



## Welcome to The Genotyping Protocol System

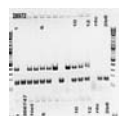
## Master Protocol

**Strain Name:** C57BL/6NJ-Neil2<sup>em1J</sup>/<sub>J</sub>  
**Stock Number:** 028972  
**Allele:** Neil2<sup>em1J</sup>  
**Protocol Name:** Neil2<sup>em1J</sup>-alternate1  
**Method:** MELT  
**Created:** 22-June -2016 (JKELMEN) **Updated:** 20-July -2016 (ESCHAAB)

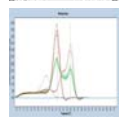
## Notes

Expected Results: Mutant = 380 bp  
 Heterozygote = 133 bp and 380 bp  
 Wild type = 133 bp

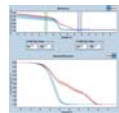
## Attachments



View Neil2-28972 Gel.jpg



View Neil2 Melt.JPG



View Neil2 HRM.JPG

- View Neil2em1j in progress 3-22-16(1).docx
- View Neil2 genomic1.gcs

## Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
27369	-	CAT CTG GAT CAA ACC GAA GG	-	Wild type Reverse	-
28622	-	TGT TGG CAG CTA GCA GTT TG	-	Common	-
28625	-	CTC CAT GCT GGT TGG TAT GT	-	Mutant Reverse	-

Number Of Reactions

Reaction A (3 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
dNTP KAPA	.26 mM	4	68	10sec	-
Primer 1	.5 uM	5	-	-	repeat steps 2-4 for 10 cycles (Touchdown)
		6	94	15sec	-

<b>Reaction A (3 primer)</b>		<b>Cycling (TouchDown 65-60_12-12-11)</b>			
Primer 2	.5 uM	7	60	15sec	-
Primer 3	.5 uM	8	72	10sec	-
Glycerol	6.5 %	9	-	-	repeat steps 6-8 for 28 cycles
Dye	1 X	10	72	2 min	-
Kapa 2G HS taq polymerase	.03 U/ul	11	10	-	hold
DNA					

Version 3.2