

# GENOTYPING PROTOCOL

## MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: CR11750 Asb13 EXDEL

Protocol: GoTaq® G2 Colorless Master Mix(Promega)

Reagent/Constituent	Volume (µL)
Water	4.5
GoTaq® G2 Colorless Master Mix,2X	7.5
Primer 1. (stock concentration is 20µM) comF	0.5
Primer 2. (stock concentration is 20µM) wtR	0.5
Primer 3. (stock concentration is 20µM) mutR	0.5
DNA (example) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	1.5
<b>TOTAL VOLUME OF REACTION:</b>	<b>15.00 µL</b>

### Comments on protocol:

- Protocol may work with other DNA extraction methods.

### Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting <span style="float: right;">HOT START? <input type="checkbox"/></span>	94	2:00	<b>1x</b>
2. Denaturation	94	0:10	
3. Annealing <span style="float: right;">steps 2-3-4 cycle in sequence</span>	65 (↓1°C/cycle)	0:30	<b>10x</b>
4. Elongation	68	2:00	
5. Denaturation	94	0:15	
6. Annealing <span style="float: right;">steps 5-6-7 cycle in sequence</span>	55	0:30	<b>25x</b>
7. Elongation	68	2:00 (↑20sec/cycle)	
8. Finish	4	∞	n/a

### Primers:

### Electrophoresis Protocol:

Name	Nucleotide Sequence (5' - 3')	Agarose: 1.5%	V: 90
1. CR_Asb13_comF	CACCACGACCAGTTCCTTATCC	Estimated Running Time: 90 min.	
2. CR_Asb13_wtR*	GCTTCCATCAAAAACAAAGGCTCC	<b>Primer Combination</b>	<b>Band (bp)</b>
3. CR_Asb13_mutR	CCAGGCAATCACAAAAGAGTCTACC	1 & 2, 1 & 3	437, 926
		1 & 3	412
			mutant

**Allele Description:** Exon 2 ENSMUSE00000256019 and flanking splicing regions were constitutively deleted from the Asb13 Gene ENSMUSG00000033781 using CRISPR Cas9 gene editing technology in mouse zygotes. The subsequent 514bp deletion from chr13: 3691864-3692377 GRCh39 was sequence verified. The selected founder animal was backcrossed to C57BL6/N to produce sequence confirmed heterozygous animals for establishing and archiving the line.

\*wtR primer untested (ePCR verified)

