

GENOTYPING BY PCR PROTOCOL
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 530-754-MMRRC

NAME OF PCR: B6.129-Eya1^{tm1Rilm}/Mmucd MMRRC # 000361-UCD

Protocol:

Reagent/ Constituent	Volume (μL)
Water	10.775
10x Buffer (contains / without 15mM MgCl ₂)	2.5
MgCl ₂ (stock concentration is 25mM)	1.7
Betaine (stock concentration is 5M) <i>Optional</i>	6.5
dNTPs (stock concentration is 10mM)	0.5
DMSO <i>Optional</i>	0.325
Primer 1 (stock concentration is 20μM) Eya1TF3	0.5
Primer 2 (stock concentration is 20μM) Eya1WR1	0.5
Primer 3 (stock concentration is 20μM) Neo220F	0.5
Taq Polymerase 5Units/μL	0.2
DNA extracted with ☒ NaOH ☐ Proteinase K ☐ Other:	1.0
TOTAL VOLUME OF REACTION:	25μL

Comments on protocol:

- Use Touch-Down cycling protocol-first 10 cycles anneal at 65° C decreasing in temperature by 1.0° C; next 30 cycles anneal at 55° C.
- Betaine/DMSO is standardized due to high GC content in promoter regions and protocol may be tested without. Also, may adjust MgCl₂ to increase reaction or decrease non specific amplifications.

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	94	5:00	1
2. Denaturation	94	0:15	} 10x
3. Annealing	65 to 55 (↓1°C/cycle)	0:30	
4. Elongation		0:40	
5. Denaturation	94	0:15	} 30x
6. Annealing	55	0:30	
7. Elongation		0:40	
8. Amplification	72	5:00	1
9. Finish	15	∞	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')
1: Eya1TF3	CAG ATT TTC TGT CTG GCT CC
2: Eya1WR1	GCT GTC TGA TGA AAC ATC ATC TAT
3: Neo220F	AAG GGC CAG CTC ATT CCT CCC ACT

Electrophoresis Protocol:

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

Expected Bands	Genotype
530 bp	WT +/+
530 / 400 bp	HET +/-
400 bp	KO -/-