

GENOTYPING PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: CR11362 Flacc1 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_Flacc1_comF	TGTGTCCACAGTCTGATTGTTGG	Estimated Running Time: 90 min.	
2. CR_Flacc1_wtR*	CTGCTTCATGTTGGAACAGCG	Primer Combination	Band (bp)
3. CR_Flacc1_mutR	GCTCTGAGTGAGAACACAGAGAC	1 & 2, 1 & 3	401, 1295
		1 & 3	384
			mutant

Allele Description: Exon 6 ENSMUSE00000441488 and flanking splicing regions were constitutively deleted from the Flacc1 gene ENSMUSG00000047528 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)

