



Welcome to The Genotyping Protocol System

Master Protocol

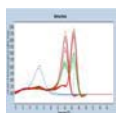
Strain Name: C57BL/6NJ-Zfp174^{em1J}J
Stock Number: 028355
Allele: Zfp174^{em1J}
Protocol Name: Zfp174^{em1J}
Method: MELT
Created: 16-October -2015 (JKELMEN) **Updated:** 20-April -2016 (LUCASM)

Notes

Notes: This allele from project Zfp174-7014J-2R1L(MP1) was generated at The Jackson Laboratory by injecting Cas9 RNA and 3 guide sequences, ATAGAGTGACCCCTTACCC, TCCCAGCAGAGACATTACAGG, TGGGAGGCTGTGATGGAAAG, which resulted in a 194 bp deletion beginning in intron 2 at Chromosome 16 positive strand position 3,849,266 bp, TAAGGGGGTCACTCTATAGC, and ending after CTCCCAGCAGAGACATTC at 3,849,459 bp (GRCm38/mm10) in exon 2. This mutation deletes 85 bp of intronic sequence and the splice acceptor as well as 109 bp of exon 2 resulting in a complete deletion of exon 2. This mutation is predicted to result in a change of amino acid sequence after amino acid 134 and early truncation 4 amino acids later.

Expected Results: Mutant = 326 bp
Heterozygote = 178 bp and 326 bp
Wild type = 178 bp

Attachments

[View 28355.JPG](#)[View 28355.jpg](#)

- [View Zfp174em1jMolDesOct2015.docx](#)
- [View Zfp174 genomic1.gcs](#)

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
25273	-	TTC TTT TTG CTC TGC CTG CT	-	Common	A
25274	-	TCC TCT GAC CTC CAT AAG TGC	-	Mutant Reverse	A
25275	-	CCT TGC ATA CAA ACA GTC ACC	-	Wild type Reverse	A

Reaction/Components A

Cycling

Reaction Components	Volume Amt	Final Concentration	Total Volume Amt	Step #	Temp°C	Time	Note
ddH2O	4.55	-	-	1	94	2 min	-
5 X Kapa 2G HS buffer	2.40	1	-	2	94	20sec	-
25 mM MgCl2	0.96	2	-	3	65	15sec	-0.5 C per cycle decrease

Reaction/Components A				Cycling			
10 mM dNTPS-kapa	0.24	.2	-	4	68	10sec	-
20 U Primer 1	0.30	.5	-	5	-	-	repeat steps 2-4 for 10 cycles
20 U Primer 2	0.30	.5	-	6	94	15sec	-
5 mM 10x Loading Dye	1.20	.5	-	7	60	15sec	-
2.5 U/ul Kapa 2G HS taq polymerase	0.05	.01	-	8	72	10sec	-
DNA	2.00	-	-	9	-	-	repeat steps 6-8 for 28 cycles
				10	72	2 min	-
				11	10	-	hold

Reaction/Components B				Cycling			
Reaction Components	Volume Amt	Final Concentration	Total Volume Amt	Step #	Temp°C	Time	Note
ddH2O	#####	-	-	1	94	2 min	-
5 X Kapa 2G HS buffer	2.40	1	-	2	94	20sec	-
25 mM MgCl2	0.96	2	-	3	65	15sec	-0.5 C per cycle decrease
10 mM dNTPS-kapa	0.24	.2	-	4	68	10sec	-
20 U Primer 1	0.30	.5	-	5	-	-	repeat steps 2-4 for 10 cycles
20 U Primer 2	0.30	.5	-	6	94	15sec	-
5 mM 10x Loading Dye	1.20	.5	-	7	60	15sec	-
2.5 U/ul Kapa 2G HS taq polymerase	0.05	.01	-	8	72	10sec	-
DNA	2.00	-	-	9	-	-	repeat steps 6-8 for 28 cycles
				10	72	2 min	-
				11	10	-	hold

Number Of Reactions 1

Version 3.2