



Welcome to The Genotyping Protocol System

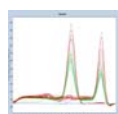
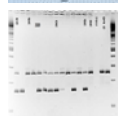
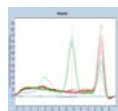
Master Protocol

Strain Name: C57BL/6NJ-Cre^{bzf^{em1J}}
Stock Number: 029209
Allele: Cre^{bzf^{em1J}}
Protocol Name: Cre^{bzf^{em1J}}
Method: MELT
Created: 04-May -2016 (LUCASM) **Updated:** 13-December -2016 (LUCASM)

Notes

Expected Results: Mutant = 162 bp
Heterozygote = 162 bp and 248 bp
Wild type = 248 bp

Attachments

[View](#) 29209.jpg[View](#) 29209.jpg[View](#) Crebzf 3 Primer Mplex Melt.jpg

- [View](#) Crebzf Genomic.gcs
- [View](#) Crebzfem1jMolDesApr2016.docx

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
27894	-	CTG GCC GAT CTG CTG GAC	-	Common	A
27895	-	GAC TTT CTC CGC CTC TTC TC	-	Wild type Reverse	A
27896	-	CTG AAA TGC GTC CGG TGA	-	Mutant Reverse	A

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	-
27894	.5 uM	5	-	-	repeat steps 2-4 for 10 cycles (Touchdown)
		6	94	15sec	-

Reaction A (3 primer)		Cycling (TouchDown 65-60_12-12-11)			
27895	.5 uM	7	60	15sec	-
27896	.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