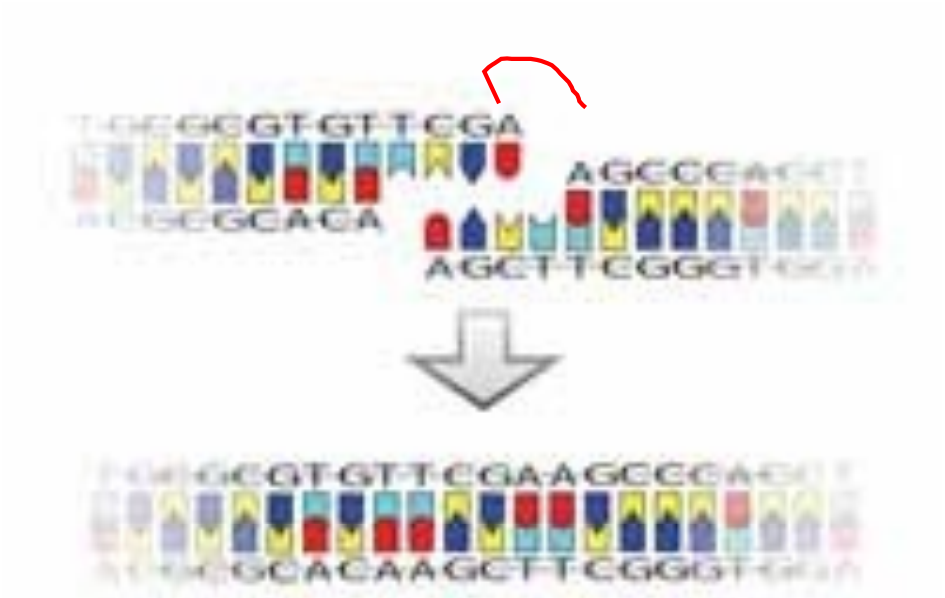
DNA Engineering: M1D4 Lab Talk

20.109 (F12)
09.25.12

About ligations…



Ligase

requires ATP

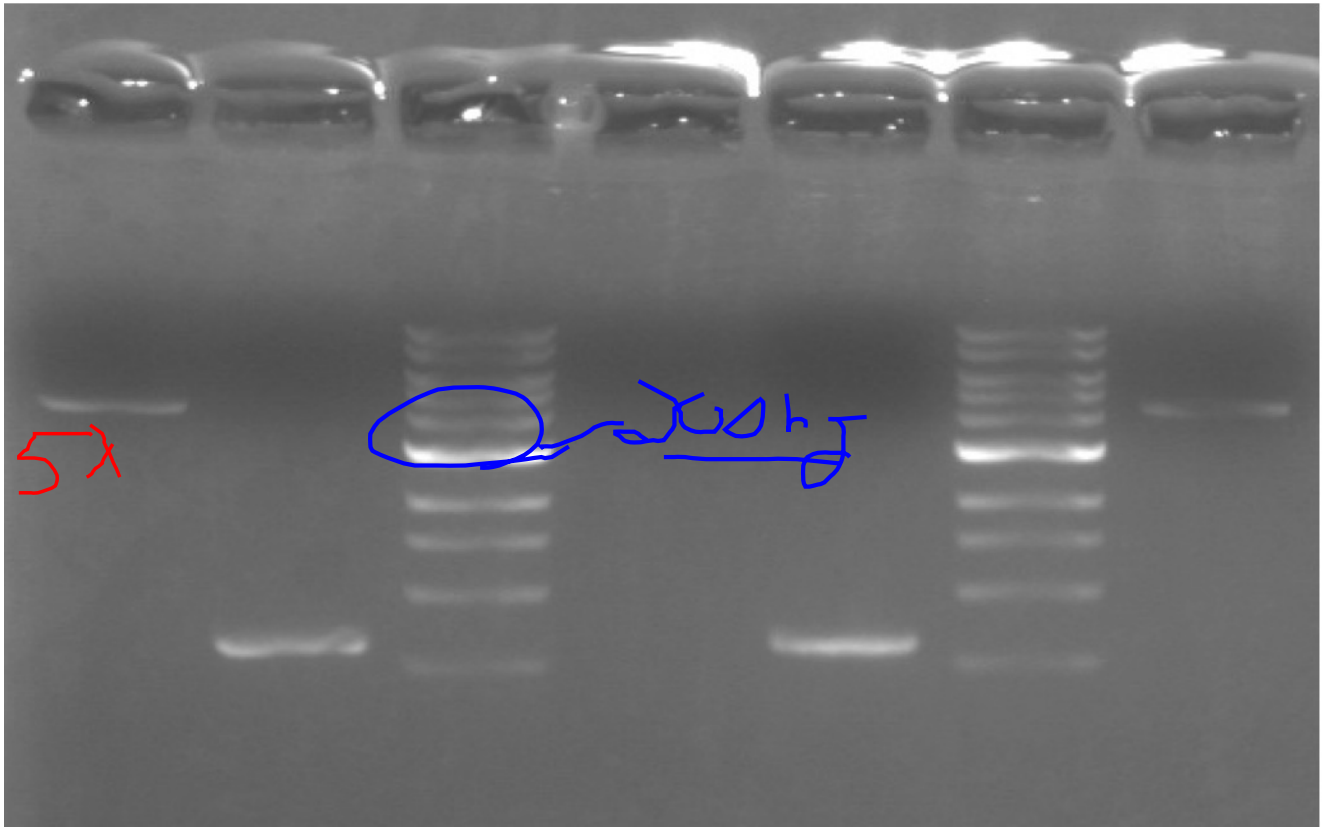
What affects efficiency?

Temp: RT / 16°C

[DNA]: blch: insert 1:4

Your Data

~100ng



1:6
1:7

Ligation Reactions

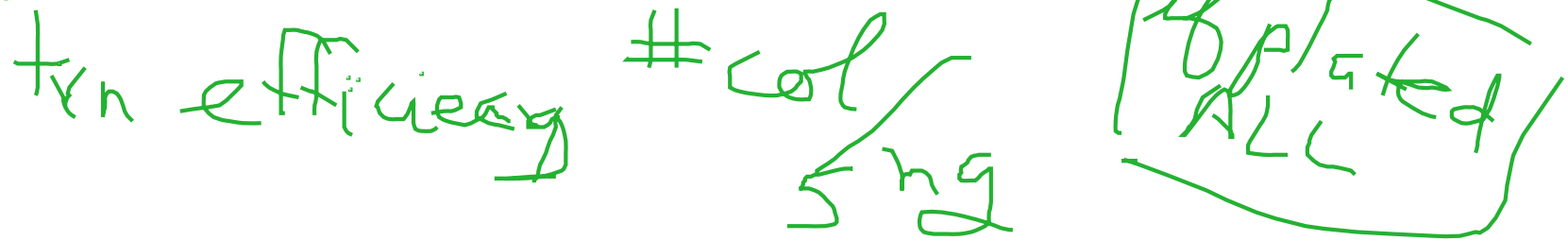
Controls for: uncut singly cut DNA Exp+

	bkb + insert, no ligase	bkb only, plus ligase	bkb + insert, plus ligase
pCX-NNX bkb	? µl	? µl	? µl
PCR insert	? µl	xxx	? µl
10X Ligation Buffer^	1.5 µl	1.5 µl	1.5 µl
T4 DNA Ligase	xxx	0.5 µl	0.5 µl
Water	To 15 µl not including volume of enzyme		

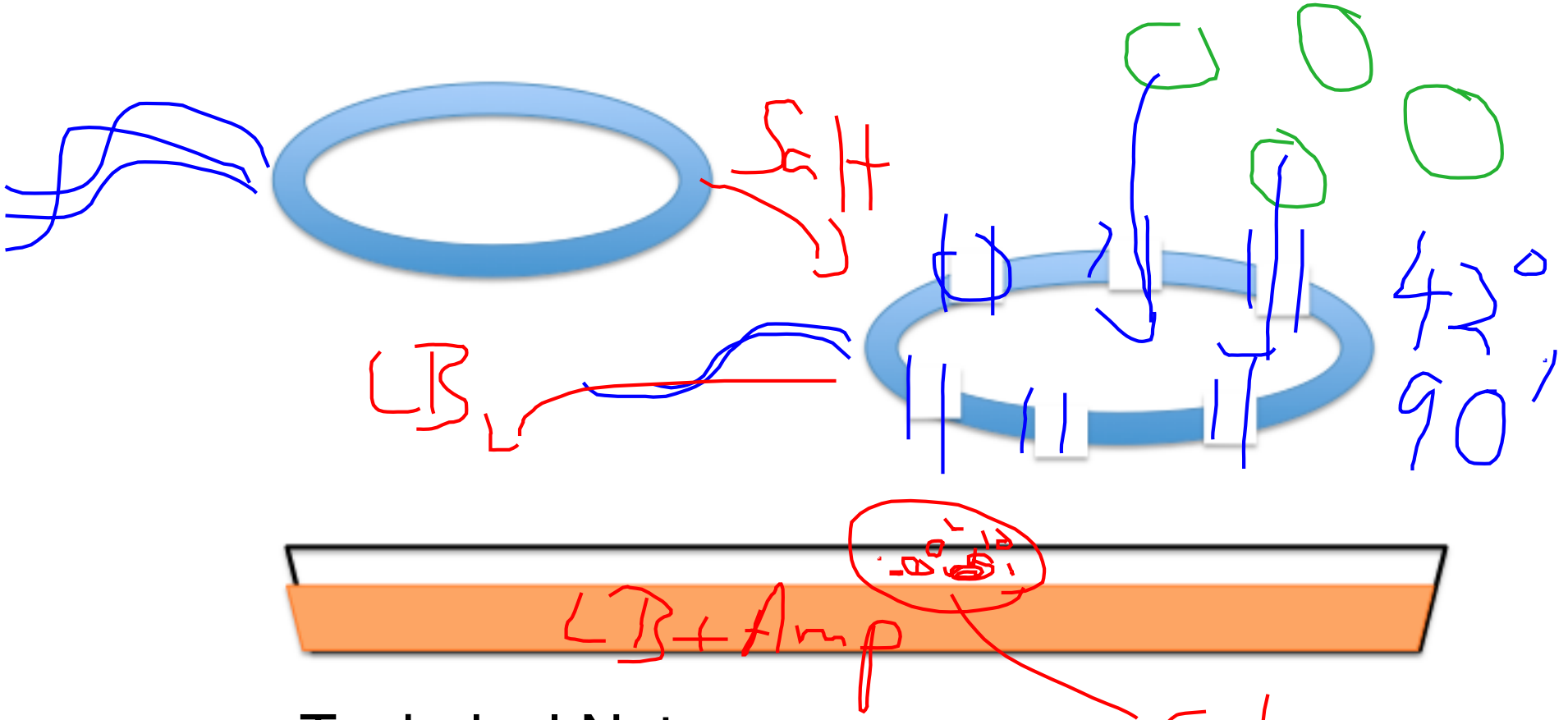
Room temp 10 minutes,
then clean up again!

Transformation Reactions

Tube	Transformation	Expectation	What if…?
Not done	Nothing		
1	pCX-EGFP 5ng	lots	
2	Bkb+insert, no ligase		lots?
3	Bkb, + ligase	few	lots?
4	Bkb + insert + ligase		



About transformations…



Technical Notes:

- Treat competent cells gently
- Use sterile technique to plate

Today and next week in lab….