

## NOD.Cg-Kit<sup>W-41J</sup> Tyr + Prkdc<sup>scid</sup> Il2rg<sup>tm1Wjl</sup>/ThomJ

Stock No: 026622

Protocol 14262: Pyrosequencing Assay - Kit<W-41J>

Version 3.0

### Notes

This genotyping assay uses pyrosequencing technology and is run on the Biotage PSQ 96MA. The Jackson Laboratory is not posting the complete details of our pyrosequencing genotyping assays as the primers for pyrosequencing cannot be used for sequencing using more traditional methods. The wild type and mutant nucleotides and the flanking DNA sequence are provided below.

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 = A/A

Heterozygote = A/G

Wild type = G/G

### Sequence

```
CAAAGCAGTCTGGGAAATGTAGTTATAGTTGGGAGAGGC
CATATATGTTGTTGACAAA
AAAAAAAAAAAAAAAAAGAAGAAGAAGAAAGTTGCTA
GTTGAGGCCATTACTAG
AAGGAAGGTTAGAACCCCTGGACTTCTCTGCTCTTAGTTT
ACTGTCC TACTGACTCA
ACACCCCTATTTTAAAGGGAGATATTAGAA TTTTGAATTAT
AAGTAGGGGAGGTGGCTG
GAGGTCACAAGGTTTAAAGTCCTCGTCTATCGCTGCTTTC
ATTAGCTGCTTGAATTTGC
TGTGTTCCGTTCTAGGCACGACTGCCC(g/a)TGAAGTGGAT
GGCACCAGAGAGCATTTT
CAGCTGCGTGTACACATTTGAAAGTGATGTCTGGTCCTAT
GGGATTTTCTCTGGGAG
CTCTTCTCCTTAGGTAAAATGTTCTTACCCAAGATGCCC
TCACTGTCTAAAAGCTTCT
GTCGTCTCCCGCCACATGGACACTGATTTGCCACCTCT
CTGCACCCAGGAAGCAGCC
CCTACCCAGGGATGCCGGTCCGACTCCAAGTTCTACAAGA
TGATCAAGGAAGGCTTCCG
GATGGTCAGCCCGGAGCACGCGCCTGCC
```

### JAX Protocol

#### Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
poIMR0124		TTG CTG TGT TCC GTT CTA GGC		Forward		
poIMR0125	Fluorophore	GCT CTC TGG TGC CAT CCA CT		Reverse		
poIMR0126		CGT TCT AGG CAC GAC TG		SEQ		

## Reaction A

COMPONENT	FINAL CONCENTRATION
ddH2O	
Kapa 2G HS buffer	1.00
MgCl <sub>2</sub>	2.00
dNTPS-kapa	0.20
poIMR0124	0.50
poIMR0125	0.50
Glycerol	5.00
Kapa 2G HS taq polym	0.01

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
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.