Chromosome Painting: Versatility of the Technique and Applications in the Present Diagnostics

Abstract
Generally, a Karyotype is the featured chromosome complement of each and every eukaryotic species. The preparation and study of the karyotype is an immensely important part if the Cytogenetic studies. An immensely important technique known as Chromosome Painting for visualizing the chromosomal aberrations with the aids provided by fluorescently labelled DNA probes that are made to hybridize with the chromosomal DNA. The technique was introduced for the first time in 1999. The technique is also called as FLUORESCENT IN-SITU HYBRIDIZATION (FISH). Various methodologies and the modifications have been developed overtime to optimize the detections in DNA and RNA. This review focuses majorly on the various techniques of Karyotyping/ Chromosome Painting and their applications.

Introduction
The past two decades have marked the advent of a novel and obviously versatile technique which is well known as Chromosome Painting or FISH. The technique refers to the use of fluorescently labelled DNA/ RNA sequences on the mitotic chromosomal preparations or sometimes in the Plate-I (Interphase cells). The technique was developed in the early 1990s and is remarked as a powerful method for the detection of the chromosomal abnormalities, homozygous/heterozygous alleles, and cell reproduction cycle. For FISH technique to be developed it is very important to isolate each studied chromosome and subsequently after the successful isolation; the DNA from their chromosome is fragmented and put into the bacterial cell to amplify. The generated fragments are used as the hybridization probes and the probes are made labelled with the fluorescent dyes and later are allowed to hybridize with the specific cells, tissues, chromosomes, which are under study.

A variety of probes and the samples can be used for the FISH technique like; probes can be developed for the complete chromosome, Centromeric regions or sometimes probes may be locus specific. Probes can be developed for the entire Genome also- GENOME IN- SITU HYBRIDIZATION (GISH). Interphasic nucleus can also be obtained from a wide range of clinical isolates. Overtime around nine different techniques of Chromosome Painting or FISH have been developed.

Interphase FISH
The technique involves the probe hybridization to the cells in Interphase. The technique is immensely beneficial when it becomes impossible to prepare the metaphasic spreads as with the case in primary tumor cells. I- FISH can also be done on the paraffin- embedded or formalin- fixed tissues/ cells thereby allowing the analysis of the cell/ tissue sections and correlate it with the chromosomal aberration along with the clinical and biologica end- points. Interphase cytogenetics allows to precisely define about the cell pool carrying chromosomal abberations, also to identify whether the abnormality carrying cell is existing in the clonal particles or exist as an isolated event. This technique also aids in observing the abnormalities on a cell to cell basis despite of a population.

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Telomeric FISH
Subtelomeric probe hybridization is quite new practice. The T-FISH is a collection of 41 different FISH probes which are made to use for the identification of rearrangements which cannot be seen with the aids of conventional cytogenetic practices. The technique targets the regions which are lying right behind the chromosome ends that enable to visualize if they are involved in any rearrangements. Each probe is made labelled with a different color so that the specific chromosome abnormality and the routine chromosomes are normal or there is any chromosomal material of unknown origin.

RISH (RNA In- situ Hybridization)
RISH is an immensely important technique because it facilitates the direct visual evidences about the gene expression occurring from a particular chromosome. House- keeping genes are the...
genes which are expressed abundantly, they can be easily and reliably detected with the help of FISH technique. Furthermore, optimization and the amplification of the signals may even allow the genes which are expressed at the baseline levels.

**PRINS (Primed In-situ Hybridization)**

PRINS technique is a good developed alternative to the In-situ Hybridization for detecting chromosomal abnormalities on the basis of the application of chromosome specific oligonucleotide primers that are followed with the process of primer extension by using Taq DNA polymerase in the presence of labelled DNA building blocks (nucleotides). PRINS is very much specific and faster than the classical FISH methods for the chromosomal identifications.

**Dynamic Molecular Combing (Fiber- FISH)**

This technique basically refers to the FISH common practice that is conducted on preparing extended chromatin fibers. The FISH technique on the DNA fibers is very helpful in assessing the length of probes and also to map up the probes relative to one another; because, as it can also reveal the degree of overlapping.

Hence, this dynamic molecular combing technique carries superior mapping resolution as compared to the I-FISH. This can also reveal out the DNA loci separated up by a few kilo bases only and can study the loci as large as of two mega bases in one single experiment.

**CGH (Comparative Genome Hybridization)**

CGH method serves as a global screening for the detection of chromosome abnormalities in a tumor genome. It requires tumor cell's genomic DNA only and the metaphasic preparations of the normal donor cell; hence it circumvent the preparations of high-quality tumor metaphase spreads. The tumor DNA isolated from the archived, formalin-fixed, embedded tissues can also be used.

The comparative genome hybridization allows the identifications about chromosomal aberration and facilitates to find up the correlation of cytogenetic findings with histologic/histochemical information, clinical course and prognosis. Analysis about the small sub-regions of the defined histologic lesion can also be done. Once the areas of gain or loss are identified, these can be defined further more with the help of FISH or any other molecular genetics techniques.

CGH which is coupled with the micro-array is called as Array CGH technique, which is approved to be highly informative in various clinical settings.

**COBRA-FISH (Combinatorial Binary Ratio Labeling)**

COBRA-FISH of 24 different human chromosomes with 5 fluorophores in the conjunction with spectral or filter-based microscopic imaging has greatly advanced the molecular cytogenetic studies of the chromosomes. The application of five different fluorophores permits the identification of up to 3 different chromosome targets based on the color combinations. The technique allows the color discrimination of all the p and q arms of every chromosome and also permits the detection & elucidation of intra or inert chromosome rearrangements.

**SKY- FISH (Spectral Karyotyping- FISH)**

SKY- FISH is a molecular cytogenetic technique which helps in the differential visualization of all the human chromosomes with different colors after a single hybridization and image exposure. After the classification and alignment of the chromosomes in a karyotype table, the interpretation and comparison of all the abnormalities is summarized in the karyogram.

**M-FISH (Multiplex- FISH)**

Multiplex- FISH carries the ability to identify the 24 different chromosomes in a metaphasic spread by hybridizing simultaneously with the chromosome-specific DNA probes; each probe is labelled with the combination of different fluorescent dyes.

M- FISH differs from the SKY- FISH only in the order that, M-FISH is a filter-based technique where separate images are acquired sequentially for the each fluorochrome applied. The individual fluorochrome files are then combined to generate the final image.

The methods of M-FISH and SKY- FISH have combined the benefits of FISH with classical banding techniques and spawned many variations resulting in diverse applications. The methods allow the detection of inter-chromosomal structural abnormalities, like translocations and insertions which results in the balanced as well as unbalanced rearrangements.SKY-FISH and M-FISH carry the potential of identifying the cryptic translocation mutations and clarify the complex aberrations (marker and ring chromosomes), that are typically unidentifiable by conventional banding methods. Additionally, other abnormalities like double minutes can be better resolved, resulting to the identification of critical oncogenes.

**References**


