M1D3:
Agarose gel electrophoresis

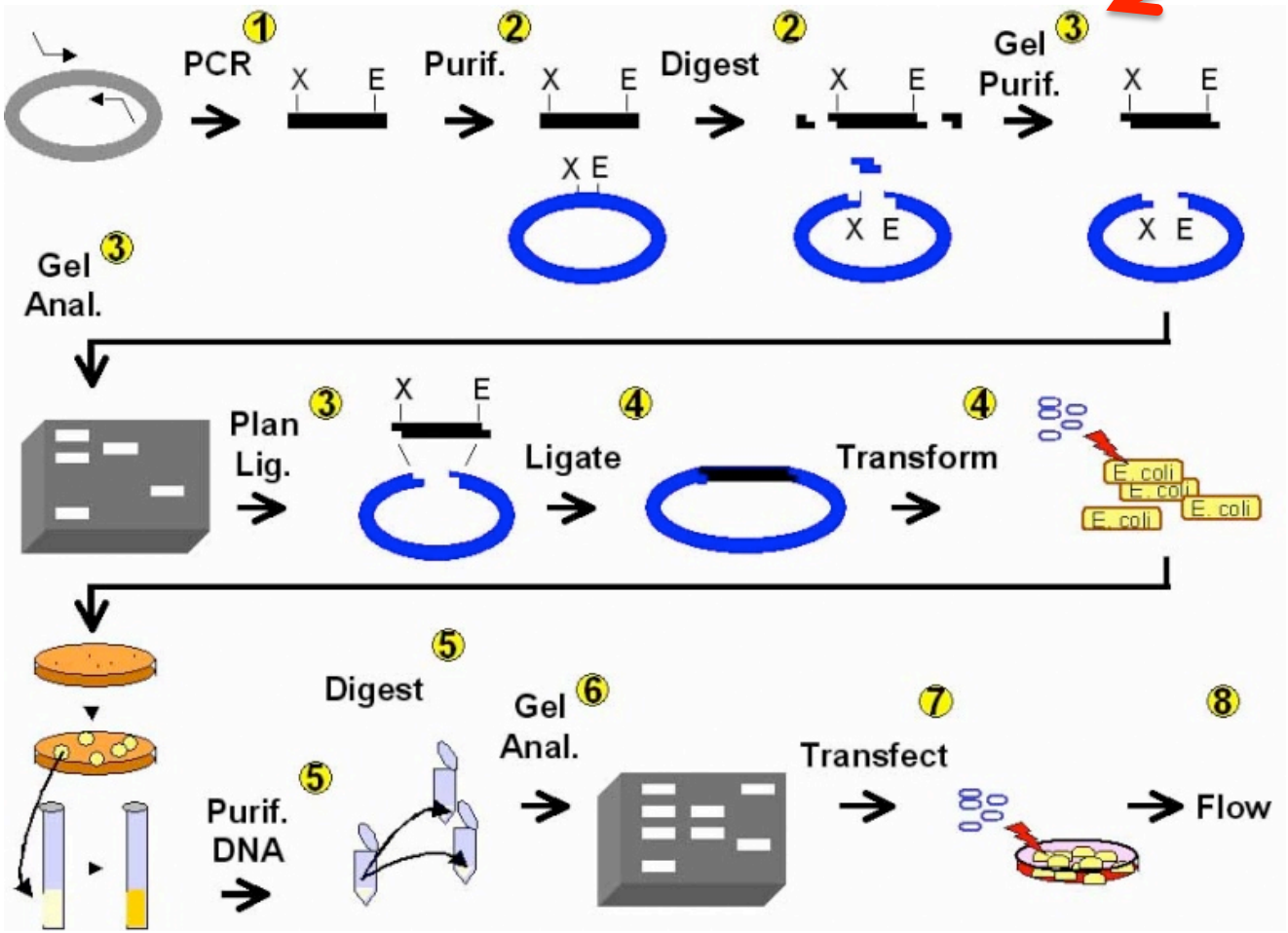
9/22/15

Lab business

- Attendance policy addendum
  - Lecture absences
  - Laboratory absences

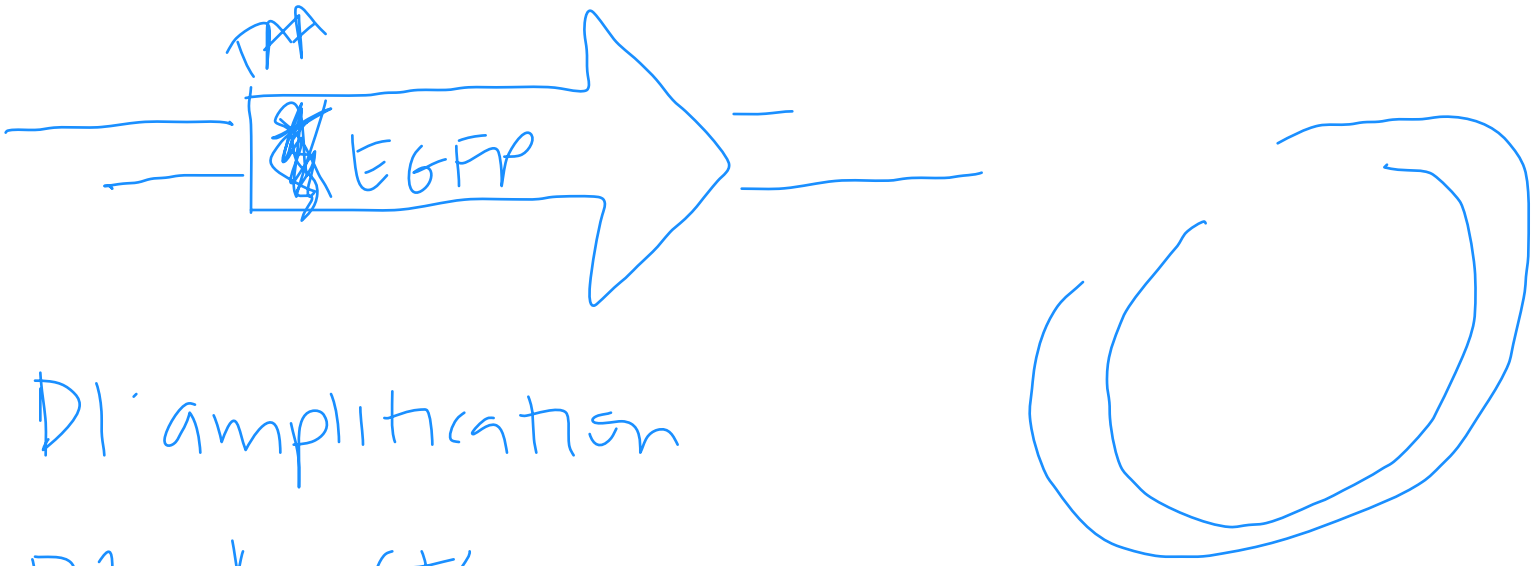
- Next time
  - Lab treat (quiz)
  - Homework due M1D4

Mod 1 overview



Complete Part 1 of the protocol, then come together for prelab discussion

From last time…



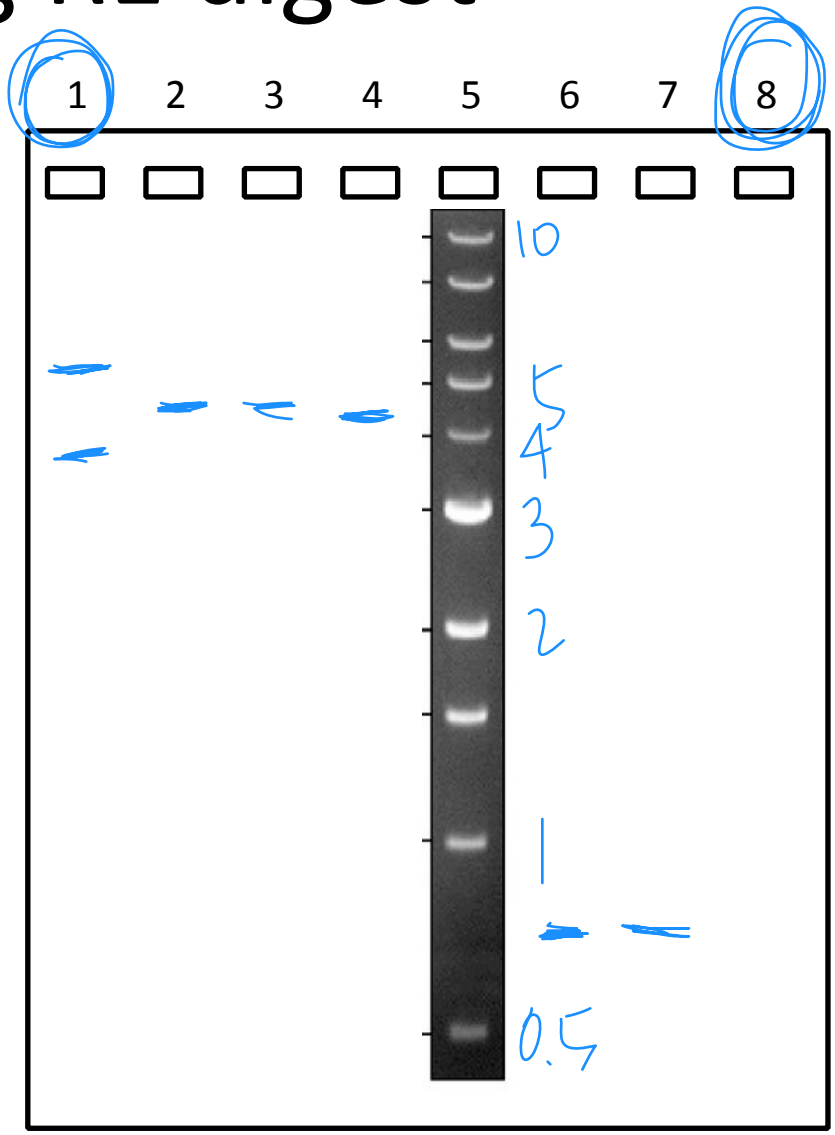
D1 amplification

D2. digests

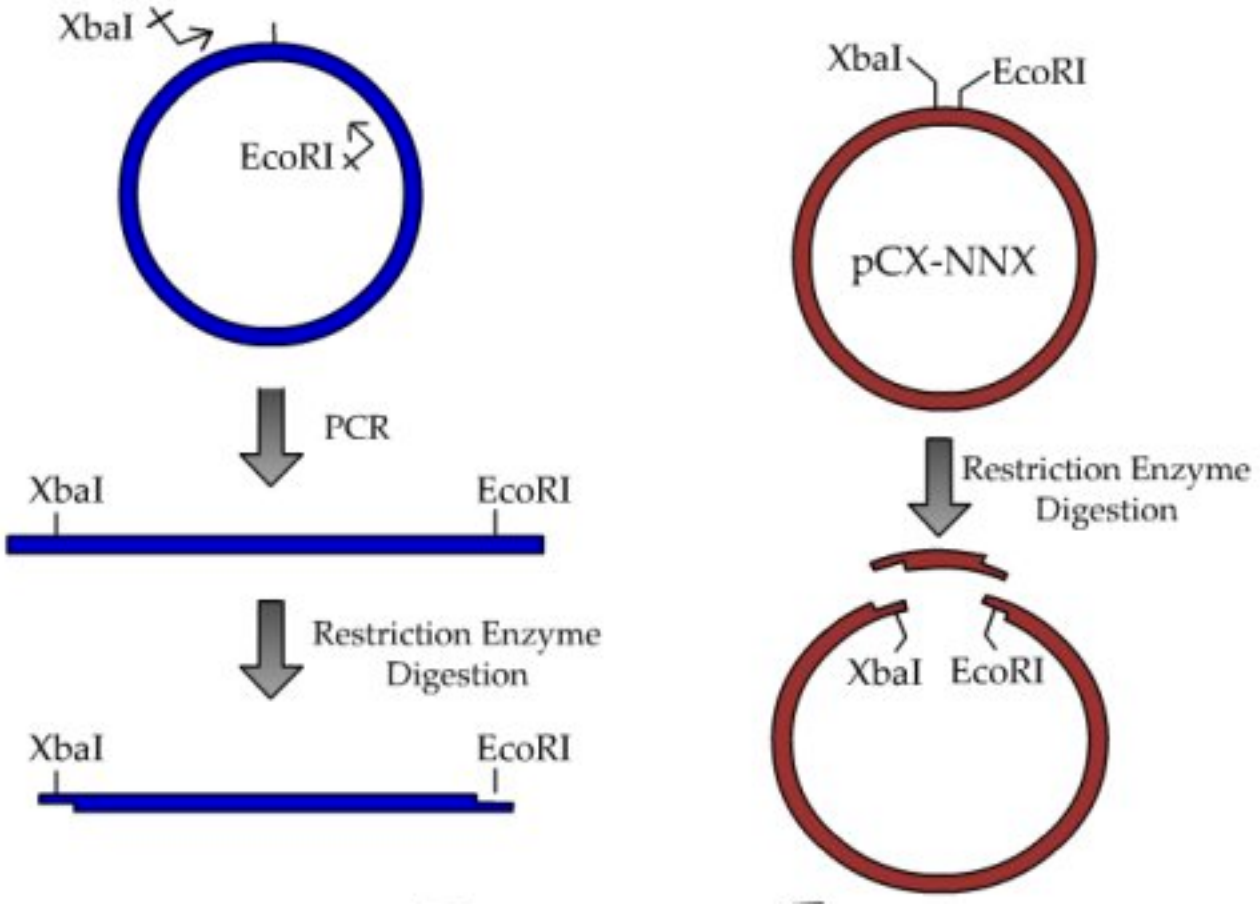
D3. How do you know the enzymes cut your DNA?

Evaluating RE digest

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



Engineering Δ5 EGFP construct



How do we recombine the DNA fragments?

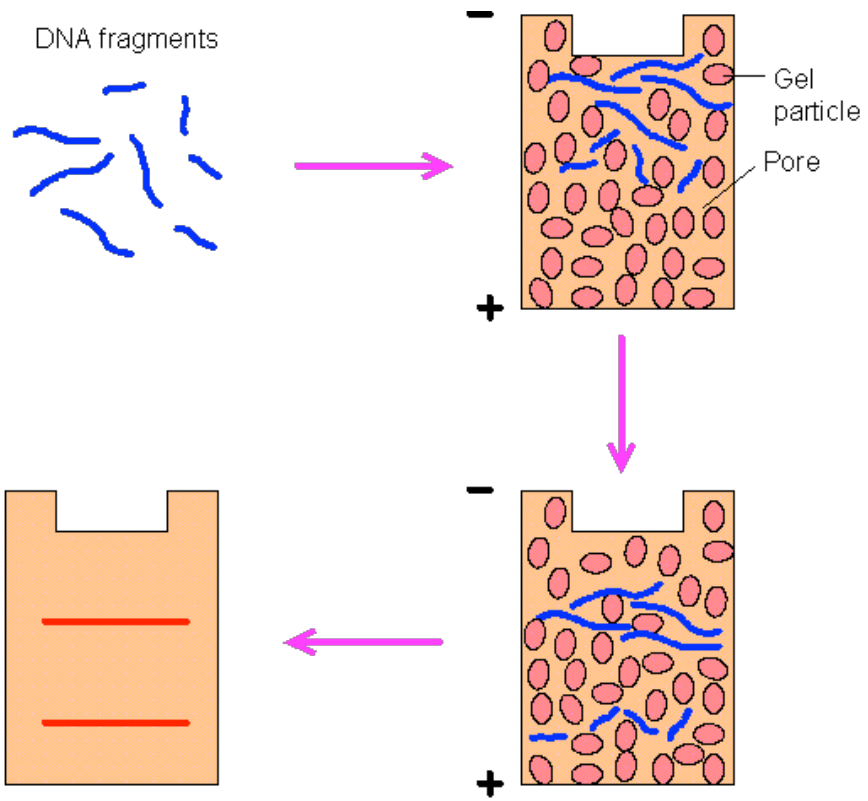
Prep for ligation: visualize DNA

- Gel electrophoresis

driving force?
charge

Separation?
Size

'Run DNA on gel'



How do we visualize DNA?

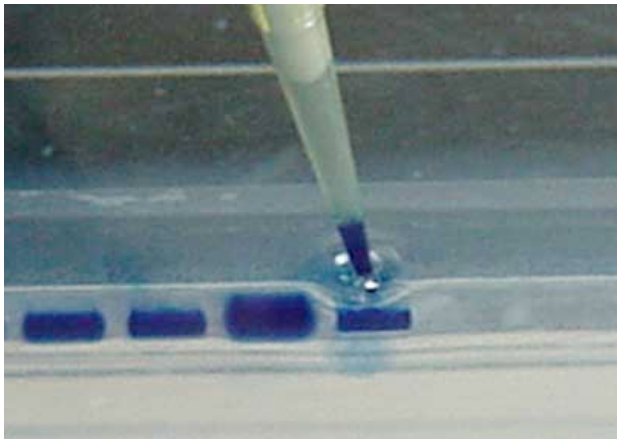
- Loading dye

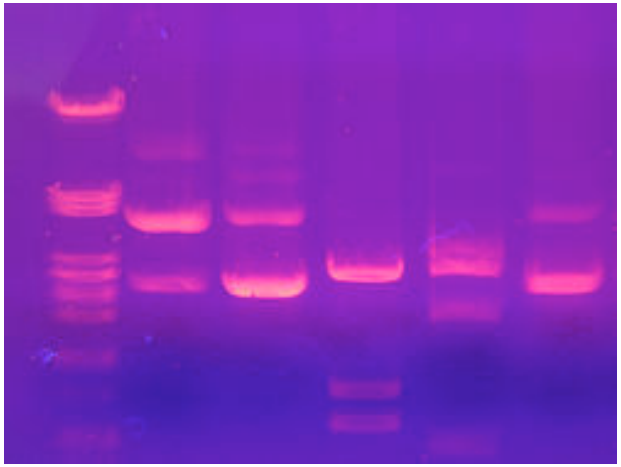
  bromophenol blue

  glycerol

- DNA stain

  SYBR Safe





Safety note: wear nitrile gloves and face shield!!

Prep for ligation: purify DNA

- Gel purification

Qiagen

QG twash

↑ salt

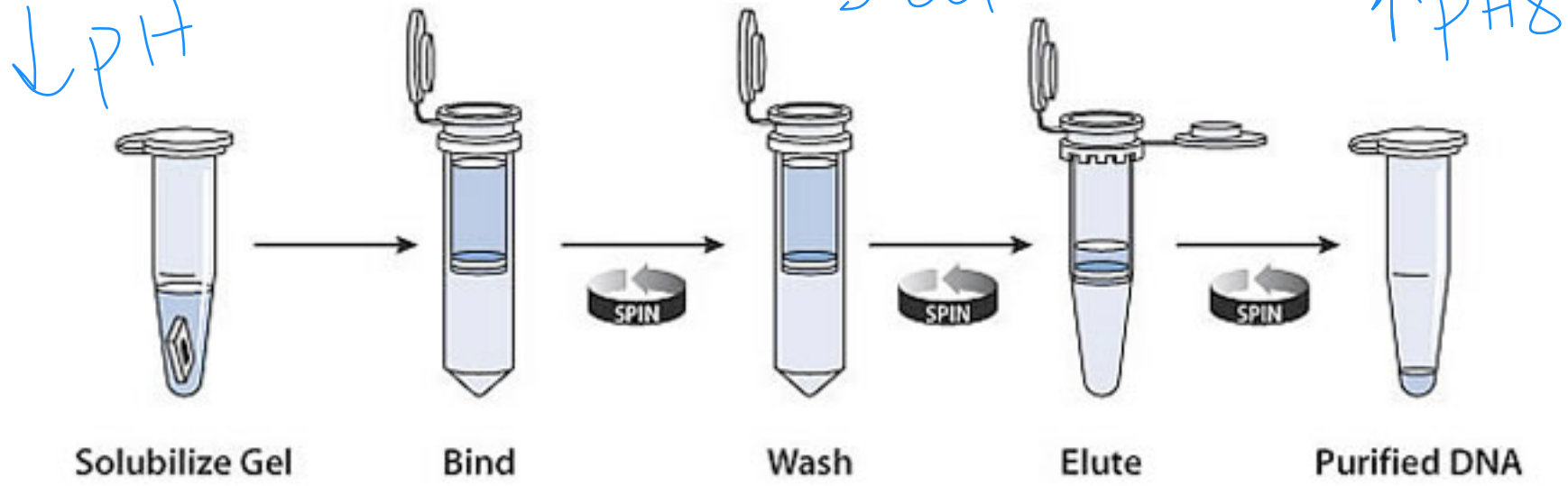
save excess

EtOH

~↑ salt

↓ pH

↑ pH8



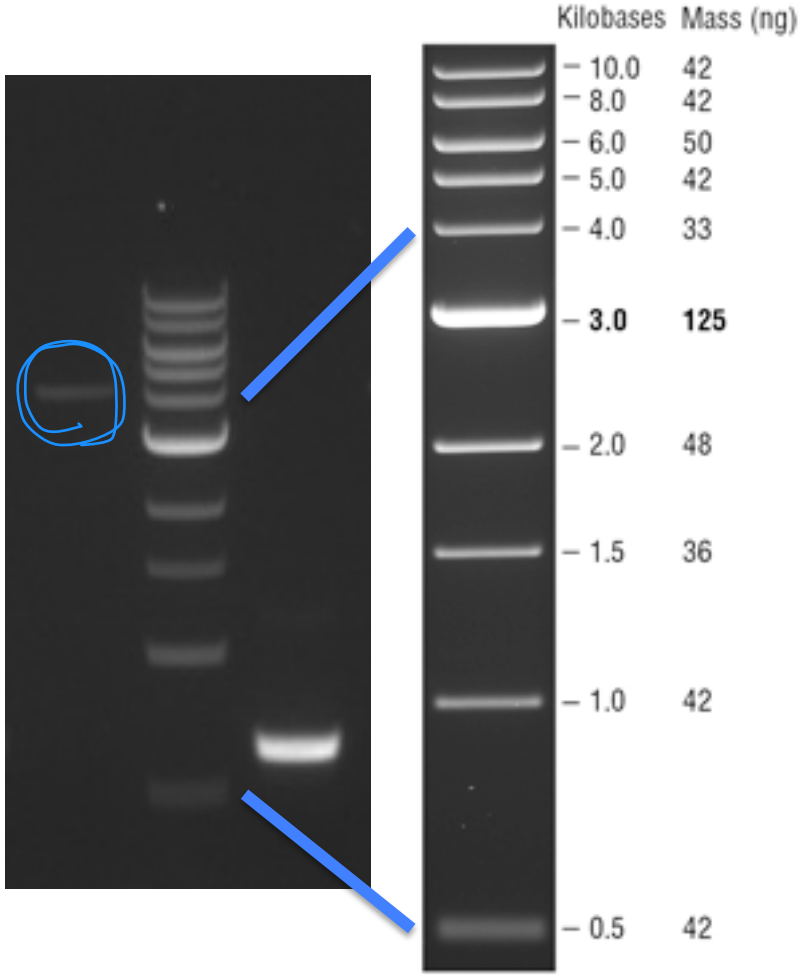
Prep for ligation: quantify DNA

- Amount (ng) of vector

115ng / X uL

Amount of DNA ≠ total amount loaded

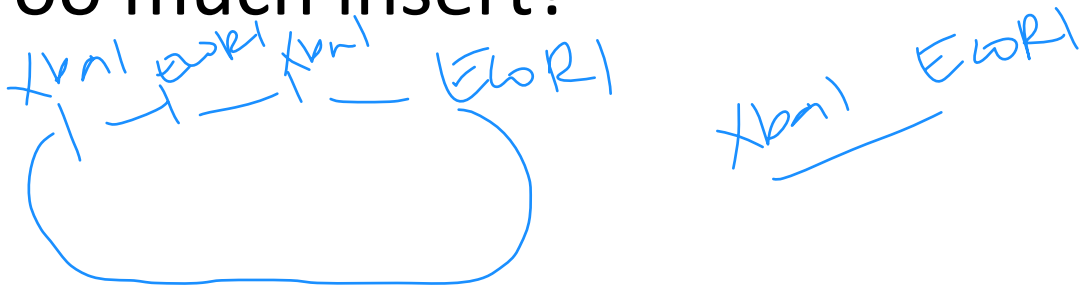
- Amount (ng) of insert



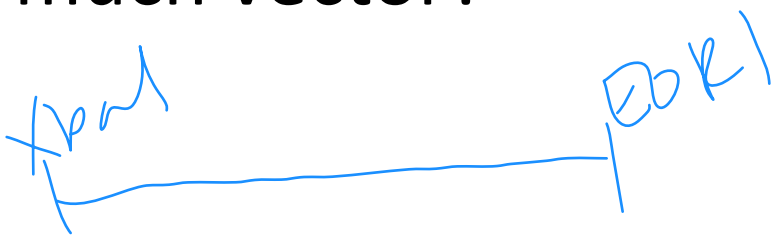
Mass of DNA ≠ molar quantity of DNA

Optimal vector-to-insert ratio

- Ideally want 1:4
  - Molar ratio, **not** volume or mass
- Too much insert?



- Too much vector?



Prep for ligation: ratio calculation

1. Examine recovery gel
   - will be posted by teaching faculty
2. Estimate mass of vector and insert
3. Determine volume of vector needed (50-100ng)
4. Calculate mol of vector
5. Determine mol of insert needed (2X-4X vector)
6. Calculate volume of insert needed

Today

- Gel electrophoresis (gloves)
- Excise vector and insert from agarose and purify (gloves and eye protection)
- Prepare vector and insert samples for gel electrophoresis
- Pour gel to examine recovery (gloves)

- Networking event
  - September 24 at 5:30p in first floor Koch lobby