



## Cloning of DNA: A Review

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### ABSTRACT

**Objective:** The advantage of molecular cloning show similarity of the chemical structure of DNA in all living creatures is fundamental as the main fact. As a result, in case any DNA fragment from any creatures is introduced into a DNA sequence essential for DNA replication, it will cause DNA replication, the foreign DNA will be reproduced alongside the host cell's DNA in the transgenic organism when the recombinant DNA is injected to the creatures to derive the replication sequences. In that, it allows the replication of DNA sequences to be comparable to a polymerase chain reaction (PCR) with molecular cloning. The primary distinction amidst two approximations is that DNA replication is one step of molecular cloning in a living microorganism, whereas *in vitro* DNA replicates in living cells PCR by PCR.

**Conclusion:** Individual DNA fragments extracted by scientists through molecular cloning infinite number of from any genome. This substance can exist utilized in applied and basic applications in biological science.

## INTRODUCTION

Molecular cloning is a set of molecular biology experimental methods for assembling recombinant DNA molecules and directing their replication in host organisms.<sup>1</sup>

Cloning refers to a technique that involves the reproduction of a single molecule to produce a population of cells with identical DNA molecules. In most cases, DNA sequences from two separate organisms are used in molecular cloning: the source of the cloned DNA and the species that will serve as the living host for recombinant DNA replication. Many aspects of modern biology and medicine rely on molecular cloning techniques.<sup>2</sup>

Molecular cloning procedure traditionally, target organism necessary present to DNA cloned and it enzymatically processed produce in the test tube to mini-DNA fragments. After that, DNA vector mixed with these pieces to make

recombinant molecules of DNA. After that, DNA recombinant molecule is implanted (*Escherichia coli* bacteria typically grow generous as laboratory strain). As a result, a colony of creatures reproduce DNA recombinant molecules alongside host DNA. These are transgenic or genetically modified microorganisms (GMOs) because they contain foreign DNA pieces.<sup>3</sup> The bacterial cell produce fact that can encouraged to take in and propagate a single DNA molecule recombinant is used in cloning technique. Cloning cell can produce many bacteria by multiplied indefinitely, copy of the original recombinant molecule each cell containing. As a result, together the recombinant DNA molecule and ensuing bacterial population are usually referred to as "clones." Recombinant DNA, strictly speaking, refers to DNA molecules, whereas molecular cloning refers to the experimental methods used to put them together. Plasmid will be content different DNA sequences, and bacteria would be taken alien sequences and digested it, according to

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the theory. In other words, plasmids can be used as cloning vector for transferring gene.<sup>4</sup>

Although practically any DNA segment may be clone and amplify, various factors may limit the procedure's success. Inverted repeats, replication origins, centromeres, and telomeres are examples of difficult-to-clone DNA sequences. DNA sequences when introduced big-sized, there is also a decreased possibility of success. Insertions more significant than 10 kbp have a low success rate, yet bacteriophages like bacteriophage can be engineered to effectively insert sequences up to 40 kbp.<sup>5</sup>

## History

The laws of Gregor Mendel explained the inheritance of biological characters in the mid-nineteenth century. The criteria are based on the premise that every heritable feature of an organism is governed through a component known as a gene, a biological molecule found someplace in the cell. Mendel's laws rediscovery in 1900 marked the beginning of genetics, the study dedicated to figuring out what genes are and how they function.<sup>6</sup>

This new science grew at an incredible rate over the first 30 years of its existence. W. Sutton advanced the hypothesis that genes existed on chromosomes in 1903, and T.H. Morgan supported it experimentally in 1910. Morgan and his colleagues then developed gene mapping techniques, and by 1922, they had completed a comprehensive investigation of the relative placements of nearly 2000 genes on *Drosophila melanogaster*'s four chromosomes.<sup>7</sup>

Despite the brilliance of this basic genetic research, the molecular nature of the gene was not fully understood until the 1940s. Indeed, no one believed that deoxyribonucleic acid (DNA) constituted the genetic material until the investigations of Avery, MacLeod, and McCarty in 1944 and Hershey and Chase in 1952; previously, it was commonly assumed that genes will translate to the protein. The discovery of the DNA importance in genetic research sparked a second great age of genetics, with many notable biologists (Chargaff, Crick, Delbrück, and Monod among the most effective) contributing to it. Between 1952 and 1966, DNA structure was deciphered, the genetic code deciphered, and the transcription and translation mechanisms were deciphered.<sup>8,9</sup>

## Steps

For any DNA fragment cloning in typical molecular cloning experiments entails VII steps: {1} Selecting host creatures and cloning vector, {2} Creation the recombinant DNA, {3} DNA clone will be producing, {4} Recombinant DNA creating, {5} DNA recombinant Introducing to the host organism, {6} Organisms harboring recombinant DNA is selection, {7} Desired DNA inserts clones screening and biological attributes.

Specific software exists for the objective, despite fact that extensive cloning planning could be achieved in any text redactor, with PCR primer creation internet utilities together, for example. {1} (open source), DNA Strider {2} ApE {3} (gratis),

and Collagens (open source), Serial Cloner are many examples of software that could be used for this purpose. Clearly, increasing capacity and fidelity synthesis platforms of DNA enables more complex molecular engineering designs. These initiatives may include testing entire libraries rather than individual sequences and comprise a very lengthy sequence of new DNA strands. These changes create complexity, necessitating a movement away from nucleotide-based impersonation and toward a more significant degree of abstracting in design.<sup>4</sup>

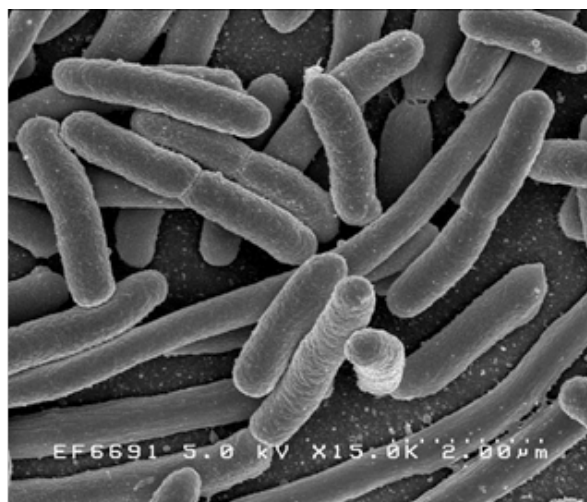
## Hosts for Cloning Vectors

The bulk of molecular cloning operations start with an *E. coli* laboratory strain as the host. Although there are many different host species and vectors for molecular cloning, the vast plurality of cloning investigations starts with *E. coli* as laboratory strain and vector cloning plasmid.<sup>10</sup>

Vectors plasmid and *E. coli* are widely utilized because they were adaptable, generally available and technically advanced, and primate to rapid recombinant organisms through minimal equipment (Figure 1). A yeast artificial chromosome vector or bacterial artificial chromosome was utilized when DNA cloned is frequently large [hundreds of thousands to millions of base pairs].<sup>11</sup>

For specific purposes, need special host-vector systems. For example, when scientists need to isolate protein from a recombinant creature, the host organism's expression vector will choose one with requisite signals for transcription and translation. A shuttle vector is multiplied host domain vector that could be utilized if DNA replication in diverse species is required (for example, transmitting DNA from microbe to plants). However, in practice, customized molecular cloning operations commonly start through molecular clone bacterial plasmid and then subcloning to the specific vector.<sup>12</sup>

Whoever vector combination and the host was utilized, the vector most contains IV DNA fragment were critical to



**Figure 1:** Classification of *E. coli*: Domain: Bacteria, Kingdom: Eubacteria, Phylum: Proteobacteria, Class: Gammaproteobacteria, Order: Enterobacteriales, Family: Enterobacteriaceae, Genus: Escherichia, Species: *E. coli*.

the function, and empirical uses– {1} Origin of replication DNA is needed to the vector, and recombinant fragment linked to it replicate in vivo, {2} restriction endonuclease recognition sites one or more unique that do as a position to restriction endonuclease {3} restriction endonuclease recognition sites one or more that do as a position for restriction endonuclease.<sup>13</sup>

### Preparation of Vector DNA

DNA breaks by a restriction endonuclease where the cloning vector is integrated into the foreign DNA. When restriction enzyme is an election to make a cleavage position arrangement that is appropriate to foreign DNA ends. This is always masterful by cleaving foreign DNA and the vector at the specific restriction enzyme, EcoRI. Many modern vectors have different appropriate cleavage position that are unrivaled through the molecule of vector (so vector could cleave only at one position) and within a gene are usually existing beta-galactosidase inactivation could be utilized to identify non-recombinant from recombinant creatures at the final step in the procedure. The enzyme can be treated with cleaved vector (alkaline phosphatase) dephosphorylates vectors end to increased range of recombinant and non-recombinant creatures. Dephosphorylated vector molecules at the ends prevent replication, and if foreign DNA was inserted to the cleavage point, replication could occur.<sup>14</sup>

### DNA Preparation

Molecular cloning is the process of inserting DNA into a vector molecule. The cloned DNA can be acquired by cutting it out of a source molecule using restriction enzymes, replicating it from exporter molecule utilized the polymerase chain reaction (PCR) or reverse transcription PCR (RT-PCR), or putting cloned DNA together to make short fragments of DNA Oligonucleotides. All approaches demand DNA supply be without impurities that can interfere with the enzyme function (polymerases, endonucleases) included in the cloning procedure.<sup>15</sup> Conventional restriction endonuclease digesting cloning can use a variety of different forms of donor DNA. To make a library of diverse inserts from the same DNA exporter, restriction enzymes can digest genomic DNA, and a suitable vector site is cloned. Through cut-off DNA by restriction enzymes and second vector cloning at matching sites, DNA cloned into one vector could be transported (sub cloned) to a new beneficiary vector. This is typically done to make protein expression or RNA transcription easier, otherwise impossible with the original vector.<sup>16</sup>

When PCR is utilized to generate DNA cloned, restriction position is usually integrated with the primer position, so the amplified DNA may be cut and cloning to the cloning vector's suitable restriction sites. The template for PCR can be any sort of DNA that has the required sequence. Cloning employing two different restriction enzymes guarantees that each molecule has non-compatible ends, prohibiting unpretentious vector re-circularization and forcing insertion to be cloning in a certain orientation.<sup>17</sup> This is critical for protein expression

because it ensures a translational open reading frame. In some cases, modifying the ends of DNA after restriction digestion can be beneficial. For example, De-phosphorylation of the cutting DNA vector would hinder re-circularization of the vector to nondirectional cloned with enzyme have a single restriction position, resulting in a higher ratio of the desired DNA recombinant molecules.<sup>18</sup>

PCR was increasing being utilized to prepare for cloning DNA. Amplified DNA could cloning either directed or after using restriction sites designed by restriction digestion of PCR primers. Alternatively, DNA amplified can be employed with Ligation Independent Cloning or Seamless Cloning techniques like NE-Builder HiFi DNA Assembly. PCR can also be used to make the vector molecules for various cloning procedures. Template-independent single adenosine (A) is added to DNA amplified by Taq polymerase in the 3' end, allowing cloned into integral T-tailed vectors. Because high dedication proofreading polymerases will not add extra bases, the DNA amplification can be cloned within a blunt-ended restriction position. Depending on the cloning process used, the ends at DNA amplified could change through A tailing, blunting, or adding or removing 5'-phosphate groups. PCR can also be used to amplify complementary DNA (cDNA) produced through reverse transcription of RNA. Cloning of sequences corresponding to gene transcripts is made possible by manufacturing DNA from RNA templates.<sup>19</sup>

### Recombinant DNA Creation with DNA Ligase

In many ways, making recombinant DNA is the most straightforward step in the molecular cloning process. DNA from the vector and a foreign source is combined at the proper concentrations and exposed to a covalently linking enzyme (DNA ligase). Ligation is a phrase used to describe the process of connecting two things together. The resulting DNA mixture, which has randomly connected ends, is now introduced into the host organism.

Only the ends of linear DNA molecules are recognized and acted upon by DNA ligase, resulting in a complicated jumble of DNA molecules with randomly connected ends. Other sequences (foreign DNA connected to itself, vector DNA linked to itself, and higher-order combinations of vector and foreign DNA) are generally present in addition to the desired results (vector DNA covalently bonded to foreign DNA). After the DNA combination is injected into cells, this complex mixture is sorted out in future steps of the cloning process.<sup>3,14</sup>

### Introduction of Recombinant DNA into Host Organism

Modified DNA combination re-introduced into a living cell formerly (known as the host creatures). Many techniques for getting DNA from cells, and molecular cloning process name of this phase significantly detectable through the experimental methods used -for example, transduction, transfection, electroporation, transformation).<sup>3,14</sup> Transformation happened when microbes can get and reproduce DNA from the environment surrounding it, and physiological state cells that



primate them to do named competent cell. The tantamount method of transporting DNA to the cells in mammalian is known as transfection. The two techniques, transfection and transformation routine, presuppose cell preparation through a specific grow regime and chemical management methods that differ depending on the cell types and species utilized.

Translocate DNA through the cell membrane by high-voltage electrical pulses are utilized if present the cell wall. Transduction, on the other hand, involves encapsulating DNA with virus derive molecules and then employing these molecules (virus-like) to transport encapsulated DNA to the cell through a technique like the viral infection. Despite the reality that transduction and electroporation are highly specialized methods, they might be the most effective methods for getting DNA to the cells.<sup>20</sup>

### Vector Sequences Selection Organisms

In any case, the techniques used, DNA recombinant introduced into the specific host creatures, are often poorly competent and have only a small percentage of cells taken up DNA. Experiential researchers recorded this problem through artificial genetic selection, selectively destroying the cells that have not taken up DNA, leaving just cells that could have active copy DNA containing selected banner gene transmitting through the vector to survival.<sup>3,14</sup>

When bacterial cells are used as hosts, a gene that gives resistance to antibiotics as the selectable marker is usually utilized will kill the cells; furthermore, common ampicillin. When exposed to an antibiotic, cells that have plasmid would survive; on the other hand, those who haven't taken plasmid sequences would die. Mammalian cells (such as mouse cells or humans) are appointed marker genes impart antibiotic Geneticin resistance.

### Desired DNA inserts Screening Clones and Biological Properties

In modern bacterial cloning vectors, the blue-white screening method is utilized, e.g., pUC19 and then derivatives, involve the pGEM vectors distinguish transgenic colonies cell clones from that had the parental vectors (i.e., DNA vector have no recombinant sequence insertion). This foreign vector DNA was inserted into the sequence that codes for a fundamental element of beta-galactosidase; any enzyme can cause a blue colony growing on the culture medium utilized in this research. Beta-galactosidase coding sequence when foreign DNA is inserted to, when the enzyme is rendered inactive and harboring altered DNA colonies remain colorless white. Researchers may become quickly detectable bacterial clones transgenic and undertake more study on them while disregarding those don't have recombinant DNA.<sup>21</sup>

The whole population of individual clones is obtained in a molecular cloning procedure called DNA library. Extremely complex libraries can be as when cloning a creature's complete genomic DNA or simplify, as when moved a formerly cloning DNA sequence to the different plasmid, but it is nearly usually needed to examine many other clones to guarantee that the

desired DNA structure is obtained. This could be accomplished utilizing various methods, such as antibody probes, nucleic acid hybridizations, polymerase chain reaction, DNA sequencing, and restriction fragment analysis.<sup>3,14</sup>

### Advantages and Applications

#### *Gene Function*

It was commonly utilized in gene and DNA research. The presence or absence of a gene can be determined simply by using gene cloning. The function of a gene can also be defined by creating a recombinant plasmid. Its uses include gene knockout, gene knockdown, and transgenic creation. However, a robust, quick, and cost-effective PCR approach replaces the aging gene cloning process of making copies of DNA. PCR can quickly amplify or synthesize genes using the Taq DNA polymerase.<sup>22</sup>

#### *Gene Structure*

Cloning can also be used to figure out a gene's structure or sequence. To learn about the sequence differences of a gene of interest, it is extracted and sequenced.<sup>22</sup>

#### *Creating Genetically Modified Organisms (GMOs)*

The technique of gene cloning is commonly used in the recombinant DNA technology of creating genetically engineered organisms. To change the characteristics, phenotype, and research the function of a gene or disease model, a transgene or recombinant DNA can be placed in bacteria, plants, mice, and other animals.<sup>23</sup>

#### *Gene Therapy*

Gene therapy to treat genetic problems is one of the most exciting applications of gene cloning. A healthy gene or DNA sequence can repair or replace a defective gene or DNA sequence.<sup>25</sup>

### Mutational Studies and Identification of Mutations

The gene cloning approach was once employed to identify mutations and conduct other mutagenesis experiments. Still, it has since been supplanted by the polymerase chain reaction technique, which is faster and less expensive. It's also employed in the research of site-directed mutagenesis.<sup>24</sup>

### Biopharmaceuticals

One of the most innovative applications of gene cloning is the production of recombinant or artificial protein products. We have already talked about how it happens.<sup>22</sup>

## CONCLUSION

The goal of gene cloning is to generate copies of DNA; however, it is a time-consuming process. After the development of the Polymerase chain reaction in 1983, the constraints of gene cloning were addressed.

The polymerase chain reaction is a quick, low-cost, precise, and high-yielding technology. The amount of DNA copies obtained after the experiment is significantly more than that obtained using traditional gene cloning methods.

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