GENOTYPING PROTOCOL
MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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Protocol Name: CR1091 Sgtb exdel **MMRRC:** 42216-UCD

Protocol: ***GoTaq® Long PCR Master Mix(Promega)***

Reagent/Constituent	Volume (µL)
Water	5.6
GoTaq® Long PCR Master Mix,2X	7.5
Primer 1. (stock concentration is 20µM) comF	0.6
Primer 2. (stock concentration is 20µM) wtR	1.2
Primer 3. (stock concentration is 20µM) mutR	0.3
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 (ºC)	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 (↓1ºC/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')
1. CR_Sgtb-comF	GACATTCTAGTGGGAAAGACCTTGC
2. CR_Sgtb-wtR	CCGGCATAGTCTCATACGAGATAGC
3. CR_Sgtb-mutR	GCACTACCACCAGAGCTAAAACAGC

Electrophoresis Protocol:

Argarose: **1.5%** **V:** **90**

Estimated Running Time: **90** min.

Primer Combination	Band (bp)	Genotype
1 & 2,1 & 3	456,1749	wildtype
1 & 3	184	mutant

Allele Description: Exon 4 ENSMUSE00000313716 and flanking splicing regions were constitutively deleted from the Sgtb Gene ENSMUST00000044385.13 using CRISPR Cas9 gene editing technology in mouse zygotes. Subsequent founders were backcrossed to C57BL6/N to produce sequence confirmed heterozygous animals.