Lab: Paper Chromatography
Biology A

Name_____Per_____

Lab Partner_____

Essential Questions:

- Why are plants green?
- What molecules capture light energy?
- How are the molecules different?

Background:

The pigments in a leaf can be separated using paper chromatography. The special paper used for this lab has an affinity with the pigment molecules, which varies between the pigment molecules. A combination of pigment properties determine how quickly the pigment moves through the paper.

The solvent moves up the paper by capillary action, which occurs as a result of the attraction of solvent molecules to the paper and the attraction of solvent molecules to one another. As the solvent moves up the paper, it carries along any substances dissolved in it. The pigments are carried along at different rates because they are not equally soluble in the solvent and because they are attracted, to different degrees, to the fibers in the paper through the formation of intermolecular bonds, such as hydrogen bonds.

Beta-carotene, an orange-yellow pigment, is carried along near the solvent front because it is very soluble in the solvent being used and because it forms no hydrogen bonds with cellulose. *Xanthophyll*, a bright yellow pigment, differs from beta-carotene in that it contains oxygen. Xanthophyll is found further from the solvent front because it is less soluble in the solvent and has been slowed down by hydrogen bonding to the cellulose.

Chlorophylls contain oxygen and nitrogen and are bound more tightly to the paper than are the other pigments.

Chlorophyll a, a blue-green pigment, is the primary photosynthetic pigment in plants, and is found at the reaction center of plant photosystems. *Chlorophyll b*, an olive green pigment, captures light energy and transfers it to the chlorophyll *a* at the reaction center.

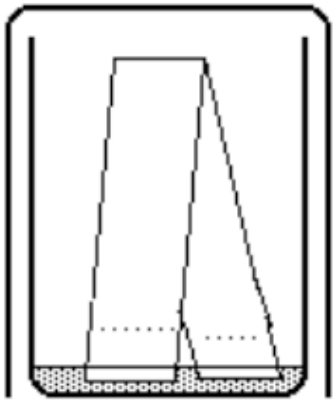
Procedure:

1. Obtain a small beaker, a larger beaker, and a precut strip of chromatography paper (handle the paper by the edges only).
2. Make sure the strip is the proper width to fit in the beaker without touching the sides. If it is too wide you will need to trim the edge of the paper.
3. Because the paper was stored on a roll, it will have a bend or curve to it. You must gently manipulate the paper in the opposite direction so that it is nearly perfectly straight. The paper must not touch the side of the test tube as it sits in the solvent during the experiment.
4. Fold the paper in half. See Figure 1.
5. Draw pencil lines 1.5 cm above the bottom of each end of the paper.
6. Obtain a portion of a leaf from the supply table.

Lab: Paper Chromatography
Biology A

7. Use a quarter or a dime to extract pigments form leaf cells by **rolling** (not sliding) the edge of the serrated coin over the pencil lines, with the leaf between the coin and the paper. Be sure the resulting pigment line is on top of the pencil line.
8. **Allow the pigment line to dry.**
9. Repeat the extraction at least 8-10 times until the pigment line is very dark. Use a new portion of the leaf each time.
10. Fold the paper and assemble the chromatography experimental setup as shown in Figure 1 **without** the solvent. Use the smaller beaker. Make sure everything fits correctly. Using an overhead pen or marker, make a mark 0.5 cm from the bottom of the beaker.

Figure 1:



11. Add just enough chromatography solvent to the small beaker to reach the line you drew in Step 10. Insert the test paper. Do NOT allow the pigment to be in the solvent or to touch the sides of the tube.
12. Place the larger beaker over the smaller beaker. See Figure 1.
13. When the solvent front (leading edge of the solvent) is about 1 cm from the fold in the paper, or if the solvent front has not moved upward for 5 minutes, remove the paper and **immediately** mark the top of the solvent front with a pencil before it evaporates.
14. Mark the **bottom** of each pigment band. Label the color of each band since the colors fade and change over time. See background information.
15. Measure the distance each band migrated from the bottom of the pigment origin to the bottom of each separated pigment band. Record the distance that each from moved, including the solvent front.
16. Record all data in Table 1. Depending on the species of plant used, you should be able to observe 3-5 pigment bands. Calculate the R_f value for each band, using the following formula:

$$R_f = \text{distance migrated by pigment} / \text{total distance migrated by solvent}$$

17. The paper strip is your data. Cut the strip at the fold. Each partner should take one side and tape it to the lab margin in the "Data Collected" section of the lab report.

Lab: Paper Chromatography
Biology A

Data Collected:

Total distance migrated by solvent front = _____ cm

Table 1:

Pigment color	**Pigment name**	**Distance pigment moved (cm)**	**R_f value**

For lab quiz: Be able to answer the following questions.

1. What two factors are involved in the separation of the pigments?
2. What pigment(s) is/are located in the reaction center of plant photosystems? Identify the color of each pigment.
3. What plant pigment(s) is/are located outside the reaction center of plant photosystems? Identify the color of each pigment.
4. What role do plant pigments outside the reaction center play in photosynthesis?
5. What does a relatively high R_f value say about the chemical properties of a pigment?
6. What does a relatively low R_f value say about the chemical properties of a pigment?