Techniques in Molecular Cytogenetics

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Cytogenetics is the branch of genetics which deals with the study of cell, structure and function of the chromosomes. Molecular cytogenetics involves the combination of Molecular biology and Cytogenetics. Molecular cytogenetical techniques are used to detect chromosomal aberrations caused due to variation chromosome number, chromosome size, number of chromosome segments, variation in chromosome morphology.

Advanced techniques in molecular cytogenetics includes

(1) Karyotyping
(2) Chromosome Banding Techniques
(3) Fluorescence In Situ Hybridization (FISH)
(4) Genomic In Situ Hybridization (GISH)
(5) Comparative Genomic Hybridization
(6) Spectral Karyotyping
(7) Virtual Karyotyping
(8) Chromosomal Painting

Karyotyping

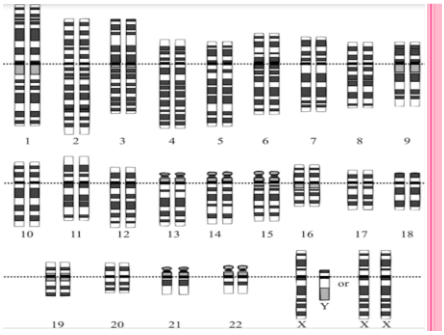
Karyotyping is the process of pairing and ordering all the chromosomes of an organism, thus providing a genome wide snapshot of an individual's chromosomes. Main purpose of the karyotyping is to locate or visualize the changes in the number of chromosomes and abnormality in the structure. Karyotype gives information on size of the chromosome, position of centromere, presence of secondary constriction and size of satellite. When haploid set of chromosomes of an organism are ordered in a series of decreasing size, it is said to be an Idiogram. Grygorii Levitsky seems to have been the first person to define the karyotype as the "phenotypic appearance of somatic chromosomes in contrast to their gene contents".

Types of karyotype

1.Symmetric Karyotype: Show lesser difference between smaller and larger chromosomes in a set. Have more metacentric chromosomes.

2. Asymmetric Karyotype: Show larger difference between smaller and larger chromosomes in a set. Have more acrocentric chromosomes.

Representation of karyotype: By arranging chromosomes of somatic complement in a descending order of size keeping their centromeres in a straight line. Longest chromosome –on extreme left. Shortest chromosome-on extreme right. Sex chromosomes-Allosomes –extreme right.



Karyotype variations

1.Variations in karyotype among different species within the same genera observed in several herbaceous genera possessing medium to large chromosomes in size. Ex. Crepis genus -Degree of symmetry and Chr. No. are negatively associated. *C. capillaris, C.sibirica*- large chromosomes and *C.leotodontoides, C.fuliginosa*- smaller chromosomes.

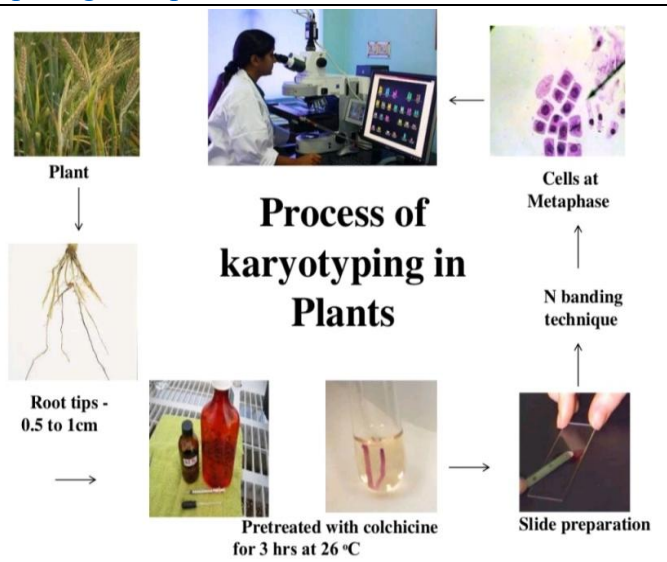
2. Spp with small chromosome asymmetry high. eg: In Clarkia asymmetry increased with increase in Chromosome number.

Factors influencing Karyotype variation

- Absolute size-Duplications
- Centromere position- Unequal translocations and pericentric inversions
- Relative size change- Segmental interchanges
- Basic no may be reduced due to unequal translocations, loss of centromere. Increase in chr. no. by addition of centric fragments and transfer of essential gene loci to them can be clearly understood in species with multiple sex chromosomes- *Rumex*
- No and position of satellites: Location and size of the nucleolar organiser regions may differ. Heterochromatic regions- may be scattered or localised.

Procedure For Karyotyping

1. Cell fixed in metaphase
2. Chromosome stained
3. Pictures taken
4. Enlarged
5. Cut out
6. Matched by banding techniques
7. Arrange them in order



Advantages of Karyotyping

- Reveals structural features of each chromosome.
- Helps in studying chromosome banding pattern.
- Helps in identification of chromosomal aberrations.
- Diagnosis of prenatal genetic defects.
- Aids in studying evolutionary changes.
- Correct size and shape of chromosomes can be visualized in this technique.
- We can identify gender of unknown sample by this technique.

Idiogram

Diagrammatic/ graphical representation of a karyotype (haploid complement) is an Idiogram. It is Prepared from measurements of somatic metaphase chromosomes. Now Individual chromosomes can be identified by Florescent staining, pulse labelling, banding, study of tertiary constrictions and chromomeres

Chromosomal Banding Techniques

BAND

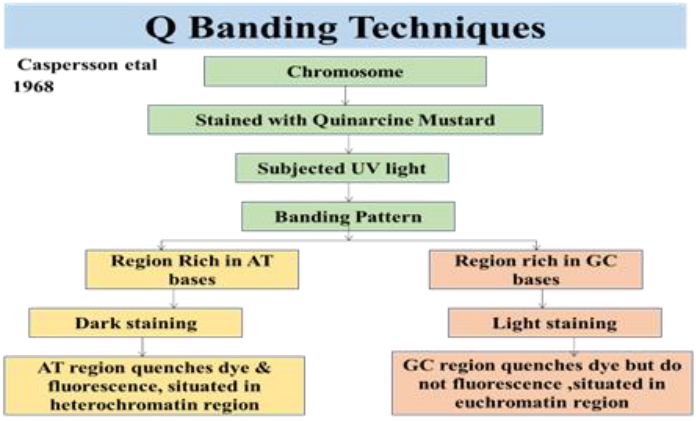
A part of chromosome which is clearly distinguishable from its adjacent segments by appearing darker or lighter with various banding methods. In 1958 Casperson *et al.*, published first paper describing the use of Quinacrine mustard to stain chromosome there by ushered in a new era of chromosome banding. Chromosomal banding allows to see smaller pieces of the chromosomes so that you could identify smaller structural chromosomes abnormalities not visible on a routine analysis**.** Always metaphase chromosomes whose size has condensed and whose diameter is increased are used for chromosome banding studies after fixing the stage.

Different banding techniques of chromosomes

- Q - Banding
- G - Banding
- R - Banding
- C - Banding
- T - Banding
- NOR - Banding
- High Resolution Banding
- Restriction Endonuclease Banding

1.Q-banding

It was first described by Casperson *et al* in 1968 and revived the search for dependable morphological characteristics for differentiation of mitotic metaphase chromosomes. This banding pattern is obtained by treating the dividing cells with fluorochrome. They can be identified by yellow fluorescence of different intensity. It stains most readily the heterochromatic region. Quinacrine binds those regions which are rich in A-T & G-C but fluorescence's only A-T quinacrine regions. Since A-T regions are seen more in heterochromatic stained more readily than euchromatin.



Advantages: It is Simple and Versatile. Used where G band is not accepted. Helps to study of chromosome heteromorphism.

Disadvantages Tendency to fade during examination. Photo-degradation. Chromopore- absorb light of a particular wavelength due to a chemical bond formed between dye and light. UV light breaks the chemical bond

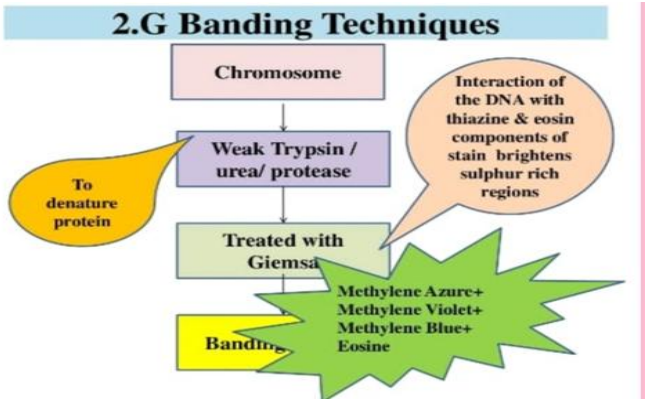
2. G-banding

Giemsa staining following pretreatment with trypsin solution, urea, protease. It gives greater details than C- banding. It was first reported by Summer *et al.* 1971 in humans. G bands- reflect stronger chromatin condensation.

Advantages: Used in identification of bands rich in Sulphur content, chromosomal abnormalities and helps in gene Mapping.

Disadvantages

It is not suitable for plant chromosomes



G banding not used in Plants. Why????

- Plant chromosomes in metaphase more DNA than G banding vertebrate of comparable length. At a such degree of condensation, contraction, even vertebrate chromosomes would not show G bands –optical reasons. The striking correspondence of pachytene chromomeres and mitotic G bands – G band equivalents. Human mitotic metaphase chromosome is 2.3 times shorter. Plant mitotic metaphase chromosome is 10 times shorter than human chromosome. Hence difficult to demonstrate the arrangement of bands at this level of saturation with G banding technique.

3. N Banding Techniques

Islam 1980- used to this technique to identify barley chromosome from wheat chromosomes and Wheat-barley hybrids. He also used in wheat substitution lines by a pair of barley chromosomes. N bands located at NOR region (Secondary constriction), satellites, cesntromere, and heterchromatic region. A modifed Giemsa N banding – Singh and Tsuchiya 1982- identification of barley chromosomes. Combination of acetocarmine staining and Giemsa N banding. Centromeric region looks like a diamond shaped structure. Early metaphase or prometaphase chromosomes are more suitable for this staining.

Advantages: Used in the identification of Nucleolar organizer region and it is a superior banding pattern for plants.

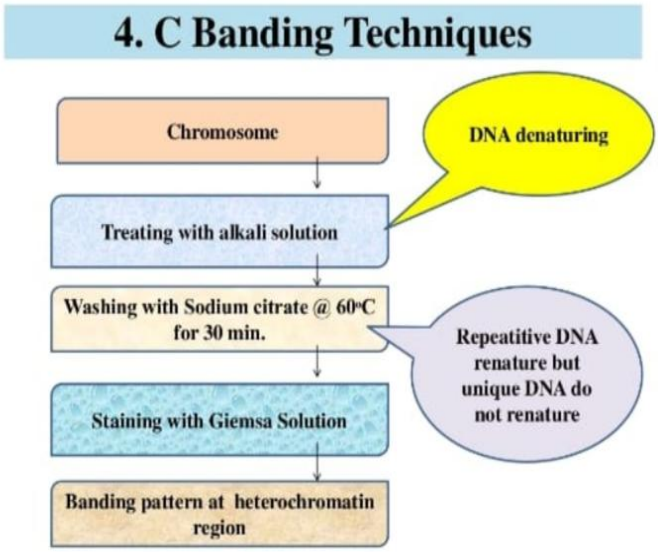
4. C-banding

C-banding represents the constitutive heterochromatin. Pardue and Gall reported constitutive heterochromatin staining by Giemsa solution. Constitutive heterochromatin presents in centromere, telomere and NOR. It is a highly repetitive DNA. Differential staining reactions of heterochromatin and euchromatin. Complex technique as it involves several treatments with acids, alkali, increased temperature for Denaturation of DNA.DNA renaturation in treatments with sodium citrate at 60 degrees Celsius. Repetitive DNA renatures, low repetitive and unique DNA do not renature. Differential staining occurs useful in identification of various plant, animal chromosome and particular Y chromosome in Humans which is the heterochromatic

In barley, Linde-Laursen, 1978 divided C bands based on their position into:

1. Centromeric bands
2. Telomeric bands
3. Intercalary bands
4. Bands adjacent to secondary constrictions in the short arm of satellite chromosome

He observed polymorphism in banding pattern in different barley lines. Through Giemsa C-banding extrachromosomes of trisomics and telotrisomics can be identified.



Advantages

- Identification of chromosomes particularly in insects and plants. Identification of bivalents at diakinesis using both centromere position. Paternity testing. Gene mapping.

5. R Banding Techniques

R-Bands shows a pattern that is reverse of G bands i.e., light banded regions of G banded chromosomes become darkly stained and vice versa

6. T Banding Techniques

It was developed by Dutrillaux and which involves the using of high temperatures (87° c) & pH at 6.7 followed by giemsa staining. It shows especially a staining of some terminal regions of chromosomes. The application of this technique to translocations allows the precise of juxta –telomeric break point

Fluorescence In-situ Hybridization: (Fish)

It is process of painting the whole chromosome or only part of the chromosomes (protein) with florescence molecule to identify chromosomal abnormality and presence of a region of DNA or RNA within the chromosome. FISH is often used for finding specific features in DNA for use in genetic counselling, medicine and species identification.

Procedure

Step 1: Denaturation of chromosome: Conversion of double stranded DNA into single stranded DNA

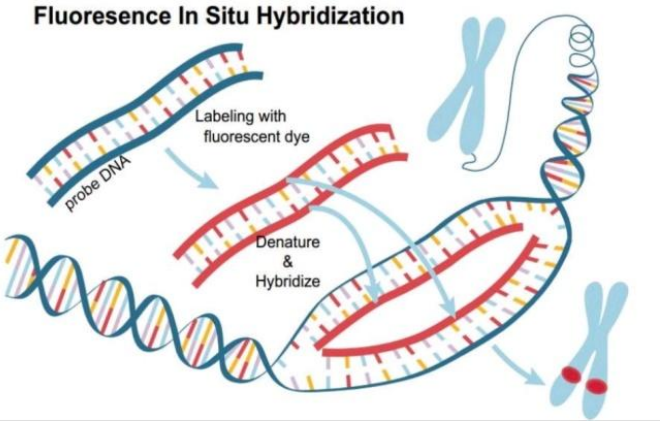
Step 2: Denaturation of probe

Step 3: Hybridization: It is the formation of duplex between 2 complementary sequences. Application of probe DNA to slide and overnight incubation at 37C. Binding of probe DNA to target DNA.

Step 4: Post hybridisation washing and detection: Washing of unbound probe DNA.

Step 5: Fluorescence staining: Application of Fluorescence stain.

Step 6: Visualization: Examination of the slide using fluorescence microscopy or store in dark.



Advantages

1. Quick and correct results save time (48-72 hr.) and money by preventing unnecessary additional diagnostics and suboptimal treatment approaches.

2. Less labour-intensive method for confirming the presence of a DNA segment within an entire genome than other conventional methods

3 Identification of marker chromosomes and gene deletions

4. It can detect numerous abnormalities (gain, losses of whole chromosome or deletion/duplication) superior to PCR.

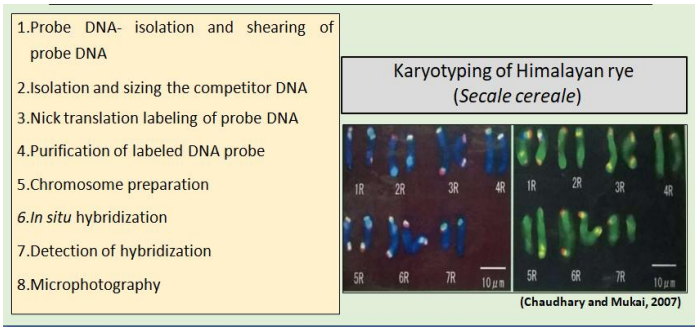
Genomic In Situ Hybridization (Gish)

Genomic in situ hybridization (GISH) is a cytogenetic technique that allows one to radiolabel parts of genome with in the cells. GISH was mainly developed for animal hybrid cell lines (1986) and later used for plants at plant breeding institute, Cambridge (1987), where this technique got its name. The GISH is quick, sensitive, accurate, informative and a comparative approach rather than absolute one. It is an advancement in the fluorescence in-situ hybridization

Principle

The technique involves the extraction and sub sequentially radio labelling of whole DNA of one organism and it is used as a probe to target the genome of another organism. The parts of genome that are sufficiently similar to the probe –target complex which is now labelled.

The main steps involved in the genomic in situ hybridization are



Comparative Genomic Hybridization (Cgh)

Comparative genomic hybridization is a molecular cytogenetic method for analysing copy number variations with the help of hybridization techniques.

Procedure

1. Metaphase Slide Preparation
2. Isolation of DNA from test tissue and reference tissue
3. Selection and application of probe
4. Hybridization
5. Fluorescence detector image analysis and software analysis

Applications

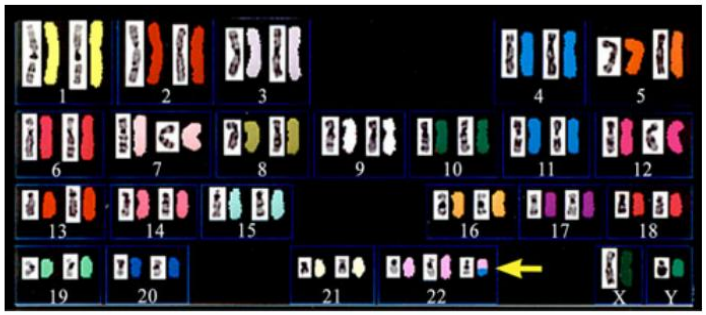
- Chromosome mapping
- Genome analyse
- Determination of phylogenetic relationships.
- Determine the positions of translocation breakpoints.
- Interspecific and intergenic crosses
- Detection of chromosomal aberration
- Spectral karyotyping (SKY) and multiple fluorescent hybridization
- Unknown genomic identification

Spectral Karyotyping

Spectral karyotyping is a cytological technique used to simultaneously visualize all pairs of chromosomes in an organism. The SKY technique is

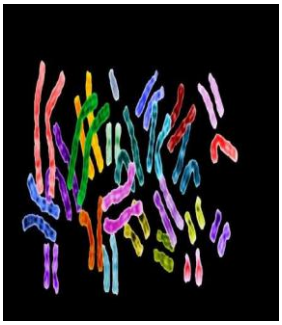
useful for identifying chromosomal abnormalities. We can arrange the chromosomes according to their number just by visualization of different colours acquire by the chromosomes. SKY can discern the aberration that can't be detected very well by conventional banding techniques and FISH. It allows visualization of all chromosomes in different colours on same platform which is very easy to detect chromosomal abnormalities. The procedure:

1.Chromosome preparation from tissue
2.Denaturation of DNA
3.Probe Denaturation
4.Hybridization
5.Application of Dye
6.Washes and detection
7.Image Acquisition
8.Visualization



Chromosome Painting

Chromosome painting refers to the hybridization of fluorescently labelled chromosome specific, composite probe pools to cytological preparations. First termed by Pinkel et al. (1988). Chromosome painting coupled with Fluorescence in situ hybridization (FISH) is used routinely for identification of chromosome. Helps in the identification of chromosomal rearrangements, chromosomal breakpoints and determination of extra chromosomal material.



Multiplex in situ hybridization

M-fish is a 24-color karyotyping technique and method of choice for studying complex intrachromosomal rearrangements. Secondly, the visualization of digital acquisition of each fluorophore using specific single band pass filter sets and dedicated M-FISH software.

Conclusion

- Chromosome genetic abnormalities are the hidden cause for the huge economic loss.
- Molecular cytogenetic techniques like FISH, CGH and SKY are the available advanced diagnostic tools to detect such chromosomal abnormalities.

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