E-Study material
For 5th semester Botany Honours (CBCS)
Course Code: BD501T
Core Course VII: Genetics
DSE Course – I: Analytical Techniques in Plant Sciences
Unit 1: Imaging and related Techniques
Topic: Chromosome banding

Debajit Saikia
Assistant Professor
Department of Botany
CNB College, Bokakhat

Chromosome banding:

Chromosome banding refers to alternating light and dark regions along the length of a chromosome, produced after staining with a dye. When subjected to different treatments before staining, the chromosomes develop different dark and light regions in form of bands. The banding pattern can be used to identify homologous chromosome and detect different types of chromosomal rearrangements abnormalities.

Giemsa staining:

Giemsa is a dye that is visible to light and it bonds to DNA through a process called intercalation. Fluorochromes are less stable than visible light dyes, which allow visible light dyes to produce bands that are more clearly defined. The Giemsa stain is a combination of anionic eosin dyes like eosin Y and cationic thiazine dyes like thiazine blue.

Because positive thiazine dye molecules are smaller than negative thiazine dye molecules, when two positive thiazine dye molecules intercalate into a negative DNA molecule, the result is a blue stain. The DNA is coloured purple as a result of the binding of the anionic eosin molecule to the two thiazine molecules. Giemsa produces a stronger stain in the hydrophobic zones.

G-bands, R-bands, C-banding, and T-banding are the four distinct varieties of banding methods that can be carried out with the assistance of Giemsa. Because of the variety of pretreatments utilised in each of the many methods of staining described above, the Giemsa stain marks distinct locations in the sample. It is common knowledge that the histone proteins are distributed evenly along the whole length of the chromosomes. The non-histone proteins are dispersed over the chromosome at a variable number of locations and are responsible for the loose or compact condition of various parts of the chromosome. The regions that are loosely packed are called euchromatin regions, whereas the sections that are firmly packed are called heterochromatin regions. These proteins are extracted in a non-uniform manner due to the pre-treatment methods, which results in distinct differences in staining.

Types of chromosome banding technique:

1. Q-banding:

The Q banding method was the first chromosomal banding technique, and it was distinguished by its use of the quinacrine stain (also known as quinacrine dihydrochloride or quinacrine mustard). Chromosomes that have been stained with quinacrine display the recognisable pattern of bright bands on a darker backdrop. Only when the chromosomes are illuminated by ultraviolet (UV) light do the bands become visible. This is due to the fact that quinacrine is a DNA intercalating agent as well as a fluorescent substance. Because the quinacrine molecules only shine when exposed to ultraviolet light, only the portions of the chromosome that have been intercalated with quinacrine are visible to the naked eye, while the unintercalated regions are invisible. This alternating light and dark banding pattern is not only very easy to reproduce, but it is also unique to each chromosome. Therefore, quinacrine banding can be utilised to identify certain chromosomes inside a cell, in addition to determining whether or not a chromosome possesses aberrant structural characteristics. The chemical compound quinocrine is known as an intercalating agent because it inserts itself between the base pairs that make up the DNA helix. Quinacrine has a stronger attraction to DNA sequences that are composed primarily of AT sequences. Because of this, the fluorescence of quinacrine is increased along AT rich sequences, making it look more brilliant than GC rich sequences.

2. 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.

Giemsa staining is an excellent nonfluorescent staining techniques. Giemsa also creates a reproducible pattern of bands on each chromosome. It is still not clear why chromosomes show bands when they are stained with quinacrine or Giemsa. Bright field microscope is used for visualization. In G banding technique, before using Giemsa stain, there is always a pretreatment step.Usually proteolytic enzyme trypsin is used for pretreatment. Therefore the process is also known as GTG banding (G-banding by trypsin with Giemsa). G-banding also produce same banding pattern as Q banding along the length of chromosome. Geimsa stain has more affinity for DNA sequence rich in AT content hence stained dark while sequence rich in GC content stain light.

3. C-banding:

Centromeric heterochromatin staining is another name for the C-banding pattern. In this method, the cell is prepared with alkali in order to prepare it for the application of the Giemsa stain. Because of this, it is also known as CBG-staining (B-banding by base with Giemsa). The C-binding approach is used to stain centromeric heterochromatin as well as the distal region of the Y chromosome, both of which include highly repetitive DNA sequences (satellite DNA). The banding is examined with a bright-field microscope after it has been stained.

4. R-banding:

Reverse chromosomal banding is often referred to as R-banding. The band pattern that is formed in the chromosome by this method of banding is inverted in comparison to the band pattern that is produced by G-banding and Q-banding. i.e. the black band that is seen with the G-banding approach seems to be quite light when using the R-banding technique, and vice versa. Giemsa stain is also used in the R-banding procedure; however, the slide is heated to 88 degrees Celsius in a buffer solution before the Giemsa stain is applied. DNA can become denatured by the process of heating. The AT rich areas of the chromosome undergo denaturation at a faster rate than the GC rich regions, which results in the loss of DNA from

these regions but not from the GC rich regions. The Giemsa stain is then used to colour the GC-rich region, resulting in a stained appearance (R band). The G-banding method is typically chosen over the R-banding method. R-banding, on the other hand, can be utilised for the purpose of chromosome identification. The use of R-banding as a supplement to G-banding can be beneficial in certain circumstances. This is because certain light and small G bands are easier to spot after being stained with R-banding. The telomere sequence at the ends of chromosomes can also be visualised with the help of R-banding, which is another application of this technique's utility. The R-banding method leaves telomeres with a black stain, while the G-banding method leaves them light.

Chromosome painting:

It is the most advanced technique. Chromosome spreads are treated with fluorescently labelled DNA fragments that have been extracted and described in the laboratory. This procedure results in the production of colourful images of chromosomes.

First, a fluorescent dye is used to identify the DNA fragment that was extracted from the gene of interest, and then the fragment is used to apply to chromosomal spread on a slide. The fluorescently labelled DNA fragment will only bind to chromosomal DNA that has a sequence that is complementary to its own. This binding causes a change in coloration on the chromatogram at the complementary sequence location.