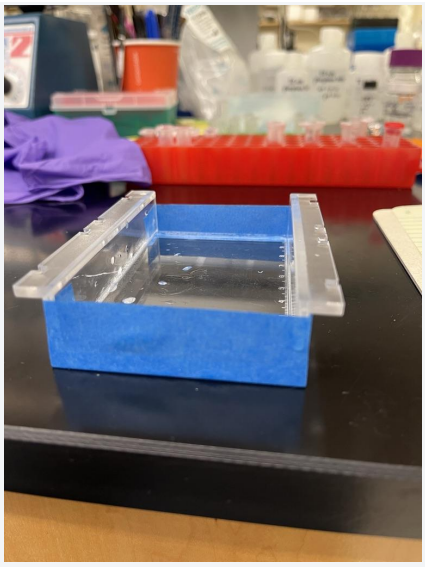
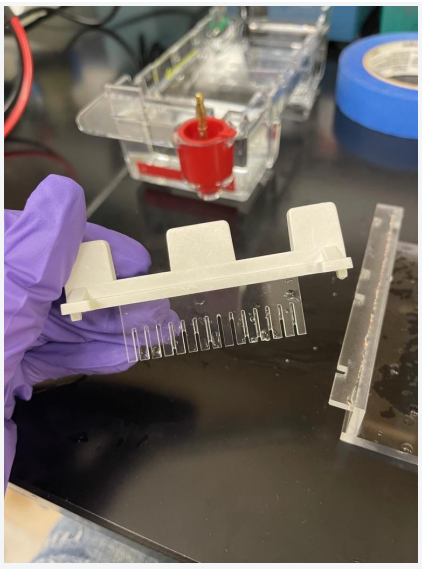
**Gel Electrophoresis Protocol**

**Note:** This protocol specifically runs for DNA-gold nanoparticle separation.

Preparing the Gel (Preparation of a 0.75% Agarose Gel)

1. Tape sides of gel casting tray and insert well comb.





2. Insert taped tray into gel caster and adjust feet of gel caster until surface is leveled.
    - Level gel casting tray with bubble leveler.
3. Add 40 mL of 0.5x TBE buffer and 300 mg agarose powder to 250 erlenmeyer flask.
    - Concentration of agarose gels are normally around 0.7% - 2%.

- Depending on the size of DNA or RNA being separated and better resolution/separation of bands, agarose percentage should be changed and adjusted as needed.
- Smaller bands are separated better with higher agarose gel percentages.
- Larger bands are resolved better with lower agarose gel percentages.

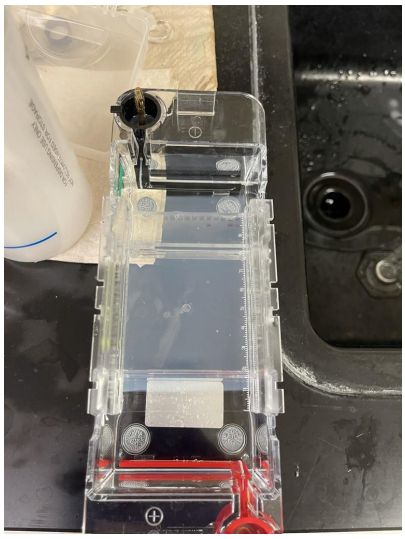
4. Microwave in 30 increments. Remove flask between each increment and swirl gently. Repeat until agarose is completely dissolved.
    - Cover top of erlenmeyer flask with tissue paper when microwaving to avoid evaporation and loss of solution.

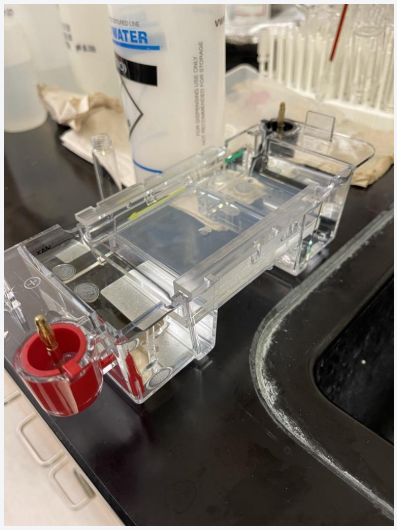
<u>Casting the Gel</u>

5. Pour molten agarose solution into the casting tray with the well comb in place.
    - If any bubbles are present, either push them to the side or get rid of them using a pipette tip.
6. Wait for the gel to completely solidify before removing the comb (typically takes around 15 min for gel to solidify once poured into the tray).

<u>Loading the Gel</u>

7. Remove tape from sides of the casting tray.
8. Place gel into electrophoresis chamber with wells on the cathode end of chamber.
9. Fill electrophoresis chamber with 0.5x TBE buffer until the gel is covered up to the max fill line on the chamber.





10. Add glycerol to each sample (total glycerol concentration of the sample should be at least 10%) so that it will settle at the bottom of the well.
11. Load samples into wells of the gel.
    - Click on link HERE for more information on how to load samples.
    - 10 µL was used to load samples into wells, but wells can hold approximately 15 µL

Running the Gel

12. Run gel at 75 V for around 30 min.
    - Bubbles should appear on cathode and anode terminals when voltage is applied.
    - Gel is normally ran between 70 - 150 V but voltage may vary based on sample.
    - Unlabeled DNA experiments are typically ran until loading dye travels 75% of the gel which normally takes 2.5 - 3 hours.
    - Depending on the size of DNA or RNA being separated and better resolution/separation of bands, voltage should be changed and adjusted as needed.
    - Running the gel at a lower applied voltage for a longer period of time can increase resolution/separation of bands.