

## Transformation

- Steven Allen

- 6/21/13

- I721002 (Lead Binding Protein) -- 2
- + Control: pUC19 -- 2

- 50x CC + 2  $\lambda$  DNA

- ICE = 2

- 42° = 30'

- ICE = 2

- add 1ml warm SOC

- NO A.B.

• 37°C = 1 hour

- plate

## PCR check of

## NEW PCR machine

## program 001

1	K824008 A	2.5 $\mu$ DNA ↓	95°C	15'	} 39x
2	B		94°C	30"	
3	K176012		62°C	30"	
4	K299009		68°C	3.5'	
5	dH <sub>2</sub> O		68°C	20'	

Uses 0.2 ml tubes

Mix:

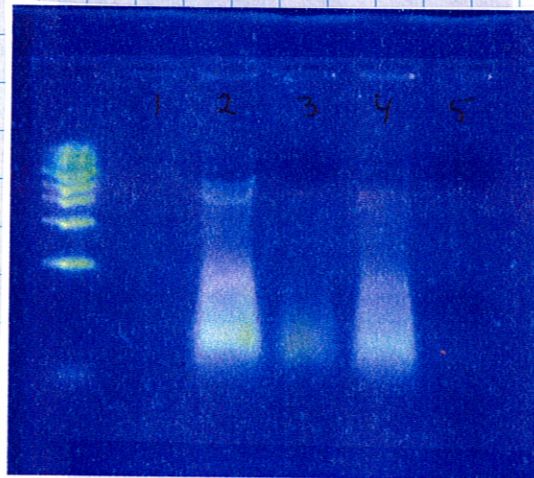
Buffer	2.5	$\times$ 5.5 =	13.75 ✓
Mg Cl <sub>2</sub>	1.5	=	8.25 ✓
dNTPs	1	=	5.5
VR	.5	=	2.75
VF2	.5	=	2.75
tag dH <sub>2</sub> O	<del>1.5</del> 1.5	=	<del>2.75</del> 8.8 ✓
	20 $\mu$ 2.5		

Tube 1 came open

Doublet in lane 2

Faint band in 4

5 is blank (neg. ctrl)



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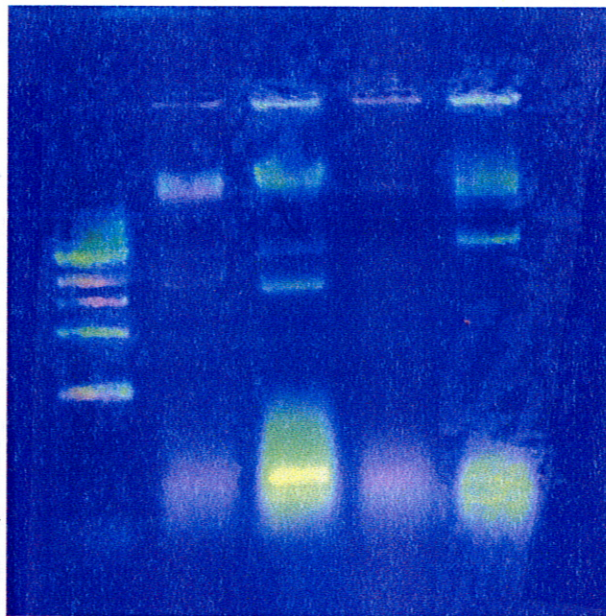
Restriction digest of: ~~K824008~~

1. K824008 A } Use 10  $\lambda$  of DNA  
2. K824008 B }  
3. K176012  
4. K299009

Mix:

Buffer 2	- 2 $\mu$ l	x 6	= 12 $\mu$ l
Eco RI	- .5 $\mu$ l		= 3 $\mu$ l
Pst I	- .5 $\mu$ l		= 3 $\mu$ l
dH <sub>2</sub> O	- 7 $\mu$ l		= 42 $\mu$ l
	<u>10</u>		<u>60</u>

1 & 2 identical



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GE plasmid prep of

K174015

K081014-2

K824012

K824009

Appeared  
infected. - ~~A~~ K081014-1

- ~~A~~ K824009-1

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Started Plasmid Prep of K824002, K824002 #2, and K824012.

1. Add 100 µl of 100 mg/ml ampicillin to 100 ml of K824002.
2. Grow overnight.
3. Harvest cells.
4. Wash cells with 100 ml of water.
5. Resuspend cells in 10 ml of water.
6. Add 100 µl of 100 mg/ml ampicillin to 100 ml of K824002 #2.
7. Grow overnight.
8. Harvest cells.
9. Wash cells with 100 ml of water.
10. Resuspend cells in 10 ml of water.