

GENOTYPING PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

mmrrc@ucdavis.edu

530-754-MMRRC

Protocol Name: CR10498 Oprl1 iDex

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_Oprl1_comF	AAGATGATGACCCTCTTGGTGGTCT	Estimated Running Time: 90 min.	
2. CR_Oprl1_wtR	CGAACATCAAGGGCACGGATA	Primer Combination	Band (bp)
3. CR_Oprl1_mutR	GCAGTCTCACTACCTGGCTGAACA	1 & 2, 1 & 3	339,825
		1 & 3	656
			Genotype
			wildtype
			mutant

Allele Description: Exon 3 [ENSMUSE00001213207](#) had 169bp deleted from the 344th coding nucleotide through the 512th coding nucleotide from the Oprl1 gene [ENSMUSG00000027584.16](#) using CRISPR Cas9 gene editing technology in mouse zygotes. Subsequent founders were backcrossed to C57BL6/N to produce sequence confirmed heterozygous animals.

