M1D3: Ligation & Transformation

9/24/13

1. Pre-lab discussion
2. Ligation -- scale as needed
3. Get rid of the salt!
4. Leslie here to talk about Abstracts
5. Transform e.coli and plate on LB/Agar + Ab

Review M1D2 FNT: Methods section

going forward. - PCR (use your primers!)
- Sub-cloning in pCX-WNX ⇒ pCX-4326 FD

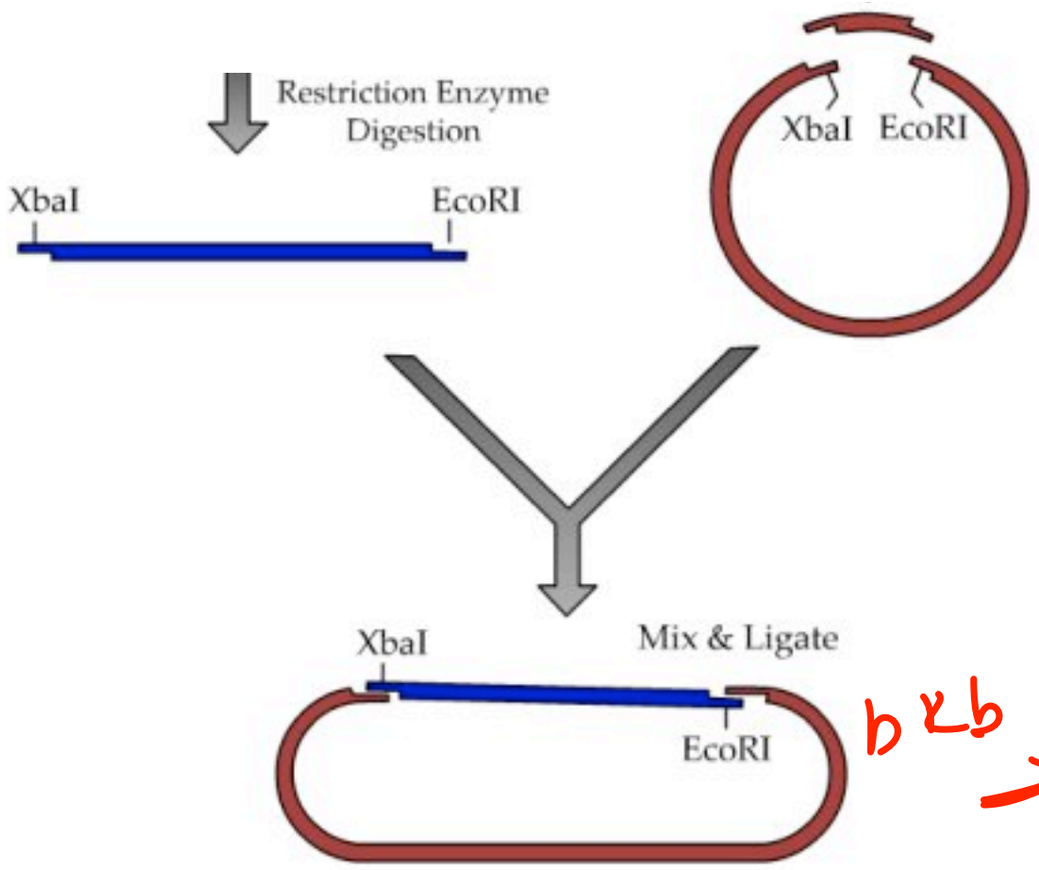
① What does the reader need to know?
- primer sequences - text cues
- PCR cycling conditions
- [final] * NaM
- RE: U(nits) of activity - HF EcoRI
  - XbaI

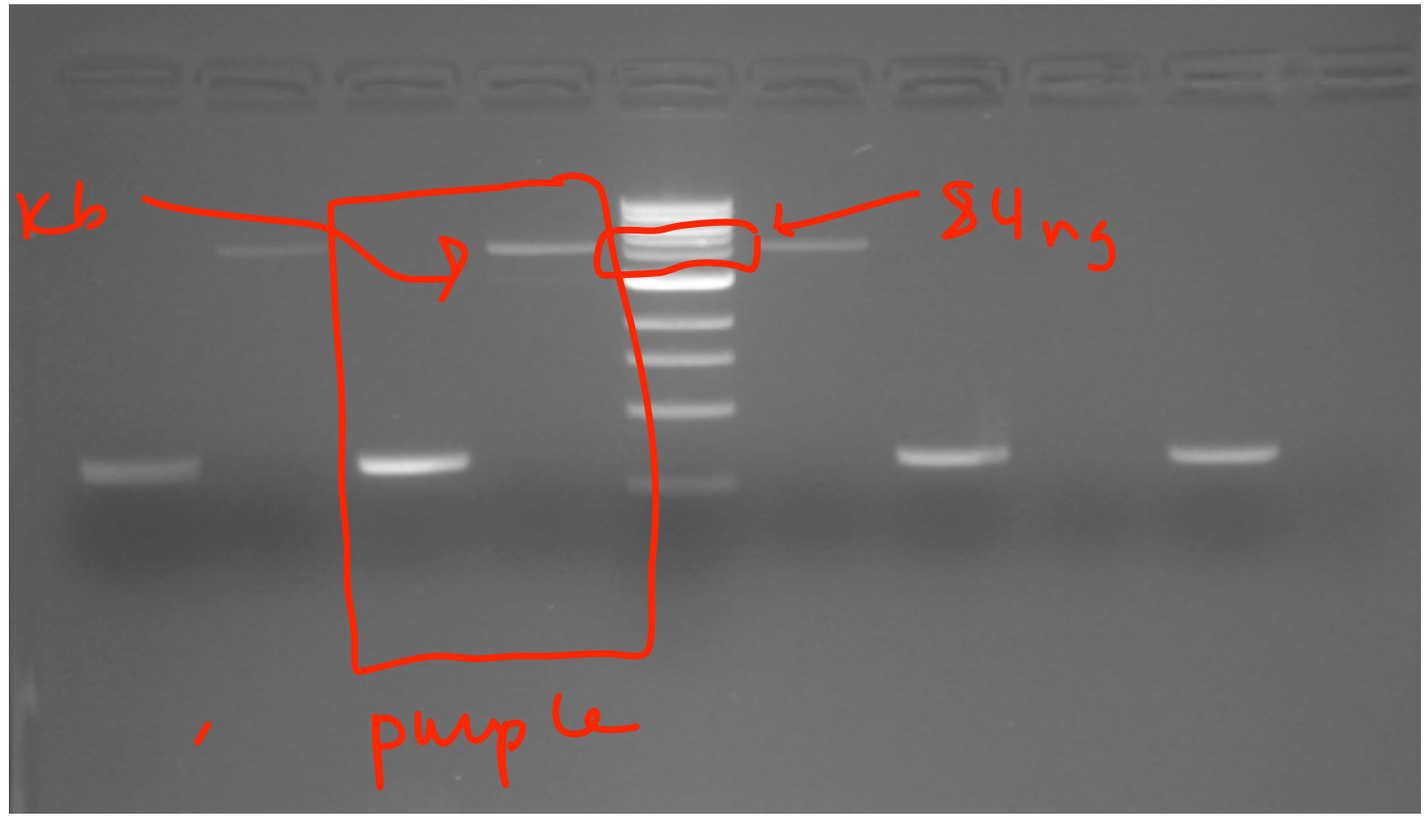
② Technical language: - flap/landing
- "clean" → purification
- "run on a gel" - evaluated
  - separated

③-④ Sub-section (more specific)

* Introductory sentence

Step 1: Build the system!





6 kb → $\frac{84\,ng}{20\,\mu L} = \frac{4.2\,ng}{\mu L} \times 4 \approx 16\,\frac{ng}{\mu L}$

~3 μL 6 kb

How to estimate the correct volumes for the ligation:

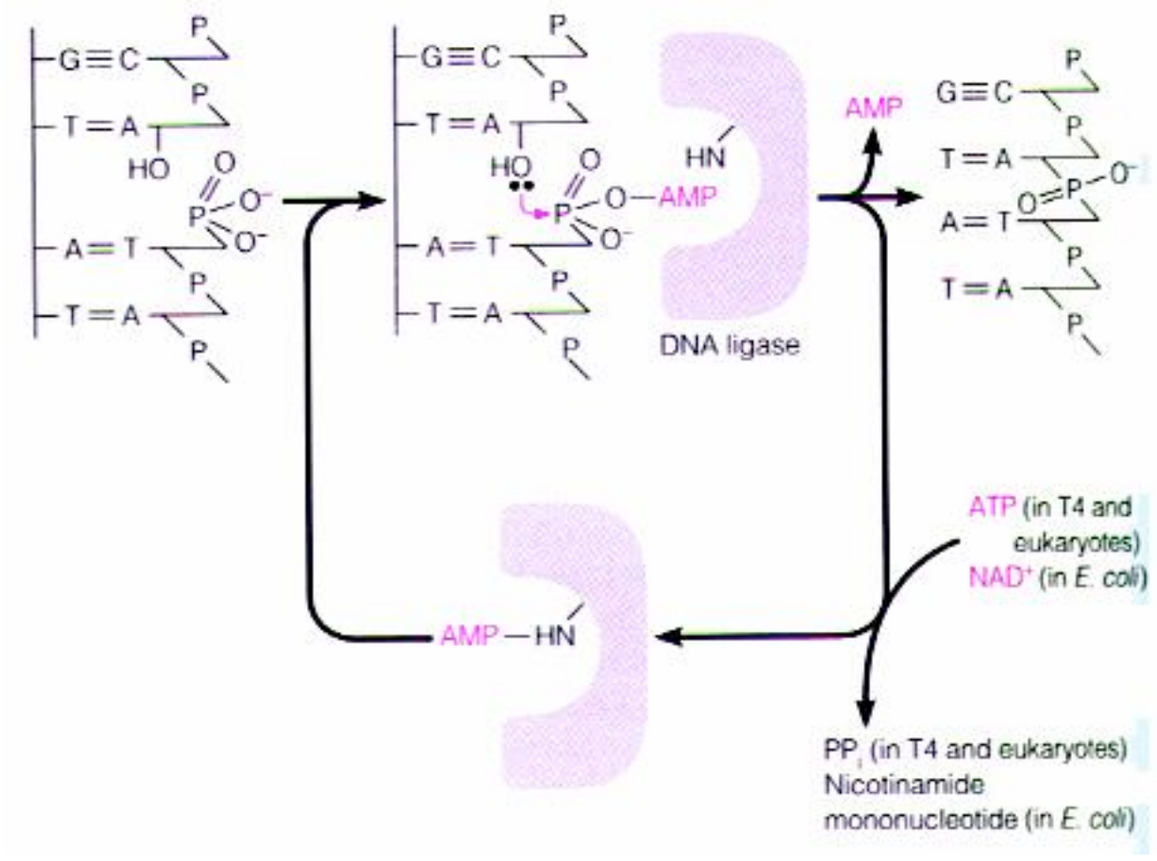
50 ng 6 kb

$500\,\frac{Da}{bp} \times 2 = \left(1000\,\frac{Da}{bp}\right)(4200\,bp) \approx 4.2 \times 10^6\,g/mol$

$\frac{50\,ng}{4.2 \times 10^6\,ng}\,|\,nmol \approx 12\,fmol \times 4 \approx 4.8\,fmol$ insert

⇓ ng ⇒ μL

Overview: Ligation



What effects the efficiency of ligation?

- [salt] – 50mM Tris
  - Mg^{2+}, ATP, DTT
    - availability of ATP
- temperature
  - 16°C O/N
  - 25°C (RT) 10 min

Your Ligations

	bkb + insert, no ligase	bkb only, + ligase	bkb + insert, + ligase
What does this control for?	bkb uncut	Single cut bkb	Expt !!
pCX-NNX bkb	? uL	? uL	? uL Sorry
Δ5 PCR product	? uL	xxx	? uL
10x buffer	1.5 uL	1.5 uL	1.5 uL
T4 DNA Liagase	xxx	0.5 uL	0.5 uL
Water*	to 15 uL	to 15 uL	to 15 uL

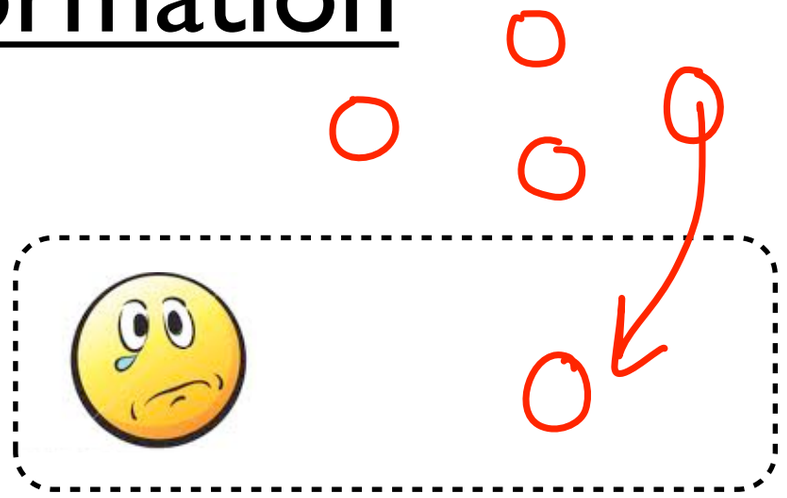
*not including enzyme volume

XL-1 Blue

Overview: Transformation

chemically competent

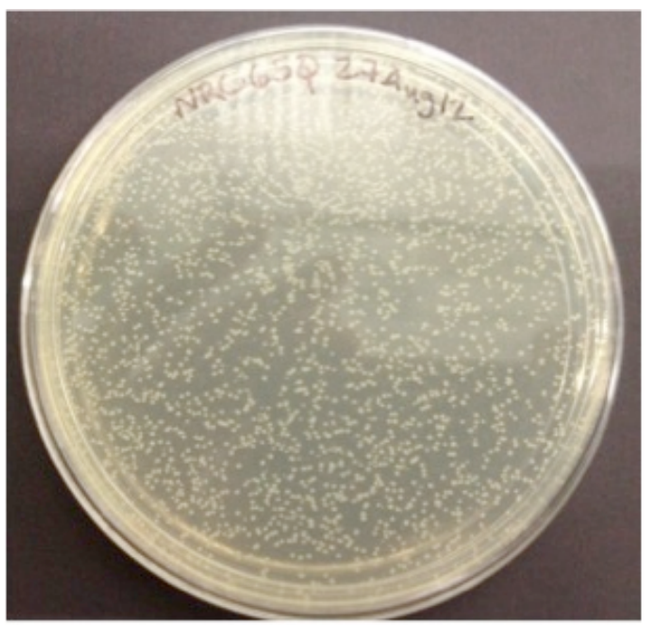
$CaCl_2$



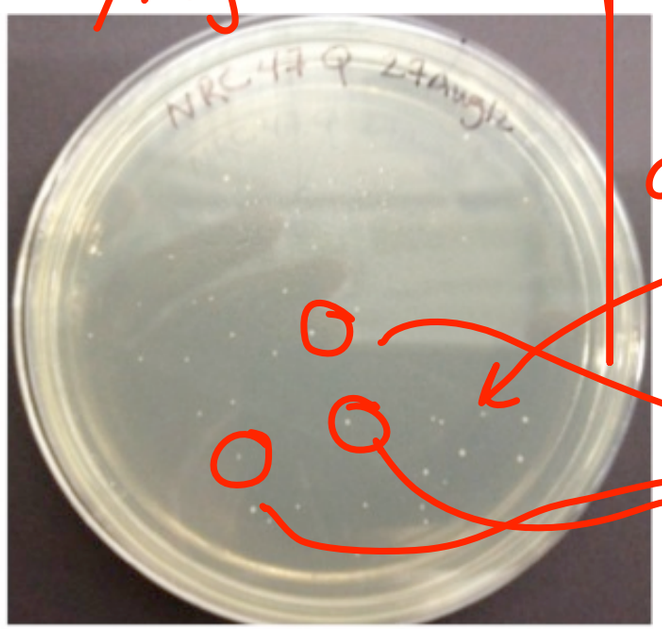
ice

heat shock @42°C - 90sec

LB/Agar + Amp



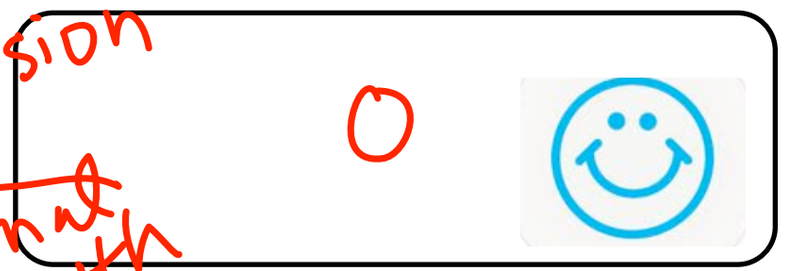
Left Pit



Right Pit

division

clonal growth



37°C LB

(No Amp)

harvest DNA MIDS

http://www.yourwildlife.org/2012/08/not-all-pits-are-equal/

Your Transformations

Tube	Transformation	Expectation:	What if?
Not doing	nothing (just the plate)	ϕ	lots? - contamination
1	pCX-EGFP (5ng)	A lot 100's	ϕ? - overheated - weren't competent - too ↑ Ab
2	bkb + insert; no ligase	ϕ - 1	A lot? - contaminating uncut plasmid
3	bkb; + ligase	10's - 100	lots? 11 ^ Single cut
4	bkb + insert; + ligase	50 - 100 > 200 - 400	Nothing? - transformation - ligation

transformation efficiency: # of colonies / amt of DNA (Not plating everything)

Today in the lab:

- Set up ligations using your calculations from the FNT -- remember that total volume of bkb + insert cannot be greater than 13.5 uL
- Clean up ligation -- talk about Abstracts
- Transform into e.coli and then plate (with fire)

Next time in the lab:

- Minipreps
- Diagnostic digests
- Intro to Tissue Culture!