Students use paper chromatography and calculate the retention factor of ink to determine what type of marker wrote a ransom note.

**OBJECTIVES**
Students will:
- Use paper chromatography to separate the pigments found in ink samples.
- Construct a reference library of ink samples and use it to identify an “unknown” marker.
- Calculate the retention factor for ink samples.

**ADVANCE PREPARATION**
1. Make copies of the Reference Library pages so each group has one page for every three markers tested.
2. Collect and distribute materials.
3. Determine which marker was used to write the ransom note and prepare a filter paper strip for each group using that marker.

**MATERIALS**
Per Group:
- Filter paper (coffee filters, paper towels, or chromatography strips)
- 4 to 6 different brands of black markers (Sharpie, Crayola, etc.)
- Scissors
- Small cups or beakers, one for every marker being tested
- Toothpicks
- Ruler
- Calculator
- Reference Library pages (at least two)
- Water

Per Student:
- Pencil

**WHAT YOU NEED TO KNOW**
Chromatography is a method for analyzing mixtures by separating them into the chemicals from which they are made. It can be used to separate mixtures like ink, blood, gasoline, and lipstick. In ink chromatography, you are separating the colored pigments that make up the color of the pen. Even though a pen will only write in one color, the ink is actually made from a mixture of different colored pigments.

To perform ink chromatography, you put a small dot of ink to be separated at one end of a strip of filter paper. This end of the paper strip is placed in a solvent. The solvent moves up the paper strip and, as it travels upward, it dissolves the mixture of chemicals and pulls them up the paper. The chemicals that dissolve best in the solvent will move up the paper strip further than chemicals that do not dissolve as well. What is produced from this method is a chromatogram.

Forensic scientists are able to use ink chromatography to solve
crimes by matching documents or stains found at a crime scene to the marker or pen that belongs to a suspect. Forensic scientists analyze the unknown ink and compare it with writing utensils collected from possible suspects.

**WARM UP**

Start class being very upset. Tell students that earlier that morning you woke to find a ransom note written in black marker. Someone had stolen your dog! You have four possible suspects: your neighbor (who hates how much your dog barks), your postal carrier, your landlord, and your ex-boyfriend/girlfriend. You have obtained one marker from each of the suspects and have samples of the note written on filter paper. Tell the students they have to solve the crime and determine who may have taken your dog!

*Optional:* If using more markers, come up with more suspects.

Make sure each student knows which marker belongs to which suspect.

**ACTIVITY**

1. Pour about 10 ml of water into a beaker or small cup.
2. Cut a strip of filter paper to form a point at one end.
3. Choose a marker to test. Record the brand of marker on your Reference Library page. Use the marker to make a good-sized dot of color (like the size of a grain of rice) about 1.5 cm up from the pointed end of the paper. Assign the marker a letter or code so you remember which marker is which.
4. Use a pencil and make a mark on the paper strip beside the ink dot.
5. Lower the pointed end of the paper into the solvent BUT make sure the color dot stays above the solvent level. Carefully push a toothpick through the top of the paper to hold the strip at just the right level in the cup.
6. The solvent should immediately start moving up the paper strip, carrying the ink pigments with it. While waiting for the solvent to rise toward the top of the paper, set up your other cups and test the other markers, recording information on your Reference Library Page.
7. When the solvent has finished moving up the paper strip, you can remove the paper from the cup and immediately mark with a pencil the highest point the solvent traveled up the paper strip.
8. Let the strip dry and tape it onto your Reference Library under its correct brand name.
9. Continue testing all the ink samples, including the unknown.

**CALCULATING Rf VALUES AND DESCRIBING YOUR INK SAMPLES**

1. You probably noticed that each marker brand uses a different combination of
inks to produce their black colors. After testing the unknown ink sample, use your Reference Library to match color combinations and determine which marker was used to write the ransom note.

2. Look closely at each marker’s chromatography strip. How many different colors are present in each ink sample? Record the Total Number of Colored Pigments number for each ink sample on your Reference Library page.

3. Look closely at each marker’s chromatography strip. Record the colors in the order that they appear on your Reference Library page. Each color represents a different pigment present in the ink. (Colored Pigment #1 = pink, Colored Pigment #2 = orange, etc.)

4. To prove that an ink sample is a certain brand, you will also need to calculate the Rf (retention factor) values of the different colored chemicals present in the marker. Rf is a calculation that compares the distance the solvent traveled up the paper strip to the distance a pigment traveled up the same strip. First, look at a chromatography strip and measure the distance in millimeters from the original color dot to the final point the solvent traveled. That distance is the solvent distance measurement. Record it on your Reference Library page.

5. Next, measure in millimeters from the original color dot to the highest point the first colored pigment (Colored Pigment #1) traveled up the strip. This is the pigment distance measurement for Colored Pigment #1. Record this measurement on your Reference Library page.

6. If there is a Colored Pigment #2 on the strip, measure in millimeters the distance from the original color dot to the highest point this colored pigment (Colored Pigment #2) traveled up the strip. This is the pigment distance measurement for Colored Pigment #2. Record this on your Reference Library page.

7. Repeat the measurement for any other colored pigments and record on your Reference Library page.

8. Calculate the Rf value for each colored pigment using the formula below:

\[
Rf \text{ Value} = \frac{\text{Distance traveled by solute (pigments in ink)}}{\text{Distance traveled by solvent (water)}}
\]

9. Record the Rf values for each colored pigment on your Reference Library page.

10. Repeat these measurements and Rf calculations for each of the markers tested.

11. Keep your Reference Library page so you can use it to determine what kind of marker was used to write the ransom note.

WHAT’S HAPPENING
Black markers are made by mixing together several different colored pigments. Paper chromatography can separate this mixture of pigments so you can see

In paper chromatography, porous paper (like filter paper, coffee filters, chromatography paper, paper towels or even newspaper) is called the stationary phase. Water or another solvent, like alcohol or acetone, is called the mobile phase.
what colors are used to make up each marker. Every marker company produces their markers using their own secret mixtures of colored compounds. Calculating the retention factor provides more information that can help forensic scientists match and determine what brand of marker or pen was left at a crime scene.

CHECK IN

Answer these questions as a group.

1. What is the purpose of the water in this experiment?
2. Do you think permanent markers would have reacted the same in the water? Why or why not?
3. Do you think linking a brand of marker or pen to the crime is enough evidence to convict a suspect? Why or why not?
4. Did any of the marker samples have the same chromatography results? If so, which ones?
5. What are some other mixtures that you think can be separated by chromatography?

ETCETERA

For early finishers, if there is extra time, have students calculate the retention factor for black water-soluble markers using different types of porous papers to see if the retention factor remains the same.
### Reference Library for Ink Samples and Their Colored Pigments

<table>
<thead>
<tr>
<th>Colored Pigment</th>
<th>Color</th>
<th>Distance measured</th>
<th>Rf</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colored Pigment #1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colored Pigment #2</td>
<td></td>
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<tr>
<td>Colored Pigment #3</td>
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</tr>
<tr>
<td>Colored Pigment #4</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

**Tape Strip Here**

<table>
<thead>
<tr>
<th>Total # of Colored Pigments</th>
<th>Total # of Colored Pigments</th>
<th>Total # of Colored Pigments</th>
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</table>

<table>
<thead>
<tr>
<th>Solvent Distance Measured</th>
<th>Solvent Distance Measured</th>
<th>Solvent Distance Measured</th>
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