

GENOTYPING PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: CR11534 Ap5b1 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_Ap5b1_comF	CCTCCAGAGTTCATCTCGGGAC	Estimated Running Time: 90 min.	
2. CR_Ap5b1_wtR*	CACTCCTTAGGTCACCTCAACAGG	Primer Combination	Band (bp)
3. CR_Ap5b1_mutR	CTGCTACCTTATGATCTGAGTTCAGC	1 & 2, 1 & 3	318,3223
		1 & 3	389
			Genotype
			wildtype
			mutant

Allele Description: Exon 2-3 (ENSMUSE00001454401, ENSMUSE00000352116) and flanking splicing regions were constitutively deleted from the Ap5b1 Gene ENSMUSG00000049562 using CRISPR Cas9 gene editing technology in mouse zygotes. The subsequent 2834bp deletion from Chr 19: 5618497 - 5621330 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)

