

**GENOTYPING BY PCR PROTOCOL**  
**MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS**  
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**NAME OF PCR:** B6;129S7-Del(11Mpo-Chad)18Brd Dp(11Mpo-Chad)18Brd/Mmcd MMRRC # 030221-UCD

**Protocol:** Run 2 simplex reactions per mouse, one for the duplication and one for the deletion.

Reagent/ Constituent	Volume (μL)
Water	11.275
10x Buffer (contains / without 15mM MgCl <sub>2</sub> )	2.5
MgCl <sub>2</sub> (stock concentration is 25mM)	1.7
Betaine (stock concentration is 5M) <i>Optional</i>	6.5
dNTPs (stock concentration is 10mM)	0.5
DMSO <i>Optional</i>	0.325
Primer 1 (stock concentration is 20μM)	0.5
Primer 2 (stock concentration is 20μM)	0.5
Taq Polymerase 5Units/μL	0.2
DNA (50-200 ng/ μL) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	1.0
<b>TOTAL VOLUME OF REACTION:</b>	<b>25μL</b>

**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.
- 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	} 35x
3. Annealing } steps 2-3-4 will cycle in sequence	65 to 55 (↓1°C/cycle)	0:30	
4. Elongation	72	0:40	
5. Amplification	72	5:00	1
6. Finish	15	∞	n/a

**Primers:**

Name	Nucleotide Sequence (5' - 3')
1: 30221-dupF2	AACCTTACCTGGTATTGAATGCTTGC
2: 30221-dupR	CGCCTGTGCTCTAGTAGCTTT
3: 30221-delF2	GTTGTGGTATAGTGGGACTCTGTAGGG
4: 30221-delR2	CAGAATAGCCAGACATACAATGCAAGC

**Electrophoresis Protocol:**

**Agarose:** 1.5% **V:** 90 **Estimated Running Time:** 90 min.

Primer Combination	Band	Genotype
1 and 2	~420 bp	duplication
3 and 4	251 bp	deletion

- Mice that are hom for the deletion or deficiency (Df/Df) are early embryonic lethal.
- Mice that are hom for the duplication (Dp/Dp) are 100% lethal by 6 weeks of age.
- "Balanced" (Df/Dp) mice that carry both the deficiency (Df) and duplication (Dp) lack a phenotype, are healthy & fertile