

GENOTYPING PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: CR11766 Haus1 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:

Electrophoresis Protocol:

Name	Nucleotide Sequence (5' - 3')	Agarose: 1.5% V: 90
1. CR_Haus1_comF	CATGTGCTCACTCATAGATCATGC	Estimated Running Time: 90 min.
2. CR_Haus1_wtR*	CCTCTGATTTTAAGGCTACCCAGG	Primer Combination Band (bp) Genotype
3. CR_Haus1_mutR	GCTAAGAAGCTAAGGACGATGACC	1 & 2, 1 & 3 450,1670 wildtype
		1 & 3 507 mutant

Allele Description: Exon 3 ENSMUSE00000303265 and flanking splicing regions were constitutively deleted from the Haus1 Gene ENSMUSG00000041840 using CRISPR Cas9 gene editing technology in mouse zygotes. The subsequent 1163bp deletion from Chr18: 77851062-77852224 GRChm39 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)

