General & MOLECULAR GENETICS

cDNA LIBRARY

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- Preparation of cDNA library
- Construction of c DNA library
- Extraction of mRNA
- Uses of cDNA
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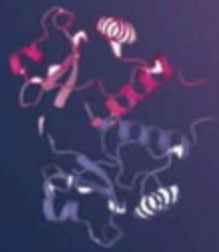
Who discovered cDNA library

The cDNA cloning method developed by Rougeon, Kourilsky and Mach. The first strand of cDNA is synthesized with an oligo(dT) primer for reverse transcription, separated from mRNA and then tailed at its 3′-end with a poly(dT) homopolymer with terminal deoxynucleotidyl transferase (TdT)

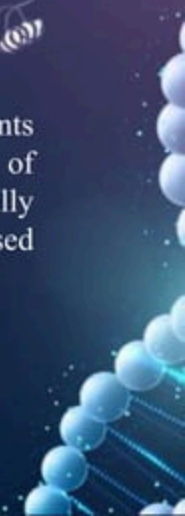
Definition

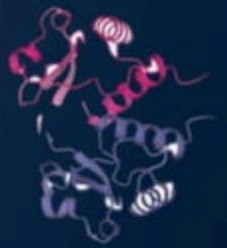
Combination of cDNA that has been inserted into host cell. In cDNA libraries we just take out only that portion of the genome which is first converted into the cDNA is now inserted into host cell.

What is cDNA library?



cDNAlibrary is a combination of cloned cDNA (complementary DNA) fragments inserted into a collection of host cells, which together constitute some portion of the transcriptome of the organism. cDNA is produced from fully transcribed mRNA found in the nucleus and therefore contains only the expressed genes of an organism.

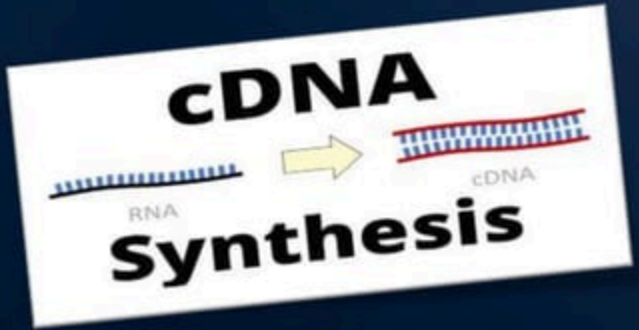




- In eukaryotic cells the mature mRNA is already spliced, hence the cDNA produced lacks introns and can be readily expressed in a bacterial cell.
- While information in cDNA libraries is a powerful and useful tool since gene products are easily identified, the libraries lack information about enhancers, introns, and other regulatory elements found in a genomic DNA library.

cDNA library preparation

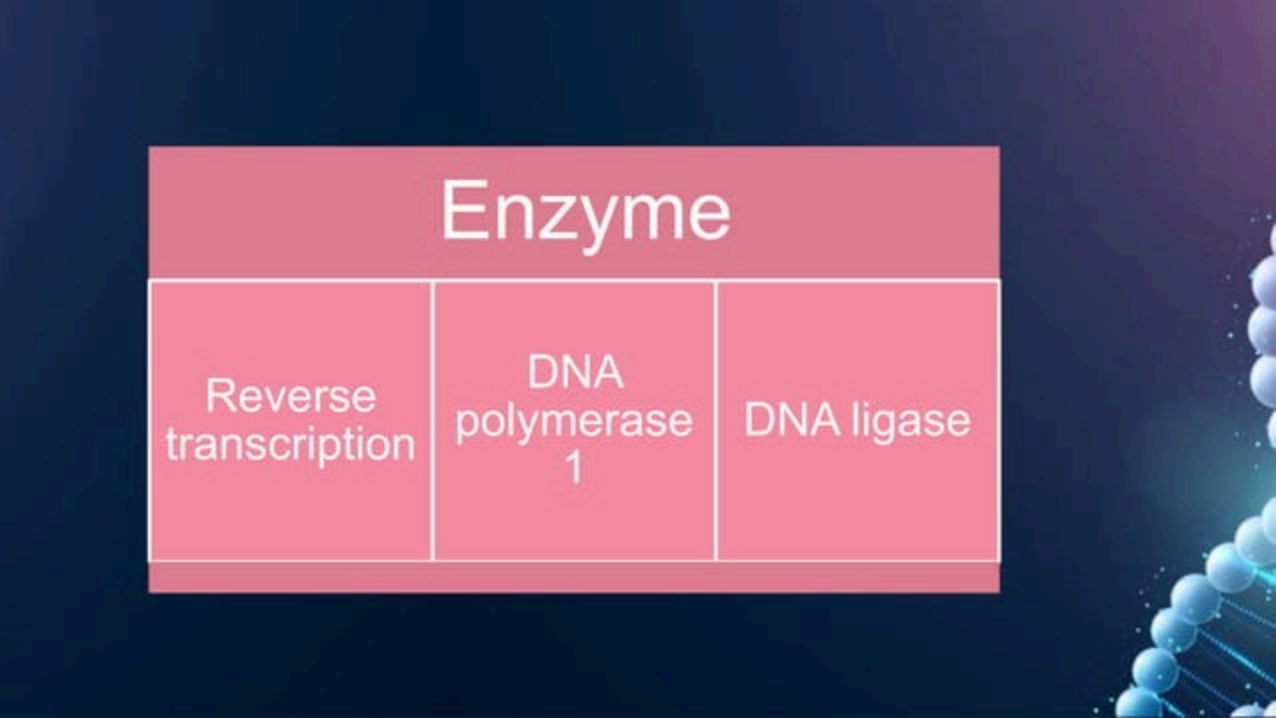
cDNA is created from a mature mRNA from a eukaryotic cell with the use of an enzyme known as reverse transcriptase. In eukaryotes, a poly-(A) tail (consisting of a long sequence of adenine nucleotides) distinguishes mRNA from tRNA and rRNA and can therefore be used as a primer site for reverse transcription.



Construction of cDNA library

- Isolation of messenger RNA
- Messenger RNA is converted into cDNA
- Complementry DNA inserted into the vector molecule
- Finally this vector molecule is introduce into host cell that may be either bacteria.

Enzyme		
Reverse transcription	DNA polymerase 1	DNA ligase



Enzyme Work

- Reverse Transcription

It can make DNA from RNA

- RNase H

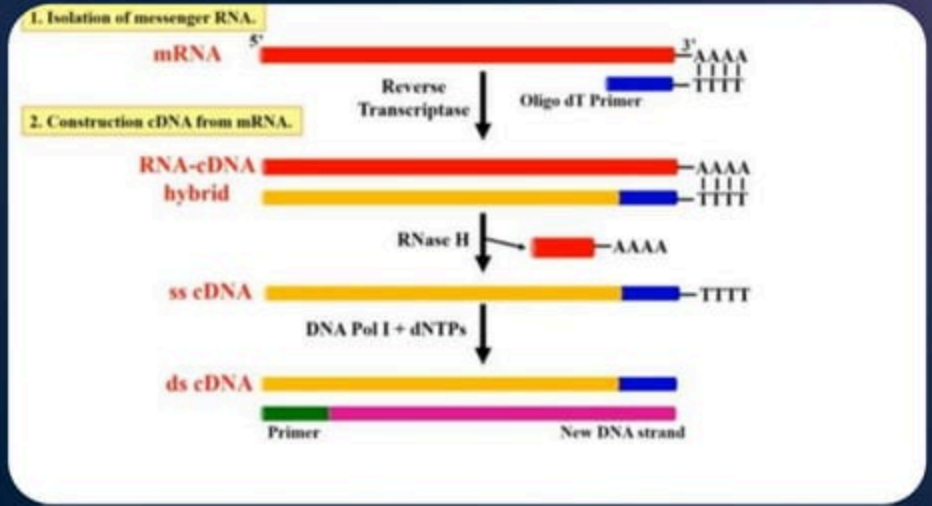
It remove the RNA fragment

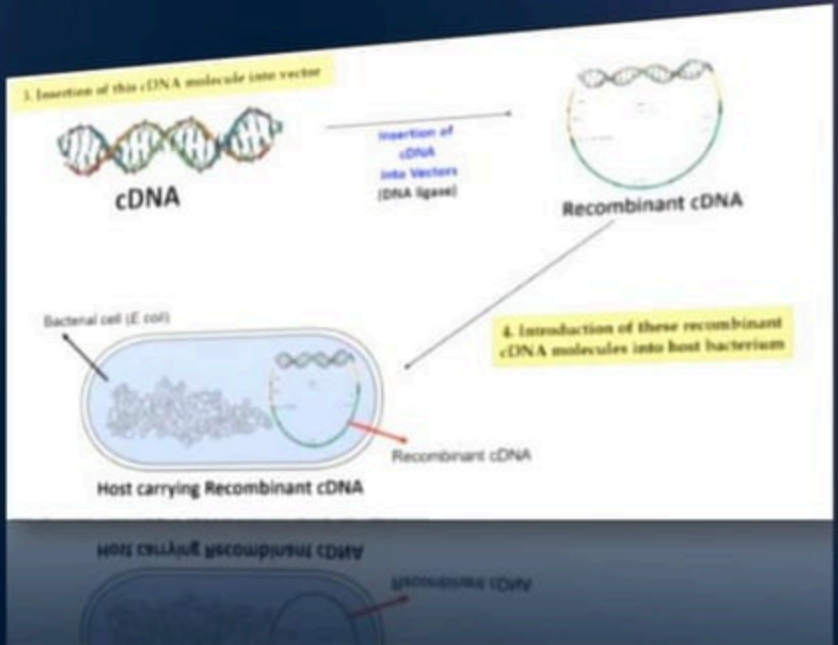
- DNA polymerase 1

It make another strand DNA by using this SScDNA molecule as a templet

- DNA ligase

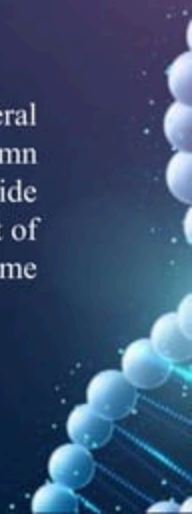
Help in joining of cDNA with vector molecule


1. Isolation of messenger RNA.
mRNA
5'
3'
AAAA
TTTT
Reverse Transcriptase
Oligo dT Primer
2. Construction cDNA from mRNA.
RNA-cDNA hybrid
AAAA
TTTT
RNase H
AAAA
ss cDNA
TTTT
DNA Pol I + dNTPs
ds cDNA
Primer
New DNA strand


3. Insertion of this cDNA molecule into vector
cDNA
Insertion of cDNA into Vectors (DNA ligase)
Recombinant cDNA
4. Introduction of these recombinant cDNA molecules into host bacterium
Bacterial cell (E coli)
Recombinant cDNA
Host carrying Recombinant cDNA

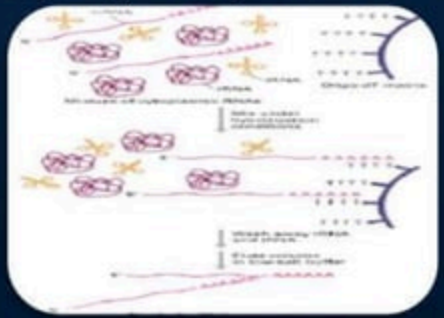
mRNA Extraction

Firstly, the mRNA is obtained and purified from the rest of the RNAs. Several methods exist for purifying RNA such as trizol extraction and column purification . Column purification is done by using oligomeric dT nucleotide coated resins where only the mRNA having the poly-A tail will bind. The rest of the RNAs are eluted out. The mRNA is eluted by using eluting buffer and some heat to separate the mRNA strands from oligo-dT.



mRNA Extraction

Purification can be performed by binding mRNAs on a solid matrix to which short strings of thymidylate residues are attached (oligo dT matrix). The mRNAs are removed again by washing in a low salt buffer.

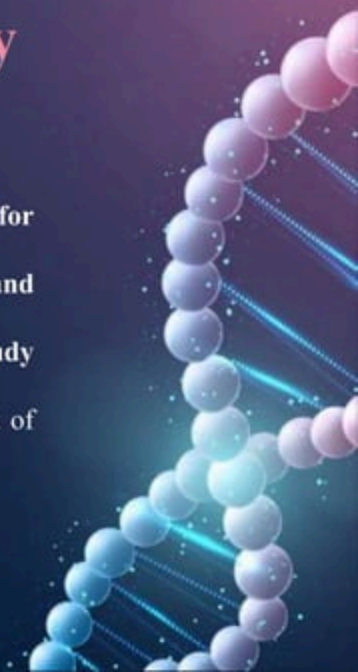


Uses of cDNA

cDNA libraries are commonly used when reproducing eukaryotic genomes, as the amount of information is reduced to remove the large numbers of non-coding regions from the library. cDNA libraries are used to express eukaryotic genes in prokaryotes. Prokaryotes do not have introns in their DNA and therefore do not possess any enzymes that can cut it out during transcription process. cDNA does not have introns and therefore can be expressed in prokaryotic cells. cDNA libraries are most useful in reverse genetics where the additional genomic information is of less use. Additionally, cDNA libraries are frequently used in functional cloning to identify genes based on the encoded protein's function. When studying eukaryotic DNA, expression libraries are constructed using complementary DNA (cDNA) to help ensure the insert is truly a gene.[1]

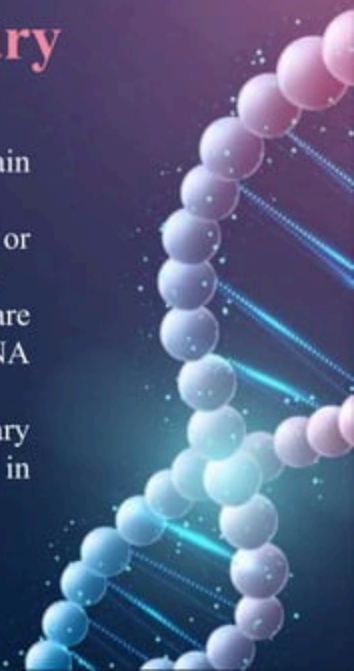
Advantage of cDNA library

- cDNA library is **useful for isolating gene that code for particular mRNA.**
- cDNA of protein **can facilitate to generate antibodies and monoclonal antibodies**.
- The most important application of cDNA library is **to study expression of mRNA.**
- By making cDNA libraries we can store reduced amount of information due to the removal of non-coding regions.



Disadvantages of cDNA library

- The disadvantage of a cDNA library is that it contain only sequences that present in mature mRNA.
- Introns and any other sequences that are altered or changed after transcription are not present in cDNA.
- Sequences such as promotors and enhencers that are not transcribed into RNA are not present in cDNA library.
- It is also important to note that the cDNA library represent only those gene sequences expressed in tissues from which the RNA was isolated.




THANK
YOU !


Any
questions