



Welcome to The Genotyping Protocol System

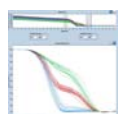
Master Protocol

Strain Name: C57BL/6NJ-Kcnc3^{em1J}/_J
Stock Number: 028540
Allele: Kcnc3^{em1J}
Protocol Name: Kcnc3^{em1J}
Method: MELT
Created: 10-December -2015 (JKELMEN) **Updated:** 16-February -2016 (ESCHAAB)

Notes

Expected Results: Mutant = 215 bp
Heterozygote = 215 bp and 283 bp
Wild type = 283 bp

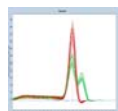
Attachments



View 28540.JPG



View 319921-28540 Kcnc3 gel.jpg



View 319921-28540 Kcnc3 tm calling.jpg

- View Kcnc3em1jMolDesNov2015.docx

- View Kcnc3 genomic1.gcs

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
24566	-	CCT GAG CCT CTG TTC CTG TC	-	Common	A
25932	-	CAC CGT CTT GTT GCT GAT GT	-	Wild type Reverse	A
25933	-	CAC CCT ACT CTG CCT GAA CC	-	Mutant Reverse	A

Number Of Reactions 1

Reaction A (3 primer)		Cycling (TouchDown 65-60_12-12-11)			
Component	Final Concentration	Step #	Temp°C	Time	Note
ddH2O		1	94	2 min	-
Kapa 2G HS buffer	1.3 X	2	94	20sec	-
MgCl2	2.6 mM	3	65	15sec	-0.5 C per cycle decrease
dNTP KAPA	.26 mM	4	68	10sec	-
24566	.5 uM	5	-	-	repeat steps 2-4 for 10 cycles (Touchdown)
		6	94	15sec	-

Reaction A (3 primer)		Cycling (TouchDown 65-60_12-12-11)			
25932	.5 uM	7	60	15sec	-
25933	.5 uM	8	72	10sec	-
Glycerol	6.5 %	9	-	-	repeat steps 6-8 for 28 cycles
Dye	1 X	10	72	2 min	-
Kapa 2G HS taq polymerase	.03 U/ul	11	10	-	hold
DNA					

Version 3.2