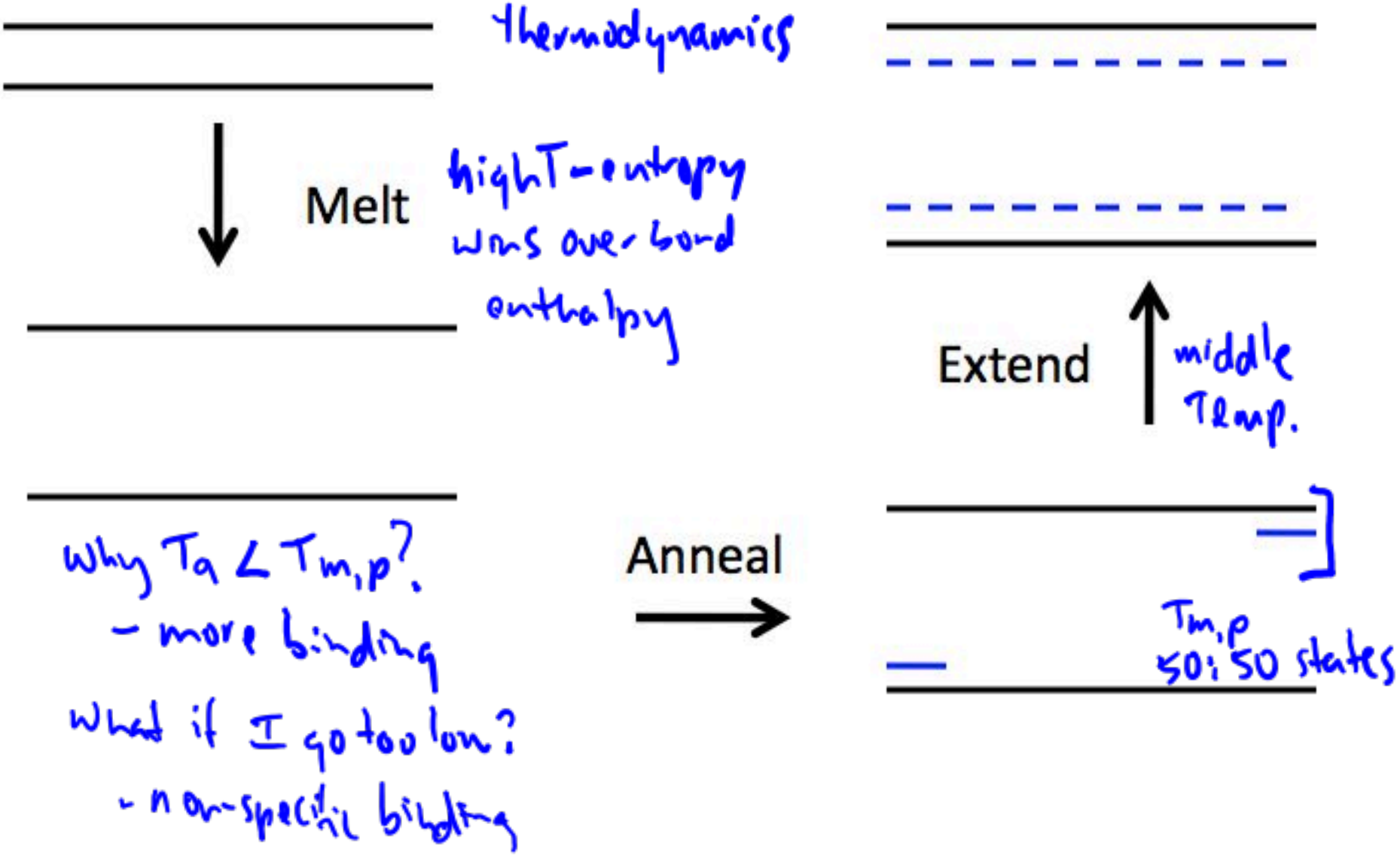
- Announcements
- Lab Quiz (on M1D1 material)
- Pre-lab Lecture
  - Writing a Methods Section
  - Gel Electrophoresis
  - DNA Purification
  - Today in Lab: M1D2

Announcements

- Discussion of orientation day quiz

Quick note: what drives PCR?



Methods section tips

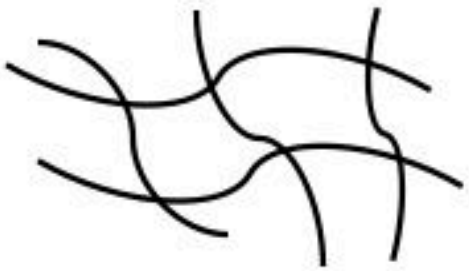
- Organizing sub-sections
  Start w/ an overview sentence → step-by-step details
- Methods should be concise and complete
  - Space-wise, avoid tables/lists when a sentence will do
  - Sentence-wise, avoid extra words
  - Content-wise, cover what's needed and only that to understand and replicate what you did
- Concentrations are more useful than volumes; or you can state amounts, plus total volume.

Methods section exercise

- Consider the following passage: "Template DNA (5 ng) and primers were mixed with 20 uL of 2.5X Master Mix in a PCR tube. Water was added to 50 uL. A tube without template was prepared and labeled control. "
  - What information is missing?
  - What information can be cut?

DNA Electrophoresis (EP): Principle

Agarose gel

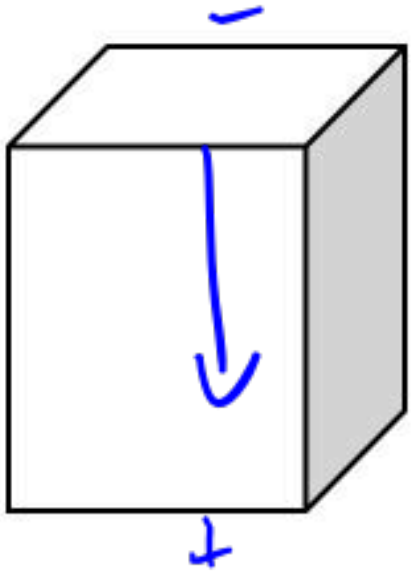


DNA

Agarose and DNA are both biopolymers
→ have molec. entanglements

Driving force for separation: charge
(mass : charge)

DNA moves − to + because of phosphate groups



Separation is according to: size

smaller DNA moves faster because

entanglements ↑ size

as wt% ↑, pore size ↓

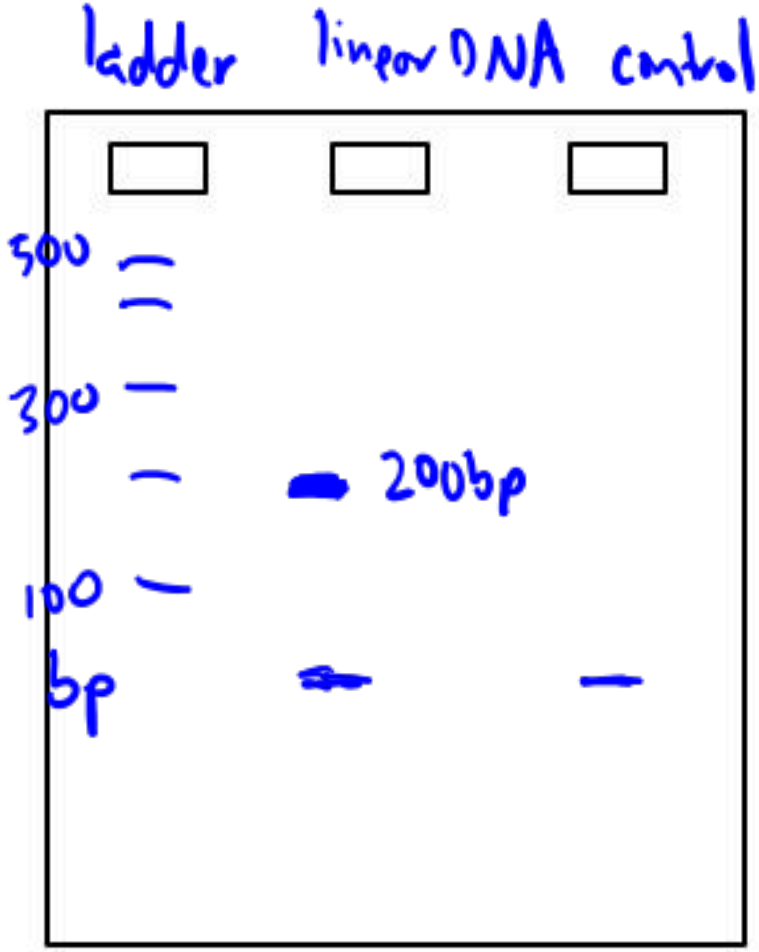
DNA EP: Visualization

Loading dye: glycerol → sink into wells

Xylene cyanol → visual tracking dye

Ethidium bromide: fluoresces under UV if DNA bound

DNA EP: Analysis



DNA ladder: known sizes and conc.
Standards

Relationship:

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? get rid of other dNTPs; switch buffers

Silica resin column

[qiagen.com]

1. bind DNA → high salt, low pH
   chaotropic salts disrupt H-bonding
   DNA sticks to the column
2. keep DNA and wash other stuff
   ethanol precipitate
3. elute DNA → low salt, high pH
   −Si−O⁻ ⁻O-P-DNA ↓ electrostatic repulsion

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.
- Meanwhile, discussion w/Neal from WAC.
- Finally, DNA extraction from gel.
- FNT: methods section, read journal article.