# * CYTO TAXONOMY

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# INTRODUCTION

- Cyto- taxonomy is the branch of biology dealing with the relationship and classification of organism using comparative studies of the number, structure and behaviour of chromosomes.
- Chromosome number is the most quoted character in cyto-taxonomy. Position of centromere and meiotic behaviour for a taxon may be constant and provides valuable taxonomic evidence.
- Chromosomal studies are useful at two different levels-
    1. they aid in the comparison of the closely related species including sibling ` species.
    2. chromosomes pattern are highly useful in establishing phyletic relationship among different organism.
- Most chromosomal events are unique one, which then characterize all descendent of ancestral populations in which the new pattern first became established. Changes in sex determination in all sorts of arrangement of chromosomes ,centromere in fission, fusion, translocations in the acquisitions of supernumeraries etc. often give unequivocal clues to relationship.
- Amongst various branches of omega taxonomy the **Cyto- taxonomy** is most fundamental because species are an object of reality of some particular genetic continuity and the chromosomes are blue print of ancestry, relationship, present and future trends of species concerned.

# * Variables of Cyto-taxonomy

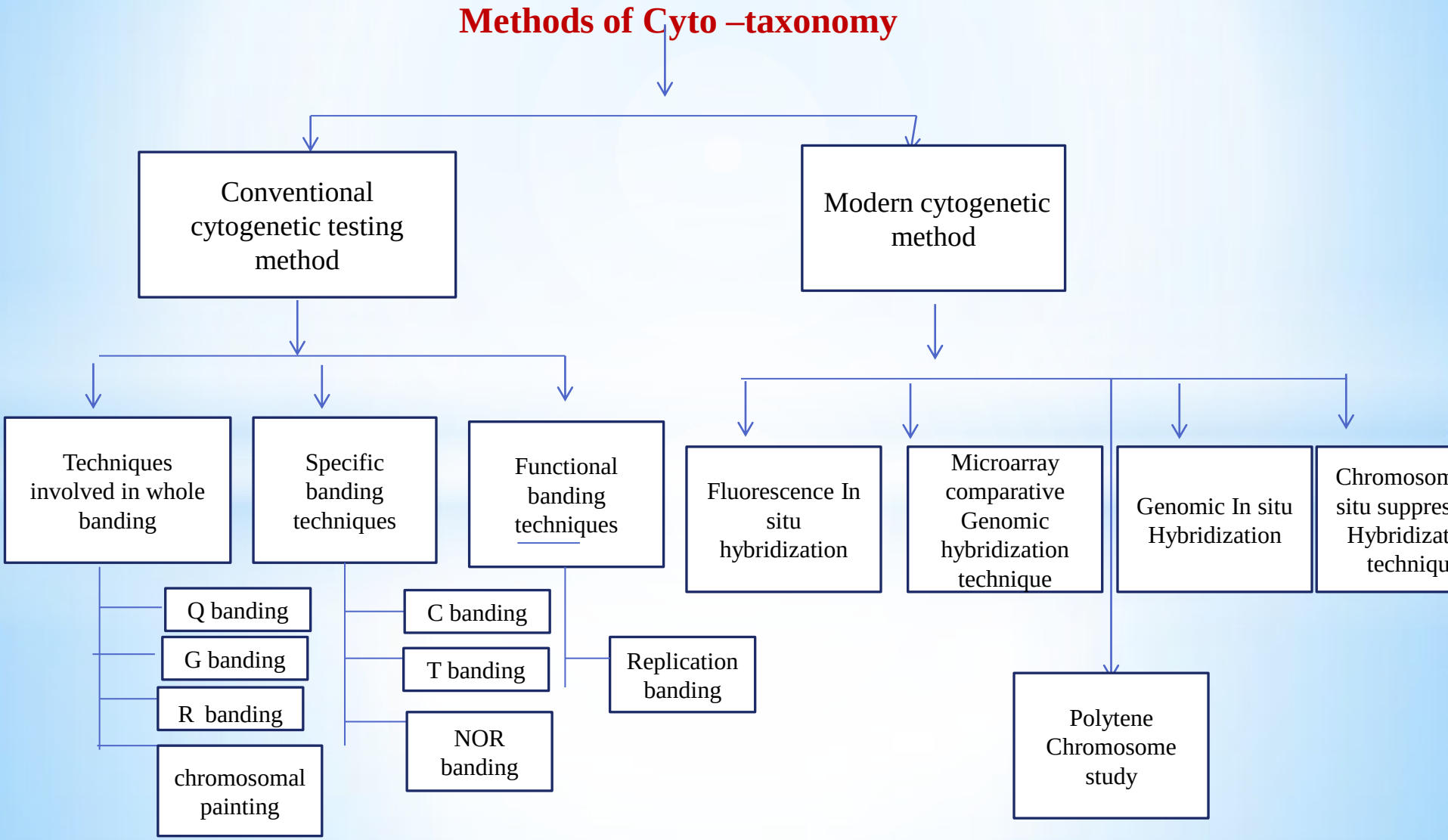
The main avenue of approach for cyto-taxonomy are-

- Banding, size and behaviour of somatic and germinal chromosome.
- Analysis of polytene and lampbrush chromosomes as special task, if present.
- M chromosomes in heteropterans.
- Macro(M) and micro(m) chromosomes in vertebrates.
- Aberrant meiosis in some scale insects, fungus and Gall midges, biting and sucking lice etc.

# Exciting examples of Cyto- taxonomy

- Based upon cyto- taxonomy we have now more valuable karyotype for about 1000 species of mammals, several hundred species of fishes, amphibians, reptiles and birds.
- Patterson and Stove(1952)differentiated 16 species of the genus drosophila on the basis of number and shape of chromosomes.
- Kianta (1968)was able to demonstrate the phylogenetic relationship among various families of the order Trichoptera on the basis of number of chromosomes.
- Mittal *et al.(*1974) were able to separate two synchronized species of the earwig genus *Labidura* on the basis of number and shape of chromosomes.
- Grewal (1982) separated some important fruit fly species of ( Diptera -Tephritidae) on the basis of shape and number of chromosomes. He also discovered a new population of *Bacterocera zonata*.

# Methods Involved in cyto- taxonomy



# * Conventional Cytogenetic Testing Methods

- Conventional cytogenetic testing or routine chromosome analysis is known as Karyotyping It is significant in cyto- taxonomy by two ways-
    1. firstly they aid in comparison of closely related species including sibling species. Individual species show a marked difference in structure of chromosome in contrast to external morphology.
    2. Chromosomal patterns are of extreme importance in establishing phyletic lines.

**Techniques involved in whole banding**

- Chromosomal banding visualize a certain aspects and region of chromosomes. Each chromosome has a unique banding pattern, which projects the structure of genome and its organization. Each band usually contains from 5-10 Mb. Nearly all banding methods rely on harvesting chromosomes in mitosis by treating cells with tubulin inhibitors (colchicine, Colcemid),which depolarizes mitotic spindle.

**Geimsa banding**

- It is a cyto -genetic technique to produce a visible karyotype by staining condensed chromosomes. It is highly useful in identifying genetic diseases through photographic representation of the entire environment.

**Steps in Geimsa banding**

- Metaphase chromosome is treated with trypsin and then stained with Geimsa stain.

# * Conventional Cytogenetic Testing Methods

- The heterochromatic regions which tend to rich in adenine and Thymine(AT rich) DNA and relatively gene poor, stains more darkly in G banding, while less condensed euchromatin (GC rich) and more transcriptionally active , incorporates less Geimsa stain and apparently these regions appear as light bands in G banding.
- The patterns of bands are numbered in each arm of the chromosome from centromere to the telomere.
- This numbering system allows any band on the chromosomes to be identified and described precisely. These bands are same in appearance on the homologous chromosomes and thus identification becomes easier and more accurate.
- **R banding**
- R banding is a cyto-genetic technique that produces reverse of the G band stains on chromosomes. R banding is obtained by incubating the slides in hot phosphate buffer at 88◦ c, followed by a subsequent treatment of Geimsa dye.
- Resulting chromosomes pattern shows darkly stained dark band in guano- cytosine rich region, while adenine thymine rich regions are more readily denatured by heat.
- This technique is useful for analysing genetic deletion or chromosomal translocation that involves the telomere of the chromosomes
- It is also used for tracing phyletic lines in the similar group of organism in sibling species.

# * Conventional Cytogenetic Testing Methods

**Q banding( Quinacridine banding)**

- It was discovered by Cespersson *et al.*(1968) as most simplest type of banding technique.
- Q banding uses fluorescent staining i.e. by an DNA intercalating agent quinacridine of metaphase chromosome.
- It is thought to reflect the distribution of relatively AT and GC rich classes of DNA throughout metaphase chromosome, while AT rich pairs increases fluorescence(bright band), GC pair suppress it. It helps in detecting either euchromatin or heterochromatin regions.

## Chromosome Painting

- Chromosomal painting involves hybridization of each chromosome using a chromosome specific with a unique combination of fluorescent dyes.
- This provides a colourful array of chromosome, each one painted a different colour and this painted chromosome is examined under fluorescent microscope.
- It allows the comparison of entire genome. This method has shown that human chromosome number 6 having hundreds of genes in the major histocompatibility complex have homologous genes in chromosomes number 5 of chimpanzee , chromosome B2 of domestic cat , chromosome 7 of pig and chromosome number 23 of cow.

Short comings:

- The incompatible fossil record in many animal groups may pore problems in solving evolutionary or phylogenetic relationship in these species.

## Methods of selective banding

### C banding

- C banding is also known as CBG staining and requires mild alkali treatment before Geimsa staining. It is used for banding of constitutive heterochromatin ,therefore it is used for identification of centromere.

# * Conventional Cytogenetic Testing Methods

It is highly useful for staining of chromosomes regions, which contain repetitive DNA sequence

(satellite DNA).These repetitive sequence are often treated adjacent to centromere and distal portion of Y chromosomes. The most significant bands are found on chromosomes 1,9,16 and Y.

**T banding**

T banding is slight modification of R banding.

It is used for visualization of telomere.

**NOR banding**

Nucleolar Organiser Region staining or silver staining is an advanced cyto-genetic technique which requires silver nitrate solution, that stains active ribosomal DNA containing nucleolar organizer region(NOR)in interphase nuclei.

It is used for identification of human acrocentric chromosomes 13, 14, 15, 21 and 22.

It is used for identification of marker chromosomes origin and detection of transcription proteins in the NOR regions.

# Conventional Cytogenetic Testing Methods

## Limitations of conventional cytogenetic testing

- Detection of chromosomal abnormalities in metaphase cell preparation by conventional banding methods is highly limited and it can be detected only up to 1000x oil immersion LM method.
- Detection of structural chromosome changes are restricted to 4-6 Mb in size.
- Such type of studies do not rule out other forms of genetic abnormalities such as sub-microscopic or molecular defects , uni-parental disomics or sub telomeric rearrangements.

# Modern Cytogenetic Testing Methods

1. **Molecular cyto genetic testing via Fluorescence *In situ* hybridization (FISH)**

FISH may be utilize to address specific focussed clinical questions and is provided for a variety of clinical applications including the assessment of both constitutional and acquired chromosomal aberration.

FISH testing is a method by which an assessment is made for the presence , absence, relative positioning and/or the copy number of specific DNA segment by fluorescent microscopy.

FISH studies can be done at following levels:

A . **Metaphase FISH studies (Micro deletion FISH)**:

Meta phase FISH studies has been designed to detect change s viz. micro-deletion or/ and micro-duplication associated with specific phenotypic findings and requires specific and focussed clinical questions to be addressed via appropriate DNA probe selection.

**Limitations**:

1. genetic changes that are detected in metaphase chromosome preparation by this type of testing are limited to position and copy number changes primarily deletions and at some instances duplications for the specific chromosomal region for which the employed DNA probes are localized.
2. such studies do not rule out other form of genotypic abnormalities, which may include low level of tissue mosaicism and/ or other form at molecular alterations i.e. single base pair mutations , uni-parental desomics etc.
3.unknown familial genetic polymorphism may result in false positive or negative FISH results, which may or may not be of phenotypic consequences.

# Modern Cytogenetic Testing Methods

**B. Interphase FISH studies**.

Enumerations and rearrangements involving specific DNA probes are the only information available from interphase studies.

It can be used as an adjunct test to standard cytogenetic analysis.

It fails to depict structural aberrations in the chromosomes detected.

**C. Dual colour/ Fusion FSH studies:**

This form of testing utilizes DNA probe system that are designed to detect well known neoplastic rearrangements affecting two specific loci but may not detect variant complexes and/or a typical rearrangements involving these loci.

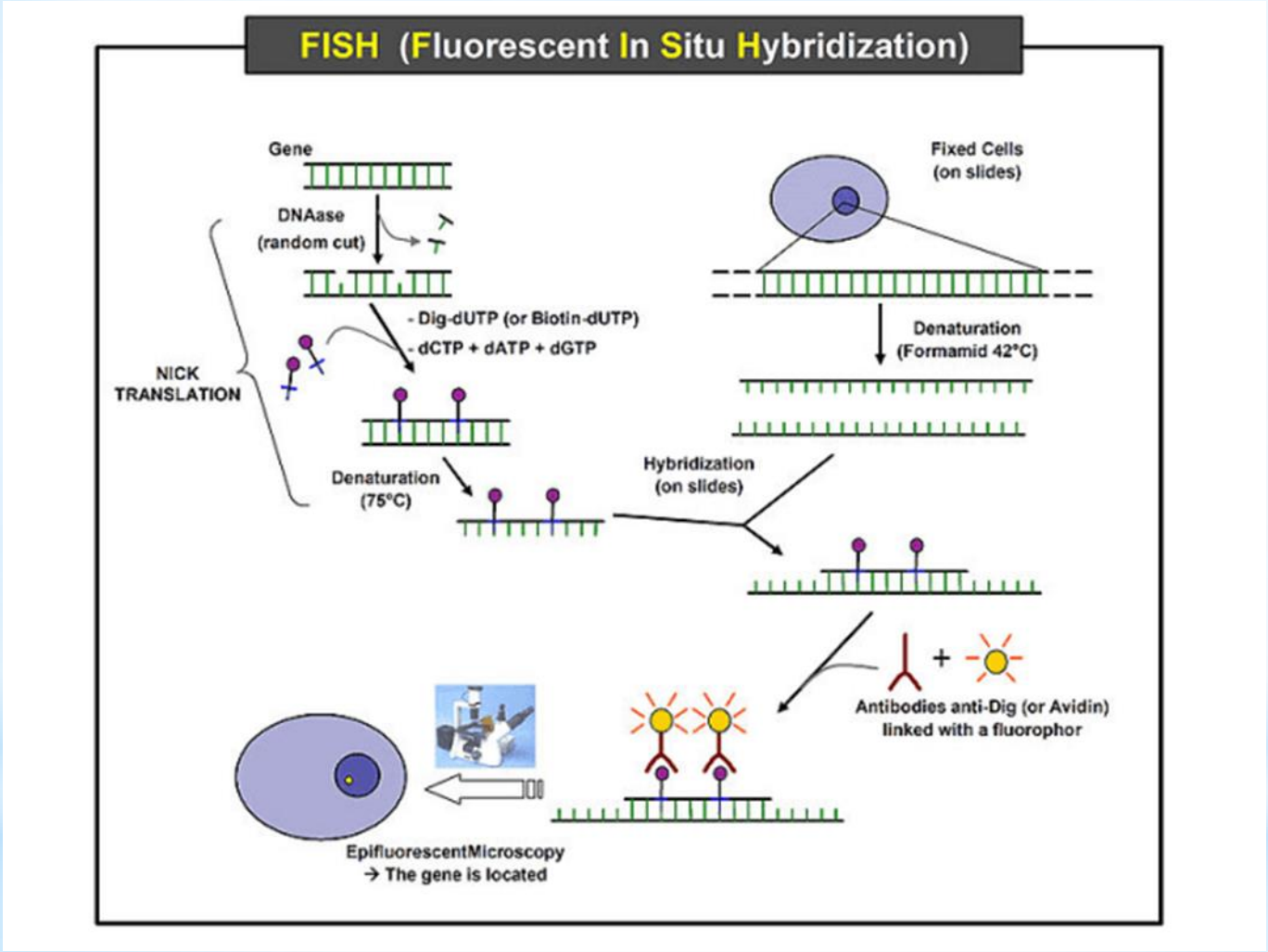
Additional secondary clonal rearrangements may not be detected in interphase nuclei.

**D. Dual colour/ Break- Apart FISH studies** :

These studies utilize DNA probe system that are designed to detect the involvement of specific loci known to participate in rearrangements involving a variety of translocation partners.

This type of DNA probe system can only identify rearrangements involving a single locus and can not identify other loci that may be involved in rearrangement by interphase analysis.

# *FLUORESCENCE *IN SITU* HYBRIDIZATION



# Modern Cytogenetic Testing Methods

## 2. Micro Array Comparative Genomic hybridization technique (a CGH):

1.Micro Array Comparative Genomic hybridization (a CGH) testing is useful for the detection of small genetic substances ( gain or loss of chromosomal materials)also known as genomic copy number changes which may not be detectable by conventional cytogenetic and/or FISH technique.

2. a CGH testing can also be useful for the identification of specific genes involved and in sizing a chromosomal abnormality detected by conventional cytogenetic and/or FISH technique. This test is highly specific to identify loci of common micro deletion or duplication syndromes as well as numerous sub telomeric and peri-centromeric regions.

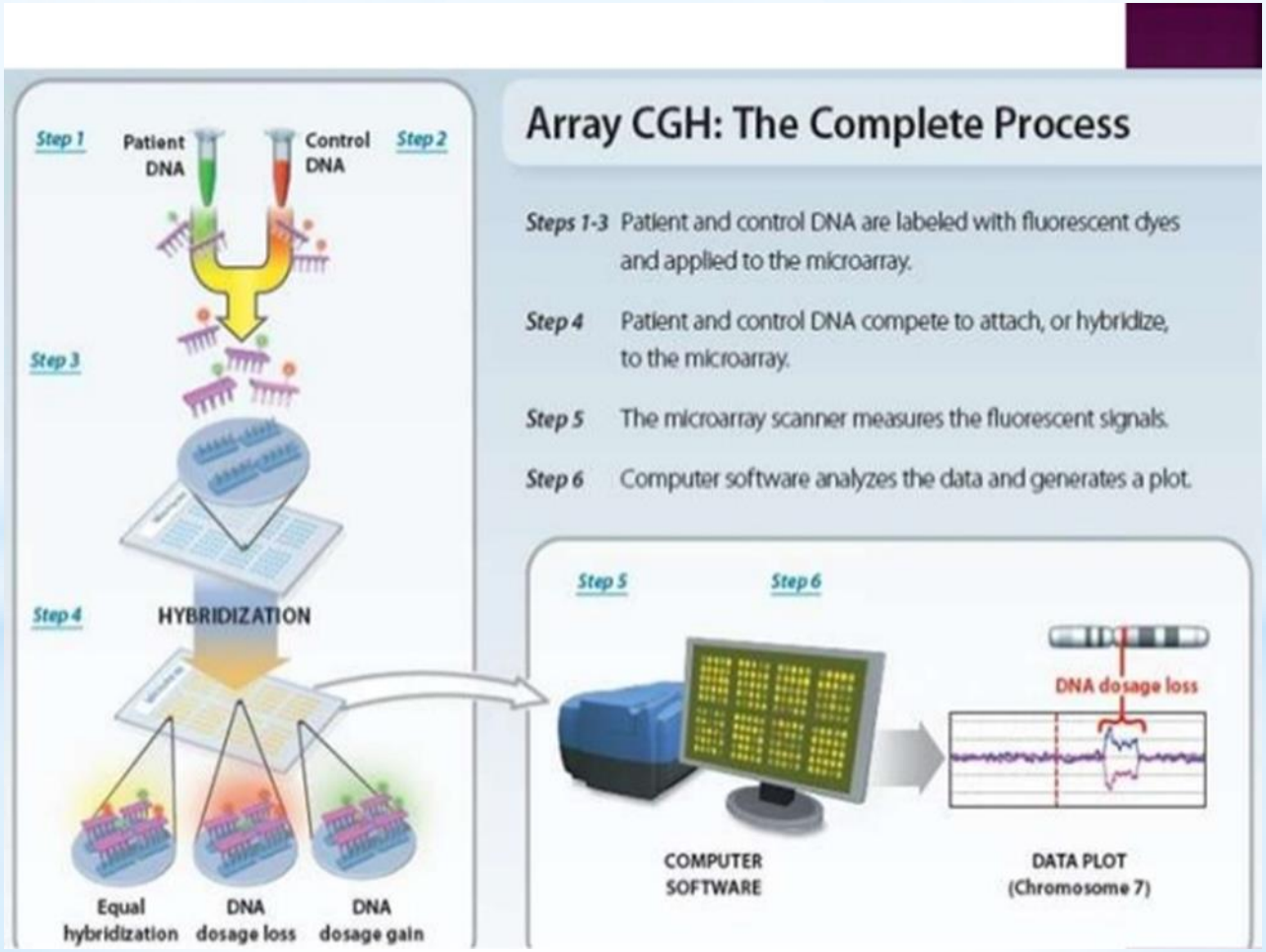
3. A aCGH testing utilizes short DNA sequences corresponding to known chromosomal loci spanning the genome that are fixed to a solid surface.

4. To conduct the test fluorescently labelled DNA from both the parent and control is hybridized to the array. After hybridization the signals are detected and software assisted interpretations of the generated data is performed to determine any copy number of changes between control and patient DNA.

**Limitations :**

1. A aCGH testing may include false negative if the patient has a copy number change not covered by specific array used in the laboratory.
2. Balanced chromosomal rearrangements ( transversion, inversions ) cannot be detected.
3. Mutational changes ( base pair substitutions, methylation status , point mutations and duplications etc.) and deletions less than those that can be resolved by particular array, can not be detected.

# Modern Cytogenetic Testing Methods



# Modern Cytogenetic Testing Methods

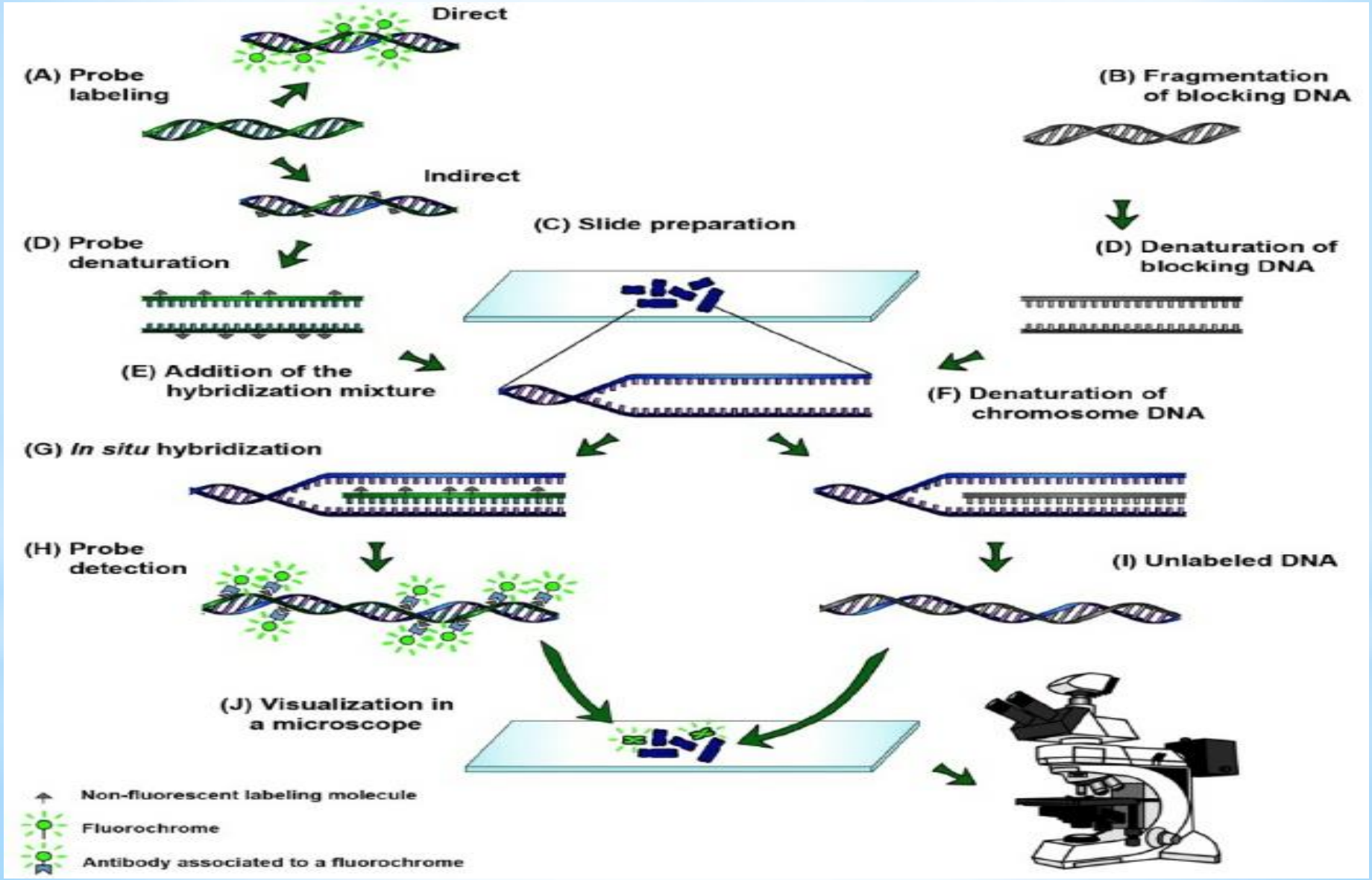
### 3. Genomic In Situ Hybridization (GISH):

GISH is a cytological technique that allows one to radio label parts of genome within the cells. It was mainly developed for the animal hybrid cell lines (1986) and later used for plants at Plant Breeding Institute, Cambridge(1987),where this techniques got its name.
The GISH is quick, sensitive accurate, informative and a comparative approach rather than absolute one . It shows advancements over FISH technique.

**Principle:**

The technique is based upon the extraction and subsequently radiolabelling of whole DNA of one organism and to use as a genome probe to target it the genome of other organism. The parts of the genome that are sufficiently similar to the probe hybridize to form a probe-target complex which is now labelled. The remaining unhybridized parts of genome can be further stained to view them.

# * GENOMIC *IN SITU* HYBRIDIZATION



# Modern Cytogenetic Testing Methods

**Applications of GISH in Taxonomy:**

1. It is highly useful in- meiotic studies, determination of phylogenetic relationship, determination of the position of translocation break points, comparative genomic studies of malignant and normal cells of individual, unknown genomic identification and to identify the hybridized genome of crop varieties.
2. GISHpermits characterization of the genome and chromosome of hybrid plants, allopolyploid species and recombinant breeding lines. Thus the ancestry of hybrid and polyploidy species can be elucidated by genomic southern and *in situ* hybridization. The analysis involves hybridization of labelled genomic DNA from suggested ancestors or relatives to chromosomes spreads or southern blots of DNA from the species under investigation . Hybridization strength, uniformity, presence of positive or negative bands are then assessed to indicate relationship.
3. It gives unique information about the similarity between DNA from related species.
4. It provides data about the physical distribution of sequence which are common or differ between the species being probed and the species used to supply the probed DNA.
5. Together information can be used to support and develop theories about phylogenetic hybridization and diversification of plant species. . These information help to plan effective breeding programme designed to transfer desired genes or gene cluster from alien species into otherwise superior cultivars of crop plants.

# Modern Cytogenetic Testing Methods

**Chromosome In Situ suppression Hybridization (CISS) technique :**

It is new emerging technique for analysing chromosomal evolution in primates.

Here Biotin labelled DNA libraries from Flow sorted human chromosomes are hybridized to chromosomal preparation of *Cattarhines, Platyrrhines* and *Prosimians*. By this approach, rearrangement of chromosomes that occurred during hominoid evolution are visualized directly at the level of DNA sequences, even in primates species with pronounced chromosomal shuffles.

Verma *et al.* studied the mapping of the homology of the human Rb1 gene to chromosome 14 of higher primates . It was found that Rb1 gene is implicated with retinoblastoma and is located on human chromosome (Chr)13q14.2. A unique sequence human Rb1 cosmid DNA probe has been used to localize this region on ape's chromosome 14 by the FISH technique.. The conservation of Rb1 gene at higher primates at the corresponding equivalent chromosome locus (14q14) of the human may serve as a phylogenetic marker to further trace the evolutionary pathway of human descent.

# Modern Cytogenetic Testing Methods

## Polytene chromosomal studies

Polytene chromosomal analysis is the basis of cyto-taxonomy in many cases.
In some of the Ciliates , polytene structure of chromosome of micronuclei are the basis of cyto-taxonomy.

Macronuclei generally originate from diploid micronucleus , divide amitotically, vary in number, structure and chemical constituents of Ciliophora. In *Nyctotherus* the chromosomes join end to end to form a single giant polytene element with two ends free ,while in *S mytilus* some 250 polytene chromosomes join in one or two chains, however *S. muscorum* show 50-60 free polytene chromosomes.

Based on polytenic chromosomes of four members of *Simulium perflavum* species group in Brazil, a standard map for the species group is presented.

# Applications of cyto-taxonomy in Animals

## Lower Invertebrates

**1.** Among lower invertebrates cyto -taxonomic information are available for lower ciliates,7 species under 5 genera indicating polyploid origin in Porifera (Makino,1956); 27 species under 17 genera of 3 families (2n=12-32) in Coelenterata (Makino,1956).

2. Among triploblastic acoelomates invertebrate 2n number is reported to between 4- 40 in 120 species of Turbullarians (Makino,1956).

## Annelida

3. The discovery of cyto- biotype in earth worm is an example of the importance of cyto- taxonomical investigation . In true earthworm four genetic system are found: **a**. Diploid sexual species, **b**. Polyploid sexual species, **c**. Automictic polyploids and **d**. Apomictic polyploids.

## Mollusca

4. Based on cytological data of living forms, White (1973) prepared a histogram of haploid number of some 430 species of between 5-36 under *Eltheneura, streptoneura, bivalvia, amphineura* and *Cephalopoda.*

## Echinodermata

**5.** Cyto-taxonomy is limited to Asteroidea (5 species of *Asterias* and 1 species of *Cribrella*, 2n=18-36), crinoidea (*Antedon*, 2n=8), Holothuroidea (*Stichopus*, 2n=28-36) and Ophioroidea (*Ophiriocome*, 2n=18).

## Arthropoda

6. The maximum cytological studies have been done exhaustively in 5 living class of arthropoda- crustacea, Insecta, Arachnida, Myriapoda and Onychophora. Among insecta the cytological data are available for Apterygota (Thysanura- *lepisma;* 2 sps. Of silver fish, 2n=34, X1x2O in males and another species with n=29XO). Among Pterygota, the data for cyto-taxonomy are available for few orders like Orthoptera, Dermaptera, Homoptera, Heteroptera, Neuroptera, Mecoptera, Lepidoptera,

# Applications of cyto-taxonomy in Animals

## Fish

**7.** The study of fish chromosome started before the beginning of 20th century . According to latest review of Manna (1989), out of 20000 taxonomic species chromosomes of about 1700 species belonging to some 774 genus under 193 families 37 orders of 02 super class Chondrichthyes and Osteichthyes are unevenly known. Among extensively studied families (30-300) of teleost are Characidae, Cyprinidae, Cyprinidontidae, Poecillidae, Channidae, Cichlidae, Gobidae, Salmonidae, . Male heterogamety for XO, XY X1X24 and female heterogamety for ZO , ZW and ZW1 W2 have been reported. some chromosomes polymorphs have been also marked in some species.

## Amphibia

**8.** Among some 250 taxonomic species of amphibians cyto-taxonomy has been done for a fewer species. A very elaborate cyto-taxonomic implications are based on number, morphology, size (M and m) and banding pattern (Morescalchi,1973 Manna, 1983b). Experimental hybridization and lampbrush chromosome analysis are of some special features in amphibian cytology.

## Reptile

**9.** Among reptiles , in Chelonia order of super order Anapsida the diploid number recorded in 03 species is 52. Among Diapsida 02 living order like Rhyncocephalia, the sole endemic Australian tuatara (*Sphenedon punctatus*) has 2n=36 including 4 pairs of m like chromosomes with no indications of sex chromosome and Squamata comprising very large number of species . Based upon karyotypes earlier Malthey put Lacertilia as–**a**. complex Gekonoidae showing no sharp distinction between M and m chromosome. **b**. complex Iguanoidae with sharp distinction between M and m chromosome **c**. Complex Scinolacertoidae. In Ophidia over 100 species under 6 families show 2n=24-50 chromosome.

# Applications of cyto-taxonomy in Animals

**Aves**

**10.** Among 8600 taxonomic species of Aves, nearly 2000 species are cytologically known (Makino,1956: Kaul and Ansari, 1983). The diploid number ranges from 78-84 chromosome . The presence of 6-8 pairs of M and more variable number of m chromosome, female heterogamety for ZW is common , ZO is very rare.

**Mammal**

**11.** Among about 4500 taxonomic species about 2600 species are cytologically known with the help of colchicine-hypotonic Geimsa technique, supplemented with banding technique.

Out of three extinct **Monotremes** 2n=63,XY:XX in *Echidna*; and 2n=53,XY1Y2:XX in *Platypus* recall reptilian affinity.

In **Marsupials** , out of 230 living species, the diploid number of about 100 species ranging from 10-24 with 1 species of 2n=32 and the highest mode at 14 and a second at 22 chromosome. Males are XY with few rare cases of X1X2Y and XY1Y2.

In **Eutherians** , out of some 2500 species, the 2n number ranges from 6-82 with frequency peak highest at 38 and two lower ones at 42 and 48 in different taxonomic groups. In tetra-specific chromosomal variation, reported in rats , mice and man, both for autosomal and sex chromosome , male heterogamety is the rule with most prevalent XY, some X1X2Y, XY1Y2, XO etc (Mathey, 1973).Most extensive cyto- taxonomically studied Eutherian families are – Phyllostomatidae and Vespertilionidae of Chiroptera, Bovidae of Artiodactyla, Cercopithocidae of Primates, Sciuridae, Cricetidae and Muridae of Rodentia.

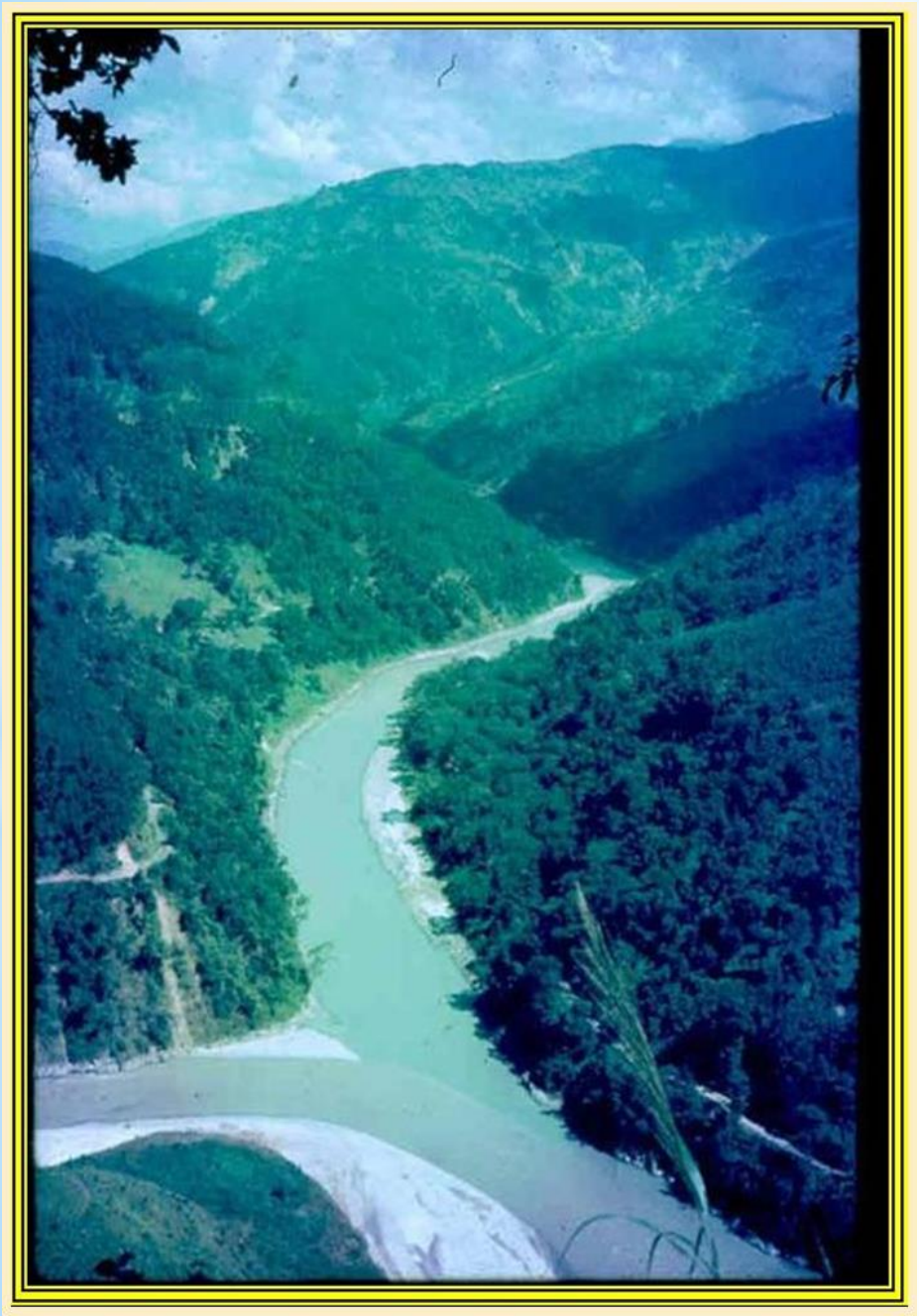
# Conclusions

**1.** Cyto-taxonomy is the most emerging branch of modern taxonomy.
2. The number, structure, meiotic behaviour of both somatic and sex chromosome , position of centromere, analysis of polytene and lampbrush chromosomes (in few species), analysis of M and m chromosomes are the basic cytological ingredients which have tremendous applications in cyto-taxonomy.

2. various conventional cytogenetic method used in cyto-taxonomy principally includes karyotype analysis which is primarily based on whole chromosomal banding technique (Q banding, G banding, R banding, chromosomal painting), selective banding (C banding, T banding , NOR banding) and functional banding (replication banding- BrdU banding).

3. Other methods involved in cyto-taxonomy are-FISH, GISH, aCGH, CISS techniques, polytene chromosomal analysis and study of chromosomal polymorphism like- macro(M) and micro(m) chromosomes are highly significant.

Among plants the cyto-taxonomy has proved its worth with full potential, however its applications in establishing phyletic lines in animals is still in its infancy stage and needs to be explored further.



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