



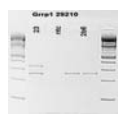
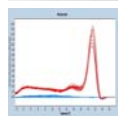
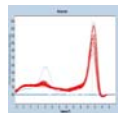
Welcome to The Genotyping Protocol System

**Master Protocol**

**Strain Name:** C57BL/6NJ-*Grrp1*<sup>em1J/J</sup>  
**Stock Number:** 029210  
**Allele:** *Grrp1*<sup>em1J</sup>  
**Protocol Name:** *Grrp1*<sup>em1J</sup>  
**Method:** SEPARATED MELT  
**Created:** 04-May -2016 (LUCASM) **Updated:** 17-August -2016 (ESCHAAB)

**Notes**

Expected Results: Mutant = 323 bp  
 Heterozygote = 247 bp and 323 bp  
 Wild type = 247 bp

**Attachments**View [Grrp1-29210 Gel.jpg](#)View [Grrp1 2 Primer MUT Melt.jpg](#)View [Grrp1 WT Melt.jpg](#)- View [Grrp1em1jMolDesApr2016.docx](#)- View [Grrp1 Genomic.gcs](#)**Protocol Primers**

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
27897	-	TGC TGT CCT CAC CCA CTA CA	-	Common	A + B
27898	-	AGG GAG TCA GGT CTT GGC TGT	-	Wild type Reverse	A
27899	-	TCA TCT CTC ACC CCA AGT CC	-	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	-
27897	.5 uM	5	-	-	repeat steps 2-4 for 10 cycles (Touchdown)
		6	94	15sec	-

<b>Reaction A (2 Primer)</b>		<b>Cycling (TouchDown 65-60_12-12-11)</b>			
27898	.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

<b>Reaction B (2 Primer)</b>		<b>Cycling (TouchDown 65-60_12-12-11)</b>			
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	-
27897	.5 uM	5	-	-	repeat steps 2-4 for 10 cycles (Touchdown)
27899	.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