



Welcome to The Genotyping Protocol System

Master Protocol

Strain Name: C57BL/6NJ-*Dbn1*^{em1J/J}
Stock Number: 027202
Allele: *Dbn1*^{em1J}
Protocol Name: *Dbn1*^{em1J}
Method: MELT
Created: 11-August -2015 (JKELMEN) **Updated:** 12-July -2016 (LUCASM)

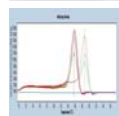
Notes

Expected Results: Mutant = 444 bp
 Heterozygote = 172 bp and 444 bp
 Wild type = 172 bp

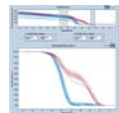
Attachments



View 346115.jpg



View 27202.JPG



View Dbn HRM.JPG

- View Dbn1em1j_final_7-31-15.docx
- View Dbn1 genomic.gcs

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
22748	-	GGA GGA TTC AGA GGC AAA GA	-	Common	-
24622	-	CTG GAG AAA CCA GGG AGA TG	-	Wild type Reverse	-
24623	-	CCA ACC GTA CCC ACT TCA GA	-	Mutant Reverse	-

Reaction/Components A

Reaction Components	Volume Amt	Final Concentration	Total Volume Amt
ddH2O	4.85	-	-
5 X Kapa 2G HS buffer	2.40	1	-
25 mM MgCl ₂	0.96	2	-
10 mM dNTP KAPA	0.24	.2	-
20 uM 22748	0.30	.5	-
20 uM 24622	0.30	.5	-

Cycling

Step #	Temp °C	Time	Note
1	94	2 min	-
2	94	20sec	-
3	65	15sec	-0.5 C per cycle decrease
4	68	10sec	-
5	-	-	repeat steps 2-4 for 10 cycles

Reaction/Components A				Cycling			
20 uM 24623	0.30	.5	-	6	94	15sec	-
2.5 U/ul Kapa 2G HS taq polymerase	0.05	.01	-	7	60	15sec	-
20 X EvaGreen	0.60	1	-	8	72	10sec	-
DNA	2.00	-	-	9	-	-	repeat steps 6-8 for 28 cycles
				10	72	2 min	-
				11	10	-	hold

Number Of Reactions 1

Version 3.2