

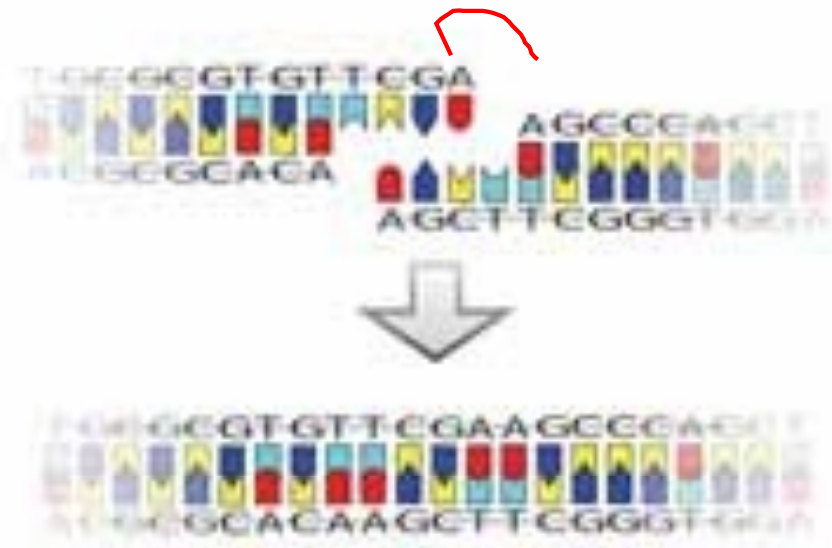
# DNA Engineering: M1D4 Lab Talk

20.109 (F12)

09.25.12

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# About ligations...



Ligase  
requires ATP

What affects efficiency?

Temp: RT / 16°C  
[DNA]: blch: insert [1:4]

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~100ng

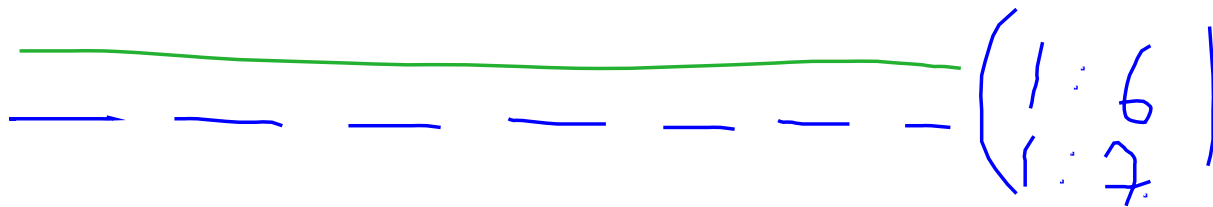
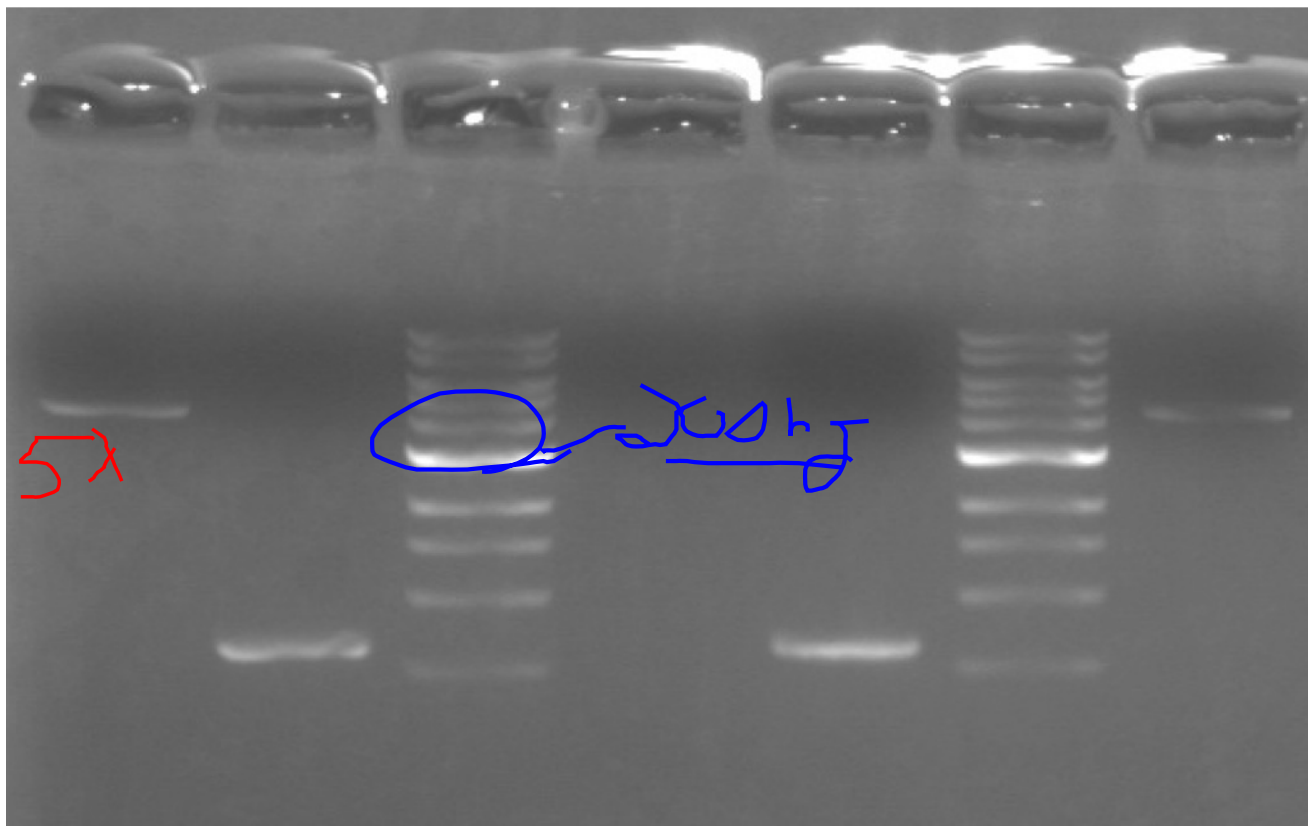
# Your Data

4.6 Kb

5x

~100ng

200






# Ligation Reactions

Controls for: uncut singly cut DNA Exp

	bkb + insert, no ligase	bkb only, plus ligase	bkb + insert, plus ligase
pCX-NNX bkb	? $\mu$ l	? $\mu$ l	? $\mu$ l
PCR insert	? $\mu$ l	xxx	? $\mu$ l
10X Ligation Buffer <sup>^</sup>	1.5 $\mu$ l	1.5 $\mu$ l	1.5 $\mu$ l
T4 DNA Ligase	xxx	0.5 $\mu$ l	0.5 $\mu$ l
Water	To 15 $\mu$ l not including volume of enzyme		

Room temp 10 minutes,  
then clean up again!

# Transformation Reactions

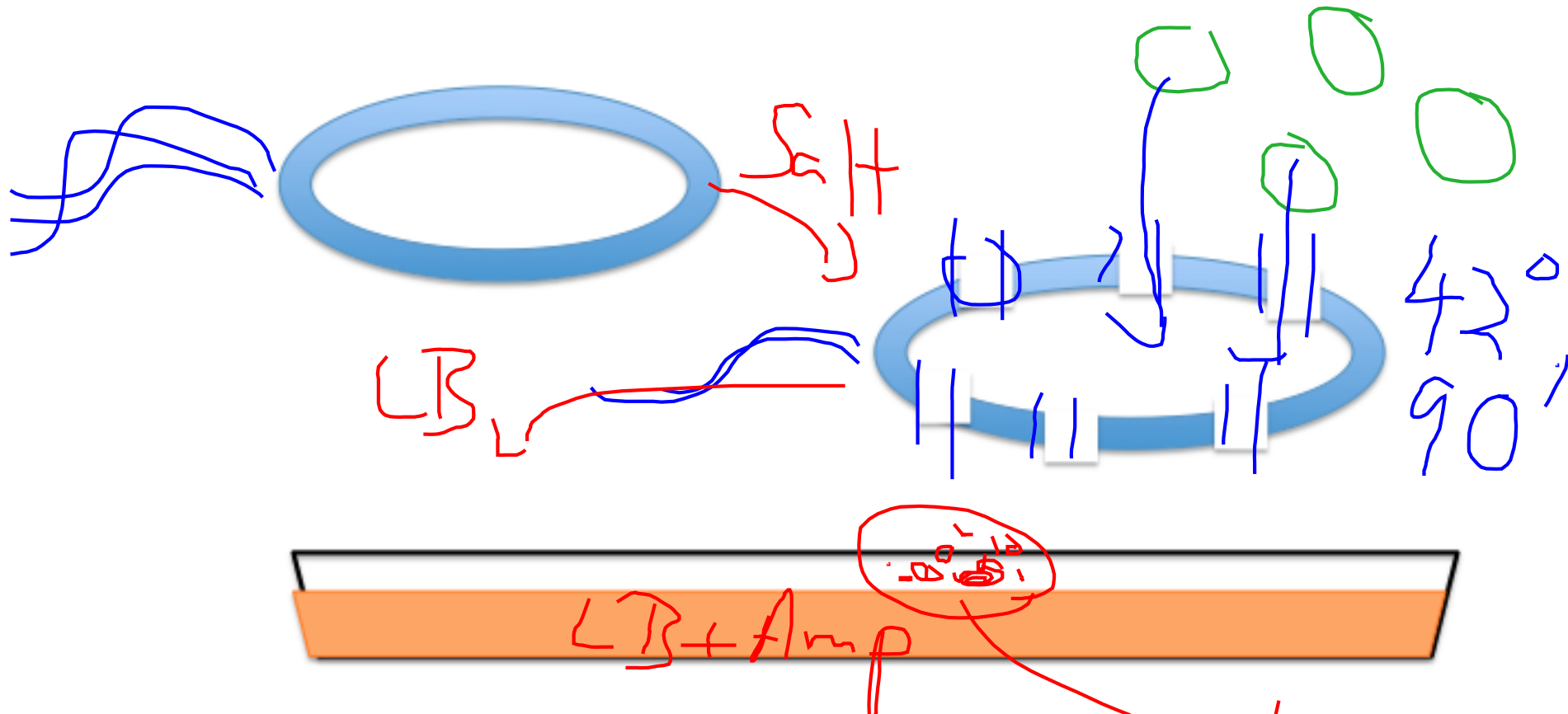
Tube	Transformation	Expectation	What if...?
Not done	Nothing	<del>0</del>	TMTZ/lawn
1	pCX-EGFP 5ng	lots	<del>0</del> ?
2	Bkb+insert, no ligase	<del>0</del>	lots?
3	Bkb, + ligase	<del>0</del> /few	lots? 
4	Bkb + insert + ligase		<del>0</del>  lots

ten efficiency

# col / 5ng

plated ALL

# About transformations...



## Technical Notes:

- Treat competent cells gently
- Use sterile technique to plate

colony  
colony  
~~colony~~

# Today and next week in lab....

- ① Ligate / rxn / EHS  
opt and HW Assignment
- ② Miniprep / Gel + intro TC  
opt and HW
- ③ Lab certifications + Lipofect  
+ 20 lab
- ④ FACS