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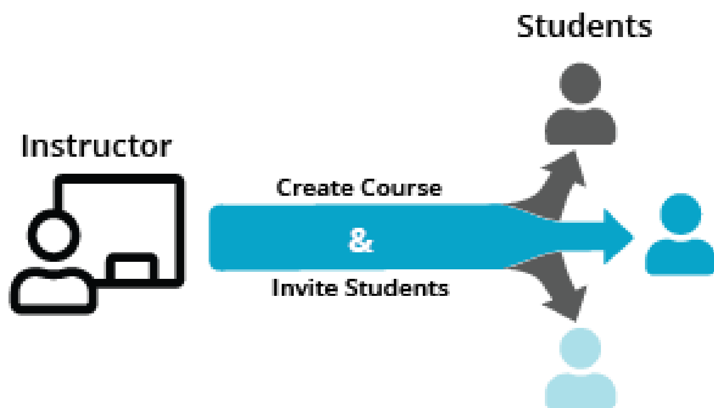

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Lab Builder

Agarose Gel Electrophoresis Protocol

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Topic

- PCR
- Electrophoresis
- DNA
- Mutations
- Genetics
- Agarose
- Molecular weight standards

Timeline

- **Prepare samples, run and image gel:** 45 minutes – 1.5 hours

Safety

- Wear gloves and safety goggles!

Equipment

- Gel casting tray
- Combs (to make the sample wells)
- Gel box to hold buffer and gel
- Voltage source
- Imager (many options)
- Microwave

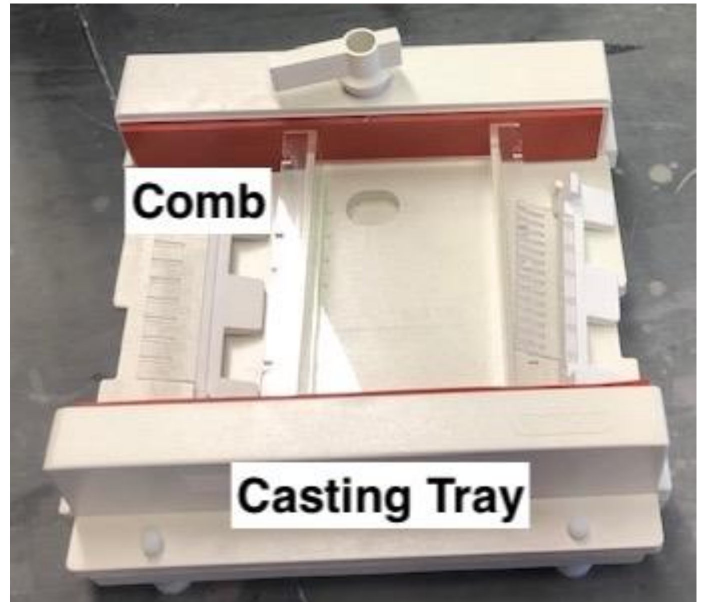


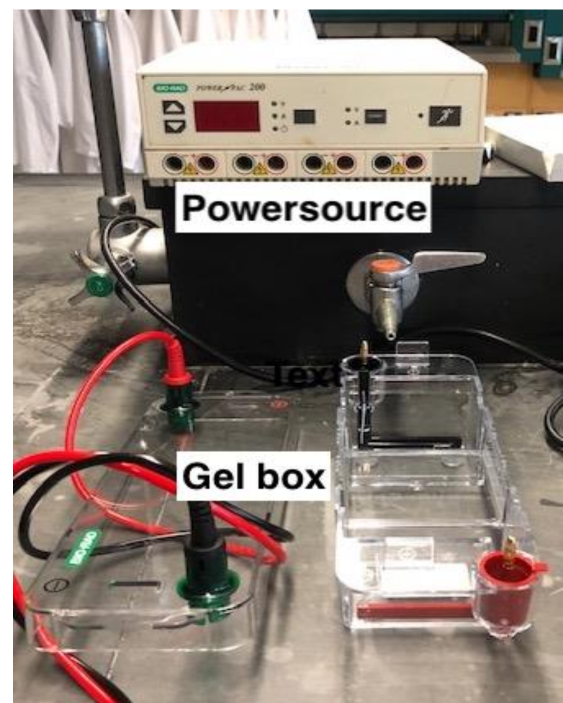
Image credit: Dan Kraut, Villanova University

Materials

- TAE or TBE
- Agarose
- Molecular weight standards
- DNA staining reagent
  - Ethidium bromide
  - SYBR Safe
  - GelRed or GelGreen

Prepare the Agarose Gel

1. Prepare casting tray
2. Prepare 0.7-1.5% agarose solution in 1X TBE or TAE buffer
   - Use the same buffer to run your gel
   - The concentration depends on the size of the DNA you need to separate
   - The final volume of agarose depends on your specific gel apparatus
3. Mix agarose with buffer in a microwavable flask



4. Microwave until the solution bubbles (without overflowing) and the agarose is completely dissolved (about a minute is a good place to start).
5. Let solution cool
   - Be sure to use paper towels or a hot pad to avoid burning yourself
   - Allow to cool until you can touch the flask with a gloved hand without it being too hot to handle; you can use a cold water bath or run the flask under tap-water to help.
6. Add DNA staining reagent to appropriate final concentration and swirl
   - *Alternative* - stain after running the gel
7. Pour the solution into the casting tray
8. Allow to solidify

Running the Gel

1. Add loading buffer to each of your samples in an eppendorf tube
2. Amount of DNA loaded on gel depends on many factors
   - Fragment size
   - Detection method
   - Well volume
   - Distribution of bands (fragment sizes)
3. Add the appropriate amount of sample to each well of your gel
4. Add the vendor's recommended volume of molecular weight standards (ie Ladder)
5. Run gel
   - Optimal voltage and time depend on fragment size and buffer choice