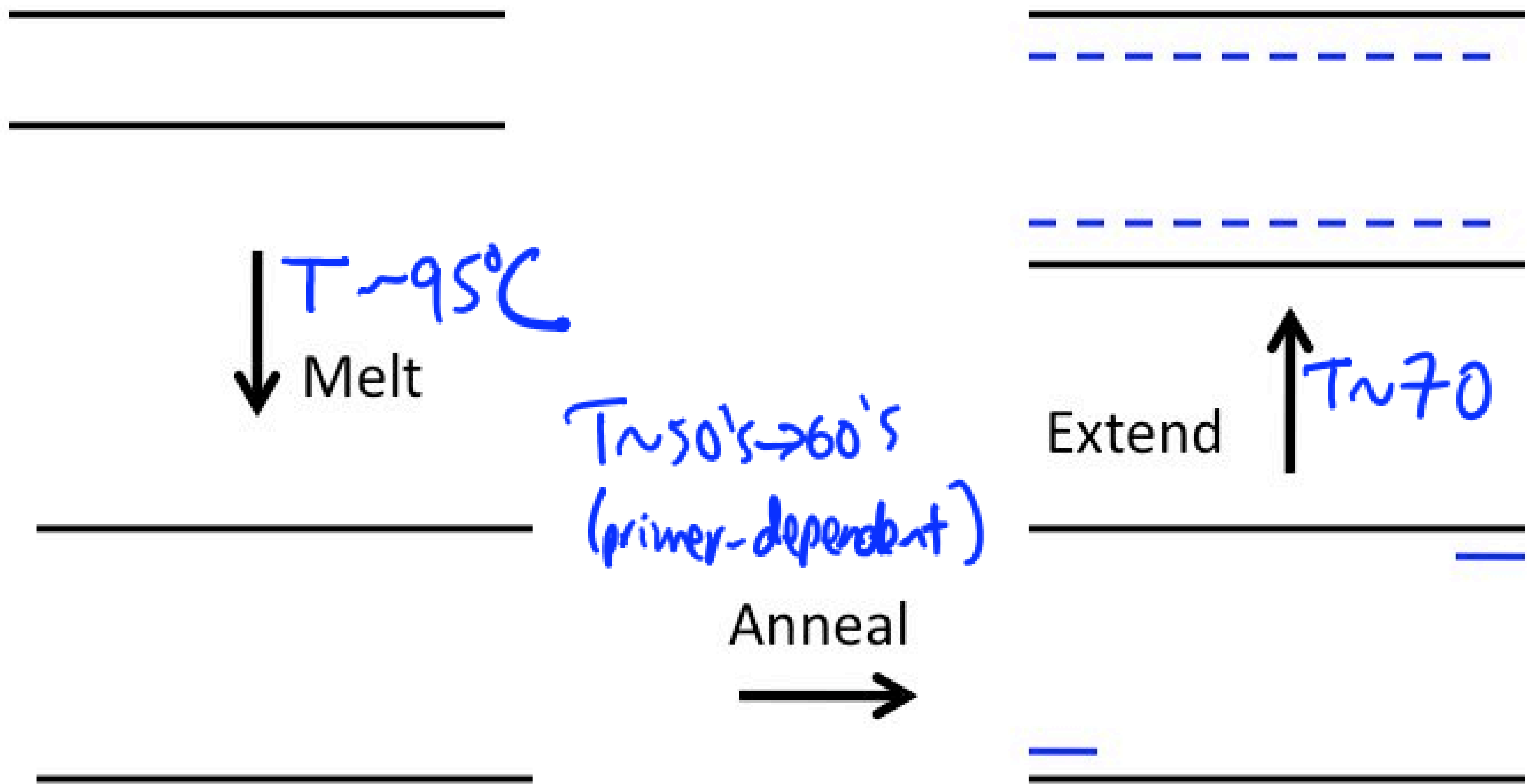
- Announcements
- Quiz
- Pre-lab Lecture
    - Writing a Methods Section
    - Gel Electrophoresis
    - Bacterial Transformation
    - Today in Lab

Announcements

- Friday: ChemE seminar about biomaterials
  - 66-110, 3 pm
- Next Thursday: BE seminar about biogels
  - 32-141, 4:05 pm

Quick note: what drives PCR?



Methods section tips

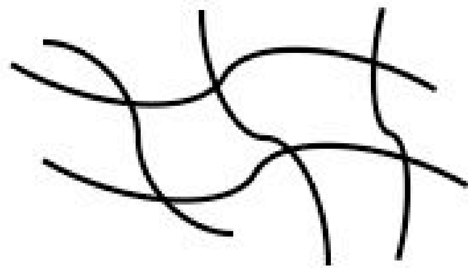
- Organizing sub-sections
  start w/an overview sentence giving the purpose of that exp.
- Methods should be concise and complete
  - Space-wise, avoid tables/list when a sentence will do
  - Sentence-wise, avoid extra/confusing words
  - Content-wise, cover what's needed and only that needed to understand and replicate your exp.
- 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 ~~5~~ 10X buffer B"?
  2% protein in (1X) ~~buffe~~ aqueous buffer B
- Which parts of an SDM are unique to a given experiment, versus standard protocol?
  # cycles ; Tannealing ; textension → 1min/1kbp DNA
  composition/concentration primers, template

DNA Electrophoresis (EP): Principle

Agarose gel



DNA

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

Driving force for separation: charge

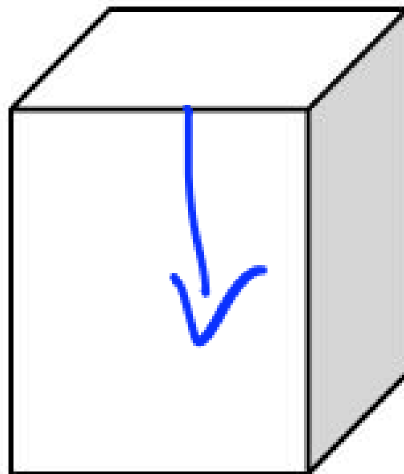
* mass : charge

DNA moves – to + because of phosphate groups

Separation is according to: size

smaller DNA moves faster because

entanglements ↑ w/size



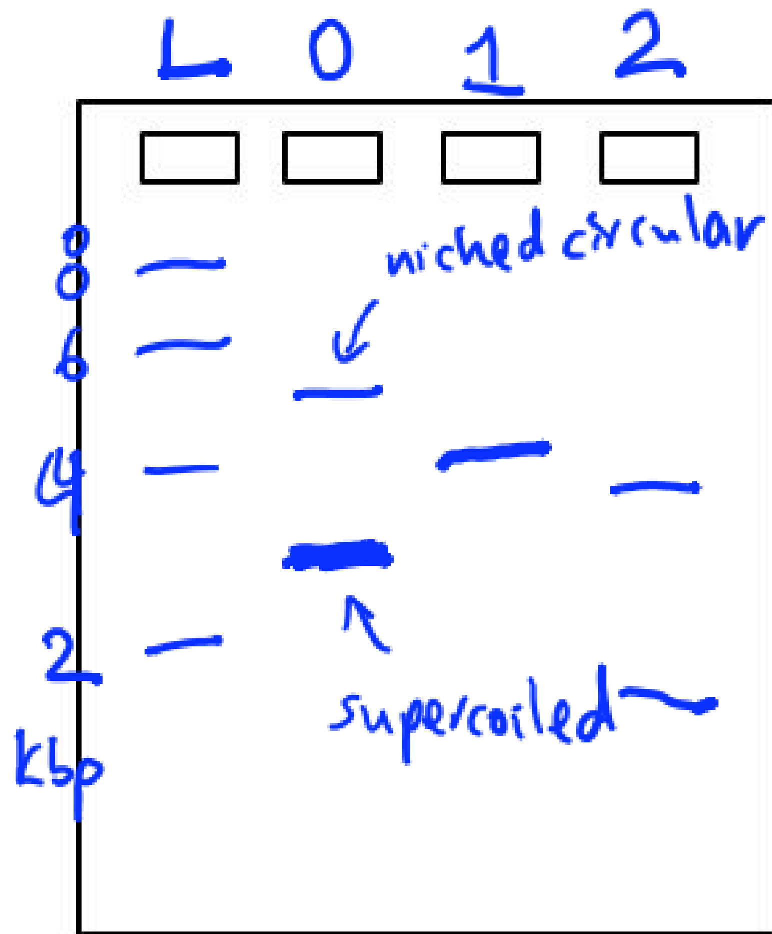
DNA EP: Visualization

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, conc.

Relationship:

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

Samples:

uncut plasmid → 2 forms

single-cut → 4kbp - linear

multi-cut → multiple bands

DNA EP: Clean-up and Safety

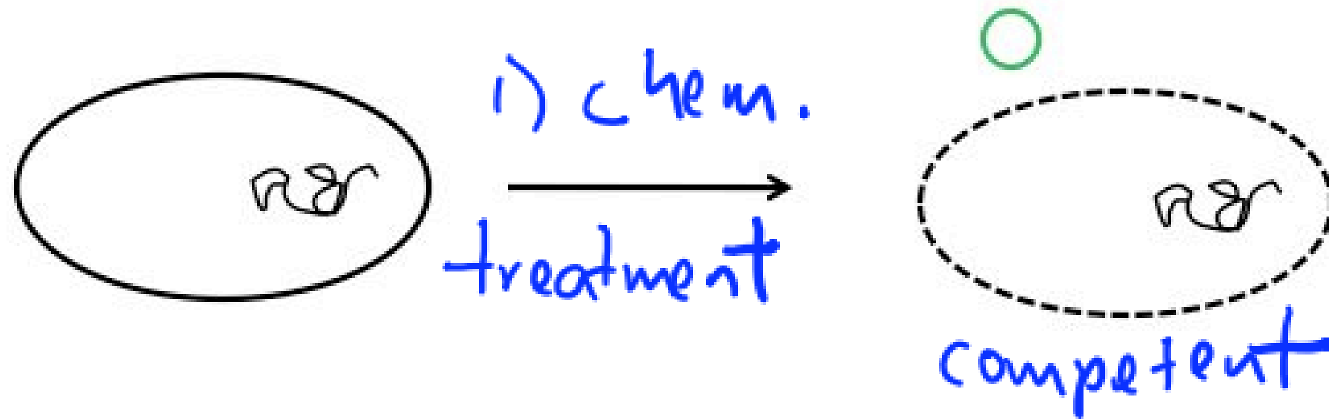
- Use nitrile gloves when handling DNA gels and all equipment used for gels.

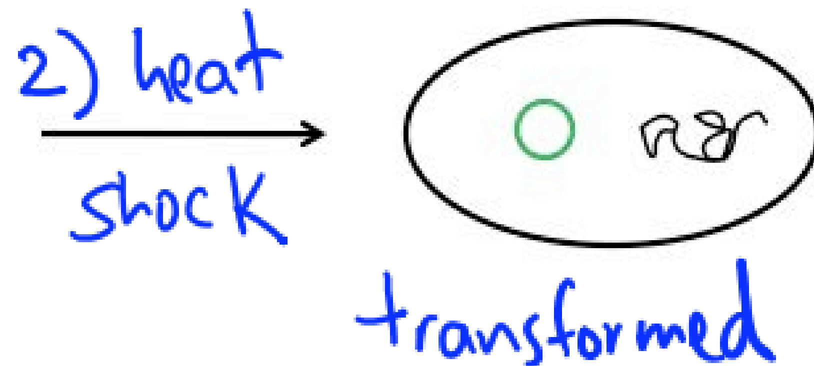
  EtBr → MUTAGEN

- Gels and gel-contaminated papers are disposed of in solid chemical waste.

- Wear eye protection/face shields if cutting DNA bands out of a gel.

Bacterial transformation

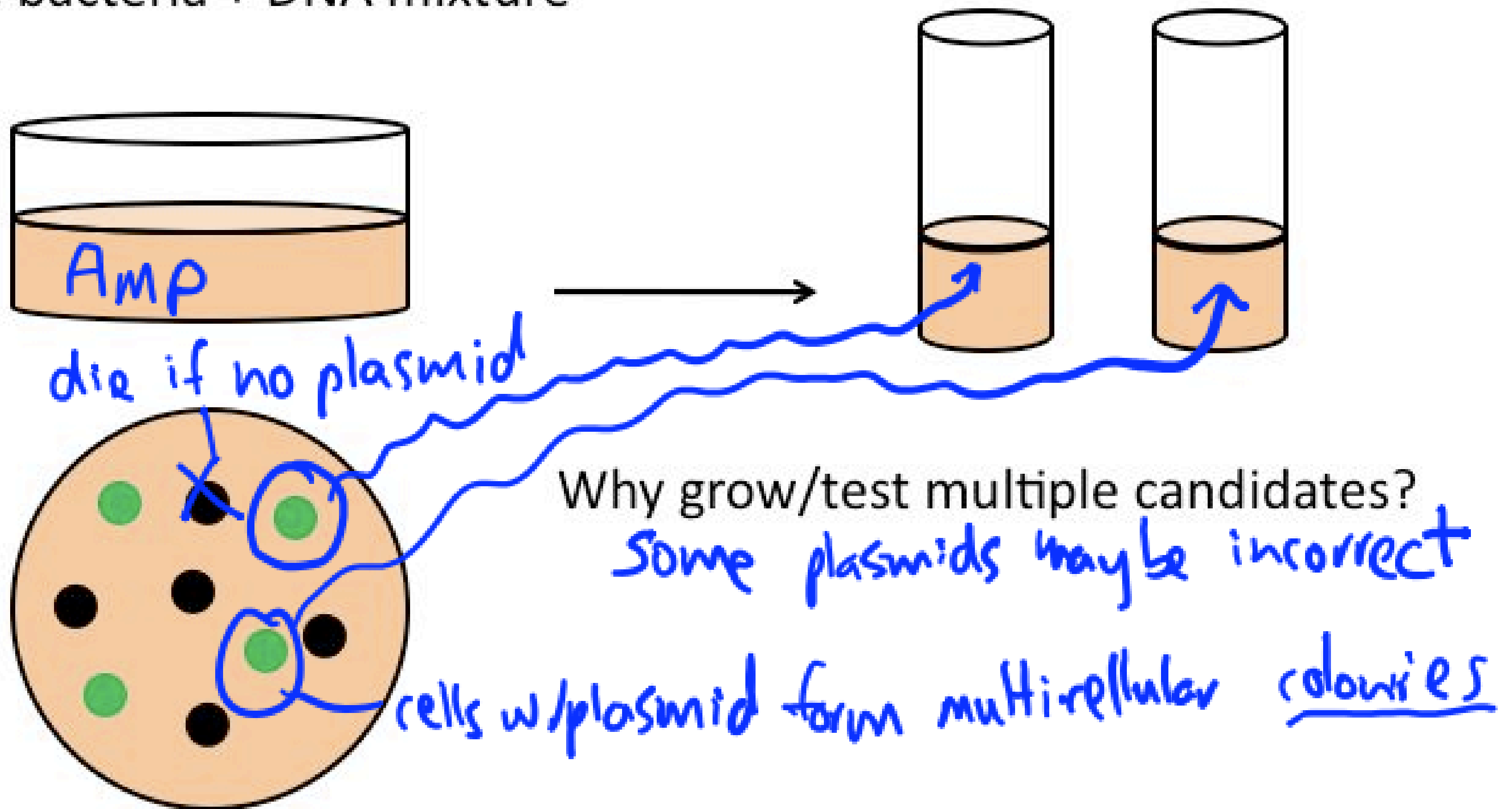




other methods (1-step)

1) electroporation
2) ballistics

DNA Amplification in Bacteria



Today in Lab

- Set up gel: runs 45 min, we will photograph it.
  - Mark your area with a coloured sticker
  - Bring your USB key up front
  - Update: in BLANK wells, teaching faculty will put concentrated parent plasmid instead (reference)
- Meanwhile, discussion w/Neal and Atissa.
- Finally, bacterial transformation – be gentle!