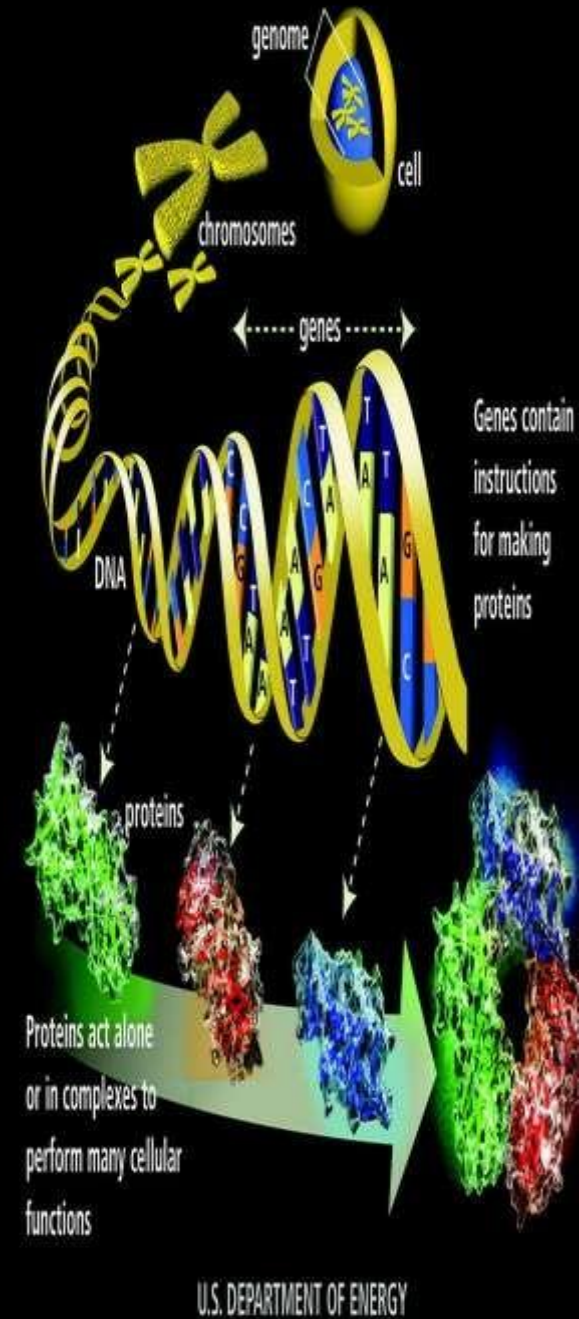


Library construction and screening

- A gene library is a collection of different DNA sequences from an organism,
- which has been Also called **genomic libraries** or **gene banks**.
- cloned into a vector for ease of purification, storage and analysis.



Uses of gene libraries

- To obtain the sequences of genes for analysis, **amplification, cloning, and expression**.
- Once the sequence is known probes, primers, *etc.* can be synthesized for further diagnostic work using, for example, **hybridization reactions, blots and PCR**.
- Knowledge of a gene sequence also offers the possibility of **gene therapy**.
- Also, gene expression can be used to **synthesize a product in particular host cells**, *e.g.* synthesis of human gene products in prokaryotic cells.

two types of gene library

depending upon the source of the DNA used.

1.genomic library.

2.cDNA library

Types of GENE library:

- **genomic library** contains DNA fragments representing the entire genome of an organism.
- **cDNA library** contains only complementary DNA molecules synthesized from mRNA molecules in a cell.

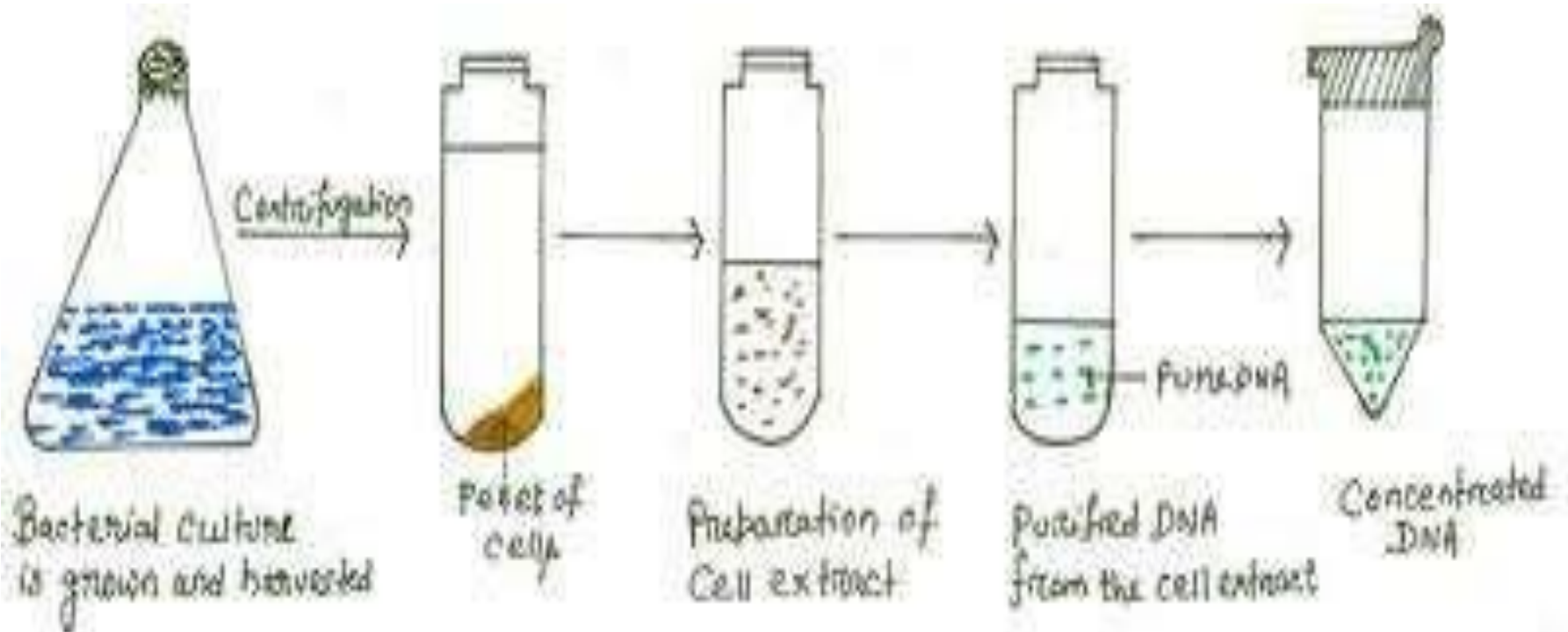
Genomic Library :

- Made from nuclear DNA of an organism or species.
- DNA is cut into clonable size pieces as randomly possible using **restriction endonuclease**
- Genomic libraries contain whole genomic fragments including gene **exons and introns, gene promoters, intragenic DNA, origins of replication, etc**

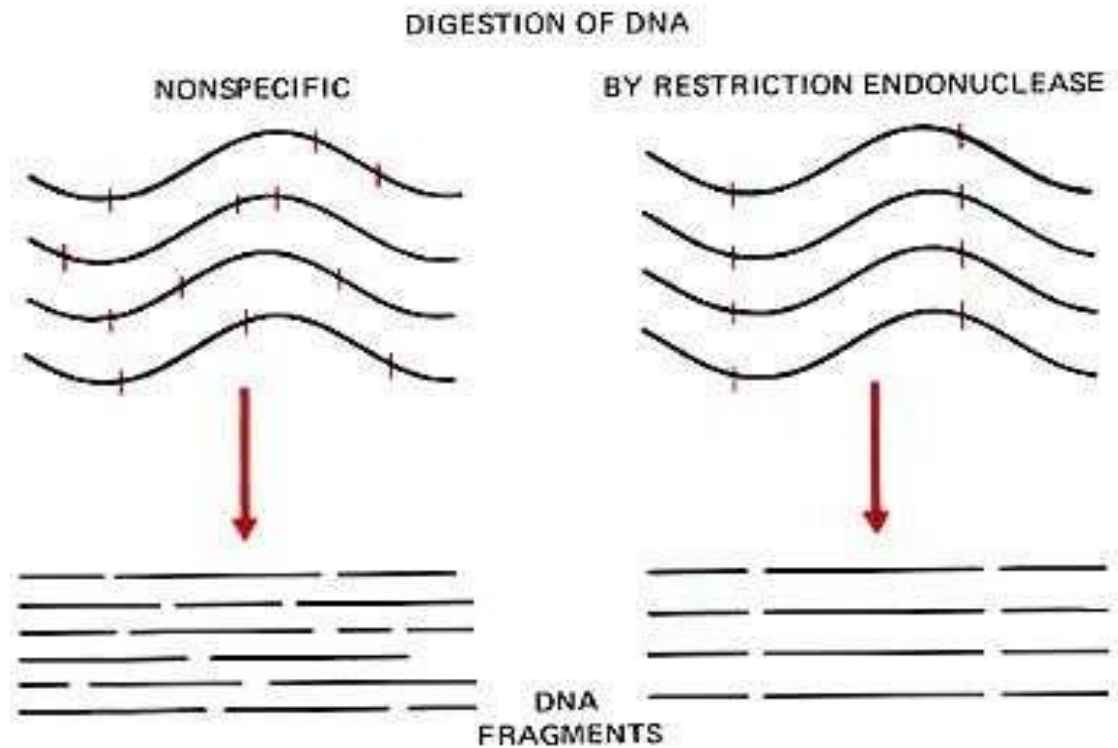
Construction of Genomic Libraries

1. Isolation of genomic DNA and vector.
2. Cleavage of Genomic DNA and vector by Restriction Endonucleases.
3. Ligation of fragmented DNA with the vector.
4. Transformation of r-DNA in the bacterial cell.
5. Amplification of the r-DNA in bacterial cells.

- Step1:- Extraction of genomic DNA

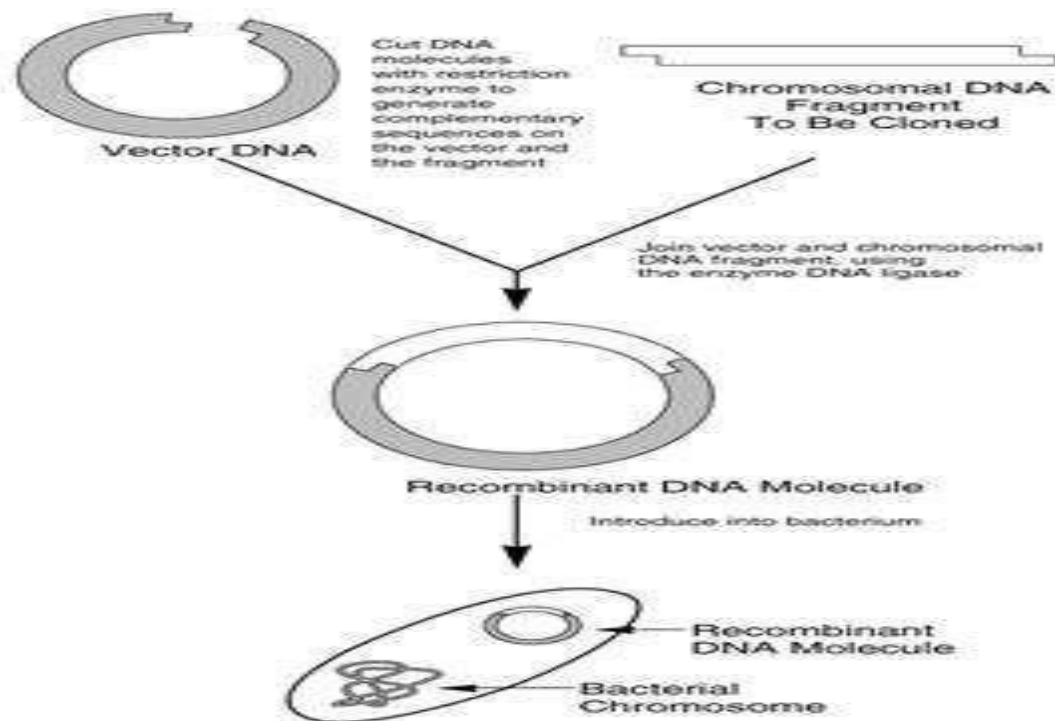


- Step2:- Cut with a restriction endonuclease enzyme



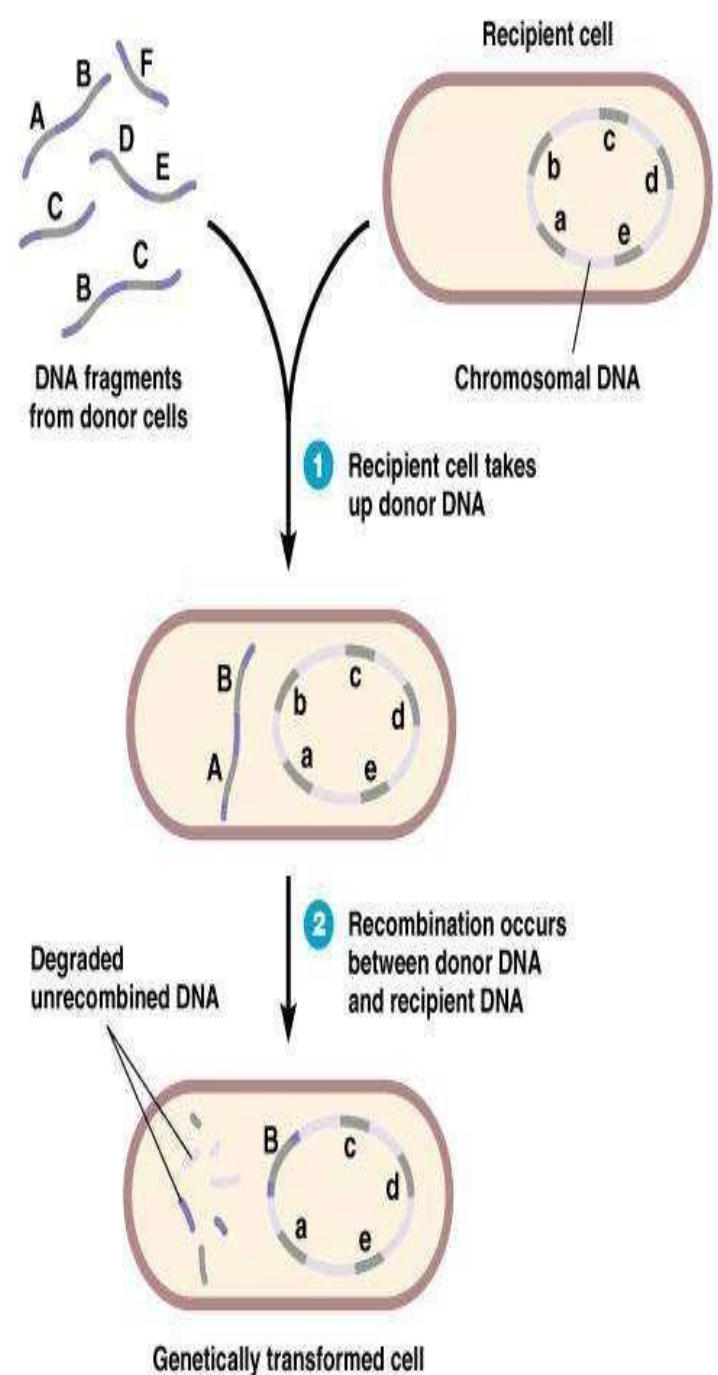
- Physical shearing (agitation or sonication)
- endonuclease enzymes (2 or more can be used)

- Step3:-Ligate the gene with the vector which cleaves by same enzyme



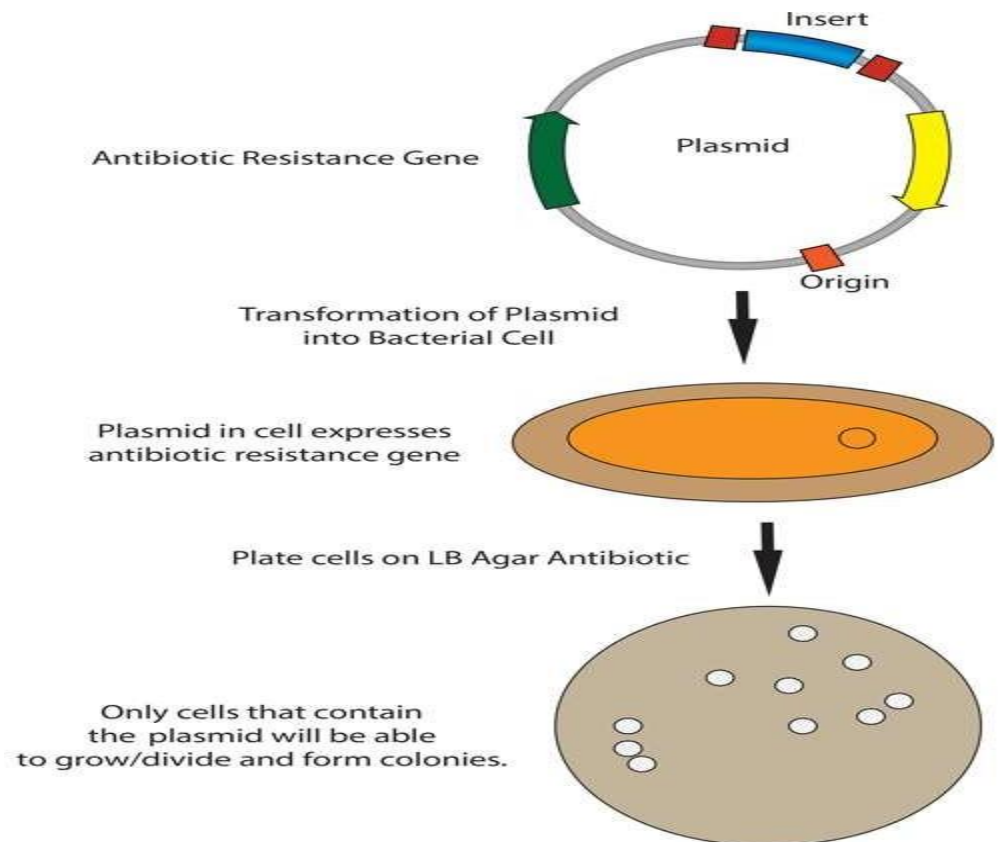
Step4:- transformation

- Transfer (transform) into bacteria Cells which are able to undergo this treatment are termed as competent cells
- CaCl_2 causes DNA to precipitate on the outside walls of bacterial cells.



Step5:- Amplification

- E.coli cells are grown in an agar medium containing ampicillin or tetracyclin at 37°C.



Screening of genomic library

- Once the genomic library has been created, it is screened to **identify the genes of interest**. One of the most common library screening technique is called **colony hybridization**.
- In the process of library construction, phage vectors are used then the process of identification of genes of interest involved is the **plaques hybridization**

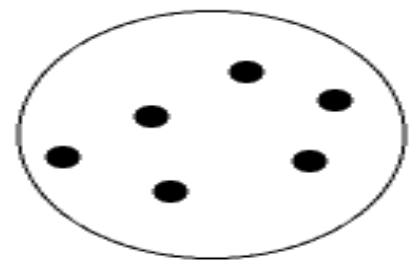
colony hybridization

- Colony Hybridization is the screening of library with a labeled probe (radioactive, etc.) to identify a specific sequence of DNA, RNA, enzyme, protein, or antibody.

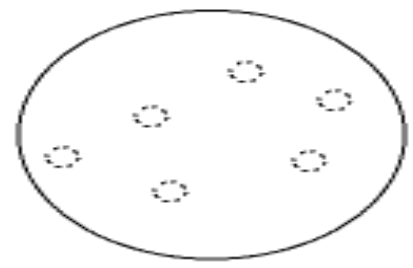
Replica plate onto nitrocellulose disk placed on agar in dish



Nitrocellulose disk removed



Break open bacteria with NaOH, neutralize, treat with protease, wash, bake at 80 C to immobilize DNA on disk



DNA print of colonies

Hybridize with radioactive probe, autoradiography

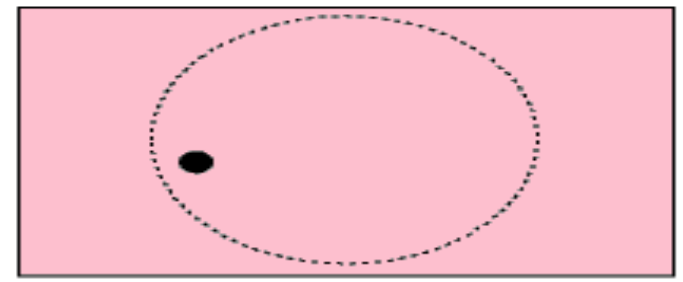


Transformant colonies growing on agar surface

Retain master plate



Reference set of colonies (Note: red colony has DNA complementary to the probe)



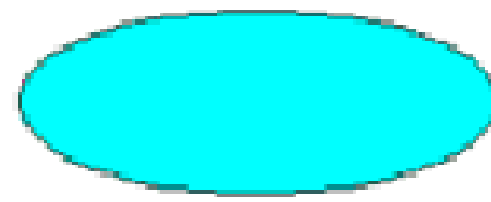
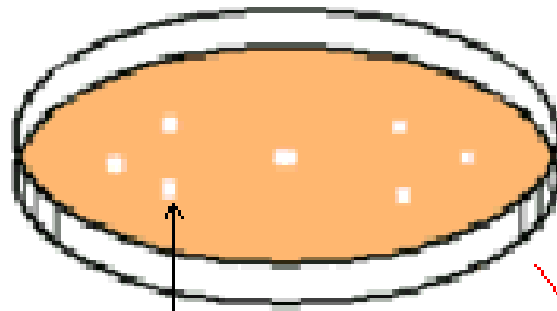
X-ray film

Plaque hybridization :

- The plaques are screened by a technique ,based on the hybridization of oligonucleotide probe to target DNA.
- DNA is transferred directly from the Petri dish to the filter,
- which is then incubated with labeled probes

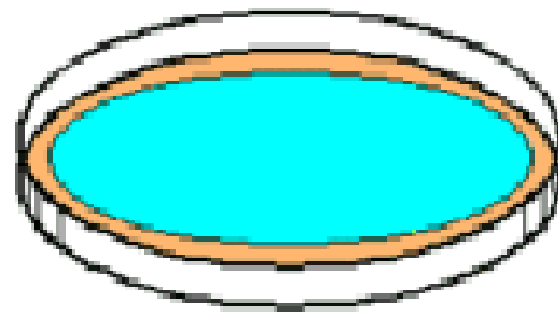
Master plate

Nitrocellulose filter

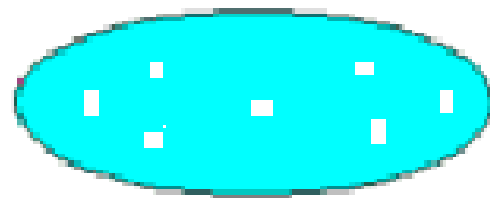


Phage plaque

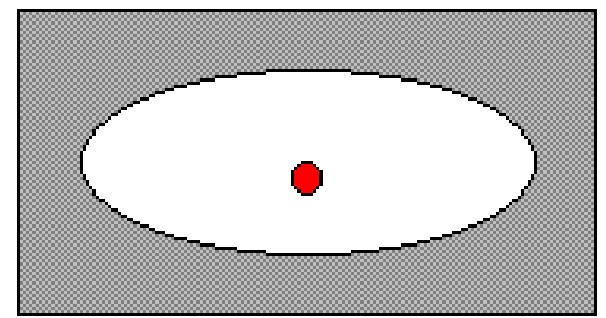
Place nitrocellulose filter on plate



Phages transferred to filter



Denature,
Hybridize with probe,
Perform autoradiography



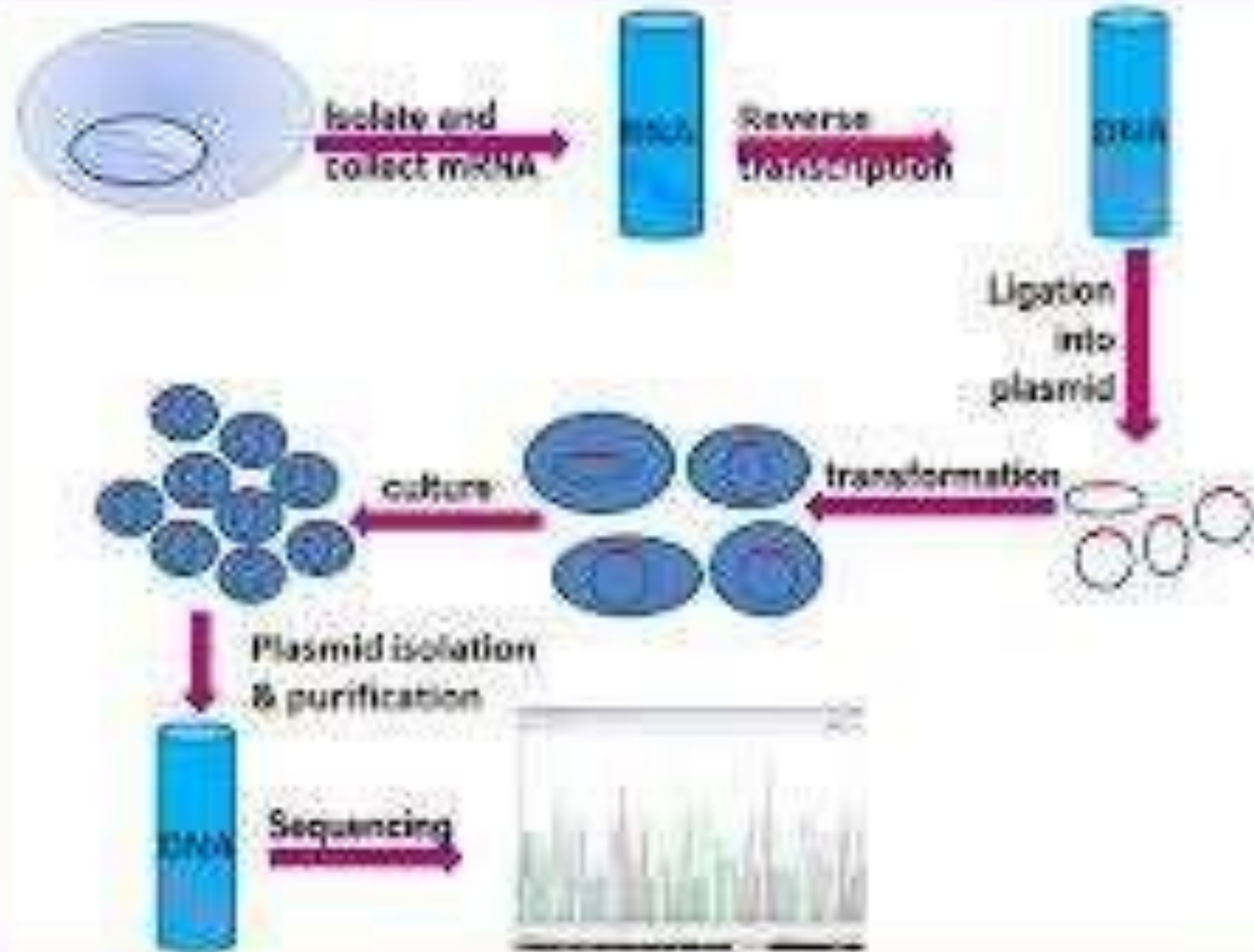
Applications of Genomic Library

- 1. Genomic library construction is the first step in any DNA sequencing projects.
- 2. Genomic library helps in identification of the novel pharmaceutically important genes.
- 3. Genomic library helps in identification of new genes which were silent in the host.
- 4. It helps us in understanding the complexity of genomes.

- 5. Serving as a source of genomic sequence for **generation of transgenic animals through genetic engineering** .
- 6. Study of the function of **regulatory sequences in vitro**.
- 7. Study of **genetic mutations in cancer tissues**.

cDNA library -

- complementary DNA molecules synthesized from mRNA molecules in a cell. ,



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

- 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 distinguishes mRNA from tRNA and rRNA and can therefore be used as a primer site for reverse transcription

Step S

1. mRNA extraction

2 methods

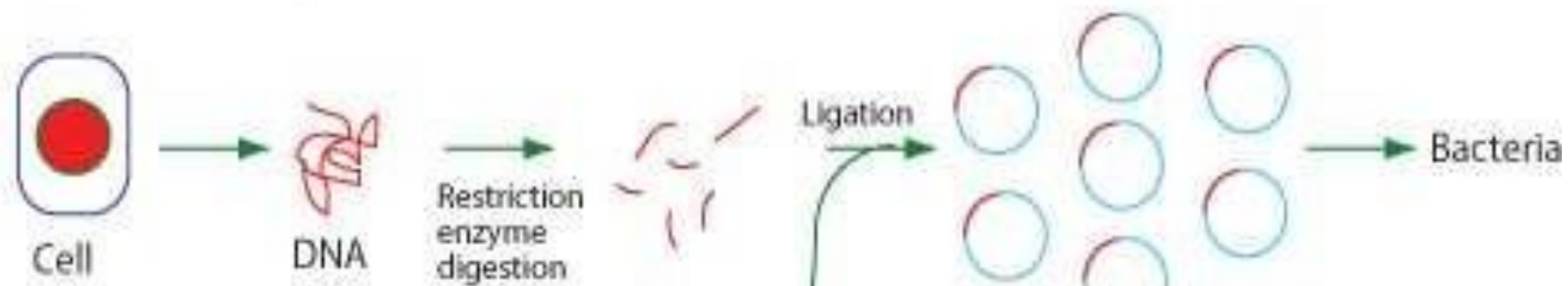
- [trizol](#) extraction
- [column purification.](#)
- ❖ by using oligomeric dT nucleotide coated resins where only the mRNA having the poly-A tail will bind.
- ❖ The rest of the RNAs are eluted out
- ❖ The mRNA is eluted by using eluting buffer and some heat to separate the mRNA strands from oligo-dT.

Once mRNA is purified,

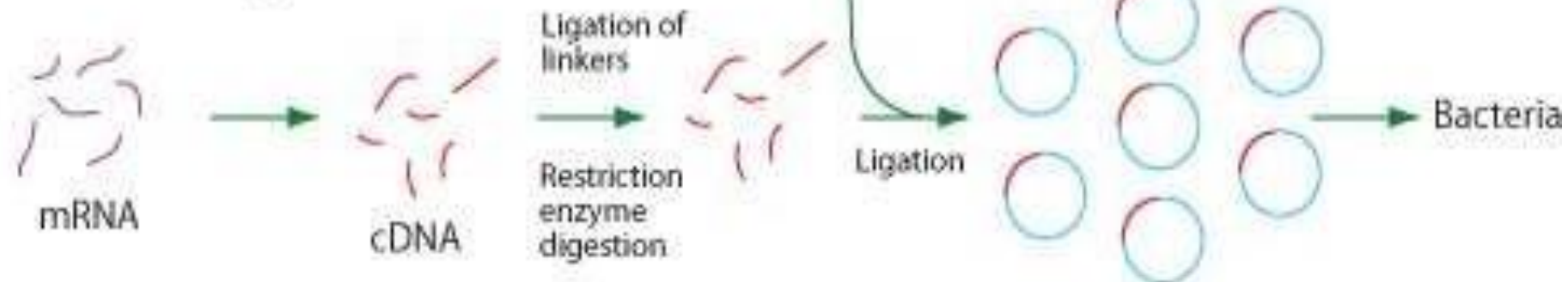
- *oligo-dT* is tagged as a complementary primer which binds to the poly-A tail providing a free 3'-OH end that can be extended by reverse transcriptase to create the complementary DNA strand.
- Now, the mRNA is removed by using a RNAse enzyme leaving a single stranded cDNA (sscDNA).
- This sscDNA is converted into a double stranded DNA with the help of DNA polymerase
- 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 hairpin loop 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 S₁ nuclease.
- 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 Library



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 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.

cDNA Library vs. Genomic DNA Library

- cDNA library lacks the non-coding and regulatory elements found in genomic DNA.
- [Genomic DNA libraries](#) provide more detailed information about the organism, but are more resource-intensive to generate and maintain.



Thank You!