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?

Melt

25-95°C

Annel

Tm50's-260's (primer-dependent)

Extend

Tm70
Methods section tips

- Organizing sub-sections
  Start with 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 what’s 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 8X buffer B”?

  270 protein in (1X) aqueous buffer B

• Which parts of an SDM are unique to a given experiment, versus standard protocol?

  # cycles ; annealing ; extension \[ \rightarrow \text{1 min/1kbp DNA} \]
  composition/concentration primers, template
DNA Electrophoresis (EP): Principle

Agarose gel

DNA

Agarose and DNA are both bio polymers $\rightarrow$ entanglements (molec.)

Driving force for separation: charge

$\times$ mass $\times$ charge

DNA moves to $+$ because of phosphate groups

Separation is according to: size

smaller DNA moves faster because entanglements $\uparrow$ w/ size
DNA EP: Visualization

Loading dye:  glycerol $\rightarrow$ sink into wells
              xykne  cyanol $\rightarrow$ 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 \( \rightarrow \) 2 forms
- single-cut \( \rightarrow \) 4Kbp-linear
- multi-cut \( \rightarrow \) multiple bands
DNA EP: Clean-up and Safety

- Use nitrile gloves when handling DNA gels and all equipment used for gels.
  \[ \text{Et} + \text{Br} \rightarrow \text{MUTAGEN} \]

- 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

1) Chemical treatment

2) Heat shock

- Competent
- Other methods (1-step)
  1) electroporation
  2) ballistics

transformed
DNA Amplification in Bacteria

Plate bacteria + DNA mixture

Amp

die if no plasmid

Why grow/test multiple candidates?

some plasmids may be incorrect

cells w/plasmid form multicellular colonies
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!