

8/15/13

- made liquid culture of transformation 2 (Cd Promoter + delta phi activator)
- digested GFP-S, ligated GFP-S + PO Promoter

### Digest

1)	plasmid DNA	5 $\lambda$	2) 37° for 10 min
	dH <sub>2</sub> O	38 $\lambda$	
	10x NE Buffer 2	5 $\lambda$	3) 80° for 20 min
	Xba-I	1 $\lambda$	
	Pst-I	1 $\lambda$	
		50 $\lambda$	

### Ligation

1)	upstream DNA	1.5 $\lambda$	2) room temp. 10 min
	downstream DNA	1.5 $\lambda$	
	backbone*	1 $\lambda$	
	10x T4 Ligase Buffer	2 $\lambda$	
	T4 DNA Ligase	1 $\lambda$	
	dH <sub>2</sub> O	13 $\lambda$	
		20 $\lambda$	

\* note - used chlor backbone

### Cadmium Tests Procedure

1) Gather X amount of tubes, fill each with 4ml of LB + Kan.

2) Labeling

A = DH5 $\alpha$ (control)	H = 1-4C2	O = 2-2C1	1 = 0mM Cadmium
B = 1-2C2	I = 1-4C3	P = 2-6C1	2 = 5mM Cadmium (209)
C = 1-2C3	J = 1-5C1	Q = 2-6C2	3 = 50mM Cadmium (205)
D = 1-3C1	K = 1-5C3	R = 2-7C1	Concentration Codes
E = 1-3C2	L = 2-1C1	S = 2-7C2	
F = 1-3C3	M = 2-1C2	<del>R</del> T = 2-7C3	
G = 1-4C1	N = 2-1C3		

Cell codes (pipet 100  $\lambda$  into each set)

3) Mix by inversion, loosen caps, incubate

\* Note, these are all Cd Prom + GFP

1 denotes normal Cd Prom, 2 denotes mutagenic Cd Prom.

# Cadmium Test Results - B

Sample	Concentration	OD 600	Fluorescence	Ratio
Control DHSa (sample A)	(1) 0 mM	0.245	0.710 (I)	28.979
	(2) 1 mM	0.362	0.187 (II)	51.657
	(3) 5 mM	0	0.272	—
	(4) 10 mM	0	0.478	—
	(5) 50 mM	0.759	0.095 (II)	12.516
	(6) 100 mM	0.888	<del>0.117 (I)</del> 0.159 (II)	17.905
Slant - K824008 (sample B)	0 mM	0.425	<del>0.020 (I)</del> 0.917 (I)	21.576
	1 mM	0	<del>0.70 (I)</del> 0.262 (I)	—
	5 mM	0	0.963	—
	10 mM	0	0.963	—
	50 mM	0.910	<del>0.149 (I)</del> 0.115 (II)	14.198
	100 mM	0.880	<del>0.064 (I)</del> 0.234 (II)	26.591
Trans 3-1 (C)	"	0.261	<del>0.055 (I)</del> 0.859 (II)	32.912
	"	0.172	<del>0.060 (I)</del> 0.848 (I)	49.302
	"	0	0.704	—
	"	0	0.487	—
	"	0.828	<del>0.141 (I)</del> 0.163 (II)	19.686
	"	0.863	0.244 (II)	28.273
mK824008A New 2 (D)	"	0.337	0.823 (I)	24.421
	"	0.501	0.196 (II)	39.122
	"	0	0.151 (II)	—
	"	0	0.397	—
	"	0.719	0.075 (II)	10.431
	"	0.779	0.113 (II)	14.506
mK824008A old 3 (E)	"	0.587	0.131 (II)	22.317
	"	0.869	0.960 (I)	16.8
	"	0	0.337 (I)	—
	"	0	0.689	—
	"	0.796	0.136 (II)	17.085
	"	0.901	0.230 (II)	25.527
mK824008A old 6 (F)	"	0.260	0.878 (I)	33.769
	"	0	0.171 (I)	—
	"	0	0.179 (I)	—
	"	0	0.672	—
	"	0.705	0.086 (II)	12.198
	"	0.601	0.278 (II)	46.256/54