



Welcome to The Genotyping Protocol System

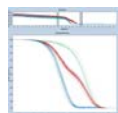
Master Protocol

Strain Name: C57BL/6NJ-Ccdc30^{em1J}/_J
Stock Number: 028995
Allele: Ccdc30^{em1J}
Protocol Name: Ccdc30^{em1J}
Method: High Resolution Melting
Created: 04-April -2016 (JKELMEN) **Updated:** 21-September-2016 (LUCASM)

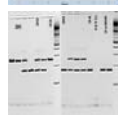
Notes

Expected Results: Mutant = 232 bp
 Heterozygote = 170 bp and 232 bp
 Wild type = 170 bp

Attachments



View 28995.JPG



View 28995.jpg

- View Ccdc30em1jin progress 3-25-16.docx
- View Ccdc30 genomic1.gcs

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
27493	-	ATG CTG TTT GCT GCC TTT CT	-	Common	A
27494	-	CAC TCC ACA TCC CTC AGG A	-	Wild type Reverse	A
27495	-	AAA TGA GTG GTA ATT CAT GAG GTC	-	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	-
27494	.5 uM	5	-	-	repeat steps 2-4 for 10 cycles (Touchdown)
27493	.5 uM	6	94	15sec	-
27495	.5 uM	7	60	15sec	-
		8	72	10sec	-

Reaction A (3 primer)		Cycling (TouchDown 65-60_12-12-11)			
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