

**GENOTYPING BY PCR PROTOCOL**  
**MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS**  
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 530-754-MMRRC

NAME OF PCR: C57BL/6-Mbtps1<sup>wrt</sup>/Mmcd, (woodrat) MMRRC # 012829-UCD

**Protocol:**

Reagent/ Constituent	Volume (μL)
Water	12.675
10x Buffer (contains 15mM MgCl <sub>2</sub> )	2.5
Betaine (stock concentration is 5M) <i>Optional</i>	6.5
dNTPs (stock concentration is 25mM)	0.5
DMSO <i>Optional</i>	0.325
Primer 1 (stock concentration is 20μM) 12829 PCR F1	0.5
Primer 2 (stock concentration is 20μM) 12829 PCR R1	0.5
Taq Polymerase	0.5
DNA sample extracted with <input type="checkbox"/> NaOH <input type="checkbox"/> Proteinase K <input checked="" type="checkbox"/> Other: Any	1.0
<b>TOTAL VOLUME OF REACTION:</b>	<b>25μL</b>

**Comments on protocol:**

- PCR products are verified to contain the correct amplicon size by running ~10μl of the reaction on a gel and the remaining 15μl purified via column based PCR purification method for sequencing.
- The *woodrat* mutation destroys an *Afl* III restriction enzyme site in the *Mbtps1* genomic sequence, and genotyping may also be performed by PCR amplification of the region containing the mutation followed by *Afl* III restriction enzyme digestion.
- 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: 12829 PCR F1	ACA CCC TCA GTG CCA GCC AGG AC
2: 12829 PCR R1	ACA AAG TCT GGG CCA CGT CAC ACA G
3: Woodrat Sequencing	Use PCR primers for sequencing

**Electrophoresis Protocol:**

Agarose: 2% mV: 80 Estimated Running Time: 90 min

Primer Combination	Band	Genotype
1 and 2	300 bp	<i>woodrat</i>
the <i>Afl</i> III site destroyed by the <i>woodrat</i> mutation is highlighted in gray		
Restriction Digest w/ <i>Afl</i> III	158 bp, 142 bp	WT

Mutation site (red) and flanking sequence:

WT tgtccctAcatgt  
*woodrat* tgtccctGcatgt