Genetic Methods for Isolating and Reading Chromosomes

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ABSTRACT

This paper offers a comprehensive examination of chromosomal abnormalities and emphasizes the pivotal role of chromosome banding in elucidating the intricate mechanisms of human cells. Various facets of chromosome structure and cytogenetic techniques are explored. The incorporation of karyotyping methods such as G-banding, Q-banding, and R-banding enriches our understanding of structural nuances and chromosomal anomalies. Moreover, by focusing on their applications in molecular cytogenetics, the research delves into contemporary approaches like silver staining (NOR), spectral karyotyping, (FISH) techniques, and genomic hybridization. The data is more accessible and comprehensible when presented in a systematic fashion and utilizing a table. In conclusion, this manuscript delivers a valuable overview of chromosome analysis for cytogeneticists, scholars, and individuals keen on expanding their knowledge of the intricacies of chromosome analysis.


1. Introduction

Chromosomes are the molecular builders of heredity they provide the elaborate framework on which life history is enacted. The voyage of chromosome research has been filled with significant discoveries, starting with the crude observations of the first cytologists and continuing through the complex genomic investigations of today [1].

In 1882, Flemming published the first report on human chromosomes, which describes twenty-two to twenty-eight chromosomes in the dividing cells of the corneal epithelium. Who revealed forty years later that there were 48 human chromosomes and that sex was based on the fact that the Y chromosome was present or absent? The nature of human chromosomes was not further established until Tjio and Levan’s 1956 report on (2n=46) [2]. Nowadays, 46 chromosomes, which are typically found in pairs in a healthy human cell, include one pair of sex chromosomes and 22 pairs of autosomes. The chromosome morphology and structure are closely linked to human health, and they contain significant material needed for human genetics. The rod-like structures known as chromosomes are created when chromatin polymerizes during mitosis or meiosis [3].

Although, the chromosomes are the physical structures that house the majority of the genome and act as carriers of eukaryotic genetic materials. Furthermore, the uncoiling of chromosomes facilitates the initiation of DNA synthesis and transcription, and their three-dimensional arrangement influences the expression of genes, consequently serving as a regulatory element in multiple processes [4].

The classification of chromosomes and the identification of structural and numerical alterations in chromosome aberrations have both benefited from the application of flow cytometric analysis of chromosomes, frequently referred to as flow karyotyping. In order to analyze chromosomes with a flowcytometer, chromosomes from mitotic cells must first be isolated and then stained with two fluorescent dyes that are specific to bases [5]. Besides of flow cytometry, the fluorescence in situ hybridization (FISH) technique is used for karyotyping chromosomal analysis. Because spectral karyotyping uses technology to paint each of the 24 human chromosomes a different color, it is possible to diagnose a wide range of diseases. The use of spectral karyotyping for research in general clinical practice has been feasible in recent years, and its applicability in the diagnosis of various diseases has drawn particular interest [6]. Thus, in this review using karyotyping techniques for isolation of chromosome abnormality to detect genetic disorders in clinical applications were taken as consideration.

2. Chromosome Structure in Human Cell

Chromosome arranged hierarchically on several levels, from the basic structure of DNA to three-dimensional architecture found in the nucleus. DNA is fundamentally twisted around histone proteins to form nucleosomes, which are the fundamental repeating units. Further compaction is facilitated by this nucleosome array, which forms the scaffold for the distinctive X-shaped chromosomes that are visible during cell division. Chromosomes manifest their most effective visualization when they undergo maximal condensation, referring to the state of highest compaction within the chromosomal structure. In cell division, the two sister
chromatids that are present on each chromosome in the nuclei of individual cells split apart [5]. As seen in Figure 1, the centromere is the only location where two chromatids can connect during metaphase. The main nucleic acid sequence of the human genome is inadequate for understanding its functions and regulatory mechanisms. The DNA is densely packed into a high concentration of heterochromatin, which is present throughout, making this easier. [1]. Furthermore, the location of each chromosomal DNA molecule within the nucleus is a specific three-dimensional territory, and this arrangement is not random; rather, it varies depending on the stage of cell differentiation. [7]. The processes of chromosome division, DNA replication, gene expression, and DNA repair are significantly influenced by the three-dimensional structure of chromatin. Furthermore, there exists a significant association between the placement of genetic loci inside the nucleus and functional characteristics such as gene expression, particularly in relation to one another, as well as nuclear structures and their protective envelopes. One crucial objective in biology is to determine the precise three-dimensional position of the six billion base pairs of DNA in each of the twenty-three chromosomes within the nucleus of a human cell. [1].

Higher-order structures, such as chromatin fibers, which are stacks of nucleosomes that are either eleven or thirty nanometers long, can be found and studied in vitro thanks to modern techniques. The existence of regular, higher-order nucleosome structures in vivo under physiological conditions has not been demonstrated. This is due to the limitation of light diffraction, which can only resolve entire chromosome regions that are a few micrometers in size, while individual nucleosomes are approximately 5 nanometers in size. Euchromatin and heterochromatin are the two main types of higher-order chromatin organization. How many genes they contain and how active they are have traditionally distinguished them. Euchromatin is less compact and has a higher gene density and activity, while heterochromatin is more compact and has a lower gene density and activity. [8]. The way that individual chromosomes are arranged causes the majority of DNA-DNA interactions to happen in cis. The organization of chromatin is characterized by the arrangement of distinct territories. Contacts across different chromatin territories, known as trans contacts, are relatively few and are usually observed on the outer surface or loops that extend beyond the territories. The generalized spatial segregation of heterochromatin and euchromatin occurs both within individual nuclei and throughout specific regions. This leads to the formation of regions surrounding nucleoli and at the nuclear periphery that have a lack of gene expression and a high concentration of heterochromatin. Only light can achieve the diffraction limit of 200 nm. Currently, there is a limited understanding of the three-dimensional structure of chromatin that is functionally significant...[9].

![Figure 1. Human metaphase chromosome (redrawn)](image)

2.1 Chromosome abnormality

According to chromosomal abnormality we have two types of chromosomal abnormalities: acquired and constitutional. Constitutive chromosomal abnormalities impact all or most of an organism's cells and develop during gametogenesis or early embryogenesis. Adopted chromosomal abnormalities impact a single clone of cells with a limited distribution throughout the body, and they typically manifest during adulthood [11]. Numerous neoplasms have acquired chromosomal abnormalities as part of their pathogenesis. Environmental exposures may, occasionally through chromosomal alteration-related mechanisms, contribute to the development of cancer. Chromosomal abnormalities can be classified into two main categories: structural abnormalities and numerical abnormalities [12]. Down syndrome, Turner syndrome, Edwards syndrome, and Klinefelter's syndrome are among the conditions that can be brought on by numerical abnormalities, which arise from the gain or loss of an entire chromosome [13, 14]. Although, A person may be born without fingers, have an unusual facial form, have epilepsy, have blood disorders, struggle with learning, and have many other ailments for which there is no known cause until an individual's chromosomes are examined. The doctors advise the affected people to get genetic testing for this reason. Since chromosomes carry genetic information, genetic testing is done to determine the cause of these abnormalities. There are 46 chromosomes in a healthy human [15]. Chromatids only bind to one another at the centromere during metaphase. Staining makes the distinct gene substructures found on these chromatids visible [10]. The chromosomes are divided into condensed and less condensed regions, which are represented by dark and light bands, respectively. As a result, after staining, each chromatid generates a distinct banding pattern based on its substructures [16, 17]. There are various staining methods available, including G-banding. [18] R-banding, C-banding [19], Q-banding [18, 20], NOR banding, and T-banding. Chromosome staining is frequently done using the fluorescent stain 4,6-diamidino-2-phenylindole (DAPI) is a fluorescent stain that's frequently applied to chromosomes. The ability to avoid the need to prepare multiple duplicate cell samples is the reason for this widespread use [21]. Several imaging techniques, including light, fluorescent, electron, and coherent x-ray diffraction imaging, are used to create a visible photographic representation of the chromosomes following staining. Flow cytogenetics, the term for the analysis of chromosomes by flow cytometry, requires the sorting and inspection of single mitotic chromosomes in suspension. The evaluation of flow cartograms can be utilized for identifying deletions, translocations, or any other kind of aneuploidy by providing information on chromosomal DNA content and chromosome number and structure [22].

3. Chromosome Banding

Methods for banding chromosomes are based on assaying for a specific function or staining chromosomes. The dye-based chromosome banding techniques that are most frequently used are G-(Giemsa), R-(reverse), C-(centromere), and Q-(quinacrine) [23]. Even so, distinct bands stain to a variety of intensities, so the staining patterns are not binary [24]. R-positive (R-) bands and G-positive (G-) bands are typically referred to as G-bands. Indicative heterochromatin is identified in positive C-bands. G-bands and Q-bands are considered to be interchangeable [23]. Certain reagents stain specific chromosomal regions more intensely after different treatments, resulting in chromosome-specific bands: G/Q bands have facultative heterochromatin, are late replicating, and are (At-R), bands are early-replicating, GC-rich, and comprise approximately 80% of known genes. In addition to numerous
al sequences, centromeric DNA tandem repeats are typically found in C bands [25]. The total number of bands varies with the resolution, but roughly speaking, prophase, "replication" banding, and prematurely condensed chromosomes can all show up to 1,250 bands [26]. Individual R bands fuse more frequently and quickly than G bands as cells enter mitosis, reducing the total [27]. G bands are typically produced by trypsin treatment, but atomic force microscopy of untreated human metaphase spreads shows a similar pattern of marginally thicker regions. Even though homologous chromosomes have similar shapes, their lengths and banding patterns are rarely exactly the same, which explains why karyotyping automation is moving so slowly. Nevertheless, these bands should be explained by any chromosomal structure model [28].

4. Karyotyping Techniques

Genetic disorders are caused by structural as well as numerical abnormalities in the chromosomes. Chromosome analysis is therefore an essential technique in the identification of genetic disease. A typical technique for analyzing chromosomes is called karyotyping [29, 30]. One of the most crucial methods in the field of genetic measurement and diagnosis is karyotyping [31]. The karyotype analysis of the human chromosome is clinically significant for the diagnosis and treatment of genetic diseases in modern medicine. Separating the banded chromosomes is extremely significant in such an analysis [3]. There are basically three primary steps in the chromosome karyotyping process. Using a light microscope, the chromosomes are first isolated and stained. Following that, all of the chromosomes were separated and implemented out of the microscopic picture of the metaphase chromosome. Lastly, the chromosomes that were extracted have been classified as arranged to create a karyotype picture with 24 different chromosome types [3]. Under a 100x microscope, figure 2a displays an image of the 46 different types of chromosomes, and figure 2b displays the karyotype image of these chromosomes in pairs.

4.1 Spectral Karyotyping Techniques

A new method for chromosomal analysis called spectral karyotyping was created based on the principles of fluorescence in situ hybridization. Because spectral karyotyping uses technology to paint each of the 24 human chromosomes a different color, it is possible to diagnose a wide range of diseases. The use of spectral karyotyping for research in general clinical practice has been feasible in recent years, and its applicability in the diagnosis of various diseases has drawn special attention [6]. Since 1996, spectral karyotyping (SKY) has been utilized to simultaneously detect and register every chromosome, in line with the definition of "karyotype," which refers to the entire set of chromosomes. A distinct fluorescent color is used to identify and record each homologous chromosomal pair [32]. The SKY technique ease of use in detecting chromosomal abnormalities. As a result, it is possible to identify chromosomal length, banding patterns, biological relationships, and modifications to chromosomal number and structure [33]. Shon in Figure 3. This offers the significant benefit of identifying a wide range of illnesses and birth abnormalities, including Down syndrome and polycystic kidney disease, in order to start the proper treatments [34]. Moreover, since the spectral karyotype is a source of diagnostic data, this approach unifies clinical and medical genetics [32]. Together with Texas Red, Cy2, Cy5.5, Cy5, and Spectrum Green, the five fluorescent dyes can theoretically be combined to create a total of 31 colors. The remaining 24 colors were created individually as fluorescence probes after the combination of colors that were similar and colors within the infrared wavelength were eliminated. Only one of the typical 24 human chromosomes is intended for attachment by each probe [35]. These probes were used to hybridize metaphase chromosomes in the absence of light. Following that, a two-dimensional imaging spectroscopy system was used to capture the spectral karyotype, or image, of every colored chromosome[6]. When a person has developmental issues that could impact multiple organs (multiple malformations), SKY is required. This may cause organs to grow and shrink disproportionately [36]. Not to mention that late-stage cancer can also affect organs. Consequently, SKY is helpful in identifying patients who have cancers like esophageal cancer, which is chromosome 8-affected [37]. Although during the pregnancy, When a mother conceives after the age of 35, amniocentesis is done because her child is more likely to have a chromosomal abnormality [32]. During the process, amniotic fluid is extracted from the uterus; complications, including bleeding, infection, and miscarriage, are exceptionally uncommon. Additionally, because the egg and sperm combine to create an embryo with an imbalanced abnormality, SKY should be tested in couples where the female partner is infertile or may miscarry. Since a reciprocal or Robertsonian translocation can result in infertility, this is typically the result of a translocation [32]. The spectral karyotype, or SKY, has been illustrated to be extremely helpful in identifying chromosomal aberrations like substitution and translocations, which occur when the derivative chromosome appears in two or more different colors. SKY has also significantly improved the efficiency of discriminating between different [38]. But because they do not cause the chromosomes' colors to change, mutations like inversion and duplication that take place without interaction between different chromosomes are challenging to visually identify through color [39].

4.2 G-Banding Technique

The most frequently employed dye-based chromosomal banding techniques are G- (Giemsa) Banding. The simplest and most common technique for chromosomal identification (karyotyping) is G-banding, which can also be used to detect chromosomal number abnormalities, material translocations between chromosomes, and deletions, inversions, or amplifications of specific chromosome segments [23]. When it
comes to identifying genetic disorders like Down syndrome, trisomy 18, and trisomy 21, chromosome analysis is crucial. To examine chromosomes using a light microscope, researchers created staining techniques, also referred to as banding [41]. Methods for banding chromosomes are based on assaying for a specific function or staining chromosomes with a dye [23]. Heterochromatic (AT-rich) and euchromatin (GC-rich) regions of the chromosomes appear darker and lighter, respectively, in G-banding images. Cytogenetists can perform karyotyping more easily because of this contrast difference. Cytogenetists use a process called karyotyping in which they arrange individual chromosomes in descending order of size horizontally. The karyotype and G-banding chromosome images are displayed in Figure 4.

**Figure 4. Image of the G-Banding chromosome and karyotyping [41]**

To detect genetic disorder, and treatment the karyotyping methods used for identification bands in the chromosomes. chromosome bands are not clearly distinguishable due to insufficient staining. In order to distinguish between the black and white bands in an input image, preprocessing is used. Sobel filtering and thresholding are then used to identify the chromosomal outer boundary. Bands are divided using a variety of segmentation algorithms following boundary detection. During this process four different segmentation algorithm are used. Finally, if the bands are not fully segmented, region merging and growing is employed. Whether the detection of outer bands shows any discontinuities [42]. Based on the segmented chromosome’s hole filling procedure, postprocessing is carried out [42]. The process of G-banding clarify-in figure 5. A traditional tool in cytogenetic and cancer research is the examination of metaphase chromosomes. The classic representation of karyotype chromosomes is made up of metaphase chromosomes. Arrangement of 23 pairs of chromosomes clearly appear on karyotyping image [42].

**Figure 5. Block diagram of suggested algorithm (redrawn) [42]**

G-banding and Q-banding pretreatments are extremely similar. The main distinction is that before being stained with Giemsa, the spread chromosomes were treated with trypsin [35]. Dark and light bands alternately form on chromosomes; the dark (positive) bands exist in the AT-rich region, but the light (negative) bands are noticed in the GC-rich region. In respect to staining position, G-banding and Q-banding are comparable. Fortunately, the former is more commonly employed now because it does not fluoresce, meaning that stains can be kept for longer periods of time and can be observed without the need for a fluorescence microscope [43]. Moreover, compared to Q-banding, G-banding shows more distinct bands. G-banding is frequently used in prenatal detection and karyotyping. It helps detect structural anomalies like translocations, insertions, and deletions, as well as aneuploidy [23].

### 4.3 Q-Banding

For various situations, several chromosome banding techniques were created. So, q-Banding is one of the classical cytogenetic techniques that used to detect chromosomal abnormalities. Instead of staining chromosomes, cells need to divide by adding mitogen to promote cell proliferation [44]. Q-banding. Staining alone produces fluorescent Q-banding; additional treatment is not required. A yellow fluorescence of varying intensities that arises from treating the chromosomes with quinacrine mustard (QM) or quinacrine fluorochromes can be used to identify Q-banding. Since quinacrine fluorochromes and QM were first used as chromosomal banding agents, a plethora of research has been done on the processes involved in the production of Q-bands and the variables influencing the fluorescence. A further area of interest is applying the knowledge of fluorochrome-DNA interactions to shed more light on the molecular makeup of metaphase chromosomes [45].

Peripheral blood lymphocytes, which are obtained from blood, are one of the best cell sources because of their easy accessibility and fast rate of division. Banding techniques required the extraction of the most condensed chromosome from metaphase. This is accomplished by giving the cultured cells agents like colcemid or colchicine that prevent the development of the mitotic spindle apparatus [44]. Figure 6 shown an example of chromosomal anomaly that detected by Q-banding technique. When the turgid cells burst inside a microscope slide, the resultant cells were further cultivated with a hypotonic solution and acetic acid (a fixative) in order to preserve the chromosomes and spread them out for better labeling [46]. Chromosomes were stained with (Quinacrine mustard )fluorochrome for Q-banding [47]. The chromosomes demonstrate alternating brilliant (positive) bands on AT (adenine and thymine)-rich fields when observed under a fluorescence microscope. When figuring out the X-Y or Y-autosome translocation and differentiating the human Y chromosome, Q-banding is particularly effective [47]. For the reason that fluorescence quenches quickly, prompt photos are needed for karyotyping following banding [48].
produced by R-banding. The GC-rich region is home to the dark disorder. Although this method, opposite of G-band ing is bands created by R-banding, while the AT-rich region is home to the light bands. This results from the two banding techniques' disparate pretreatments. Before applying Giemsa stain for R-banding, chromosomes were treated with a hot buffer solution. Denaturation of the AT regions takes place during incubation because their melting point is lower than the GC regions. As a result, chromosomes, AT regions have lighter bands. Telomere abnormalities are frequently identified using R-banding because telomeres are prominent in GC and are highlighted as dark bands following staining. This method can be used to identify chromosomal abnormalities such as Jacobsen syndrome (11q terminal deletion disorder) [35]. Several techniques can be used to achieve R-banding, one of which involves heating slides in a buffer to 88°C and then staining them with Giemsa (basic protocol, RHG technique). Other techniques for creating R-bands rely on some dyes' specificity for GC-base pairs. Chromomycin-A3 and distamycin A double-dye fluorescent staining (alternate protocol, CA3/DA technique) is one of these techniques [50].

4.5 C-Banding Techniques

Pardue and Gall (1970) made the initial observation of C-Banding, when they noticed that slides stained with Giemsa for situ hybridization only displayed noticeable staining in the areas close to the centromere, after denaturation and renaturation. It was highly established that highly repetitive, quickly renaturing satellite DNAs were found in these same regions [51]. Centromeric heterochromatin staining, also known as (CBG-Banding), uses Giemsa staining and mild alkaline treatment to highlight chromosomal regions that are known to contain highly repetitive DNA sequences (satellite DNA). Example of this regions include those on digital region on Y chromosome and those that are located at or near centromere of chromosomes (Centromeric Heterochromatin). Although bright-field microscope is used to view this kind of banding [50]. C-Banding can be useful in the assessment of rearranged and marker chromosomes, as well as polymorphic variants of all centromeres, particularly those chromosomes (1-9 and 16). It also highlights centromere-associated heterochromatin, and the heterochromatic portion of the human Y chromosome [50]. The C-banding methods primarily used for chromosome

4.6 FISH

The availability of fluorescently labeled region-specific DNA probe hybridization greatly increased the versatility of cytogenetic techniques. Today, this molecular cytogenetic method is commonly referred to as FISH [52]. The modern era of molecular genetics replaced the classical era of cytogenetics with the invention of the fluorescence in situ hybridization (FISH) technique [52]. Using the complementary base pairing principle, FISH hybridizes fluorescently labeled nucleic acid fragments (probes) to denature genomic DNA. Chromosomes are identified by counting and arranging the signals on the probes, which are then visible under a fluorescence microscope [53]. FISH became well-known as a physical mapping method to aid in the massive mapping and sequencing projects associated with the human genome project; nevertheless, its precision and versatility were simultaneously, or shortly after, utilized in other fields of biological and medical research. Consequently, numerous varied applications and FISH-based diagnostic assays have been created in various domains of study, such as toxicology, microbiology, clinical genetics, neuroscience, reproductive medicine, evolutionary biology, comparative genomics, cellular genomics, and chromosome biology [54]. The diagnosis of submicroscopic chromosomal aberrations by classical cytogenetics has always been hampered by its low resolution. The development of in situ hybridization (ISH) results in a breakthrough in the molecular visualization of chromosomes [55]. The same basic idea was then used to develop fluorescence in situ hybridization (FISH), a variation of in situ hybridization (ISH), in which fluorescence probes are used instead of radioactive isotopes as a marker to label chromosomes [35]. The high resolution and safety features enable its application in the diagnosis of different chromosomal aberrations. For FISH, a microscope slide must be prepared by attaching fresh tissue to it, adding acetic acid or methanol, and exposing DNA sequences to hydrochloric acid to improve probe penetration and lower background noise. Afterwards, when the temperature reaches 70°C the DNA undergoes denaturation and is then combined with haptic probes. This mixture is left to hybridize for a period of 6 to 12 hours, at a temperature of 37°C [35]. The slide is cleaned to remove loose probes and then immersed in an antifade solution. Using a fluorescence microscope, labeled chromosomes can be viewed [56]. The field of prognostic and cancer genomics relies heavily on fluorescence in situ hybridization. FISH can be used to show aneuploidy abnormalities like trisomy 21 (Down syndrome) and monosomy X (Turner syndrome) using centromeric enumeration probes (CEP) [57]. In addition, FISH can be used to analyze cells that are challenging to culture, such as solid tumor cells, because there is no requirement to culture the cells. The erythroblastic leukemia viral oncogene can be hybridized with fluorescence probes to identify cancer, such as HER2 breast cancer [58].
of structural and numerical anomalies. The fundamental comparative genomic hybridization (CGH) in the investigation of cancer. By eliminating the need for metaphase cell culture, the possibility of a chromosomal loss in the individual with the lower number of probes binding to the tumor DNA, indicating occurring. Alternatively, if the ratio is below 1, it suggests a process of chromosomal duplication been linked to the tumor DNA compared to the normal DNA. It indicates that there is a high fluorescence intensity of tumor and normal DNA in order to detect copy number changes in chromosomes. Once tumor DNA has been extracted from the patient, it is detected using green fluorochrome. Subsequently, it undergoes hybridization with regular DNA obtained from a healthy volunteer, which has been marked with red fluorochrome. This method produces an emission that is a ratio of two distinct fluorescence colors, namely green and red. If the ratio is greater than 1, a higher number of probes have been linked to the tumor DNA compared to the normal DNA. It indicates that there is a process of chromosomal duplication occurring. Alternatively, if the ratio is below 1, it suggests a lower number of probes binding to the tumor DNA, indicating the possibility of a chromosomal loss in the individual with the cancer. By eliminating the need for metaphase cell culture, the use of Comparative Genomic Hybridization (CGH) has significantly advanced the analysis of solid tumors, particularly those containing cancerous cells. The identification of chromosomal abnormalities has made it easier to understand cancer subtypes and how tumors progress.

4.7 Comparative Genomic Hybridization

A recently developed molecular-cytogenetic test called comparative genomic hybridization (CGH). A new method for looking for genetic material imbalances in genomes is comparative genomic in situ hybridization, or CGH. Manage DNA that has been marked differently from cells that have normal chromosome complements is combined with genomic test DNA that has been labeled and extracted from clinical or tumor specimens. The groundbreaking application of comparative genomic hybridization (CGH) in the investigation of structural and numerical anomalies. The fundamental idea behind CGH is to compare the fluorescence intensity of tumor and normal DNA in order to detect copy number changes in chromosomes. Once tumor DNA has been extracted from the patient, it is detected using green fluorochrome. Subsequently, it undergoes hybridization with regular DNA obtained from a healthy volunteer, which has been marked with red fluorochrome. This method produces an emission that is a ratio of two distinct fluorescence colors, namely green and red. If the ratio is greater than 1, a higher number of probes have been linked to the tumor DNA compared to the normal DNA. It indicates that there is a process of chromosomal duplication occurring. Alternatively, if the ratio is below 1, it suggests a lower number of probes binding to the tumor DNA, indicating the possibility of a chromosomal loss in the individual with the cancer. By eliminating the need for metaphase cell culture, the use of Comparative Genomic Hybridization (CGH) has significantly advanced the analysis of solid tumors, particularly those containing cancerous cells. The identification of chromosomal abnormalities has made it easier to understand cancer subtypes and how tumors progress.

5. Silver Staining (NOR)

One of the methods used to visualize and separate chromosomes in cytogenetics is known as the silver staining technique. This method gives scientists a special tool to examine the nucleolar organizer regions (NORs) found within chromosomes, revealing information about the structure, function, and anomalies of chromosomes. We will go over the fundamentals, uses, and importance of silver staining (NOR) in the process of isolating chromosomes linked to genetic diseases and cancer in this review. Because silver staining techniques can bind to particular cellular components selectively, they are frequently used in a wide range of biological applications. AgNOR staining is used in the field of cytogenetics to identify the organizer regions (NORs), which are chromosomal segments responsible for the production of ribosomal RNA. Because NORs are in the of the chromosomes, change in their or structures are linked to genetic and malignant condition or cancer. To use this method, you have to stain the nucleolar organizing region (NOR). This is on the satellite stalks of acrocentric chromosomes and has genes that make ribosomal RNA. Structural non-histone proteins that bind to ammoniacal silver and are specifically linked to NOR are known as NOR-bands. Studying specific chromosomal polymorphisms, like double satellites, and recognizing satellite stalks, which are occasionally observed on non-acrocentric chromosomes, are beneficial in clinical practice. A few crucial steps are involved in the silver staining procedure. Silver nitrate is applied after cells are prepared and fixed on slides, causing a reaction with the proteins linked to NORs. As a result, silver grains are specifically deposited at the NORs, where they become visible under a microscope. The method makes it possible to recognize and measure NORs, giving important insights into the structure of the chromosomes. According to all techniques that used for karyotyping techniques, they have some advantages as shown in Table 1.
Table 1: Karyotyping Techniques and their Advantages

<table>
<thead>
<tr>
<th>Karyotyping Techniques</th>
<th>Advantages</th>
<th>Ref.</th>
</tr>
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<tbody>
<tr>
<td>Spectral Karyotyping (SKY)</td>
<td>The power of spectral karyotyping lies in its capacity to yield detailed information in a single experiment regarding chromosomal abnormalities, such as translocations, deletions, and duplications</td>
<td>[67]</td>
</tr>
<tr>
<td>G-Banding</td>
<td>The capacity of G-banding to expose distinctive banding patterns on chromosomes makes it possible to identify specific chromosomes and identify structural abnormalities. Consist of its ease of use, consistency, and high resolution, which make it an essential tool in cytogenetics.</td>
<td>[68]</td>
</tr>
<tr>
<td>Q-Banding</td>
<td>The capacity of Q-banding to generate characteristic banding patterns on chromosomes makes it easier to identify individual chromosomes and analyze chromosomal structure.</td>
<td>[69]</td>
</tr>
<tr>
<td>R-Banding</td>
<td>Its use in specific cytogenetic applications and its ability to reveal distinct chromosomal regions in comparison to G-banding. It's the particular needs of a cytogenetic analysis frequently influence the choice of banding technique.</td>
<td>[70]</td>
</tr>
<tr>
<td>C-Banding</td>
<td>C-banding identifies constitutive heterochromatin regions with repeating DNA sequences near the centromeres and telomeres. This enables cytogeneticists to distinguish between chromosomes and identify structural anomalies such as inversions or translocations.</td>
<td>[71]</td>
</tr>
<tr>
<td>FISH</td>
<td>Fluorescence in situ hybridization (FISH) techniques excel at precisely visualizing specific DNA sequences within cells, allowing for the examination of gene expression and the detection of genetic anomalies. FISH is extensively employed in molecular biology, cytogenetics, and genetics.</td>
<td>[72, 73]</td>
</tr>
<tr>
<td>CGH</td>
<td>The capacity of comparative genomic hybridization (CGH) techniques to fully identify chromosomal copy number variations offers important new understandings of genetic diseases and cancer.</td>
<td>[74]</td>
</tr>
<tr>
<td>NOR</td>
<td>Visualizing nucleolar organizing regions (NOR) on chromosomes, particularly with silver staining, offers a key advantage by aiding in the analysis of chromosomal structure and the study of ribosomal RNA genes.</td>
<td>[35, 75]</td>
</tr>
</tbody>
</table>

1. Limitation of Karyotyping Techniques

The use of karyotyping techniques helps to detect genetic diseases, but each of these techniques has some limitation such as, using fluorescently labeled probes, spectral karyotyping (SKY) is a potent cytogenetic technique that makes it possible to see and identify every chromosome in a single cell. But the primary drawback of SKY is its intricacy and the need for specific tools and knowledge. Technically difficult and time-consuming, the procedure entails hybridizing a large number of probes with different colors with the entire chromosomal complement. Sophisticated image analysis software and skilled workers are also needed for the interpretation of the intricate spectral patterns produced by the fluorescent signals. Hence, the application of SKY may be limited to specialized laboratories possessing the requisite resources and abilities, thereby limiting its applicability for standard clinical settings and routine cytogenetic analysis [6]. Furthermore, the relatively low resolution of G-banding techniques limits their ability to provide detailed information about specific genetic changes and makes it difficult to detect small-scale chromosomal abnormalities [76]. G-banding’s ability to identify the precise genes involved in chromosomal regions that have been identified may be limited, making it less effective in determining the molecular causes of genetic disorders [77, 78]. Like G-Banding techniques, Q-Banding have a lower resolution and are therefore limited in their ability to detect subtle chromosomal abnormalities. Accurately identifying small-scale genetic changes may be difficult for these methods [79, 80]. Although, another techniques for detecting genetic disorder that we mentioned is, R-banding techniques have a limited sensitivity to specific chromosomal rearrangements and small genetic abnormalities, which makes it difficult to accurately identify and characterize particular genetic changes [81]. Heterochromatin regions, which are usually found close to chromosome centromeres and telomeres, are visualized using C-banding, which helps identify structural chromosomal abnormalities associated with specific disorders, but it is not directly linked to the diagnosis of specific genetic diseases [71, 82]. C-banding methods have a propensity to prioritize constitutive heterochromatin over dynamic alterations in facultative heterochromatin or euchromatic regions. This specificity may limit the identification of certain chromosomal abnormalities or structural variations other than constitutive heterochromatin [10, 83, 84]. In addition to classical techniques, modern techniques are not without shortcomings such as, the application of fluorescence in situ hybridization (FISH) may result in signal overlap and ambiguity when dealing with repetitive elements or closely spaced DNA sequences. FISH may have issues separating and correctly identifying individual signals when the target regions are close together on the chromosome or contain highly repetitive sequences. This restriction may affect the accuracy and consistency of FISH results, especially in circumstances where accurate localization is essential [76, 85, 86]. CGH is another modern, techniques to identify balanced chromosomal rearrangements, CGH is very good at finding copy number variations (amplifications or deletions) all over the genome, but it can't tell you anything about how the chromosomes are physically arranged. Balanced rearrangements that neither gain nor lose genetic material, for example, inversions or translocations, may be undetected by CGH. An in-depth comprehension of genomic alterations often requires combining CGH with other techniques that focus on structural rearrangements, such as fluorescence in situ hybridization (FISH) or karyotyping [35, 87]. The specificity of (NOR) silver staining in karyotyping lies in its ability to highlight specific chromosomal regions. Associated with ribosomal RNA genes, nucleolus organizer regions (NORs)
exhibit heterogeneous distribution patterns and may not be found on all chromosomes. Consequently, the NOR silver staining technique may not consistently mark every chromosome, leading to an inadequate depiction of the full karyotype. The chromatin’s overall structure and stage of the cell cycle can also affect the stainability, which consequently determines the absence or presence of nucleolar organizer regions (NORs). Given that the NOR silver staining could result in underrepresentation and variabilities in stain patterns, Scientists should use it with care. [88-91].

2. Conclusion

This review paper examines various facets of chromosomal structure and cytogenetic methodologies. The resource provides a detailed account of chromosomal abnormalities and underscores the significance of chromosome banding in comprehending human cells. Procedures such as Giemsa banding, Q-banding, and Reverse banding in karyotyping facilitate a deeper insight into chromosomal arrangement. The study also delves into the latest techniques, emphasizing their utilization in molecular cytogenetics. These methodologies encompass genetic hybridization, spectral karyotyping, fluorescence in situ hybridization (FISH), and silver staining (NOR). The methodological utilization and presentation of a table augment the comprehensibility and availability of data. Consequently, this article is poised to offer benefits to cytogeneticists, scholars, or individuals keen on gaining further knowledge regarding the complexities associated with chromosome analysis.

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