

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 H<sub>2</sub>O 13

- mix. then pulse

room temp = 10<sup>-</sup>

Transformation:

- 5  $\mu$ l DNA + 50  $\mu$ l c.c.

- Ice = 2<sup>-</sup>

- 42<sup>o</sup> = 30<sup>-</sup>

- Ice = 2<sup>-</sup>

- 1 ml warm SOL

- 37<sup>o</sup>C 60<sup>-</sup>

- spin down

- resuspend

- plate

48

Mutagenic PCR

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10x Buffer	2.5	x 8 = 20
Unsolymed dNTP	1.25	10
VF <sub>2</sub>	.5	4
VR	.5	4
25mm MgCl <sub>2</sub>	5	40
1mm MnCl <sub>2</sub>	3.75	30
d H <sub>2</sub> 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)

↳ have on left  
 ↳ on right