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

Old HW, problem 2

Enzyme activity measure in arbitrary units U

1 U = amount of enzyme to digest __1 μg__ DNA within __1 hr.__ at __37°C__ in total of __50 μL__

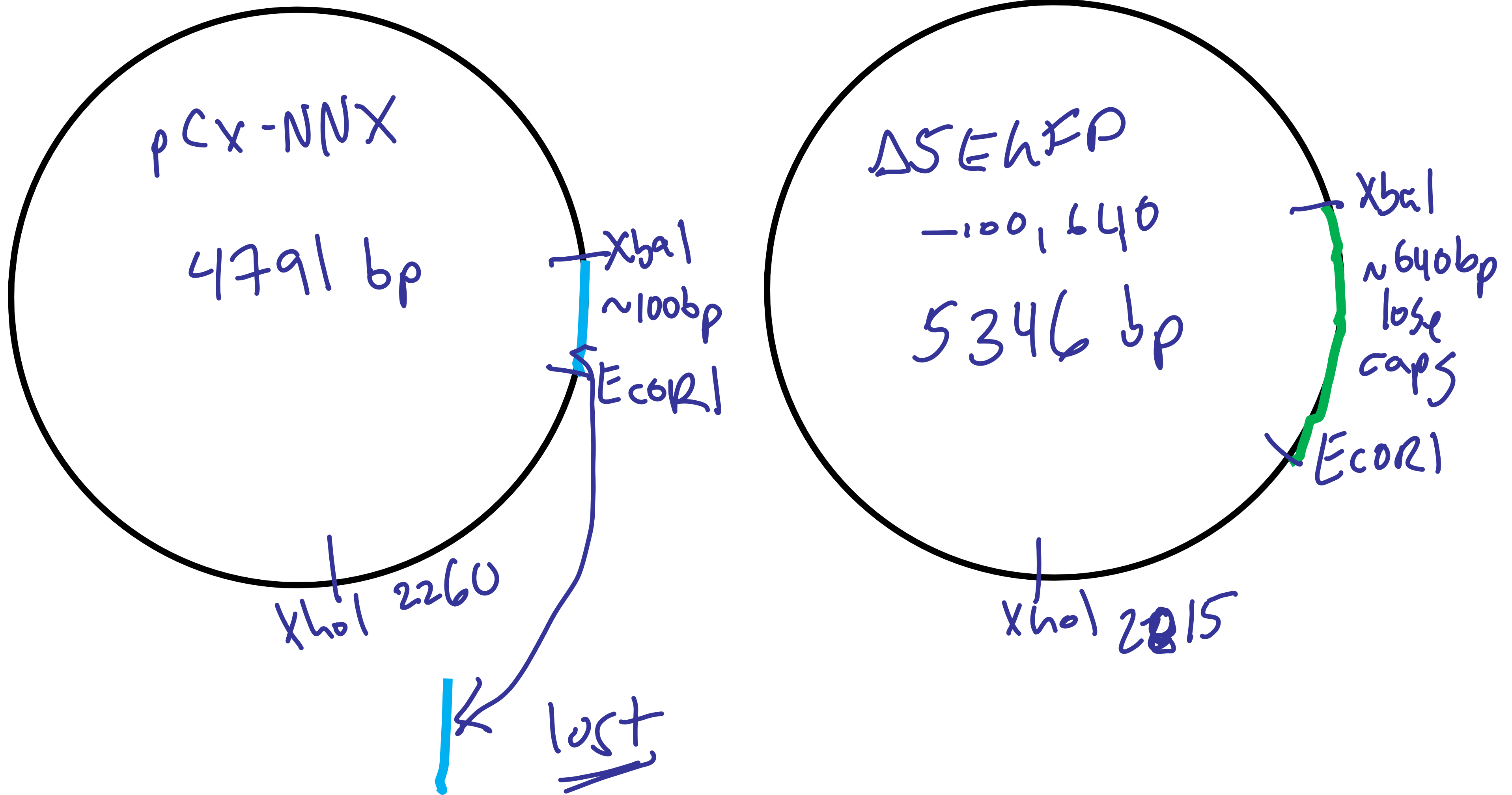
Will 75% XbaI and 100% EcoRI activity always give a 3:1 ratio of double-cut to single-cut product?

extreme cases

v. little DNA (<<1 μg) ~all double-cut

a lot DNA not even all single-cut

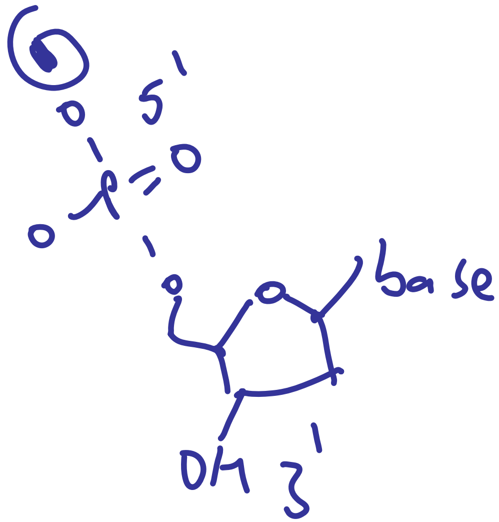
Old HW, problem 1



Where we are/going

- make the desired clone (ligation)
- amplify and select the clone in E. coli
- next time: test candidate clones

DNA Ligation

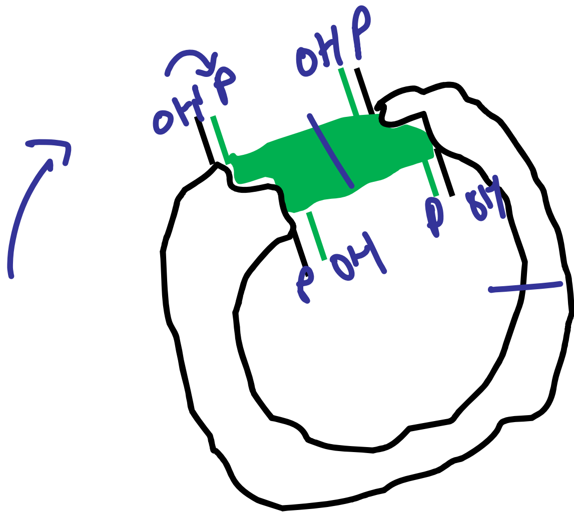


Reaction creates new phosphodiester bond

Reaction requires ATP

What factors affect yield?

- conc. of DNA
- ratio of bkb:ins
- enzyme (conc., T, age)
- time for rxn.

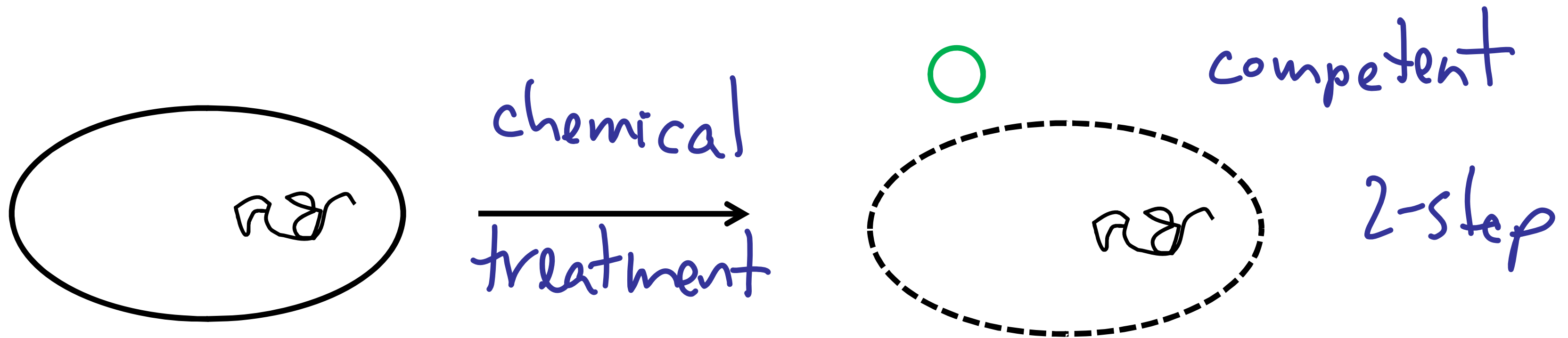


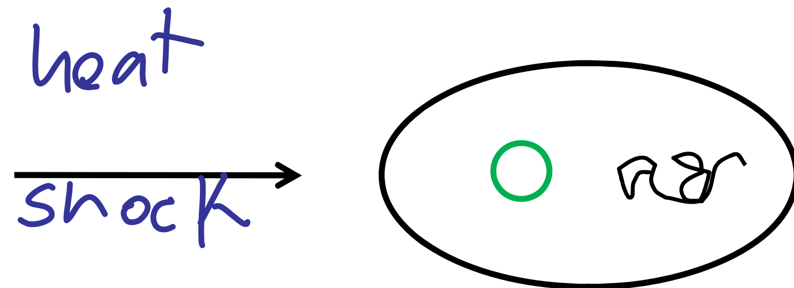
How do we assess if it worked?

* diagnostic digest

(sequencing)

Bacterial transformation



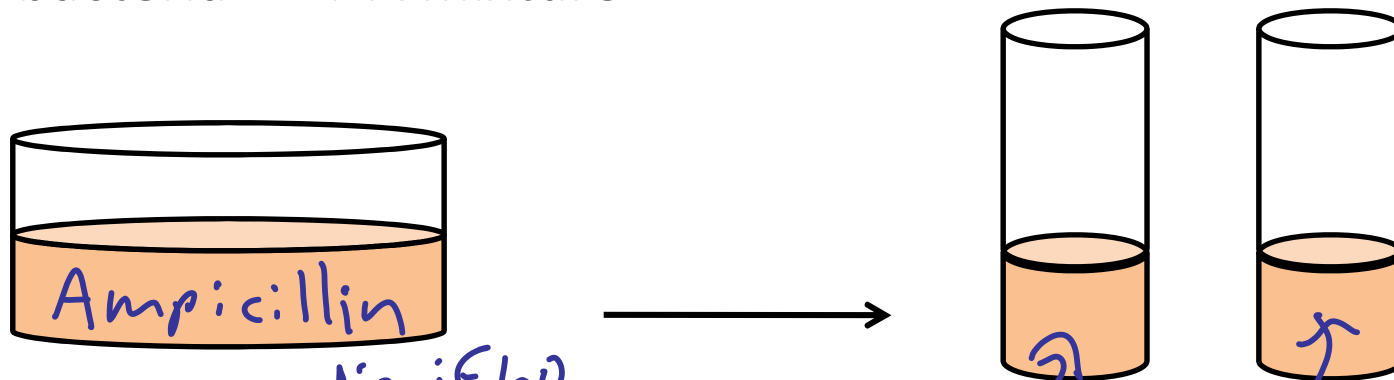


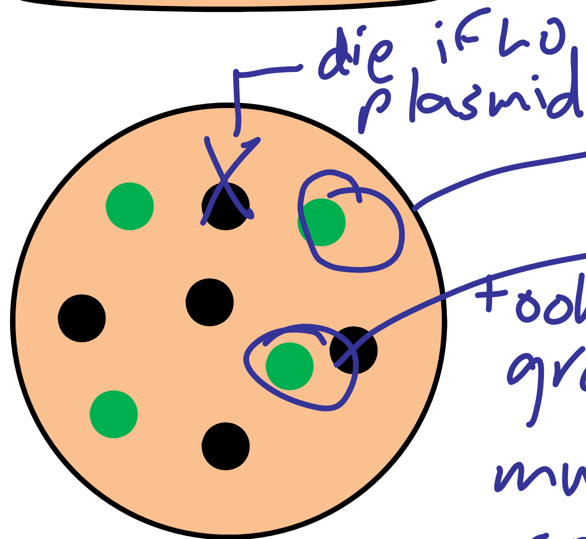
other methods (1-step)

- electroporation
- ballistics

DNA Amplification in Bacteria

Plate bacteria + DNA mixture





Why grow/test multiple candidates?

took up plasmid, grow into multi-cell colonies

not all are correct

Ligation Controls + Outcomes

Sample	Expectation	Role
pCX-EGFP	lots (of colonies)	positive control
no DNA	none	neg. control
bkb + ins, no ligase	some	control for uncut plasmid
bkb + ligase	some (exist)	control for single-cut plasmid
bkb + ins, + ligase	some–many	experiment

digestion efficiency

Ligation Controls + Outcomes

Sample	What if?
pCX-EGFP	none — wrong DNA, bad cells, bad plate
no DNA	some — wrong cell line
bkb + ins, no ligase	many — wrong DNA, bad digestion (enzymes bad)
bkb + ligase	many — wrong DNA, bad digestion (enzymes bad)
bkb + ins, + ligase	none — exp. stuff or new plasmid kills bacteria (not in our case)

In general, keep in mind:

* consider all outcomes 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
  - Ethanol → precipitates DNA
- Be gentle with competent cells keep cold, don't vortex
- Sterile technique for transformations – demo