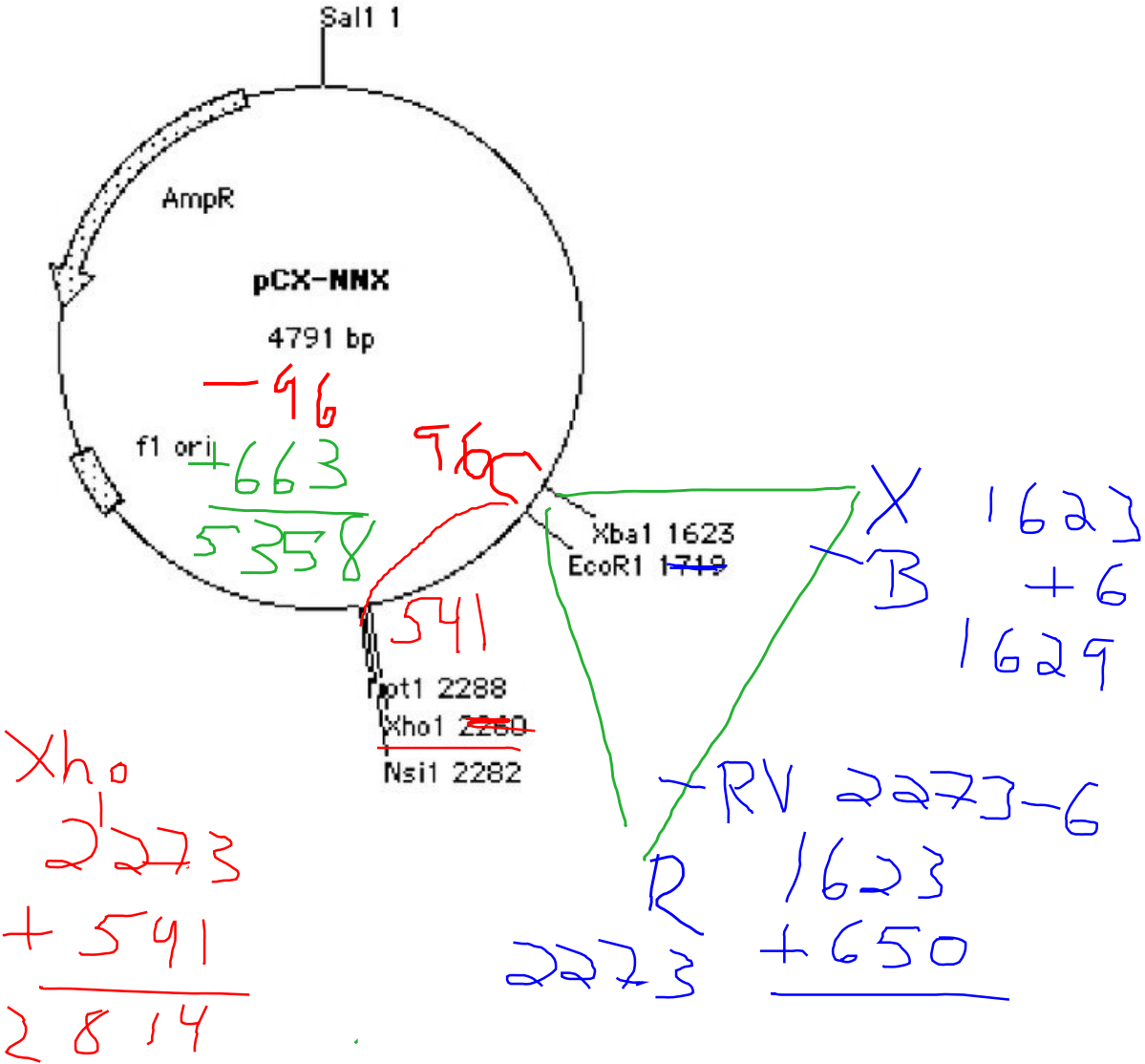


DNA Engineering: M1D4 Lab Talk

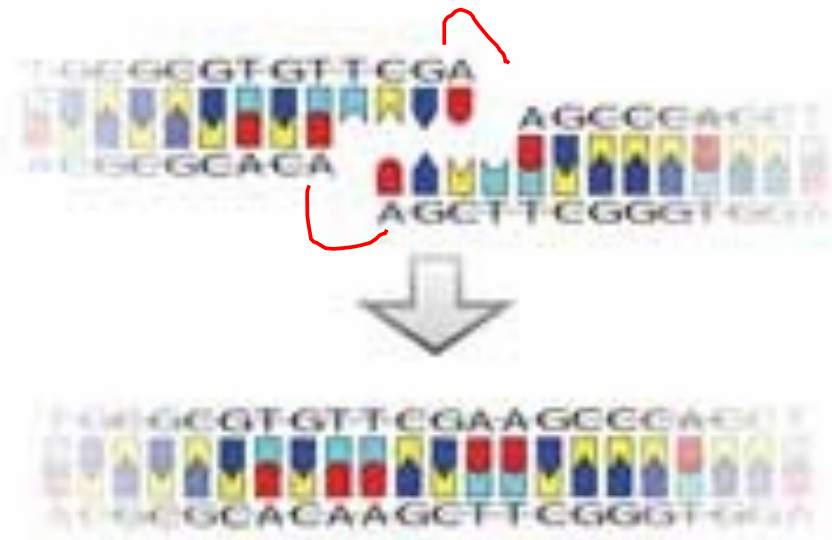
20.109 (F11)

09.27.11

Quick review of FNT...



About ligations...



Ligase

requires ATP

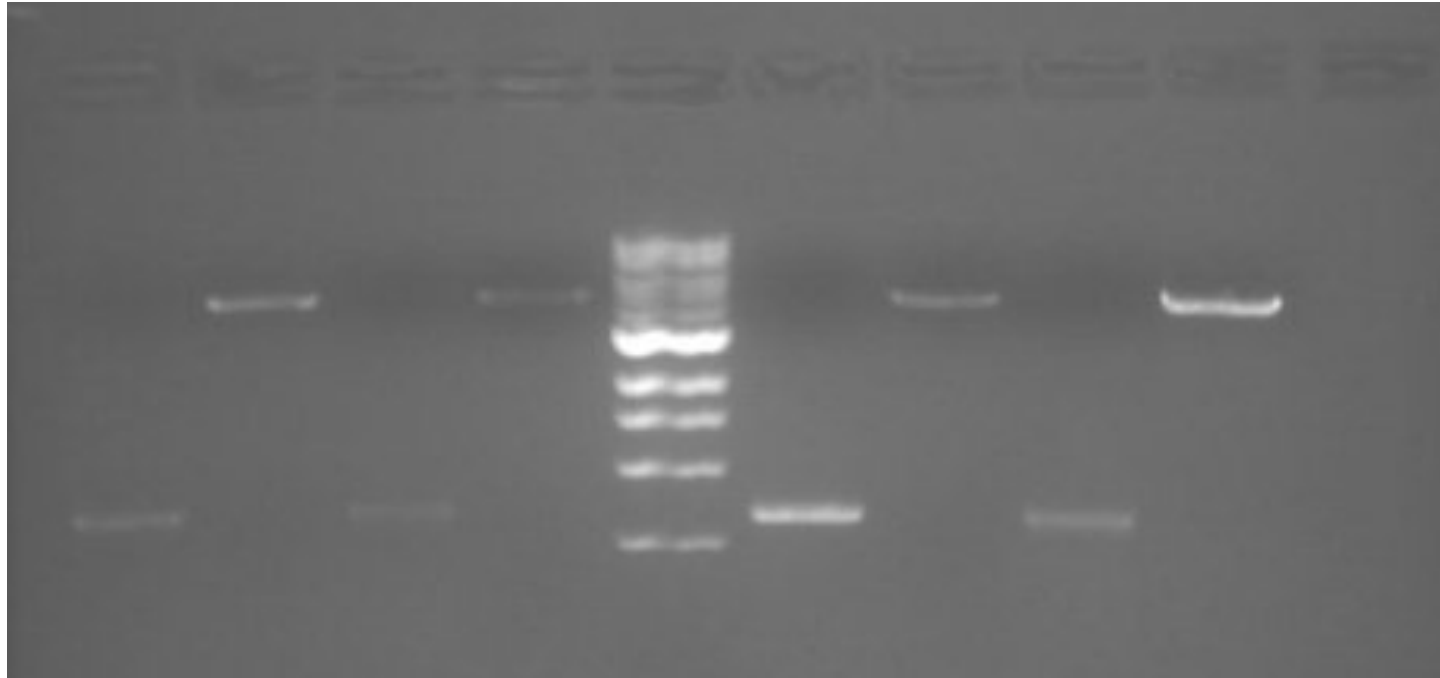


What affects efficiency?

Temp 16°/RT

[DNA] Ratio Bkb:insert

Your Data



Ligation Reactions

Controls for: UNCUT single cut Exp +

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

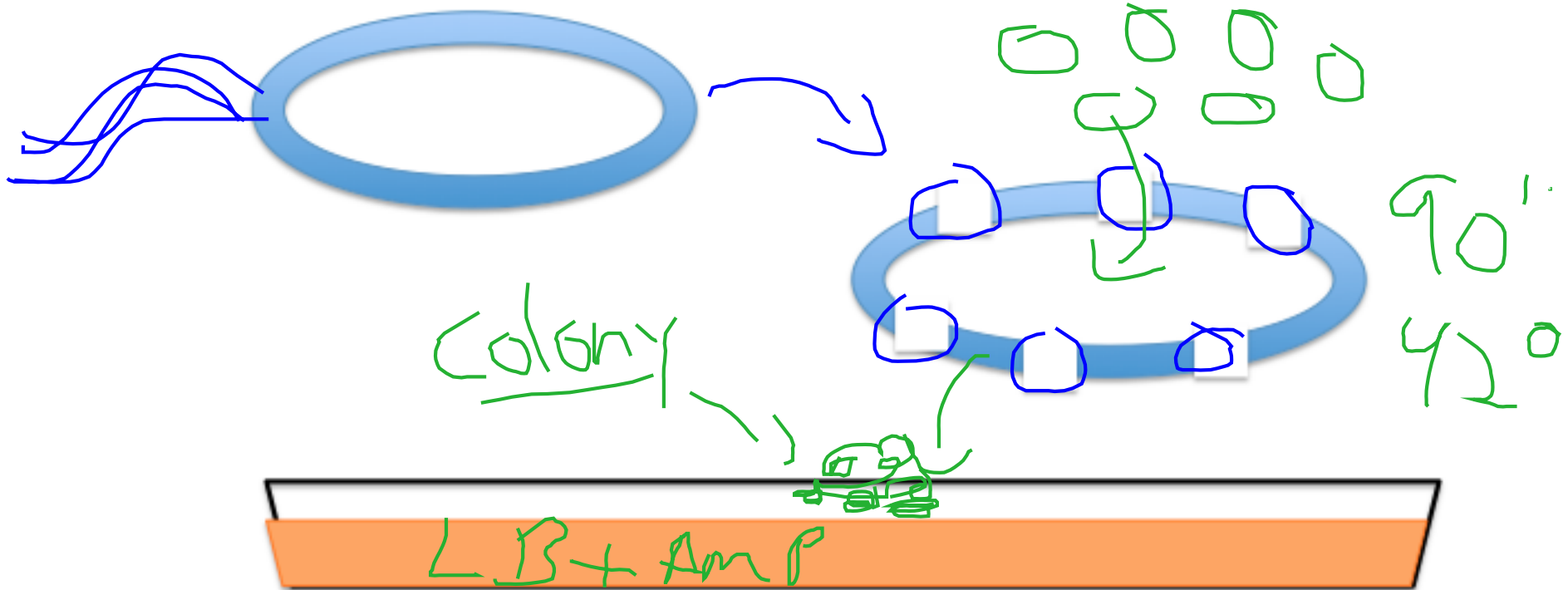
Room temp 10 minutes,
then clean up again!

Transformation Reactions

Tube	Transformation	Expectation	What if...?
Not done	Nothing	⊗	contam plates
1	pCX-EGFP 5ng	LOTS	cells?
2	Bkb+insert, no ligase	⊗	uncut
3	Bkb, + ligase	⊗ few	lots of / cut
4	Bkb + insert + ligase	⊙	

|

About transformations...



Technical Notes:

- Treat competent cells gently
- Use sterile technique to plate

Cold
don't vortex

Today and next week in lab....