



# Fluorescent *in-situ* Hybridization

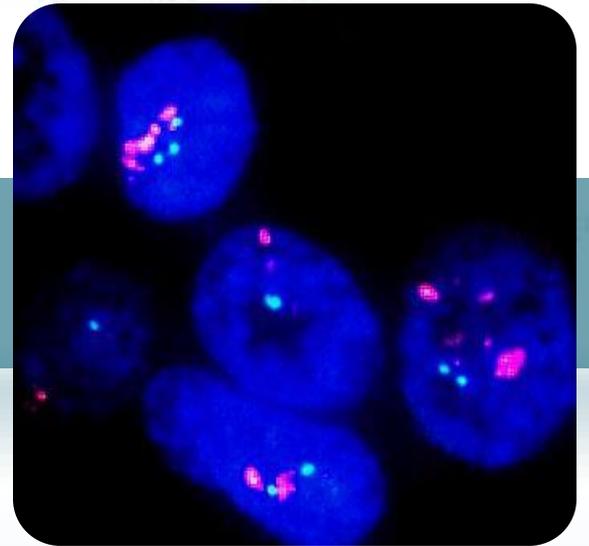
Presented for:

Presented by:

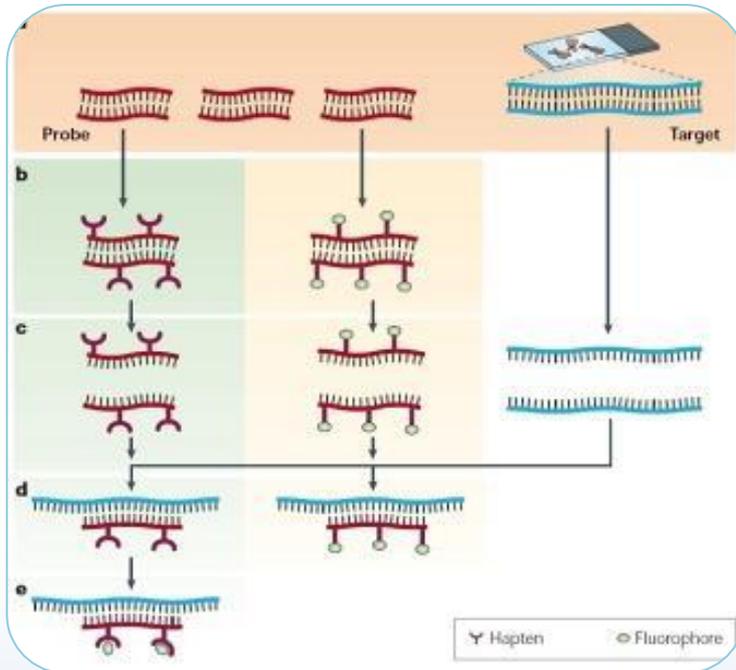
Date:



# Fluorescent *in-situ* Hybridization



# Definition



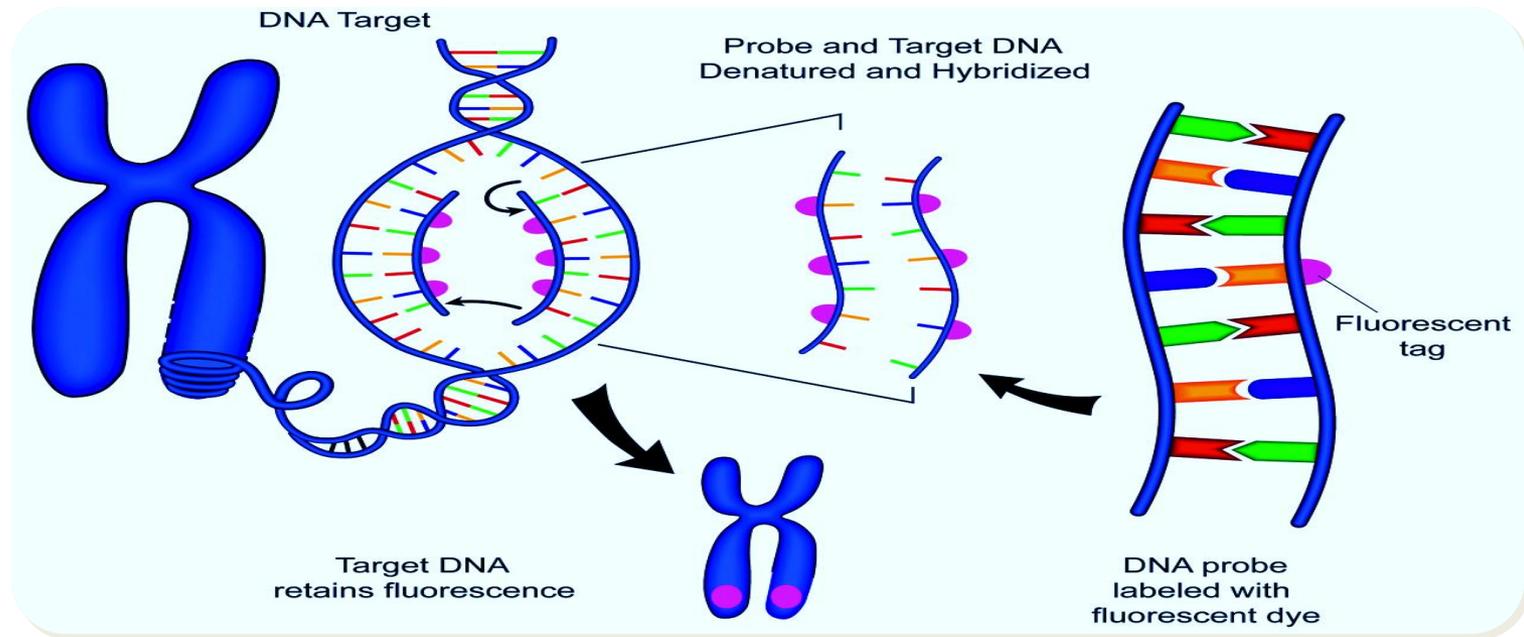
- In situ hybridization is the method of localizing/ detecting specific nucleotide sequences in morphologically preserved tissue sections or cell preparations by hybridizing the complementary strand of a nucleotide probe against the sequence of interest.
- If nucleic acids are preserved in a histological specimen, then it can be detected by using a complementary probe

# Principle

---

- Principle is same as that of ISH
- Use of a fluorescent labeled probe differentiates ISH & FISH
- FISH is a cytogenetic technique used to detect and localize the presence or absence of specific DNA sequences on chromosomes

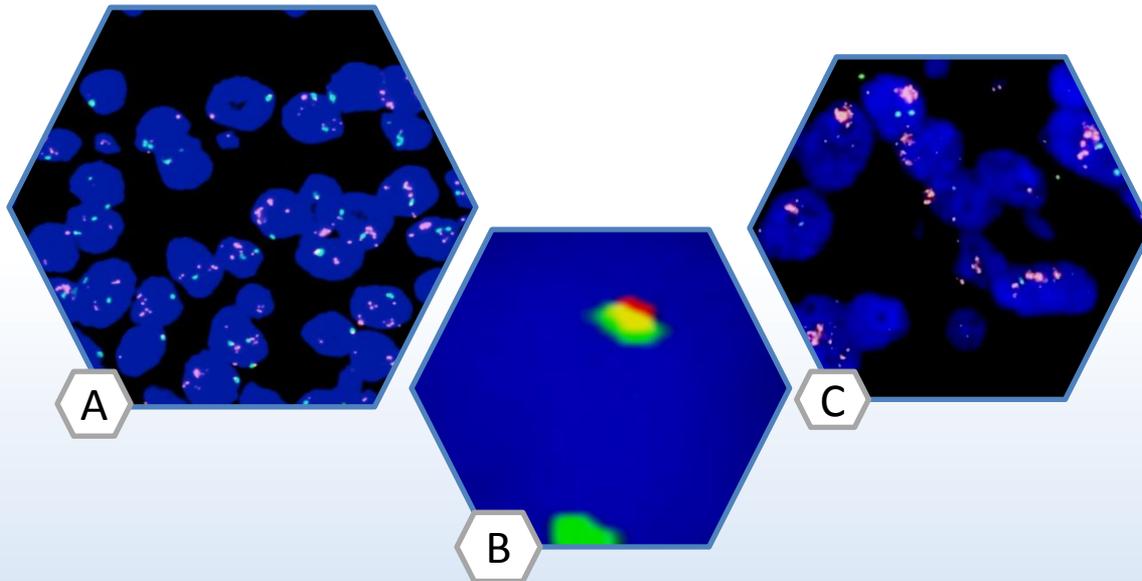
# FISH technique



Schematic representation of FISH technique. A DNA probe is tagged with a fluorescent marker. The probe and target DNA are denatured, and the probe is allowed to hybridize with the target. The fluorescent tag is then detected with a fluorescent microscope.

# Types of Samples Used

- Fixed cell suspension
- Formalin fixed paraffin embedded tissues



## FISH

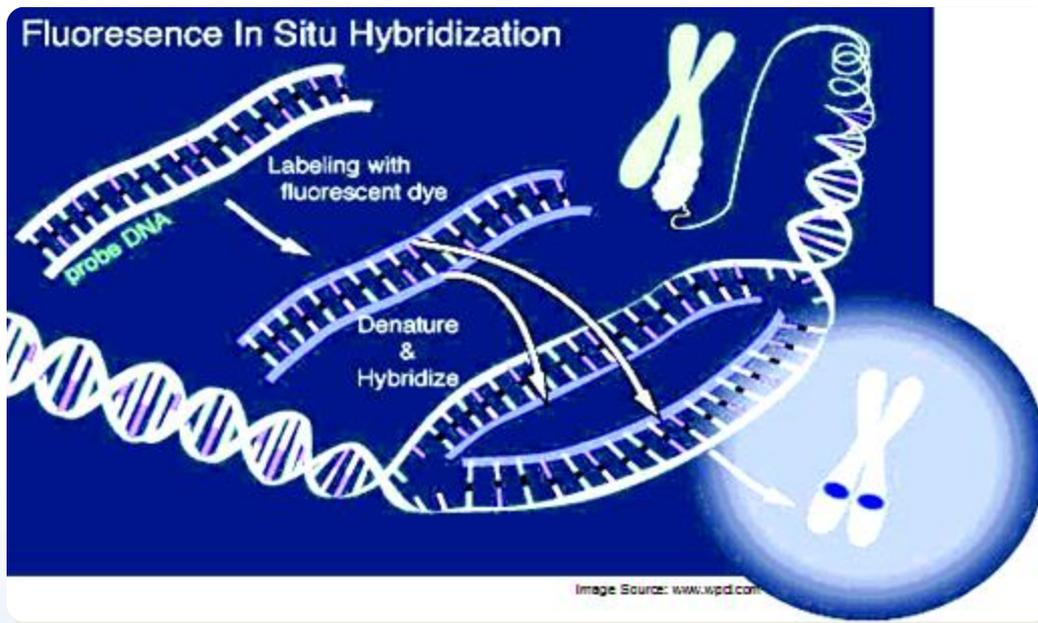
- A. FISH EGFR
- B. FISH Break apart ALK
- C. FISH HER2

# Diagnostic Applications of FISH

---

- Prenatal diagnosis
- Cancer diagnosis
- Molecular cytogenetic of birth defects and mental retardation
- The identification of specific chromosome abnormalities
- The characterization of marker chromosomes
- Interphase FISH for specific abnormalities in cases of failed
- Cytogenetic
- Monitoring the success of bone marrow transplantation

# Protocol Outline



- Preparation of the fluorescent probes
- Denaturation of the probe and the target
- Hybridization of the probe and the target
- Detection

# FISH Procedure

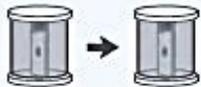
## Paraffin embedded tissue

### 1. Deparaffinized



Xylene 5 min×3  
Room temperature

### 2. Dehydrate



100% EtOH 5 min×2  
Room temperature

### 3. Air dry



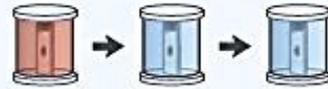
### 4. Pre-treatment



Paraffin  
Pretreatment  
Solution  
95°C 30 min

Wash buffer  
(2×SSC)  
5 min×2

### 5. Protease treatment



Protease Solution 37°C 10~20min  
Wash buffer (2×SSC) 5 min×2

\*Protease Solution  
Add 500µl protease in 50ml  
protease buffer

\*Protease preservation  
One month : 4°C  
Over one month : -20°C

### 6. Dehydrate (Room temperature)



70% EtOH 1 min  
100% EtOH 1 min

### 7. Air dry



## FISH protocol

### 1. Mark hybridizing area



### 2. Apply 10µl FISH probe for 22mm x 22mm area



### 3. Cover with cover glass and seal with rubber cement



### 4. Denature



75°C 5 min

## Hybridization

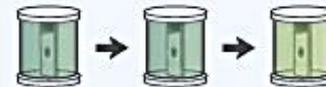
### 1. Incubation



Humidified box  
37°C 16 ~ 72 hrs

## Wash procedure

Remove rubber cement  
Slide into 2X SSC and remove  
cover glass



2X SSC  
Room temp.  
5 min

2X SSC  
0.3% NP-40  
73~75°C  
1-2min

2X SSC  
Room temp.  
1 min

## Counter stain

### 1. Apply 10µl DAPI Solution to target area



\*DAPI Paraffin embedded  
tissue 1500ng/ml

### 2. Put on cover glass Seal with manicure



## Examine



# FISH Procedure

## Frozen tissue

1. Frozen tumour tissue

2. Air dry



Positive charged slides

3. Fix and Dehydrated



95% EtOH  
20min

4. Air dry



5. Protease treatment



Protease Solution 37°C 10~20min  
Wash buffer (2xSSC) 5 min x2

\*Protease Solution  
Add 50µl protease in protease buffer

\*Protease preservation  
One month : 4°C  
Over one month : -20°C

6. Dehydrate (Room temperature)



70% EtOH 1 min  
100% EtOH 1 min

7. Air dry



touch preparations of unfixed tumour tissue/cell smears/cytospins of cultured or blood cells are possible

## FISH protocol

1. Mark hybridizing area

Diamond pen



2. Apply 10µl FISH probe for 22mm x 22mm area



3. Cover with cover glass and seal with rubber cement



4. Denature



75°C 5 min

## Hybridization

1. Incubation



Humidified box  
37°C 16 ~ 72 hrs

## Wash procedure

Remove rubber cement  
Slide into 2X SSC and remove cover glass



2X SSC Room temp. 5 min  
2X SSC / 0.3% NP-40 73~75°C 1-2min  
2X SSC Room temp. 1 min

## Counter stain

1. Apply 10µl DAPI Solution to target area



\*DAPI  
Frozen tumour tissue  
150ng/ml

2. Put on cover glass  
Seal with manicure



## Examine



# FISH Procedure



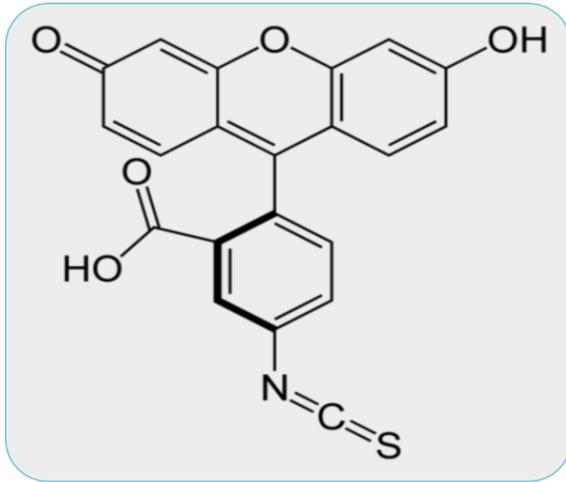
**Additional steps for  
paraffin pretreatment**



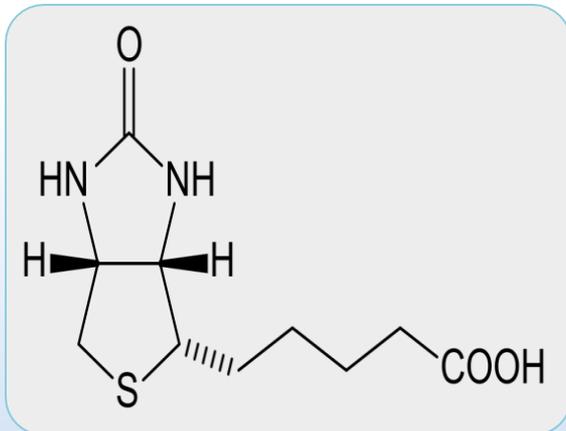
**Suspension pretreatment steps**

# Probes

**Fluorescein**



**Biotin**



- Complementary sequences of target nucleic acids
- Designed against the sequence of interest
- Probes are tagged with fluorescent dyes like biotin, fluorescein, Digoxigenin
- Size ranges from 20-40 bp to 1000bp

# Types of Probes

- **Centromere probes**
  - Alpha and Satellite III probes
  - Generated from repetitive sequences found in centromeres
  - Centromere regions are stained brighter
- **Telomere**
  - Specific for telomeres
  - Specific to the 300 kb locus at the end of specific chromosome
- **Whole chromosome**
  - Collection of probes that bind to the whole length of chromosome
  - Multiple probe labels are used
  - Hybridize along the length of the chromosome
- **Locus**
  - Deletion
  - Translocation probes
  - Gene detection & localization probes
  - Gene amplification probes

# Denaturation & Hybridization

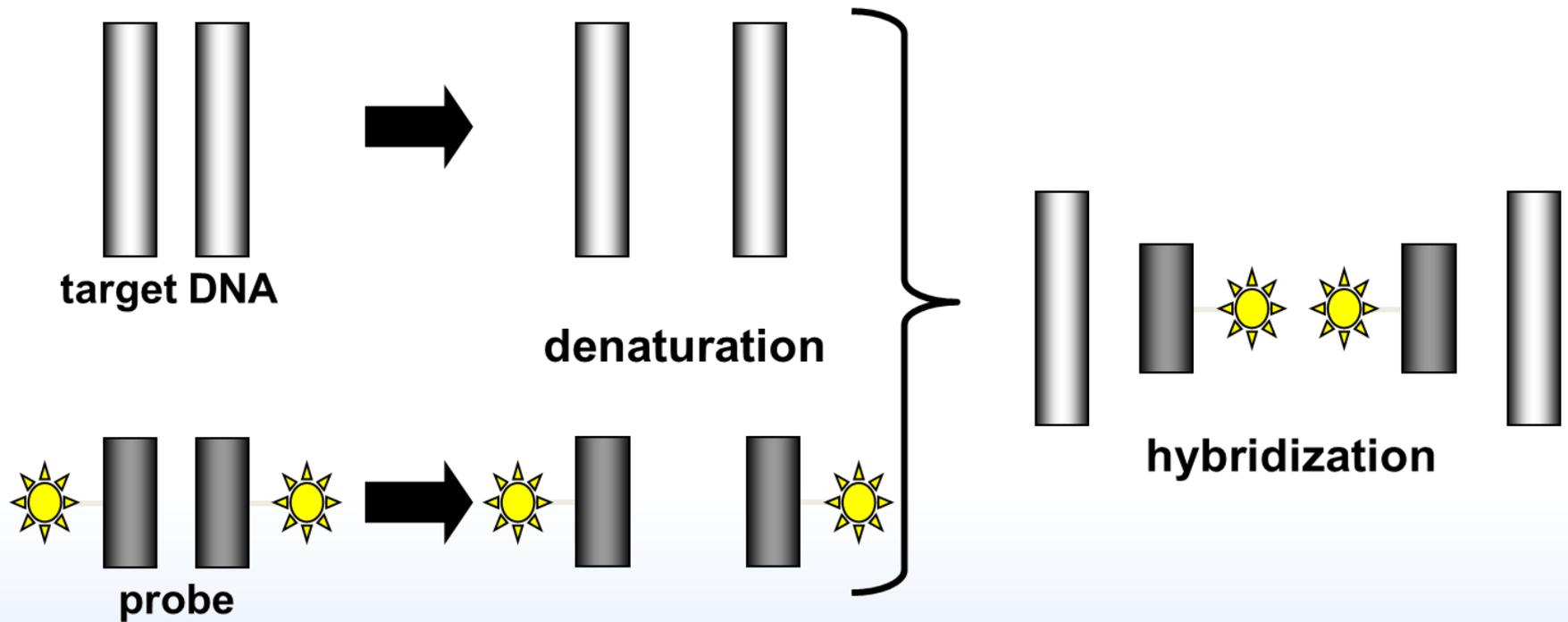
## Denaturation

- Either by heat or alkaline method
- A prerequisite for the hybridization of probe and target

## Hybridization

- Formation of duplex between two complementary nucleotide sequences
- Can be between
  - DNA-DNA
  - DNA-RNA
  - RNA-RNA

# Hybridization



# Detection & Visualization

## Detection

- Direct labelling:
  - Label is bound to the probe
  - Less sensitive
- Indirect labelling:
  - Require an additional step before detection
  - Probe detected using antibodies conjugated to labels like Alkaline phosphatase
  - Results in amplification of signal

## Hybridization

- Fluorescent probe attaches to the target sequence during hybridization
- This is visualized through a microscope with appropriate filters



# Molecular Pathology Workflow Solution

Please visit [www.biogenex.com](http://www.biogenex.com) for more details on our product portfolio

