



Welcome to The Genotyping Protocol System

Master Protocol

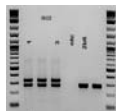
Strain Name: C57BL/6NJ-*Dock6*^{em1J}J
Stock Number: 026122
Allele: *Dock6*^{em1J}
Protocol Name: *Dock6*^{em1J}alternate1
Method: Standard PCR
Created: 15-October -2015 (JKELMEN) **Updated:** 21-October -2015 (ESCHAAB)

Notes

Notes: This allele from project Dock6-6207J FP2(B) was generated at The Jackson Laboratory by injecting Cas9 RNA and guide sequence TGGTGACAGTTAACGTGGCC, which resulted in a 145 bp deletion and a T insertion in exon12 (CGGCCACGTTAACTGTCACCA) beginning at Chromosome 9 negative strand position 21839387bp and ending after (TACCTTGGGGAATCTGTATC) at 21839244bp (GRCm38/mm10). This mutation is predicted to cause amino acid sequence changes after residue 448 and early truncation 24 amino acids later.

Expected Results: Mutant = ~320 bp
Heterozygote = 272 bp and ~320 bp
Wild type = 272 bp

Attachments



View 26122.jpg

- View Dock6_em1j__ver2_6-30-15.docx

- View Dock6 genomic.gcs

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
24331	-	TCG AGG CTA TAG TGG GGA TG	-	Forward	-
24333	-	CTC AGC AGG GAT GAT GGT C	-	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 24331	0.30	.5	-
20 uM 24333	0.30	.5	-
5 mM 10x Loading Dye	1.20	.5	-
2.5 U/ul Kapa 2G HS taq polymerase	0.05	.01	-

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	-

Reaction/Components A				Cycling			
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