Nucleus Protocols

Protein Gel

- ☐ **Denature samples.**
  - ☐ Label one tube per sample.
  - ☐ Add 4 µL of 6x Laemmli sample buffer and 20 µL of sample (∼1 µg total protein) per tube. Mix by pipetting.
  - ☐ Incubate samples at 90 °C / 10 min.
- ☐ **Set up a gel box.**
  - ☐ Open an individually wrapped protein gel (we use 10%–20% Tris Gly gels).
  - ☐ Remove plastic tape at the bottom of gel. This exposes a strip of the gel to the running buffer, allowing current to flow from one electrode through the gel to the other electrode.
  - ☐ Place gel in gel box and seal tightly. Check the seal by pouring running buffer (we use Tris Gly) into the front half of the reservoir and checking that no buffer leaks into the back half. If so, remove the gel, empty the reservoir and try again.
  - ☐ Pour running buffer into the back half of the reservoir.
  - ☐ Carefully, remove comb from top of gel.
- ☐ **Load 10 µL protein ladder onto the outermost lanes.**
- ☐ **Load each sample onto its own lane.** Target between 1 µg to 2 µg purified protein and between 10 µL and 20 µL per lane.
- ☐ **Run the gel at 200 V / 40 min to 50 min,** or until the dye front touches the bottom of the gel.
- ☐ **Stain and destain the gel.**
  - ☐ Using a gel knife, crack open the plastic cassette holding the gel.
  - ☐ Remove the gel carefully into a waterproof container. We find this is easier if you cover the bottom of your container with Ultrapure water (≥ 3 mm).
  - ☐ Cover the gel with Ultrapure water (∼40 mL), microwave for 30 s, and rock for 5 min. Decant and repeat this step twice, washing a total of three (3) times.
  - ☐ Cover gel in a rapid protein stain (∼40 mL) in the same container. We use PageBlue Protein Staining Solution. Microwave for 30 s, then incubate at room temperature with rocking for ∼30 min.
  - ☐ Decant staining solution, rinse gel at least once, then cover in Ultrapure water. Incubate gel at room temperature with rocking until you can see distinct protein bands (up to overnight).
- ☐ **Observe gel**. High purity protein preps should have distinct bands at the correct molecular weight, with no unexpected additional bands.