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Molecular cloning and confirmation of MSH2, an essential gene of human, involved in DNA mismatch repair

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Abstract
Mismatch repair (MMR) is one such pathway that repair mismatches generated during DNA replication. Errors in MMR cause certain types of cancer including hereditary non-polyposis colorectal cancer, genome instability, abnormalities in meiosis, sterility in mammalian systems and resistance to certain chemotherapeutic agents. In eukaryotes, *MSH2* (MutS homologue 2) is a major player in MMR, acting in combination with *MSH3* or *MSH6* as a heterodimer. *MSH2* has been involved in a variety of processes which protect the integrity of the genome. In this study h*MSH2* gene was cloned to identifying their role and understanding the functional diversity of protein that will contribute towards the repair mechanisms. Using in frame designed primers through PCR strategy and cloned full-length cDNA fragment h*MSH2* gene into pET32b expression vector. The sequence contained an open reading frame of 2805 bp coding for a putative protein of 935 amino acids. Recombinant C-3 clone (pET32b-h*MSH2*) observed as positive after restriction digestion with expected band size (~8.6kb). This recombinant clone of h*MSH2* gene, further used to express the protein and purified protein used to understand the role of mismatch repair protein *MSH2*.

Keywords: DNA damage, DNA repair, mismatch repair (MMR), MSH2

Introduction

DNA damage is well known as a vital factor in the growth of cancer. An irregular nucleotide or nucleotide fragment is formed by DNA lesions, resulting a break in single and both DNA strand chains. DNA damage is one of the most significant factors contributing to the development of cancer is genomic instability. The critical function of DNA repair pathways is to correct the DNA lesions that arise from harmful agents or carcinogens and preserving genomic stability. Ineffective DNA repair is a key powerful process to the development and evolution of cancer [1].

DNA damage caused by endogenous and exogenous mutagens attacks occurs in chromosomes [2]. DNA damage is unrepaired has the ability to produce mutations in somatic or germline cells mutations that may alter the cellular phenotype and cause dysfunction or disease [3]. There are a variety of factors known to cause DNA harm, such as Ultraviolet radiation, ionizing radiation, acridine dye, mustard gas and bleomycin. Damage in DNA affect both strands and cause to defeat of genetic information. There are a variety of repair mechanisms that work in organisms to enable them to resist the damage [4].

DNA repair pathways that correct the damages can be classified into six categories such as direct repair (DR), base excision repair (BER), nucleotide excision repair (NER), mismatch repair (MMR), double-strand break repair (DSBR) and repair of cross-links between DNA strand. Mismatch Repair (MMR) pathway repair mismatches that occur in eukaryotes during the many rounds of mitotic DNA replication preceding meiosis. Mismatch repair should be required to be necessary for gene mutation prevention during mitotic expansion and germline maintenance [5]. Mismatched bases generated during the DNA replication processes in which the incorrect DNA base is stitched into place of corrected DNA base or inserted inaccurately are cause defects in DNA. Mismatched base pairs which resulted from replication errors are contribute to the genetic instability of human cells [6]. MMR preserves genomic integrity by restoring base-base mismatches and insertion/deletion loops (IDLs) occurring during DNA replication as well as recombination heteroduplexes [7]. Mismatch repair pathway is responsible for the correction of base substitution mismatches and insertion-deletion mismatches created from bacteria to mammals during DNA replication in species. Mismatch repair mechanism also identifies such DNA lesions caused by normal intracellular metabolism as well as external physical and chemical insults including certain chemotherapeutic agents [8]. MMR mechanism implicated in the prevention of cancer in mammalian cells is also known to increase TNR expansion, a condition linked to a variety of human neurological disorders, including Huntington's disease, myotonic dystrophy and fragile X syndrome. Defects in mismatch repair pathway caused genome instability, certain forms of cancer and sterility disorders in mammalian systems [3].

The *MSH2* protein linked with MutS complex forms a heterodimer with either *MSH6* or *MSH3* for mismatch recognition which depending upon the type of DNA lesion to be repaired. *MSH6* required for the correction of single-base mispairs requires, while both *MSH3* and *MSH6* can contribute to the correction of IDLs. A MutL complex form heterodimer *MLH1* and PMS2 co-ordinates the interplay between the recognition of mismatch and other MMR necessary proteins, including exonulease1 (*EXO1*), helicase(s), proliferating nuclear antigen (*PCNA*), single-stranded DNA-binding protein A (*RPA*)and DNA polymerasesδ and ε. *PMS2* is important to correct single-base differences and *MLH3* both contribute to the

improvement of IDLs. That *PCNA* interacts with *MSH3* and *MSH6* to increase the mismatch recognition specificities. Other protein- protein interactions mediate subsequent repair steps after initiation of the MMR. A direct association between the excision of the 5'-3'exonuclease *EXOI* and the resynthesize of the DNA with the help of polymerase δ and ε. DNA interaction between MMR proteins will regulate this process. At last, DNA ligase I, seals the scratch delivered by DNA polymerase δ and *PCNA* [9].

The protein product h*MSH2* is a component of the DNA mismatch repair pathway which has a well-established function in bacteria and yeast [10]. The modifications of replication errors are significant among all MutS and MutL like human proteins, h*MSH2*, h*MSH3*, h*MSH6*, h*MLH1*and h*PMS2* [11]. *MSH2* function as "spell checker" in mismatch repair and involved in a generalized cellular response to DNA disruption which activating signaling cascade that triggers cell cycle checkpoints or apoptosis [12]. MMR proteins are likewise engaged with random hereditary pathways through connections with various proteins. MMR proteins also participate in diverse genetic processes through interactions with various proteins [13]. *MSH2* protein loss is associated with *MSH2* inactivation, hypermutation, and higher tumor-infiltrating lymphocyte and is most common among very high-grade primary tumors. As calculated by immunohistochemistry (IHC), the concept that *MSH2* protein loss is closely associated with the primary genomic inactivation of *MSH2* and hypermutation [14]. An integration of HR and MMR pathways is suggested in the discovery of the MutSα complex. *MSH2* and *MSH6* are involved in the detection of DNA mismatches and insertion/deletion through the MMR mechanism. The results first reported that the *RAD51* paralogs interact with MMR-related proteins (*MSH2)* and proteins with a suggested role in the repair mediated by non-homologous end joining (*SFPQ-NONO*). Western blot analysis of precipitates eluates confirmed the interactions of the four-candidate protein (*SFPQ*, *NONO*, *MSH2* and mini chromosome maintenance protein 2) to form ex-vivo complexes with *RAD51D*. Cell division, embryo formation, protein, carbohydrate metabolism, cellular trafficking, protein synthesis, alteration or folding and cell structure or motility functions have been correlated with additional interacting proteins [15]. This study demonstrated the successful cloning and confirmation of h*MSH2* gene in pET32b expression vector. Cloning h*MSH2* gene into bacterial strain is easier to create several exact copies of a segment of a DNA fragment that encodes h*MSH2* protein. h*MSH2* gene can be an important component for correction of mismatches involved during DNA replication process in mismatch repair pathways. This study further used to understand the role of mismatch repair h*MSH2* gene which encodes *MSH2* protein in other repair pathways.

Materials and Methods

Bacterial Strains, Reagents and Kits

Host strain *Escherichia coli* DH5α and pET32b-TEV expression vector was obtained from Invitrogen. Commercial DNA clone (pBLuescript-hMSH2-E3) was ordered from Addgene (GeneID-16453) Company. 1kb DNA ladder, DNA Taq polymerase and T4 DNA ligase were purchased from New England Biolabs (NEB). PCR purification kit was purchased from QIAGEN. Restriction enzymes were purchased from NEB and QIAGEN. Oligonucleotide primers were purchased from Invitrogen. Chemicals, growth medium and other salts used in the laboratory work were of good analytical grade and of HIMEDIA and New England Biolabs.

Primer designing for PCR amplification

Sequence mining and analysis of h*MSH2* gene done by using NCBI database for primer designing to amplify the complete cDNA sequence. Retrieved the gene sequences of h*MSH2* from NCBI "Gene" database. Enter the respective gene names to search and use *HomoSapiens* sequence filters respectively. Designed in-frame primers for amplification of cDNA sequence of h*MSH2* for cloning into pET32 vector. Select the sequence of the verified and longest isoform for further analysis. Analyzed the primers using UCSC Genome Browser Software. Restriction analysis of the gene transcript for primer designing and analyzed the vector's multiple cloning sites (MCS) to select the restriction enzyme that can be used to designed the primers for successful cloning of the gene. Design the primers for obtaining the required PCR products manually, such that each primer pair has similar Tm, contains the required restriction endonuclease site and the base length between 18 to 24 base pairs. Analyze the primer pairs further using Oligo -Analyzer Software.

Isolation of Commercial cDNA clone (pBluescript-h*MSH2*)

For isolation of plasmid DNA, bacterial cultures were grown in 100 µg of ampicillin per ml. *E.coli* competent cells were prepared by Calcium chloride method and culturing the cells till an OD of 0.6 is obtained. Transformed the plasmid DNA contained cDNA insert are spread on the plates containing ampicillin (100µg/ml). Plasmid isolation was done using alkaline lysis method as follow Sambrook *et al*, by harvested overnight bacterial culture by centrifugation. The DNA yield was 1.0 to 1.7mg per liter of bacterial culture. Restriction enzyme analysis of the plasmid DNA was done to check the quality of DNA. Run the digested plasmid vector on 1.2% agarose gel. Observed the bands under UV light in an UV-illuminator.

Amplification of hMSH2 gene using plasmid DNA (pBluescript-h*MSH2*)

Polymerase chain reactions (PCR) were carried to make full length DNA sequence using Taq DNA polymerase (New England Biolabs) in 50µl final volume. Stage1, the initial denaturation occurs at 94° C for 5 minutes, during denaturation the double stranded DNA melts to form single strands and all enzymatic reactions stop. Annealing at 50° C for 15 seconds in stage 2, hydrogen bonds are constantly formed between single stranded primer and the single stranded template. Stage3, Extension at 72° C for 3 min and then final extension at 72° C for 7 min the bases are added to the template DNA on 3'side through the help of DNA polymerase. Components used for reaction mixture for PCR amplification, 10X Standard Taq Reaction Buffer, 10 mM dNTPs, 10 µM Forward Primer, 10µM Reverse Primer, Taq DNA Polymerase and Template DNA.

Cloning of full-length cDNA encoding the hMSH2 protein

The amplified fragments of h*MSH2* gene and plasmid vector (pET32b) were digested with the restriction enzymes (New England Biolabs). Restriction enzyme BamHI and XhoI are used to digest the amplified gene product of h*MSH2* and pET32b expression vector for cloning purpose. Incubated-the eppendorf at 37˚C for 1 hour. Run the digested plasmid vector on 1.2% agarose gel.

Observe the bands under UV light in an UV-illuminator. Following the New England Biolabs standard procedure, DNA ligation reaction was performed to fuse DNA fragments in a final volume of 20µl using T4 DNA ligase (NEB). For the ligation process add 10X rapid ligation buffer, digested vector, gene of interest and T4 DNA ligase in an eppendorf. The T4 DNA ligase processes a remarkably ability to attach sticky and blunt ends of DNA fragments. The ligase enzyme catalyzes the formation of phosphodiester bonds between 5' phosphate and 3' hydroxyl terminus of dsDNA. The digested fragments were mixed at molar ratio (1:1) respectively. The reaction was incubated at 20ºC for 2 hours. The ligation reaction mixture used directly for bacterial transformation by calcium chloride transformation method. 4µl aliquot of the ligated mixture was used to transformed into 50µl of competent cells. The transformed ligated clone was confirmed on 1.2% agarose gel electrophoresis.

Selection and Screening of recombinant clones

Competent cells were prepared by using *E.coli* (DH5ά) strain for the transformation of ligated samples. The ligated samples were dispensed into the eppendorf tubes containing competent cells separately and incubated. The selection of transformants was accomplished by including 100 µg/ml ampicillin in the LB agar medium, due to which only *E. coli* cells transformed with vector appeared on cultured plates. All the colonies on plate were further screened for the identification of recombinant clones by plasmid isolation, restriction digestion and plasmid restriction double digestion with restriction enzymes.

Isolation and restriction digestion of plasmid DNA

The plasmid DNA was extracted from positive clones by alkaline lysis method. The quality of plasmid DNA was checked by electrophoresis on 1.2% agarose gel. The recombinant plasmid DNA digested with the help of restriction enzymes. The extent of restriction digestion was analyzed after run of 4µl samples on agarose gel electrophoresis along with standard 1 kb DNA marker (New England Biolabs). Double and single restriction digestion done for the confirmation of recombinant plasmid DNA (pET32b-h*MSH2*) clones with the help of restriction enzymes and reaction mixture incubated for 2 h at 37˚C. Expected band size of recombinant plasmid DNA was evaluated by gel electrophoresis.

Results and Discussion

Cloning Strategy

Commercial clone with gene of interest, approximately ~2800bp fragment of h*MSH2* contained full length cDNA was used to construct recombinant clone into expression vector pET32b-TEV. In frame primers were designed to amplify the full cDNA sequence, then both expression vector and full amplified sequence are digested using restriction enzymes BamHI and XhoI. To obtain sticky end cloning, which produce 5' or 3' overhangs in both plasmid vector and PCR product. Finally, DNA fragment and expression vector with complimentary overhangs at the both ends, which were able to anneal and sealed by DNA ligase. The inserted genes were confirmed by restriction analysis (Figure-1).

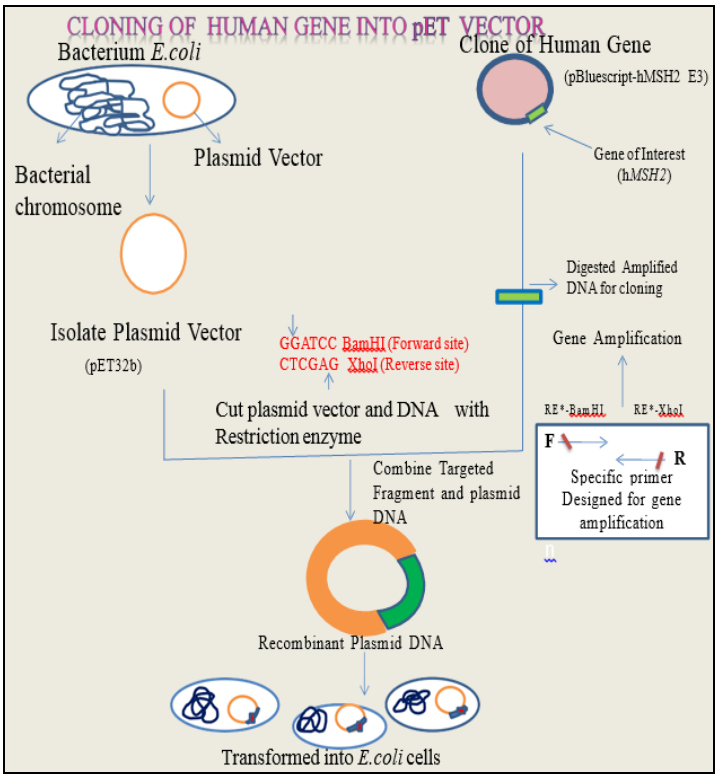

Fig 1: Schematic representation of cloning strategy human (*MSH2*) gene into pET expression vector. Refer to the text for detailed explanations

Molecular cloning strategy (Figure-1) used commercial clone which contains cDNA that encodes gene of interest (h*MSH2*), insert gene was amplified using designed primer specific for this particular gene, or pET32b original expression vector used for directional cloning. Digested the fragment of gene and vector by using restriction enzymes to create cohesive ends of both gene and vector. Both gene insert and vector overhangs ends were joined by ligation and transformed into *E.coli.*

Plasmid Isolation and restriction analysis of cDNA clone

Plasmid DNA (pBluescript-h*MSH2*-E3) which contains full length cDNA sequence of h*MSH2* were used for transformation to obtain higher concentration of the respective plasmid DNA for cloning. Competent cells were prepared using calcium chloride and these plasmids DNA were used for transformation. Successfully transformed the clone (pBluescript-h*MSH2*) and plasmid vector (pET32b-TEV) using Luria agar plates containing ampicillin (100µg/ml) incubate at 37ºC. The transformation of these plasmids resulted in ~3000 colonies per plate. Thus, the transformation resulted in an efficiency of 1.5 X 108 cells/µg of plasmid DNA which was considered as good transformation efficiency. The plasmids DNA were isolated from the transformed cells and digested using restriction enzymes to authenticate the plasmid isolation, quality and specificity of DNA, then were analyzed on 1.2% agarose gel. The agarose gel shows the successive plasmid isolation after using the complete steps of the procedure. Plasmid DNA contained full length of cDNA sequence of h*MSH2* was confirmed using restriction digestion. Single and double restriction analysis done at different restriction sites in plasmid DNA (pBluescript-h*MSH2*) and obtained precise band that was expected. Total size of commercial clone pBluescript-h*MSH2* (6100bp) which h*MSH2* (3000bp) inserted into pBluescript 3.1 vector (3100bp). Plasmid DNA (pBluescript- h*MSH2*) digested with HindIII restriction enzyme and observed the expected band size (~4.8kb and ~1.2kb). Figure-2 completely confirmed that this plasmid DNA was contain the complete sequence of h*MSH2* gene and further used for amplification of h*MSH2* gene for cloning.

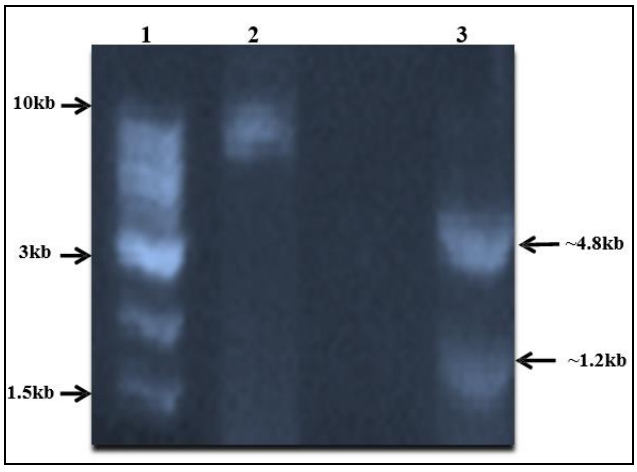

Fig 2: Gel electrophoresis of isolated plasmid DNA (pBluescript-h*MSH2*) through transformation. Confirmed the full-length cDNA sequence of h*MSH2* by restriction digestion analysis: Lane1- 1kb ladder, Lane2-undigested plasmid DNA (pBluescript-h*MSH2*), and Lane3- Plasmid DNA digested with HindIII, expected band size (~4.8kb and ~1.2kb).

Primer Analysis for PCR amplification

Primers designing for cloning of human MSH2 gene, primers were designed using different software and contain the restriction sites for BamH1and Xho1. In frame primer designed by retrieving the sequence from NCBI for MSH2 (*HomoSapiens*) with forward primer hMSH2BamHIF "5'-GTGGATCCGAAGGAGACG-3" and reverse primer name hMSH2XhoIR "5'-AGTGAGCTCTATTCGTGAAAT-3". The primers designed for the amplification of h*MSH2* was analyzed for their specificity, to avoid any non-specific binding and any unwanted effects, using UCSC Genome Browser's In-Silico PCR software. The primers with a single binding site within the required transcript producing the product of sufficient size were selected. The oligo-analyzer software used to evaluate physical properties of primers. Determine primer properties like Tm, GC contents and compare unlimited number of primers to each other. Total length of forward primer was 18bp long, Tm-53, GC content 61.11% and reverse primer 21bp long, Tm 49, GC content 38.11% obtained after analyzing the primer sequence.

PCR amplification

For *HomoSapiens MSH2* gene specific PCR primers were designed from the predicted human sequences NM_000251 on chromosome location 2p21 (GeneID-4436, Exon count-17) that represents the human orthologue of the MSH gene and contain the restriction sites for BamHI and XhoI. Amplification was carried out in a thermal cycler using h*MSH2* specific set of primers to amplify of full cDNA. PCR amplification of h*MSH2* from isolated plasmid DNA using taq- polymerase was done for cloning of h*MSH2* into pET32b expression vector. Using transformed clones of plasmids DNA (pBluescript-h*MSH2*) which contains full sequence of h*MSH2* gene were used for PCR amplification. Amplified fragment showed a single band of ~2.8kb as expected after PCR amplification. Plasmid DNA (pET32-TEV) were isolated from the transformed cells and were digested using restriction HindIII enzymes to authenticate the quality of plasmid DNA. Figure-3 agarose gel analysis of both amplified sequence of h*MSH2* gene and plasmid vector confirmed by obtained the expected band size of amplified h*MSH2* (~2.8kb) and plasmid vector size after restriction digestion (~5.9kb). These results are used for further experiments for cloning of h*MSH2* gene.

Construction of a Recombinant Plasmid Coding for the Full-length h*MSH2* gene

The clone coding for the full-length protein h*MSH2* was constructed as using amplified full cDNA sequence of h*MSH2* using in frame designed primers and plasmid DNA (pET32b) that was analysed previously for cloning purpose and confirmed by restriction digestion. The resulting PCR fragment was digested with BamHI and XhoI restriction enzymes and cloned into pET32b expression vector that also digested with the same enzymes to finally obtain the construct pET32b- h*MSH2* clone. In Figure-3 both the inserted gene and the selected vector digested with same restriction enzymes i.e. BamHI and XhoI. Double restriction digestion of insert DNA fragment and vector which produce compatible ends that can ligate each other. The quality of DNA inserts and expression vector was characterized by electrophoresis and bands appeared same size as expected. To ligate restrict digested fragments using T4 DNA ligase

enzyme with three different (Vector: Insert DNA) molar ratio for cloning of h*MSH2* into pET32b expression vector. Positive clones were obtained after transformation and colonies were randomly selected from each transformation. Recombinant clones were analyzed after isolation on 1.2% agarose gel. Restriction digestion analysis was performed following the single and double restriction digestion protocol using restriction enzymes.

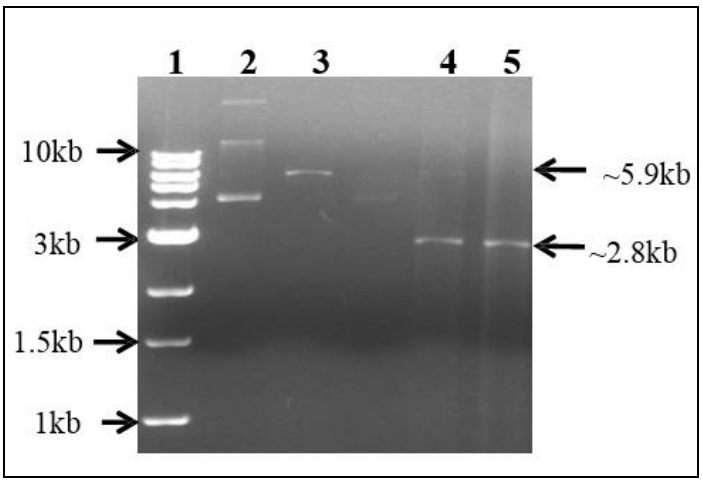

Fig 3: Restriction digested of insert DNA fragment and pET32b expression vector with BamHI and XhoI for cloning: Lane1-1kb Ladder, Lane2- Undigested pET32b, Lane3- Digested pET32b, Lane4-Undigested amplified h*MSH2* and Lane5-digested h*MSH2* were analyzed on gel electrophoresis, expected band size h*MSH2* (~2.8kb) and pET32b vector (~5.9kb).

Isolation and Restriction Analysis of recombinant clones (C-3)

Sixteen positive clones were isolated after transformation of ligation mix and after single digestion, clones were found to be positive clones. For confirmation the positive clones named as C-3 (pET32b-h*MSH2*) clone observed as a positive recombinant clone showing the expected band size after single digestion with MluI restriction enzyme approximately ~8.6kb (Figure-4A). Again, single restriction digestion of pET32b vector used as control and clone C-3 by using another restriction enzymes XbaI (Figure-4B). Expression vector (pET32b) was used as control to authenticate the quality of DNA, the expected band size for pET32b (~5.9kb) and Clone C-3 (~8.6kb) was observed (Figure-4B). Complete recombinant plasmid DNA (pET32b-h*MSH2*) was characterized by electrophoresis through 1.2% agarose gels, the band appeared as expected with the same size of constructed clone.

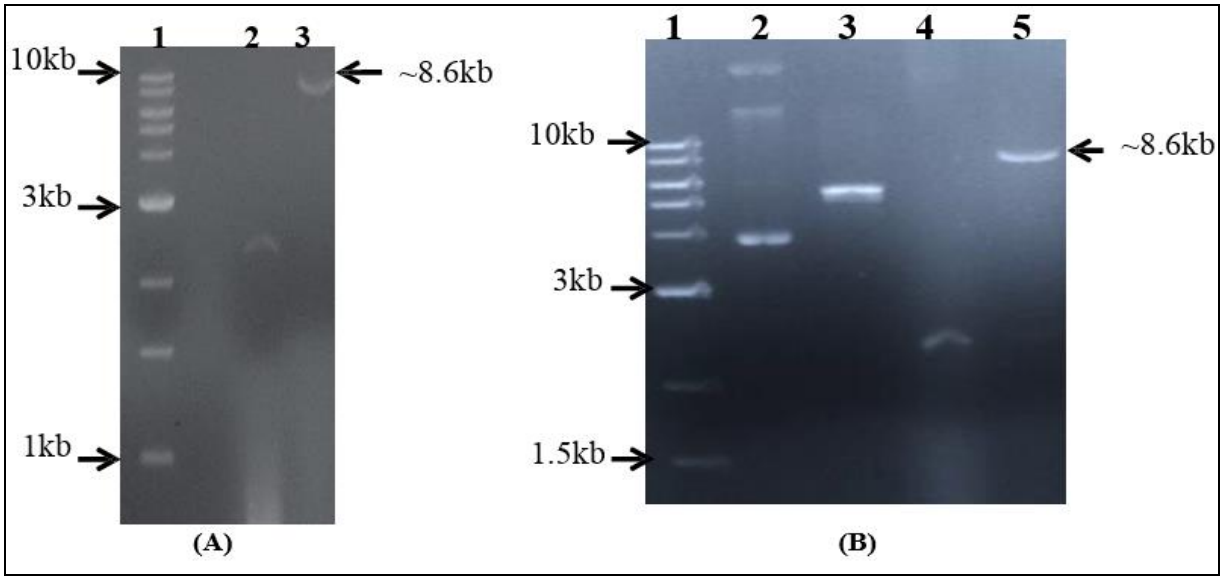

Fig 4: Restriction digestion of clone C-3 (pET32b-h*MSH2*) and pET32b vector with two different restriction enzymes. (A) Single restriction digestion C-3 (pET32b-h*MSH2*) using MluI restriction enzyme: Lane1 -1kb Ladder, Lane2- Undigested Clone(C-3) and Lane3- Digested Clone(C-3) with MluI. (B) Restriction digestion of pET32b vector (Control) and clone C-3 (pET32b-h*MSH2*) with XbaI restriction enzyme: Lane1 -1kb Ladder, Lane2 -Undigested pET32b, Lane3- pET32b digested with XbaI, Lane4- Undigested Clone(C-3), and Lane5-Clone (C-3) digested with XbaI, in this pET32b digestion used as control and approximately ~8.6kb band was observed after digestion.

Restriction Digestion Confirmation

Finally, double restriction digestion of clone C-3 for conformation of recombinant plasmid DNA with XhoI & KpnI restriction enzymes. Expected bands were obtained after double digestion of C-3 clone (~ 2.8kb and 5.8kb) (Figure-5). For confirmation of successful cloning, double

restriction of constructed clone C-3 done with BamHI and XhoI, for 2 h incubation at 37˚C. Figure- 5 agarose gel analysis showing the expected bands were obtained after double digestion of clone C-3 (~2.8kb and ~5.8kb) and confirmed that the clone was contain the complete sequence of h*MSH2* gene.

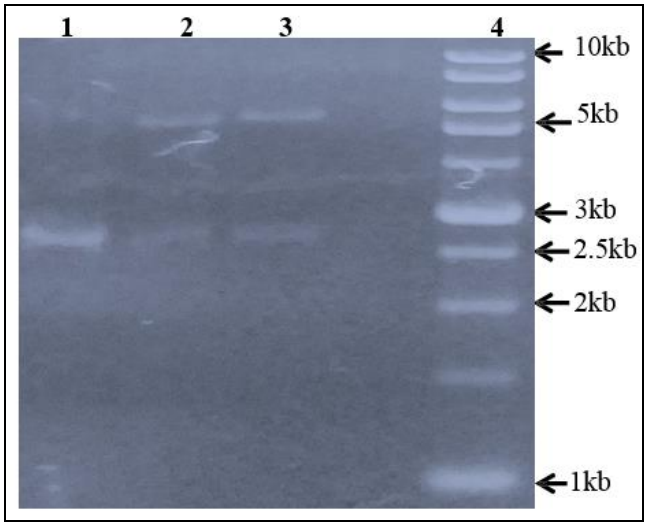

Fig 5: Double restriction enzyme digestion of clone C-3 (pET32b-h*MSH2*) for confirmation. Isolated C-3 clone positive recombinant digested with BamHI & XhoI and analyzed on 1.2% agarose gel: Lane1 - Undigested Clone(C-3), Lane2 - Clone (C-3) digested with XhoI and KpnI, Lane3-Clone (C-3) digested with BamHI and XhoI and Lane1 -1kb Ladder. Refer the text for detail.

Gene cloning or genetic cloning for a molecular biologist has much different consequence. Cloning a gene involves making several exact copies of a portion of a gene encoding DNA molecule. Cloned genes that have also been used to research the proteins they encode that were introduced into a bacterium may also guide the bacterium to generate its protein product. Since the bacteria are genetic code is similar with that of eukaryotes. This study reports the cloning of human MutS homologue, *MSH2* that seems to signify the MSH homologue. Use PCR dependent homology strategy among formerly established *E.coli* mutS gene homologues. Probes obtained for the cloning of Xenopus and mouse *Msh2* genes. Thus, these probes were used to clone cDNA of both species whole Msh2 genes [16]. A rat cDNA clone encoding the *Msh2* mismatch repair protein was isolated and characterized. The cDNA of *Msh2* contains open reading frame of 2802 nucleotides with length coding for protein having 933 amino acids (100kDa). It demonstrates strong homology to *MSH2* for humans and mouse [17]. In order to get several copies of gene of interest in vitro, molecular cloning based technique was used. There are many applications of gene cloning such as early detection of genetic disorders, for gene probes, forensic investigations and routine diagnosis. The industrial application of this technology develops new antibiotics which can be used as therapeutic agents in the form of antimicrobial peptides and recombinant cytokines. Cloning is commonly used for protein formation, such as industrial processing of recombinant antigens and cytokines. A number of advanced cloning vectors are available to empower the development of protein expression, tagging, single-stranded RNA and DNA. Recombinant DNA based technique is easy step in which specific DNA fragment was cloned or subcloned by inserting gene of interest into a specific target DNA region by replacing the related DNA fragments and constructing it simple, effective, enabling high throughput processes [18]. Genes connected with the DNA mismatch repair mechanism are thought to be possible candidates for involvement in the pathogenesis of human tumors. Thus, the identification of the human MutS homologue2 genes and their structural characterization now open the path for the investigations of the molecular mechanisms involved in this human MutS homologue and also allow the study of the functions.

Conclusion

The overall aim of the study was molecular cloning and confirmation of sequence of DNA mismatch repair gene h*MSH2* into pET32b expression system generated to understand the role of mismatch repair proteins during repair of DNA damages. In-frame primers have been designed to clone the gene sequences of h*MSH2* into pET expression system. Commercial cDNA clones to be used to amplify the gene sequences of *MSH2* using designed primers. Amplify the complete cDNA sequences of *MSH2* using designed primers from the commercial clones. Positive clones were confirmed after analysis of clones (pET32b-h*MSH2*) by obtaining the expected band size (~8.6kb). After confirmation of recombinant construct C-3 which encodes h*MSH2* proteins further used for expression of h*MSH2* gene for deciphering their role *in vitro* for resolving various DNA damages.

References

1. Alhmoud JF, Woolley JF, Al Moustafa AE, Malki MI. DNA Damage/Repair Management in Cancers. Cancers,2020:12:1050.
2. Yokoyama H, Sarai N, Kagawa W, Enomoto R, Shibata T, Kurumizaka H, *et al*. Preferential binding to branched DNA strands and strand-annealing activity of the human Rad51B, Rad51C, Rad51D and Xrcc2 protein complex. Nucleic Acids Research,2004:32:2556-2565.
3. Li GM. Mechanisms and functions of DNA mismatch repair. Cell Research,2008:18:85-98.
4. Kumari S, Rastogi RP, Singh KL, Singh SP, Sinha RP. DNA Damage: Detection strategies. Experimental and Clinical Sciences,2008:7:44-62.
5. Painter RB. DNA Damage and repair in Eukaryotic cells. Genetics,1974:18:139-1443.
6. Genschel J, Littman SJ, James T, Drummond JT, Modrich P. Isolation of MutSβ from Human Cells and Comparison of the Mismatch Repair Specificities of MutSβ and MutSα. The Journal of Biological Chemistry,1998:273:19895–19901.
7. Bellacosa A. Functional interactions and signaling properties of mammalian DNA mismatch repair proteins. Cell Death and Differentiation,2001:8:1076–1092.
8. Kunkel TA, Erie DA. DNA Mismatch Repair. Annual Review of Biochemistry,2005:74:681–710.

9. Shilpa V, Lakshmi K. Molecular Mechanisms of Mismatch Repair Genes in Cancer – A Brief Review. Journal of Proteomics and Genomics,2014:1:101-109.
10. Mitchell RJ, Farrington SM, Dunlop MG, Campbell H. Mismatch Repair Genes *hMLH1* and *hMSH2* and Colorectal Cancer: A HuGE Review. American Journal of Epidemiology,2002:156:885-902.
11. Darmanin SS, Neyton S, Lespinasse F, Gaudray AP, Flucklinger VP. The DNA mismatch-repair MLH3 protein interacts with MSH4 in meiotic cells, supporting a role for this MutL homolog in mammalian meiotic recombination. Human Molecular Genetics,2002:11:1697–1706.
12. Smith JA, Waldman BC, Waldman AS. A Role for DNA Mismatch Repair Protein Msh2 in Error-Prone Double-Strand-Break Repair in Mammalian Chromosomes. Genetics,2005:170:355–363.
13. Jun SH, Kim TG, Ban C. DNA mismatch repair system Classical and fresh roles. Federation of European Biochemical Societies,2006:273:1609–1619.
14. Varlet I, Pallard C, Random M, Moreau J, Wind ND. Cloning and expression of the Xenopus and mouse Msh2 DNA mismatch repair genes. Nucleic Acids Research,1994:22:5723-5728.
15. Guedes LB, Antonarakis ES, Schweizer MT, Mirkheshti N, Almutairi F, Park JC, *et al*. MSH2 Loss in Primary Prostate Cancer. Clinical Cancer Research,2017:23:6863-6874.
16. Rajesh C, Gruver AM, Basrur V, Pittman DL. The interaction profile of homologous recombination repair proteins RAD51C, RAD51D and XRCC2 as determined by proteomic analysis. Proteomics,2009:9:4071–4086.
17. Vani RJ, Rao MRS. Cloning of the cDNA encoding rat homologue of the mismatch repair gene MSH2 and its expression during spermatogenesis, An International Journal on Genes and Genomics,1997:185:19-26.
18. Sharma K, Mishra AK, Mehraj V, Duraisamy GS. Advances and applications of molecular cloning in clinical microbiology. Biotechnology and Genetic Engineering Reviews,2014:30:65-78.