



Welcome to The Genotyping Protocol System

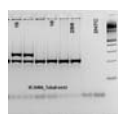
Master Protocol

Strain Name: C57BL/6NJ-Tuba8^{em1J}/J
Stock Number: 029406
Allele: Tuba8^{em1J}
Protocol Name: Tuba8^{em1J}
Method: MELT
Created: 21-July -2016 (LUCASM) **Updated:** 03-November -2016 (ESCHAAB)

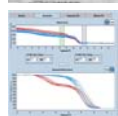
Notes

Expected Results: Mutant = 285 bp
Heterozygote = 216 bp and 285 bp
Wild type = 216 bp

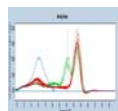
Attachments



View 29406_GEL.jpg



View 364477_Tuba8_genescan a_11-1-16.JPG



View 364477_Tuba8_Mplex a_11-1-16.jpg

- View Tuba8 genomic.gcs
- View Tuba8em1jMolDesJuly2016.docx

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
28932	-	CTT CAG TGA GAC TGG CAA CG	-	Wild type Forward	A
28933	-	CCC TAA CTA GCT CCC CAA GG	-	Common	A
28934	-	TGA CCT TGA TGA TCT ATT TCT GC	-	Mutant Forward	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	-
28932	.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)			
28933	.5 uM	7	60	15sec	-
28934	.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