



Welcome to The Genotyping Protocol System

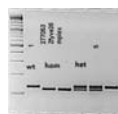
Master Protocol

Strain Name: C57BL/6NJ-Zfyve26^{em1J}J
Stock Number: 026273
Allele: Zfyve26^{em1j}
Protocol Name: Zfyve26^{em1j}
Method: Standard PCR
Created: 28-April -2015 (DTG) **Updated:** 03-June -2015 (JKELMEN)

Notes

Expected Results: Mutant = 106 bp
 Heterozygote = 106 bp and 117 bp
 Wild type = 117 bp

Attachments



View [277053-ZfyveCRISPR.jpg](#)

- View [Zfyve26 genomic-1.gcs](#)

- View [Zfyve26em1J_1-27-15.docx](#)

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
21880	-	AGA CTG GGA GTT GGC ACA AG	-	Forward	-
23439	-	CGG GGA TCG CTT ACC TTA G	-	Reverse	-

Reaction/Components A

Reaction Components	Volume Amt	Final Concentration	Total Volume Amt
ddH2O	4.55	-	-
5 X Kapa 2G HS buffer	2.40	1	-
25 mM MgCl ₂	0.96	2	-
10 mM dNTPS-kapa	0.24	.2	-
20 uM 21880	0.30	.5	-
20 uM 23439	0.30	.5	-
5 mM 10x Loading Dye	1.20	.5	-
2.5 U/ul Kapa 2G HS taq polymerase	0.05	.01	-
DNA	2.00	-	-

Cycling

Step #	Temp°C	Time	Note
1	94	2 min	-
2	94	20sec	-
3	65	15sec	-0.5 C per cycle decrease
4	68	10sec	-
5	-	-	repeat steps 2-4 for 10 cycles
6	94	15sec	-
7	60	15sec	-
8	72	10sec	-
9	-	-	repeat steps 6-8 for 28 cycles
10	72	2 min	-
11	10	-	hold

Number Of Reactions 1

Version 3.2