

7-8-13

Mix for Digest:

Buffer	1 $\lambda$	x	(10 + 1)	11
BSA	.5			5.5
EcoRI	.5			5.5
Pst	.5			5.5
Plasmid	5			55
dH <sub>2</sub> O	<u>2.5</u>			<u>27.5</u>
	10			110

Plasmids:

1. + control Puc 19
2. K824009 #2
3. K824002
4. #2 K824002
5. K081014
6. Lead binding protein
7. K824012
8. K081014
9. K824009 #1
10. K174015

Mix dH<sub>2</sub>O, Buffer, BSA, Enzymes - S<sub>x</sub> mix to S<sub>x</sub> DNA

Incubate

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Master mix (PCR)

✓ Buffer	2.5	x 11	=	27.5
✓ MgCl <sub>2</sub>	1.5		=	16.5
✓ dNTP	1		=	11
✓ VA	.5		=	5.5
✓ VF <sub>2</sub>	.5		=	5.5
✓ Taq	.5		=	5.5
✓ dH <sub>2</sub> O	13.5		=	148.5

20  $\mu$ l MM

5  $\mu$ l dNA

samples

1. lead binding protein
- 2 K174015
- 3 K824012
- 4 + control PUC19
- 5 K824002
- 6 K824002 #2
- 7 K824009
- 8 K081014 #2a
- 9 K081014 #2b
- 10 K824009 #2