

Alpha-Sarcoglycan Genotyping

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

- Mfd1: CCCAGGGCCTTGATGCCT
- CKO-2: CAGGGCTGGGAGCTGGGTTCTG
- NeoTR: GCTATCAGGACATAGCGTTGGCTA

The wild-type product will be a 1000 bp band

The mutant (knock out) product will be a 600 bp band

Heterozygous products will contain both the 1000 bp and the 600 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 Mfd1 (10 uM stock)
1 ul CKO-2 (10 uM stock)
1 ul NeoTR (10uM stock)
0.5 ul TAQ polymerase (5U/ul)
35.5 ul dH₂O

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

55°C 1 min

72°C 1.5 min

Final extension: 10 min 72°C