



Research Article

In-silico approach for the identification of mirror repeats within Glucagon, Sox17, Gapdh and PKLR diabetic genes of *Rattus norvegicus*

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Article Information

Received: 23 December 2025

Revised version received: 21 March 2026

Accepted: 23 March 2026

Published: 20 April 2026

Cite this article as:

Babli and P. Kumar (2026) *Int. J. Appl. Sci. Biotechnol.* Vol 14(1): 28-43. DOI: [10.3126/ijasbt.v14i1.92261](https://doi.org/10.3126/ijasbt.v14i1.92261)

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Peer reviewed under authority of IJASBT

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Keywords: *Rattus norvegicus*; Repetitive sequences; Mirror repeat; FPCB; Blast; Diabetic genes.

Abstract

Repetitive sequences are immensely distributed in the genomes of all viruses, bacteria, plants and animals. Repetitive sequences throughout the genome in both coding and non-coding regions are a major source of endogenous DNA damage, due to the propensity of many of them to form alternative non-B DNA structures that can interfere with replication, transcription and DNA repair. The alternative non-canonical structures include triple-helices, hairpins, cruciforms and slipped structures and they are more likely to form at particular repetitive sequences such as mirror repeats, inverted repeats, direct repeats, and short tandem repeats. Although mirror DNA repeats were discovered in genomic DNA, the ability of a subset of lengthy homopurine-homopyrimidine mirror repetitions (H-motifs) to form a triple-helical DNA secondary structure was the only functional property that was thoroughly identified. Over the past decade, biological databases have expanded significantly due to developments in genomics, computational biology, and bioinformatics. A computational method was used in this study to identify mirror repetitions in the *Rattus norvegicus*'s diabetic genes. The Fast Parallel Complement Blast (FPCB) tool, a rapid and efficient bioinformatics method for identifying gene and genome repeats, was used to do this. Mirror repeats were found 199 in Glucagon, 135 in Sox17, 116 in Gapdh and 167 in PKLR genes during this study. Through this study, we can better understand the function of mirror repeats in the *Rattus norvegicus*'s genes, which may be further linked to mirror repeats in the human genome.

Introduction

DNA is both a dynamic, three-dimensional molecule that frequently deviates from the traditional double helix and a sequence of letters that may be broken down linguistically. Since 1953, B-DNA, the classic right-handed DNA double-helical structure, has been identified. Although B-DNA is the most common arrangement within cells, over 20 alternative non-canonical structures have been identified (Ghosh and Bansal, 2003). Alternative structures like cruciforms, hairpins, slipped structures and triple helices are more likely to occur at specific repeated sequences like

short tandem repeats, mirror repeats, inverted repeats, and direct repetitions (Wells, 2007). Based on in vitro investigations of prokaryotic (Todd and Glickman 1982; Hoede *et al.*, 2006) and eukaryotic cells (Biffi *et al.*, 2013; Bacolla *et al.*, 2016; Kouzine *et al.*, 2017), non-canonical secondary structures are linked to higher mutability. The rate at which complimentary strands re-associate after being heated and disassociated should be inversely related to the amount of haploid DNA present in each cell. This pattern is true for bacteria and viruses, but it was found that in eukaryotes, large portions of the genome reassociated far

more quickly than their cellular DNA quantity would have indicated. That insight led to the startling discovery that repetitive DNA made up more than half of the genomes of many eukaryotes. From a few to several thousand nucleotide sequences, repeated DNA sequences are genomic segments that recur throughout the genome and are found in the genomes of higher animals (Britten and Kohne, 1968; Jurka *et al.*, 2007; Biscotti *et al.*, 2015).

Reassociation kinetics led to the initial discovery of repetitive DNA, which was categorized as "highly" and "middle" repetitive sequences, roughly corresponding to tandem and interspersed repeats. Interspersed repeats are DNA fragments that are introduced into host DNA more or less randomly and have a maximum size of 20–30 kb. On the other hand, tandem repeats are collections of DNA segments that are arranged head-to-tail next to one another. Interspersed repeats are transposable elements (TEs) that have been inserted into genomic DNA; they are primarily inactive and frequently incomplete copies. A sufficiently long repeat may cause the results of mutational events to preferentially change from contractions to expansions. Strand slippage and/or hairpin formation during replication or repair (Ruggiero and Topal, 2004), replication fork stalling that varies by strand orientation (Gerhardt *et al.*, 2016; Rastokina *et al.*, 2023), impairments in Okazaki fragment maturation (Tsutakawa *et al.*, 2017), homologous recombination (Tang *et al.*, 2011), error-prone DNA repair (Du *et al.*, 2012; Ezzatizadeh *et al.*, 2014) and challenges processing single-stranded DNA nicks or gaps within H-DNA structures (Li *et al.*, 2024; Masnovi *et al.*, 2024) are some of the known mechanisms that cause DNA repeat instability. Even though these structures are crucial to human physiology, it is well known that their unusual forms make it difficult for DNA machinery to process them.

Two-dimensional gel-electrophoresis, which shows the change in writhe on formation of these non-B DNA structures, can be used to monitor the superhelical stress-induced transition for purine-pyrimidine conformations. A regionally underwound DNA structure changes the molecule's topology, making it less compact and hence as a result there is decrease in mobility in an agarose gel (Collier and Wells, 1990). The magnitude of this mobility shift can reveal how many helical translocally unwound helices and how much superhelical stress are needed to induce the transition to the alternative structure. Non-B motifs do not have a uniform thermodynamic capacity to form secondary structures. Studies using biophysical simulations and experiments indicate that hairpin formation is optimal at certain spacer and arm lengths (Nag and Petes, 1991; Goddard *et al.*, 2000). In particular, for spacer and arm lengths that are particularly suited to hairpin/cruciform formation, we would expect to observe higher mutability if the physical formation of a secondary structure affected mutability (Sinden *et al.*, 1991). Spacer-to-arm mutation

enrichment is indeed variable for different spacer sizes and various arm lengths, for example, structure-forming repeats have been shown to stall replication (Dahan *et al.*, 2018). Intramolecular triple helix, H-DNA, which requires a sequence of all purines on one strand and all pyrimidines on the other strand arranged in mirror symmetry (Mirkin *et al.*, 1987). This subset of mirror repeats (H-motifs) forms a triplex when a strand from one half of the repeat (whether purine or pyrimidine) folds back to bond with the duplex half of the repeat, while its complement remains single-stranded (Hisey *et al.*, 2024). To this day, formation of H-DNA is the only known functional property of mirror repeats.

Notably, H-DNA is a target for multiple DNA repair enzymes (Khristich and Mirkin, 2020; Wang and Vasquez, 2023) and develops in vivo (Matos-Rodrigues *et al.*, 2022), limiting important genetic events by halting transcription and DNA replication. The length of the repeat motif causes an exponential rise in H-DNA stability (Lyamichev *et al.*, 1989). As a result, stable H-DNA structures significantly increase H-motif repeat expansion and induce genomic instability (Wang and Vasquez, 2023; Hisey *et al.*, 2024). This phenomenon has been associated with several human disorders because sufficiently lengthy H-motifs affect gene activity (Depienne and Mandel, 2021). The first known H-motif in disease, (GAA)_n repeats in Friedreich's ataxia, has been joined by the recent discoveries of (CCCTCT)_n repeat expansions in X-linked dystonia parkinsonism (Bragg *et al.*, 2017), (AAGGG)_n repeat expansions in CANVAS disease (Rafehi *et al.*, 2019), and (GAA)_n repeats in FGF14-related ataxia (Pellerin *et al.*, 2023).

Expansions of H-motif STRs were also observed in several human cancers (Bacolla *et al.*, 2016). In these known examples, carriers of a relatively long H-motif transmit an even longer allele to their offspring, and this allele continues to expand somatically throughout life, progressing the disease. Therefore, knowing how H-motifs modify to lengthen is essential. Slipped-strand structures with looped out bases can result from misalignment of direct repeat motifs, which are made up of two copies of the repeating unit separated by a non-repetitive spacer. (Sinden *et al.*, 2007).

Inverted repeat motifs, which consist of repetitive sequences complementary to each other (e.g., 5'-GACTGC and GCAGTC-3') separated by a non-repetitive spacer, are capable of forming hairpins and cruciform structures (Nag and Petes 1991). Mirror repeat motifs that consist of stretches of homopurines: homopyrimidines arranged in a mirrored fashion, separated by a spacer, can form triple-helix (H-DNA) structures (Htun and Dahlberg, 1988). Finally, Z-DNA motifs, which consist of alternating pyrimidines and purines, such as (CG:CG)_n or (CA:TG)_n, can form left-handed zig-zag DNA structures (Wang *et al.*

1979; Singleton *et al.*, 1982). The frequency of different repetitions in bacterial genomes and organelles can be either random or enriched for direct or inverted tandem repeats. Nonetheless, eukaryotic genomes have an excess of mirror repeats of homopurines and homopyrimidines.

Whereas it has long been known that direct repeats are abundant in eukaryotes, the dramatic overrepresentation of such mirror repeats as H palindromes was a surprise. In the human genome, the majority of mirror repeats are also direct repeats. Up to 85% of all H palindromes are direct repeats in the worst scenario. Just 20% of inverted repeats

are also straight repeats, for contrast. Similar ratios were found in yeast DNA, with AT-rich regions showing the largest overlap between mirror and direct repetitions. On the other hand, prokaryotic DNA rarely coincides mirror and direct repetitions. While the percentage of the genome that corresponds to coding sequences varies greatly, ranging from 67% in yeast DNA to 4% in a sample human sequence, the overrepresentation for eukaryotic genomes is identical for all of them each structure for overlap with the GenBank annotation's coding features.

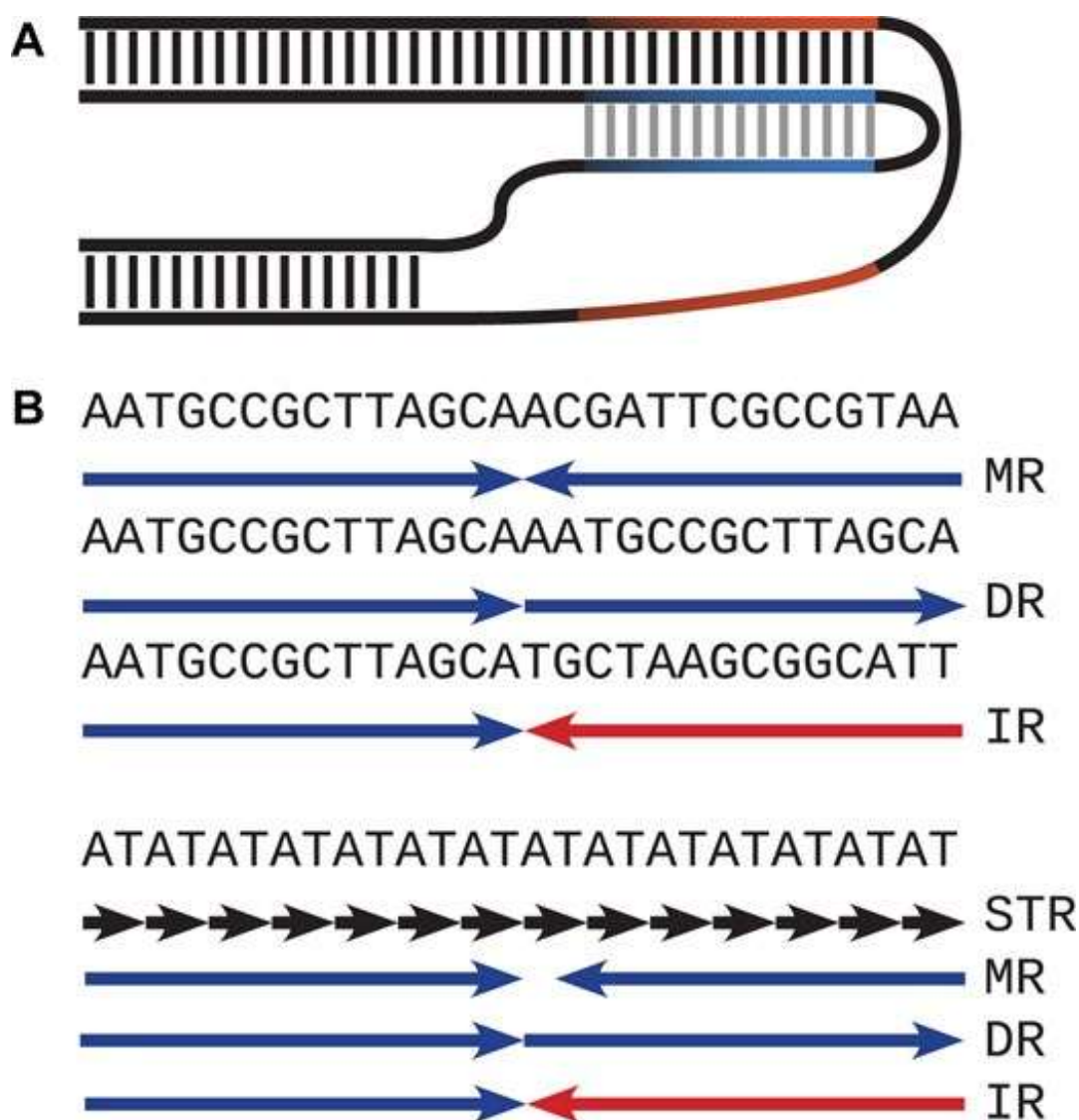


Fig. 1: Illustration of H-DNA secondary structure and repeat symmetry. (A) Example of H-DNA secondary structure, which requires a mirror repeat. Triple-helical DNA is stabilized through non-Watson–Crick base pairing in addition to typical Watson–Crick base pairing (B) Various DNA sequences containing axes of symmetry, as indicated. MR, mirror repeat; DR, direct repeat; IR, inverted repeat; STR, simple tandem repeat.

Diabetes is a chronic noncommunicable disease (Unnikrishnan *et al.*, 2016) defined by chronic hyperglycemia caused by abnormalities in the secretion and function of the pancreatic hormone insulin (Kharroubi *et al.*, 2015). Diabetes mellitus is classified into type 1, type 2, and gestational diabetes depending on its cause and clinical aspects (Rayburn *et al.*, 1997). Animal models can be developed through two main mechanisms: disease induction or genetic modification. *Rattus norvegicus* is one of the most well-established experimental model organisms, with use of the species dating back to the mid-19th century (Modlinska and Pisula, 2020). The long-standing use of *Rattus norvegicus* in the laboratory as a model organism has led to a multitude of discoveries, providing insight into human physiology, behaviour and disease. For sequence data, one may use DNA, RNA or Protein data depending on your interest. All the completed sequences can be retrieved from the NCBI public website: <http://www.ncbi.nlm.nih.gov/>. The database for the DNA sequence is called GenBank. The manual bioinformatics approach is used to identify mirror repeats where FPCB (FAST PARALLEL COMPLEMENT BLAST) is the most commonly used method for analyzing or locating mirror repeats in various prokaryotes and eukaryotes. In addition, it was used to characterize maleless (mle) gene, a sex determination gene in *Drosophila melanogaster* (Saini *et al.*, 2023). The existence of highly divergent repeats could be associated with the presence of a single-type triplet periodicity in various genes or with the packing of bacterial DNA into a nucleoid (Korotkov *et al.*, 2023).

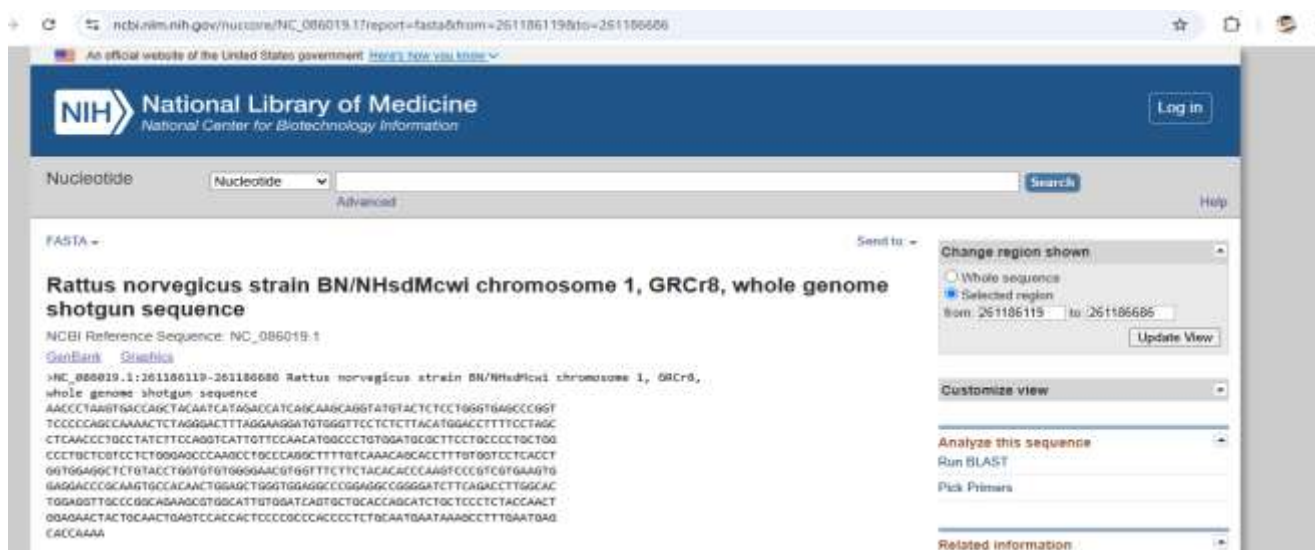
Mammalian Glucagon (Gcg) is primarily expressed in the alpha cells of the pancreas, L-cells of the intestine, and some

neurons of the brainstem (Lafferty *et al.*, 2021). Sox17 is expressed at high levels in the human fetal pancreas and at lower levels in adult islets (McDonald *et al.*, 2009). Embryos from diabetic rats and embryos cultured in high glucose concentrations (Deveze-Alvarez *et al.*, 2001) showed decreased activity of Gapdh (by 40–60%) and severe dysmorphogenesis on gestational days 10.5 and 11.5. Pyruvate kinase is a key glycolytic enzyme. Isoforms that are expressed in the red cell, liver, pancreatic beta-cells, small intestine, and proximal renal tubule are encoded by the 12 exons of the PKLR gene, which maps to chromosome 1q23 (Yamada and Noguchi, 1999).

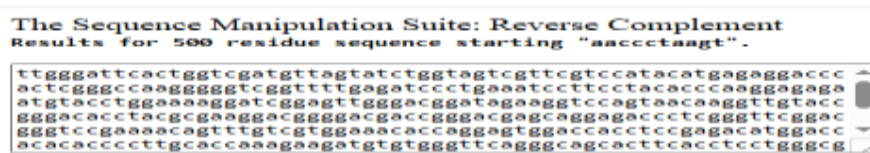
Methodology

The coding sequence of the Glucagon, Sox 17, Gapdh and PKLR genes of *Rattus norvegicus* retrieved from NCBI website (National Centre for Biotechnology Information) in FASTA format (<https://www.ncbi.nlm.nih.gov/>). The nucleotides sequences of gene were splitted into different regions of 500 nucleotides. Using the reverse complement tool, a parallel complement of each region of gene was contrived. Using the BLAST tool, the original query sequence and its parallel complement were aligned for homology by selecting a word size limit of 7, and expected thresholds (E value) where maximum numbers of hits were observed. Mirror repeats can be identified easily as those hits where position number of the query sequence and subject sequence are exactly reversed. The identified mirror repeats were classified as perfect mirror repeats, perfect mirror repeats with one spacer and imperfect mirror repeats (Fig. 2) (Bhardwaj *et al.*, 2013).

Step 1. Retrieve the original nucleotide sequence from NCBI, divide the whole sequence into fragments (1-500), that represents Query sequence.



Step 2. Using the Reverse Complement Tool, make the Parallel Complement of Query sequence. The Parallel Complement represents Subject sequence.



Step 3. Align the both Query and Subject sequence using Blast Tool.



Set program selection - Somewhat similar sequences, Algorithm parameters – expected threshold 20 and word size limit 7.



Step 4. Mirror repeats can be detected by examining the nucleotide which are reversed in Query and Subject sequence.

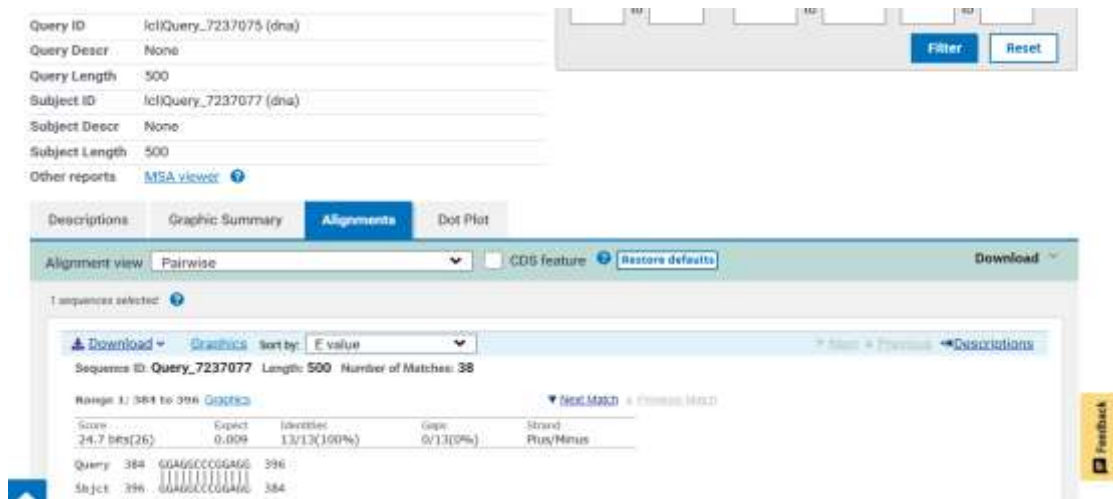
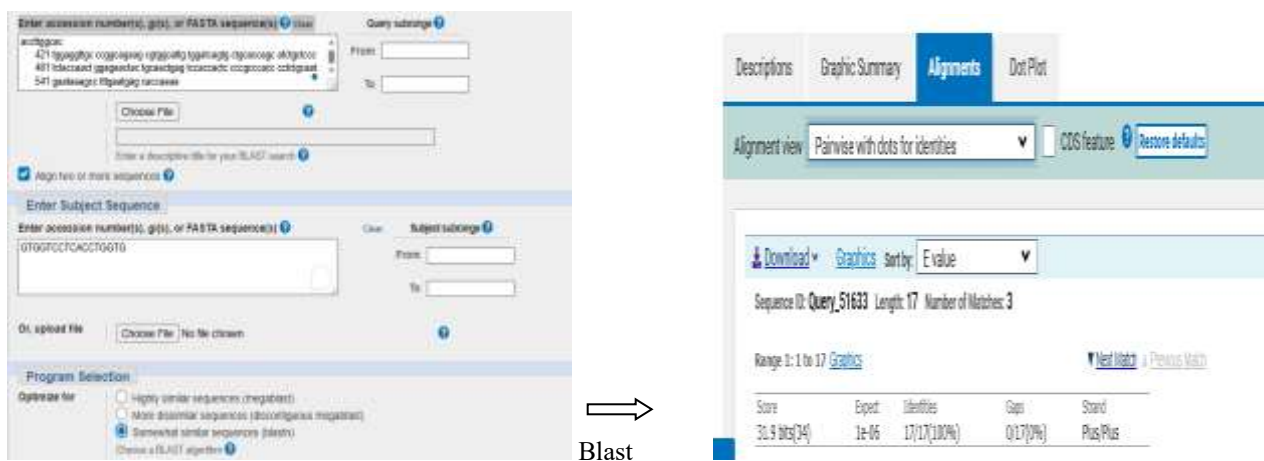


Fig. 2: FPCB approach to find out mirror repeats.

Further, identified mirror repeats were again searched within the complete gene and genome of *Rattus norvegicus* and Homo sapiens genome using MegaBLAST tool. Distribution of perfect mirror repeats of gene is studied. Firstly, perfect mirror repeat was examined within complete gene by program selection (somewhat similar sequences) Blast at expected threshold 20 and word size was set to 7.



Secondly, perfect mirror repeats examined within *Rattus norvegicus* and Homo sapiens genome by program selection (highly similar sequences) MegaBLAST at threshold limit 20 and word size was set to 16.

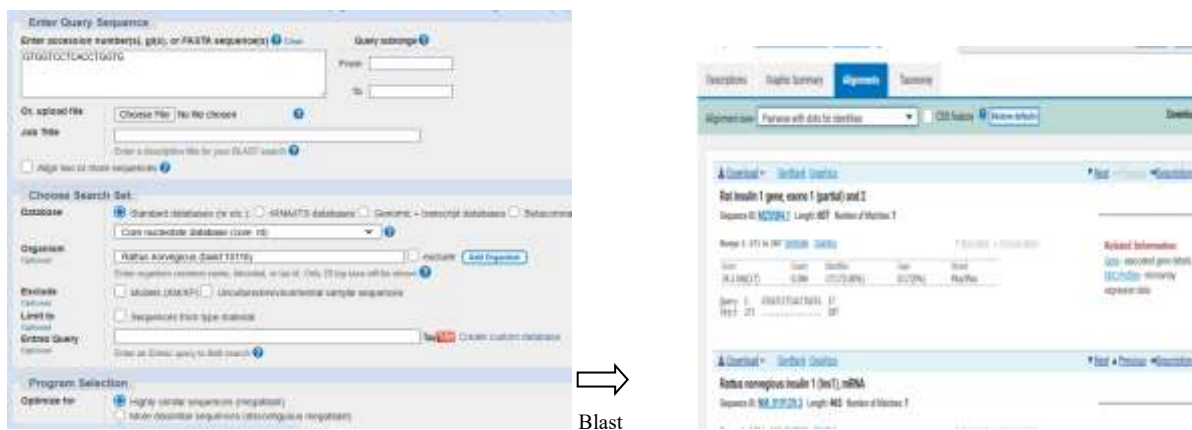


Fig. 3: MegaBLAST tool used to find distribution of mirror repeats in different genomes.

Results and Discussion

Mirror repetitions are found in numerous organisms, including bacteria, viruses, and plants, according to several previous studies (Yadav *et al.*, 2022; Yadav *et al.*, 2022). But no one selected the model organism *Rattus norvegicus* for their study for identification of mirror repeats. So, we have first time identified mirror repeats within the diabetic Glucagon (Gene Id: 24952), Sox17 (Gene Id: 312936), Gapdh (Gene Id: 24383) and PKLR (Gene Id: 24651) genes of *Rattus norvegicus*. In the present research work, a simple bioinformatic approach, was used to contrive mirror repeats present within the genes of *Rattus norvegicus* and its exons and introns. This recent developed method is very efficient to characterize mirror repeats. At the E-value of 20, a maximum number of hits were observed, so we fixed this value for identification of mirror repeat. Identified mirror repeats have different length varying from 7 base pairs to the largest mirror repeat of 58 base pairs. Further, the classification of identified mirror repeats was done on the basis of number of spacer element and the arrangement of nucleotides. Perfect mirror repeats have identical sequence around center of symmetry whereas imperfect mirror repeats have mismatch around center of axis. Perfect mirror repeats were further divided on the basis of spacer elements single spacer, double spacer and multispace. Similarly, imperfect mirror repeats may also have a spacer or no spacer elements. The frequency of occurrence of imperfect mirror repeats within *Rattus norvegicus* gene was less than the

frequency of perfect mirror repeats. A total of 199 mirror repeats were found in different regions of the Glucagon gene (Fig. 4) out of 199 mirror repeats, 122 were perfect with one spacer, 50 were perfect and 27 were imperfect repeats. A maximum number of hits 60 observed in fragment 4001-4500 and the corresponding mirror repeats were 13 with 6 perfect, 1 imperfect and 6 perfect with one spacer type.

We obtained 23 mirror repeats within exons and 79 mirror repeats within introns and of *Rattus norvegicus*'s Glucagon gene. The exon 1st, being the largest, contain 19 mirror repeats, whereas intron 2nd and 3rd, the largest have 23 mirror repeats as shown in Table 1.

Complete gene mirror repeats in the Sox17 gene:

A total of 199 mirror repeats were found in different regions of the Glucagon gene (Fig. 4) out of 199 mirror repeats, 122 were perfect with one spacer, 50 were perfect and 27 were imperfect repeats. A maximum number of hits 60 observed in fragment 4001-4500 and the corresponding mirror repeats were 13 with 6 perfect, 1 imperfect and 6 perfect with one spacer type..

We obtained 27 mirror repeats within exons and 35 mirror repeats within introns and of *Rattus norvegicus*'s Sox17 gene. The exon 5th, being the largest, contain 14 mirror repeats, whereas intron 3rd, the largest one_has 16 mirror repeats as shown in Table 2.

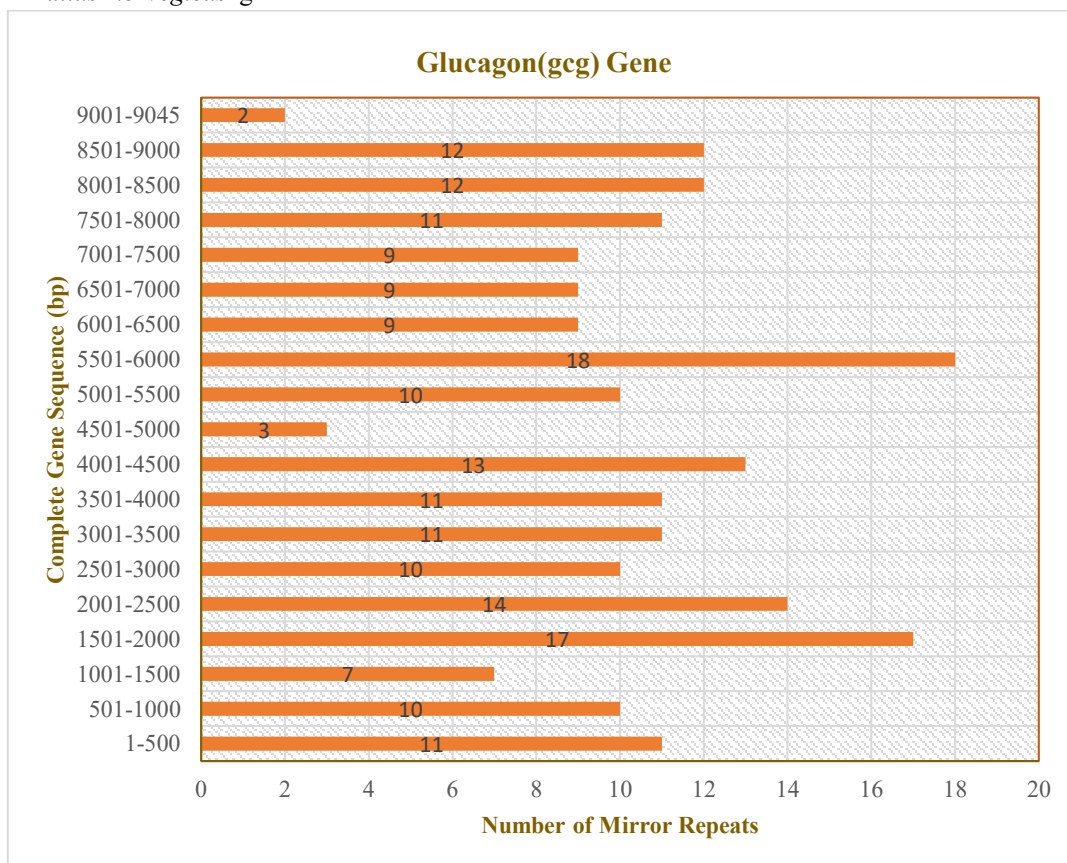


Fig. 4: Distribution of MR's (mirror repeats) in Glucagon gene.

Table 1: Total number of mirror repeats observed within the exons and introns of Glucagon gene

Exons	Length of Exons	Number of mirror repeats	Introns	Length of Introns	Number of mirror repeats
Exon1 (63-3134 bp)	3072	19	Intron 1(1-62 bp)	62	1
Exon2 (4886-5047 bps)	162	2	Intron 2 (3135-4885 bp)	1751	23
Exon 3(6616- 6753 bp)	138	0	Intron 3 (5048-6615 bp)	1568	23
Exon 4(7724- 7867 bp)	144	2	Intron 4 (6754-7723 bp)	970	19
Exon 5(8594-9045 bp)	452	0	Intron 5 (7868-8593 bp)	726	13

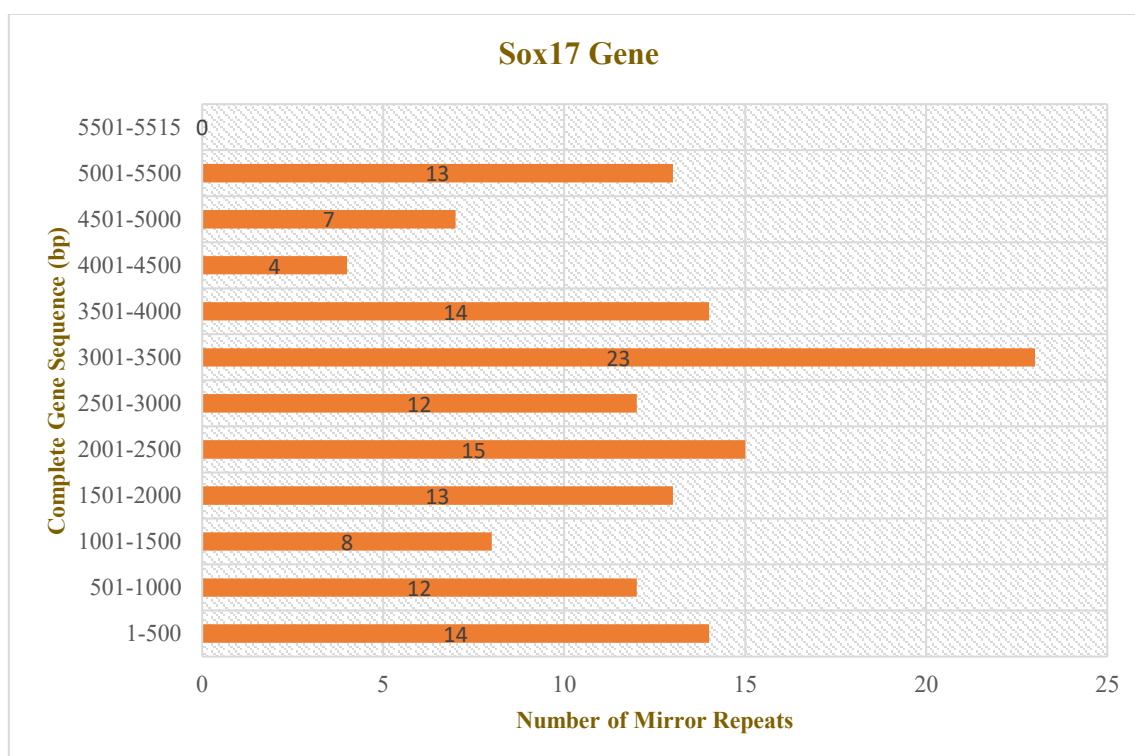


Fig. 5: Distribution of MR's (mirror repeats) in Sox17 gene.

Table 2. Total number of mirror repeats observed within the exons and introns of Sox 17 gene

Exons	Length of Exons	Number of mirror repeats	Introns	Length of Introns	Number of mirror repeats
Exon 1(1-140 bp)	140	0	Intron 1 (141-305 bp)	165	3
Exon 2 (306-677 bp)	372	0	Intron 2 (678-1008 bp)	331	5
Exon 3(1009-1296 bp)	288	0	Intron 3(1297-2967 bp)	1671	16
Exon 4(2968-3358 bp)	391	13	Intron 4(3359-3795 bp)	437	11
Exon 5(3796-5513 bp)	1718	14			

Complete Gene Mirror Repeats in The Gapdh Gene

A total of 116 mirror repeats were found in different regions of the Gapdh gene (Fig. 6). Out of 116, 70 mirror repeats were perfect with one spacer, 29 were perfect and 17 were imperfect repeats. A maximum number of hits 52 was observed in fragment 1501-2000 and the corresponding mirror repeats were 14 with 2 perfect, 2 imperfect and 10 perfect with one spacer type.

We obtained 26 mirror repeats within exons and 62 mirror repeats within introns and of *Rattus norvegicus*' s Gapdh gene. The exon 8th, being the largest, contain 9 mirror

repeats, whereas intron 3rd, the largest one have 24 mirror repeats as shown in Table 3.

Complete gene mirror repeats in the PKLR gene:

A total of 167 mirror repeats were found in different regions of the PKLR gene (Fig.7). Out of 167, 131 mirror repeats were perfect with one spacer, 22 were perfect and 16 were imperfect repeats. A maximum number of hits 47 were observed in fragment 3501-4000 and the corresponding mirror repeats were 13 with 3 perfect, no imperfect and 10 perfect with one spacer type.

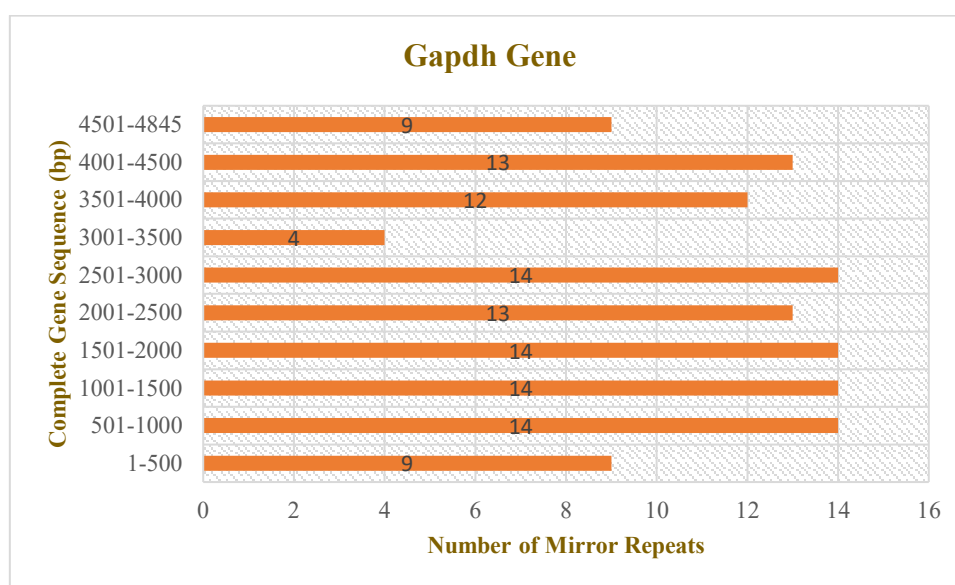


Fig. 6: Distribution of MR's (mirror repeats) in Gapdh gene.

Table 3: Total number of mirror repeats observed within the exons and introns of Gapdh gene

Exons	Length of Exons	Number of mirror repeats	Introns	Length of Introns	Number of mirror repeats
Exon 1(1-57 bp)	57	0	Intron 1 (58-972bp)	915	20
Exon 2 (973-1172 bp)	200	0	Intron 2 (1173-1260 bp)	88	2
Exon 3 (1261-1301 bp)	41	0	Intron 3(1302-3180 bp)	1879	24
Exon 4(3181-3387 bp)	207	3	Intron 4(3388-3467 bp)	80	1
Exon 5(3468-3558 bp)	91	2	Intron 5 (3559-3654 bp)	96	4
Exon 6 (3655-3852 bp)	198	4	Intron 6(3853-3964 bp)	112	1
Exon 7(3965-4195 bp)	231	7	Intron 7 (4196-4284 bp)	89	7
Exon 8(4285-4466 bp)	182	9	Intron 8 (4467-4576 bp)	110	3
Exon 9(4577-4845 bp)	269	1			

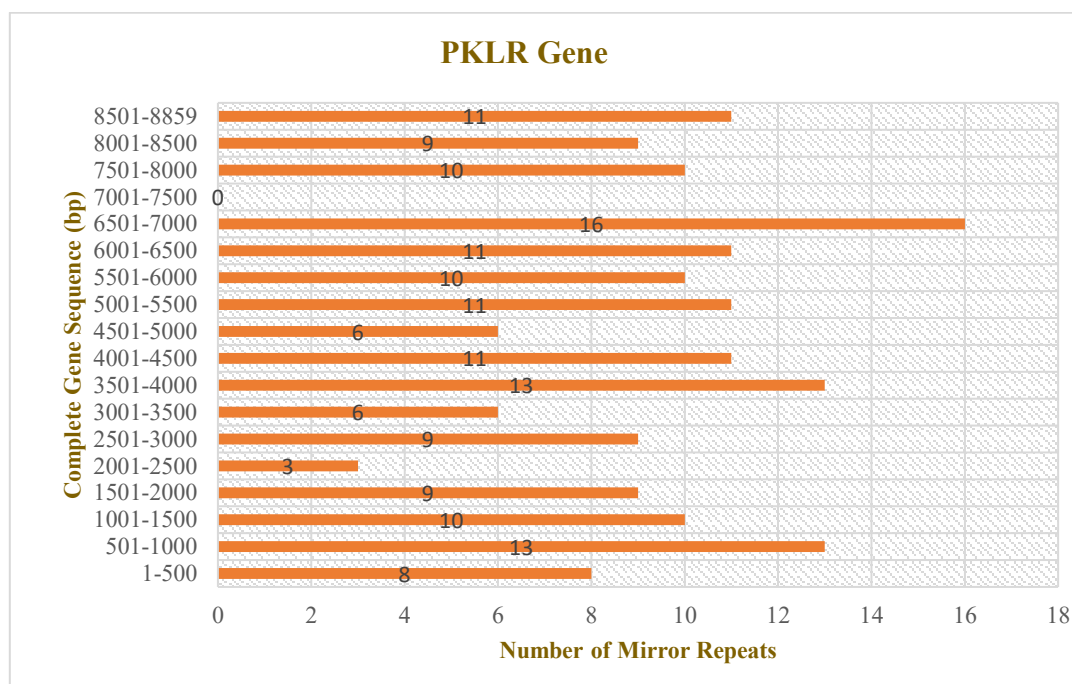


Fig. 7: Distribution of MR's (mirror repeats) in Gapdh gene.

Table 4. Total number of mirror repeats observed within the exons and introns of PKLR gene

Exons	Length of Exons	Number of mirror repeats	Introns	Length of Introns	Number of mirror repeats
Exon 1(1-134 bp)	134	0	Intron 1 (135-1091bp)	957	23
Exon 2(1092-1274 bp)	183	3	Intron 2(1275-3238 bp)	1964	12
Exon 3(3239-3330 bp)	92	0	Intron 3(3331-3471 bp)	141	2
Exon 4(3472-3603 bp)	132	1	Intron 4(3604-3737 bp)	134	5
Exon 5(3738-3924 bp)	187	3	Intron 5(3925-4240 bp)	316	13
Exon 6(4241-4511 bp)	271	2	Intron 6(4512-4623 bp)	112	2
Exon 7(4624-4774 bp)	151	1	Intron 7(4775-4986 bp)	214	1
Exon 8(4987-5139 bp)	153	2	Intron 8(5140-5232 bp)	93	3
Exon 9(5233-5399 bp)	167	4	Intron 9(5400-6619bp)	1220	24
Exon 10 (6620-6801 bp)	182	6	Intron 10(6802-7477 bp)	676	15
Exon 11 (7478-8859 bp)	1382	11			

We obtained 33 mirror repeats within exons and 100 mirror repeats within introns and of *Rattus norvegicus*'s PKLR gene. The exon 11th, being the largest, contain 11 mirror repeats, whereas intron 9th, the largest one have 24 mirror repeats as shown in Table 4.

Megablast is intended for comparing a query to closely related sequences and works best if the target percent identity is 95% or more. Using MegaBLAST tool, we further investigated identified mirror repeats among the genome of *Rattus norvegicus* and Homo sapiens of gene

Glucagon in Table 5, Sox17 in Table 6, Gapdh in Table 7 and PKLR in Table 8. This result confirms the ubiquitous distribution of mirror repeats of size 10-14 base pairs in the genera mentioned. These repeat sequences must have some important role in the genome; therefore, they are being maintained during course of evolution. Here we wish to mention that we were unable to look distribution of mirror repeats having a length less than 7 or equal to 7 bps and even more than 7 within *Rattus norvegicus* genome as well as in another genome.

Table 5: Distribution of perfect mirror repeats of Glucagon gene among different genera, here +ve sign depicts presence of mirror repeats and –ve sign depicts absence of mirror repeats.

CDS length	Mirror repeats	Within Gene	Within <i>Rattus norvegicus</i> genome	Within <i>Homo sapiens</i> genome
1-500	TTTAATTT	+	-	-
	CCATTACC	+	-	-
501-1000	AAAACCAAAA	+	-	-
1001-1500	TTTTAATTTT	+	-	-
	TTATTTTATT	+	-	-
	AAAGGAAA	+	-	-
1501-2000	CTGTTTGTC	+	-	-
	TGGAAGGT	+	-	-
	AGAAAAGA	+	-	-
	AAACCAAA	+	-	-
	TGAGGAGT	+	-	-
2501-3000	GATAAAATAG	+	-	-
	TATAATAT	+	-	-
3001-3500	ACCCCCA	+	-	-
4001-4500	TAATTTAAT	+	-	-
	TGTTTTTGT	+	-	-
	CATTTAC	+	-	-
	TTTAATTT	+	-	-
	ATTAATTA	+	-	-
4501-5000	ATGAAGTA	+	-	-
5501-6000	AACCTCCAA	+	-	-
	GGAAAAGG	+	-	-
	AAAGGAAA	+	-	-
6001-6500	CTTGCCG TTC	+	-	-
	AGTCCTGA	+	-	-
	.TATCCTAT	+	-	-
-6501-7000	CTTGCCG TTC	+	-	-
	AGTCCTGA	+	-	-
	TATCCTAT	+	-	-
	TGAAGTT	+	-	-
7001-7500	AATAATAA	+	-	-
7500-8000	TTTTAGTTGATTTT	+	+	+

	CGATTAGC	+	-	-
8001-8500	TTAAAATT	+	-	-
	AATTTTAA	+	-	-
8501-9000	ACAGAAGACA	+	-	-
	GTCTTCTG	+	-	-
	TTGAAGTT	+	-	-
	AGTGGTGA	+	-	-

Table 6: Distribution of perfect mirror repeats of Sox17 gene among different genera, here +ve sign depicts presence of mirror repeats and –ve sign depicts absence of mirror repeats.

CDS length	Mirror repeats	Within Gene	Within <i>Rattus norvegicus</i> genome	Within <i>Homo sapiens</i> genome
1-500	GAGAAGAG	+	-	-
	GTAAAATG	+	-	-
501-1000	TTCCAACCTT	+	-	-
	TAAGGAAT	+	-	-
1501-2000	GTCTTTTCTG	+	-	-
	TCTGGTCT	+	-	-
2001-2500	TATTTTAT	+	-	-
	TTTAATTT	+	-	-
	AGTGGTGA	+	-	-
3000-3500	CCGGGTTGGGCC	+	+	+
	GCGGGGCG	+	-	-
	ACACCACA	+	-	-
3501-4000	CCCTTCCC	+	-	-
4001-4500	CCGGCCGGCC	+	-	-
	CAGGGGAC	+	-	-
5001-5500	GTTAATTG	+	-	-
	GTGGGGTG	+	-	-

Table 7: Distribution of perfect mirror repeats Gapdh gene among different genera, here +ve sign depicts presence of mirror repeats and –ve sign depicts absence of mirror repeats.

CDS length	Mirror repeats	Within Gene	Within <i>Rattus norvegicus</i> genome	Within <i>Homo sapiens</i> genome
1-500	CCCCGGGGCCCC	+	+	+
	GGCCCCGG	+	-	-
	CTTTTTTC	+	-	-
501-1000	CTCCCCTC	+	-	-
	GAGCCGAG	+	-	-
	GCGGGGCG	+	-	-
1001-1500	GAGGCCGGAG	+	-	-
	TCGGGGCT	+	-	-
	GACCCCAG	+	-	-
1501-2000	CCGCCGCC	+	-	-
	CTTCCTTC	+	-	-
2001-2500	CCTTTTCCC	+	-	-
	TCTGGGGTCT	+	-	-
	GGGATTAGGG	+	-	-
	CCCAACCC	+	-	-
2501-3000	TTAAAGGAAATT	+	+	+
	ACAAAACA	+	-	-
3001-3500	TGTCCTGT	+	-	-
3501-4000	TCAGAAGACT	+	-	-
	AAGGGGAA	+	-	-
4001-4500	GCCTCCG	+	-	-
	TGTTTTGT	+	-	-
	TATGGTAT	+	-	-
	TTTGGTTT	+	-	-
	GGACCAGG	+	-	-
4501-4845	CTGAGGAGTC	+	-	-
	AGAAAAGA	+	-	-
	GGTGGTGG	+	-	-

Table 8: Distribution of perfect mirror repeats of PKLR gene among different genera, here +ve sign depicts presence of mirror repeats and -ve sign depicts absence of mirror repeats

CDS length	Mirror repeats	Within Gene	Within <i>Rattus norvegicus</i> genome	Within <i>Homo sapiens</i> genome
1-500	CACGGGGCAC	+	-	-
	GTAGGATG	+	-	-
501-1000	TGGAAGGT	+	-	-
1001-1500	ATGTTGTA	+	-	-
1501-2000	GTTTTG	-	-	-
2501-3000	GAACCAAG	+	-	-
	GAAGGAAG	+	-	-
	AGGAAGGA	+	-	-
3001-3500	GTACCATG	+	-	-
3501-4000	TCCAAAACCCT	+	+	+
	CTTTGGTTTC	+	-	-
	CACCCAC	+	-	-
	CTCCCCTC	+	-	-
6001-6500	GAGAAGAG	+	-	-
	AGGTTGGA	+	-	-
6501-7000	TTCCCCTT	+	-	-
	AGTGGTGA	+	-	-
8001-8500	CCCCAACCCC	+	-	-
8501-8859	CAGGGGAC	+	-	-
	TCCCCCT	+	-	-
	AATAATAA	+	-	-

These identified mirror repeats have any role in within the genome of *Rattus norvegicus* is still mysterious. However, to understand the function, our future goal is to identify proteins that bind to these identified mirror repeats. We strongly believe identification of mirror repeat binding proteins will open a new chapter of molecular biology.

Summary and Conclusion

Repetitive sequences play crucial roles in biological processes with both functional and non-functional implications. Certain repeats, like promoters and enhancer repeats, regulate gene expression, by acting as a binding site for regulatory proteins. They also serve as structural elements, such as centromeres and telomeres, which are vital for genome stability and cell division. By using a simple computational approach, we have identified 199 mirror repeats in Glucagon, 135 in Sox17, 116 in Gapdh and 167 in PKLR genes of *Rattus norvegicus*. These identified

mirror repeats are not restricted only in the genome of *Rattus norvegicus*, but also scattered among the genome of *Homo sapiens*. The frequent existence of mirror repeats (shorter as well as larger length) in analyzed genes gives a hint about their significant roles in the genes or genomes of *Rattus norvegicus* and *Homo sapiens*. These types of studies will be exploring new trends and tools of molecular biology research as well as development of new concept for mirror repeat identification.

Author Contribution Statement

Parveen Kumar: Conceptualization, Methodology, Software, Supervisor, Reviewing, Editing.

Babli: Data curation, Investigation, Writing-Original draft preparation, Writing-Review Editing.

Conflict of Interest

Authors declare that they have no conflict of interest.

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