

Lindberg Lab Tips 2/12

- **DATE EVERYTHING!!** Especially anything you put in the freezers or refrigerators, but also all data. The year too! The first sample in any long series bears the date and exptl details.
- **BACK UP everything!** (especially cells; freeze them down before doing any experiments)
- File a plasmid map in the Blue Book within 2 weeks of construction. Make sure the location of your plasmid is accurately specified. **Label with backbone and species -as well as insert** (“mPOMC-pcDNA3”)
- Leave 10% of your tube contents and freeze. There are very few experiments where leaving 10% of your sample behind will cause total loss of data. This permits reassay!
- **Add 0.1% sodium azide to most solutions** (but not solutions that ever touch animals or cells). It doesn't affect our enzymes at all (but mold will!)
- Samples and standards should **always be in the same buffer.**
- Dilute your unknowns so that **they are in the middle of the standard curve** (or repeat the assay to get them there).
- **Don't accept replicate variation of greater than 15%.** Something is wrong!
- Use different standard colors for control tubes and experimental tubes- I always label control tubes black, and exptl. tubes red.
- **Put a standard lane on the left side of every gel-** this way you will never have a direction problem. Use 5 ul of the colored standards for blots and 2 ul of the non-colored standards for Coomassie stains. Make sure the standards used correspond to the gel percentage: the higher the percentage, the lower Mr the standards used.
- Always put the handle of the Eppendorf tube on the outside when centrifuging; the pellet will then always be right below the handle.
- Use a sponge to soak up Coomassie dye during destains. Heat solutions in the microwave if you want destain or stain to go faster.
- **Thaw samples completely and mix gently before pipetting by inversion or pipetting.** Vortexing denatures proteins.
- Make sure you know how to seat a rotor, the allowed speeds of each rotor, and the allowed speeds of each type of plasticware.
- Polystyrene is very protein-adsorptive. Don't use it with dilute protein solutions in the absence of carrier protein. Polypropylene is less absorptive, but many of our sticky proteins will still need detergent or carrier protein.
- **Always do positive and negative controls** to make sure your assay is working and is well-controlled.
- When working with a new protocol, read to the bottom before starting! Make sure you interrupt at approved places if you stop in the middle.
- Always do **feasibility calculations the** day before; also, make and chill your buffers the day before. Write out (typing is best) your protocols the day before.
- If you deviate from your protocol on the day of the experiment, write it down **on the same sheet as the protocol!** You never know when you will want to repeat the deviation -it may work better!
- Don't work at night on new things you might need help with-- if no one is around.
- **Always write the PURPOSE of the experiment in a sentence at the top of the page**
- **Always write the overall RESULT/CONCLUSIONS of an experiment on the first page** so you can easily see what happened --years later. This need only be a sentence!