

GENOTYPING BY PCR PROTOCOL
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530-754-MMRRC

NAME OF PCR: C.Cg-Msx2^{tm1Rilm}/Mmucd MMRRC # 000069-UCD

Protocol:

Reagent/ Constituent	Volume (μL)
Water	17.1
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 15μM) Sxg-2	0.8
Primer 2 (stock concentration is 15μM) Sxg-4	0.4
Primer 3 (stock concentration is 15μM) Sxg-5	0.8
Taq Polymerase 5Units/μL	0.2
DNA extracted with ☒ NaOH ☐ Proteinase K ☐ Other:	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	1:00	} 4x
3. Annealing	62	1:30	
4. Elongation	72	2:00	
5. Denaturation	94	1:00	} 32x
6. Annealing	60	2:00	
7. Elongation	72	2:00	
8. Amplification	72	5:00	1
9. Finish	4	∞	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')
1: Sxg-2	TCT GGA CGA AGA GCA TCA GG
2: Sxg-4	CCC TCT CTG TCC TCT AGG AC
3: Sxg-5	GCC TGA GGG CAG CAT AGG CT

Electrophoresis Protocol:

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

Primer Combination	Band	Genotype
Sxg-4 and Sxg-5	350 bp	WT +/-
Sxg 2 and Sxg-5	450 bp	KO -/-