

GENOTYPING BY PCR PROTOCOL

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NAME OF PCR: B6.129P2-H2-K^{btm1} H2-D^{btm1}/TacMmucd MMRRC: 037399-UCD

Protocol: Two Separate Reactions

Reagent/Constituent	Volume (μL)
Water	10.275
10x Buffer	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)	0.5
Primer 2. (stock concentration is 20μM)	0.5
Primer 3. (stock concentration is 20μM)	0.5
Primer 4. (stock concentration is 20μM)	0.5
Taq Polymerase 5Units/μL	0.2
DNA (example) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	1.0
TOTAL VOLUME OF	25.000 μL

Comments on protocol:

- Protocol may work with other DNA extraction methods.
- 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 and DMSO have been standardized due to high GC content. Protocol may be tested without. Also, may adjust

Strategy:

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

Primers:

Name		Nucleotide Sequence (5' - 3')		Electrophoresis Protocol:			
1. 37400-Mut-F		CAGACTGCCTTGGGAAAAGCG		Argarose: 1.5% V: 90			
2. 37400-wt-F		GGGGAGAGAGTGTCTGCTGGT		Estimated Running Time: 90 min.			
3. 37400-R3		CAGCCAGGGCTATACAGAGAAACC		Primer Combination		Band (bp)	Genotype
4. 37401-wt-F2		AACATGGACTCCTATACACTAGCCTGG		H2-K1 PCR	1 & 2	583	Mutant
5. 37401-wt-R		CCATAATCCCAGCACTCAGGAG			2 & 3	472	WT
6. 37401-H2Db-F		CAGCAGAAACATACAAGCTGTC		H2-Db PCR	4 & 5	357	WT
7. 37401-R		AAGGCCAGGCAGTCCATAATCC			6 & 7	244	Mutant

