

GENOTYPING PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: CR11327 Clcn6 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')	Electrophoresis Protocol:		
1. CR_Clc6_comF	CTCCTGTAAGTCCAGTCCAAGGA	Agarose: 1.5%	V: 90	
2. CR_Clc6_wtR*	GTGCTCCACATACAACAGCTTTC	Estimated Running Time: 90 min.		
3. CR_Clc6_mutR	GGTACTGAGTTCAAAGCCAGTAAG	Primer Combination	Band (bp)	Genotype
		1 & 2, 1 & 3	302, 3684	wildtype
		1 & 3	327	mutant

Allele Description: Exon 6-8 (ENSMUSE00000630346, ENSMUSE00000184533, ENSMUSE00000356527) and flanking splicing regions were constitutively deleted from the Clcn6 gene ENSMUSG00000029016 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)

