

GENOTYPING PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: CR11549 Cct8l1 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:

Name	Nucleotide Sequence (5' - 3')	Agarose: 1.5%	V: 90
1. CR_Cct8l1_comF	GTGACTTCATTCCCTATCTCTGTTCC	Estimated Running Time: 90 min.	
2. CR_Cct8l1_wtR*	CACTCTGGAAATGGCAGTAGGC	Primer Combination	Band (bp)
3. CR_Cct8l1_mutR	GGTCTACAAAGCCAGTTTCAGAATAGC	1 & 2, 1 & 3	273, 2242
		1 & 3	341
			wildtype
			mutant

Electrophoresis Protocol:

Allele Description: Exon 1 ENSMUSE00000258735 was constitutively deleted from the 5'UTR through the 3' UTR from the Cct8l1 Gene ENSMUSG00000038044 using CRISPR Cas9 gene editing technology in mouse zygotes. The subsequent 1901bp deletion from Chr 5: 25721152-25723052 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)

