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gbf1$^{tsu3994/+}$ (CZRC catalog ID: CZ401)

Nature of the mutation

tsu3994 mutant embryos carry a T to G single nucleotide substitution in the 23rd exon. This T to G mutation is predicted to cause a leucine to arginine substitution (L1246R for X5 isoform of Gbf1) in Gbf1 protein, which is highly conserved across animal species from Caenorhabditis elegans to human. According to the NCBI database, zebrafish gbf1 may produce five transcription variants (X1– X5), which all encode an identical protein except for a few amino acids in the non-conserved linker regions. The full-length Gbf1 X5 isoform (WT) consists of 1846 residues and the L1246R mutation resides in the highly conserved HDS2 domain.

Sense Strand Sequence

gagtgtgaaccaggcagtcaagctcaatttgaaggtagtctaagaattgtcgttgtactctccacagTACGGCTTGCATGAGCT
GCTTAAAACTAACGCTGCCAACATCCACAGCACTGACGATTGGTACACTCTCTTTTCCC
TCCTGGAGTGCATCGGCGCTGGGATCAAACCTgtgattcaaggaattcccacttatttccaaccattttatctgtttct
gtaccgcaagatgcagtcgaatgtgccgtgatgtttcttttccagattggcttatcttgtgtaatccctttgtttcagCCGGCTGCTCTGC
AGTTCGCCAACACTAACCCAGACAATGACACAGGCGCGCAGTCAGACAGTGAACTCA
GCTCATATCATCAGAGTGAAGTTAGTCTTGACCGGGGATACACGTCCGATTCTGAGATT
TACAACGAGCATGGCAAATCCAGA

Uppercase: Exon/coding sequence
Lowercase: intron/noncoding sequence
atcg: Forward/Reverse primer

Genotyping assay

Primers:
CZ401_forward:5'　CAAGCTCAATTTGAAGGTAG　3'
CZ401_reverse: 5' TGTAAATCTCAGAATCGGAC 3'

PCR program:

95℃	5min	
95℃	30 sec	30 Cycles
58℃	30 sec	
72℃	30 sec	
72℃	8min	
4℃	hold	

Product size: 415 bp

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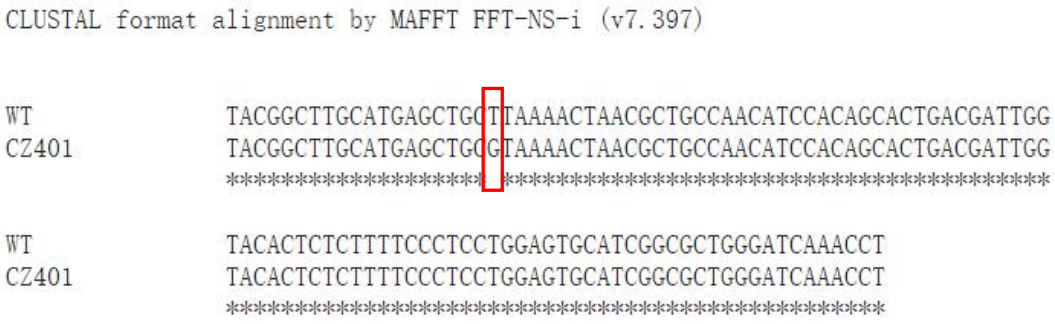
The sequencing results of the CZ401:



Reference:

Chen, J., Wu, X., Yao, L., Yan, L., Zhang, L., Qiu, J., Liu, X., Jia, S., and Meng, A. (2017). Impairment of cargo transportation caused by gbf1 mutation disrupts vascular integrity and causes hemorrhage in zebrafish embryos. J. Biol. Chem. 292, 2315–2327.