BIOTECHNOLOGY TOPIC: C DNA LIBRARY

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cDNA LIBRARY

cDNA library -

 complementary DNA molecules synthesized from mRNA molecules in a cell.

- constitute some portion of the <u>transcriptome</u> of the organism.
- cDNA is produced from fully transcribed <u>mRNA</u> found in the <u>nucleus</u>
- therefore contains only the expressed genes of an organism.
- So ,tissue specific cDNA libraries can be produced

- cDNA is created from a mature <u>mRNA</u> from a eukaryotic cell with the use of an enzyme known as <u>reverse transcriptase</u>.
- In eukaryotes, apoly-(A)
 <u>tail</u> distinguishes <u>mRNA</u> from <u>tRNA</u> and <u>rRNA</u>
 and can therefore be used as a <u>primer</u> site for reverse transcription

Steps

1. mRNA extraction

2 methods

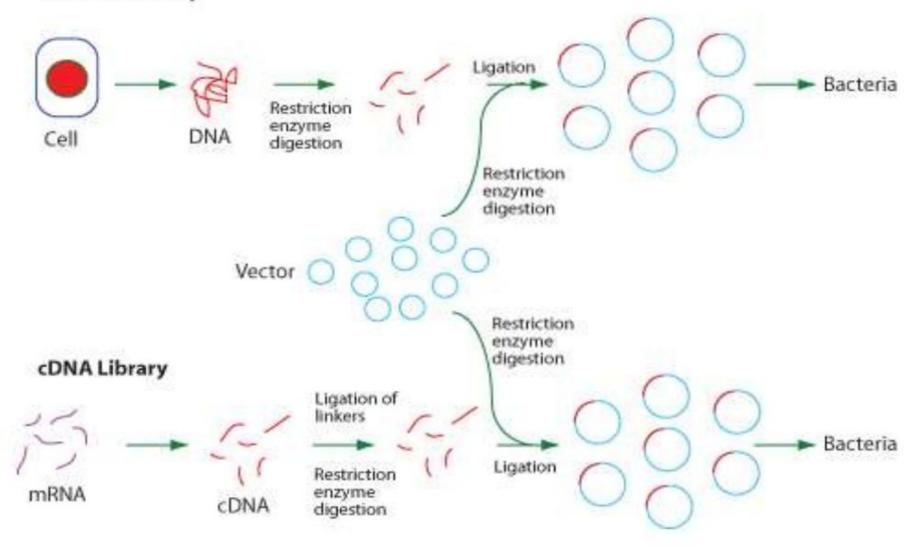
- <u>trizol</u> extraction
- column purification.
- by using oligomeric dT nucleotide coated resins where only the mRNA having the poly-A tail will bind.
- The rest of the RNAs are eluted out
- The mRNA is eluted by using eluting buffer and some heat to separate the mRNA strands from oligo-dT.

Once mRNA is purified,

- oligo-dT is tagged as a complementary primer which binds to the poly-A tail providing a free 3'-OH end that can be extended by reverse transcriptase to create the complementary DNA strand.
- Now, the mRNA is removed by using a <u>RNAse</u> enzyme leaving a single stranded cDNA (sscDNA).
- This sscDNA is converted into a double stranded DNA with the help of <u>DNA polymerase</u>
- However, for DNA polymerase to synthesize a complementary strand a free 3'-OH end is needed

- This is provided by the sscDNA itself by generating a <u>hairpin loop</u> at the 3' end by coiling on itself.
- polymerase extends the 3'-OH end and later the loop at 3' end is opened by the scissoring action of <u>S</u>₁ <u>nuclease</u>.
- Restriction endonucleases and DNA ligase are then used to clone the sequences into bacterial plasmids.
- The cloned bacteria are then selected, commonly through the use of antibiotic selection.
- Once selected, stocks of the bacteria are created which can later be grown and sequenced to compile the cDNA library

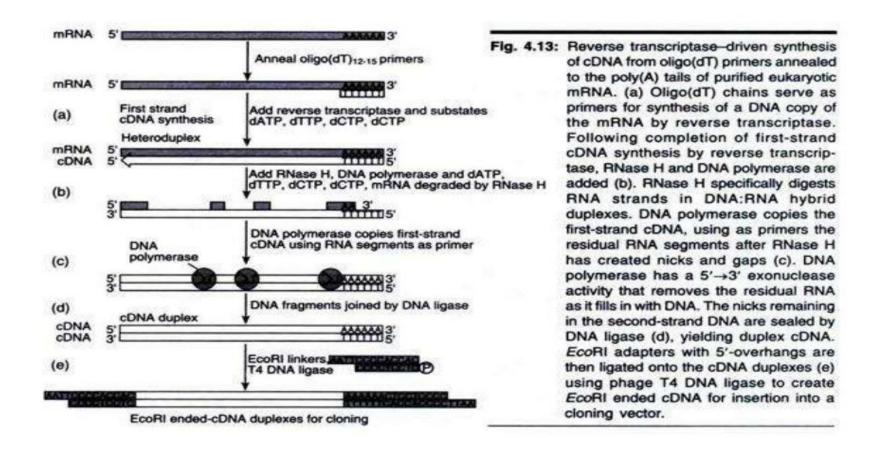
Genomic Library



cDNA Libraries:

- •cDNAs are DNA molecules copied from mRNA templates. cDNA libraries are constructed by synthesizing cDNA from purified cellular mRNA.
- These libraries present an alternative strategy for gene isolation, especially eukaryotic genes.
- •Because most eukaryotic mRNAs carry 3'-poly(A) tails, mRNA can be selectively isolated from preparations of total cellular RNA by oligo(dT)-cellulose chromatography.
- •DNA copies of the purified mRNAs are synthesized by first annealing short oligo (dT) chains to the poly(A) tails.

•These oligo(dT) chains serve as primers for reverse transcriptase-driven synthesis of DNA (Fig. 4.13). Reverse transcriptase is an enzyme that synthesizes a DNA strand, copying RNA as the template. DNA polymerase is then used to copy the DNA strand and form a double-stranded (duplex DNA) molecule.



- Lastly Linkers are added to the DNA duplexes rendered from the mRNA templates, and the cDNA is cloned into a suitable vector.
- Once a cDNA derived from a particular gene has been identified, the cDNA becomes an effective probe for screening genomic libraries for isolation of the gene itself.
- •Because different cell types in eukaryotic organisms express selected subsets of genes, RNA preparations from cells or tissues in which genes of interest are selectively transcribed are enriched for the desired mRNAs.
- cDNA libraries prepared from such mRNA are representative of the pattern and extent of gene expression that uniquely define particular kinds of differentiated cells.

Complementary DNA (cDNA) Libraries

- * cDNA Library is a population of mRNAs
- * Generated by <u>Reverse Transcription</u> of cellular mRNA, reveals expression profiles in different cell types and developmental stages
- * Cloned eukaryotic cDNAs have their own special uses
- * Advantage: Size of cDNA clone is significantly lower than the Genomic DNA library

Early cDNA cloning Strategy

*** 3 Major Steps:**

- First-strand DNA synthesis on the mRNA by Reverse transcriptase
- Removal of mRNA template
- ▲ Second-strand DNA synthesis using Ist strand DNA as a template by DNA pol-I
- * cDNA cloning was based on the Homo-polymer tailing method (1970s)



*** Drawbacks:**

- Generally 3'end bias in the resulting library
- Native enzymes have poor processivity & intrinsic RNase activity
- © Optimal activity for native enzymes at 37°C

cDNA Library uses

- 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.
- cDNA libraries are most useful in <u>reverse</u> genetics where the additional genomic information is of less use.
- Also, it is useful for subsequently isolating the gene that codes for that mRNA.