

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: C57BL/6N-Spns2tm1a(KOMP)Wtsi/Mmucd **MMRRC:** 049846-UCD

Protocol:

Reagent/Constituent	Volume (μL)
Water	5.6
GoTaq® G2 Colorless Master Mix, 2X	7.5
Primer 1. (stock concentration is 20μM)	0.45
Primer 2. (stock concentration is 20μM)	0.45
DNA (example) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	1.0
TOTAL VOLUME	
15	

Comments on protocol:

- Protocol may work with other DNA extraction methods.
- Use Touch-Down cycling protocol-first 10 cycles anneal at 65°C decreasing in temperature by 1.0°C; next 30 cycles anneal at 55°C.
- The mutant PCR is a general LacZ PCR. The wild type is specific for this strain.

Strategy:

Steps	Temp (°C)	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 (↓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)	

Primers:

Electrophoresis Protocol:

Name	Nucleotide Sequence (5' - 3')	Argarose: 1.5% V: 90		
1. CSD-lacF	GCTACCATTACCAGTTGGTCTGGTGTC	Estimated Running Time: 90 min.		
2. CSD-neoF	GGGATCTCATGCTGGAGTTCTTCG	Primer	Band (bp)	Genotype
3. CSD-loxF	GAGATGGCGCAACGCAATTAATG	3 & 5	270	floxed
4. CSD-Spns2-ttR	GGGACTCCAAGTCTTCTATCACCCC	2 & 4	663	PreCre
5. CSD-Spns2-R2	GACATTTGCCTTCCTGGGAC	1 & 5	568	PostCre
6. CSD-Spns2-F2	CCTGTAATCCTAGAACTTGAGAGAGG	6 & 4	370	Wildtype
7. CSD-Spns2-wtF	ACCTGACTTTCTGAGCAGCCTC	6 & 4	462	PostFlp
		6 & 5	416	PostFlp & PostCre
		3, 5 & 7	270, 212	Floxed, wildtype

*alternative, untested 3 primer protocol

Please note, these primers are auto-designed and may not have been verified by the repository, and as such may require optimization or redesign by your facility.

We recommend running primers singleplex. For screening of pups prior to any Flp or Cre recombination, the Floxed or PreCre primers may be used to identify the mutant mice. The Floxed primers test for the distal LoxP site. The PostCre primers should be used if mutant mice were crossed with a Cre recombinase line (without any FLP recombination). The PostFlp primers should be used if mutant mice were crossed with a Flp recombinase line. The PostFlp & Cre primers should be used if mutant mice were crossed with a Flp recombinase line and then a Cre recombinase line. The wildtype primers should be used for zygosity testing of all mutant mice (pre or post recombination).

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