

8-7-13

Ligation:

	(up)		+	(down)
1.	Cd Prom.		+	Ogr. Act.
2.	Cd Prom.		+	delta Phi

on ice ---

~~Block~~

upstream 1.5 x

downstream 1.5

Backbone (kan) 1

10 x Buffer 2

Ligase 1

d H₂O 13

- mix. then pulse

room temp = 10⁻

Transformation:

- 5 μ l DNA + 50 μ l c.c.

- Ice = 2⁻

- 42^o = 30⁻

- Ice = 2⁻

- 1 ml warm SOL

- 37^oC 60⁻

- spin down

- resuspend

- plate

48

Mutagenic PCR

8-7-13

10x Buffer	2.5	x 8 = 20
Unsolvent dNTP	1.25	10
VF ₂	.5	4
VR	.5	4
25mm MgCl ₂	5	40
1mm MnCl ₂	3.75	30
d H ₂ O	6	48
top	.5	4
	<u>20</u>	

plasmid: 5x

1. K174015 (blue w/ink)
2. K174015 (red)
3. K174015 (blue)
4. K174015 (red)
5. 0805-16 p.46 (blue)
6. 0805-17 p.46 (blue)
7. K824008 #1 (brown)

✓
 Samples = Slab #5 (1-7)

↳ large on left
 ↳ on right

8/7/13

- completed 2 ligations

ligation 1: Cd Promoter digest + cgr activator digest +
Kan. backbone digest

ligation 2: Cd Promoter digest + phi R73 delta activator digest +
Kan. backbone digest

1) On ice

2) upstream part - 1.5 λ

downstream part - 1.5 λ

backbone - 1 λ

10x Ligase Buffer - 2 λ

T4 DNA Ligase - 1 λ

dH₂O - 13 λ

3) vortex then pulse (centrifuge)

4) room temp. for 10 min.

- completed 2 transformations using fresh DH5 α competent cells

*note: 5 λ of ligation DNA used

Cadmium test Results-2

Sample	Concentration	OD600	Fluorescence	Ratio
Control - DHSd (sample A)	1. 0 mM	.342	.678	1.9825
	2. 5 mM	.020	.263	13.15
	3. 50 mM	.141(1)	.761(1)	5.3972
K824008 (sample B)	0 mM	.886	.683(1)	7.7088
	5 mM	.040	.277	6.925
	50 mM	1.336	.695(1)	5.2021
mK824008A old-1 (C)	0 mM	.980	.645(1)	6.5816
	5 mM	.590	.064	.1085
	50 mM	.370	.040	.1081
mK824008A old-2 (D)	"	.974	.337(1)	3.46
	"	.062	.091	1.4677
	"	.102	.690	6.7647
mK824008A old-3 (E)	"	.892	.327(1)	3.6659
	"	.183	.123	.6721
	"	1.325	.111(1)	8.3774
mK824008A old-4 (F)	"	.269	.425(1)	15.7993
	"	.545	.415	.7615
	"	.000	.198(1)	19.8
mK824008A old-5 (G)	"	.216	.345(1)	15.9722
	"	.587	.407	.6934
	"	.121	.827(1)	683.4711
mK824008A old-6 (H)	"	.186	.1514(1)	27.6344
	"	.508	.194	.3819
	"	.112	.779(1)	69.5536
mK824008B new-1 (I)	"	.179	.273(1)	15.2514
	"	.531	.281	.5292
	"	.174	.933(1)	53.6207
mK824008B new-2 (J)	"	.206	.351(1)	17.0388
	"	.489	.382	.7812
	"	.167	.054(1)	32.3353
mK824008A new-1 (K)	"	.351	.452(1)	12.8775
	"	.216	.242	1.1204
	"	.303	.270(1)	89.1089

Sample	Concentration	OD600	Fluorescence	Ratio
mK8240084 new-2 (L)	0 mM	.353	.393	1.1133
	5 mM	.068	.322	4.7353
	50 mM	1.32	.240 (1)	1.8181
Trans. 3-1 (M)	0 mM	.860	.120 (1)	1.3953
	5 mM	.044	.341	7.75
	50 mM	.246	.600 (1)	24.3902