STOCK Tg(THY1-Snca)M1mSud/J

Stock No: 008132
Protocol 23862: Standard PCR Assay - Tg(THY1-SNCA*A53T)M53Sud, F53Sud
Version 4.2

Notes

This assay will NOT distinguish hemizygous from homozygous transgenic animals.

Melting curve analysis is done using a Roche Light Cycler 480.

The genotyping protocol(s) presented here have been optimized for reagents and conditions used by The Jackson Laboratory (JAX). To genotype animals, JAX recommends researchers validate the assay independently upon receipt of animals into their facility. Reaction cycling temperature and times may require additional optimization based on the specific genotyping reagents used.

Expected Results

Transgene Tm = 86.5°C +/- 1°C
Internal positive control Tm = 83°C +/- 1°C

Transgene = 500 bp

JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
oIMR8584		GGC ACC TAG AGG ATC TCG ACT AGT GG		Transgene Forward	A	
oIMR8585		GGA CCT CGA CGC TTA GGC TTC AGG		Transgene Reverse	A	
oIMR8744		CAA ATG TTG CTT GTC TGG TG		Internal Positive Control Forward	A	
oIMR8745		GTC AGT CGA GTG CAC AGT TT		Internal Positive Control Reverse	A	

Reaction A

COMPONENT	FINAL CONCENTRATION
ddH2O	
Kapa 2G HS buffer	1.30 X
MgCl2	2.60 mM
dNTP KAPA	0.26 mM
oIMR8584	0.50 uM
oIMR8585	0.50 uM
oIMR8744	0.50 uM
oIMR8745	0.50 uM
Glycerol	6.50 %
Dye	1.00 X
Kapa 2G HS taq polym	0.03 U/ul
DNA	

Cycling

STEP	TEMP °C	TIME	NOTE
1	94.0	--	
2	94.0	--	
3	65.0	--	-0.5 C per cycle decrease
4	68.0	--	
5		--	repeat steps 2-4 for 10 cycles (Touchdown)
6	94.0	--	
7	60.0	--	
8	72.0	--	
9		--	repeat steps 6-8 for 28 cycles
10	72.0	--	
11	10.0	--	hold

JAX uses a very high speed Taq (~1000 bp/sec), use cycling times recommended for your reagents.

JAX uses a 'touchdown' cycling protocol and therefore has not calculated the optimal annealing temperature for each set of primers.