

# GENOTYPING BY PCR PROTOCOL

## MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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530-754-MMRRC

NAME OF PCR: B6;129S7-*Hprt1*<sup>tm2(Pgk1-Pac/Tk)Brd</sup>/Mmcd MMRRC # 010860-UCD

**Protocol: Simplex reaction works best.**

Reagent/ Constituent	Volume (μL)
Water	14.29
10x Buffer (contains 15mM MgCl <sub>2</sub> )	2.5
MgCl <sub>2</sub> (stock concentration is 25mM)	1.5
Betaine (stock concentration is 5M) <i>Optional</i> at final concentration of 1M	
dNTPs (stock concentration is 10mM) Qiagen DNA only	0.12
DMSO <i>Optional</i> at final concentration of 1%	
Primer 1 (stock concentration is 20μM) Sense2 com	0.12
Primer 2 (stock concentration is 20μM) Antisense1 wt	0.12
Primer 3 (stock concentration is 20μM) pudtkR2 mut	0.12
Taq Polymerase 5Units/μL Amplitaq (AB)	0.15
DNA extracted with <input type="checkbox"/> NaOH <input type="checkbox"/> Proteinase K <input checked="" type="checkbox"/> Other: Qiagen	1.0
<b>TOTAL VOLUME OF REACTION:</b>	<b>20μL</b>

### Comments on protocol:

- Simplex works best. Use Qiagen DNA only.
- Primers used in this protocol should be kept frozen until used as they tend to degrade quickly, do not leave out for extended periods on lab bench, remove required aliquot and refreeze immediately. **If you notice problems obtaining a reaction it is recommended to re-order new primers.**
- Betaine and DMSO have been standardized due to high GC content. Protocol may be tested without. Also, may adjust MgCl<sub>2</sub> to increase reaction or decrease non specific amplifications.

### Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? <input type="checkbox"/>	94	5:00	1
2. Denaturation	94	0:15	} 10x
3. Annealing	65 to 55 (↓1°C/cycle)	0:30	
4. Elongation		0:40	
5. Denaturation	94	0:15	} 30x
6. Annealing	55	0:30	
7. Elongation		0:40	
8. Amplification	72	5:00	1
9. Finish	15	∞	n/a

### Primers:

Name	Nucleotide Sequence (5' - 3')
1: Sense2 com	AGG ACT GAA AGA CTT GCT CGA
2: Antisense1 wt	GAT AAA ACT AGA ATG GCC CT
3: pudtkR2 mut	CCC AAC GGC GAC CTG TAC AA

### Electrophoresis Protocol:

Agarose: 1.5% V: 100 Estimated Running Time: 60 min

Primer Combination	Expected Bands	Genotype
1 and 2	237 bp	WT +/+
1, 2 and 3	237 / 600 bp	HET +/-
1 and 3	600 bp	KO -/-