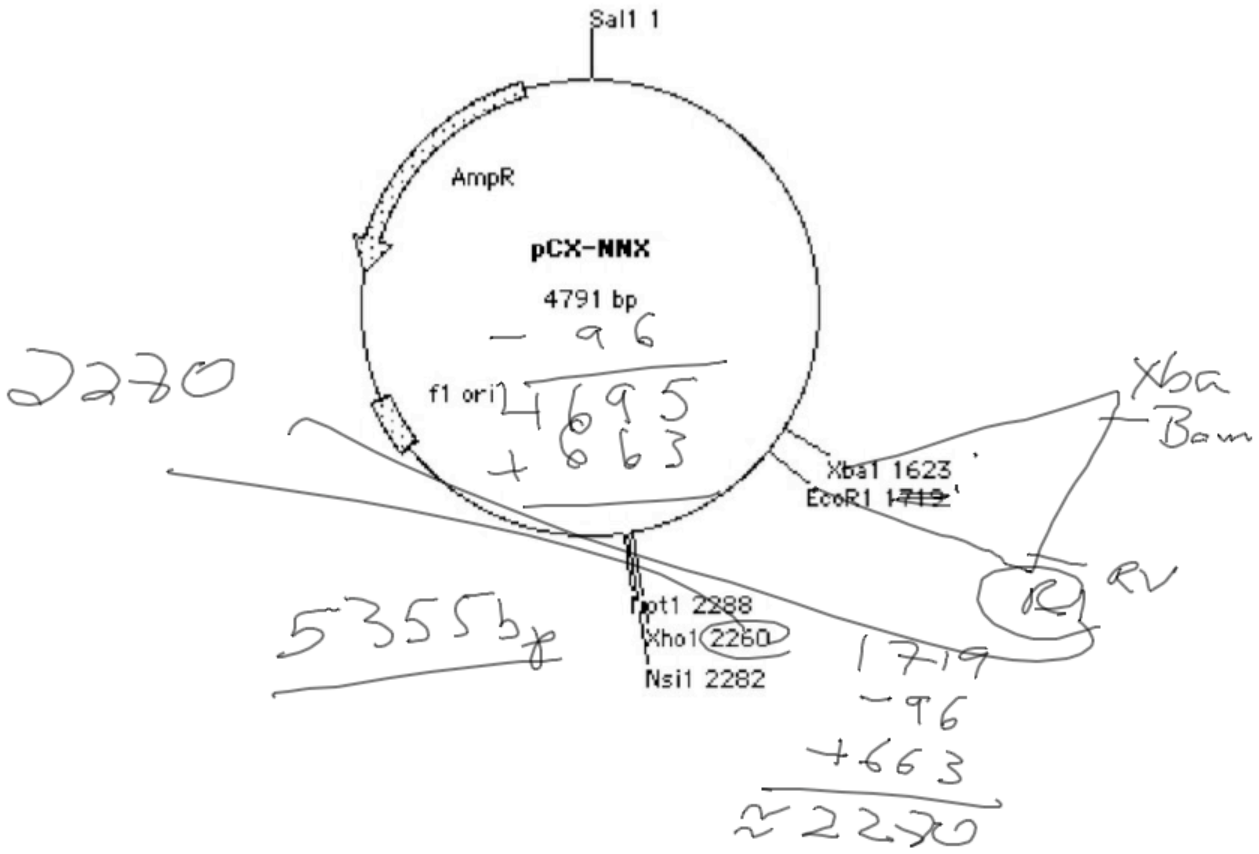
DNA Engineering
M1D4

20.109

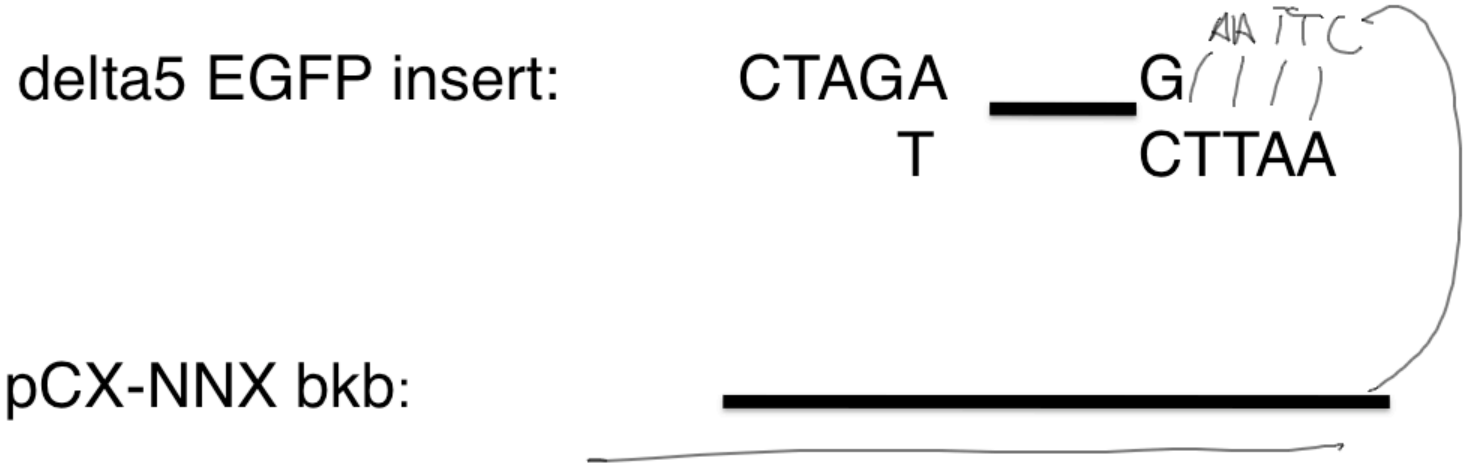
09.23.10

Quick review of FNT…



Last time in 20.109…

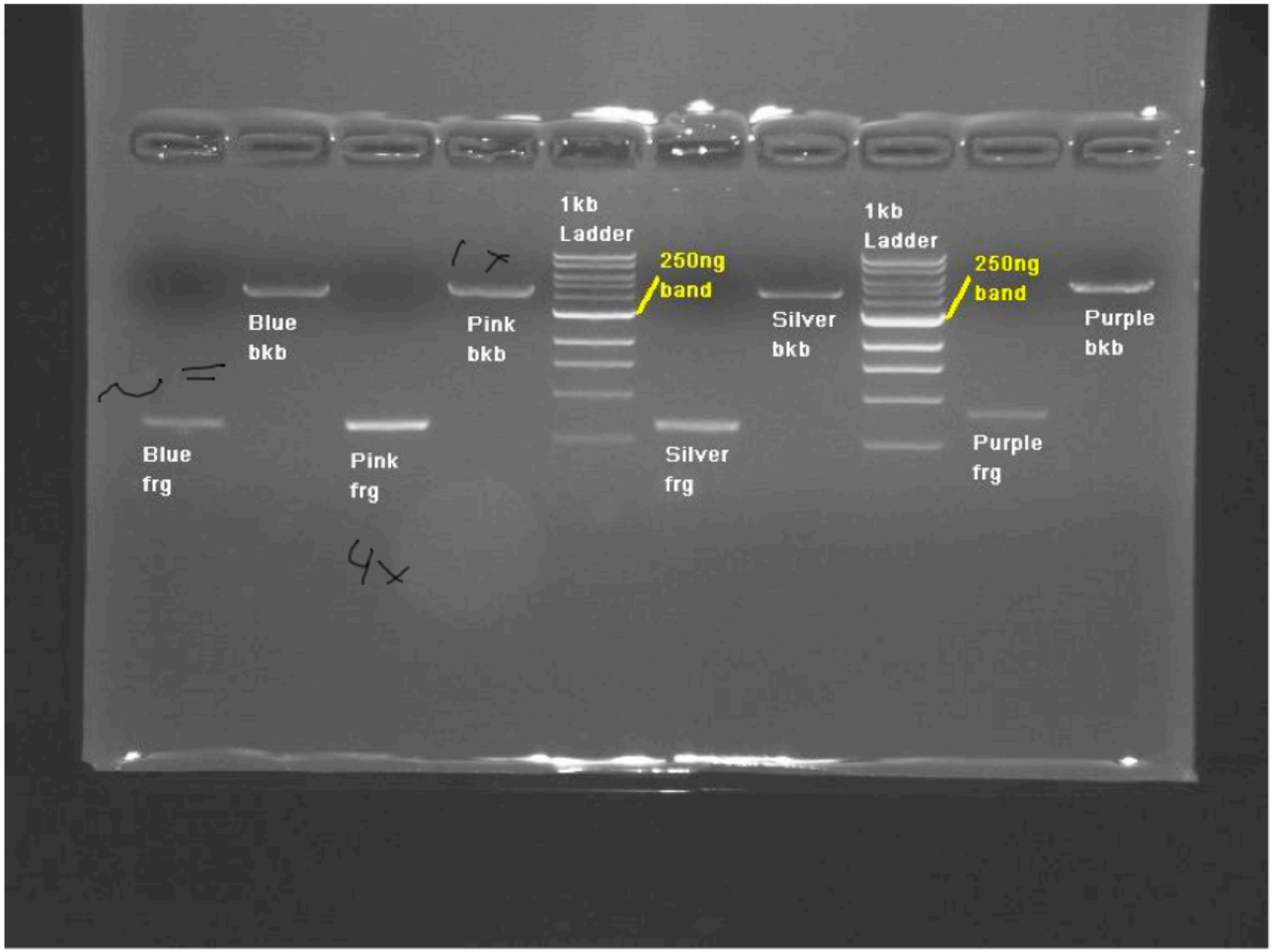
delta5 EGFP insert:



pCX-NNX bkb:

Today: ligation and transformation

Your Data


1kb
Ladder
250ng
band
Blue
bkb
Pink
bkb
Silver
bkb
1kb
Ladder
250ng
band
Purple
bkb
Blue
frg
Pink
frg
Silver
frg
Purple
frg
1x
4x

Ligation Reactions

Controls for:

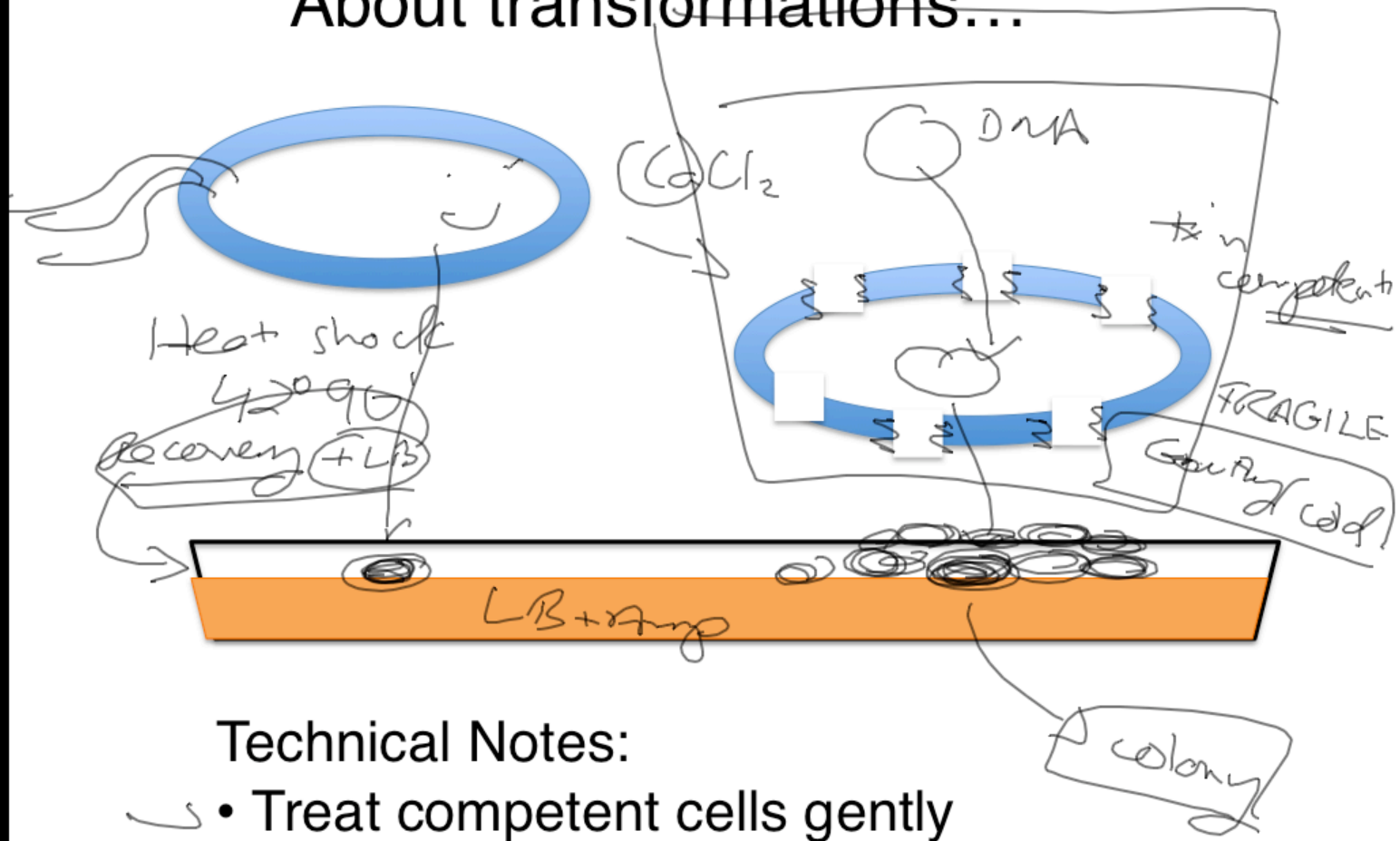
	bkb + insert, no ligase	bkb only, plus ligase	bkb + insert, plus ligase
pCX-NNX bkb	? µl	? µl	? µl
PCR insert	? µl	xxx	? µl
10X Ligation Buffer^	1.5 µl	1.5 µl	1.5 µl
T4 DNA Ligase	xxx	0.5 µl	0.5 µl
Water	To 15 µl not including volume of enzyme		

Room temp 10 minutes,
then clean up again!

Transformation Reactions

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

About transformations…



Technical Notes:

- Treat competent cells gently
- Use sterile technique to plate