



Welcome to The Genotyping Protocol System

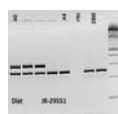
Master Protocol

Strain Name: C57BL/6NJ-Dlat^{em1J}/
Stock Number: 029551
Allele: Dlat^{em1J}
Protocol Name: Dlat^{em1J}
Method: SEPARATED MELT
Created: 12-August -2016 (LUCASM) **Updated:** 23-November -2016 (ESCHAAB)

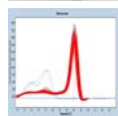
Notes

Expected Results: Mutant = 315 bp
 Heterozygote = 252 bp and 315 bp
 Wild type = 252 bp

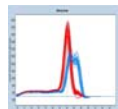
Attachments



View 29551 Dlat.jpg



View dlat wt melt JW.jpg



View dlat mut melt JW.jpg

- View Dlat genomic.gcs
- View Dlatem1j in progress 8-9-16.docx

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
29162	-	ACT GGG AAA GCA ATT AGT ACA GAC	-	Common	A + B
29163	-	GGC CTT GTC GGT CTC TAT CTC T	-	Wild type Reverse	A
29164	-	GAC ATC CAT GGC ACT TTC AG	-	Mutant Reverse	B

Number Of Reactions

Reaction A (2 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
dNTPS-kapa	.26 mM	4	68	10sec	-
29162	.5 uM	5	-	-	repeat steps 2-4 for 10 cycles (Touchdown)
		6	94	15sec	-

Reaction A (2 Primer)		Cycling (TouchDown 65-60_12-12-11)			
29163	.5 uM	7	60	15sec	-
Glycerol	6.5 %	8	72	10sec	-
Dye	1 X	9	-	-	repeat steps 6-8 for 28 cycles
Kapa 2G HS taq polymerase	.03 U/ul	10	72	2 min	-
DNA		11	10	-	hold

Reaction B (2 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
dNTPS-kapa	.26 mM	4	68	10sec	-
29162	.5 uM	5	-	-	repeat steps 2-4 for 10 cycles (Touchdown)
29164	.5 uM	6	94	15sec	-
Glycerol	6.5 %	7	60	15sec	-
Dye	1 X	8	72	10sec	-
Kapa 2G HS taq polymerase	.03 U/ul	9	-	-	repeat steps 6-8 for 28 cycles
DNA		10	72	2 min	-
		11	10	-	hold

Version 3.2