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Biotechnology

التهجين الجزيئي للأحماض النووية

Molecular hybridization of nucleic acids

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قسم التقنيات الاحيائية

Molecular hybridization of nucleic acids

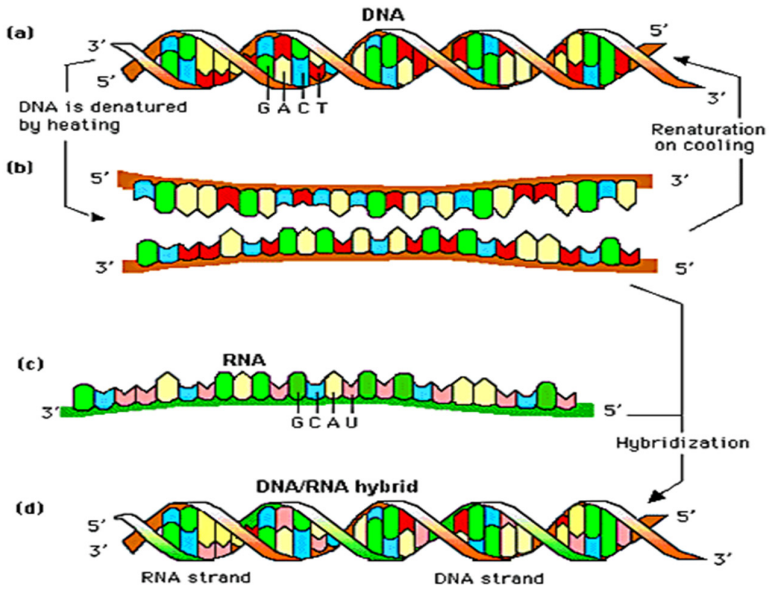
Molecular hybridization of nucleic acids is the process in which two single-stranded nucleic acid molecules with complementary base sequences form a double-stranded nucleic acid molecule.

Nucleic acid hybridization technology is a fundamental tool in molecular biology, and has been applied in various fields such as

- Detection of gene expression
- Screening specific clone from cDNA or genomic library
- Determining the location of a gene in chromosome
- Diagnosis of diseases

Principles Of Nucleic Acid Hybridization

The technique of nucleic acid hybridization is established and developed on the basis of the denaturation and renaturation of nucleic acids. Hydrogen bonds in double stranded nucleic acids can be disrupted by some physicochemical elements, and two strands of nucleic acids are separated into single strand.



Nucleic Acid Hybridization

If different single-stranded DNA molecules, or DNA and RNA molecules, or RNA molecules are mixed together in a solution, and the renaturation is allowed to occur under proper conditions, single stranded DNA or RNA will bind with each other to form a local or whole molecule of double-stranded structure as long as the single-stranded molecules are complementary, no matter what kind of sources they come from.

Nucleic acid hybridization as a technique involves using a labelled nucleic acid probe, which is a known DNA or RNA fragment, to bind with the target nucleic acids, which is usually a poorly understood, heterogeneous population of nucleic acids. A probe labelled with detectable tracer is the prerequisite for determining a specific DNA sequence or gene in a sample or genomic DNA by nucleic acid hybridization.

The target nucleic acids to be analysed are usually denatured, and then mixed with the labelled probe in the hybridization system. The probe will bind to the segment of nucleic acid with complementary sequence under proper conditions. The hybridization can be identified by the detection of the tracer labelling the probe. Thus, the existence or the expression of specific gene can be determined.

Preparation and Labelling Of Nucleic Acid

1- Preparation of probes

Probes may be single-stranded or double stranded molecules, but the working probe must be single-stranded molecules. The probes used in hybridization of nucleic acids include

a) oligonucleotide(15-50 nucleotides)
b) genomic DNA fragment
c) cDNA fragment
d) RNA.

a- **Oligonucleotide probes** are short single stranded DNA fragments designed with a specific sequence complementary to the given region of the target DNA. They are usually synthesized in vitro.

b- **Genomic DNA probes** can be prepared from the cloned DNA fragment in plasmid.

c- **cDNA probes** can be prepared from the cloned cDNA in plasmid, or amplified directly from mRNA by RT-PCR.

d- **RNA probes** are usually transcribed *in vitro* from a cloned cDNA in a proper vector.

The size of genomic DNA probes, cDNA probes and RNA probes may be 0.1 kb to 1 kb.

2- Labelling of probes

Probe is usually labelled with a detectable tracer, which is either isotopic or non-isotopic. The purified oligonucleotide is labelled *in vitro* by using a suitable enzyme to add the labelled nucleotide to the end of the oligonucleotide.

For the preparation of the labelled **RNA probes**, RNA probes are usually synthesized by RNA polymerase in the presence of ATP, GTP, CTP and the labelled UTP, with specific fragment of a gene or cDNA in a proper vector as template. RNA probes can then be generated and be labelled at the same time.

Genomic DNA probes and **cDNA probes** are usually labelled in the process of DNA synthesis *in vitro*. In the reaction of DNA synthesis with **random priming** (random primers and Klenow fragment) or **nick translation** (DNase I and DNA polymerase), if a labelled-dNTP, which can be incorporated into newly-synthesized DNA chain, is added as a substrate, the labelled DNA probe will be formed.

There are different, sensitive detecting methods for each of the labels used in nucleic acid hybridization. After hybridization, the location and the quantity of the hybrid molecules can be determined. The labels in common use include radioactive (^{32}P and ^{35}S) and nonradioactive (digoxigenin, biotin, fluorescein) substances which are used to label dNTP.

Southern blot hybridization

Southern blot hybridization is an assay for sample DNA by DNA-DNA hybridization which detects target DNA fragments that have been size-fractionated by gel electrophoresis (Figure below).

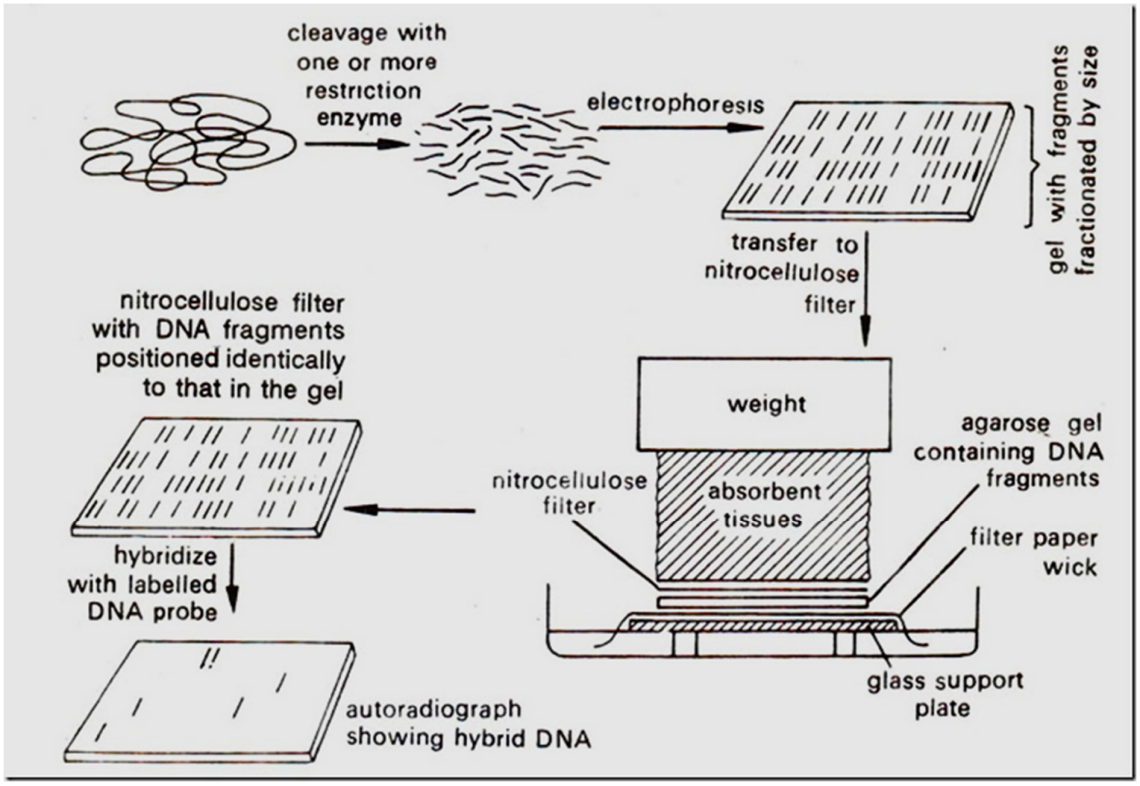
In Southern blot hybridization, the target DNA is digested with restriction endonucleases, size-fractionated by agarose gel electrophoresis, denatured and transferred to a nitrocellulose or nylon membrane for hybridization.

DNA fragments are negatively charged because of the phosphate groups so to migrate towards the positive electrode, and sieved through the porous gel during the electrophoresis. Shorter DNA fragments move faster than longer ones. For fragments between 0.1 and 20kb in length, the migration speed depends on the length of fragment. Thus, fragments in this size range are fractionated by size in a conventional agarose gel electrophoresis system.

Following electrophoresis, the sample DNA fragments are denatured in strong alkali, such as NaOH. Then, the denatured DNA fragments are transferred to a nitrocellulose or nylon membrane and become immobilized on the membrane. Subsequently, the immobilized single stranded target DNA sequences are allowed to interact with labelled single stranded probe DNA.

The probe will bind only to complementary DNA sequences in the target DNA to form a target-probe hetero duplex. As the positions of the immobilized single stranded target DNA fragments on membrane are faithful records of the sieve separation achieved by agarose electrophoresis, they can be related back to the original gel to estimate their size.

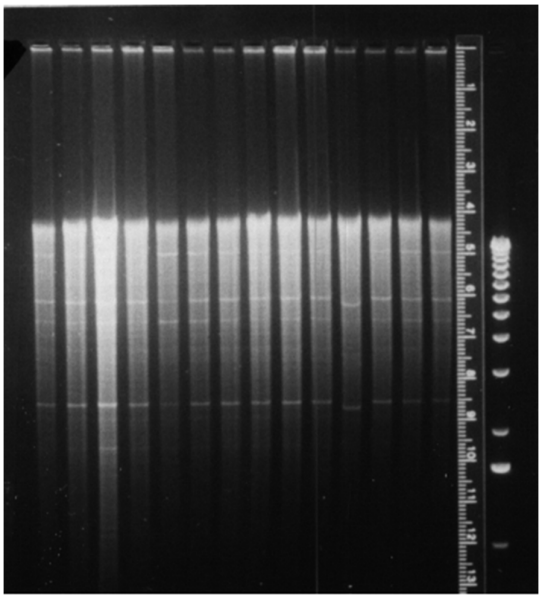
Southern Blotting

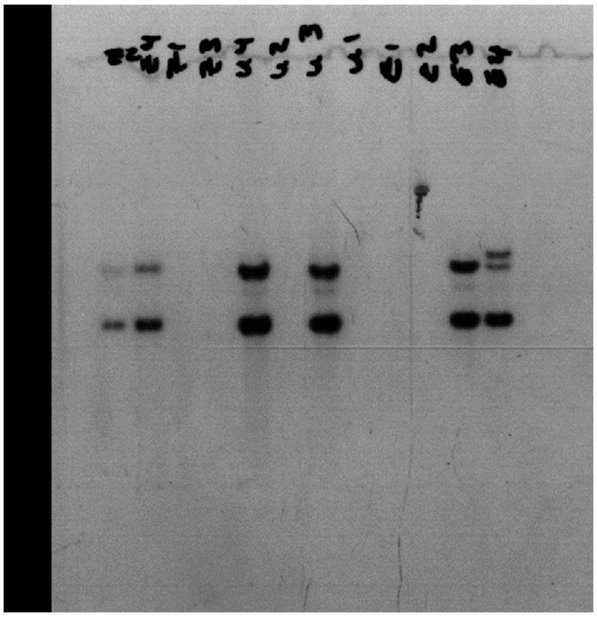


Autoradiography

X-ray film is placed over the membrane and left until radiation from the probe has exposed the film. Fragments complimentary to the probe appear as bands on the film.

Gel **Autoradiograph**





Southern blot hybridization technique is widely applied in researches since its invention. It could be applied for analysis of

1. **Gene expression**
2. **Screening of recombinant plasmids**
3. **Analysis of gene mutation**
4. **Identification of the existence of a given DNA such as DNA from pathogenic microorganism**.
5. **Detect deletion of gene by restrictions mapping**.

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