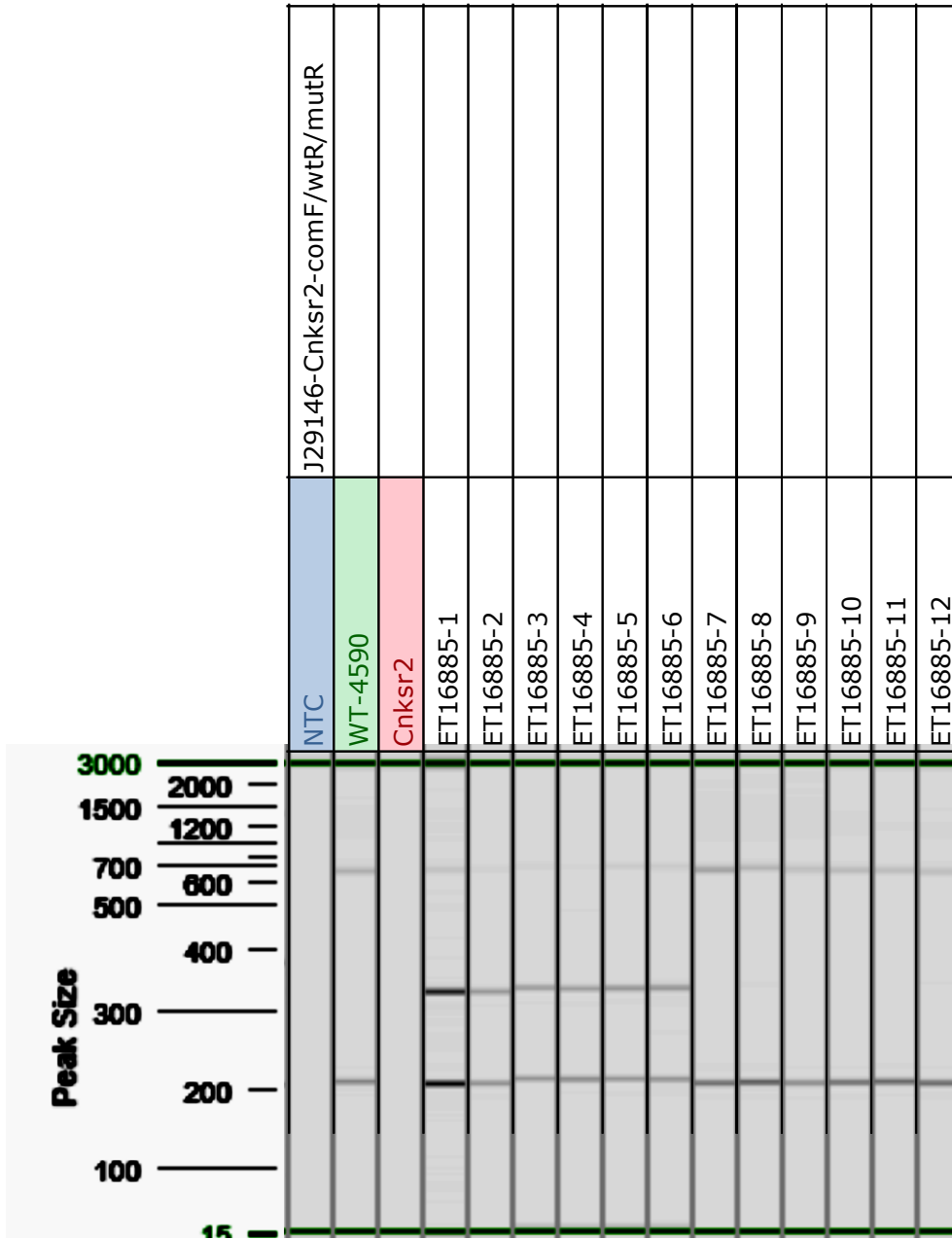


**GENOTYPING BY PCR PROTOCOL
MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS**

mmrrc@ucdavis.edu
530-754-MMRRC





Welcome to The Genotyping Protocol System

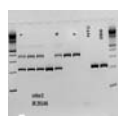
Master Protocol

Strain Name: C57BL/6NJ-Cnksr2^{em1J}J
Stock Number: 029146
Allele: Cnksr2^{em1J}
Protocol Name: Cnksr2^{em1J}
Method: SEPARATED MELT
Created: 29-April -2016 (ESCHAAB) **Updated:** 16-September-2016 (JKELMEN)

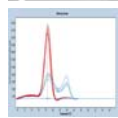
Notes

Expected Results: Mutant = 352 bp
 Heterozygote = 221 bp and 352 bp
 Wild type = 221 bp

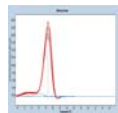
Attachments



View 29146 cnksr2.jpg



View cnksr2 mut melt JW.jpg



View cnksr2 wt melt JW.jpg

- View 29146 sup.docx
- View 29146 Cnksr2 genomic.gcs

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
27814	-	CCA GCA TCA GGC ATT TGA GT	-	Common	A + B
27815	-	CAG ATG CAT TCA ACT TGT GAG A	-	Wild type Reverse	A
27816	-	TTT TGC TCT AAC AGG CAA TGA G	-	Mutant Reverse	B

Number Of Reactions 1

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	-
27814	.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)			
27815	.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	-
27814	.5 uM	5	-	-	repeat steps 2-4 for 10 cycles (Touchdown)
27816	.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