

GENOTYPING PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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Protocol Name: CR10765 Fah 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:

Electrophoresis Protocol:

Name	Nucleotide Sequence (5' - 3')	Agarose: 1.5%	V: 90
1. CR_Fah_comF	TGTCCATGAACCTCCACATTCAGC	Estimated Running Time: 90 min.	
2. CR_Fah_wtR	TGCCAGTACCCTCCTCTCATTTTGTA	Primer Combination	Band (bp)
3. CR_Fah_mutR	AGCAGGATGCCTCTACCAAATGTCA	1 & 2, 1 & 3	476, 1320
		1 & 3	696
			Genotype
			wildtype
			mutant

Allele Description: Exon 5 ENSMUSE00000201432 and flanking splicing regions were constitutively deleted from the Fah gene ENSMUST00000032865.16 using CRISPR Cas9 gene editing technology in mouse zygotes. Subsequent founders were backcrossed to C57BL6/N to produce sequence confirmed heterozygous animals.

*wtR primer untested (ePCR verified)

