



Welcome to The Genotyping Protocol System

Master Protocol

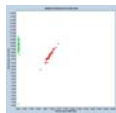
Strain Name: C57BL/6NJ-Krt90^{em1J}J
Stock Number: 029496
Allele: Krt90^{em1J}
Protocol Name: Krt90^{em1J}
Method: Probe
Created: 26-August -2016 (LUCASM) **Updated:** 07-October -2016 (ESCHAAB)

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: Muant= 124 bp
Wild Type = 107 bp

Attachments



View [krt90 EP JW.jpg](#)

- View [Krt90 genomic.gcs](#)
- View [Krt90em1jMolDesJuly2016_edited 7-29-16.docx](#)

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
29400	-	CAA GCC CAC TGT ACC AAC AC	-	Mutant Forward	A
29401	-	CGG AGC AGG CTG GAG A	-	Wild type Forward	A
29402	-	CCT GGT TCC ATG GCT CTG	-	Common	A
29403	Hex	CGT GGT GGA GGA CTT CAA GA	Black Hole Quencher 1	WT Probe	-
29404	6-FAM	CAG AAG CCA CCA CCT GAG T	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	-
29401	.4 uM	3	60	30sec	-
29400	.4 uM	4	-	-	repeat steps 2-3 for 40 cycles
29402	.4 uM	5	4	-	Forever
Wt Probe	.15 uM				

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

Version 3.2