M1D5: Ligation & Transformation

9/23/14

1. Lab Treat! (For real — there are treats in the lunch room)

2. Pre-lab discussion

3. Ligation -- scale as needed — remove salts

4. Leslie here to talk about Abstracts

5. Transform e.coli and plate on LB/Agar + Ab

6. Prepare for diagnostic digest next time

Review M1D2 FNT: Methods section

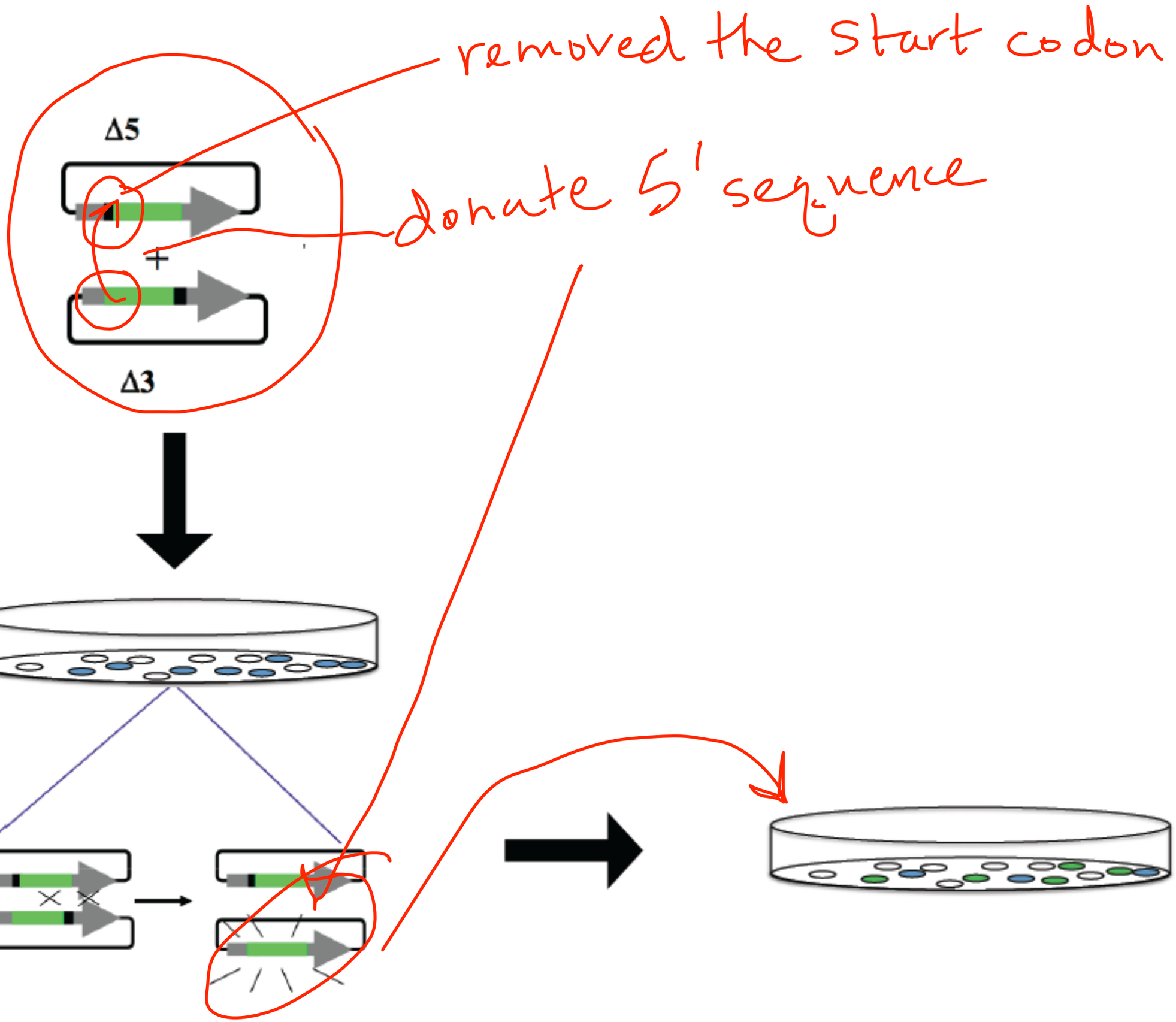
① Name your intermediates product → "PCR product" → D32N-EGFP
→ cloned → your name here

② What does your audience need to know?
A) primer sequences
B) PCR cycling conditions
C) Final concentrations → RE → units of activity (we buy small)

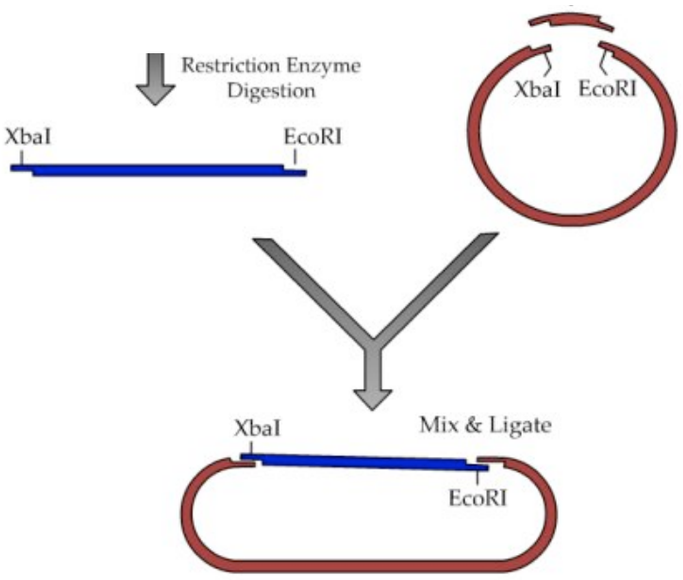
③ Technical Language → flup/landing

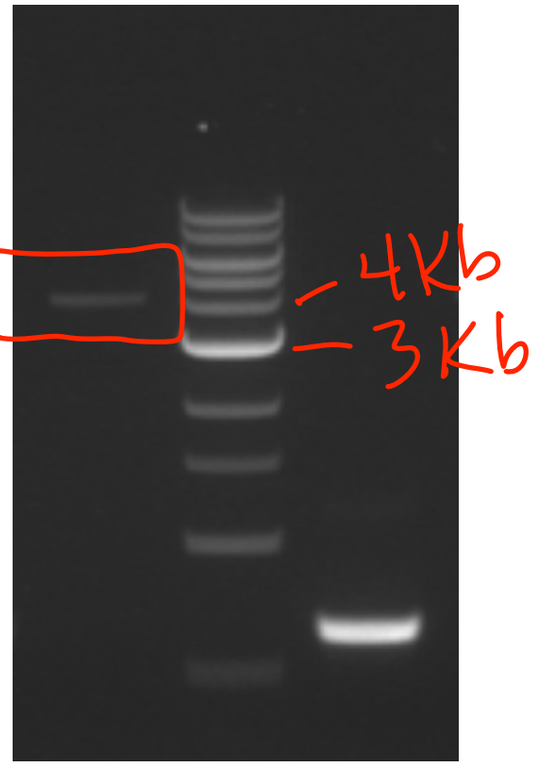
④ sub-section grouping → "clean" → purify
[primers → amplify via PCR → purified] → "run" → perform - PCR
Cloning/Construction of ....
[Digest → ligation → transformation →
→ evaluate
→ separating } gels
→ purify

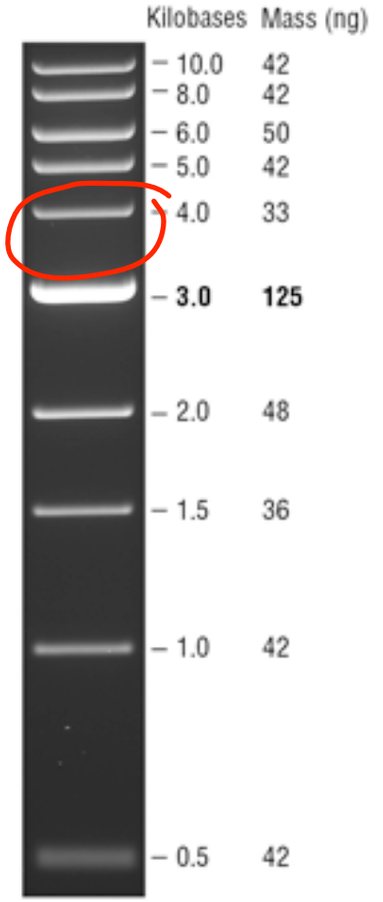
Let's revisit the overall goal of the module:



Step 1: Build the system!







How to estimate the correct volumes for the ligation:

dilution factor

(bKb)

$$\sim \frac{33\,ng}{20\,\mu L} = \left(\frac{\sim 1.65\,ng}{\mu L}\right)\left(\frac{23\,\mu L}{5\,\mu L}\right) \sim \frac{8\,ng}{\mu L} \sim 6.3\,\mu L/50\,ng$$

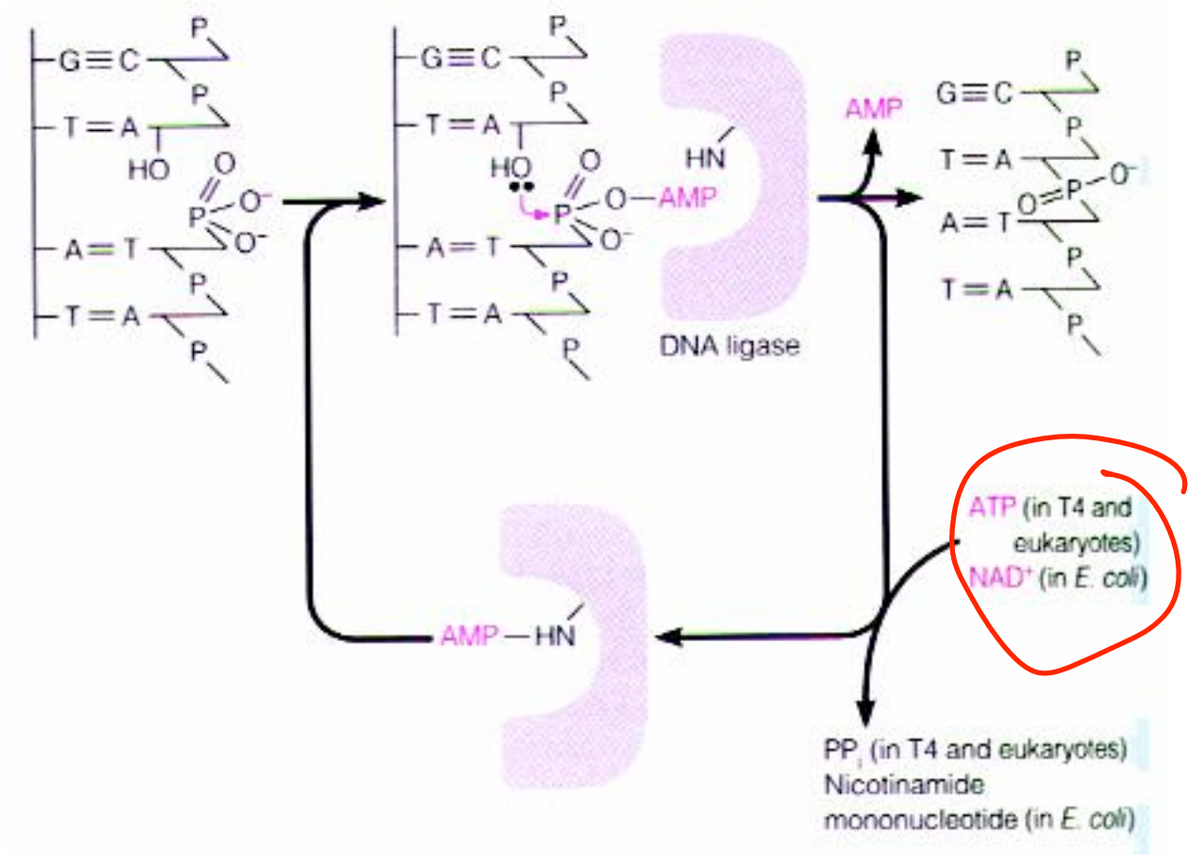
$$(4200\,bp)\left(\frac{500\,Da}{b}\right)(2) \sim 4.2\times10^6\,g/mol$$

$$\frac{50\,ng}{4.2\times10^6\,ng}\,\frac{|\,nmol}{} \Rightarrow 1.2\,fmol \times 4 \Rightarrow 4.8\,fmol\ of\ insert$$

↳ calc ng

↳ use the gel to cal

Overview: Ligation



What effects the efficiency of ligation?

1) Salt concentration
- 50mM Tris

2) co-factors → Mg^{2+}
→ ATP
→ DTT

3) Temperature
25°C → 10 min
16°C ⇒ O/N

Your Ligations

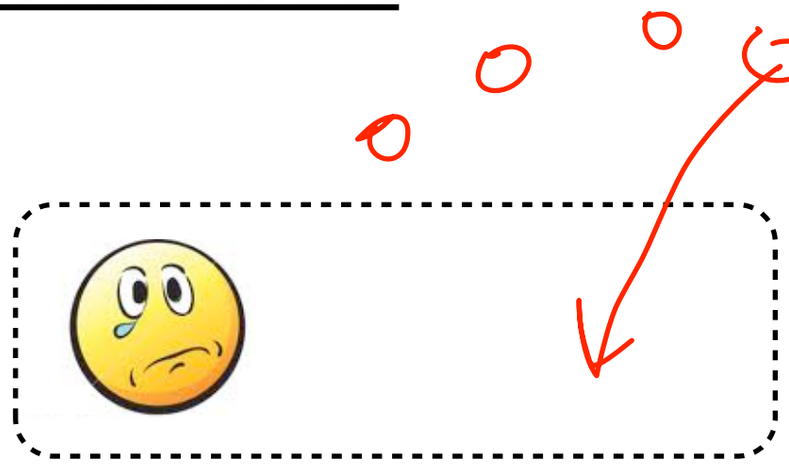
	bkb + insert, no ligase	bkb only, + ligase	bkb + insert, + ligase
What does this control for?	uncut bKb	Single cut bKb	:) product
pCX-NNX bkb	? uL	? uL	? uL
Δ 5 product	? uL	xxx	? uL
10x buffer	1.5 uL	1.5 uL	1.5 uL
T4 DNA Liagase	xxx	0.5 uL	0.5 uL
Water*	to 15 uL	to 15 uL	to 15 uL

*not including enzyme volume

Overview: Transformation

CaCl2 chemically

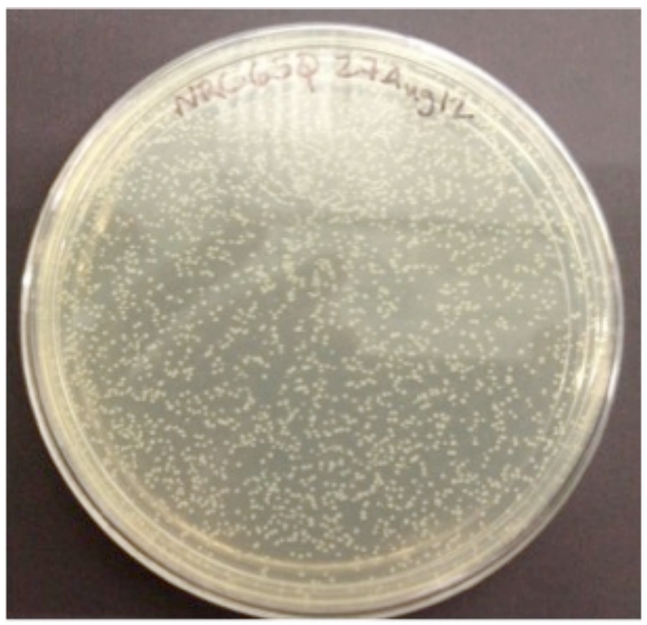
competent



heat shock 42°C for 90sec

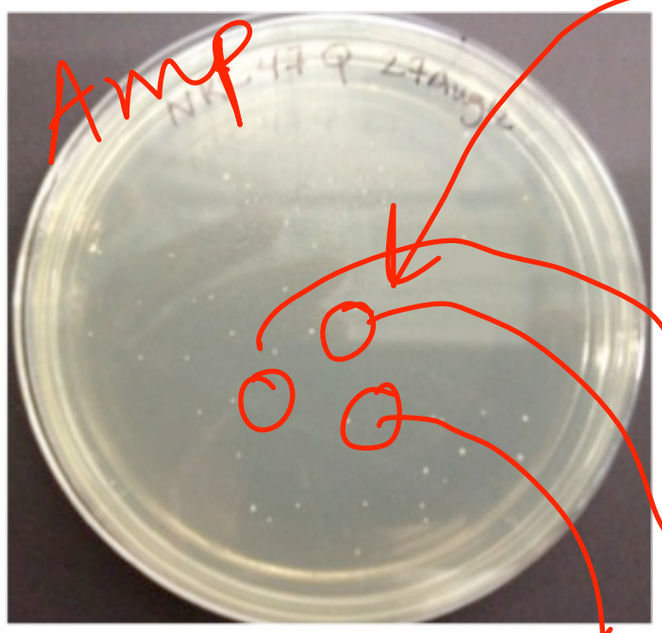
LB/Agar

37° O/N

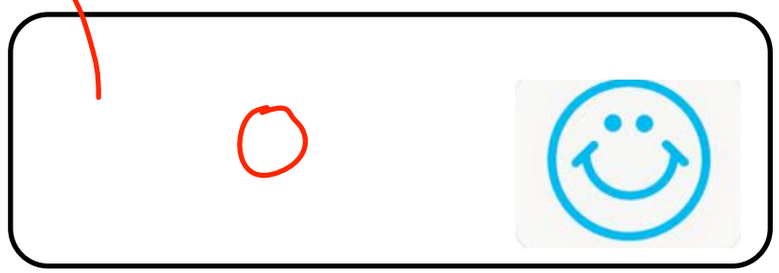


Left Pit

Amp



Right Pit



Incubate w/ LB

(37°C, 60min)

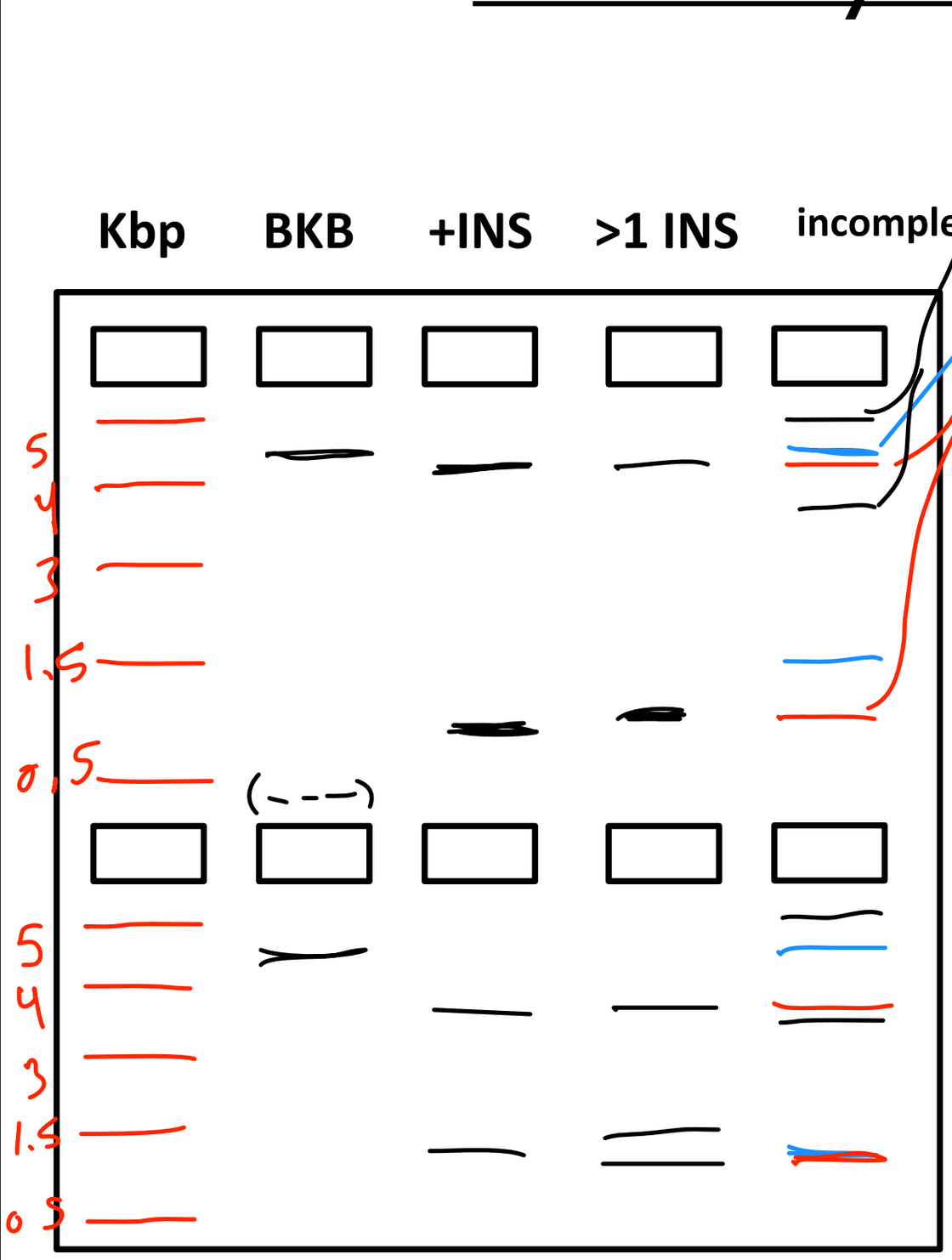
→ miniprep

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

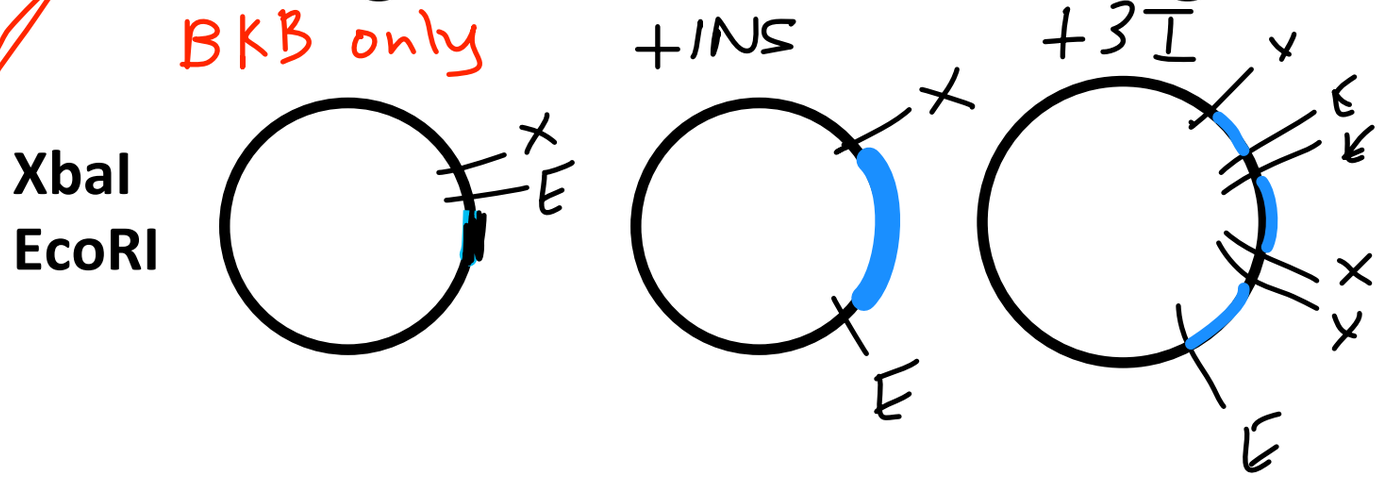
Your Transformations

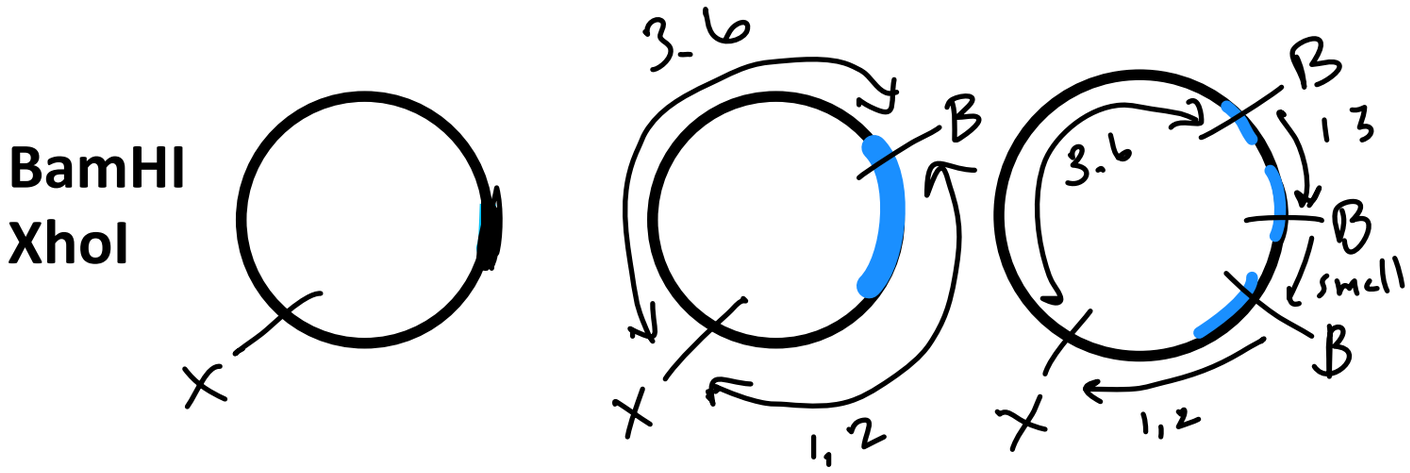
Tube	Transformation	Expectation:	What if?
Not doing	nothing (just the plate)	ϕ	!! contamination
1	+ control pCX-EGFP	100's	ϕ cells ≠ ~~competent~~ competent
2	bkb + insert; no ligase	ϕ → 10's	A lot ~RE bad
3	bkb; + ligase	10 → 100	A lot ~ single cut
4	bkb + insert; + ligase	~50	Nothing? competent cells ligase

How do you know it worked?



Choosing restriction sites for digest





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)
- Plan diagnostic digests for next time

Next time in the lab (M1D5 is a long day):

- Minipreps — harvest the plasmid DNA from the e.coli
- Diagnostic digests
- Intro to Tissue Culture!