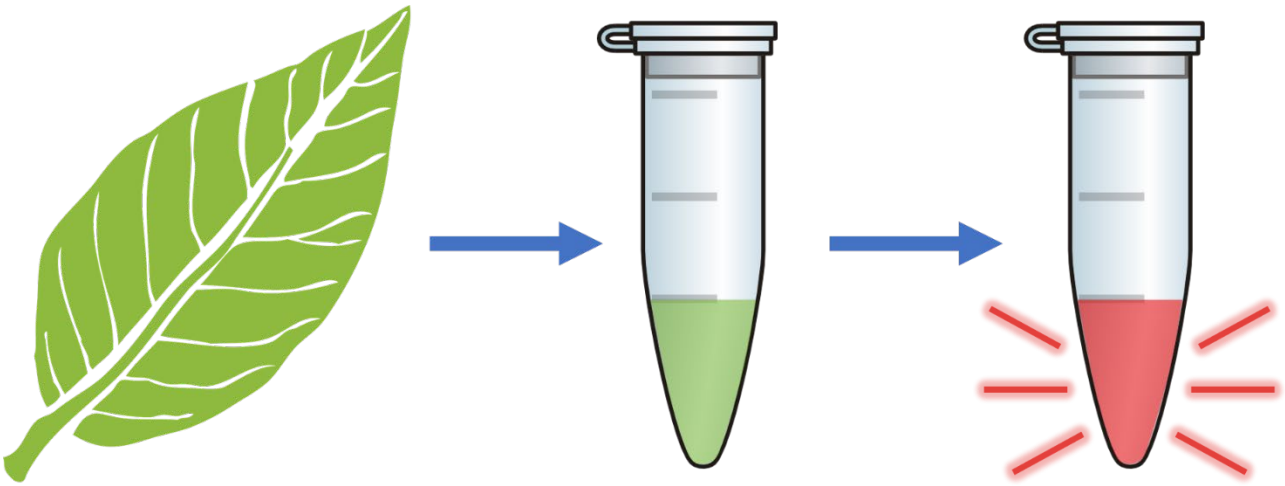
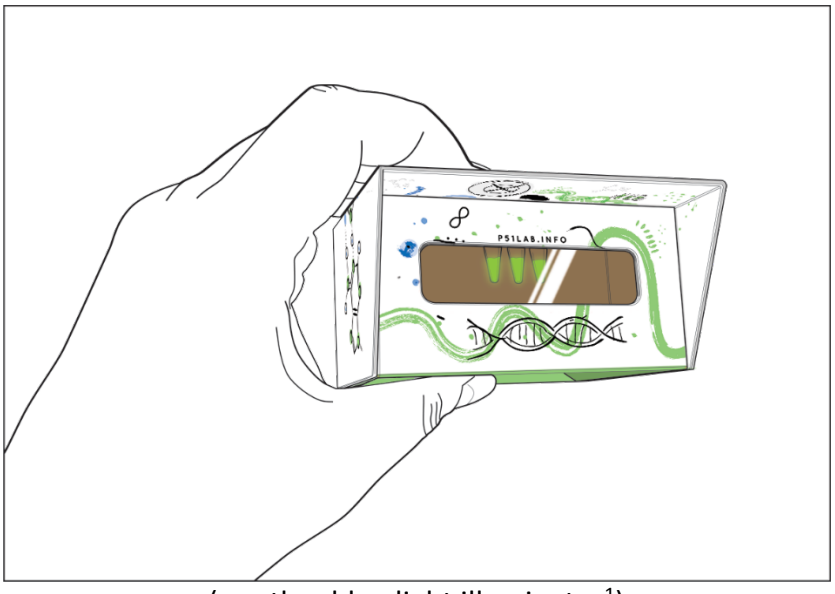
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P51™ Chlorophyll Lab: Green Glows Red!



For use with P51™ Molecular Viewer



(or other blue light illuminator[1])

[1] Compatible with blue light transilluminators such as blueGel™, blueBox™ and other 460-480 nm illuminators.

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Lab overview

Why do most plants look green? And how do they capture energy from light?

These and more questions about the properties of chlorophyll are explored through this simple hands-on investigation, which we present here as a free resource. In this activity, students will isolate plant pigments from leaves, separate the pigments using paper chromatography and then investigate whether the different pigments will fluoresce when suspended in a solvent. Using the background information provided with this lab, students will be able to describe the physiological role of chlorophyll and will be able to explain why fluorescence is a property of chlorophyll only when removed from its cellular surroundings.

This lab is designed to be performed with the P51™ Molecular Viewer, but can be carried out with any blue light illumination system, including blueGel™ and blueBox™.

- **Techniques utilized:** Micropipetting, paper chromatography, fluorescence detection.
- **Time required:** One 45-minute period.
- **Reagents needed:** Acetone, isopropyl alcohol, or other organic solvent, chromatography paper/filter paper, leaves.
- **Suggested skill level:** Familiarity with chlorophyll, photosynthesis and pipetting technique.

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1. Learning goals and skills developed

Student Learning Goals – students will be able to:

- Separate and identify plant pigments.
- Describe the role of chlorophyll in the cell.
- Relate observations to molecular properties of chlorophyll.

Scientific Inquiry Skills – students will be able to:

- Create hypotheses and make prediction about results.
- Relate observations to scientific principles.
- Make conclusions based on their experimental results about their hypothesis.

Molecular Biology Skills – students will be able to:

- Manipulate liquids using a micropipette.
- Separate of molecules using chromatography.
- Detect fluorescence.

Analyzing and Interpreting Data – students will be able to:

- Collect and organize qualitative data.
- Relate results to specific molecular properties.

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2. 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/p51-chlorophyll-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

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/p51-chlorophyll-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, worksheet, and hands-on 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/

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4. Background and significance

When we start a fire for warmth and burn wood, we are releasing energy stored in the bonds of plant molecules. When we look for nourishment by eating sugar or other carbohydrates, we are doing the same thing, releasing stored chemical energy from a plant for our own use. But energy can never be created nor destroyed, meaning the plant that we are using for energy had to obtain that same energy from somewhere else. So where did the plant matter we use for heat and nourishment get its energy in the first place? Plants obtain energy from the sun, and store that energy in the bonds of carbohydrates. In fact, virtually all energy used by any living organism on Earth started out as solar energy captured by plants or other organisms that perform photosynthesis. This ability to convert light energy into usable chemical energy provides nearly all of the energy used by life on this planet.

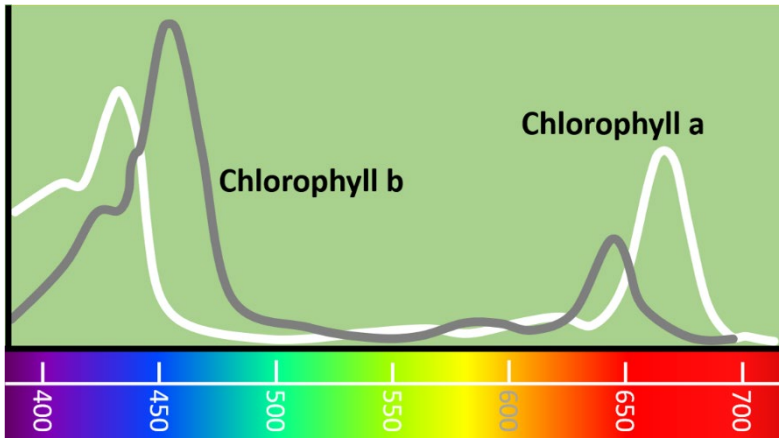
Photosynthesis is the process whereby light energy is absorbed and converted into chemical energy that can be used by organisms. The key first step of photosynthesis, therefore, is to efficiently absorb light energy. In plants and algae, this job is done primarily by the molecule *chlorophyll*. Chlorophyll is a pigment, a molecule that absorbs light. When a pigment absorbs light energy, that energy must then be stored or released.

When light hits a pigment in photosynthesis, the energy from the light is absorbed, and it causes an electron of the pigment molecule to raise in energy level. But that electron can't stay in that excited state forever; it will quickly pass that energy off, returning to its original state. If you know what it feels like to wear a black shirt on a bright sunny day, you know one place that energy can go; the dark pigments of the black shirt absorb the light energy but then release it as heat. The plant pigment chlorophyll will absorb light energy like any pigment, but instead of releasing that energy as heat, it will pass that energy from light off to other components of the chloroplast, where it will eventually be stored as chemical energy.

Chlorophyll located in the chloroplast thylakoid membrane is arranged in such a way that, when light is absorbed, the energy in the electron will be passed down a chain from one chlorophyll molecule to the next until it reaches the photosystem reaction center. In the reaction center, the energy is transferred down the electron transport chain, eventually contributing to the production of sugar molecules in the cell. In this way, energy enters the plant leaf in the form of light, but is transformed into stored chemical energy.

There are two main forms of chlorophyll, *chlorophyll a* and *chlorophyll b*. Both chlorophylls best absorb wavelengths in the blue to violet ranges of the spectrum and also in the red ranges of the spectrum, but each is best at absorbing slightly different wavelengths of light. Chlorophyll's inability to absorb green wavelengths is the reason plants are green—when white light from the sun hits a plant, the short violet and blue wavelengths are absorbed along with the long red wavelengths, but the intermediate green wavelengths are reflected. Plants also have other pigments that help absorb some of this light that chlorophyll misses. Carotenoids are yellow to red pigments present in almost every leaf that absorb some of the green that chlorophyll can't. It is these carotenoids that give fall foliage much of their yellow and orange colors.

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Light energy that is absorbed by a pigment can be released as heat energy, as in a black shirt on a sunny day, or it can be transferred into chemical energy as in chlorophyll in a leaf, but there is another way that this energy can also be released. In some cases, absorbed light energy can be released back again as light. When this happens, because some energy has been lost to heat and other processes, the light that is released is of a longer, lower-energy wavelength than when it was first absorbed. This is known as the Stokes shift and it is the basis of the phenomenon known as fluorescence. Chlorophyll absorbs light best in the violet to blue areas of the visible spectrum. This blue and violet light will be absorbed by chlorophyll, but if the chlorophyll cannot transfer the absorbed energy to be stored as chemical energy, that energy must go somewhere: it is released back as light of a longer wavelength—red light.

So why aren't most plants emitting red light? In a living plant, fluorescing chlorophyll would be a problem, as energy that is released as light cannot be used to make sugar. A fluorescent red plant would be very bad at storing energy in the form of sugar and would essentially starve to death. In fact, measuring the amount of red fluorescence produced by chlorophyll in a plant is actually a tool that scientists use to measure the efficiency of the chloroplast electron transport chain. When the electron transport chain is operating at high efficiency, very little fluorescence is produced because a high proportion of the energy absorbed by chlorophyll is being efficiently transmitted into chemical energy. If more fluorescence is produced, less of the absorbed sunlight is being transmitted into chemical energy.

In this lab, you will be able to observe and separate the pigments found in a leaf. You will then observe the appearance of those pigments—both in their normal physiological surrounding within a leaf, as well as when they are separated from the leaf and dissolved in solution.

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5. Materials needed

Reagent or equipment needed	Amount needed per group	Checklist
Fresh leaves *Baby spinach works well, or try a leaf of your choice.*	*A few leaves per group*	
Acetone or **90%+ isopropyl alcohol**	*About 2 ml. Volume depends on the extraction method*	
70% isopropyl alcohol	*~.5 cm deep covering of chromatography chamber*	
Filter paper for chromatography	*1*	
Mortar and pestle	*1*	
Paper chromatography set up: **Beaker and glass stirring rod (or similar)**	*1*	
Pipettes (micropipettes or plastic transfer pipettes can be used in this experiment)	*1 P20 or P200 with tips or* *Several plastic transfer pipettes*	
Funnel and filter paper **or**	*1*	
Microcentrifuge		
200 µl tubes	*About 5*	
1.7 ml microcentrifuge tubes **(if using a centrifuge)**	*1-3*	
P51™ Molecular Viewer or other blue light illumination system	*1*	

Available at miniPCR.com

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6. Laboratory guide

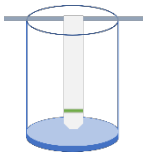
Planning your time

In this lab, groups will begin by making an extract of leaf tissue by grinding leaves in a mortar and pestle. They will then filter or centrifuge their leaf extracts to remove particulates from the solution. Using the purified extract, students will set up a paper chromatography experiment. While the chromatography is running, students will observe their extract in P51™ to observe fluorescence. Following chromatography, students will isolate different pigments from their chromatography paper and test each for fluorescence.

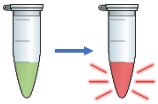
Experimental timeline

Preparation:
Dispense reagents and prepare equipment
- *10 min*

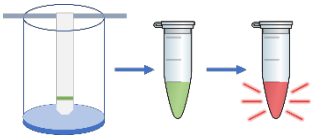
A Preparing extract and setting up chromatography
- 15 min



B Observing fluorescence during chromatography
- 15 min



C Isolating plant extracts from chromatography paper and observing in P51™
- 15 min



Note: *There are many variations on paper chromatography using leaf pigments. Methods using petroleum ether in a closed chromatography apparatus tend to give the best separation of pigments. Here we describe using easily attainable 70% isopropyl alcohol. This method tends to separate two distinguishable bands of pigment. If you have a protocol that you are familiar with and prefer to use, we encourage you to do so.*

This lab calls for the use of either acetone or 90%+ isopropyl alcohol as extraction solvents and 70% isopropyl alcohol as a chromatography fluid.

- *We have found that **acetone** works best as extraction solvent. Alternatively, use **90+% isopropyl alcohol**.*
- *For chromatography, commercial **70% isopropyl alcohol** is effective for separating pigments in a classroom environment.*
- *All three liquids (acetone, 90% isopropanol, and 70% isopropanol) can typically be purchased for under $10 at a drug store.*

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Acetone and isopropyl alcohol are highly flammable. Keep away from heat sources or open flame. Protect eyes and skin. Gloves and protective eyewear should be worn during the entirety of this lab. Plant extract will stain clothing and other materials. Be careful while handling.

1. *Extracting plant pigments*

For extraction we recommend 100% acetone or at least 90% isopropyl alcohol



1. **Place a few leaves in a mortar.**
   - Any green leaf, from baby spinach to pine needles will work.
   - If using baby spinach 1-3 leaves should be plenty, depending on the size of your mortar.

2. **Add isopropyl alcohol or acetone as a solvent.**
   - Add just enough that the leaves are wet.
   - Adding too much liquid can make crushing with the pestle more difficult.



3. **Use the pestle to grind and crush the leaves.**
   - Grind the leaves using the pestle until they are thoroughly crushed and very few intact pieces of leaf are left.

4. **Add some more solvent if needed.**
   - Add more acetone or alcohol until there is just enough liquid to be able to pour.
   - Adding too much liquid will cause the extract to become too dilute.

If using a centrifuge:

5. **Add at least 200 µl of the ground leaf to a 1.7 ml microcentrifuge tube.**
   - Use a plastic transfer pipette or carefully pour some of the liquid into the tube.



6. **Using a microcentrifuge, spin for at least 20 seconds at 10,000 rpm.**
   - Make sure that the centrifuge is balanced with equal volumes in all tubes.

7. **Carefully remove tube from centrifuge. The top liquid phase is your leaf extract.**
   - There should be a transparent green liquid at the top of the tube and an opaque pellet of plant matter at the bottom of the tube.
   - The transparent green liquid is your plant extract.
   - Handle the tube carefully so you don't disturb the pellet.

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If using filter paper:

5. **Fold a piece of filter paper and place it inside a funnel.**

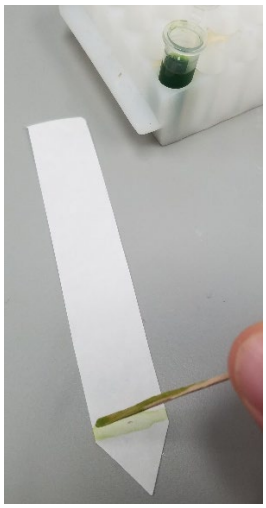
6. **Place the funnel in a beaker, test tube, or other collection container.**

7. **Pour the entirety of the liquid in the mortar into the filter paper.**
   - Wait for the liquid to filter through the paper (a few minutes).

8. **The liquid that collects in your beaker is your leaf extract.**

2. Paper chromatography

If you have a paper chromatography protocol that you are familiar with and prefer to use, we encourage you to do so.



1. **Obtain a clean dry piece of filter paper and cut it into a long strip.**
   - The strip should be between 1 and 3 centimeters wide and at least as tall as the beaker you intend to perform chromatography in.
   - Cut the tip of the paper into the shape of a point.

2. **Use <u>pencil</u> to draw a line on the paper about 2-3 cm from the tip.**
   - The pencil line should be perpendicular to the length of the paper.

3. **Use a toothpick to draw a line of leaf extract on top of your pencil line.**
   - Dip toothpick in the leaf extract and drag it across the pencil line.
   - Repeat until the entire line is wet.
   - Do not get too wet, or the extract will bleed away from the line.

4. **Let the leaf extract dry and reapply until there is a sufficient amount of leaf extract dried on the paper.**
   - The solvent will air-dry quickly – usually in ~15 seconds.
   - When dry, use a toothpick to reapply plant extract on the same line as in step 3.
   - When finished the line should be a solid green color.
   - 5-8 applications are usually enough, depending on the concentration of your leaf extract.

5. **Add chromatography solvent your beaker.**
   - Use 70% isopropyl alcohol so that the total amount of liquid is no more than 1 cm deep.

6. **Rest a glass stirring rod, pencil or other similar object across the top of the beaker.**

7. **Hang your paper off of the stirring rod so that the tip of the paper is in the solvent, but the line of dried plant extract is <u>*above*</u> the solvent.**
   - Fold the paper so that it can hang from the rod. Alternatively attach it to the rod using tape.
   - The point of the paper should be in the alcohol, but the dried plant extract should be <u>above</u> the solvent.

8. **Wait until you see significant separation of pigments (15-20 minutes). Draw your results on pg. 14.**

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3. Observing pigment fluorescence



1. **Obtain a 200 µl tube and label the tube "PE" for leaf extract.**

2. **Add up to 50 µl leaf extract to your tube.**
   - If using a 200 µl pipette, transfer 50 µl to the tube.
   - If using a 20 µl pipette, transfer 20 µl several times.
   - If using a plastic transfer pipette, fill the tube ¼ to ½ full.

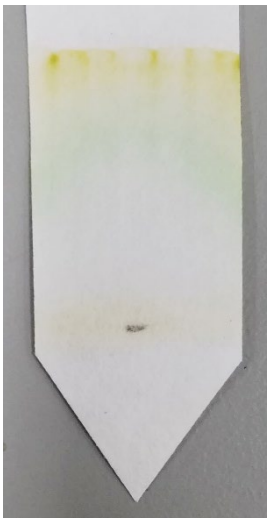
3. **Prepare controls.**
   - Add approximately the same volume of your solvent without any leaf extract added. Label the tube "S" for solvent.
   - Tear or cut a small piece of leaf and place it in a tube alone.
   - Optional: Add green food coloring to water and dilute it until is a similar color to your plant extract.

4. **Place the tubes in P51 or other blue light illuminator and record your results in the table on pg. 14.**

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4. Isolating pigments for fluorescence observation

For isolation we recommend 100% acetone or at least 90% isopropyl alcohol.



1. **Remove the chromatography paper from the beaker.**

2. **Use scissors to cut distinct bands of pigment out of the chromatography paper.**
   - As best as you can, cut strips of paper so that each strip contains only a single color of extracted pigment.
   - The strips should be narrow enough to fit in a 200 µl tube.

3. **Place each strip of paper in a different 200 µl tube.**
   - Label the tubes 1, 2, 3 and so on for as many strips you have cut out.
   - #1 should correspond to the band on your paper that ran the farthest on your chromatography paper. #2 should have run the second highest and so on.
   - You will likely have about 2-3 discernible pigments, but as many as 5 are possible depending on your chromatography protocol.

4. **Add ~50 µl of acetone (or 90%+ isopropyl alcohol) to each tube.**

5. **Cap the tube and flick the tube several times to mix well**.
   - The pigment should be washed from the paper and into the solvent in the tube.

6. **Remove the paper from each tube.**
   - You may use forceps, a toothpick or a pipette tip to pull the strip of paper out of the tube.

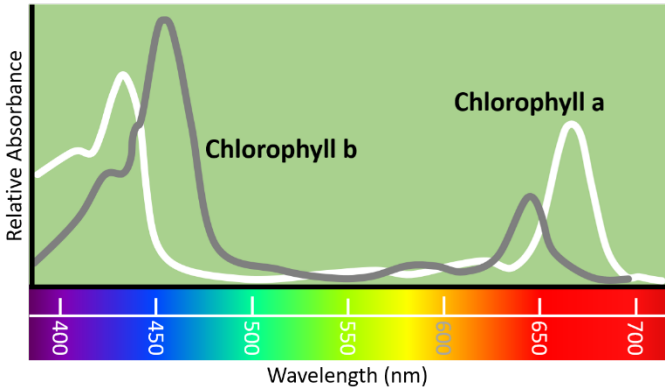
7. **Place the tubes in P51™ or other blue light illuminator and record your results in the chart on pg. 15.**

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7. Study Questions

Pre-lab questions

1. The graph to the right shows the approximate relative absorbance of chlorophyll a and b. For reference, approximate color ranges of different wavelengths are listed below the graph.
   a. If you were designing an extremely efficient light to grow plants, what wavelengths or colors would it emit? Justify your answer.



   b. What wavelengths or colors would it be inefficient for your grow light to produce? Justify your answer.

Violet: 380-450 nm	**Blue**: 450-495 nm
Green: 495-570 nm	**Yellow**: 570-590 nm
Orange: 590-620 nm	**Red**: 620-750 nm

2. Plants also contain pigments known as carotenoids. Carotenoids also absorb light and pass that energy to chlorophyll. Carotenoids absorb light best in the range of about 450 nm to about 530 nm. Why might it be helpful for a plant doing photosynthesis to use carotenoids along with chlorophyll?

3. Imagine that you left a leaf that was performing photosynthesis in the sun, and next to it, you left a material that was the same size, shape, mass, and color, but did not perform photosynthesis and contained pigments other than chlorophyll. And after half an hour you measured the temperature of both materials. Which material would you expect to be warmer? Justify your answer.

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Post-lab questions

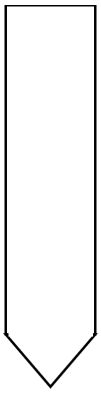
Interpreting results

1. Record information about the type of leaf that you use to extract chlorophyll.

2. Record the results of your pigment fluorescence observations in the table below.

Tube	Color of solution in ambient light	Color of solution in blue light illuminator	Notes
Plant extract			
Solvent only			
Leaf only			
Food coloring			

3. Sketch your paper chromatography results on the template below.



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4. Record the results of your isolated pigment fluorescence observations in the table below.

Tube#	Distance traveled on paper	Color of band on paper	Color of solution under blue light	Notes
1				
2				
3				
4				
5				

5. What color was the plant extract in ambient light, and what color was the extract in blue light?

6. For each control tube that used, explain why it was important to include that tube as part of your experiment. If you had not included that control in your experiment, what alternate conclusion could you have made?

Control	Without this control, what alternate conclusion regarding the red fluorescence could you make?
Solvent only	
Unextracted leaf	
Green food coloring	

7. Based on the graph of chlorophyll absorbance, is the red color you observed more likely from chlorophyll a or chlorophyll b? For reference, P51™ emits light with a wavelength of 465 nm. Explain your answer.

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8. If you wanted to more clearly test the *other* chlorophyll molecule for fluorescence, what wavelength/color light would you use?

9. How many pigments did you see on your chromatography paper?

10. Of those pigments, which fluoresced and which didn't?

11. Red light emitted by the chlorophyll is a form of energy. Where did that energy come from?

12. If a pigment does not fluoresce, there are two possible reasons why. (1) It did not absorb the light, or (2) after absorbing the light it released the light energy in a form other than fluorescence. Can you propose a simple experiment to see if either of those two explanations is accurate?

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CER Table

Fill in the table based on your results from the lab.

Question:

Why do you observe red fluorescence under certain circumstances?

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.	

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	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.