

**GENOTYPING BY PCR PROTOCOL  
MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS**

2795 2nd Street, Suite 400, Davis, CA 95618

[mmrrc@ucdavis.edu](mailto:mmrrc@ucdavis.edu)

530-754-MMRRC

**NAME OF PCR:** B6.129S2-Dnnt<sup>tm1Gfn</sup>/Mmcd

**MMRRC #** 000045-UCD

**Protocol:**

Reagent/ Constituent	Volume (µL)
Water	11.75
10x Buffer (contains 15mM MgCl <sub>2</sub> )	2.5
dNTPs (stock concentration is 2mM)	2.5
Primer 1 (stock concentration is 10µM) Tdt B	2.5
Primer 2 (stock concentration is 10µM) Tdt A	1.25
Primer 3 (stock concentration is 10µM) Tdt C	1.25
Taq Polymerase 5 Units/µL	0.25
DNA Sample	2.5
<b>TOTAL VOLUME OF REACTION:</b>	<b>25µL</b>

**Comments on protocol:**

Note: *Tdt* is an older synonym of the *Dnnt* gene.

**Strategy:**

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting <span style="float:right">HOT START? <input type="checkbox"/></span>	94	5:00	1
2. Denaturation	94	0:30	} 30x
3. Annealing } steps 2-3-4 will cycle in sequence	55	0:30	
4. Elongation	72	0:30	
5. Amplification	72	5:00	1
6. Finish	4/15	∞	n/a

**Primers:**

Primer Name	Nucleotide Sequence (5' - 3')
1: Tdt B	CTC TAT GAT ACT CTT CAC CTT G
2: Tdt A	GAT GCC CTT GAT ATC CTG G
3: Tdt C	ACG CAC GGG TGT TGG GTC G

**Electrophoresis Protocol:**

% Agarose: 3-4      V: 160

Estimated Running Time (min): \_\_\_\_\_

Expected Bands	Genotype
170 bp	WT +/+
170 / 210 bp	Het +/-
210 bp	KO -/-