Fluorescent *In Situ* Hybridization (FISH) Assay
What is FISH?
Definition, Principle and Sample Types

Probes
The core of FISH technology

FISH Procedure
A quick and simple FISH protocol

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**
- **Cooling**
- **Hybridisation**

Probe with fluorescent label
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

**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

<table>
<thead>
<tr>
<th>Probes</th>
<th>dsDNA probes</th>
<th>ssDNA probes</th>
<th>RNA probes</th>
<th>Synthetic oligonucleotides probes</th>
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<tbody>
<tr>
<td></td>
<td>Stable, available, easier to obtain</td>
<td>Stable, easier to work with, more specific, resistant to RNases, better tissue penetration, without self-hybridize</td>
<td>• Higher thermal stability,</td>
<td>• Economical, stable, available, easier to work with, more specific, resistant to RNases, better tissue penetration, better reproducibility.</td>
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<td>• Better tissue penetration,</td>
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<td>• More specific,</td>
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<td>• Low background noise by RNase</td>
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Probes Labeling

- Radioactive isotopes
  - $^{32}$P
  - $^{35}$S
  - $^3$H

- Non-Radioactive isotopes
  - Biotin
  - Digoxigenin
  - Fluorescent dye (FISH)
Protocol Outline

- Denaturation of the probe and the target
- 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. \(\alpha\)-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

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

**Morphology**  Morphology and population structure of microorganisms

**Pathology**  Pathogen profiling, abnormal gene expression

**Developmental biology**  Gene expression profiling in embryonic tissues

**Karyotyping and phylogenetic analysis**  Unique FISH patterns on individual chromosomes, chromosomal aberrations
We provide comprehensive commercial probes and FISH Kits for easy to use.

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