



Welcome to The Genotyping Protocol System

Master Protocol

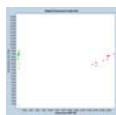
Strain Name: C57BL/6NJ-*Tubgcp4*^{em1J}/J
Stock Number: 029689
Allele: *Tubgcp4*^{em1J} Probe
Protocol Name: *Tubgcp4*^{em1J} Probe
Method: Probe
Created: 22-September-2016 (LUCASM) **Updated:** 13-December -2016 (ESCHAAB)

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= 93 bp
Wild Type = 129 bp

Attachments



View [Tubgcp4-29689 EP.jpg](#)

- View [Tubgcp4 genomic.gcs](#)
- View [Tubgcp4em1jMolDesSep2016.docx](#)

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
29839	-	CAT AGC TGT GGG GGC TTG	-	Wild type Forward	A
29840	-	CAC TTG ACT CCC TCC CCT A	-	Common	A
29841	-	GAT ATA ACC CTT TCC CCA GCA	-	Mutant Forward	A
29842	Hex	CCG AAG CGC ATT AGA AAA GT	Black Hole Quencher 1	WT Probe	-
29843	6-FAM	ACA CTG AGG GCG GAA GAG T	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	-
29839	.4 uM	3	60	30sec	-
29841	.4 uM	4	-	-	repeat steps 2-3 for 40 cycles
29840	.4 uM	5	4	-	Forever
Wt Probe	.15 uM				

Reaction A (Endpoint 3 primer)

Mutant Probe	.15 uM
DNA	

Version 3.2