

EXP. NUMBER	EXPERIMENT/SUBJECT	DATE	60
NAME	LAB PARTNER	LOCKER/DESK NO.	COURSE & SECTION NO.

7-26-12  
 #s 5-6 grew (plates)  
 - Started liquid cultures

Plasmid Preps:  
 - N.D. (Recovered)  
 - A-D  
 - J04450 (2010)  
 - J04450 (2011)

Started Cultures from stabs:  
 - 2 for each of following (all Amp.)  
 - I13401  
 - K174015  
 - K081014

PCR: (6)  
 - N.D. 1 for DNA  
 - A, B, C, D  
 - J04450 (2010)  
 - J04450 (2011)

Buffer	2.5	* 6.6 =	16.5
MgCl <sub>2</sub>	1.5	=	9.9
dNTP <sub>3</sub>	1	=	6.6
VF <sub>2</sub>	1	=	6.6
VR	1	=	6.6
Taq	.5	=	3.3
dH <sub>2</sub> O	16.5	=	108.9
	<u>24</u>		

6el:  
 Ladder | A | B | C | D | J10 | J11

7-27-12  
 - Growth in all liquid cultures  
 - ∴ 4 N.D.S

- Started Culture at: (2)  
 - I765000

- Kit Plates:

- J33201 -- Arsenic Promoter (1)  
 - 2012 plate 4 -- 2K

- Ampicillin  
 - I721001 -- Lead Promoter (2)  
 - 2012 plate 2 -- 166

- Transformation for those 2:  
 50 x cells + 1 x DNA  
 - 2° ice  
 - 30° 42°  
 - 2° ice (SOC)  
 - 1 mL LB (no AB) 1 hour 37°  
 - Plate

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EXP. NUMBER	EXPERIMENT/SUBJECT	DATE	61
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7-27-12 (cont.)  
 Making Cadmium Sensor -- Cod: GFP (2), Cod: RFP (2)

- Digest:
- I13401 (2) -- GFP
  - Kog104 (2) -- RFP
  - K174015 (2) -- Cadmium Promoter
  - Chlor Backbone

	Prom.	RFP	B.B.
dH <sub>2</sub> O	3.5 ml	3.5 ml	3.5 ml
Buffer 2	1	1	1
BSA	.5	.5	.5
EcoRI	.5	-	.5
XbaI	-	.5	-
SpeI	.5	-	-
Pst	-	.5	.5
Plasmid	4	4	4
	10	10	10

★ Plasmid Before Enzymes!

Transformation  
 - after Ligation

Sol Comp Cells + 10<sup>1</sup>  
 PNA  
 (1:9)

Ice 2'  
 42° 30'  
 ice 2'  
 - add 1 ml SOC  
 - no A.B.  
 - 37°C - 1 hour  
 - plate

Cut -- 37° for 15' (or more)

Inactivate -- 80° for 20'

Ligation Protocol:

Promoter	2 <sup>1</sup>	
Reporter	2	
Backbone	2	
10x Buffer	2	MM=18
Ligase	1	
dH <sub>2</sub> O	11	MM=97
	20 <sup>1</sup>	

Room Temp -- 10'  
 80° -- 20'

Ligations: (8)

- Cd Promoter 1	RFP 1 (C1R1)	1
- Cd Promoter 1	RFP 2 (C1R2)	2
- Cd Promoter 1	GFP1 (C1G1)	3
- Cd Promoter 1	GFP2 (C1G2)	4
- Cd Promoter 2	RFP 1 (C2R1)	5
- Cd Promoter 2	RFP 2 (C2R2)	6
- Cd Promoter 2	GFP1 (C2G1)	7
- Cd Promoter 2	GFP2 (C2G2)	8

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