MEDICAL ACADEMY NAMED AFTER S.I.GEORGIEVSKY OF VERENADSKY CFU

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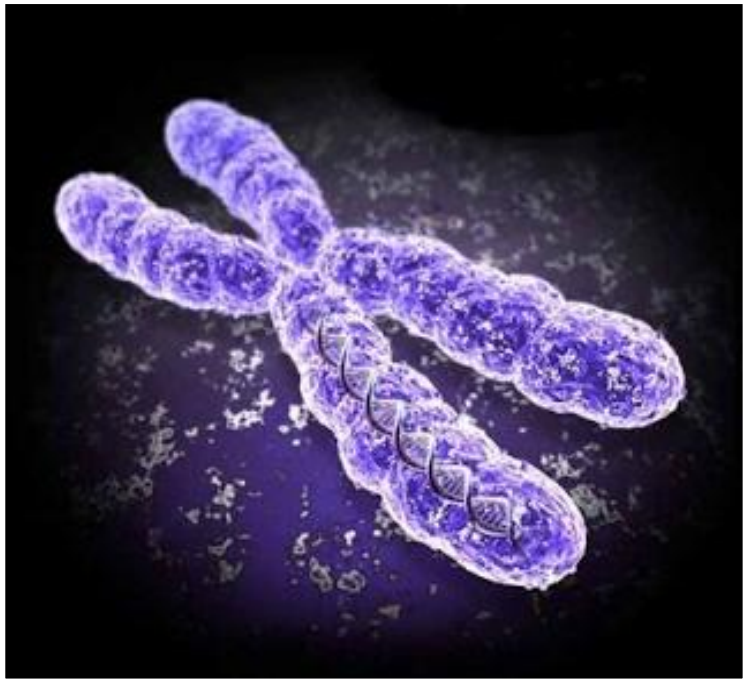
GROUP : LA3 -204(2)

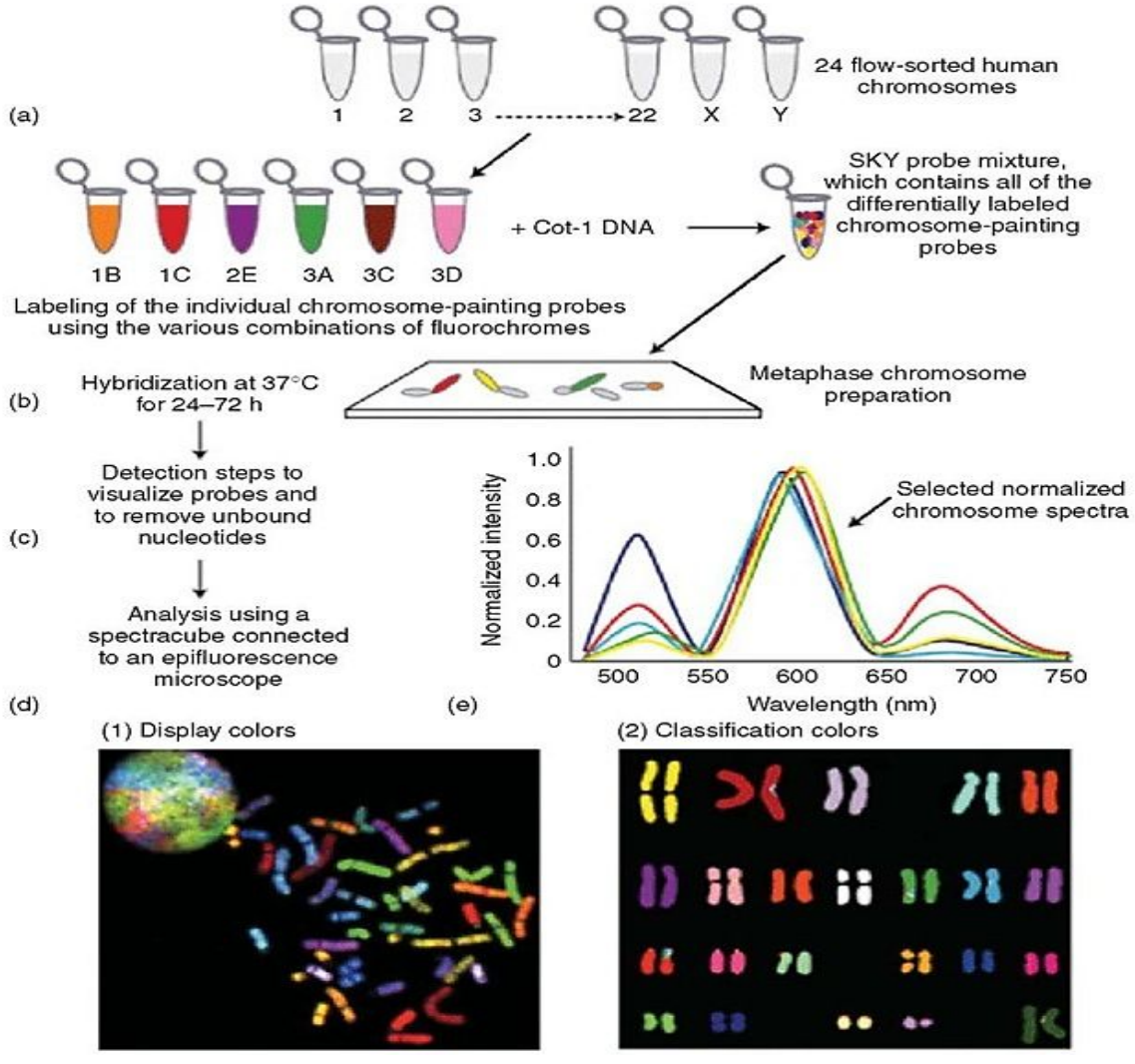
TOPIC :CYTOGENETIC METHOD

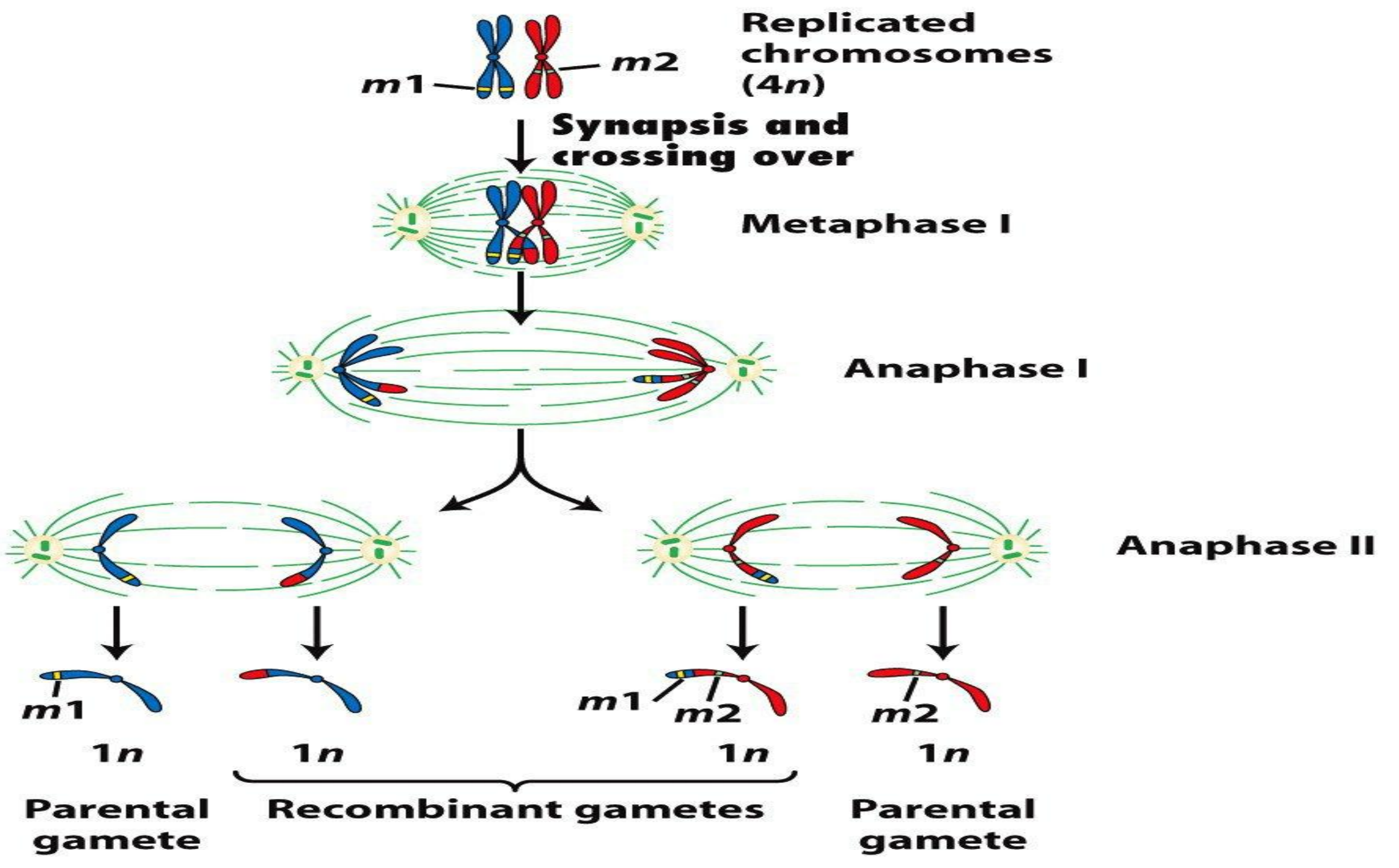
TEACHER NAME :MAM SVETLANA SMIRNOVA



CYTOGENETIC

- **CYTOGENETICS IS ESSENTIALLY A BRANCH OF GENETICS, BUT IS ALSO A PART OF CELL BIOLOGY/CYTOLOGY (A SUBDIVISION OF HUMAN ANATOMY), THAT IS CONCERNED WITH HOW THE CHROMOSOME RELATE TO CELL BEHAVIOUR, PARTICULARLY TO THEIR BEHAVIOUR DURING MITOSIS AND MEIOSI**



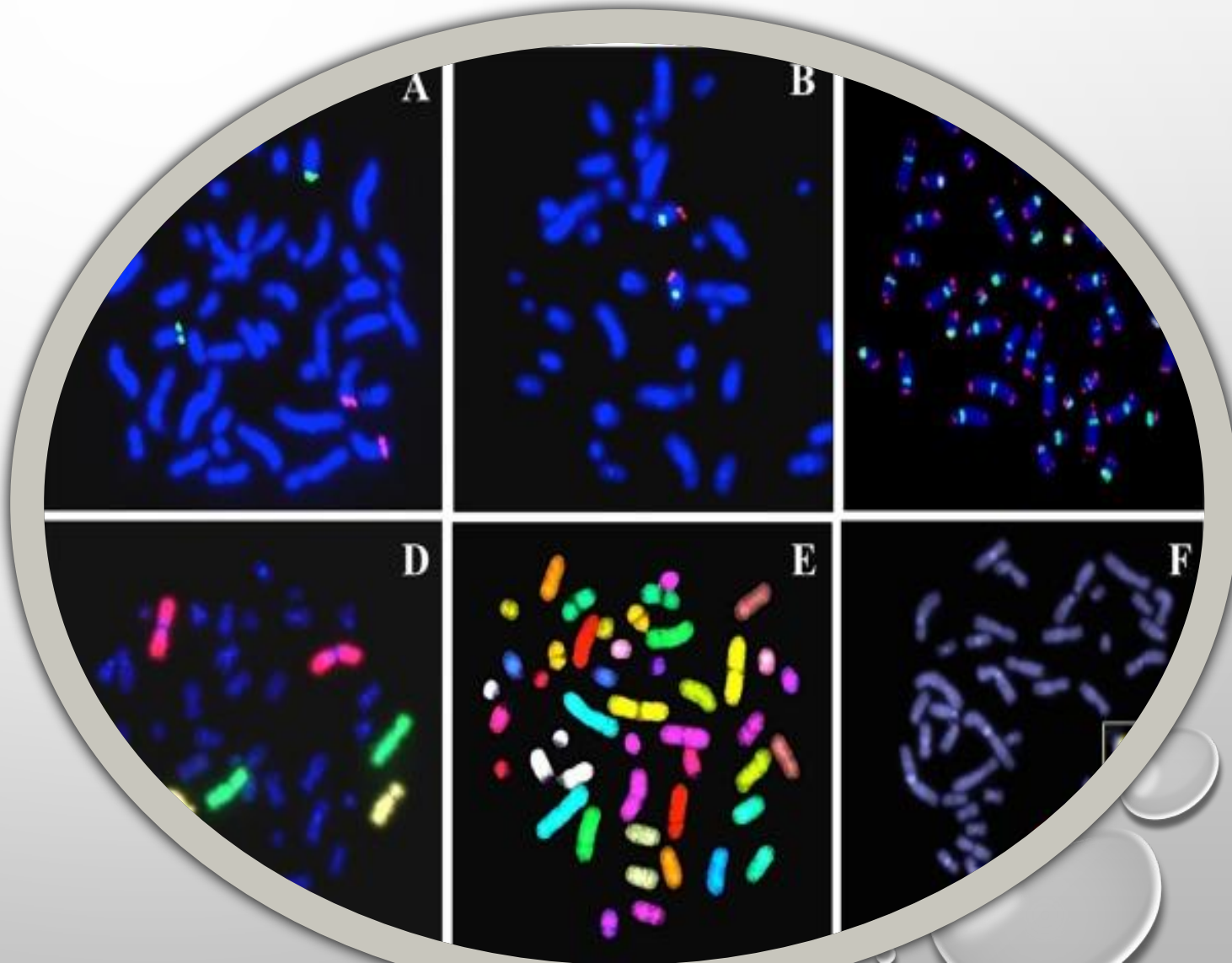

m1
m2
Replicated chromosomes (4n)
Synapsis and crossing over
Metaphase I
Anaphase I
Anaphase II
m1
1n
1n
m1
m2
1n
m2
1n
Parental gamete
Recombinant gametes
Parental gamete

HISTORY AND EVOLUTION OF CYTOGENETICS

- has been a key part of biology since 1842, when Swiss botanist Karl Nägeli first discovered chromosomes in pollen. In the decades since, the science has been defined as the study of chromosomes, including their behavior, mechanics, and role in inheritance. Ever since Nägeli's discovery, methods for examining chromosomes have become more and more effective, further illuminating their roles in cell biology and human and animal health in ways undreamed-of when chromosomes were first discovered. Their behavior in animal (salamander) cells was described by Walther Flemming, the discoverer of mitosis, in 1882. The name was coined by another German anatomist, von Waldeyer in 1888.

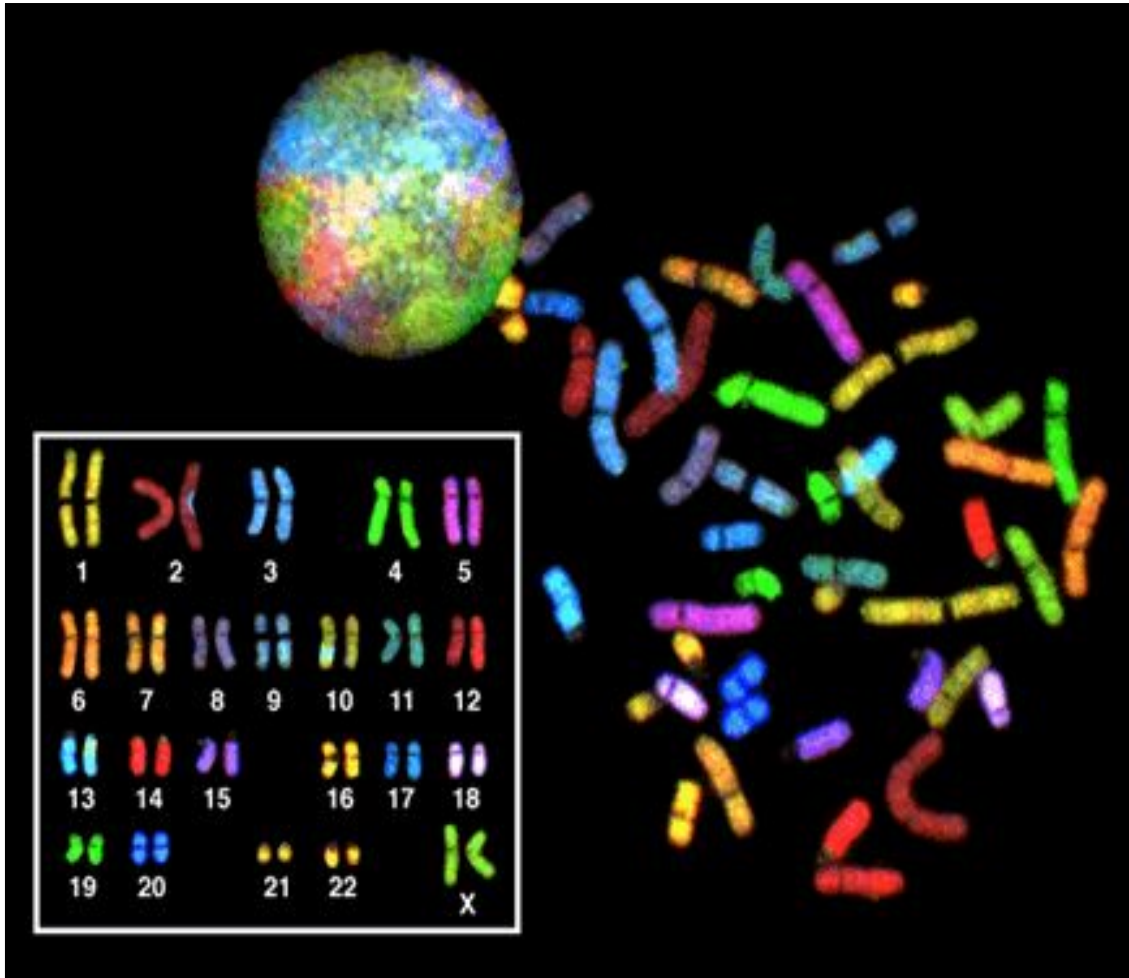
Cytogenetic methods

- 1 – KARYOTYPING
- 2 - FLUORESCENT IN SITU HYBRIDIZATION

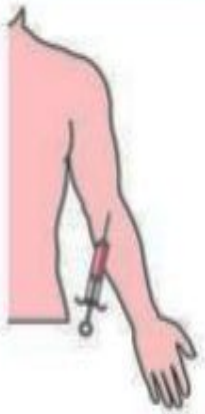


KARYOTYPING

The routine chromosome analysis (Karyotyping) refers to analysis of metaphase chromosomes which have been banded using trypsin followed by Giemsa, Leishmanns, or a mixture of the two. This creates unique banding patterns on the chromosomes. The molecular mechanism and reason for these patterns is unknown, although it likely related to replication timing and chromatin packing.



Procedure of karyotyping



5 mL venous blood

Add phytohemagglutinin and culture medium

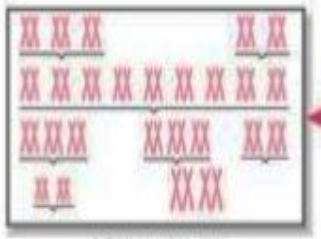
Culture at 37°C for 3 days

Add colchicine and hypotonic saline → Cells fixed

Spread cells onto slide by dropping

Digest with trypsin and stain with Giemsa

Analyze "metaphase spread"



Karyotype

Process of karyotyping in Plants

Plant

Root tips - 0.5 to 1cm

Pretreated with colchicine for 3 hrs at 26 °C

Slide preparation

N banding technique

Cells at Metaphase

STAINING

The study of karyotypes is made possible by staining. Usually, a suitable dye, such as Giemsa is applied after cellshave been arrested during cell division by a solution of colchicine usually
in metaphase or prometaphase when most condensed. In order for the Giemsa stain to adhere correctly, all chromosomal proteins must be digested and removed. For humans, white blood cells are used most frequently because they are easily induced to divide and grow in tissue culture Sometimes observations may be made on non-dividing (interphase) cells. The sex of an unborn fetus can be determined by observation of interphase cells (see amniotic centesis and Barr body).

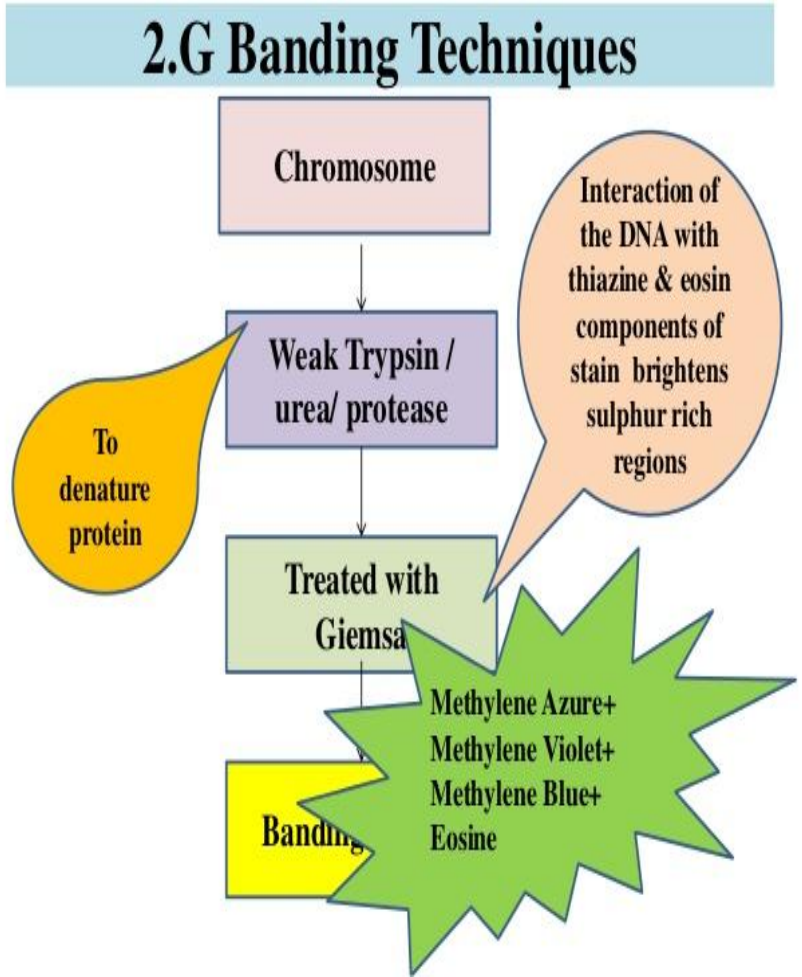


TABLE 5-2 Common Inherited Human Diseases

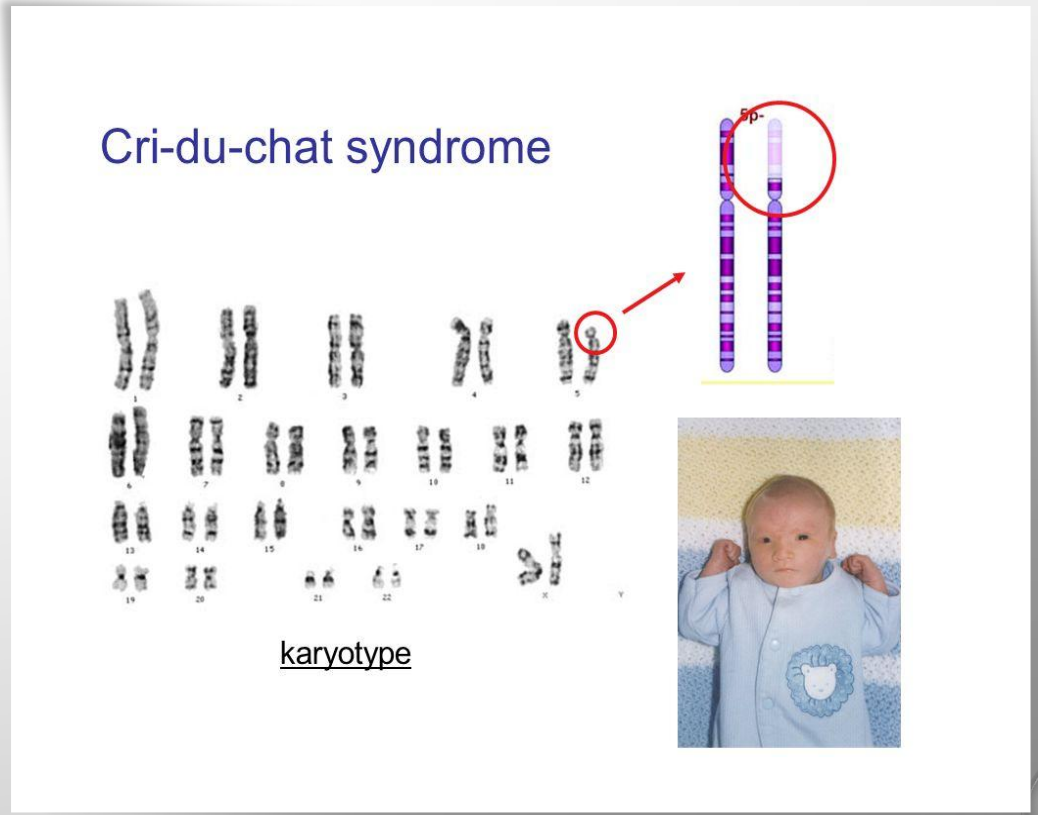
DISEASE	MOLECULAR AND CELLUAR DEFECT	INCIDENCE
AUTOSOMAL RECESSIVE		
Sickle-cell anemia	Abnormal hemoglobin causes deformation of red blood cells, which can become lodged in capillaries; also confers resistance to malaria.	1/625 of sub-Saharan African origin
Cystic fibrosis	Defective chloride channel (CFTR) in epithelial cells leads to excessive mucus in lungs.	1/2500 of European origin
Phenylketonuria (PKU)	Defective enzyme in phenylalanine metabolism (tyrosine hydroxylase) results in excess phenylalanine, leading to mental retardation, unless restricted by diet.	1/10,000 of European origin
Tay-Sachs disease	Defective hexosaminidase enzyme leads to accumulation of excess sphingolipids in the lysosomes of neurons, impairing neural development.	1/1000 eastern European Jews
AUTOSOMAL DOMINANT		
Huntington's disease	Defective neural protein (huntingtin) may assemble into aggregates causing damage to neural tissue.	1/10,000 of European origin
Hypercholesterolemia	Defective LDL receptor leads to excessive cholesterol in blood and early heart attacks.	1/122 French Canadians
X-LINKED RECESSIVE		
Duchenne muscular dystrophy (DMD)	Defective cytoskeletal protein dystrophin leads to impaired muscle function.	1/3500 males
Hemophilia A	Defective blood clotting factor VIII leads to uncontrolled bleeding.	1–2/10,000 males

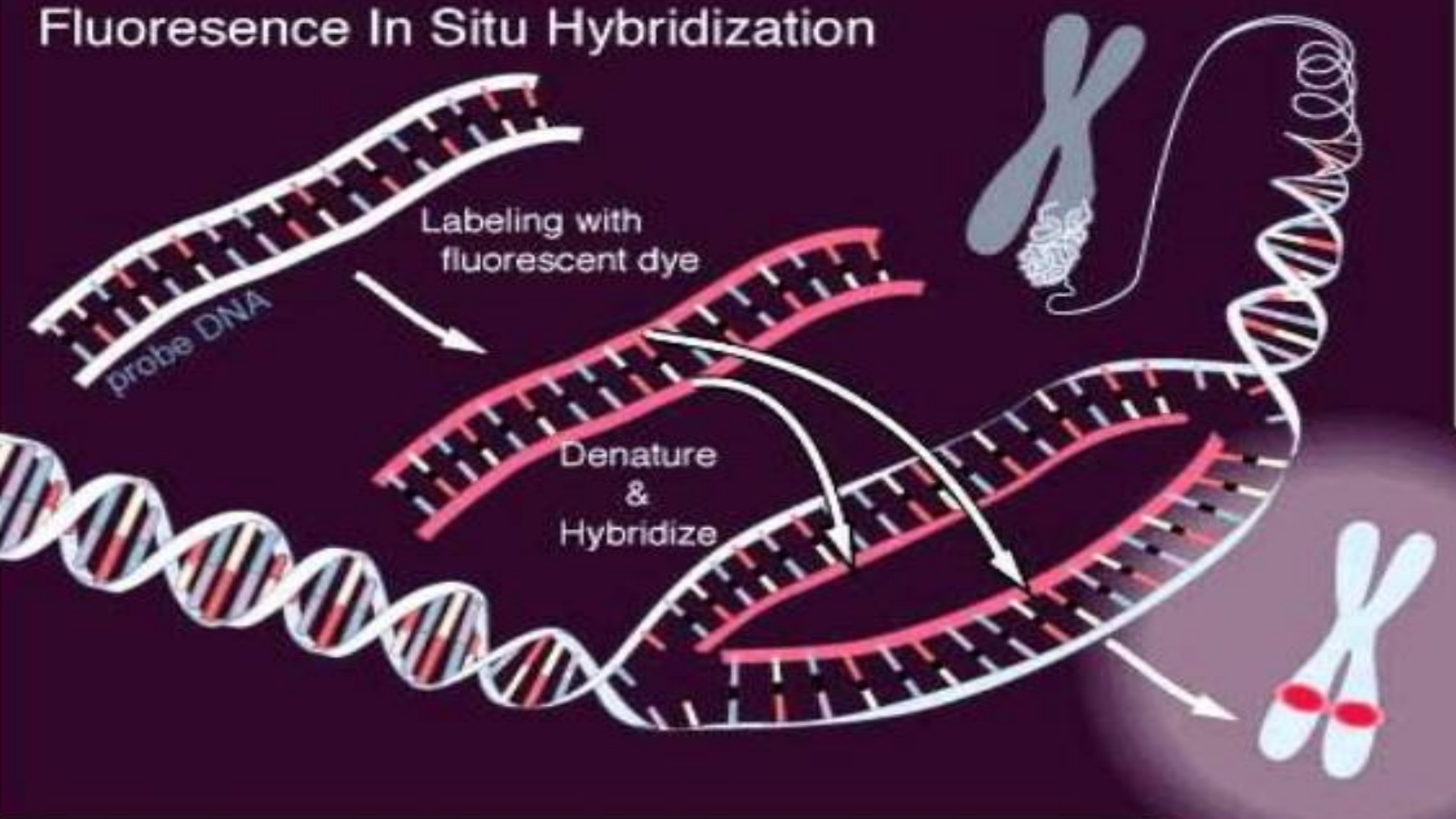
Chromosome abnormalities

- CHROMOSOMAL ABNORMALITIES THAT LEAD TO DISEASE IN HUMANS INCLUDE
- **TURNER SYNDROME** RESULTS FROM A SINGLE X CHROMOSOME (45,X OR 45,X0).
- **KLINEFELTER SYNDROME,** THE MOST COMMON MALE CHROMOSOMAL DISEASE, OTHERWISE KNOWN AS 47,XXY, IS CAUSED BY AN EXTRA **X** CHROMOSOME.
- **EDWARDS SYNDROME** IS CAUSED BY TRISOMY (THREE COPIES) OF CHROMOSOME 18.
- **DOWN SYNDROME,** A COMMON CHROMOSOMAL DISEASE, IS CAUSED BY TRISOMY OF CHROMOSOME 21.
- **PATAU SYNDROME** IS CAUSED BY TRISOMY OF CHROMOSOME 13.
- **TRISOMY 9,** BELIEVED TO BE THE 4TH MOST COMMON TRISOMY, HAS MANY LONG LIVED AFFECTED INDIVIDUALS BUT ONLY IN A FORM OTHER THAN A FULL TRISOMY, SUCH AS TRISOMY 9P SYNDROME OR MOSAIC TRISOMY 9. THEY OFTEN FUNCTION QUITE WELL, BUT TEND TO HAVE TROUBLE WITH SPEECH.

SOME DISORDERS ARISE FROM LOSS OF JUST A PIECE OF ONE CHROMOSOME, INCLUDING

- **Cri du chat (cry of the cat), from a truncated short arm on chromosome 5. The name comes from the babies' distinctive cry, caused by abnormal formation of the larynx.**
- **<u>1p36 Deletion syndrome</u>, from the loss of part of the short arm of chromosome 1.**
- **Angelman syndrome – 50% of cases have a segment of the long arm of chromosome 15 missing; a deletion of the maternal genes, example of imprintingdisorder.**
- **Prader-Willi syndrome – 50% of cases have a segment of the long arm of chromosome 15 missing; a deletion of the paternal genes, example of imprinting disorder.**




Fluoresence In Situ Hybridization
Labeling with
fluorescent dye
probe DNA
Denature
&
Hybridize

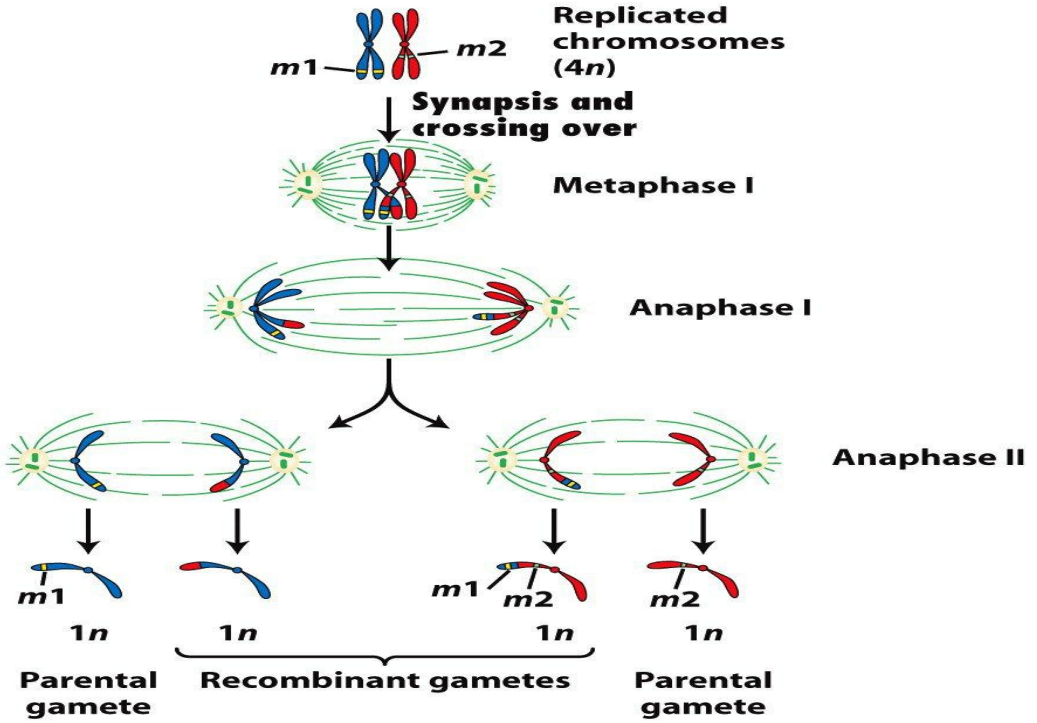
FLUORESCENCE IN SITU HYBRIDIZATION (FISH) IS A LABORATORY TECHNIQUE FOR DETECTING AND LOCATING A SPECIFIC DNA SEQUENCE ON A CHROMOSOME. THE TECHNIQUE RELIES ON EXPOSING CHROMOSOMES TO A SMALL DNA SEQUENCE CALLED A PROBE THAT HAS A FLUORESCENT MOLECULE ATTACHED TO IT. THE PROBE SEQUENCE BINDS TO ITS CORRESPONDING SEQUENCE ON THE CHROMOSOME.

LINKAGE MAPPING USING MOLECULAR MARKERS

- THE NEXT STEP IN GENE ID IS TO GENETICALLY MAP ITS POSITION WITH RESPECT TO KNOWN GENETIC MARKERS IN THE GENOME. THIS METHOD CAN BE PERFORMED BY BREEDING STUDIES IN SIMPLE EXPERIMENTAL ORGANISMS IN WHICH GENETIC MARKERS CONFER READILY DETECTABLE PHENOTYPES. HOWEVER SUCH PHENOTYPIC MARKERS ARE UNCOMMON IN HUMANS, AND INSTEAD DNA-BASED MOLECULAR MARKERS ARE USED. MOLECULAR MARKERS CAUSED BY DNA POLYMORPHISMS (SEQUENCE DIFFERENCES) OCCUR AT A FREQUENCY OF ABOUT 1/1,000 NUCLEOTIDES. POLYMORPHISMS ARE USED AS LANDMARKS IN LOCATING THE POSITION OF A DISEASE GENE. IN SOME CASES, POLYMORPHISMS CHANGE THE LOCATIONS OF RESTRICTION SITES. THIS RESULTS IN RESTRICTION FRAGMENT LENGTH POLYMORPHISMS (RLFPS) WHICH CAN BE USED IN LINKAGE STUDIES. OTHER DNA POLYMORPHISMS DO NOT AFFECT RESTRICTION SITES. THESE MOLECULAR MARKERS--CALLED SINGLE NUCLEOTIDE POLYMORPHISMS (SNPS) AND SIMPLE SEQUENCE REPEATS (SSRS)--CAN BE IDENTIFIED AND STUDIED BY PCR AMPLIFICATION AND SEQUENCING OF GENOMIC DNA.

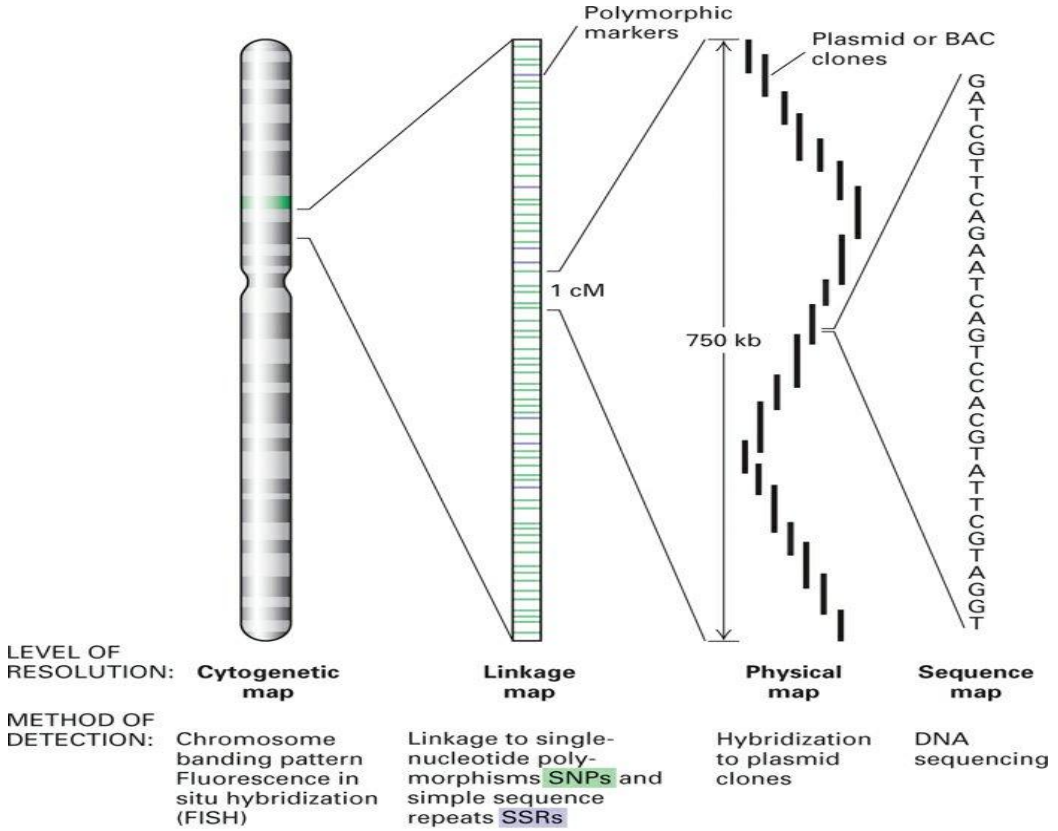
Linkage Mapping of Disease Gene Location by Recombination Analysis

- Although it is not commonly used in analysis of human diseases, it is instructive to consider the procedure known as recombination analysis (Fig. 5.10), which is often applied in linkage analysis in simple model organisms. This method relies on the facts that, phenotypic traits that segregate together during meiosis more frequently than expected based on random segregation typically are specified by genes residing on the same chromosome. In addition, the less frequently recombination occurs between two markers on a chromosome, the more tightly they are linked and the closer together they are. One genetic map unit is defined as the distance between two genes along a chromosome that results in a 1% (1/100 gametes) recombination frequency (1 centimorgan, cM). In humans, 1 cM corresponds to a physical distance of ~750,000 bp.



Final Steps of Mutant Gene Isolation

- Often mutations can be mapped only to 1 cM regions of human DNA using the methods discussed above (Fig. 5.38). Regions of this length can contain dozens of genes. The final identification of the disease gene typically involves sequencing and mapping of all SNPs, etc. in a long region of DNA. The responsible gene is likely to be located in regions where SNPs associated with the disease consistently are found in a number of affected individuals. The mutation itself eventually is identified by DNA sequencing. The analysis of gene expression by Northern blotting and in situ hybridization in affected tissues also may help in identifying a disease gene in cases where grossly defective mRNA transcripts are produced from a gene.



Common Hereditary Human Diseases

- Most inherited diseases are caused by preexisting mutant alleles that have been passed down from one generation to the next. Examples of common autosomal recessive, autosomal dominant, and X-linked recessive human diseases of monogenic origin are listed in Table 5.2. At least some fraction of diseases such as cancers, diabetes, obesity, and heart disease are hereditary and polygenic in origin. The molecular bases of these diseases are even harder to solve than those of monogenic diseases.

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