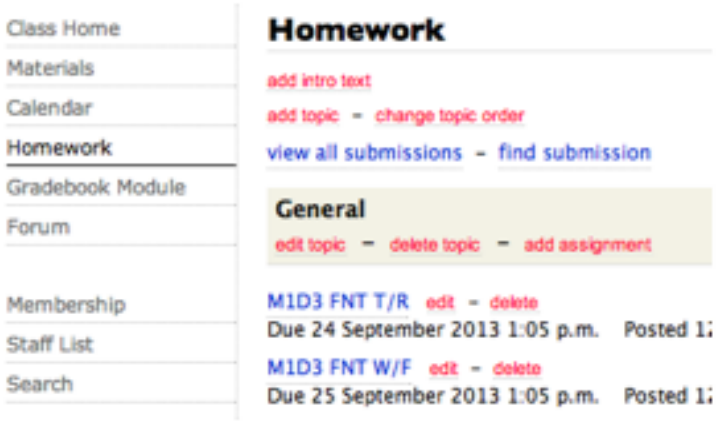
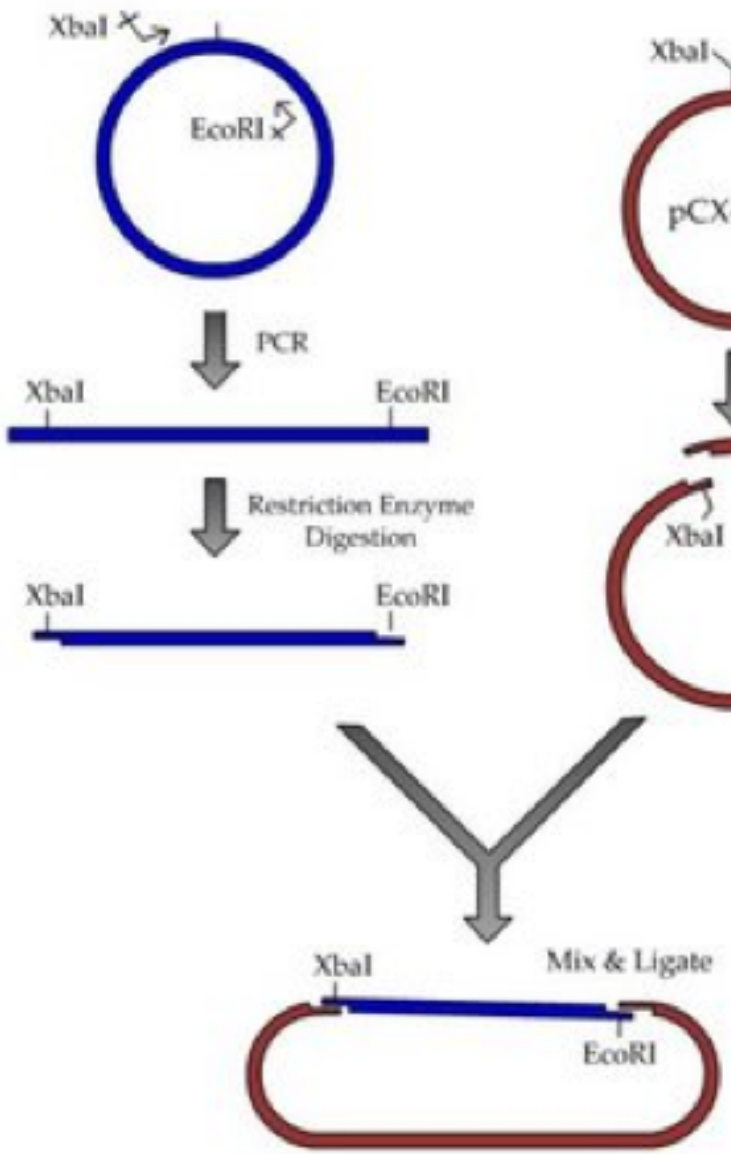
- Announcements
- Lab Quiz
- Start gels running
- Pre-lab Lecture
  - DNA electrophoresis
  - DNA ligation, part 1
  - Today in Lab: M1D3

Announcements

- About lab notebooks
  - M1 collection on D7: either D3, 4, or 6 evaluated
  - Text highlights (for changes) – helps Lizzie *and* you
  - Calculations – "get creative"
- My OH: Tue 4-5 pm, 16-319 (see me if conflict)
- Next time: lecture but no lab!
- Figure caption titles: okay to preview conclusion
- Part of next assignment submitted on Stellar
- Briefly: jump to slide 11



Roadmap for Plasmid Construction



D2-digest

* D3-purify + check

D4-ligate

DNA electrophoresis (EP): principle

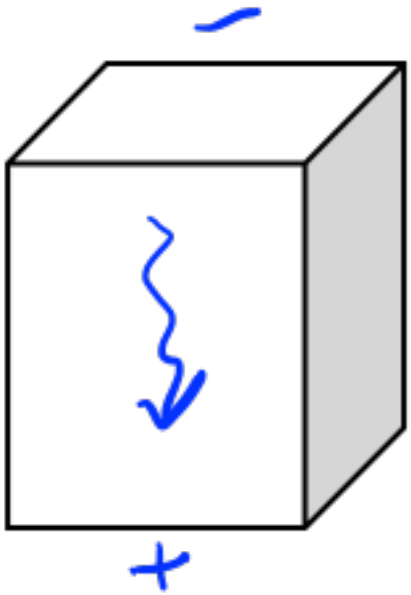
Agarose gel

DNA

Agarose and DNA are both polymers
→ have molecular entanglements

Driving force for separation: charge
(≠ mass : charge)

DNA moves – to + because of phosphates



Separation is according to: size

smaller entanglements → DNA moves faster because ↑ size

(note: high % gel, small pores, ↑ resolution)

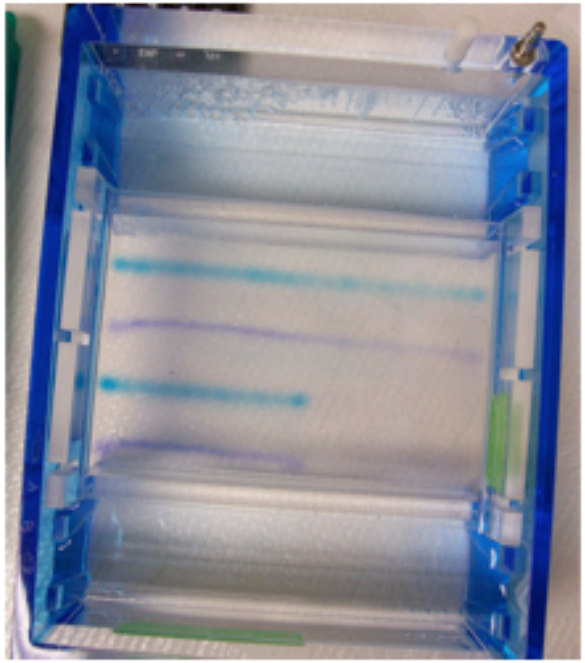
DNA EP: visualization

Loading dye:

- glycerol → DNA sinks into wells
- XC or BPB: real time tracking dye
  - single band, independent of DNA
- later - RNase → cleaner view

DNA stain:

- ethidium bromide or SYBR safe, etc.
- fluoresces under UV or blue light if bound to DNA

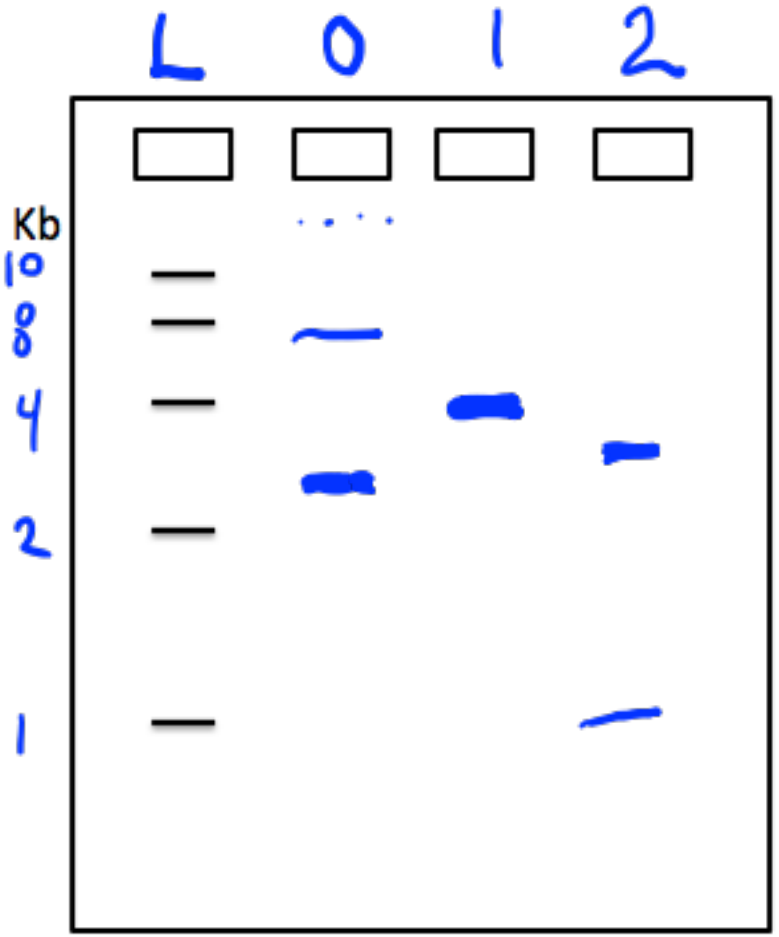


sr.wikipedia.org

BPB runs smaller

Thanks to Shannon for image!

DNA EP: analysis



DNA ladder: standards of known size (and concentration)

Example: 4 Kbp plasmid

Controls: single-cut: linear
uncut: supercoiled - faster
relaxed or nicked circular - slower

Samples: for collection: M103
for analysis: M103 and M105

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

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
  - else needs pH adjustment
- Qiagen waste stream: chaotropic salts/EtOH

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.

Determining bkb:ins ratio

- What if bkb:ins 1:100?
  multiple inserts
- What if bkb:ins 100:1?
  plasmid dimers

  ↑ more "background" (partially cut plasmid reclosing)
- Why have insert in slight excess?
  contact frequency typical 1:1 — 1:10

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
  - pre-weigh two eppendorfs afterward!!!
- Isolate and set aside DNA
  - 2 groups simultaneously view gel with me
  - 1 group at a time isolates DNA slices
- Prepare to pause ~ 3 pm for Biosafety talk

Toward next time

- FNT 1
  - gel images: figures/captions plus summary below
  - read full assignment description for context!
- FNT 2
  - we post recovery gel
  - you estimate DNA masses (cf ladder), and then…
  - calculate backbone volume for 50-100 ng
  - calculate insert volume for 1:4 molar ratio
  - ready to do ligation when you get to lab! (no lecture)