

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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Protocol Name: B6.129-Tg(ITGAL-Ptprca)LP5HRasch Ptprctm1Holm/Mmucd MMRRC: 046268-UCD

Protocol:

Reagent/Constituent	Volume (µL)
Water	5.6
GoTaq® G2 Colorless Master Mix, 2X	7.5
Primer 1. (stock concentration is 20µM)	0.45
Primer 2. (stock concentration is 20µM)	0.45
DNA (example) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	1.0
TOTAL VOLUME	
15	

Comments on protocol:

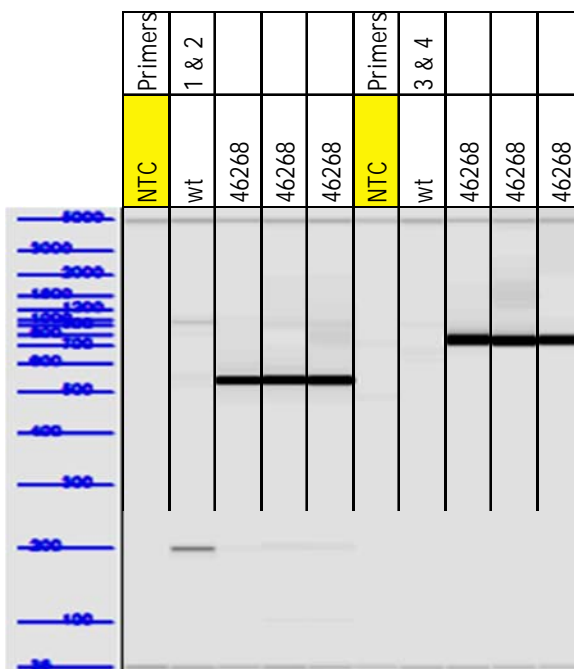
Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? <input type="checkbox"/>	94	2:00	1x
2. Denaturation	94	0:10	
3. Annealing steps 2-3-4 cycle in sequence	65 (↓1°C/cycle)	0:30	10x
4. Elongation	68	2:00	
5. Denaturation	94	0:15	
6. Annealing steps 5-6-7 cycle in sequence	55	0:30	30X
7. Elongation	68 (+20s/cycle)	2:00	

Primers:

Electrophoresis Protocol:

Name	Nucleotide Sequence (5' - 3')	Argarose: 1.5% V: 90		
1. 46268-CD363-F	GCCAGCTACATTGATGGCTTC	Estimated Running Time: 90 min.		
2. 46268-CD365-R	CCTGTATGAAGGAAGTCTCTGG	Primer Combination	Band (bp)	Genotype
3. 46268-321-F	GGTCACTGGAATGAAAACCTCCCG	1 & 2	~520	mutant
4. 46268-332-R	GCATAGGAAATGGCCATAGTC	3 & 4	~750	mutant



PCR protocol developed by MMRRC at University of California, Davis

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PstI digest of primers 1 & 2 PCR

