Genomic and cDNA Library

Programme: B.Sc (H) Botany
Course Title: Plant Biotechnology
Course code: BOTY 3014

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Genomic library

- A genomic library is a collection of clones sufficient in number to contain all of the DNA present in a particular organism.

Genomic library

- An *E. coli* genomic library, for example, contains all the *E. coli* genes as well as the non coding region of *E. coli* genome, so any desired gene can be withdrawn from the library and studied.

- Genomic libraries are prepared by purifying total cell DNA, and then making a partial restriction digest, resulting in fragments that can be cloned into a suitable vector, usually a λ replacement vector, a cosmid, or possibly an yeast artificial chromosome (YAC), bacterial artificial chromosome (BAC) or P1 vector.

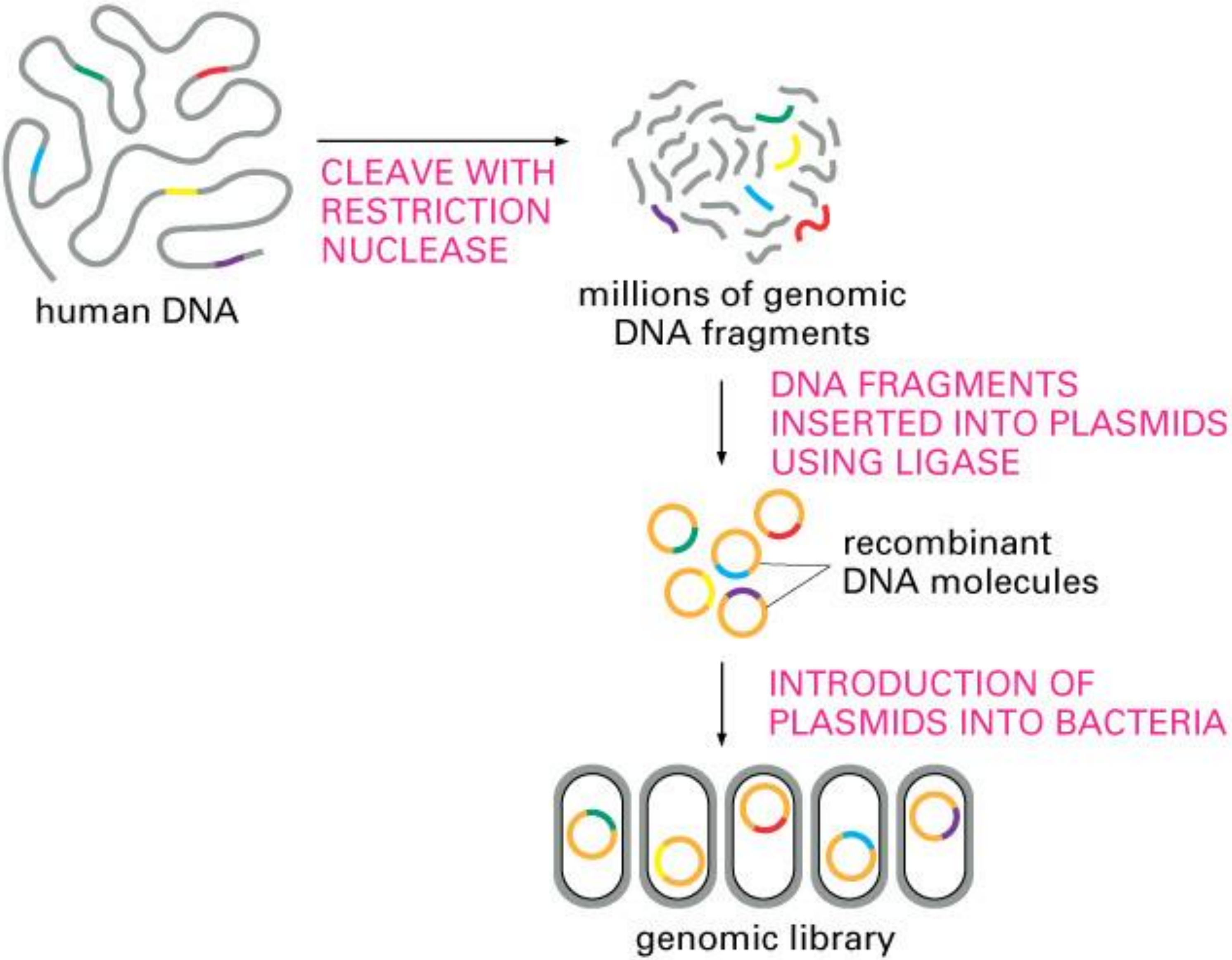


Figure 10-23 **Essential Cell Biology, 2/e. (© 2004 Garland Science)**

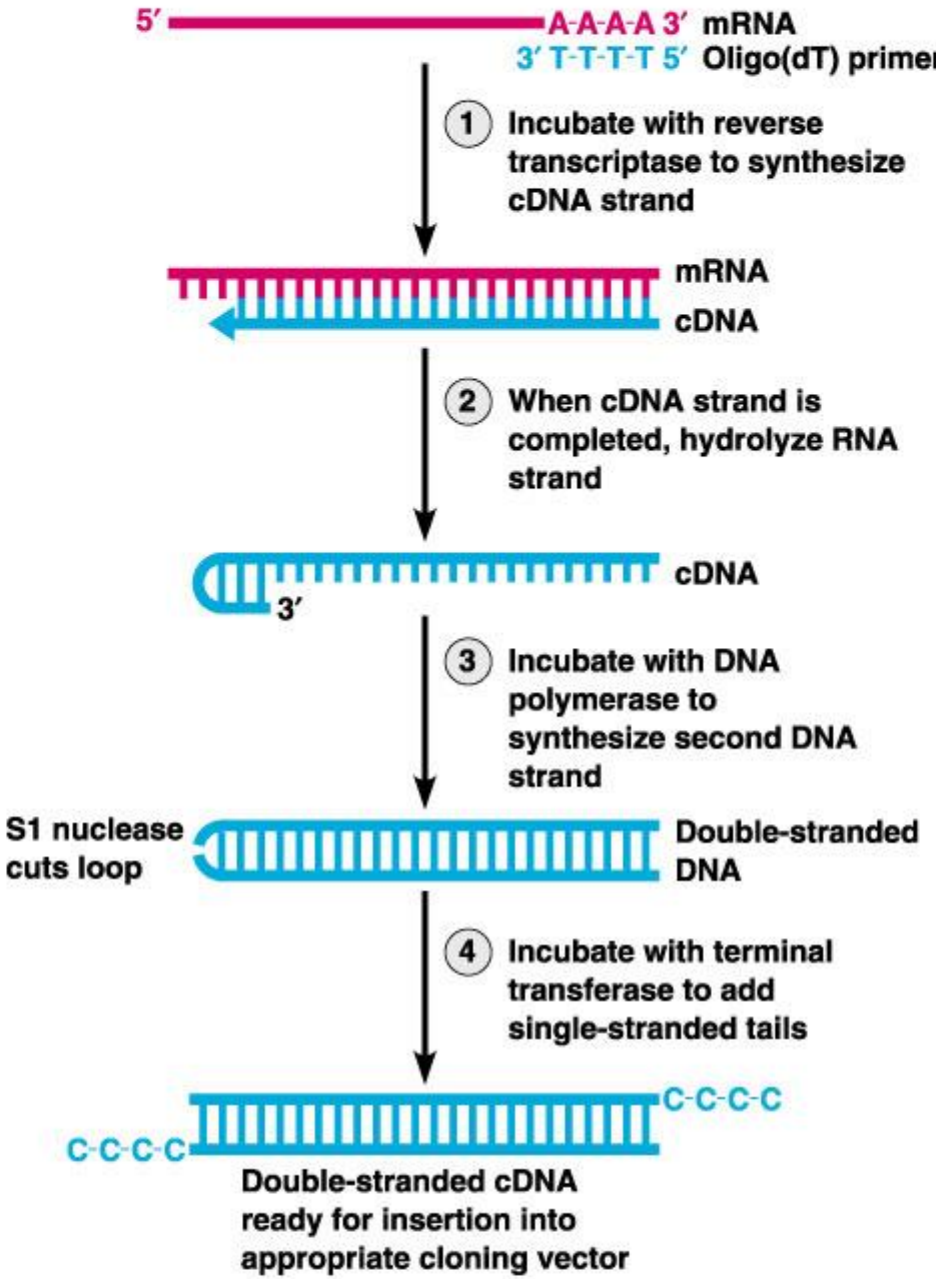
- Genomic libraries (colonies containing the fragment of DNA) can be retained for many years, and propagated so that copies can be sent from one research group to another.

- For bacteria, yeast, and fungi, the number of clones needed for a complete genomic library is not so large as to be unmanageable.
- But with plants and animals it becomes so large that identifying a desired gene becomes very difficult.

- With these organisms a second type of library, specific not to the whole organism but to a particular cell type, may be more useful.

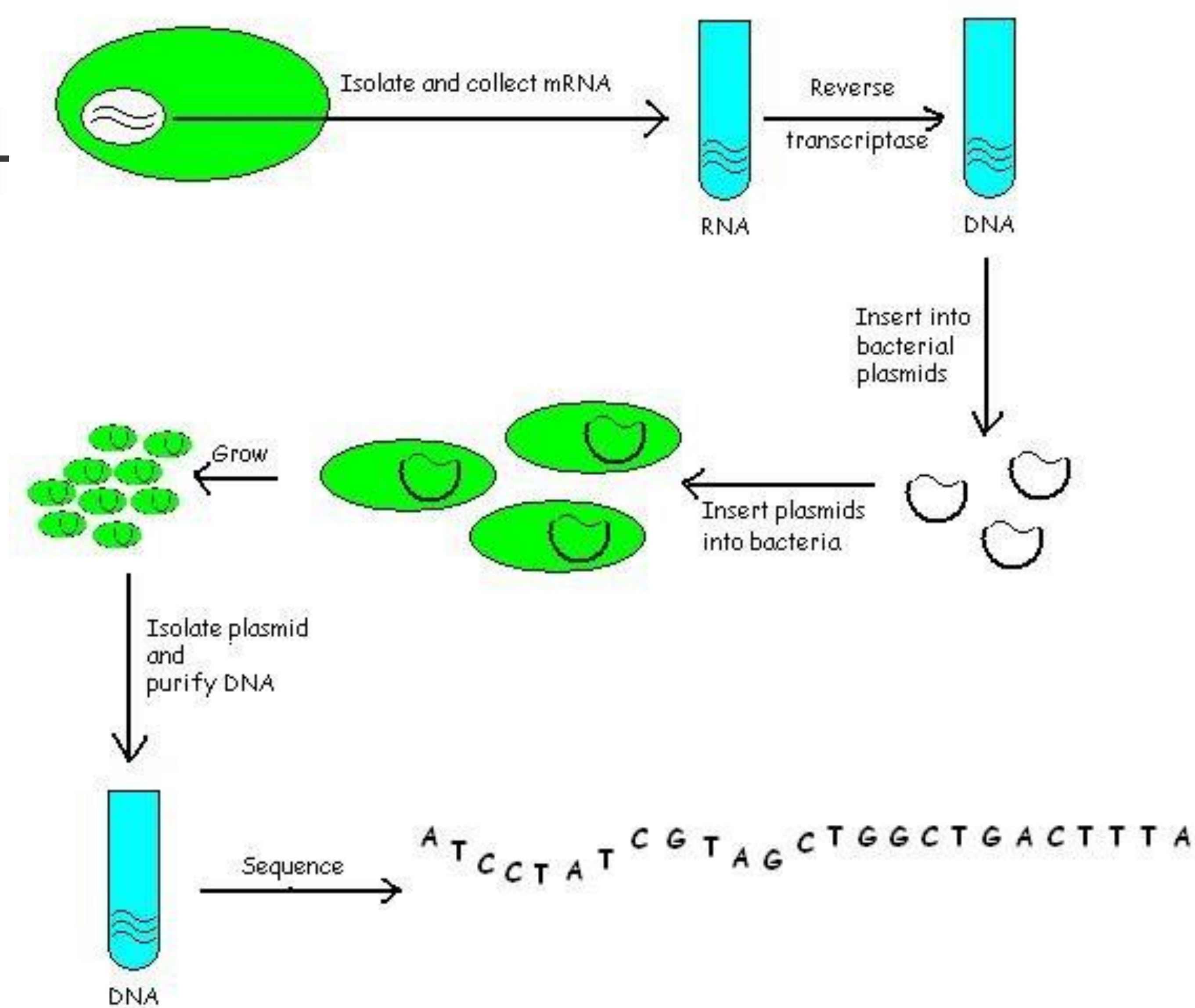
cDNA library

- As we all know that each cell has a different collection of transcripts.
- This fact can be utilized in preparation of a library if the material that is cloned is not DNA but messenger RNA or (mRNA).


5′
A-A-A-A 3′ mRNA
3′ T-T-T-T 5′ Oligo(dT) primer
1 Incubate with reverse transcriptase to synthesize cDNA strand
mRNA
cDNA
2 When cDNA strand is completed, hydrolyze RNA strand
cDNA
3′
3 Incubate with DNA polymerase to synthesize second DNA strand
S1 nuclease cuts loop
Double-stranded DNA
4 Incubate with terminal transferase to add single-stranded tails
C-C-C-C
C-C-C-C
Double-stranded cDNA ready for insertion into appropriate cloning vector

- Messenger RNA cannot itself be ligated into a cloning vector. However, mRNA can be converted into DNA by **complementary DNA (cDNA)** synthesis.
- This resulting in a double-stranded DNA fragment that can be ligated into a vector and cloned

Formation of a cDNA Library



https://en.wikipedia.org/wiki/CDNA_library

Screening of Libraries

Screening

After preparation of genomic DNA library or a cDNA library we may require to find out a clone that may contain our gene of interest or a regulatory sequence. In order to isolate clones that contain regions of interest from a library, the library must first be screened.

Two of the screening strategies are:

(1) Screening by DNA Hybridization

(2) Screening by PCR

Screening by Hybridization

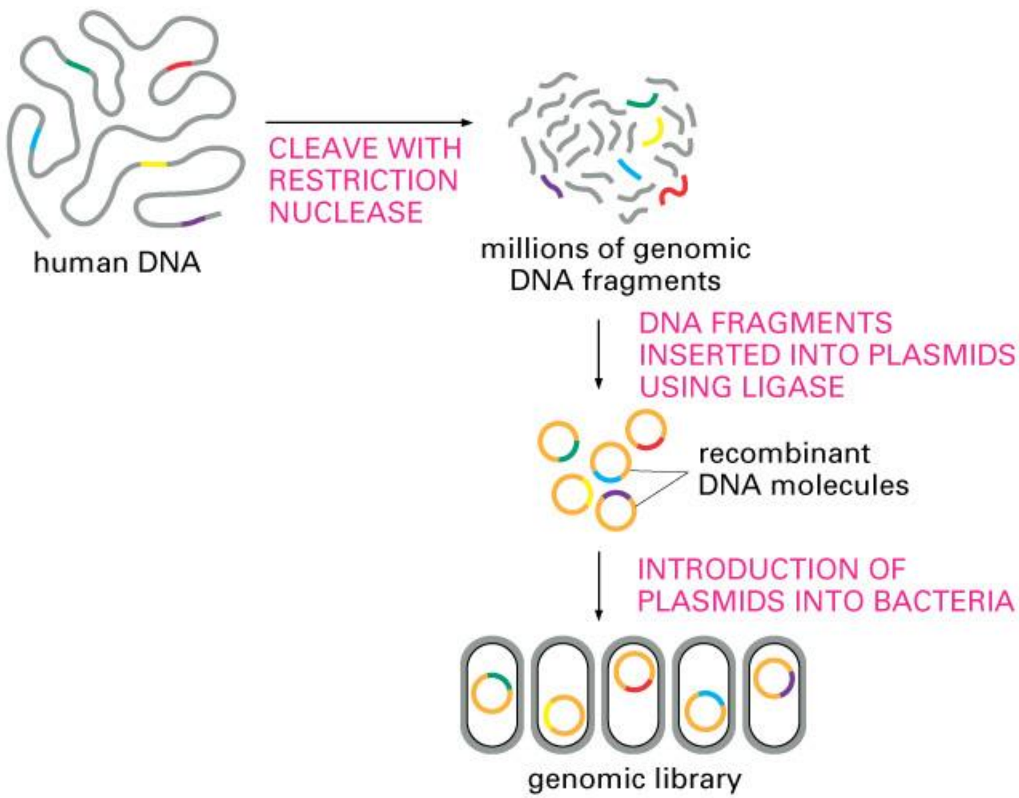


Figure 10-23 **Essential Cell Biology, 2/e. (© 2004 Garland Science)**

Methods for clone identification

- Although a few of these procedures are based on detection of the translation product of the cloned gene, it is usually easier to identify directly the correct recombinant DNA molecule.
- This can be achieved by the important technique of **hybridization probing**.

- Principle behind it is Complementary nucleic acid strands hybridize to each other.
- Not only can this occur between single-stranded DNA molecules to form the DNA double helix, but also between a pair of single-stranded RNA molecules or between combinations of one DNA strand and one RNA strand

- After hybridization, the location of the bound probe is detected by **autoradiography**. A sheet of X-ray-sensitive photographic film is placed over the membrane. The radioactive DNA exposes the film, which is developed to reveal the positions of the colonies or plaques to which the probe has hybridized

Colony and plaque hybridization probing

- Hybridization probing can be used to identify recombinant DNA molecules contained in either bacterial colonies or bacteriophage plaques

- Colonies or plaques are transferred to a nitrocellulose or nylon membrane by placing the membrane on the colonies

↓

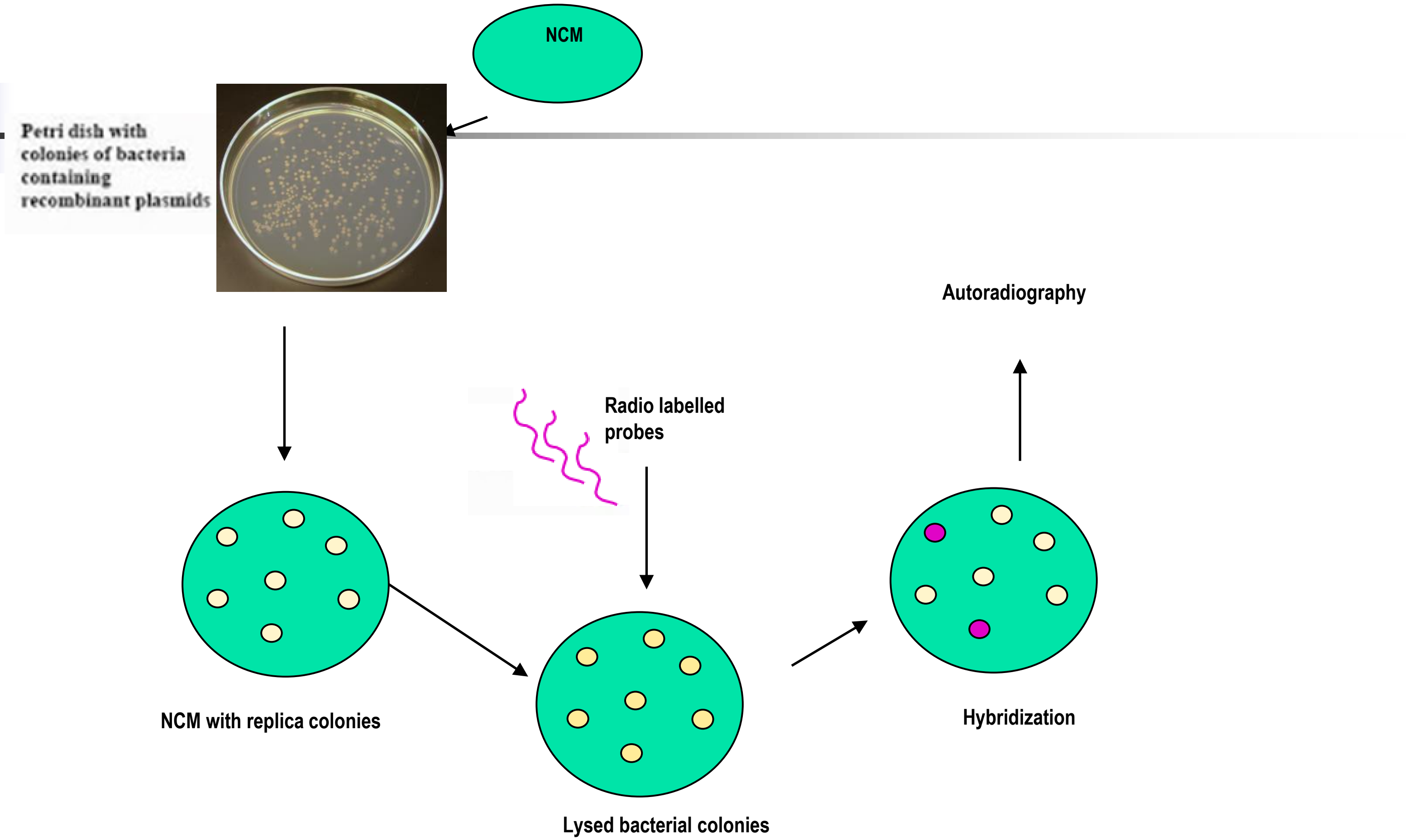
- Then the membrane is treated to remove all contaminating material, leaving just DNA

↓

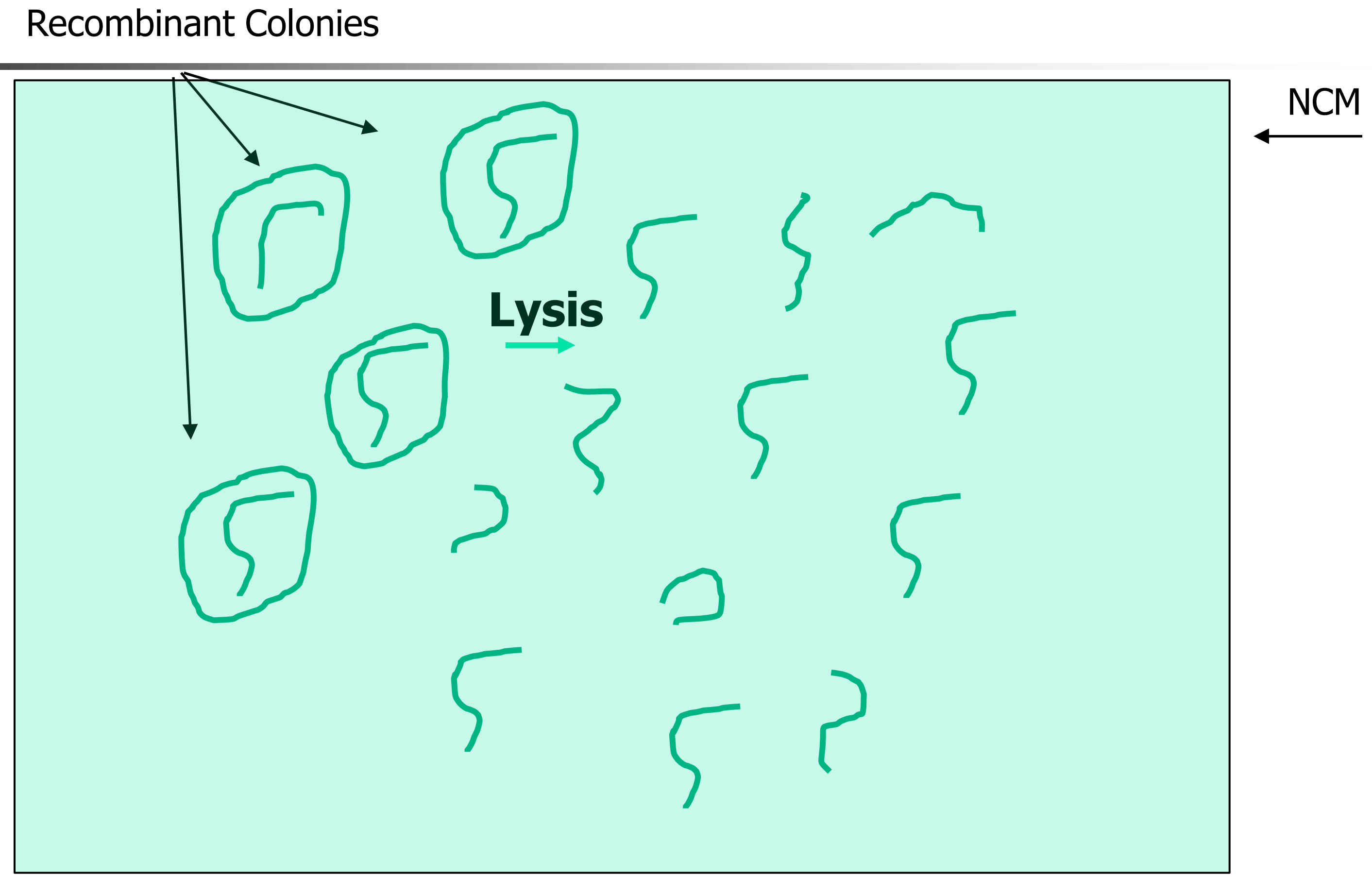
- This treatment also results in denaturation of the DNA molecules

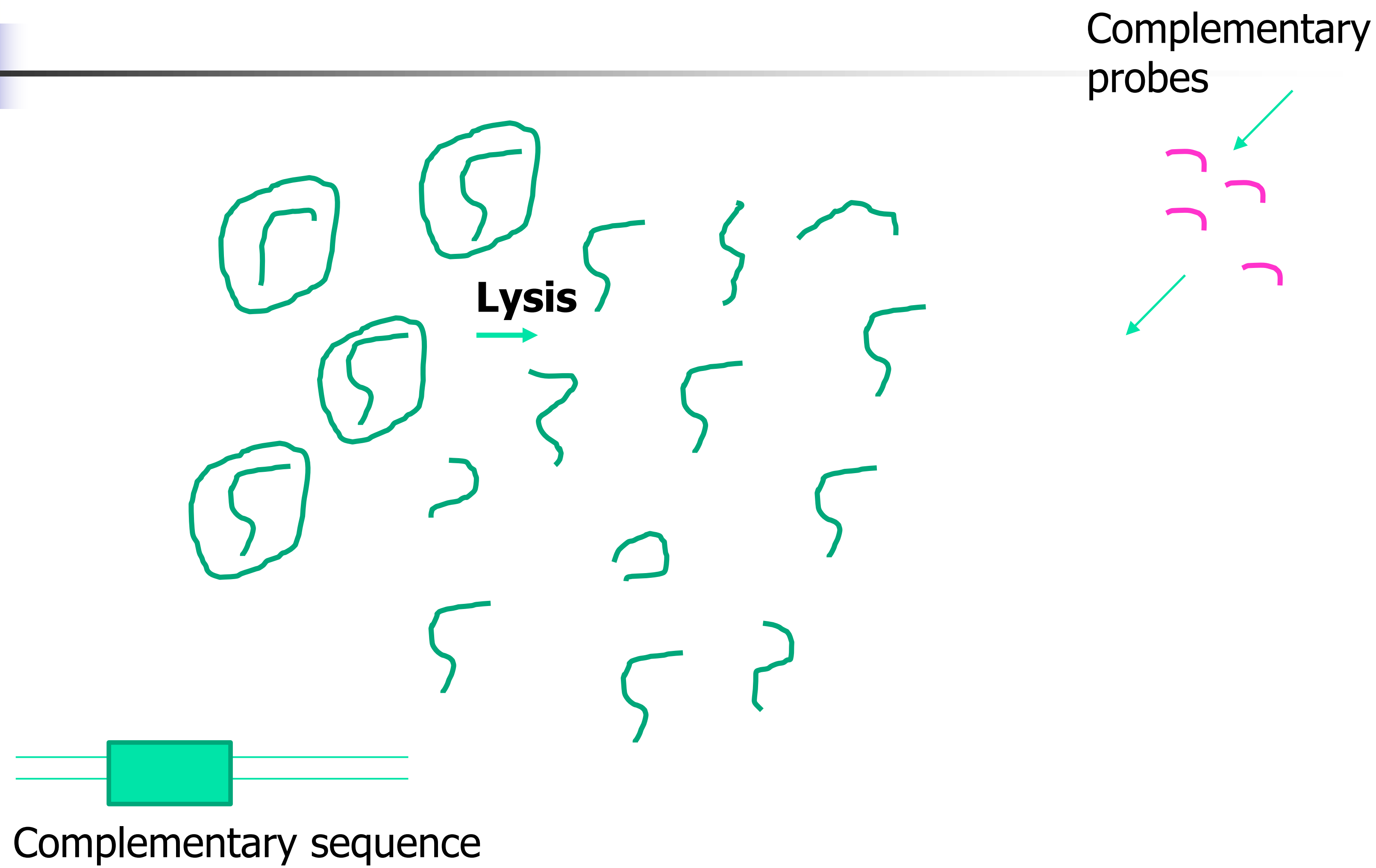
- Single-stranded molecules are then bound tightly to the membrane by heating for a short period at 80°C if a nitrocellulose membrane is being used, or with a nylon membrane by ultraviolet irradiation ↓

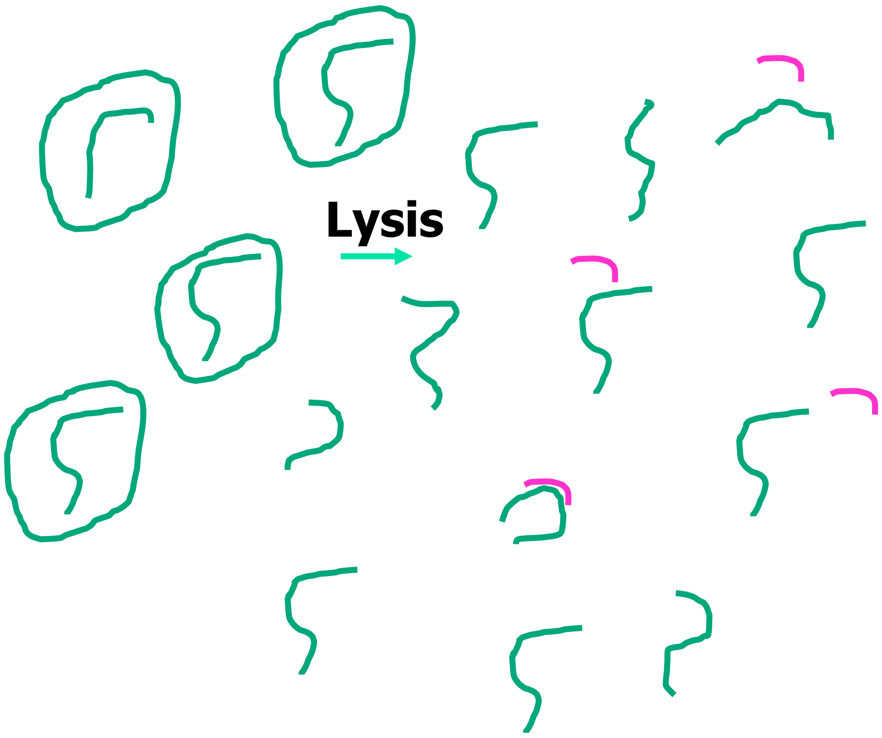
- The molecules become attached to the membrane through their sugar–phosphate backbones, so the bases are free to pair with complementary nucleic acid molecules

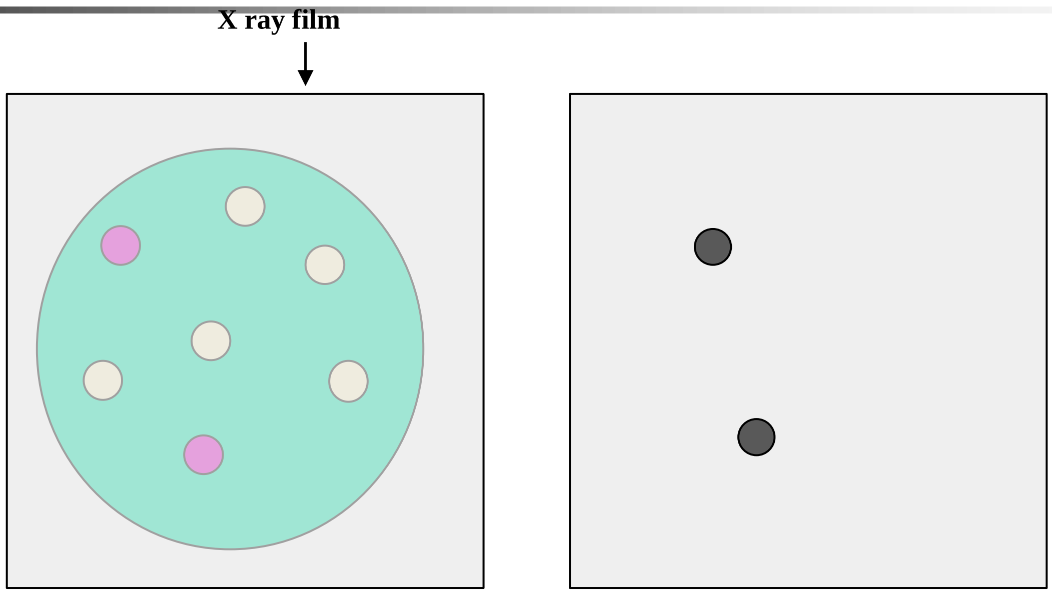


Colony picture https://en.wikipedia.org/wiki/Agar_plate#/media/File:Ecoli_colonies.png;


Recombinant Colonies
NCM
Lysis


Complementary
probes
Lysis
Complementary sequence


Lysis



Colonies on NCM with hybridized probes

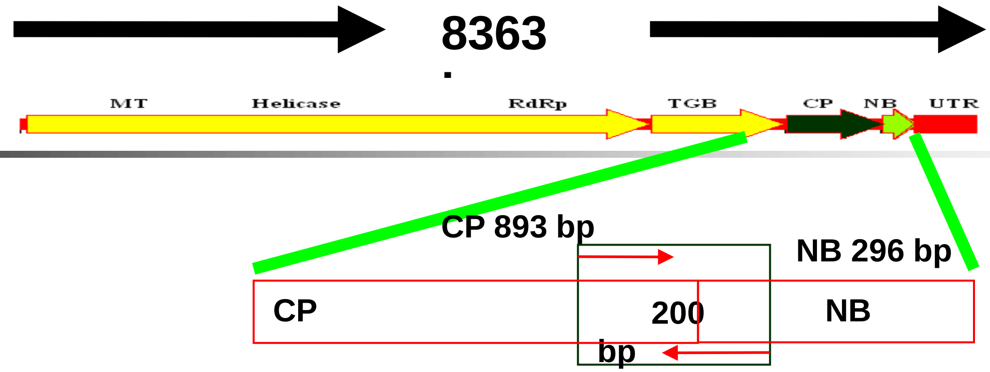
After hybridization and autoradiography

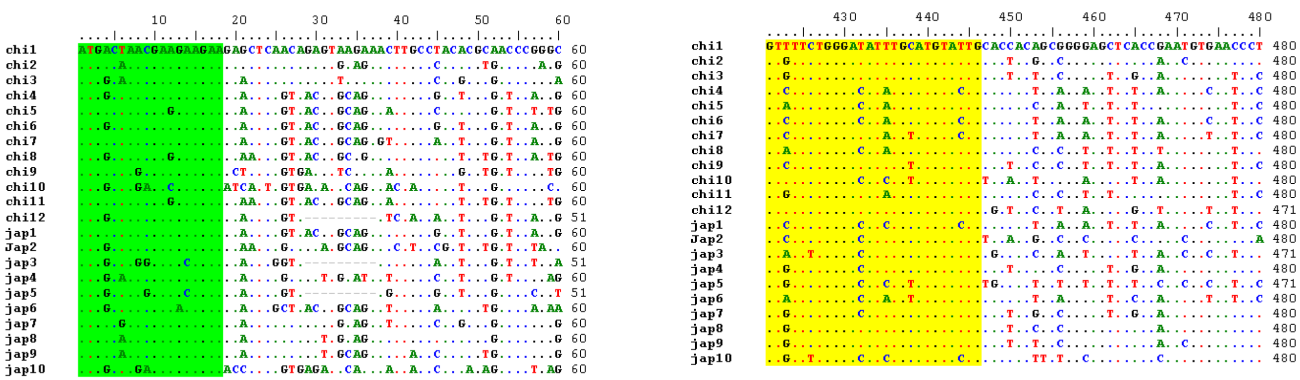
- The probe must now be **labeled** with a radioactive or other type of marker, denatured by heating, and applied to the membrane in a solution of chemicals that promote nucleic acid hybridization

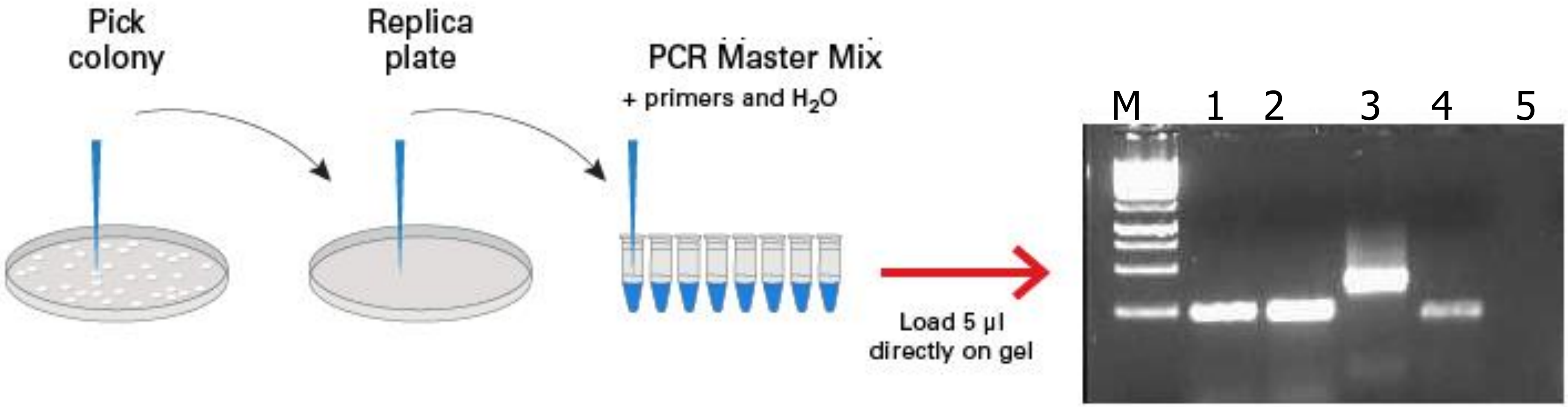
Screening by PCR: Colony PCR

- Polymerase chain reaction (PCR) is as good as hybridization technique for screening DNA libraries. But adequate information (on the flanking sequences of target DNA) must be available to prepare primers for this method. The colonies are maintained in multiwell plates, each well is screened by PCR and the positive wells are identified.

- Colony PCR is a method used to screen for plasmids containing a desired insert directly from bacterial colonies without the need for culturing or plasmid purification steps.
- Primers are designed as follows


8363
MT
Helicase
RdRp
TGB
CP
NB
UTR
CP 893 bp
NB 296 bp
CP
200
bp
NB





Amplified DNA can be visualised on agarose gel containing Ethidium bromide

https://www.takarabio.com/learning-centers/pcr/technical-notes/colony-pcr-in-under-an-hour

Probe

- In molecular biology, a **hybridization probe** is a fragment of DNA or RNA of variable length (usually 100-1000 bases long) and with a complementary sequence of the NA to be probed
- Short synthetic DNA molecules similar to those used as primers in PCR and sequencing can be used as probes in hybridization procedures

- Oligonucleotide probes can be designed from known DNA sequence but more usually are derived from amino acid sequence.

Probes can be of different origins

DNA

Cell based DNA PCR

RNA

Transcript from insert DNA cloned in suitable vectors

Oligonucleotide

Chemical synthesis

- The probe must now be **labeled** with a radioactive or other type of marker, denatured by heating, and applied to the membrane in a solution of chemicals that promote nucleic acid hybridization

Labeling with a radioactive marker

- A DNA molecule is usually labeled by incorporating nucleotides that carry a radioactive isotope of phosphorus, 32P
- Nucleic acid probes may be made as single-stranded or double-stranded molecules, but the working probe must be in the form of single strands.

Resources

- NCBI book shelf
- Free web resources