



Welcome to The Genotyping Protocol System

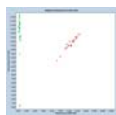
Master Protocol

Strain Name: C57BL/6NJ-Kif15^{em1J}J
Stock Number: 029556
Allele: Kif15^{em1J}
Protocol Name: Kif15^{em1J}
Method: End Point Analysis
Created: 25-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= 166 bp
Wild Type = 130 bp

Attachments

View kif15.jpg

- View Kif15em1j in progress 8-10-16.docx
- View Kif15 genomic.gcs

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
29369	-	AGG CAA GCT GAG ATT CTG GT	-	Common	A
29370	-	CCC TCT TCA GCA GGA CGA A	-	Wild type Reverse	A
29371	-	GTC TTA AAT TAG GGT GTT TGT CCA G	-	Mutant Reverse	A
29372	Hex	CTT TGA TAG CGT CAC CTT CAT T	Black Hole Quencher 1	WT Probe	-
29373	6-FAM	TGT CAC TTT TTA GCT TGG CAG A	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	-
29369	.4 uM	3	60	30sec	-
29370	.4 uM	4	-	-	repeat steps 2-3 for 40 cycles
29371	.4 uM	5	4	-	Forever
Wt Probe	.15 uM				

Reaction A (Endpoint 3 primer)

Mutant Probe	.15 uM
DNA	

Version 3.2