

# GENOTYPING BY PCR PROTOCOL FORM

## MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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530-754-MMRRC

NAME OF PCR: B6(B10.BR)-*fas*<sup>theo</sup>/Mmcd (*Theoden*), MMRRC# 031680-UCD

### Protocol:

Reagent/ Constituent	Volume (μL)
Dd H <sub>2</sub> O	4.95
dNTPs (2.5mM each –amplifluor kit)	0.8
20x amplifluor SNP FAM Primer (amplifluor kit)	0.5
20x amplifluor SNP JOE Primer (amplifluor kit)	0.5
20x Theoden specific primer mix (allele 1&2 are 0.5μM, reverse is 7.5μM)	1.5
10x Reaction Mix S Plus	1.0
Platinum Taq (5u/μl)	0.05
DNA Sample (ear punch)	0.7
<b>TOTAL VOLUME OF REACTION:</b>	<b>10μl</b>

**Comments on protocol:** Perform reactions at room temperature with reagents added in order.  
Final MgCl<sub>2</sub> conc. is 2.5mM.

### Strategy: program Amp2

Steps	Temp (°C)	Time (min)	# of Cycles
1. Initiation/Melting <span style="float: right;">HOT START? [√]</span>	94	4:00	1
2. Denaturation	94	0:10	
3. Annealing } steps 2-3-4 will cycle in sequence	60	0:20	35x
4. Elongation	72	0:40	
5. Amplification	72	3:00	1
6. Finish	10	hold	hold

### Primers: Nucleotide sequence (5' - 3')

Primer Name	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
1:Theoden-1	G	A	A	G	G	T	C	G	G	A	G	T	C	A	A	C	G	G	A	T	T	G	A	T	T	C	T	C	T	G	G	C	
	T	G	T	G	A	A	C	A	C	T	A																						
2:Theoden-2	G	A	A	G	G	T	G	A	C	C	A	A	G	T	T	C	A	T	G	C	T	A	T	T	C	T	C	T	G	G	C	T	
	G	T	G	A	A	C	A	C	T	G																							
3:Theoden-R	T	C	A	G	A	A	G	G	C	A	G	G	A	T	G	T	T	C	C	A	T												

