What you need to do:

Procedure: Part A: Preparing the chromatogram

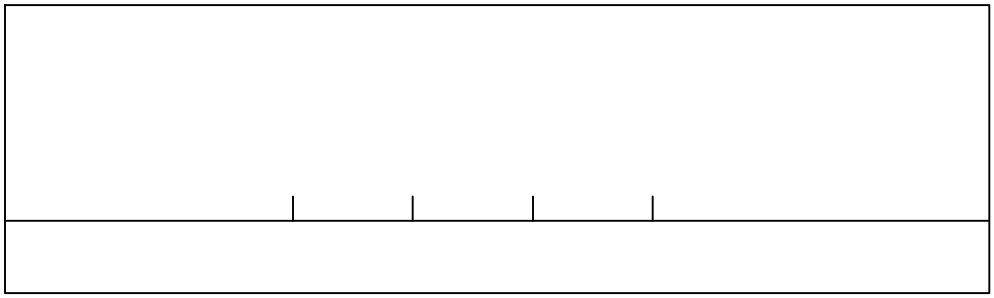
1. Put on the gloves provided.

2. Collect a precut piece of chromatographic paper from the front counter.

3. Using a pencil, draw a line across the width of the paper ~1.5 cm from the bottom. In the upper right hand corner write your name or initials.

4. Mark 7 spots with a small vertical line ~2 cm apart across the chromatogram. These are the places where you will "load" your sweat.

5. Below the marks label (in pencil) the following: AA1, AA2, and AA3 (standing for the known amino acids 1, 2, and 3 and , Left Elbow, Right Elbow, Left Palm, Right Palm. Below is a diagram of a typical chromatogram setup. Obviously, you will need 7 lines rather than four but this should give you an idea of how to mark the chromatogram.



Procedure: Part B: Preparing the Amino Acids

1. Place a drop of distilled water on a clean watch glass.
2. Using a Q-tip, first wet the Q-tip and then rub the Q-tip gently in the inside crease of your left elbow to collect any surface sweat that may exist there. Rub the Q-tip for at least 30 seconds to accumulate as much residue as possible and then return it to the drop of water to deposit the residue. Repeat this action several times.
3. Now using a toothpick so that the spots on your chromatogram are small, dip the toothpick in the water containing the sweat residue and spot your chromatogram on the line marked "Left Elbow".
4. Repeat the process for your Right Elbow, Right and Left palms. (When you remove your gloves be sure not to touch the chromatograms with your fingers).
5. Your instructor will have a set of three known amino acids we will be using as standards available to you. Clean and dry your watch glass and take it to your

instructor. S/He will dispense a small drop of each standard amino acid for you to use.

6. Use a toothpick as before to spot the correct amino acid to the lines marked "AA1, AA2, and AA3". Be sure to note the actual identities of these amino acids in your notebook.
7. Allow the chromatogram to dry completely until you can no longer see the spots you have marked as wet spots.
8. Using a stapler, coil the chromatogram and staple the ends so that it is a cylinder capable of "standing up" on its own. Make sure the edges are even.

Procedure: Part D: Running the chromatogram

1. Using a graduated cylinder measure out 30 mL of the solvent provided. The solvent is 8:2:1 v/v ethyl acetate: pyridine: water.

2. Collect a 1000 mL beaker from the front counter and add the 30 mL of solvent.

3. Take the beaker as well as your chromatogram to the hood.

4. Once at the hood, carefully set the chromatogram into the beaker.

5. Note the time you started your chromatogram in your notebook.

6. Cover the beaker with saran wrap and seal with a rubber band.

7. Allow the chromatogram to run for 30 minutes.

Procedure: Part E: Developing the chromatogram

1. Carefully remove the chromatogram from the beaker (make sure you are wearing your gloves when handling the chromatogram)

2. Gently remove the staples and lay the chromatogram flat on a paper towel.

3. Quickly use a pencil to draw a line to mark the position reached by the solvent front.

4. Allow the chromatogram to dry.

5. Once dry, take your chromatogram to the hood and allow the TA to spray it with ninhydrin.

6. Use the hotplates (***set very low***) to dry the ninhydrin and develop your amino acid spots.

a) Turn the hotplate on low.
b) Gently rub the chromatogram back and forth across the top of the hotplate for about 5 to 10 minutes.

Procedure: Part F: Calculating the R_f values

1. Use a ruler to mark the center of all the amino acid spots.
2. Measure in cm the distance between the starting line and each spot.
3. Since you have four spots containing the same unknown, your amino acid spots should be relatively the same. Report the averages of any spots you consider to be the same amino acid.

4. Measure the ***average*** distance from the starting line to the solvent front. (Your average should be based on at least 3 measurements)