- Announcements
- Lab Quiz (on M1D1 material)
- Pre-lab Lecture
  - ❖ Writing a Methods Section
  - ❖ Gel Electrophoresis
  - ❖ DNA purification
  - ❖ Today in Lab: M1D2

Announcements

- Christina will be back by Day 4, is currently contributing from home
- Discussion of orientation day quiz

Methods section tips

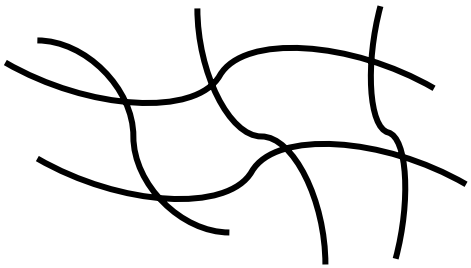
- Organizing sub-sections
  - Start with an overview sentence, then detailed steps
- Methods should be concise and complete
  - Space-wise, avoid tables/lists if a sentence suffices
  - Sentence-wise, avoid extra/confusing words
  - Content-wise, cover what's needed and only that which is needed to understand and replicate your experiments.
- Concentrations are more useful than volumes; or you can state amounts, plus total volume.

Methods section exercises

- Which is more readable: “To the Y were added the X” or “The X were added to the Y”?
- How can I more quickly express “1 g of protein in 45 mL of water and 5 mL of 10X buffer B”?
  - 2% protein in aqueous buffer B
- Which parts of a PCR are unique to a given experiment, versus standard protocol?
  - T_{anneal}; $t_{extension}$ (1min/kbp plasmid); # cycles; composition; concentration of template, primers

DNA Electrophoresis (EP): Principle

Agarose gel



DNA

Agarose and DNA are both

Biological polymers → have molecular entanglements

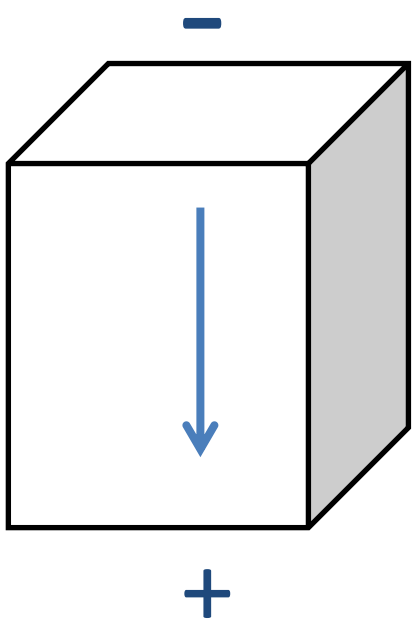
Driving force for separation:

Electrostatic charge, mass

DNA moves - to + because of

Phosphate groups

Separation is according to: Size



Shorter DNA moves faster because

Entanglements increase with size
wt. % increases, pore size decreases

DNA EP: Visualization

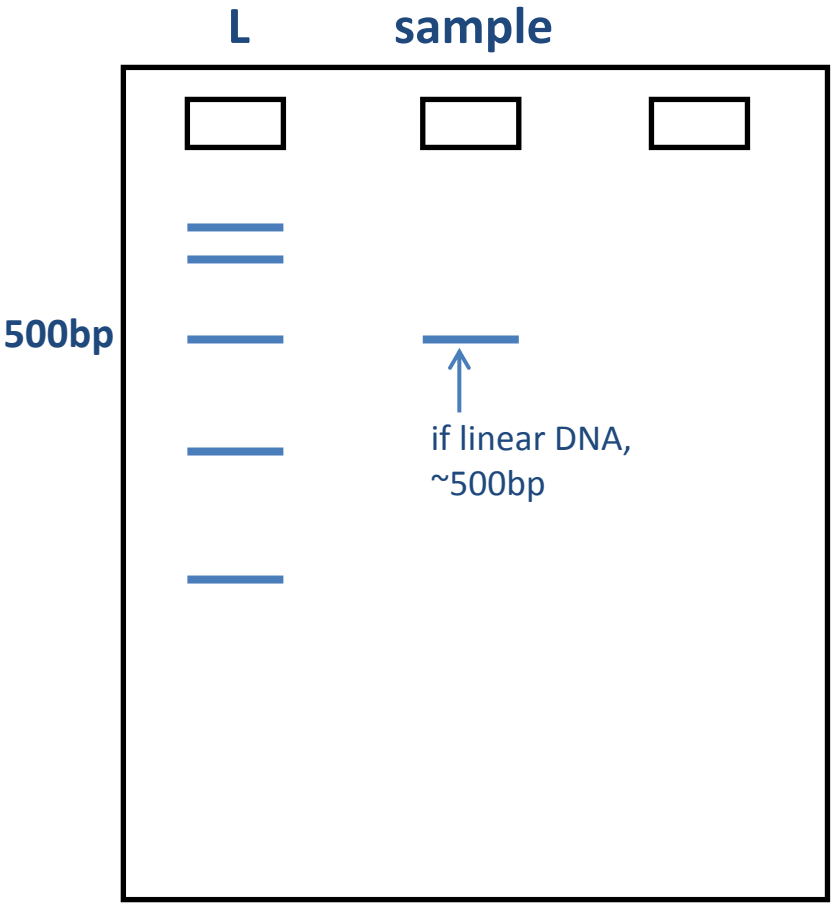
Loading dye:

glycerol → sink into wells
xylene cyanol → visual tracking dye

Ethidium bromide:

fluoresces under UV if bound to DNA

DNA EP: Analysis



DNA ladder: standards of known size (and concentration)

Relationship:

$$\text{distance} \propto \frac{1}{\log(MW)}$$

more details in Mod 2

DNA EP: Clean-up and Safety

- Use nitrile gloves when handling DNA gels and all equipment used for gels.
- Gels and gel-contaminated papers are disposed of in solid chemical waste.
- Wear eye protection/face shields when cutting DNA bands out of a gel.

DNA extraction from agarose gel

Silica resin column

why? isolated desired DNA, change buffer

1. bind DNA → high salt, low pH
   chaotropic salt disrupts H-bond
   DNA sticks to silica column

2. keep DNA wash else
   ethanol – precipitates DNA

3. elute DNA → low salt, high pH
   → electrostatic repulsion $-Si-O^-$ $^-O-P-DNA$

[qiagen.com]

Note: initial mixture should look yellow, not blue

Today in Lab

- Set up gel: runs 60 min, we will photograph it.
  - Mark your area of the gel box with coloured tape.
  - Bring your USB key up front.
- Meanwhile, discussion w/Neal and Linda.
- Finally, DNA extraction from gel.
- FNT: methods section, read journal article.

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