

GENOTYPING BY PCR PROTOCOL FORM

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DNA Extraction Method: NaOH _____ Proteinase K ☒ Other _____

Protocol: NAME OF PCR: C57BL/6J-Rorb^{m1Btr}/Mmcd (4-limb clasper), MMRRRC #031040-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): *4-limb clasper* 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 amplification and 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: Rorb_4-limb clasper_F	AACAGCCAGTGGTCTGTCTCACTTC
2: Rorb_4-limb clasper_R	TGCCCAATTACCCATAGCACTTAGTTC
3: Rorb_4-limb clasper_seqF	GTGACTTTAACAACACTGAAGACCTC
4: Rorb_4-limb clasper_seqR	GAAAGATCCTGTGAATTGCC

Electrophoresis Protocol:

% Agarose: _____ V : _____

Estimated Running Time (min): _____

Primer combination	Band (bp)	genotype
(i.e. 1&2)	1000	
(i.e. 3&4)		
(i.e. 1&2&3)		

Mutation site (red) and flanking sequence:

WT ctaggaatg**t**caagagatg
4-limb clasper ctaggaatg**c**caagagatg