cDNA Library: A Tool for Molecular Plant Breeding

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Abstract

By virtue of the powerful technology developed, it is possible to isolate after reverse transcription, any transcribed gene in the form of cDNA. cDNA library is a collection of cloned DNA fragments inserted into a collection of host cells, which together constitute some portion of the transcriptome of the organism. In recent years with development of molecular biology technology, the methods of construction of cDNA libraries have improved and enhanced greatly. In this article cDNA library construction was summarized and the applications cDNA library was described.

Introduction

In higher eukaryotes, gene expression is tissue-specific. Only certain cell types show moderate to high expression of a single gene or a group of genes, e.g. the genes encoding globin proteins are expressed only in erythrocyte precursor cells, called reticulocytes. Using this information a target gene can be cloned by isolating the mRNA from specific tissue. The specific DNA sequences are synthesized copies from mRNA of particular cell type, and cloned into bacteriophage vectors. cDNA is produced from a fully transcribed mRNA which contains only the expressed genes of an organism. Clones of such DNA copies of mRNAs are called cDNA clones.

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Construction of a cDNA library (overview)

Figure 1: Overview showing construction of cDNA library

Procedure of Construction of cDNA Library

1. Isolation of mRNA

It involves the isolation of total mRNA from a cell type or tissue of interest. The amount of desired mRNA can be increased by following ways:
• Chromatographic purification of mRNA using oligo-dT column, which retains mRNA molecules, resulting in their enrichment.
• Spinning down mRNA by density gradient centrifugation.
• When the protein produced by a gene is known, it is purified and used to produce antibodies specific to it. These antibodies are used to precipitate the polysomes (mRNAs associated with ribosomes and newly synthesized polypeptide chains) engaged in synthesis of the concerned polypeptide.

2. Synthesis of First and Second Stand of cDNA

A poly-T oligonucleotide primer is annealed with the poly (A) tail on the mRNA. Reverse transcriptase extends the 3’ end of the primer using mRNA molecule as a template producing a cDNA:mRNA hybrid. The mRNA from the cDNA:mRNA hybrid can be removed by RNase H or alkaline hydrolysis to give a ss-cDNA molecule. The 3’ end of this ss-cDNA serves as its own primer generating a short hairpin loop at this end. The ss-cDNA is then converted into double stranded (ds) cDNA by either reverse transcriptase or E. coli DNA polymerase. The hairpin loop is cleaved by a S1 nuclease to obtain blunt-ended cDNA.

3. Incorporation of cDNA into Vector

The blunt ended cDNA are modified in order to ligate into a vector to prepare ds cDNA for cloning. Short restriction site linkers are first ligated to both ends. Linker is double stranded DNA segment with a recognition site for a particular restriction enzyme. It is 10-12 base pairs long prepared by hybridizing chemically synthesized complementary oligonucleotides. The blunt ended ds DNAs are ligated with the linkers by the DNA ligase. The resulting ds cDNAs with linkers at both ends are treated with a restriction enzyme specific for the linker generating cDNA molecules with sticky ends. The vectors (e.g. plasmid or bacteriophage) should be restricted with the same restriction enzyme used for linkers. Adding DNA ligase to the plasmid-linker cDNA mixture produces recombinant DNA.

4. Cloning of cDNA

The recombinant DNA molecules are now ready for ‘cloning’. They are transforming into suitable host (E. coli or λ-phage). Xiaohui Pan et al., 2020 has cloned cDNA of four Hsp genes from Agarophytonvermiculophyllum and transcription analysis was also done in different phases.

Applications
• Discovery of novel genes. The gene function in stress tolerance of wheat was confirmed by study of an overlapping gene TaPR-1-1 which was detected through over expression in Arabidopsis and yeast (Jingyi Wang et. al., 2019).
• Elucidation of gene function. Differential expression of selected genes (hsp101 and CRT) from the SSH library were validated by qRT-PCR analysis. The ESTs generated are rich source of heat stress responsive genes, which can be utilized in improving thermotolerance of other food crops (Donald James et. al., 2015).
• In vitro study of gene function.
• To obtain pure sample of a gene.
• They are commonly used for the removal of various non-coding regions from the library.
• Study of alternative splicing.

Conclusion

As a concluding mark we can say that cDNA libraries are used as a powerful tool for molecular biology and biotechnology studies, which ultimately helps the plant breeder for identification, characterization and mapping of desirable genes in plant breeding programmes against different biotic and abiotic stresses.

References

