- Announcements, Review HW
- Lab Quiz
- Pre-lab Lecture
  - Where we are/going
  - DNA Ligation, part 2
  - Bacterial Transformation
  - Controls, Expected Outcomes
  - Safety + Technical Tips

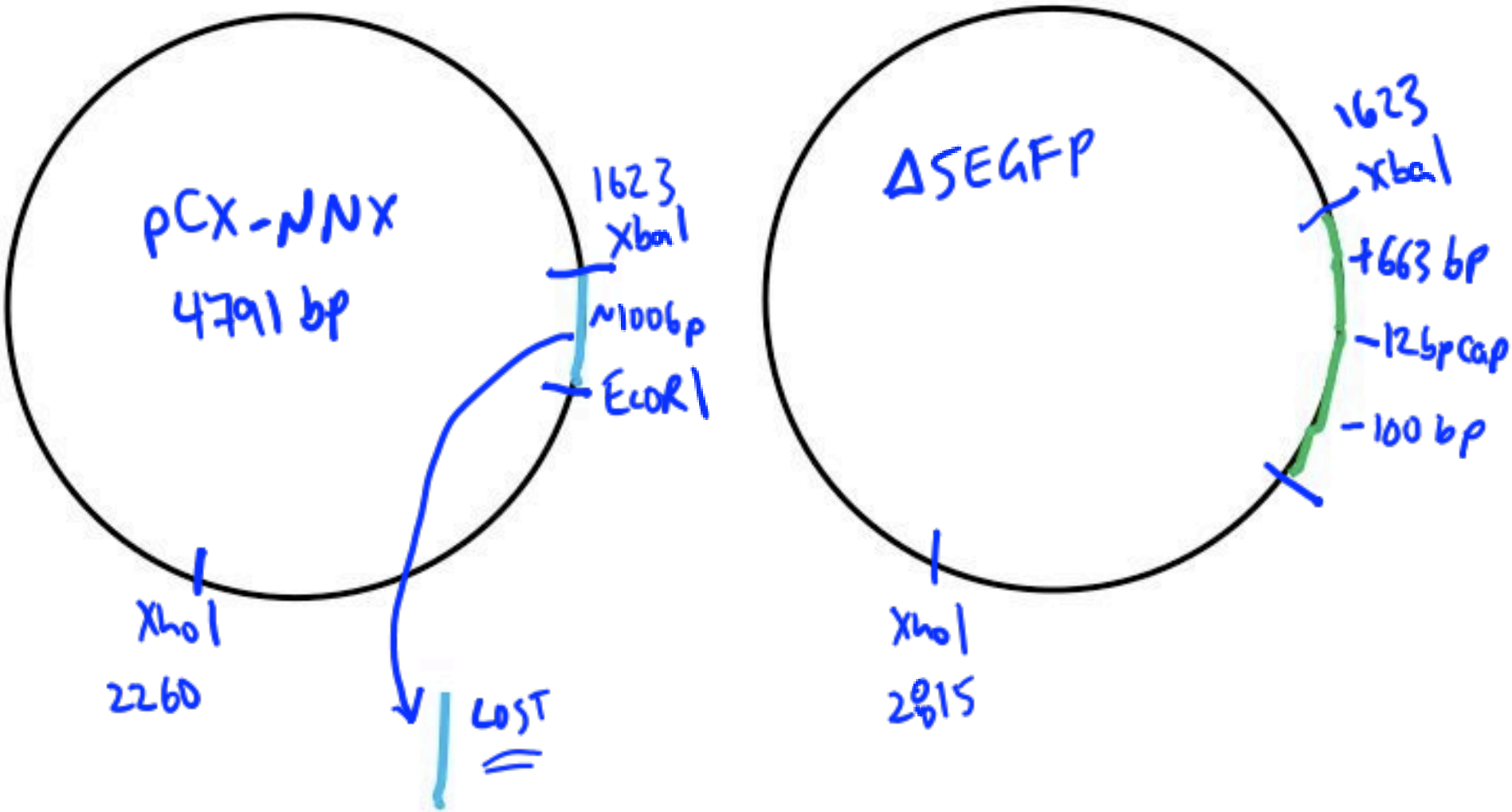
Mon. OH 1 2 4 5

56 6 55

1:30-2:30 pm

16-319 Mon.

Old HW, problem 1



Old HW, problems 2+3

#3 Agarose gels have limited resolution/range.

Note: Enzyme activity measure in arbitrary units U

1 U = amount of enzyme to digest 1ug DNA within 1 hr. at 37°C in total of 50uL

+ think of extreme cases

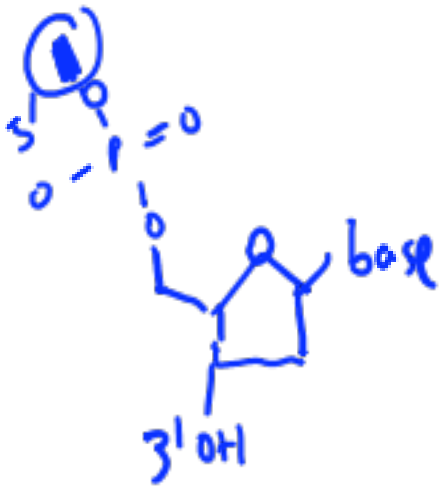
Where we are/going

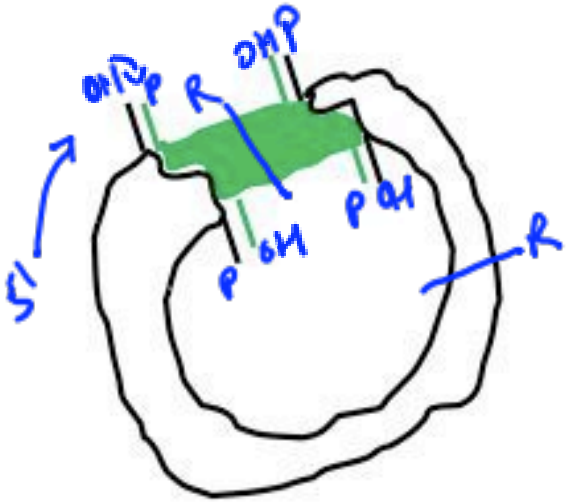
D4: make the desired clone by ligation

D4-5: amplify and select the clone in E.coli

D5+: test candidate clones

DNA Ligation





Reaction creates new phosphodiester bond

Reaction requires ATP

What factors affect yield?

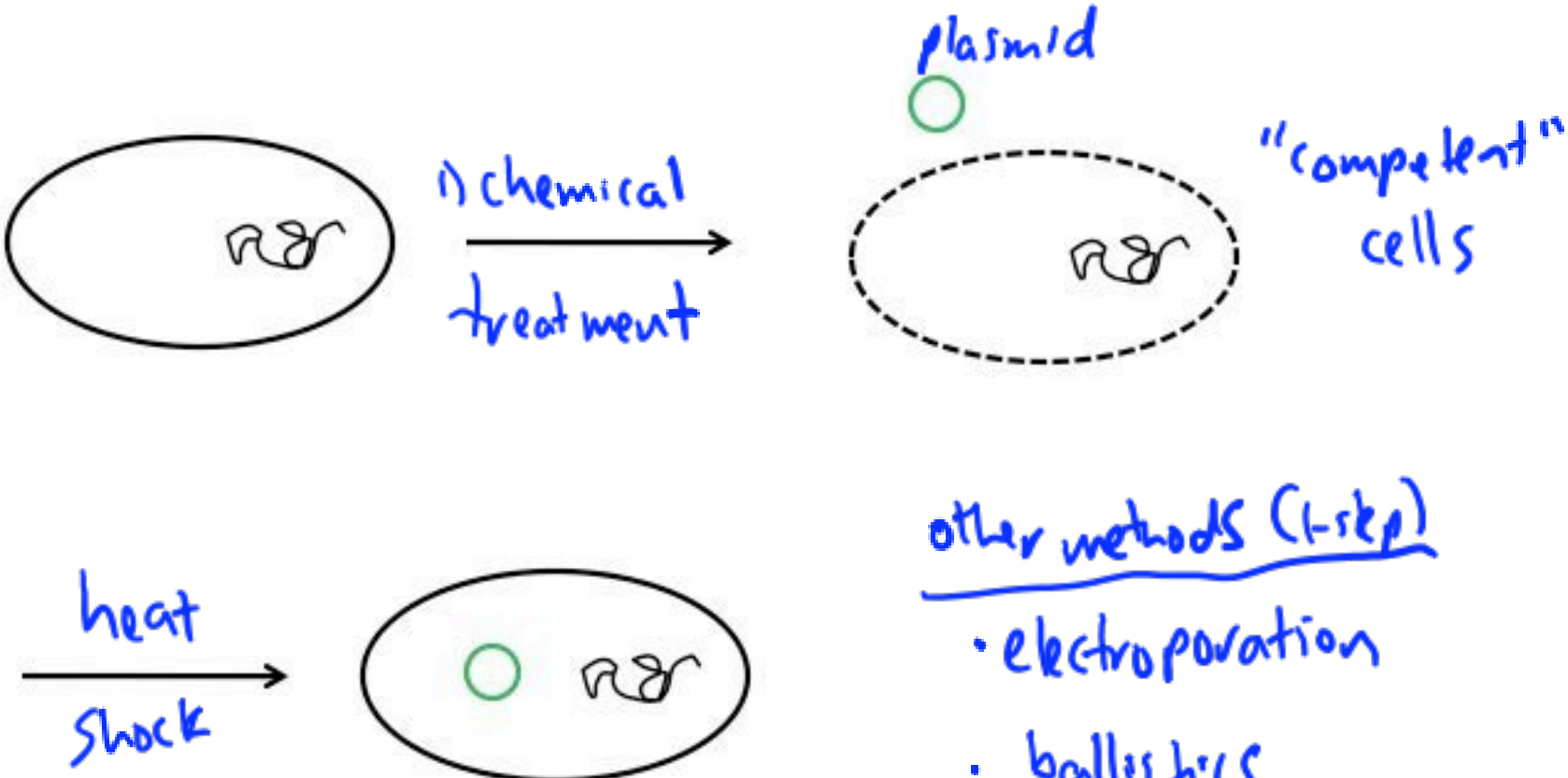
- Temperature, pH
- concentrations of DNA
- conc., quality of ligase

* ratio of bk bj: ins
→ think about extreme cases

How do we assess if it worked?

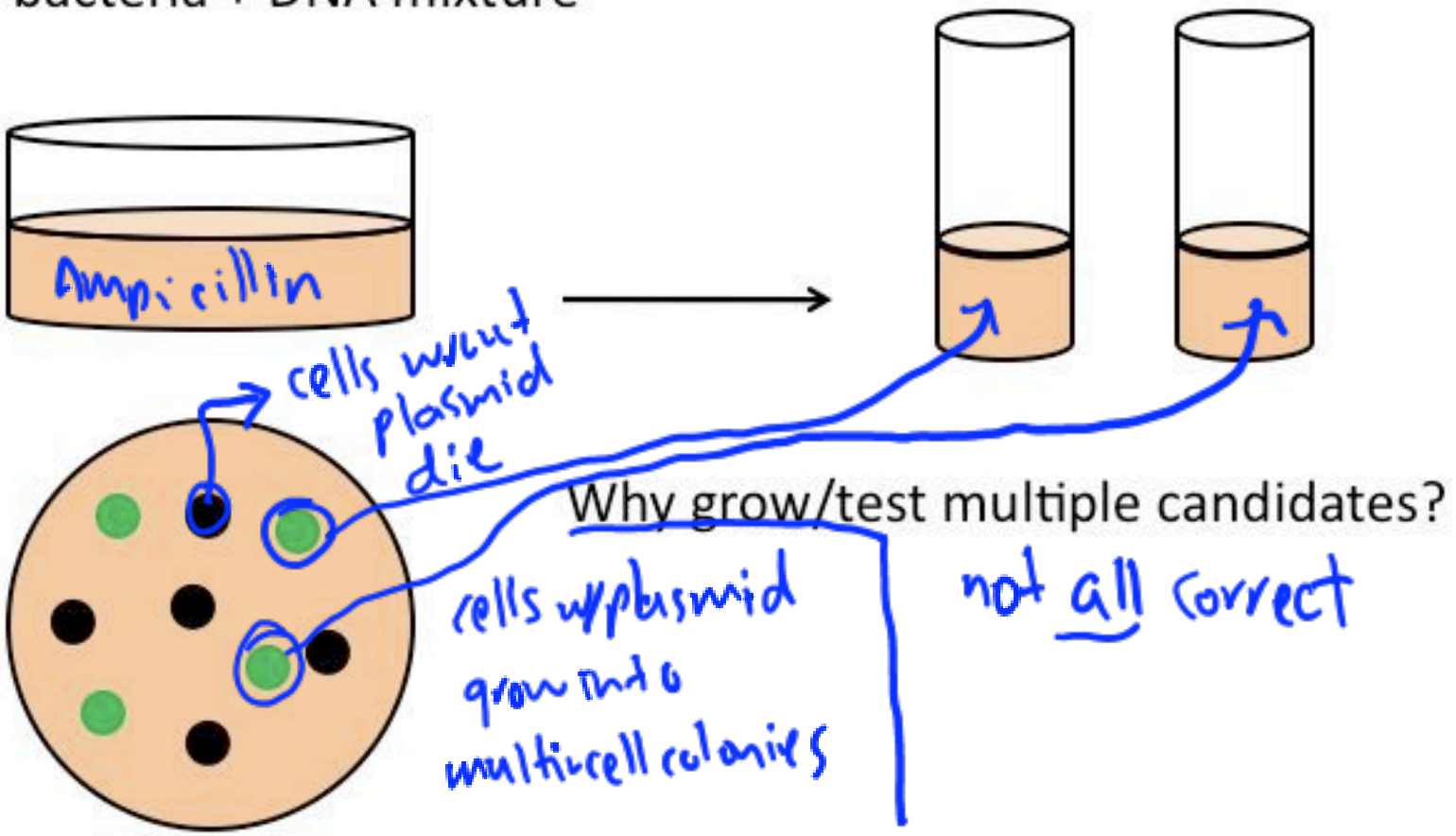
* diagnostic digest

Bacterial transformation



DNA Amplification in Bacteria

Plate bacteria + DNA mixture



Ligation Controls + Outcomes

Sample	Expectation	Role
pCX-EGFP	lots of (colonies)	(+) control
no DNA	none	(-) control
bkb(+ ins) no ligase	few-some	control for uncut plasmid
bkb + ligase	some	control for singly cut backbone
bkb + ins, + ligase	some-many	exp

digestion efficiency

Ligation Controls + Outcomes

Sample	What if?
pCX-EGFP	none
no DNA	some
bkb + ins, no ligase	many
bkb + ligase	many
bkb + ins, + ligase	none

poor cells
too little, wrong DNA
wrong plate (not Amp)

contamination by other DNA
wrong plate, etc.

poor digestion
etc.

exp. issue → insert design
or new plasmid is lethal (not common!)

In general, keep in mind:

* Consider all exp'l. samples together

* rxns. do not go to completion

Today in Lab

- Keep ligase *and* ligase buffer (ATP) cold
- DNA precipitation after ligation reaction
  - Yeast tRNA - "carrier" to visualize DNA, improve yield
  - Ethanol - precipitates (along w/salt)

  * get rid of liquid!
- Be gentle with competent cells keep cold; don't vortex
- Sterile technique for transformations – demo