**Agarose gel electrophoresis protocol**

Update: 10/1/2025

This protocol describes how to create and run an agarose gel in the Stenglein lab to analyze a DNA sample.

**Making the gel**

1. Prepare the gel casting apparatus in the fume hood in the chemical room. Place combs in the apparatus and make sure it is level by rotating the leveling screws.
2. Into a 500 mL flask, weigh out an appropriate amount of agarose for a certain percentage gel. Our typical gel casting apparatus takes a volume of ~125 mL and concentration is defined in units of g/100 mL of gel. So for 125 mL of a 1.25% agarose gel, you would need 1.56 g of agarose.
3. Add an appropriate volume of 1x TAE from carboy (e.g. 125 mL)
4. Swirl the agarose to mix. Plug the flask opening with a wadded up paper towel.
5. Microwave the gel for 1-2 minutes until fully disolved, gently swirling every 20 seconds or so to mix. Hold the flask with a hot pad: it will get hot. Wear safety goggles and always point the flask away from yourself: the vapor in the flask can become superheated, causing the gel to shoot out of the flask.
6. Add ethidium bromide (10 mg/mL) to the agarose/TAE solution at 5 µL per 100 mL of gel (so 6.25 µL for 125 mL). Swirl to mix. Dispose of the EtBr contaminated tip in the EtBr solid waste.
7. Pour the molten gel in the prepared casting apparatus.
8. Wait ~20 minutes for the gel to cool and transfer it to a labeled plastic bag for storage at 4˚C.
9. Wash and rinse the gel casting apparatus and combs.

**Running the gel**

1) Make an agarose gel prior to running. Gel should have enough wells to run products and a ladder.

2) Place a gel carefully in the buffer tank against the edge so the gel does not float away. Make sure clean 1x TAE is covering the gel and wells. Add more if needed. If the TAE is old, replace it entirely.

3) Mix samples with 1/5 volume 6x orange G loading buffer. For example, mix 5 µL of PCR product with 1 uL of loading buffer and load 6ul.

4) Load ladder and samples into wells.

5) Run the gel at a voltage of 150 VDC and a run time of 50 min.

6) Image the gel on the BioRad GelDoc.