

EXP. NUMBER	EXPERIMENT/SUBJECT	DATE	63
NAME	LAB PARTNER	LOCKER/DESK NO.	COURSE & SECTION NO.

7/31/12 Ars. Prom.  
 Digest: K174015 (Cld Prom) - 1+2  
 (b) I13401 (GFP) - 1+2  
 K081014 (RFP) - 1+2  
 Chlan Backbone  
 Lead Prom

	Prom	Rep	Backbone
dH <sub>2</sub> O	3.5 $\lambda$	3.5 $\lambda$	7 $\lambda$
Buffer 2	1	1	2 $\lambda$
BSA	.5	.5	1
Eco RI	.5	-	1
Xba I	-	.5	-
Spe I	.5	-	-
<del>RSF Pst</del>	-	.5	1
Plasmid	4	4	8
Total	10 $\lambda$	10 $\lambda$	20 $\lambda$

370 -- 15', 800 -- 20'

Ligation Protocol:

Prom.	2 $\lambda$
Rep.	2
BB.	2
10x Buffer	2
Ligase	1
dH <sub>2</sub> O	11
	<u>20 <math>\lambda</math></u>

Room Temp: 10'  
 80°: 20'

Transformation:

1-5: Standard Soft 10  $\lambda$  DNA

\* 6-12: old cont cells -  
 - procedure from r. 46

Ligations:

- 1-8 are same as page 61

- Arsenic Prom: GFP1 (9)

- Lead Prom: GFP1 (10)

if enough B.B.

Present...

- Ars: RFP1 (11)

- PL: GFP1 (12)

Plasmid Prep: Arsenic Prom  
 Lead Prom  
 (H1) ~~1~~ 1 x high trans. 2  
 (H2) ~~1~~ 1 x high trans. 2  
 (L1) ~~1~~ 1 x low trans. 2  
 (L2) ~~1~~ 1 x low trans. 2

PCR: 6 samples above

dH <sub>2</sub> O	16.5	* 6.6 = <del>16.9</del> 16.9
Buffer	2.5	= 16.9
MnCl <sub>2</sub>	1.5	= 9.9
dNTPs	1	= 6.6
VF <sub>2</sub>	1	= 6.6
VR	1	= 6.6
Taq	.5	= 3.3

24  $\lambda$

cc: Ars/PB/H1/H2/L1/L2

- on left (back of previous page)

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7-31-12  
 Nitrate Detector Test:  

	Control	Calcium $\text{NO}_3^-$ (0.5M)
H1		
H2		
L1		
L2		

Control                      +  
 5 mL LB                      5 mL LB  
~~100 x~~ 10 x cells              10 x cells  
    200 x  $\text{CaNO}_3$  (0.5M)

8-1-12  
 - Started cultures of all transformations that grew  
 - also streaked on plate

Testing OLDER Transformations (7-30-12)  
 - 1<sup>st</sup> trans.

- 11 trans. in liquid culture  
 - 4 mL LB rich  
 - 1M Calcium Nitrate (400 to 500)  
~~100~~ - 100 x of cells  
 - Positive & Negative Control

8/1/12

- IT worked!  
 -3D →  
 K824002

8/2/12

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