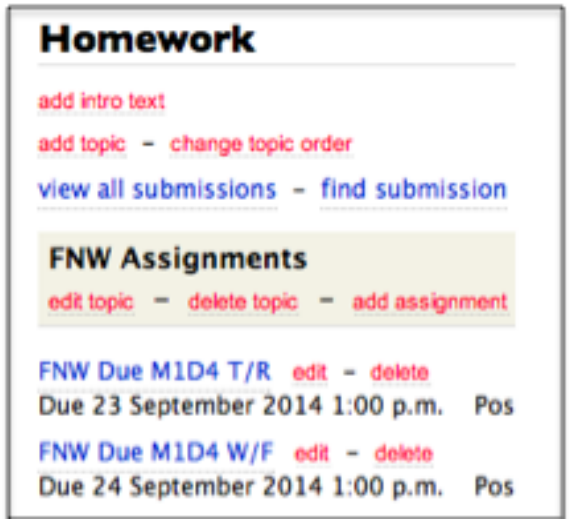
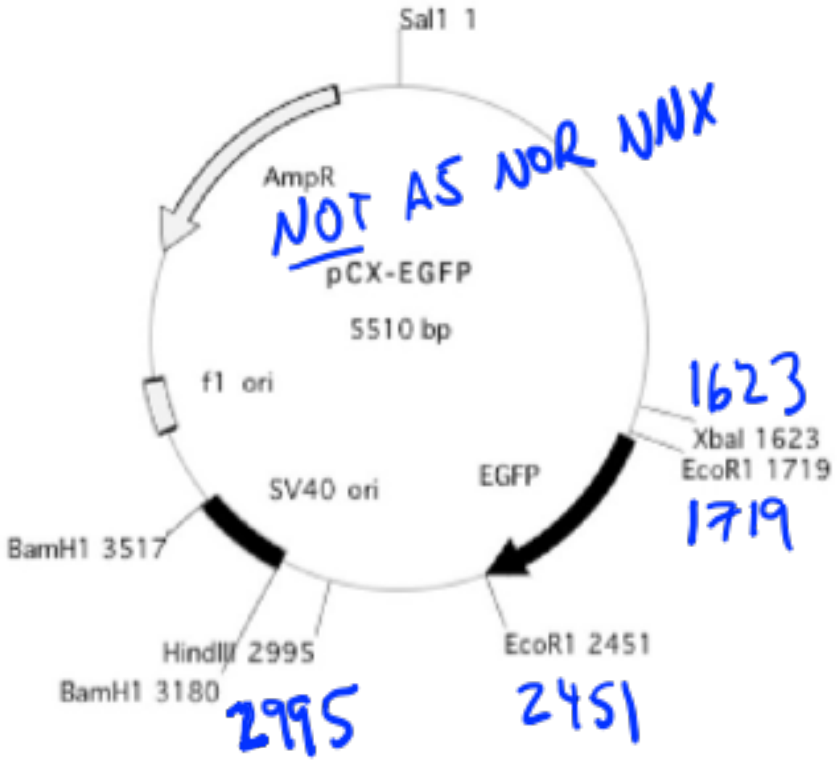
- Announcements
- Lab Quiz
- Start gels running
- Pre-lab Lecture
  - DNA electrophoresis
  - DNA ligation, part 1
  - Today in Lab: M1D3

Announcements

- NO lecture Thu or lab Fri this week (this S-k!)
- My OH: Tue 4-5 pm, 16-319 + some Sundays
- About lab notebooks
  - M1 collection on D7: either D3, 4, or 6 evaluated
  - Text highlights (for changes) helps Isaak *and* you
  - Calculations – "get creative"
- Part of next assignment submitted on Stellar → (hardcopy)
- Briefly: jump to slide 11



Restriction enzyme analysis example



Enzyme(s)	# bands	Size(s)
XbaI	1	~5500
EcoRI	2	~700 ~4800
XbaI + HindIII	2	~1400 ~4100
XbaI + EcoRI	3	~100* ~700 ~4700

DNA electrophoresis (EP): principle

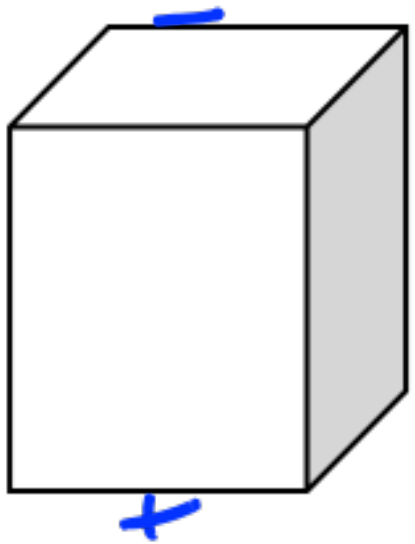
Agarose gel (buffer)

DNA

Agarose and DNA are both polymers
→ have molec. entanglements

Driving force for separation: (mass:) charge

DNA moves − to + because of phosphates



Separation is according to: size

Smaller
entanglements ↑ w/ size ↑
DNA moves faster because

(note: high wt% gel, ↓ pore size, ↑ small DNA resolution)

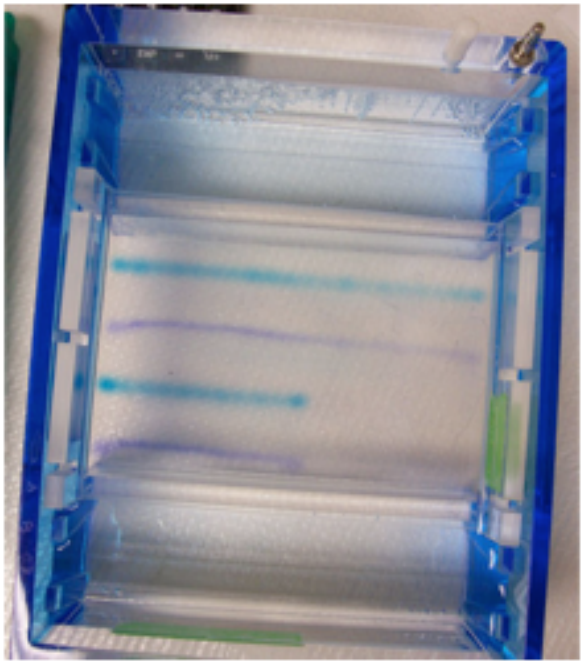
DNA EP: visualization

Loading dye:

- glycerol → DNA sinks into wells
- XC or BPB → real-time tracking dye
  - single band, DNA-independent
- later RNase for cleaner prep (D5)

DNA stain:

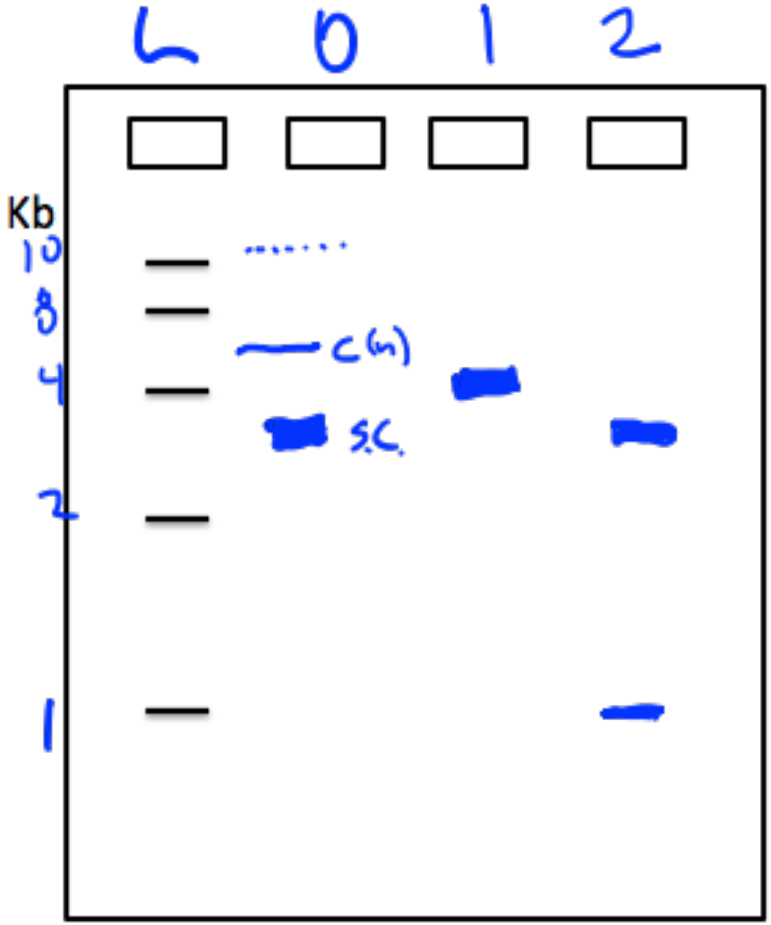
- ethidium bromide or SYBR safe
- fluoresces under UV or blue light
  if bound to DNA [→ intercalates, conformation change (π-stacking?)]



sr.wikipedia.org

Thanks to Shannon for image!

DNA EP: analysis



DNA ladder: standards of known size + concentration

Example: 4 Kbp plasmid

Controls:

single-cut: linear, runs w/ L

uncut: nicked circular
supercoiled

cut-twice: add up to 4, linear

Samples:

for collection: D3

for analysis: D3 (eve), D5

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.

amber glasses, blue UV (+ glasses)

- 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.

goal = 4:1 molar ratio of ins:bkb for ligation (DW)

Determining bkb:ins ratio

- What if bkb:ins 1:100?
  multiple insert products
- What if bkb:ins 100:1?
  plasmid dimer

  ✯ more background: re-closure of partly cut backbone (pCk-NNK)
- Why have insert in slight excess?
  contact frequency (1:1 – 1:10)

  and note: 2x inserts (vs. 3) = impossible

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
- If time, set up your own recovery gel

Toward next time (=in a week!)

- 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!