Fluorescence In Situ Hybridization (FISH)

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Course: Genetics II (MA 206)
Summer Class
Lecture 4
Date 30-8-2026

Outline

- What is FISH.
- Principle of FISH
- Materials Required for FISH
- Types of probes
- Procedure of FISH
- Expected Results
- Safety Precautions

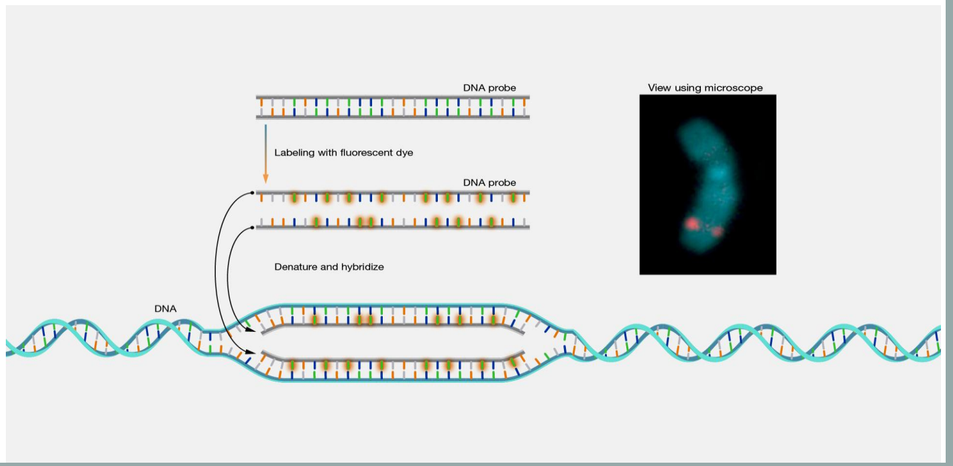
Objectives

To acquaint students with the concepts of Fluorescence In Situ Hybridization.

❖ What is FISH

- Is a **laboratory technique** for **detecting and locating specific DNA** sequences on **chromosomes**.

- It is widely used in **research and clinical diagnostics** to identify **genetic abnormalities**, such as **chromosomal rearrangements**, **deletions**, **duplications**, **and aneuploidies**.



❖ Principle of FISH

- FISH is a technique that involves the hybridization of a fluorescently labelled **DNA or RNA probe** to its complementary target sequence within a biological sample.

- The hybridized probes are subsequently visualized using fluorescence microscopy, which enables the identification of **genetic abnormalities**, **gene mapping**, and **pathogen detection**.

❖ Materials Required:

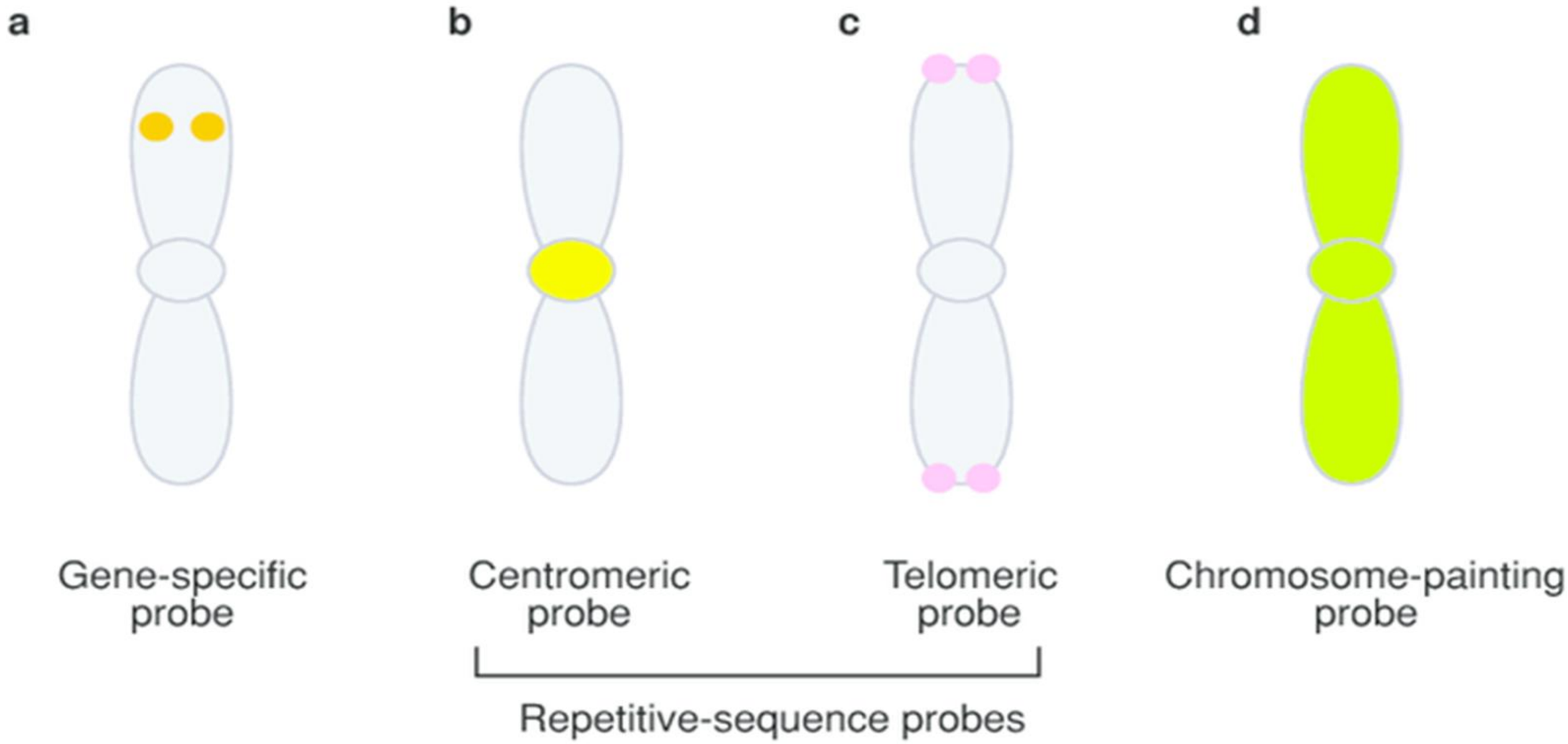
1. **Sample:** Metaphase chromosomes from cells (blood, tissue, or cultured cells).

2. **Probe:** Fluorescently labeled DNA or RNA probe specific to the target sequence.

3. **Reagents:** Formamide or NaOH, Hybridization buffer, Wash buffers, Counterstain

4. **Equipment:** Fluorescence microscope, Water bath, Incubator and Slides and coverslips.

❖ Types of probes:



Examples of different types of fluorescence in situ hybridisation (FISH) probes

❖ Procedure of FISH:

1. **Sample Preparation**: Prepare metaphase chromosomes.

2. **Denaturation**: Denaturation solution (e.g., 70% formamide) at 70–75°C for 2–5 minutes, Dehydrate the slide using an ethanol series (70%, 85%, 100%) and air-dry.

3. **Probe Denaturation**: By heating it at 75°C for 5 minutes, then place it on ice.

4. **Hybridization**: Apply the denatured probe, incubate the slide in a humid chamber at 37°C for 4–16 hrs.

5. **Post-Hybridization Washes**:

- Wash in formamide at 72°C for 2–5 minutes and Wash in formamide at room temperature for 1–2 minutes.

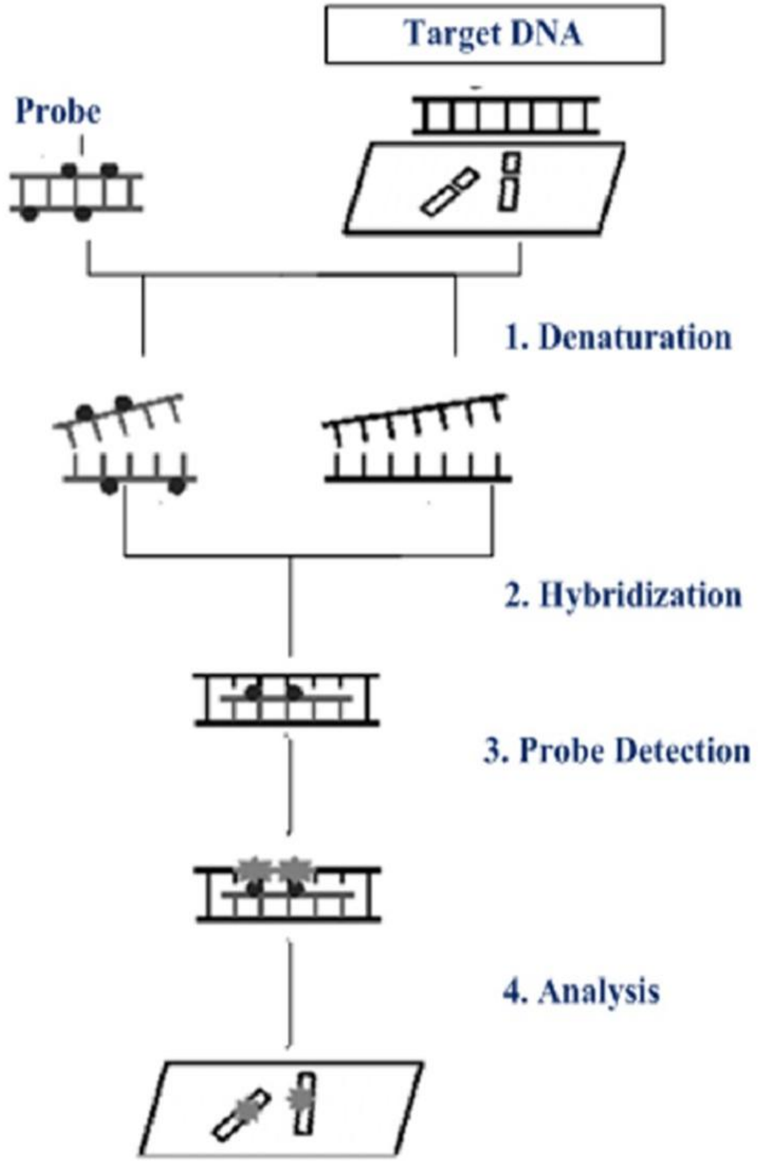
6. **Counterstaining**: .

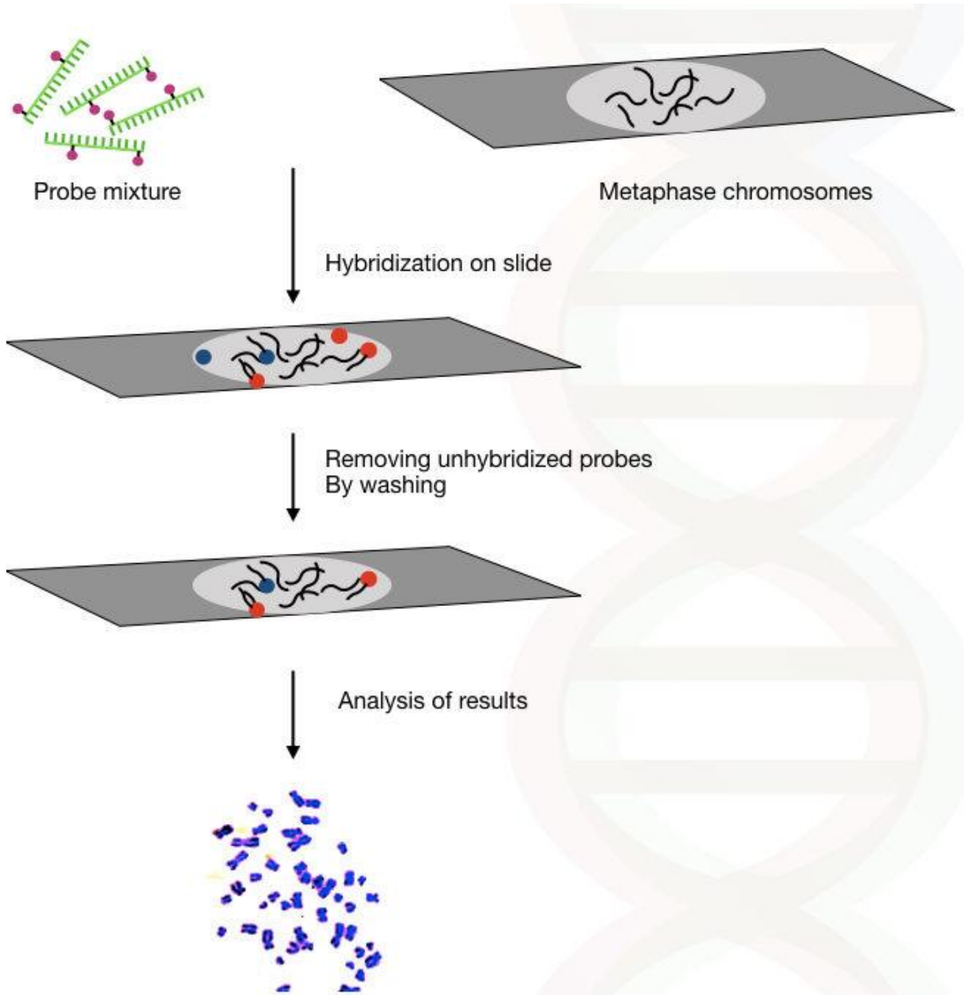
- Apply a counterstain, Mount the slide.

7. **Visualization:**

- Observe the slide under a fluorescence microscope

Procedure of FISH:

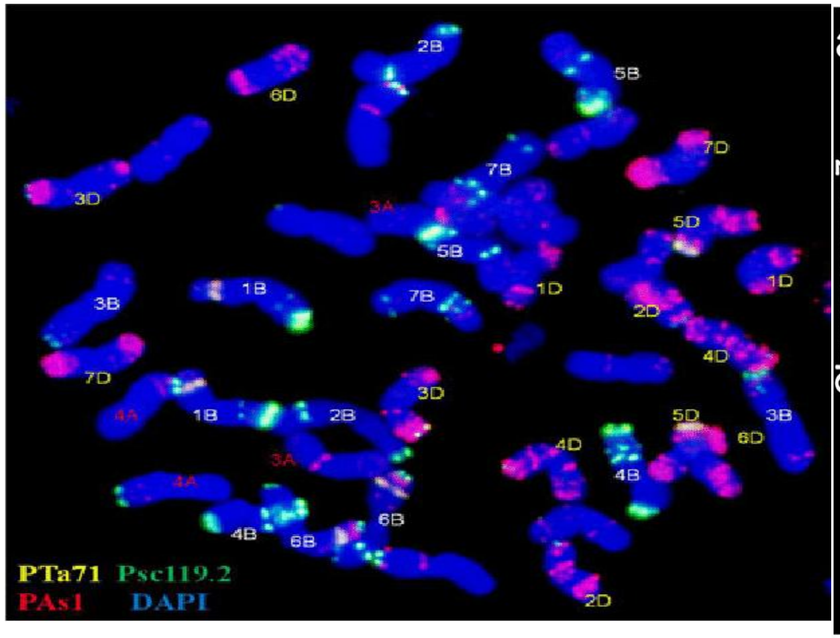


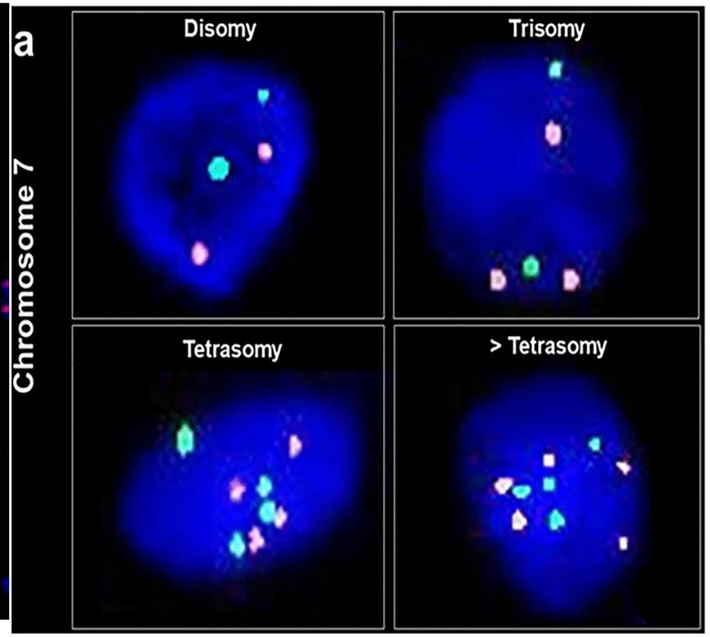


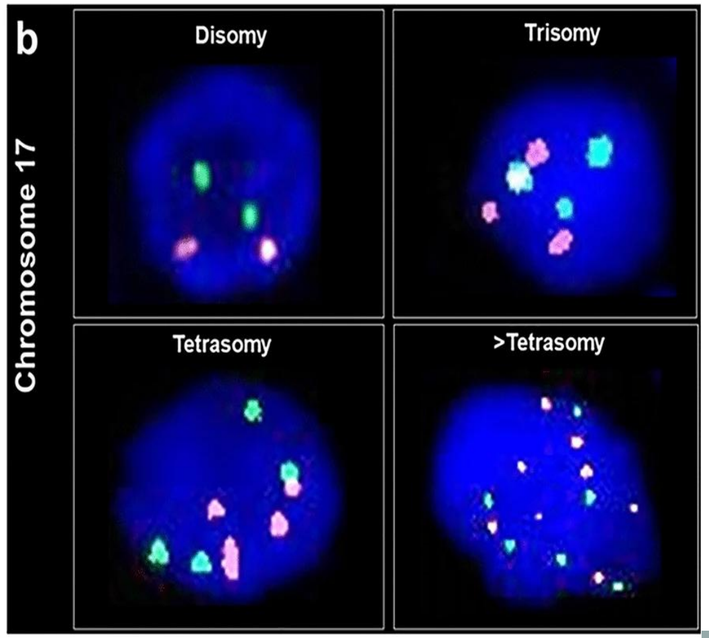
❖ Expected Results:

1. Positive Signal:

- A fluorescent signal at the specific chromosomal location indicates the presence of the target sequence.



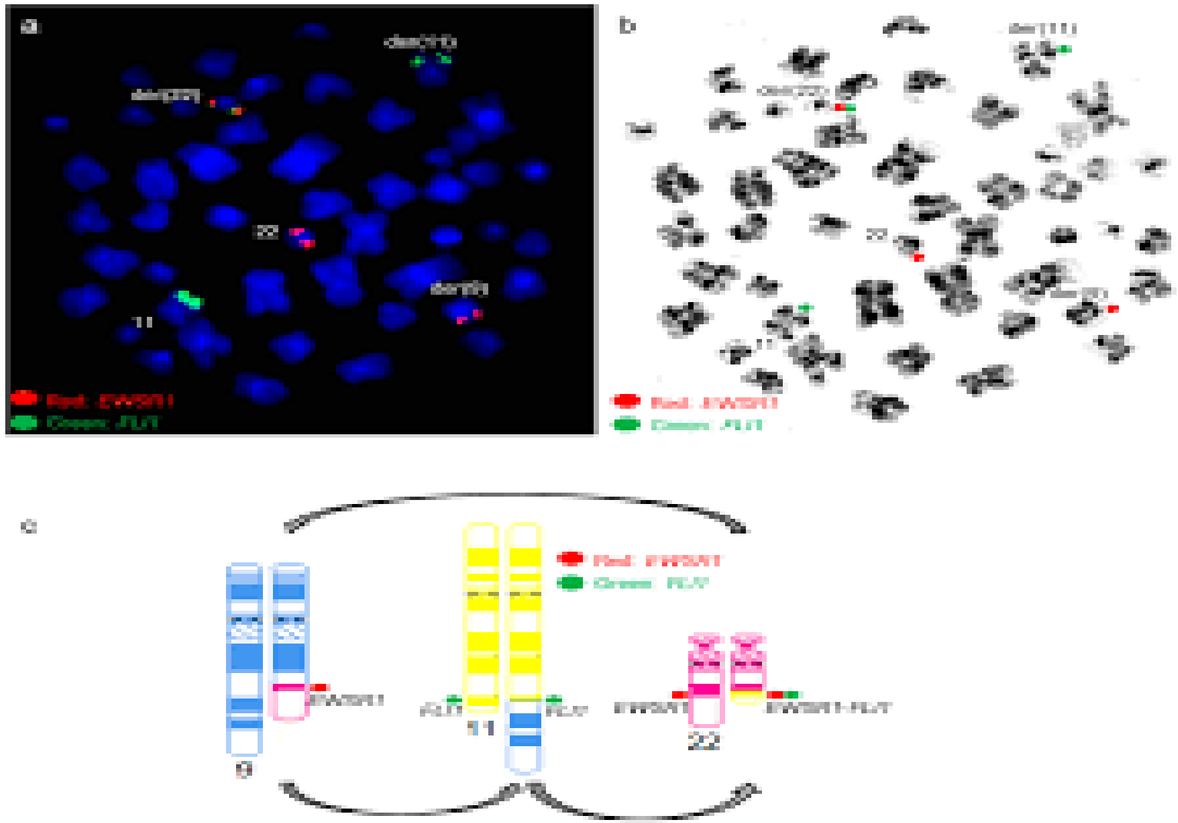




❖ Expected Results:

2. Negative Signal:

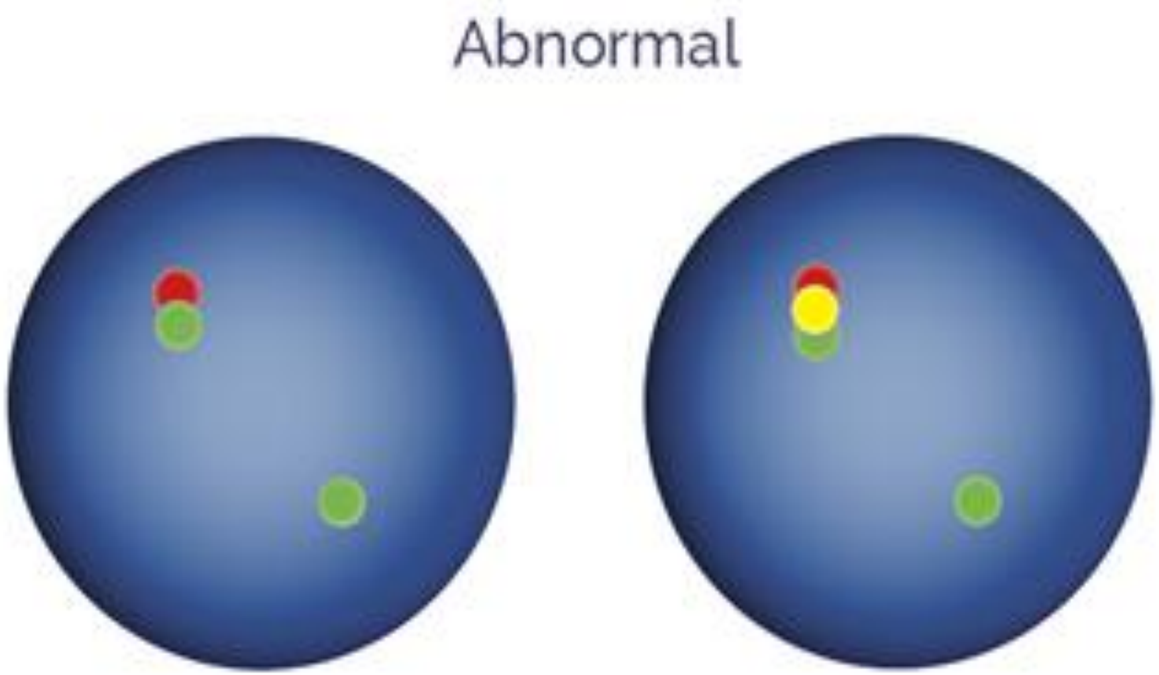
- Absence of a fluorescent signal indicates the absence of the target sequence.



❖ Expected Results:

3. Abnormal Patterns:

- Abnormal patterns, such as split signals or additional signals, indicate chromosomal rearrangements or amplifications.



❖ Applications of FISH:

1. Cancer Diagnostics.

2. Prenatal Diagnosis

3. Genetic Research

4. Microbial Detection

❖ Advantages:

1. High specificity and sensitivity.

2. Can be used on non-dividing cells

3. Multiplexing capability

❖ Limitations:

1. Requires specialized equipment.

2. Time-consuming and labor-intensive

3. Probe design must be highly specific

❖ Safety Precautions:

- Handle fluorescence dyes with care as they may be hazardous.
- Use appropriate personal protective equipment .
- Dispose of reagents properly according to laboratory regulations.

References

- Babcock, E. B., & Collins, J. L. (1918). Genetics laboratory manual. McGraw-Hill Book Company Incorporated.

- Blair, C. (2018). BIO2450L Genetics Laboratory Manual.

- VENNISON, S. (2009). Laboratory manual for genetic engineering. PHI Learning Pvt. Ltd..

Thanks