



Identification Of Mirror Repeats Within The Trp53 (P53) Gene Of *Mus Musculus*

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Abstract

Various types of repetitive elements have been identified within the genome of organism such as direct repeat, indirect repeat and tandem repeat. One such repeats which is not widely examined in *Mus musculus* is mirror repeats. The present investigation aims to determine the mirror repeats in *Trp53* gene of *Mus musculus*. *Trp53* gene plays a significant role in cell cycle arrest, maintenance of stem cell through development, apoptosis, DNA repair and cellular senescence. By using a simple approach we have identified mirror repeats within the *Trp53* gene. As a result 281 mirror repeats were recognized within the complete gene and 27 mirror repeats in the eleven exons of *Trp53* gene of *Mus musculus*. We concluded that these mirror repeats is well distributed in within genome of *Mus musculus* and other organism genome.

Keywords: *Trp53* (*p53*) gene, Mirror repeats, *Mus musculus*, Tumor suppressor gene.

Introduction:

The term “repetitive sequences” denotes homologous DNA fragments which exist in multiple copies in the genome (Mehrotra and Goyal 2014; Dangi et al., 2022). Repetitive DNA was initially identified based on re-association kinetics and classified into “highly” and “middle” repetitive sequences (Britten and Kohne 1968; Jurka et al., 2007). Repetitive DNA sequences appear in whole higher plants wherein they account for approximately 90% of the genome size in certain species (Mehrotra and Goyal, 2014). Hence, these repetitive DNA sequences have been instrumental in variety of differences between genomes, which may imitate evolutionary changes amongst species. At times, the repetitive sequences were also assessed as “selfish elements” or “junk DNA” (Orgel and Crick 1980; Doolittle and Sapienza 1980). Based on the length of the repeated unit and arrangement size, tandem repeated DNA sequences can be categorized into three groups: (i) microsatellites, (ii) minisatellites repeats and (iii) satellite DNA (Mehrotra and Goyal 2014). Having three main classes of repeat: transposable elements,

tandem repeats, and high copy number genes (Bustos et al., 2016). Depending upon their positions and symmetry, DNA repeats can be sub-divided as direct repeats, inverted repeats & mirror repeats (Mirkin 2001; Bhardwaj et al., 2013; Dangi et al., 2022).

The genomes of maximum eukaryotes encompass a wide variety of repetitive DNA sequences (Harrison et al., 1998; Jiang et al., 2003). In the early 1900s, *Mus musculus* (house mouse) was acknowledged as pioneer genetic model organisms, resultant to its tiny generation span, reasonably sized litters, ease of husbandry, and evident phenotypic variants (Phifer-Rixey and Nachman 2015). Mice were extensively used in experimental study in the 17th and 18th centuries as well (Morse HC 1981). The three extensively established subspecies which have been widely used as distinct species by few scholars are (Mitchell et al., 1999; Musser et al., 2005), *Mus musculus castaneus*, *Mus musculus domesticus*, *Mus musculus musculus*. Despite being small animals with a short lifespan and high metabolic rate, mice can develop tumors in the same tissues as humans, often following a remarkably similar histopathological course

(Balmain and Harris., 2000). It's worth mentioning that 99% of mouse genes have a homolog in the human genome, with 80% of these genes having the best match in the human genome (Jean Louis Guénet in 2005). Furthermore, the comprehensive genome sequence of *Mus musculus* was published in 2002 (Gregory SG et al; 2002, Waterston RH et al; 2002).

Trp53 (also known as p53) is commonly referred to as the "guardian of the genome" and is a well-known tumor suppressor gene. The transformation-related protein (*Trp53*), which it produces, is one of the most researched tumor suppressor genes in biomedical research (Jane et al., 2020). The p53 protein was first discovered as a protein that binds to the SV40 large T antigen in altered cells (reviewed in Levine et al., 1991; Lane and Benchimol 1990). Approximately 50% of human cancers have mutations in the tumor suppressor gene p53. Apart from its tumor suppression role, p53 also plays a critical role in the response of malignant and non-transformed cells to many anti-cancer therapeutics, especially those that cause DNA damage (Aubrey et al., 2017). Its functions include transcriptional regulation, DNA repair, cell cycle check-point control, apoptosis, autophagy, and senescence, (Jane et al., 2020; Aubrey et al., 2017; Basu and Murphy 2016). Mice deficient for this gene are developmentally normal but are susceptible to spontaneous tumors. Evidences available reflect that this gene contains one promoter, in contrast to alternative promoters of the human gene, and transcribes a few of splice variants which encode different isoforms, although the biological validity or the full-length nature of some variants has not been ascertained. *Trp53* gene is positioned at chromosome number 11 and is 11,526 bp in length containing eleven exons. Aim of this study is to ascertain mirror repeats in *Trp53* gene of the *Mus musculus*. 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 *Trp53* gene of *Mus musculus* (house mouse) by using a simple manual bioinformatics methodology.

Material and Method:

Genomic sequence of *Trp53* gene having accession number NC_000077.7 and gene ID-22059 was retrieved from the National Center for Biotechnology Information (NCBI) link: (<https://www.ncbi.nlm.nih.gov/>). The *Trp53* gene is present on chromosome number 11 and has eleven exons with a total of 11526bps; it was subdivided into 500 base pair regions. Exons sequence represents the query sequence. By using the Reverse complement tool (<https://www.bioinformatics.org/sms/revcomp.html>), parallel complement of each subdivided regions and exons were retrieved. The mirror repeats of each sequence (query sequences) were analyzed by using the same approach as used by (Dangi et al., 2022; Dhankhar et al., 2022; Yadav et al., 2022). Using the mega BLAST tool, we searched the identified mirror repeats within the *Mus musculus* genome and other organism's genome (*Danio rerio*, *Drosophila melanogaster*, *Caenorhabditis elegans* and *Homo sapiens*).

Results and Discussion

By using a simple computational-based approach, we identified the mirror repeats within the *Trp53* gene which is involved in cell cycle arrest, maintenance of stem cell through development, apoptosis and DNA repair of *Mus musculus*. The 11,526bp long *Trp53* gene was subdivided into different parts of 500 bps each to identify maximum number of mirror repeats. The maximum numbers of hits were observed at the threshold value of 10, so we identify mirror repeats at this value in each 500bp. In total, 27 mirror repeats (MR's) were found in eleven exons of *Trp53* gene and 281 mirror repeats were found within the whole gene sequence. Identified mirror repeats were classified into Perfect mirror repeat, Perfect mirror repeat with 1 spacer, and 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. These repeats may or may not have any spacer elements. Table no. 1 shows all exons of *Trp53* (Transformation related protein 53) gene in which total 27 mirror

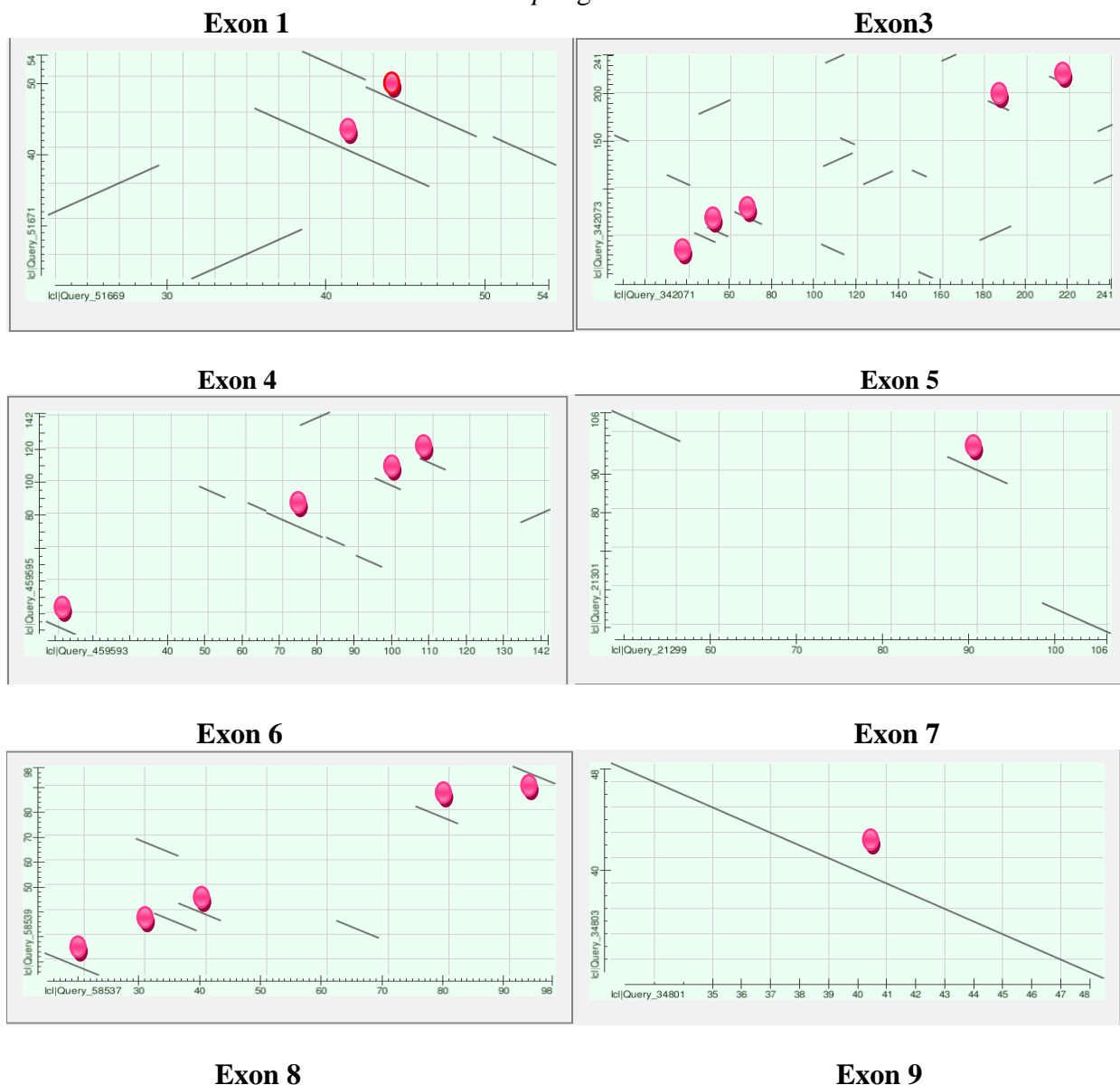
repeats were identified. We could not detect any mirror repeat within exon 2 having small size length i.e. 22bp. Figure (A) represents the number of mirror repeats in each dot plot

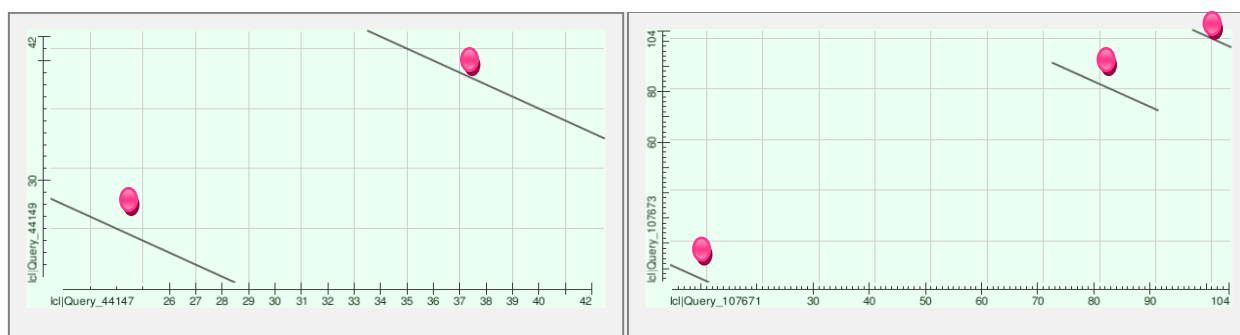
graph of exons of *Trp53* gene. The ‘*o*’ symbol represent the mirror repeat in each dot plot graph or *Trp53* gene.

Table 1: shows the number of mirror repeats with position and length within eleven exons of the *Trp53* gene.

Name of gene	Exons	Position	Length	Threshold Value	No. Of hits	No. Of Mirror repeats
<i>Trp53</i> (Transformation related protein 53)	Exon 1	6544-6626	83bp	10	4	2
	Exon 2	6910-6931	22bp	10	0	0
	Exon 3	7025-7285	261bp	10	19	5
	Exon 4	8017-8200	184bp	10	8	4
	Exon 5	8279- 8391	113bp	10	3	1
	Exon 6	8794-8903	110bp	10	7	5
	Exon 7	9226-9362	137bp	10	1	1
	Exon 8	9442-9515	74bp	10	2	2
	Exon 9	10309-10415	107bp	10	3	3
	Exon 10	10907-10961	55bp	10	1	1
	Exon 11	11003-11084	82bp	10	3	3
Total						27

Figure A: Represents the number of hits and mirror repeats on dot plot graphs of exons of *Trp53*gene





Exon 10

Exon 11

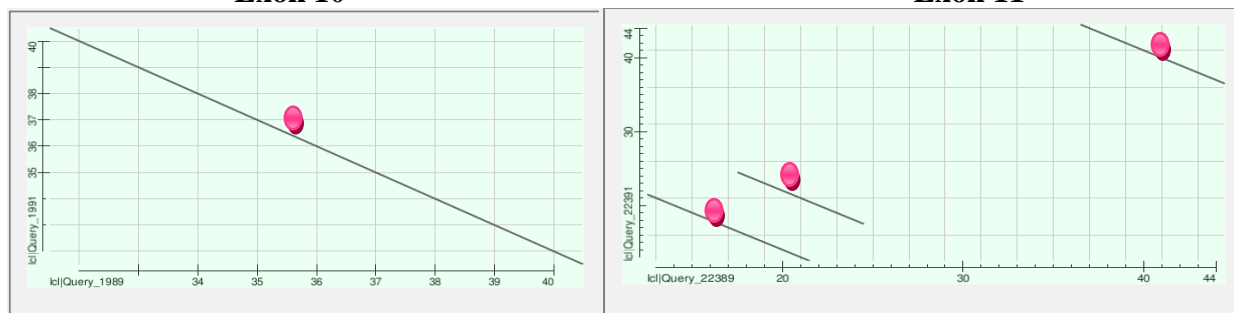


Table (2) depicts the position, length and types of all mirror repeats. The identified mirror repeats were classified into perfect mirror repeat, imperfect mirror repeat and perfect mirror repeat with single spacer. Out of 27 mirror repeats in all eleven exons of *Trp53* gene 5 Perfect mirror repeat, 5 imperfect mirror repeat and 17 perfect with

single spacer mirror repeats were identified. The largest mirror repeat within the exons of the gene is 26bp (imperfect) while the smallest mirror repeat is of 7bp (perfect mirror repeat with single spacer). Figure (B) shows the graphic representation of identified mirror repeats in all exons of *Trp53* gene.

Table 2: Shows the number of Mirror repeats, their positions, length and types within exons of the *Trp53* gene.

Exons	No. Of Mirror repeats	Mirror repeats	Position	Length of Mirror repeats	Types
Exon 1 6544-6626	2	1.CCTCGAGCTCC 2.CGAG-CTCCCTCTGAGC	36-46 39-54	11bp 16bp	Perfect with 1 spacer Imperfect
Exon 2 6910-6931	Not detected				
Exon 3 7025-7285	5	1.TTGAGGAGTT 2. AGTTTTTTGA 3. TCAAAAAACT 4. CCCAAGTGAAGCC 5. TCCACCT	44-53 50-59 183-192 63-75 212-218	10bp 10bp 10bp 13bp 7bp	Perfect Perfect Perfect Imperfect Perfect with 1 spacer
Exon 4 8017-8200	4	1. CTCCCCTC 2.GGGTC-AGCGCCACACCTCCAGCTGGG 3. CCGCGCC 4. CATCTAC	8-15 62-87 96-102 108-114	8bp 26bp 7bp 7bp	Perfect Imperfect Perfect with 1 spacer Perfect with 1 spacer
Exon 5 8279- 8391	1	1. TGGTGGT	88-94	7bp	Perfect with 1 spacer
Exon 6 8794-8903	5	1. TACCACCAT 2. GTACATG 3. ATGTGTA 4. CCTATCC 5. TCACACT	15-23 33-39 37-43 76-82 92-98	9bp 7bp 7bp 7bp 7bp	Perfect with 1 spacer Perfect with 1 spacer Perfect with 1 spacer Perfect with 1 spacer Perfect with 1 spacer
Exon 7 9226-9362	1	1. GTTCGTGTTTGTGCCTG	32-48	17bp	Imperfect
Exon 8 9442-9515	2	1. AAAAGAAAA 2. CCTCTCC	34-42 22-28	9bp 7bp	Perfect with 1 spacer Perfect with 1 spacer
Exon 9 10309-10415	3	1. ACAGAGGAGTCTGGAGACA 2. GCGGGCG 3. CTCACTC	73-91 5-11 98-104	19bp 7bp 7bp	Imperfect Perfect with 1 spacer Perfect with 1 spacer
Exon 10	1	1. AAGGAGGAA	32-40	9bp	Perfect with 1 spacer

10907-10961					
Exon 11 11003-11084	3	1. CTCCCCTC 2. GACTTCAG 3. CCTCTCC	13-21 37-44 18-24	9bp 8bp 7bp	Perfectwith1spacer Perfect Perfectwith1spacer

Figure B:Shows the graphic representation of mirror repeats within exons of *Trp53* gene

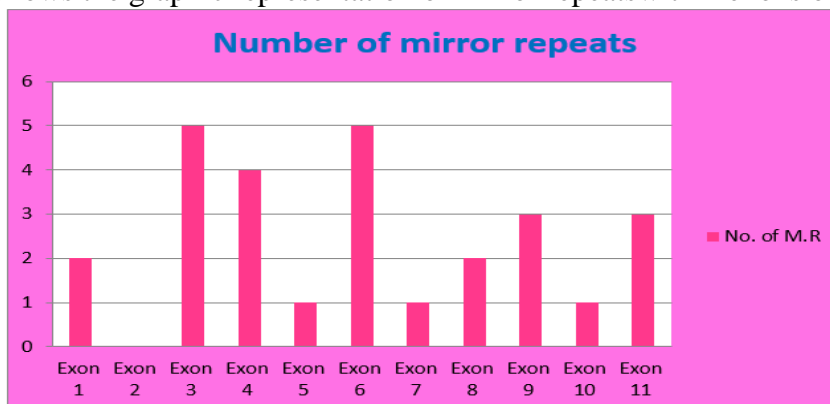


Table (3) shows presence of mirror repeats within complete *Trp53* gene. The whole gene sequences were subdivided into 500bp length, so that we can identify the maximum number of mirror repeats in each 500bp sequence. Total 281 mirror repeats were identified within complete *Trp53* gene. Out of 281 mirror repeats, 49 are Perfect mirror repeats, 184 are Perfect mirror repeats with one spacer, and 48 are Imperfect mirror repeats were observed. Largest mirror repeat is present in region 10 (35bp) while smallest

mirror repeat is of 10bp length which is present in almost all the regions. The percentage occurrence of Perfect mirror repeats is 9%, Perfect mirror repeats with one spacer is 33% and Imperfect mirror repeats is 8% within the *Trp53* gene are shown in figure(C). The classification of selected mirror repeats in the different regions of *Trp53* gene was depicted in table 3. The complete details of the mirror repeat in different regions of the gene are provided in the supplementary file.

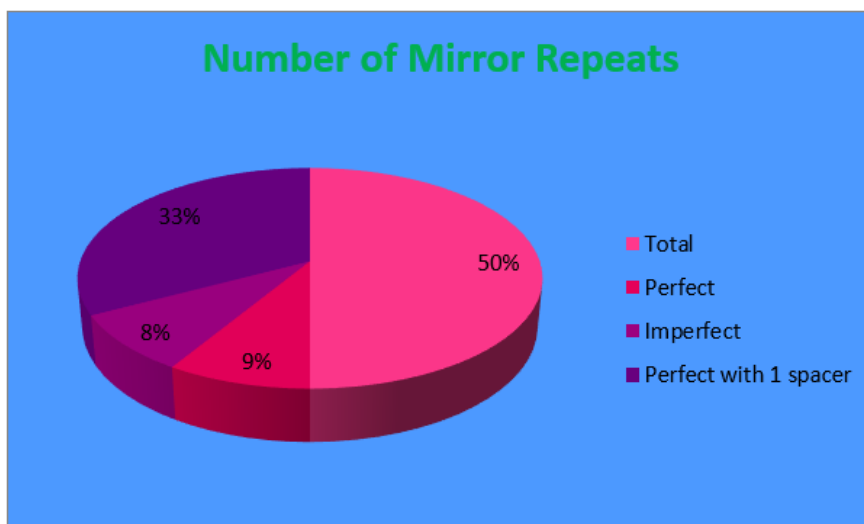
Table 3:Shows the classification of selected mirror repeats distributed in different regions of *Trp53* gene.

Name of gene and Id	Gene Sequence	Mirror repeats	Position	Length of Mirror repeats	Types
<i>Trp53</i> gene Gene id: 22059	Region 1(1-500)	1. CTCTCGGTCACTGGCTCTC 2. TCCTCCTCCT 3. GAGTGTATATGTCAG 4. CCCTCATCCTCCTCCTCC 5. GGGAGTGTATATGTCAGATGC TGTA—GTGAGGG	196-214 125-134 328-342 119-137 326-357	19bp 10bp 15bp 19bp 32bp	Perfect with 1spacer Perfect Imperfect Imperfect Imperfect
	Region2 (501-1000)	1. CCGACTCAGCC	293-303	11bp	Perfect with 1spacer
	Region3 (1001-1500)	1. GGGCCCCGGG 2. TTGGTTATTGCTT	178-187 15-27	10bp 13bp	Perfect Imperfect
	Region4 (1501-2000)	1. AAATGTAGATATATGTAAA 2. TCTCTTCTCT	264-282 353-362	19bp 10bp	Imperfect Perfect
	Region5 (2001-2500)	1. AAAACA- GAGAAAGAGTACAAAA 2. AAAAAGAAAA	320-341 125-135	22bp 11bp	Imperfect Perfect with 1spacer
	Region6 (2501-3000)	1. ACAAAAAACA 2. GCCACCACCG 3. CCTAGGATCC	61-70 365-374 419-428	10bp 10bp 10bp	Perfect Perfect Perfect
	Region7 (3001-3500)	1. TTGGTTGGTTGGtt 2. AATA--- AAACTAAATCAAACATAA 3. ATGTTCTCTTGTA 4. AAAGGGGGAAA 5. CAAAATAAAAC	193-206 460-481 310-322 1-11 457-467	14bp 22bp 13bp 11bp 11bp	Imperfect Imperfect Perfect with 1spacer Perfect with 1spacer Perfect with 1spacer

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		6. TTCCAAGGT- GCGAAAGCGATGGTTCCTT 7. GGAAGGAAGG	32-59 157-166	28bp 10bp	Imperfect Perfect
Region8 (3501-4000)		1. TTTTGAGTTTT 2. gagtgtgtgt-CGAG-- CTCATGCATACTCATTAGCATG TGTGTCAG	227-237 76-119	11bp 44bp	Perfect with 1spacer Imperfect
Region9 (4001-4500)		1. ATTCCCTTCCCTTA 2. CCCCCACCCCC 3. CCTTTTTTCC 4. TTTGGGGTTT	303-316 7-17 23-32 229-238	14bp 11bp 10bp 10bp	Perfect Perfect with 1spacer Perfect Perfect
Region10 (4501-5000)		1. TTCTTGTGAAAAGTTTCATATTG GCAACTGTTCTT 2. AGTTCCTTGA 3. GGCGTGGGGTGGGG	5-39 212-221 337-350	35bp 10bp 14bp	Imperfect Perfect Imperfect
Region11 (5001-5500)		1. TTCTCAAACCTCTT 2. CTCTGGGTCTC 3. CTCATTACTC 4. ATACAAGTGAATATA	238-250 7-17 44-53 278-292	13bp 11bp 10bp 15bp	Perfect with 1spacer Perfect with 1spacer Perfect Imperfect
Region12 (5501-6000)		1. AGAGGGAAGGGAAGAGAGA 2. GTGGTGGTGGTG 3. GTTAGTAACAAGAGAA- AATGAGTG	426-444 71-82 14-37	19bp 12bp 24bp	Imperfect Perfect Imperfect
Region13 (6001-6500)		1. TTTGTTTGTGTTGATGTTT 2. GTTTGTGTTGTTG 3. GTTTGTTGTTGTTGATG 4. TTGTTTGTATGTTTTT	473-491 472-484 472-488 478-494	19bp 13bp 17bp 17bp	Imperfect Perfect with 1spacer Imperfect Imperfect
Region14 (6501-7000)		1.CAGTACACAA- TCTCTTCTCTACAGATGAC 2. GGGCGGGGTGGCgggg 3. CCTCGAGCTCC 4. TGAGAGAAACAAAAACAGT	18-48 251-267 79-89 189-207	31bp 17bp 11bp 19bp	Imperfect Imperfect Perfect with 1spacer Imperfect
Region15 (7001-7500)		1. TGCCTCTTTTTCTCTGT 2. TGAGTGGGCCCGGG-GAGT 3. TTGAGGAGTT 4. AGTTTTTTGA 5. TCAAAAAACT 6. CCTCTTCTCC	328-344 287-305 68-77 74-83 207-216 416-425	17bp 19bp 10bp 10bp 10bp 10bp	Imperfect Imperfect Perfect Perfect Perfect Perfect
Region16 (7501-8000)		1. CAAAAACAAAAAC 2. AGGAAGAAGGA	115-127 357-367	12bp 11bp	Perfect with 1spacer Perfect with 1spacer
Region17 (8001-8500)		1. CTCTCCTCTC 2. CTCCTCTCTCCAGTACTCTCCTC	1-10 3-26	10bp 24bp	Perfect Imperfect
Region18 (8501-9000)		1. GTTCCCTCCCATG	106-118	13bp	Imperfect
Region19 (9001-9500)		1. CCAAGGAGGCGGAGGAGCC 2. GTCTCCTCCTCTG 3.GTTCGTGTTTGTGCCTG 4.CTTGCTCTCTCCTTC	377-395 33-45 257-273 422-436	19bp 13bp 17bp 15bp	Imperfect Perfect with 1spacer Imperfect Imperfect
Region20 (9501-10000)		1. AAAGTAACCCAAGGAAA 2. CCCCAGGATTAGGTCCC 3. GCCTCCTCCG 4. AGGCCCCGGA	388-404 464-479 177-186 461-470	17bp 16bp 10bp 10bp	Imperfect Imperfect Perfect Perfect
Region21 (10001-10500)		1. GACCTCCCTCCAG 2. GTGACCTTGCCAGTG	458-470 265-280	13bp 16bp	Perfect with 1spacer Imperfect
Region 22 (10501-11000)		1. GAAGTTTGAAG	145-155	11bp	Perfect with 1spacer
Region 23 (11001-11500)		1. CCACCCCCACC 2. GTTGATAGTTG	436-447 252-262	12bp 11bp	Perfect Perfect with 1spacer

Figure C: Shows the percentage occurrence of mirror repeats within the *Trp53* gene



With the help of Mega BLAST tool, the identified selected mirror repeat within the whole gene sequence of p53 gene were searched within genome of *Mus musculus* and other organisms genome such as *Drosophila melanogaster*, *Danio rerio*, *Homo sapiens* and *Caenorhabditis elegans*. In table4 ‘+’ sign

shows the presence of mirror repeats and the ‘-’ sign shows the absence of mirror repeats. Based on the mega BLAST results, we could not identify 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: Distribution of identified mirror repeats of different regions of *Trp53* gene within the genome of *Mus musculus*, *Drosophila melanogaster*, *Danio rerio*, *Homo sapiens* and *Caenorhabditis elegans*.

Mirror Repeats	length	M.musculus	C.elegans	D.rerio	H.sapiens	D.melanogaster
CTCTCGGTCACTGGCTCTC	19bp	+	+	+	+	+
TCCTCCTCCT	10 bp	-	+	-	-	-
GAGTGTATATGTCAG	15 bp	+	+	+	+	+
CCCTCATCCTCCTCCTTCC	19 bp	+	+	+	+	+
GGCGGGCGG	9 bp	+	+	+	+	+
CCGACTCAGCC	11bp	+	+	+	+	+
TGTATTGT	9bp	-	-	-	-	-
ACCCCCA	8bp	-	-	-	-	-
GGGCCCCGGG	10bp	-	-	-	-	-
CGTGCGTGC	9bp	-	-	-	-	-
TTGGTTATTGCTT	13bp	+	+	+	+	+
AAATGTAGATATATGTAA A	19bp	+	+	+	+	+
TCTCTTCTCT	10bp	-	-	-	-	-
GGGTTTGGG	9bp	-	-	-	-	-
AAAACA- GAGAAAGAGTACAAAA	22bp	+	+	+	+	+
AAAAAGAAAAA	11bp	+	+	+	+	+
AACCCCAA	8bp	-	-	-	-	-
ACAAAAACA	10bp	-	-	-	-	-
GCCACCACCG	10bp	-	-	-	-	-
TTGGTTGGTTGGtt	14bp	+	+	+	+	+
AATA--- AAACTAAATCAAACATAA	22bp	+	+	+	+	+
ATGTTCTCTTGTA	13bp	+	+	+	+	+
TTTTGAGTTTT	11bp	-	+	-	-	+
gagtgtgtg-CGAG— CTCATGCATACTCATTAGC	44bp	+	-	+	+	+

ATGTGTGTCAG						
ATCCCTCCCTTA	14bp	+	+	+	+	+
CCCCACCCCC	11bp	-	+	-	-	+
TTCTTGTAAGGTTTCAT ATTGGCAACTGTTCTT	35bp	+	+	+	+	+
AGTTCCTTGA	10bp	-	-	-	-	-
TTCTCAAACCTTT	13bp	+	+	+	+	+
CTCTGGGTCTC	11bp	-	+	-	-	+
AGAGGGAAGGGAAGAGAG A	19bp	+	+	+	+	+
GTGGTGGTGGTG	12bp	-	+	-	+	+
GTTAGTAACAAGAGAA- AATGAGTG	24bp	+	+	+	+	+
TTGTTTGTGTTGATGTTT	19bp	+	+	+	+	+
GTTTGTGTTGTTT	13bp	+	+	+	+	+
CAGTACACAA- TCTCTTCTCTACAGATG AC	31bp	+	+	+	+	+
CTCCTCTCTCCAGTACTC TCCTC	24bp	+	+	+	+	+
GTTCCCTCCCATG	13bp	+	+	+	+	+
CCACCCCCACC	12bp	-	+	-	+	+
GTTGATAGTTG	11bp	-	+	-	-	+

Conclusion:

Our investigation has revealed that the *Trp53* gene of *Mus musculus* contains a significant number of mirror repeats. Specifically, we identified 281 mirror repeats distributed across different regions of the *Trp53* gene, as well as 27 mirror repeats located in all eleven exons of the gene. By using the BLAST tool, we were able to determine that these mirror repeats vary in length and type. However, the precise function of these mirror repeats remains unknown, and it would be highly significant to gain a deeper understanding of their role at the molecular level. Additional studies may therefore be necessary to elucidate the exact function of mirror repeats within the genome of *Mus musculus* and other organisms.

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