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

Announcements

(4), cont'l: sanity check — as insert size ↓, mole amount per same mass concentration ↑

- Lab quiz next time
- Regular OH in 16-319, 4-5 pm on Tuesdays
- Methods FNT: content (concentrations!); organization/telegraphing; formal language (+ identity (e.g., primers))
- (4) Ligation sample calculation: See T/R Prp. "run" → "evaluated, electrophoresed, separated"

ladder-20uL DNA = 15 x 20/23 = 4.35uL 6kb ~36ng marker ins ~100ng

ex ≅ 36x2ng ÷ 4.35uL = 16.6 ng/uL ins ~ 46 ng/uL

50ng 6k * uL/16.6ng ≅ 3uL 6kb

$$50\text{ng}\ 6\text{kb}\ \frac{\text{mol·bp}\ 6\text{kb}}{500\text{ng}} \times \frac{4\ \text{mol ins}}{1\ \text{mol 6kb}} \times \frac{660\text{bp ins}}{4300\text{bp 6kb}} \times \frac{500\text{ng}}{\text{mol·bp ins}} \times \frac{4.35\text{uL}}{200\text{ng}} \approx \underline{0.68\text{uL}}$$

Where we are/going

D4: make the desired clone

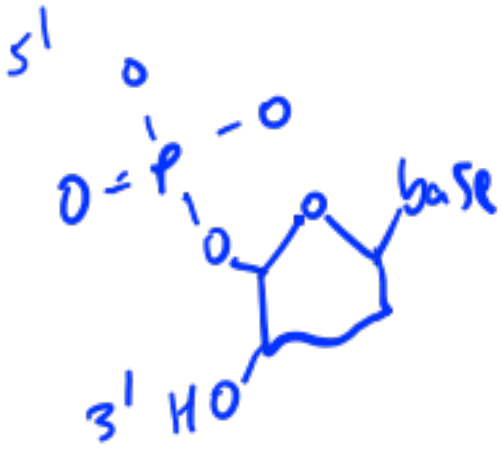
D4-5: amplify and select DNA in E. coli

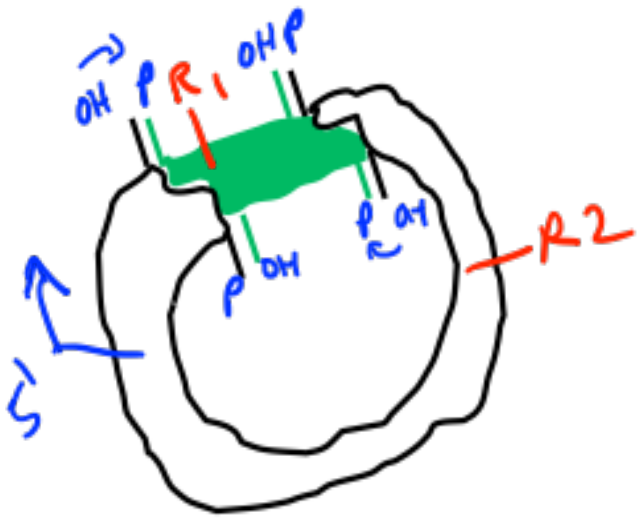
D5t: test candidate Δ5 clones

→ for correctness

→ for HR (w/ Δ3)

DNA ligation





Reaction creates new phosphodiester bond

Reaction requires ATP

What factors affect yield?

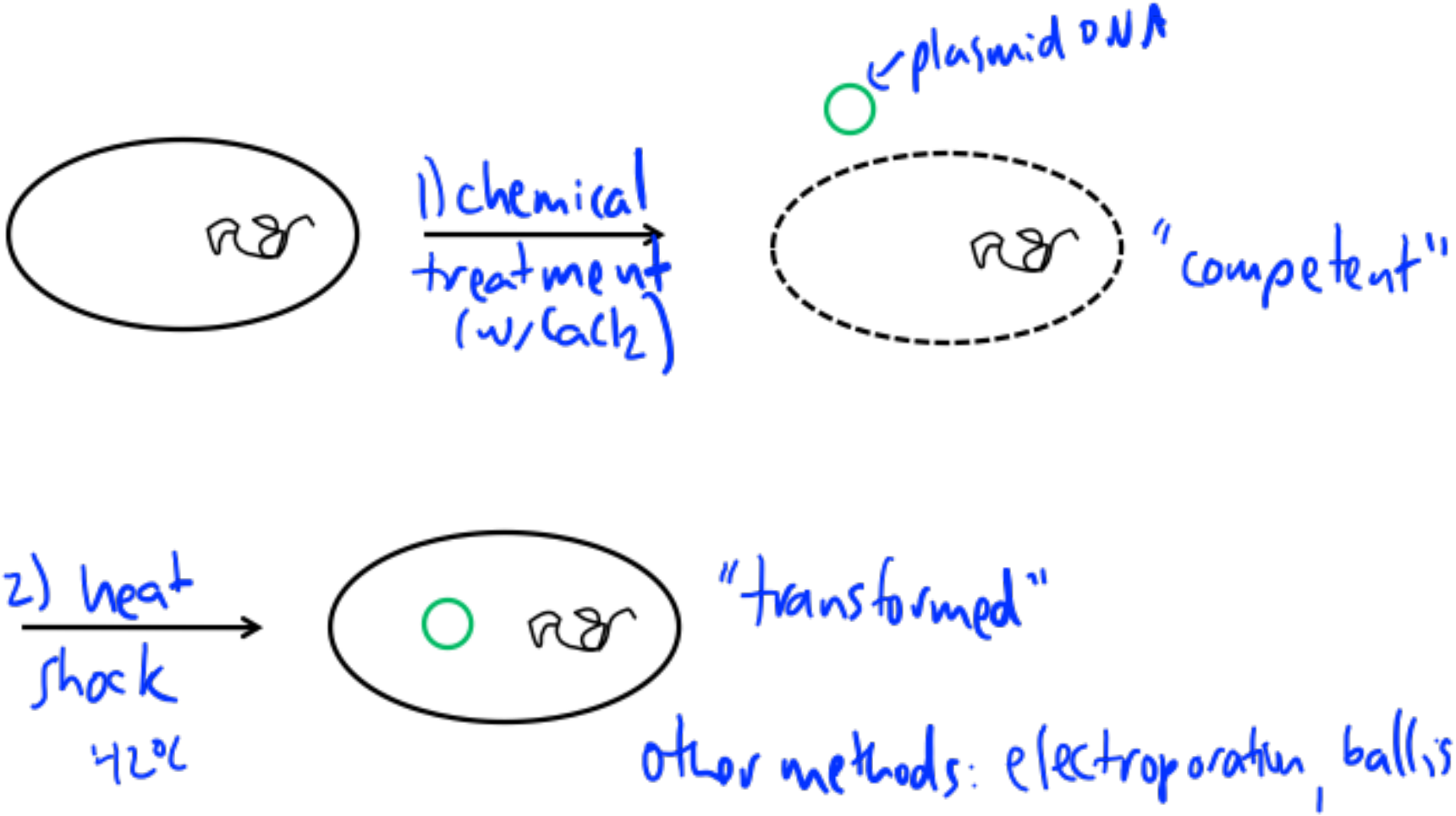
- [ATP], quality ligase quality
- pH, salt
- T, t
- bkb:ins ratio, [DNA]

How do we assess if it worked?

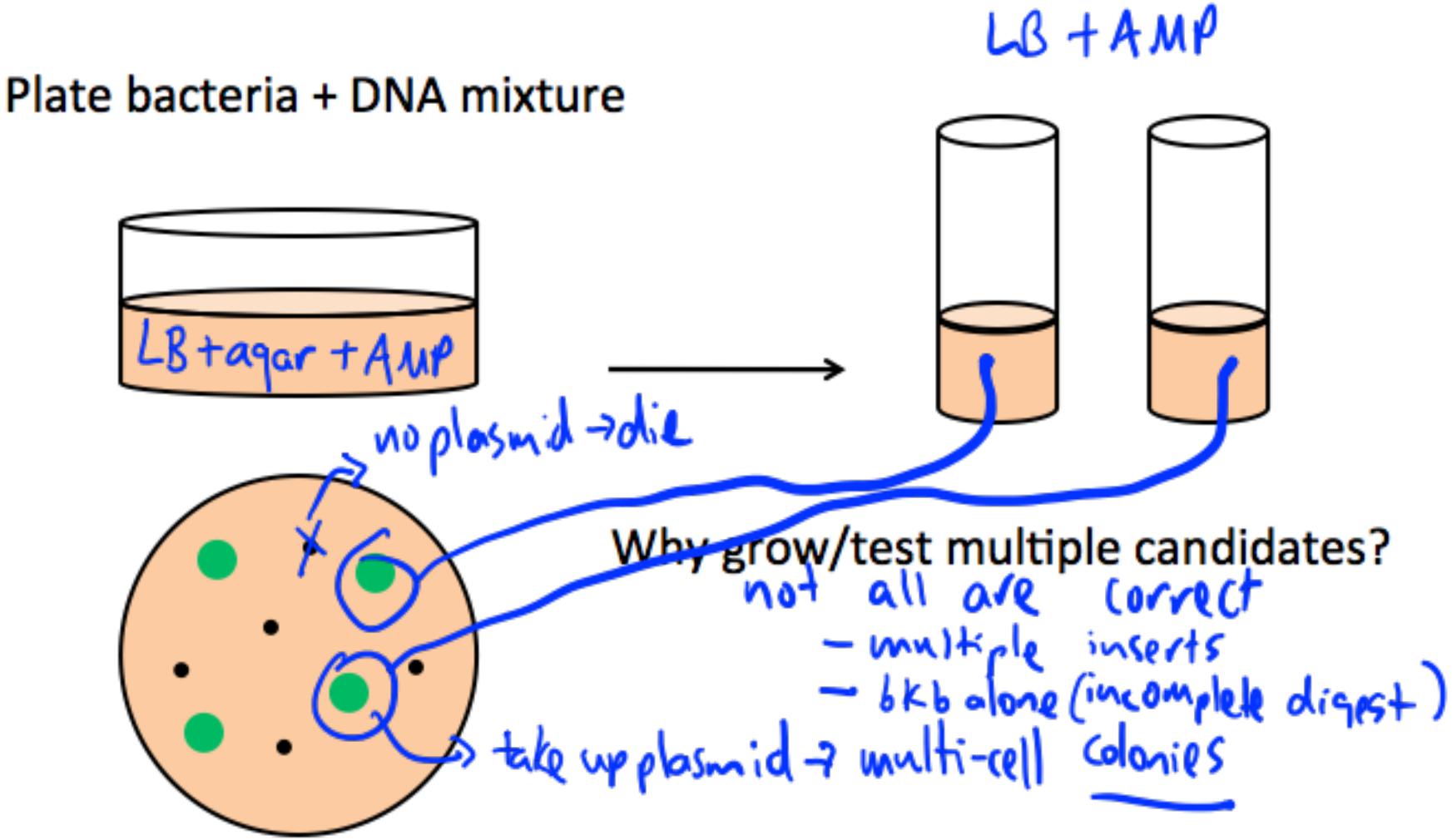
diagnostic digest ★plan for FNT

(sequencing)

Bacterial transformation



DNA amplification in bacteria



Interpreting transformation data

* view data holistically
* transformation efficiency (+), take cells w/ amounts

Sample	Role	Expectation... what if?
no DNA (skip today)	(−) control for contamination	E: none W1: LOTS { contamination w/ resistant bacteria or DNA; wrong plates (incl. dead/old) }
pCX-EGFP	(+) control for transformation	E: LOTS W1: none { killed cells; wrong ~~antibiotic~~ antibiotic; low [DNA] }
bkb + ins, no ligase	uncut plasmid	E: none, few W1 many? poor digestion efficiency
bkb + ligase	singly cut plasmid (+ uncut)	E: few, some
bkb + ins, + ligase	expt'l	E: some, many W1 << (+)? rxn issues; too low [DNA]

Today in Lab: M1D4

- Keep ligase *and* ligase buffer (ATP) cold
- DNA precipitation after ligation reaction
  - Yeast tRNA – "carrier" – see DNA, improve yield
  - Ethanol – precipitates XNA ★ ask us if you removed enough EtOH ★
- WAC visit at 3:20 pm – Leslie on abstracts
- Be gentle with competent cells
- Sterile technique for transformations: demo
  - ↳ during incubations, 2–4 groups @ a time

borrow FNT, revise but make clear (hybrid grading)

{ keep cold, don't vortex