

Ca Genotyping protocol (PCR)

Oligos

SM Ca6: CTGACCTTTGAGTATCTGCAC

SMCa7:GTCCCACACAAGGTCCAAGTA

Ca-3 intron: AGACTACTGCTCTATCACTGA

SM Neo-3: GTGGTTTGTCCAAACTCATCAATGT

We run two different reactions. One reaction for the wt (250 bp product) and one for the knockout (270 bp product). We run the reaction on 7/5% acrylamide gel and can separate the two products if you load a small volume (less than 10 ul).

The program we run on the thermocycler is

95° x2 min followed by 30 cycles of (94° x 30 sec, 62°x30 sec, 72°x60 sec)

We typically use about 150 ng of tail digested DNA suspended in low TE (1 mM Tris, 25uM EDTA) in each (30 ul) reaction as follows:

H2O	24.5 ul
RP-PCR (10X)	3 ul
BSA (10mg/ml)	0.1958 ul
dNTP's(10 mM each)	1.5 ul
Amp Taq (5u/ul)	0.3 ul

Plus approximately 200 ng of each oligo for each reaction.

Use Ca-3 intron and SM-Neo-3 for the knockout reaction

Use Ca-6 and Ca-7 for the wild type reaction.

Buffers

10XRP-PCR Buffer

166 mM (NH₄)SO₄

670 mM Tris pH 8.8

67 mM MgCl₂

10X Buffer 9

100 mM Tris.HCl pH 9.2 (with KOH)

15 mM MgCl₂

250 mM KCl