- Announcements
- Lab Quiz (on M1D1 material)
- Pre-lab Lecture
  - Writing a methods section
  - Gel electrophoresis
  - DNA purification
  - Today in Lab: M1D2

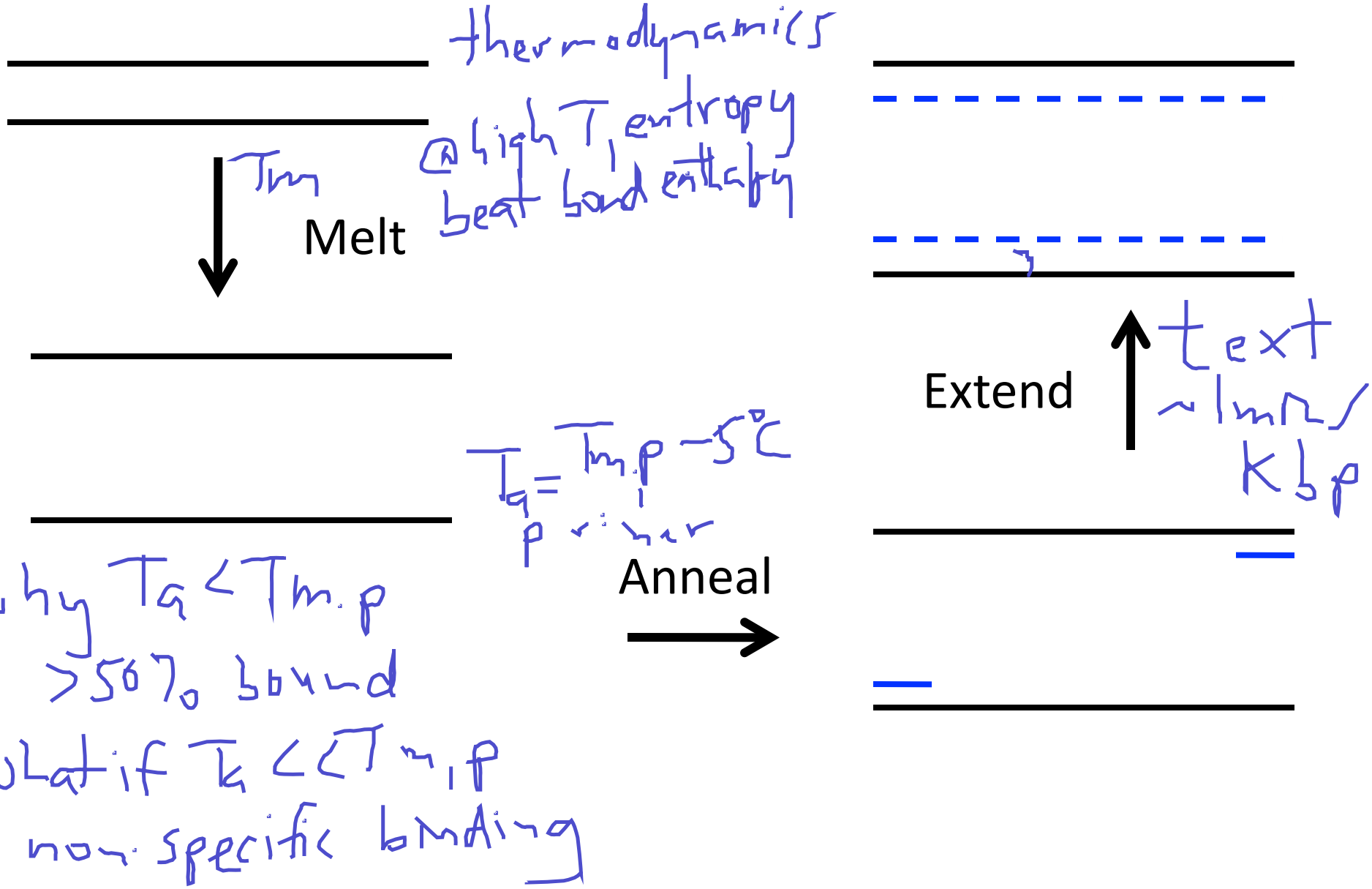
Announcements

- Brief discussion of orientation day quiz
- Remember, *Assignments* + *Schedule* = our syllabus
- A few FNTs due on Stellar

MODULE	DAY	DATE	FNT DUE TODAY
		Tue Feb 7	N/A
1	1	Thur Feb 9	Due M1D1
1	2	Tue Feb 14	Due M1D2
1	3	Thu Feb 16	Due M1D3

wiki FNT list Stellar →

Quick note: what drives PCR?



Methods section tips

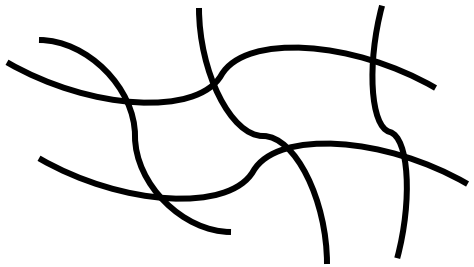
- Organizing sub-sections
  - Often start with an overview/introductory sentence (*what*, not why) → then give step-by-step details
- Methods should be concise and complete
  - Space-wise, avoid tables/lists when a sentence will do
  - Sentence-wise, avoid extra words
  - Content-wise, cover what's needed and only that needed to understand and replicate your work
- 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

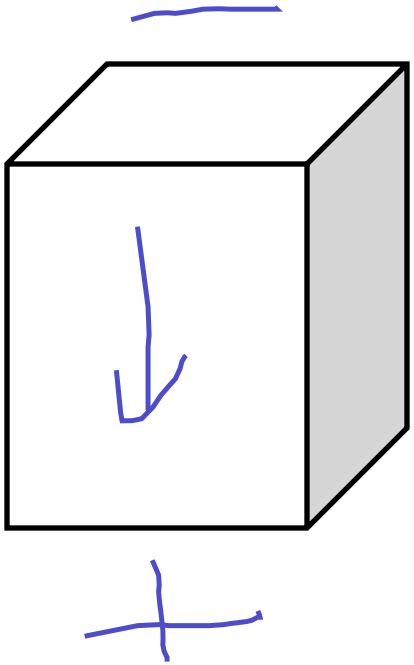


DNA

Agarose and DNA are both polymers: have molecular entanglements

Driving force for separation: mass: charge

DNA moves − to + because of phosphate groups



Separation is according to: size

Smaller DNA moves faster because

entang. ↑ size

as wt% ↑, pore size ↓

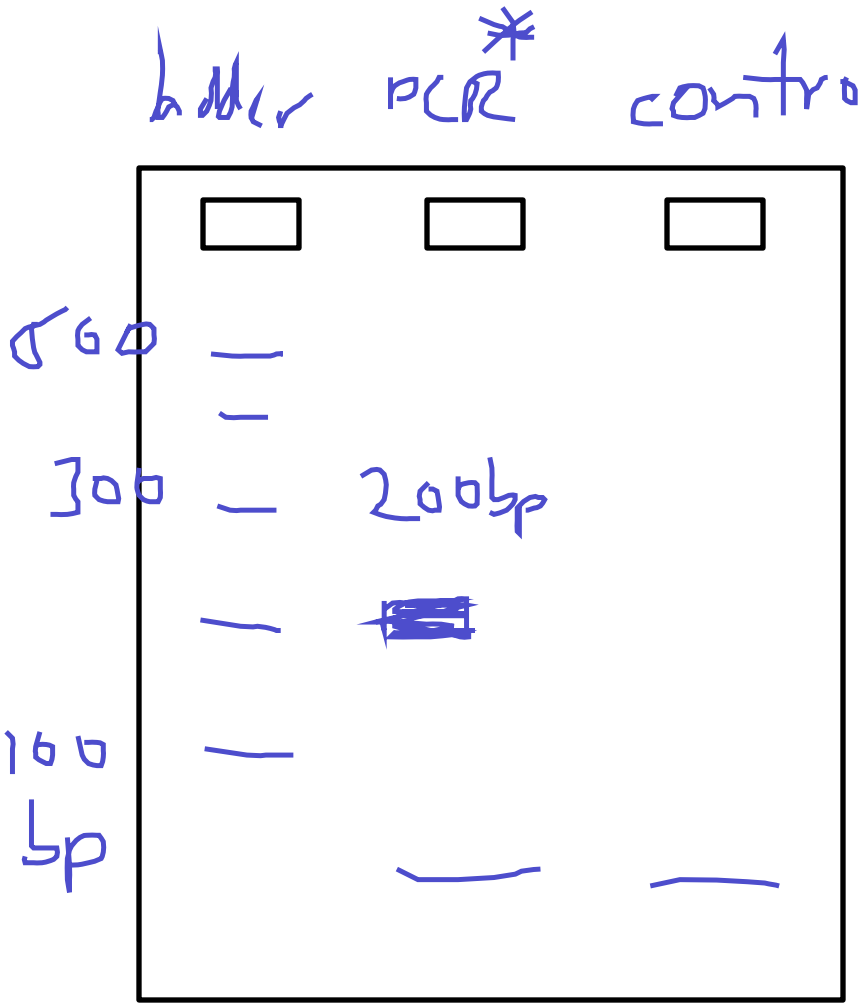
DNA EP: Visualization

Loading dye: glycerol: sink into wells

xylene cyanol: visual/realtime tracking dye

Ethidium bromide (or SYBR Safe, etc.): fluoresces under UV/blue if DNA-bound

DNA EP: Analysis



DNA ladder: known size and conc. standards

Relationship:

distance of $\frac{1}{\log(MW)}$

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 amber glasses (blue light) or face shields (UV) when cutting DNA bands out of a gel.

DNA extraction from agarose gel

why? get rid of dNTPs, etc.; switch buffers

1. bind DNA → high salt, low pH
   chaotropic salts disrupt H-bonds
   DNA sticks to column

2. Keep DNA, wash rest
   ethanol precipitate

3. Elute DNA → low salt, high pH

Silica resin column

O–Si–O⁻   –O–P–DNA   e-static repulsion

[qiagen.com]

Note: initial mixture should look yellow, not blue

Today in Lab (M1D2)

- Prepare PCR products for running on gel.
- Gel runs 60 min.
  - Mark your area of the gel box with colored tape.
  - After, learn to photograph and cut out bands.
- Meanwhile, WAC discussion on lab reports.
  - T/R Leslie 2:30-3:15
  - W/F Marilee 2:45-3:30
- Finally, DNA extraction from gel.
- FNT: methods section, read journal article.

prep tubes!