GENOTYPING PROTOCOL
MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

Protocol Name: CR11685 Surf6 EXDEL   Stock # 75990

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: **Electrophoresis Protocol:**

Name	Nucleotide Sequence (5' - 3')
1. CR_Surf6_comF	GTGTCAGAACAAAAGGCTCAGAAGC
2. CR_Surf6_wtR*	CCACCATTAAGAAGTTCTACCCAAGC
3. CR_Surf6_mutR	GCGGGAATCAGGAAAGGAAATGG

Agarose: **1.5%**	V: **90**
Estimated Running Time:	**90** min.

Primer Combination	Band (bp)	Genotype
1 & 2,1 & 3	418, 1241	wildtype
1 & 3	420	mutant

Allele Description: Exon 3 ENSMUSE00001229256 and flanking splicing regions were constitutively deleted from the Surf6 Gene ENSMUSG00000036160 using CRISPR Cas9 gene editing technology in mouse zygotes. The subsequent 821bp deletion from Chr 2: 26788859 - 26789679 GRCm39 was screened by PCR analysis. 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**)**