

**Dll-1 PCR Protocol: Screening for targeted (*tm*) vs. wild-type (*w*) allele**  
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**PCR reagents:**

1. oligo primers at 20 uM  
**Dll1 + Dll2:** 567 bp targeted (tg) and/or a ~464 bp “wt” alleles  
Dll1 5'-CTA GCT CTT AGG CCT TGG TTG-3' (top)  
Dll2 5'-TTC AAT GAT CAG AGA GAA GGT-3' (bottom)
2. Perkin Elmer PCR buffer with MgCl<sub>2</sub>
3. dNTP premix (I make my own dNTP premix using 100 mM NEB dNTP's. The premix contains 250 ul of each dNTP - A, C, G, & T - and 19 ml sterile water and is stable at -20°C. I freeze this in 1 ml aliquots and thaw and refreeze as needed.)
4. Perkin Elmer Amplitaq Gold
5. genomic DNA samples diluted to 50 ng/ul with sterile water

**PCR reaction mixture:**

15.8 ul sterile water  
2.5 ul 10X PCR buffer  
4 ul dNTP premix  
0.75 ul primer Dll1  
0.75 ul primer Dll2  
1 ul dil. DNA template  
0.2 ul Amplitaq Gold  
25 ul total volume

**Cycling conditions:**

1 cycle - 94° x 6 min.  
40 cycles - 94° x 1 min., 60° x 30 sec., 72° x 30 sec.  
1 cycle - 72° x 7 min.  
hold at 4°C.

**Analysis of PCR products:**

Load 10 ul aliquots of reactions + 2 ul gel loading buffer in a 1% mini-agarose gel (using a 15-well comb). Run gel approximately half-way down.

**Sample gel:**

Standards = Hind III digested λDNA + Hae III digested PhiX 174 DNA  
PCR using primers Dll-1 + Dll-2 showing targeted (*Tg*) & wild-type (*wt*) alleles.

**Dll1 + Dll2**

567 bp *Tg* band  
464 bp *wt* band

**Results:**

Gel #1: samples 34-36 are *wt* animals (w/w)

37-41 are heterozygous for the *Dll-1* allele (*Dll-1*/w)