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# cDNA Library: A Molecular Plant Breeding Tool

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**Abstract**

Due to the advanced technology, it is now possible to isolate any transcribed gene in the form of cDNA after reverse transcription. A cDNA library is a collection of cloned DNA fragments introduced into a variety of host cells, which collectively make up a component of the organism's transcriptome. The techniques for creating cDNA libraries have significantly advanced and improved in recent years with the advancement of molecular biology technology. The process of creating a cDNA library was discussed in this article, along with some of its potential uses.

**Keywords**- cDNA, Construction, Molecular biology, Transcriptome.

## Introduction

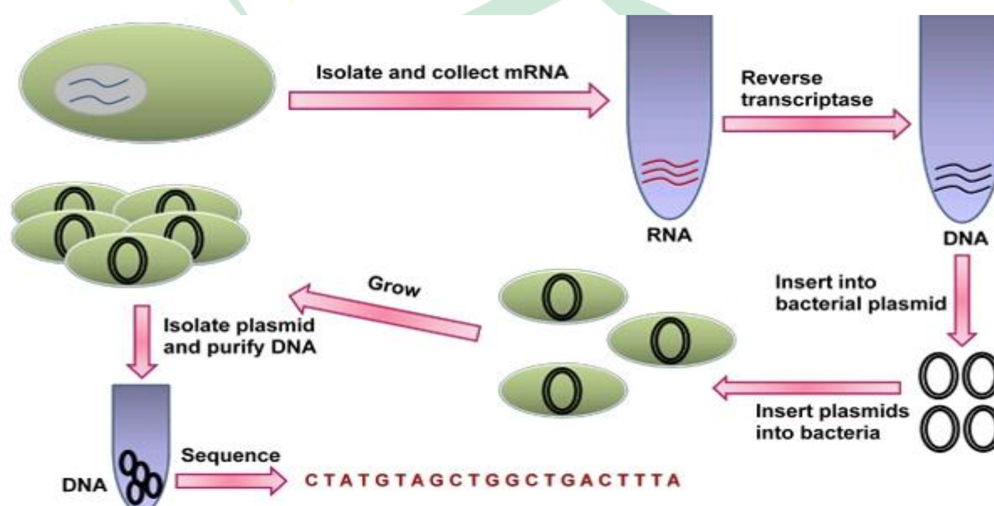


**Figure 1: A general picture illustrating the creation of a cDNA library**

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Gene expression in higher eukaryotes is tissue-specific. A single gene or a collection of genes are only moderately to highly expressed in certain cell types, for example, the globin protein-coding genes are only found in reticulocytes, which are erythrocyte precursor cells. By extracting the mRNA from a particular tissue, a target gene can be cloned using this knowledge. From the mRNA of a given cell type, copies of the precise DNA sequences are created and then cloned into bacterio-phage vectors. A fully transcribed mRNA that solely contains an organism's expressed genes can be converted into cDNA. Clones of these mRNA DNA copies are known as cDNA clones.

**Procedure of Construction of cDNA Library**

**1. Isolation of mRNA**

Total mRNA is isolated from an interest cell type or tissue. The amount of desired mRNA can be increased by following ways:

- Chromatographic purification of mRNAusing oligo-dTcolumn, which retains mRNA molecules, resulting in their enrichment.
- Density gradient centrifugation for the spinning down of mRNA.
- 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 sscDNA 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. 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.

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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 readyfor '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**

- Novel genes have been discovered. An analysis of the overlapping gene TaPR-1-1, which was discovered through over expression in Arabidopsis and yeast, verified the gene's role in wheat's ability to withstand stress (Jingyi Wang *et al.*, 2019).
- Determining the role of genes. By using quantitative real-time PCR (qRT-PCR), the differential expression of two genes (hsp101 and CRT) from the SSH library was confirmed. The produced ESTs contain a wealth of genes that are responsive to heat stress and can be used to increase the thermo tolerance of other food crops (Donald James *et al.*, 2015).
- Gene function is being studied in vitro.
- Getting a gene's pure sample.
- Investigation into alternative splicing.
- They frequently serve as a means of deleting different non-coding portions from libraries.

**Conclusion**

As a final point, we can state that cDNA libraries are employed as a potent tool for molecular biology and biotechnology studies, which ultimately helps the plant breeder in the

identification, characterization, and mapping of desirable genes in plant breeding programmes against various environmental stresses.

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