

GENOTYPING BY PCR PROTOCOL FORM

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DNA Extraction Method: NaOH _____ Proteinase K ☒ Other _____

Protocol: NAME OF PCR: C57BL/6J-Ptprc^{m1Btlr}/Mmcd (*belittle*), MMRRC #031764-UCD

Reagent/ Constituent	Volume (uL)
DNA Sample	1
10x Buffer (contains 15mM MgCl ₂)	5
dNTPs (stock concentration is 25mM)	0.4
Primer 1 (stock concentration is 20 uM)	1
Primer 2 (stock concentration is 20 uM)	1
Taq Polymerase	2.5
water	39.1
TOTAL VOLUME OF REACTION:	50 uL

Comments on protocol (e.g., different concentration of MgCl₂, etc): *Belittle* genotyping is performed by amplifying the region containing the mutation using PCR, followed by sequencing of the amplified region to detect the single nucleotide transversion. Use primers 1 & 2 for PCR amplification and primers 3 & 4 for sequencing.

Strategy:

Steps	Temp (°C)	Time (min)	# of Cycles
1. Initiation/Melting HOT START?..CHECK HERE []	94	2	1
2. Denaturation	94	30 seconds	
3. Annealing } steps 2-3-4 will cycle in sequence	56	30 seconds	30
4. Elongation	72	1 min	
5. Amplification (i.e., 72°C, 10 min)	72	7	1
6. Finish (i.e., 4°C, indefinite)	4	n/a	n/a

Primers:

Primer Name	Nucleotide Sequence (5' - 3')
1: Ptprc_belittle_F	TGTCTTCTCTACGCACAATTAAGCCC
2: Ptprc_belittle_R	AGTCACAGATTCCACCTTACTCCAGG
3: Ptprc_belittle_seqF	TACACACACCTGAAGATTGGAG
4: Ptprc_belittle_seqR	GGACATCATTAAGTGTCTAGCTGC

Electrophoresis Protocol:

% Agarose: _____ V : _____

Estimated Running Time (min): _____

Primer combination	Band (bp)	genotype
(i.e. 1&2)	1039	
(i.e. 3&4)		
(i.e. 1&2&3)		

Mutation site (red) and flanking sequence:

WT gaaaaatgt**a**aaagtttgc
belittle gaaaaatgt**t**aaagtttgc