

GENOTYPING PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: CR11752 AW554918 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
TOTAL VOLUME OF REACTION:	15.00 μL

Comments on protocol:

- Protocol may work with other DNA extraction methods.

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	94	2:00	1x
2. Denaturation	94	0:10	
3. Annealing steps 2-3-4 cycle in sequence	65 (↓1°C/cycle)	0:30	10x
4. Elongation	68	2:00	
5. Denaturation	94	0:15	
6. Annealing steps 5-6-7 cycle in sequence	55	0:30	25x
7. Elongation	68	2:00 (↑20sec/cycle)	
8. Finish	4	∞	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')	Agarose: 1.5%	V: 90
1. CR_AW554918_comF	GTGAAGTCTTAATGGAGAACAGGAGC	Estimated Running Time: 90 min.	
2. CR_AW554918_wtR*	CCTGATTCATTCAAACAGTGACACTCC	Primer Combination	Band (bp)
3. CR_AW554918_mutR	CTAAGTGCAGGATTCCCAATAGAGG	1 & 2, 1 & 3	801, 1541
		1 & 3	911
			mutant

Electrophoresis Protocol:

Allele Description: Exon 4 ENSMUSE00001296985 and flanking splicing regions were constitutively deleted from the AW554918 Gene ENSMUSG00000033632 using CRISPR Cas9 gene editing technology in mouse zygotes. The subsequent 630bp deletion from Chr18:25314842-25315471 GRCm39 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)

