

GENOTYPING PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: CR1771 Paqr8 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? <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:

Name	Nucleotide Sequence (5' - 3')	Electrophoresis Protocol:		
1. CR_Paqr8-comF	GCCATCATTCCCACTGAAAGC	Agarose: 1.5%	V: 90	
2. CR_Paqr8-wtR*	CTGCTGCCCACTCATTGACAGG	Estimated Running Time: 90 min.		
3. CR_Paqr8-mutR	AATGGCCTGCATTTGAAATTCC	Primer Combination	Band (bp)	Genotype
		1 & 2, 1 & 3	502, 1887	wildtype
		1 & 3	733	mutant

Allele Description: Exon 3 [ENSMUSE00000453394](#) was constitutively deleted from the 56th coding nucleotide through the 3' UTR from the Paqr8 gene [ENSMUSG00000025931](#) 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)

