

* Genomic & cDNA libraries

→ DNA library → collection of cloned restriction fragments of DNA of an organism

→ 2 type → genomic libraries

Complementary DNA [cDNA] libraries

→ genomic libraries → contain copy of every DNA nucleotide sequence in genome

- Introns & control region of gene present

→ cDNA libraries → contain those DNA sequence that only appear as processed messenger (mRNA) molecules

- Differ according to cell type & environmental condition

- Lack intron & control region of gene.

→ Genomic DNA library :-

Digestion of total DNA of organism with restriction endonucleases

↓

ligation to appropriate vector

↓

recombinant DNA molecule replicate within host bacteria

↓

cloned DNA fragments collectively represent entire genome of organism

↓

called genomic library.

Disadvantage :- If digestion is allowed to go to completion &

↓

if gene of interest contain more than one restriction site, it is fragmented

To avoid this - partial digestion performed by either amount or time of action of enzyme is limited.

Complementary DNA libraries:

Use mRNA as template

↓ reverse transcriptase

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cDNA - double stranded

- Template mRNA isolated from tRNA & rRNA by presence of its poly A tail.

- Amplify cDNA by PCR (or) by biologic cloning.

- cDNA can be used as a probe to locate gene that encode original mRNA in mixture containing many unrelated DNA fragment.

- cDNA - No introns

- Can be cloned into expression vector for synthesis of eukaryotic proteins by bacteria.

- Expression vector [special plasmid]

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Contain bacterial promoter for transcription of cDNA & SD [Shine-Dalgarno] sequence that allow bacterial ribosome to initiate translation of resulting mRNA molecule.

- cDNA is inserted downstream of promoter & within a gene for protein that is expressed in bacterium, such that mRNA

produced contain SD sequence, a few codons for bacterial protein & all codon for eukaryotic protein.

↓
allows more efficient expression & results in production of fusion protein.

e.g. Therapeutic human insulin is made in bacteria through this technology.