NEW ERA AGRICULTURE MAGAZINE

Fluorescence *In Situ* Hybridization: Method and Applications

Syed Kulsoom Fatima Jafri[1], Devesh Yadav[2], Ajeet Kumar Gupta[1], Rishabh Gupta[1] and Piyusha Singh[1]

Introduction:

Advances made over the last two decades in techniques and approaches used in molecular cytogenetics research contributed immensely to the crop improvement. These include the use of chromosome banding, fluorescence in situ hybridization (FISH), multicolor FISH (McFISH), Q-FISH, Flow-FISH, RNA-FISH, genomic in situ hybridization (GISH) and flow cytometry. These tools have been used both for identification of individual chromosomes and for physical localization of DNA sequences on individual chromosomes of plant cell. Multiplexing fluorescence in situ hybridization (FISH) enables to assay multiple targets simultaneously and visualize co-localization within a single plant specimen. Construction of genetic and physical maps of crop plants involving molecular markers including FISH mapping of genes for agronomic traits has been done. These maps have extensive practical use in comparative genomics. GISH technology can be applied in the analysis of the meiotic behavior in hybrids and polyploids and thus helpful in study of hybrid lineages, genetic improvement programs, and studies of the evolution of polyploidy species.

In the mid-1980s, the technique of fluorescence in situ hybridization (FISH) using fluorescently labeled DNA probes enabled the staining of specific sequences within chromosomes. FISH has largely replaced specialized staining of chromosomal regions. FISH can be performed using different types of probes, which are cloned portions of the genome labeled with fluorescent reporter molecules. FISH probes can be unique genomic segments or probe collections, such as chromosome-specific painting probes, all telomere-specific probes, and all centromere-specific probes. FISH was widely used in the late 1980s and early 1990s, providing a link between classical cytogenetics and human genome sequencing, and was also useful in studying the nuclear localization of genes during interphase.

Since its creation more than 30 years

Syed Kulsoom Fatima Jafri[1], Devesh Yadav[2], Ajeet Kumar Gupta[1], Rishabh Gupta[1] and **Piyusha Singh[1]**
[1]Acharya Narendra Dev University of agriculture and technology Kumarganj Ayodhya
[2]C. S. Azad University of agriculture and technology, Kanpur

ago, fluorescence in situ hybridization (FISH) has revolutionized cytogenetic study. FISH has been utilized to provide information about the structure, mutation, and evolution of both whole genomes and individual chromosomes. In many plant species, FISH has been a useful method for chromosomal identification. The creation and use of probes based on artificial oligonucleotides (oligos) is the most important recent advancement in FISH. It is possible to identify, parallel synthesis, and fluorescently label oligos specific to a repetitive DNA sequence, a particular chromosomal region, or an entire chromosome using computational methods. A significant development in the study of cytology is the macromolecule recognition method known as fluorescence in situ hybridization (FISH). It was initially created as a physical mapping method to distinguish between genes on different chromosomes. Biological and medical studies subsequently benefited from the precision and adaptability of FISH. An intermediate level of clarity between DNA analysis and chromosomal research is offered by this aesthetically appealing method. A hybridising DNA probe used in FISH can be either directly or indirectly labelled. Fluorescent nucleotides are employed for direct labelling, whereas reporter molecules used for indirect labelling are afterwards recognised by fluorescent antibodies or other affinity molecules. Nucleic acids can be spatially detected and quantified in their biological surroundings using the technique of fluorescence in situ hybridization. FISH is regarded as the industry standard cytogenetic technique in diagnostics for the identification of sick or malignant cells that exhibit chromosomal rearrangements or gene abnormalities. Since its inception in the 1960s, FISH has benefited greatly from advancements in probe-labeling methods and particular probe design tactics that have increased its sensitivity. The sharp increase in the number of studies and diagnostics using it demonstrates its widespread application in both.

Principle

"FISH is based on the ability of single-stranded DNA (probes) to hybridize to complementary DNA sequence"

Methodology of FISH

The first step is to create short single-stranded DNA sequences that closely match a region of the gene that we're searching for. Probes are what they are. Numerous techniques, including nick translation, random primed labelling, and PCR, are used to label the DNA probe. Indirect labelling and direct labelling are the two labelling techniques employed. In the case of direct labelling, fluorophore-containing nucleotides are used to label the probes. The modified nucleotides that contain a hapten are where probes are labelled

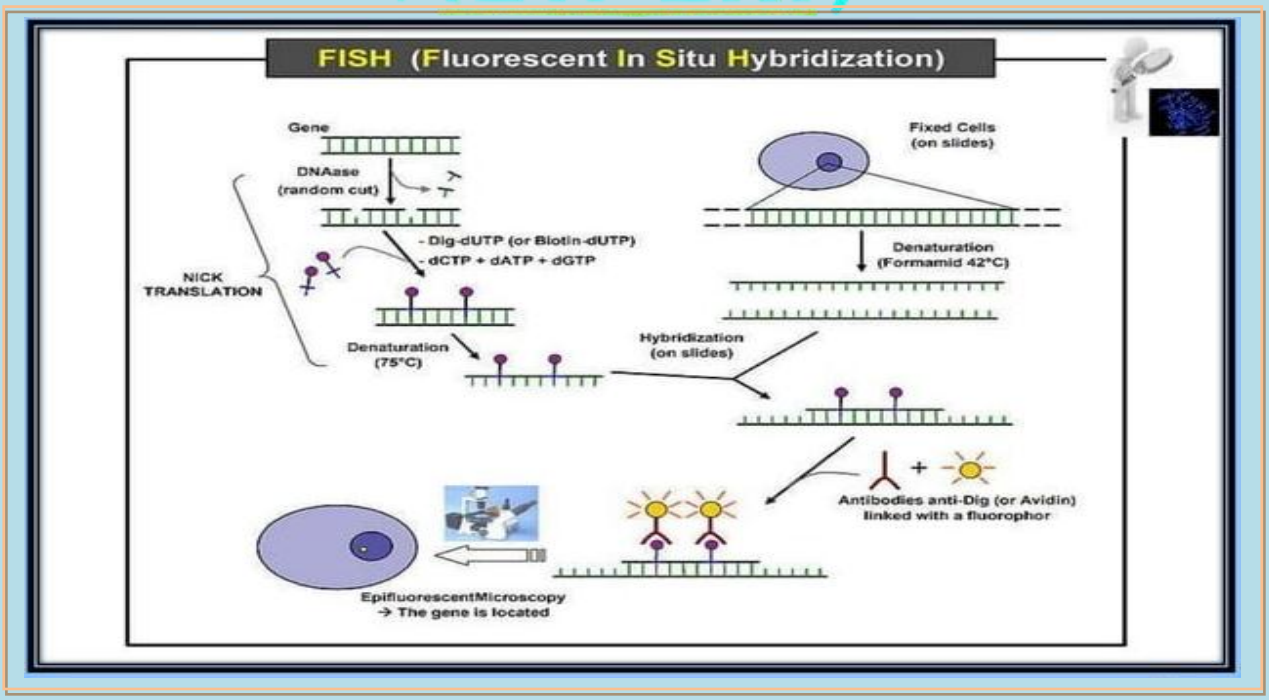
during indirect labelling. The target DNA and the labelled probe are then both denatured. Due to the combined effects of denatured probe and target DNA, complementary DNA sequences anneal. The visualisation of the non-fluorescent hapten in the case of indirect labelling requires an additional step and either an enzymatic or immunological detection technique.

Steps

1. Probe DNA- Characterization
2. Nick translation labelling of probe DNA
3. Purification of labelled DNA probe
4. Chromosome preparation.
5. *In Situ* hybridization
6. Washing
7. Fluorescent microscope

Applications of FISH

1. **Identify the position of genes.** FISH provides a powerful tool for identifying the location of a cloned DNA sequence on metaphase chromosomes. Figure 2a shows the results of a typical FISH experiment, in which a cloned DNA sequence was hybridized to normal metaphase chromosomes. Red bands are detected at hybridization sites on two homologous chromosomes, which can be identified by their characteristic banding patterns. Closer examination shows that each red band actually consists of two spots, corresponding to the two sister chromatids in a mitotic chromosome. A skilled cytogeneticist would be able to use these hybridization data together with the



banding pattern to place the probe sequence within a few megabases of other known genes on the chromosome.

2. **An effective method for locating a cloned DNA sequence on metaphase chromosomes is FISH.** On two homologous chromosomes, red bands are seen at the hybridization sites, which can be distinguished by their distinctive banding patterns. Each red band is actually made up of two spots, which represent the two sister chromatids in a mitotic chromosome, upon closer inspection. These hybridization results, along with the banding pattern, could be used by an expert cytogeneticist to locate the probe sequence on the chromosome within a few megabases of other known genes. FISH and other in situ hybridization results have historically been important.

Challenges of current FISH assay

The spatial and cellular context of genomic and transcriptomic data is provided by fluorescence in situ hybridization. It has found uses in oncology, preventive and reproductive medicine, clinical diagnostics, and genomic research because of its distinctive advantages. A few significant drawbacks of the traditional FISH method, however, become clear, particularly in the context of clinical applications.

For multicolor FISH, tremendous advancements have been made in recent years. The FISH signal is detected using two different combinatorial labeling techniques, spectral karyotyping and Mc-FISH depending on the spectral characteristics or the presence or absence of particular fluorophores. Both techniques can be used to specifically label all 24 human chromosomes, find translocations, insertions, or other genetic changes.

Uses of FISH technology

- ❑ Identification and characterisation of numerical and structural chromosome abnormalities
- ❑ Detection of microscopically invisible deletions
- ❑ Parental diagnosis of the common aneuploids

Advantages of FISH technology

- ❑ Higher spatial resolution and speed
- ❑ High efficiency of hybridisation and detection
- ❑ Whole chromosomes, chromosomal segments or single copy sequences can be highlited.
- ❑ Physical location along chromosomes.
- ❑ Hybridization with multiple probes enable detection of translocation products.

Disadvantages of FISH technology

- Limited number of commercial probes available.
- Probe must be available - given sequence of DNA.
- Unsuspected variation in nuclear organization cannot be detected.
- Need specialized camera and image capture system.
- Only provide information about the probe being tested.