الجامعة المستنصرية

كلية العلوم

قسم علوم الحياة

المرحلة الثالثة

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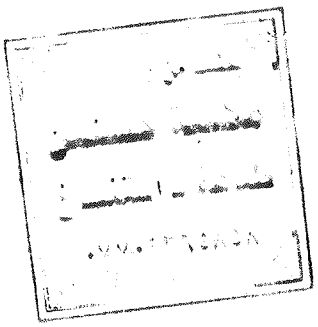

وراثة خلوية–عملي

CYTOGENETICS

LAB- 7-8

للعام الدراسي

يطلب حصريا من

مكتبة حسنين

السعر: 500

G-Banding

Chromosomes are G-banded to facilitate the identification of structural abnormalities.

Procedure

1. Prepare the staining solution the day prior to use. Also, slides should be aged at least 7-10 days or placed in a 55-65°C oven for 45 minutes before staining, to ensure excellent banding patterns. Aging the slides helps to eliminate fuzzy banding and increases contrast of the bands.

2. Exact timing is important; therefore, a maximum of 5 slides should be stained at one time. Optimum time in the stain appears to be between 2.5-4 minutes. It is necessary to determine the approximate staining times for each bottle of stain solution. The exact time will vary by several seconds depending on the source of cells, age of slides, the cell concentration on the slide, etc. (refer to the table below).

	Trypsin Time (seconds)	Staining Time (minutes)
Cell Source		
Lymphoblastoid	30	4.0
Blood Lymphocytes	15	3.0
Age of Oven Dried Slides		
0-3 days	15	3.0
3-20 days	30	3.5
20+ days	45	4.0
Previously Banded	45	4.0
Cell Concentration		
< 20 mitosis	15	3.0
20-50 mitosis	30	3.5
50+ mitosis	45	4.5

3. Mix 1ml 0.25% stock trypsin with 49ml 0.85% NaCl. Wait 4 minutes before beginning to stain to allow the trypsin to dissolve.

4. Dip oven-dried slides that have cooled to room temperature in the trypsin solution for 5-30 seconds. The time in trypsin is dependent on slide preparation conditions, harvesting conditions, material being banded, etc. Stain a test slide first to determine optimum conditions.

5. Rinse slides in 50ml working salt solution.

6. Stain the slides for 3-4 minutes with Giemsa stain.

7. Rinse slides in distilled water and air dry.

8. Check the slides with microscope, 100X oil objectives.

   - Over-trypsinized chromosomes appear fuzzy; somewhat difficult to recognize exact bands.

   - Under-trypsinized chromosomes will have indistinct bands, decreased contrast; very difficult or impossible to determine bands.

   - Adequately trypsinized chromosomes will show telomeres not overly digested and G-Bands will appear sharp.

9. If slides are under-treated with trypsin, destain the slides before rebanding. Quickly dip the slides in 3:1 methanol : acetic acid 2-3 times, or until all stain is removed, rinse in distilled water, air dry and reband.

✓ Be careful not to over rinse slides since over rinsing will fade stain.

✓ Wright's stain or Leishman's stain may be used in place of Giemsa stain.

C-Banding

To specifically stain the centromeric regions and other regions containing constitutive heterochromatin, i.e., the secondary contrictions of human chromsomes 1, 9, 16, and the distal segment of the Y chromosome long arm.

Procedure

1. Treat the slides in 0.2 N HCl for one hr at room temperature.
2. Rinse in de-ionized water.
3. Immerse in 1% barium hydroxide at 50°C for 5-15 min.
4. Rinse in deionized water.
5. Incubate at 60°C in 2XSSC buffer for one hr.
6. Rinse in deionized water and stain in 4% Giemsa stain for 90 min.
7. Rinse in deionized water, dry and examine under oil immersion.

Q-banding

Chromosomes are treated with quinicrine mustard solution, a fluorescent stain, to identify specific chromosomes and structural rearrangements.

Procedure

1. Dehydrate the slides by dipping in alcohol with decreasing concentration 90%, 70% and 50% one minute each.
2. Rinse in distilled water.
3. Wash the slide in phosphate buffer at pH 6.8
4. Stain the slide in quinacrine mustard (5%) or in quinacrine dihydrochloride (5%) for 20 min.
5. Rinse in phosphate buffer.
6. Examine under fluorescent microscope.

R-banding

R-banding methods are useful for analyzing deletions or translocations that involve the telomeres of chromosomes.

Procedure

1. Age the slides for 7 -10 days.
2. Place the slides in a Coplin jar containing phosphate buffer of pH 6.5 at 85°C and incubate for 20-25 min.
3. Stain the slides in 0.01% acridine orange in the phosphate buffer pH 6.5 for 4-6 min. Rinse in phosphate buffer and mount in the same buffer.
4. Examine under fluorescent microscope.

T -banding

T-banding is used to stain the telomeric regions of chromosomes for cytogenetic analysis.

Procedure

1. Age the slide for 7 days.
2. Place the slides in PBS pH 5.0 for 20-60 min at 87°C.
3. Rinse in PBS.
4. Stain in 3% Giemsa in phosphate buffer pH 6.8 at 87°C, leave for 5-30 min and rinse.
5. Slides are stained in Hoechst 33258 stain for 10 min (Hoechst stain 0.5 pg/ml of phosphate buffer). Rinse in phosphate buffer and examine in fluorescent microscope.
6. Alternatively, the stained slides are covered with a cover slip and placed in a wet chamber under UV lamp for 2 to 3 hrs or under direct sunlight for 2 hrs.
7. Remove the cover slip and stain in Giemsa stain for 10 min.
8. Rinse in buffer, dry and mount in DPX.

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Chromosome karyotyping

Ayat M. Al-Sudany

A karyotype is an organized profile of a person's chromosomes. In a karyotype, chromosomes are arranged and numbered by size, from largest to smallest. This arrangement helps scientists quickly identify chromosomal alterations that may result in a genetic disorder.

To make a karyotype, scientists take a picture of someone's chromosomes, cut them out and match them up using size, banding pattern and centromere position as guides.

Procedure:

1. Count the number of chromosomes. Solid stained chromosomes or chromosomes treated with a trypsin and giemsa stain can be counted at the microscope with a 100X magnification. However, for analysis such as identification of marker chromosomes or determination of the number of copies of individual chomosomes, it is usually necessary to photograph and print the chromosome spreads. Two prints should be made of each spread. One will be cut for karyotyping; the uncut print serves as a reference if questions arise about the interpretation of a certain chromosome.

2. Cut out each individual chromosome and arrange on a karyotype sheet. Chromosomes are ordered by their length, the position of the centromere, the position of the chromosome bands, and the relative band sizes and distributions.

3. In the construction of the karyotype the autosomes are numbered 1 to 22, in descending order of length. The sex chromosomes are referred to as X and Y. The symbols p and q are used to designate, respectively, the short and long arms of each chromosome.

4. Secure chromosomes in place with glue. Pair the chromosomes closely together and align the centromeres (for easier band comparison and checking for structural chromosome aberrations).

5. A description of the karyotype should be recorded on the karyotype sheet. First record the number of chromosomes, including the sex chromosomes, followed by a comma (,). The sex chromosome constitution is given next. Any structural rearrangements and additional or missing chromosomes are listed next. Other information such as the cell line number, the date karyotype was prepared, the specimen type, and the technologist also should be recorded on the karyotype sheet.

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Chromosomal Abnormalities

Ayat M. Al-Sudany

Chromosome abnormalities describe alterations in the normal number of chromosomes or structural problems within the chromosomes themselves. Both kinds of chromosome abnormalities may result from an egg (ovum) or sperm cell with the incorrect number of chromosomes, or with a structurally faulty chromosome uniting with a normal egg or sperm during conception.

Some chromosome abnormalities may occur shortly after conception. In this case, the zygote, the cell formed during conception that eventually develops into an embryo, divides incorrectly. Other abnormalities may lead to the death of the embryo. Zygotes that receive a full extra set of chromosomes, a condition called polyploidy, usually do not survive inside the uterus, and are spontaneously aborted (a process sometimes called a miscarriage).

Chromosomal abnormalities can cause serious mental or physical disabilities.

There are two major categories of chromosomal abnormalities:

1. irregular number of chromosomes
2. structural modification in a chromosome

Numerical chromosomal abnormalities

Polyploidy

Between 1 and 3% of recognized human pregnancies are triploid. The most usual cause is two sperm fertilizing a single egg (dispermy); sometimes the cause is a diploid gamete. Triploids very seldom survive to term, and the condition is not compatible with life. Tetraploidy is much rarer and always lethal. It is usually due to failure to complete the first zygotic division: the DNA has replicated to give a content of 4C, but cell division has not then taken place as normal. Although constitutional polyploidy is rare and lethal, all normal people have some polyploid cells.

Aneuploidy

Euploidy means having complete chromosome sets (n, 2n, 3n, etc.). Aneuploidy is the opposite, that is, one or more individual chromosomes extra or missing from a euploid set. Trisomy means having three copies of a particular chromosome in an otherwise diploid cell, for example trisomy 21 (47, XX or XY, + 21) in Down syndrome. Monosomy is the corresponding lack of a chromosome, for example monosomy X (45, X) in Turner syndrome. Cancer cells often show extreme aneuploidy, with multiple chromosomal abnormalities. Aneuploid cells arise through two main mechanisms:

- **Nondisjunction:** failure of paired chromosomes to separate (disjoin) in anaphase of meiosis I, or failure of sister chromatids to disjoin at either meiosis II or at mitosis. Nondisjunction in meiosis produces gametes with 22 or 24 chromosomes, which after fertilization by a normal gamete make a trisomic or monosomic zygote. Nondisjunction in mitosis produces a mosaic.
- **Anaphase lag:** failure of a chromosome or chromatid to be incorporated into one of the daughter nuclei following cell division, as a result of delayed movement (lagging) during anaphase. Chromosomes that do not enter a daughter cell nucleus are lost.

Sex Chromosome Abnormalities

Genotype	Gender	Syndrome	Physical Traits
XXY, XXYY, XXXY	male	Klinefelter syndrome	sterility, small testicles, breast enlargement
XYY	male	XYY syndrome	normal male traits
XO	female	Turner syndrome	sex organs don't mature at adolescence, sterility, short stature
XXX	female	Trisomy X	tall stature, learning disabilities, limited fertility

Structural chromosomal abnormalities

Abnormalities in chromosome structure follow a chromosome break and, during the repair process, the reunion of the wrong segments of the chromosome. If, following repair, there is a loss or gain of chromosomal material (an **unbalanced rearrangement**) there can be significant clinical consequences. If there is no loss or gain of chromosomal material (a **balanced rearrangement**), then the individual is mentally and physically normal. However, there is an increased risk of having chromosomally abnormal offspring because individuals who carry balanced chromosome rearrangements may produce chromosomally unbalanced gametes.

Types of structural chromosomal abnormalities:

Chromatid break

Breakage of single chromatids.

Chromosome break

Breakage of both chromatids at an identical site.

Dicentric chromosome

When the broken end of one chromosome may join with a broken end of another, resulting in the formation of a dicentric chromosome. A dicentric chromosome is one that contains two centromeres instead of one normally present in one chromosome.

Acentric

Fragment when centromere is lost, an acrocentric chromosome is created which is usually lost during meiosis (death of cell or loss of fertility).

Gap

Is a chromatic lesion smaller than the width of one chromatid, and with minimum misalignment of the chromatids.

Ring chromosome

Could happen in two ways. First the end of the p and q arm breaks off and then stick to each other resulting in loss of information. Second, the ends of the p and q arm stick together (fusion), usually without loss of material.

Delesions

Deletion refers to the loss of a segment of a chromosome. This can be **terminal** (close to the end of the chromosome on the long arm or the short arm), or it can be **interstitial** (within the long arm or the short arm). Deletions have been described on all chromosomes. A deletion can occur on any chromosome, at any band, and can be any size (large or small). What a deletion causes depends on how big a piece is missing and what genes are missing in the section.

Duplications

Duplication refers to an extra chromosomal segment within the same homologous chromosome or an extra chromosomal segment on another nonhomologous chromosome.

Translocations

Translocations: When a portion of one chromosome is transferred to another chromosome. There are two main types of translocations.

- In a **reciprocal translocation**, segments from two different chromosomes have been exchanged.

- In a **robertsonian translocation**, involving the reciprocal transfer of the long arms of two of the acrocentric chromosomes: 13, 14, 15, 21 or 22 (on rare occasions, other non-acrocentric chromosomes undergo Robertsonian translocation), a transfer of the whole long or short arms close to the centromere.

Inversions

Inversions involve only one chromosome in which two breaks occur and, in the process of repair, the intervening segment is rejoined in an inverted or opposite manner. Since there is no loss or gain of chromosomal material, inversion carriers are normal.

An inversion is **paracentric** if the inverted segment is on the long arm or the short arm and does not include the centromere. The inversion is **pericentric** if breaks occur on both the short arm and the long arm and the inverted segment contains the centromere.

Isochromosome

Formed by the mirror image copy of a chromosome segment including the centromere.

How do chromosome abnormalities happen?

Chromosome abnormalities usually occur when there is an error in cell division mitosis and meiosis. In both processes, the correct number of chromosomes is supposed to end up in the resulting cells. However, errors in cell division can result in cells with too few or too may copies of a chromosome. Errors can also occur when the chromosomes are being duplicated.

Other factors that can increase the risk of chromosome abnormalities are:

- *Maternal Age:*

  Women are born with all the eggs they will ever have. Therefore, when a woman is 30 years old, so are her eggs. Some researchers believe that errors can crop up in the eggs' genetic material as they age over time. Therefore, older women are more at risk of giving birth to babies with chromosome abnormalities than younger women. Since men produce new sperm throughout their life, paternal age does not increase risk of chromosome abnormalities.

- *Environment:*

  Although there is no conclusive evidence that specific environmental factors cause chromosome abnormalities, it is still a possibility that the environment may play a role in the occurrence of genetic errors.