

GENOTYPING BY PCR PROTOCOL
MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS
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 530-754-MMRRC

NAME OF PCR: B6.Cg-Siglec15^{tm1.2Cfg}/Mmucd

MMRRC # 032722-UCD

Protocol:

Reagent/ Constituent	Volume (μ L)
Water	10.275
10x Buffer	2.5
MgCl ₂ (stock concentration is 25mM)	1.7
Betaine (stock concentration is 5M) Optional	6.5
dNTPs (stock concentration is 10mM)	0.5
DMSO Optional	0.325
Primer 1 (stock concentration is 20 μ M)	0.5
Primer 2 (stock concentration is 20 μ M)	0.5
Primer 3 (stock concentration is 20 μ M)	0.5
Taq Polymerase 5Units/ μ L	0.2
DNA (50-200ng/ μ L) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	1.0
TOTAL VOLUME OF REACTION:	24.500 μL

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.
- Betaine and DMSO have been standardized due to high GC content. Protocol may be tested without. Also, may adjust MgCl₂ to increase reaction or decrease non specific amplifications.

Strategy:

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

Primers:

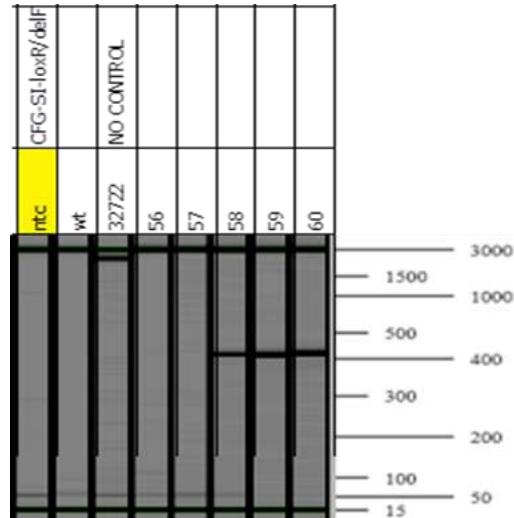
Name	Nucleotide Sequence (5' - 3')
1. 32722-delF	CTTCAGGTGAATCACAGCTTCATGC
2. CFG-SI-loxR	GGAGACCCGACCTTACAGGACAC
3. CFG-SI-wtR	GAAGCGTCTCTTAGTTTCACAAGGG

Electrophoresis Protocol:

Agarose: 1.5% V: 90

Estimated Running Time: 90 min.

Primer Combination	Band	Genotype
1 and 2	433 bp	Mutant
1 and 3	203 bp	WT



PCR protocol developed by MMRRC at University of California, Davis