- 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 w/ an overview sentence, then detailed steps
- Methods should be <u>concise</u> 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 needed to understand and replicate your exps.
- 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

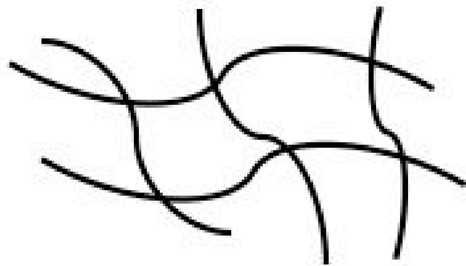
- Which parts of a PCR are unique to a given experiment, versus standard protocol?

  anneal; extension (1min/kbp plasmid); # cycles

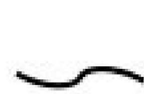
  composition, concentration of template, primers

DNA Electrophoresis (EP): Principle

Agarose gel



DNA



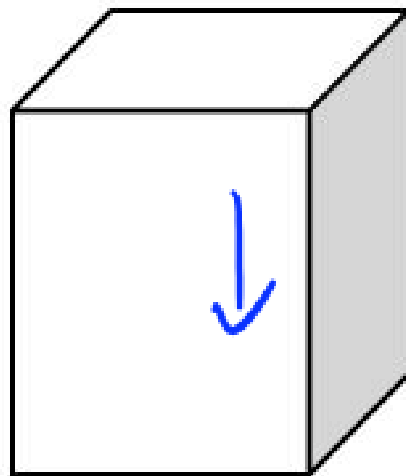
Agarose and DNA are both (bio)polymers → have molec. entanglements

Driving force for separation: e-static

* mass : charge *

DNA moves – to + because of phosphate groups

Separation is according to: size



shorter DNA moves faster because

entanglements ↑ w/ size

wt. % ↑, pore size ↓

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 conc.)

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

why? isolated desired DNA, change buffer

Silica resin column

[qiagen.com]

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
   → e'static repulsion - Si - O⁻ ⁻O-P-DNA

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.