

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: C57BL/6N-Nsun5tm2a(EUCOMM)Wtsi/JMmucd **MMRRC:** 048863-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.

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? <input type="checkbox"/>	94	5:00	1
2. Denaturation	94	0:15	
3. Annealing steps 2-3-4 cycle in sequence	65 to 55 (↓1°C/cycle)	0:30	40x
4. Elongation	72	0:40	
5. Amplification	72	5:00	1
6. Finish	15	∞	n/a

Primers:

Electrophoresis Protocol:

Name	Nucleotide Sequence (5' - 3')	Argarose: 1.5% V: 90		
1. 48863-lacF	GCTACCATTACCAGTTGGTCTGGTGTC	Estimated Running Time: 90 min.		
2. 48863-neoF	GGGATCTCATGCTGGAGTTCTTCG	Primer Combination	Band (bp)	Genotype
3. 48863-loxF	GAGATGGCGCAACGCAATTAAT	3 & 5	462	floxed
4. 48863-ttR	GCTGTCCAATGAGGTGTTGTGG	2 & 4	547	PreCre
5. CSD-Nsun5-SR2	TAACCTGGCAACTCACTCTGTAGGC	1 & 5	760	PostCre
6. 48863-F	GGACAGGGTGATGATGGCAAGG	6 & 4	296	Wildtype
		6 & 4	426	PostFlp
		6 & 5	688	PostFlp & PostCre

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).