Fluorescent *in-situ* Hybridization

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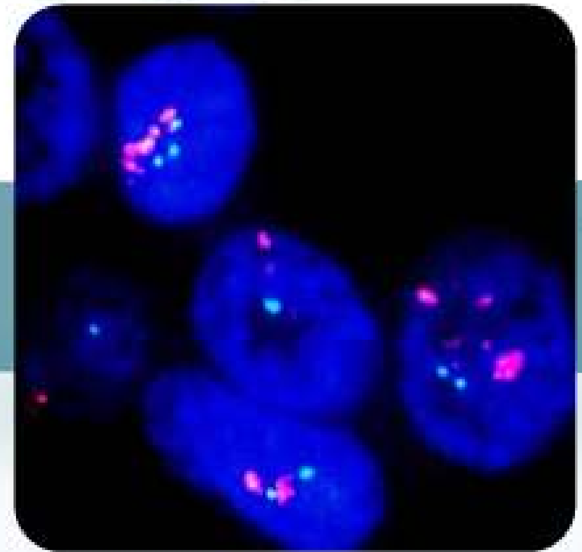

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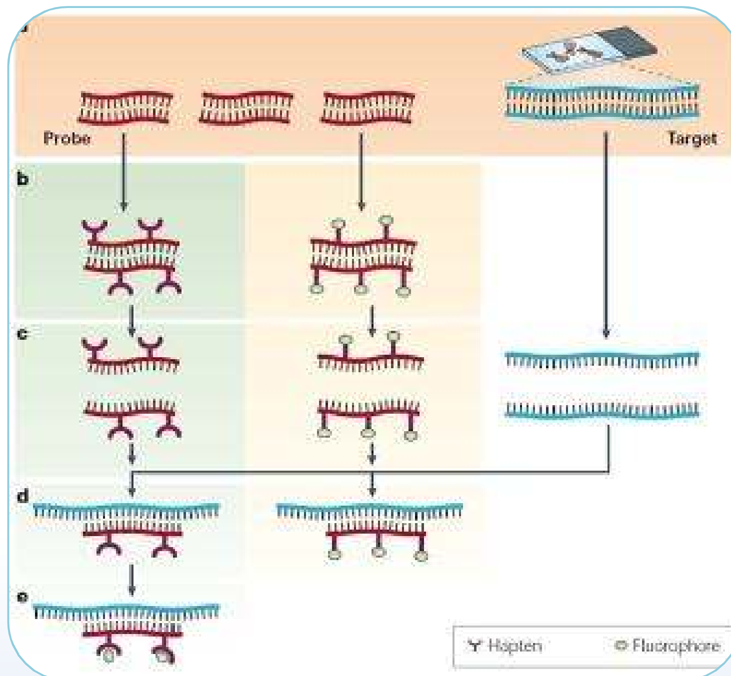
Definition



- 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

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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

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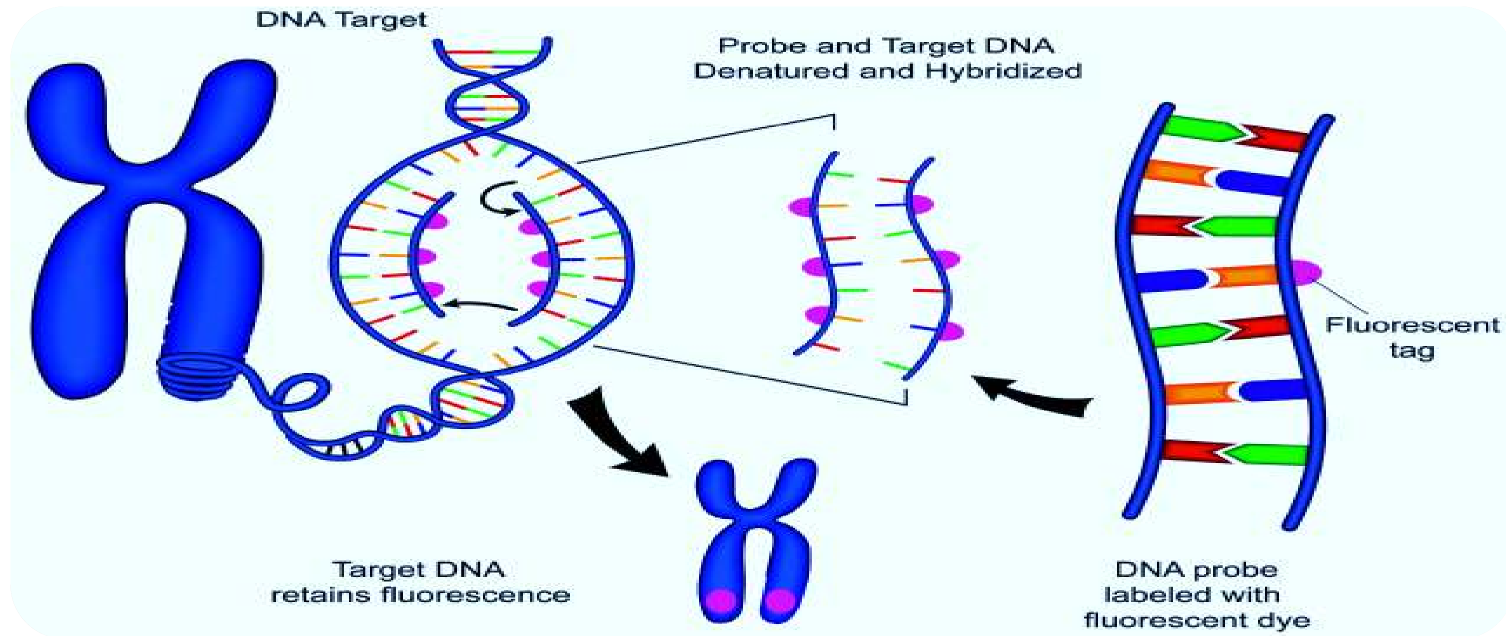
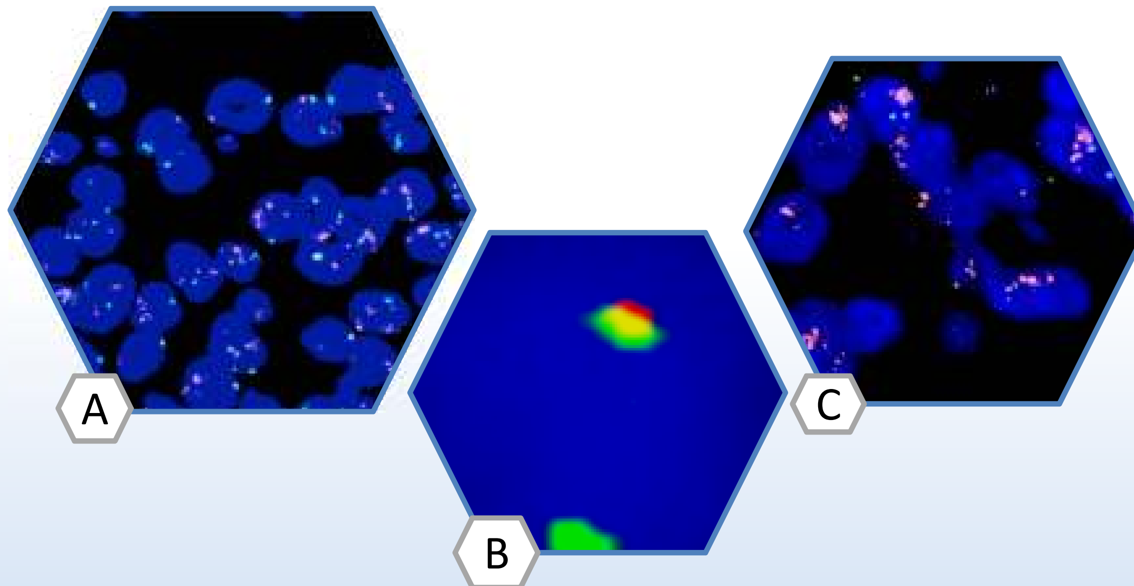
FISH technique



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

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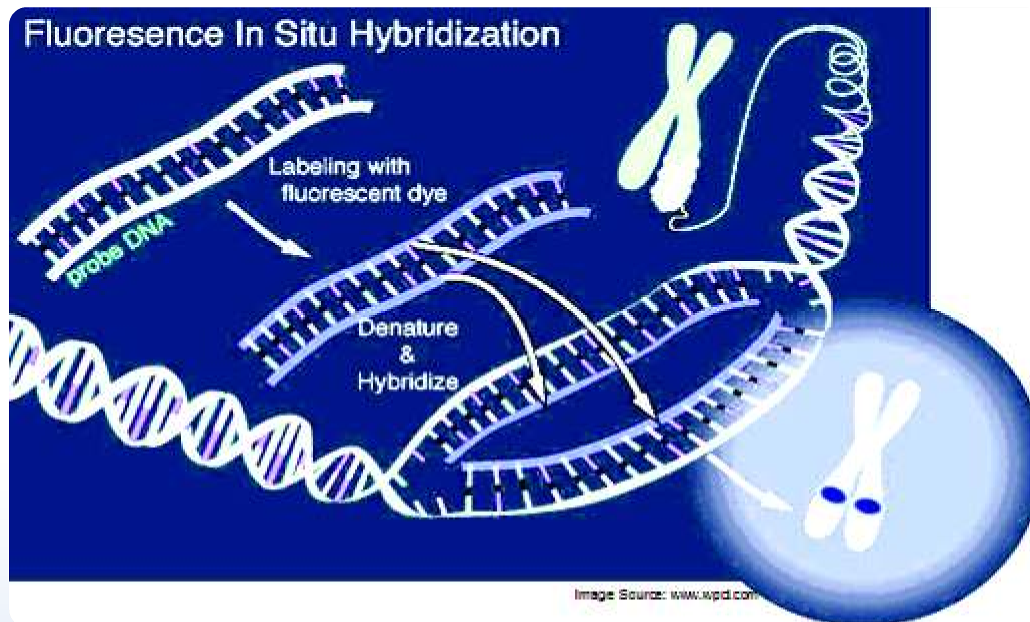
Diagnostic Applications of FISH

- 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

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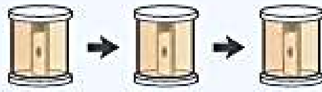
FISH Procedure

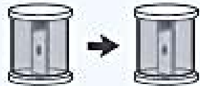
Paraffin embedded tissue

1. Deparaffinized



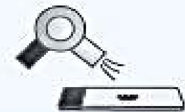
Xylene 5 min×3
Room temperature

2. Dehydrate



100% EtOH 5 min×2
Room temperature

3. Air dry

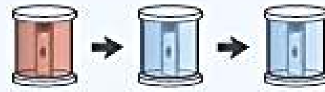


4. Pre-treatment

Paraffin Pretreatment Solution 95℃ 30 min

Wash buffer (2×SSC) 5 min×2

5. Protease treatment



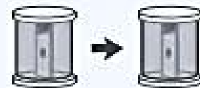
Protease Solution 37℃ 10~20min

Wash buffer (2×SSC) 5 min×2

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

*Protease preservation
One month : 4℃
Over one month : -20℃

6. Dehydrate (Room temperature)



70% EtOH 1 min

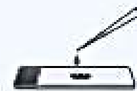
100% EtOH 1 min

7. Air dry

FISH protocol

1. Mark hybridizing area

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



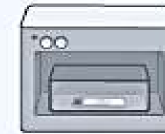
3. Cover with cover glass and seal with rubber cement

4. Denature

75℃ 5 min

Hybridization

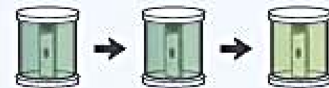
1. Incubation



Humidified box
37℃ 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℃ 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

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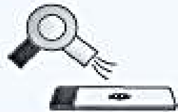
FISH Procedure

Frozen tissue

1. Frozen tumour tissue
2. Air dry

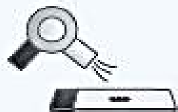


Positive charged slides

3. Fix and Dehydrated

95%EtOH
20min

4. Air dry



5. Protease treatment

Protease Solution 37℃ 10~20min
Wash buffer (2xSSC) 5 minx2

*Protease Solution
Add 50µl protease in protease buffer

*Protease preservation
One month : 4℃
Over one month : -20℃

6. Dehydrate (Room temperature)

70% EtOH 1 min
100% EtOH 1 min

7. Air dry

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

FISH protocol

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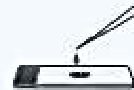
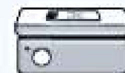


3. Cover with cover glass and seal with rubber cement

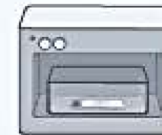
4. Denature



75℃ 5 min

Hybridization

1. Incubation



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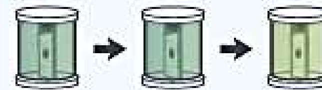

2X SSC Room temp. 5 min
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*DAPI
Frozen tumour tissue
150ng/ml

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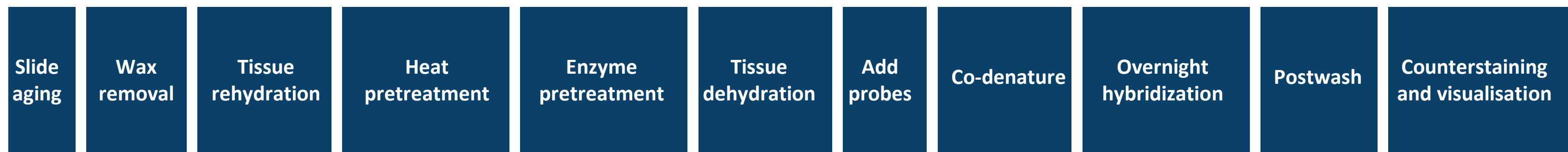
FISH Procedure



Additional steps for paraffin pretreatment

Suspension pretreatment steps

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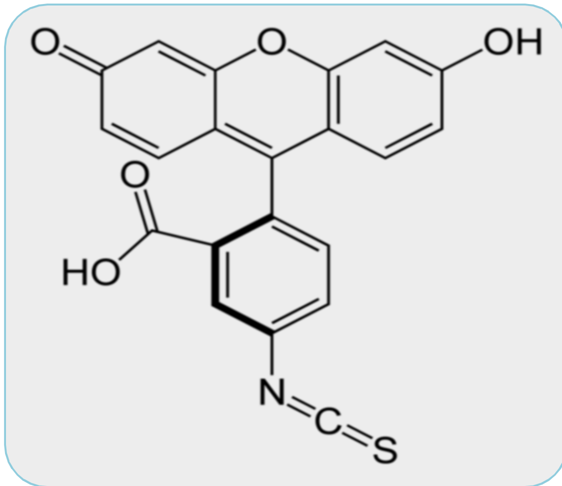
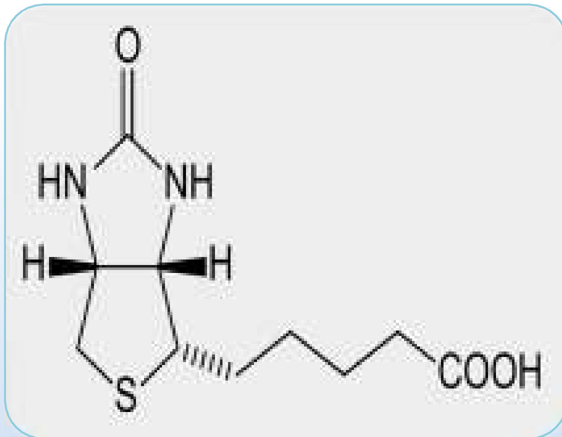
Probes

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

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Denaturation & Hybridization

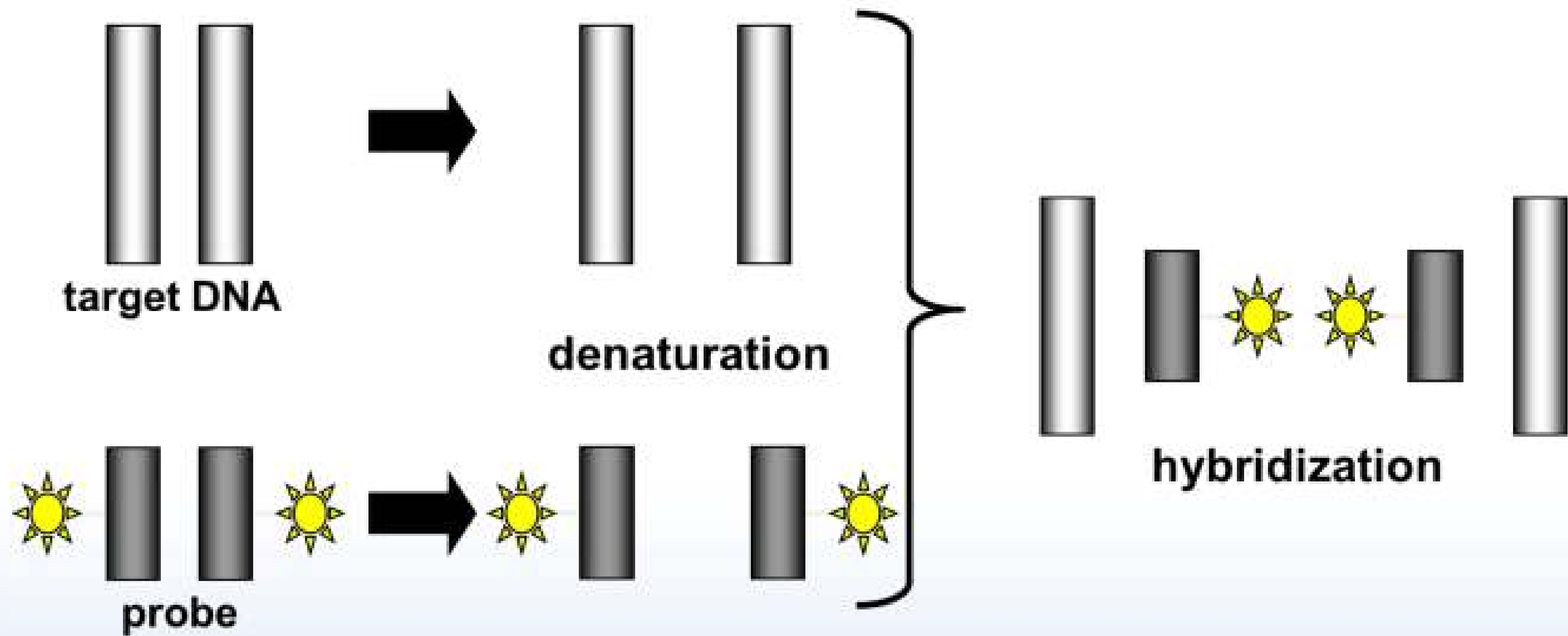
Denaturation

- 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



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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

Thank You

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