

Mutant Mouse Regional Resource Center: UC Davis

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530-754-MMRRC

NAME OF PCR: B6.Cg-Siglec15^{tm1.1Cfgr}/Mmucd MMRRC # 032723-UCD

Protocol:

Reagent/ Constituent	Volume (μL)
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 <i>Optional</i>	0.325
Primer 1 (stock concentration is 20μM)	0.5
Primer 2 (stock concentration is 20μM)	0.5
Primer 3 (stock concentration is 20μM)	0.5
Primer 4 (stock concentration is 20μM)	0.5
Taq Polymerase 5Units/μL	0.2
DNA (50-200ng/μL) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	1.0
TOTAL VOLUME OF REACTION:	25.000 μL

Comments on protocol:

- Protocol may work with other DNA extraction methods.
- Use Touch-Down cycling protocol-first 10 cycles anneal at 65o 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? ☐	94	5:00	1
2. Denaturation	94	0:15	} 40x
3. Annealing	65 to 55 (↓1°C/cycle)	0:30	
4. Elongation		0:40	
5. Amplification	72	5:00	1
6. Finish	15	∞	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')
1. CFG-SI-loxR	GGAGACCCGACCTTTACAGGACAC
2. LR-CFG-lox2	CCGGGATCATAACTTCGTATAGCATAC
3. 32722-delF	CTTCAGGTGAATCACAGCTTCATGC
4. CFG-SI-wtR	GAAGCGTCTCTTCTAGTTTTTCACAAGGG

Electrophoresis Protocol:

Agarose: 1.5% V: 90

Estimated Running Time: 90 min.

Primer Combination	Band	Genotype
1 and 2	272 bp	Floxed
3 and 4	203 bp	WT

