AUCTORES
Globalize your Research
Journal of Clinical Surgery and Research
Fattma A. Ali *
Open Access
Review Article

The Influence of Restriction Endonucleases for Cloning

Fattma A. Ali [1]*, Ahmed Akil Khudhair Al-Daoody [1], Muna Muhammad Najeeb [1], Media Azeez Othman [2], Maryam Nihad Philip [3]

[1]Medical microbiology department /College of Health Sciences/ Hawler Medical University/ Kurdistan region /Erbil/Iraq.

[2]Midwifery department, Erbil technical medical institute, Erbil polytechnic university, Kurdistan region/Erbil/ Iraq.

[3]Catholic university in Erbil.

***Corresponding Author:** Fattma A. Ali., Medical microbiology department /College of Health Sciences/ Hawler Medical University/ Kurdistan region /Erbil/Iraq.

Received Date: 18 November, 2024 | **Accepted Date:** 16 December, 2024 | **Published Date:** 14 January, 2025

Citation: Fattma A. Ali, Khudhair Al-Daoody AA, Muna M. Najeeb, Media A. Othman, Maryam N. Philip, (2025), The Influence of Restriction Endonucleases for Cloning, *Journal of Clinical Surgery and Research*, 6(1); **DOI:**10.31579/2768-2757/156

Copyright: © 2025, Fattma A. Ali. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Abstract:

Background: Restriction//endonucleases, also referred to as restriction//enzymes, are enzymes that identify and cleave DNA at or in close proximity to certain DNA//sequences known as recognition//sites. Enzymes play a crucial role in molecular//biology by facilitating the manipulation of DNA, including tasks like cloning, gene//mapping, and DNA//sequencing.). There are four primary categories of restriction//enzymes, namely types I,//II,//III//and//IV. These categories vary in terms of enzyme composition, //cofactor//needs, and mode//of//action.

Objectives: Our study aimed to carry out the role of the restriction endonucleases in the molecular microbiology mainly in cloning.

Main body: Historically, restriction//endonucleases were obtained in a pure form from the organism they naturally occur in. The advancement of gene//cloning//vectors and selection procedures facilitated the cloning//of //REases. Cloning facilitated the creation of huge amounts of meticulously purified enzymes and enabled the manipulation of restriction//endonucleases Presently. Traditional//cloning. When used together with DNA//ligases, REases///enable a reliable process of cutting and pasting DNA, allowing for the transfer of a specific DNA//fragment from one organism to another. Stanley Cohen and his colleagues utilized this technology to introduce foreign//DNA into native plasmids, resulting in the creation of cloning-plasmid vectors that can self-replicate in E.coli.

Conclusion: restriction endonucleases have greatly transformed the field of molecular biology by enabling progress in recombinant//DNA//technology, gene cloning, DNA//fingerprinting, and gene mapping. An example frequently encountered in molecular biology is the utilization of restriction//enzymes in the plasmid employed for the manufacture of human insulin. Restriction enzymes in plasmid DNA manipulation work by identifying and cutting certain DNA//sequences, called restriction sites, in the plasmid//DNA. Plasmids are compact, ring-shaped//DNA molecules that are frequently employed as carriers in molecular biology for the transportation and duplication of foreign DNA pieces. Restriction enzymes identify and attach to particular palindromic DNA sequences, referred to as restriction sites, inside the plasmid// DNA. Upon binding, these enzymes hydrolyze the plasmid //DNA at or in close proximity to their specific recognition sites, resulting in the production of linear DNA//fragments with either cohesive or blunt ends.

keywords: restriction endonucleases; cloning; dna mapping; molecular biology; engineering

1.Introduction

Restriction endonucleases, also referred to as restriction enzymes, are enzymes that identify and cleave DNA at or in close proximity to certain DNA sequences known as recognition sites. Enzymes play a crucial role in molecular biology by facilitating the manipulation of DNA, including tasks like cloning, gene mapping, and DNA sequencing [1] . Scientists faced difficulties in isolating and studying individual genes until the 1970s. The initial breakthrough involved the identification of restriction enzymes and the DNA ligase enzyme. The identification of this crucial breakthrough, together with the elucidation of scientific methodologies, empowered scientists to utilize these techniques for the purpose of isolating specific genes from a genome. An important breakthrough in the discipline occurred with the creation of plasmid cloning vectors, which enabled the reception and replication of isolated segments of DNA. The creation of these instruments resulted in the release of the initial

recombinant DNA molecules in 1972.Genes are replicated by cloning techniques in order to facilitate the production of the proteins they carry, allowing for their abundant expression and subsequent purification. This is done for several purposes, including medical, experimental, and commercial uses [2]. Cloning in molecular biology is the act of creating several duplicates of a certain DNA sequence through the use of recombinant DNA technologies. The process entails the incorporation of a DNA segment of interest into a vector, such as a plasmid or a viral genome, and then amplifying it within a host cell, usually bacteria or yeast. Cloning allows for the separation, examination, and alteration of particular genes, which aids in a range of molecular biology tasks such as gene mapping, sequencing and functional analysis [3]. The elucidation of the mechanism by which restriction endonucleases, a group of bacterial enzymes, function, marked a significant advancement in the science of genetic engineering. Within a living organism, these enzymes participate in the recognition and fragmentation of foreign DNA that enters the cell. Therefore, their primary function is to safeguard the bacterium from phage infection. The relevant aspect for us is that these enzymes have the ability to identify specific DNA sequences. The enzymes utilized in DNA manipulation are specifically referred to as Class II restriction endonucleases. These enzymes cleave the DNA at a specific location within the recognition sequence. When DNA is treated with these enzymes, each molecule will be cut at the identical locations, resulting in the production of consistent pieces [4].Restriction endonucleases are a crucial part of restriction-modification (RM) systems, which primarily serve to safeguard bacterial cells against the intrusion of foreign DNA molecules [5]. There are 4 primary categories of restriction enzymes, namely types I, II, III and IV, as illustrated in table (1). These categories vary in terms of enzyme composition, cofactor needs, and mode of action [6].

TYPES AND ACTIVITIES OF RESTRICTION ENZYMES

Type	Activity
Type I	Cleaves DNA at random sites far from its recognition sequence
Type II	Cleaves DNA at defined positions close to or within its recognition sequence
Type IIG	Cleaves outside its recognition sequence with both REase and MTase enzymatic activities in the same protein
Type IIP	Cleaves symmetric targets and cleavage sites
Type IIS	Recognizes asymmetric sequences
Type III	Cleaves outside its recognition sequence and require two sequences in opposite orientations within the same DNA
Type IV	Cleaves modified (e.g., methylated) DNA

Table 1: types and activities of restriction enzymes.

The initial restriction endonucleases (REases) that were identified had the ability to recognize particular DNA sequences, but their cutting sites were varying distances away from the recognition sequence (referred to as Type I). Consequently, these enzymes were not very useful for DNA editing purposes. Following this, the identification and refinement of restriction endonucleases that could recognize and cleave specific DNA sequences (known as Type II REases) enabled scientists to carry out accurate modifications of DNA under laboratory conditions. This included tasks like inserting foreign genes into DNA and developing effective cloning tools. Currently, there are over 4,000 Restriction Endonucleases that have been identified. These enzymes are capable of detecting and binding to more than 300 different DNA sequences. The introduction of Polymerase Chain Reaction , real time-PCR, and PCR-based mutagenesis techniques revolutionized biological research in the subsequent decades. The type II representatives are the most extensively studied. They have a special ability to identify DNA sequences that are 4-8 base pairs long and can break the DNA either inside these sequences or in close proximity to them. Type II enzymes are extremely precise, making them essential for manipulating DNA [7]. Type II restriction endonucleases cleave specifically at the sites within their recognition sequences. Ligations of two strands cleaved by the same Type II enzyme are typically vulnerable to recognition and cleavage by the same enzyme. Therefore, it is necessary to remove that enzyme (via gel filtration or heat denaturation) before ligation takes place [8]. It is worth mentioning that there have been approximately 5000 restriction endonucleases identified so far, with nearly 4900 of them being type II enzymes [9] . Simultaneously, a total of 625 commercially accessible type II enzymes are capable of identifying only 239 distinct particular locations within DNA. Due to the limited number of specific sites available, continuous efforts are made to extensively search for new variations of restriction enzymes in natural sources and to artificially modify the specificity of known enzymes. While there are already hundreds of known DNA restriction enzymes , there is a need for enzymes that can recognize new DNA targets. Discovering new restriction enzymes that are highly specific for a unique nucleotide sequence at a "restriction site" and do not have nonspecific effects will increase the range of enzymes available for biotechnology. These new enzymes may also be able to replace existing ones and be more effective in their function [10].

2. Main body

2.1. Biotechnology of restriction enzymes (REases)

Historically, restriction endonucleases (REases) were obtained in a pure form from the organism they naturally occur in. The advancement of gene cloning vectors and selection procedures facilitated the cloning of

REases. Cloning facilitated the creation of huge amounts of meticulously purified enzymes and enabled the manipulation of restriction endonucleases Presently [10].

2.2. Enhancing Performance in Engineering

Some restriction endonucleases have been discovered to exhibit cleavage activity at sites that are not their intended target sites, which is referred to as "star activity". This phenomenon has been well-documented. Among these enzymes, certain ones demonstrate star activity when subjected to less-than-ideal reaction circumstances, whilst others have a limited range of enzyme units that may fully digest a specific amount of substrate without showing any star activity. Scientists at NEB conducted extensive research to develop restriction enzymes that demonstrate limited or no star activity when used over extended periods of time and at large concentrations of the enzyme [11].

2.3. Developing novel sequence specificities in engineering

Efforts to modify them sequence specificities of Type IIP restriction endonuclease has mostly ineffective, perhaps due to the structural integration of sequence specificity determinant with them active sites of these enzymes. MmeI is a Type IIG restriction endonuclease that possesses both methyltransferase and REase activities within the same polypeptide. It specifically detects the target sequence TCCRAC using the target recognition domain located within its methyltransferase component . This gave a great chance to manipulate the REase's sequence specificity. The sharing of target recognition domain between them REasen and methyltransferase activities has the additional benefit of causing an equal change in methyltransferase activity regardless of the specific target sequence cleavage ,This protects the new target site from being cleaved in recombinant host cells. By conducting bioinformatics analysis on protein sequences that are similar, researchers at NEB were able to identify the precise amino acid residues that can recognize particular bases in the target sequences. They then developed MmeI mutants that had modified specificities in terms of sequence recognition [12].

2.4. Engineering Nicking Endonucleases

Research focused on restriction endonucleases has uncovered unexpected discoveries regarding the apparently simple process of cleavage. Typically, Type IIP REases function as homo dimers, where each monomer cleaves one half of the palindromic site. On the contrary, Type IIS restriction endonucleases demonstrate a wide variety of ways for cutting both strands of DNA. These mechanisms include hetero dimerization, as seen in enzymes like BtsI and BbvCI as well as sequential cleavage of the double-stranded DNA as a single molecule, as seen in the case of FokI. The unique characteristics of these traits have been utilized to develop strand.-specific nicking enzymes (NEases) [13].

2.5. Applications involving the use of restriction enzymes include:

2.5.1. Traditional cloning.

When used together with DNA ligases, REases enable a reliable process of cutting and pasting DNA, allowing for the transfer of a specific DNA fragment from one organism to another (Figure 1). Stanley Cohen and his colleagues utilized this technology to introduce foreign DNA into native plasmids, resulting in the creation of cloning.-plasmid vectors that can self-replicate in E.coli. These vectors have become essential tools in modern biology, allowing for the cloning of DNA and the synthesis of recombinant proteins. Restriction enzymes serve as valuable instruments for post-cloning confirmation, ensuring the accurate occurrence of insertions. The conventional process of cloning, in conjunction with DNA amplification techniques like PCR and RT-PCR, has become a widely used method for studying molecular mechanisms, particularly in relation to restriction endonucleases [14].

Figure 1. Traditional Cloning Workflow

PCR is employed to introduce restriction sites at both ends of a double-stranded DNA molecule. Subsequently, the DNA molecule is cleaved by the matching restriction enzymes. The fragmented DNA can subsequently be joined to a plasmid vector that has been fragmented by the same or compatible restriction endonucleases using T4 DNA ligase. DNA fragments can be transferred across vectors by digesting them using restriction endonucleases and then ligating them to the compatible ends of the target vector.

2.5.2. DNA Mapping

Daniel Nathans, armed with a limited number of restriction endonucleases in the earlym1970s, successfully identified the functional units of SV40 DNA. This marked the beginning of the era of "restriction mapping" and the comparison of complicated genomes. Over time, it has developed into advanced techniques that enable the identification of individual variations in DNA sequences and the presence of genetic mutations. These methods are used for various purposes such as identifying locations associated with genetic disorders, evaluating the genetic variability within populations, and conducting parental testing [15].

2.5.3. Understanding Epigenetic Modifications

The heightened responsiveness of REases to the methylation state of certain bases has been utilized to accurately locate changed bases in genomes. Restriction Landmark Genome Scanning is a mapping approach that uses 2-dimensional gel electrophoresis and specific enzymes (NotI, AscI, EagI, or BssHII) to analyze alterations in the methylation patterns of the genome in normal and cancer cells. Methylation.-Sensitive Amplification is a technique used to amplify DNA fragments that are sensitive to methylation. Polymorphism is utilized by using the varying sensitivity of MspI and HpaII enzymes towards the methylation status of the second C in the sequence CCGG. This allows for the identification of 5-methylcytosine or 5-hydroxymethylcytosine[16]. Furthermore, the recently discovered restriction enzymes (REases) MspJI, FspEI, and LpnPI have the ability to recognize and cleave DNA at specific sites containing 5-methylcytosine and 5-hydroxymethylcytosine. Additionally, there are enzymes such as PvuRts1I and AbaSI that have a preference for cleaving 5-hydroxymethylcytosine or 5-ghmC rather than 5-methylcytosine or C. These enzymes [17], have the potential to be used for efficiently mapping cytosine-based epigenetic markers in genomes that have undergone cytosine methylation [18].

2.5.4. In vitro DNA Assembly Technologies

Synthetic biology is an expanding discipline that utilizes specific elements to construct biological systems for the purpose of investigating biological processes and developing practical biological devices. Novel technologies like BioBrick™ were first developed to simplify the construction of biological systems. In recent times, the synthetic biology community has extensively embraced more resilient methods, such as Golden Gate. Assembly and Gibson Assembly™. Both methods enable the simultaneous and smooth joining of numerous DNA fragments

without the need for unconventional bases [19]. The BioBricks group aimed to develop a large collection of "standardized parts" of DNA molecules to facilitate quick gene assembly. The biennial International Genetically Engineered Machines competition fostered the growth of the BioBricks community and generated significant interest among university students in the field of synthetic biology. Utilizing the conventional REase-ligation technique. However, they do result in the presence of scar sequences at the junctions. In order to develop a functional biological system, it is necessary to undergo numerous cloning cycles [20].

2.5.5. Golden Gate Assembly

Golden Gate Assembly and its derivative technologies utilize the capacity of Type IIS restriction endonucleases to cleave DNA outside of the recognition sequence . The inserts and cloning vectors are specifically designed to position the Type IIS recognition site away from the cleavage site, allowing the Type IIS REase to eliminate the recognition sequence from the assembly (Figure 2). There are three advantages to such an arrangement:

1. The sequence that extends beyond the restriction enzyme recognition site is not determined by the restriction enzyme, and as a result, no more sequence is added.

2.The precise sequencing of the overhangs enables the systematic assembly of numerous fragments at the same time.

3. The ligated product undergoes elimination of the restriction site, allowing for simultaneous digestion and ligation [21].

The ultimate outcome is the organized and uninterrupted combination of DNA fragments in a single reaction. The precision of the assembly relies on the length of the overhang sequences .Hence, Type IIS restriction endonucleases that generate four-bases overhangs (such as BsaI/BsaI-HF, BbsI, BsmBI, and Esp3I) are favored. A limitation of these Type IIS REase-based approaches is that the presence of a limited number of overhanging bases can result in the incorrect joining of fragments that have comparable overhang sequences [22] . Additionally, it is crucial to confirm that Type IIS REase sites utilized are absent in fragments used for assembling the intended result. However, Golden Gate Assembly is a reliable and efficient approach that produces several targeted alterations [23] and combines various DNA fragments [24] . With the growing availability of open source methodologies and reagents, the use of Golden Gate Assembly has become widespread in the creation of custom-specific TALENs for in vivo gene editing, among other applications [25].

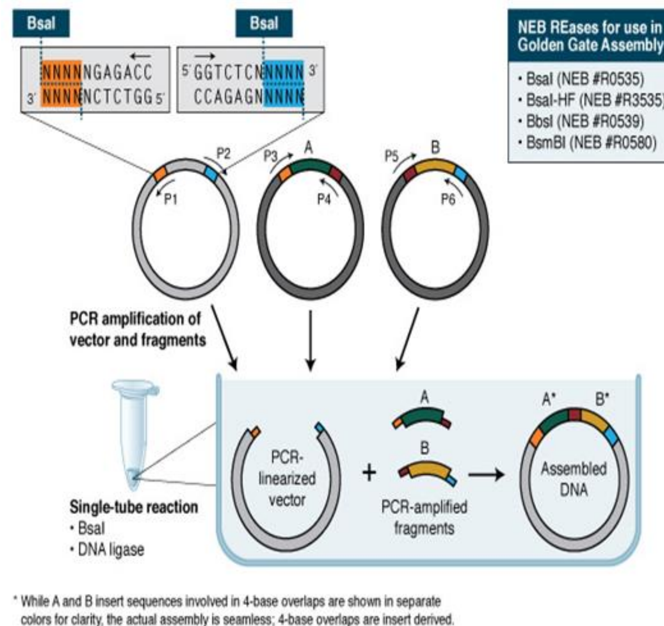

Figure 2: Golden Gate Assembly Workflow.

Golden Gate Assembly involves the addition of a BsaI recognition site (GGTCTC) to both ends of a double-stranded DNA fragment located away from the cleavage site. This allows for the removal of the BsaI site by digesting it with BsaI or BsaI-HF (GGTCTC 1/5). After being split, the protruding sequences of the adjacent fragments bind together. DNA ligase then closes the gaps in order to generate a novel DNA molecule that is chemically bonded. It is possible to concurrently cut and ligate multiple sections of DNA.

2.5.6. Gibson Assembly

Daniel G. Gibson, from the J. Craig Venter Institute, explained a reliable technique that uses exonucleases to assemble DNA without any gaps and in the proper sequence. The reaction is performed at a constant temperature utilizing three enzymatic processes: a 5' exonuclease creates extended ends, a polymerase fills in the gaps of the single-stranded areas that have been joined together, and a DNA ligase closes the gaps that have been joined and filled in (Figure 3). The process involved assembling the mouse mitochondrial genome, which has a size of 16.3 kb, using 600 overlapping 60-mers [26]. Gibson Assembly was employed alongside in vivo assembly in yeast to synthesize the 1.1 Mbp Mycoplasma mycoides genome. A recipient cell of M. capricolum was created by transplanting the synthesized genome, resulting in the production of new self-replicating M. mycoides cells. Gibson Assembly can also be employed for cloning purposes. This involves combining a DNA insert with a vector that has been digested by restriction enzymes, followed by a process called transformation. Using the Gibson Assembly Cloning Kit, this entire procedure can be accomplished in just under two hours. Gibson Assembly has various other uses, such as facilitating the incorporation of numerous

mutations, constructing plasmid vectors using chemically genera oligonucleotides, and generating libraries of genes and pathways with combinatorial diversity [27].

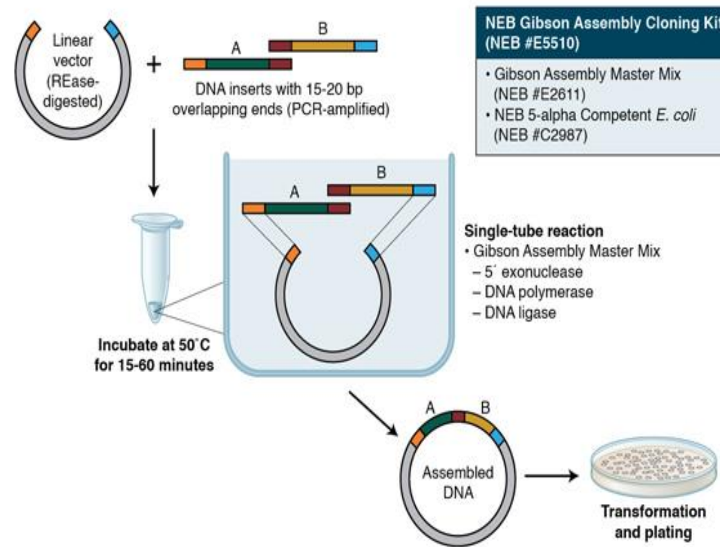

Figure 3: Gibson Assembly Workflow.

Gibson Assembly utilizes a combination of three enzymatic activities in a single-tube reaction: 5´mexonuclease, the 3´ extension activity of a DNA polymerase, and DNA ligase activity. The 5´exonucleasemactivity removes nucleotides off the 5´ end of the sequence, revealing the complementary sequence for annealing. The polymerase activity subsequently completes the missing sections on the areas that have been annealed. Following the nick, a DNA ligase enzymatically plugs the gap and forms covalent bonds between the DNA fragments, effectively joining them together. The length of the overlapping sequence of adjacent fragments is significantly greater than that employed in Golden Gate Assembly, leading to a higher proportion of accurate assemblies. The NEB Gibson Assembly Master Mix and Gibson Assembly Cloning Kit facilitate fast assembly at a temperature of 50˚C.

2.5.7. Construction of DNA Libraries

The technique of Serial Analysis of Gene Expression has facilitated the detection and measurement of a substantial quantity of RNA transcripts. It has been extensively utilized in cancer research to detect mutations and investigate gene expression. Restriction enzymes play a crucial role in the Serial Analysis of Gene Expression procedure. NlaIII serves as a crucial anchoring enzyme due to its distinctive ability to identify a four-bp sequence CATG and generate a four nucleotide overhang with the same sequence. Type IIS enzymes, such as FokI, BsmFI, MmeI, and EcoP15I, are used as tagging enzymes in Serial Analysis of Gene Expression analyses. These enzymes cleave DNA sequences that are located further away from the recognition sequence. This allows for a higher amount of information to be obtained in SAGE analyses. Examples of SAGE studies that have utilized Type IIS enzymes include [28] for FokI and BsmFI [29] for MmeI [30] for EcoP15I for Deep Serial Analysis of Gene Expression [31] .

Chromosome conformation capture (3C) and related techniques enable the precise mapping of the spatial arrangements of genomes with exceptional resolution and efficiency. REases are essential for generating compatible DNA ends that are cross-linked to their interacting proteins. This allows for the ligation and identification of spatially related sequences by high-throughput sequencing. Despite the fact that restriction endonucleases cannot facilitate the random fragmentation of DNA, they are being utilized in innovative target enrichment techniques such as hairpin adaptor ligation and HaloPlex™ enrichment (Agilent) [32]. The long-reach REase, AcuI, and USER™ Enzyme are utilized for the purpose of incorporating tags into sample DNA. This DNA is subsequently amplified through rolling circle amplification, resulting in the formation of long, single-stranded DNA "nanoballs". These nanoballs serve as templates in the high density, chip-based sequencing-by-ligation methodology, which was developed by Complete Genomics [33]. The DNA library for genotyping-by-sequencing technique in the investigation of maize sequence diversity was generated using ApeKI [34].

2.5.8. Creation of Nicks in DNA

Prior to the availability of NEases, non-hydrolyzable phosphorothioate groups were added to a particular strand of the target DNA. This allowed REases to create specific nicks in the DNA, based on the sequence and strand. These nicks were used in applications like strand displacement amplification, where a DNA polymerase (such as Bstm2.0 DNA Polymerase) extends from the newly formed 3'-hydroxyl end and replicates the complementary sequence [35]. Repeated rounds of nicking and extension amplify specific single-stranded portions of the sample DNA without requiring thermocycling, as the nicking site is regenerated. Nucleases significantly simplify the process of such applications and enable the development of applications that cannot be accomplished by restriction endonucleases. The utilization of nicking enzyme-based isothermal DNA amplification technologies, such as RCA, NESA, EXPAR and other comparable amplification systems, has demonstrated the ability to detect extremely low quantities of DNA [36]. The technique of nicking-based DNA amplification has been integrated in to molecular beacon technologies in order to enhance the signal amplification [37]. Implementing these sample and or signal amplification approaches can result in straightforward yet very sensitive and specific methods for

detecting target DNA molecules in the field (NEAR, EnviroLogix™). The process involves attaching adaptors with nicking sites to the ends of blunt-ended DNA. This allows for the rapid amplification of a specific region of double-stranded DNA by the combined actions of NEases and strand-displacing DNA polymerase [38] .Nicking-extension cycling amplification is capable of multiplexing and has the potential to attain greater fidelity than PCR. The collective action of NEases and Bst DNA polymerase has been utilized to include specific fluorescent markers into long chromosomal DNA in vitro for the purpose of viewing (nanocoding) [39] . The utilization of nicking enzymes in novel ways includes the production of reporter plasmids with altered bases or structures and the development of a DNA motor that delivers DNA payload without the need for additional energy [40].

2.5.9. In vivo Gene Editing

The capacity to manipulate DNA using restriction endonucleases in a laboratory setting has naturally prompted efforts to achieve the same process within living organisms, with the aim of rectifying genetic mutations responsible for causing hereditary disorders. The utilization of restriction endonucleases and homing endonucleases in Restriction Enzyme Mediated Integration (REMI) enabled the creation of transgenic embryos in higher organisms [41]. Unfortunately, there is no regulation or supervision of the integration site. Zinc Finger Nucleases and Transcription Activator-like Effector Nucleases have been successfully used to edit genes by creating precise double stranded breaks in complicated genomes. The success of gene editing in model organisms and livestock has led to the testing of its therapeutic potential in Phase I/II clinical trials. These trials involve the use of Zinc Finger Nucleases to improve T helper cells counts by suppressing the expression of the CCR5 gene in autologous T-cells from HIV patients. The clinical trial with this approach has been registered under the identifier NCT00842634 on ClinicalTrials.gov. The latest studies on CRISPR, the adaptive defense mechanism found in bacteria and archaea, have shown the capabilities of the Cas9-crRNA complex as programmable RNA-guided DNA endonucleases and strand-specific nicking endonucleases for gene editing within living organisms [42].

2.6. Moving forward

Restriction enzymes have played a significant role in facilitating gene cloning and revolutionizing the field of molecular biology. Emerging technologies like Golden Gate Assembly and Gibson Assembly are continuously advancing our capacity to generate novel DNA molecules. The ability to establish new recognition specificity in the/ MmeI family restriction endonucleases , the development of additional non-specific endonucleases and the identification of more modification-specific REases, are continuously leading to the creation of novel tools for DNA manipulation and study of the epigenome. The utilization of these enzymes in novel ways will expand the role of REases beyond molecular cloning, further expediting the advancement of biotechnology and introducing us to fresh prospects and obstacles [10].

2.7. The commercialization and availability of restriction enzymes

The commercialization and accessibility of restriction enzymes have had a substantial impact on the progress of molecular biology research. Due to the growing need for these enzymes in different fields, numerous businesses have successfully brought a diverse array of restriction enzymes to the market, offering researchers easy access to top-notch reagents. The commercialization of restriction enzymes commenced during the initial stages of molecular biology research, as corporations identified the need for these indispensable instruments. Currently, there is a diverse selection of restriction enzymes that can be purchased from several providers, including New England Biolabs, Thermo Fisher Scientific, and Promega Corporation. The companies New England Biolabs, Thermo Fisher Scientific, and Promega Corporation provide a diverse range of restriction enzymes. These enzymes have distinct recognition sequences, cleavage patterns, and optimal reaction conditions. As a result, researchers have access to a comprehensive set of tools for manipulating and analyzing DNA [1].

3.Conclusion

In conclusion restriction endonucleases, also known as restriction enzymes, are fundamental instruments in the field of molecular biology, serving as essential agents in the manipulation, analysis and cloning of DNA. These enzymes have the ability to identify and cut DNA at particular nucleotide sequences, allowing for accurate manipulation of DNA fragmentation and recombination. Since being discovered in the late 1960s, restriction endonucleases have greatly transformed the field of molecular biology by enabling progress in recombinant DNA technology, gene cloning, DNA fingerprinting, and gene mapping. An example frequently encountered in molecular biology is the utilization of restriction enzymes in the plasmid employed for the manufacture of human insulin. Restriction enzymes in plasmid DNA manipulation work by identifying and cutting certain DNA sequences, called restriction sites, in the plasmid DNA. Plasmids are compact, ring-shaped DNA molecules that are frequently employed as carriers in molecular biology for the transportation and duplication of foreign DNA pieces. Restriction enzymes identify and attach to particular palindromic DNA sequences, referred to as restriction sites, inside the plasmid DNA. Upon binding, these enzymes hydrolyze the plasmid DNA at or in close proximity to their specific recognition sites, resulting in the production of linear DNA fragments with either cohesive or blunt ends. Subsequently, the linearized plasmid DNA can be merged with a DNA fragment of interest, and then joined together using DNA ligase, resulting in the formation of a recombinant plasmid. The recombinant plasmid can be placed into a host organism, such as bacteria, where it can duplicate and produce the inserted DNA fragment. Restriction enzymes possess a high degree of specificity and precision when it comes to cutting DNA sequences. This characteristic allows for accurate manipulation and cloning of DNA fragments within plasmid vectors. As a result, different applications in molecular biology, such as gene cloning, gene expression research, and functional genomics, are made possible. The broad use and importance of restriction enzymes in research laboratories worldwide is further emphasized by the fact that they are commercially available from a variety of sources. Restriction endonucleases are essential tools in molecular biology that play a crucial role in driving innovation and making discoveries in biological research. They contribute significantly to our understanding of genetics, disease mechanisms, and the creation of new therapeutic approaches.

4. References

1. Roberts, R. J., Vincze, T., Posfai, J., & Macelis, D. (2015). "REBASE—enzymes and genes for DNA restriction and modification". *Nucleic Acids Research*, 43(Database issue), D298–D299).

2. Watson JF, Garcia-Nafria J. (2019). In vivo DNA assembly using common laboratory bacteria: a re-emerging tool to simplify molecular cloning. *J. Biol. Chem.* 294(42), 15271–15281.
3. Sambrook, J., & Russell, D. W. (2010). "Molecular Cloning: A Laboratory Manual". *Cold Spring Harbor Laboratory* Press).
4. Gingold, E.B. (2015). The use of restriction endonucleases. *Methods in Molecular Biology* (Clifton, N.J.), [online] 2, pp.217–223.
5. Wilson, G.G.; Murray, N.E. (2010). Restriction and Modification Systems. *Annu. Rev. Genet*. 25, 585–627.
6. Roberts, R.J. (2013). A Nomenclature for Restriction Enzymes, DNA Methyltransferases, Homing Endonucleases and Their Genes. *Nucleic Acids Res*. 31, 1805–1812.
7. Thielking, V.; Alves, J.; Fliess, A.; Maass, G.; Pingoud, A. (2010). Accuracy of the EcoRI Restriction Endonuclease: Binding and Cleavage Studies with Oligodeoxynucleotide Substrates Containing Degenerate Recognition Sequences. *Biochemistry*. 29, 4682–4691.
8. Matsumura, I. (2022). Golden Gate assembly of BioBrick-compliant parts using Type II restriction endonucleases. *BioTechniques*.
9. Roberts, R.J.; Vincze, T.; Posfai, J.; Macelis, D. (2023). REBASE: A Database for DNA Restriction and Modification: Enzymes, Genes and Genomes. *Nucleic Acids Res*. 51, D629–D630.
10. Jinek, M., Chylinski, K., Fonfara, I., Hauer, M., Doudna, J.A. and Charpentier, E.(2012). A programmable dual-RNA–guided DNA endonuclease in adaptive bacterial immunity. *science*, 337(6096), pp.816-821.
11. Wei, H., Therrien, C., Blanchard, A., Guan, S. and Zhu, Z.(2010). The Fidelity Index provides a systematic quantitation of star activity of DNA restriction endonucleases. *Nucleic acids research*, 36(9), pp.e50-e50.
12. Morgan, R.D. and Luyten, Y.A.(2009). Rational engineering of type II restriction endonuclease DNA binding and cleavage specificity. *Nucleic acids research*, 37(15), pp.5222-5233.
13. Chan, S.H., Stoddard, B.L. and Xu, S.Y.(2011). Natural and engineered nicking endonucleases—from cleavage mechanism to engineering of strand-specificity. *Nucleic acids research*, 39(1), pp.1-18.
14. Cohen, S.N.(2013). DNA cloning: a personal view after 40 years. *Proceedings of the National Academy of Sciences*, 110(39), pp.15521-15529.
15. Kudva, I.T., Griffin, R.W., Murray, M., John, M., Perna, N.T., et al., (2010). Insertions, deletions, and single-nucleotide polymorphisms at rare restriction enzyme sites enhance discriminatory power of polymorphic amplified typing sequences, a novel strain typing system for Escherichia coli O157: H7. *Journal of Clinical Microbiology*, 42(6), pp.2388-2397.
16. Mastan, S.G., Rathore, M.S., Bhatt, V.D., Yadav, P. and Chikara, J.(2012). Assessment of changes in DNA methylation by methylation-sensitive amplification polymorphism in Jatropha curcas L. subjected to salinity stress. *Gene*, 508(1), pp.125-129.
17. Zhu, Z., Zheng, Y., Guan, S., Wang, H., Quimby, A., et al. (2015). New England Biolabs Inc. Compositions, methods and related uses for cleaving modified DNA. *U.S. Patent* 8,969,061.
18. Cohen-Karni, D., Xu, D., Apone, L., Fomenkov, A., Sun, Z., et al. (2011). The MspJI family of modification-dependent restriction endonucleases for epigenetic studies. *Proceedings of the National Academy of Sciences*, 108(27), pp.11040-11045.
19. Ellis, T., Adie, T. and Baldwin, G.S. (2011). DNA assembly for synthetic biology: from parts to pathways and beyond. *Integrative Biology*, 3(2), pp.109-118.
20. Anderson, J., Dueber, J.E., Leguia, M., Wu, G.C., Goler, J.A., et al. (2010). BglBricks: A flexible standard for biological part assembly. *Journal of biological engineering*, 4, pp.1-12.
21. Nour-Eldin, H.H., Geu-Flores, F. and Halkier, B.A. (2010). USER cloning and USER fusion: the ideal cloning techniques for small and big laboratories. Plant secondary metabolism engineering: methods and applications, pp.185-200.
22. Sarrion-Perdigones, A., Falconi, E.E., Zandalinas, S.I., Juárez, P., Fernández-del-Carmen, A., et al. (2011). GoldenBraid: an iterative cloning system for standardized assembly of reusable genetic modules. *PloS one*, 6(7), p.e21622.
23. Engler, C., Gruetzner, R., Kandzia, R. and Marillonnet, S. (2009). Golden gate shuffling: a one-pot DNA shuffling method based on type IIs restriction enzymes. PloS one, 4(5), p.e5553.
24. Yan, P., Gao, X., Shen, W., Zhou, P. and Duan, J. (2012). Parallel assembly for multiple site-directed mutagenesis of plasmids. *Analytical biochemistry*, 430(1), pp.65-67.
25. Werner, S., Engler, C., Weber, E., Gruetzner, R. and Marillonnet, S. (2012). Fast track assembly of multigene constructs using Golden Gate cloning and the MoClo system. *Bioengineered*, 3(1), pp.38-43.
26. Sanjana, N.E., Cong, L., Zhou, Y., Cunniff, M.M., Feng, G. and Zhang, F. (2012). A transcription activator-like effector toolbox for genome engineering. *Nature protocols*, 7(1), pp.171-192.
27. Gibson, D.G., Glass, J.I., Lartigue, C., Noskov, V.N., Chuang, R.Y., et al. (2009). Creation of a bacterial cell controlled by a chemically synthesized genome. *science*, 329(5987), pp.52-56.
28. Gibson, D.G., Young, L., Chuang, R.Y., Venter, J.C., Hutchison, C.A. et al. (2010). Enzymatic assembly of DNA molecules up to several hundred kilobases. *Nature methods*, 6(5), pp.343-345.
29. Høgh, A.L. and Nielsen, K.L. (2010). SAGE and LongSAGE. *Serial Analysis of Gene Expression (SAGE) Methods and Protocols*, pp.3-24.
30. Matsumura, H., Reuter, M., Krüger, D.H., Winter, P., Kahl, G. et al. (2010). SuperSAGE. In Serial Analysis of Gene Expression (SAGE) Methods and Protocols (pp. 55-70). Totowa, NJ: Humana Press.
31. Nielsen, K.L. (2010). DeepSAGE: higher sensitivity and multiplexing of samples using a simpler experimental protocol. *Serial Analysis of Gene Expression (SAGE) Methods and Protocols*, pp.81-93.
32. Singh, P., Nayak, R. and Kwon, Y.M. (2011). Target-enrichment through amplification of hairpin-ligated universal targets for next-generation sequencing analysis. *High-Throughput Next Generation Sequencing: Methods and Applications*, pp.267-278.

33. Drmanac, R., Sparks, A.B., Callow, M.J., Halpern, A.L., Burns, N.L., et al. (2010). Human genome sequencing using unchained base reads on self-assembling DNA nanoarrays. *Science*, 327(5961), pp.78-81.
34. Elshire, R.J., Glaubitz, J.C., Sun, Q., Poland, J.A., Kawamoto, K., et al. (2011). A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. *PloS one*, 6(5), p.e19379.
35. Dawson, E.D., Taylor, A.W., Smagala, J.A. and Rowlen, K.L. (2010). Molecular detection of Streptococcus pyogenes and Streptococcus dysgalactiae subsp. equisimilis. *Molecular biotechnology*, 42, pp.117-127.
36. Murakami, T., Sumaoka, J. and Komiyama, M. (2009). Sensitive isothermal detection of nucleic-acid sequence by primer generation–rolling circle amplification. *Nucleic acids research*, 37(3), pp.e19-e19.
37. Joneja, A. and Huang, X. (2011). Linear nicking endonuclease-mediated strand-displacement DNA amplification. *Analytical biochemistry*, 414(1), pp.58-69.
38. Zhang, P., Too, P.H.M., Samuelson, J.C., Chan, S.H., Vincze, T., et al. (2010). Engineering BspQI nicking enzymes and application of N. BspQI in DNA labeling and production of single-strand DNA. *Protein expression and purification*, 69(2), pp.226-234.
39. Lühnsdorf, B., Kitsera, N., Warken, D., Lingg, T., Epe, B. and Khobta, A. (2012). Generation of reporter plasmids containing defined base modifications in the DNA strand of choice. *Analytical biochemistry*, 425(1), pp.47-53.
40. Bath, J., Green, S. and Turberfield, A. (2010). A free-running DNA motor powered by a nicking enzyme. *Angewandte Chemie (International ed. in English)*, 44(28).
41. Ishibashi, S., Love, N.R. and Amaya, E. (2012). A simple method of transgenesis using I-sce I meganuclease in Xenopus. *Xenopus Protocols: Post-Genomic Approaches*, pp.205-218.
42. Joung, J.K. and Sander, J.D. (2013). TALENs: a widely applicable technology for targeted genome editing. *Nature reviews Molecular cell biology*, 14(1), pp.49-55.
43. Gasiunas, G., Barrangou, R., Horvath, P. and Siksnys, V. (2012). Cas9–crRNA ribonucleoprotein complex mediates specific DNA cleavage for adaptive immunity in bacteria. *Proceedings of the National Academy of Sciences*, 109(39), pp.E2579-E2586.

This work is licensed under Creative Commons Attribution 4.0 License

To Submit Your Article Click Here: **Submit Manuscript**

DOI:10.31579/2768-2757/156

Ready to submit your research? Choose Auctores and benefit from:

- fast, convenient online submission
- rigorous peer review by experienced research in your field
- rapid publication on acceptance
- authors retain copyrights
- unique DOI for all articles
- immediate, unrestricted online access

At Auctores, research is always in progress.

Learn more https://www.auctoresonline.org/journals/journal-of-clinical-surgery-and-research