

CBA/CaJ

Stock No: 000654

Protocol 31378: Standard PCR Assay - Generic Pde6b Alternate1

Version 1.0

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 = ~550 bp

Heterozygote = 318 bp and ~550 bp

Wild type = 318 bp

 >[chr5:108819374+108819691](#) 318bp CATGTCCTACAGCCCTCTC ACCATTTGCAAGGAAAGCAC

JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
36758		CAT GTC CTA CAG CCC CTC TC		Common	A	
36759		ACC ATT TGC AAG GAA AGC AC		Wild type Reverse	A	
oIMR2093		AAG CTA GCT GCA GTA ACG CCA TTT		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
36758	0.50 uM
36759	0.50 uM
oIMR2093	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.

