

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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NAME OF PCR: B6;129-Upp1^{tm1Gp}/Mmucd MMRRC: 037119-UCD

Protocol: *PCR protocol provided by Donating Investigator*

Reagent/Constituent		Volume (μL)
Water		16.75
10x Buffer		2.5
MgCl ₂	(stock concentration is 25mM)	2.0
dNTPs	(stock concentration is 10mM)	0.5
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		0.25
DNA (example) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"		1.0
TOTAL VOLUME OF REACTION:		25.00 μL

Comments on protocol:

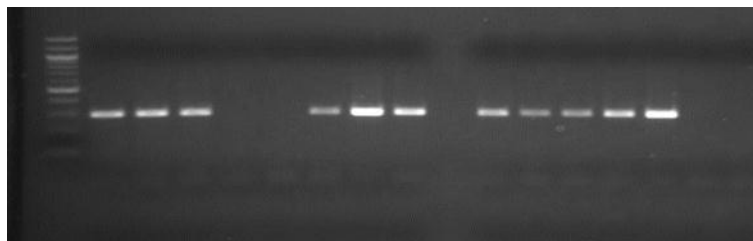
Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	95	2:00	1
2. Denaturation	95	0:30	
3. Annealing steps 2-3-4 cycle in sequence	55	0:30	30x
4. Elongation	72	1:00	
5. Amplification	72	5:00	1
6. Finish	4	∞	n/a

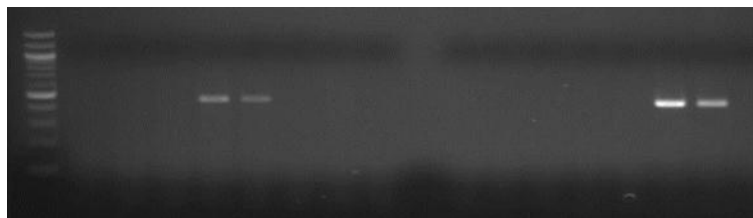
Primers:

Electrophoresis Protocol:

Name	Nucleotide Sequence (5' - 3')	Argarose: 1.6% V: 100		
1. P500 (wt)	CAGACCCCCGATGACACAGTATACTAATGG	Estimated Running Time: 24 min.		
2. P500a (wt)	GTTCAGCTGTTCCCATATGCAAAAGTGTGG	Primer Combination	Band (bp)	Genotype
3. PNEO (Mutant)	AGAAAGCGAAGGAACAAAGCTGCTATTGGC	1 and 2	300	WT
4. p500a (Mutant)	GTTCAGCTGTTCCCATATGCAAAAGTGTGG	3 and 4	500	Mutant



UP1 WT Positive 300 bp



UP1 Mutant Positive 500 bp

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