Agarose Gel Electrophoresis

1. Mix 100ml of 1X TAE buffer with 1g of regular or LMP agarose (for a 1% gel). For a small gel, mix 30ml of 1X TAE with 0.3g agarose.
2. Microwave covered for about 1.5 minutes or until agarose is dissolved
3. Cool and add 4µl SYBR safe. For a small gel, add 1.5µl SYBR safe.
4. Set up gel tray and balance properly. Pour and cast gel. LMP gels should be cast in the fridge.
5. Run sample at 100V (regular gel) or 80V (LMP gel) until sample is ¾ of the way down the gel (20-30min)c

Note:

- Small combs fit ~20µL of sample
- Larger/wider combs can fit more sample
    - If you need to fit even more sample you can look into making your gel thicker (i.e. using more agarose+TAE mix), but be careful as gels that are too thick can run weirdly

Loading the gel:

Small gel V_{total}: 30 mL

- 2 uL of ladder
- Sample + loading dye (5 uL + 1 uL) = V_{total} = 6 uL

Big gel V_{total}: 150 mL

- 4 uL ladder
- Sample + loading dye (10 uL + 2 uL) = V_{total} = 12 uL

Troubleshooting:

1. Squished bands
    a. Try decreasing the the amount of agarose to 0.8% for DNA >1.5kB
    b. Running the gel for longer
    c. Lower voltage (for the whole time or a period of time)
2. No bands
    a. Forgot SYBR Safe/RedSafe/whatever nucleic acid stain you're using
    b. Not enough DNA was present to be visible
    c. Your previous experiment (ex. PCR, digestion) failed

3. Appearance of smudged bands at the bottom of the gel (below ladder) = these are primer dimers

4. Smudged bands (this may or may not be a desired result)
    a. DNA shearing → particularly in genomic and plasmid DNA
        i. Note: If visualizing plasmid DNA it can produce different bands based on the state of the plasmid (i.e. open circular, supercoiled, nicked, linear)
    b. Try replacing TAE buffer → this is likely not the main/only problem though