

7-25-12

- Charlotte Observer Reporter

- Gel from PCR

- N.D. 1-6  $\approx$  100 bp

- N.D. 7  $\approx$  1000

- N.D. 8  $\approx$  1000

- I 13401  $\approx$  -

- K176011  $\approx$  -

- K216005  $\approx$  -

- K174015  $\approx$  -

- Digest/cut of Chlor backbone  
\* from NEB B.B. assembly kit

b.b.	
dH <sub>2</sub> O	3.5 $\lambda$
Buffer 2	1
BSA	.5
Eco RI	.5
Xba I	
Spe I	
Pst	.5
Plasmid	4
	10 $\lambda$

Cut 37° for 15' (or more)  
80° for 20' (inactivate)

Ligation:	
Promoter	2 $\lambda$
Reporter	2 $\lambda$
Backbone	2 $\lambda$
10x Buffer	2 $\lambda$
Ligase	1 $\lambda$
dH <sub>2</sub> O	11 $\lambda$
	20 $\lambda$

Room Temp.  
10'  
then  
80° for 20 min.

Transformation:

50  $\lambda$  cells + 5  $\lambda$   
10  $\lambda$  ligation from left

ice 2'  
42° 30'  
ice 2'  
- add 1 mL LB -- 1 hour at 37°C  
(NO A.B.)

Plate

1 - Cl <sup>-</sup> GFP 5 $\lambda$	4 - NO <sub>3</sub> <sup>-</sup> GFP 10 $\lambda$
2 - Cl <sup>-</sup> GFP 10 $\lambda$	5 - 1 $\lambda$ low N.D.
3 - NO <sub>3</sub> <sup>-</sup> GFP 5 $\lambda$	6 - 1 $\lambda$ high N.D.