

EXP. NUMBER	EXPERIMENT/SUBJECT	DATE	44	
NAME	LAB PARTNER	LOCKER/DESK NO.	COURSE & SECTION NO.	

6/25/12

- Made digest of plasmid backbone, K174015, I13401
- Made cultures NRP-1... NRP-6, K174015, I13401, J04450

6/26/12

- ~~BA~~ did ligation of K174015, I13401, and plasmid backbone

Ligation

- 1) Incubate at 37°C for 30 min
- 2) heat shock at 80°C for 20 min
- 3) d H₂O 11 λ

Ligase Buffer	2 λ
Backbone	2 λ
Promoter	2 λ (Cd promoter - K....)
GFP	2 λ (GFP+stop - I....)
Ligase	1 λ
	<u>20 λ</u>

- 4) room temperature for 10 min
 - 5) heat shock at 80°C for 20 min
- started growth of comp. cells.

6/27/12

- Made competent cells, used in transformation with the ligation

- 1) Centrifuge cells at 8000 rpm for 5 min.
- 2) Resuspend in 1 mL ice-cold CaCl₂ by vortexing.
- 3) Leave on ice for at least 30 min.
- 4) Spin for 30 sec. in microcentrifuge tube
- 5) Resuspend ~~ea~~ pellet in ~~150 λ~~ ice-cold CaCl₂ by flicking tube.
- 6) Distribute 50 λ into each tube
- 7) Add 5 λ of ligation DNA to each tube
- 8) Incubate on ice for 30 min
- 9) Heat shock at 42°C for 30 sec., then chill on ice for 5 min
- 10) Add 1 mL of broth to each, incubate for 1 hour at 37°C, and plate.

SIGNATURE	DATE	WITNESS/TA	DATE
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