



Welcome to The Genotyping Protocol System

Master Protocol

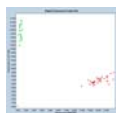
Strain Name: C57BL/6NJ-*Tbc1d31*^{em1J}/_J
Stock Number: 029728
Allele: *Tbc1d31*^{em1J} Probe
Protocol Name: *Tbc1d31*^{em1J} Alternate1
Method: Probe
Created: 14-December -2016 (LUCASM) **Updated:** 22-December -2016 (JKELMEN)

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= 191 bp
Wild Type = 168 bp

Attachments



View [Tbc1d31-29728 EP.jpg](#)

- View [Tbc1d31 genomic.gcs](#)
- View [Tbc1d31em1j in progress 9-27-16.docx](#)

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
30176	-	GCT CTC AGA TCT TCT GTC TGG T	-	Common	A
30178	-	CTG TGC ACG CTT GTG CT	-	Wild type Reverse	A
30179	-	TCG TGT GAG CAA CCT TGA AC	-	Mutant Reverse	A
30180	Hex	AGA AAT GGC TGT ATT AGT ATC AGG A	Black Hole Quencher 1	WT Probe	-
31053	6-FAM	CAC TGG TAG ACT TCT GAA GC	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	-
30179	.4 uM	3	60	30sec	-
30178	.4 uM	4	-	-	repeat steps 2-3 for 40 cycles
30176	.4 uM	5	4	-	Forever
Wt Probe	.15 uM				

Reaction A (Endpoint 3 primer)

Mutant Probe	.15 uM
DNA	

Version 3.2