**Brad's TLC protocol**
-adapted from various sources
-for use in analyzing hydrolysis of labeled terminal phosphate from NTP's

Preparing the TLC plates

- Use PEI-cellulose TLC plates (plastic backing). I recommend plates made by JT Baker (Bakerflex). Cut the plates to an appropriate size – generally the size of the beaker you are planning to run the plate in (I cut it to around ~15 cm).
- Mark lanes at the bottom of the plate using a pencil, being careful not to score the plate. I find that lane widths of 1.2 cm are convenient, although you may be able to reduce this.
- Mark the spot line (the region of the plate where you will be applying your samples) along the sides of the plate. I find that putting the spot line approximately 3 cm from the bottom of the plate to be convenient.
- Pre-run the plate in ddHOH to remove impurities:
    - This step is absolutely crucial to get meaningful results, regardless of what the manufacturer says.
    - Fill a beaker with ddHOH so that the level of the water is 1/3 to 1/2 the distance from the bottom of the plate to the spot line.
    - Place the TLC plate into the beaker so that the bottom portion of the plate is submerged.
    - Cover the beaker with plastic wrap, making sure that there is a tight seal – this is critical to prevent excessive evaporation of water from the plate.
    - Run the plate until the water front is near but not at the top edge of the plate.
    - Romeve the plate and allow to air dry.
- The pre-run plate will show a yellow stain where the water front was. Cut off this portion of the plate and discard.

Running the TLC plates

- Spot 2 µL of sample onto the TLC plate at the spot line
    - It is important to make sure the plate is completely dry before you spot. The drier the plate, the smaller the spot size which means the more samples you can get on a plate.
    - I like using 2 µL sample sizes because they are large enough that you do not have to worry about pipetting error, making quantitation more reliable, yet small enough to produce a compact spot.
- Allow spots to air dry.
- Run the plate as before, using 0.75 M $KH_2PO_4$ pH=4.2 instead of ddHOH.
- Remove plates and allow to air dry completely.
- Wrap the plates in plastic wrap and analyze using phosphor-imaging.