



Welcome to The Genotyping Protocol System

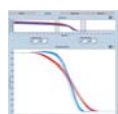
Master Protocol

Strain Name: C57BL/6NJ-Ttc32^{em1J}/J
Stock Number: 029111
Allele: Ttc32^{em1J}
Protocol Name: Ttc32^{em1J}
Method: High Resolution Melting
Created: 05-May -2016 (DLINDNER) **Updated:** 22-July -2016 (ESCHAAB)

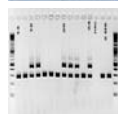
Notes

Expected Results: Mutant =430 bp
Heterozygote = 430 bp and 270 bp
Wild type = 270 bp

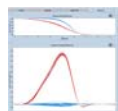
Attachments



View 29111 Ttc32 hrm.JPG



View 29111 Ttc32 gel.jpg



View 29111 Ttc32 hrm B6.JPG

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View Ttc32-29111.gcs

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
27930	-	AGC AAG GGC GTT GAA GAC T	-	Common	A
27931	-	TCC TCA GGG CTA CAT TTG CT	-	Wild type Reverse	A
27932	-	GTC TAA ATC GGG GAC GGA GA	-	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	-
27930	.5 uM	5	-	-	repeat steps 2-4 for 10 cycles (Touchdown)
27931	.5 uM	6	94	15sec	-
		7	60	15sec	-

Reaction A (3 primer)		Cycling (TouchDown 65-60_12-12-11)			
27932	.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