Lecture-
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COURSE BSc (BIOTECHNOLOGY) III YEAR

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PAPER TITLE: RECOMBINANT DNA TECHNOLOGY

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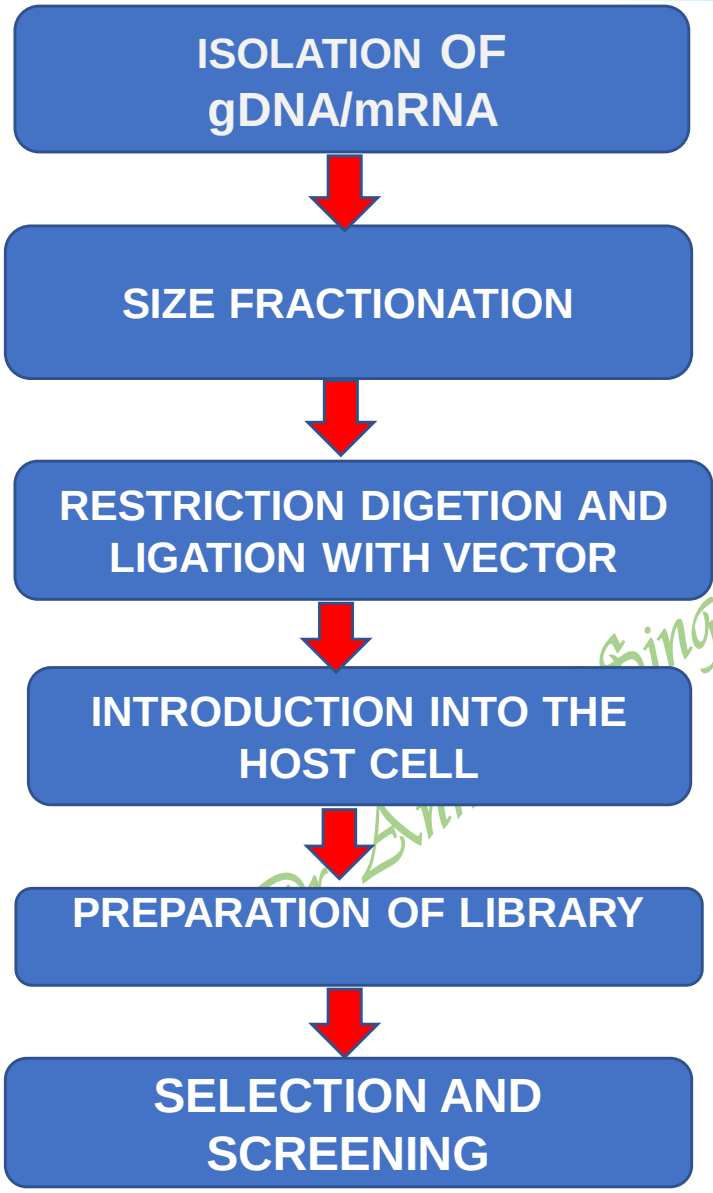
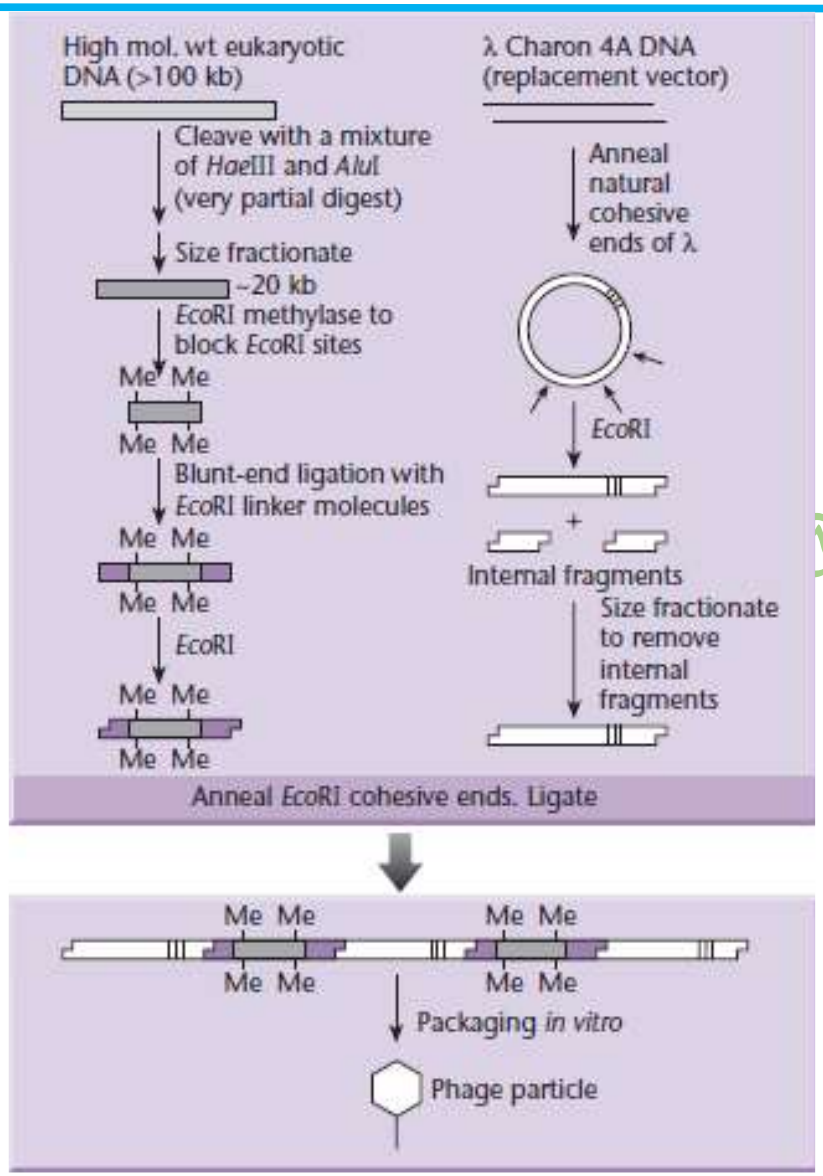
DNA Libraries



- There are two major approaches for isolating sequences from complex sources such as genomic DNA or cDNA
- A collection of clones, representative of the entire starting population, is known as a *library*.
- **Genomic DNA libraries** are generated by fragmenting the genome and cloning overlapping fragments in vectors
- **Complementary DNA (cDNA) libraries** are generated by the reverse transcription of mRNA
- cDNA is representative of the mRNA population, and therefore reflects mRNA levels and the diversity of splice isoforms in particular tissues

Genomic DNA Library



* Maniatis' strategy for producing a representative gene library.

- A *Genomic Library is* a very large number of recombinants, which together contain a complete collection of all of the DNA sequences in the entire genome.
- Clarke & Carbon (1976) derived a formula that relates the probability (*P*) of including any DNA sequence in a random library of *N* independent recombinants:

$$N = \frac{\ln(1-P)}{\ln\left(1-\frac{1}{n}\right)}$$

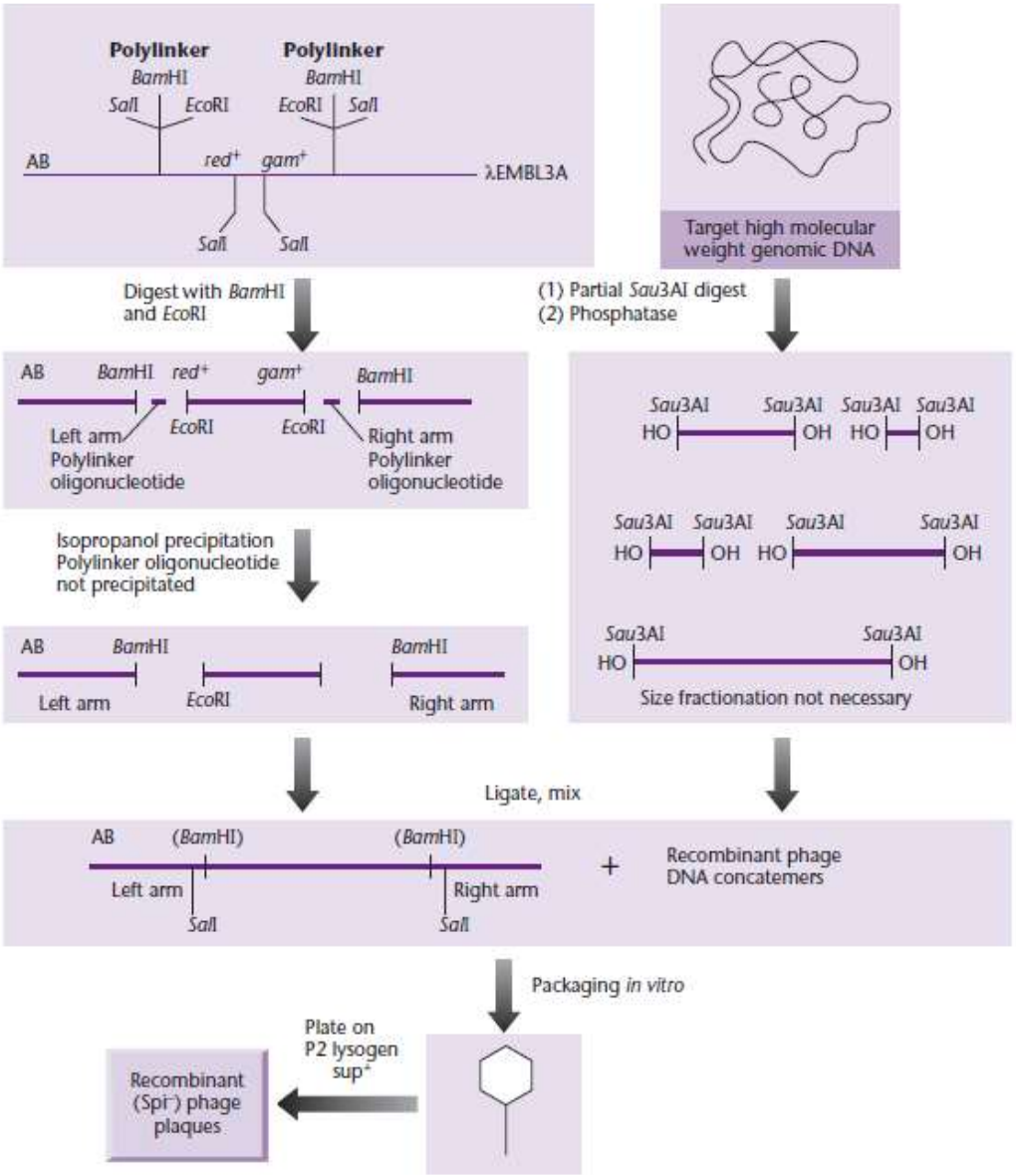
**n* be the size of the genome relative to a single cloned fragment.

- to achieve a 95% probability (*P* = 0.95) of including any particular sequence in a random human genomic DNA library of 20 kb fragment size:

$$N = \frac{\ln(1-0.95)}{\ln\left(1-\frac{1}{1.4\times10^5}\right)} = 4.2\times10^5$$

human genome (2.8 × 106 kb) and an average cloned fragment size of 20 kb, $n = 1.4 \times 10^5$

DNA Libraries



- Creation of a genomic DNA library using the phage-λ vector EMBL3A. High-molecular-weight genomic DNA is
- partially digested with *Sau*3AI. The fragments are treated with phosphatase to remove their 5′ phosphate groups.
- The vector is digested with *Bam*HI and *Eco*RI, which cut within the polylinker sites.
- The tiny *Bam*HI/*Eco*RI polylinker fragments are discarded in the isopropanol precipitation, or alternatively the vector arms may be purified by preparative agarose gel electrophoresis.
- The vector arms are then ligated with the partially digested genomic DNA. The phosphatase treatment prevents the genomic DNA fragments from ligating together.
- Non-recombinant vector cannot reform because the small polylinker fragments have been discarded.
- The only packageable molecules are recombinant phages. These are obtained as plaques on a P2 lysogen of *sup*+ *E. coli*.
- The Spi− selection ensures recovery of phage lacking *red* and *gam* genes.
- A *sup*+ host is necessary because, in this example, the vector carries amber mutations in genes *A* and *B*.
- These mutations increase biological containment, and can be applied to selection procedures, such as recombinational selection, or tagging DNA with a *sup*+ gene.

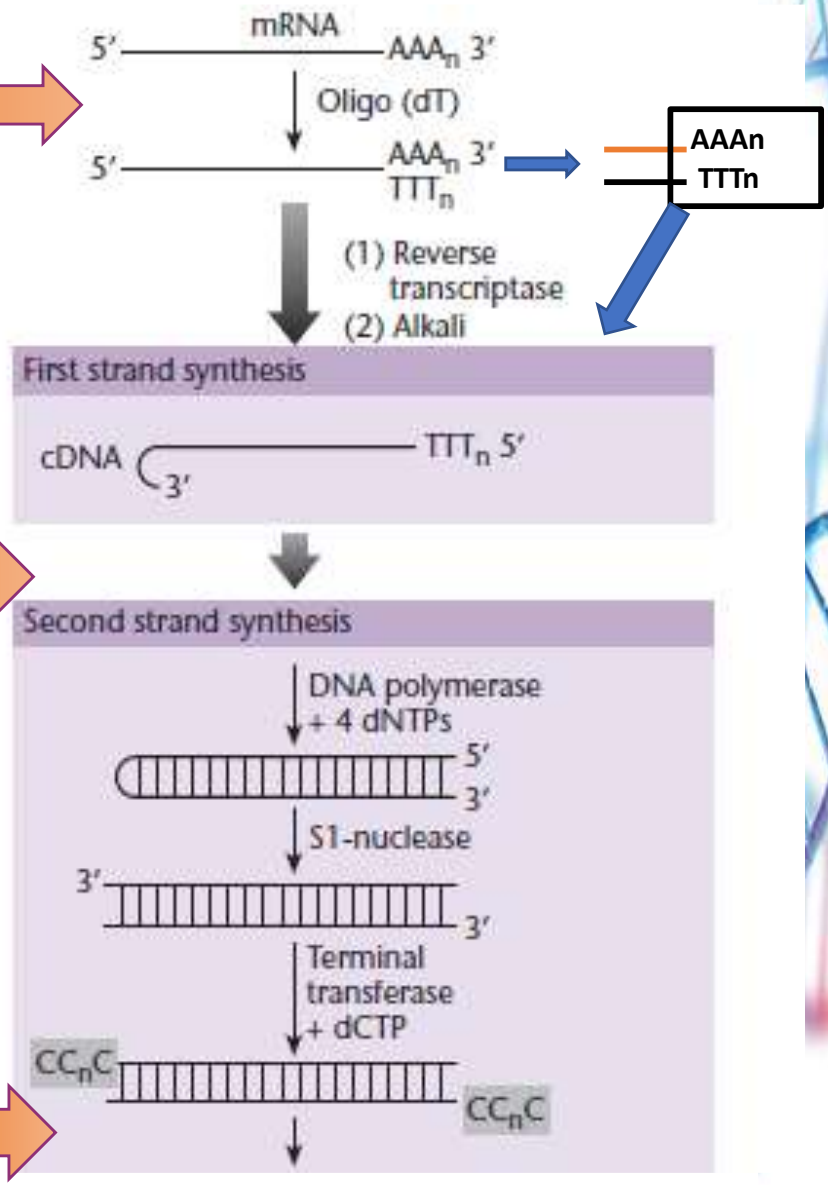
CONTRUCTION OF cDNA LIBRARY

Isolation of mRNA

- cDNA library represents mRNA population present at a particular stage in a organism into multiple clones containing small DNA fragments.

Preparation of complementary DNA fragments-

Cloning in suitable vector system And Transformation in suitable host



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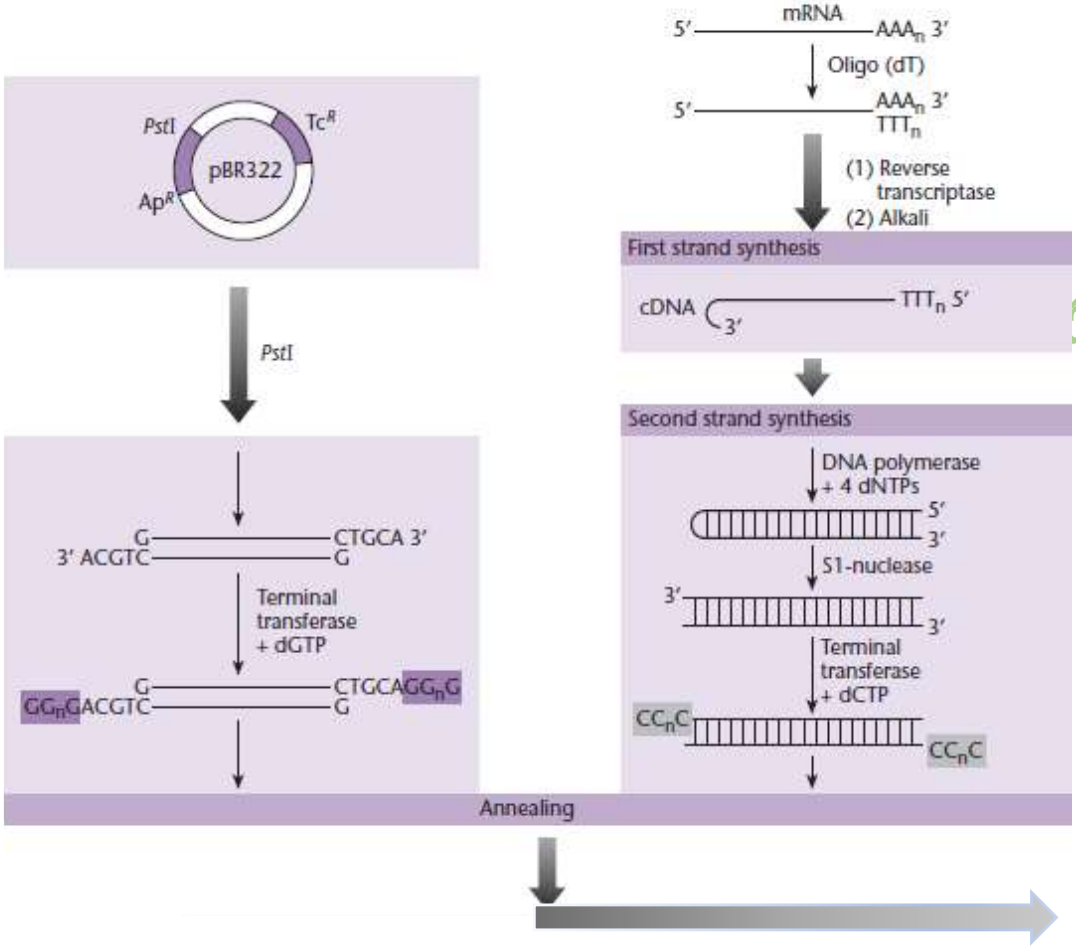
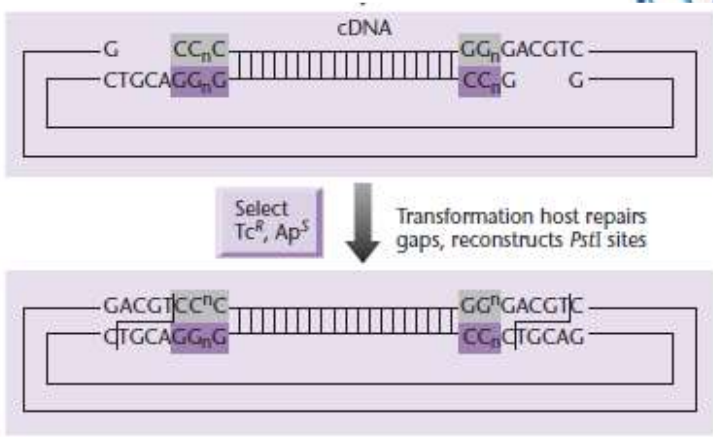




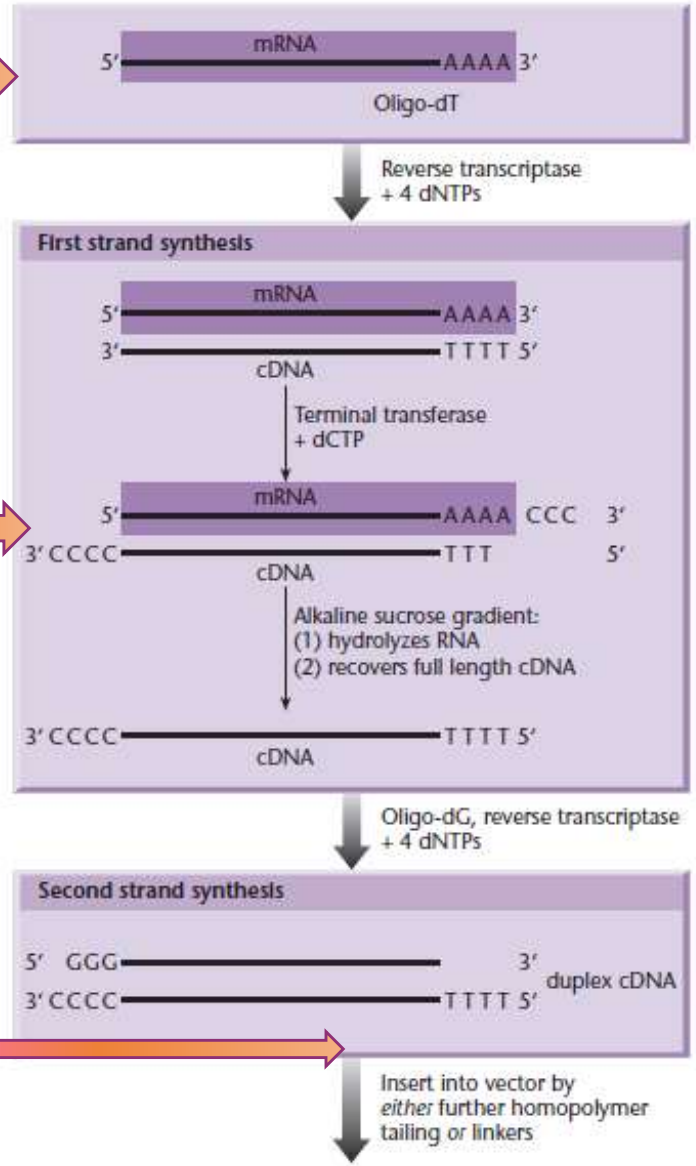
- cDNA cloning strategy, involving hairpinprimed second-strand DNA synthesis and homopolymer tailing to insert the cDNA into the vector.

CONTRUCTION OF cDNA LIBRARY

Isolation of mRNA

Preparation of complementary DNA fragments-

Cloning in suitable vector system And Transformation in suitable host



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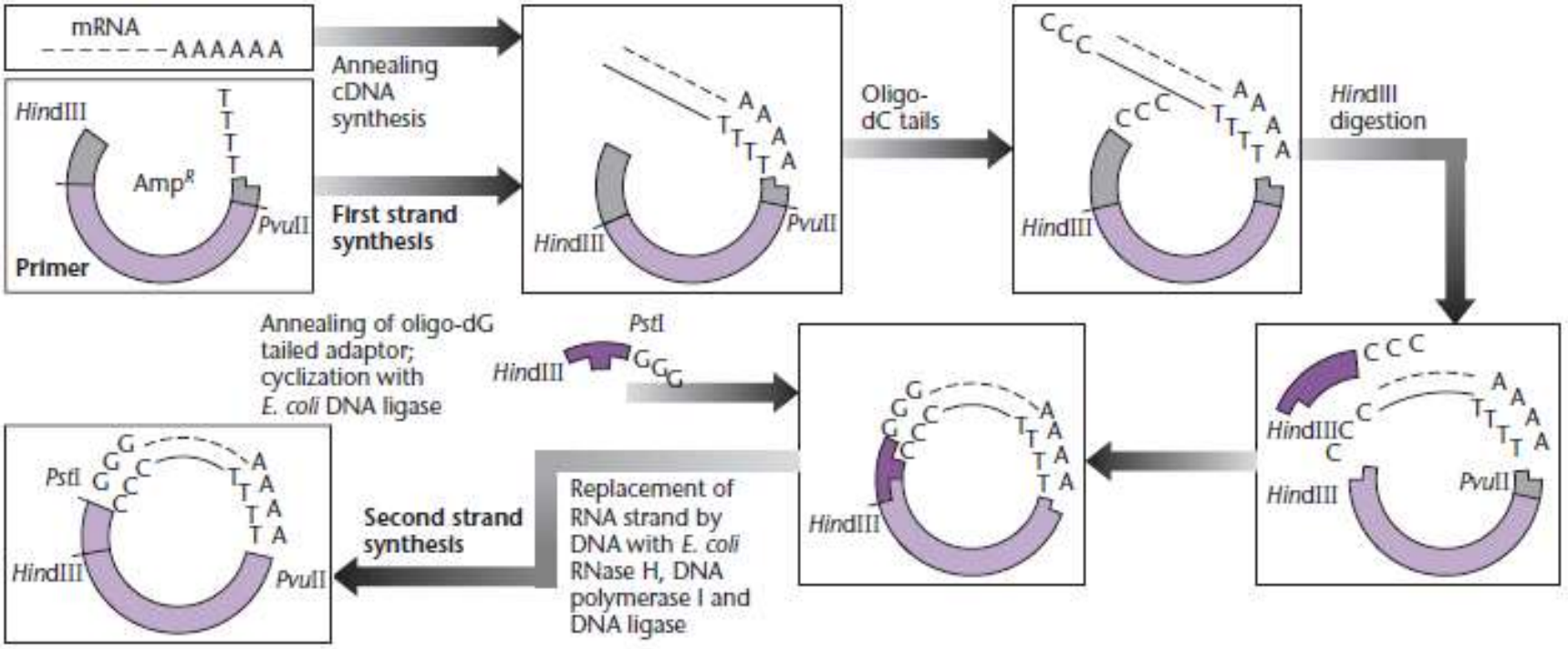
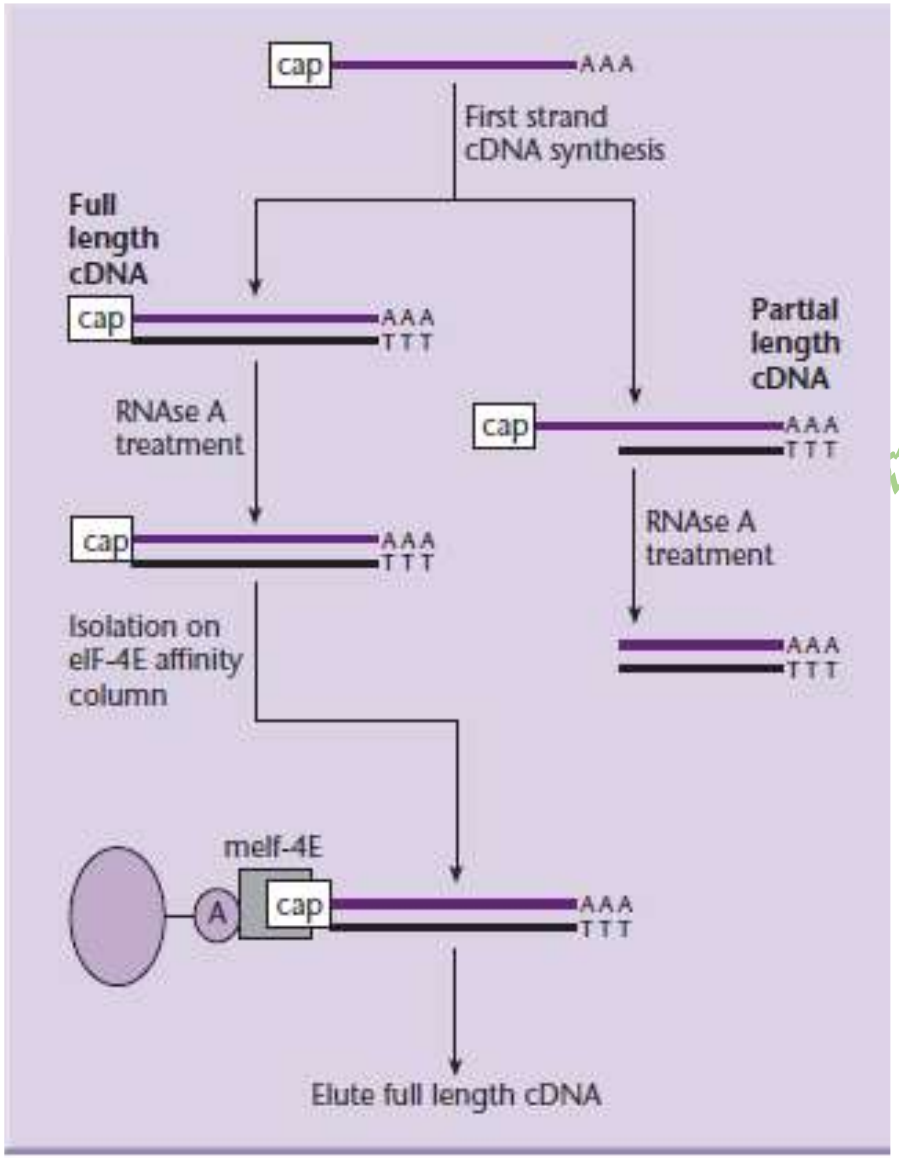


The strategy of Okayama & Berg (1982), where the mRNA is linked unidirectionally to the plasmid cloning vector prior to cDNA synthesis, by virtue of a cDNA tail.

CONTRUCTION OF cDNA LIBRARY



- The CAPture method of full-length cDNA cloning, using the eukaryotic initiation factor eIF-4E to select mRNAs with caps protected from RNase digestion by a complementary DNA strand.

The PCR can be used as an alternative to cDNA cloning

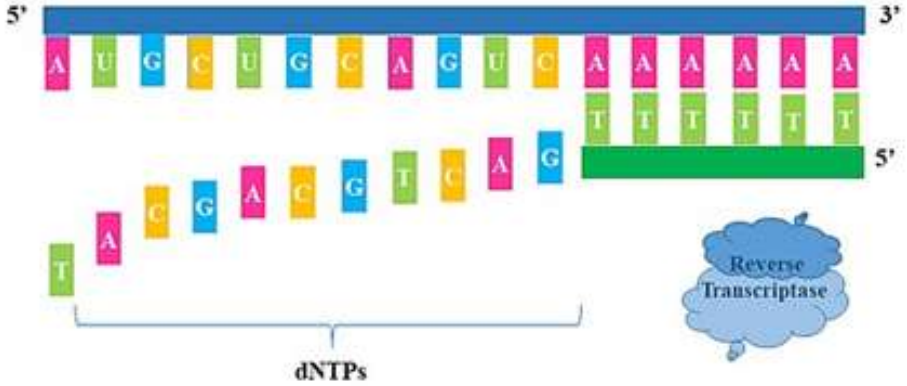
- Reverse transcription followed by the polymerase chain reaction (RT-PCR) leads to the amplification of RNA sequences in cDNA form.
- Using gene-specific primers, RT-PCR is a sensitive means for detecting, quantifying, and cloning specific cDNA molecules.
- Reverse transcription is carried out using a specific 3′ primer that generates the first cDNA strand, and then PCR amplification is initiated following the addition of a 5′ primer to the reaction mix.
- The sensitivity is such that total RNA can be used as the starting material, rather than the poly(A)+ RNA which is used for conventional cDNA cloning.
- Due to the speed with which RT-PCR can be carried out, it is an attractive approach for obtaining a specific cDNA sequence for cloning.
- Instead of gene-specific primers, universal primers can be used to amplify all mRNAs, which can then be subcloned into suitable vectors.
- A disadvantage of PCR-based cDNA library construction is that the DNA polymerases used for PCR are more error-prone than those used conventionally for second-strand synthesis,

Reverse transcription polymerase chain reaction (RT-PCR)

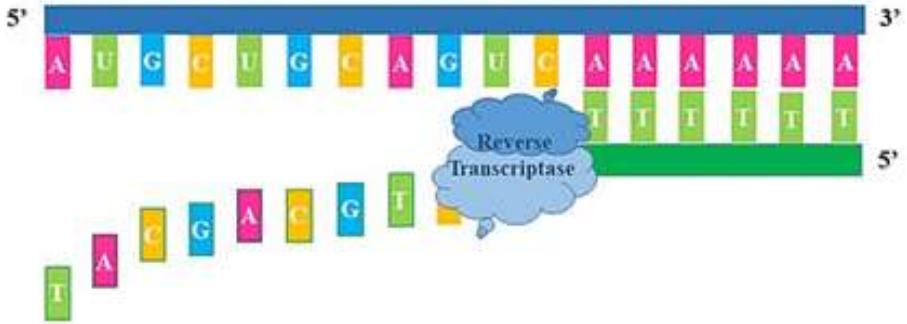
1 a. **RNA**
RNA consist of Start codon AUG and ends with poly A tail

b. **Oligo dT Primer**
Oligo dT Primer is binding to RNA poly A tail

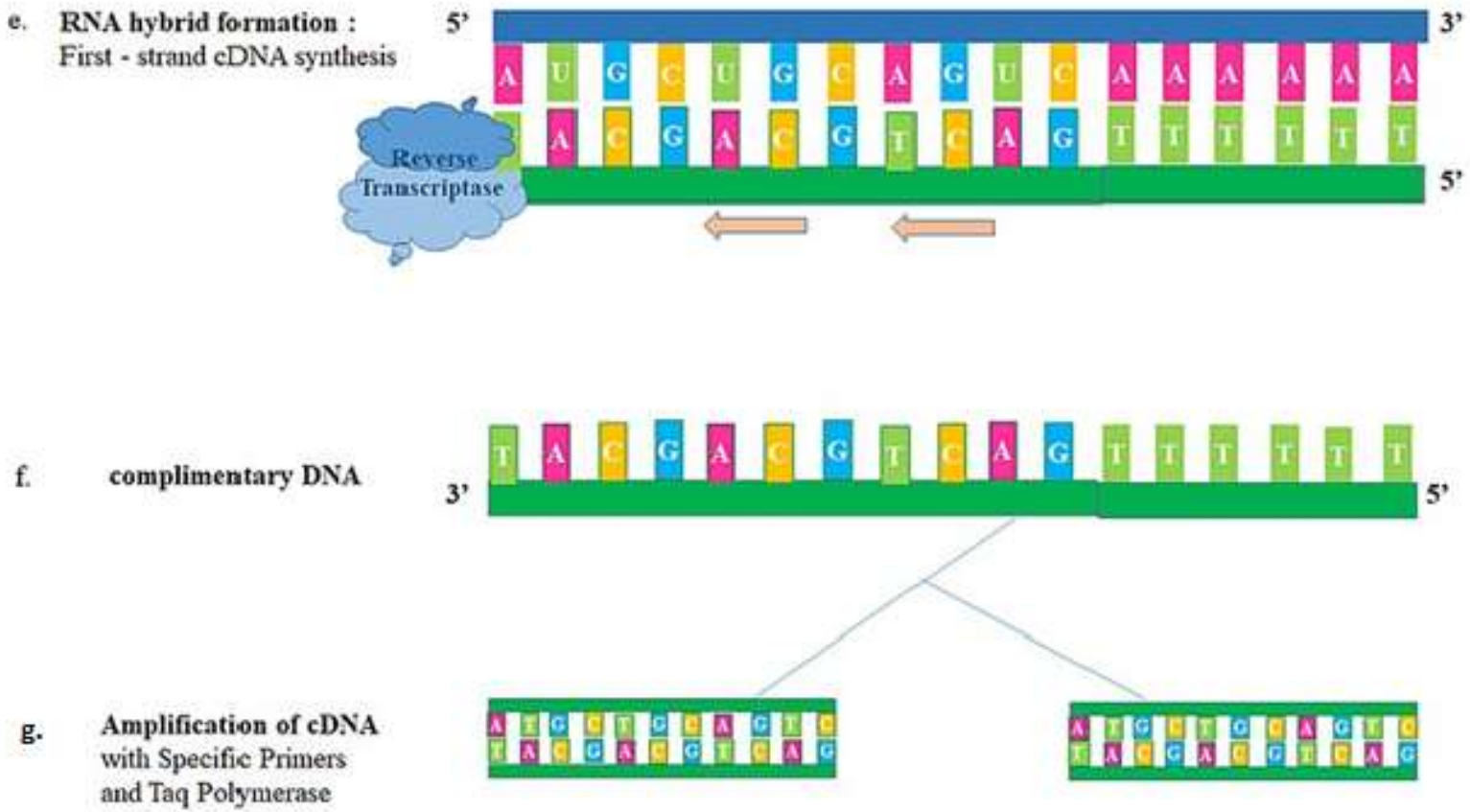
c. **Reverse Transcriptase and dNTPs**



d. **Reverse Transcriptase** is an enzymes binds to oligo dT primer and synthesises the cDNA by adding dNTPs



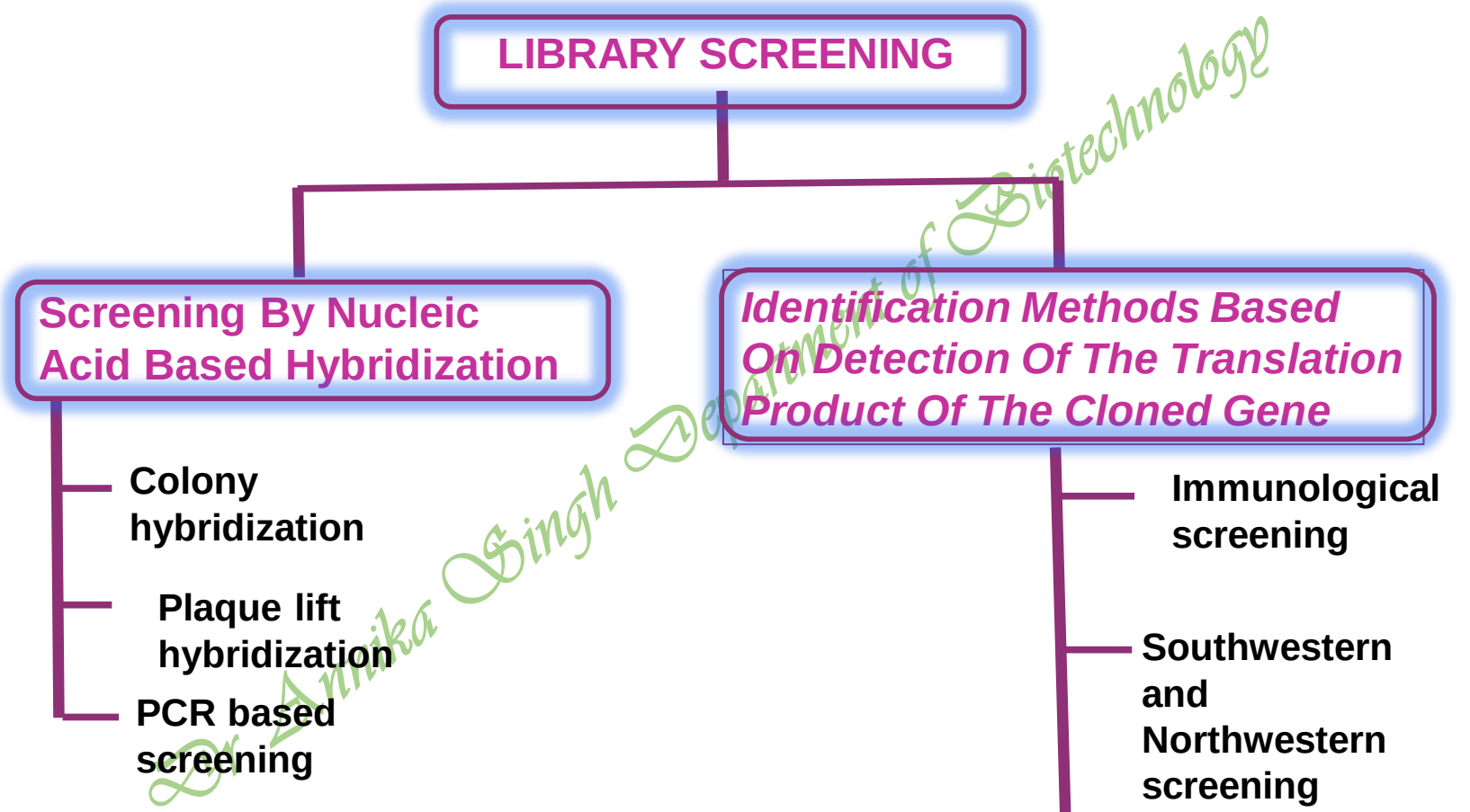
Reverse transcription polymerase chain reaction (RT-PCR)



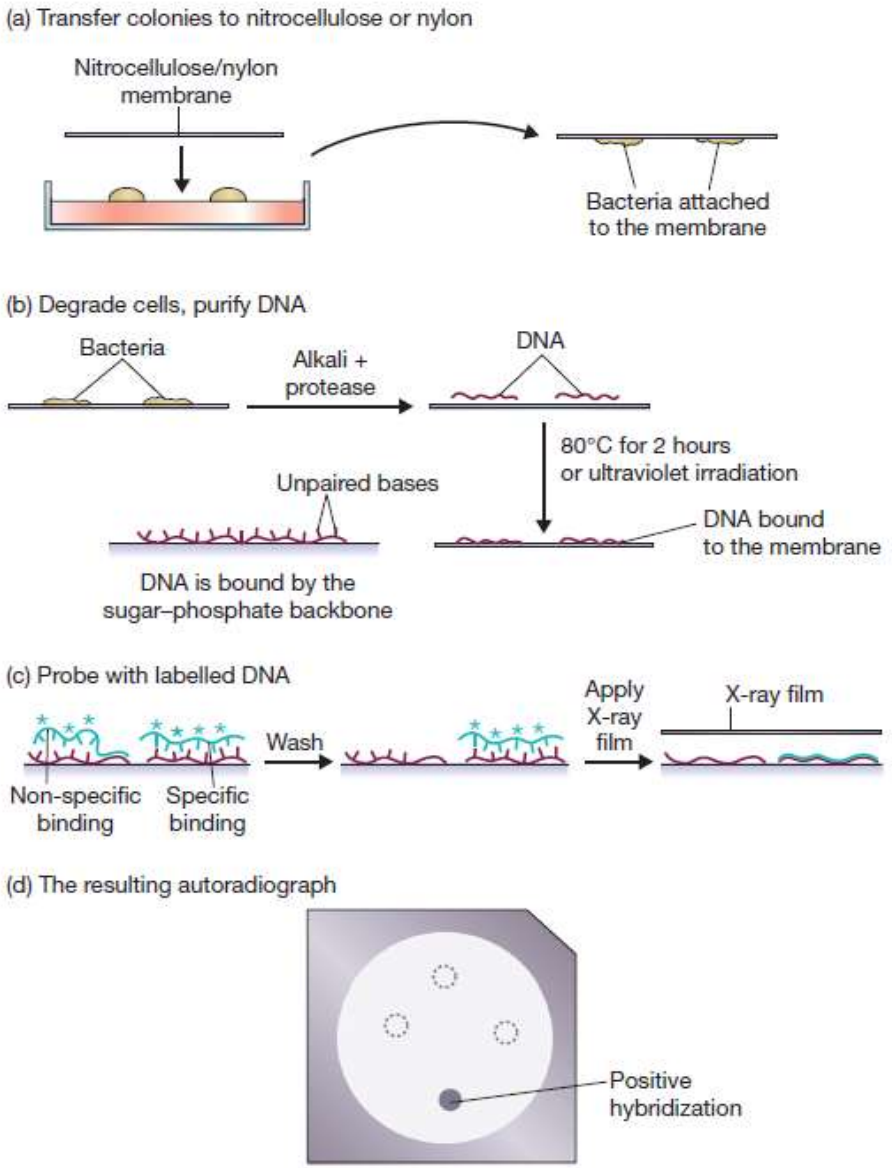
Rapid amplification of cDNA ends (RACE)

- **Rapid amplification of cDNA ends** (**RACE**) is a technique used in molecular biology to obtain the full length sequence of an RNA transcript
- RACE results in the production of a cDNA copy of the RNA sequence of interest, produced RT-PCR. The amplified cDNA copies are then sequenced
- RACE is commonly followed up by cloning before sequencing of what was originally individual RNA molecules.
- A more high-throughput alternative which is useful for identification of novel transcript structures, is to sequence the RACE-products by next generation sequencing technologies.
- RACE can provide the sequence of an RNA transcript from a small known sequence within the transcript to the 5' end (5' RACE-PCR) or 3' end (3' RACE-PCR) of the RNA.
- This technique is sometimes called *one-sided PCR* or *anchored PCR*.
- The first step in RACE is to use reverse transcription to produce a cDNA copy of a region of the RNA transcript, an unknown end portion of a transcript is copied using a known sequence from the center of the transcript.
- The copied region is bounded by the known sequence, at either the 5' or 3' end.

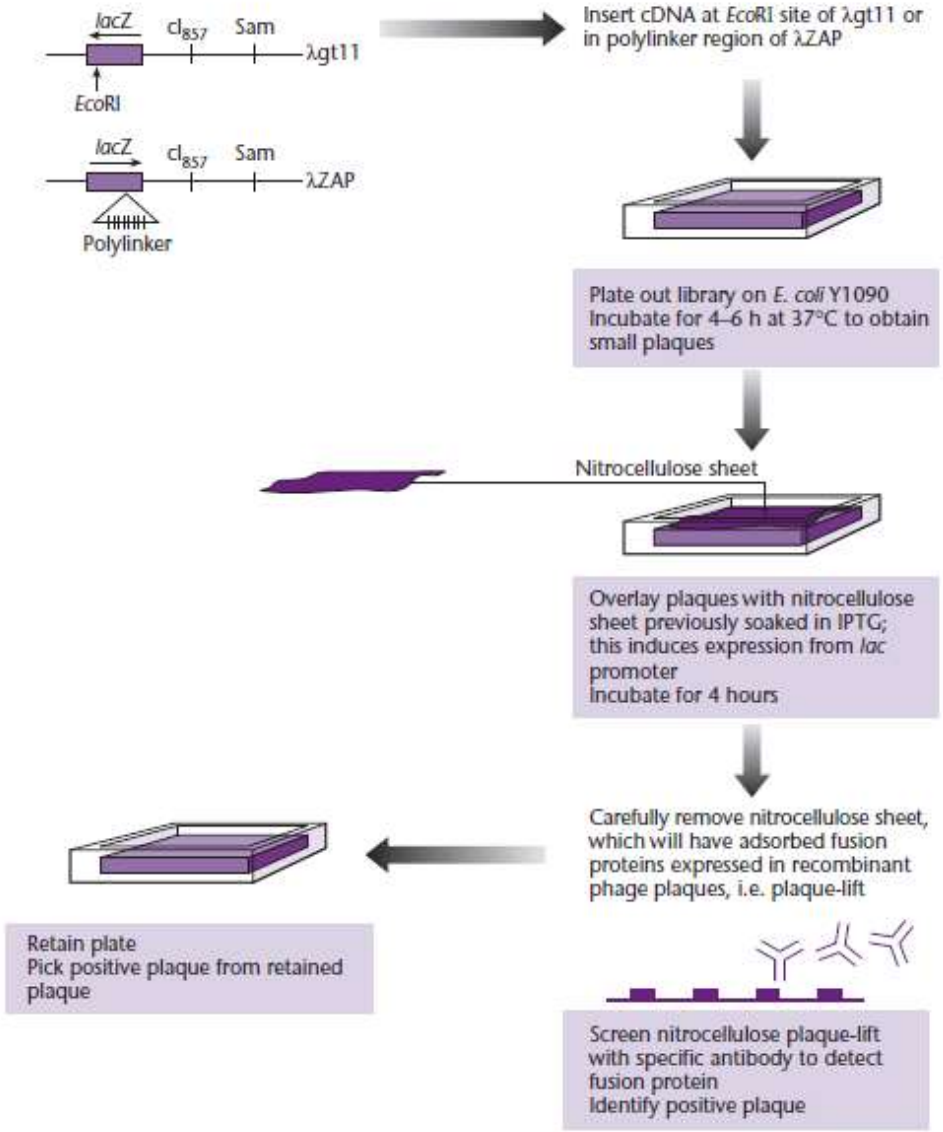
DIFFERENT STRATEGIES FOR LIBRARY SCREENING



Colony hybridization



Immunological screening



Immunochemical screening of λgt11 or λZAP recombinant plaques.