

GENOTYPING BY PCR PROTOCOL
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NAME OF PCR: B6;129-Kif3c^{tm3Gsn}/Mmucd MMRRC # 000134-UCD

DNA Extraction Method: ☐ NaOH ☐ Proteinase K ☒ Other: Qiagen

Protocol:

Reagent/ Constituent	Volume (μL)
Water	12.15
10x Buffer (contains 15mM MgCl ₂)	2.5
MgCl ₂ (stock concentration is 25mM)	1.7
dNTPs (stock concentration is 10mM)	0.5
Primer 1 (stock concentration is 20μM) 000134 KO F	0.5
Primer 2 (stock concentration is 20μM) 000134 KO R	0.5
Primer 3 (stock concentration is 20μM) 000134 WT F	0.5
Primer 4 (stock concentration is 20μM) 000134 WT R	0.5
Taq Polymerase 5 Units/μL	0.15
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? ☐	95	15:00	1
2. Denaturation	94	0:45	} 35x
3. Annealing } steps 2-3-4 will cycle in sequence	65	0:45	
4. Elongation	72	1:00	
5. Amplification	72	10:00	1
6. Finish	4	∞	n/a

Primers:

Primer Name	Nucleotide Sequence (5' - 3')
1: 000134 KO F	5' –GAT GGA TTG CAC GCA GGT TCT– 3'
2: 000134 KO R	5' –AGG TAG CCG GAT CAA GCG TAT– 3'
3: 000134 WT F	5' –GGT CAT GAG CAG ATT CTG AC– 3'
4: 000134 WT R	5' –GAG ACG TGA CCT CAT TCA TG– 3'

Electrophoresis Protocol:

Agarose: 2% V: 100 Estimated Running Time: 60 min

Expected Bands	Genotype
~400 bp	KO -/-
400 / 550 bp	Het +/-
~550 bp	WT +/+