

7-16-13

PCR w/ Primers : D15d

Mix

<del>Buffer</del>	<del>2.5</del>
<del>MgCl<sub>2</sub></del>	<del>1.5</del>
<del>dNTP</del>	<del>1</del>
<del>WDR</del>	<del>.5</del>
<del>WDR</del>	<del>.5</del>
<del>top</del>	<del>.5</del>
<del>dH<sub>2</sub>O</del>	<del>13.5</del>

Buffer	2.5	23.5 =	8.75
MgCl <sub>2</sub>	1.5		5.25
dNTP	1		3.5
DNA	5		17.5
top	.5		1.75
dH <sub>2</sub> O	13.5		47.25
	<u>24</u>		

results: Absolutely Nothing!

- no bands whatsoever

- Primers in tube
- M.M.
- top = lost
- PCR on
- put MM in hot

- 5 λ DNA
- 5 X primers

- using white MCF tube of D15d DNA

1  
Ace E  
-for  
-rev

2  
Ace F  
-for  
-rev

3  
1pt  
-for  
-rev

Green Arden  
Transformation  
7-16-13

- Lead Binding Protein -- 1
- + Ctrl -- PUC 19 -- 2

- 50  $\mu$ l C.C. + 5  $\times$  DNA (only  $\approx$  3 for C.B.P)
- ICE = 2'
- 42° = 30'
- ICE = 2'
- add 1ml warm SOC
- NO A.B.
- 37° = 1 hour
- plate

+ 6 plasmid prep  
- new l:8. culture

Spin down  
resuspension  
in LB

7/16/13

Gordon Ellison

Mix for Digest			
d H <sub>2</sub> O	2.5	x9	22.5
Buffer	1.1		9
BSA	.5		4.5
EcoRI	.5		4.5
Pst	.5		4.5
Plasmid	5		
<hr/>			
	10		

✓  
✓  
✓  
✓  
✓

Plasmids

- 1 J23100
- 2 J23102
- 3 J23104
- 4 J23104
- 5 J23100 #2
- 6 K174015
- 7 CD
- 8 K824008
- 9 LBP (Lead Binding Protein)
- 10 LBP