A NOTE ON

SOUTHERN & FLUORESCENCE IN SITU HYBRIDIZATION

Submitted BY

Dr. Namrata Kahar (Guest Faculty)

Department of Biotechnology

Govt. Digvijay Autonomous College Rajnandgaon
(C.G.) 491441, INDIA

CONTENTS :-

INTRODUCTION :-

- **Annealing of the probe with target DNA by hydrogen bonding is called hybridization.**
- **DNA probe is a short piece of labelled single stranded DNA. It is also known as hybridization probes.**
- **Probe DNAs are used to detect the presence of certain genes in the DNA isolate, such probe DNAs are called gene probes.**
- **The base sequence of the DNA probe is complementary to the DNA strand to be detected.**
- **The formation of the double helix from two complementary strands of nucleic acids is the basis of nucleic acid hybridization technique.**

HISTORY :-

1961	**Marmur & Doty**	**Describe the nucleic acid hybridization**
1969	**Gall & Pardue**	***In situ* hybridization was 1st introduced**

1975	**Southern**	**Southern *in situ* hybridization**
1980s	**Biomedical researchers**	**Develop the fluorescence *In situ* hybridization**

DEFINITION :-

- ***In situ* hybridization is the annealing of the probe DNA with complementary regions on chromosomes.**
- **Southern *in situ* hybridization is a nucleic acid hybridization method used to identify and separate a particular gene from a pool of DNA fragments.**
- **Fluorescence *in situ* hybridization (FISH) is a molecular diagnostic technique utilizing fluorescently labelled DNA probes to detect or confirm gene or chromosome abnormalities.**

PRINCIPLE OF FISH :-

- **The sample DNA (metaphase chromosomes or interphase nuclei) is first denatured, a fluorescently labelled probe of interest is then added to the denatured sample mixture and hybridizes with the sample DNA at the target site as it re-anneals back into a double helix.**
- **The probe signal can then be seen through a fluorescent microscope and the sample DNA can be scored for the presence or absence of the signal.**
- **Unlike most other techniques used to study chromosomes, FISH does not have to be performed on cells that are actively dividing.**
- **FISH uses fluorescent probes that bind to only those parts of the chromosome with which they show a high degree of sequence complementarities.**

- **Fluorescence microscopy can be used to find out where the fluorescent probe is bound to the chromosomes.**

PROTOCOL FOR FISH :-

- **Make a probe complementary to the known sequence.**

- **When making the probe, label it with a fluorescent marker, e.g. fluorescein, by incorporating nucleotides that have the marker attached to them.**

- **Put the chromosomes on a microscope slide and denature them.**
- **Denature the probe and add it to the microscope slide, allowing the probe hybridize to its complementary site.**

- **Wash off the excess probe and observe the chromosomes under a fluorescent microscope.**

- **The probe will show as one or more fluorescent signals in the microscope, depending on how many sites it can hybridize to.**

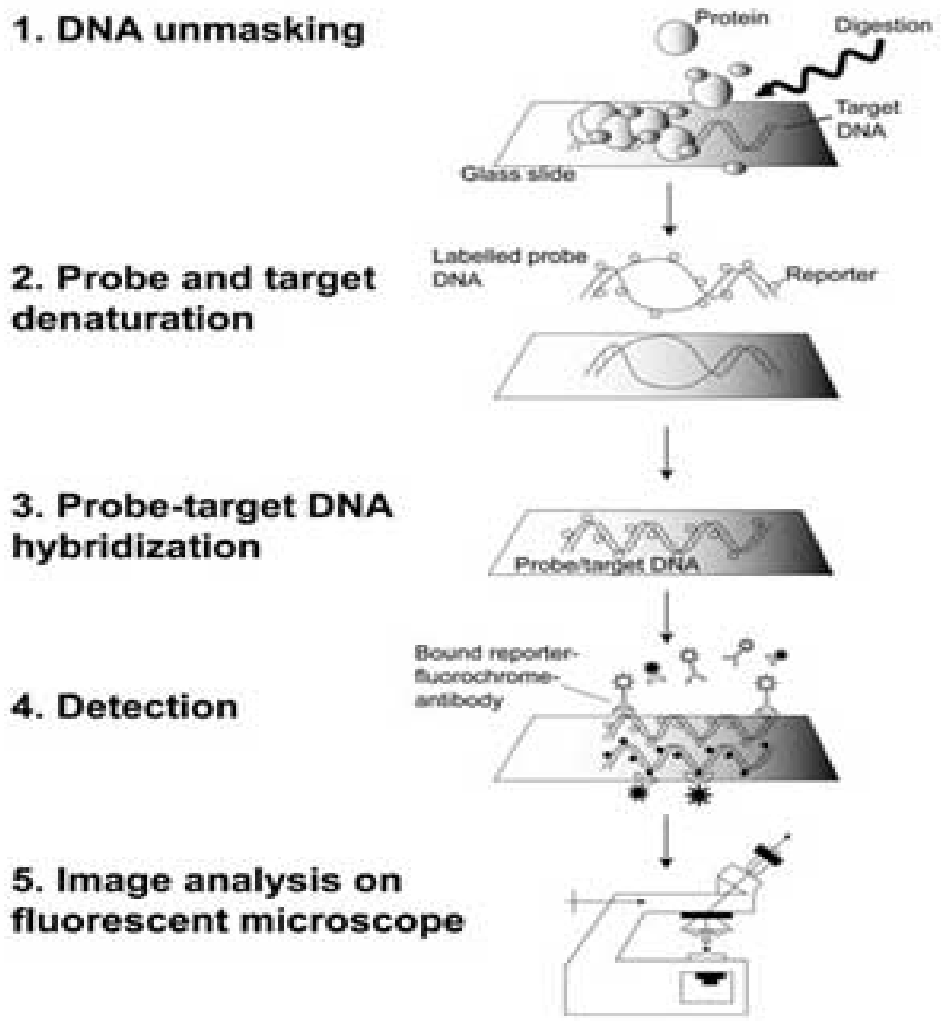

FIG. 1: FISH

PRINCIPLE OF SOUTHERN BLOT HYBRIDIZATION :-

- **Southern blot hybridization refers to the detection of specific DNA fragments that have been separated by gel electrophoresis.**

- **After the electrophoresis the separated DNA fragments are denaturated and transferred to a nitrocellulose (or nylon) membrane sheet by blotting.**

- **In the blotting the gel is supported on a sponge in a bath of alkali solution, and buffer is sucked through the gel and the sheet by paper towels stacked on top of the nitrocellulose sheet.**

- **The buffer denaturates the DNA and transfers the single stranded fragments from the gel to the surface of the sheet, where they adhere firmly.**

- **The nitrocellulose sheet containing the bound single-stranded DNA fragments is pealed off the gel and placed in a sealed plastic bag or a box together with buffer containing labelled DNA probe specific for the target DNA sequence**

- **The sheet is exposed to the probe under conditions favouring hybridization.**

- **After the hybridization, the sheet is removed from the bag, washed thoroughly to remove unhybridized probes and viewed using autoradiography or ultraviolet light depending on the labels used (radioactive of fluorescent).**

- **An adaptation of Southern blotting is Northern blotting, in which RNA molecules are electrophoresed through the gel instead of DNA.**

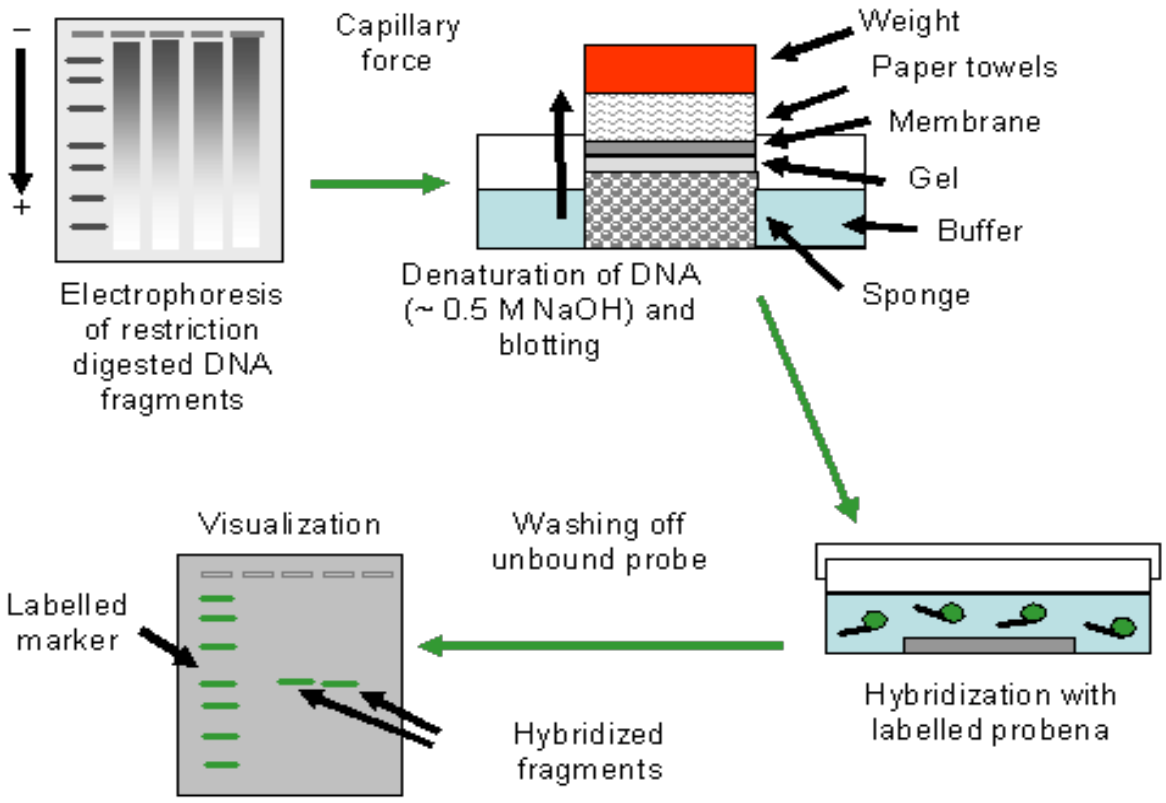

FIG. 2: SOUTHERN *IN SITU* HYBRIDIZATION

APPLICATIONS :-

- **FISH is used to detect and localize the presence or absence of specific DNA sequences on chromosomes.**
- **FISH is often used for finding specific features in DNA for use in genetic counselling, medicine, and species identification.**
- **FISH can also be used to detect and localize specific RNA targets (mRNA, lncRNA and miRNA) in cells, circulating tumour cells, and tissue samples.**
- **Southern blotting is used to identify desired genes and rDNA and in DNA fingerprinting.**
- **Southern blotting can also used in genetic mapping, identification of genetic markers etc.**

LIMITATIONS :-

- ***In situ* hybridization also has limitations. Frequently encountered problems include no signals or low signal intensity.**
- **This can be caused by non complementarities of probe and target, ineffective probe labelling or non optimal hybridization conditions.**
- **Low signal intensity can be caused by small numbers or insufficient accessibility of the target molecules (rRNA).**

SUMMARY :-

- **Southern *in situ* hybridization is a nucleic acid hybridization method used to identify and separate a particular gene from a pool of DNA fragments.**
- **Southern blotting can also used in genetic mapping, identification of genetic markers etc.**

- **Fluorescence *in situ* hybridization (FISH) is a molecular diagnostic technique utilizing fluorescently labelled DNA probes to detect or confirm gene or chromosome abnormalities.**

- **FISH is often used for finding specific features in DNA for use in genetic counselling, medicine, and species identification.**

CONCLUSION :-

- ***In situ* hybridization is the annealing of probe DNA fragments with the complementary sections of chromosomes.**

- **It is DNA hybridization in the cells.**

- **This technique is used to locate specific genes in chromosomes.**

- ***In situ* hybridization is also a form of colony hybridization.**

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