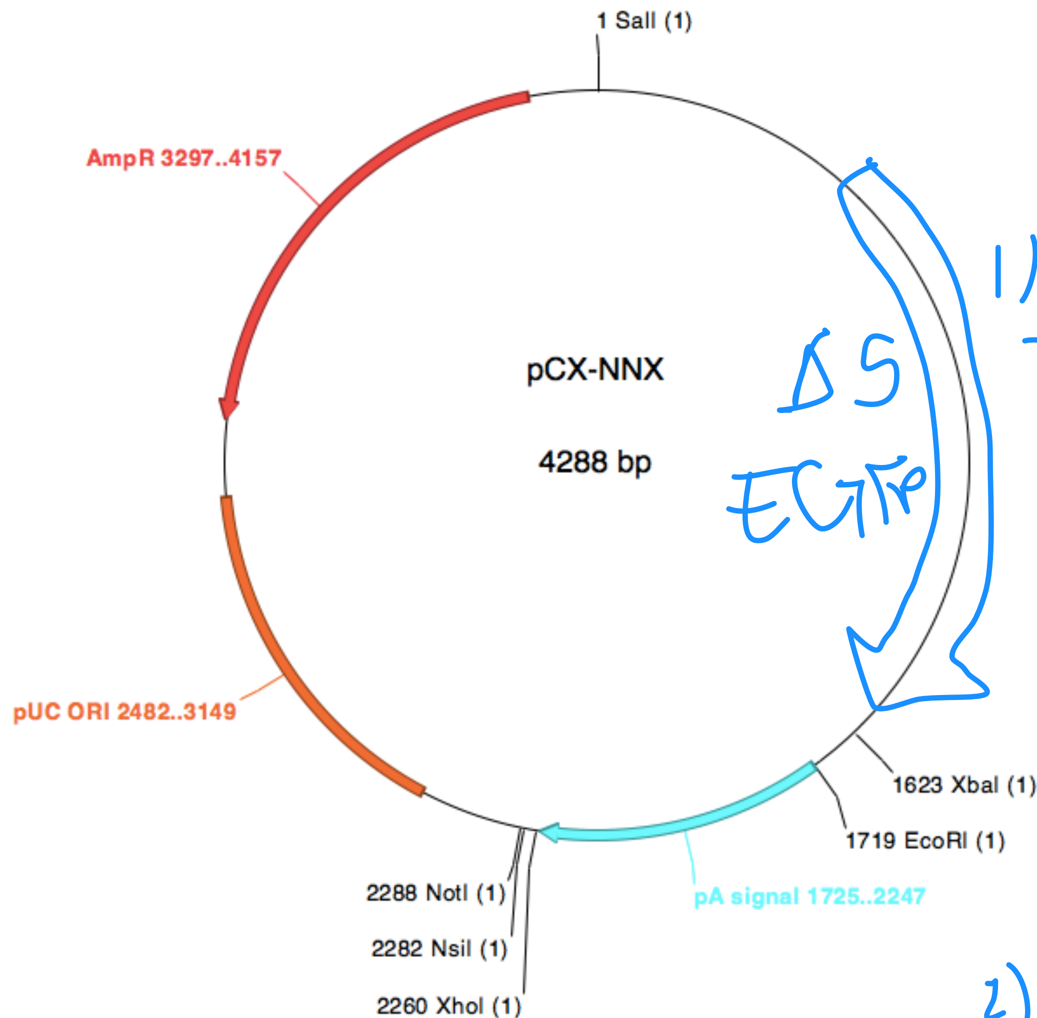
M1D4:Ligation and transformation

9/25/15

1. Visit from Vivian to discuss Abstracts
2. Lab Treat
3. Prelab Discussion
   - Follow-up on homework due M1D3
   - Homework due M1D5
4. FYI: M1D5 will be long!

Follow-up on homework due M1D3



What to include in the caption?

1) features of plasmid
- Amp^R
- restriction sites of interest
- Δ5 EGFP
- name/size

2) relevance of plasmid

→ No methods

Background and Motivation: HW M1D5

citations

choose one

Disease/Evolution

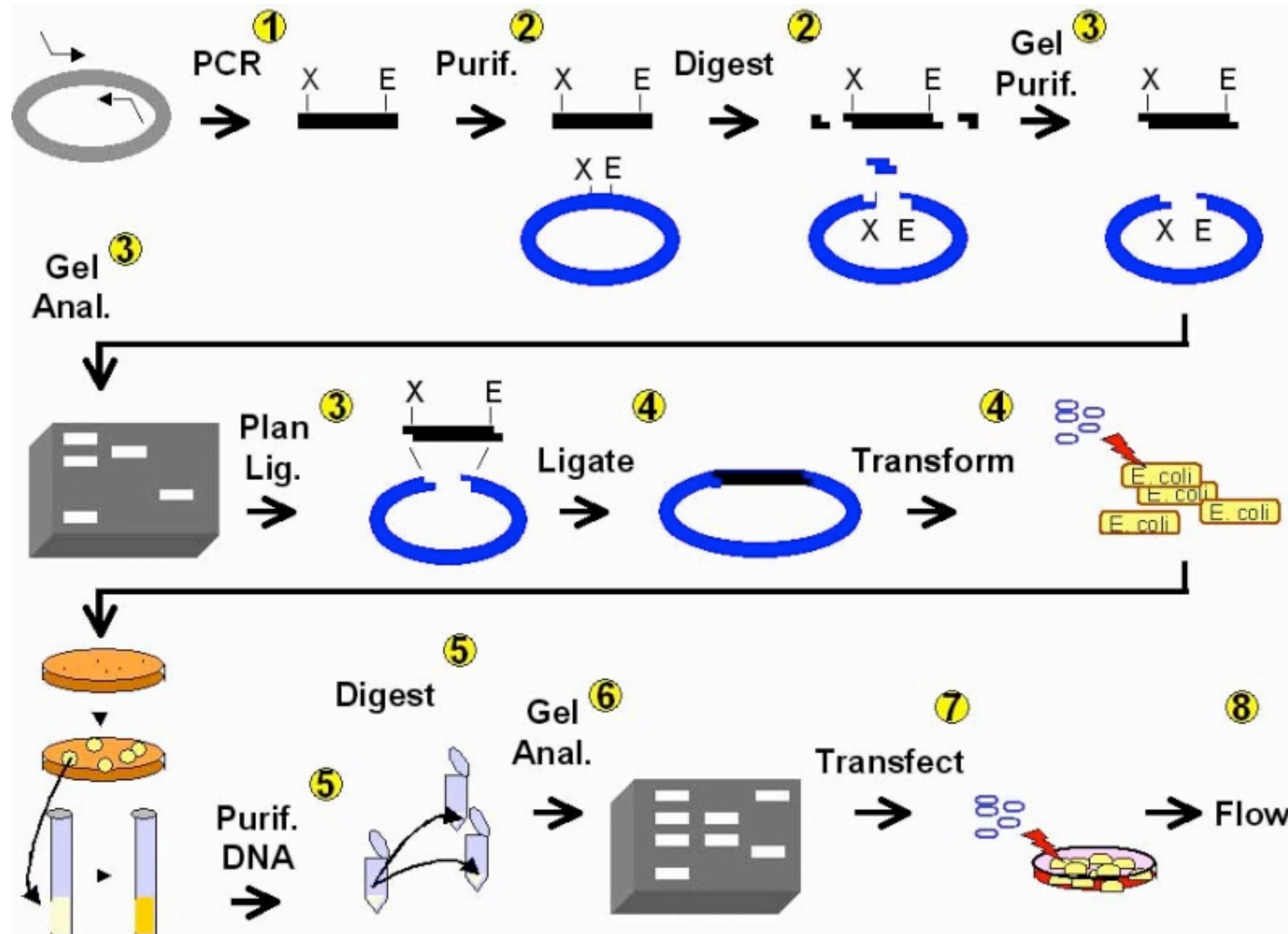
HR

How we approached problem/assay

key results of study

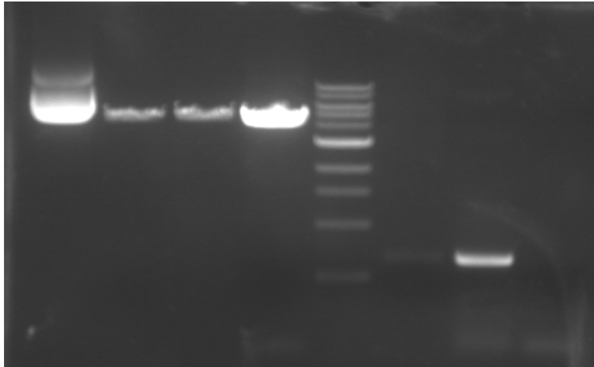
3-7 topic sentences + citations

Mod 1 overview



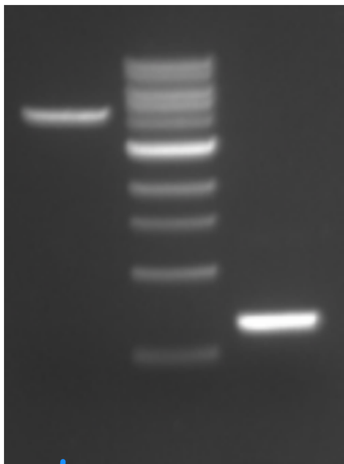
From last time…

- PCR/digest troubleshooting



• lost DNA @ PCR purification
• PCR Rxn sucessful

- Ligation calculation



6kb   frag

6kb = 2x brighter 66ng

~132ng/5ul = 26 4ng/ul

(want 50ng) / 26.4ng/ul ~2ul (52.8ng)

- 1 bkb : 4 frag molar ratio
- Dalton = 1 g/mol

MW bkb } $4200 \cancel{bp} \cdot \frac{500\ Da}{\cancel{b}} \cdot 2 = 42 \times 10^{6}\ g/mol$

mol of bkb } $50 \cancel{ng} \cdot \frac{nmol}{42 \times 10^{6} \cancel{ng}} = 1.2 \times 10^{-5}\ nmol$ 1 : 4

4.8×10^{-5} nmol frag

MW frag } $660 \cdot 500 \cdot 2 = 6.6 \times 10^{5}\ g/mol$

ng of frag } $4.8 \times 10^{-5} = \frac{nmol}{6.6 \times 10^{5}} \cdot X$

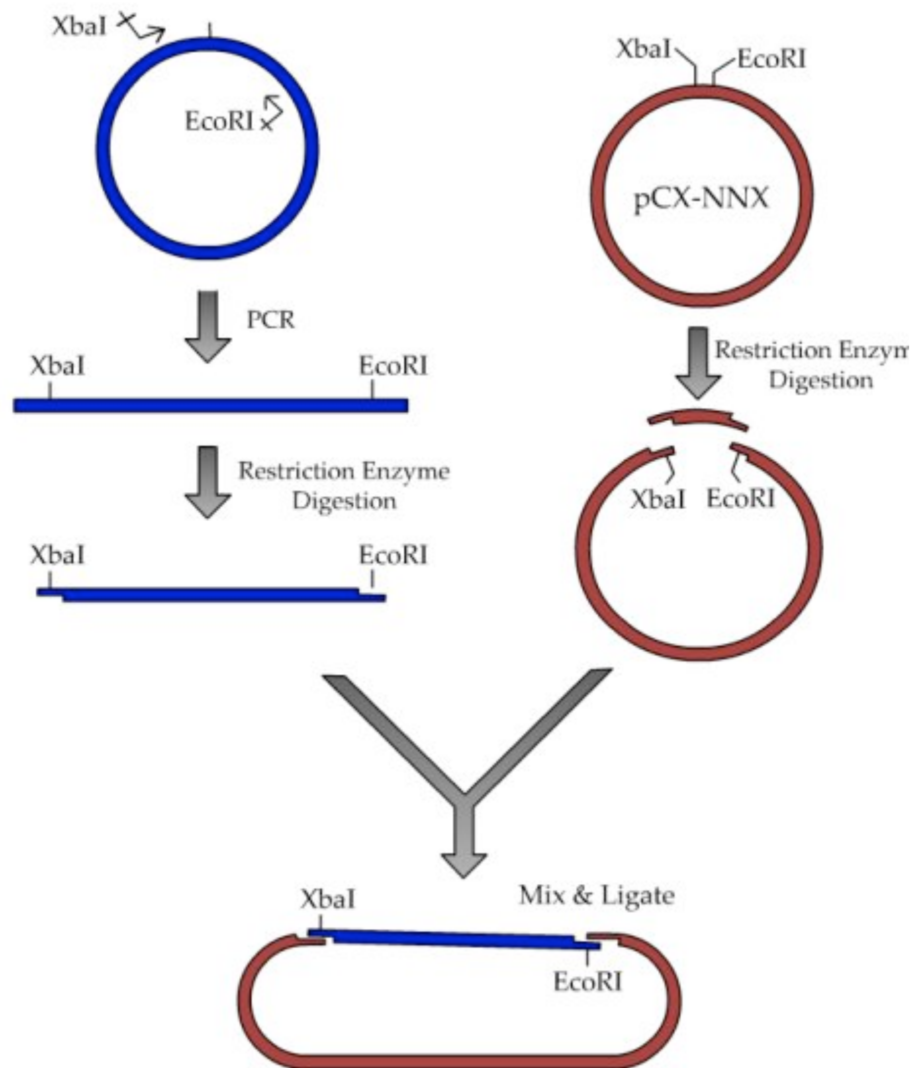
want $X = 31.68$ ng frag

constraints

1) Reasonable volumes
   - less than 13.5
   - no very small volumes

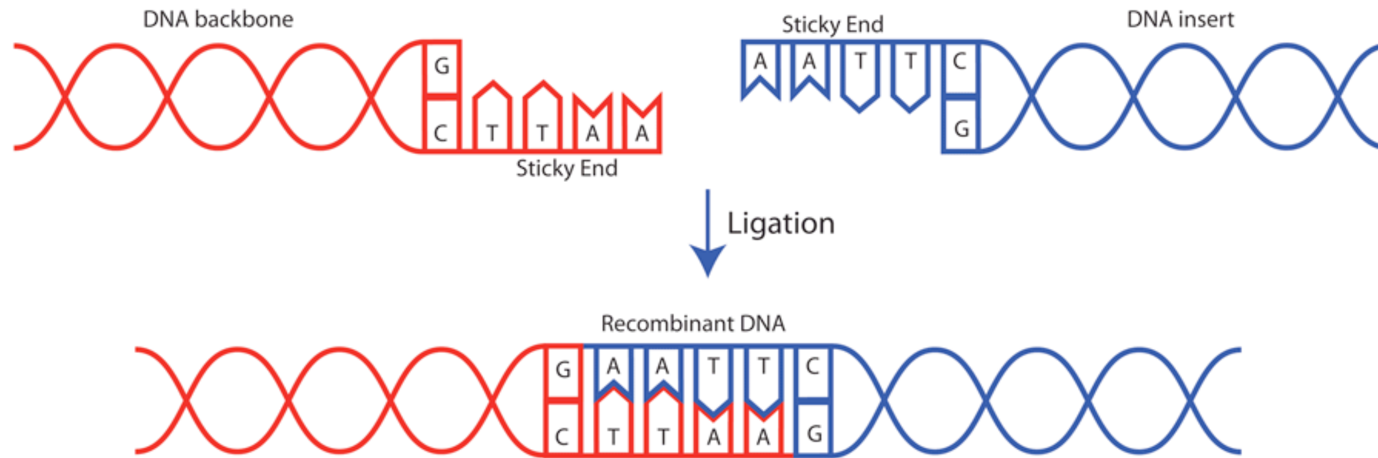
2) consider how much DNA

Using ligase to build Δ5 EGFP construct



- T4 DNA ligase
  - ATP
  - attach AMP → good leaving group

Ligation



What effects efficiency of ligation reaction?

quality ATP
salt
pH
} buffer
temperature

Ligation setup: What do we expect?

1. Backbone/vector, fragment/insert, no ligase
   (+) if uncut bkb
2. Backbone/vector, plus ligase
   (+) 2 plasmids ligated 1) single cut 2)
3. Backbone/vector, fragment/insert, ~~no~~ plus ligase
   (++ → +++) cloning product!

Legend: (+++) a lot of circular DNA, (++) , (+) a little circular DNA

DNA purification, again

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

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

① tRNA = bigger DNA pellet = see it

② tRNA stabilizes

Transformation

Keep competent cells on ice!

Antibiotic-sensitive bacterial cell

$CaCl_2$ treatment to permeabilize cell walls

Add Plasmid DNA

"Transformed" bacterial cell

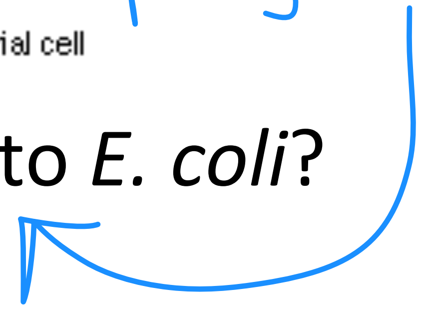
Selection on bacterial growth medium containing appropriate antibiotic

• no vortex
• minimize pipettes

heat shock + recover + plate

LB AMP

• Select for circular
• amplify DNA



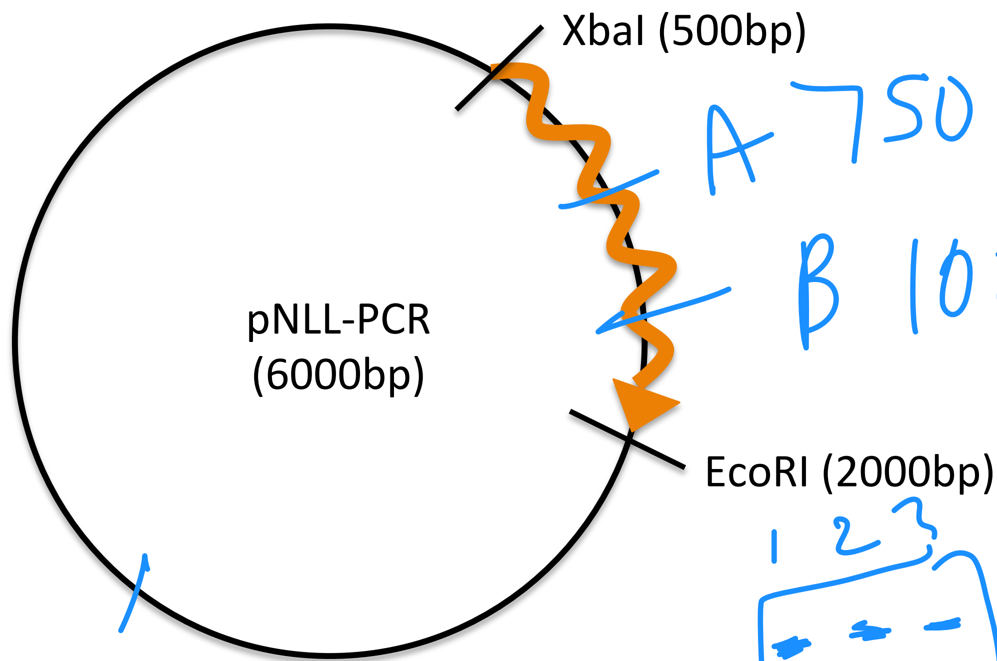
Why transform ligation product into *E. coli*?

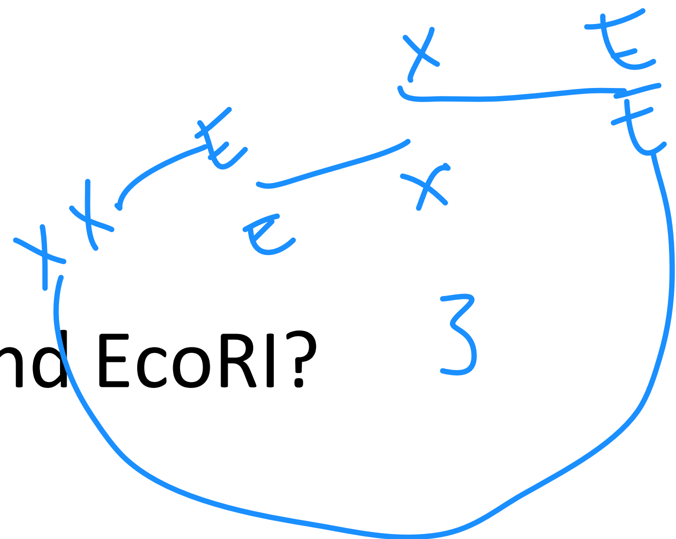
Transformation setup: What do we expect?

1. Uninoculated plate
   AMP 0
2. pCX-EGFP
   +++
3. Backbone/vector, fragment/insert, – ligase
   (+) uncut plasmid
4. Backbone/vector, + ligase
   (+) uncut, single cut plasmid
5. Backbone/vector, fragment/insert, + ligase
   (++) cloning worked!

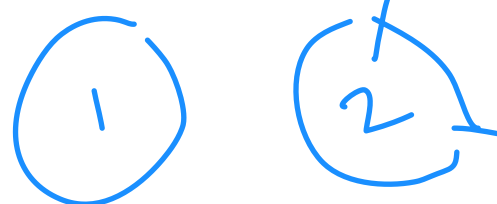
Legend: (+++) a lot of colonies, (++) , (+) a few colonies

Confirmation of your clones





Why not use XbaI and EcoRI?



Today!

- Confirm your ligation calculations
- Complete ligation reaction
- Purify DNA in ligation reaction
- Transform ligation product (safety glasses)
- Plan diagnostic digest approaches (M1D5 HW)