

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

- ~~did~~ 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 H<sub>2</sub>O 11  $\lambda$ 

Ligase Buffer	2 $\lambda$
Backbone	2 $\lambda$
Promoter	2 $\lambda$ (Cd promoter - K....)
GFP	2 $\lambda$ (GFP+stop - I....)
Ligase	1 $\lambda$
	20 $\lambda$

- 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 1ml ice-cold CaCl<sub>2</sub> by vortexing.
- 3) Leave on ice for at least 30 min.
- 4) Spin for 30 sec. in microcentrifuge tube
- 5) Resuspend ~~ca~~ pellet in ~~150~~ <sup>150</sup>  $\lambda$  ice-cold CaCl<sub>2</sub> by flicking tube.
- 6) Distribute 50  $\lambda$  into each tube
- 7) Add 5  $\lambda$  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 1ml of broth to each, incubate for 1 hour at 37°C, and plate.

SIGNATURE	DATE	WITNESS/TA	DATE
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• Slants for: \_\_\_\_\_ Continued... 6/27/12

- I 13401 (Amp)
- I 13401 (Amp)
- J04450 (Amp)
- K 174015 (Amp)

• Started Cultures of:

- K 176012 (Amp)
- K 299009 (Amp)
- K 176011 (Amp)

• Poured New Plates (chlor)

• ~~Plasmid Preps for:~~ 6/28/12

- ~~- K 174015 (Amp)~~
- ~~- I 13401 (Amp)~~
- ~~- I 13401 (Amp)~~
- ~~- J04450 (Amp)~~

• Replenished Plasmid Prep Materials

6/28/12

• Plasmid Preps for:

- K 29909      - J04450
- I 13401      - I 13401
- K 176011    - ~~K 29909~~ 174015
- K 176012

• Made liquid cultures for:

- same 7 as above

• Plates for:

- same 7 as above

• Slants for:

- same 7 as above

6/29/12

• Results

• Lots of growth for all 7 except...

- K 174015 (very minimal)

- leaving it over wknd

- J04450 most likely contaminated

- green fluorescence... supposed to be red

- liquid culture/plates discarded.

- The following showed green fluorescence

- K 29909

- K 176012

- K 176011

- The following did not:

- K 174015

- I 13401

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