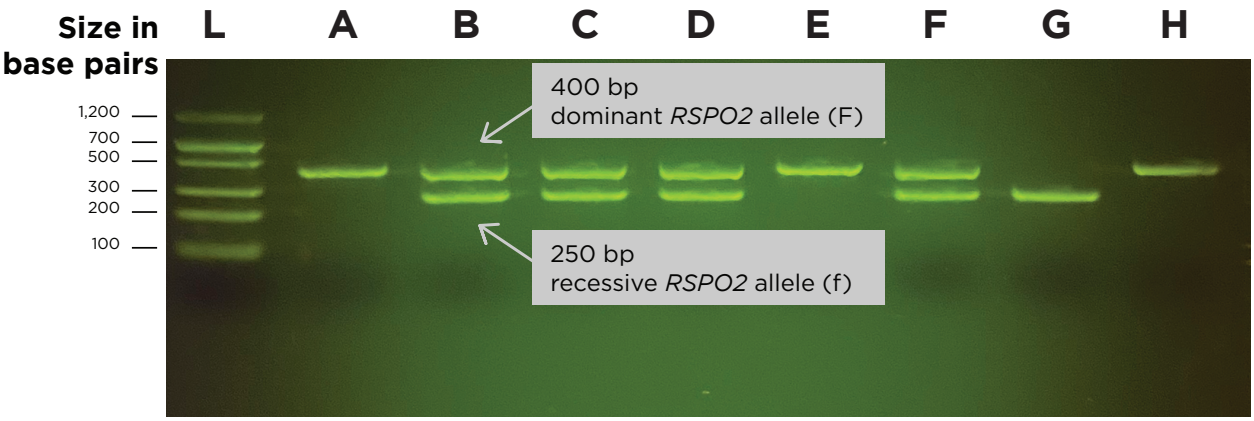
c. Fur has to be a certain length in order to curl. So even if a dog's genotype for the *KRT71* gene specifies curly fur, if the dog's fur is short then it won't display the curly fur phenotype (Cadieu *et al.,* 2009). Knowing this, what are the actual predicted phenotypic ratios for the cross between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

Expected results

Gel electrophoresis results are expected to resemble the photo below.



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

Results and interpretation

	Astro	Buster	Chewy	Daisy	Elsa	Flora	Ginger	Hugo
Dominant F allele (400 bp)	✓	✓	✓	✓	✓	✓		✓
Recessive f allele (250 bp)		✓	✓	✓		✓	✓	
RSPO2 genotype (FF/Ff/ff)	FF	Ff	Ff	Ff	FF	Ff	ff	FF
Predicted phenotype (Furnishings/ no furnishings)	Furnish	Furnish	Furnish	Furnish	Furnish	Furnish	No Furnish	Furnish

For technical support, contact **support@minipcr.com**

For answers to the student questions, email **answers@minipcr.com**
Please include in the body of the email:

- The name of the lab
- Your name, school, and job title

Unexpected results and troubleshooting

If fluorescent DNA bands are not visible on the gel, the following may have occurred:

- Failure to use a fluorescent DNA stain: This lab requires agarose gels made with a fluorescent DNA stain (*e.g.,* SeeGreen™ or GelGreen®). DNA stains that reveal DNA with a visible blue compound are less sensitive and are not compatible with this lab kit.
- Incorrect visualization conditions: Fluorescent DNA stains (*e.g.,* SeeGreen™ or GelGreen®) must be viewed using a blue light or UV transilluminator. The blueGel electrophoresis system has an integrated blue light transilluminator. For DNA visualization, ensure that you have turned on the blueGel's blue light by pressing the light bulb button and that the orange lid is in place.
- Samples were run off the gel: If you run the gel too long, the DNA samples will migrate the entire length of the gel and off the far end. You should always monitor the progress of your gel run by occasionally visualizing the DNA samples using a transilluminator or tracking the migration of the loading dye. The DNA samples contain colored dyes that migrate through the gel and are visible to the naked eye. Stop the gel run before the loading dye reaches the end of the gel.
- Reagents were stored improperly and/or are expired: DNA samples can be stored in the freezer for up to twelve months after receipt or in a refrigerator for six months. Storage under different conditions or in excess of this guidance may impair performance.

If some or all of the bands appear faint, the following may have occurred:

- Failure to load the DNA samples: Loading DNA samples for gel electrophoresis takes a little practice. The bands will appear faint if students do not successfully deposit the full sample volume into the well. Refer to https://www.minipcr.com/how-to-load-a-gel-electrophoresis/ for gel loading tips.
- Non-optimal visualization conditions: Dimming the lights in the room can make the fluorescent DNA stain easier to see in the transilluminator. If using the blueGel, viewing the gel using the Fold-a-View documentation hood and a smartphone camera will provide the best results.
- Old or improperly stored gels: Agarose gels can generally be prepared in advance, but the storage time and conditions depend on the fluorescent DNA stain being used. If using SeeGreen™ or GelGreen® DNA stain, gels can be prepared up to five days in advance. Store gels at room temperature in an airtight container protected from light. Do not soak the gels in buffer or wrap them in paper towels.
- Old or incorrectly prepared buffer: Gel visualization defects not directly ascribable to other causes may be remedied by using freshly prepared buffer compatible with your gel electrophoresis system. If using the blueGel, TBE buffer is recommended.

For tips on picture-perfect gels, see https://www.minipcr.com/gel-electrophoresis-troubleshooting/.

For additional technical support, contact support@minipcr.com.

Notes on lab design

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

- 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.

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.

Bearded Collie

Gene	Genotype
RSPO2	FF
FGF5	aa

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

Citations

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.

Andl, T., Reddy, S.T., Gaddapara, T., and Millar, S.E. (2002). WNT Signals Are Required for the Initiation of Hair Follicle Development. *Developmental Cell* 2, 643–653. https://doi.org/10.1016/S1534-5807(02)00167-3.

Additional student supports

E-worksheets: The student questions accompanying this lab are available for download here as editable text documents you can customize and upload to your LMS. E-worksheets can also be accessed from the Curriculum Downloads tab at https://www.minipcr.com/product/dog-genetics-lab/.

miniPCR tutorials: Access an extensive set of free resources to help your students succeed in molecular biology techniques. Visit https://www.minipcr.com/tutorials/. The resources 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.

miniPCR Digital: Interactive tools for experiment-based learning with or without hands-on lab kits. Visit https://digital.minipcr.com/.

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).

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:

- Identify or pose a testable question
- Formulate hypotheses
- Follow detailed experimental protocols
- Use data to evaluate a hypothesis
- Make a claim based in scientific evidence
- Use reasoning to justify a scientific claim

Molecular biology skills:

- Micropipetting
- Principles of PCR
- Agarose gel electrophoresis

Standards alignment

The standards alignment document for this activity is available for download here. This document can also be accessed from the Curriculum Downloads tab at https://www.minipcr.com/product/dog-genetics-lab/.

This activity is aligned to the following standards:

- Next Generation Science Standards: High School Life Science
- Advanced Placement Biology
- Texas Essential Knowledge and Skills: Biology
- Texas Essential Knowledge and Skills: Biotechnology
- Biotechnician Assistant Credentialing Exam
- Common Core ELA/Literacy Standards (9-10)

For additional information on alignment to state standards, please contact support@minipcr.com.