



Welcome to The Genotyping Protocol System

Master Protocol

Strain Name: C57BL/6NJ-Cercam^{em1J}J
Stock Number: 029560
Allele: Cercam^{em1J}
Protocol Name: Cercam^{em1J}
Method: Probe
Created: 15-August -2016 (LUCASM) **Updated:** 30-November -2016 (DLINDNER)

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= 120 bp
Wild Type = 186 bp

Attachments



View Cercam-29560 EP.jpg



View Cercam-29560 EP Zoomed.jpg

- View Cercam genomic.gcs

- View Cercamem1j_in progress 8-11-16.docx

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
29175	-	CTT CTT CAG CAG GCC TTG G	-	Wild type Reverse	A
29176	-	ACA GAT GGG GAC AGC CAC T	-	Common	A
29177	-	GGG TCC CAG AGA CTG GAT TC	-	Mutant Forward	A
29179	Hex	TCC ATC CCA CAT TTC ATC CT	Black Hole Quencher 1	WT Probe	-
29180	6-FAM	TGG CCC TGG TTA GAT CTC TGT	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	-
29177	.4 uM	3	60	30sec	-

Reaction A (Endpoint 3 primer)		Cycling (EndPoint)			
29175	.4 uM	4	-	-	repeat steps 2-3 for 40 cycles
29176	.4 uM	5	4	-	Forever
Wt Probe	.15 uM				
Mutant Probe	.15 uM				
DNA					

Version 3.2