

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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530-754-MMRRC

NAME OF PCR: B6;129S5-Gpr89^{tm1Lex}/Mmcd MMRRC # 032343-UCD

Protocol:

Reagent/ Constituent	Volume (μL)
Water	10.275
10x Buffer (15mM MgCl ₂)	2.5
MgCl ₂ (stock concentration is 25mM)	1.7
Betaine (stock concentration is 5M)	6.5
dNTPs (stock concentration is 10mM)	0.5
DMSO	0.325
Primer 1 (stock concentration is 20μM) DNA449-21	0.5
Primer 2 (stock concentration is 20μM) DNA449-2	0.5
Primer 3 (stock concentration is 20μM) UNQ192-R2	0.5
Primer 4 (stock concentration is 20μM) Neo3a	0.5
Taq Polymerase 5 Units/μL	0.2
DNA Sample	1.0
TOTAL VOLUME OF REACTION:	25μL

Comments on protocol:

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? CHECK HERE ☐	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	4	∞	n/a

Primers:

Primer Name	Nucleotide Sequence (5' - 3')
1: DNA449-21	CTA AGA GCG TGT AAG CGT CTT
2: DNA449-2	CTG GTG CAG GTT TTC TAT GTG
3: UNQ192-R2	CCA TAG TGT GGT CTT TGA GCC ATC TTA G
4: Neo3a	GCA GCG CAT CGCCTT CTA TC

Electrophoresis Protocol:

% Agarose: 1.5 V: 90

Estimated Running Time (min): 90

Primers	Expected Bands	Genotype
1 and 2	313 bp	Wild-type
3 and 4	430 bp	Mutant