M1D4:
Ligation and transformation

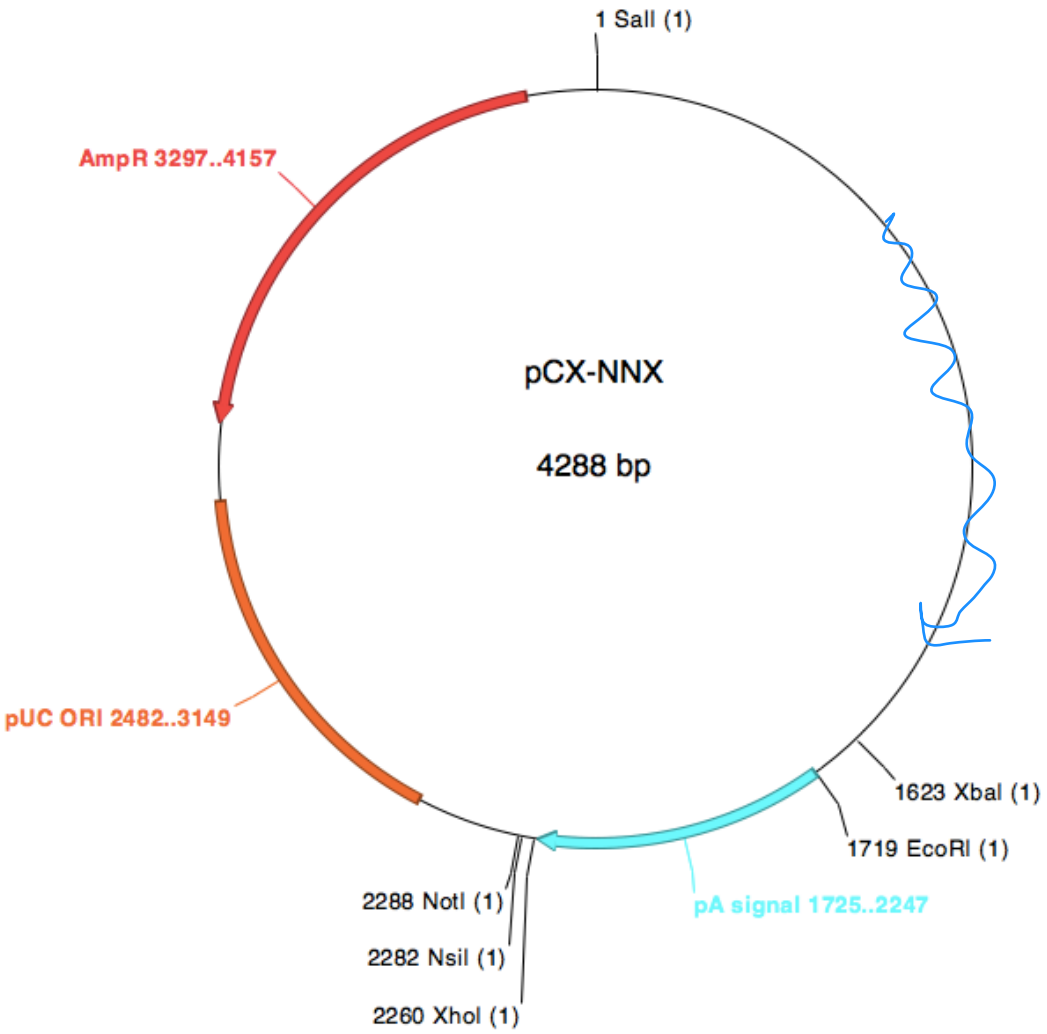
9/24/15

Lab business

- Lab treat…

- Visit from Vivian to discuss Abstracts
- Follow-up on homework due M1D3
- Homework due M1D5
- M1D5 will be long!

Follow-up on homework due M1D3



What to include in the caption?

features

ampR

origin

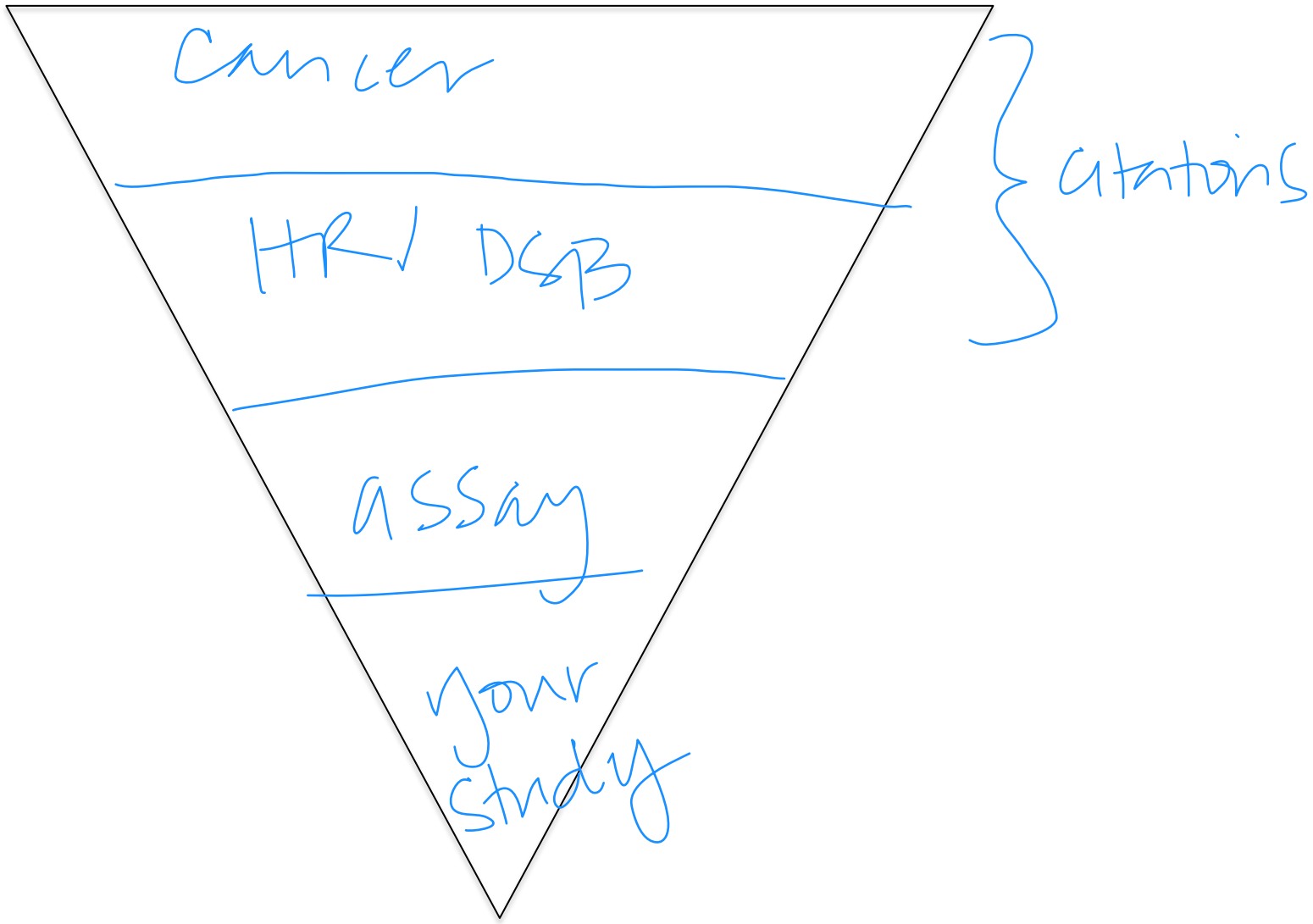
$\Delta 5'$ EGFP

restriction sites

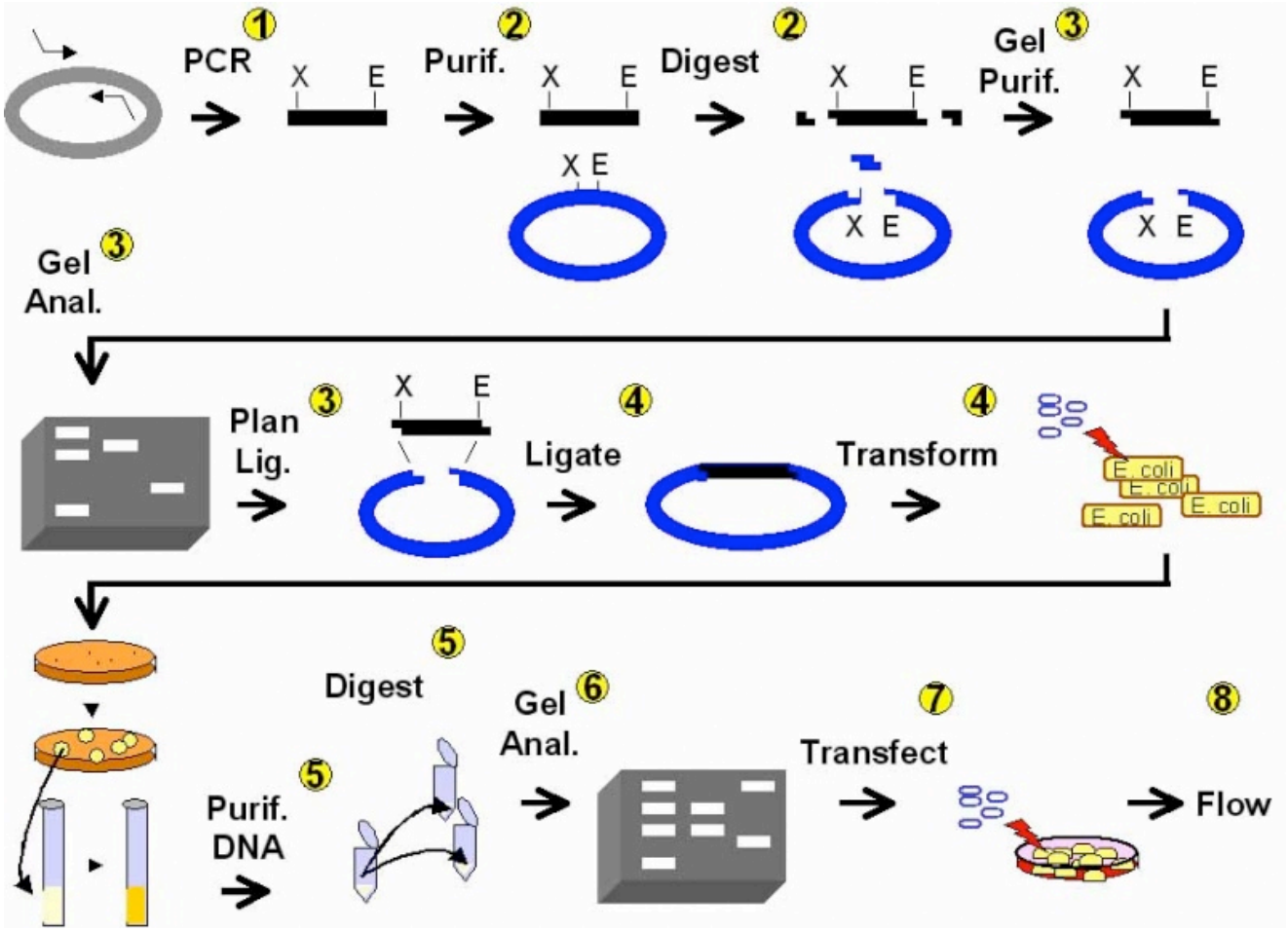
→ cloning

→ diagnostic digest

Homework due M1D5

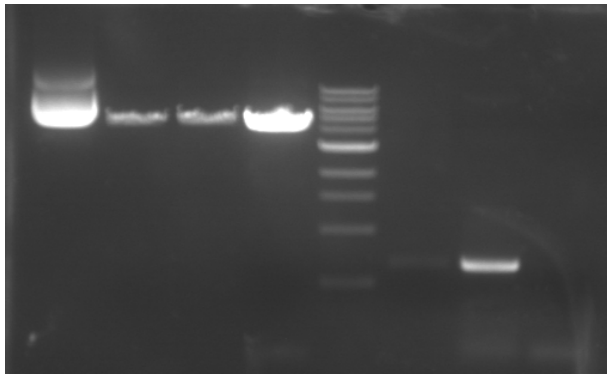


Mod 1 overview

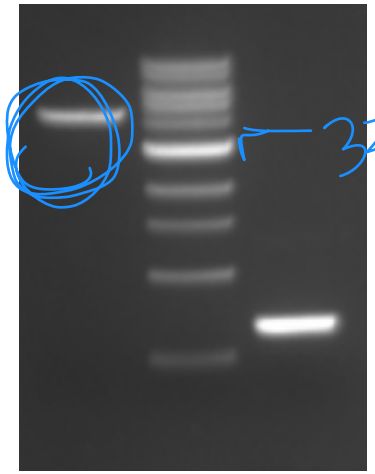


From last time…

- PCR/digest troubleshooting



- Ligation calculation



Vol of vector 50ng → $\frac{50ng}{6ng/\mu L} = 8.3\mu L$

MW vector → $4200bp \cdot \frac{500Da}{bp} \cdot 2 = 4.2 \cdot 10^6 \frac{g}{mol}$

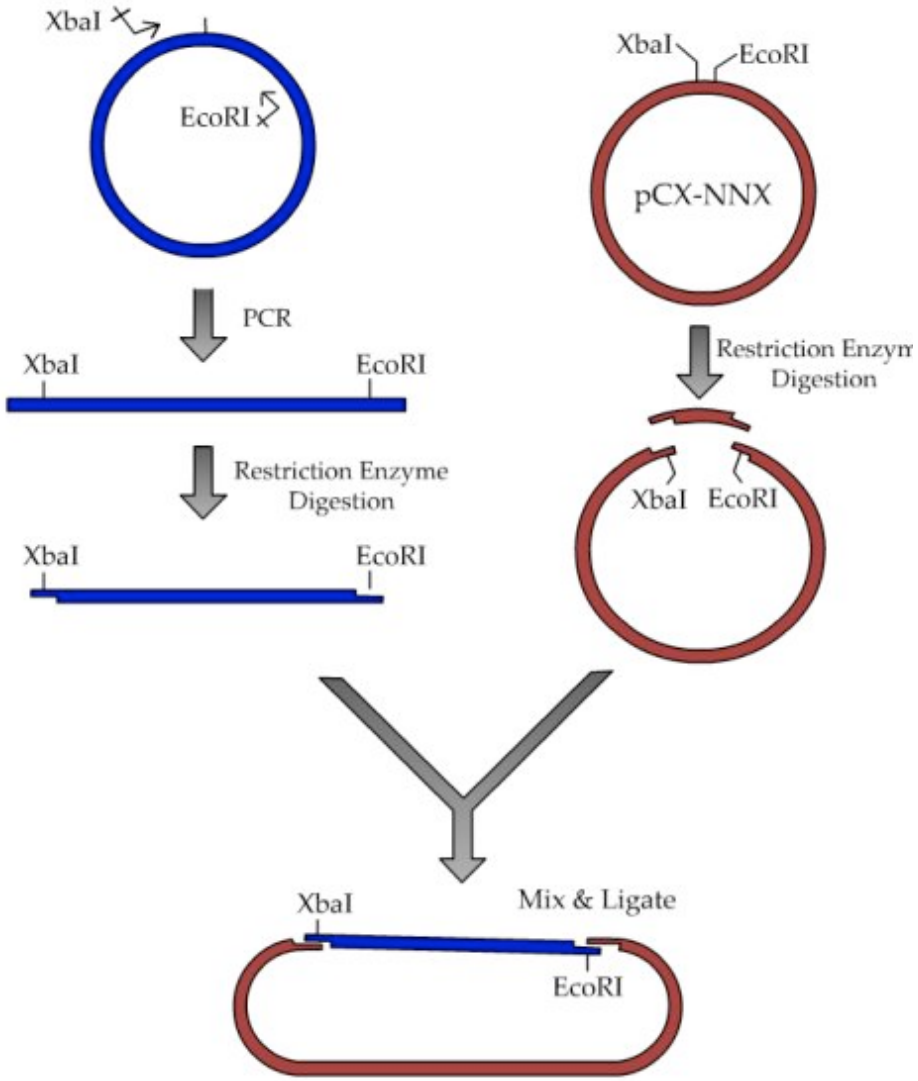
mol of vector → $50ng \cdot \frac{1mol}{4.2\,10^6 ng} = 1.2\,tmol$ vector

$\Downarrow$

48 tmol insert

→ MW of insert
→ ng of insert
→ use gel - vol of insert

Using ligase to build Δ5 EGFP construct



- T4 DNA ligase

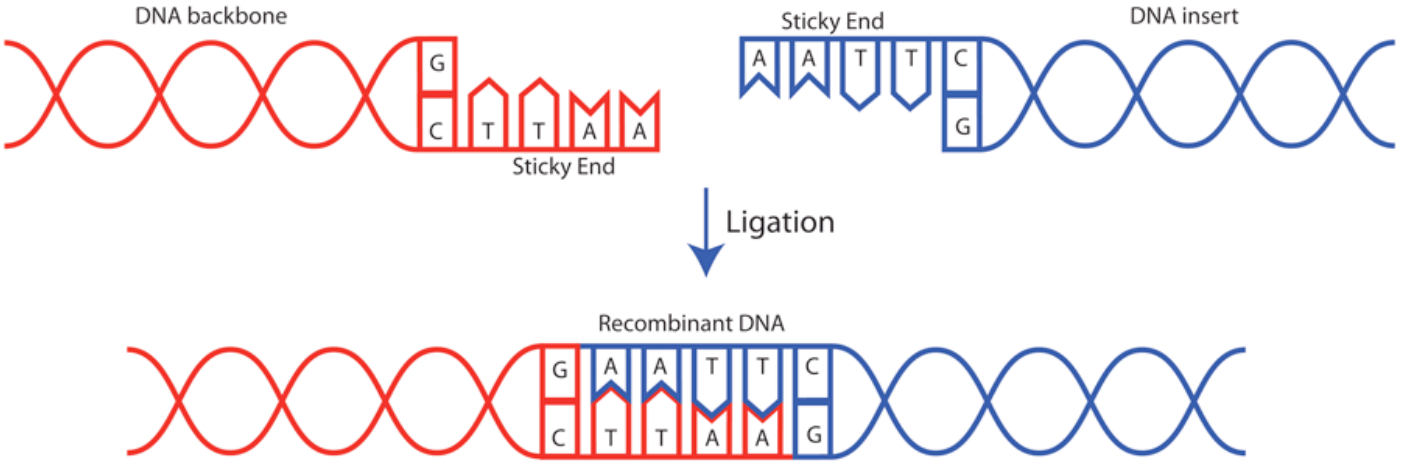
uses ATP

attach AMP

↓

leaving group

Ligation



What effects efficiency of ligation reaction?

ATP
pH
Salt
temperature
buffer

Ligation setup

1. Backbone/vector, fragment/insert, – ligase

   uncut backbone → colonies

2. Backbone/vector, + ligase

   Single cut bkb → colonies

3. Backbone/vector, fragment/insert, + ligase

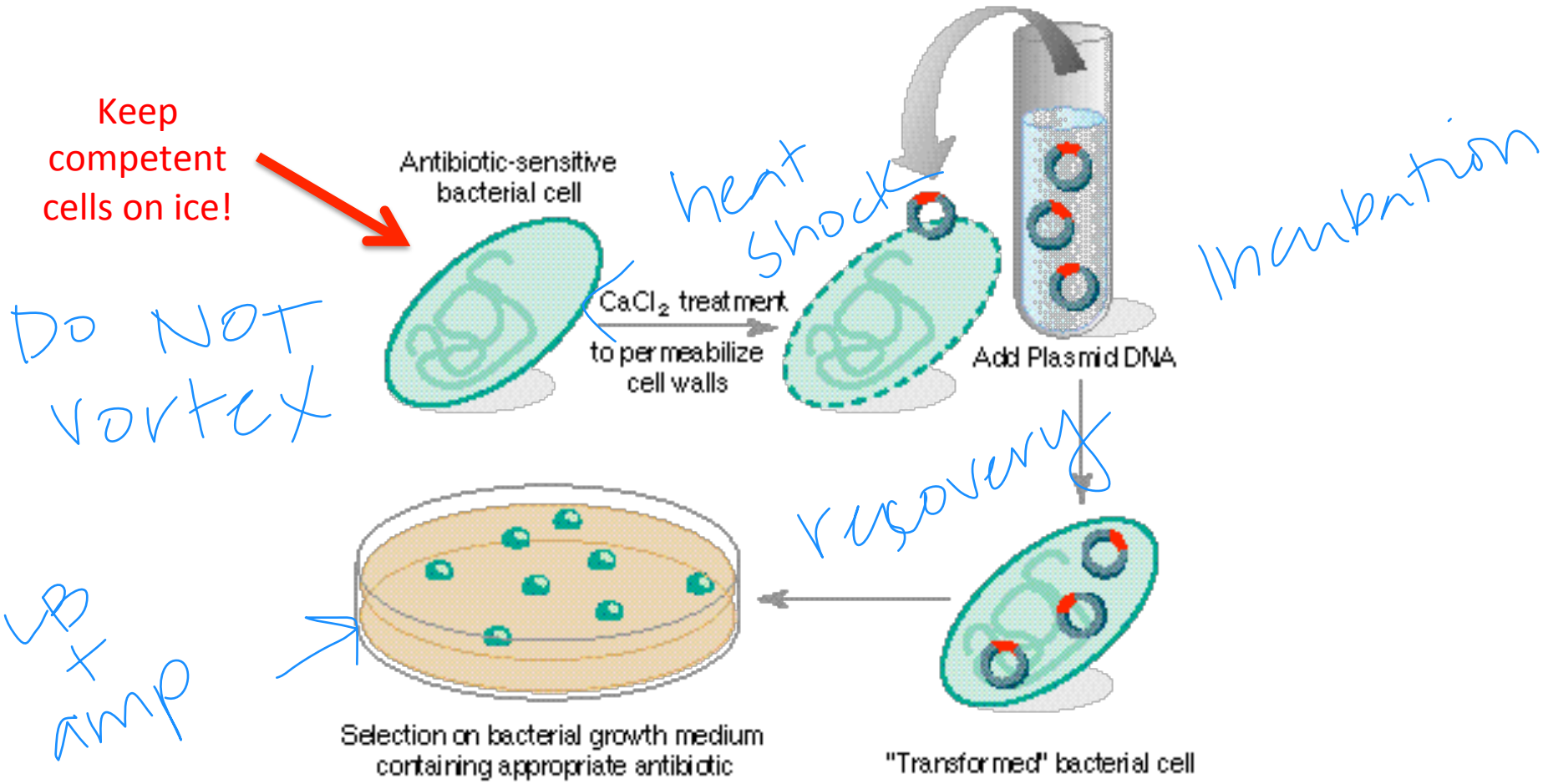
   product ☺

DNA purification, again

"…increased overall transformation efficiency by 70-fold."

"…mechanism may involve altering or stabilizing the topographical form of the DNA molecules."

Transformation



Why transform ligation product into *E. coli*?

Transformation setup

1. Uninoculated plate

   Contamination check

2. pCX-EGFP

   + control

   +++ colonies

3. Backbone/vector, fragment/insert, – ligase

   + colonies

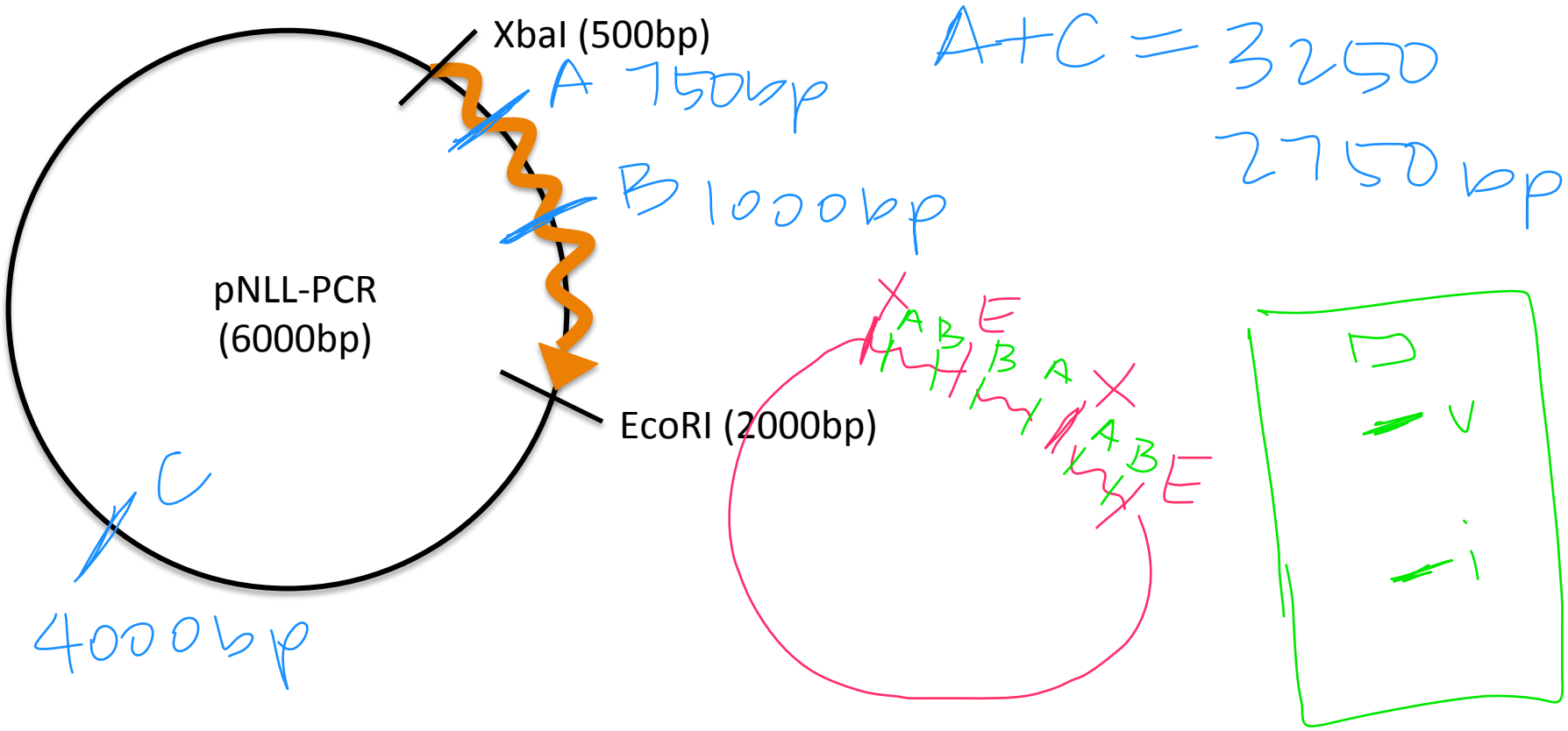
4. Backbone/vector, + ligase

   + colonies

5. Backbone/vector, fragment/insert, + ligase

   ++ colonies

Confirmation of your clones



Why not use XbaI and EcoRI?

Today

- Confirm your ligation calculations
- Complete ligation reaction
- Transform ligation product (safety glasses)
- Plan diagnostic digest approaches

- Networking event
  - September 24 at 5:30p in first floor Koch lobby