(actually a compilation of several different protocols)

Preparing the sheets

- I use commercial sheets and do not go through the trouble of making my own: Baker-Flex PEI (polyethyleneimine-cellulose) plates (J. T. Baker, Phillipsburg, N.J.) work fine.
- Mark the plate with a pencil before pre-running; the dry plate is easier to draw on. I usually mark the plate this way: a dashed horizontal line 2 cm from the bottom to mark the sample origin and vertical lines down the plate every 2 cm to mark the lanes. With the horizontal line, I leave a space in the center of each lane for the sample origin. Usually I mark the plate in half, vertically, to get two plates, drawing another sample origin line 2 cm above this halfway mark (leave about 2 cm at the very top to cut off impurities). Running the sample farther achieves higher resolution but resolution is often degraded by diffusion.
- Pre-run the plate in milliQ H2Oin a humidified, enclosed tank or chamber. You will probably see yellow streaks in the solution front. Run water up to the top of the plate.
- Remove the plate and cut off the top 2 cm or so containing yellow streaks. The plate is much less flakey and easier to cut after pre-running. I usually cut the plate in half, vertically (see above).
- Air-dry the plate completely (about 1-2 hours). At this point, plates can be wrapped in foil and stored stably at -20°C for 2-4 months. Note that if the plates are dried overnight, the chromatographic characteristics can supposedly change because of PEI breakdown. Caution: If the sheets are placed in the freezer slightly damp, the PEI cellulose may separate from the vinyl backing later during chromatography.

- Another protocol (which I haven't tried) lists an alternative method for removing impurities from the plates. Shake the sheets gently for 2-4 minutes in reagent grade methanol then shake in distilled water for 10 minutes. The washed sheets are air dried thoroughly and wrapped with aluminum foil or placed in a sealed container for storage.

Loading samples:

- Spot ~0.5 ul of sample at the sample origin (the blanks left in your origin line). I have spotted samples of much larger volumes. The resulting chromatographic spots are large but often resolvable from each other and are not as large as you might think from the wet spot at the origin. For large volume samples, I load 5 ul at a time, letting the sample dry between loads.
- Always load standards, ideally at least one per plate. I often find it useful, especially for radioactive experiments, to load a cold control which will be visible by handheld UV lamp. 5 ul of a 1 mM nucleotide stock is sufficient.
- Let the samples air-dry before running.
- Make sure that the sample origin is above the level of buffer in your chamber.

- If you are loading large sample volumes, you may need to be aware of excessive urea, salt, or glycerol, all of which will make your samples smear out and run irreproducibly. For example, to check the efficiency of an enzymatic reaction, I will take samples and dilute them 1:1 in a denaturing solution (8 M urea, 20 mM Tris-Cl, pH 7.5, 5 mM EDTA). Denaturing proteins forces them to release nucleotides; protein should then remain at the origin during TLC. To get rid of the urea, I will pre-run post-load in water: that is, run the plate in milliQ H2O again. If you are pre-running post-load, it is probably not necessary to have pre-run pre-load in milliQ H2O (as in above). Once I had to pre-run twice post-load to get rid of a large amount of 60% glycerol in my samples.

Running samples:

- Develop the sheets in an enclosed, humidified chamber. For separating nucleotides, I usually use either 1.4 M LiCl and 0.75 M potassium phosphate, pH 4.2.
- Remove plates and mark the buffer front with a pencil. I usually run the plates about 5-6 cm but of course one can run them farther. You can check the resolution and progress of your spots with a hand-held UV lamp if you have loaded enough standard solution.
- For the potassium phosphate buffer system, you should get Rf values of 0.24 and 0.71, respectively, for ATP and Pi. For theLiCl system, those Rfs are between 0.4 and 0.5 for ATP and 0.9 for Pi. Protein should stay at the origin. Rf is the distance traveled by the spot divided by the total distance traveled by the solvent front. For large spots, I tend to get more consistent Rfs by measuring from the origin to the middle of the spot, rather than the top or bottom.
- Let the plates air-dry, then wrap in saran wrap. I usually quantitate radioactive spots by phosphorimaging.
- If the Pi is too diffuse, try running for a shorter distance in these buffers and/or lowering the salt concentration.