- Announcements
- Lab Quiz
- Pre-lab Lecture
  - DNA electrophoresis
  - DNA ligation, part 1
  - Today in Lab: M1D3

Announcements

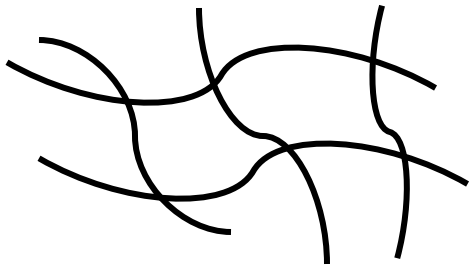
no lec/lab this R/F

- Lab certification coming up Tue, Oct. 2nd
- Ditto cloning defense (oral concentration)
- Lab notebooks due today (yellow sheets)
- Reminder: office hours poll!
- Importance of optional HWs

lets run gel now :)

DNA electrophoresis (EP): principle

Agarose gel

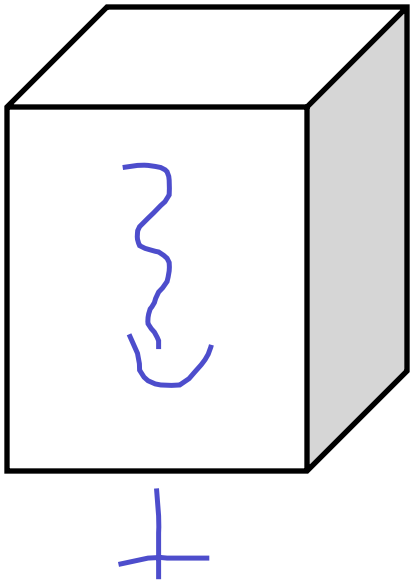


DNA

Agarose and DNA are both polymers → have molec. entanglements

Driving force for separation: charge (< mass)

DNA moves − to ~~+~~ because of phosphate groups



Separation is according to: size

smaller
DNA moves faster because

entanglements ↑ w/ size

(note: high η, small pores, ↑ resol.)

DNA EP: visualization

Loading dye:

glycerol → DNA sinks into wells

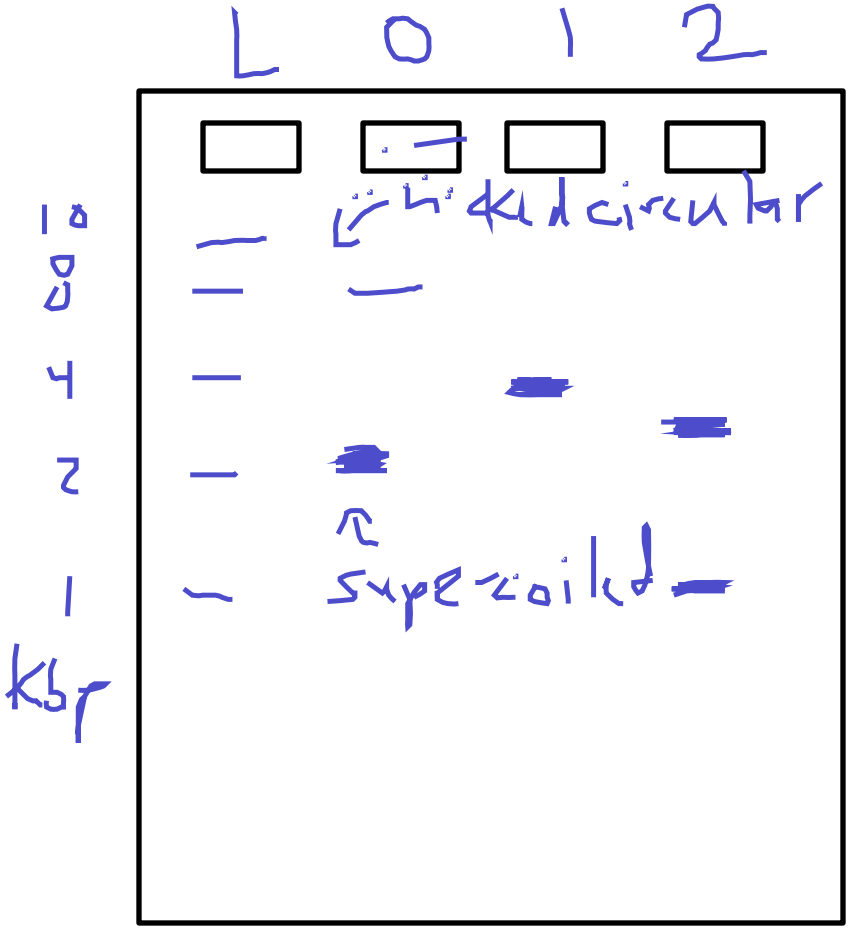
xylene cyanol → real-time visual tracking dye (single band, indep. of DNA)

RNase → cleaner prep.

Ethidium bromide:

fluoresces under UV/blue light if bound to DNA

DNA EP: analysis



DNA ladder: standards of known size/conc.

Controls:
uncut plasmid → 2 forms
single-cut p. → linear

Samples:
for collection (M103)
for analysis (M105, M107)

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

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***.

Backbone  Length = X bp

Insert  Length = X/4 bp

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

goal = 4:1 molar ratio ins:bkb
for ligation

Determining bkb:ins ratio

- What if bkb:ins 1:100?

  multiple inserts

- What if bkb:ins 100:1?

  (plasmid dimers)

  ☆more background (not fully cut)

- Why have insert in slight excess?

  contact frequency

Today in Lab: M1D3

- Load agarose gels
  - bring own pipets, piece of tape, but no tips
  - can train 1-2 groups at a time, queue up
- While gel runs, presentation on figure-making
- Isolate and set aside DNA
  - 1-2 groups simultaneously view gel with me
  - one group at a time isolates DNA

before pre-lab ✓

3+ pm

pd

→ small piece ⟹ to fridge till after writing talk

★ Turn in 4 tubes ★