CHROMOSOME BANDING

Study of chromosome number and structure by staining the dividing cells with certain dyes and then examining them under microscope for cytogenetic analysis is called chromosome banding.

Most cytological and cytogenetic analysis is performed on dividing cells (metaphase of mitosis). Therefore, meristematic cell of root or shoot tip of plant and embryo cell of animals are used. However, the development of cell-culturing techniques has made it possible to study chromosomes in other types of cells. For example, human white blood cells can be collected from peripheral blood, separated from the non-dividing red blood cells, and put into culture. The white cells are then stimulated to divide by chemical treatment, and midway through division a sample of the cells is prepared for cytological analysis. The dividing cell on treatment when treated with chemical disables spindle fibre formation, such mitotically arrested cell are then immersed in a hypotonic solution that causes cell to take up water. On preparation for microscopic examination such cells are squashed on a microscopic slide, such that the chromosomes are spread out in an uncluttered fashion which can be observed by staining. The stains such as Feulgen's reagent or aceto-carmine stains the chromosome uniformly making difficult to distinguish one chromosome form other. Now a days, cytogeneticists use dyes that stain chromosomes differentially along their lengths.The staining of chromosome is known as banding technique because stains give rise to pattern of bands along the length of chromosome.

Types of chromosome banding technique:

. There are various techniques to stain chromosomes and achieve different types of banding. Here we mention some of them:

1. Giemsa staining:- Giemsa is a visible light dye, which binds DNA through intercalation. Visible light dyes are more stable and capable of producing clearer bands than fluorochromes. Giemsa stain is a mixture of cationic **thiazine dyes** and anionic **eosin dyes** such as eosin Y. Positive thiazine dye molecules are smaller and two molecules of the same quickly intercalate into the negative DNA molecule, and stains it blue. The anionic eosin molecule then binds the two thiazine molecule and stains the DNA purple. Giemsa stains the hydrophobic regions better.

There are four different types of banding techniques, which can be done using Giemsa: **G-bands**, **R-bands**, **C-bands** & **T–banding**. In each of the above mentioned staining techniques, Giemsa stain stains different regions due to difference in the pretreatment. As we all know, the histone proteins are uniformly spread through the length of the chromosomes. The non-histone proteins are spread at variable sites and responsible for loose or condensed state of different regions of the chromosomes. The loosely packed regions or euchromatin regions and tightly packed region are the heterochromatin regions. The pre-treatment processes differentially extract these proteins resulting into differently stained regions.

1. G-banding:

This is the most commonly used banding method for cytogenetic analysis using Giemsa stain. The technique was first developed by Dr. Marina Seabright in 1971.

The cells are arrested in metaphase, swollen (made turgid), fixed, dropped and bursted. Then the chromosome spreads are air dried and chromosomes are pretreated before Giemsa staining.

Pretreatment:

During the standard G-banding the chromosomes are mildly treated with proteolytic enzymes (trypsin) before staining with Giemsa. Standard protocol G-band staining when followed gives around 400 and 600 bands to be seen on metaphase chromosomes.

The two types of bands which are observed are

• **Positive G-bands**:

Positive G-bands are the darkly stained bands. These regions are hydrophobic, and favour the formation of the thiazine-eosin precipitate. The hydrophobicity is due to the hydrophobic proteins. The proteins are difficult to extract as they have more of disulfide cross-links. These proteins keep the regions more condensed . They form the late replicating **heterochromatin** and are generally **AT-rich** region.

• **Negative G-bands**:

The lightly stained bands are called negative G-bands. These regions are rich in **GC base** pairs. These are early replicating **euchromatin** and are less condensed. The proteins that bind these regions have more of **sulfhydrils** (fig 4) and are easily removed during pretreatment. These regions are less hydrophobic and less favorable for the formation of the thiazine-eosin precipitate.

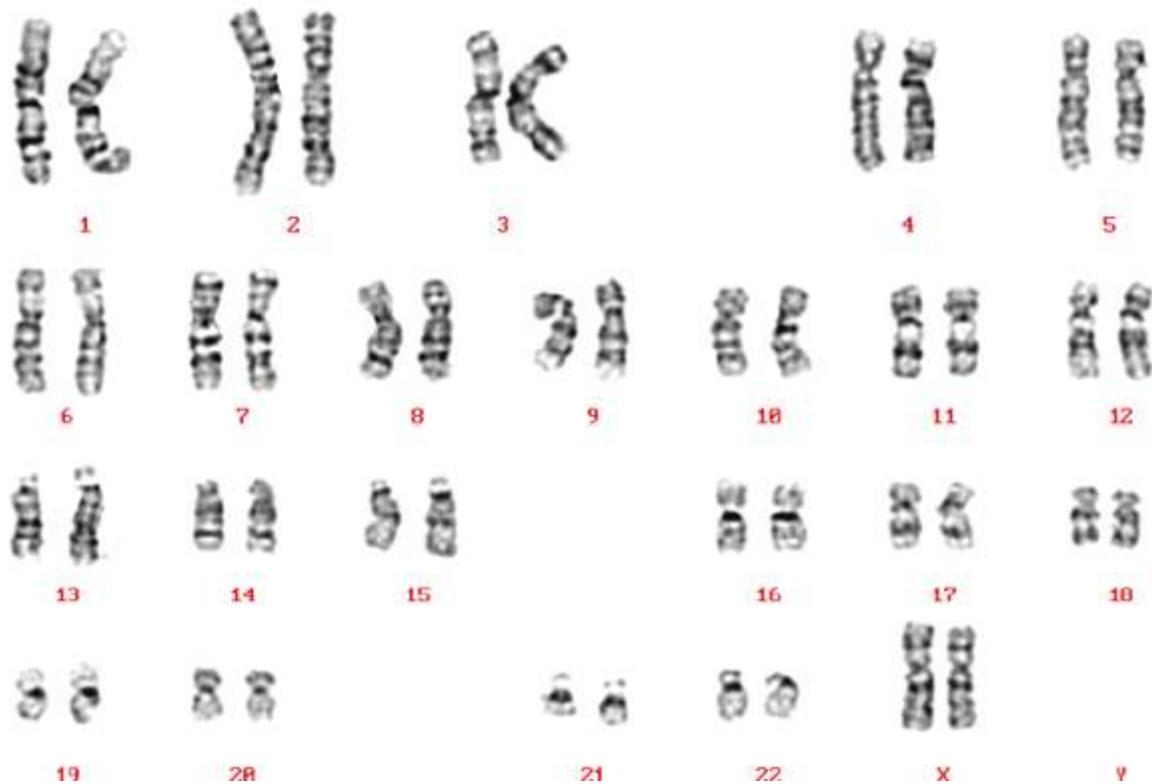

Fig 5: Normal Human (Female) Karyotype.

2. R-banding:

This banding technique reveals the GC-rich euchromatin and produces positive bands that correspond to the negative G-bands and vice versa. This gives results reverse of the standard G-banding.

Pre-treatment:

In this technique, banding is produced by metaphase chromosomes in hot phosphate buffer (~87°C) before staining with giemsa stain. The incubation causes the denaturation of the AT regions of the chromosomes because of the low melting point of these regions (~65°C) as compared to that of the GC regions (~105°C). R-banding is helps analyse the structure of chromosome ends, which stain light with G-banding but darker with R-Banding.

3. C-banding:

C-banding stains constitutive heterochromatin which is present around the centromeres of all human chromosomes, and is most abundant around the centromeres of human chromosomes 1, 9, 16 and the distal long arm of the Y-chromosome.

Pre-treatment:

The pretreatment involves three successive steps; treatment with acid (HCl), followed by a alkaline treatment (barium hydroxide) and finally treatment with hot salts [saline- sodium citrate (SSC)]. Treatment with acid (HCl) brings about the removal of the purines. The alkaline treatment (barium hydroxide or Sodium borohydride) reduces the apurinated sugars. Chain breakage of the depurinated sites occur during treatment with hot salts solution [60°C, saline- sodium citrate (SSC)]. In this final treatment, the sites with highly repetitive sequence resist the breakage and get renatured. Also the sites with proteins having strong interaction are protected. Therefore, the sites protected by protein or having highly repetitive sequences like centromeres get stained.

T-Banding:

T-banding involves the staining of telomeric regions of chromosomes. The chromosomes (slides) are incubated in a phosphate or PBS buffer at 87°C followed by staining with Giemsa solution or acridine

orange(OA) . T-bands are heat-resistant regions, particularly rich in C-G pairs. They make up around 15% of all the bands but contain around 65% of all the genes mapped.
Banding techniques using other stains:

2. Q-Banding:

Quinacrine mustard is an alkylating agent which fluoresces brightly under UV light. These bands are visible under a fluorescence microscope. The alternating bands of bright and dull fluorescence are called Q bands. The bright bands are AT rich region and the dull bands are GC rich region (similar to G banding). Q bands are useful in distinguishing the human Y chromosome and various chromosome polymorphisms involving satellites and centromeres of specific chromosomes.

NOR banding

NOR banding involves the staining of "nucleolar organizing region" by silver stain (silver nitrate solution).The NOR contains rRNA genes. It is thought that the silver nitrate attaches to the nucleolar proteins and not the rDNA in itself. In humans, the NORs are found on the short arms of the chromosomes 13, 14, 15, 21 and 22, the genes RNR1, RNR2, RNR3, RNR4, and RNR5 respectively. They code for 5.8S, 18S, and 28S rRNA. The silver stain usually stains the transcriptionally active rRNA genes. Changes in the NOR number and size help explain changes in transcriptional activity in different environment and conditions.

DAPI/Distamycin A Staining:

This is a fluorescent staining technique for labelling a specific subset of C bands. DAPI/Distamycin A staining is used in identification of peri-centromeric breakpoints in chromosomal rearrangements and chromosomes that are too small for standard banding techniques. 4'-6-diamidino-2-phenylindole (DAPI) is a DNA-binding AT-specific fluorochrome. which gives blue fluorocence. Distamycin A is an DNA-binding AT-specific oligopeptide antibiotic. Distamycin A pretreatment results in decrease in fluorescence by DAPI staining, allowing only some specific regions of constitutive heterochromatin to brightly fluoresce.

Hence different banding technique help in staining certain regions darkly or brightly. These regions can be compared with that in homologous chromosomes or chromosomes of different individuals or species to obtain information on diseases, evolution and parentage.

CHROMOSOME PAINTING

Chromosome painting involves the use of fluorescent-tagged chromosome specific DNA sequences to visualize specific chromosomes or chromosome segments by in situ DNA hybridization and fluorescence microscopy. Chromosome painting refers to the hybridization of fluorescently labelled chromosome-specific, composite probes to cytological preparations.

Chromosome painting allows the visualization of individual chromosomes in metaphase or interphase stages and the identification of both numerical and structural chromosomal aberrations with high sensitivity and specificity. The simultaneous hybridization of multiple chromosome painting probes, each tagged with a specific light-emitting fluorochrome has resulted in the differential colour display of human and mouse chromosomes, which is also called colour karyotyping.

Fluorescent in situ hybridization **(FISH)** has been used to detect the location of specific genomic targets using probes that are labelled with specific fluorochromes. That is the reason chromosome painting is also called **M-FISH** or **multicolour FISH**. The technique allowed detection of simple and complex chromosomal rearrangements. In addition, complex chromosomal abnormalities could also be identified that could not be detected by the conventional cytogenetic banding techniques.
Almost a decade ago, chromosome painting was developed independently by research teams at Lawrence Livermore National Laboratories and at Yale University. Both groups had taken advantage of the availability of cloned DNA libraries that were derived from flow-sorted human chromosomes. The first generation of probes, based on chromosome-specific **phage libraries,** were rather cumbersome to use, due to low insert-to-vector ratios, which frequently resulted in a relatively high background staining. Some of these limitations were overcome with the availability of **plasmid libraries**, where an improved insert-to-vector ratio and easier probe generation enhanced the painting quality considerably.

Chromosome painting has improved the efficiency of screening cells for chromosome abnormalities, in testing chemicals for mutagenacity and for rearrangements associated with tumours. Painting probes detect chromosome rearrangements. Use of the same chromosome paints for chromosomes of different species reveals the extent of chromosome rearrangements since divergence of the species.

Chromosome painting probes are now also available for an ever increasing number of species, most notably for the mouse and the rat, allowing the expansion of chromosome painting analyses to animal models for human diseases. FISH techniques have been developed and applied to identify the origin of the markers and other structural chromosomal aberrations. The use of chromosome painting probes in one, two or three colour FISH experiments has significantly improved the definitive diagnosis of chromosomal aberrations.

The introduction of chromosome painting to the field of comparative cytogenetics has added significantly to the understanding of chromosome changes that occurred during the evolution of species. Chromosome painting can be used to identify homologous chromosome segments in different species and to map probes of different complexities and chromosome rearrangements in a single experiment. In recent years, the complete karyotypes of various mammals including primates, carnivores and artiodactyls have been analyzed by chromosome painting.

Chromosome Painting Probes, available in liquid format, are directly labelled in either a red or green fluorophore. They can be mixed together to label a number of chromosomes in a single reaction. The probes come in the form of ready to use hybridization solution in a five-test kit format, and are supplied complete with DAPI counter stain. The protocol is rapid and simple and follows simultaneous co-denaturation of the FISH probe and target DNA.

The origin of marker chromosomes that were unidentifiable by standard banding techniques could be verified by **reverse chromosome painting.** This technique includes micro-dissection, followed by *in vitro* DNA amplification and fluorescence *in situ* hybridization (FISH). The chromosomal material is amplified by a degenerate oligonucleotide-primed polymerase chain reaction (DOP-PCR). The resulting PCR products are labelled by nick-translation with biotin-11-dUTP and used as probes for FISH. Homologies between the chromosomes of different species can be detected by chromosome painting.

A more detailed description of how this method of chromosome painting works is given below:

- Initially suspensions of chromosomes from dividing cells are sorted by a method known as flow cytometry.
- DNA from one chromosome is then labelled with a fluorescent dye using a technique called fluorescence in situ hybridisation **(FISH).** This labelled DNA paints the chromosome and allows homologous regions of DNA of other species, from great apes to mice, to be identified. Homologous regions show up in the same colour on the chromosome charts.
- If we take a paint made from human chromosome **2** and hybridise it onto the chromosomes of another species, then you'll see segments of paint on different chromosomes, each being homologous to part of human chromosome **2**, so with the gibbon karyotype, regions on chromosome **2** in human can be tracked to 5 different chromosomes in gibbon. The **fluorescent-labelled DNAs** will attach to the analogous chromosomes from which they were derived. DNA fragments with the same base sequences have the characteristic of attaching to each other.
- This tells us that the lineage that produced gibbon and the lineage that produced human have diverged over several million years and during this time rearrangements have occurred between the two species which can be tracked using the painting technique.
- If a part of a painted chromosome (yellow, for example) had undergone an exchange with another, non-painted chromosomes (stained red), it is possible to detect the aberration as reciprocal translocation, because the aberrant chromosome contains both yellow and red segments. Usually, a pair of bi-coloured chromosomes can be detected in one metaphase, because two chromosomes typically exchange a part of their DNA. Reciprocal translocations are difficult to detect by simple staining technique that stains the entire set of chromosomes with a single material such as with Giemsa.

- When human chromosome probes are hybridised to the chromosomes from other species, the same set of blocks, dispersed across multiple human chromosomes, are often located together on one chromosome in other species. For example, parts of human chromosome 3 and 21, or 14 and 15, or 12 and 22, tend to be located together on one chromosome in other animals. This is evidence that an ancestral block of genes has been split apart and moved to different chromosomes during human evolution.
- Collating all these patterns of linked regions of chromosome across various mammalian species has enabled to assemble a picture of genomic commonalities across multiple species.

Comparison between chromosome banding and paiting

Attribute	Chromosome banding	Chromosome painting
Definition	Chromosome banding is a technique that displays parts of chromosomes in dark and light bands, which are distinguishable.	Chromosome painting is a technique that Paints specific regions of chromosomes with sequence specific probes which are fluorescently labeled.
Outcome	Produces alternating dark and light bands along the length of the chromosome.	Produces fluorescently labeled regions of chromosomes.
Basis	Staining with a dye.	Molecular hybridization with sequence specific probes on chromosomes.
Techniques used	G-banding, C-banding, Q-banding, R-banding, T-banding, etc.	FISH
Use of probes	No	Yes