

USING PAPER CHROMATOGRAPHY



(Adapted from: Forensic Science Activities. University of Colorado Boulder Hughes Initiative. <<http://www.colorado.edu/Research/hughes/web.forensics.html>>.)

DESCRIPTION:

Students will use paper chromatography to separate ink molecules and identify the pen used on an unknown sample of handwriting. Students will graph and analyze data they collect using paper chromatography.

PURPOSE/GOAL:

Students will be able to:

- Gain understanding of the purpose of chromatography.
- Measure and graph pigment separation.
- Use evidence gathered from the chromatograms to support their conclusions about which suspects can be excluded from suspicion based on evidence.
- Understand that the separation of dye molecules in chromatography is a physical property of the dye and movement of the dye is unique for each different dye molecule.
- Defend their conclusions about the dyes based on the evidence that they gathered.

TIME ESTIMATE:

Prep: 60 minutes (cutting paper stripes and marking the crime scene strips takes a lot of time.)

Activity: 90 minutes + answering the questions as homework

MATERIALS:

- Hydroville Science Journal
- Pencil
- Ruler

Per group of four students:

- Test tubes or culture tubes (20 X 150 mm) in test tube rack
- Strips of chromatography paper (1 x 16 cm)
- Strip of chromatography paper with crime scene ink (1 x 16 cm)

Class Material on Tool Table:

- set of black ink pens labeled “A”, “B”, “C”, and/or
- set of black ink pens labeled “D”, “E”, “F”
- Paper towels
- Colored pencils
- Wash bottle with rubbing alcohol

MATERIALS TO PHOTOCOPY:

- *Using Paper Chromatography - Instructions*
- *Team Worksheet*
- *Student Worksheet*

BACKGROUND INFORMATION:**What is Chromatography?**

Chromatography is a laboratory method that is widely used for the separation, identification, and determination of chemical components of a complex mixture. More specifically, chromatography separates compounds based on differences in their structure, size, and/or composition. Analytical chemistry uses chromatography to conduct qualitative analysis (identify the components) and quantitative analysis (determine the concentration) of unknown substances. No other separation method is as powerful and generally applicable as chromatography.

Qualitative Analysis – identifying chemicals (what kind?)

- Species identification , e.g., "killer" bees can be distinguished from native bees by comparing gas chromatograms of cuticle extracts
- Tracing contraband sources and detecting drugs in urine

Quantitative Analysis – finding concentrations (how much?)

- Each peak corresponds to a separate component in the mixture
- Area of each peak is proportional to concentration

How Does Chromatography Work?

There are numerous different chromatography techniques; however, they all use a stationary phase and mobile phase. The part that stays in one place is called the stationary phase, and the part that moves is called the mobile phase. Substances have different attractions for the stationary and the mobile phases and can be separated by these different attractions. Components of a mixture are carried through the stationary phase by a flow of a gaseous or liquid mobile phase. Each sample will migrate through the stationary phase at a different rate.

Using Paper Chromatography

In paper chromatography, a small amount of the substance to be analyzed (analyte) is placed on a strip of paper (the stationary phase) above the level of the solvent (mobile phase). In this activity, you will be using ink as the analyte and alcohol as the solvent. As the alcohol moves up the paper, the dye molecules from the ink mixture will move with it. If they are more strongly attracted to the alcohol molecules (mobile phase) than to the paper molecules (stationary phase), the dyes will continue to move up the paper. If the dye molecules are more strongly attracted to the paper than the alcohol, they will move more slowly than the alcohol or not at all. If two or more dyes have been mixed to form ink, then they may move at different rates as the alcohol moves up the paper. If this happens, they will separate out as different bands of color and can be identified by analyzing the paper chromatograms. Each paper chromatogram displays a unique pattern formed by the separation of the visible bands of dyes. Different mixtures of ink are specific for each brand of pen.

Retention Factors

After running the chromatogram, each separated band can be assigned a Retention Factor (R_f) which is characteristic of each specific dye(s). The R_f is a ratio of the distance the band travels to the distance the solvent (alcohol) travels. The R_f is calculated by dividing the band distance by the solvent distance. This ratio should be a constant that is characteristic of the dye(s) in a particular spot under a particular set of chromatographic conditions (i.e. paper chromatogram, alcohol solvent, etc.).

R_f (Retention Factor) = Distance Traveled by Band \div Distance Traveled by Solvent

Each component of the mixture will move a definite distance on the paper in proportion to the distance that the solvent moves. This ratio, R_f = distance component moves \div distance solution moves, can be calculated for each component to aid in identification. R_f values are dependent upon the paper, the solvent, and the amount of sample used.

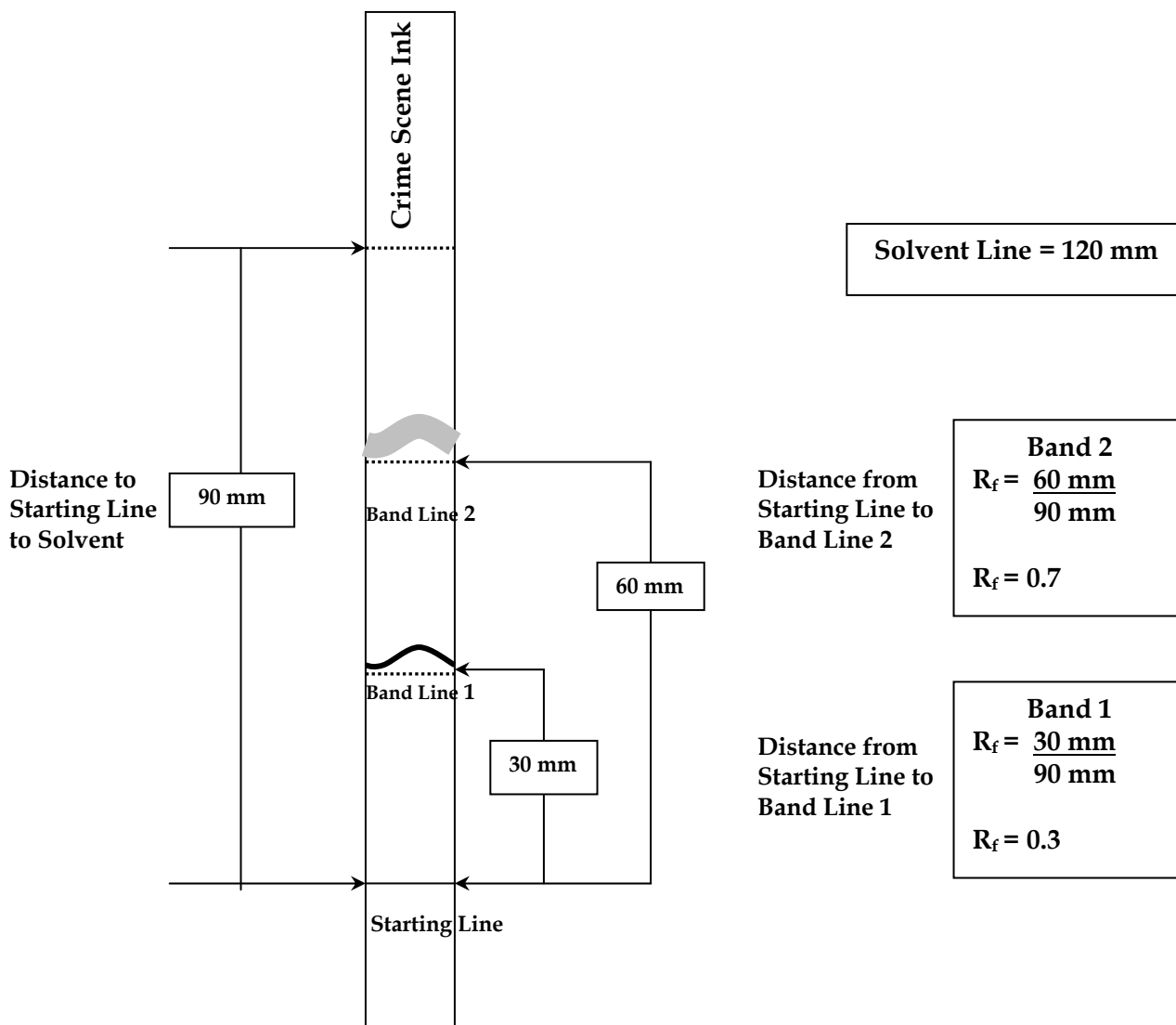


Figure 2. Explanation of Retention Factor

TEAM-BUILDING SKILL:

Everyone contributes and helps

Application: Each team member is involved in the lab.

Assessment: Students share something that another team member contributed to completing the lab.

TERMINOLOGY:

Chromatography	Molecule
Chromatogram	Analytical chemistry
Paper chromatography	Mobile phase
Qualitative analysis	Stationary phase
Quantitative analysis	Retention factor

SUGGESTED LESSON PLAN:*Getting Started*

1. The lab will go much faster if you pre-cut strips of chromatography paper (1 cm x 16 cm) and place them in an envelope. Chromatography paper can be purchased from Fisher in rolls of 100 yards (300 feet). Since students will be working in groups of 3 or 4, you will need to cut 30-32 strips of chromatography paper, one for each student.
2. Obtain brands of ink that will yield good results. Pens that work well in this lab include:
 - Sanford Calligraphic Pen, 1.5 mm fine point, waterbased ink
 - Sanford Uniball Micro
 - Sanford Sharpie, micro point, permanent marker
 - Papermate Ball Point, medium point
 - Papermate Flexgrip Ultra, medium point
 - Marks-alot, Dual Sharp Two Tip Permanent Marker, fine and ultrafine
3. Select three pens and label them "A," "B," and "C." Use one of these pens as the *Crime Scene* pen, the one that the suspect used. With the chosen *crime scene* pens, mark and label strips of chromatography paper, *Crime Scene*. Give one strip to each team. If you would like a second set of pens to complicate the experiment, use additional pens and label them "D," "E," and "F."
4. Present a crime scene scenario. For example: Police discover a forged check at a crime scene with the signature signed in an unusual type of black ink. Police confiscate black ink pens from three possible suspects (Suspect A, B, and C) in the forgery case. Ideas from other teachers:
 - Name the suspects after each of the 6 characters from the board game, CLUE, e.g., Colonel Mustard, Professor Plum, etc. or teachers in the school.
 - Relate the ink back to ink on the anthrax letters. Kids got very interested in "solving the crime"
 - Another teacher suggests these two scenarios:

I get more pens and put a different student's name on each so they are the suspects. I also do two chromatograms, one in water and one in alcohol. I do both chromatograms for each pen ahead of time and pick one that was close to several others. Maybe all were the same with alcohol, but one had a slight difference with water or vice versa. I tell them that I have been taking pens from each of them over the last couple of weeks! Some even believe me! Then

they compare with crime scene and they need to prove who did it (or if it is them, prove someone else did it).

I have also done a whole production with something like who *kidnapped the principal*. The teachers are suspect and there was a ransom note. Prepare teachers ahead of time to have a certain type of pen. Have intercom announcements every day giving clues (“so and so was seen exchanging pens with so and so”). It ended up being really fun.

5. The initial lab set up does not take very long, but students must wait 30 - 45 minutes until all of the dyes are separated. While students are waiting, have them read *Step 1. Background Information* in the student worksheet. While they are reading, have them underline words and phrases that are new to them. Other teachers have shown a video on forensic science during the wait time.
6. **Journal Prompt:** “List the words or phrases that you underlined. Consider these new vocabulary words and write definitions for them.” If students do not identify these words as new, make sure that they are aware of all of these vocabulary words. Help the students understand the meaning of these new vocabulary words, either by having them look them up or providing them definitions. Here is a list of possible vocabulary words and definitions:
 - Chromatography is a process that separates a mixture of compounds into separate components, i.e., molecules, based on differences in their structure, size, and/or composition. A liquid (mobile phase) carrying a chemical mixture or gas is separated into components as a result of differential distribution of the solutes as they flow around or over a stationary liquid or solid.
 - Paper Chromatography uses paper strips or sheets as the adsorbent stationary phase through which a solution flows and that is used especially to separate amino acids.
 - Analytical Chemistry is the science of making quantitative and qualitative measurements.
 - Quantitative Analysis - chemical analysis designed to identify the components of a substance or mixture
 - Qualitative Analysis - chemical analysis designed to determine the amounts or proportions of the components of a substance
 - Compound - something formed by a union of elements or parts; especially : a distinct substance formed by chemical union of two or more ingredients in definite proportion by weight.
 - Analyte - a small amount of the substance to be analyzed.
 - Stationary phase - the part that stays in one place. In paper chromatography, it is the paper and in gas chromatography it is the inert gases.
 - Mobile phase -the part that moves is called the mobile phase. In paper chromatography, it is the alcohol, and in gas chromatography, it is the column in the instrument.
 - Molecule - the smallest particle of a substance that retains all the properties of the substance and is composed of one or more atoms.
 - Chromatogram - the pattern formed on the adsorbent medium by the layers of components separated by chromatography and a time-based graphic record (as of concentration of eluted materials) of a chromatographic separation

Doing the Activity

1. Students will work in groups of 3 or 4 so that each student can conduct his or her own paper chromatography experiment.
2. Have students set up the lab at the very beginning of the period so that there is time for the solvent and inks to rise up the paper.
3. Refer to the student worksheet for specific lab instructions. Emphasize that following the instructions is critical to the success of the lab. Suggest that one of the team members read the instructions while the others do each step in order.
4. Remind students to use **PENCIL** when marking on the paper chromatograms. This is critical to the success of the experiment.
5. **Safety issues:** The chromatography solvent, rubbing alcohol, can produce unpleasant, noxious fumes. Use in a well-ventilated area under a hood. **Rubbing alcohol is flammable.**
6. Remind the students that they are conducting this lab to prove without a doubt that the suspect is guilty. Encourage them to make accurate observations and measurements.
7. To be able to present both quantitative and qualitative data, students will calculate **Retention Factor (R_f)** for the crime scene pen and suspect pen chromatograms. This is the distance that ink molecules traveled relative to the solvent front traveled. The *Retention Factor* is comparable to the *Retention Time* in gas chromatograms.
8. **Hint from Pilot School Teachers:** These calculations are difficult for some students and you may want to lead the students through these calculations as a class.
9. The crime is solved when the students match the chromatogram from the suspect's pen to chromatogram from one of the unknown pens. Compare individual retention factors for each pen and compile class data. It is likely that the R_f values will not be identical. But the separations are sufficiently distinct that students can identify the components qualitatively.

Wrap-up

Journal Prompt: "Briefly describe how the colors of the dyes mix to determine the color of the ink. Are some dyes found in more than one ink? Were you able to determine who the suspect was? If so, how?"

ASSESSMENT:

- Students complete the student worksheet and turn in a lab report.
- Students research chromatography procedures on the internet and explain in their own words how it works. See "Resources" for recommended web sites.

EXTENSION:

1. At the same time that the “suspect” inks are separating, have students perform paper chromatography on ink samples from Sanford brand Uniball Vision pens, and then have students explain why the ink does not separate into its component pigment molecules.
2. If you want to make the mystery more complex, add another clue. Perhaps the pen or check also has lipstick on it. Be creative! Here is a list of other chromatography labs:
 - Lipstick Analysis – Hughes Undergraduate Biological Science Education Initiatives. www.colorado.edu/UCB/Research/hughes/for_teacherguide.pdf
 - Separate Colors in a Green Leaf Using Chromatography. photoscience.la.asu.edu/photosyn/education/colorchange.html
 - Candy Chromatography (testing FD&C dyes from M&M's and Skittles). www.woodrow.org/teachers/chemistry/institutes/1986/exp3.html
 - Grape Kool-Aid Column Chromatography. www.chem.vt.edu/RVGS/ACT/lab/Experiments/Exp_14-Kool-Aid.html
 - Analysis of Mr. Sketch Ink by Paper Chromatography. wwwchem.csustan.edu/chem1002/msketch3.htm

RESOURCES:

- The Slightly Irreverent Guide to the World of Gas Chromatography and Analytical Chemistry. home.att.net/~gcresource/GCmain.html
- What is Gas Chromatography? members.kr.inter.net/guesu/gs/a_introduction/whatisgc.html
- General Chemistry On-line. Companion Notes: Matter: “Chromatography”. antoine.frostburg.edu/chem/senese/101/matter/chromatography.shtml
- The Chemistry HyperMedia Project. www.chem.vt.edu/chem-ed/scidex.html
- Welcome- Start GC. www.sciencesolutions4all.com/GC/
- Gas Chromatography. Sheffield Hammond University. www.shu.ac.uk/schools/sci/chem/tutorials/chrom/gaschr.htm

TEACHER KEY**Step 1. background Information**

Addressed as Learning Log prompt.

Step 2. Paper Chromatography Lab

Data appears in, "Paper Chromatograms of Ink Pens". Drawings should be similar for the same pens.

Step 3. Paper Chromatogram Analysis

Chromatograms will vary. Compare class data for accuracy.

Step 4. Determining Retention Factors

Retention factors will vary. Compare class data for accuracy.

Step 5. Conclusion Questions: Critical Thinking Questions (Students should record their answers in their Learning Logs)

1. Compare the patterns on the four chromatograms. Do any of the patterns appear to match? Which ones? Explain your answer.
Only one of the unknown strips should match the chromatogram from the crime scene ink. Other chromatograms may have similar bands, but not all will be identical.
2. What can you conclude about the suspects in this case? Which suspect(s) can you exclude (if any)? Which suspect(s) can you link to the crime scene?
Answers will vary depending on the crime set-up. All of the suspects are innocent, except the one whose pen matches the chromatogram.
3. If you were an expert witness in a jury trial, how would you explain your ink chemical analysis evidence to a jury? Include information about the Retention Factor.
Typically, an expert would try to provide quantitative evidence in court. Unfortunately this lab activity only provided qualitative evidence. Showing a jury the matching chromatograms based on observational data alone may be insufficient to persuade them. If each of the bands of dye are measured and the retention factor is calculated for each and compared to the retention factor of the crime scene ink, then it appears as more concrete evidence.
4. Why do some molecules move further than others do on a chromatogram in the same amount of time? Use your new vocabulary words in your answer.
Molecules have different affinities for the mobile phase and thus are separated out based on this attraction.



**STUDENT PAGES
FOR
USING PAPER CHROMATOGRAPHY**

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INSTRUCTIONS

Step 1. Background Information

In this activity, you will use paper chromatography to distinguish among different brands of ink pens used by each of the suspects and compare them to ink on a forged check found at a crime scene.

There are many types of chromatography. In this lab, you will be using paper chromatography. In the analysis of the pesticide spill, you will be using gas chromatography. But all chromatography works on the same principles. Chromatography separates a mixture of substances by the affinity or attachment of the substance to a stationary phase (in this case the paper) and a mobile phase (the solvent alcohol). The ink molecules that are most attracted to the solvent will travel up the paper with the solvent while leaving others behind. This results in a paper strip with different colors of ink going up the paper. The same inks will produce the same patterns so that you can match the chromatogram of a know pen with the crime scene ink to make a positive identification.

Step 2. Paper Chromatography Lab

In this experiment, you will conduct a qualitative analysis of various dyes in ink.

Materials

- Hydroville Science Journal
- Pencil
- Ruler

Materials (per group of 4 students):

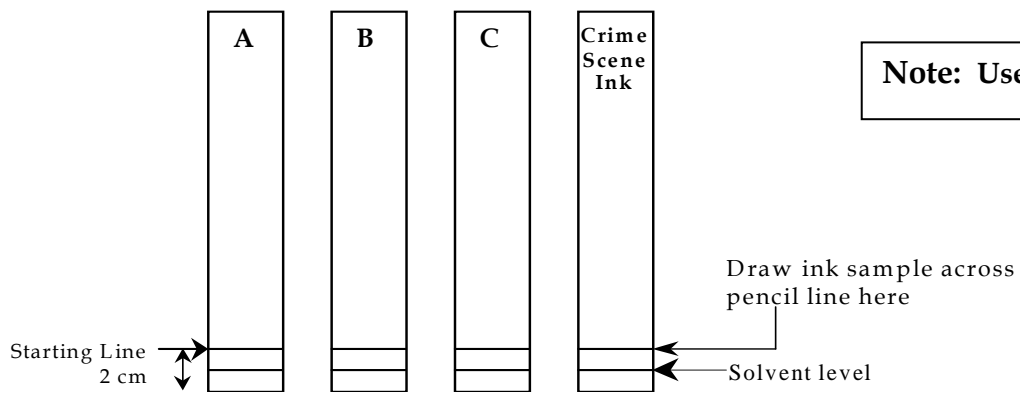
- Test tubes or culture tubes (20 X 150 mm) in a test tube rack
- 3 strips of chromatography paper (1 x 16 cm)
- 1 strip of chromatography paper with crime scene ink (1 x 16 cm)

Instructions

1. Each group receives a plastic bag of supplies with three strips of chromatography paper.
2. Using a pencil (**NOT a pen!**), draw a horizontal line 2 cm from one end of each strip and label it, "Starting Line". (See Figure 1)
3. Using a pencil (**NOT a pen!**), label the four strips, "A," "B," and "C" to represent each of the suspects (See Figure 1)

Figure 1. Set up for Paper Chromatography

INSTRUCTIONS



INSTRUCTIONS

4. Draw a thin, horizontal line of the appropriate black ink sample on top of the pencil line on each strip of filter paper labeled A, B, and C from pens labeled "A," "B," "C".
5. Obtain a crime scene ink chromatography paper from your instructor.
6. With a ruler, measure 1 cm from the bottom of the test tube and draw a line with a marking pen or wax pencil. This is your fill line mark. Now fill each test tube with solvent (rubbing alcohol) to this fill line.

WARNING: Rubbing alcohol can produce unpleasant, noxious fumes. Use in a well-ventilated area or under a hood. **Rubbing alcohol is flammable.**

Read this entire step before continuing

7. Place paper chromatogram strips in the test tubes, one per test tube. Place the ink line toward the bottom of the test tube. The paper should be submerged into the alcohol, but the ink **must not touch the solvent**. Fold the top of the strips over the lip of the test tube. Make sure the strips are not flattened against the side of the test tube.
8. Leave the strips in the test tube for 30-45 minutes.

Step 3. Paper Chromatogram Analysis

1. Remove paper strips from test tubes. Before the solvent dries, use a **PENCIL** to draw a line on the filter paper that will indicate where the solvent front reached. Label line, *Solvent Line*. Then allow the filter paper to dry.
2. Tape the paper strips on the drawing on the worksheet.

Step 4. Determining Retention Factors

When comparing the unknown samples of ink to the ink used at the crime scene, you must show concrete evidence of an ink match. Therefore, it is important to calculate the Retention Factor (R_f) for each band in every ink sample. We can use retention factors as a tool to help answer several questions about the inks. In this investigation, you will analyze the paper chromatograms of the Crime Scene Ink and the one you identified as from the Suspect.

1. On the crime scene chromatogram, measure the distance from the starting line to the solvent line. Record this measurement in the column labeled, *Distance Traveled by Solvent (mm)*.
2. To determine the exact location of each band on the crime scene ink chromatogram, measure the distance in millimeters from the starting line to the **bottom of each band**. Record this measurement in the column labeled, *Distance Traveled by Band (mm)*.

Note: Measure all of the bands that appear on the chromatogram.

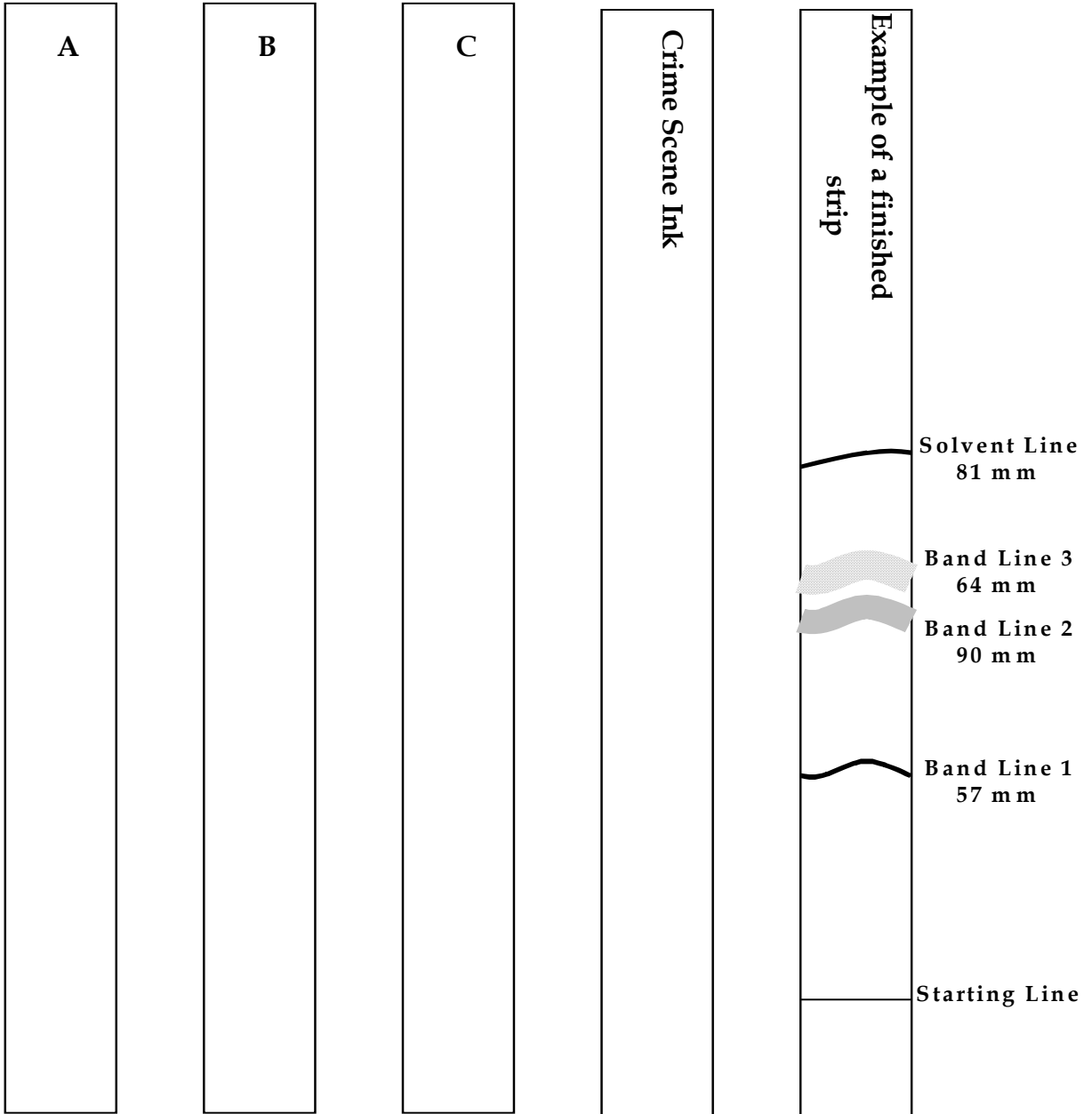
INSTRUCTIONS

3. Calculate the R_f for each band by dividing the *Distance Traveled by Band* by the *Distance Traveled by Solvent*.
4. Repeat steps 3-5 for the chromatogram of the ink you identified as from the suspect.
5. Compare the R_f values of the bands from the *Crime Scene Ink* chromatogram and the *Suspect* chromatogram. If they are similar, then you've found your suspect.

TEAM WORKSHEET

Step 3. Paper Chromatogram Analysis

Tape chromatography paper strips here



STUDENT WORKSHEET

NAME _____

Step 4. Determining Retention Factors

Crime Scene Ink

Band #	Distance Traveled by Band (mm)	Distance Traveled by Solvent (mm)	R_f (Retention Factor) = Distance Traveled by Band/ Distance Traveled by Solvent
1			
2			
3			
4			

Suspect Pen

Band #	Distance Traveled by Band (mm)	Distance Traveled by Solvent (mm)	R_f (Retention Factor) = Distance Traveled by Band/ Distance Traveled by Solvent
1			
2			
3			
4			

Conclusion Questions

1. Compare the patterns on the four chromatograms. Do any of the patterns appear to match? Which ones? Explain your answer.
2. What can you conclude about the suspects in this case? Which suspect(s) can you exclude (if any)? Which suspect(s) can you link to the crime scene?
3. If you were hired as an expert witness in a jury trial, how would you explain your ink chemical analysis evidence to a jury? Include information about the Retention Factor.
4. Why do some molecules move further than others do on a chromatogram in the same amount of time? Use your new vocabulary words in your answer.