

GENOTYPING PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

mmrrc@ucdavis.edu

530-754-MMRRC

Protocol Name: CR10978 Fa2h EXDEL Stock #: 67444

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_Fa2h_comF	TCCTGCTGAAGACCTTCCACTGG	Estimated Running Time: 90 min.	
2. CR_Fa2h_wtR	CTGGTTGGTGAACCCACTCATCAT	Primer Combination	Band (bp)
3. CR_Fa2h_mutR	GAGAGCTGTGGTCCAAAGGGAC	1 & 2, 1 & 3	843, 1355
		1 & 3	581
			Genotype
			wildtype
			mutant

Electrophoresis Protocol:

Allele Description: Exon 3 [ENSMUSE00001283086](#) and flanking splicing regions were constitutively deleted from the Fa2h gene [ENSMUSG00000033579.16](#) using CRISPR Cas9 gene editing technology in mouse zygotes. Subsequent founders were backcrossed to C57BL6/N to produce sequence confirmed heterozygous animals.

