



Welcome to The Genotyping Protocol System

Master Protocol

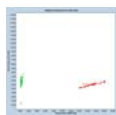
Strain Name: C57BL/6NJ-*Nim1k^{em1J}*
Stock Number: 029629
Allele: *Nim1k^{em1J}*
Protocol Name: *Nim1k^{em1J}*
Method: End Point Analysis
Created: 31-August -2016 (LUCASM) **Updated:** 23-November -2016 (JKELMEN)

Notes

Notes: Taqman qPCR protocols are run on a real time PCR instrument. Use an appropriate instrument specific Fluorophore/Quencher combination. The transgene genotype is determined by comparing ΔC_t values of each unknown sample against known homozygous and hemizygous controls, using appropriate endogenous references.

Expected Results: Mutant= 94 bp
Wild Type = 112 bp

Attachments



View [nim1k val.jpg](#)

- View [Nim1 genomic.gcs](#)
- View [Nim1kem1j in progress 8-29-16.docx](#)

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
29449	-	CTT CTC CCA GAT CCT GTC TGC	-	Wild type Forward	A
29450	-	TGG GAT GAT GAT GTG AAA GTG A	-	Common	A
29451	-	CAG GTT AAA GGG GAA AGT TGG	-	Mutant Forward	A
29453	Hex	AAG CAG GGG CTC CCA TTC	Black Hole Quencher 1	WT Probe	-
29454	6-FAM	CAG GGA CCA CAG ACA GTG AC	Black Hole Quencher 1	MUT Probe	-

Number Of Reactions 1

Reaction A (Endpoint 3 primer)		Cycling (EndPoint)			
Component	Final Concentration	Step #	Temp°C	Time	Note
Kapa Probe Fast QPCR	1 X	1	95	3min	-
ddH2O		2	95	5sec	-
29449	.4 uM	3	60	30sec	-
29451	.4 uM	4	-	-	repeat steps 2-3 for 40 cycles
29450	.4 uM	5	4	-	Forever
Wt Probe	.15 uM				

Reaction A (Endpoint 3 primer)	
Mutant Probe	.15 uM
DNA	

Version 3.2