

Stem Allen's ~~part~~ ~~part~~

8-22-13

Digest:

- Phi Delta 9
- 3-up { 1. Cd Prom. + Activator  
 2. Cd Prom. + Ogr activator (colony 1)  
 3. Cd prom. + Ogr activator (colony 2) for each person
- 2-down { 4. PO prom. + GFP-S (colony 1)  
 5. PO prom. + GFP-S (colony 2)

Plasmid	DNA	UP (λ)	down (λ)	<del>140λ</del>	<del>20λ</del>	<del>4λ</del>	<del>4λ</del>
	dH <sub>2</sub> O	8	8	<del>140λ</del>	<del>20λ</del>	<del>4λ</del>	<del>4λ</del>
	10x NE Buffer 2	35	35	<del>140λ</del>	<del>20λ</del>	<del>4λ</del>	<del>4λ</del>
	EcoRI	5	5	<del>140λ</del>	<del>20λ</del>	<del>4λ</del>	<del>4λ</del>
	Xba-I	1	1	<del>140λ</del>	<del>20λ</del>	<del>4λ</del>	<del>4λ</del>
	Spe-I	1	1	<del>140λ</del>	<del>20λ</del>	<del>4λ</del>	<del>4λ</del>
	Pst-II	1	1	<del>140λ</del>	<del>20λ</del>	<del>4λ</del>	<del>4λ</del>

37° --- 10 mins.

80° --- 20 mins

upstream parts (3)

DNA	8λ	x 4	
dH <sub>2</sub> O	35λ		140λ
10x NE Buffer 2	5λ		20λ
Eco RI	1λ		4λ
Spe -I	1λ		4λ

8-22-13  
Skeneaden

cont'd:

Ligations:

1. (Cd Prom + Phi Delta Act.) + (Po Prom. + 6FP-5 C1)
2. " " " " " " C2
3. (Cd. Prom + ogr Act. C1) + (Po Prom. + 6FP-5 C1)
4. " " " " " " C2
5. (Cd Prom. + ogr Act. C2) + (Po Prom. + 6FP-5 C1)
6. " " " " " " C2

UPstream <del>plasmid</del> DNA	1.5	1	
downstream DNA	1.5		
backbone (ohbr)	1	* 6.6 =	6.6 ✓
10xTY Ligase Buffer	2		13.2
TY DNA Ligase	1		6.6
dH <sub>2</sub> O	13		85.8 ✓
	<hr/>		<hr/>
	20		17

Transformations (8-23-13)

- 1-6 -- same as above
- 50ul cells + 5 ul DNA
- ice water = 2 mins.
- 42° = 30 sec.
- 2 mins = ice
- 1 ml warm LB
- incubate 1 hour
- plate