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cDNA Library: A Molecular Plant Breeding Tool

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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.

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.

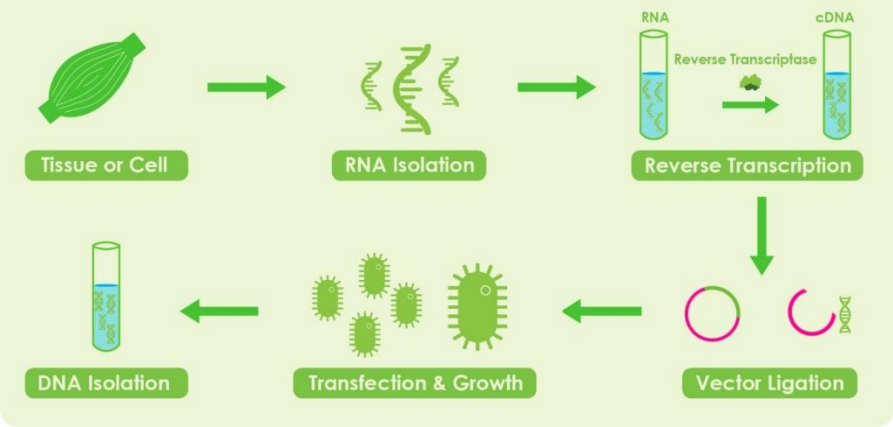

Fig: Schematic representation of cDNA library construction
Source: (https://www.goldbio.com/articles/article/Reverse-Transcriptase-cDNA-Overview-Applications)

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 sscDNA is then converted into double stranded (ds)cDNS by either reverse transcriptase or E. coliDNA 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 is 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 *Agarophytonvermiculophyllumand* 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 (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 (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 st udies, which ultimately helps the plant breeder for identification, characterization and mapping of desirable genes in plant breeding programmes against different biotic and abiotic stresses.

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