

NOD/ShiLt-Tg(Ins2-TAg)1Lt/J

Stock No: 002033

Protocol 30011: QPCR Assay - TAg

Version 4.0

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

JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
oIMR1544		CAC GTG GGC TCC AGC ATT		Internal Positive Control Forward	A	
oIMR3580		TCA CCA GTC ATT TCT GCC TTT G		Internal Positive Control Reverse	A	
oIMR9161		CAT GAA CAG ACT GTG AGG ACT GAG		Forward	A	
oIMR9162		GGA AGA CTC AGG GCA TGA AAC		Reverse	A	
TmoIMR0105	Fluorophore-1	CCA ATG GTC GGG CAC TGC TCA A	Quencher-1	IC Probe		
TmoIMR0133	Fluorophore-2	CCT GAA ATG AGC CTT GGG ACT GTG A	Quencher-2	MUT Probe		

Reaction A

COMPONENT	FINAL CONCENTRATION
ddH ₂ O	
Kapa Probe Fast QPCR	1.00 X
oIMR1544	0.40 uM
oIMR3580	0.40 uM
oIMR9161	0.40 uM
oIMR9162	0.40 uM
Tg Probe	0.15 uM
IC Probe	0.15 uM
DNA	

Cycling

STEP	TEMP °C	TIME	NOTE
1	95.0	--	
2	95.0	--	
3	60.0	--	repeat steps 2-3 for 40 cycles

JAX uses a very high speed Taq (~1000 bp/sec), use cycling times recommended for your reagents.