

## B6;SJL-Tg(Thy1-TARDBP)4Singh/J

Stock No: 012836

Protocol 25492: Standard PCR Assay - Tg(Thy1-TARDBP)4Singh

Version 2.2

### Notes

Donating Investigator reports this transgene had integrated at locus 6qB3 of the mouse genome (nucleotide 56,524,796) and did not interrupt any known gene.

WT F and Common R primers amplify a PCR product from mouse of 303 bp. Tg F primer is specific for the Tg.

This assay will distinguish hemizygous from homozygous transgenic animals.

Changed common from .13791 (no amplification with Tg) to .13790 per Kai (successful amplification with Tg) SC 3-1-12.

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

Mutant = ~500 bp

Heterozygote = 303 bp and ~500 bp

Wild type = 303 bp

### JAX Protocol

#### Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
13790		TGA AAT CCG GGT GGT ATT GG		Common	A	
13791		GGT GAG TTT AAC CTT CAA GGG CT		Wild type	A	
13792		AGC TTG CTA GCG GAT CCA GAC		Transgene	A	

#### Reaction A

COMPONENT	FINAL CONCENTRATION
ddH <sub>2</sub> O	
Kapa 2G HS buffer	1.30 X
MgCl <sub>2</sub>	2.60 mM
dNTP KAPA	0.26 mM
13790	0.50 uM
13791	0.50 uM
13792	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.

