# FLUORESCENT *IN SITU* HYBRIDIZATION (FISH)

- **Fluorescent *in situ* hybridization (FISH)** is a molecular cytogenetic technique that uses fluorescent probes that bind only those parts of the chromosome with a high degree of sequence complementarily.

**OR**

- **Fluorescent *in situ* hybridization (FISH)** is a cytogenetic technique that can be used to detect and localize the presence or absence of specific DNA sequences on chromosomes.

# Main Features of FISH

1. It is a laboratory technique which is used to determine how many copies of a specific segment of DNA are present or absent in a cell.
2. It is a form of DNA testing for genetic diagnosis in which a special region of a chromosome is stained with a fluorescent dye.
3. FISH technique that is used to detect nucleic acids in cells or tissues by using probes that are coupled to a fluorescent dye.
4. FISH is used to localize & detect specific mRNA sequences in preserved tissue sections or cell preparations by hybridizing the complementary strand of a nucleotide probe to the sequence of interest.

# Preparation of Material

- There are three types of tissue section.

1. **Frozen sections**
2. **Paraffin embedded sections**
3. **Cells in suspension**

- This methods are most commonly used for *in situ* hybridization.

# PROBES

- Probes are complimentary sequences of nucleotide bases to the specific mRNA sequences of interest.

- These probes can be as small as 20-40 base pairs or be up to 1000 bp.

# Types of Probes

1) Double Stranded DNA Probes.
2) Single Stranded DNA Probes.
3) RNA Probes.
4) Oligonucleotide Probes.

**CONT.**

1) **Double Stranded DNA Probes:-** This type of probes can be produced in two ways, *viz.*, *(i)* by bacteria, and *(ii)* PCR. This types of probes are rarely used.

2) **Single Stranded DNA Probes:-** This type of probes can be produced in two ways, *viz.*, *(i)* by reverse transcription of RNA or *(ii)* PCR.

3) **RNA Probes:-** RNA Probes also known as cRNA probes. RNA probes are still most probably the most widely used probes for *in situ* hybridization.

4) **Oligonucleotide Probes:-** Oligonucleotides are short sequences of nucleotides (RNA or DNA), typically with 20-30 bases in size that are synthesized in vitro.

   This is ideal for *in situ* hybridization because their small size allows for easy penetration into the cells or tissue of interest

# Step Involved in FISH

1. Selection of probe.
2. Probe generations.
3. Labelling of probe.
4. Fixation of tissues.
5. Hybridization and Washing.
6. Detection.
7. Observation.

**1) Probe Selection:-** The first step of *in situ* hybridization is selection of probe types.

- ✓ There are several probes like double stranded DNA (dsDNA), single stranded DNA (ssDNA), single stranded complimentary RNA (sseRNA) and synthetic oligonucleotides.

**2) Probe Generation:-** There are two methods of generation of probes.

A) Nick translation

B) PCR using tagged nucleotides

**3) Probe Labeling:-** Both radioactive and non-radioactive probe labels are commonly used for *in situ* hybridization. Radioisotopes that are used for labeling include $^{3}H$, $^{32}P$, or $^{35}S$, but $^{14}C$ and $^{125}I$ have also used.

- ✓ In non-radioactive labelling Biotin and Digoxigenin are commonly used.

**CONT.**

**4) Fixation of Tissue:-** There are several variables that need to be considered when fixing tissue for *in situ* hybridization.

- ✓ **A) Acetic acid-alcohol mixture:-** Best probe penetration, but may permit the loss of RNA from tissue.
- ✓ **B) Glutaraldehyde:-** Provide best RNA retention and tissue morphology, but because of extensive protein cross-linking the probe penetration is low.
- ✓ **C) Paraffin & Formalin:-** Leads to decreased sensitivity possibly resulting from increased cross-linking or loss of mRNA during embedding.
- ✓ **D) 4% paraformaldehyde solution:-** The most widely successful fixative solution. This provides a satisfactory compromise between the variable and good sensitivity.

**CONT.**

**5) Hybridization and Washing:-** The hybridization is done by simply mixing the single strand probes with the denaturated target DNA.

- ✓ Denaturation of the DNA is obtained by heating the DNA, which separate the two strands, and allows access of the single strand.
- ✓ Hybridization is performed by placing a small amount of solution containing the hybridization probe on a cover slip, which is then placed on the slide containing tissue sections to incubate overnight.
- ✓ The next day, washes are serially applied to the slides to remove the probe that is not bound to target DNA/RNA.
- ✓ There are several parameters such as (*i*) probe construction (*ii*) temperature (*iii*) pH (*iv*) formamide and (*v*) salt concentration in the hybridization buffer which play a crucial role in the specificity of target labeling during hybridization and washing.

**CONT.**

# Control Procedure

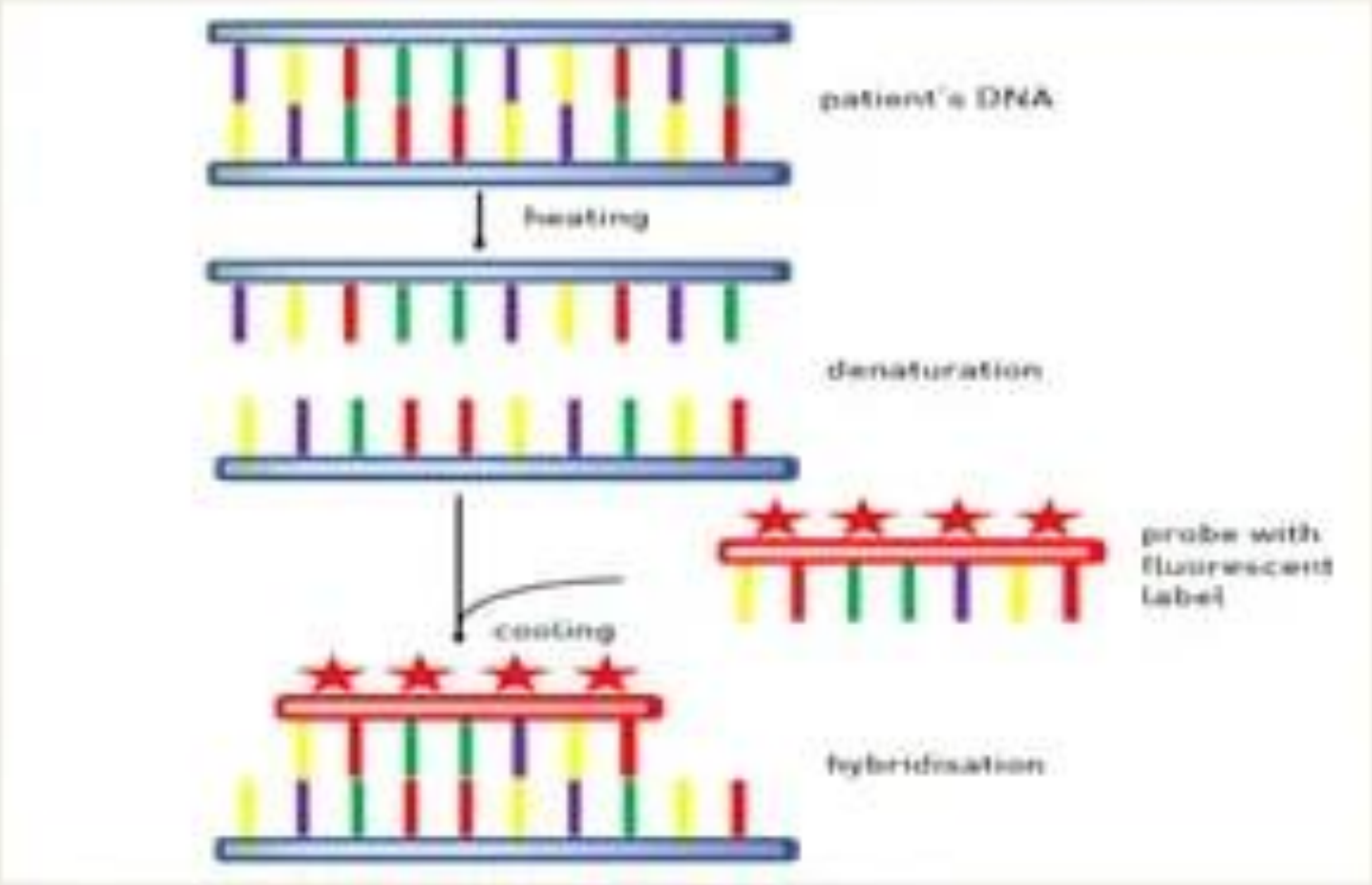
i. Use of Alternate Sequences

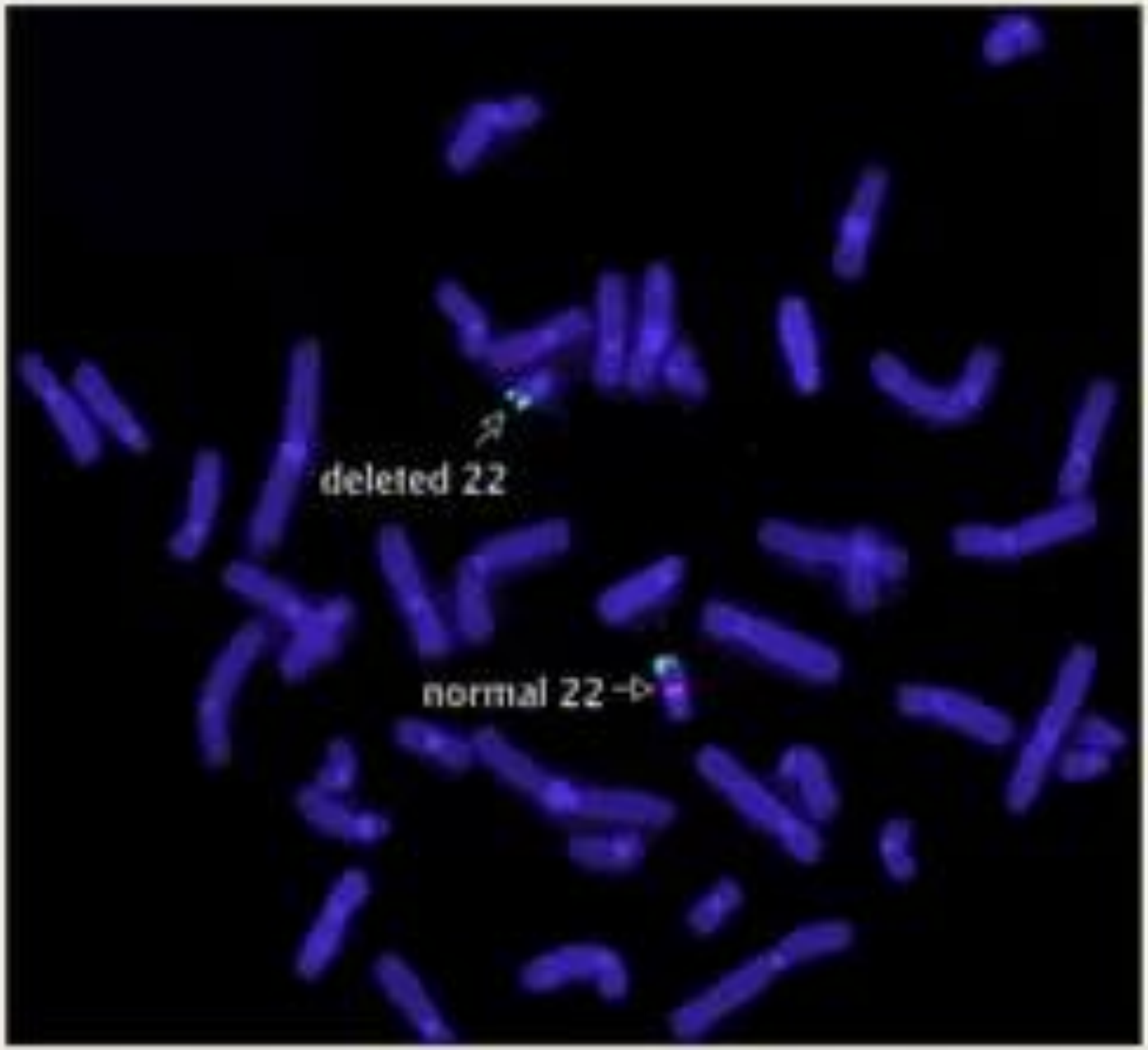
ii. Use of Multiple Probes

iii. Use of non-labeled Probes

iv. Use of Northern Analysis

# An overview of the FISH test




deleted 22
normal 22

# FIBER FISH

- Fiber fish is a cytogenetic (chromosome) laboratory technique in which FISH (fluorescence *in situ* hybridization) is done on chromosomes that have been mechanically stretched.
- Fiber FISH provides a higher resolution of analysis than convectional FISH.
- Here the word fiber refers to a chromosomes fiber.
- In fiber FISH, *interphase* chromosomes are attached to a slide in such a way that they are stretched out in straight line, rather than being tightly coiled.

➢ Chromosome Combing:- A technique used to produce an array of uniformly stretched DNA that is highly suitable for nucleic acid hybridization studies such as FISH; also known as molecular combing or DNA combing.

# Application of FISH

1) **Applications in Medicines**
2) **Species identification**
    i. *Bacterial species*
    ii. *Genome identification*

# Various FISH Techniques

1) **M-FISH**
2) **Double Fusion FISH or D-FISH**
3) **Break ap**

## Application of FISH

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   i. *Bacterial species*
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# Advantages of FISH

- FISH is able to detect many small deletions, duplications and rearrangements that are not visible with standard microscope analysis.
- A diagnosis from FISH may avoid your child having to undergo many other tests.

# Benefits of FISH

- It may help you and your doctor watch for common health problems associated with your child's chromosome imbalance.
- It may help to predict what to expect as your child gets older.
- It may show which specific genes are included in your child's deletion or duplication. If the gene(s) has been associated with a particular feature or health problem it may help to guide management or treatment for your child.
- It can help you to obtain specialist services for your child.
- You can choose to join a support group to meet other parents facing similar challenges.
- Parents and other family members can be tested to see if they are carriers of changes in their DNA that put them at risk of having more children with a chromosome change.

# Limitations of FISH

1. It is very expensive, hence it would be difficult for the average laboratory to undertake this technique.
2. It is time consuming and requires detailed molecular biological knowledge of sub cloning, in-vitro transcription and bacterial expression.
3. FISH has limited ability to precisely define which genes and breakpoints are involved in an imbalance.


THANK YOU