



Welcome to The Genotyping Protocol System

Master Protocol

Strain Name: C57BL/6NJ-*Rps6ka1^{em1J}*J
Stock Number: 029658
Allele: *Rps6ka1^{em1J}* Probe
Protocol Name: *Rps6ka1^{em1J}* Probe
Method: Probe
Created: 21-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= 121 bp
Wild Type = 91 bp

Attachments



View [Rps6ka1-29658 EP.jpg](#)

- View [Rps6ka1em1jMolDesSep2016.docx](#)
- View [Rps6ka1 genomic.gcs](#)

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
29819	-	GAA CTG AGG CTG GGC TTA GG	-	Wild type Forward	A
29820	-	CCA GAG ACT CCT GAG AAC GAG	-	Common	A
29821	-	GGG TCT CAC AGG TCG TGT CT	-	Mutant Forward	A
29822	Hex	CAG GCT GCA GGA TCA CAG A	Black Hole Quencher 1	WT Probe	-
29823	6-FAM	TGG AAT GTC AGG GGT TAC CT	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	-
29820	.4 uM	3	60	30sec	-
29819	.4 uM	4	-	-	repeat steps 2-3 for 40 cycles
29821	.4 uM	5	4	-	Forever
Wt Probe	.15 uM				

Reaction A (Endpoint 3 primer)	
Mutant Probe	.15 uM
DNA	

Version 3.2