GENOTYPING PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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Protocol Name: **CR11514 Spata45 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_Spata45_comF	CTACCTTGTCTCCTGAATGCTGAG
2. CR_Spata45_wtR*	CCTCATAACCACATCTACCAGGAC
3. CR_Spata45_mutR	CGTGTTCATTCCTAAATCATACCGA

Electrophoresis Protocol:

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

Estimated Running Time: **90** min.

Primer Combination	**Band** (bp)	**Genotype**
1 & 2,1 & 3	412,4535	wildtype
1 & 3	559	mutant

Allele Description: Exon 2-3(ENSMUSE00000288478, ENSMUSE00000288465) were constitutively deleted from 5'UTR through the 3' UTR from the Spata45 Gene ENSMUSG00000057072 using CRISPR Cas9 gene editing technology in mouse zygotes. The subsequent 3976bp deletion from Chr 1: 190771543 - 190775518 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**)**