



Welcome to The Genotyping Protocol System

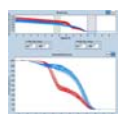
Master Protocol

Strain Name: C57BL/6NJ-Mcmbp^{em1J/J}
Stock Number: 029126
Allele: Mcmbp^{em1J}
Protocol Name: juhMcmbp^{em1J}
Method: High Resolution Melting
Created: 05-May -2016 (LUCASM) **Updated:** 29-July -2016 (ESCHAAB)

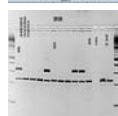
Notes

Expected Results: Mutant = 321 bp
 Heterozygote = 224 bp and 321 bp
 Wild type = 224 bp

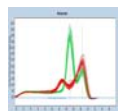
Attachments



View Mcmbp HRM.JPG



View Mcmbp-29126 Gel.jpg



View Mcmbp Mplex Melt.jpg

- View Mcmbp Genomic.gcs

- View Mcmbpem1JMolDesApr2016-1.docx

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
27927	-	GTG CTG CTA TGT CTG GGT GA	-	Common	A
27928	-	GCC AGC AGA TTA TGT TCA GGT	-	Wild type Reverse	A
27929	-	CTC CTA AGC CCC CAC ACT G	-	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	-
27927	.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)			
27929	.5 uM	7	60	15sec	-
27928	.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