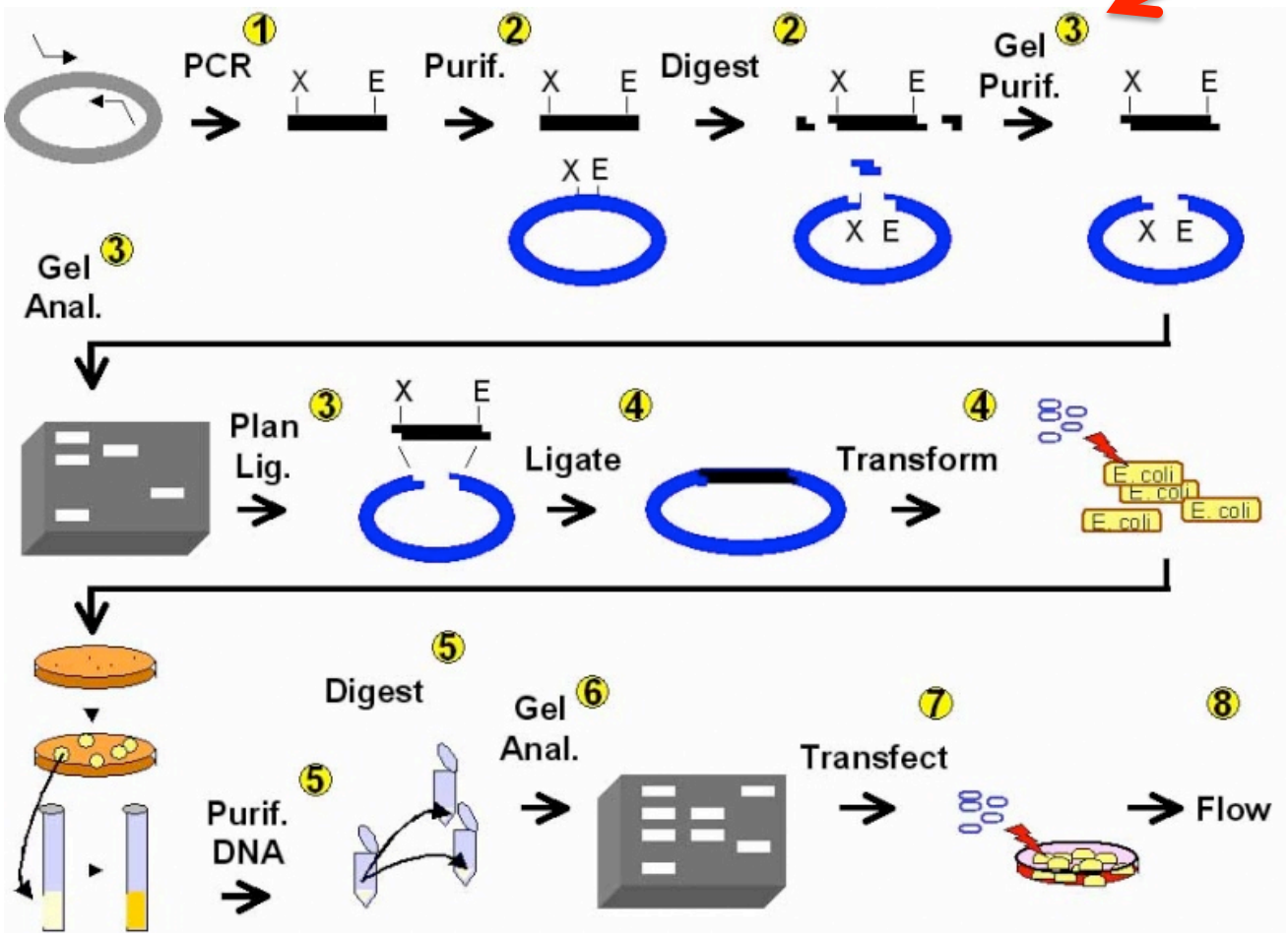
M1D3: Agarose gel electrophoresis 9/23/15

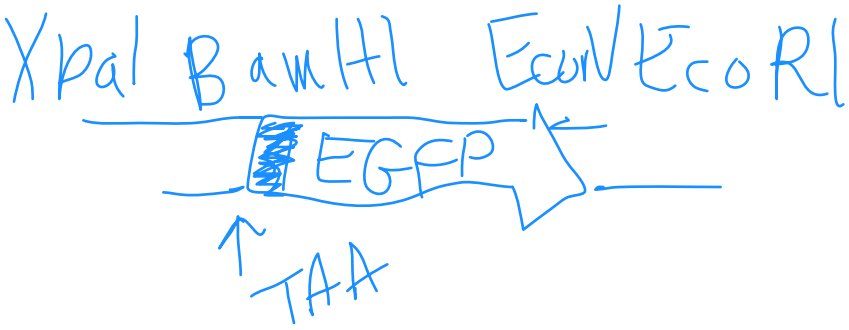
1. Attendance policy addendum
   - Lecture absences
   - Laboratory absences
2. Next time
   - Lab treat (quiz)
   - Homework due M1D4

Mod 1 overview



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

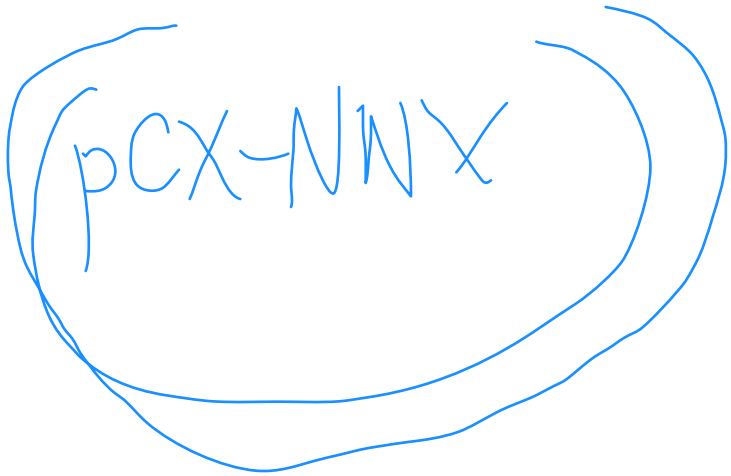
From last time…



D1: amplify

D2: digest

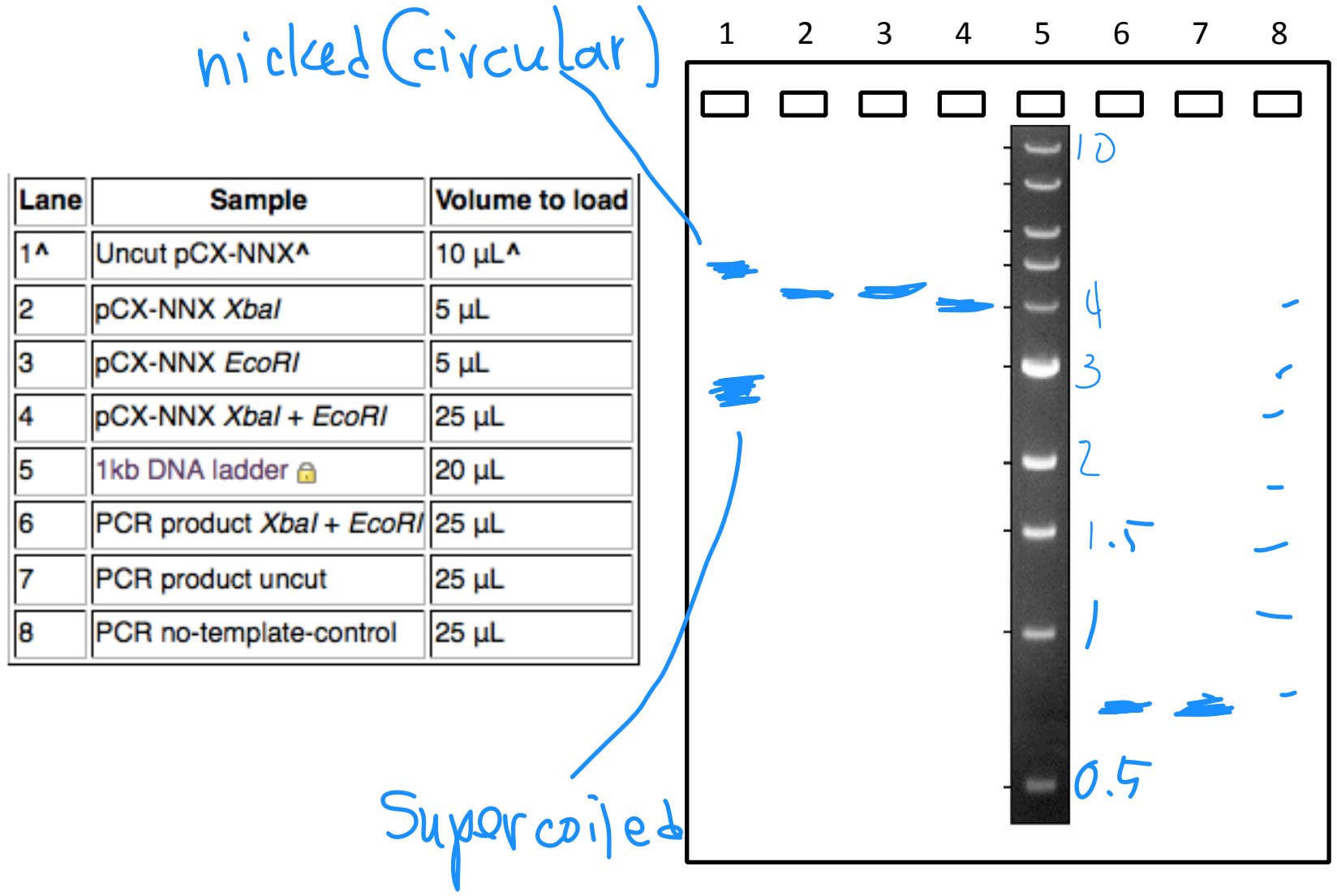
D3: purify + verify



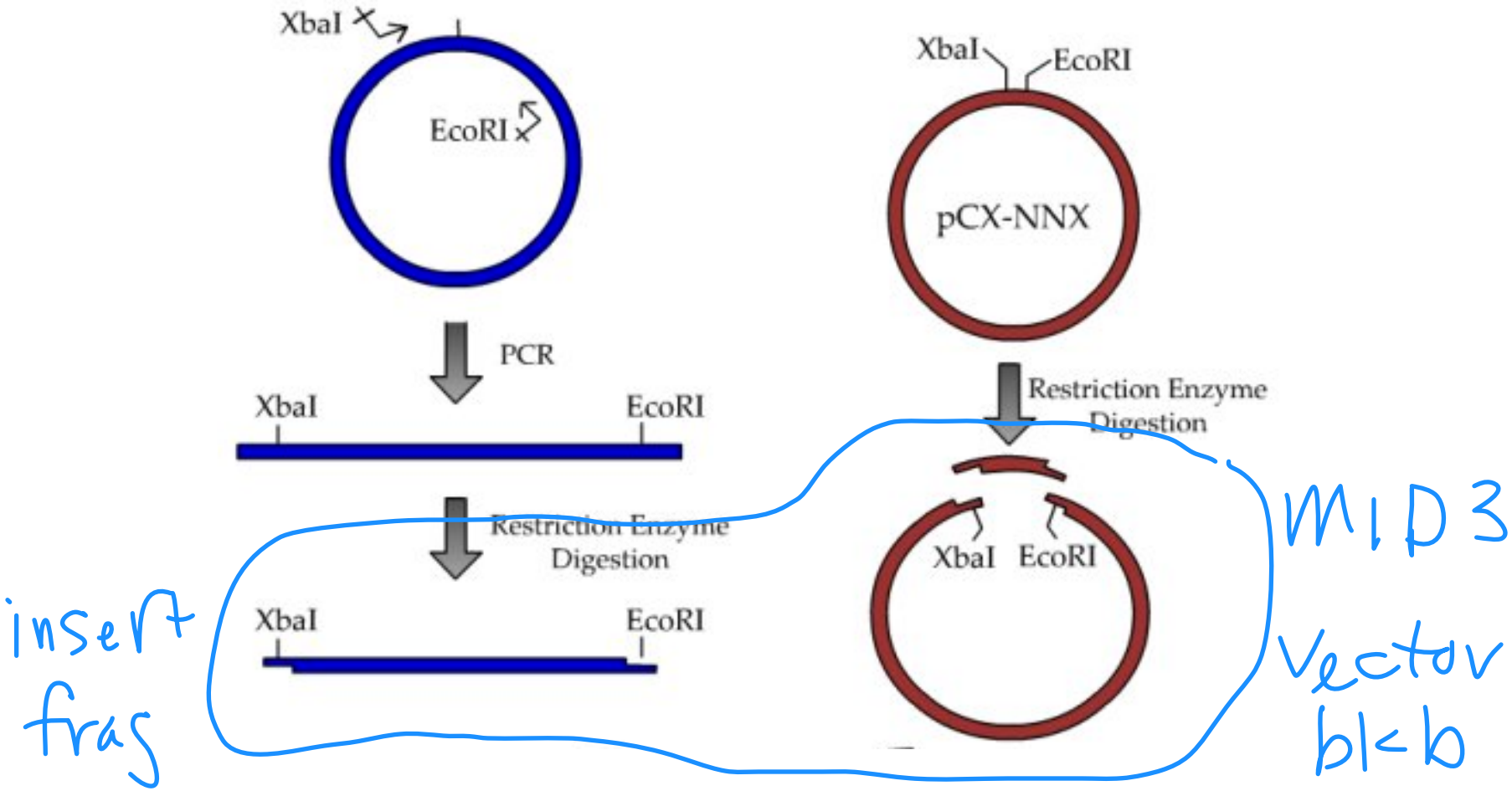
How do you know the enzymes cut your DNA?

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?

LIGASE

Prep for ligation: visualize DNA

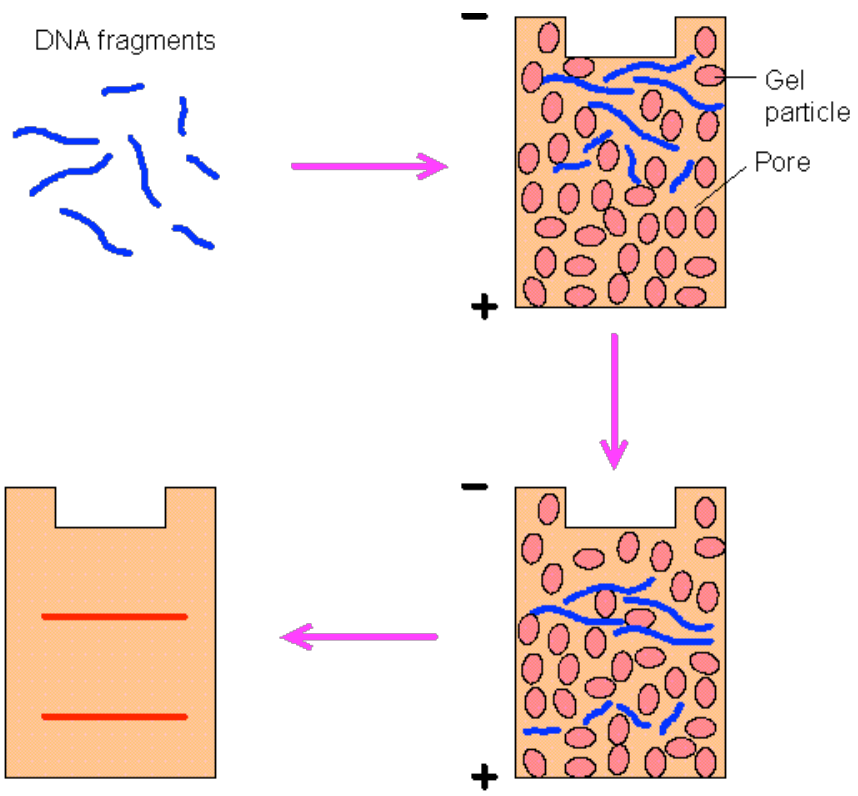
- Gel electrophoresis

• seperated by size charge

driving force? charge

DNA charge? (−)

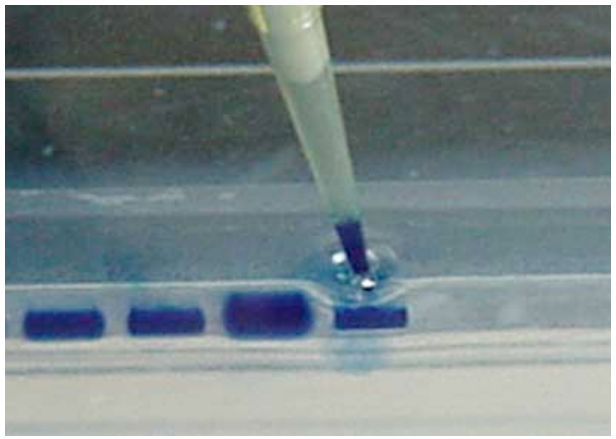
Seperate? size



How do we visualize DNA?

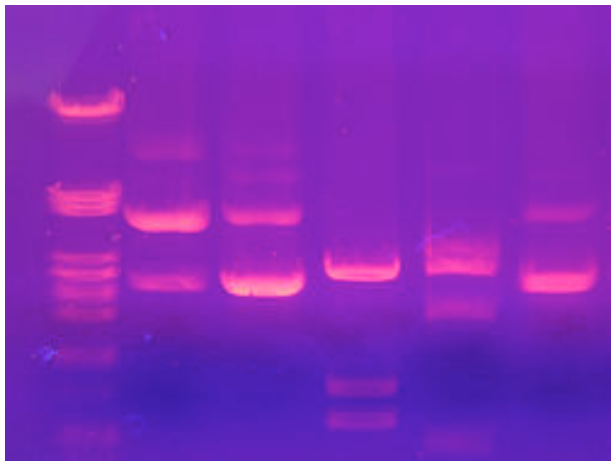
- Loading dye

bromophenol blue · ~500bps
glycerol viscosity



- DNA stain

Sybr-safe
DNA intercalator
visualize w UV



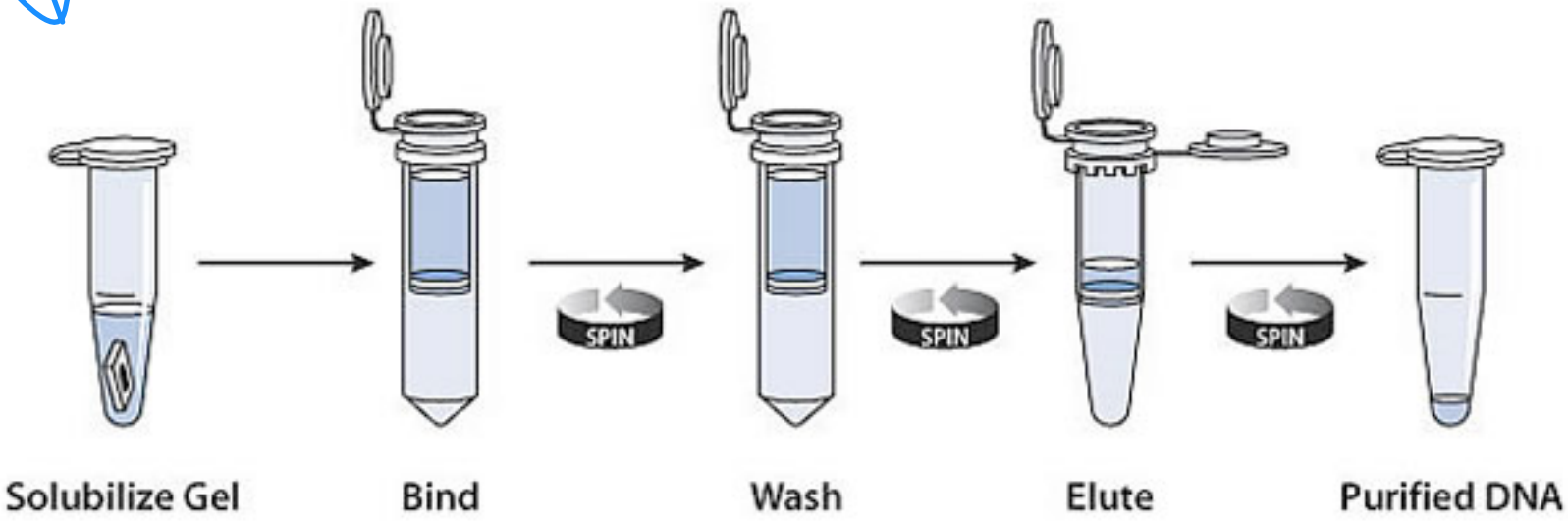
Safety note: wear nitrile gloves and face shield!!

Qiagen

Prep for ligation: purify DNA

- Gel purification

QG: ↑salt ↓pH
↓ Wash: EtoH ↑salt
Elution: ↑pH(a) ↓salt



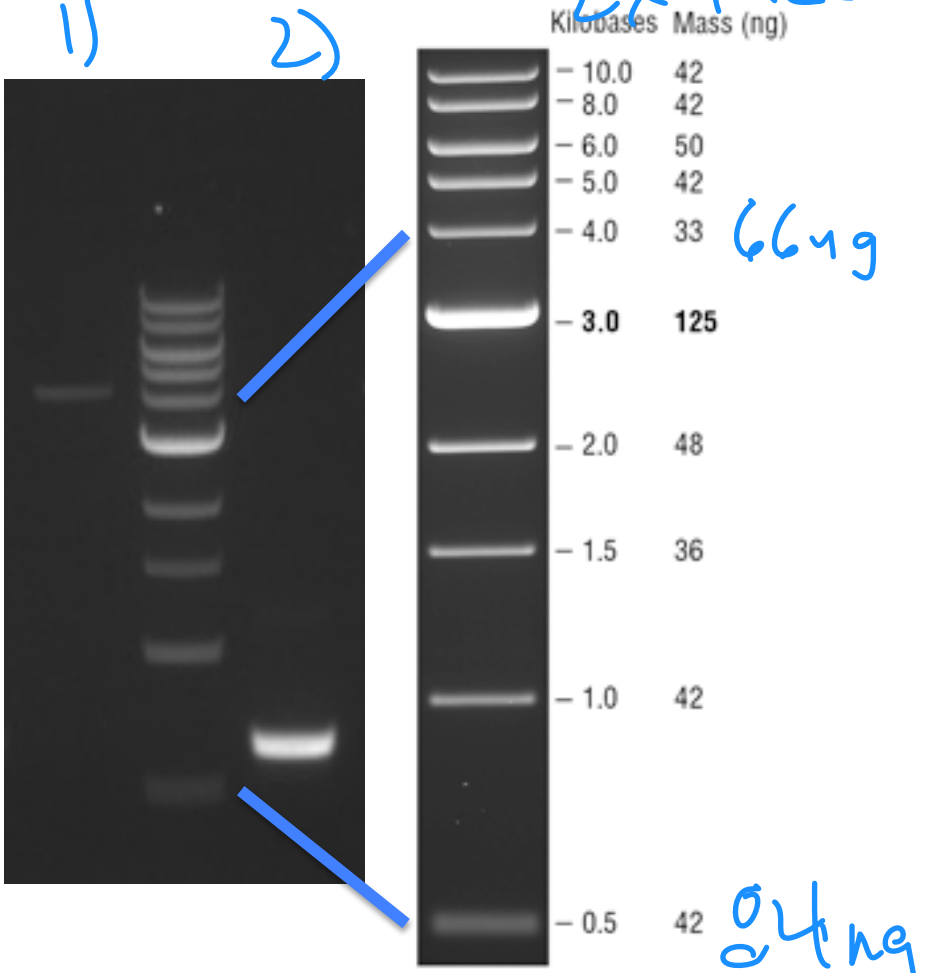
Prep for ligation: quantify DNA

1) • Amount (ng) of vector

band 1/2 intense
~ 33 ng / X volume of DNA

2) • Amount (ng) of insert

~ 33 ng / X volume of DNA



* we load 2x ladder

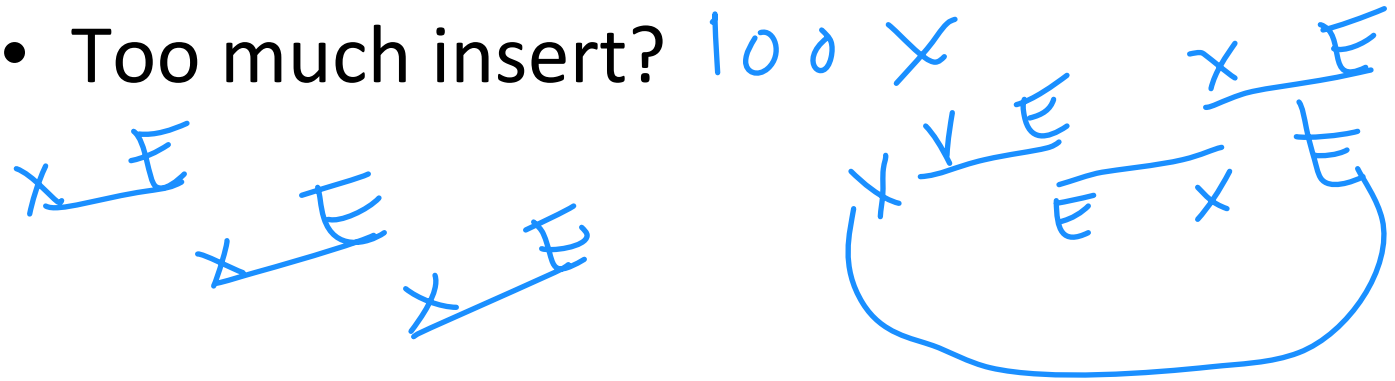
66 ng

24 ng

Mass of DNA ≠ molar quantity of DNA

Optimal vector-to-insert ratio

- Ideally want 1:4 backbone : frag
  - Molar ratio, **not** volume or mass
- Too much insert? 100 x



- Too much vector? 100x

giant vector

linear DNA = no colonies

Prep for ligation: ratio calculation

1. Examine recovery gel
   - will be posted by teaching faculty tonight
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