International Journal of Management, Sciences, Innovation, and Technology IJMSIT

Review Paper

Vol.3, Issue.2, pp.17-19, March (2022) **ISSN:** 0000-0000

Molecular Cloning

Sweta Shivani [1]

[1*] Biotechnology first year student from (Banasthali vidyapith university)

[*]*Corresponding Author:* e-mail: shwetashivani17@gmail.com

Available online at: https://ijmsit.com/volume-3-issue-2/

Received: 09 March 2022 Revised: 13 March., 2022 Accepted: 22 March, 2022

***Abstract*—** The idea of using molecular cloning to produce recombinant DNA was invented by (Paul Berg)molecular cloning is fundamentals technology in molecular biology Molecular clones can be used to generate many copies of the DNA for analysis of the gene sequence, and/or to express the resulting protein for the study or utilization of the protein's function. The clones can also be manipulated and mutated in vitro to alter the expression and function of the protein. Standard molecular cloning experiments, the cloning of any DNA fragment essentially involves seven steps: (1) Choice of host organism and cloning vector, (2) Preparation of vector DNA, (3) Preparation of DNA to be cloned, (4) Creation of recombinant DNA, (5) Introduction of recombinant DNA into host organism. Plasmids can transfer genes (such as those for antibiotic resistance) that occur naturally within them, or plasmids can act as carriers (vectors) for introducing foreign DNA from other bacteria, plasmids, or even eukaryotes (animals, plants, fungi, protists) into bacterial cells. Restriction endonucleases (enzymes) can be used to cut and insert pieces of foreign DNA into the plasmid vectors. If these plasmid vectors also carry genes for antibiotic resistance, transformed cells containing plasmids that carry the foreign DNA of interest in addition to the antibiotic resistance gene can be easily selected from other cells that do not carry the gene for antibiotic resistance. "Obtaining the molecular clone of a gene can lead to the development of organisms that produce the protein product of the cloned genes, termed a recombinant protein. In practice, it is frequently more difficult to develop an organism that produces an active form of the recombinant protein in desirable quantities than it is to clone the gene. This is because the molecular signals for gene expression are complex and variable, and because protein folding, stability and transport can be very challenging. Many useful proteins are currently available as recombinant products. These include—(1) medically useful proteins whose administration can correct a defective or poorly expressed gene (e.g. recombinant factor VIII, a blood-clotting factor deficient in some forms of hemophilia,[17] and recombinant insulin, used to treat some forms of diabetes[18]), (2) proteins that can be administered to assist in a life-threatening emergency (e.g. tissue plasminogen activator, used to treat strokes[19]), (3) recombinant subunit vaccines, in which a purified protein can be used to immunize patients against infectious diseases, without exposing them to the infectious agent itself (e.g. hepatitis B vaccine[20]), and (4) recombinant proteins as standard material for diagnostic laboratory tests.

***Keywords*—** Subunits Vaccine, Antibiotics Resistant, Recombinant Factor(8) ,Hepatitis B,Infectious Agent.

I. INTRODUCTION

Molecular cloning, which is one of the most fundamental procedures available for modern molecular biology research, has been critical for driving biotechnological advances. One of the main objectives in the post-genomics era is to functionally map gene expression data. Thus, developing methods for the rapid and efficient construction of various vectors for transgenic research is more critical now than ever before.

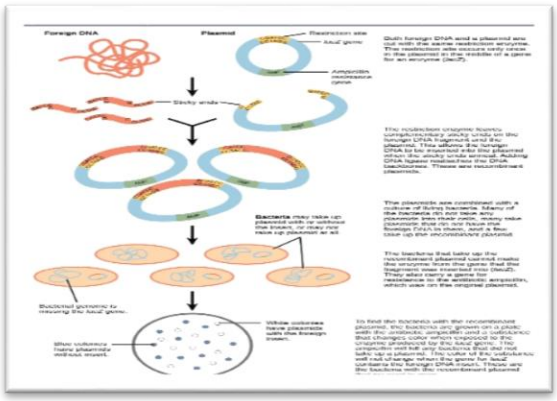
Molecular cloning is a set of experimental methods in molecular biology that are used to assemble recombinant DNA molecules and to direct their replication within host organisms. Traditionally, molecular cloning is defined as the isolation and amplification of a specific DNA fragment. Most of these fragments are created either by digesting an existing piece of DNA with restriction enzymes or by targeting it via PCR. Short inserts of ~ 100 bp can also be commercially synthesized as complementary single-stranded oligos, which are subsequently annealed to form a double-stranded fragment.

After successful isolation, the DNA of interest is ligated into a vector plasmid, a double-stranded circular piece of DNA that can be propagated in E. coli. Vectors used in the laboratory represent a smaller version of naturally occurring plasmids that include several basic features: a replication origin, a drug-resistance gene, and unique restriction sites to facilitate the insertion of DNA fragments. Often, several different restriction sites are clustered together in so-called 'polylinker regions' or 'multiple cloning sites,' making it

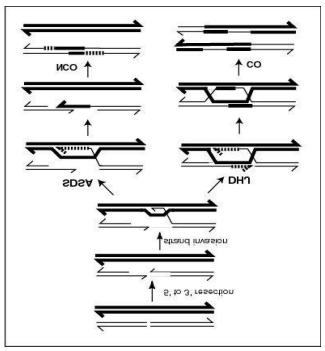
easier to choose convenient and unique restriction enzyme combinations for a variety of inserts.

II. PRACTICES

The choice of restriction enzymes is critical when designing a cloning strategy. While some sever the double-stranded DNA in one place, creating 'blunt' ends, others leave an overhang of a few bases at the cut site. These complementary 'sticky' ends find one another easily, increasing the efficiency of the ligation reaction and thus the chances for a successful cloning event. Thoughtful combination of restriction enzymes can also help to control the directionality of the insert, which is critical to many applications.

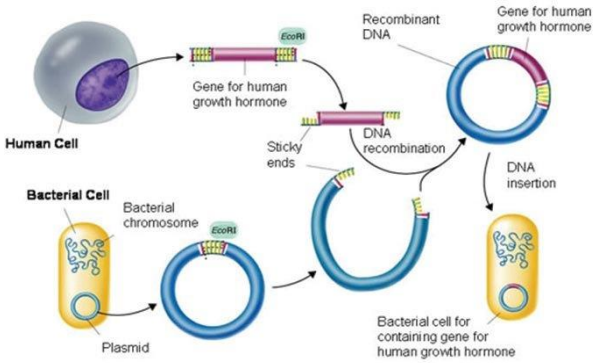


Escherichia coli strains DH5α and DB3.1 (Transgen Biotech, Beijing, China) were used for cloning. Agrobacterium tumefactions strain GV3101 was used for transforming plants. The E. coli cultures were grown at 37°C in Lysogeny Broth (LB) selection medium. The SfiI, T5 exonuclease, and Phusion polymerase enzymes as well as the Gibson Mix were obtained from New England Biolabs (Ipswich, MA, USA). Primers were ordered from ThermoFisher (Guangzhou, China).



Plasmid Construction

The destination and entry vectors of the Nimble Cloning system were generated by inserting their cloning cassettes into the desired vector via Gibson assembly. The cloning cassette for the destination vector was "adapter 1–SfiI site–ccdB gene–SfiI site–adapter 2" (NC frame), whereas that for the entry vector was "SfiI site–adapter 1–XcmI–ccdB gene–XcmI–adapter 2–SfiI site." Details regarding the primers used to construct these vectors are listed in Supplementary Table S1. The "SfiI–adapter 1–XcmI–ccdB gene–XcmI–adapter 2–SfiI" fragment was inserted into the cloning sites of the pENTR/D-TOPO (Invitrogen) and pMD-18T (Takara, Dalian, China) vectors to produce the entry vectors with kanamycin and ampicillin resistance genes, respectively.



III. SYNTHESIS OF DOUBLE-STRAND CDNA AND QUALITY DETECTION OF PHAGE LIBRARY

After detecting ultraviolet spectrophotometry, the A260/A280 of total RNA is 1.96. The electrophoresis results showed that the double-stranded cDNA is located between 0.3 and 2.5 kb, presenting an elongated waterfall-like band, which agreed well with the distribution of plant tissue DNA. According to the number of bacterial colony and bacteria liquid PCR identification results, the recombination rate was 95.17%. The capacity of the original library is 8.38 × 106 pfu/mL−1. DS Gene Cloning and Sequence Analysis. After electrophoresis, the PCR amplification product of DS gene obtained a specific band about 2310 bp, which agreed well with the expected results. pMD-18T-DS plasmid was digested by XbaI and PstI and an insert size of 2310 bp, the size which corresponds to the target strap was obtained. BLAST analysis found that the DS cDNA carried a 2310 bp full open reading frame (ORF) fragment.

The amino acids of DS (769 amino acids with a predicted molecular mass of 84.6 kDa) were found to be 56, 50, and 48% identical to those of β-amyrin synthase, cycloartenol synthase, and lanosterol synthase in P. ginseng. Identification of Recombinant Expression Vector and

Induced Expression of Recombinant Specific band with the size about 2310 bp, consistent with expectation, was obtained by the application of NcoI and NotI double digestion pET-30a-DS. The results found that DS gene was inserted into pET-30a vector, and the recombinant plasmid pET-30a-DS was constructed.

IV. CONCLUSION

Gene cloning starts the new age of modern Bio-Technology. Gene cloning is being used to address problem in all areas of agriculture production, Pharmacology, Environmental issues, Food and nutrition field etc.

Authors Profile

[1] Department of Pharmacy, Banasthali Vidyapith , India.

