

## NOD.Cg-Rag1<sup>tm1Mom</sup> Dysf<sup>prmd</sup> Il2rg<sup>tm1Wjl</sup>/McalJ

Stock No: 029663

Protocol 27761: Standard PCR Assay - Rag1&lt;tm1Mom&gt;Alternate1

Version 1.2

### Notes

To run this assay on agarose you will need to run two separate reactions; one for the wild type Rag1 allele (Wild type forward and common primers) and one for the Rag1 knockout allele (mutant forward and common primers).

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 = 197 bp

Heterozygote = 197 bp and 192 bp

Wild type = 192 bp

If running on a gel run separated:

23267 and 23268 for Wt

23268 and oIMR8162 for Mut

### JAX Protocol

#### Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
23267		TCT GGA CTT GCC TCC TCT GT		Wild type Forward	A	
23268		CAT TCC ATC GCA AGA CTC CT		Common	A	
oIMR8162		TGG ATG TGG AAT GTG TGC GAG		Mutant Forward	A	PGK R

#### Reaction A

COMPONENT	FINAL CONCENTRATION
ddH <sub>2</sub> O	
Kapa 2G HS buffer	1.30 X
MgCl <sub>2</sub>	2.60 mM
dNTP KAPA	0.26 mM
23267	0.50 uM
23268	0.50 uM
oIMR8162	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.



