- Announcements
- Lab Quiz
- Pre-lab Lecture
  - Where we are/going
  - DNA ligation, part 2
  - Bacterial transformation
  - Today in Lab: M1D4

Announcements

- Office hours: varying
  - some combination of Mon 2-3 and Tue 4-5 pm
  - in 16-319 by default, 16-336 when announced
- Upcoming assignments, M1D6
  - lab certification (M1D1-D5) – read description!
  - oral defense of online lab modules
  - part of Sonoda paper discussed in lecture

Where we are/going

D4: make the desired clone

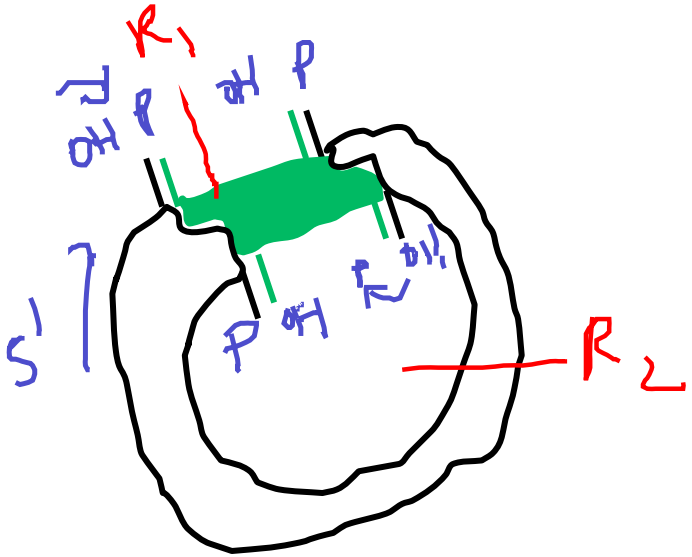
D4-5: amplify and select DNA in E. coli

D5+: test candidate clones (Δ5)

→ for correctness

→ for HR (w/Δ3)

DNA ligation



Reaction creates new phospho-diester bond

Reaction requires ATP

What factors affect yield?

T, pH, t ≠ ratio of blebs

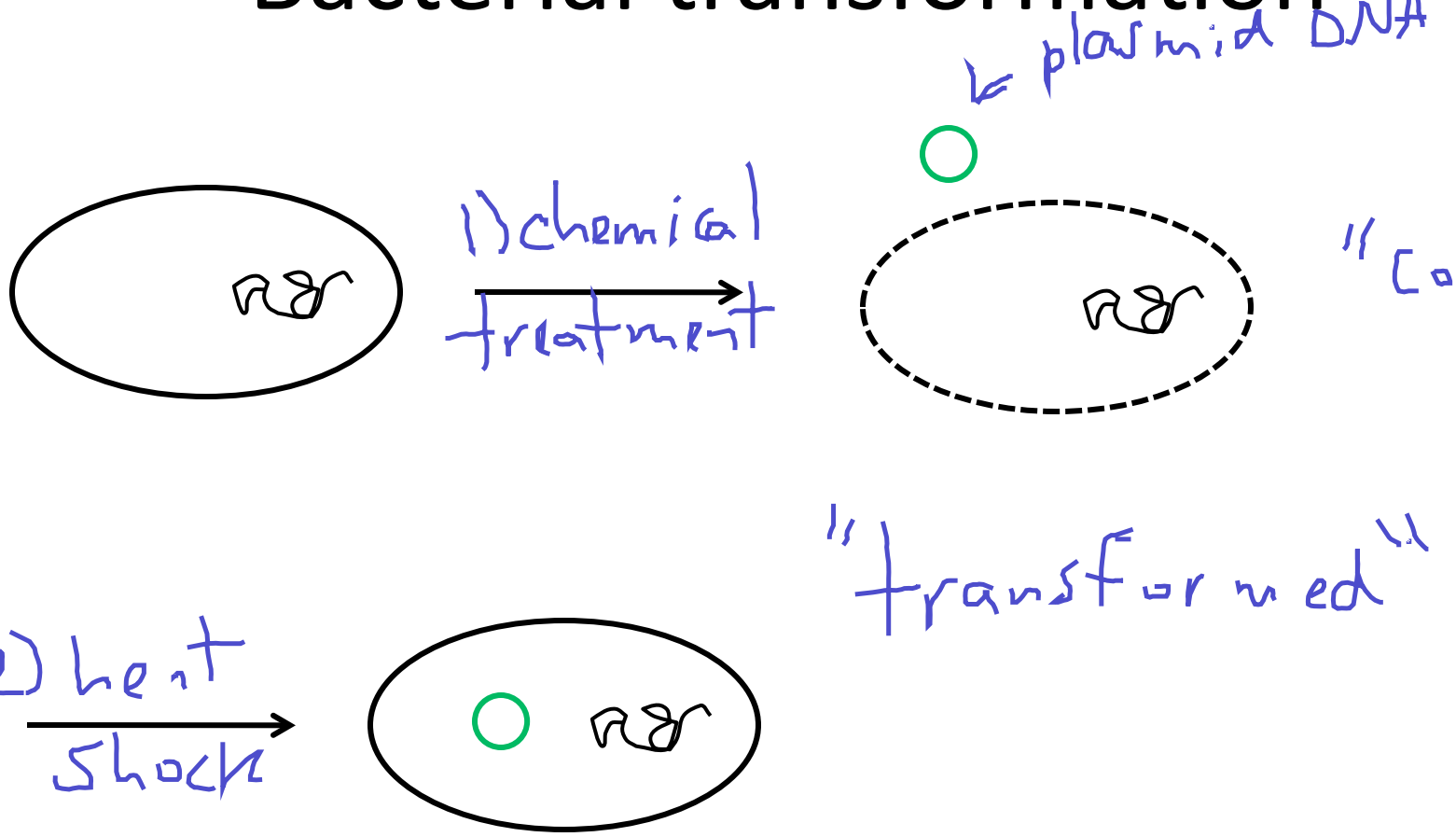
[DNA] ligase → quality

How do we assess if it worked?

diagnostic digest

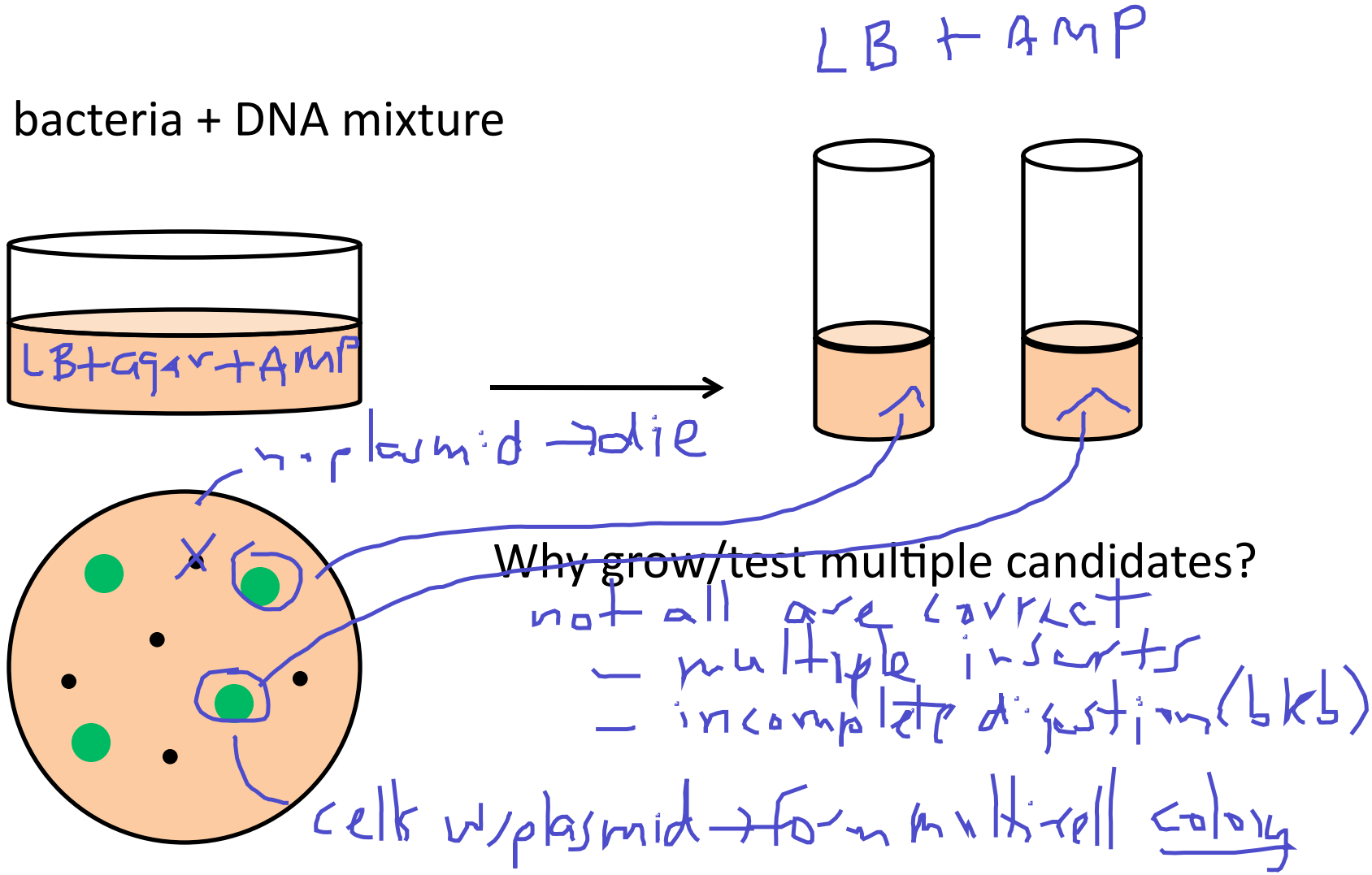
(Sequencing)

Bacterial transformation



DNA amplification in bacteria

Plate bacteria + DNA mixture



Interpreting transformation data

Sample	Role	Expectation… what if?
pCX-EGFP	(+) control for transformation	E: lots. W: none — killed cells; wrong antibiotic or plate; too low [DNA]
no DNA	(–) control for contamination	E: none — contam w/other cells or DNA; W: lots — no antibiotic
bkb + ins, no ligase	for uncut	E: none-few — W: many? poor digestion efficiency
bkb + ligase	for fully cut (+uncut)	E: few-some — W: many? poor digestion efficiency
bkb + ins, + ligase	expt'l	E: some-many W: <<A)? low [DNA]

Today in Lab: M1D4

- Keep ligase *and* ligase buffer (ATP) cold
- DNA precipitation after ligation reaction
  - Yeast tRNA "carrier" - see DNA, improves yield
  - Ethanol precipitates RNA - ask if you removed enough
- EHS visit at 3 pm
- Be gentle with competent cells } keep cold } don't vortex
- Sterile technique for transformations – demo
  - during incubation steps
  - 1-2 per group