

GENOTYPING PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: CR11680 Prkrip1 EXDEL

Stock # 75963

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_Prkrip1_comF	GCTCCTCTTACCTTATCTTCCCTACG	Estimated Running Time: 90 min.	
2. CR_Prkrip1_wtR*	GGAATTGGAAGCTTTGTCCTGG	Primer Combination	Band (bp)
3. CR_Prkrip1_mutR	GGTGGATGTTTCTGATGCCAGC	1 & 2, 1 & 3	642, 1450
		1 & 3	666
			wildtype
			mutant

Allele Description: Exon 2 ENSMUSE00000241207 and flanking splicing regions were constitutively deleted from the Prkrip1 Gene ENSMUSG00000039737 using CRISPR Cas9 gene editing technology in mouse zygotes. The subsequent 784bp deletion from Chr 5:136226148-136226931 GRCm39 was screened by PCR analysis. The selected founder animal was backcrossed to C57BL6/N to produce sequence confirmed heterozygous animals for establishing and archiving the line.

*wtR primer untested (ePCR verified)

