B6CBA-Tg(Prnp-TBP*)105Xjl/J

Stock No: 008075
Protocol 23526: Standard PCR Assay - (Prnp-TBP*)105Xjl
Version 1.2

Notes

AM Bufer: 670 mM TrisHCl pH 8.8, 166 mM (NH4)2SO4, 20 mM MgCl2, 1.7 mg/ml BSA, 10 mM 2-mercaptoethanol (Add 2-mercaptoethanol to 10 mM just prior to use. Do not store with 2-mercaptoethanol in buffer.)

Size on ABI3730.

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 = 545 bp
C57BL/6J = 276 bp
Human = 341 bp

JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
TBP-A629		AGA AGC TGG TGT GGC AGG AGT GAT			A	
TBP-S365	Fluorophore	CCA CAG CCT ATT CAG AAC ACC			A	

Reaction A

COMPONENT	FINAL CONCENTRATION
ddH2O	
Kapa 2G HS buffer	1.30 X
MgCl2	2.60 mM
dNTPS-kapa	0.26 mM
TBP-A629	0.50 uM
TBP-S365	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.