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# A Review on Principles of FISH and GISH and its Role in Cytogenetic Study

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**Abstract:**
This review article was reviewed at Bahir Dar University College of Agriculture and Environmental science in 2018. A genome can be considered as all the DNA sequences of an organism together. However, we also can see it as all its chromosomes and all its genes. Conventional cytogenetic technique always studies at a particular stage of cell division. Classical cytological studies using chromatin staining still contribute much to the present appreciation of chromosomal diversity in wild species. This approach allows the detection of gross karyotypic alterations such as changes in chromosome number and morphology and gives an overview of chromosome behavior in mitosis and meiosis. However, many recent cytogenetic studies have used FISH to map single cloned or Polymorphic Chain Reaction-amplified sequences to chromosomes or GISH with total genomic DNA probes to identify the parental origin of chromatin in hybrids and allopolyploids. Fluorescence In situ hybridization and Genome In situ hybridization's role in improving our understanding of interspecific hybridization, and polyploidy, genetic mapping, and plant breeding. These methods also have a role in clarifying patterns of chromatin folding, interphase nuclear organization, and chromatin distribution in different cells of the cell cycle and development. Thus, this review was reviewed to create an understanding of the principles of GISH and FISH and their applications in Cytogenetic study.

*Keywords: Cytogenetic, DNA, FISH, GISH, Hybridization*

## INTRODUCTION

The age of classical cytogenetic has, however, been largely superseded by the implementation of DNA techniques during the past few decades. In situ hybridization (FISH) is now recognized as an important technique in many areas of molecular biological research. The technique is used to locate the physical position of a known DNA sequence on a chromosome (Devi et al., 2005). Cytogenetic techniques have become necessary components of studies of the organization of the genome and its association with chromatin.

Cytogenetic research and chromosome analysis are the main aspects of genomics and genetic sciences. Molecular cytogenetic techniques, such as in situ hybridization methods, are admirable tools to analyze the genomic structure and function, chromosome constituents, recombination patterns, alien gene introgression, genome evolution, aneuploidy, and polyploidy (Andres et al., 2013 Younis et al., 2015).

After in situ hybridization technique development by John et al (John et al., 1969) and Gall and Pardue (John et al., 1969), various approaches were achieved such as radioactively labeled probes improved into nonradioactive probes labeled with biotin (Rayburn and Gill, 1985) and detection by indirect (antibody-fluoro chrome conjugate) and direct (fluoro chrome detection) staining.

Genomic in situ hybridization (GISH) was first used to discriminate the genomes of the intergeneric hybrid between parental genomes, that is, Hordeum chilense and Scale africanum (Schwarzacher et al., 1989). Genomic in situ hybridization (GISH) is an efficacious technique that used for genome differentiation of one parent from the other by utilizing special chromosome labeling techniques. GISH has a tip role in cytogenetics for the investigation of the evolutionary relationship of crops and identification of an inserted region in the parent from the alien species. GISH technique follows the same protocol as the fluorescent in situ hybridization (FISH) technique. However, genomic and blocking DNA utilization in GISH differentiate it from FISH analysis. In plant species, genome organization and homology study are carried out by the use of genomic in situ hybridization (GISH). In addition, karyotype analysis of many plant species has been performed by the GISH technique (Ksiazczyk et al., 2010).

**Objective**

To Review the Principles of GISH and FISH and its Application for Cytogenetics Study

## LITERATURE REVIEW

### Applications of FISH and GISH

Classical cytological studies using chromatin staining still contribute much to the present appreciation of chromosomal diversity in wild species. This approach allows the detection of gross karyotypic alterations such as changes in chromosome number and morphology and gives an overview of chromosome behavior in mitosis and meiosis. However, many recent cytogenetic studies have used FISH to map single cloned or PCR-amplified sequences to chromosomes or GISH with total genomic DNA probes to identify the parental origin of chromatin in hybrids and allopolyploids. FISH and GISH together have shed much light on many biological phenomena. These methods also have a role in clarifying patterns of chromatin folding, interphase nuclear organization, and chromatin distribution in different cells of the cell cycle and development (Leitch, 2000).

Some very useful studies have been conducted utilizing these techniques in both animals and plants. Initially, studies made involved repeated DNA including sat-DNA from Drosophila and mouse. The application of in situ hybridization techniques in plants has lagged compared to its use in mammalian cytogenetics.

***Chromosome Mapping:***

The utilization of in situ hybridization technology is of particular interest to those engaged in chromosome walking or genome mapping projects. FISH has been utilized in many plants to identify chromosomes accurately, using species-specific repetitive sequences, ribosomal genes, and even unique sequences. Because of their universal occurrence and redundancy, ribosomal genes are of great value for karyotype analysis and comparative studies of genome organizations. FISH techniques using fluorochrome allow the visualization of multigenic families, such as 5s and 18s-5.8-26s ribosomal RNA genes for their location chromosomes Physical localization of multicopy gene families, such as 5S and 18S-26 rRNA genes have been reported in wheat2, tomato3, barley4, garlic5 and in Aegilops umbellulata6. In cotton, multi-copy genes were mapped on specific chromosomes in meiosis7. Recently, FISH has been used for the physical mapping of ribosomal genes, microsatellites, and transposable DNA sequences on the sugar beet chromosome (Schmidt et al., 1994; Schmidt et al., 1996).

***Genome Analysis:***

GISH permits the characterization of the genome and chromosome of hybrid plants, allopolyploid species, and recombinant breeding lines. Thus, the ancestry of hybrid and polyploid species can be elucidated by genomic southern and in situ hybridization. In essence, the analysis involves the hybridization of labeled genomic DNA from suggested ancestors or relatives to chromosome spreads or Southern blots of DNA from the species under investigation. Hybridization strength, uniformity, and presence of positive or negative bands are then assessed to indicate relationships. Traditionally, genome relationship was analyzed by the study of chromosome painting but there may be several limitations of chromosome pairing. The amount of pairing not only depends on the degree of homology between the pairing chromosomes but also on genetic and environmental factors. A multicolor FISH (MFish) using total genomic DNA probe is a promising approach for simultaneously discriminating each genome in natural or artificial amphidiploids. It uses various fluorescence dyes to represent different painting probes at the same time. Moreover, this technique is a powerful tool for investigating genome homology between polyploid species and their diploid progenitors. Using fluorescent probes produced by shearing the total genomic DNA of a particular progenitor species, it may be possible to identify all chromosomes belonging to a particular genome of the amphidiploids. Multicolor in situ hybridization has been used to distinguish three genomes in hexaploid wheat20. Biotinylated total genomic DNA of the diploid A-genome progenitor Triticum Urartu, digoxigenin- a belled total genomic progenitor Aegilops squares, and non-labeled total genomic DNA of one of the possible B genome progenitors Ae. speltoides were hybridized in situ to metaphase chromosome spreads of Triticum aestivum cv. Chinese Spring. For detection, only two fluorochromes, fluorescein, and rhodamine, were used. The A, B, and D genomes were simultaneously detected by their yellow, blue, and red fluorescence, respectively. (Bennett et., 1999) by using genomic in situ hybridization, demonstrated the allopolyploids origin of Milium montianum (2n=22) and the homology between eight large chromosomes of this species and M. vernal (2n=8).

***Transgene Detection:***

Chromosomal position and local chromatin structure are thought to have a profound effect on the level of gene expression. Variable transgene expression and silencing occur frequently in transgenic plants. In the past few years, there has been much interest in the correlation between transgene expression and cytogenetic position (Kohli et al., 2003). In plants that have been genetically engineered by particle bombardment or Agrobacterium-mediated transformation, the integration mechanism does not appear to be sequence-dependent and presumably occurs via illegitimate recombination at double-strand breaks in the genomic DNA (Svitashev et al., 2002).

***Analysis of Soma Clonal Variation:***

Soma-clonal variations arising in tissue culture have been looked upon as a novel source of genetic variation for crop improvement. Tissue culture phases may impose stress, and induce instability (chromosome breakage and DNA transposition) leading to karyotyping changes. Genetic instability may be associated with the fraction of repeated sequences of DNA present in the plant genome (Bebeli et al., 1990). Analysis of genetic variation in regenerated plants is necessary for the identification and utilization of the proper soma clonal variation for crop improvement. Examination of the chromosomal distribution of 5S and 18S-26S rRNA is useful in identifying the types of genomic changes that might occur during in vitro culture (Maluszynska et al., 1993).

***Karyotyping Using FISH:***

Rather than distinguishing chromosomes from different genomes in hybrids or allopolyploids, karyotyping techniques are used to differentiate the non-homologous chromosomes within a genome. Such procedures are important for detecting chromosomal aberrations, defining which chromosomes are involved in cases of aneuploidy, for studies of chromosomal behavior, and the genomic localization of repetitive DNA sequence arrays, individual loci, or transgene insertion sites. The most commonly used probes for fluorescence in situ hybridization (FISH) karyotyping in plant species are the 5S and 25S rRNA genes, tandemly repeated sequences near telomeres, and centromere-specific repeats. A limited number of tandemly repeated sequences, which provide a large target for hybridization by a single probe, make chromosome identification possible by FISH procedures in Arabidopsis thaliana (Koorneef et al., 2003). Pinus species (Hizume et al., 2002) and Norway spruce (Vischi et al., 2003); however, in the latter two cases there are some ambiguities in the ability to distinguish different members of the karyotype. By combining these FISH landmarks with other chromosomal characters such as arm ratio and heterochromatic regions, precise karyotyping has been reported for the cytologically well-characterized inbred line of maize (Sadder et al.,2001; Chen et al., 200) and selected lily species (Lim et al., 2000).

***Phylogenetic Applications of GISH:***

A new area of GISH experiments is its utilization as a tool in phylogenetic studies, a new application of GISH that we propose to term GISH-phylogenetic. Molecular systematics based on DNA sequences is sometimes limited by the lack of sequence data. Moreover, different algorithms have been used to construct phylogenetic trees that provide for different assumptions and treat data differently. As a result, various phylogenetic approaches applied to the same dataset may generate different phylogenetic trees (reviewed in Whelan et al., 2001). In this case, an independent control system could help to decide, which tree represents the true species relationships. Lim et al. (2007) applied a combination of GISH and FISH with tandem repeat probes to study the evolution of Nicotiana polyploid species. Allopolyploid divergence in Nicotiana is associated with several phenomena, e.g., parental genome DNA losses, retro element activity, and intergenomic homogenization (Leitch et al., 2008). A consequence of these homogenization processes is that GISH works on natural Nicotiana allopolyploids only if allopolyploidy occurred less than 5 million years ago. In natural polyploid species, of this age and older, distinguishing parental genomes using GISH was not possible. The authors argued that GISH failure represented a near-complete genome turnover associated with long-term genome diploidization. By comparing GISH patterns in allopolyploids of different ages, including synthetic allopolyploids that mimic natural polyploid species, Lim et al. (2007) attempted to reconstruct the evolutionary time scales for "genome turn-over".

***Genomic Constitution:***

Horticultural crop genomic compositions and parental chromosomes can be investigated by utilizing GISH methods such as in strawberries (Nathewet and Yanagi, 2014; Choi et al., 2007). GISH analysis can significantly distinguish the different genomes, foremost the evaluation based on chromosome size in each genome. Discrimination of the different genomes proved the incidence of rearrangements after interspecific hybridization (Reis et al., 2014). Karyotype and genomic study of sour cherry proved that GISH could differentiate chromosomes between parental species chromosomes. Based on size and centromere position, karyotype results of Prunus a valium and Prunus cerasus clearly distinguish chromosomes from one another. The utilization of genomic DNA as a probe helps in hybridization with species-specific repetitive sequences which are dispersed over the genome. Hybridization distribution in 32 chromosomes

of sour cherry showed that 16 chromosomes came from P. avium, while the rest of the 16 chromosomes from P. fruticose. These findings ensured that P. cerasus genome constituents are composed of P. fruticose and P. avium (Schuster and Schreiber, 2000).

***Karyotyping by GISH:***

Karyotypes of many plant species are characterized by chromosomes of rather a uniform size, shape, and banding patterns (Schubert et al., 2001). Considering classical banding methods only N banding (in only a limited number of species) and C banding are feasible in plants. Besides FISH, the whole gDNA can be used in some cases to karyotype chromosomes. Genome-specific repeats have frequently a non-random distribution, forming clusters within heterochromatin blocks. As the hybridization signals often coincide with C-bands, this approach is referred to as GISH-banding. Based on GISH-banding, a universal reference karyotype for rye has been created (Zhou et al., 2008). The GISH banding pattern coincided not only with Giemsa C-banding but also with DAPI patterns (DAPI C-banding (Markova et al., 2006), whereby the fluorescence was brighter at the GISH bands, suggesting the presence of AT-rich DNA sequences at these loci. In some species with relatively small genomes, GISH probes label preferentially (peri) centromeric regions, leaving chromosome arms mostly unlabelled (Fig. 2a). A critical 1C genome size of 0.6 pg has been proposed, below which GISH labeling of entire chromosomes usually fails (Raina and Rani, 2001). In some species, GISH labeling seems to be almost exclusively based on centromeric satellite repeats (centromeric GISH, English). On the other hand, the hybridization pattern of some repetitive sequences can mimic GISH. One particular repetitive sequence could be dispersed along the whole genome of only one parent; hence it can recognize parental genomes in hybrid (e.g., Cermak et al., 2008). For example, only pericentromeric regions were revealed in hybridogenous Boechera genotypes (Brassicaceae) upon GISH using gDNA of B. holboellii and B. stricta. This pattern has been caused by genome-specific centromeric repeats (Kantama et al., 2007). GISH-based labeling of entire chromosomes could be potentially achieved by the use of exceptionally high concentrations of hybridizing gDNA and longer hybridization times, as shown for the allopolyploid species Arabidopsis suecica (Ali et al., 2004), having a comparable genome size as Boechera species.

**Difference Between FISH and GISH**

***Fluorescence In-situ Hybridization:***

A laboratory technique for detecting and locating a DNA sequence or a gene on a chromosome within the genome. It is the process of painting/staining/ the whole or part of a chromosome with fluorescence molecule to identify chromosomal abnormality and the presence of a region of DNA or RNA within the chromosome. Chromosomes can be seen if they are stained and magnified under the microscope. The technique relies on exposing chromosomes to a small DNA sequence (probe) that has a fluorescent molecule attached to it. So that scientists can visualize the location of a particular gene to check for a variety of chromosomal abnormalities. (Source: https://www.majordifferences.com/2015/10/difference-between-fish-andgish.html#. wx04btqvziv).

On the other hand, a molecular cryptogenic technique uses fluorescent probes that bind to only those parts of the chromosome with a high degree of sequence complementarity. It was developed by biomedical researchers in the early 1980s (Langer et al., 1982) and is used to detect and localize the presence or absence of specific DNA sequences on a chromosome. Fluorescence microscopy can be used to find out where the fluorescent probe is bound to the chromosomes. FISH is often used for finding specific features in DNA for use in genetic counseling, medicine, and

species identification. FISH can also be used to detect and localize specific RNA targets (mRNA and miRNA) in cells, circulating tumor cells, and tissue samples. In this context, it can help define the spatial-temporal patterns of gene expression within cells and tissues (Amann et al., 2008).

***Basic Features of FISH:***

The detection of chromosomal DNA in cytological preparations was initially based on the application of isotopic labeled RNAs and DNAs. A breakthrough came with the introduction of fluorescent labels, (in) directly linked to DNA probes and visualized under the fluorescence microscope. The technology of FISH appeared superior to previous in situ technologies in providing better spatial resolution along with the potential of simultaneously using different fluorescence systems for multi-probe analysis. The number of targets to be analyzed simultaneously depends on the number of fluorochromes with different excitation and emission wavelengths (Nederlof et al. 1990). In a large-scale study involving all major classes of repeats, patterns that are representative and unique for each chromosome can be produced. In a comprehensive study of the sugar beet (Beta vulgaris) genome, the positions of major classes of repeats and gene-rich regions were assessed concerning the heterochromatic pericentromeric regions and gene-rich distal euchromatin regions of the chromosomes (Schmidt et al., 1998). Thus, in summary, FISH adds a powerful new tool to the existing battery of cytological techniques, and will play an important role in future efforts to determine genome structure.

***Genomic In-situ Hybridization:***

The first procedure to use fluorescent labels to distinguish chromosomes in the plant kingdom involved the process of genomic in situ hybridization (GISH) (Schwarzacher et al., 1989). This widely applied cytogenetic technique provides a direct visual method for distinguishing parental genomes and analyzing genome organization in interspecific hybrids, allopolyploid species, and interspecific introgression lines (Jiang and Gill, 1994.) In GISH, labeled total genomic DNA is used as a probe with unlabeled genomic DNA from another species as a blocking agent. Because the chromosomal sequences that are common to the two species contributing to the analyzed specimen are hybridized with unlabeled DNA, the labeled probe, especially the portion containing species-specific dispersed repetitive sequences (such as transposable elements), hybridizes to only one of the two sets of chromosomes.

**Procedures of FISH and GISH**

***FISH Procedure:***

1. Cells cultured, harvested, prepared on microscopic slides and are denatured (now DNA is single stranded for probe attachment)
Cells on metaphase stage of division is selected (as maximum condensation on metaphase stage)
2. Fluorescently labeled hybridization probe is added
(The hybridization probe is a short fragment of DNA that has a fluorescent dye attached that enable scientist to visualize the site of probe attachment. A typical FISH probe would be 10 - 100 kb long)
3. If the DNA corresponding to the probe is present in the sample, then the fluorescently labeled probe will attach to the DNA and will be visible under a fluorescent microscope.
4. This allows deletions (no fluorescent spot at the expected position) and rearrangements (spot present, but in an unexpected chromosomal location) to be detected. Thus helps in diagnosis of genetic diseases.

Source: https://www.majordifferences.com/2015/10/difference-between-fish-and gish.html#. WxO4BTQvzIV.

***DNA and RNA Probes***:
RNA probes can be designed for any gene or any sequence within a gene for the visualization of mRNA (Louzada et al., 2012; Lee et al., 2011) lncRNA and miRNA in tissues and cells. FISH is used by examining the cellular reproduction cycle, specifically the interphase of the nuclei for any chromosomal abnormalities (Bernasconi et al., 2008). FISH allows the analysis of a large series of archival cases much easier to identify the pinpointed chromosome by creating a probe with an artificial chromosomal foundation that will attract similar chromosomes. The hybridization signals for each probe when a nucleic abnormality is detected. Each probe for the detection of mRNA and lncRNA is composed of 20 oligonucleotide pairs, each pair covering a space of 40–50 bp. For miRNA detection, the probes use proprietary chemistry for specific detection of miRNA and cover the entire miRNA sequence (Bernasconi et al., 2008).

Probes are often derived from fragments of DNA that were isolated, purified, and amplified for use in the Human Genome Project. The size of the human genome is so large, compared to the length that could be sequenced directly, that it was necessary to divide the genome into fragments. (In the eventual analysis, these fragments were put into order by digesting a copy of each fragment into still smaller fragments using sequence-specific endonucleases, measuring the size of each small fragment using size-exclusion chromatography, and using that information to determine where the large fragments overlapped one another.) To preserve the fragments with their DNA sequences, the fragments were added into a system of continually replicating bacteria populations. Clonal populations of bacteria, each population maintaining a single artificial chromosome, are stored in various laboratories around the world. The artificial chromosomes can be grown, extracted, and labeled, in any lab containing a library. Genomic libraries are often named after the institution in which they were developed. An example is an RPCI-11 library, which is named after the Roswell Park Cancer Institute in Buffalo New York. These fragments are about 100 thousand base pairs and are the basis for most FISH probes.

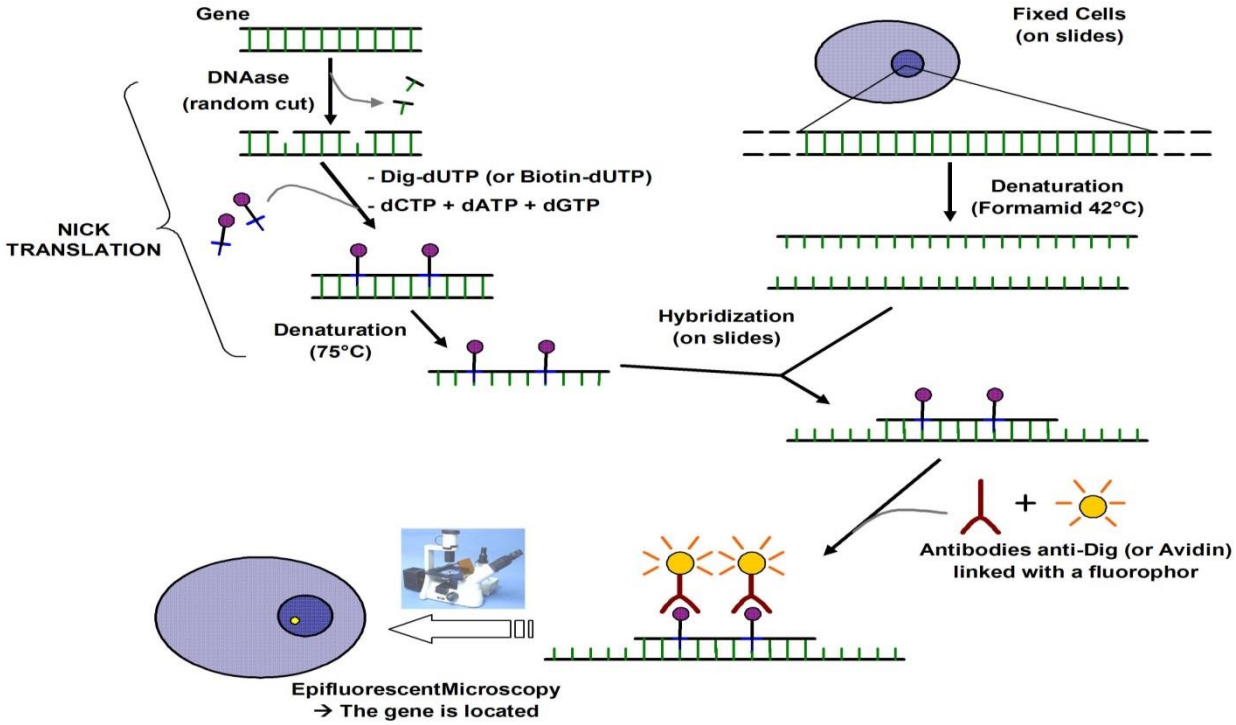


**Figure 1: Scheme of the principle of the FISH Experiment to localize a gene in the nucleus.**
(Source: https://en.wikipedia.org/wiki/Fluorescence_in_situ_hybridization#Probes_%E2%80%93_RNA_and_DNA).

***GISH Procedure:***

Extracting a total of genomic DNA from one of the species (to be used as a probe). Chromosome preparation of species two being studied repeated sequences in both species anneal quickly than the unique sequences of the genome. This helps in assessing genome relationship between species

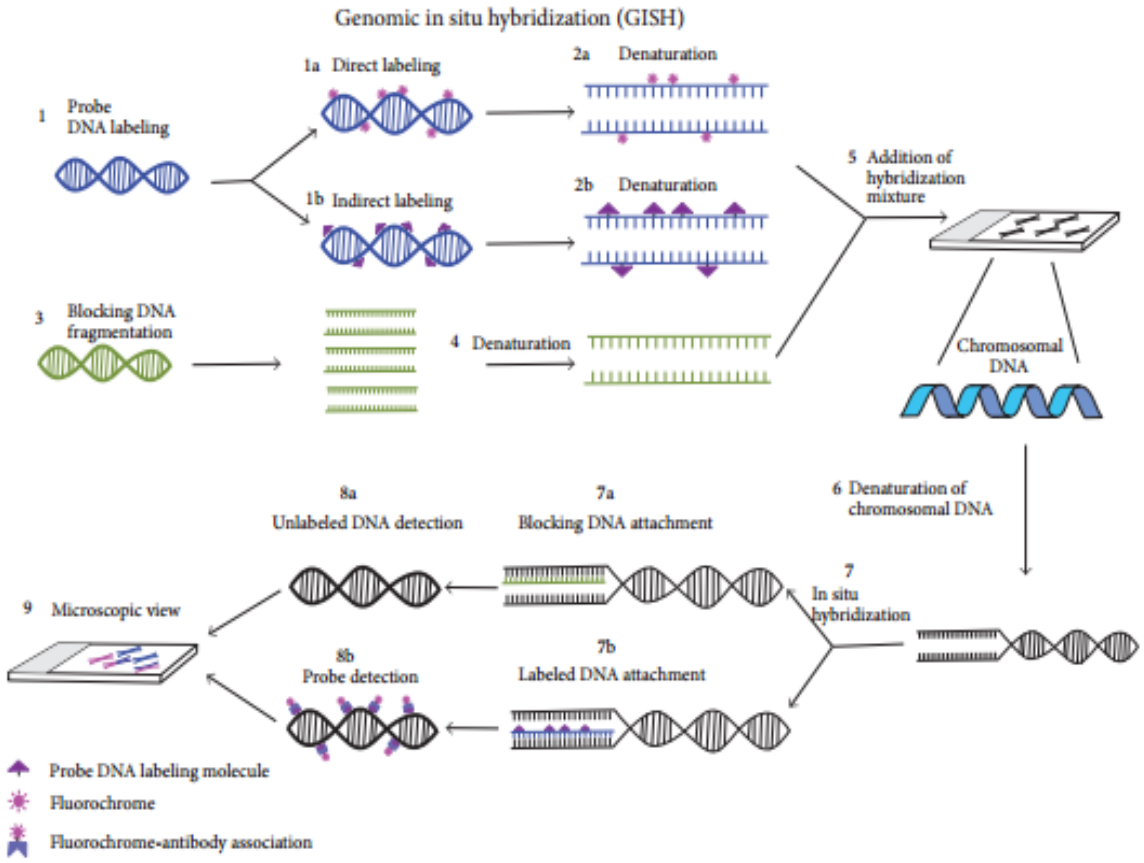


**Figure 2: Genomic in situ hybridization (GISH) mechanism diagram (Pendinen *et al.*, 2012).**
Source: https://www.majordifferences.com/2015/10/difference-between-fish-and-gish.html#.WxO4BTQvzIV.

**Advantages and Disadvantages of FISH**

FISH does not require the cultivation of the organisms or any technology-based gene amplification which can lead to false negatives and positives. In contrast to some other EMDs, FISH allows visualization of whole cells that are important to environmental remediation activities. FISH can thus provide complementary information to other EMDs, such as the morphology of the cells or the association of groups of microorganisms with relationship to one another. FISH can target several different genes simultaneously, for example, genes associated with specific degrading species of interest (e.g., Dehalococcoides) and broader microbial groups, such as methane-producing organisms. Depending on the species, and in combination with other appropriately validated activity-targeted approaches, FISH can provide general information about the activity of the organisms or populations of interest. FISH enables single-cell microbial studies and allows for subsequent studies, such as gene sequencing.
Source: https://www.itrcweb.org/documents/team_emd/FISH_Fact_Sheet.pdf.

The detection limit of FISH is high (~106 cells/mL). However, in some cases, high detection limits can be corrected by sample concentration or cell extraction methods which lower the detection limits to a few hundred cells per concentrated sample. Validated probes and FISH procedures are

not available for a wide range of organisms within the bioremediation field. Additionally, standard protocols for sample collection and storage before FISH analysis have not yet been developed. FISH can also be used to target not only ribosomal genes (which indicate the type of organism) but also functional genes (via mRNA) relevant to bioremediation. These other genes indicate what the microorganisms can do with regards to contaminant biodegradation, for example, naphthalene dioxygenase or reductive dehalogenase. However, laboratory protocols are often time-consuming and complicated and not yet validated for field applications.

The FISH method is not widely commercially available. Currently, mainly specialized research laboratories are performing these analyses to explore and optimize the potential of FISH for validated and cost-effective applied studies. The FISH method is currently expensive because of the expertise and labor needed for the development of validated FISH protocols and direct microscopic counting. Once validated protocols have been developed, FISH can be automated to some extent by using flow cytometers to count target cells more efficiently, reducing the analytical costs. However, when using flow cytometers for cell counting, all information regarding spatial relationships (among and between the cells) is lost.

Source: https://www.itrcweb.org/documents/team_emd/FISH_Fact_Sheet.pdf.

## SUMMARY

The recent developments in cytogenetic described above will provide new tools for the analysis of plant genomes. These techniques allow the study of the fine details of chromosome structure and will permit sophisticated analyses of chromosomal behavior. As more genomes become sequenced, tools to study chromosomal organization and behavior will play a greater role in investigating the function of those genomes. Chromatin staining for classical cytological studies still contributes much to the present appreciation of chromosomal diversity in wild species. This approach allows the detection of gross karyotype alterations such as changes in chromosome number and morphology and gives an overview of chromosome behavior in mitosis and meiosis.

The FISH and GISH techniques present an authentic model for analyzing the individual chromosome, chromosomal segments, or the genomes of natural and artificial hybrid plants. These have become the most reliable techniques for studying allopolyploids because most cultivated plants have been developed through the hybridization of polyploidization. FISH is the technique that relies on exposing chromosomes to a small DNA sequence (probe) that has a fluorescent molecule attached to it. GISH is an in-situ hybridization method that uses whole genomic DNA as a probe to study the relationship, divergence, and evolution of the genome between different species.

Cytogenetic research and chromosome analysis are the main aspects of genomics and genetic sciences. Molecular cytogenetic techniques, such as in situ hybridization methods, are admirable tools to analyze the genomic structure and function, chromosome constituents, recombination patterns, alien gene introgression, genome evolution, aneuploidy, and polyploidy (Andres et al., 2013 Younis et al., 2015).

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