

GENOTYPING BY PCR PROTOCOL

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Protocol Name: B6.129-Tg(ITGAL-Ptprca)LP4Rasch Ptprctm1Holm/Mmucd **MMRRC: 046266-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:

- 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.
- Strains 44051, 46054, 46256, 46257, 46258, 46266, 46268, 46269, 46270 share similarities. PstI digest distinguishes between Ptprca and Ptprca*. Primers 3 and 4 are to detect ΔC strain which is present in 26258.

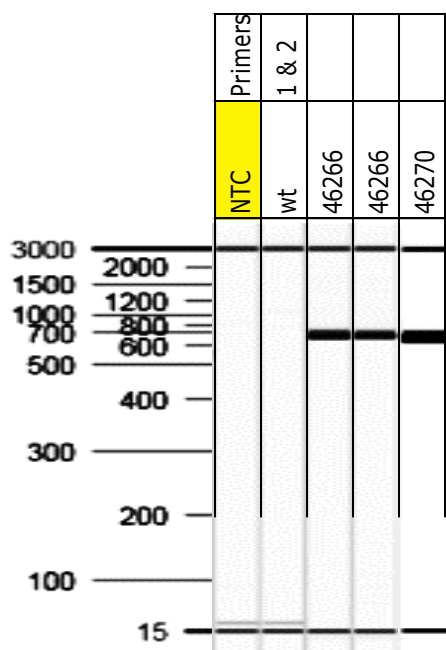
Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? <input type="checkbox"/>	94	5:00	1x
2. Denaturation	94	0:15	
3. Annealing steps 2-3-4 cycle in sequence	65 (↓1°C/cycle)	0:30	10x
4. Elongation	72	0:40	
5. Denaturation	94	0:15	
6. Annealing steps 5-6-7 cycle in sequence	55	0:30	30X
7. Elongation	72	0:40	

Primers:

Electrophoresis Protocol:

Name	Nucleotide Sequence (5' - 3')	Argarose: 1.5%	V: 90
1. 46266-CD363-F	GCCAGCTACATTGATGGCTTC	Estimated Running:Time: 90 min.	
2. 46266-CD365-R	CCTGTATGAAGGAAGTCTCTGG	Primer	Band (bp)
3. 46266-321-F	GGTCACTGGAATGAAAACCTCCCG	1 & 2	734
4. 46266-332-R	GCATAGGAAATGGCCATAGTC	3 & 4	0
			Genotype
			mutant



PstI digest
431bp
301bp

