

GENOTYPING PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: CR11698 Atp5me 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? <input type="checkbox"/>	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')	Electrophoresis Protocol:		
1. CR_Atp5me_comF	CAGCTTACACTAACAGCCTAGACTTCC	Agarose: 1.5%	V: 90	
2. CR_Atp5me_wtR*	CAGTGTCACACAGCCTTTACC	Estimated Running Time: 90 min.		
3. CR_Atp5me_mutR	CAGTAAAGAGAGAGGAAGCCACAGC	Primer Combination	Band (bp)	Genotype
		1 & 2, 1 & 3	582, 1327	wildtype
		1 & 3	671	mutant

Allele Description: Exon 2-3 (ENSMUSE00000365706, ENSMUSE00001227820) and flanking splicing regions were constitutively deleted from the Atp5me Gene ENSMUSG00000050856 using CRISPR Cas9 gene editing technology in mouse zygotes. The subsequent 656bp deletion from Chr 5: 108581418 - 108582073 GRCm39 was screened by PCR analysis. 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)

