



Welcome to The Genotyping Protocol System

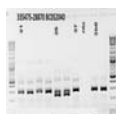
Master Protocol

Strain Name: C57BL/6NJ-BC052040^{em1J}/_J
Stock Number: 028870
Allele: BC052040^{em1J}
Protocol Name: BC052040^{em1J}
Method: High Resolution Melting
Created: 29-February -2016 (JKELMEN) **Updated:** 12-May -2016 (ESCHAAB)

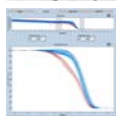
Notes

Expected Results: Mutant = 236 bp
 Heterozygote = 236 bp and 276 bp
 Wild type = 276 bp

Attachments



View 335475-28870 BC052040 gel.jpg



View 335475-28870 BC052040 hrm.JPG

- View BC052040MolDesFeb2016.docx
 - View BC052040 genomic.gcs

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
26979	-	GGT GTG GCT AAA CCT CTT GC	-	Common	A
26980	-	GTA CAA AGG GAC CAG GGA ACA	-	Mutant Reverse	A
26981	-	CTC ATT GGC CAG CTC CAA G	-	Wild type Reverse	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	-
26979	.5 uM	5	-	-	repeat steps 2-4 for 10 cycles (Touchdown)
26981	.5 uM	6	94	15sec	-
26980	.5 uM	7	60	15sec	-
Glycerol	6.5 %	8	72	10sec	-
		9	-	-	repeat steps 6-8 for 28 cycles

Reaction A (3 primer)		Cycling (TouchDown 65-60_12-12-11)			
Dye	1 X	10	72	2 min	-
Kapa 2G HS taq polymerase	.03 U/ul	11	10	-	hold
DNA					

Version 3.2