



Welcome to The Genotyping Protocol System

Master Protocol

Strain Name: C57BL/6NJ-*Tbc1d30^{em1J}*/*J*
Stock Number: 029630
Allele: *Tbc1d30^{em1J}*
Protocol Name: *Tbc1d30^{em1J}*
Method: Probe
Created: 31-August -2016 (LUCASM) **Updated:** 15-November -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= 132 bp
Wild Type = 108 bp

Attachments



View Tbc1d30-29630 EP.jpg

- View Tbc1d30 genomic.gcs
- View Tbc1d30em1jin progress 8-29-16.docx

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
29455	-	TCA GCA CCT CGA CAC ACT TC	-	Wild type Forward	A
29456	-	GCA GAG CAG AAC CAG ACC AG	-	Common	A
29457	-	TCG CAG CAT AGC TGA CTT TG	-	Wild type Forward	A
29458	6-FAM	TCT CAT TGC AGG AGG GTG A	Black Hole Quencher 1	MUT Probe	-
29459	Hex	CGA ACA AAG AGA GCG GAG GT	Black Hole Quencher 1	WT 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	-
29455	.4 uM	3	60	30sec	-
29456	.4 uM	4	-	-	repeat steps 2-3 for 40 cycles
29457	.4 uM	5	4	-	Forever
Wt Probe	.15 uM				

Reaction A (Endpoint 3 primer)

Mutant Probe	.15 uM
DNA	

Version 3.2