



Welcome to The Genotyping Protocol System

Master Protocol

Strain Name: C57BL/6NJ-*Plk5*^{em1J/J}
Stock Number: 026274
Allele: *Plk5*^{em1J}-Endpoint
Protocol Name: *Plk5*^{em1J}-Endpoint
Method: End Point Analysis
Created: 21-January -2015 (JKELMEN) **Updated:** 10-February -2015 (ESCHAAB)

Notes

Notes: This allele from project Plk5-5922J-4194 was generated at The Jackson Laboratory by injecting Cas9 RNA and guide sequence CGAGCCTGGGTCGCGCAGGA, which resulted in a 1 bp deletion G in exon 1 beginning at Chromosome 10 positive strand position 80356646 bp (GRCm38). This mutation is predicted to cause amino acid sequence changes after residue 28 and an early truncation 3 amino acids later.

Expected Results: Mutant =TCGGGGCGCGTGTACCG-CGCGGGAAGCTGATCG
Heterozygote = TCGGGGCGCGTGTACCG(**G/-**)CGCGGGAAGCTGATCG
Wild type = TCGGGGCGCGTGTACCG**G**CGCGGGAAGCTGATCG

Attachments

[View](#) plk-validation.jpg[View](#) Plk5em1JSept2014.docx[View](#) Plk5 genomic1.gcs

Sequence

Sequence

```
GCGCAGTCCCCGCGCGCTGCGCACTCAGGCCAACCTGCGCAGATGGAGCCCCGG
TCCCC
GCGGCGGCGGAGCCGCCAGCTTGTGGCCACCTTCTGCGCGACCCAGGCTCGGGGCG
CGTGT
ACCGgCGCGGGAAGCTGATCGGCAAGGTGGGAGTTCCCGCGTCCGTACACACACAC
ACGT
ACACAAACACACGCGCGCGCGAGTTCAAAGCGCGGGAACGGAGAGAGTTGAGGGGGC
GGGG
GTCTCGGGTAGGACGGGACCCCGCGCGCCATAGCTGGAGCCTTGGGTCCCCGGAGTTT
GG
```

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
22511	-	CTT GTG GCC ACC TTC CTG	-	Forward	-
22512	-	ACG TGT GTG TGT GTG TAC GG	-	Reverse	-
22515	Hex	TGT ACC GGC GCG GGA	Black Hole Quencher 1	WT Probe	-
22516	6-FAM	GTG TAC CGC GCG GGA A	Black Hole Quencher 1	MUT Probe	-

Reaction/Components A				Cycling			
Reaction Components	Volume Amt	Final Concentration	Total Volume Amt	Step #	Temp°C	Time	Note
2 X Kapa Probe Fast QPCR	2.50	.42	-	1	95	10min	-
ddH2O	7.44	-	-	2	92	15sec	-
100 uM 22511	0.02	.17	-	3	60	1 min	-
100 uM 22512	0.02	.17	-	4	-	-	repeat steps 2-3 for 40 cycles
100 uM 22515	0.01	.08	-	5	4	-	Forever
100 uM 22516	0.01	.08	-				
DNA	2.00	-	-				

Number Of Reactions 1

Version 3.2