

GENOTYPING BY PCR PROTOCOL
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 530-754-MMRRC

NAME OF PCR: B6.129P2-Hfe^{tm1.1Gfn}/Mmucd MMRRC # 000046-UCD

Protocol:

Reagent/ Constituent	Volume (μL)
Water	15.75
10x Buffer (contains 15mM MgCl ₂)	2.5
dNTPs (stock concentration is 10mM)	0.5
Primer 1 (stock concentration is 10μM)	2.5
Primer 2 (stock concentration is 10μM)	1.25
Primer 3 (stock concentration is 10μM)	1.25
Taq Polymerase 5 Units/μL	0.25
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? ☐	94	5:00	1
2. Denaturation	94	0:30	} 30x
3. Annealing } steps 2-3-4 will cycle in sequence	60	0:30	
4. Elongation	72	0:30	
5. Amplification	72	0:30	1
6. Finish	25	∞	n/a

Primers:

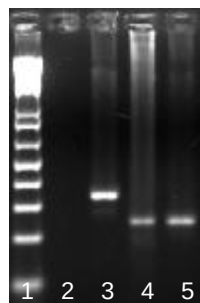
Primer Name	Nucleotide Sequence (5' - 3')
1: HFE Ext 5'	GAA TTA ACA GGC CGT TTC TAA AG
2: HFE Int II	CTT GGA GTA GTG GCT CAC ACT
3: HFE Del	CTT AGG AAG CCG AGG CTG TGT T

Electrophoresis Protocol:

% Agarose: 2 V: 100

Estimated Running Time (min): 60

Expected Bands	Genotype
333 bp	WT +/+
333 / 240 bp	Het +/-
240 bp	KO -/-



Lanes:
 1. 1Kb+ ladder
 2. H₂O
 3. Wild-type +/+ sample
 4-5. Hom -/- samples