

GENOTYPING PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: CR11725 Slc66a2 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? <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')	Agarose: 1.5%	V: 90
1. CR_Slc66a2_comF	CTGGCTGCTCTTTCTGTAGTGG	Estimated Running Time: 90 min.	
2. CR_Slc66a2_wtR*	ATGACTATGCTCTGCCACAGG	Primer Combination	Band (bp)
3. CR_Slc66a2_mutR	GGGTAAGGATGTGATGGATACTGC	1 & 2, 1 & 3	575, 1117
		1 & 3	509
			wildtype
			mutant

Electrophoresis Protocol:

Allele Description: Exon 2 ENSMUSE00001268918 and flanking splicing regions were constitutively deleted from the Slc66a2 Gene ENSMUSG00000034006 using CRISPR Cas9 gene editing technology in mouse zygotes. The subsequent 608bp deletion from Chr 18: 80306434- 80307041 GRCm39 was sequence verified. 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)

