GENOTYPING PROTOCOL
MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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Protocol Name: **CR11721 Sbp 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 (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_Sbp_comF	CTGAAGTCTCAGGAGCCGATGG
2. CR_Sbp_wtR*	GGCTCCAGTGTGAGATGAGTACC
3. CR_Sbp_mutR	TGTAAAGGAAGAGGGTGAGCAGG

Electrophoresis Protocol:

Agarose: **1.5%** **V:** **90**

Estimated Running Time: **90** min.

Primer Combination	**Band** (bp)	**Genotype**
1 & 2,1 & 3	491, 3677	wildtype
1 & 3	531	mutant

Allele Description: Exon 2-4 (ENSMUSE00001276045, ENSMUSE00001028131, ENSMUSE00000138570) and flanking splicing regions were constitutively deleted from the Sbp Gene ENSMUSG00000024128 using CRISPR Cas9 gene editing technology in mouse zygotes. The subsequent 3146bp deletions from Chr 17 (Deletion: 17:24161390 - 17:24164583 and Deletion: 17:24164273 - 17:24164844) 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**)**