BLM 2: Plant Pigment Chromatography Instructions

1. Using a pencil, mark a line on the paper strip 2.0 cm from one end. This is the ***origin*** - it represents where the pigments start from at the beginning of the experiment (Figure 1). If you use a pen or marker the pigments in the ink will also dissolve and move along the paper, so make sure you use a pencil!

2. Use pencil to print the name of the sample at the other end of the paper strip. For Tomatosphere™ samples indicate which seed group the leaf is from (e.g. group A or B leaf) (Figure 1).

3. Place one of the two leaves on top of the paper strip so that the leaf covers the pencil mark (Figure 2). Gently roll a coin back and forth across the leaf to transfer a "smudge" of pigments from the leaf onto the paper strip (Figure 2).

4. Allow pigments to dry for one minute, reposition the leaf and repeat this pigment transfer process 3 to 4 more times until a dark strip of pigments is transferred (Figure 3).

5. Label each cup so that you know which leaf sample is being tested in each (Figure 4). Once you have prepared your cups and strips, ask your teacher to add the isopropyl alcohol; the depth of the alcohol should be 1.0 cm (Figure 4). This is the ***solvent***. The alcohol will dissolve the plant pigments allowing the pigment molecules to travel up the filter paper.

6. Tape each prepared paper strip to a pencil or coffee stir stick (Figure 5). The coffee stir stick should rest of the rim of the glass to hold the paper strip upright. **The paper strip should be long enough to dip the end into the solvent, but not so long that the pigments go into the solvent.**

Note: Depending on the height of the cup, you may need to adjust how the paper strip is taped to the pencil or coffee stir stick. Try this out before using your actual sample.

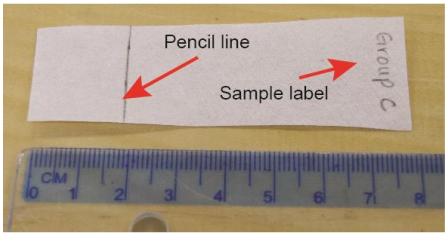

Figure 1: Mark origin and sample name on paper strip.

Figure 2: Roll the coin over the leaf on the origin line.

Figure 3: There should be one solid band of pigment.

Figure 4: Label the cup and add alcohol.

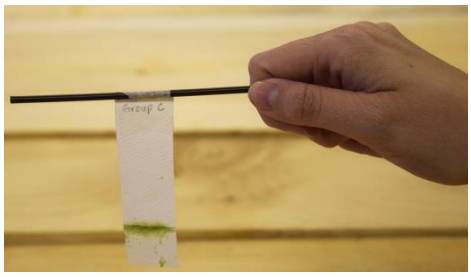
Figure 5: Tape the paper strip to the pencil or stir stick.

7. Gently lower the paper strip into the cup. The pigments from the leaf sample must be above the isopropyl alcohol (Figure 6). **Be careful not to dip the pigment band in the alcohol; the pigments will dissolve away into the alcohol and you will lose your sample!**

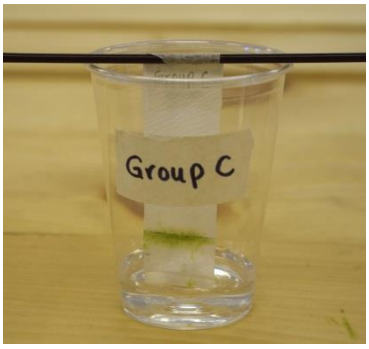

Figure 6: Rest pencil or stir stick on the rim of the cup.

8. Place a cover, such as a piece of aluminum foil, over the cup.

9. Repeat steps 1 to 8 for the second leaf sample. To save time it is best to prepare both the samples and then run them at the same time in separate cups.

   The chromatography will take approximately 30 to 40 minutes to complete (the alcohol has travelled about ¾ of the way up the paper strip).

NOTE: Make sure you are careful not to jostle or move the paper once the experiment is running.

10. After the chromatography is complete, carefully remove the strips of filter paper and lay them out on **BLM1: Pigment Chromatography Inquiry Summary** to dry. The paper strips should be removed from the alcohol before the solvent reaches the top of the strips of the paper.

11. Immediately mark the position and note the colour of each band (e.g., light green, dark green, etc.) on **BLM1**. You should also note the position of the pencil line and how high up the filter paper strip the solvent travelled (i.e., the highest point on the paper that is wet) – this is called the ***solvent front***.

Note: The colours will fade as the paper dries, so this is much easier to do as soon as possible, and of course, the solvent front will disappear quite quickly as the alcohol evaporates.

12. Complete the remaining sections on **BLM1: Pigment Chromatography Inquiry Summary**.