Original Article

IDENTIFICATION OF MIRROR REPEATS WITHIN THE Pdgf-Aa GENE OF DANIO RERIO

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Abstract

Compared to other organs, the adult human heart lacks the capacity to regenerate after injury. In contrast, a zebrafish's (*Danio rerio*) heart renews after amputation. It is one of the most relevant model organisms which is used to study regenerative biology. Various types of repetitive elements have been identified within the genome of different organisms. The repetitive elements can be direct, inverted, tandem, mirror repeats, etc. One such repeat which is not widely explored in *Danio rerio* is mirror repeats. The present study aims to determine the mirror repeats within the *pdgf-aa* gene of *Danio rerio*. The *pdgf* signalling plays a crucial role in initiating cardiomyocyte proliferation during *Danio rerio* (zebrafish) heart regeneration. Using a simple approach, we have identified mirror repeats within the *pdgf-aa* gene. We concluded that these mirror repeats are limited to the *pdgf-aa* gene and well distributed within the *Danio rerio* genome and other organism genomes.

Keywords: Zebrafish, Heart regeneration, BLAST tool, mirror repeats, megaBLAST tool.

INTRODUCTION

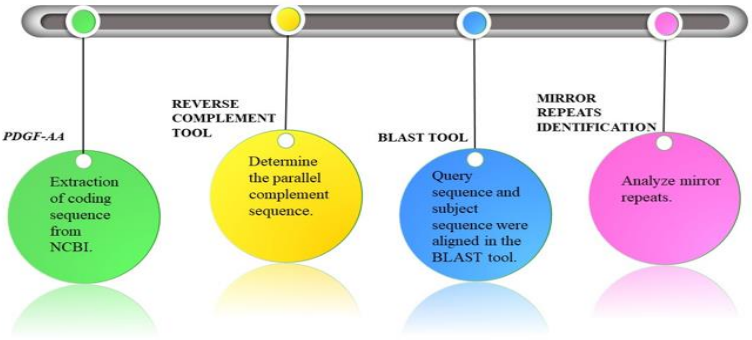
The DNA molecules act as a central repository for genetic data. Genetic data held within the DNA regulates a number of biological processes. DNA can exist in a variety of forms in addition to the natural B-DNA form. These forms can develop as a result of mutations, strand slippers, & unequal crossing over which causes repetitive sequences in the genome (Gurusaran *et al.*, 2013; Zattera *et al.*, 2020; Yadav *et al.*, 2022). "Repetitive sequences are the DNA fragment present in the multiple copies of the genome" (Mehrotra and Goyal, 2014; Dhankhar *et al.*, 2022; Dangi *et al.*, 2022). They play a crucial role in cell cycle regulation, gene regulation, determination of chromosome structure, karyotypic evolution & chromosomal rearrangements events like deletions, duplications, & inversions (Jurka 2007; Zattera *et al.*, 2020; Yadav *et al.*, 2022). The repetitive sequence can be divided into 2 classes based on their distribution, namely tandem repeats and interspersed repeats (Pathak and Ali 2012; Zattera *et al.*, 2020). Mini-satellites & micro-satellites are the most common types of tandem repeats while interspersed repeats including LINES and SINES (Gurusaran *et al.*, 2013; Dhankhar *et al.*, 2022; Dangi *et al.*, 2022). Based on their positions and symmetry, DNA repeats can be divided into Direct repeats, inverted repeats & mirror repeats (Mirkin 2001; Bhardwaj et al., 2013; Dangi *et al.*, 2022).

Various types of repetitive elements are found in eukaryotic organisms (Jurka *et al*., 2007; Dangi *et al*., 2022). In recent years, zebrafish (*Danio rerio*) have become a potent model organism in scientific research. The zebrafish (*Danio rerio*) is a tropical freshwater fish of size about 2.5 cm to 4 cm long that lives in rivers (mostly in Ganga) in the Himalayan region of South Asia, particularly in India, Nepal, and Pakistan. It is a teleost fish that is a member of the Actinopterygii subclass of the Cyprinidae family (Kinth *et al*., 2013; Khan and Alhewairine 2018). *Danio rerio* (Zebrafish) is commonly used as a potent model organism to study developmental biology (Kinkel and Prince, 2009; McCluskey and Postlethwait, 2015; Lowe *et al*., 2021) and more recently regeneration biology (Kikuchi *et al*., 2010; Gemberling *et al*., 2013; Marques *et al*., 2019). It can regenerate many organs including the heart (Lien *et al*., 2006; Shoffner *et al*., 2020; Morejón and Mercader, 2020). Zebrafish are capable of regenerating their hearts even after having up to 20% of their ventricles amputated (Poss *et al*., 2002; Rosa *et al*., 2017, Morejón and Mercader, 2020). One of the important genes involved in the heart regeneration of Zebrafish (*Danio rerio*), is the *pdgf-aa* gene. PDGF (Platelet-derived growth factor) signalling plays an important role in initiating cardiomyocyte proliferation during *Danio rerio* (zebrafish) heart regeneration (Lien *et al*., 2006). The *PDGF-aa* gene is located at chromosome 22 and consists of 14,793 base pairs in length with six exons. Our aim is to study mirror repeats in various genes of the *Danio rerio* organism. Mirror repeats (MR's) are widely distributed in eukaryotic organisms (Mehrotra and Goyal, 2014; Yadav *et al*., 2022). In this investigation, we have identified mirror repeats within the *pdgf-aa* gene of *Danio rerio* (Zebrafish) using a simple manual bioinformatics approach.

MATERIAL AND METHODS

We retrieved the coding sequence (query subject) of *pdgf-aa* gene of *Danio rerio* from the website of NCBI (https://www.ncbi.nlm.nih.gov/). The *pdgf-aa* gene (Gene ID: 378480), having a length of 14,793bp was divided into different parts of 500 bps each. With the help of reverse complement tool available at (https://www.bioinformatics.org/sms/rev_comp.html), the parallel complement of the coding sequence (subject sequence) of *pdgf-aa* gene and its exons were retrieved. The mirror repeats of each sequence were analyzed using the same approach as used by (Dangi *et al*., 2022; Dhankhar *et al*., 2022; Yadav *et al*., 2022). Using the megaBLAST tool, the identified mirror repeats were searched within the *Danio rerio* genome and other organism's genomes (*Mus musculus domesticus* and *Sus scrofa domesticus*).

Figure a: Infographic representation of the methodology used to Identify mirror repeats (i) The coding sequenceof *pdgf-aa* gene of *Danio rerio* was retrieved from the NCBI website (ii) Parallel complement sequence was retrieved using reverse complement tool (iii) Analyze hits where position numbers are same in both query and subject sequence.



RESULT AND DISCUSSION:

In the current investigation, a simple computational-based approach was applied to identify mirror repeats within the *pdgf-aa* gene involved in the heart regeneration of *Danio rerio*. The *Pdgf-aa* gene is located at chromosome

22 and consists of 14,793 base pairs in length with six exons sequences. The 14,793bp long *pdgf-aa* gene was divided into different parts of 500 bps each to identify a maximum number of mirror repeats. The maximum number of hits is observed at the threshold value of 100 so, we identify mirror repeats at this value. In total, 17 mirror repeats (MR's) were found in six exons of *pdgf-aa* gene and 359 mirror repeats were found in different parts of the gene of *Danio rerio*. The identified mirror repeats have been randomly distributed in all the parts of the gene irrespective of their sizes. The identified mirror repeats were classified as PMR (Perfect mirror repeat), PMRSS (Perfect mirror repeat with single spacer), and IMR (Imperfect mirror repeat). Perfect mirror repeats have identical sequences around the central axis of symmetry while Imperfect mirror repeats have mismatched sequences around the central axis of symmetry. A perfect mirror repeat may have one spacer element or more depending upon the spacer element between them.

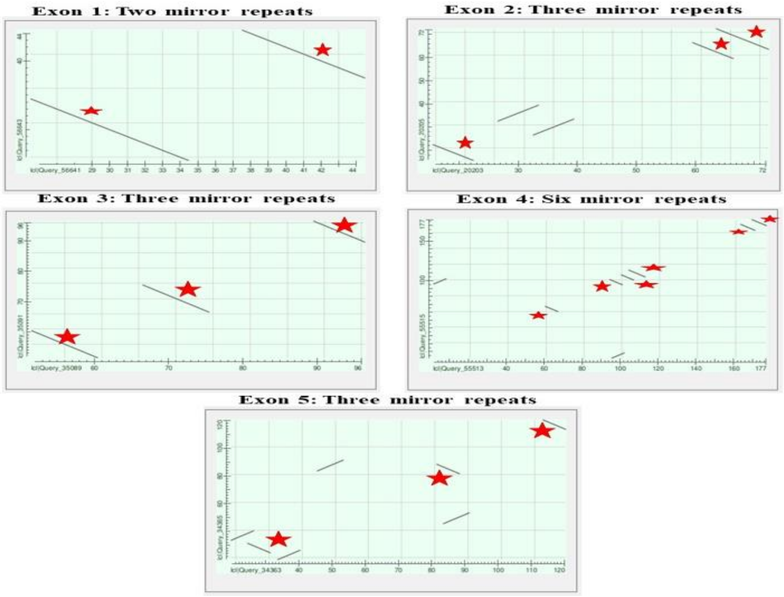
In exons, out of 17 mirror repeats, 2 are PMR, and 15 are PMRSS. The identified mirror repeats present in all six exons of the *pdgf-aa* gene with their types, positions & length are shown below in table 1. The largest mirror repeat within the exons of the gene is of 9bp (PMSS) while the smallest mirror repeat is of 7bp (PMSS). We could not detect the small-sized mirror repeat as the minimum word size limit of the BLAST tool is 7. Figure b represents the dot plot graph of exons with mirror repeats. The "★" symbol represents the number of mirror repeats in each exon of *pdgf-aa* gene.

Table 1: shows the number of mirror repeats with their types, length & positions within six exons of the *Pdgf-aa* gene.

Exons	**No. of mirror repeats**	**Mirror repeats**	**Positions of mirror repeats**	**Length of mirror repeats**	**Types**
Exon-1 (440-502)	2	1.TCGTCTGCT 2.TCTCTCT	26-34 38-44	➢ 9bp ➢ 7bp	➢ PMRSS ➢ PMRSS
Exon-2 (1130-1226)	3	1.GACCTCCAG 2.CGAGAGC 3.CAGCGAC	64-72 16-22 60-66	➢ 9bp ➢ 7bp ➢ 7bp	➢ PMRSS ➢ PMRSS ➢ PMRSS
Exon-3 (5757-5858)	3	1.ACGACAGCA 2.TCAAGAACT 3.AAGAGAA	52-60 67-75 90-96	➢ 9bp ➢ 9bp ➢ 7bp	➢ PMRSS ➢ PMRSS ➢ PMRSS
Exon-4 (8239-8426)	6	1.GTGGAGGTG 2.CAAAAAAC 3.ACACCACA 4.AGGTGGA 5.GCCTCCG 6.GTGTGTG	105-113 164-171 170-177 61-67 95-101 101-107	➢ 9bp ➢ 8bp ➢ 8bp ➢ 7bp ➢ 7bp ➢ 7bp	➢ PMRSS ➢ PMR ➢ PMR ➢ PMRSS ➢ PMRSS ➢ PMRSS
Exon-5 (12615-12735)	3	1.CGGAGGC 2.TGCACGT 3.ACACACA	25-31 82-88 114-120	➢ 7bp ➢ 7bp ➢ 7bp	➢ PMRSS ➢ PMRSS ➢ PMRSS

Exon-6 (14312-14325)	0	Not detected			--------

Figure b: shows the dot plot graph of exons of the *pdgf-aa* gene.



Out of 359 mirror repeats in different parts of *pdgf-aa* gene, 76 are PMR, 233 are PMRSS, and 50 are IMR were observed. The frequency of PMR and PMRSS within the *pdgf-aa* gene are more than the IMR shown in figure c. The classification of selected mirror repeats in the different parts of *pdgf-aa* gene were depicted in table 2. The complete details of the mirror repeat in different regions of the gene are provided in the supplementary file.

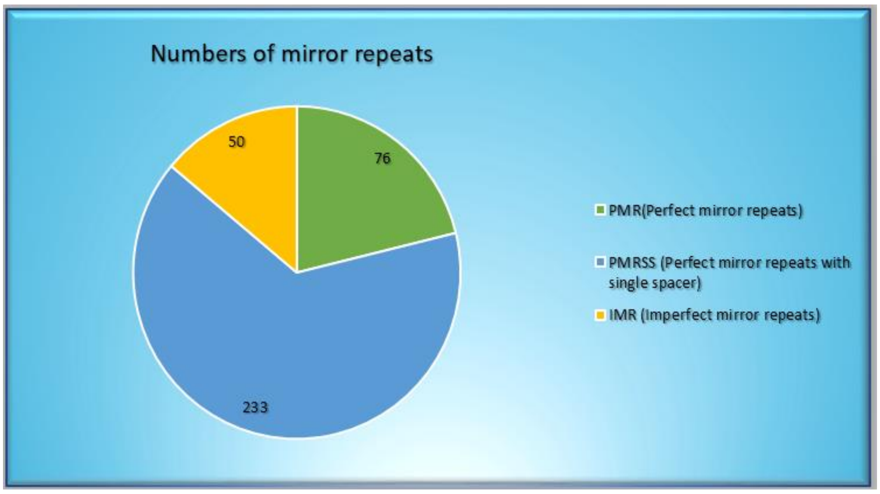
Table 2: shows the classification of selected mirror repeats distributed in different parts of *pdgf-aa* gene.

Symbol& Gene Id	**Gene sequence**	**Mirror repeats**	**Length (bps)**	**Types of mirror repeats**
Pdgf-aa **Gene ID:** **378480**	1-500	1.TTTTGGATTTAGATTTT 2.TCGTCTGCT	➢ 17 ➢ 9	❖ IMR ❖ PMRSS
	501-1000	1.TTTTAATTAATTTT 2.ACTTCCATACACGTGCACTTATATTCA	➢ 14 ➢ 27	❖ PMR ❖ IMR
	1001-1500	1.ACAAATAAACA 2.GAAATGGACTTCCT--AGGTAAAG 3.CATTTTTTAC	➢ 11 ➢ 24 ➢ 10	❖ PMRSS ❖ IMR ❖ PMR
	1501-2000	1.TTCAAAAACTT	➢ 11 ➢ 9	❖ PMRSS ❖ PMRSS

		2.TAATGTAAT 3.TAACCCAAT	➢ 9	❖ PMRSS
	2001-2500	1.TCTTTTTATATATTTTGCT 2.TAAAAGTTTTAGTTTGACTCAAGAACTC---TGGCTTTTTAAAAT	➢ 19 ➢ 45	❖ IMR ❖ IMR
	2501-3000	1.AGACTACTTGTTAAATAGTTACTTTTATTTATAAATTATTCATAAGA 2.AATTTCA--TGTTTCATTTTTAATTTGTCCACTTTAA	➢ 47 ➢ 37	❖ IMR ❖ IMR
	3001-3500	TAATCAAATTAAATTAAT	➢ 18	❖ IMR
	3501-4000	1.GAAAGTTGTTAATGTTGAAAG 2.GTTTAAAAAATTTG	➢ 21 ➢ 14	❖ IMR ❖ PMR
	4001-4500	1.ATGTGAGTTAATTTTTAGTTTAGTTTA 2.ATTGTTTTGTTA	➢ 27 ➢ 12	❖ IMR ❖ PMR
	4501-5000	1.AATATTTATAA 2.TACTTTCAT	➢ 11 ➢ 9	❖ PMRSS ❖ PMRSS
	5001-5500	AGTGTTTGCGTTTGTGA	➢ 17	❖ PMRSS
	5501-6000	ATGTGCGTGTA	➢ 11	❖ PMRSS
	6001-6500	CCCAAAACCC	➢ 10	❖ PMR
	6501-7000	AATAAATAA	➢ 9	❖ PMRSS
	7001-7500	1.CTGGGTAAAATGTGGTACTAACAGAACTTTCAA---ATTACTGGTGTGAAACGTGTC	➢ 57	❖ IMR
	7501-8000	GTGTAAAATGTG	➢ 12	❖ PMR
	8001-8500	1.TACACATACACAT 2.TGTAATTTAATGT	➢ 13 ➢ 13	❖ PMRSS ❖ PMRSS
	8501-9000	TATTAGCCCCCCTGAATTATTA-GACCCCCTGAATTATT-AGACCCCCTGTTTAt	➢ 55	❖ IMR
	9001-9500	atatatGTGTGTATGTA	➢ 17	❖ IMR
	9501-10000	ATATAAAATATA	➢ 12	❖ PMR
	10001-10500	TAACAAGAGCAAA—CCGTGAAACGTGCCAAAAAGGAGGACAAT	➢ 44	❖ IMR

	10501-11000	TTGTTTTAATTCGGCTAAAATAAAAGCAGTTTAAATTTTTT	➢ 41	❖ IMR
	11001-11500	AATGTAGATCAAGCATGATGTaa	➢ 23	❖ IMR
	11501-12000	1.TCACGTCAAAAACAGGACT 2.TAATTTAAT	➢ 19 ➢ 9	❖ IMR ❖ PMRSS
	12001-12500	AAATAAATAAA	➢ 11	❖ PMRSS
	12501-13000	TCTCTGTCTCT	➢ 11	❖ PMRSS
	13001-13500	ATAATGATTATTGTATTTTAAAAGTTATTATGTTTACATTTTTTAAGATC-AATGCAATGTAGTCCTCAAGattttaaatttgtaatattttagattatct-ttaataataata	➢ 114	❖ IMR
	13501-14000	-------------------		
	14001-14500	TCTCCCCTCT	➢ 10	❖ PMR
	14501-14793	1.GTATATATG 2.ACTTCTGTCTCCA	➢ 9 ➢ 13	❖ PMRSS ❖ IMR

Figure c: shows the frequency of mirror repeats within the *pdgf-aa* gene.



We also used the non-BDNA motif search tool to identify mirror repeats within the exons of *pdgf-aa* gene. With this tool, we could not find mirror repeats in all six exons of the gene. By comparing the results obtained from both tools, we can say that our strategy is more efficient in searching mirror repeats in the gene because the non-BDNA motif search tool could not search mirror repeats in the exons of *pdgf-aa* gene are shown in table 3 below.

Table 3: shows the number of mirror repeats in both BLAST and non-BDNA motif search tools.

Exons	**No. of mirror repeats in the BLAST tool**	**No. of mirror repeats in the non-BDNA motif search tool**
Exon-1	2	0
Exon-2	3	0
Exon-3	3	0
Exon-4	6	0
Exon-5	3	0
Exon-6	0	0

Using the Mega BLAST tool, the identified mirror repeat within the exons of the *pdgf-aa* gene were searched among the genome of *Danio rerio*, *Mus musculus* & *Sus scrofa*. The result of exons is summarized in table 4 below. In this, + sign shows the presence of mirror repeats and the – sign shows the absence of mirror repeats. The distribution of selected identified mirror repeats in different parts of *pdgf-aa* gene were analyzed among different genomes are shown in table 5. Based on the megaBLAST results, we could not detect small-sized mirror repeats. The complete Mega BLAST result for all identified mirror repeats in different parts of the gene is provided in the supplementary file.

Table 4: Illustrates the distribution of identified mirror repeat of all six exons within the genome of *Danio rerio*, *Mus musculus domesticus* & *Sus scrofa domesticus*.

Exons	**Mirror repeats**	***Danio rerio* (taxid:7955)**	***Mus musculus domesticus* (taxid:10092)**	***Sus scrofa domesticus* (taxid:9825)**
Exon-1	TCGTCTGCT	-	-	+
	TCTCTCT	-	-	+
Exon-2	GACCTCCAG	-	-	+
	CGAGAGC	-	-	+
	CAGCGAC	-	-	+
Exon-3	ACGACAGCA	-	-	+
	TCAAGAACT	-	-	+
	AAGAGAA	-	-	+
Exon-4	GTGGAGGTG	-	-	+
	CAAAAAAC	-	-	+
	ACACCACA	-	-	+
	AGGTGGA	-	-	+
	GCCTCCG	-	-	+

	GTGTGTG	-	-	+
Exon-5	CGGAGGC	-	-	+
	TGCACGT	-	-	+
	ACACACA	-	-	+
Exon-6	Not detected			

Table 5: Distribution of selected identified mirror repeats in the different parts of the gene among different genera by using the megaBLAST tool.

S.no.	**Mirror repeats**	***Danio rerio*** **(taxid:7955)**	***Mus musculus domesticus*** **(taxid:10092)**	***Sus scrofa domestica*** **(taxid:9825)**
1.	TAATATATTTTTTAATTAAT	+	+	+
2.	ACGATAATAATATCA	+	+	+
3.	TCTTTTTATATATTTTGCT	+	+	+
4.	AGACTACTTGTTAAATAGTTACTTTTATTTATAAATTATTCATAAGA	+	–	+
5.	ATGTGAGTTAATTTTTAGTTTAGTTTA	+	+	+
6.	TTGTTTTAATTCGGCTAAAATAAAAGCAGTTTAAATTTTTT	+	+	–
7.	TCACGTCAAAAACAGGACT	+	+	+
8.	GAAATGGACTTCCT—AGGTAAAG	+	+	+
9.	AGTGTTTGCGTTTGTGA	+	+	+
10.	GTTTAAAAAATTTG	+	+	+
11.	ACGATAATAATATCA	+	+	+
12.	TTTTAATTAATTTT	+	+	+
13.	TTTTGGATTTAGATTTT	+	+	+
14.	ACTTCTGTCTCCA	+	+	+

CONCLUSION:

In the current investigation, we found that the *pdgf-aa* gene of *Danio rerio* is enriched in mirror repeats. A total of 359 mirror repeats were identified in different regions of the *pdgf-aa* gene & 17 mirror repeats were identified in all six exons of the gene of *Danio rerio*. The mirror repeat of the *pdgf-aa* gene was determined by using the BLAST tool. We also use another tool like the non-BDNA motif search tool. By comparing the results of both tools, we can say that our method is more sufficient in searching for mirror repeats in the gene. This gene consists of mirror repeats of various lengths & types. It will be highly significant to discover the exact role of mirror repeat at the molecular level. The exact role of mirror repeat has not been elucidated. Future studies may be required to determine the exact role & function of mirror repeat within the genome of *Danio rerio* and other organisms.

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CONFLICTS OF INTEREST

The authors don't have any conflicts of interest.

Credit author statement

Vikash Bhardwaj[*]: Conceptualization, Methodology, Software, Supervisor, Reviewing, Editing.

Namrata Dangi[1]: Data curation, Investigation, Writing-Original draft preparation.

Kavita Saini[2]: Writing- Review Editing.

Madhuri[3]: Writing-Review Editing.

Anu Bhargva[3]: Writing-Review Editing.

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