



## Welcome to The Genotyping Protocol System

## Master Protocol

Strain Name: C57BL/6NJ-Cuedc1<sup>em1J/J</sup>

Stock Number: 028988

Allele: Cuedc1<sup>em1J</sup>Protocol Name: Cuedc1<sup>em1J</sup>

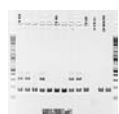
Method: MELT

Created: 03-May -2016 (ESCHAAB) Updated: 10-August -2016 (ESCHAAB)

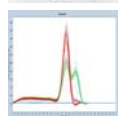
## Notes

Expected Results: Mutant = 296 bp  
 Heterozygote = 157 bp and 296 bp  
 Wild type = 157 bp

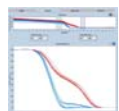
## Attachments



View 28988 Cuedc1 gel.jpg



View 28988 Cuedc1 mca.jpg



View 28988 Cuedc1 hrn.JPG

- View 28988 Cuedc1 genomic.gcs

- View 28988 sup.docx

## Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
27853	-	TGC TTG GAT AGA TGC TGC TG	-	Common	A
27854	-	GAG TTT TGT GCA TTG CTG CT	-	Wild type Reverse	A
27855	-	TCT GCA ACT CCA GCT ATT GG	-	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	-
27853	.5 uM	5	-	-	repeat steps 2-4 for 10 cycles (Touchdown)
		6	94	15sec	-

<b>Reaction A (3 primer)</b>		<b>Cycling (TouchDown 65-60_12-12-11)</b>			
27855	.5 uM	7	60	15sec	-
27854	.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