

GENOTYPING BY PCR PROTOCOL
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 530-754-MMRRC

NAME OF PCR: C.Cg-Msx1^{tm1Rilm}/Mmucd MMRRC # 000068-UCD

Protocol:

Reagent/ Constituent	Volume (μL)
Water	18.55
10x Buffer (without 15mM MgCl ₂)	2.5
MgCl ₂ (stock concentration is 25mM)	1.0
dNTPs (stock concentration is 10mM)	0.25
Primer 1 (stock concentration is 15μM) Sxg-1	0.2
Primer 2 (stock concentration is 15μM) Sxg-2	0.4
Primer 3 (stock concentration is 15μM) Sxg-3	0.4
Taq Polymerase 5Units/μL	0.2
DNA extracted with ☒ NaOH ☐ Proteinase K ☐ Other:	1.5
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	0:15	} 35x
3. Annealing } steps 2-3-4 will cycle in sequence	62	1:30	
4. Elongation	72	1:00	
5. Amplification	72	5:00	1
6. Finish	4	∞	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')
1: Sxg-1	CCA GCA TGC ACC CTA CGC AA
2: Sxg-2	TCT GGA CGA AGA GCA TCA GG
3: Sxg-3	AGC AGG CGG CAA CAT GGG TT

Electrophoresis Protocol:

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

Primer Combination	Band	Genotype
Sxg-1 and Sxg-3	270 or 350 bp	WT +/+
Sxg 2 and Sxg-3	450 bp	KO -/-