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Principle and Techniques of Chromosome Banding: A Primitive Method for Chromosome Analysis

Anjana Chauhan*
School of Agriculture, Dev Bhoomi Uttarakhand University, Dehradun, Uttarakhand, India..

**Corresponding author e mail: anjanachauhan618@gmail.com*

Abstract
Chromosomal banding is described as alternating light and dark patches along the length of a chromosome created on staining with a dye. A band is a portion of a chromosome that can be distinguished from its neighbouring portion by appearing darker or lighter using one or more banding techniques. Different banding techniques produce bands that reflect a certain region or structure of a chromosome, which can be Heterochromatin or Euchromatin. Heterochromatin regions are condensed and inactive in transcription throughout the cell cycle, whereas Euchromatin regions are decondensed and active in transcription during interphase. The procedures for banding chromosomes are either dye staining or assaying for a specific function. G(Giemsa), R(reverse), C(constitutive), and Q(quinacrine) banding are the most popular banding techniques. In G-banding, when protease-treated chromosomes are stained with Giemsa dye dark bands occur on the heterochromatin region (AT-rich). R-banding is a reverse-Giemsa staining technique in which AT-rich regions are preferentially denatured to stain GC-rich regions that are under-denatured. In C-banding, bands are observed upon the constitutive heterochromatin region (AT). In Q banding, fluorescence in AT-rich DNA is induced using quinacrine dye. Chromosome banding techniques are a helpful tool in analyzing karyotypes for establishing evolutionary relationships and discovering chromosome polymorphism. In cytogenetic research, band polymorphism enables the use of bands in conjunction with genetic markers. These procedures are less commonly used these days since the results are highly dependent on the cytogeneticist's experience and cytogenetic preparation. FISH, GISH, SKY and CSH are some of the latest chromosome analysis techniques.

Keywords: *Chromosome, Banding, Techniques, Analysis, Primitive*

Introduction

Chromosome identification has traditionally been dependent on their morphological characteristics such as relative lengths, arm ratio, and presence or absence of secondary constrictions. Then the differential banding patterns of chromosomes, usually observed at specific regions on particular levels, were initially developed for the analysis of human chromosome segments. Treatment of chromosomes to reveal characteristics **pattern of horizontal bands** is called **chromosome banding**.

A band is a part of a chromosome that is distinguishable from its adjacent segments by appearing darker or lighter with various banding techniques **(Paris conference 1971)**
These bands are made visible through the fluorescence microscope or as differentially stained areas under the light microscope.). These methods were then extended first to different animals and later to plant chromosomes.

. The development of chromosome banding techniques, however, the development of chromosome banding techniques during the last more than two decades provided a very useful additional tool for the identification of individual chromosomes within the complement. These techniques, not only allow the identification of chromosomes that differ morphologically with a greater degree of confidence but also allow the identification of chromosomes that possess similar morphological attributes. It also permits us to establish a correlation between linkage maps and cytological maps.

The banding techniques are based on the identification of chromosome segments that predominantly consist of either GC or AT-rich regions or of constitutive heterochromatin. There is also general agreement that the techniques, which involve denaturation of DNA followed by slow renaturation, permit the identification of constitutive heterochromtin because it mainly consists of repetitive DNA. **Casperson et al., (1969)** while working with the plant *Vicia faba* used highly fluorescent Quinacrine Mustard to produce the first chromosome banding pattern.

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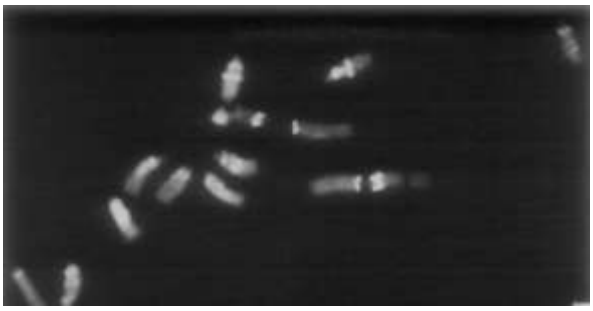
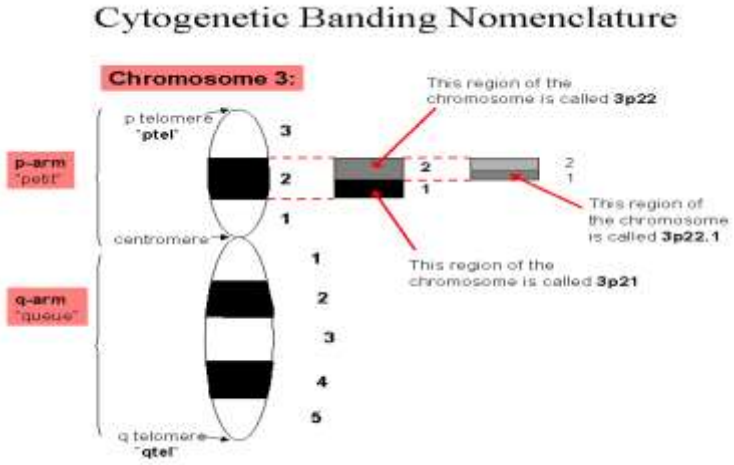


The chromosome of *vicia faba* when treated with Quinacrine Mustrad

Paris Conference 1971: Standardization in human cytogenetics was the first attempt to provide the nomenclature for chromosome banding for various species.

According to International System for Human Cytogenetics Nomenclature **(ISCN)** a chromosome can be named by dividing it into two arms the shorter ar known as "p" arm and the longer arm known as "q" arm. Each band on a chromosome is numbered, band lying closest to the centromere is given the lowest number while the highest number is for the band at the distal ends of chromosome.



A decade of **1970-1980** is well known as **" Banding Revolution"** because of the landmark acheivments over the past.

Stain or Banding Technique	**Investigator**	**Year**
Q-banding	Caspersson, Zech, Johansson	1970
G-banding (by trypsin)	Seabright	1971
G-banding (by acetic-saline)	Sumner, Evans, Buckland	1971
C-banding	Arrighi, Hsu	1971
R-banding (by heat and Giemsa)	Dutrillaux, Lejeune	1971
G-11 stain	Bobrow, Madan, Pearson	1972
Antibody bands	Dev. et al	1972
R-banding (by fluorescence)	Bobrow, Madan	1973
In vitro bands (by actinomycin D)	Shafer	1973
T-banding	Dutrillaux	1973
Replication banding	Latt	1973
Silver (NOR) Stain	Howell, Denton, Diamond	1973
High resolution banding	Yunis	1975
DAPI/distamycin A stain	Schweizer, Ambros, Andrle	1978
Restriction endonuclease banding	Sahasrabuddhe, Pathak, Hsu	1978

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Causes of banding

Various causes have been ascribed to the occurrence of the chromosome bands. Of them four important factors are:

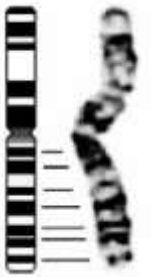
1) The occurrence of repetitive DNA.
2) Differences in the base composition of DNA,
3) Difference in the protein component
4) Difference in the degree of packing of DNA or DNP-complex.

Principle of banding

It is based on the principle that single strands of RNA or DNA are able to recognize and pair with their complementary base sequences. A denatured DNA duplex, on renaturation, undergoes pairing at complementary sequences. Highly repeated sequences show rapid rate of reannealing. The reassociation kinetics gives an indication of sequence complexity of DNA. There are three related objectives in studying mechanism of chromosome banding obtaining and understanding of chromosome structure, understanding the behaviors of chromosome in terms of their substructure; and improving the reliability of banding techniques. Similarities in organism are commonly interpreted as the result of common ancestry. Since chromosomes are the carriers of heredity, similarities in chromosomes could have special significance in studying the ancestry and relationships of species. Many studies comparing chromosome banding have been conducted.

Staining and Banding

Staining is used to enhance the contrast between different cellular components thus allowing proper visualization of chromosomes with different imaging techniques. Differential staining along the length of a chromosome leads to the production of bands.



Banding in chromosome

It is necessary to understand how stain bind to the chromosome. There are several ways in which stain bind to the DNA.

Modes of stain Binding

A stain can bind to the DNA through **intercalation, minor groove or major groove binding, or external binding.** Binding of stain depend on the type of interaction stain have with DNA. The interaction can be covalent or non-covalent.

The covalent interactions involve metal stains wherein the metal coordinates with the nitrogen atoms of the DNA base pairs**.** The coordination of metal with nitrogen often occurs at position 7 of adenine or guanine. Some platinum stains exhibit this type of interaction since they have affinity for nitrogen. **This type of interaction results into major groove binding.**

The non-covalent interactions include hydrogen bonding and electrostatic interaction. Hydrogen bonding (H-bonding) occurs with stains that have a functional group containing an electronegative atom such as nitrogen or oxygen. The electronegative atom cans hydrogen bond with the H-donor sites of the base pairs. These sites are position 6 of adenine, position 4 of cytosine, and position 2 of guanine. Furthermore, the electronegative atom can be attached to a hydrogen and hydrogen bond with the H atom acceptor sites of the base pairs. These sites are positions 3 and 7 of adenine and guanine and position 2 of thymine. H-bonding leads to major groove or minor groove binding.

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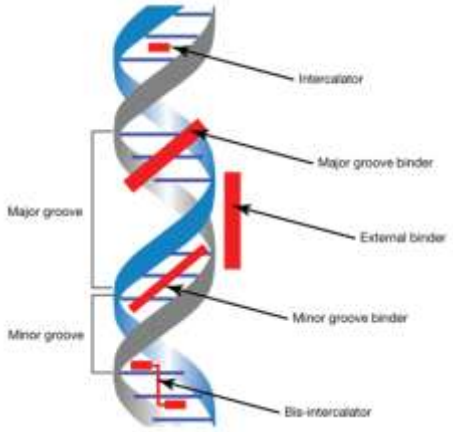
Electrostatic interactions involve stains that have cationic groups. The cationic groups form an ionic interaction with the negatively charged phosphate groups of the DNA. Electrostatic interactions involve stains that have cationic groups. This type of interaction results to **external binding.**



Modes of Binding

Classification of bands

Constant heterochromatic bands, visible in interphase and throughout division and relatively constant in size. Heterochromatin are chromatin regions that remain condensed throughout the cell cycle and are inactive in transcription. They are late replicating and have a low density of genes, which are mostly inactive. These bands are equivalent to C, G11 and N bands, and certain bands revealed by G, Q and R banding techniques.

Facultative heterochromatin bands is a condensed and transcriptionally silent chromatin region like constitutive heterochromatin but can decondense into euchromatin if triggered by several factors like hypoacetylation.

There are different approaches to study the chromosomes. Chromosome banding techniques proved to be one of the best method. There are different types of chromosome banding techniques on the basis of chemical agent used for creation of that differential pattern on a chromosome.

1. Visible light dye based
2. Flurochrome based

1.1 Giemsa Staining

Giemsa is a visible light dye that binds to DNA through intercalation and thus, is used for chromosome staining. It is a mixture of cationic thiazine dyes, most importantly azure B,and anionic eosin dyes such as eosin Y. Staining of the chromosomes involves the formation of a thiazine-eosin precipitate in a 2:1 molar ratio. Two molecules of the small, fast diffusing thiazine dye first intercalate between the base pairs of the DNA in a configuration that favors the binding of the large, slow diffusing eosin molecule. The chromosomes stain blue as a result of this.The eosin molecule then forms a precipitate with the thiazine molecules thus causing the chromosomes to stain purple. The formation of this precipitate is favored on a hydrophobic environment.

Giemsa banding (G banding)

G-banding is the most widely used banding method for cytogenetic analysis that was first developed by Seabright in 1971. This technique, which is nonfluorescent, advantageous in the aspect of stability and resolution of the bands produced. Visible light dyes are more stable and capable of producing clearer bands than fluorochromes. In order for banding to occur, the chromosomes must first undergo a pre-treatment process before staining with Giemsa. The most common pre-treatment method used is the digestion of the chromosomes with a protease such as trypsin. These pre-treatment methods are known to extract a characteristic subset of proteins from the chromosomes. This differential extraction of the proteins throughout the length of the chromosome is responsible for the banding and is a reflection of the difference in the structure of the various chromosomal regions. The positive G-bands, which are the dark bands, corresponding to the hydrophobic regions of the chromosomes that favour the formation of the thiazine-eosin precipitate. These regions are identified as the late replicating heterochromatin, which are characterized as condensed and rich in protein

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disulfide cross-links. the negative G-bands, which are the light bands, corresponding to the less hydrophobic regions of the chromosomes that do not favor the formation of the thiazine-eosin precipitate.
These regions are identified as the early replicating euchromatin that have relatively loose structure and have their protein sulfur predominantly as sulfhydrils.

Other types of bands, such as R- and C-bands, can be obtained using the giemsa stain. The type of bands produced depends on the extent of denaturation induced on the chromosome structure. **R-banding** reveals the GC-rich euchromatin and produces positive bands that correspond to the negative G-bands. Banding is produced by incubating the chromosomes in an ionic solution at a high temperature (~ 87oC) followed by staining with giemsa. The incubation process causes the denaturation of the AT regions of the chromosomes because of the low melting point of these regions (~ 65oC) as compared to that of the GC regions (~ 105ºC).

C-banding on the other hand, reveals the AT-rich centromere, which consists of constitutive heterochromatin. This technique involves acid treatment, hot saline incubation, and alkali treatment of the chromosomes. These treatments depurinate the DNA and break the DNA backbone, which then cause the extraction of the DNA from certain regions of the chromosomes. C-bands are produced due to this differential extraction of the DNA. It was observed that the DNA in the C-bands is more resistant to extraction than the DNA in the other regions of the chromosomes. This is due to the stronger interaction of the proteins, which protects the DNA from extraction, with the DNA in the C-bands than in the other regions of the chromosomes.

G11 banding, N banding and T banding are some other banding techniques based on giemsa stain.

Staining with Fluorochromes

Fluorochromes are organic molecules that are capable of undergoing fluorescence. These molecules contain large conjugated systems such as aromatic or heterocyclic groups and are characterized by rigid and planar structures. The fluorescence of a fluorochrome is affected by the fluorochrome structure and environment.The quantum efficiency of a fluorochrome increases as the size of the conjugated system increases. Meanwhile, the environmental factors to consider include pH, ionic strength, temperature, viscosity of the medium, and presence of macromolecules. pH and ionic strength can change the structure of a fluorochrome thus affecting its fluorescence. Temperature, viscosity, and presence of macromolecules can affect the competition between radiantionless processes and fluorescence.

Q Banding

Chromosome banding using fluorochromes was first achieved by Caspersson in 1970 with the use of quinacrine mustard, an aminoacridine dye. Quinacrine has a positive charge that is capable of interacting with the negatively charged phosphate groups of the DNA. Quinacrine is observed to bind uniformly throughout the length of the chromosome.Hence, banding is produced due to differential fluorescence. The amino group at position 2 of the guanine bases of the DNA quenches the fluorescence of quinacrine thus causing the AT-rich regions of the chromosomes to fluoresce more brightly than the GCrich regions. The bands produced are called Q-bands. The bright yellow-green positive Q bands corresponds to the positive G-bands. Q-bands represent the facultative heterochromatin.

Commonly used flurochromes for chromosome banding

Flurochromes	Binding mode	Mechanism of Banding	selectivity
Quinacrine	Intercalation	Differential fluorescence	AT
Daunomycin	Intercalation	Differential fluorescence	AT
DAPI	Minor groove	Differential fluorescence	AT
Hoechst 33258	Minor groove	Differential binding	AT

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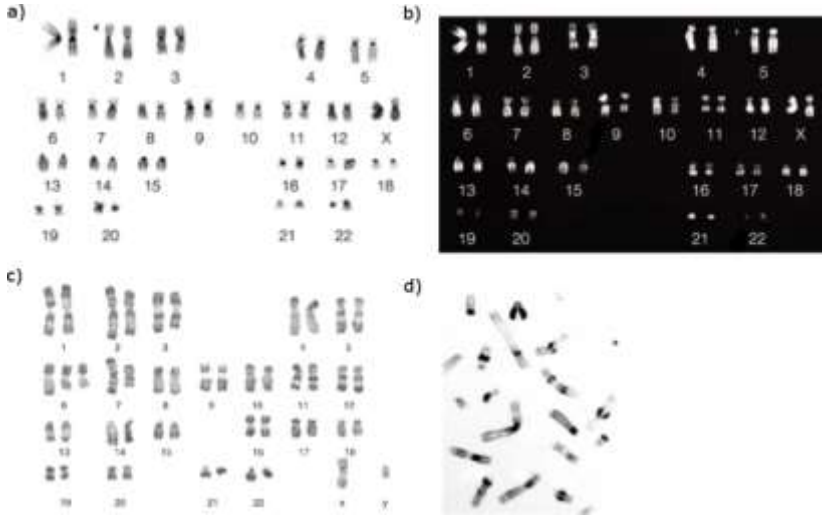


Comparison of different banding
(a-G Banding, b- Q Banding, c- R Banding and d- N Banding)

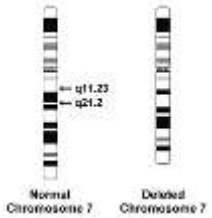
Counterstaining

Counterstaining is a technique that is used to induce banding with fluorochromes that bind and fluoresce uniformly throughout the chromosome. It is also used to enhance banding patterns that do not have a very high resolution. This technique involves the use of a primary fluorescent stain and a fluorescent or a nonfluorescent counterstain.However, if a fluorescent counterstain is used, its emission wavelength must differ from that of the primary stain.

Primary stain	Counterstain	Type of bands
DAPI	Actinomycin D	G
DAPI	Distamycin A	C
Chromomycin A3	Methyl green	R
DAPI	Chromomycin A3	G

Applications of chromosome banding techniques

1.Study of chromosome abnormalities and alterations.



Deletion of a DNA sequence in human chromosome 7

It can be used to identify individual chromosomes since each chromosome number produces unique bands. Thus, Chromosome banding can be used to study abnormalities in the chromosome such as deletions, insertions, or translocations

2.Investigation and understanding of plant organisation.

As we know that chromosome is the carrier of heredity information which ulmitately makes the living ones the way they are. So by studying the chromosome we can have better understanding about the organization of plant body and investigate for the difference that separate two species.

3. Important tool in analyzing karyotype and detecting polymorphism, that may have been important in speciation. The pattern of chromosome banding is highly specific in each chromosome of a species. Thus, bands showing polymorphism can be used simultaneously with genetic markers in cytogenetic study**.**

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4. **Confirm the evolutionary relationship between species**
By studying the banding pattern of two species, similarities in chromosome bands can tell us about the evolutionary relationship between the two.

B.S.Gill and Kimber in 1974 studied the evolution of wheat by using C banding pattern. On comparing the C banding pattern of *Triticum aestivum* with other diploid species they found that *Triticum monococcum* show the similarity in banding pattern.

5. Sequential Banding
Nowdays banding techniques are used along with modern method of chromosome analysis like FISH and SKY to get more filtered results.
6. Alien substitution and alien addition
Addition a pair of chromosome into a new background or a pair being substituted in a species can be studied by using the chromosome banding techniques.
Islam 1980, by making use of N banding pattern isolated a pair of barley chromosome substituted for a pair of wheat chromosome in a wheat- barley hybrid.

Conclusion
Chromosome banding technique is a preliminary way to understand the chromosome structure in a better way. Chromosome banding techniques used with modern techniques of chromosome analysis like Fluroscence *in situ* Hybridisation(FISH), Genomic *in situ* Hybridisation(GISH), Spectral Karyotyping(SKY) and Comparitive Genomic *in situ* Hybridisation(CSH) gives the refined results of chromosomal abnormalities. Band can be considered as a local address for a gene. Thus chromosome banding techniques can be used to locate a gene on a chromosome.

Chromosome banding is an important tool for the analysis of animal and plant chromosomes. Karyotyping can be used for detection of chromosome deletions associated with disorders.
Comparisons of chromosome banding patterns can confirm evolutionary relationships between species and also reveal changes in karyotype that may have been important in speciation.

The mechanism of staining and banding reveals the relation of the chromosomal bands with the chromosomal substructures. Higher resolution of band can be achieved through counterstaining and it also enhance the fluorescence and sequence specificity of metal stains.

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