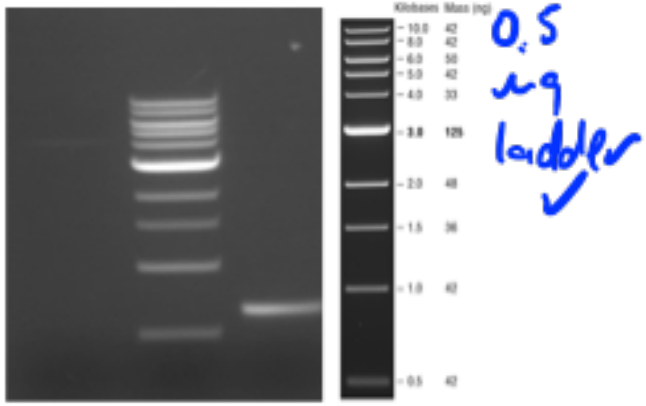
- Announcements
- Lab Quiz
- Pre-lab Lecture
  - Where we are/going
  - DNA ligation, part 2
  - Bacterial transformation
  - Today in Lab: M1D4

Announcements

RE - we always buy smallest

- My OH (T 4-5) now in 16-336 except 109 orals days
- Methods FNW common issues (11/4, 12, 9)
  - informative introductory sentences NOT intermediate stock [conc] or vols.
  - efficient amounts: [x] g/L & [y] M OR *x* g & *y* mol in *z* L ← final rxn. ✓
- Ligation sample calculation:



0.5 ug ladder ✓

- [DNA] $5\,\mu L \times \frac{20}{23} = 4.35\,\mu L$
- [I]: $50\,ng \div 4.35\,\mu L = 11.5\,ng/\mu L$
- goal $50\,ng \times \frac{\mu L}{2.3\,ng} = \boxed{21.7\,\mu L\ B}$

ins ~50ng
4kb ~ 10^7 ng
B: 2.3 "

- $50\,ng\ B \times \frac{mol \cdot bp\ B}{500\,g} \times \frac{4\,mol\ I}{1\,mol\ B} \times \frac{660\,bp\ I}{4300\,bp\ B} \times \frac{500\,g}{mol \cdot bp\ I} \times \frac{\mu L}{11.5\,ng} = \boxed{2.7\,\mu L\ I}$
- $21.7 + 2.7 > 13.5\,\mu L \Rightarrow$ so scale down

↳ sanity check on direction

Where we are/going

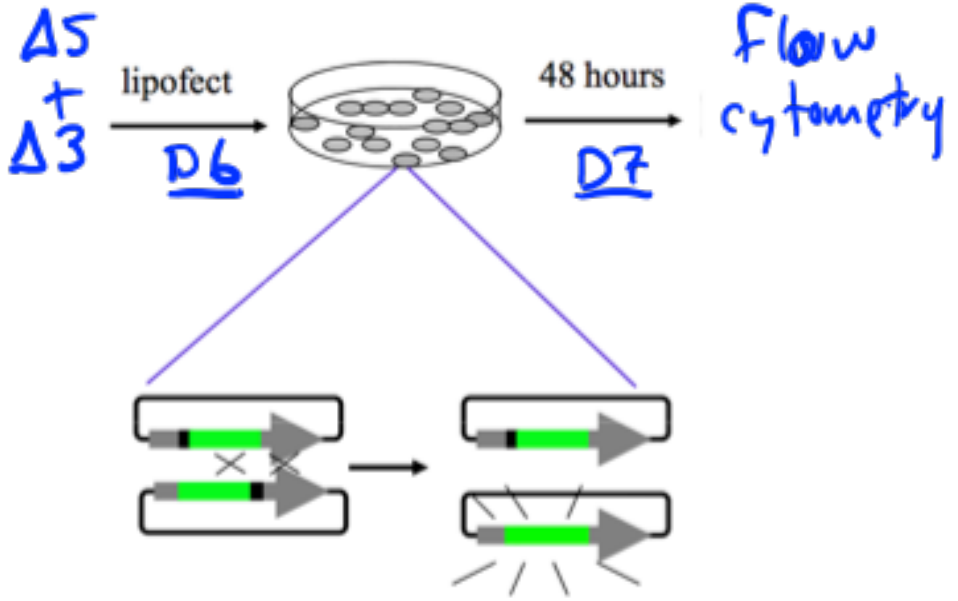
D4: make the desired clone

D4-5: amplify + select DNA in E.coli + isolate

D5+: test Δ5 candidate clones

→ for correctness

→ for extent of HR



DNA ligation basics

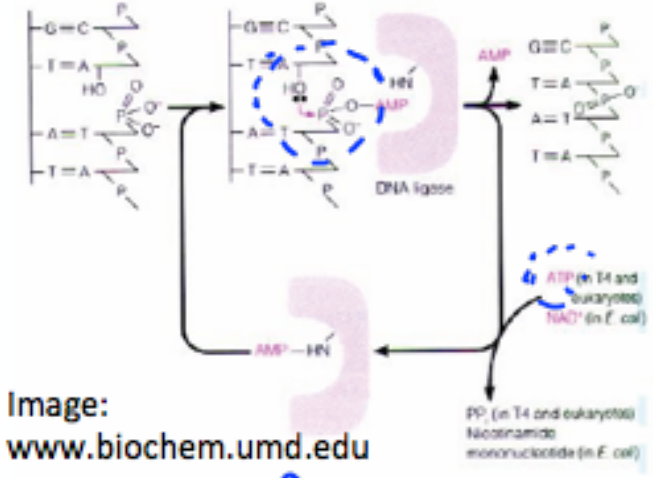
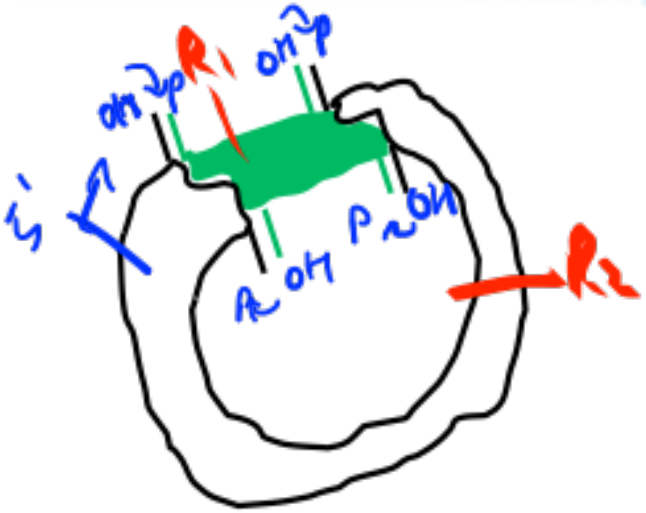


Image:
www.biochem.umd.edu



Reaction creates
new phosphodiester bond

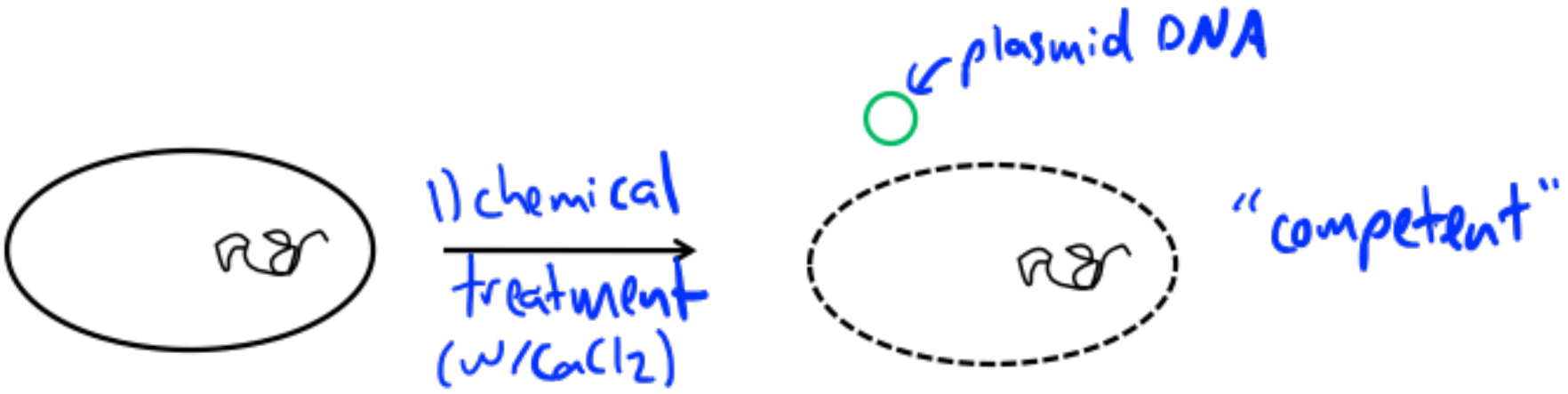
Reaction requires
ATP, Mg^{++}, DTT(SH)

What factors affect yield?

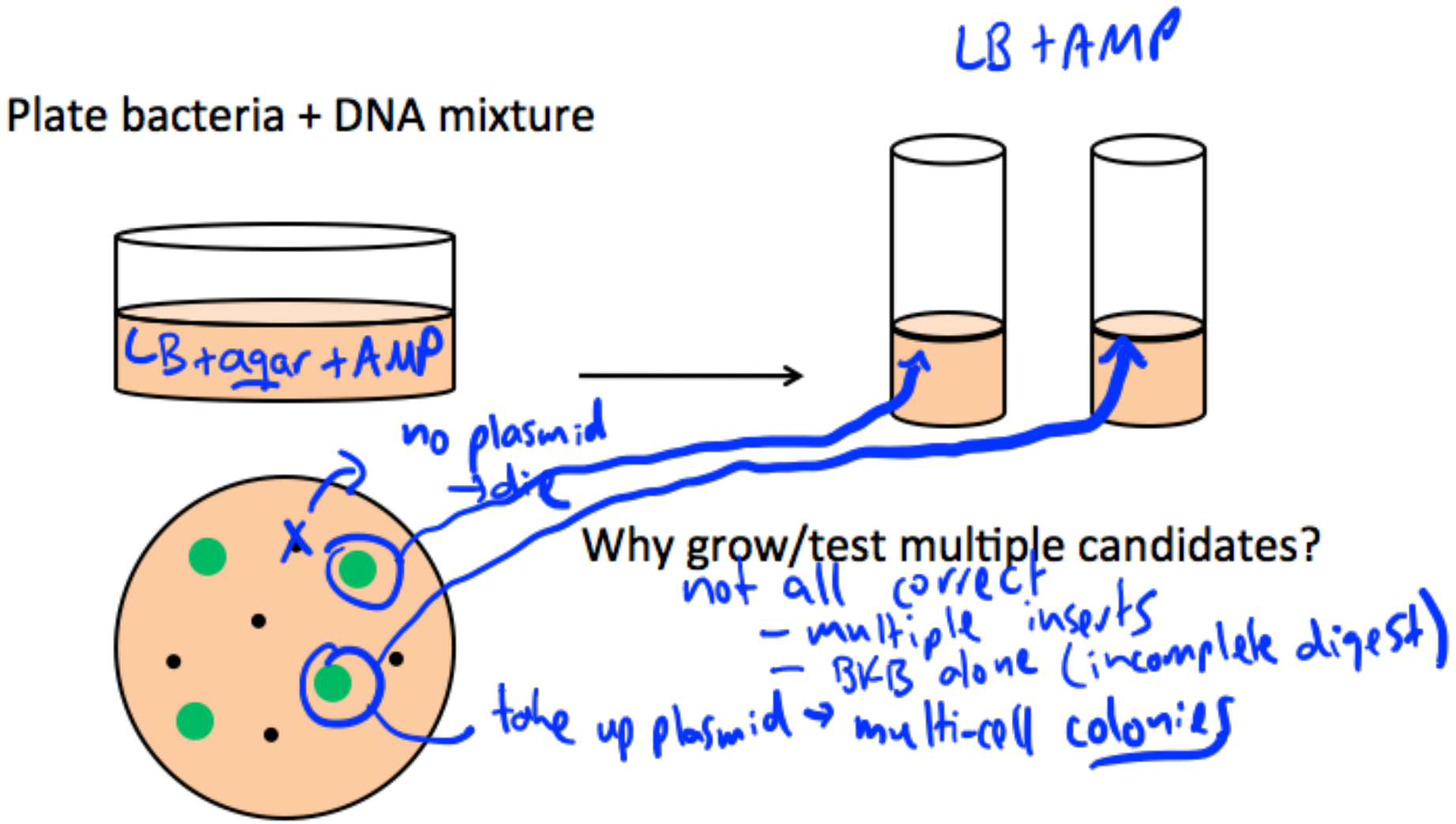
- DNA : ligase ratio
- [B], [I] & ratio
- time, Temp
  → optimal 16°C, RT is ok.
- pH
- salts
- [ATP] + [ligase]
  + quality (denatured?)

How do we assess if it worked?
sequencing or diagnostic digest
⇒ plan today!

Bacterial transformation



DNA amplification in bacteria



Why grow/test multiple candidates?

not all correct
- multiple inserts
- BKB alone (incomplete digest)

Interpreting transformation data

★ view data holistically ★ transformation efficiency = DNA used / CFU ⇑ calculating

Sample	Role	Expectation... what if?
no DNA (skip today)	(–) control for contamination	E: ∅ WI: LOTS { • contamination w/resistant bacteria or DNA • wrong plates (incl. expired or ∅ antibiotic or wrong antibiotic on plate)
pCX-EGFP	(+) control for transformation	E: LOTS { • wrong antibiotic • low [DNA] • killed cells WI: ∅
bkb + ins, no ligase	uncut plasmid (or contam.)	E: none or few WI LOTS? poor digestion
bkb + ligase	singly cut plasmid (+uncut)	E: few or some efficiency ↓
bkb + ins, + ligase	expt'l	E: some-many too low [DNA] e.g., rxn. conditions WI << < (+)?

Today in Lab: M1D4

- Keep ligase *and* ligase buffer (ATP) cold
- DNA precipitation after ligation reaction
  - Yeast tRNA - "carrier" - see pellet, improve yield → (vs. tube walls)
  - Ethanol precipitate XNA (low dielectric constant + high salt give charge screening + solubility)
    ★ Check w/ us about EtOH amount ★
- WAC visit at 3:15 pm – Jessie on abstracts
- Be gentle with competent cells } - keep cold - don't vortex!
- Sterile technique for transformations: will demo during your incubation step
- Reduce volume of cells added ★ plate 50uL (NOT 200) of cells ★