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Welcome to The Genotyping Protocol System

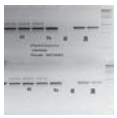
Master Protocol

Strain Name: C57BL/6NJ-*Zfp422*^{em1J}/J
Stock Number: 028308
Allele: Zfp422^{em1J}
Protocol Name: Zfp422^{em1J}
Method: Standard PCR
Created: 01-December -2015 (EST) **Updated:** 02-December -2015 (ESCHAAB)

Notes

Notes:	This allele from project Zfp422-7040J M#1223 was generated at The Jackson Laboratory by injecting Cas9 RNA and 2 guide sequences: CCACACTCATCACAGCAGTA, GTGGCCATTCGCTTCGACTC, which resulted in a 301 bp deletion beginning in exon 2 at Chromosome 6 negative strand position 116,626,917 bp (CTCGGGCTTGAGTCGACGGA) and ending after (ACCGGCGAGAAGCCCTACTGC) at 116,626,617bp (GRCm38/mm10) in exon 2. This mutation results in the deletion in exon 2 of 301 bp and results in an amino acid sequence change after residue 40 and early truncation 41 amino acids later.
Expected Results:	Mutant = 557 bp Heterozygote = 420 bp and 557 bp Wild type = 420 bp

Attachments



View Mplex gel 28308 12-1-15.jpg

\- View Zfp422 genomic1.gcs

\- View Zfp422em1j_moldes_9-24-15_in progress.docx

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
25157	-	TTG AAG AGG TGC TAG TGC TGA	-	Common	A
25158	-	CAC AAT CGG CAC ATT TAT GG	-	Wild type Reverse	A
25159	-	TTT TAA AAA GGC CGG AAG TG	-	Mutant Reverse	A

Number Of Reactions 1

Reaction A (3 primer)

Component	Final Concentration
ddH2O	
Kapa 2G HS buffer	1.3 X
MgCl2	2.6 mM
dNTP KAPA	.26 mM
25158	.5 uM
25159	.5 uM

Cycling (TouchDown 65-60_12-12-11)

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 (Touchdown)
6	94	15sec	-

Reaction A (3 primer)	
25157	.5 uM
Glycerol	6.5 %
Dye	1 X
Kapa 2G HS taq polymerase	.03 U/ul
DNA	

Cycling (TouchDown 65-60_12-12-11)			
7	60	15sec	-
8	72	10sec	-
9	-	-	repeat steps 6-8 for 28 cycles
10	72	2 min	-
11	10	-	hold

Version 3.2