



Dye Electrophoresis Lab



Cat Genetics



Student's Guide



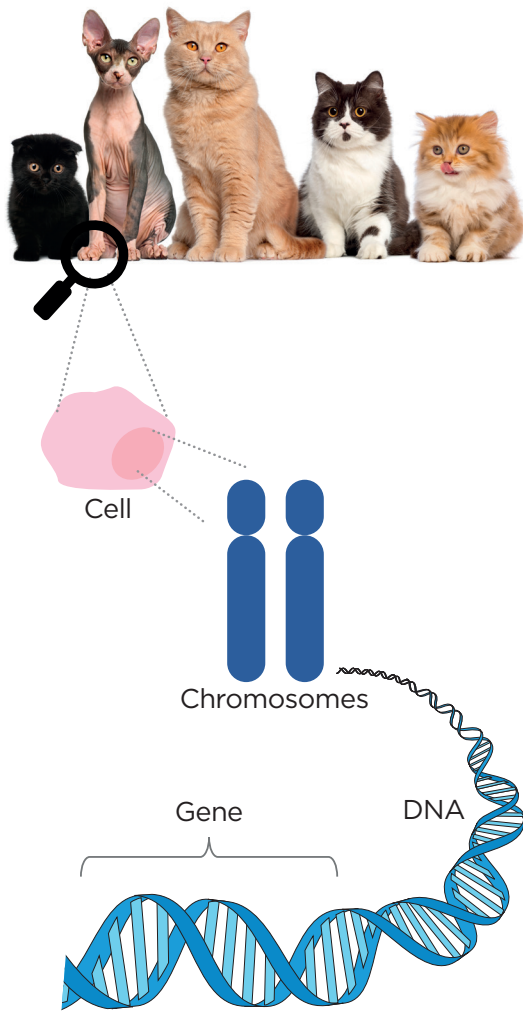
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Background information

DNA and genes

You know that cats come in many shapes, sizes, and colors. But have you ever wondered why cats can look so different from each other? Much of what determines what a cat will look like is **inherited**, or passed from parents to offspring, through DNA.



1

- **DNA** contains the instructions for the cell.
- DNA is found in structures called **chromosomes**.

2

- A **gene** is a section of DNA that contains a specific instruction for the cell.
- Like humans, cats have two copies of each chromosome, one inherited from each parent.
- Because of this, cats have two copies of each gene, one inherited from each parent.

3

- Cats have 19 pairs of chromosomes (38 total) that carry all their genes.
- The instructions in a cat's genes determine its inherited traits, including some of the cat's appearance.
- Today we will focus on a specific inherited trait related to a cat's fur color.



Background: Stop and think

- Q1. Human cells have 23 pairs of chromosomes. How does this compare to cats?
- Humans have fewer chromosomes because cats have 38 total.
 - Humans have more chromosomes because cats only have 19 pairs of chromosomes.
 - It's impossible to know from the information given.



Alleles

1

- Some cats have patches of white fur. This pattern is called **white spotting**.
- White spotting is inherited, so we know that a cat's genes determine if it will have white spotting.

2

- Genes can come in different versions. The different versions have differences in the DNA.
- We call different versions of the same gene **alleles** (pronunciation: "uh-leel").
- Whether or not a cat has white spotting depends on which alleles they inherit from their parents.

White spotting



Chromosome pair



Dominant
B allele

Recessive
b allele

No White spotting



Chromosome pair



Recessive
b allele

Recessive
b allele

3

- People have been breeding cats for a long time. From this, we already know there are two alleles of the gene that controls white spotting in cats.
- The white spotting allele is **dominant**. If a cat has one copy of the dominant allele, the cat will have white spotting, no matter what the other allele is.
- The no-white spotting allele is **recessive**. If a cat does not have any white spotting, it means that both alleles must be the no-white spotting allele.
- We will use a capitalized "B" to represent the dominant allele and a lowercase "b" to represent the recessive allele.



Background: Stop and think

Circle the word(s) that complete each sentence:

Q2. (Alleles/DNA molecules) are different versions of the same (gene/chromosome).

Q3. White spotting in cats is (dominant/recessive). If a cat has Bb alleles, then the cat will (have white spotting/not have white spotting).



Investigating the *KIT* gene in cats

1

- Scientists know that in animals such as horses, white spotting is controlled by a gene called *KIT*.
- Cats also have the *KIT* gene and scientists think it might control white spotting too.
- Even though we have known for a long time that white spotting is dominant, it can be difficult to link differences in traits to the actual differences in the DNA of specific genes.

2

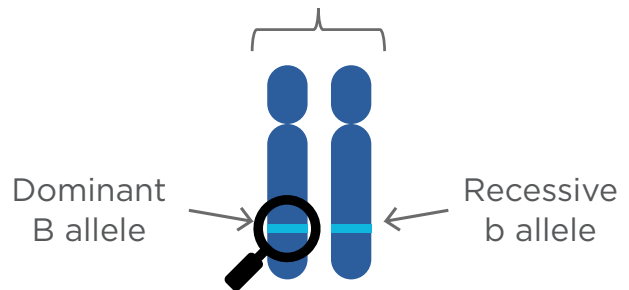
- In cats, there are two alleles of the *KIT* gene that scientists suspect control white spotting.
- When comparing the two *KIT* alleles, one allele is longer than the other; in other words, it contains extra DNA. Otherwise the alleles are identical.
- We'll call these two alleles the "long allele" and the "short allele".
- But we don't know which allele (long or short) causes white spotting.

3

Today you will test DNA from cats to determine which allele of the *KIT* gene is the dominant white spotting allele!



Chromosome pair



Short allele of *KIT* gene

or

Long allele of *KIT* gene

Which allele of the *KIT* gene is the dominant white spotting allele?



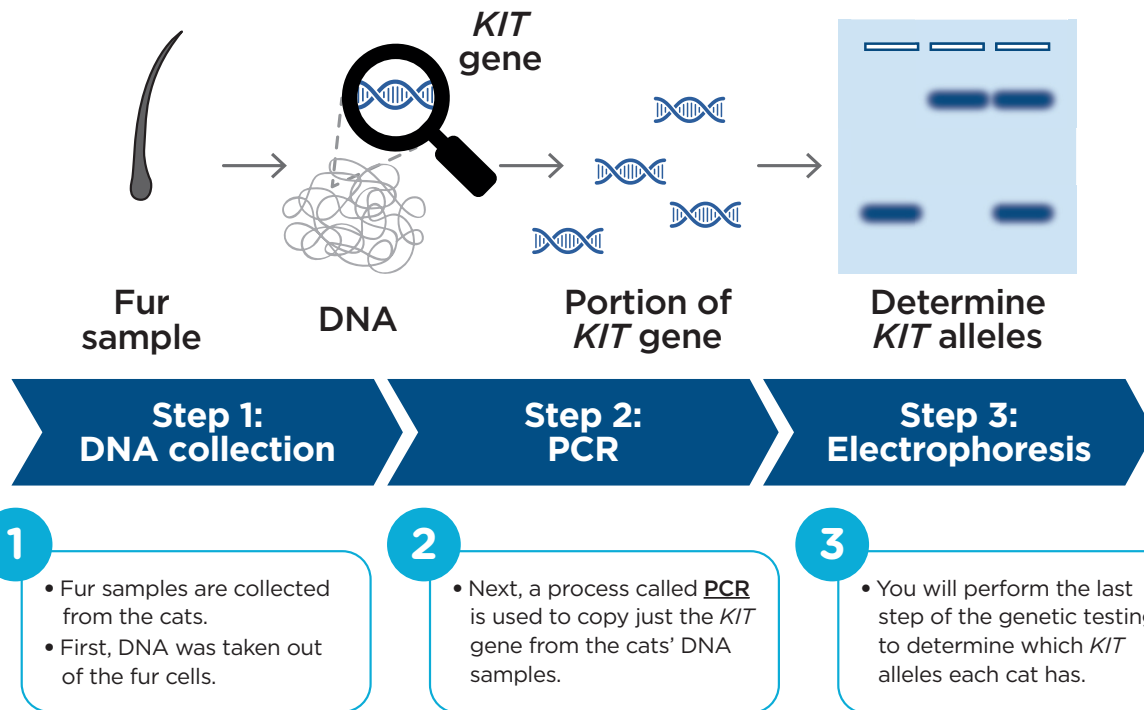
Background: Stop and think

Q4. Describe the difference between the two alleles of the *KIT* gene that scientists suspect controls white spotting in cats.



Genetic testing workflow

DNA testing involves multiple steps. To study the cats' *KIT* alleles, their DNA samples will be tested as follows.

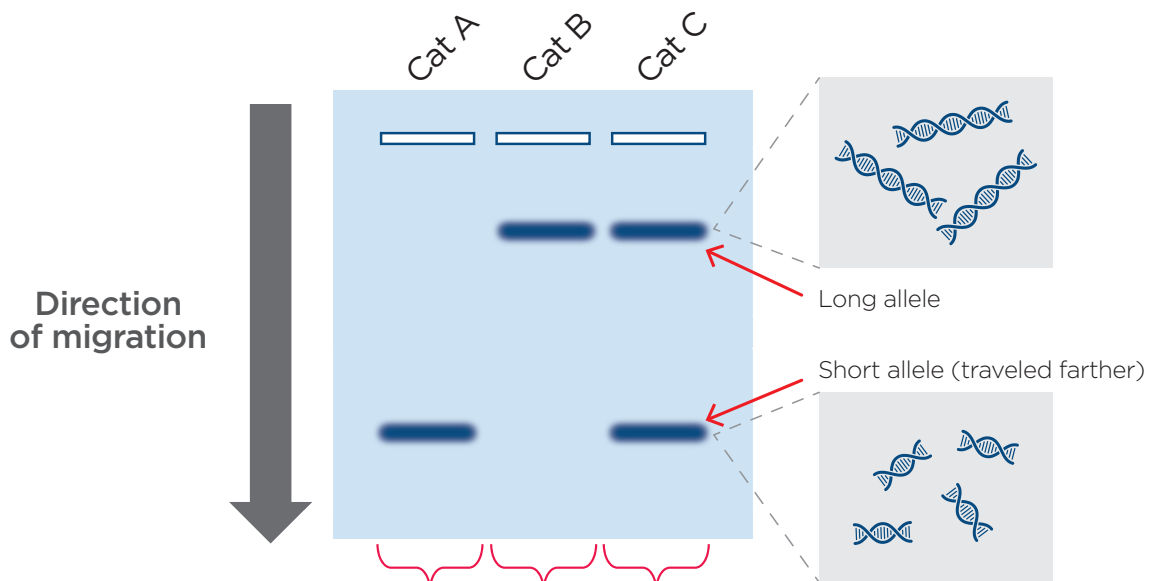




Interpreting gel results

1

- You will use gel electrophoresis to study the *KIT* gene in cats.
- Gel electrophoresis** separates pieces of DNA by size. Smaller pieces of DNA travel farther through the **agarose gel**.
- Because the alleles of the *KIT* gene in cats are different lengths, you can use gel electrophoresis to identify which *KIT* alleles a cat has.
- For a detailed explanation of gel electrophoresis, refer to <https://www.minipcr.com/tutorials/>



2

- Cat A only has one band. This tells us that all of the DNA was the same size.
- Because the DNA traveled farther, we know for Cat A, both chromosomes have the short *KIT* allele.

3

- Cat B only has one band. This tells us that all of the DNA was the same size.
- Because the DNA traveled less far, we know for Cat B, both chromosomes have the long *KIT* allele.

4

- Cat C has two bands. This tells us that the DNA was two sizes.
- Because Cat C has both bands, we know for Cat C, one chromosome has the short *KIT* allele and the other chromosome has the long *KIT* allele.

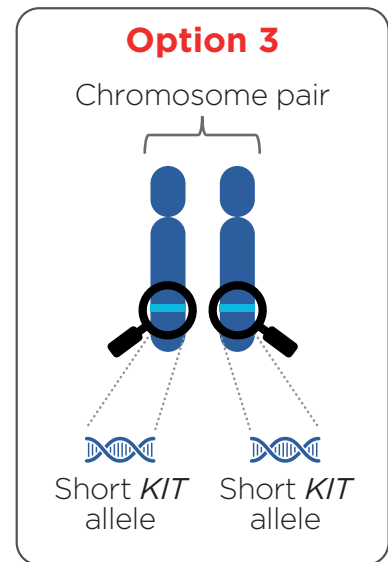
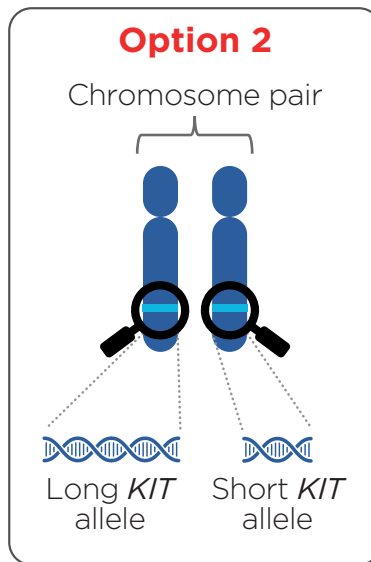
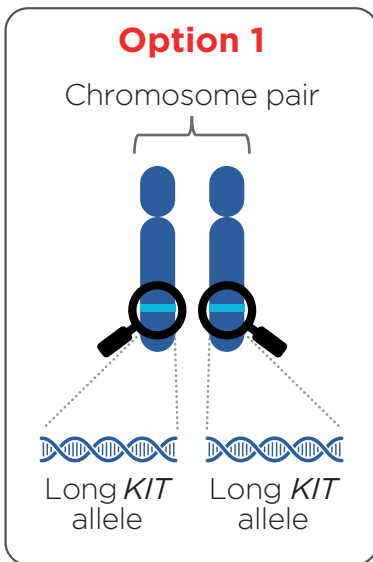
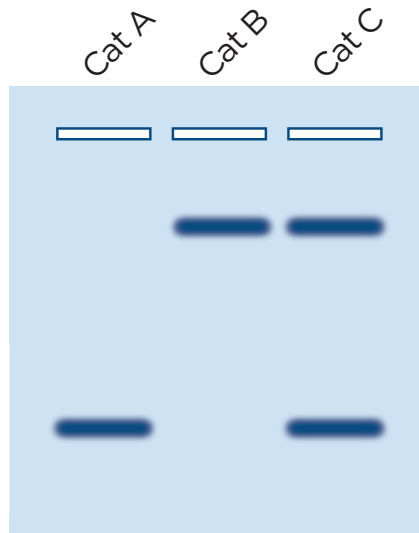


Background: Stop and think

Q5. Why is gel electrophoresis a good tool for studying the different alleles of the *KIT* gene in cats?



Q6. Match the potential allele combinations shown below with the gel results for Cats 1, 2, and 3.





Glossary

Inherited: A trait is inherited when it is passed from parents to offspring through DNA.

DNA: DNA contains the instructions for the cell and is passed down from parent to offspring.

Chromosome: The structures that store DNA in the cell. Organisms like humans and cats have pairs of chromosomes, with one copy of each chromosome inherited from each biological parent.

Gene: A region of DNA that contains a single set of instructions. Different genes correspond to different traits.

White spotting: The presence of patches of white fur on a cat.

Allele: One of two or more alternative versions of the same gene. Different alleles of the same gene have differences in the DNA.

Dominant: Some alleles have a relationship called dominant/recessive. A single copy of a dominant allele of a gene will produce the corresponding dominant trait.

Recessive: Some alleles have a relationship called dominant/recessive. A recessive trait will only be present if both alleles of a gene are recessive.

***KIT* gene:** A gene scientists suspect controls white spotting in cats. The *KIT* gene has been shown to control white spotting in other animals.

Polymerase chain reaction (PCR): A method used to make many copies of a DNA segment you are interested in studying. For more detailed information on electrophoresis, refer to <https://www.minipcr.com/polymerase-chain-reaction/>.

Gel electrophoresis: A method that separate pieces of DNA by length. For more detailed information on electrophoresis, refer to <https://www.minipcr.com/gel-electrophoresis/>.

Agarose gel: A type of gel commonly used for gel electrophoresis. Agarose is a sugar from seaweed. At the microscopic level, the inside of an agarose gel looks like a web or a sponge. Small molecules can move through the holes with relative ease, but larger molecules get slowed down. This allows scientists to separate molecules of different sizes.



Today's lab

You work in a lab that studies cat genetics. A fellow scientist has asked for your help with their research on the link between the *KIT* gene and white spotting. You already know that white spotting is dominant, and you will be investigating two alleles of the *KIT* gene: the short allele and the long allele.

Your goal is to determine which *KIT* allele is responsible for white spotting. To do this, you will use gel electrophoresis analyze the *KIT* gene in a family of cats.

For each cat, you will need to:

- Determine which *KIT* alleles the cat has
- Compare those alleles to whether or not the cat has white spotting

Meet the cats!



Astrid
(mother)



Beau
(father)



Cara

Diego



Ezra



Freya



Laboratory guide



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

See detailed assembly and gel pouring instructions for the Bandit™ STEM Electrophoresis Kit
<https://www.minipcr.com/bandit-assembly/>

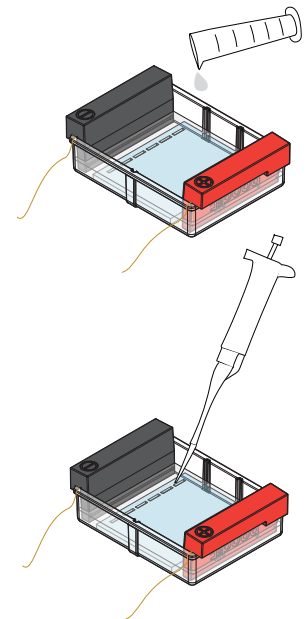


1. Submerge your gel in enough TBE buffer to just cover the gel and fill the wells

- If using a Bandit™ or blueGel™ electrophoresis system you will need approximately 30 ml of TBE buffer.

2. Use a micropipette to load samples onto the gel from the corresponding tubes in your Load Ready™ Strip

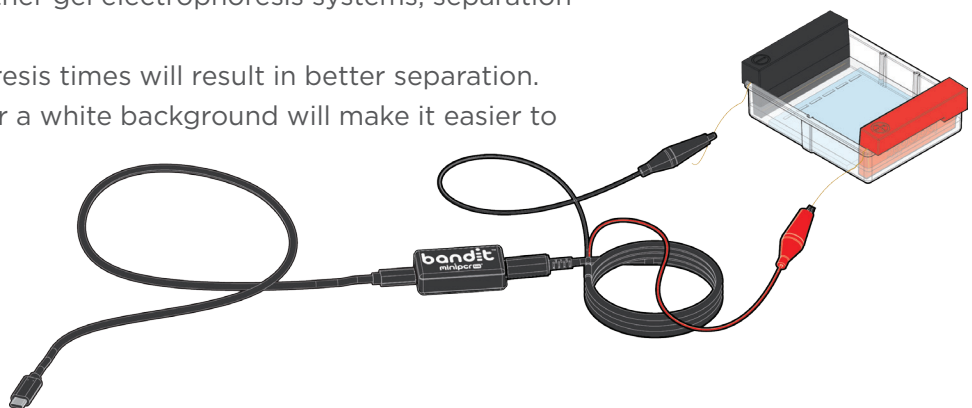
- **Lane 1:** 10 µl Astrid DNA
- **Lane 2:** 10 µl Beau DNA
- **Lane 3:** 10 µl Cara DNA
- **Lane 4:** 10 µl Diego DNA
- **Lane 5:** 10 µl Ezra DNA
- **Lane 6:** 10 µl Freya DNA



3. Connect the electrodes and turn on your gel electrophoresis system

4. Run the gel for 15-20 minutes or until there is sufficient separation between the bands

- Times are based on Bandit™ and blueGel™ electrophoresis systems. If using other gel electrophoresis systems, separation time may vary.
- Longer electrophoresis times will result in better separation.
- Placing the gel over a white background will make it easier to see your results.





Pre-lab study questions

Critical thinking

Refer to the picture of the cat family on page 18. By looking at the cats, you already know whether they have white spotting, but what can you deduce about their DNA?

1. Astrid has white spotting. Based only on what she looks like, which combination(s) of alleles could Astrid have for the gene that controls white spotting? Select as many answers as are correct.
 - a. BB
 - b. Bb
 - c. bb

Explain your reasoning:

2. Beau does not have white spotting. Based only on what he looks like, which combination(s) of alleles could Beau have for the gene that controls white spotting? Select as many answers as are correct.
 - a. BB
 - b. Bb
 - c. bb

Explain your reasoning:

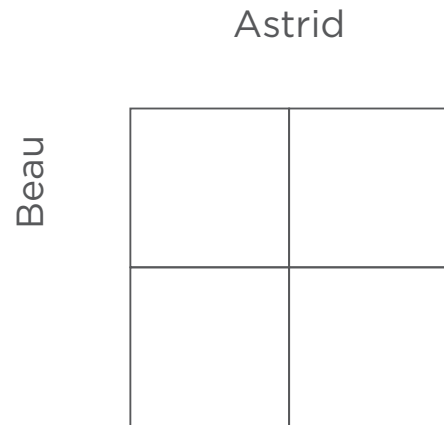
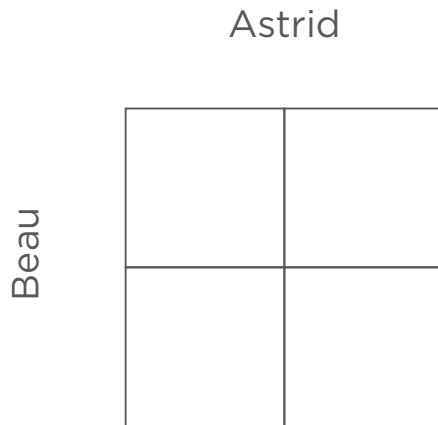


3. Now let's look at kittens that could be born to Astrid and Beau. You know which alleles Beau has, but Astrid is still uncertain.

Complete both Punnett squares below using each of Astrid's possible allele combinations.

A) Cross: _____ x _____
 Astrid's alleles Beau's alleles

B) Cross: _____ x _____
 Astrid's alleles Beau's alleles



4. If Astrid has BB alleles:

a. What allele combination(s) could kittens born to Astrid and Beau have?

b. Based on their allele combinations, what could these kittens look like in terms of having white spotting or not?



5. If Astrid has Bb alleles:

a. What allele combination(s) could kittens born to Astrid and Beau have?

b. Based on their allele combinations, what could these kittens look like in terms of having white spotting or not?

6. Compare your answers in questions 4 and 5 with Astrid and Beau's actual kittens (Cara, Diego, Ezra, and Freya). Based on this information, can you say for sure which allele combination Astrid has? Explain your reasoning.

7. Summarize what you know about the cats in the table below. Use the letters B and b to represent the alleles.

	Cat	Appearance	Alleles
Parents {	Astrid	White spotting	
	Beau	No white spotting	
Kittens {	Cara	No white spotting	
	Diego	No white spotting	
	Ezra	White spotting	
	Freya	White spotting	



Post-lab study questions

Interpreting results

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



2. Label each band as either the short allele or the long allele of the *KIT* gene.

3. Record your results in the table below

- You should have already completed the first part of this table with the cats' white appearance and alleles in pre-lab question 7 on page 22.
- The first row has been filled out for you as an example.

Cat	Appearance	Alleles	<i>KIT</i> gel results
Astrid	White spotting	Bb	Long allele, short allele
Beau			
Cara			
Diego			
Ezra			
Freya			



Critical thinking

Compare the results in the “Alleles” column and the “*KIT* gel results” column to answer the following questions.

4. When you see a “short allele” in the gel results column, which allele does it seem to match up with?
- A. B allele
 - B. b allele

Does this suggest that the short allele is the dominant or recessive allele? Explain your reasoning.

5. When you see a “long allele” in the gel results column, which allele does it seem to match up with?
- A. B allele
 - B. b allele

Does this suggest that the long allele is the dominant or recessive allele? Explain your reasoning.

6. Based on your answers to questions 4 and 5, does this support the idea that the *KIT* gene controls white spotting in cats? Explain your reasoning.



Connections to mutations:

7. The ancestors of the domestic cats that live with us as pets were wild cats that lived about 10,000 years ago in what we now call Egypt and the Middle East. The white spotting trait only exists in domestic cats and not in any wild populations. Scientists think a mutation in a domestic cat's DNA led to the white spotting trait. Based on what you learned in this lab, describe how this mutation changed the cat's DNA.

Connections to natural selection:

8. Some changes in the DNA will give an organism an advantage and will spread because of natural selection. Some mutations will put an organism at a disadvantage and will be removed by natural selection. Sometimes domestic cats with white spotting will mate with wild cats and their offspring will grow up to live in the wild. But white spotting is not common in wild cats. Can you think of any reasons having large white spots might put wild cats at a disadvantage? Why may that not be true for domestic cats?

9. Can you think of an environment where white spotting might give wild cats an advantage and become more common in the population?



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:

Is the long allele or the short allele of the KIT gene responsible for the white spotting trait?

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 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	55	60	65	70	75	80	85	90	95	100