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# Protocol for DNA Gel Electrophoresis

*Adapted from protocol by Alice Walsh*

### *Preparation of Agarose Gel*

1. Prepare 1X TAE buffer by adding 20 mL of 50X TAE Buffer to 980 mL water.
2. Chose % agarose for gel. A 0.9 or 1% agarose gel will work for most applications.

| Range of separation | % agarose | Amount of agarose for 50 mL gel |
|---|---|---|
| 5 kb – 60 kb | 0.3 | 0.15 g |
| 1 kb – 20 kb | 0.6 | 0.30 g |
| 500 bp – 7 kb | 0.9 | 0.45 g |
| 400 bp – 6 kb | 1.2 | 0.60 g |
| 200 bp – 3 kb | 1.5 | 0.75 g |

3. Add desired amount of ultra-pure agarose to 1X TAE buffer in a flask. For a standard gel, use 50 mL of 1X TAE Buffer. Swirl the flask to mix.
4. Microwave solution for 1 min. Remove with gloves and swirl. Microwave for longer if there is undissolved agarose. Allow the solution to cool for 1 min.
   - Note: Add a folded paper towel to the opening of the flask when microwaving to prevent steam from escaping.
5. Add 1 µL of ethidium bromide to solution and swirl.
   - Note: Ethidium bromide is a carcinogen and must be handled with care. Dispose of ethidium bromide tips in the designated biohazard bin.
6. Place the gel tray into the cassette and pour the solution into the tray. Insert the comb into the top of the gel and allow the gel to solidify for 30 min. Avoid bubbles in the gel.
   - Choose either an 8- or 16-well gel depending on application. If performing gel extractions, use the 8- well comb to accommodate a larger mass of DNA.
7. Rinse with water and dry the flask to prevent residual gel from solidifying in the flask.

### *Running Gel Electrophoresis*

1. Once the gel has solidified, carefully remove the comb by pulling straight up.
2. Ensure the gel is in the correct orientation, with the negative/black electrode above the wells so that the DNA runs toward the positive/red electrode.
3. Prepare the samples by adding 6X loading buffer to each. For a 16-well gel, combine 5 µL of DNA with 1 µL of 6X loading buffer in order to load 5 µL. [For most applications, load 20-100 ng of DNA/lane.] If doing a gel extraction in an 8-well gel, combine 30 µL DNA with 6 µL 6X loading buffer to load 36 µL.
   - Note: These samples can be prepared on the reverse side of paraffin paper since the volume is so small.
4. Load 5 µL of DNA ladder into one lane of your gel.
   - Note: Choose a ladder that contains the weights of your sample.
5. Load samples into wells. Avoid bubbles.
6. Place lid on cassette and ensure the red and black wires are connected to the matching red and black electrodes on the cassette.
7. Gel can be run at a variety of time and voltage settings depending on the size of samples and desired separation. For most samples, 90V for 30-50 min will work.
8. Remove the tray with the gel and image with UV. Exercise caution when imaging with UV, especially if doing a gel extraction over the UV box.
9. If doing gel extraction, proceed with gel. If not, dispose of the gel in the gel biohazard bin.