

Chapter 8 Lab- Chromatography and Photosynthesis

Purpose: Part 1
To determine the chlorophyll composition in spinach leaf and understand the importance of chlorophyll in photosynthesis.

Part 2
To determine which gases (CO₂ or O₂) are given off when a plant is exposed to light or placed in the dark.

Part 3
To observe how different cells of a Zebrina leaf have specialized functions during photosynthesis.

Materials:	test tubes	bromothymol blue indicator	chromatography paper
	distilled water	Zebrina plant	coin
	elodea	ring stand	ruler
	straw	spinach leaf	paper clip
	stereoscope	chromatography solvent	slide
			slide coverslip

Procedure:

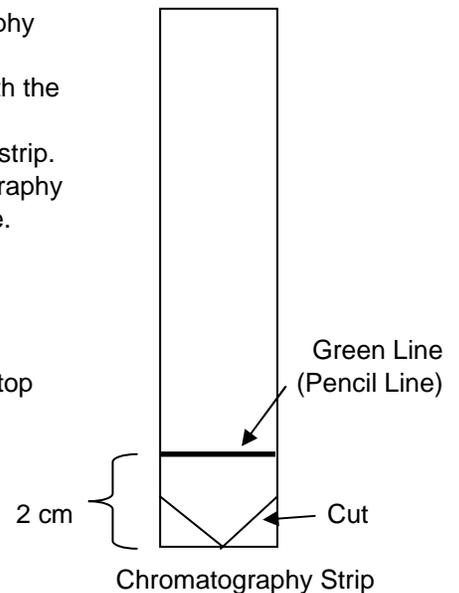
Part 1- Paper Chromatography

- 1 Obtain a strip of chromatography paper.
- 2 Use a ruler to measure and draw a light pencil line 2 cm above the bottom of the chromatography strip. See drawing.
- 3 Just below your pencil line, cut the bottom of the chromatography paper creating a point. See drawing.
- 4 Obtain a spinach leaf and dry it. Wrap a leaf around a coin with the waxy side of the leaf facing outward.
- 5 Rub the leaf along the light pencil line on the chromatography strip. Use another part of the leaf and rub the leaf on the chromatography strip again. Continue to do this until you have a dark green line.

**Do not rub the leaf above or below the line!!
Rub the leaf on the line only!!**

- 6 Hang the chromatography strip on the test tube by folding the top of the strip so the strip hangs down.

The paper should not be touching the bottom of the test tube!!



- 7 Remove the chromatography strip from the test tube.
- 8 Carefully add chromatography solvent to the test tube. You should only add enough solvent so that the tip of your chromatography paper gets wet. Do not let the chromatography solvent run down the side of the test tube.
- 9 Hang your chromatography strip in your test tube so that the tip of the strip dips into the chromatography solvent.

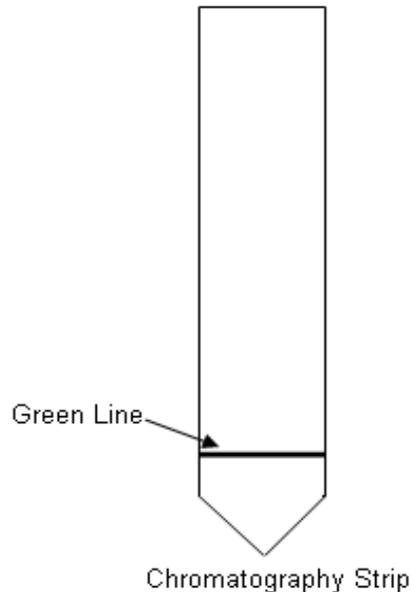
Make sure the level of the solvent is below the green line. If the solvent is going to cover your green line, pour out some solvent or adjust your fold at the top of your chromatography strip.

Do not get the green line wet!!

- 10 Leave the chromatography strip in the solvent for 30 minutes. **Do not touch or move your experiment!**

- 11 **Begin answering your Analysis Questions.**
- 12 At the end of 30 minutes remove the paper from the test tube and use a pencil to mark a line at the highest point the solvent reached. This is your solvent front.
- 13 Examine the chromatography strip for the presence of different bands of color. Each color band is a different pigment. Chlorophyll a appears blue-green, chlorophyll b appears yellow-green, carotene appears bright yellow, and xanthophyll appears pale yellow-green. You may not see all of these bands.
- 14 Draw, color and label as many pigment bands that your group can identify. Be sure to label your solvent front.
- 15 Staple your chromatography strip next to your drawing.

Staple Chromatography Strip Here



Analysis Questions:

- 1 Define photosynthesis.

- 2 Explain the important role of chlorophyll in photosynthesis.

- 3 Using the list and description of pigments in procedure #13, which pigments were present on your chromatography strip?

Using pg. 207, answer questions 4, 5 and 6.

- 4 What color light does chlorophyll a absorb? Reflect?

- 5 What color light does chlorophyll b absorb? Reflect?

- 6 List the 4 things needed by photosynthesis to create high-energy sugars.

Part 2- CO₂ Absorption During Photosynthesis

- 1 Fill 1 test tube 1/4 of the way with distilled water.
- 2 Add 1-2 dropper full of bromthymol blue to your test tube.
- 3 Draw and color your initial observations of your test tube.
- 4 Covering the top of your test tube with a paper towel and using a straw, gently blow into your test tube until the solution turns yellow in color.
- 5 Add a sprig of elodea to your test tube.
- 6 Draw and color your initial observations of your test tube after you blew into your test tube.
- 7 Using tape, label your test tube with your period and group table #.
- 8 Place your test tube in the test tube rack labeled light.
- 9 Let your test tube sit for 48 hours.
- 10 Begin answering your Analysis Questions.
- 11 After 48 hours, draw and color your final observations of your test tube.

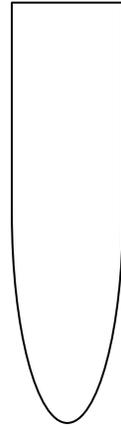
Initial Observations **(Before blowing into straw)**



Initial Observations **(After blowing into straw)**



Final Observations **(After 48 hours)**



Analysis Questions:

- 7 After you blew into the straw, what caused the color in your test tube to change from green/blue to yellow?
- 8 What is the role of carbon dioxide in photosynthesis?
- 9 List the products and reactants of the Light-Dependent reaction.
Reactants:

Products:
- 10 List the products and reactants of the Calvin Cycle (Light Independent reaction).
Reactants:

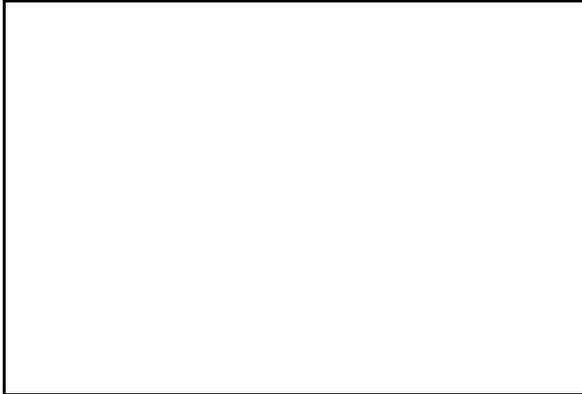
Products:
- 11 Based on your lab results, what color did your test tube change to after 48 hours? Explain why this color change occurred. Be sure to include the role of the elodea plant.
- 12 What is the overall equation for photosynthesis?

Part 3- Zebrina Leaf Stomata

- 1 Cut a small piece of Zebrina leaf.
- 2 Place leaf on stereoscope stage with the underside of the leaf up (purple side up).
- 3 Observe, draw and color what you see in the box below labeled stereoscope.
- 4 Remove a smaller piece of the leaf and prepare a wet mount.
- 5 Place slide under the light microscope with the underside of the leaf up (purple side up).
- 6 Observe, draw and color what you see in the box below labeled light microscope
- 7 Read **page 597** in your textbook for information on the role of stomata and guard cells.

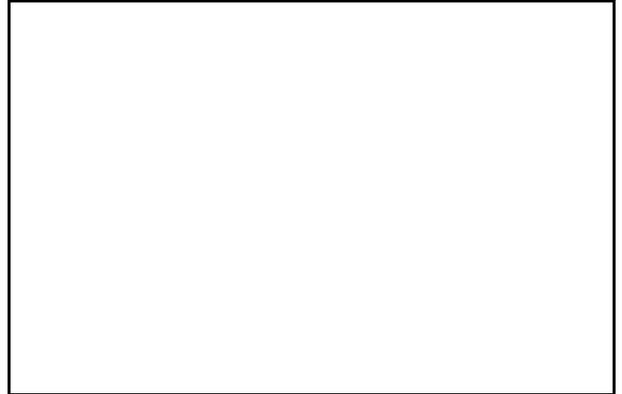
Observations:

Stereoscope



3x magnification

Light Microscope



400x magnification

Analysis Questions:

13 What color are the epidermal (skin) cells of the Zebrina plant?

14 What color are the cells forming the stomata?

15 What are the functions of the following?

Stomata:

Guard cells: