# In-situ hybridization, GISH, FISH

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# *Introduction*

**In-situ hybridization techniques, including Fluorescence In Situ Hybridization (FISH) and Genomic In Situ Hybridization (GISH), are pivotal in cytogenetic research for identifying and mapping chromosomes and specific DNA sequences. FISH uses fluorescent probes to bind to specific chromosome regions, while GISH differentiates between genomes using total genomic DNA from different species. These techniques have been instrumental in plant and animal genome research, providing insights into chromosome structure, gene mapping, and hybridization processes. Below are the key aspects of these techniques:**

# *Principle of FISH*

- **Fluorescence In Situ Hybridization (FISH)：is a powerful molecular cytogenetic technique that combines the principles of cytogenetics and molecular biology. It operates through a specific mechanism to detect genetic variations within a cell. FISH utilizes fluorescently labeled probes to identify specific nucleic acid sequences within cells, enabling the visualization of chromosomal structures and the detection of genetic variations, such as deletions, inversions, duplications, and translocations (Hu and Wu, 2021).**
- **This technique is considered an important tool in diagnosing genetic disorders and studying chromosomal organization as well as alterations associated with hereditary diseases and cancers.**

## Mechanisms of Fluorescence In Situ Hybridization (FISH):

FISH is a precise technique used to detect genetic variations within cells.

The following section describes the detailed mechanisms through which FISH operates.

**Core Mechanism: Fluorescent Probes and Nucleic Acid Detection**

•FISH primarily functions by employing fluorescent probes to identify particular nucleic acid sequences within cells and pinpoint their exact location. These probes are designed to bind specifically to target DNA or RNA sequences.

**Detection of Cytogenetic Variations**

•The method is capable of detecting a range of cytogenetic variations. This includes identifying chromosomal deletions, amplifications, and translocations. By visualizing these changes, FISH provides critical insights into the genetic makeup of cells.

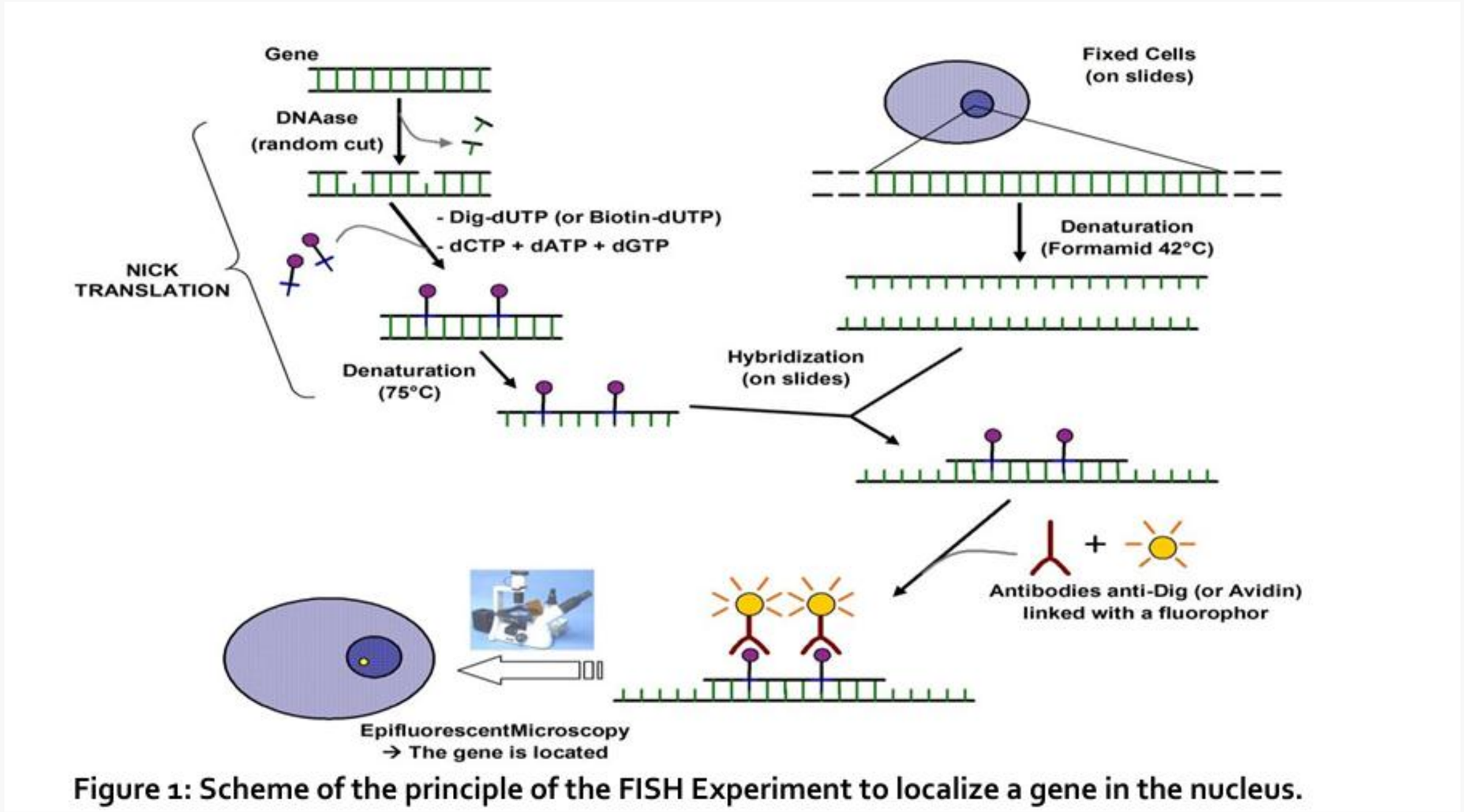
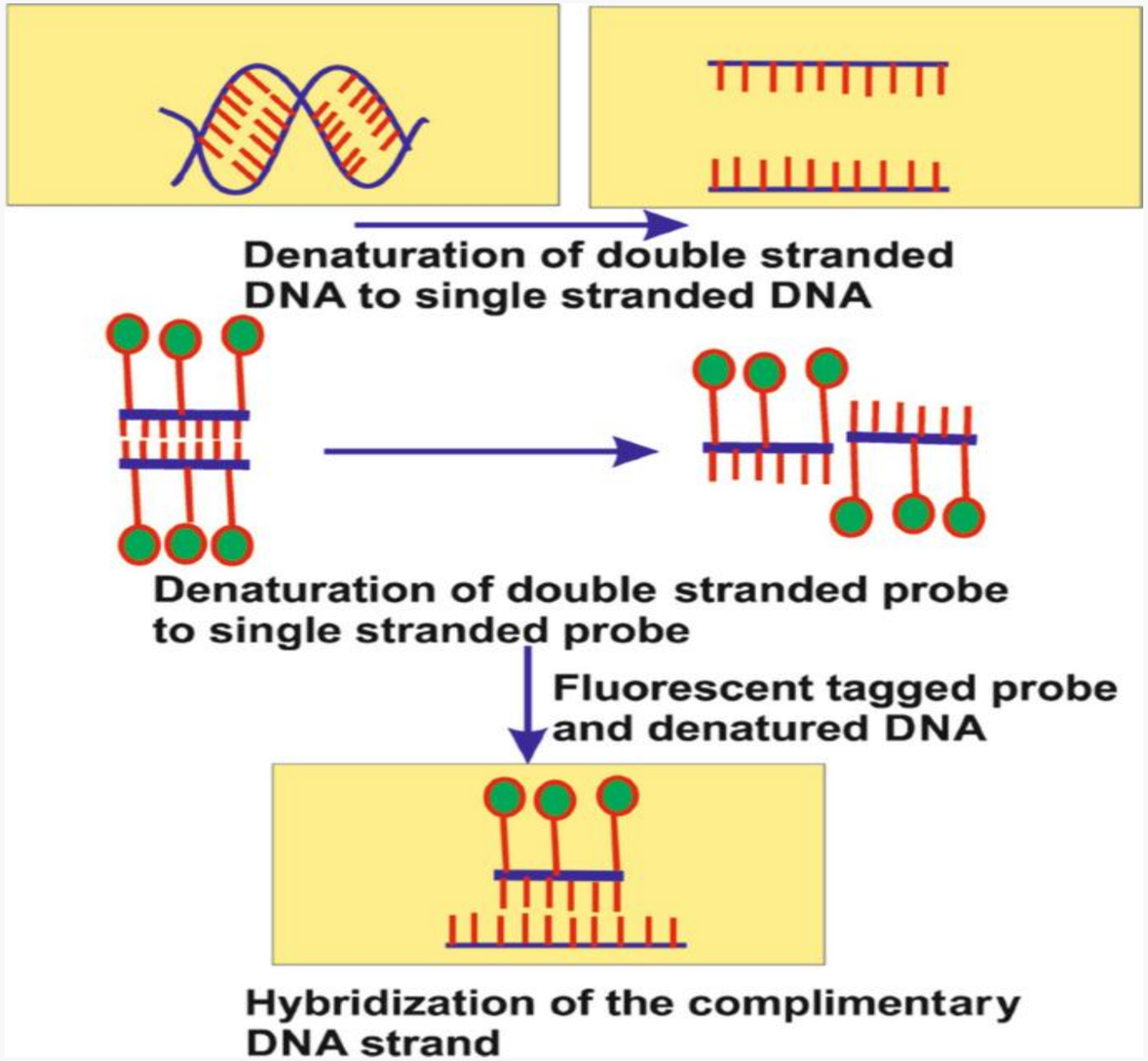



Figure 1: Scheme of the principle of the FISH Experiment to localize a gene in the nucleus.

(Mekonen and Ali, 2023)




Fluorescent in situ hybridization (FISH) technique

# *Principle of GISH*

**Genomic In Situ Hybridization (GISH) is a specialized type of in situ hybridization used to map the complete parental genomes, particularly in diploid and polyploid hybrids. The effectiveness of this technique depends on several factors, including the age of the polyploid and the similarity between repetitive DNA regions in the parental genome, as high overall similarity between parental genomes reduces GISH efficiency. The main principle of this technique is to enable researchers to distinguish the origin of chromosomes in hybrid organisms, that is, to determine which chromosomes came from which parent, thereby facilitating the study of genome organization and structure in hybrids. (Weiss-Schneeweiss and Jang, 2023).**



# *Working Mechanism of GISH*

**1.Selective Hybridization of Genomic DNA:**
Genomic in situ hybridization (GISH) is an efficacious technique 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.

**2.Blocking DNA to Prevent Non-Specific Hybridization:**
However, genomic and blocking DNA utilization in GISH differentiate it from FISH analysis.

**3.Visualization of Parental Genomes:**
In plant species, genome organization and homology studies 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(Mekonen and Ali, 2023) .

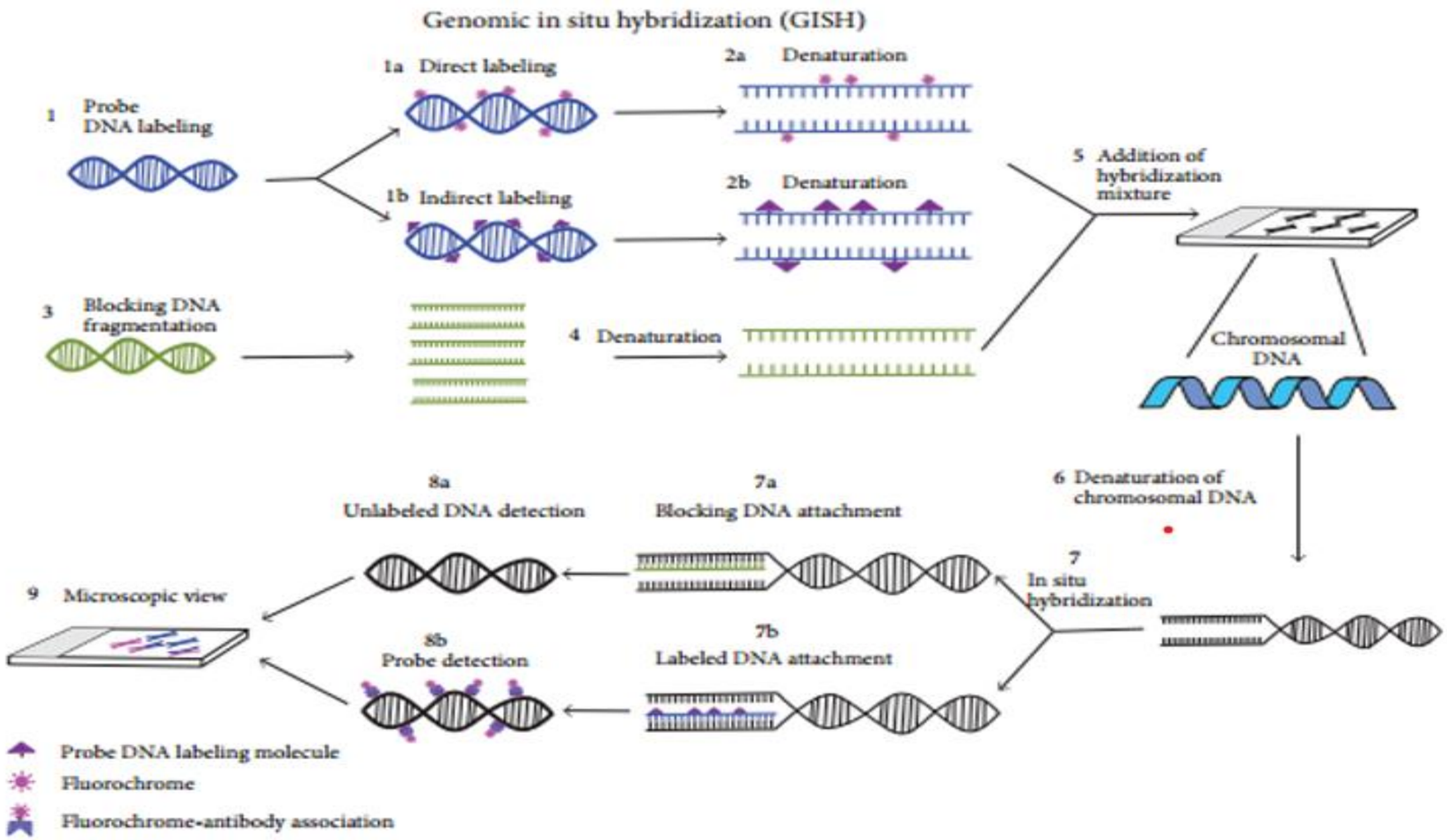
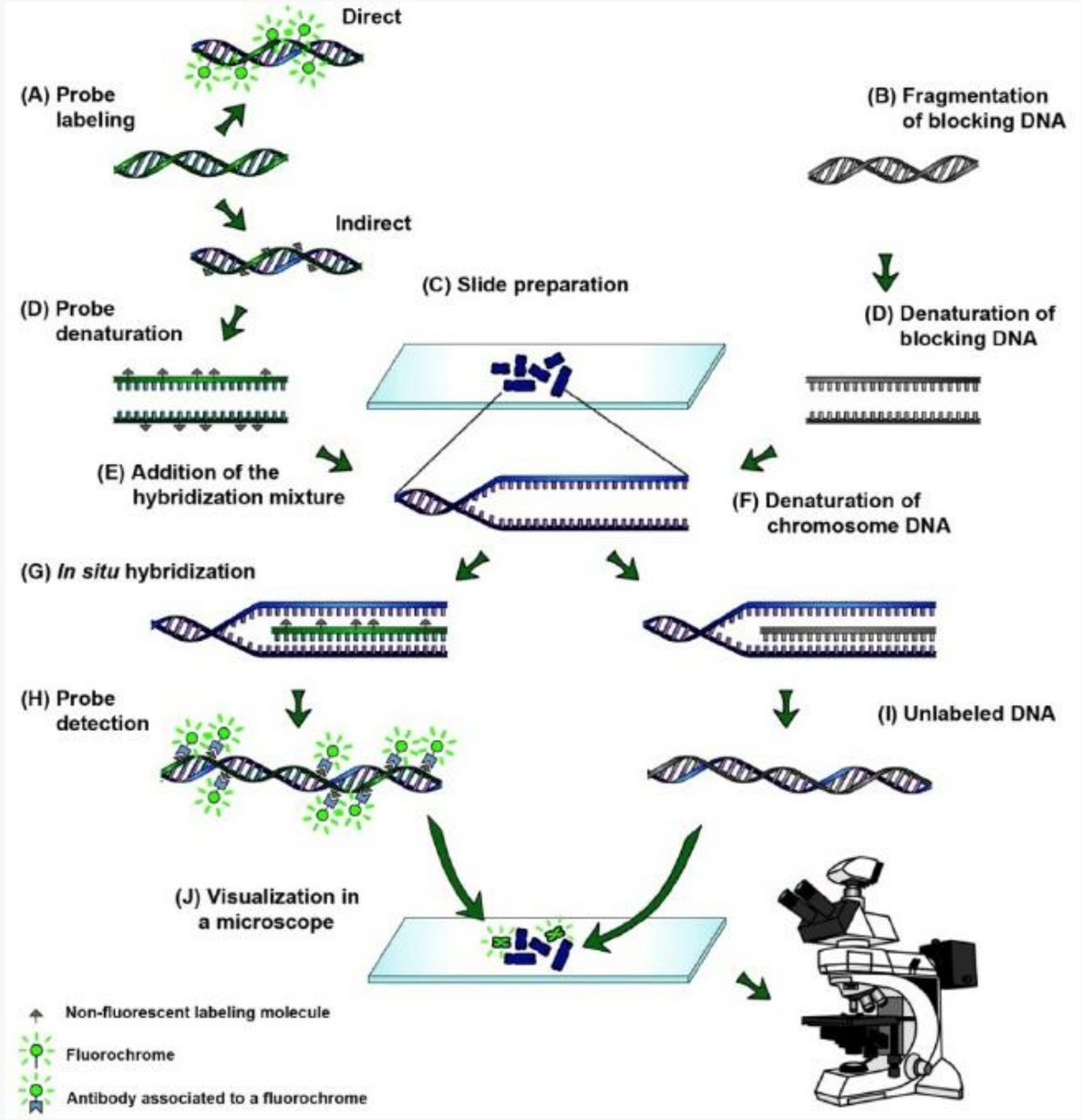



**Figure 2: Genomic in situ hybridization (GISH) mechanism diagram (Pendinen *et al.*, 2012).**




Main steps of the genomic in situ hybridization (GISH).

# Comparison Between FISH and GISH Techniques

| Feature / Comparison | GISH | FISH |
|---|---|---|
| **Definition** | **Genomic In Situ Hybridization:** Uses total genomic DNA as a labeled probe with blocking DNA to differentiate .parental genomes in hybrids and polyploids | **Fluorescence In Situ Hybridization:** Uses fluorescent probes to detect and localize specific DNA or RNA .sequences on chromosomes or inside cells |
| **Type of probe / Principle** | Uses labeled total genomic DNA as a probe with unlabeled genomic DNA from another species as a .blocking agent | Detects and locates DNA sequences using fluorescent .probes that bind to specific targets on chromosomes |
| **Purpose / Application** | Distinguishes parental genomes and analyzes genome organization in interspecific hybrids, allopolyploid .species, and introgression lines | Detects presence/absence of DNA sequences on .chromosomes; can also target RNA (mRNA, miRNA) |
| **Visualization / Specificity** | Hybridizes only to one chromosome set due to species- .specific repetitive sequences | Visualized under a fluorescence microscope with high .spatial resolution and multi-probe capability |
| **History / Origin** | .First applied in plants in 1989 (Schwarzacher et al., 1989) | Developed by biomedical researchers in the early 1980s .(Langer et al., 1982) |
| **Common Uses / Applications** | Used for distinguishing parental genomes and studying .genome organization | Used for genetic counseling, medicine, species identification, and defining spatial-temporal gene .expression |

# *Applications of FISH and GISH*

- Classical cytological studies using **chromatin staining** help understand **chromosomal diversity** in wild species.
- Detect **major karyotypic changes**, e.g.:
  - Variations in **chromosome number**
  - Changes in **chromosome morphology**
- Provide an overview of **chromosome behavior** during **mitosis and meiosis**.
- Modern cytogenetics:
  - **FISH:** maps **single cloned or PCR-amplified sequences**, detects **specific DNA/RNA targets**.
  - **GISH:** uses **total genomic DNA probes** to determine the **parental origin of chromatin** in **hybrids** and **allopolyploids**.

- **FISH and GISH together** help clarify:
  - **Chromatin folding**
  - **Nuclear organization**
  - **Chromatin distribution** during **cell cycle and development**
- Applied in both **animals and plants**:
  - Early studies used **repeated DNA sequences** (e.g., sat-DNA in Drosophila and mice).
  - Plant studies initially lagged behind mammalian cytogenetics, but are now widely used for:
    - **Hybrid analysis**
    - **Genome organization studies**
    - **Chromosomal behavior research**

# *Advantages and Disadvantages of FISH*

## Advantages of FISH:

- Does **not require cultivation** of organisms.
- Allows **visualization of whole cells**, showing morphology and cell associations.
- Can **target multiple genes simultaneously** (e.g., species-specific and broad microbial groups).
- Provides **information on microbial activity**, especially when combined with other approaches.
- Enables **single-cell studies** and further analysis، like **gene sequencing**.
- **Complementary to other environmental molecular diagnostics (EMDs).**

# *Advantages and Disadvantages of FISH*

## Disadvantages of FISH:

- **High detection limit (~$10^6$ cells/mL)**; requires sample concentration or extraction for rare cells.
- **Validated probes not available** for all microorganisms.
- **Standard protocols for sample collection and storage** are not fully developed.
- **Targeting functional genes** is complex, time-consuming, and not fully validated for field use.
- **High cost and limited commercial availability**; mainly used in specialized research labs.
- **Flow cytometry automation loses spatial information** about cells.

# *Advantages and Disadvantages of GISH*

## Advantages of GISH:

- **High Specificity**: GISH allows for the precise localization of specific DNA sequences, making it invaluable for identifying chromosomal abnormalities and studying genetic diversity.
- **Visualization of Chromosomal Structures**: This technique enables the observation of chromosomal arrangements and interactions, which is crucial for understanding complex genetic traits.
- **Application in Plant Breeding**: GISH is particularly useful in plant genetics, facilitating the identification of alien chromatin in hybrid species, thus aiding in the development of new cultivars.

# *Advantages and Disadvantages of GISH*

## Disadvantages of GISH

•**Technical Complexity**: The procedure requires meticulous preparation and optimization, which can be time-consuming and may lead to variability in results.
•**Limited Resolution**: While GISH provides valuable information, its resolution may not be sufficient for detecting small-scale genetic variations compared to other methods like sequencing.
•**Dependence on Probe Design**: The effectiveness of GISH is heavily reliant on the design of DNA probes, which can limit its applicability if suitable probes are not available.
In contrast, while GISH offers significant advantages in genetic research, alternative methods such as next-generation sequencing provide higher resolution and broader applications, albeit at a higher cost and complexity. This highlights the ongoing need for balancing the benefits and limitations of various genetic analysis techniques in research(Xia et al., 2025 ؛Azuma, 2024).

https://youtu.be/MqubuIdR8NY?si=k5UjD_S6tyyoW54q

https://youtu.be/jAMsKynZWNo?si=qDaS-oEteqFsTjMH

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*Thank you*