

NOD.129P2(B6)-I2^{tm1Hor}/DvsJ

Stock No: 002573

Protocol 26241: Standard PCR Assay - I2<tm1Hor>

Version 2.2

Notes

Validation/LC Conversion data attached to rec 165561

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

Evagreen

HT-TD65-60 cycling RS Macro Genescanning analysis

DO NOT USE TM Calling analysis, cannot differentiate between Het/Hom

Mutant = 500 bp

Heterozygote = 324 bp and 500 bp

Wild type = 324 bp

JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
oIMR0041		TCG AAT TCG CCA ATG ACA AGA CGC T			A	
oIMR7338		CTA GGC CAC AGA ATT GAA AGA TCT			A	
oIMR7339		GTA GGT GGA AAT TCT AGC ATC ATC C			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
oIMR0041	0.50 uM
oIMR7338	0.50 uM
oIMR7339	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.

