KARYOTYPING

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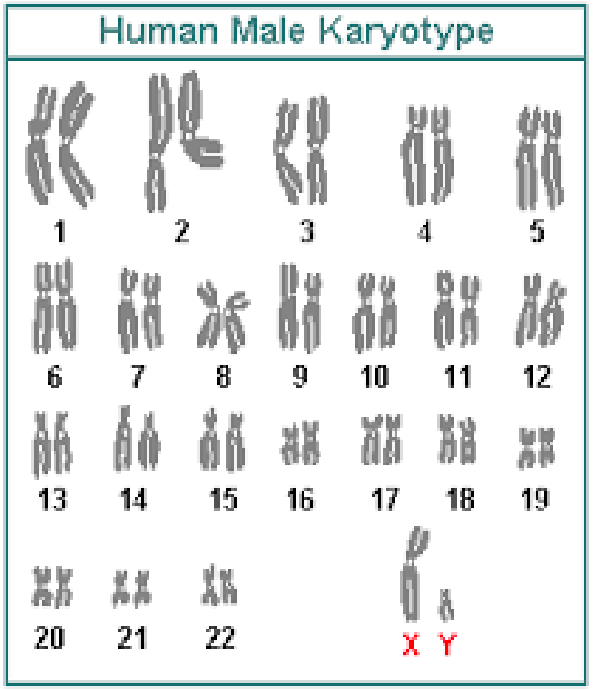
What is karyotyping?

- **Karyotyping** is the process by which photographs of chromosomes are taken in order to determine the chromosome complement of an individual, including the number of chromosomes and any abnormalities. The term is also used for the complete set of chromosomes in a species or in an individual organism and for a test that detects this complement or measures the number.

The chromosomes are depicted (by rearranging a photomicrograph) in a standard format known as a ***karyogram* or *idiogram***: in pairs, ordered by size and position of centromere for chromosomes of the same size.



Procedure of karyotyping

5 mL venous blood

Add phytohemagglutinin and culture medium

Culture at 37°C for 3 days

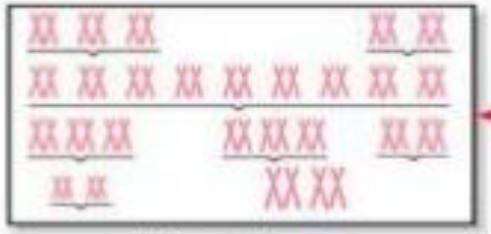
Add colchicine and hypotonic saline

Cells fixed

Spread cells onto slide by dropping

Digest with trypsin and stain with Giemsa

Analyze "metaphase spread"

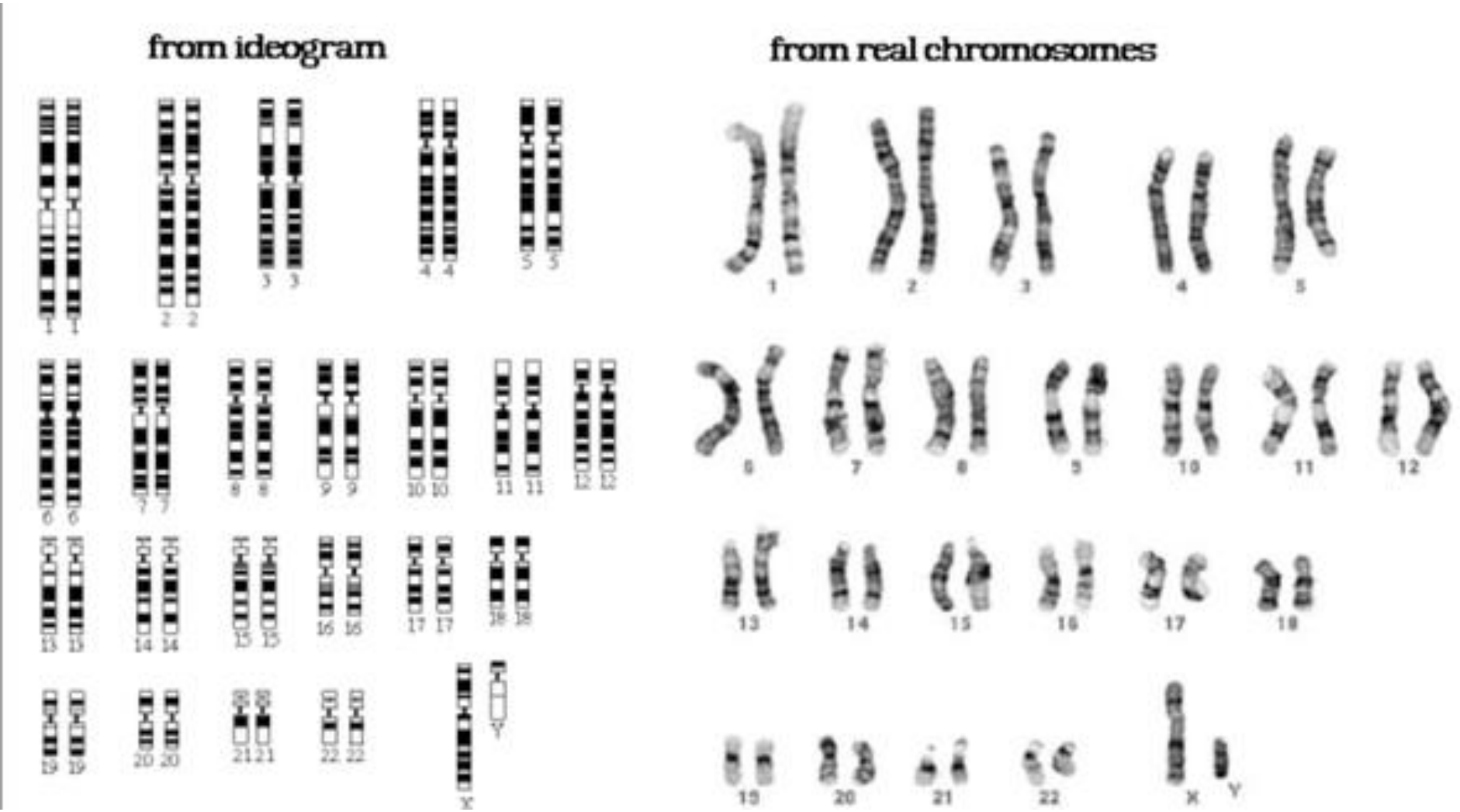


Karyotype

- Samples used in clinical karyotyping:
  - Biopsies
  - Bone marrow
  - Blood cells
  - Cells from amniotic fluid
  - Cells from chorionic villus
- Artificial media are used for cell culture
- Colchicine treatment arrests the cells at mitotic metaphase
- Giemsa stain (contains a mixture of Azure, Methylene blue, and Eosin dye and is specific for the phosphate groups of DNA and attaches itself to where there are high amounts of adenine-thymine bonding)
- Photographs of chromosomes are taken
- The photographs of chromosomes are arranged in descending order as per size
- Sex chromosomes are placed after the autosomes
- Chromosomes depicted with short p arms at the top and long q arms at the bottom

IDEOGRAM

KARYOGRAM



Significance & Importance of Karyotype and karyotyping

- Karyotypes of different species can be compared
- Similarities in the karyotype represent the evolutionary relationship
- Karyotype can be used to solve taxonomic disputes
- Karyotype can indicate primitive and advanced features
  - Symmetric karyotype- Primitive
  - Asymmetric karyotype- Advanced (e.g. Zymographic flowers)
- Species with special characteristics in their karyotype
  - Mouse: Acrocentric chromosomes
  - Amphibians: Metacentric chromosomes

Contd.

- Provides structural features of chromosomes
- Clinical cytologists can determine the genetic changes
- Karyotype also reveals the numerical anomalies in chromosomes
  - Trisomy at 21st chromosome (Down Syndrome)
  - Trisomy at sex chromosome- XXY (Klinefeltners syndrome)
  - Monosomy at sex chromosome- XO (Turner syndrome)
- Karyotype also helps in detection of some cancers

Modern method of Karyotyping

- Fluorescence in- situ hybridization technique (FISH) is used for karyotyping

  Fluorescence in situ hybridization (FISH) is a technique that uses fluorescent probes which bind to special sites of the chromosome with a high degree of sequence complementarity to the probes. The fluorescent probes are nucleic acid labeled with fluorescent groups and can bind to specific DNA/RNA sequences. Thus, we can understand where and when a specific DNA sequences exist in cells by detecting the fluorescent group. It was developed in the early 1980s. Fluorescence microscopy can be used to find out where the fluorescent probe is bound to the chromosomes and flow cytometry can be used to detect the binding quantitatively. This FISH protocol is for a Cy5 and FAM labeled probe used in flow cytometry detection and fluorescence microscopy detection.

FISH (Fluorescent In Situ Hybridization)

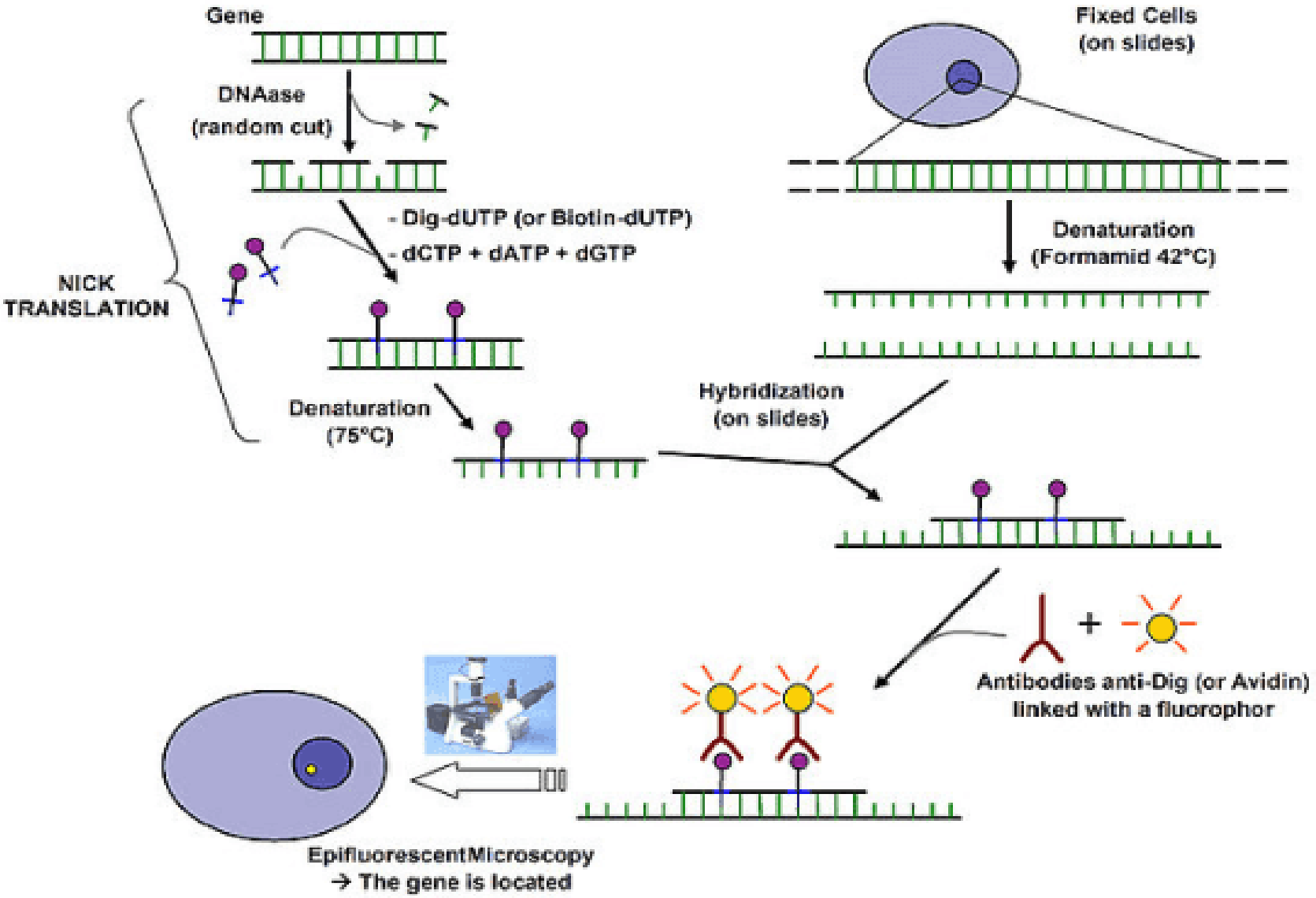
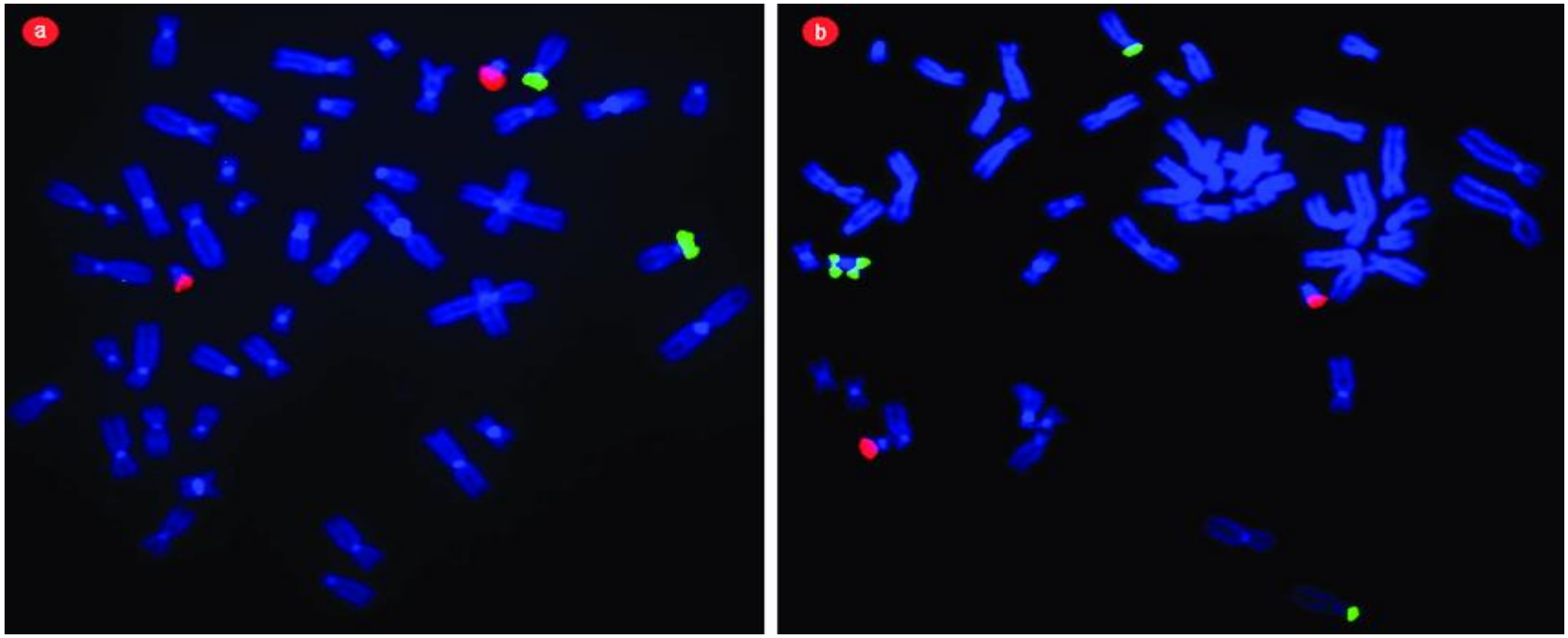


Figure A

Figure B



(A) FISH technique applied in a normal pattern of chromosomes obtained from peripheral lymphocytes of a blood sample from a normal/healthy child. The dual color translocation probes [TEL/AML1] were used to show two green signals (TEL) at chromosome 12p and two orange signals (AML1) at chromosome 21p22 as contrast.

(B) FISH technique applied to a pattern of chromosomes obtained from peripheral lymphocytes of a blood sample from patient with Pallister-Killian syndrome. There were four green signals and two orange signals indicating the presence of 4-arms of chromosome 12p [green] and two signals for chromosome 21p22 [orange].

Oligo Paint Fish Platform

- Researchers at the Harvard Medical School and the University of Michigan in 2012 described the design and synthesis of a platform useful for the **visualization of genomes** with **oligopaint FISH probes** (Beliveau et al.).
- This technique allows a scientist to study any sequenced organism by interrogating the relationship between nuclear architecture or chromosome position and processes such as gene expression by its ability to visualize chromosomes in situ.
- The method combines an oligonucleotide- and PCR-based strategy for fluorescence in situ hybridization (FISH) with a bioinformatic platform. The proper design of the probes enables the technology to be used for the study of any organism whose genome has been sequenced.
- Additionally, it provides researchers a precise control over the sequences they target and allows for single and multicolor imaging of regions ranging from tens of kilobases to megabases.
- Therefore the technique leads to an enhanced ability to visualize interphase and metaphase chromosomes in the future.

Oligo Paint FISH Platform

Each oligonucleotide in the library is composed of 32 bases of genomic sequence flanked by 21-base primer sequences. One of the primers carries a 5′ fluorophore, whereas the other contains a recognition site for a nicking endonuclease (NE). A nicking reaction followed by denaturing gel electrophoresis yields 53-base ssDNAs.