PCR genotyping protocol for *Sst*$^{rtTA.LCA}$ mouse line

PCR reagents:

1. oligo primers at 20 µM

   Amplification across lox71 (5') site:

   Sst.HA1F 5'- GAGCAGTTACTTTGAAAGCGG -3'
   rtTA-R1 5'- CAGGCCTTCGATACCGAC -3'

   Sst.HA1F + rtTA-R1 yields a **1364bp TM** (targeted mutant allele) band

   Amplification across lox2272 site (3'):

   5NeoR 5'- TCTATCGCCTTCTTGACGAGTTCT -3'
   Sst-HA2R2 5'- CCTGACTCCTATGTTGCTAAAACC -3'

   5NeoR + Sst-HA2R2 yields a **994 bp TM** (targeted mutant allele) band

2. Perkin Elmer PCR buffer with $MgCl_2$
3. 1.25mM dNTP premix (dNTP premix is made by using 100 mM NEB dNTP's. The premix contains 250 µl 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/µl with sterile water

PCR reaction mixture:

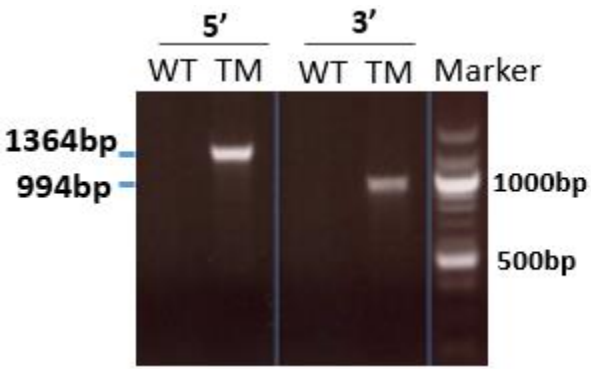
15.8 µl sterile water
2.5 µl 10X PCR buffer
4 µl dNTP premix
0.75 µl primer #1
0.75 µl primer #2
1 µl dil. DNA template
0.2 µl Amplitaq Gold
25 µl total volume

Cycling conditions:

1 cycle - 94°C x 6 min.
40 cycles - 94°C x 1 min., 60°C x 30 sec., 72°C x **2** min.
1 cycle - 72°C x 7 min.
hold at 4°C.

Analysis of PCR products:

Load 10 µl aliquots of reactions + 2 µl of 6X gel loading buffer in a 1% mini-agarose gel (using a 15-well comb). Run gel approximately half-way down.


5'
3'
WT TM
WT TM
Marker
1364bp
994bp
1000bp
500bp