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Fluorescence *in-Situ* Hybridization (FISH) and its Importance in Bio-Medical Sciences

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Abstract

There are many critical chromosomal aberrations present at the site of nucleotide sequences. For example - tuner syndrome, down syndrome, Cystic Fibrosis, Tay - Sachs disease [13], so in the testing method of fluorescence in situ hybridization (FISH). The labelled DNA probes reciprocal, to regions of the chromosome in question are allowed to hybridize to the preparation of the test sample's chromosomes. A hybridization signal verifies the presence of that chromosomal material in the test sample, whereas the absence of a signal indicates the absence of the material. Diagnostic FISH for Down syndrome would involve the use of labelled probes with sequences complementary to regions traverse chromosome number 21. The presence of three labelled chromosomes would, therefore, be diagnostic of Down syndrome [6].

Keywords: Fish; Probes; Cytogenetic; Chromosomal aberrations; Syndromes.

Introduction

The need to identify the chromosomal aberrations leads to the origin of Fluorescence In situ Hybridization (FISH) which is a cytogenetic technique. The identification of chromosomal aberrations was in-turn needed to diagnose the diseases at gene levels (Genetic disorders). Further advancements in the cytogenetic techniques helped to achieve a more sensitive approach towards the detection of chromosomal abnormalities [5].

FISH was successfully first used in an experiment which involved the use of H3_ labeled RNA probe in 1986 [8]. This cytogenetic technique was introduced ~30 years later after the development of immunofluorescence-based protein detection methods [9]. Modern FISH basically uses the fluorescence probes which binds to their complementary sites in the target DNA sequence. The fluorescence emitted by the probes is the key to identify the position of a specific sequence in the target DNA molecule. Cytogenetic deals with the relationship between chromosomes

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and cell behavior during the phase of meiosis and mitosis. It includes the karyotyping (metaphase chromosome analysis), banding techniques and molecular cytogenetic techniques [7].

Working principle: The working principle is totally based on the hybridization of the fluorescent probe to its complementary sequence in the target DNA sequence [8] The diagrammatic process of Fluorescent in-situ hybridization Technique is given in (Fig. 1), which is easy to perceive the working process of this technique.

The steps are as followed [12]:

- The very first step in the process includes the synthesis of a fluorescent probe.
- The DNA sequence to be tested (chromosome) is then fixed onto a microscopic slide and denatured.
- Followed by the addition of denatured probe to the slide.
- The probe hybridizes to the DNA sequence at its complementary sites.
- After a round of washing the chromosome is observed under a fluorescence microscope to identify the site of hybridization.

Probes

A variety of probes are used in the process of FISH. Both double stranded and single stranded probes are used which include cDNA, cRNA, and other artificially synthesized oligonucleotide probes. A probe is 50-300 base pair in size. A number of things are considered during making the choice of a probe such as - sensitivity, specificity, the stability of hybrids, and ease of application.

In a broader view two different kinds of labelling methods are used which are listed as follows:

- Radioactive or radio-isotopic labelling
- Fluorescence or non-isotopic labelling

Labelling by radio-isotopic methods is considered as the most sensitive method. Moreover, results are easily quantified with radio-isotopic labelling. Non-isotopic probes include labelling with fluorescein, biotin, alkaline phosphatase, digoxigenin. This method is relatively less sensitive then the radio-isotopic labelling and quantification of hybridized results are also difficult. The drawbacks of using radio-isotopic labelling methods are - poor spatial resolution, risk of exposure to radioactivity, and disposal of radioactive material.

Types of probes used

Three different types of probes are used [14]:

- *Whole chromosome probes:* these probes are libraries of small DNA sequences which are complimentary for different sequences of a chromosome. These probes are used for the

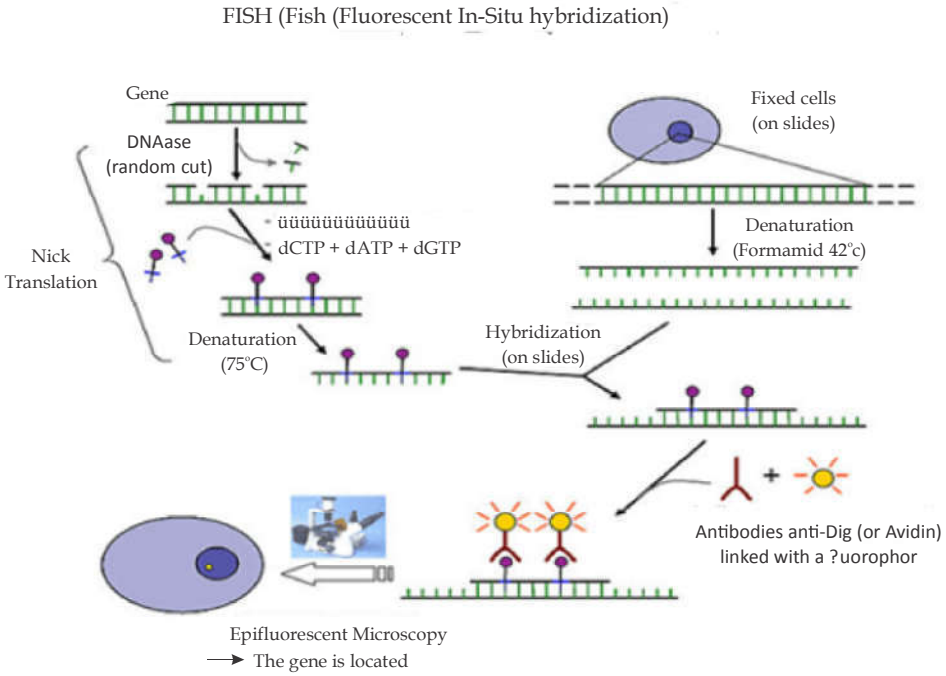

Fig. 1: Diagrammatic representation of FISH

process of chromosome painting in order to identify the numerical aberrations in chromosomes.

- *Alphoid or centromeric repeat probes:* they are complementary to the centromere region of the chromosome more specifically to the repetitive alpha satellite DNA sequences. Use of these probes let us know the number of chromosomes and thus the number of a specific chromosome.
- *Locus-specific probes:* as the name suggest this type of probes are complementary to a specific position in the chromosome. These probes are useful in determining whether a gene has a normal copy number or not.

Types of FISH

Variations in the different FISH techniques are on the basis of the probes used in them.

Different FISH techniques are:

- Single-molecule RNA FISH
- Fibre FISH
- Q-FISH
- MA-FISH
- Hybrid Fusion FISH

- *Single-molecule RNA FISH* - technique is also called as Stellaris RNA FISH. The technique is used for the detection and quantification of the mRNA along with some other long RNA molecules in a sample. Probes used in this technique are multiple oligonucleotide probes which are singly labelled. The technique finds its potential application in the cancer diagnostics, gene expression analysis, and neuroscience.
- *Fibre FISH* - technique adds on into conventional in situ hybridization in a way that it uses a stretched chromosome as a target sequence (mostly the interphase chromosome). The stretched chromosomes help us to attain a better resolution even up to a few kilobases [3].
- *Q-FISH (Quantitative FISH)* - is related to the analysis of telomere portion of the chromosome. Telomere act as the best biomarkers and its length signify the ageing process of the cell. Q-FISH combined with PNA telomere probes is used for the quantitative measurement of the DNA fragments [4].
- *MA-FISH or Microfluidics assisted* - FISH uses a microfluidic flow for the hybridization of the FISH probes to the target DNA. It gives a better hybridization efficiency by decreasing the time of DNA hybridization. Applicable for the detection of the HER2 gene in breast cancer [11].
- *Hybrid Fusion FISH* - uses a combination of fluorophores in order to create a new spectrum through the process of DOT (Dynamic Optical Transmission). Used in the diagnosis of cancer.

Applications

FISH has its major applications in the identification of the chromosomal abnormalities be it numerical or be it functiona1 [l]. Some of them are listed below:

- To localize a specific gene sequence in a target DNA [4].
- To find out the gene markers or biomarkers[1].
- For chromosome painting by the use of differently labelled short fluorescent probes [8].
- To diagnose the chromosomal rearrangements, translocation, addition, and deletions in the genetic material [2].
- For the routine analysis of interphase and metaphase chromosomes [1].
- For the detection of cancer in the various tissue samples.
- For the identification of genetic disorders such as Down's syndrome, Turner's syndrome [2].

Negative aspects of FISH

- Nonetheless, FISH procedures have a single major disadvantage: Interphase (and metaphase) FISH furthermore, to a lesser degree SKY (spectral karyotyping FISH technique, basically used for analysis of metaphase preparation through spectral microscopy) can just identify known hereditary variations, giving the specific probe is accessible (in another sense, a probe for a known genetic aberration has to be hybridized to the target sequence in order to have the results that are, to indicate the presence or absence of that specific genetic aberration alone). FISH investigation with locus-specific probes or chromosome-specific

DNA libraries is confined to the focused chromosome or chromosomal sub-region. Consequently, and in solid difference to chromosome banding-based karyotype examination, while most significant in the confirmation of recently portrayed chromosomal deviations, FISH can't fill in as a screening test for chromosomal adjustments since most FISH procedures can just recognize known imbalances [10].

- FISH techniques are not valuable for distinguishing intrachromosomal modifications, for example, duplications, deletions and inversions.

Conclusion

Fish enables the position of the marker on a chromosome and extended DNA molecule to be directly visualized. In optical mapping, the marker is a restriction site, and it is visualized at a gap in an extended DNA fibre, in the marker is a DNA sequence. That is visualized by hybridization with a fluorescent probe. The probe can be locus-specific probe and centromeric repeat probe, or whole chromosome prob. So the first process is In Situ Hybridization for making of Probs. So first we extracted the DNA, and we should have fluorescently labelled nucleotide, then we create some nick regions at a single strand of extracted DNA fragment at certain regions, then we add the fluorescent labelled nucleotide randomly at the gap region of the fragmented strand. Now further we isolate the single strand labelled with fluorescent nucleotide. This is called a fully formed probe.

The second process is to isolate the DNA sequence needed to be hybridized, after the denaturation of dsDNA, we took the single strand of DNA for the binding with its complementary sequence of the isolated labelled probe.

After that, we add the primary Antibody to the probe. The addition of the primary antibody is followed by the addition of a secondary antibody or else by the addition of a fluorescent molecule. The fluorescent molecule binds to the primary antibody and gives the fluorescence. The emitted fluorescence leads to the identification of the site where exactly the desired DNA sequence is located.

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