

GENOTYPING PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: CR1249 Spag11a exdel

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 (°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_Spag11a-comF	CACCTACCATCTTACCACAGCACC	Argarose: 1.5%	V: 90	
2. CR_Spag11a-wtR	CCTGAGCCTCAGAGTGGTCCAAC	Estimated Running Time: 90 min.		
3. CR_Spag11a-mutR	AGCAATTTGATTGGCCTCATGC	Primer Combination	Band (bp)	Genotype
		1 & 2, 1 & 3	507,2390	wildtype
		1 & 3	309	mutant

Allele Description: Exon 1 through 2 ([ENSMUSE00000359348](#), [ENSMUSE00000464570](#)) and flanking splicing regions were constitutively deleted from the Spag11a gene [ENSMUST00000058955.4](#) using CRISPR Cas9 gene editing technology in mouse zygotes. Subsequent founders were backcrossed to C57BL6/N to produce sequence confirmed heterozygous animals.

