

EXP. NUMBER	EXPERIMENT/SUBJECT	DATE	39
NAME	LAB PARTNER	LOCKER/DESK NO.	COURSE & SECTION NO.

6/1/12  
PCR Amplify

Cd Promoter (1)  
GFP+stop (2)  
NRP-1 (3)  
J04450 (4)  
Water (5)

- Placed tubes into pre heated block  
- Reduced extension time to 2"  
- Reduced cycles to 35

#7 → 8 → 10 → 9

PCR Enzyme Solution

dH <sub>2</sub> O	17λ	× 5.5 =	93.5
Buffer	2.5λ	=	13.75
MgCl <sub>2</sub>	1.5λ	=	8.25
VR	1λ	=	5.5
VF <sub>2</sub>	1λ	=	5.5
dNTP	1λ	=	5.5
Tag	1λ	=	5.5
	<u>24λ</u>		

~~6-4~~

Add 24λ solution to 1λ DNA  
Vertex - Start Program 7  
Add 1 drop Mineral Oil  
Tightly cap + put in PCR machine  
Run overnight

6-4-12

Run Gell with PCR  
Amplified

Cd Promoter (1)  
GFP + stop (2)  
NRP-1 (3)  
J04450 (4)  
Water (5)

Picture Shaky

- Look @ pic before getting rid of gel  
- Try setting up on ice  
- Reduce cycles to 30  
- Up annealing to 63°C

Run ~~NRP-1~~ + water  
Cd Prom.

SIGNATURE	DATE	WITNESS/TA	DATE
-----------	------	------------	------

EXP. NUMBER	EXPERIMENT/SUBJECT	DATE	38	
NAME	LAB PARTNER	LOCKER/DESK NO.	COURSE & SECTION NO.	

6/1/12

- made new cultures of J04450, NRP-1, I13401, K174015, also made plates

- Started Making Competent Cells (p. 15)

- obtained previous competent E. coli cells
- 1) Add 4 mL of ice-cold  $CaCl_2$ . Store on ice (good in 3 min)
- 2) Recover cells through centrifugation. (microcentrifuge 1 min.)
- 3) Decant fluid. Resuspend in ~~200~~ 200  $\mu$ l of  $CaCl_2$ .
- 4) Transferred 50  $\lambda$  of suspension into 4 different tubes. Each tube had different amounts and concentrations of ligation mix.

Tubes:	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>
	3 $\lambda$ of Steven Ligation mix	7 $\lambda$ Steven's mix	3 $\lambda$ Will's mix	7 $\lambda$ Will's mix
	50 $\lambda$ competent cells	50 $\lambda$ competent cells	50 $\lambda$ comp. cells	50 $\lambda$ comp. cells

\* For concentration of ligation mixes refer to page 36 (p. 18)

↓ 5) Put on ice (started 11:30 A.M.)  
2pm - Heat shock 45°C 2'  
ice  
Add 1ml LB - 37° 1hr

Plate on chlor plates

SIGNATURE	DATE	WITNESS/TA	DATE
-----------	------	------------	------