

## **Delta-Sarcoglycan Genotyping**

This reaction uses 3 oligo primers to identify wild type or mutant delta-SG genotypes.  
The primers used are:

- Mdint1F: GCAAACCTTGGAGAGTGAAGAGGC
- Mdint1R: GAGGCATATAAAGTTTGCACGAC
- NeoTR: GCTATCAGGACATAGCGTTGGCTA

The wild-type product will be a ~650 bp band

The mutant (knock out) product will be a ~750 bp band

Heterozygous products will contain both the 650 bp and the 750 bp bands

Run 20-30 ul of PCR reaction on 1.5% agarose gels for nice separation

### **PCR REACTION CONDITIOS:**

5 ul 10X PCR Reaction Buffer  
5 ul dNTPs (2mM stock)  
1 ul Mdint1F (10 uM stock)  
1 ul Mdint1R (10 uM stock)  
1 ul NeoTR (10uM stock)  
0.5 ul TAQ polymerase (5U/ul)  
35.5 ul dH<sub>2</sub>O

1 ul genomic DNA (processed by isopropanol precipitation method)

### **PCR RXN Program:**

Hot start: 2 min 94°C

40 cycles of:

94°C 1 min  
60°C 1 min  
72°C 1.5 min

Final extension: 5 min 72°C