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Creating cDNA Libraries

By
Jessica Vasquez

DEFINITIONS

Complementary DNA (cDNA)

mRNA cannot itself be ligated into a cloning vector so cDNAs are complementary DNA copies of RNA that are generated by the enzyme Reverse Transcriptase then the cDNA produced can be ligated to a vector to produce a cDNA library.

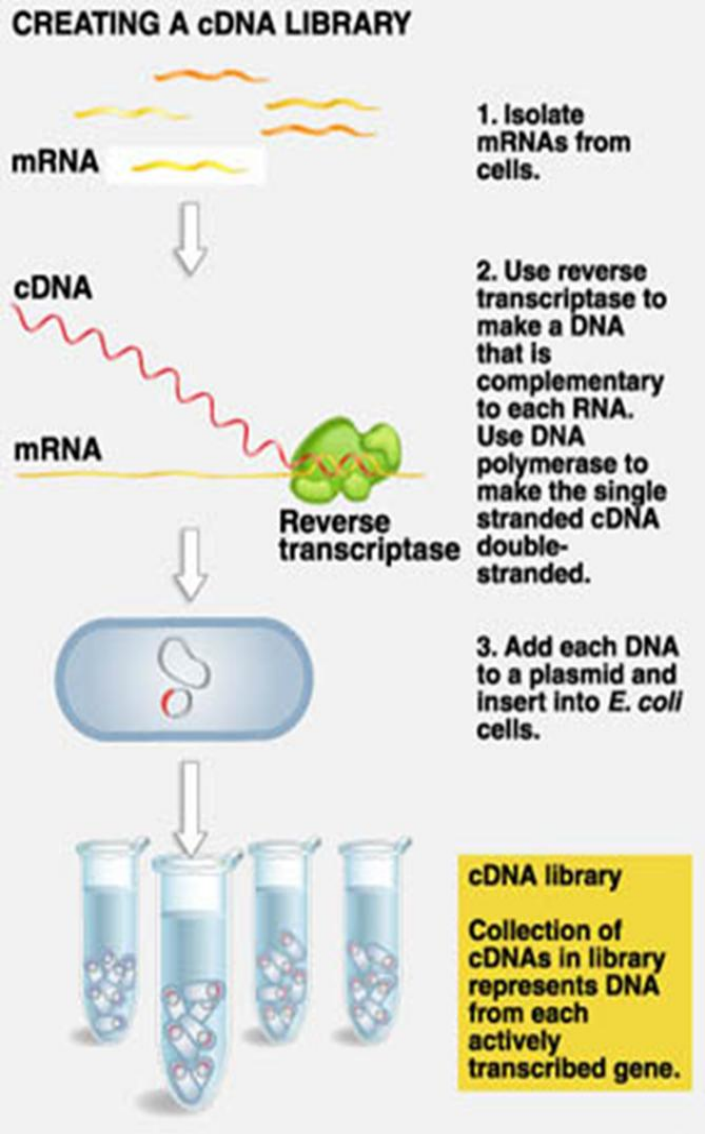
Complementary DNAs libraries

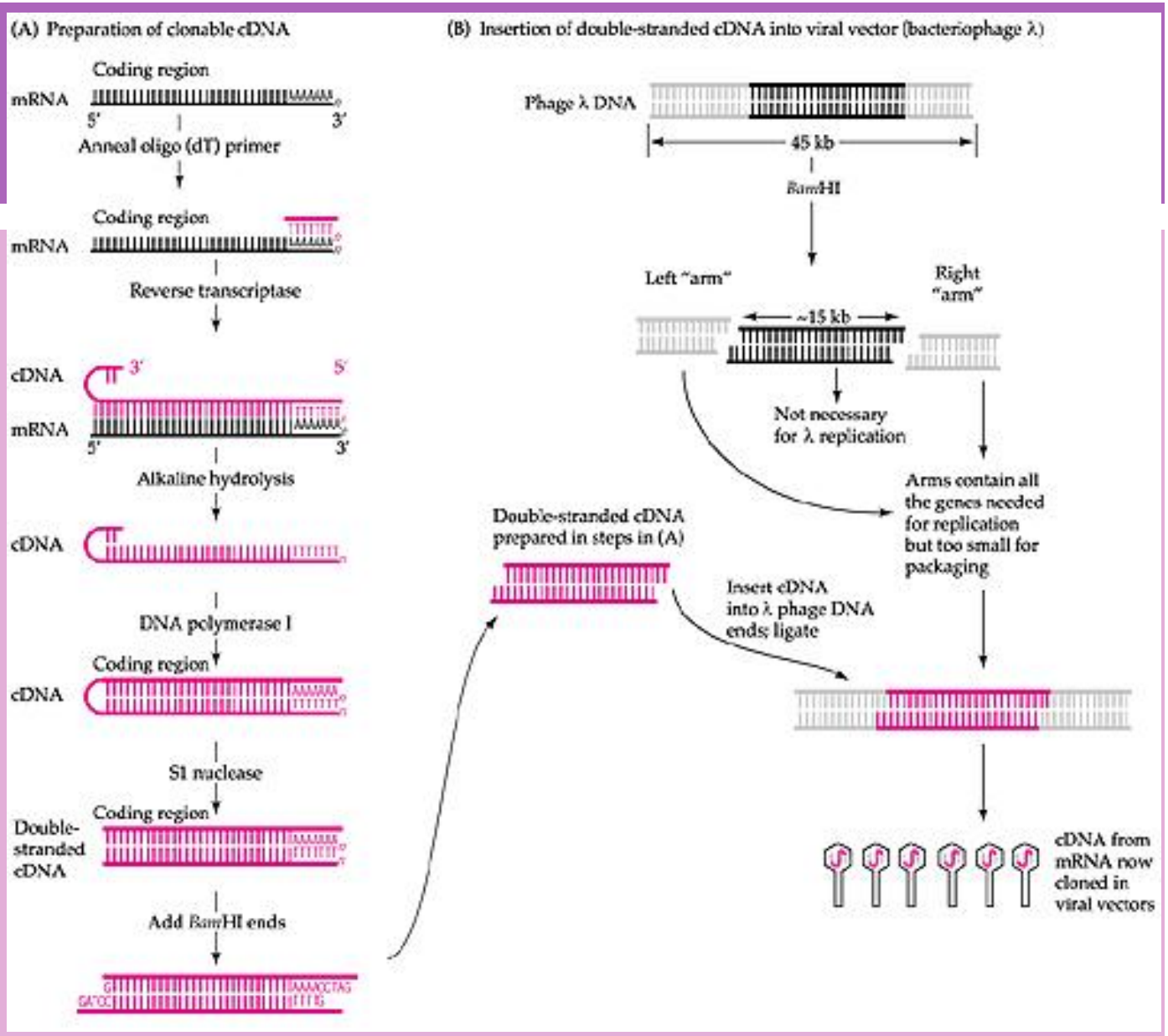
Are processed nucleic acid secuences harvested from the RNA pools of cells or tissues and represent all the cDNA sequence prepared at a certain time for genes expressed in certains cells or tisues.

DIFFERENCES BETWEEN GENOMIC AND COMPLEMENTARY DNA LIBRARIES

- The genomic library_contains DNA fragments representing the entire genome of an organism. More commonly used in bacterias.
- The c DNA library contains only complementary DNA molecules synthesized from mRNA molecules in a cell. The size of a c DNA clone needed to contain the complete gene is much smaller than the corresponding length of genomic DNA. Used for animals and other eukaryotes. Lack information about enhancers, introns, and other regulatory elements found in a genomic DNA library.

FIG. 01. FORMATION OF CDNA LIBRARIES




(A) Preparation of clonable cDNA
Coding region
mRNA
5'
3'
Anneal oligo (dT) primer
Coding region
mRNA
Reverse transcriptase
cDNA
3'
5'
mRNA
5'
3'
Alkaline hydrolysis
cDNA
DNA polymerase I
Coding region
cDNA
S1 nuclease
Coding region
Double-stranded cDNA
Add BamHI ends
(B) Insertion of double-stranded cDNA into viral vector (bacteriophage λ)
Phage λ DNA
45 kb
BamHI
Left "arm"
Right "arm"
~15 kb
Not necessary for λ replication
Arms contain all the genes needed for replication but too small for packaging
Double-stranded cDNA prepared in steps in (A)
Insert cDNA into λ phage DNA ends; ligate
cDNA from mRNA now cloned in viral vectors

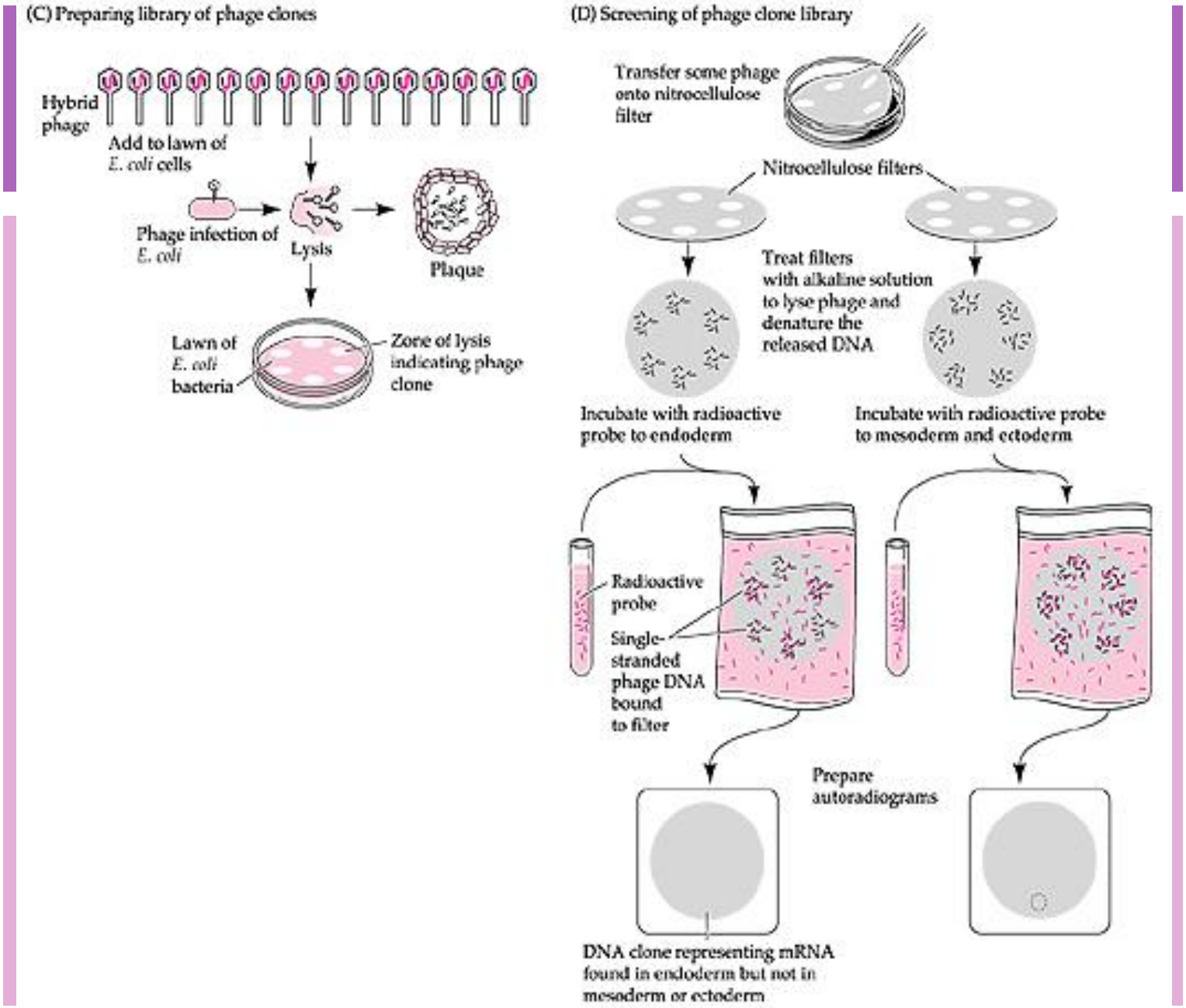

(C) Preparing library of phage clones
Hybrid phage
Add to lawn of E. coli cells
Phage infection of E. coli
Lysis
Plaque
Lawn of E. coli bacteria
Zone of lysis indicating phage clone
(D) Screening of phage clone library
Transfer some phage onto nitrocellulose filter
Nitrocellulose filters
Treat filters with alkaline solution to lyse phage and denature the released DNA
Incubate with radioactive probe to endoderm
Incubate with radioactive probe to mesoderm and ectoderm
Radioactive probe
Single-stranded phage DNA bound to filter
Prepare autoradiograms
DNA clone representing mRNA found in endoderm but not in mesoderm or ectoderm

TABLE 01. GENOMIC AND CDNA PROCESS OF CLONING LIBRARIES

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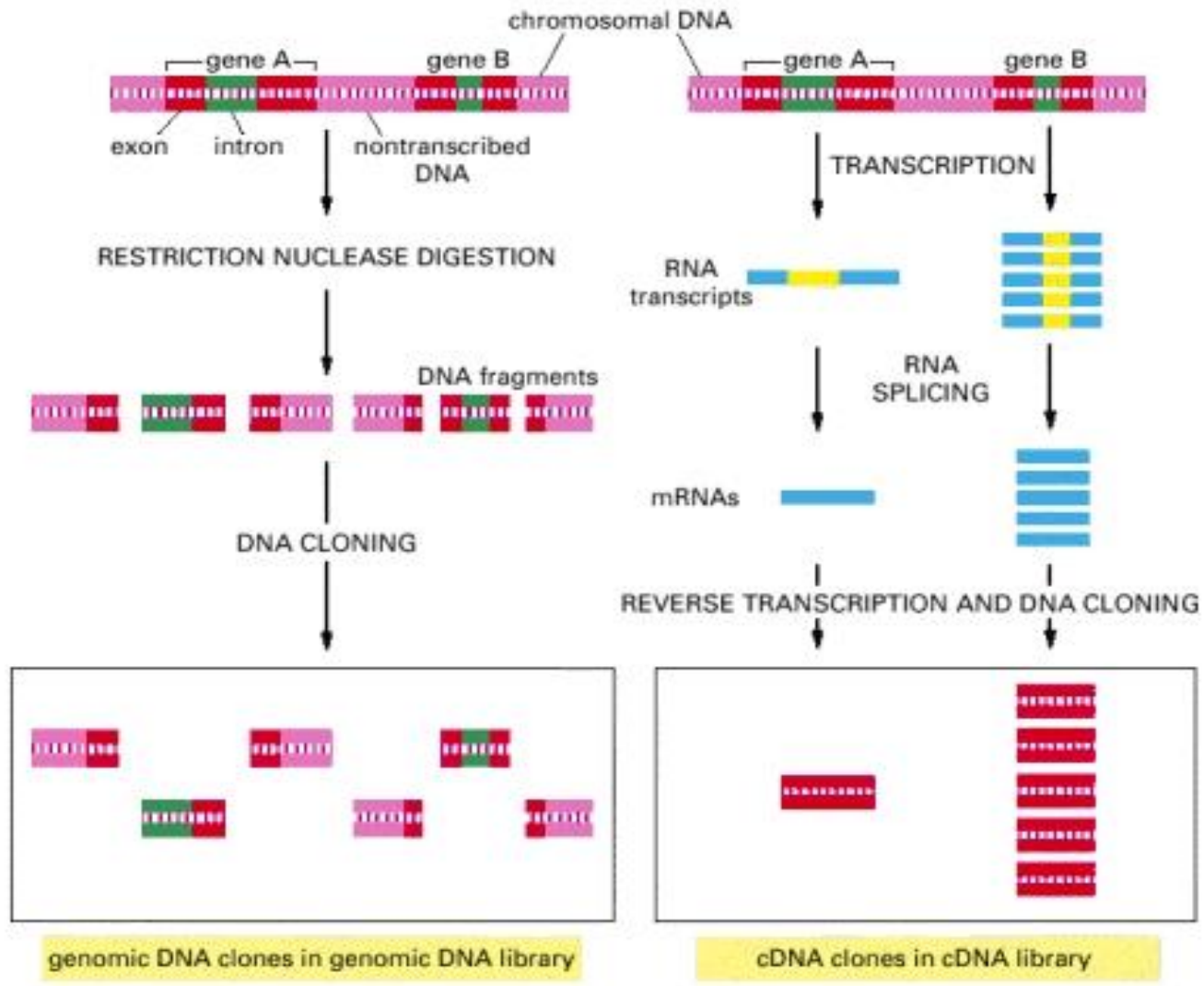

CDNA LIBRARY USES

- 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.
- 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 do 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. Also, it is useful for subsequently isolating the gene that codes for that mRNA.
- To monitor a large number of genes to provide a powerfull tool for assesing diferential mRNA expression levels for the identification of dissease-associated genes.

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MUCHAS GRACIAS POR SU ATENCION!