

GENOTYPING PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: CR1314 Ptchd2 EXDEL

Protocol: GoTaq® G2 Colorless Master Mix(Promega)

Reagent/Constituent	Volume (µL)
Water	3.9
GoTaq® G2 Colorless Master Mix,2X	7.5
Primer 1. (stock concentration is 20µM) comF	0.6
Primer 2. (stock concentration is 20µM) wtR	1.2
Primer 3. (stock concentration is 20µM) mutR	0.3
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_Ptchd2-comF	GAATAGCACTGACCCCAGTGGG	Estimated Running Time: 90 min.	
2. CR_Ptchd2-wtR	CTTCCTACCTAGTGGACTGCTTGG	Primer Combination	Band (bp)
3. CR_Ptchd2-mutR	GAAACACAGTCCCATCTTACAAGTAGG	1 & 2, 1 & 3	689, 1045
		1 & 3	462
			Genotype
			wildtype
			mutant

Allele Description: Exon 3 [NM_001083342.1.3](#) and flanking splicing regions were constitutively deleted from the Ptchd2 gene [NM_001083342.1.1](#) using CRISPR Cas9 gene editing technology in mouse zygotes. Subsequent founders were backcrossed to C57BL6/N to produce sequence confirmed heterozygous animals.

