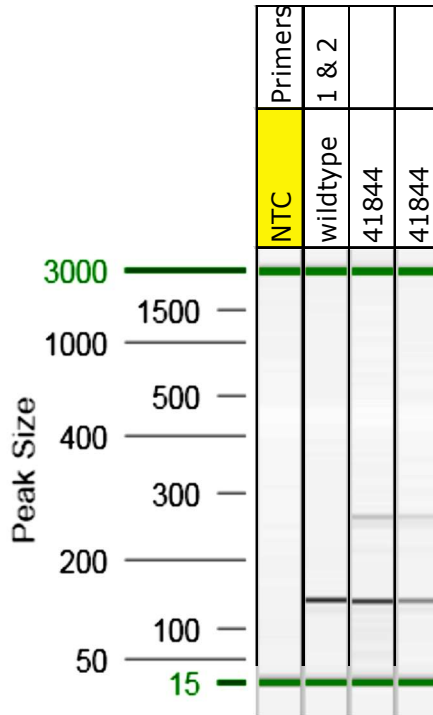




# GENOTYPING BY PCR PROTOCOL MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC



Sample ID	Assay	Well
ntc		A1
wt		B1
10466		A2
2		B2
3		A3
4		B3
5		A4
6		B4
7		A5
8		B5
9		A6
ntc		B6
wt		A7
32358		B7
3		A8
4		B8
5		A9
6		B9
7		A10
8		B10
9		A11
10		B11
11		A12
12		B12
13		C1
14		D1
15		C2
ntc		D2
wt		C3
31665		D3
3		C4
4		D4
5		C5
6		D5
7		C6
ntc		D6
wt		C7
13807-REG		D7
15		C8
16		D8
17		C9
18		D9
19		C10
20		D10
21		C11
22		D11
23		C12
ntc		D12

# GENOTYPING BY PCR PROTOCOL FORM

Mouse Biology Program: UC DAVIS

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## PCR Protocol: cKO

Reagent/ Constituent	Volume (µL)
Water	11.275
10x Buffer	2.5
25 mM MgCl <sub>2</sub>	1.7
5 M Betaine	6.5
10 mM dNTPs	0.5
DMSO	0.325
Primer 1: (20uM)	0.5
Primer 2: (20uM)	0.5
Taq Polymerase-5 Units/µl	0.2
DNA Sample	1.0
<b>TOTAL VOLUME OF REACTION:</b>	<b>25 µl</b>

### Comments on protocol:

- Use Touch-Down cycling protocol-first 10 cycles anneal at 65° C decreasing in temperature by 1.0° C; next 30 cycles anneal at 55° C.
- Betaine/DMSO is standardized due to high GC content in promoter regions and protocol may be tested without. Also, may adjust MgCl<sub>2</sub> to increase reaction or decrease non specific amplifications.

### Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting	94	5:00	1
2. Denaturation	94	0:15	\
3. Annealing steps 2-4 will cycle in sequence	65 to 55 (↓1°C/cycle)	0:30	> x 40
4. Extension	72	0:40	/
5. Final Extension	72	5:00	1
6. Finish	4	Hold	--

### Primers:

Primer Name	Nucleotide sequence (5'– 3')
1. Apex1-loxF	ggcaagttcagggcttactagacc
2. Apex1-loxR	GTAGTAAGAGCTGGCAGTTCCAACC

### Electrophoresis Protocol:

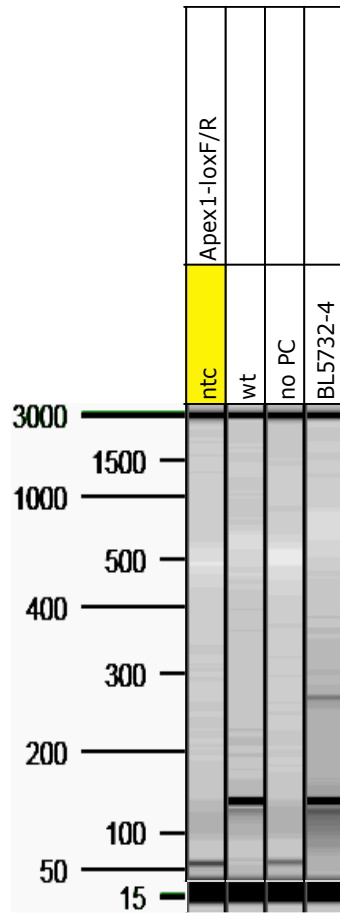
% Agarose: 1.5 Volts : 90

Estimated Running Time (min): 90

Primer Combinations	Band size (bp)	Genotype
1 & 2	150	wildtype
1 & 2	281	Floxed

\*Targeting Confirmed

Gel Image



Allele Map

