

GENOTYPING BY PCR PROTOCOL
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 530-754-MMRRC

NAME OF PCR: B6.129-*Ptk2*^{tm1Lfr}/Mmucd MMRRC # 009967-UCD

Protocol:

Reagent/ Constituent	Volume (μL)
Water	12.25
10x Buffer (without 15mM MgCl ₂)	2.5
MgCl ₂ (stock concentration is 25mM)	2.5
Q-solution	5.0
dNTPs (stock concentration is 10mM)	0.5
Primer 1 (stock concentration is 10μM) P1	0.5
Primer 2 (stock concentration is 10μM) GenoRV	0.5
Taq Polymerase 5 Units/μL	0.25
DNA Sample	1.0
TOTAL VOLUME OF REACTION:	25μL

Comments on protocol:

See Beggs et al (2003) Neuron 40: 501-513 for other primers.

Strategy:

Steps		Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting	HOT START? CHECK HERE ☐	95	5:00	1
2. Denaturation	} steps 2-3-4 will cycle in sequence	94	0:30	} 35x
3. Annealing		62	0:30	
4. Elongation		72	1:00	
6. Finish		25	∞	

Primers:

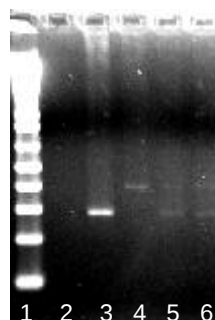
Primer Name	Nucleotide Sequence (5' - 3')
1: P1	GAG AAT CCA GCT TTG GCT GTT G
2: GenoRV	GAA TGC TAC AGG AAC CAA ATA AC

Electrophoresis Protocol:

% Agarose: 2 mV: 110

Estimated Running Time (min): 60

Expected Bands	Genotype
290 bp	Wild-type +/+
400 bp	Floxed
290 / 400 bp	F/+



Lanes:
 1. 1Kb+ ladder
 2. H₂O
 3. Wild-type +/+
 4. Floxed
 5, 6. F/+