M1D3: Agarose gel electrophoresis

9/16/14

✡ No lecture or lab Thursday

1. Lab Treat!

2. Set-up agarose gel — Read Part I Now

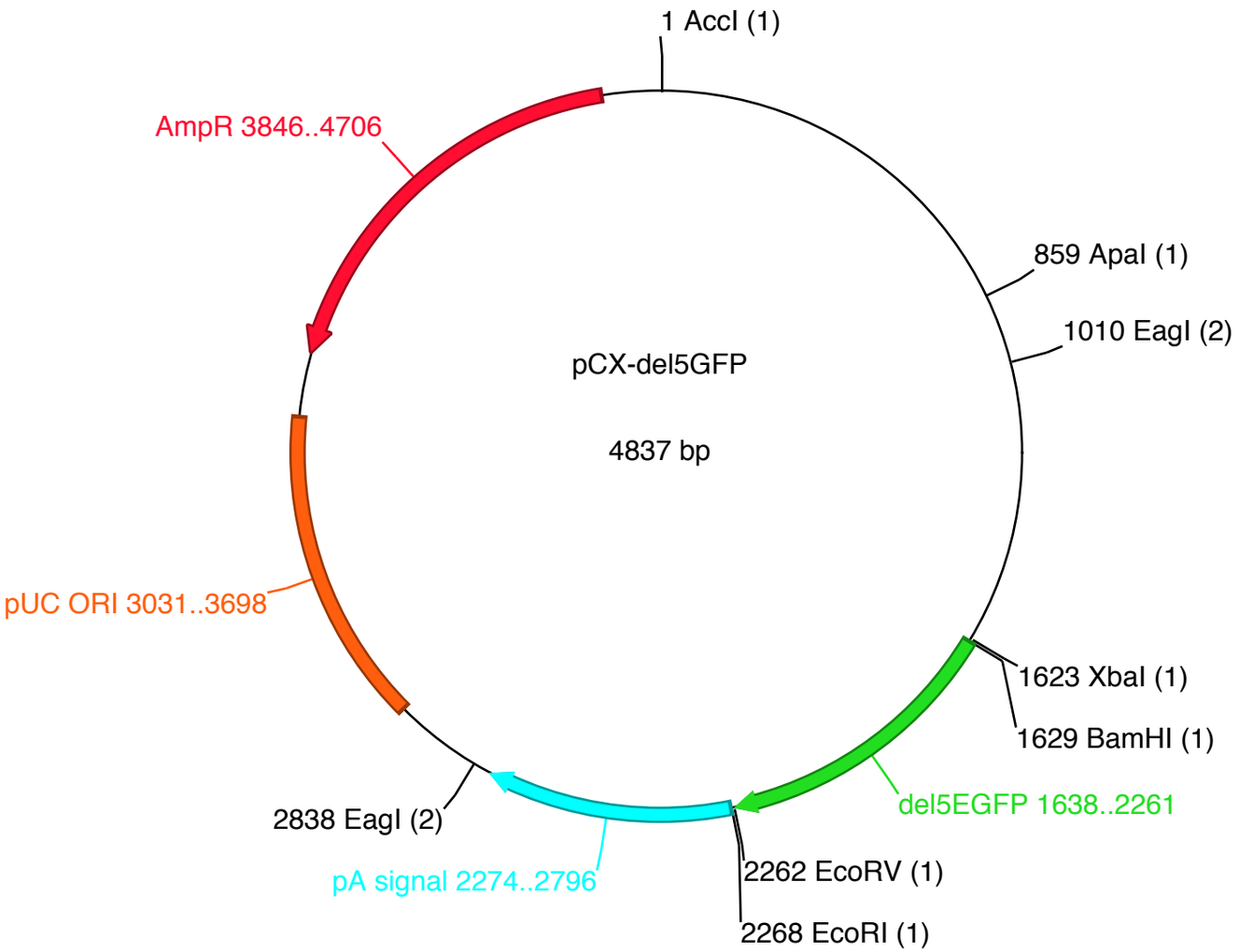
3. Pre-lab lecture — today I'm limited by gel time!

4. Image gel and extract DNA

5. Set-up another gel!

6. Use results to calculate ligation ratios for next time

Thought experiment:

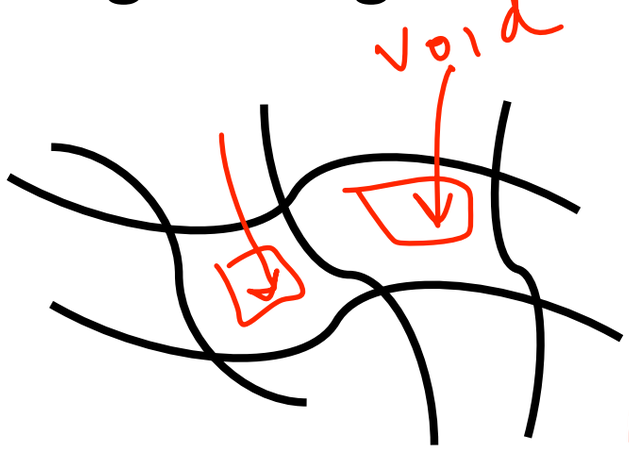


RE	Frag Size
AccI	(1) ~4800 bp
EagI	(2) ~1800 bp ~3000 bp
XbaI EcoRI	(2) ~650 bp ~4200 bp
EagI BamHI	(3) ~600 bp ~1200 bp ~3000 bp

designing your own diagnostic digest

DNA Electrophoresis (EP): Principle

Agarose gel



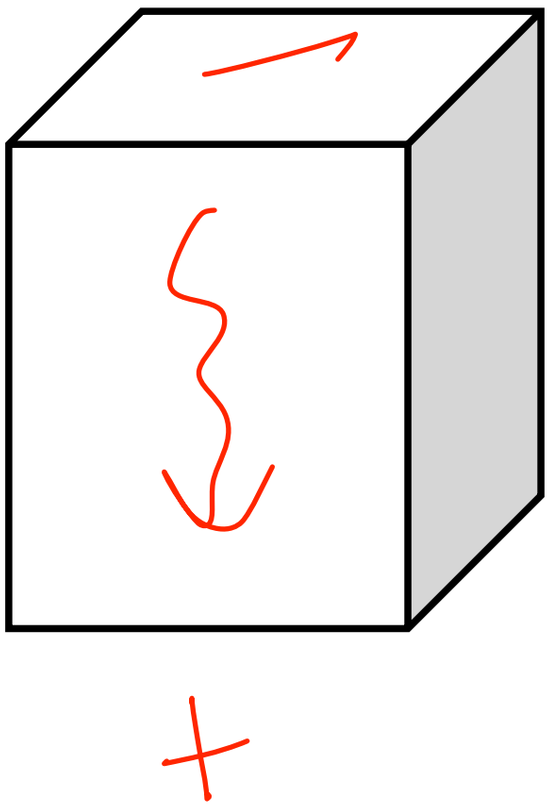
DNA

Agarose and DNA are both polymers

Driving force for separation: electric charge

"run to red" — +

DNA moves to + because of DNA has a neg charge



Separation is according to: size

Smaller DNA moves faster because

porosity + entanglements

Thanks to Agi for this slide!

DNA Electrophoresis:

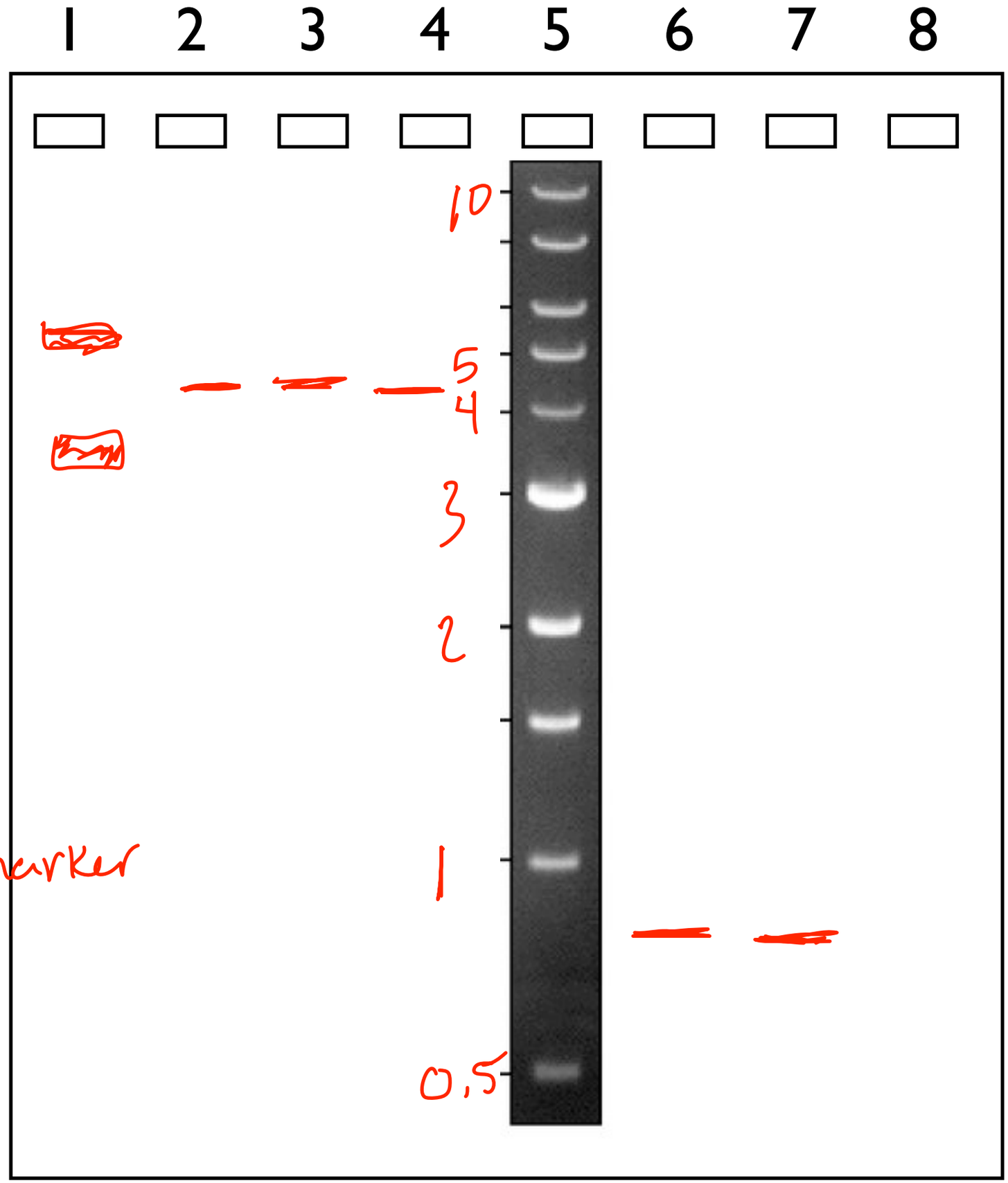
Lane	Sample	Volume to load
1^	Uncut pCX-NNX^	10 µL^
2	pCX-NNX XbaI	5 µL
3	pCX-NNX EcoRI	5 µL
4	pCX-NNX XbaI + EcoRI	25 µL
5	1Kb DNA Ladder	20 µL
6	PCR Product XbaI + EcoRI	25 µL
7	PCR Product Uncut	25 µL
8	PCR no-template-control	25 µL

Uncut DNA: (2 bands)

○ ← may appear larger

❀ ← be located near

Cut DNA: a smaller MW marker



How do we visualize the DNA?

Loading Dye: Glycerol/Ficoll – ↑ viscosity

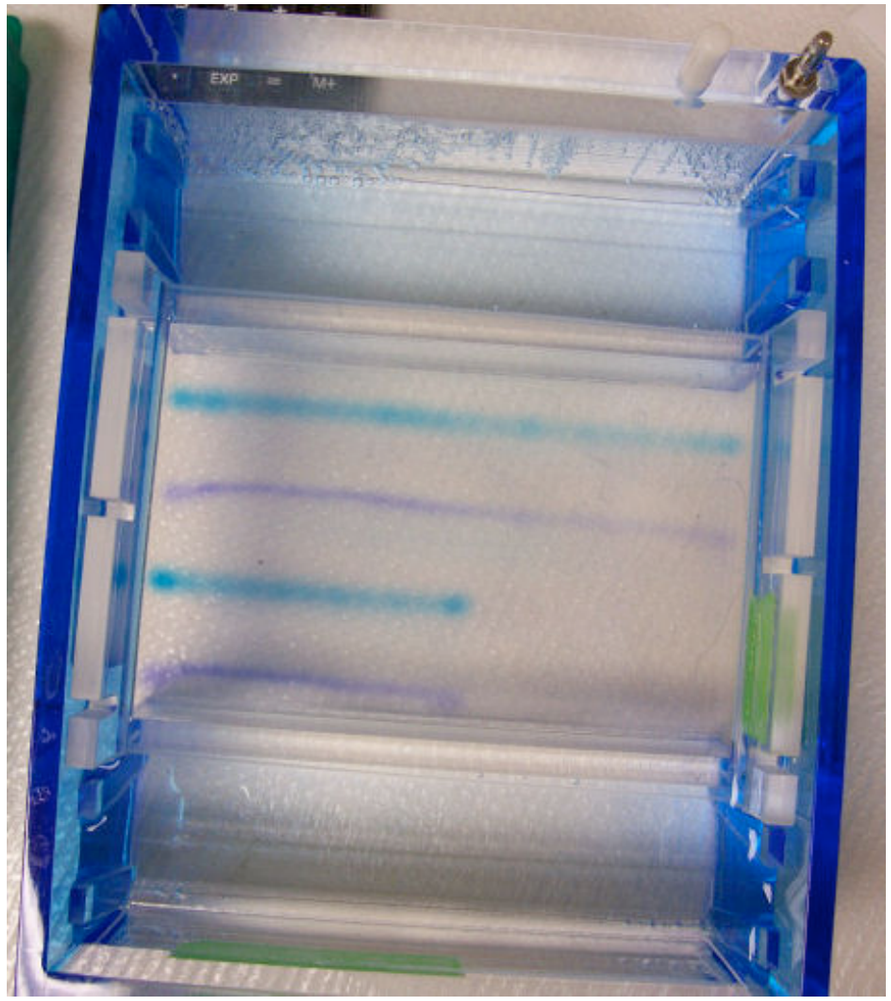
Bromophenol Blue

(similar %C) ~ 300bp

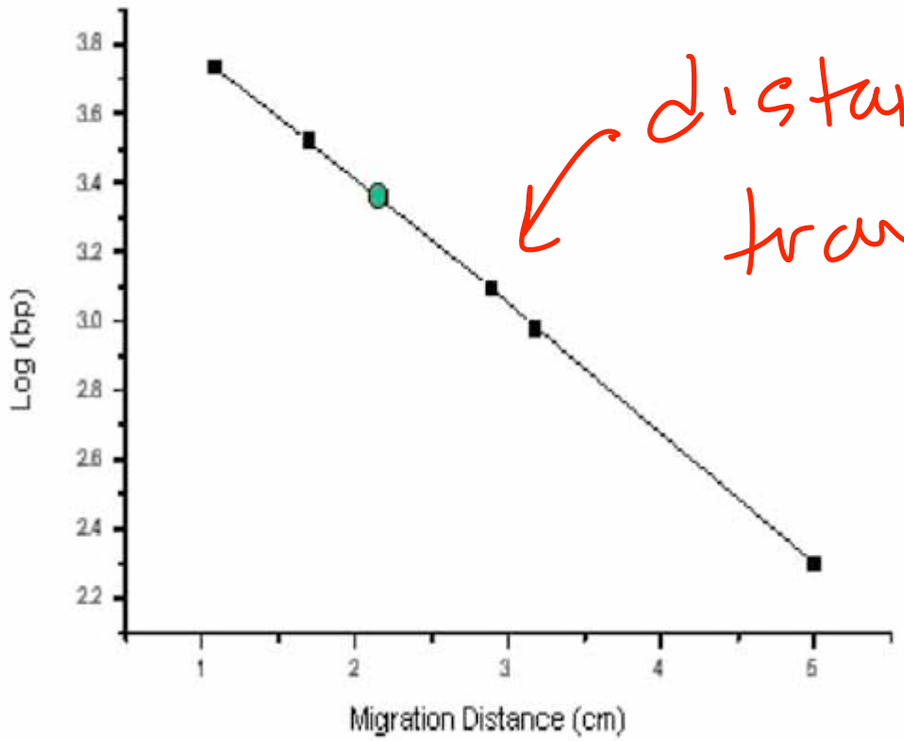
[RNase → remove RNA]

DNA stain: SybrSafe

DNA intercalating dye



sr.wikipedia.org



distance DNA travels $\propto \frac{1}{\log(MW)}$

From MIT OCW

awkward photo with your advisor

Extract DNA from agarose gel:

- mix w/ QG (yellow)

Another silica column from Qiagen:

different size silica

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

Safety Note: You must wear *UV glasses* or a *face shield* while cutting out your DNA band. Also, like last time, save waste from Qiagen kit.

DNA EP: clean-up and safety

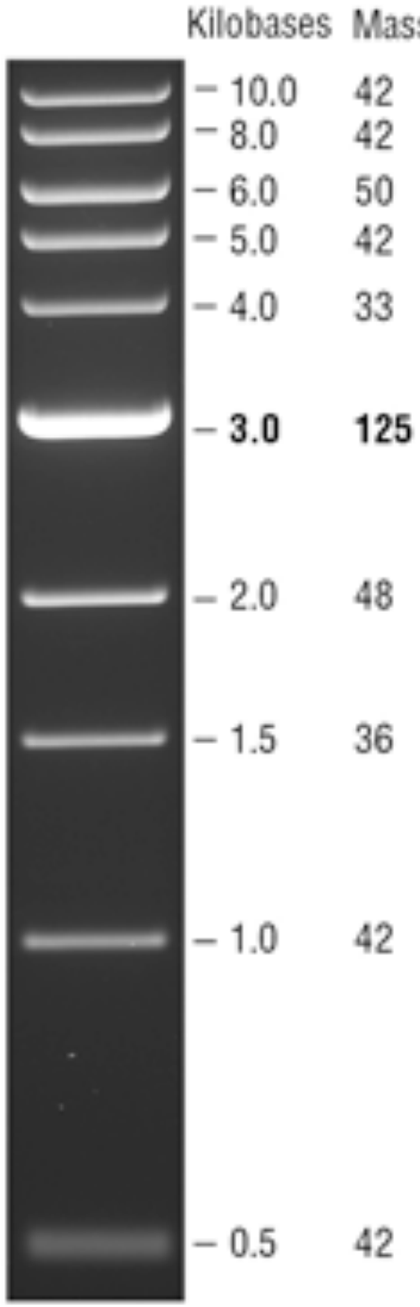
- Use nitrile gloves when handling DNA gels and all equipment used for gels.

- 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!

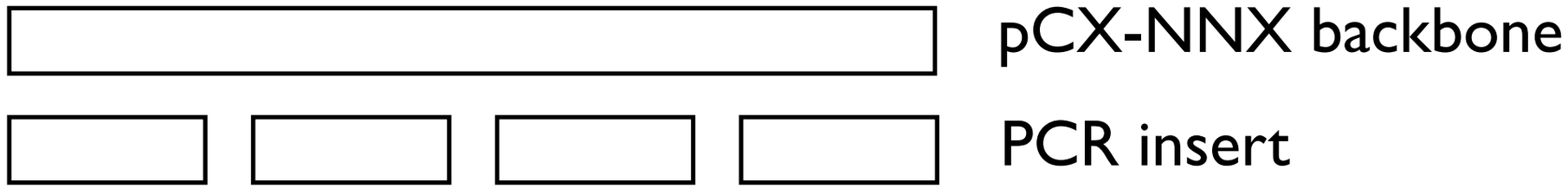
Next time: DNA Ligation -- some up front work.



neb.com: 0.5 ug
MW Marker

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

mass of DNA ≠ molar quantity of DNA ???



(1) Imaging gel
(2) Estimate mass of both bKb & insert
(3) Calc vol of bKb required for 50-100ng
(4) cal vol of insert

Goal: 1:4 molar bKb:insert

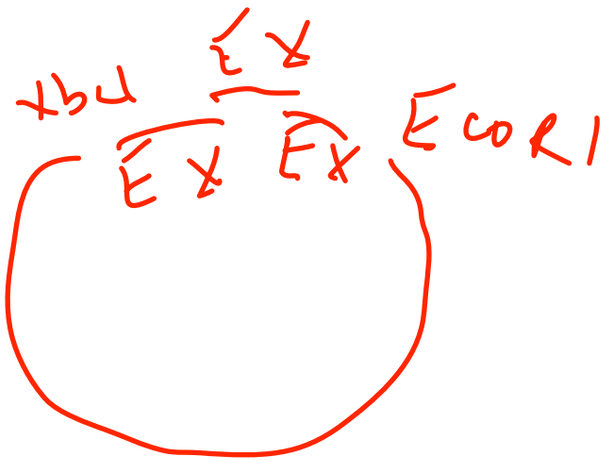
Why?

Determining optimal backbone:insert ratio:

What happens with a backbone:insert ratio of:

1 bkb
100 insert

1:100



100:1

1:4 (or slightly increased insert)

1 1
1:2
1:10
} common

1:0
bkb
no insert

Today in the lab:

- Gel purify digest reactions -- bring pipettes & samples (no tips) -- 2 groups at a time
- Cut out bands (safety first!)
- Purify DNA from agarose
- Isaak runs your evaluation gel — look on Talk page later

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

- Ligations — complete calcs ahead of time
- Transformation