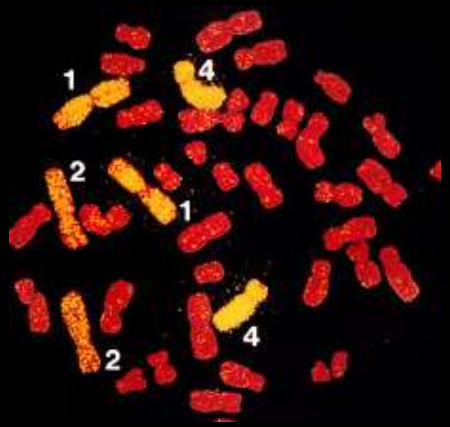

Fluorescent *in situ* Hybridization (FISH)

A Presentation by

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Definition

FISH (Fluorescent *in situ* hybridization) is a cytogenetic technique that can be used to detect and localize the presence or absence of specific DNA sequences **on chromosomes**. It uses fluorescent probes that specifically bind to parts of the chromosome with which they show a high degree of sequence complimentarity. Fluorescence microscopy can then be used to find out where the fluorescent probe is bound on the chromosome.

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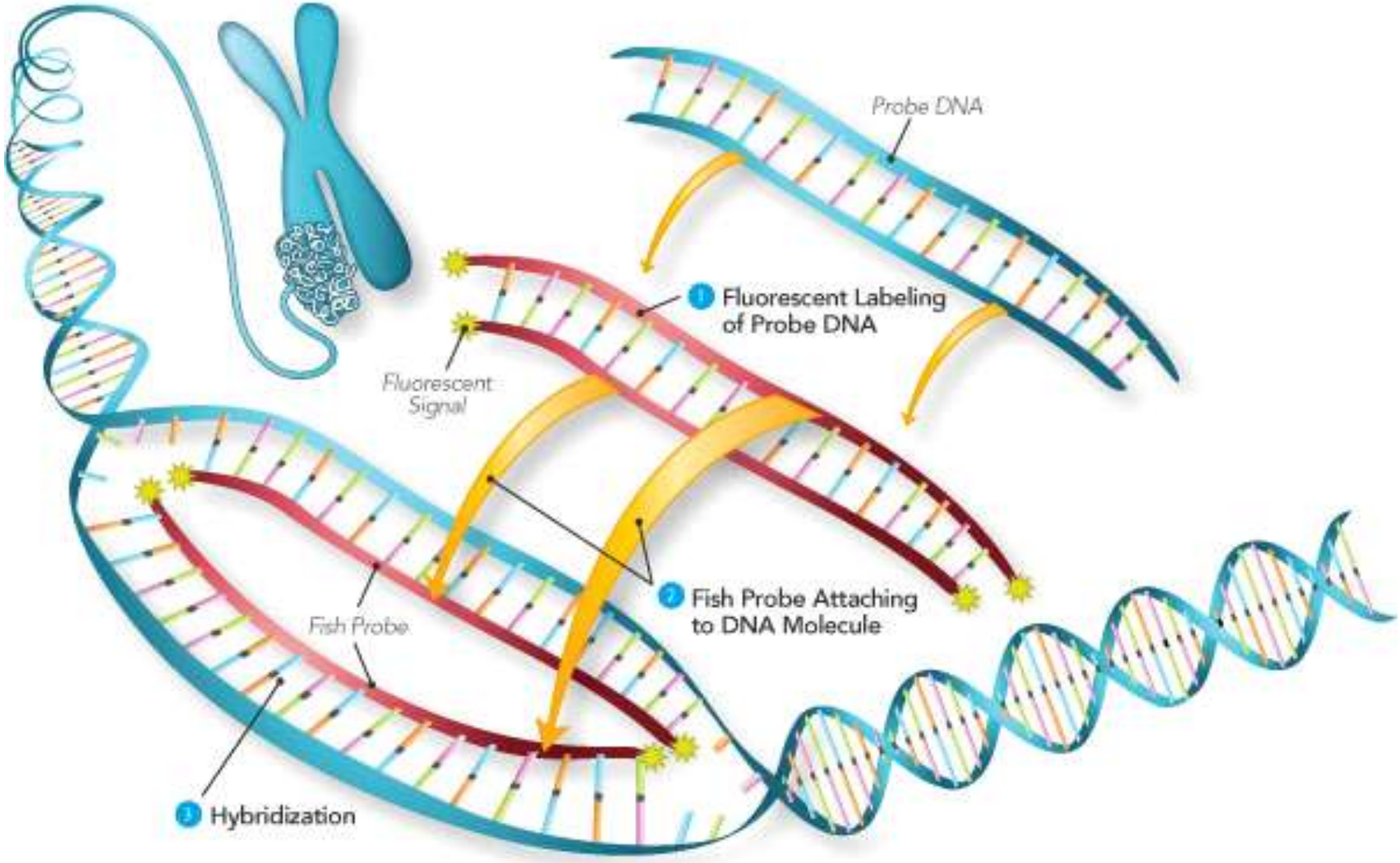

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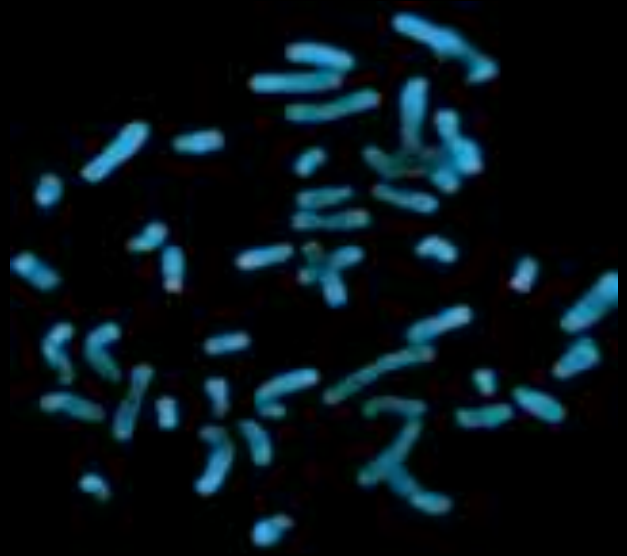
Cytogenetics entered the molecular era with the introduction of *in situ* hybridization, a procedure that allows researchers to locate the positions of specific DNA sequences on chromosomes. Since the first *in situ* hybridization experiments (Gall & Pardue, 1969), many variations of the procedure have been developed, and its sensitivity has increased enormously.



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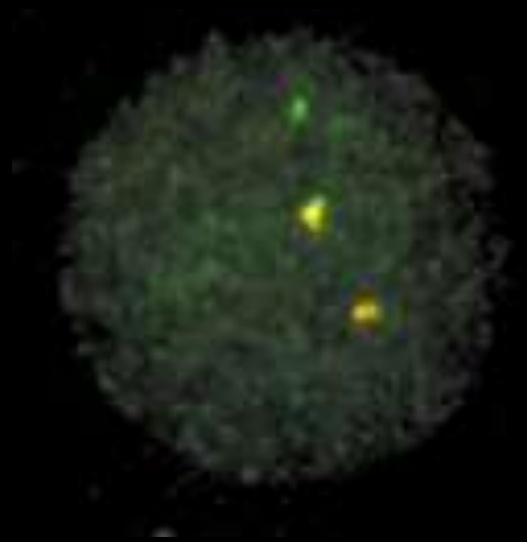
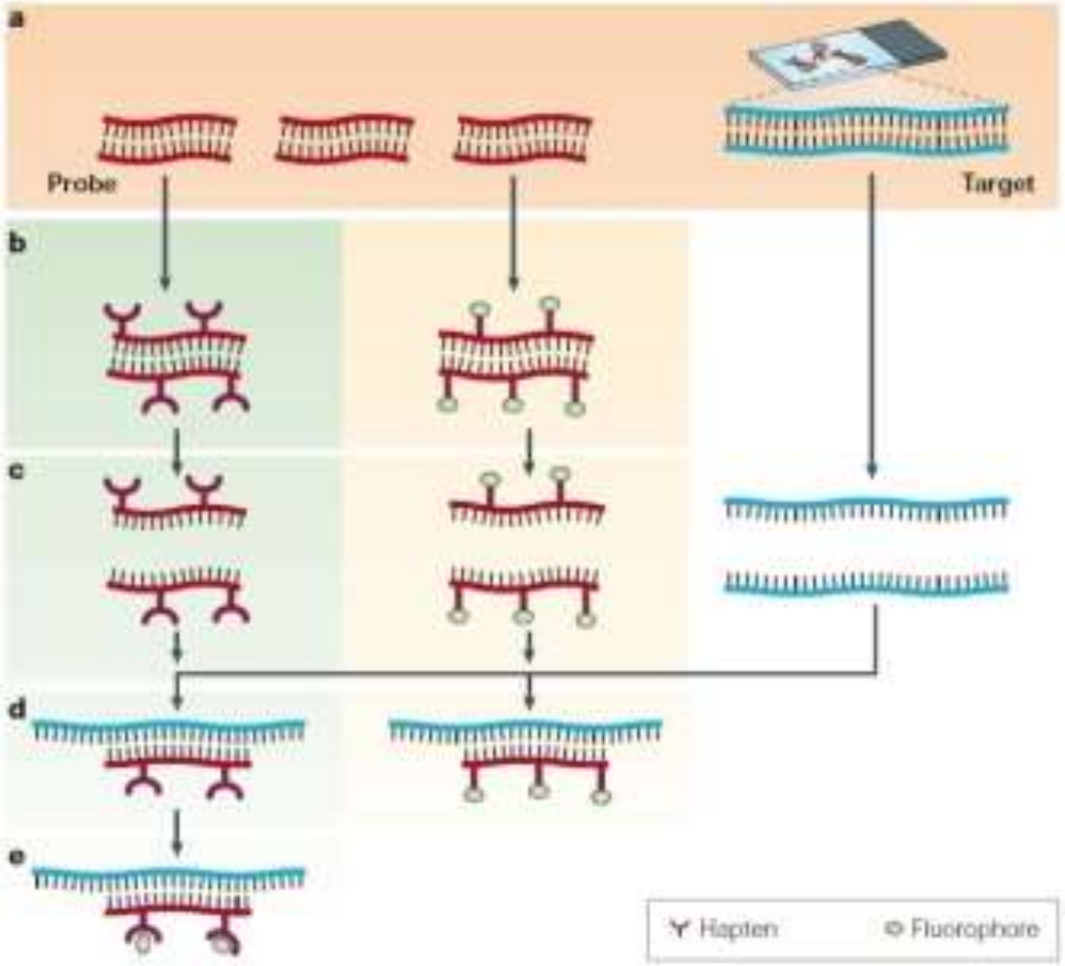


In the sixties, Joseph Gall and Mary Lou Pardue realized that molecular hybridization could be used to identify the position of DNA sequences *in situ* (*i.e.*, in their natural positions within a chromosome). In 1969, the two scientists published a landmark paper demonstrating that radioactive copies of a ribosomal DNA sequence could be used to detect complementary DNA sequences in the nucleus of a frog egg.

Probe

- The probe has to be long enough to hybridize specifically to its target but not to a similar sequences in the genome.
- At the same time, should not be that large as to impede the hybridization process.
- It should be tagged directly with **fluorophores**, with targets for antibodies or with biotin. This can be done in various ways, for example nick translation and PCR using tagged nucleotides.



The DNA probe is labeled by various means, such as nick translation, random primed labeling, and PCR. Two labeling strategies are commonly used: indirect labeling (left panel) and direct labeling (right panel). For indirect labeling, probes are labeled with modified nucleotides that contain a hapten, whereas direct labeling uses nucleotides that have been directly modified to contain a fluorophore.

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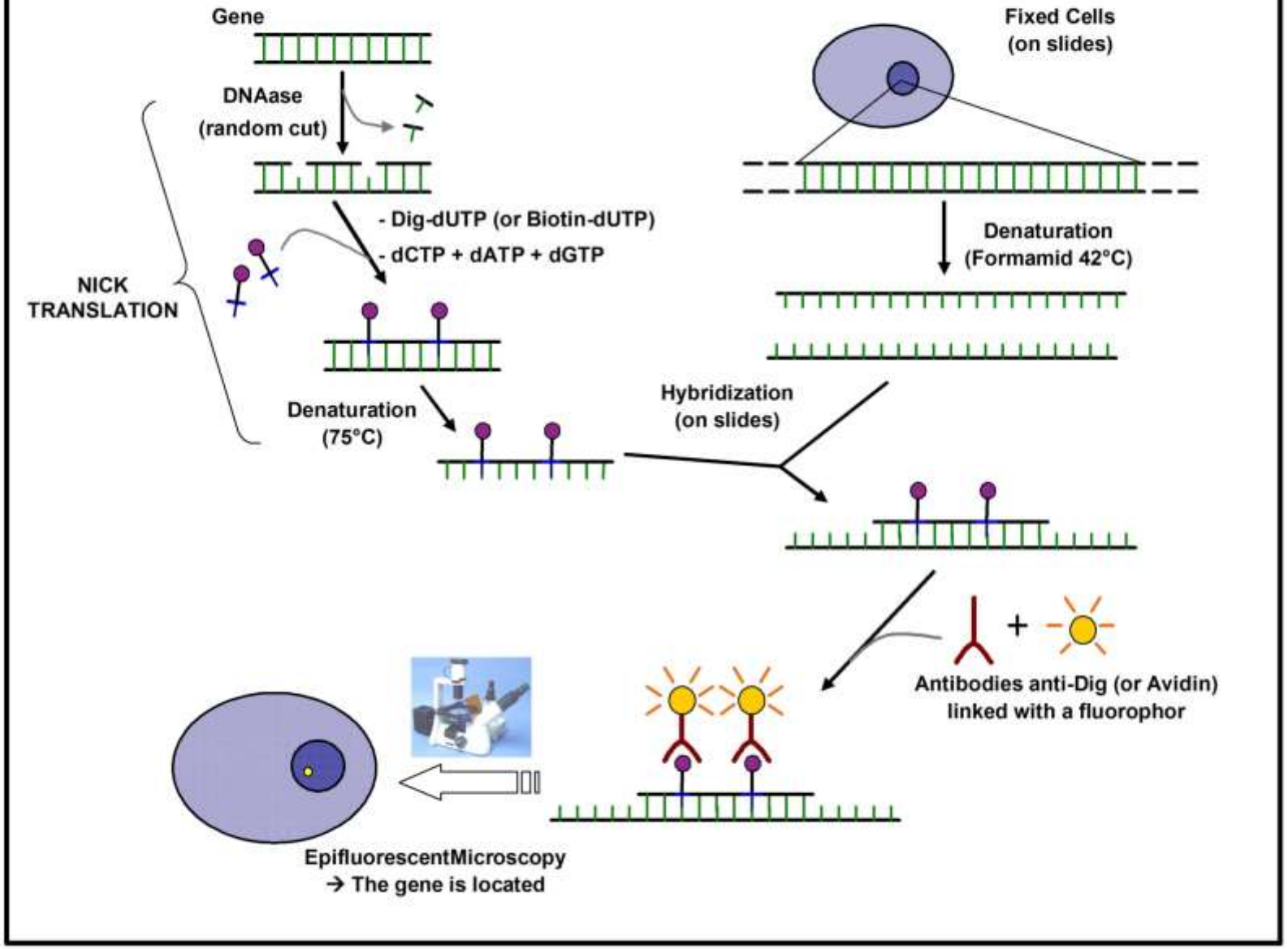
Procedure

- The first job is to make either a fluorescent probe sequence or a modified copy of the probe sequence (*e.g.* with a hapten) that can be rendered fluorescent later in the procedure.
- An interphase or metaphase chromosome preparation is done. The chromosomes are firmly attached to a substrate, usually glass slides.
- Repetitive DNA sequences are blocked by adding short fragments of DNA to the sample.
- Before any hybridization can occur, both the target and the probe sequences must be denatured.
- The probe is then applied to the chromosomal DNA and incubated for about 12 hours for hybridization.
- Several washing steps are required to remove all unhybridized or partially hybridized probes.
- The results are then visualized and quantified using a microscope that is capable of exciting the dye and recording images.

FISH (Fluorescent In Situ Hybridization)



Variations of Probe

- FISH is a very general technique. But it can be divided into specific categories based on the type of application.
- The differences between the various FISH techniques are usually due to the construction and content of the fluorescently-labeled DNA probe.
- The size, overlap, colour, and mixture of the probes make possible all different types of FISH techniques.

Variations of Probe [cont.]

- Probes size is important because longer probes hybridize more specifically than shorter probes.
- The mixture of probes determines the type of feature the probe can detect.
- Probes that hybridize along an entire chromosome are used to count the number of a certain chromosome, show translocations, or identify extra-chromosomal fragments of chromatin. This is often called "chromosome painting."
- If every possible probe is used, every chromosome, (in essence the whole genome) would be marked fluorescently, which would not be particularly useful for determining features of individual sequences.
- A mixture of smaller probes can be created that are specific to a particular region (locus) of DNA; these mixtures are used to detect deletion mutations.
- When combined with a specific colour, a locus-specific probe mixture is used to detect very specific translocations.
- Special locus-specific probe mixtures are often used to count chromosomes, by binding to the centromeric regions of chromosomes, which are unique enough to identify each chromosome (with the exception of Chromosome 13, 14 21, 22).

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Variation of FISH Techniques

- Because modern microscopes can detect a range of colours in fluorescent dyes, by using whole-chromosome probe mixtures and a variety of colours, each human chromosome can be identified (M-FISH).
- There are currently twice as many chromosomes than fluorescent dye colours. However, ratios of probe mixtures can be used to create additional colours. The probe mixture for the secondary colours is created by mixing the correct ratio of two sets of differently-labeled probes for the same chromosome.
- Colours that are adjacent appear to overlap, and a secondary colour is observed.
- Differently-coloured probes can be used for the detection of translocations.

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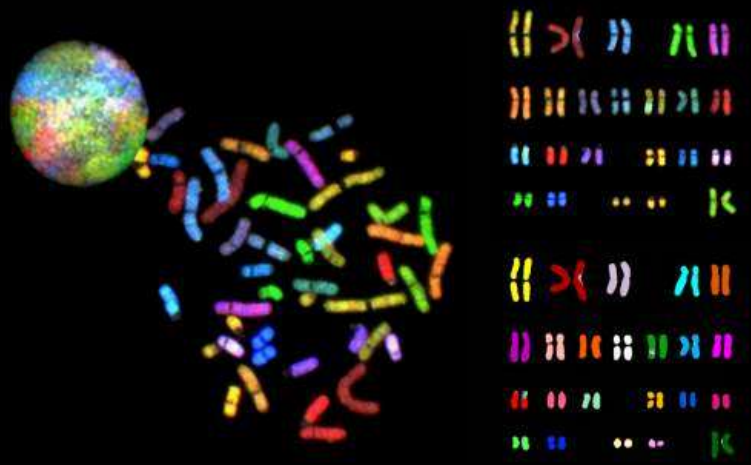
Multifluor FISH

- Cytogeneticists now have the option of using multifluor FISH, or spectral karyotyping, to quickly scan a set of metaphase chromosomes for potential rearrangements (Speicher *et. al*., 1996; Schrock *et al*., 1996). Multifluor FISH generates a karyotype in which each chromosome appears to be painted with a different color. Each "paint" is actually a collection of hybridization probes for sequences that span the length of a particular chromosome.



- To human eyes, several of the metaphase chromosomes appear to have the same color, but digital processing of the image would distinguish spectral differences between the chromosomes. A normal human chromosome (Figure) will have a uniform color along its length, but a rearranged chromosome will have a striped appearance. Figure 3c shows chromosomes from bladder cancer cells that have undergone numerous such chromosomal rearrangements (indicated by arrows). Digital processing of the image allows investigators to identify the chromosomes that have been involved in the translocations.

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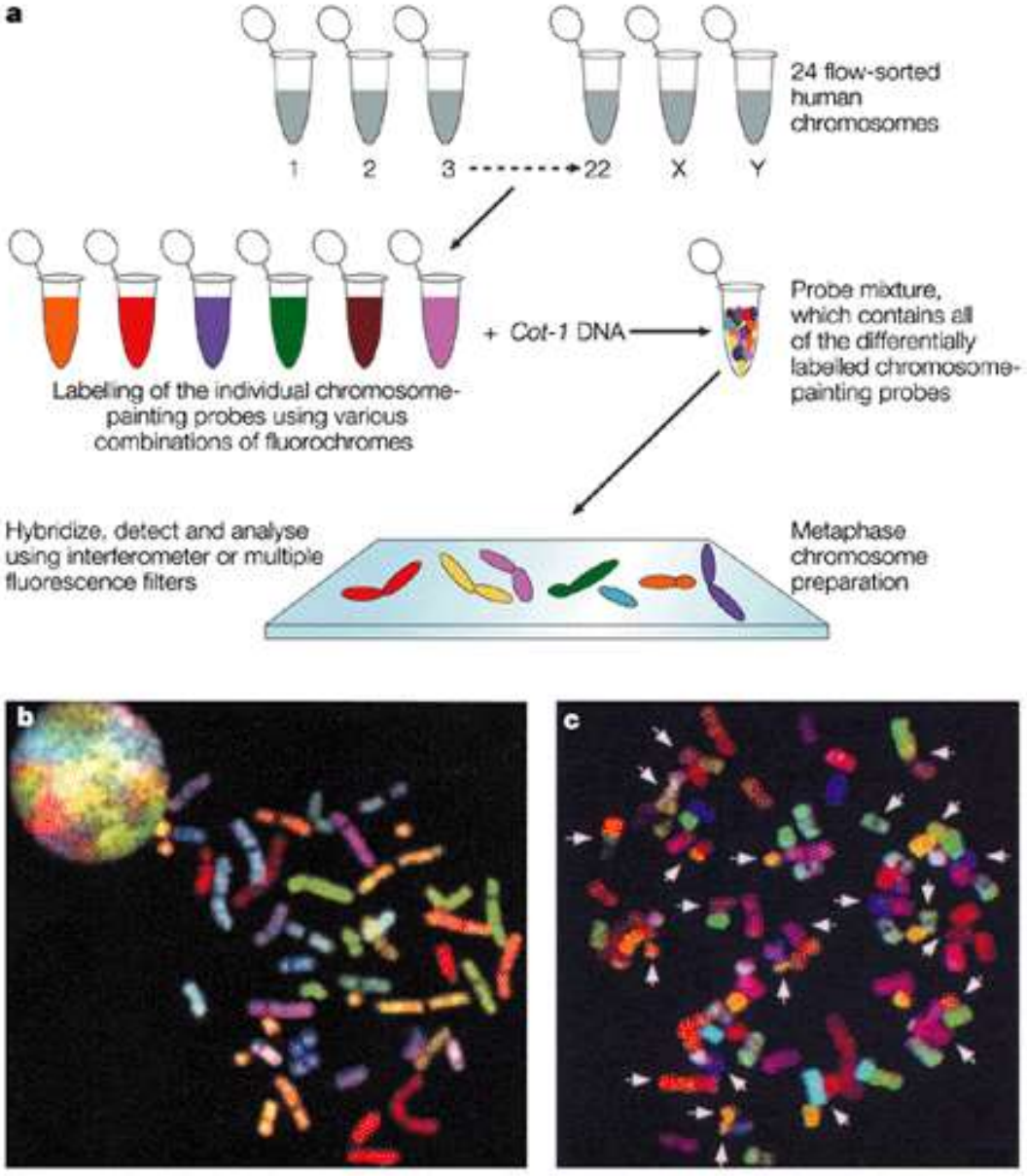
a
24 flow-sorted human chromosomes
1
2
3
22
X
Y
+ Cot-1 DNA
Probe mixture, which contains all of the differentially labelled chromosome-painting probes
Labelling of the individual chromosome-painting probes using various combinations of fluorochromes
Hybridize, detect and analyse using interferometer or multiple fluorescence filters
Metaphase chromosome preparation
b
c

Applications of FISH

Mapping Genes in HGP

- Historically, FISH and other *in situ* hybridization results played a primary role in mapping genes on human chromosomes. Results from these experiments were collected and compiled in databases, and this information proved useful during the annotation phase of the Human Genome Project (HGP).

- In species for which the genome has not been sequenced, however, FISH and related *in situ* hybridization methods continue to provide important data for mapping the positions of genes on chromosomes.

Medical Applications

- FISH and other *in situ* hybridization procedures are important in the clinical diagnosis of various chromosomal abnormalities, including deletions, duplications, and translocations resulting in diseases.

- Examples of diseases that are diagnosed using FISH include Prader-Willi syndrome, Angelman syndrome, 22q13 deletion syndrome, chronic myelogenous leukemia, acute lymphoblastic leukemia, Cri-du-chat syndrome, Velocardiofacial syndrome, and Down's syndrome.

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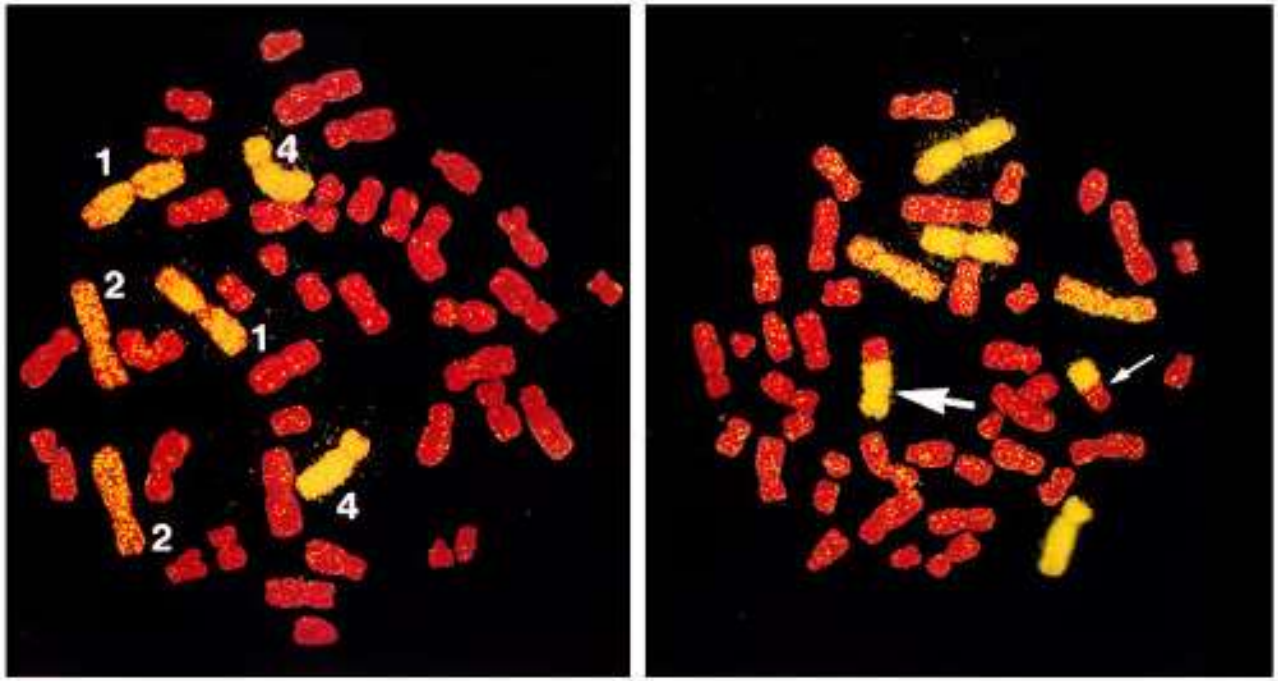
Medical Applications [cont.]

- In medicine, FISH can be used to form a diagnosis, to evaluate prognosis, or to evaluate remission of a disease, such as cancer. Treatment can then be specifically tailored. A traditional metaphase chromosome analysis is often unable to identify features that distinguish one disease from another, due to subtle chromosomal features; FISH can elucidate these differences.

- FISH can also be used to detect diseased cells more easily than standard cytogenetic methods which require dividing cells and requires labour- and time-intensive manual preparation and analysis of the slides by a technologist. FISH does not require living cells and can be quantified automatically, a computer counts the fluorescent dots present.



An example of FISH-treated metaphase chromosomes

Here, chromosomes 1, 2, and 4 were labeled yellow with FISH and the other chromosomes were stained red. Translocations between yellow and red chromosomes are detected. The left picture represents a normal cell (the numbers in the figure indicate chromosome numbers) and the right picture is an example of reciprocal translocation with two bi-color chromosomes (indicated by two arrows).

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- **Since the introduction of FISH, cytogeneticists have been able to analyze interphase chromosomes as well as the metaphase chromosomes used in karyotypes (Trask, 2002). This offers a real practical advantage, in that cells do not need to be cultured for several days or weeks before chromosomes can be prepared for analysis. In addition, FISH can be used to analyze chromosomes from specimens such as solid tumors, which are of great clinical interest but do not divide frequently.**

Species Identification

- FISH is often used in clinical studies in identification of pathogens. Traditionally if a patient is infected with a suspected pathogen such as bacteria, these are collected from patient's tissues or body fluids and typically grown on agar to determine the identity of the pathogen. However, many bacteria, even well-known species, do not grow well under laboratory conditions. FISH can be used to detect directly the presence of the suspect on small samples of patient's tissue.

- FISH can also be used compare the genomes of two biological species to deduce evolutionary relationships. A similar hybridization technique is called a zoo blot. Bacterial FISH probes are often primers for the 16s rRNA region.

- FISH is widely used in the field of microbial ecology, to identify microorganisms. Biofilms, for example, are composed of complex (often) multi-species bacterial organizations. Preparing DNA probes for one species and performing FISH with this probe allows one to visualize the distribution of this specific species within the biofilm. Preparing probes (in two different colors) for two species allows visualization/study co-localization of these two species in the biofilm, and can be useful in determining the fine architecture of the biofilm.

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