

**GENOTYPING BY PCR PROTOCOL
MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS**

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530-754-MMRRC

NAME OF PCR: CFG ES cell line Clec10a and Mgl2

MMRRC # 031980-UCD

Protocol:

Reagent/ Constituent	Volume (µL)
Water	11.275
10x Buffer	2.5
MgCl ₂ (stock concentration is 25mM)	1.7
Betaine (stock concentration is 5M) <i>Optional</i>	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
Taq Polymerase 5Units/µL	0.2
DNA (50-200 ng/ µL) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	1.0
TOTAL VOLUME OF REACTION:	25µL

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	1
2. Denaturation	94	0:15	} 40x
3. Annealing } steps 2-3-4 will cycle in sequence	65 to 55 (↓1°C/cycle)	0:30	
4. Elongation	72	0:40	
5. Amplification	72	5:00	1
6. Finish	15	∞	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')
1: 31980-comR	GTGACGTCTCCACCACCTTCTTCC
2: 31980-wtF	TGTACAGTGTGCAGCTTATTGTAGGC
3: 31980-loxF	CCTCAGCCGGGATCATAACTTCG

Electrophoresis Protocol:

Agarose: 1.5% V: 90 Estimated Running Time: 90 min.

Primer Combinations	Expected Bands	Genotype
1 and 2	529 bp	WT +/+
1 and 3	272 bp	floxed