



Fluorescent *In Situ* Hybridization (FISH) Assay



Creative Bioarray

A Division of Creative Dynamics Inc

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What is FISH ?

Definition, Principle and Sample Types

2

Probes

The core of FISH technology

3

FISH Procedure

A quick and simple FISH protocol

4

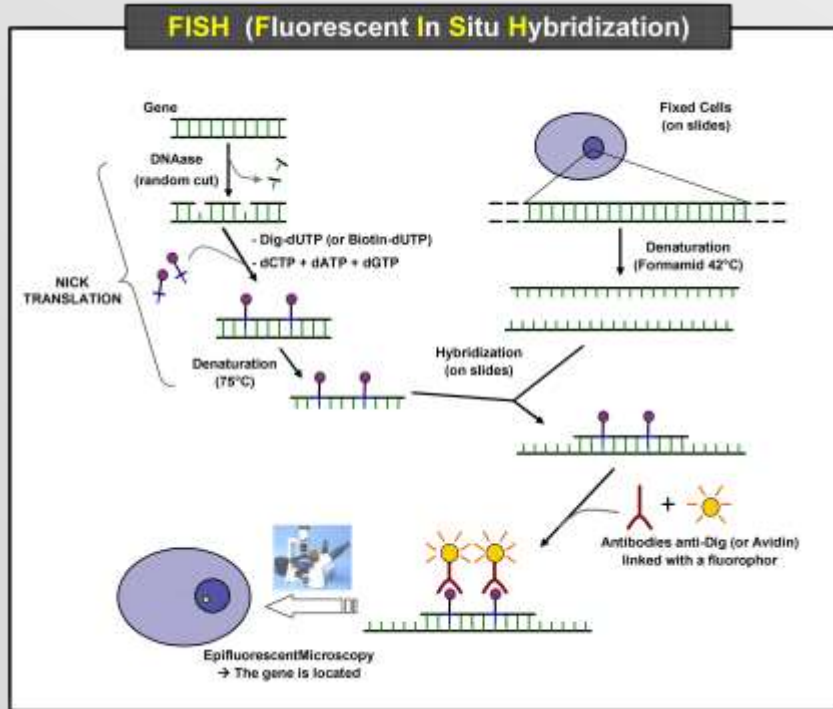
Application

Wide range of uses and bright prospects





What is Fluorescent *in situ* hybridization (FISH) ?



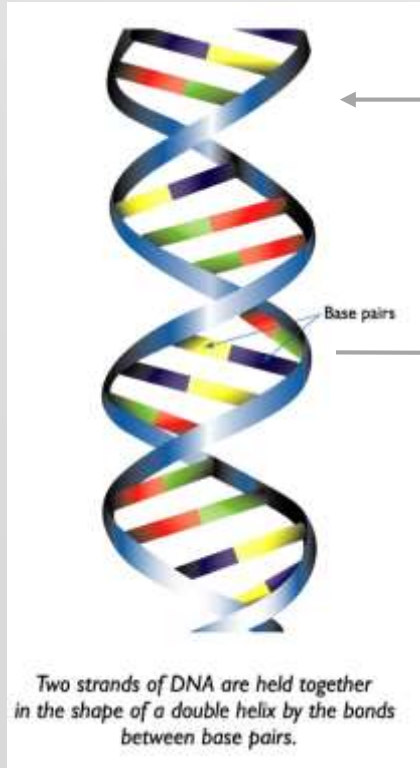
Definition

- **Fluorescent *in situ* hybridization (FISH)** is a molecular cytogenetic technique that uses fluorescent probes that bind to only those parts of the chromosome with a high degree of sequence complementarity.
- It is used to detect and localize the presence or absence of specific DNA sequences on chromosomes.





How does FISH work ?



The Structure of DNA

The DNA contains two strand-like molecules coiled together into a structure known as a double helix.



The principle of complementary base pairing

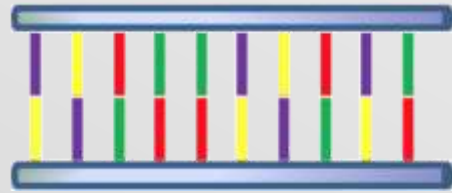
When two complementary sequences find each other they will bind together, or hybridise.

FISH works by exploiting the ability of one DNA strand to hybridise specifically to another DNA strand.

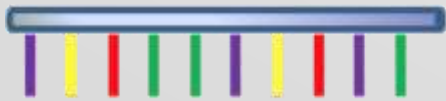




FISH Technology



heating



denaturation



probe with
fluorescent label

cooling



hybridisation





What Kind of Probes Can Be Used ?

Centromere probes

- Alpha and Satellite III probes
- Generated from repetitive sequences found in centromeres
- Centromere regions are stained brighter

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

Probes

Telomere

- Specific for telomeres
- Specific to the 300 kb locus at the end of specific chromosome

Locus

- Deletion
- Translocation probes
- Gene detection & localization probes
 - Gene amplification probes

Characteristics of Different Probes Types

dsDNA probes

Stable, available, easier to obtain

ssDNA probes

Stable, easier to work with, more specific, resistant to RNases, better tissue penetration, without self-hybridize

Probes

RNA probes

- Higher thermal stability,
- Better tissue penetration,
- More specific,
- Low background noise by RNase

Synthetic oligonucleotides probes

- Economical, stable, available, easier to work with,
- more specific, resistant to RNases,
- better tissue penetration, better reproducibility.

Probes Labeling



- ^{32}P
- ^{35}S
- ^3H

- Biotin
- Digoxigenin
- Fluorescent (FISH)

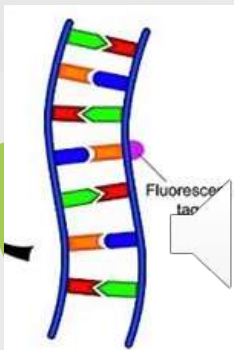


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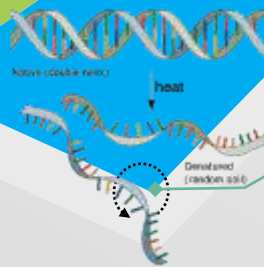
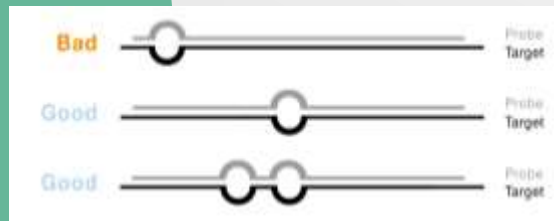


Protocol Outline

Hybridization of the probe and the target



Preparation of the fluorescent probes



Denaturation of the probe and the target





The Sample Preparation and Sample Types

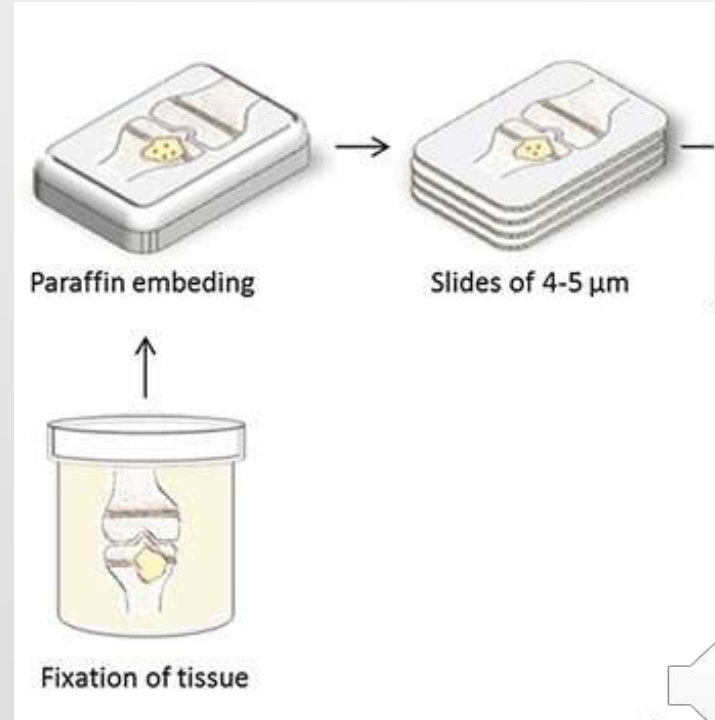
Sample Types

- Fixed cell suspension
- Formalin fixed paraffin embedded tissues



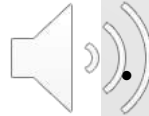
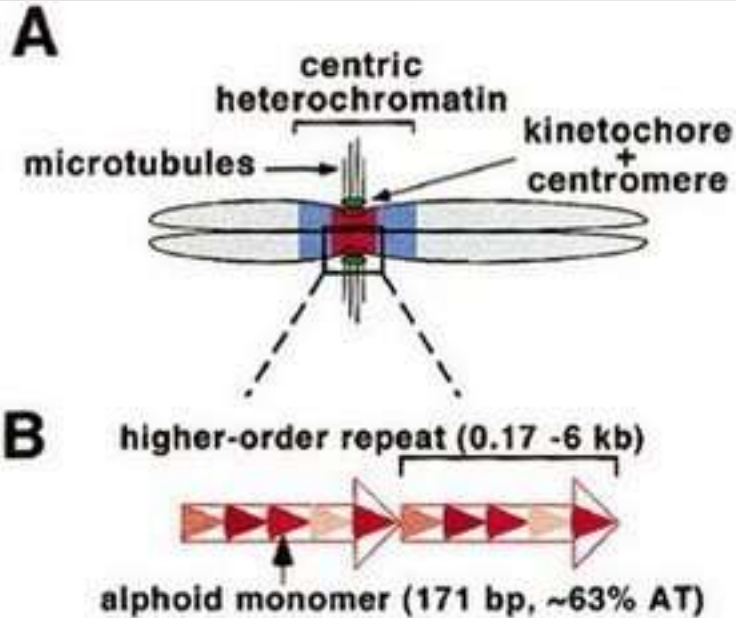
Preparation

- Sample fixation
- Slide tissue





Preparation of the fluorescent probes

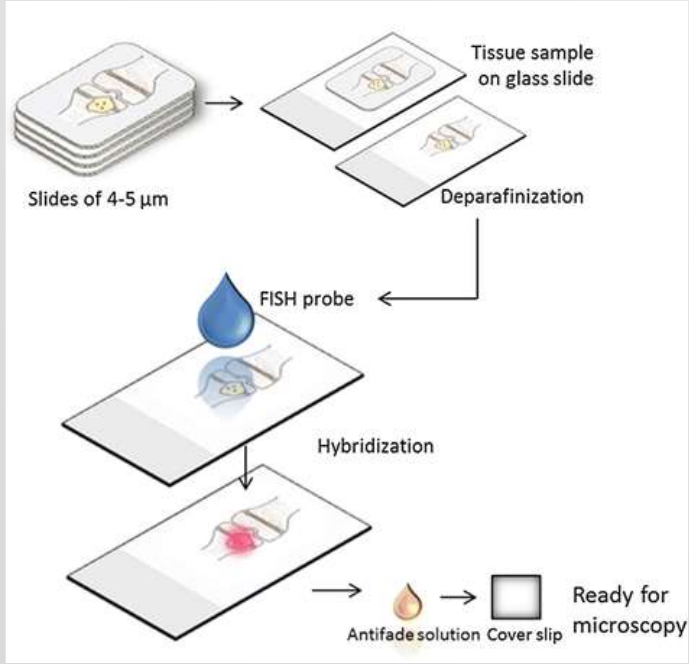


- Commercial Probes can be provided by many biotech companies
- Based on your special needs, custom probes are also synthesized
- e.g. *a*-satellite DNA is often chosen as the source of centromeric probes.





Denaturation of the probe and the target



Dehydration

- (1) Place the suitable amount of fixed sample on the slide,
- (2) put into 46° C oven drying for 10 minutes
- (3) Followed by immersion 50%, 80%, 96% ethanol solution, each for 3 minutes
- (4) dry in the air

Hybridization

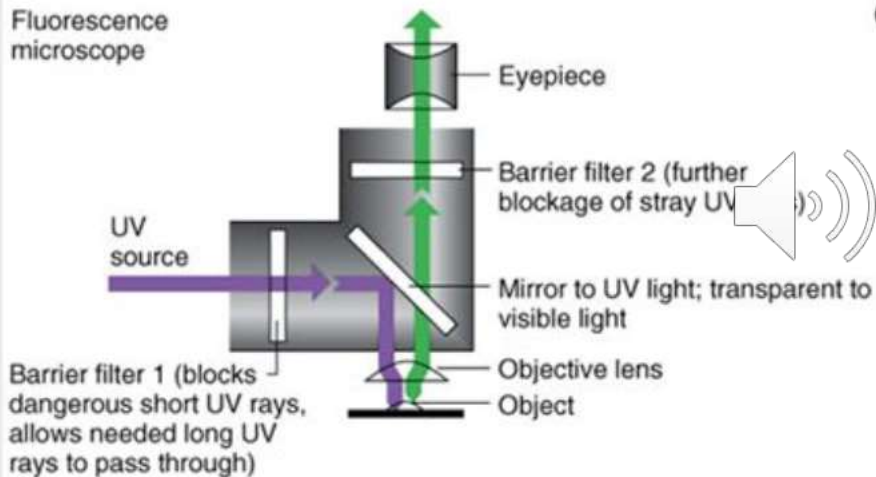
- (1) Add 10 μl of Hybridization buffer to the sample on the glass plate and try to cover the entire sample
- (2) Add 1 μl probe to Hybridization buffer
- (3) Put a wet absorbent paper in a 50ml centrifuge tube, put the glass pieces in, then place them in a 46° C incubator for 1.5 hours
- (4) Preheat the Washing buffer to 48° C for the next step





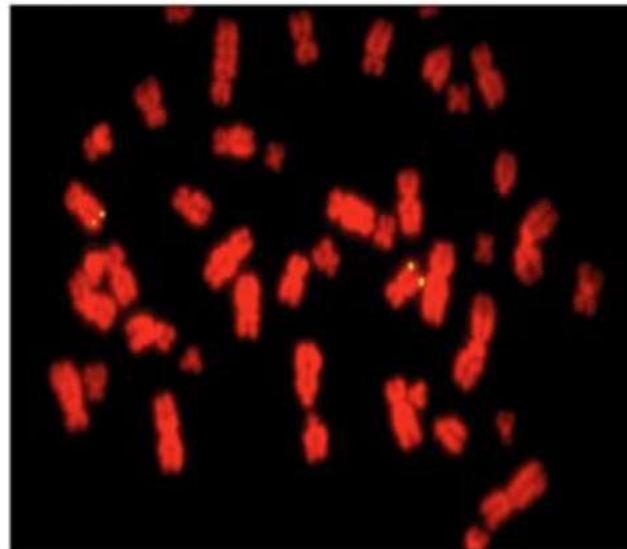
Detection by Microscope

Fluorescence microscope



4. Expose to ultraviolet (UV) light.
Take picture of fluorescent chromosomes.

(b)





Applications

Morphology Morphology and population structure of microorganisms

1

Pathology Pathogen profiling, abnormal gene expression

2

Developmental biology Gene expression profiling in embryonic tissues

3

Karyotyping and phylogenetic analysis Unique FISH patterns on individual chromosomes, chromosomal aberrations

4

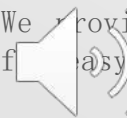


Creative Bioarray

01

Products

We provide comprehensive commercial probes and FISH Kits for easy use.



02

FISH Services

In addition to products, high-quality services are also available for our clients.



For more info please contact us:

E-mail: info@creative-bioarray.com

Go to our website:

www.creative-bioarray.com



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