

B6.Cg-Apoe^{tm1.1(APOE*4)A}Adiuj Abca7^{em1A}Adiuj Trem2^{em1A}Adiuj/J

Stock No: 030320

Protocol 28934: Standard PCR Assay - Apoe^{tm1.1(APOE*4)A}Adiuj

Version 1.2

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 = 148 bp

Heterozygote = 148 bp and 224 bp

Wild type = 224 bp

Gel on 3% due to nonspecific bands close to wt.

JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
24463		AAT TTT TCC CTC CGC AGA CT		Common	A	
24464		ACA GCT GCT CAG GGC TAT TG		Wild type Reverse	A	
24465		AGG AGG TTG AGG TGA GGA TG		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
24463	0.50 uM
24464	0.50 uM
24465	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.

