05.22.17 MLS

Genotyping Protocol: MMRRC 29870

Assay Type: PCR - can distinguish heterozygous animals from homozygous animals

DNA Extraction: DNA from tail snips was extracted using Sigma's Extract-N-Amp Tissue PCR Kit (Cat# XNAT2R). Kit directions for animal tissues were performed with a few minor modifications as follows: Use only 50 µl of Extraction Solution, 12.5 µl Tissue Preparation Solution and 50 µl of Neutralization Solution B.

Mutation: This strain carries a floxed allele of *Sprouty1* on a B6 background.

Primer Information:
1) Name: Sprouty1.71 Sequence: 5'-CTC AAT AGG AGT GGA CTG TGA AAC TGC-3'
2) Name: Sprouty1.72 Sequence: 5'-GGG AAA ACC GTG TTC TAA GGA GTA GC-3'

Assay Name: Sprouty1 PCR

PCR Master Mix Components:

component	manufacturer	concentration	µl/rxn
Extract-n-amp solution	Sigma (Cat# XNAT2R)	2X	10
Sprouty1.71	Sigma or IDT	25 uM	0.3
Sprouty1.72	Sigma or IDT	25 uM	0.3
sterile water			5.4

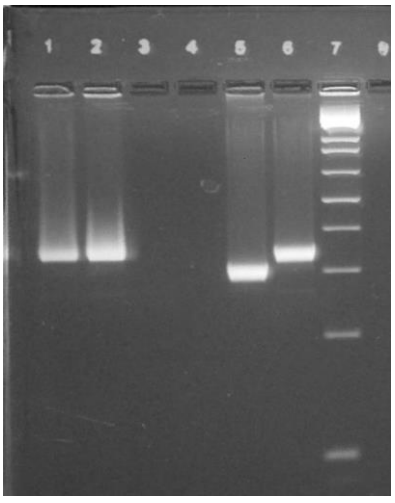
PCR Setup:
Final Reaction: 16µl master mix & 4µl DNA template (10-20ng/µl)

All reactions were performed in 200µl thin walled PCR tubes and were run in an Applied Biosystems 2700 thermocycler.

Cycle Parameters:
1) 94°C 3 minutes
2) 94°C 30 seconds
3) 58°C 30 seconds
4) 72°C 45 seconds
5) Repeat steps 2-4 34 times for a total of 35 cycles
6) 72°C 10 minutes
7) 4°C hold until refrigerate product

Product Analysis:
All products were analyzed on a 3% agarose gel with ethidium bromide staining. Run at 80 V for 90 min.
Wild type allele = 311 bp
Floxed allele= 342 bp

Example of Gel:



Wells 1 and 2 are homozygous for the floxed allele.
Well 5 is a wild-type control.
Well 6 is a homozygous floxed allele control.
Well 7 is 1 Kb Plus DNA ladder (Invitrogen Cat. # 10787-018).