



Welcome to The Genotyping Protocol System

Master Protocol

Strain Name: C57BL/6NJ-Gabarapl1^{em1J}/J
Stock Number: 029750
Allele: Gabarapl1^{em1J} Probe
Protocol Name: Gabarapl1^{em1J}
Method: Probe
Created: 13-October -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= 98 bp
Wild Type = 105 bp

Attachments



View Gabarapl1-29750 EP.jpg

- View Gabarapl1 genomic.gcs
- View Gabarapl1em1jMolDesOct2016.docx

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
30237	-	CCG TAG GGT GAA TGA CTT TTA TAG	-	Common	A
30238	-	CCC ATG CTG TCC AGT CTC A	-	Wild type Reverse	A
30239	-	GGG TAC ATG AGT ATG GCA TTG G	-	Mutant Reverse	A
30240	Hex	AGT CCC CAT TTC CAA TAC TCC	Black Hole Quencher 1	WT Probe	-
30241	6-FAM	AGA ACT AGA AAC AGG AGG GCA GA	Black Hole Quencher 1	MUT Probe	-

Number Of Reactions 1

Reaction A (3 primer)		Cycling (EndPoint)			
Component	Final Concentration	Step #	Temp°C	Time	Note
ddH2O		1	95	3min	-
Kapa 2G HS buffer	1.3 X	2	95	5sec	-
MgCl2	2.6 mM	3	60	30sec	-
dNTP KAPA	.26 mM	4	-	-	repeat steps 2-3 for 40 cycles
30238	.5 uM	5	4	-	Forever
30239	.5 uM				

Reaction A (3 primer)	
30237	.5 uM
Glycerol	6.5 %
Dye	1 X
Kapa 2G HS taq polymerase	.03 U/ul
DNA	

Version 3.2