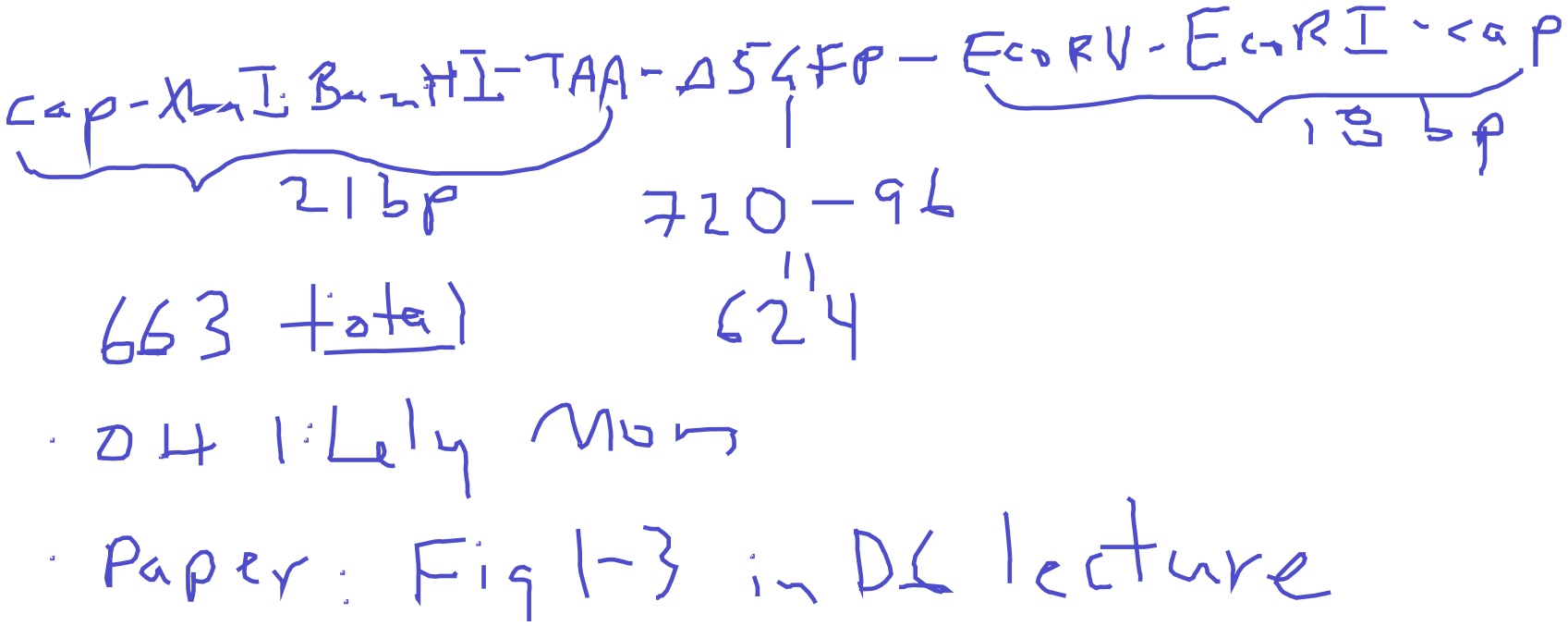
- Announcements
- Lab Quiz
- Pre-lab Lecture
  - Review PCR and homework
  - DNA Electrophoresis
  - DNA Ligation, part 1
  - Today in Lab: M1D3

Announcements

- Lab practical coming up Tue, Oct. 4th
- Close look at PCR product:



Fixing FNT M1D1

would like resubmission on index card

Primer design

- How to retrieve seq, in gen'l
- How to find relevant part
- Design of landing seq (length? Tm? GC?)
- Design of flap seq (cloning?)
- Hints for reverse primer
- Other things to check

PCReaction details

- Components:
  - what?
  - volume mass or conc?
  - how to assemble?
- Cycling conditions:
  - anneal temp?
  - extension time?

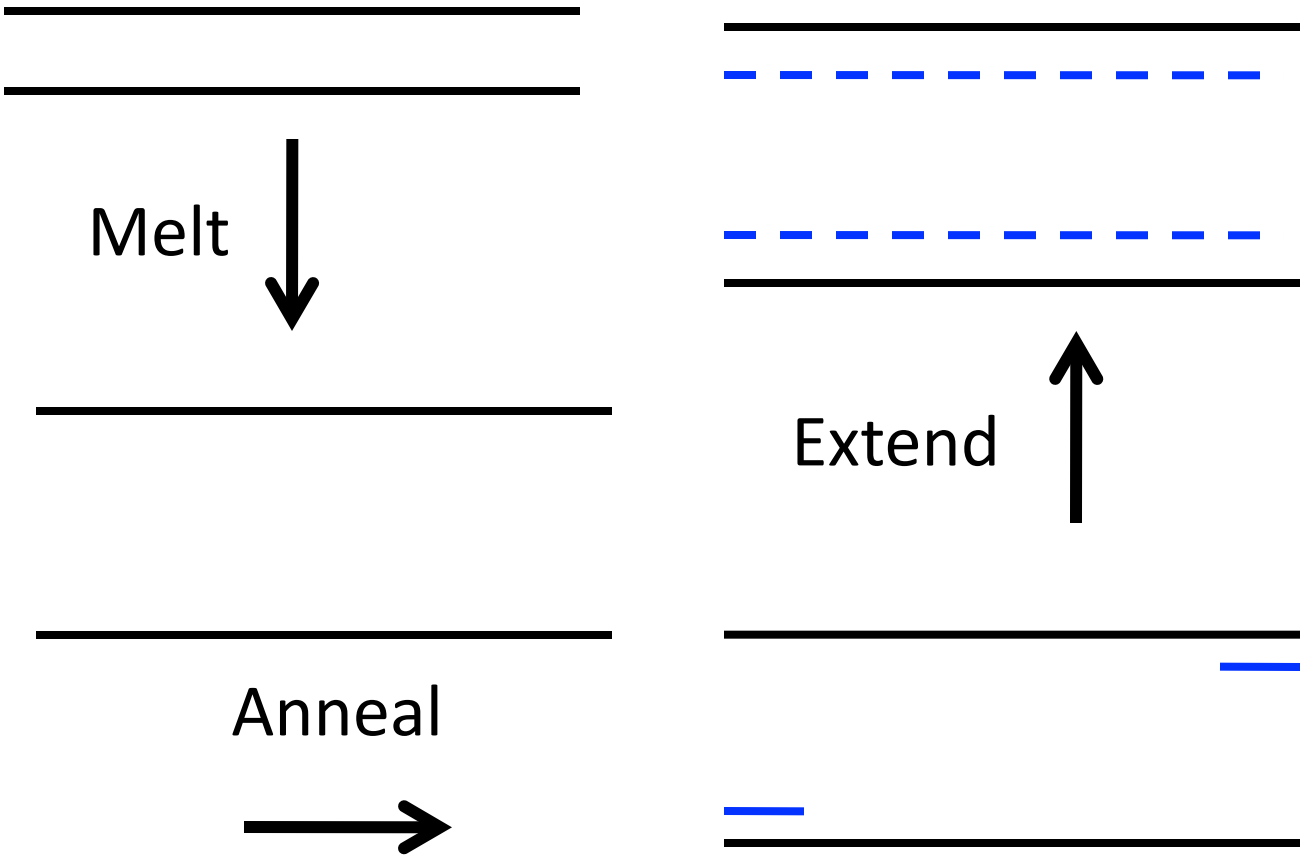
General note: be cautious with wording taken directly from wiki

Hard in next time w/old HW

Slide from N. Kuldell

What drives PCR?

thermodynamics



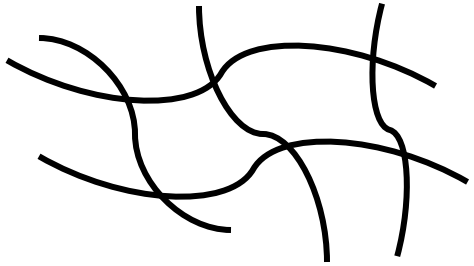
M - @ high T
s > H

A $T_a = T_{mp} - 5°C$
T_{mp} = 50% bound
$T_a < T_{mp}$
more bound
$T_a << T_{mp}$
non-specific binding

E text
~1 min/kbp

DNA Electrophoresis (EP): Principle

Agarose gel

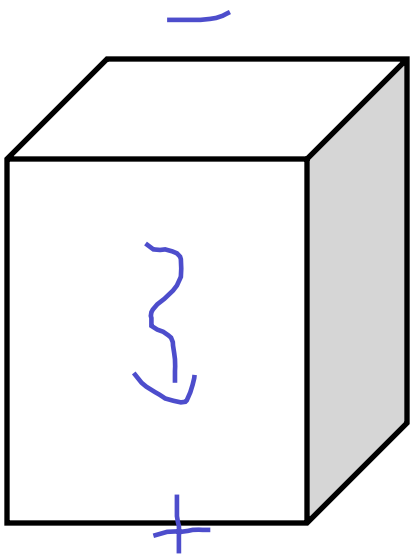


DNA

Agarose and DNA are both biopolymers → have molec. entanglements

Driving force for separation: charge

DNA moves − to + because of phosphate groups



Separation is according to: size

smaller entanglements ↑ size DNA moves faster because

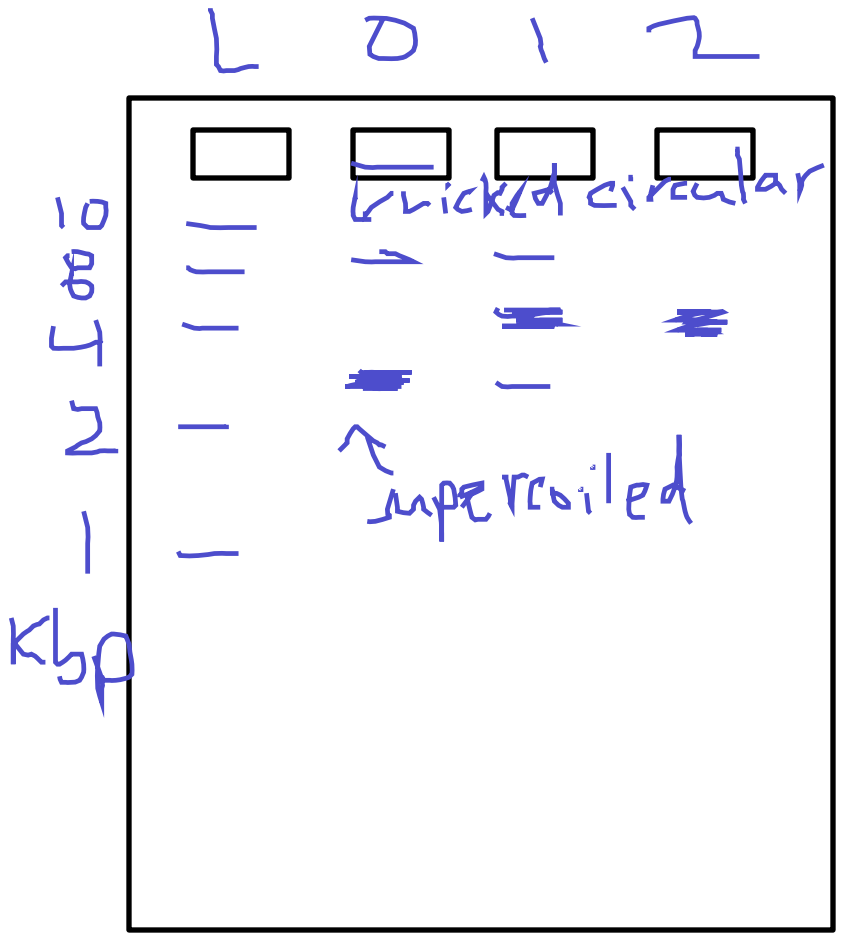
DNA EP: Visualization

Loading dye: glycerol → DNA 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

Controls:
uncut plasmid → 2 forms
single cut → linear

Sample:
for collection (purify)
for analysis (MIDS)

Relationship: 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.

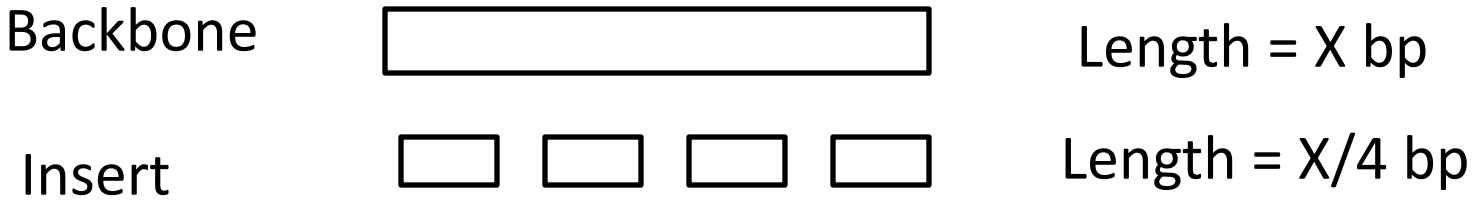
DNA extraction from agarose gel

- 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***.



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.

Determining bkb:ins ratio 1:4

- What if bkb:ins 1:100?

  multiple inserts

- What if bkb:ins 100:1?

  plasmid dimers

  more background (not cut)

- Why have insert in slight excess?

  contact frequency

Today in Lab: M1D3

★Turn in 4 tubes★

- Load agarose gels
  - Bring pipets, piece of tape, but no tips
  - Can train two groups at a time, queue up
- While gel runs, presentation on figure-making
- Isolate and set aside DNA
  - Two groups simultaneously view gel with me
  - One group at a time isolates DNA small pieces!
  - Help keep everyone moving through this part!