

You may talk about your genes from time to time - 'Oh, I have the gene for that.' But how do you see your genes? A gene is a functional unit of DNA, and your DNA is organized onto chromosomes. **Chromosome banding** is a little like tie-dyeing your chromosomes.

A **chromosome** is a unit of tightly-packed DNA. DNA has to wrap tightly around itself, because you have quite a lot of it. In fact, if you unrolled all the DNA in a single one of your cells, it would be about three meters long. Humans have 46 chromosomes - 23 from Mom and 23 from Dad.

In **chromosome banding**, we treat chromosomes with chemicals to stain them and learn about a chromosome by how it stains. There are several different types of stains we can use.

There are several types of **chromosome banding**. Here, we will list a few of the most common types.

- **G-banding** uses a stain called Giemsa stain. G-banding gives you a series of light and dark stripes along the length of the chromosome. We will discuss G-banding in the most detail, because you will likely see G-banding if you take a genetics class.

G-banding is useful because the patterns of stripes on the chromosomes are unique enough that you should be able to confidently identify each chromosome.

Giemsa staining was named after the German scientist **Gustav Giemsa**, who worked in the early part of the 20th century. Giemsa's immediate goal was to find a stain that would work on *Plasmodium*, the parasite that causes malaria. Giemsa stain, however, was quickly found to have many uses. Dr. Giemsa lamented the fact that he would be known for his staining procedure rather than for his work on tropical diseases.

Giemsa stain is a mixture of a stain called methylene blue and one called azure, which form a type of stain called an eosin compound. Researchers will typically wash a sample in Giemsa stain for around seven minutes. You would typically stain chromosomes during the early parts of the cell cycle (prophase or metaphase), because the chromosomes are partially but not fully condensed.

- **Q-banding** uses a stain called quinacrine. Q-banding yields a fluorescent pattern. It is similar in pattern to G-banding, but glows yellow.

Quinacrine banding (or Q-banding) is the first chromosome banding pattern reported. It is done by treating the [chromosomes](#) with quinacrine dihydrochloride. The resulting pattern is similar to the G-banding pattern in a way that the bright and dull regions produced along the chromosome correspond to the dark and light regions in G-banding patterns, respectively. This means that the brightly fluorescing regions implicate adenine- and thymine-rich regions. There are exceptions though. Characteristic Q-banding patterns are found at the distal long arm of the Y chromosome (which fluoresces extremely brightly), at the heterochromatic regions of chromosomes 1, 9, and 16, and at the satellite regions of the acrocentric chromosomes. Q-banding is very useful in the examination of the heteromorphism associated with the [Y chromosome](#) and the satellite regions of the acrocentric chromosomes as well as the characterization of structural abnormalities associated with Y chromosome material.

Prepared by---Sovanjan Sarkar

- **C-banding** only stains the centromeres. Centromeres are little constricted portions of chromosomes. That's where sister chromatids (two copies of the same chromosome) will attach to each other when the cell is getting ready to divide.
- **R-banding** is the opposite of C-banding. R-banding stains non-centromeric regions.

Chromosome Banding: Technique # 1. C-Bands:

The technique of C-banding originated after the work of Pardue and Gall who reported that constitutive heterochromatin can be stained specifically by Giemsa-solution. Each chromosome possesses a different degree of constitutive heterochromatin which enables the identification of individual chromosomes.

Constitutive heterochromatin is located near the centromere, at telomeres and in the nucleolar organizer regions; it is composed of highly repetitive DNA. C-banding represents the constitutive heterochromatin, and the banding is caused by differential staining reactions of the DNA of heterochromatin and euchromatin.

The banding method is a complex technique that involves several treatments with acid, alkali or increased temperature. Denaturation of DNA is caused by these treatments. Subsequently, DNA renaturation occurs in treatments with sodium-citrate at 60°C.

By these treatments, the repetitive DNA (heterochromatin) re-natures but low repetitive and unique DNAs do not re-nature. This results in differential staining of the specific chromosome regions. Giemsa-C-banding technique has been used to identify chromosomes of various plant and animal species including human. The Y chromosome of mammals is mostly heterochromatic and therefore, the technique of C-banding is quite useful for its identification.

In barley chromosomes, Linde-Laursen in 1978, divided the C-bands into the following classes based on their position:

- (i) Centromeric bands situated at one or both sides of the centromere,
- (ii) Intercalary bands,
- (iii) Telomeric bands and
- (iv) Bands beside the secondary constriction in the short arm of satellited chromosomes.

He observed polymorphism in C-banding pattern in different barley lines, Giemsa-C-banding patterns may also be used to identify the extra-chromosomes of trisomies and telotrismics.

Chromosome Banding: Technique # 2. G-Bands:

The technique of G-banding involves Giemsa staining following pretreatment with weak trypsin solution, urea or protease. It provides greater detail than C-banding. It was first used for human chromosomes by Summer et al. in 1971. G-bands may reflect a stronger chromatin condensation. However, this technique is not suitable for plant chromosomes.

Chromosome Banding: Technique # 3. Q-Bands:

The method of Q-banding was developed by Caspersson et al. in 1968. The chromosomes stained with Quinacrine mustard show bright and dark zones under UV light. This technique is used to identify human and mice chromosomes.

Chromosome Banding: Technique # 4. N-Bands:

The technique of N-banding was originally described by Matsui and Sasaki in 1973. Briefly, air-dried chromosomes slides are stained for 90 minutes with Giemsa (diluted 1 : 10 in 1/15 M phosphate buffer at pH 7.0) following extraction with 5% trichloroacetic acid at 95°C for 30 minutes and then 0.1 NHCl at 60°C for 30 minutes.

The N-bands are generally located at the secondary constriction, satellites, centromeres, telomeres and heterochromatic segments. It is suggested that the N-bands represent certain structural non-histone proteins specifically linked to the nucleolar organizer region of the eukaryotic chromosomes.

The N- banding patterns have been used for the location of nucleolar regions in the different organisms, such as, mammals, birds,

amphibians, fishes, insects and plants. N-banding patterns differ in the chromosomes of different species.

In 1980, Islam used this method to identify the barley chromosomes from those of wheat in the reciprocal wheat-barely F₁ hybrids, and to detect translocations between the wheat and barley chromosomes. He also used this technique to isolate lines possessing a pair of barely chromosomes substituted for particular pair of wheat chromosomes.

A modified Giemsa-N-banding technique was developed by Singh and Tsuchiya in 1982 for the identification of barley chromosomes. This method is a combination of acetocarmine staining and Giemsa-N-banding. After processing according to this method, the centromeric region looks like a “**diamond-shaped**” structure; this is not seen in other techniques.

Early metaphase or prometaphase chromosomes are more suitable for this staining as they show better banding pattern than the chromosomes at mid-metaphase in somatic cells.