

GENOTYPING PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: CR11722 Scgb2a2 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')	Electrophoresis Protocol:		
1. CR_Scgb2a2_comF	GAACTAAAAGGAAGCAGAGGAGAATGG	Agarose: 1.5%	V: 90	
2. CR_Scgb2a2_wtR*	GTACACTCTACACACCACACTGC	Estimated Running Time: 90 min.		
3. CR_Scgb2a2_mutR	GCTTATGATGACTGTTGGCACTCTCG	Primer Combination	Band (bp)	Genotype
		1 & 2, 1 & 3	817, 1879	wildtype
		1 & 3	452	mutant

Allele Description: Exon 2 ENSMUSE00000981960 and flanking splicing regions were constitutively deleted from the Scgb2a2 Gene ENSMUSG00000096872 using CRISPR Cas9 gene editing technology in mouse zygotes. The subsequent 1427bp deletion from Chr 19: 9827776-9829202 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)

