M1D3: Agarose gel electrophoresis

9/17/13

1. Lab treat

2. Pre-lab discussion

3. Gel electrophoresis

4. ~3pm: EHS presentation

5. Purify DNA from agarose

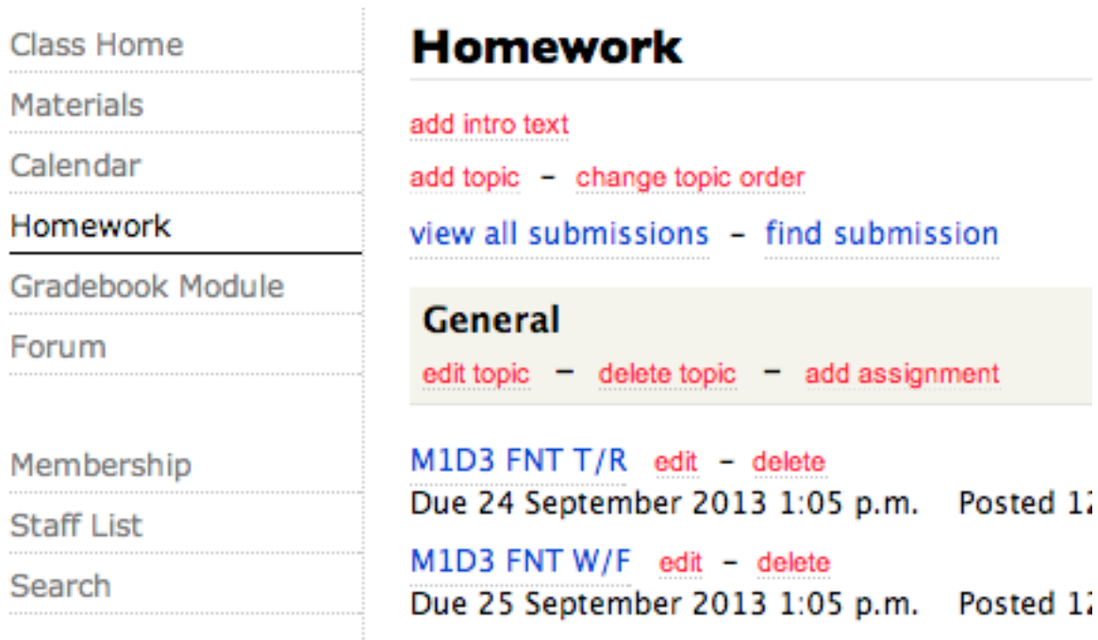
6. Evaluate recovery via electrophoresis

Clarification. Figure Caption titles.

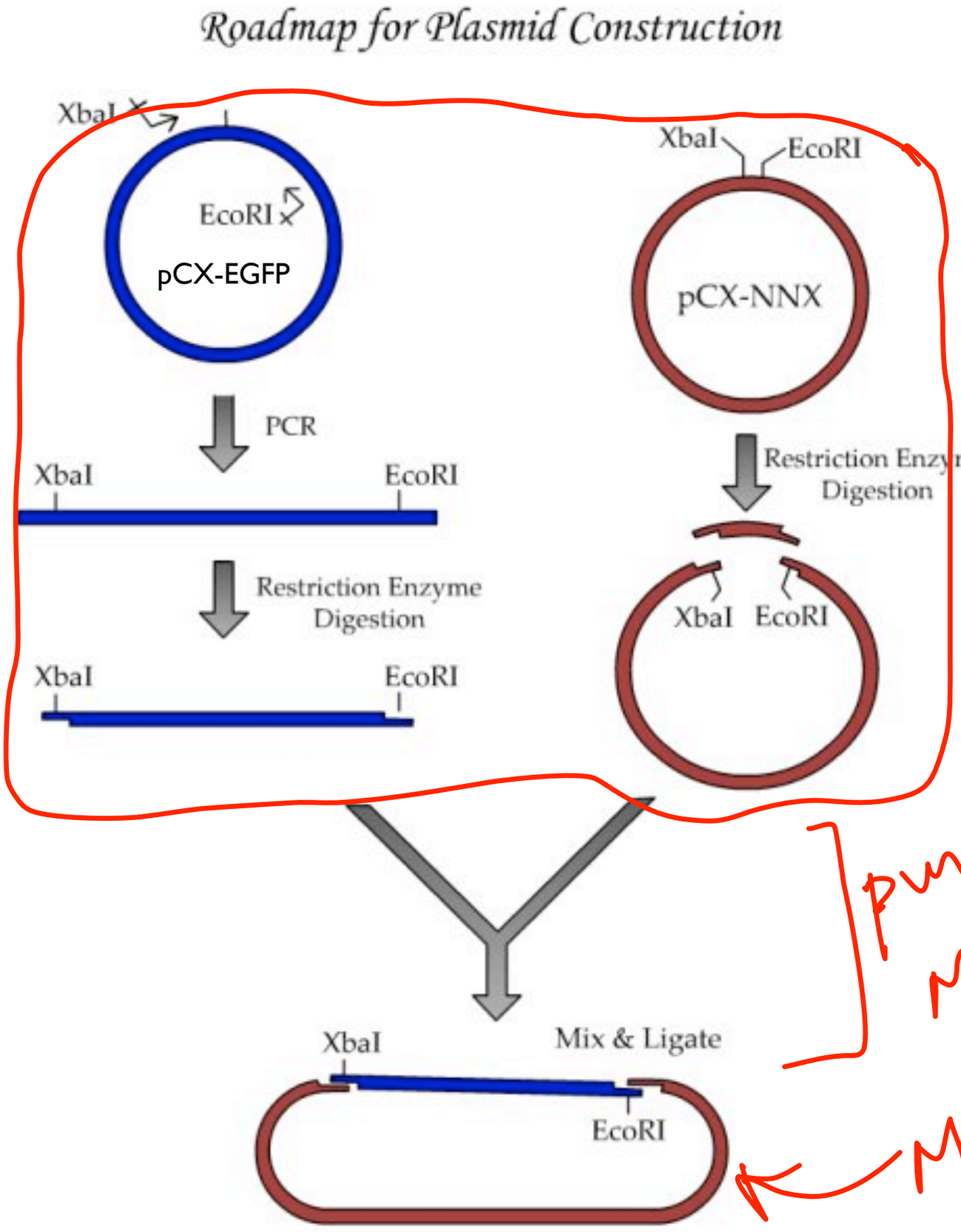
Conclusion preview OK!

A few announcements:

1. Notebooks:
   a. Collection day: M1D7
   b. Text highlights -- for you and for us
   c. Calculations -- get creative
2. Office hours: Monday 2-3pm (16-429b) & 7-8pm (Lab)
3. Next assignment is submitted to Stellar
4. Waste (as in, please empty).



Step 1: Build the system!



Expected product sizes:

PCR = ~650 bp

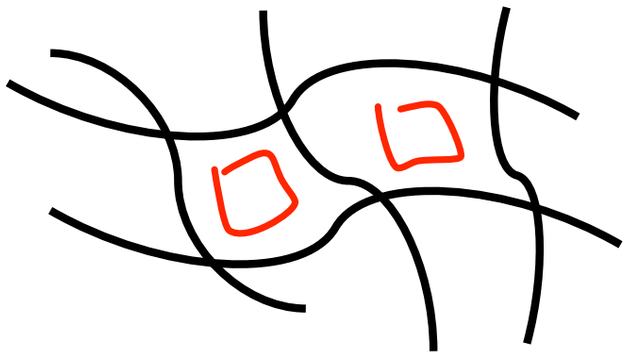
pCX-NNX = 4300 bp

purification MID3

MID4

DNA Electrophoresis (EP): Principle

Agarose gel



DNA

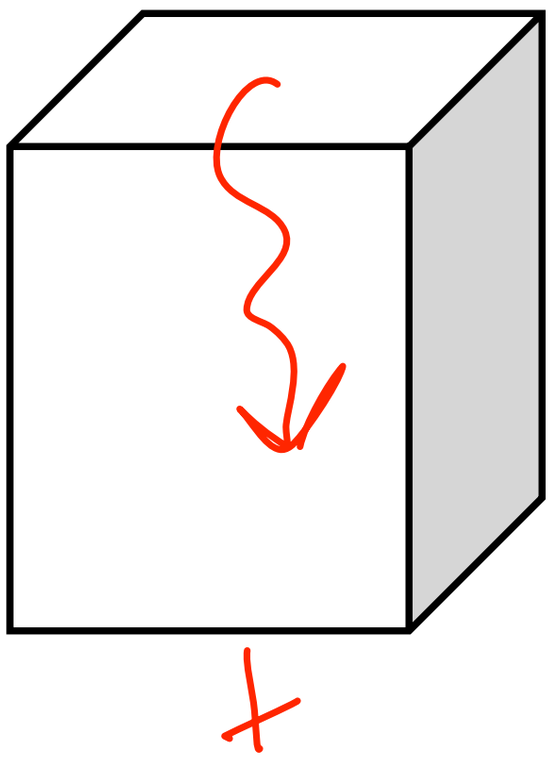
Agarose and DNA are both polymers

Driving force for separation: electric charge

DNA moves − to + because of negative charge

Separation is according to: size!

smaller DNA moves faster because ↑ porosity + entanglements



Thanks to Agi for this slide!

DNA Electrophoresis:

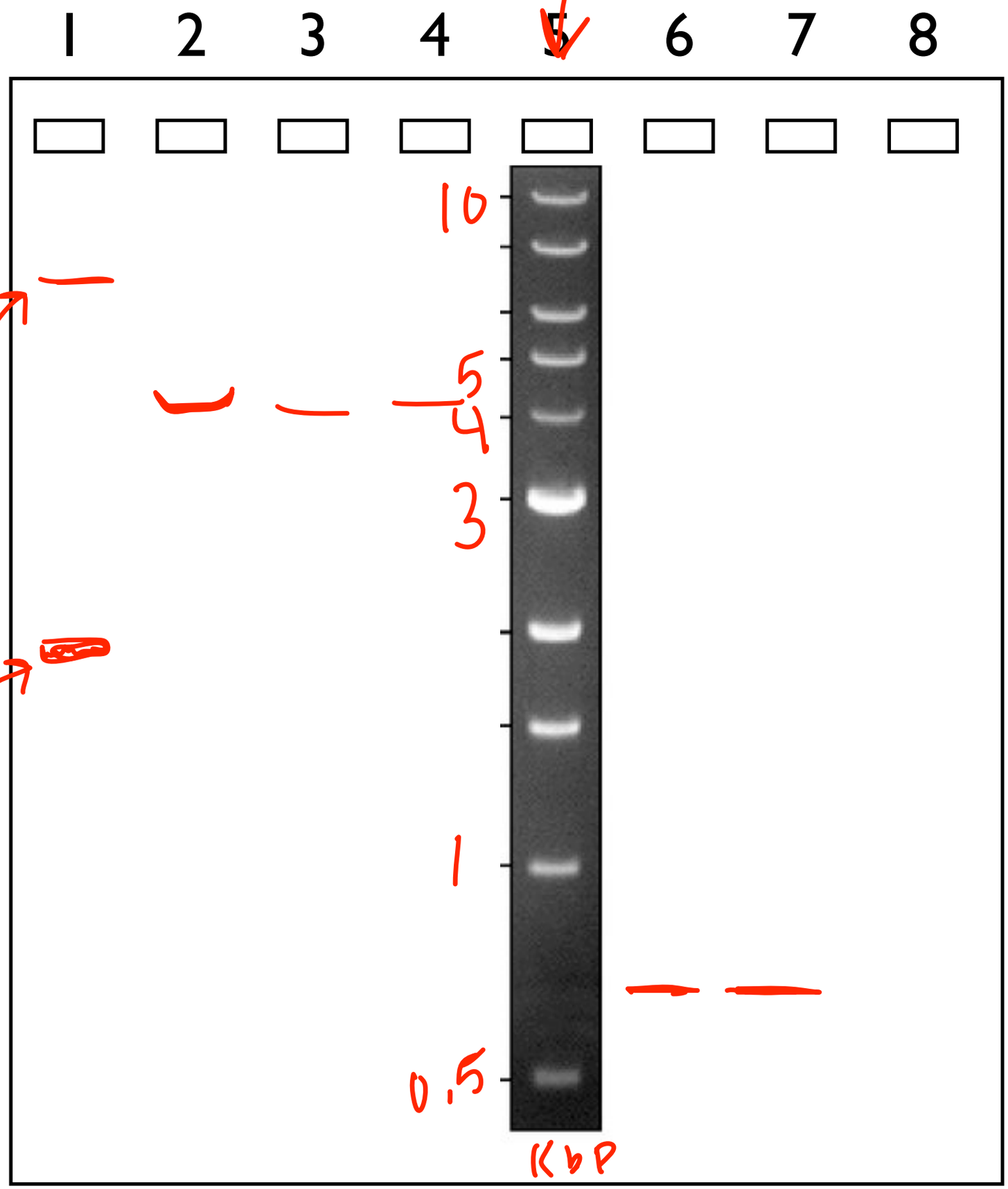
Lane	Sample	Volume to load
1^	Uncut pCX-NNX^	10 µL^
2	pCX-NNX XbaI	5 µL
3	pCX-NNX EcoRI	5 µL
4	pCX-NNX XbaI + EcoRI	25 µL
5	1Kb DNA Ladder	20 µL
6	PCR Product XbaI + EcoRI	25 µL
7	PCR Product Uncut	25 µL
8	PCR no-template-control	25 µL

Uncut DNA:

plasmid circle

supercoiled

Cut DNA:



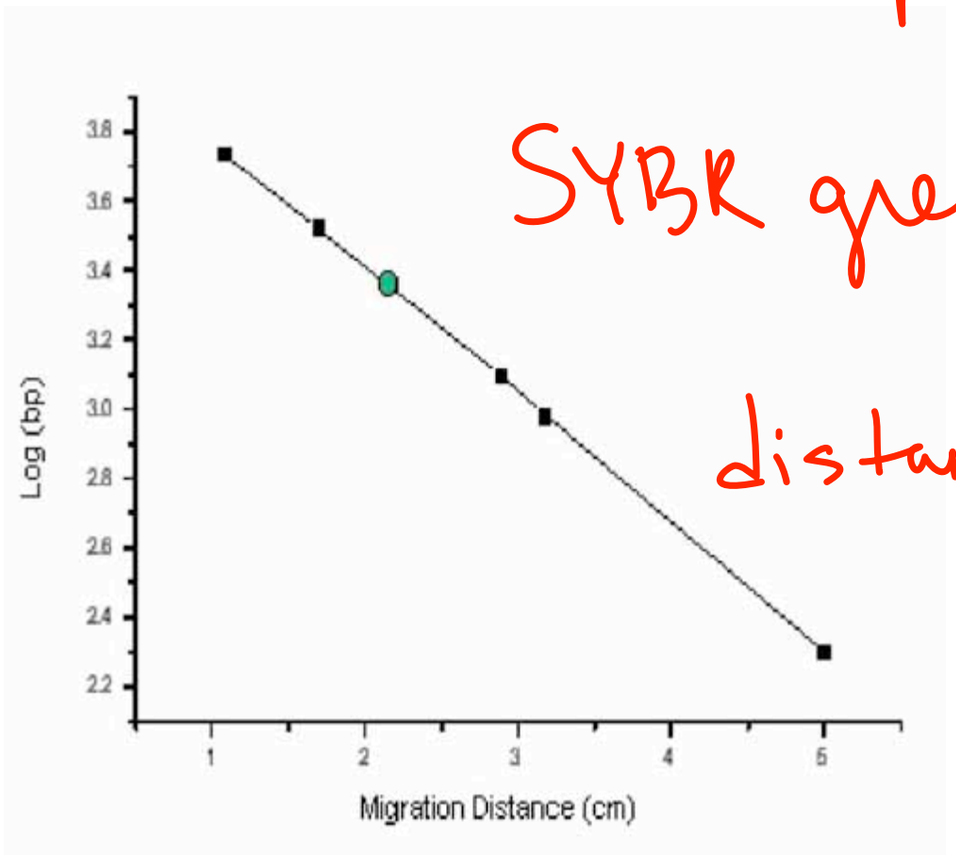
How do we visualize the DNA?

Loading Dye:
- bromophenol blue
- glycerol / Ficoll-400
- RNase

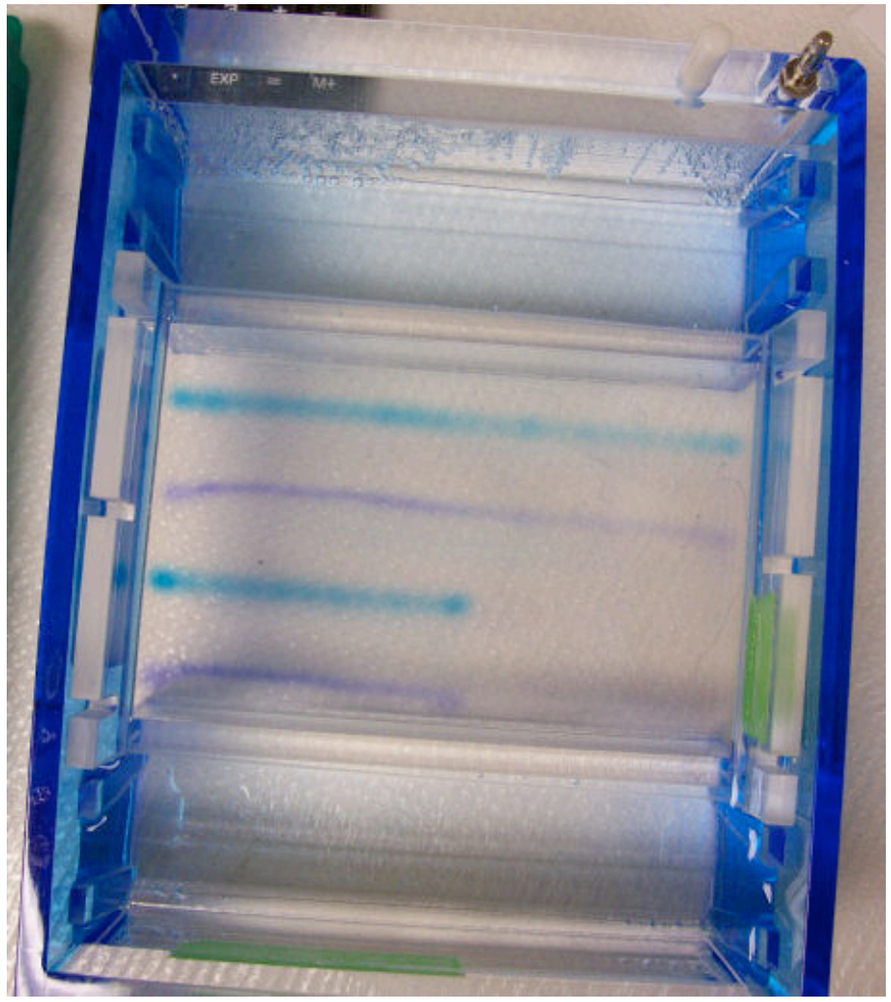
DNA stain:
ethidium bromide
- complexed w/ DNA ⇒ signal

SYBR green

distance ∝ $\frac{1}{\log(MW)}$



From MIT OCW



sr.wikipedia.org

awkward photo with your advisor

Extract DNA from agarose gel:

Another silica column from Qiagen:

- different Salt (yellow)
  - and in melting agarose
- different size silica

Note: solution should be yellow. If it turns blue, we'll have to adjust the pH - unlikely

Safety Note: You must wear *UV glasses* or a *face shield* while cutting out your DNA band.

DNA EP: clean-up and safety

- Use nitrile gloves when handling DNA gels and all equipment used for gels.

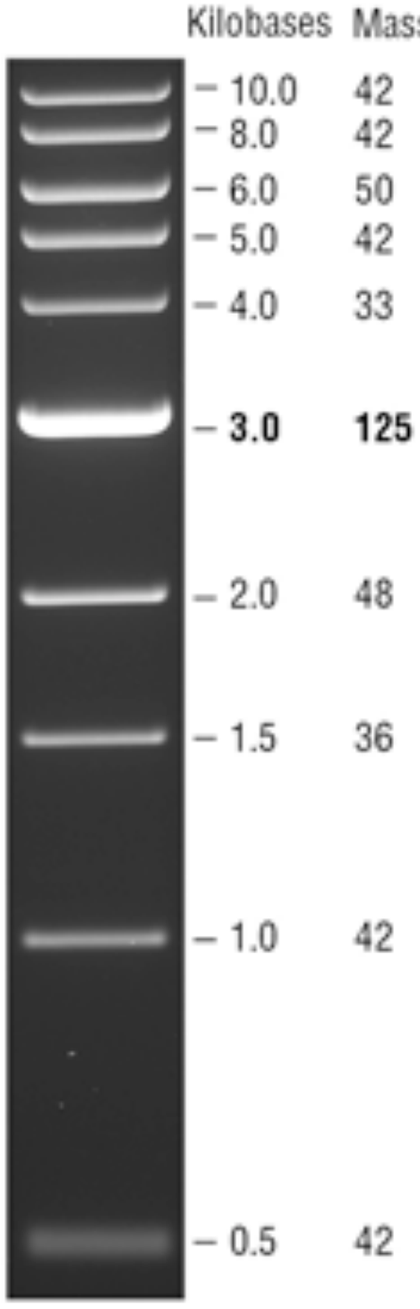
- Wear eye protection/face shields when cutting DNA bands out of the gel.

- Gels and gel-contaminated papers are disposed of in solid chemical waste.

Thanks to Agi for this slide!

Next time: DNA Ligation -- some up front work.

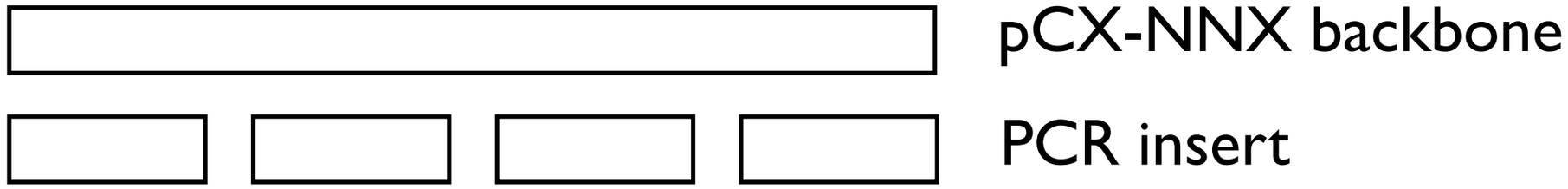
★ with evaluation gel → Recovery after gel extraction



neb.com: 0.5 ug
MW Marker

Dye intensity reflects absolute ***mass of DNA.***

mass of DNA ≠ molar quantity of DNA ???



Goal: 1 4 bkb : insert (Molar)

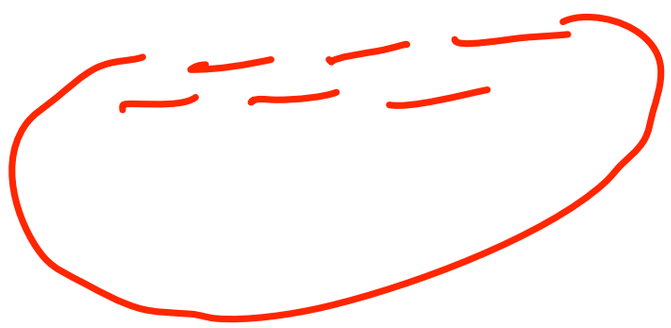
Why? Increase chance of success

(1) Imagel
(2) estimate mass - DNA
(3) calc. vol 50-100ng cut plasmi
(4) calc. volume of insert

Determining optimal backbone:insert ratio:

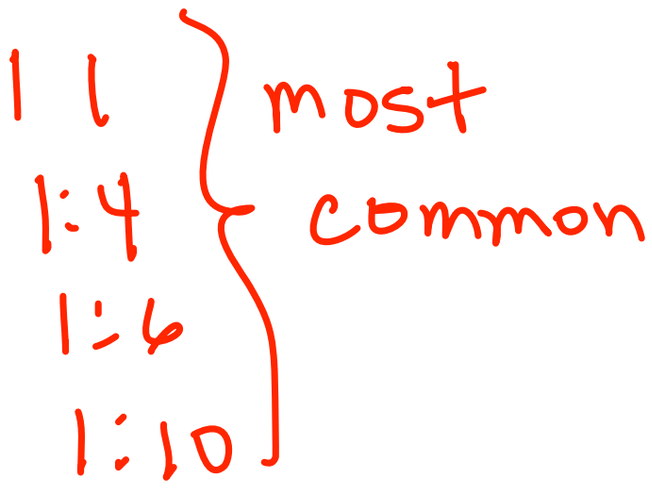
What happens with a backbone:insert ratio of:

1:100



100:1

1:4 (or slightly increased insert)



1:0 - uncut plasmid control

Today in the lab:

- Gel purify digest reactions -- bring pipettes & samples (no tips) -- 2 groups at a time (white/platinum start) – me
- Cut out bands (safety first!) red/orange - lizzie
- EHS visit ~3pm
- Purify DNA from agarose
- Lizzie runs your evaluation gel

yes! lecture

Next time in the lab (No lab Thursday!):

- Ligations
- Transformation
- Longer FNT