



Welcome to The Genotyping Protocol System

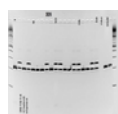
Master Protocol

Strain Name: C57BL/6NJ-*Clstn2*^{em1J/J}
Stock Number: 029216
Allele: *Clstn2*^{em1J}
Protocol Name: *Clstn2*^{em1J}
Method: SEPARATED MELT
Created: 04-May -2016 (LUCASM) **Updated:** 17-August -2016 (ESCHAAB)

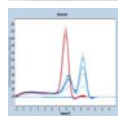
Notes

Expected Results: Mutant = 257 bp
 Heterozygote = 215 bp and 257 bp
 Wild type = 215 bp

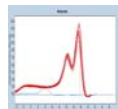
Attachments



View [Clstn2-29216 Gel.jpg](#)



View [Clstn2 2 Primer MUT Melt.jpg](#)



View [Clstn2 WT Melt.jpg](#)

- View [Clstn2em1j_in progress 5-2-16-1.docx](#)
- View [Clstn2 Genomic.gcs](#)

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
27875	-	TCC AGG CCT ATG ACT GTG GT	-	Wild type Reverse	A
27876	-	AGG CAA AGG GAT GCT AAT AGA	-	Common	A + B
27877	-	TGG CCT TTG TGT CTT TCC A	-	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	-
27876	.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)			
27875	.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	-
27876	.5 uM	5	-	-	repeat steps 2-4 for 10 cycles (Touchdown)
27877	.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