CHROMOSOMES

Dr. Fern Tsien, Dept. of Genetics, LSUHSC, NO, LA

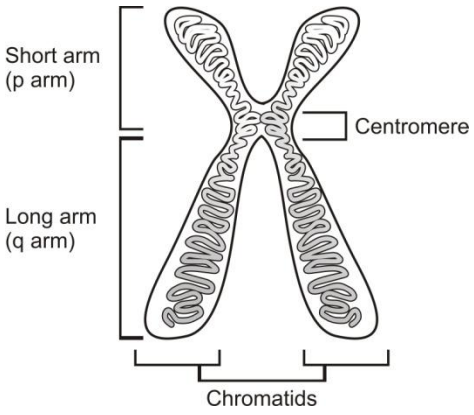
Cytogenetics is the study of chromosomes and their structure, inheritance, and abnormalities.

Chromosome abnormalities occur in approximately:

- 1 per 150 live births
- 60-80% of all miscarriages
- 10% of stillbirths
- 13% of individuals with congenital heart disease
- most types of cancer
- in many patients with developmental delay, birth defects, abnormal sexual development, and/or mental retardation
- 3-6% in cases of infertility

What are chromosomes?

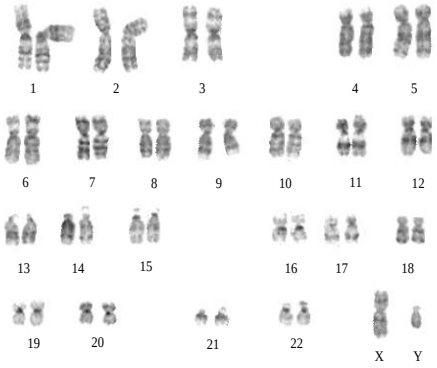
A chromosome is the organized form of DNA found in cells. After DNA replication, each chromosome contains two chromatids which are joined at the centromere.



Chromosome structure. A chromosome is made up of DNA and held together at the centromere.

We have a total of 46 chromosomes (23 pairs) in each of our cells; 23 originate from our father's sperm and 23 from our mother's egg. Two of these are the sex chromosomes, X and Y. The rest of the chromosomes are called autosomes. A normal female karyotype has two X chromosomes and a normal male has one X and one Y chromosome. The organized chromosome pattern in each cell is called a **karyotype**. This is organized during chromosome analysis in the cytogenetics laboratory.

G-banded metaphase spread from a cell of a male individual as seen through the light microscope.



G-banded karyotype of a male individual showing the normal number of 46 chromosomes, including the X and Y chromosomes.

COMMON TECHNIQUES UTILIZED IN CYTOGENETIC DIAGNOSIS

The fresh sample is collected, and the cells are cultured, or allowed to grow under normal body conditions (in a solution containing vitamins, serum, and normal amino acids at body temperature of 37°C/98°F). The cells are collected after approximately 72 hours, and the chromosomes isolated at the metaphase stage of the cell cycle, when they are most condensed and therefore more clearly visible.

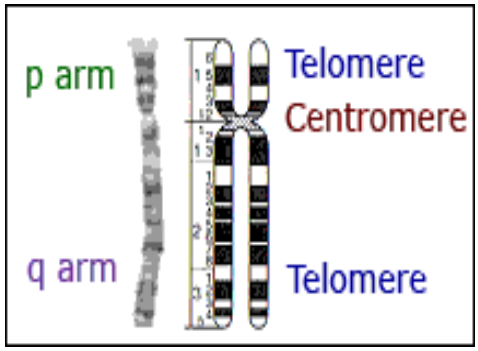
Types of samples obtained for chromosome analysis can include:

1. Blood sample
    - to evaluate the cause of birth defects, mental retardation, or developmental delay.
    - to detect the cause of infertility or repeated miscarriages.
    - to evaluate couples with chromosomally abnormal children.
    - to evaluate women who aren't menstruating.
    - to examine abnormal sexual development, particularly when there is doubt about true gender.
2. Amniotic fluid or chorionic villus sampling to detect chromosomal disorders in the fetus.
3. Bone marrow or tumor biopsy samples to diagnose certain cancers or evaluate their course and the effectiveness of treatment.
4. Miscarriage tissue to determine the cause of the pregnancy loss.

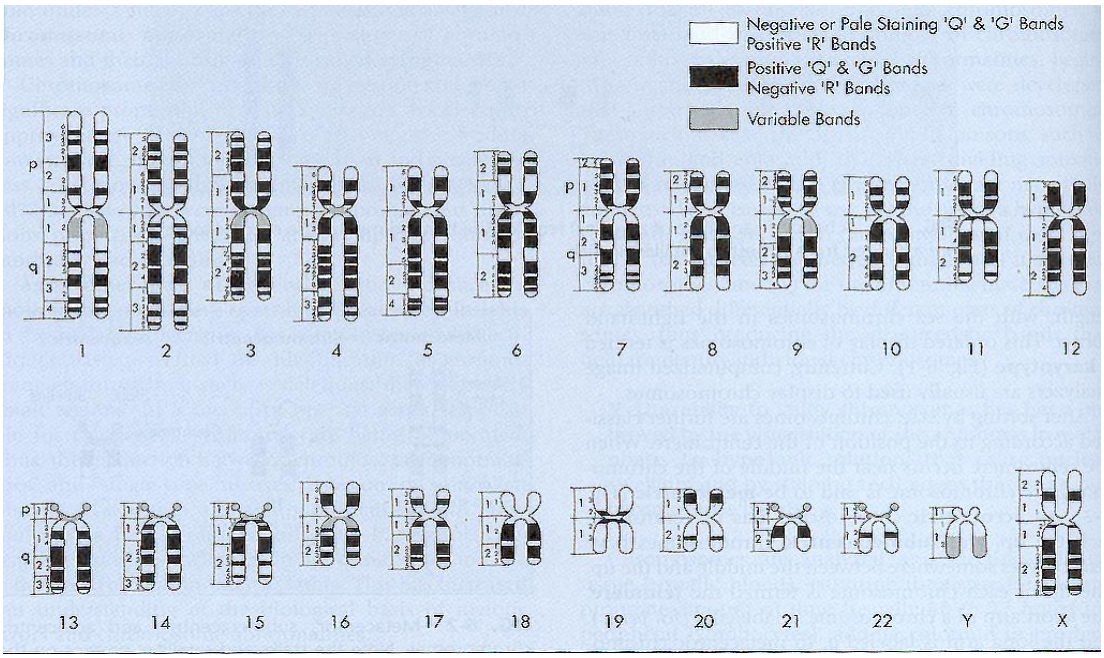
Once the chromosomes are obtained, they need to be further processed with one or several staining techniques. The entire process takes from one week up to three weeks if multiple tests are performed following cell culture.

G-banding

Giemsa banding (G-banding) is the standard technique used to identify of individual chromosomes by producing characteristic light and dark bands. Giemsa banding involves the treatment of the cells on the slides with trypsin followed by Giemsa stain. Chromosomes are classified by the position of the centromere in relation to the chromosome. The short arm is called the “p” or petite arm, and the long arm is the “q” arm. All chromosomes have a centromere and two telomeres at the ends of the chromosomes. In a chromosome lab report, abnormalities often have a description of the piece of chromosome which is affected.

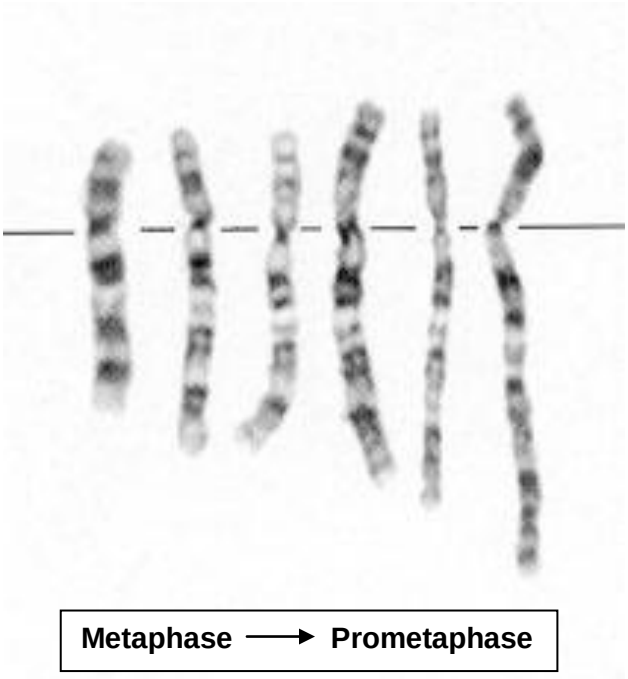


Each individual chromosome can be identified by its characteristic banding pattern. An **ideogram** is a chromosome map which describes specific regions of each of the chromosomes. A classification system has been established in which each chromosome and chromosome band is assigned a number, starting from the centromere and moving towards the chromosome tip. All cytogenetic reports sent to the doctor from obtained from the chromosome lab are written using this International System for Human Cytogenetic Nomenclature (ISCN), a system which is continuously updated.

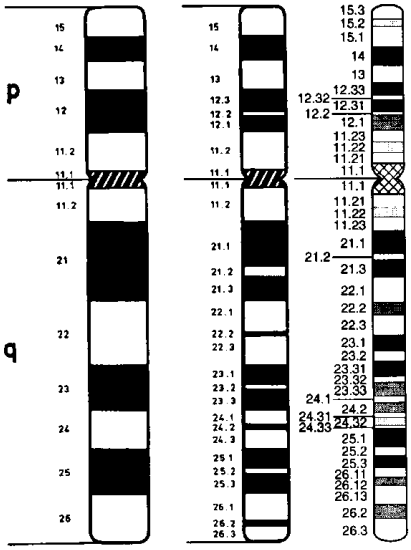


Ideogram or “chromosome map”.

Prometaphase banding, or high resolution banding, is sometimes used by the chromosome lab to look for small abnormalities. This technique provides more accurate visualization of chromosomes. Bands are divided into sub-bands, and are therefore used for detection of microdeletion syndromes and other small chromosome rearrangements. The procedure is longer to perform and therefore it takes at least two weeks for the doctor to receive the results.



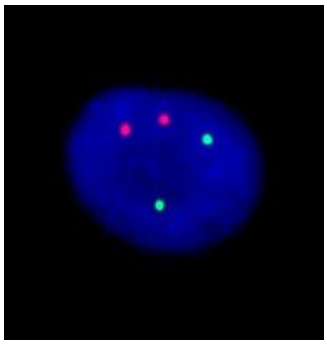
Metaphase ⟶ Prometaphase



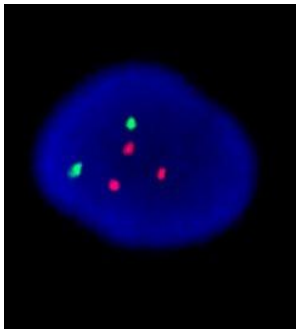
Metaphase ⟶ Prometaphase

Chromosome 10 at metaphase and various lengths leading to the prometaphase stage (high resolution), demonstrating the division of bands into sub-bands, allowing the detection of small rearrangements..

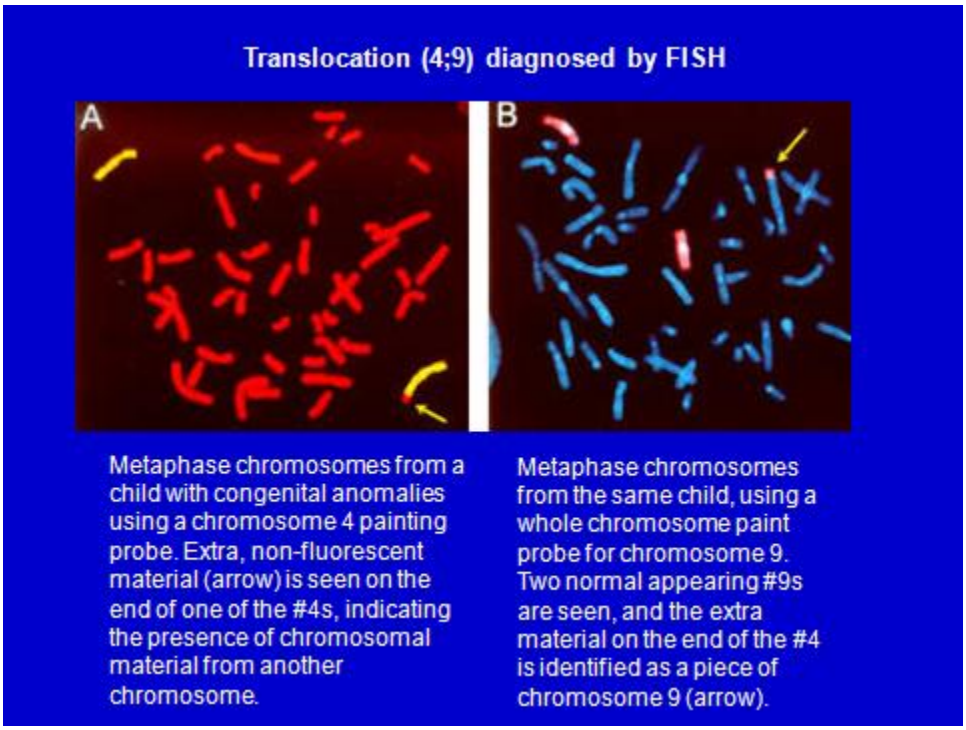
Fluorescence *in situ* hybridization (FISH) is a procedure which combines basic principles of molecular biology and cytogenetics. Fluorescently-radioactively labeled fragments of DNA or genes of interest (the probe) are allowed to find their match on the patient's metaphase or interphase cells directly on the microscope slide. This procedure provides a much higher degree of resolution than classical cytogenetics. FISH is frequently used for fast prenatal diagnosis if a particular abnormality is suspected based on the ultrasound, when a specific microdeletion syndrome is suspected, and for cancer diagnosis and follow-up.



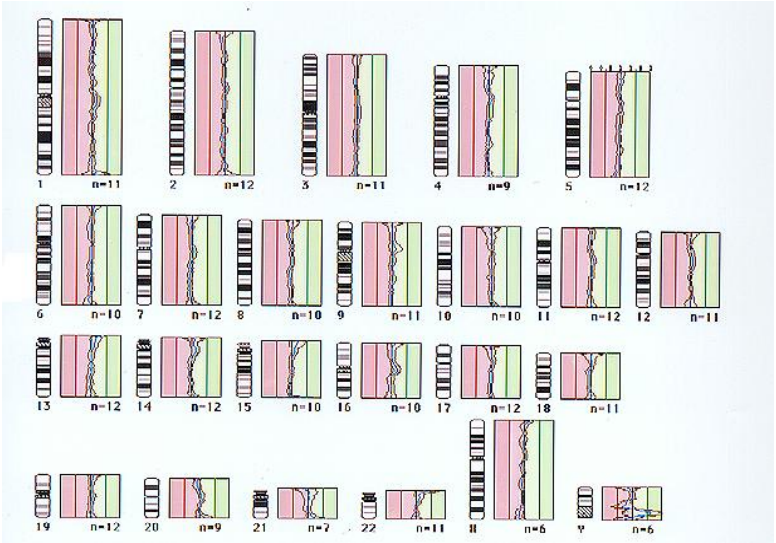
Fluorescence *in situ* hybridization (FISH) of a normal interphase cell from a fetus, using probes for chromosome 13 (green) and chromosomes 21(red)



Fluorescence *in situ* hybridization (FISH) of an interphase cell from a fetus with Trisomy 21 (Down syndrome) using probes for chromosome 13 (green) and chromosomes 21(red).



Comparative Genomic Hybridization (CGH) is a procedure which allows determination of DNA sequence copy number changes throughout the entire genome. Fluorescently-labeled DNA extracted from clinical samples is used as a probe. This DNA is mixed with normal labeled reference DNA and hybridized to normal metaphase spreads. The laboratory utilizes a specific computer program to view the ratio between the sample DNA (green) and the reference DNA (red) to determine gains or losses of DNA. It is used to detect amplifications, deletions, and chromosome gains and losses, especially for cancer cytogenetic studies.



How accurate is ch

Comparative Genomic Hybridization (CGH) showing a normal red vs. green profile.

Cytogenetic analysis is highly accurate. Not just one cell, but at least 20 cells are examined whenever a chromosome analysis is done. For cancer analysis, 100 interphase cells are routinely scanned. This is to determine whether only a few or all the cells have the same chromosome pattern. The analyses are performed by highly skilled cytogenetic technologists with multiple years of experience. Laboratory directors and supervisors are licensed, and the laboratories must pass periodic inspections and proficiency testing.

However, since genetic conditions may also be caused by changes that are too small to be seen under a microscope, normal results of chromosome analysis do not guarantee that there are no genetic problems. The geneticist can discuss these issues more thoroughly, on an individual basis, after obtaining a detailed family history and reviewing any relevant medical information.