



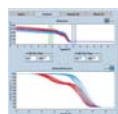
Welcome to The Genotyping Protocol System

**Master Protocol**

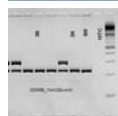
**Strain Name:** C57BL/6NJ-Fam120a<sup>em1J/J</sup>  
**Stock Number:** 029488  
**Allele:** Fam120a<sup>em1J</sup>  
**Protocol Name:** Fam120a<sup>em1J</sup>  
**Method:** MELT  
**Created:** 26-July -2016 (LUCASM) **Updated:** 03-November -2016 (ESCHAAB)

**Notes**

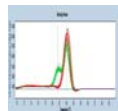
Expected Results: Mutant = 297 bp  
 Heterozygote = 230 bp and 297 bp  
 Wild type = 230 bp

**Attachments**

View 364485\_Fam120a\_genescan a\_11-1-16.JPG



View GEL\_29488.jpg



View 364485\_Fam120a\_Mplex a\_11-1-16.jpg

- View Fam120aem1jMolDesJuly2016.docx
- View Fam120a genomic.gcs

**Protocol Primers**

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
28977	-	CGG GAA GAG TCT CAC CAC A	-	Wild type Forward	A
28978	-	GCC AGC AGA AAA CAA AAC CA	-	Common	A
28979	-	CCA ACA CTT TTG AAG AGA TTG TGT C	-	Mutant Forward	A

Number Of Reactions 1

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	-
28979	.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>			
28978	.5 uM	7	60	15sec	-
28977	.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