- Announcements, Review HW
- Lab Quiz
- Pre-lab Lecture
  - Writing a Methods section
  - DNA Electrophoresis
  - Safety + Technical Tips
  - DNA Ligation, part 1

# Announcements, old HW

- First BE seminar tomorrow: Pamela Silver on cell behaviour from an engineering perspective

  modeling, building    lab has own site

- HW: when figuring out a DNA product, be careful not to double-count or omit anything

  landing sequence is part of EGFP    stop codon

* read HW before you leave

# Methods section tips

- Organizing sub-sections
  start w/an overview sentence that explains the purpose of that experiment
- Methods should be concise and complete
  - Space-wise, avoid tables/lists when a sentence will do
  - Sentence-wise, avoid extra (or confusing) words
  - Content-wise, cover what's needed and only what's needed ~~for~~ to understand and replicate your exp.
- Concentrations are more useful than volumes; or you can state amounts, plus total volume

# Methods section exercises

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

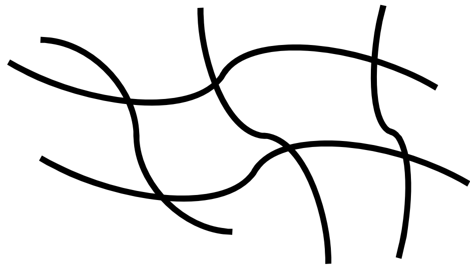
primers + template
- extension time (cycle #)
- annealing Temp.

- 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 5X buffer B”?

1g or 2% } protein in aqueous (1x) buffer B

# DNA Electrophoresis (EP): Principle

Agarose gel



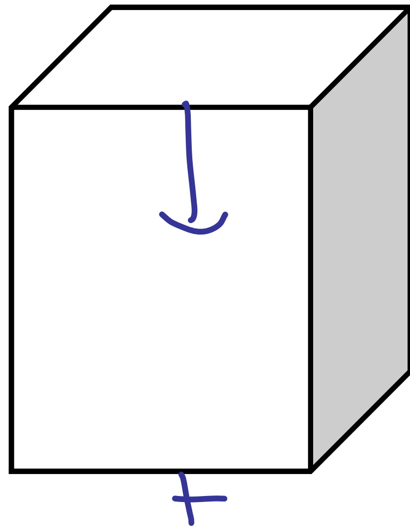
DNA

Agarose and DNA are both bio polymers

molecular entanglements (pasta)

Driving force for separation: charge

DNA moves ⁻ to ⁺ because of phosphate groups




Separation is according to: size

smaller DNA moves faster because

entanglements (↑ size) compete w/ charge

# DNA EP: Visualization

Loading dye: glycerol → DNA sinks

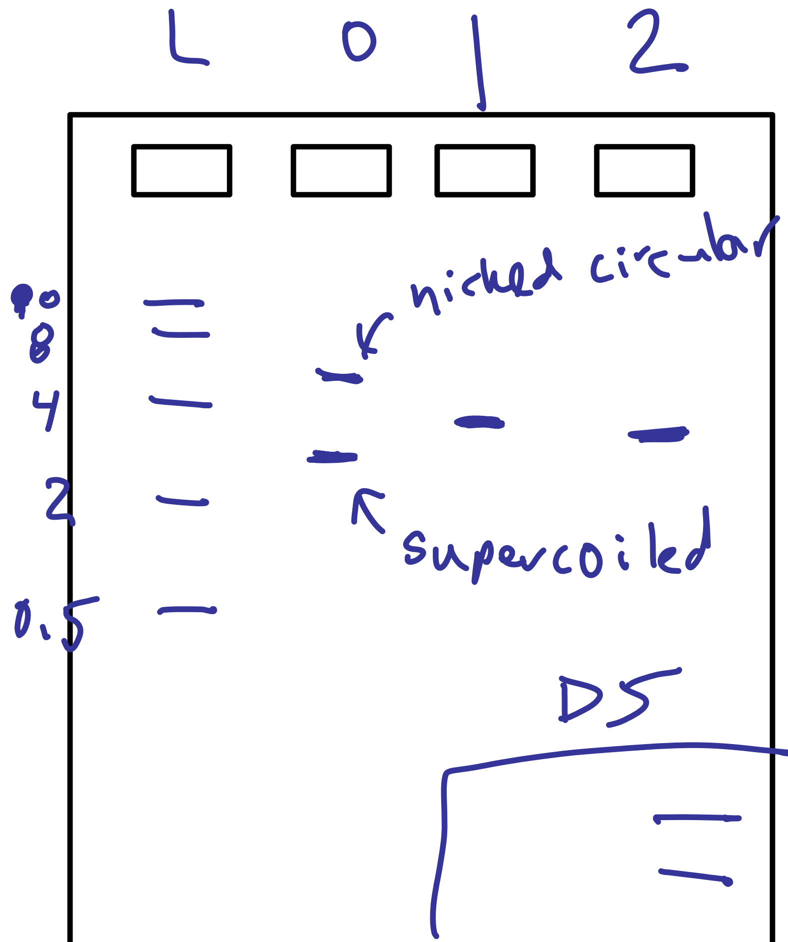
xylene cyanol → visual tracking dye (don't run off end of gel)

Ethidium bromide:

shows up under UV

fluoresces only if bound to DNA ($\pi,\pi$-stacking)

# DNA EP: Analysis




DNA ladder: standards of known size

Controls:
uncut → plasmid (2 forms)
single-cut → linear

Sample: for collection or analysis
↓
DS

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

# DNA EP: Clean-up and Safety

- Use nitrile gloves when handling DNA gels and all equipment used for gels.
- Wear eye protection/face shields when cutting DNA bands out of the gel.
- Gels and gel-contaminated papers are disposed of in solid chemical waste.

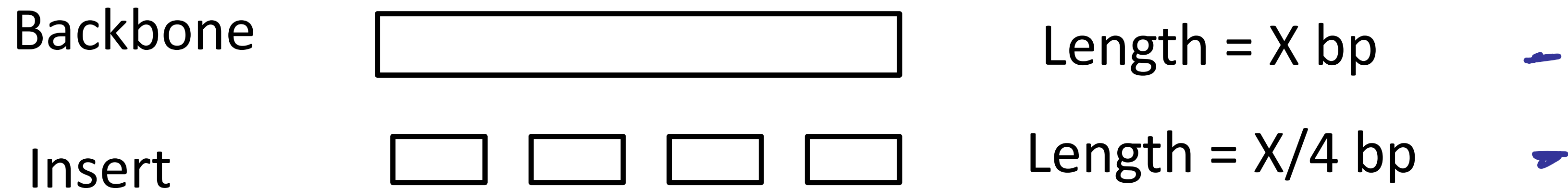
# DNA extraction from agarose gel

- Another Qiagen kit: similar principles but different buffers
  - In addition to buffer composition, size of the silica  beads can affect what is retained

- Mixture should ideally look yellow, not blue

# Preparing for DNA ligation

Ethidium intensity reflects ***absolute DNA amount***.




Equal intensity of insert and backbone means that the DNA amounts in the two lanes are __equal__. This means an equal __weight__ ratio and unequal __molar__ ratio of DNA.