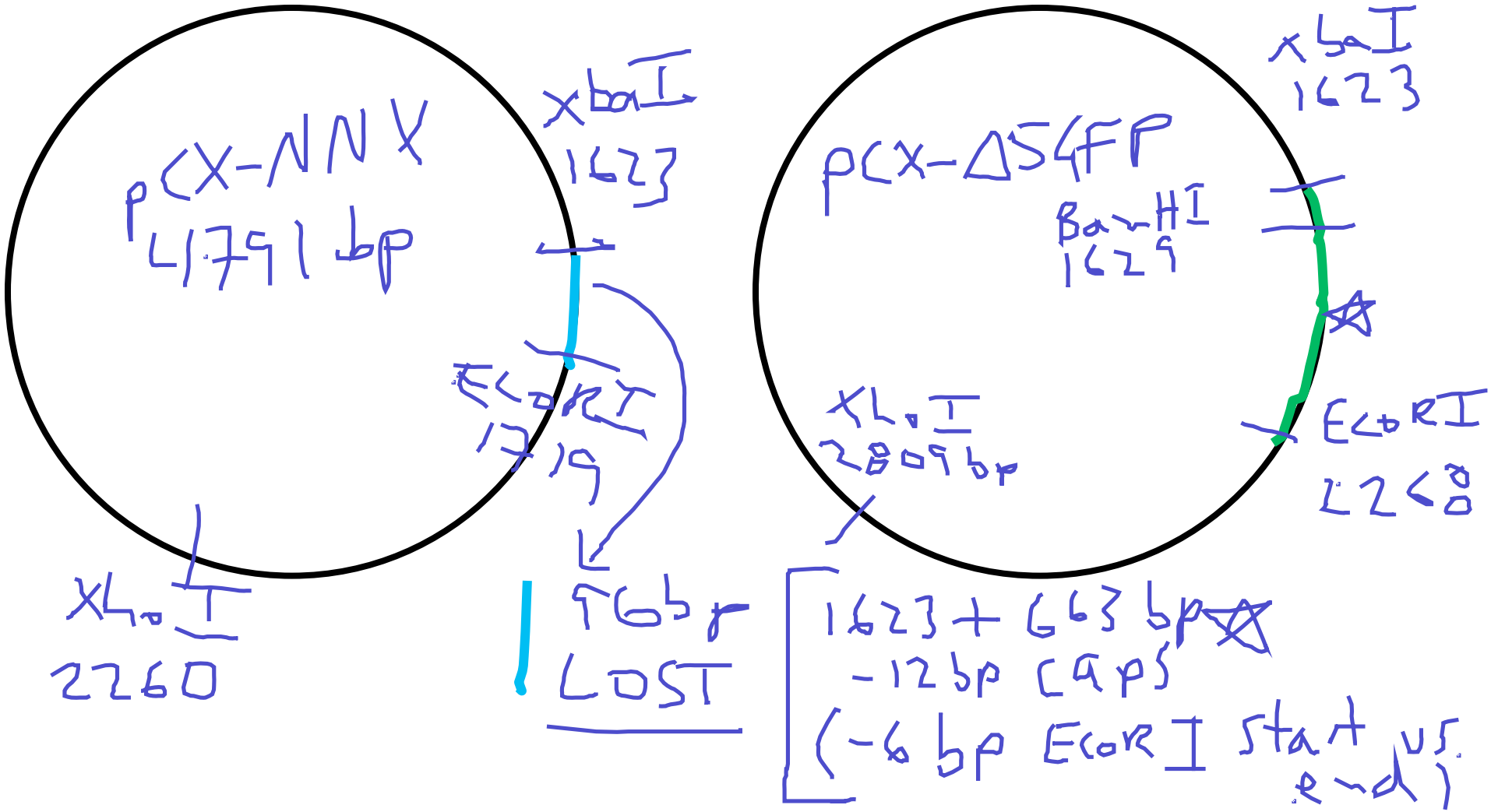
- Announcements, Review HW
- Lab Quiz
- Pre-lab Lecture
  - Where We Are/Going
  - DNA Ligation, Part 2
  - Bacterial Transformation
  - Today in Lab: M1D4

# Announcements

- Office hours: 2-3 pm on Mondays, 16-319
- Figures 1-3 of Sonoda paper for next lecture



# Where we are/going

P4: make the desired clone

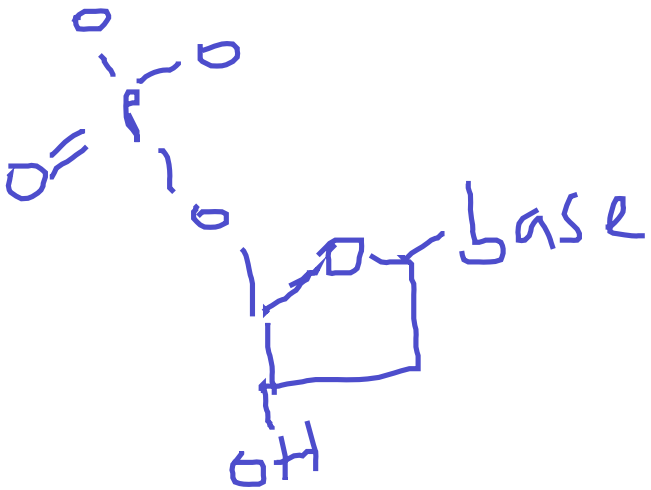
P4-5: amplify and select in E. coli

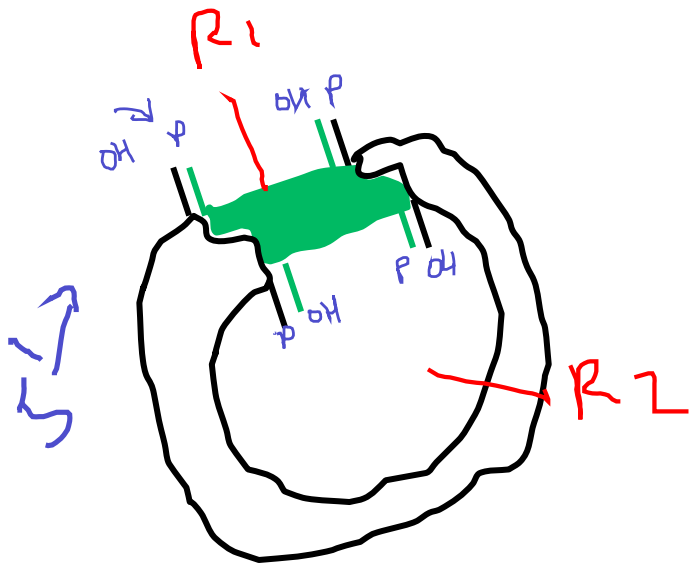
D5+: test candidate clones

→ for correctness

→ for HR

# DNA Ligation




Reaction creates new phospho-diester bond

Reaction requires ATP

What factors affect yield?

t, T, pH ★ ratio of blks : ins

[DNA] [ligase] quality

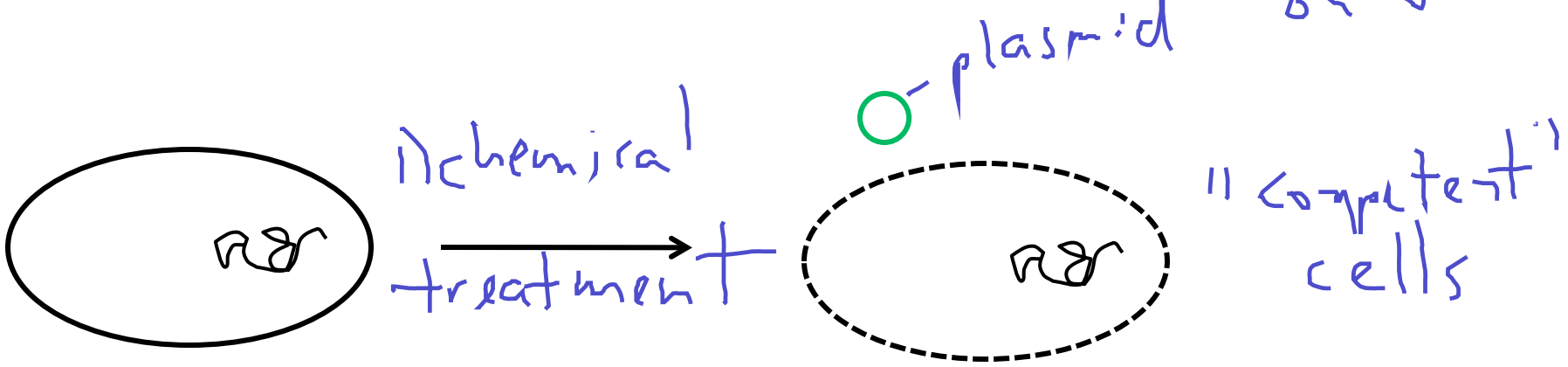
How do we assess if it worked?

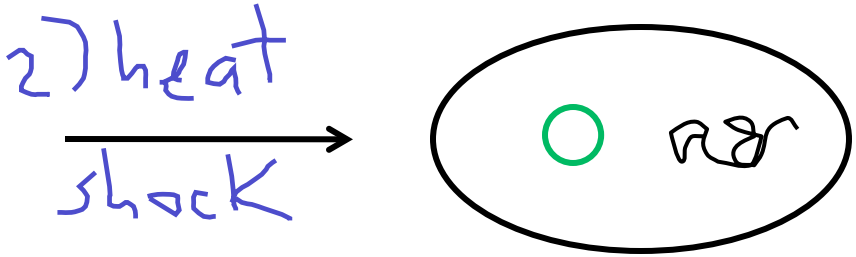
diagnostic digest

(sequencing)

# Bacterial transformation

see also animation on wiki



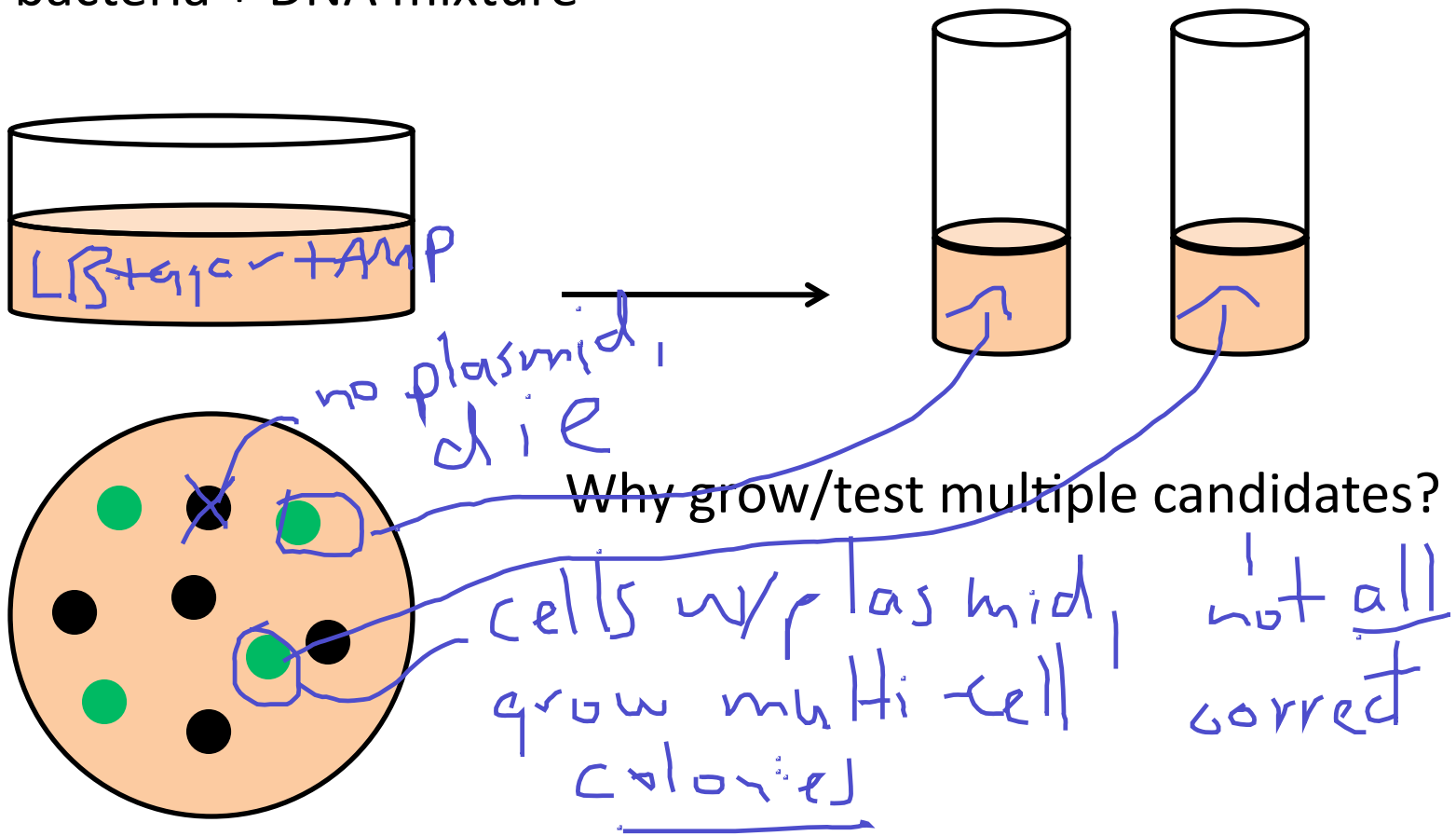

other methods

electroporation

ballistics

# DNA Amplification in Bacteria

Plate bacteria + DNA mixture




Why grow/test multiple candidates?

# Interpreting transformation data

| Sample | Role | Expectation… what if? |
|---|---|---|
| pCX-EGFP | (+) control transformation | LOTS. wrong plates. kill cells w/ low [DNA] |
| no DNA | (−) control contamination | NONE, w/ LOTS? {contam. w/ other cells or w/ DNA. wrong antibiotic on plates. |
| bkb + ins, no ligase | for uncut plasmid | FEW |
| bkb + ligase | simple cut plasmid | SOME } w/ many! poor digest efficiency |
| bkb + ins, + ligase | expt'l | some-many w/ high low [DNA] |

# Today in Lab: M1D4

Ligation calcs 3kb 125ng x (1:5/1:10)

- Keep ligase *and* ligase buffer (ATP) cold
- DNA precipitation after ligation reaction
  - Yeast tRNA "carrier" sheared DNA, improve yield
  - Ethanol precipitates → ask us if you removed enough
- EHS visit at 3 pm
- Be gentle with competent cells Keep cold don't vortex
- Sterile technique for transformations – demo