

B6.Cg-Tg(GFAP-APOE_i2)14Hol *Apoe^{tm1Unc}/J*

Stock No: 004632

Protocol 22364: Standard PCR Assay - *Apoe^{tm1Unc}*

Version 1.3

Notes

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 = ~245 bp

Heterozygote = ~155 bp and ~245 bp

Wild type = ~155 bp

Separated by gel electrophoresis on a 1.5% agarose gel.

JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
oIMR0180		GCC TAG CCG AGG GAG AGC CG		Common	A	
oIMR0181		TGT GAC TTG GGA GCT CTG CAG C		Wild type Reverse	A	
oIMR0182		GCC GCC CCG ACT GCA TCT		Mutant Reverse	A	

Reaction A

COMPONENT	FINAL CONCENTRATION
ddH ₂ O	
Kapa 2G HS buffer	1.30 X
MgCl ₂	2.60 mM
dNTP KAPA	0.26 mM
oIMR0180	0.50 uM
oIMR0181	0.50 uM
oIMR0182	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.

