

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

mmrrc@ucdavis.edu

530-754-MMRRC

NAME OF PCR: C.129P2(Cg)-Igh-8^{tm1Schr}/Mmucd MMRRC # 015506-UCD

Protocol:

Reagent/Constituent	Volume (μL)
Water	10.775
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 (50-200ng/ μL) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	1.0
TOTAL VOLUME OF REACTION:	25.000 μL

Comments on protocol:

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? ☐	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. 015506 igh F1	TCAAACCTAGCTGCTAATTC	Estimated Running:Time: 90 min.	
2. 015506 igh R1	TGGATATGATCATTGACAGG	Primer Combination	Band
3. 015506 ighneo F1	CTTGGGTGGAGAGGCTATTC	1 and 2	750 bp
4. 015506 ighneo R1	CAACGCTATGTCCTGATAGC	3 and 4	628 bp
			Genotype
			WT
			MUTANT

