



Welcome to The Genotyping Protocol System

Master Protocol

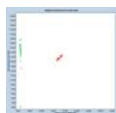
Strain Name: C57BL/6NJ-Sara^{fem1J}/_J
Stock Number: 029729
Allele: Sara^{fem1J} Probe
Protocol Name: Sara^{fem1J} Probe
Method: Probe
Created: 30-September-2016 (LUCASM) **Updated:** 21-December -2016 (JKELMEN)

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= 106 bp
Wild Type = 91 bp

Attachments



View Saraf probe JW.jpg

- View Saraf genomic.gcs
- View Sarafem1jMolDesSep2016.docx

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
29995	-	CAC AGG TAT GTT TCC CTG CTC	-	Wild type Forward	A
29996	-	TCA GCA ATG TTA ACC TCC TCT TC	-	Common	A
29997	-	GCC ATC TGG GGA ATA AGG A	-	Mutant Forward	A
29998	Hex	CGG GTC CAT GGG TGT GT	Black Hole Quencher 1	WT Probe	-
30000	6-FAM	CAG AGT GAG TGC TCG TGT ACC	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	-
29996	.4 uM	3	60	30sec	-
29997	.4 uM	4	-	-	repeat steps 2-3 for 40 cycles
29995	.4 uM	5	4	-	Forever
Wt Probe	.15 uM				

Reaction A (Endpoint 3 primer)

Mutant Probe	.15 uM
DNA	

Version 3.2