



Welcome to The Genotyping Protocol System

Master Protocol

Strain Name: C57BL/6NJ-Tomm22^{em1J}/J
Stock Number: 029159
Allele: Tomm22^{em1J}
Protocol Name: Tomm22^{em1J}
Method: Probe
Created: 16-August -2016 (LUCASM) **Updated:** 12-September-2016 (ESCHAAB)

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= 200 bp
Wild Type = 194 bp

Attachments



View Tomm22-29159 EP.JPG

- View Tomm22 Genomic-1.gcs
- View Tomm22em1jMolDesApr2016-1.docx

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
27879	-	ACC CCC TCT TCT GGA GTG TC	-	Common	A
29229	-	CGG GTG CTG TCA AAG GA	-	Wild type Forward	A
29230	-	GAA GAA GAC GAC GAC GAC GA	-	Mutant Forward	A
29231	Hex	TGT CAT ACC CCG AGT GTG GT	Black Hole Quencher 1	WT Probe	-
29232	6-FAM	AGA GGG CGG CTT CTC C	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	-
29229	.4 uM	3	60	30sec	-
27879	.4 uM	4	-	-	repeat steps 2-3 for 40 cycles
29230	.4 uM	5	4	-	Forever
Wt Probe	.15 uM				

Reaction A (Endpoint 3 primer)

Mutant Probe	.15 uM
DNA	

Version 3.2