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Mutagenesis Analysis of *ABCB8* Gene Promoter of *Danio rerio*

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ABSTRACT

The *ABCB8* is one of the members under the ABCB subfamily of ATP-Binding Cassette (ABC) transporter which possess the ability in regulating the intracellular iron and heme transport. The loss of function mutation of *ABCB8* gene leads to iron and heme accumulation in the cell which is highly toxic to human. However, the information regarding the expression regulation of this gene remains scarce. Hence, the objectives of this project are to determine the transcription factors binding site (TFBS) of *ABCB8* and to identify the transcriptional roles of the *cis*-elements through mutagenesis analysis. To examine this, total genomic DNA was extracted from *Danio rerio* and the promoter sequence was isolated by using specific pair of primers through polymerase chain reaction (PCR). The sample was sent for DNA sequencing and the result showed 98% similarities to the zebrafish DNA sequence from clone DKEYP-87A6 in linkage group 24. Besides, the TFBS was studied in aspect of TFBS abundance, TFBS composition and TFBS distribution. The two most abundant TFBSs based on liver-specific profile were HNF-3β and C/EBPβ, with 38 and 39 binding sites, respectively. The sequence of *ABCB8* promoter gene was mutated through substitution of the AP-1 binding site at location 535 with other nucleotides by using a pair of mutagenic primers (forward primer: 5'-TGGGGGTTTAGATATTGAAAC-3'; reverse primer: 5'-AACTCGC ATACATTTCAGTCATC-3'). This result may benefit the development of new diagnostics and therapeutics for iron-associated disorder.

Keywords: ABC transporter, *ABCB8* promoter sequence, *Danio rerio*, mutagenesis, TFBS