

# Karyotype & Chromosome banding pattern

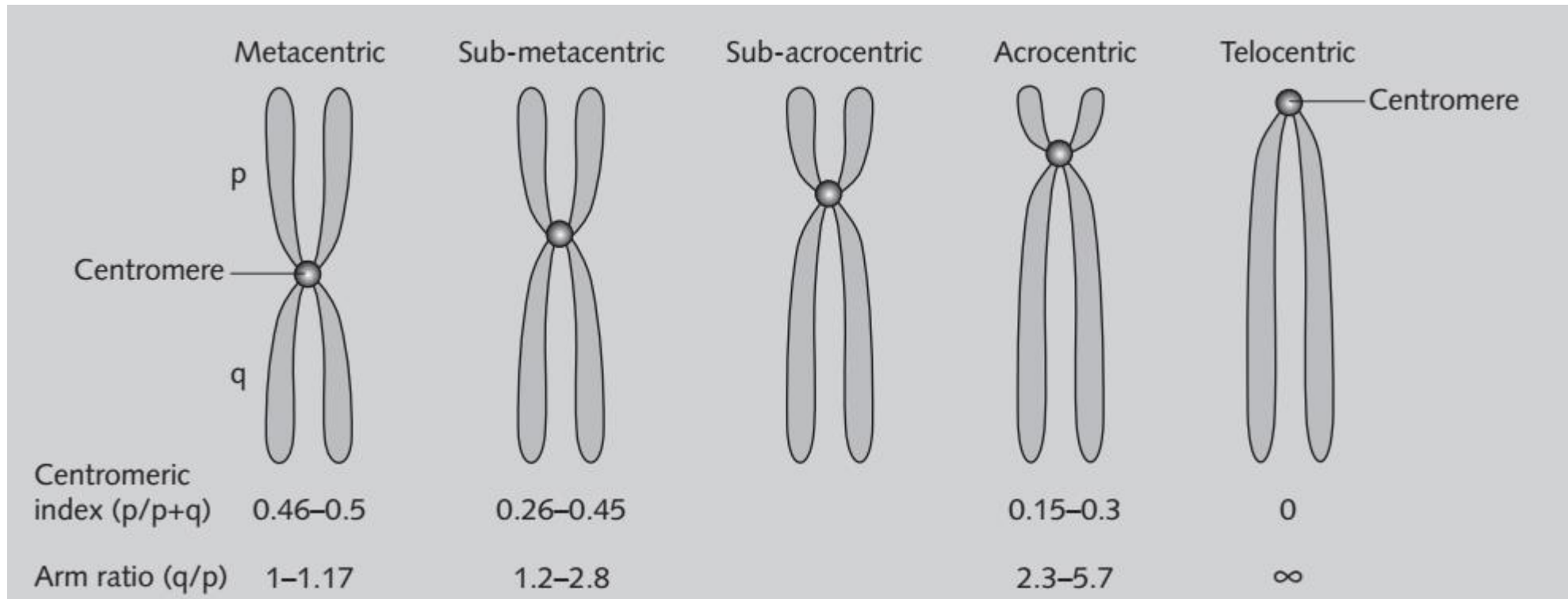
**Zoo-102, Unit III**

**By**

**Dr. Sudhansu Sekhar Nishank**  
**Dept. of Zoology, Utkal University**

**karyotype** A summary of the chromosome constitution of a cell or person, such as 46,XY. Often used more loosely to mean an image showing the chromosomes of a cell sorted in order and arranged in pairs (strictly, a karyogram).

**karyogram** A display of the chromosomes of a cell, sorted into pairs.



Chromosome shape can also be defined in terms of the **centromeric index** or the arm ratio. The centromeric index is the length of the shorter arm divided by the total chromosome length, and thus varies from 0.5 for a truly metacentric chromosome to zero for a telocentric one. The arm ratio is the length of the long arm divided by the length of the short arm, and thus ranges from unity for a truly metacentric chromosome to infinity for a truly telocentric chromosome.

## HUMAN CHROMOSOME GROUPS

Group	Chromosomes <sup>a</sup>	Description
A	1–3	largest; 1 and 3 are metacentric <sup>b</sup> but 2 is submetacentric <sup>c</sup>
B	4, 5	large; submetacentric with two arms very different in size
C	6–12, X	of medium size; submetacentric
D	13–15	of medium size; acrocentric <sup>d</sup> with satellites <sup>e</sup>
E	16–18	small; 16 is metacentric but 17 and 18 are submetacentric
F	19, 20	small; metacentric
G	21, 22, Y	small; acrocentric, with satellites on 21 and 22 but not on Y

<sup>a</sup>Autosomes are numbered from largest to smallest, except that chromosome 21 is slightly smaller than chromosome 22. <sup>b</sup>A *metacentric* chromosome has its centromere at or near the middle.

<sup>c</sup>A *submetacentric* chromosome has its centromere placed so that the two arms are of clearly unequal length. <sup>d</sup>An *acrocentric* chromosome has its centromere at or near one end. <sup>e</sup>A satellite, in this context, is a small segment separated by a non-centromeric constriction from the rest of a chromosome; these occur on the short arms of most acrocentric human chromosomes.

## Euchromatin vs Heterochromatin

- Euchromatin is a part of chromatin which **takes less stain, loosely packed, genetically active, involved in active transcription**, dispersed appearance with more DNA content than RNA.
- Heterochromatin is slightly opposite to the euchromatin with **dark stained region, tightly packed, genetically inactive**, not involved in the active transcription, thick appearance with more RNA content than DNA.
- Heterochromatin could be of two type's **constitutive** and **facultative heterochromatin**.
- Constitutive heterochromatins are permanently conserved or condensed and in stable form i.e. not changed from heterochromatin to euchromatin and vice-versa. It consists of multiple repeats of DNA sequences with quite **less density of genes in this region** which are transcriptionally inactive.
- Thick and condensed state of the constitutive heterochromatin, **replicates late in S-phase** with reduced frequency of genetic recombination.

## Euchromatin vs Heterochromatin

- Facultative heterochromatins are unstable - easily changes to euchromatin
- Heterochromatic regions could be easily recognized on chromosome structure in the form of *chromomeres*, *chromocentres* and *knobs*.
- Chromomeres are regular features of all prophase chromosomes but their number, size, distribution and arrangements are specific for a particular species at a particular stage of development.
- Chromocentres are the regions with varying size near the centromere in the proximal regions of chromosome arms. They consist of several strings of chromomeres of varying sizes.
- Knobs are considered to be a spherical bodies or regions with spherical in shape and sometimes diameter of these spherical bodies is equal in width to chromosome arm, but the size may vary i.e. less or more than the diameter of chromosome arm.
- For example, a very distinct such type of chromosome knob could be observed in maize (*Zea mays*) at pachytene stage of meiosis I. It could be considered as a valuable chromosome marker for distinguishing chromosome of related species and races

# KARYOTYPE ?

- Karyotype is defined as the study of chromosome morphology of a chromosome complement in the form of size, shape, position of primary constriction or centromere, secondary constriction, satellite, definite individuality of the somatic chromosomes and any other additional features.
- Karyotype highlights closely or distantly related species based on the similarity or dissimilarity of the karyotypes.
- **Asymmetric karyotype** is defined as the huge difference between the largest and smallest chromosome as well as **less number of metacentric chromosomes** in a chromosome complement.
- Similarly, **symmetric karyotype** is defined as the small difference between the largest and smallest chromosome as well as **more number of metacentric chromosomes in a chromosome complement**.

## Banding technique ?

- This is a technique for the identification of chromosomes and its structural abnormalities in the chromosome complement.
- Chromosome identification depends on their morphological characteristics such as relative length, arm ratio, presence and absence of secondary constrictions on the chromosome arms.
- it is an additional and useful tool for the identification of individual chromosome within the chromosome complement.
- it could be used for identification of chromosome segments that predominantly consist of either GC or AT rich regions or constitutive heterochromatin.
- On banded chromosome, darkly stained or brightly fluorescent transverse bands (positive bands) alternate with the lightly stained or less fluorescent (negative bands).
- The bands are consistent, reproducible and are specific for each species and each pair of homologous chromosomes.

## Banding techniques ?

- Banding techniques also revealed the **extensive genetic polymorphism** manifested as inter-individual differences in the size and stain ability of certain chromosomal segments.
- Initially four basic types of banding techniques were recognized for the identification of Human chromosome complement (**Q, C, G and R bands**) and later on two additional major type of bands were developed (**N and T bands**) for complete identification of the chromosome complement.
- These bands are widely used in animals and plants for the identification of chromosome complement, chromosome aberrations as well as traces of phylogeny.
-

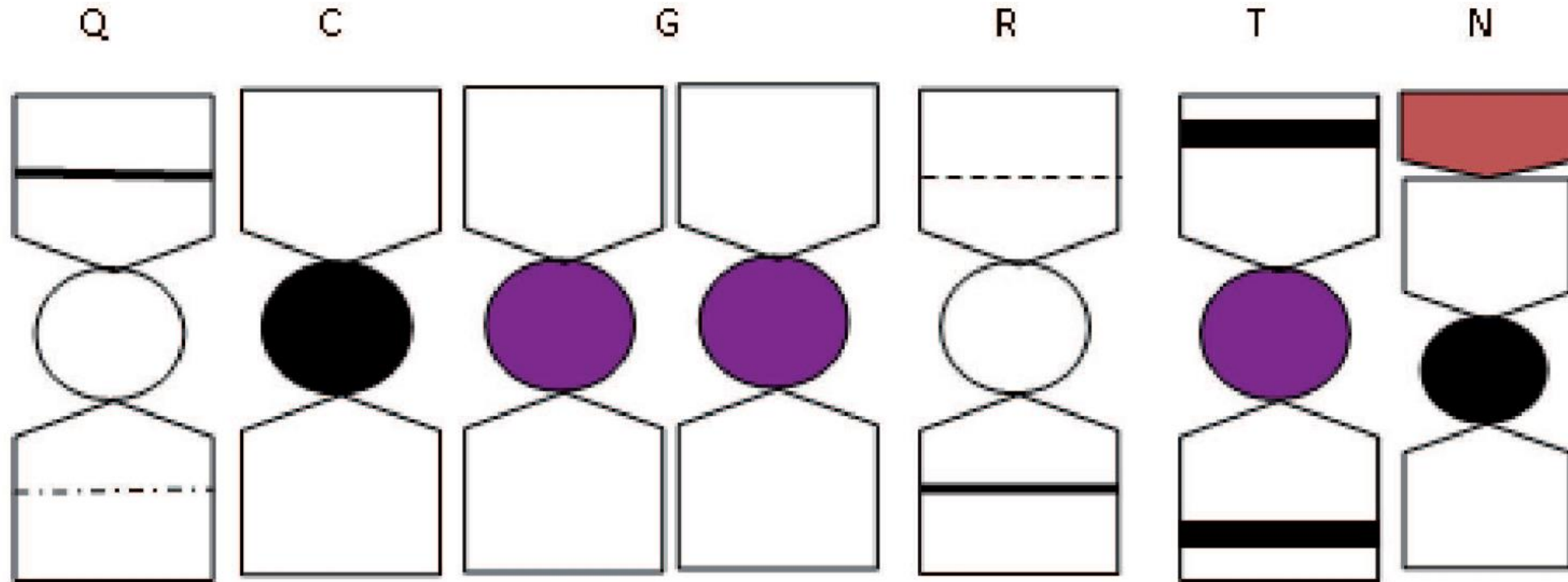
# Classification of Banding of Chromosomes

Banding techniques fall into the following two groups

- ***Bands distributed along the length of the whole chromosome***
  - 1. Giemsa banding (G-banding)
  - 2. Quinacrine banding (Q-banding)
  - 3. Reverse banding (R-banding)
- ***Bands that stain specific chromosome structures***
  - 1. Centromeric heterochromatin staining (C-banding)
  - 2. Nucleolar-organizer-region staining (NOR staining)
  - 3. T-banding
  - 4. FISH

## Banding techniques ?

- 



*Characteristics of various basic banding techniques.*

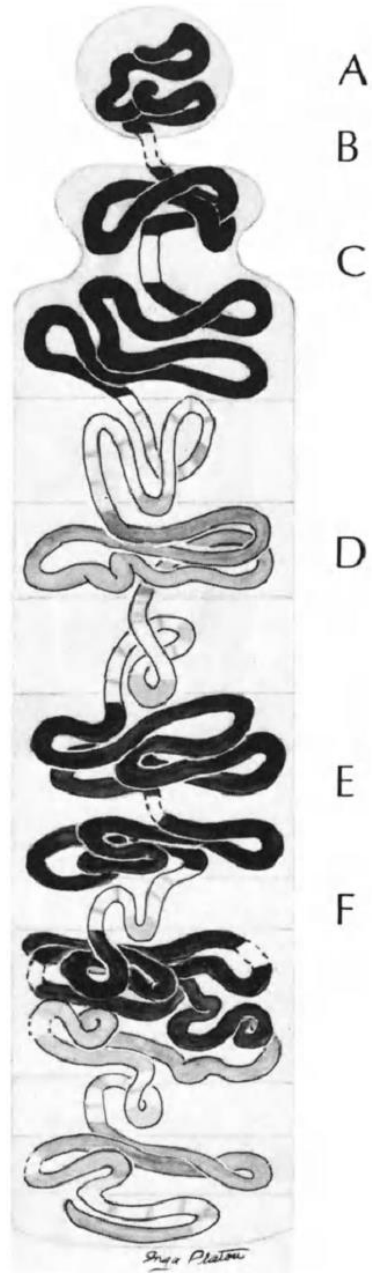


Fig. 4. Chromosome model in which the fine structure of chromosomes, the G-banding pattern, and the localization of repetitive DNA are combined to illustrate various structural elements of the chromosome. A, perinucleolar and pericentromeric constitutive heterochromatin, rich in the highly repetitive satellite DNA; B, secondary constriction of nucleolar organizer, site of 18 and 28 S rRNA cistrons; C, primary constriction or centromere; D,E, minor and major dark G-bands, rich in intermediate repetitive DNA, spacing vital genes such as tandem gene duplicates for 5 S rRNA, 4 S tRNA, and 9 S histone IV; F, light G-bands, where the bulk of the structural genes spaced by intergenic segments are believed to be localized.

## Banding pattern of Q, G, R and C bands on Human chromosome complement

- Chromosome band C and G clearly identifies the secondary constrictions of chromosome number 1, 9 and 16.
- C-band clearly stains and identifies peri-centromeric region on the chromosomes, while band Q slightly stains peri-centromeric region of chromosome 3.
- Both C and Q bands are equally important for staining the distal part of long arm of Y chromosome
- Partial Q band staining was reported for chromosome 3, 13 and 21 while other chromosomes were recorded with intense staining.
- 3 letter coding system for the banding procedure, for example, first letter codes for the type of banding to be done; second letter codes for the general technique to be used and third letter codes for the stain to be used.
- For instance, code QFQ indicates the Q-band to be done, fluorescence technique to be used and quinacrin mustard stain to be used during banding procedure.

## Q (quinacrine) band

- The band stains the chromosome with fluorochrome quinacrine mustard or quinacrine dihydrochloride (atebrin), observed under fluorescence microscope, and shows a specific banding pattern
- The AT-rich regions enhance the fluorescence while GC-rich regions quench the fluorescence.
- the fluorescence of Q band is not permanent and fades rapidly, therefore, the banding must be observed on fresh preparation and selected metaphases photographed immediately for further analysis.
- Q banding could also be achieved by fluorochromes other than quinacrine or its derivatives e.g. daunomycin, hoechst33258, BrdU etc which enhances AT-rich regions and quenches GC-rich regions. Acridine orange stains AT-rich regions red and GC rich regions green.
- Bright Q-bands correspond to dark G-bands.

## C (constitutive heterochromatin) banding

- Centromeric regions with constitutive heterochromatin where satellite DNA was located stained more deeply with Geimsa than the rest of the chromosome.
- The C banding technique is based on the denaturation and renaturation of DNA and the regions containing constitutive heterochromatin stain dark (C band) and could be visible near the centromere of each chromosome.
- The C bands are polymorphic in size which is believed to correspond to the content of the satellite DNA in those regions.
- C banding allows precise analysis of abnormalities in the centromeric regions and detection of isochromosomes (An isochromosome is an unbalanced structural abnormality in which the arms of the chromosome are mirror images of each other).
- The C banding in combination with simultaneous T-banding in particular, extends to easy detection of dicentric rings
- Sometimes, C banding also permits to ascertain the parental origin of foetal chromosomes and distinguish between maternal and foetal cells in amniotic fluid cell culture.

## Giemsa banding (G-banding, GTG)

- GTG-banding uses the proteolytic enzyme trypsin as a pretreatment, followed by staining with Giemsa.
- G-banding can be visualized with a brightfield microscope.
- In general, Giemsa positive bands (dark bands) are AT-rich, late replicating, and gene-poor, whereas Giemsa negative bands (light bands) are CG-rich, early replicating, and relatively gene-rich
- Abnormalities detected by G-banding are whole chromosome aneuploidies, balanced rearrangements, deletions, duplications, >20% mosaicism, sSMC (nonmosaic), and polyploidy.
- G bands correspond exactly to chromomeres of meiotic chromosomes
- The chromosomes are subjected to controlled digestion with trypsin before being stained with Giemsa stain. Positively-staining dark bands are known as G-bands. Pale bands are G-negative. G-banding reveals the same patterns as Q-banding, without the complications of fluorescence microscopy, and unlike Q-bands, G-bands do not fade away.

## R (Reverse) banding

- R banding (AT-rich regions) patterns are based on the thermal treatment of chromosomes and in general the reverse of the Q and G bands.
- This permits the observation of minor abnormalities in the terminal regions of chromosomes and the precise determination of chromosomal lengths.
- The technique is performed on a fixed chromosomal preparation and is based on heat denaturation of chromosomal DNA.
- Giemsa stained R bands can be observed under phase contrast microscope while acridine orange stained R bands require fluorescence microscope.
- This produces the reverse of the G-banding pattern. The chromosomes are heat-denatured in saline before being stained with Giemsa. The heat treatment denatures AT-rich DNA, and dark R-bands correspond to pale G-bands.
- R-banding is useful for studying the telomeres of chromosomes, which are pale and hard to make out on G-banded preparations.

## T (Telomeric) banding

- T bands are, in fact, the segments of the R bands that are most resistant to the heat treatment
- The clear marking of telomeric regions of chromosome with T banding enables the detailed analyses of the structural rearrangements at the ends of chromosomes.
- It also allows the detection of human chromosome 22 and its involvement in translocation. The usefulness of this method is for the detection of dicentric rings that were undetectable by other procedures.

## N (Nucleolar organizing regions) banding

- Nucleolar-organizer-region staining (NOR staining) is a technique that stains NOR regions that contain genes for ribosomal RNA.
- NOR are located in the satellite stalks of acrocentric chromosomes 13, 14, 15, 21, and 22.
- Acrocentric chromosomes have long and short arms with stalks and satellite regions without euchromatic regions.
- This stain uses a silver nitrate solution and is viewed with a brightfield microscope.
- The NOR regions could be selectively stained by techniques involving either giemsa or silver staining.
- NOR banding is useful to study some chromosome polymorphisms and to identify satellite stalks in nonacrocentric chromosomes.

# Chromosome Banding Techniques and their Applications<sup>a</sup>

Banding type	Stain (technique)	Microscope used	Uses and advantages
Q-banding	Quinacrine (QTQ)	F	ID of all chromosomes and bands; reveals polymorphisms on chromosomes 3, 4, 13, 14, 15, 21, 22, and Y; easily destained for sequential staining
G-banding	Giemsa (GTG)	B	ID of all chromosomes and bands; permanent stain; simple photography
	Wrights	B	ID of all chromosomes and bands; permanent stain; simple photography
R-banding	Giemsa (RHG)	B	ID of all chromosomes and bands; visualization of ends of chromosomes and small positive R-bands
	CH3/DA	F	ID of all chromosomes and bands; visualization of ends of chromosomes and small positive R-bands
Replication band-Hoechst ing <sup>b</sup>		F	ID of all chromosomes and bands, and of inactive, late-replicating X chromosome
	Hoechst and Giemsa	B	ID of all chromosomes and bands, and of inactive, late-replicating X chromosome
C-banding	Giemsa (CBG)	B	ID of all centromeric and distal Y heterochromatin; reveals polymorphisms including heterochromatin inversions; evaluation of ring and dicentric chromosomes
NOR banding	AgNO <sub>3</sub>	B	ID of active NOR; reveals polymorphisms and rearrangements of acrocentric chromosomes

<sup>a</sup>Abbreviations: B, bright-field; CH3/DA, chromomycin A/distamycin A; DA, distamycin A; DAPI, 4',6-diamidino-2 phenylindole; F, fluorescent; ID, identification; NOR, nuclear organizer region.

<sup>b</sup>Depending on timing of BrdU incorporation, a G- or Q-type banding pattern can be obtained with highlighting of late-replicating X chromosome.

## Sequential banding

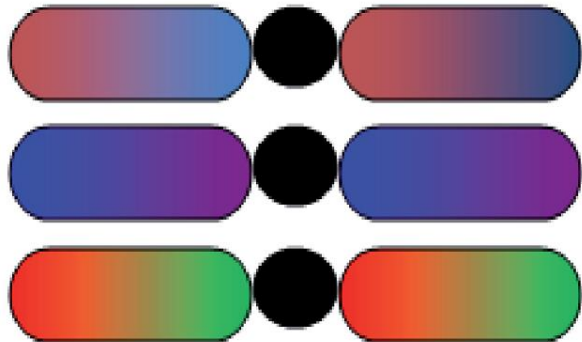
- In routine cytogenetic diagnosis, a single banding technique is usually sufficient for the detection of chromosomal abnormalities e.g. G banding or R banding, but sometimes, more complicated chromosomal rearrangements often require sequential staining of the same metaphase by several banding techniques and the process is known as sequential banding.
- The quality of chromosomes in sequential banding deteriorates with each staining therefore; it restricts the sequential banding up to 3 or 4 different staining techniques.
- For example, single metaphase → First procedure, Q banding → Second procedure, G banding → Third procedure, C banding → deteriorates the chromosome quality → therefore, **restricts up to 3 or 4 staining procedures.**



## Simultaneous banding

- This is the technique that produces simultaneously two types of banding on the same metaphase or on one slide but different metaphases.
- For example, same metaphase → first procedure, G banding → second procedure, C banding OR single slide with different metaphases → first procedure, C banding → second procedure, T banding.
- Simultaneous banding restricts up to two staining procedures in different or single metaphase and results in precise estimation of chromosomal aberrations.

Simultaneous staining (2 or more metaphase of the same slide)



2—3 staining techniques

- Differential staining along the longitudinal axis of mitotic chromosomes. are referred to as **chromosome-banding** techniques because the staining patterns resemble the bands of polytene chromosomes.
- **Chromosome banding techniques produce** a series of consistent landmarks along the length of metaphase chromosomes that allow for both recognition of individual chromosomes within a genome and identification of specific segments of individual chromosomes.
- First chromosome-banding techniques by Mary Lou Pardue and Joe Gall (chromosome preparations from mice if heat denatured and then treated with Giemsa stain, : Only the centromeric regions of mitotic chromosomes took up the stain! The staining pattern was thus referred to as **C-banding** .

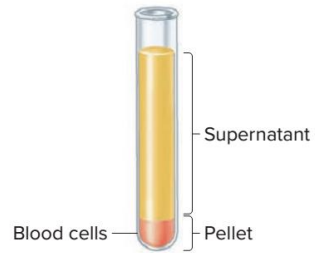
- *G-banding*—the chromosomes are subjected to controlled digestion with trypsin before being stained with Giemsa, a DNA-binding chemical dye. Positively staining dark bands are known as G bands. Pale bands are G negative.
- *Q-banding*—the chromosomes are stained with a fluorescent dye that binds preferentially to AT-rich DNA, notably quinacrine, DAPI (4', 6-diamidino-2-phenylindole), or Hoechst 33258, and viewed by ultraviolet fluorescence. Fluorescing bands are called Q bands and mark the same chromosomal segments as G bands.

- *R-banding*—this is essentially the reverse of the G-banding pattern. The chromosomes are heat-denatured in saline before being stained with Giemsa. The heat treatment denatures AT-rich DNA, and R bands are Q negative. The same pattern can be produced by binding GC-specific dyes such as chromomycin A<sub>3</sub>, olivomycin, or mithramycin.
- *T-banding*—identifies a subset of the R bands that are especially concentrated close to the telomeres. The T bands are the most intensely staining of the R bands and are revealed by using either a particularly severe heat treatment of the chromosomes before they are stained with Giemsa, or a combination of standard dyes and fluorescent dyes.
- *C-banding*—this is thought to demonstrate constitutive heterochromatin, mainly at the centromeres. The chromosomes are typically exposed to denaturation with a saturated solution of barium hydroxide, before Giemsa staining.

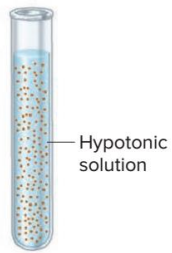
Characteristics of euchromatic bands in mammalian chromosomes.

<b>G-Bands</b>	<b>R-Bands</b>
Positive G-bands	Negative G-bands
Positive Q-bands	Negative Q-bands
Negative R-bands	Positive R-bands
A+T-rich DNA	G+C-rich DNA
Late replicating DNA	Early replicating DNA
Early condensation	Late condensation
Pachytene chromomeres	Interchromomeric regions
Little recombination	Meiotic pairing and recombination
Nuclease insensitive	Nuclease hypersensitive
Low concentration of genes	High concentration of genes
Low level of histone acetylation	High level of histone acetylation
High level of H1 subtypes	Low level of H1 subtypes
HMGA1a present	HMGA1a absent
Rich in LINEs (long intermediate repetitive DNA sequences)	Rich in SINEs (short intermediate repetitive DNA sequences)
Low level of chromosome breakage	High level of chromosome breakage

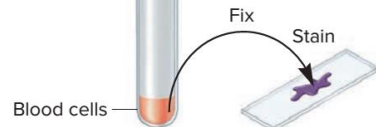
A sample of blood is collected and treated with chemicals that stimulate the cells to divide. Colchicine is added because it disrupts spindle formation and stops cells in mitosis where the chromosomes are highly compacted. The cells are then subjected to centrifugation.



The supernatant is discarded, and the cell pellet is suspended in a hypotonic solution. This causes the cells to swell.



The sample is subjected to centrifugation a second time to concentrate the cells. The cells are suspended in a fixative, stained, and placed on a slide. As shown in part (b), the chromosomes within leukocytes (white blood cells) are observed under a microscope.

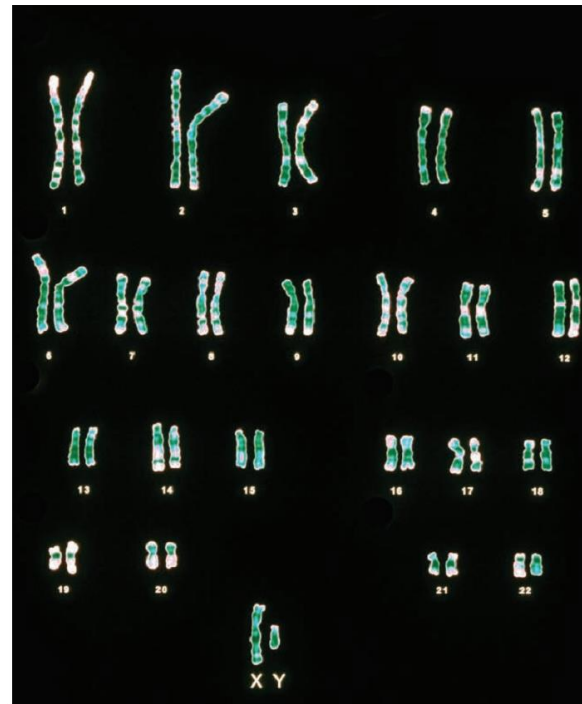


**(a) Preparing cells for a karyotype**



**(b) The slide is viewed by a light microscope; the sample is seen on a video screen. The chromosomes can be arranged electronically on the screen.**

## The procedure for making a human karyotype.



**(c) For a diploid human cell, two complete sets of chromosomes from a single cell constitute a karyotype of that cell.**

# Cell culture protocol for standard karyotype.

## *Cell culture*

Blood collected in sterile tubes containing anticoagulant **heparin**

Whole blood leukocytes separated from red blood cells or purified lymphocytes put in a tube with **culture medium** supplemented with serum and antibiotics

Mitogen [**phytohemagglutinin (PHA)**] added to induce mitosis ☐. PHA is a lectin extracted from *Phaseolus vulgaris* that interacts with glycoproteins present on the surface of T cells and stimulates proliferation

Cultures incubated at **37°C for 72 or 96 hours in an incubator**, shaken at least twice daily to increase mitosis

**Colchicine or colcemid** added to cultures a few hours before harvesting to arrest cells in metaphase. Colcemid is a synthetic analogue of colchicine, an alkaloid derived from *Autumn crocus*, that exerts an antimitotic effect by blocking formation of the mitotic spindle, preventing cells from advancing to anaphase

# Cell culture protocol for standard karyotype.

## *Harvest*

Tubes centrifuged after incubation for 72 hours

Supernatant discarded and cells resuspended

**Hypotonic treatment** of fresh potassium chloride solution (KCl, 0.075 M) added. Osmosis causes inflammation of cells, dispersing chromosomes [25,26]

Tubes centrifuged, supernatant discarded, and pellet resuspended

## *Fixation*

**Carnoy's solution** (3:1 methanol and glacial acetic acid) used for fixation and several washings

## *Extension*

Cell suspension dropped on a clean slide to fix chromosomes

Slides aged at room temperature for a few days or in an oven

Prepared slides are stained (banding)

# QUINACRINE BANDING (Q-BANDING)

- Materials
- Air-dried slides of metaphase chromosomes
- Quinacrine staining solution
- McIlvaine buffer, pH 5.6
- Immersion oil
- Coverslips, no. 0 or no. 1

## QUINACRINE BANDING (Q-BANDING)

- 1. Place air-dried slide of metaphase chromosomes in a Coplin jar containing quinacrine staining solution, 5 min at room temperature.
- 2. Rinse slide by dipping several times into Coplin jar filled with water. Discard rinse water, refill jar with fresh water, and repeat rinse. Air dry slide.
- 3. Mount slide using McIlvaine buffer, pH 5.6. Add a coverslip and gently squeeze excess buffer from under coverslip by blotting gently with paper towel.
- 4. View and photograph using fluorescence microscope with appropriate filters

## GTG Technique for G-Banding

- Materials
- HBSS (APPENDIX 2)
- Trypsin solution
- 70% and 90% (v/v) ethanol
- 2% Giemsa staining solution
- Aged slides of metaphase chromosomes
- Xylene

## GTG Technique for G-Banding

- 1. Prepare a series of Coplin jars containing the following at room temperature:  
jar 1—HBSS  
jar 2—trypsin solution  
jar 3—HBSS  
jar 4—70% ethanol  
jar 5—90% ethanol  
jar 6—2% Giemsa staining solution  
jar 7—H<sub>2</sub>O.
- 2. Place aged slide of metaphase chromosomes briefly (~10 sec) in jar 1.
- 3. Transfer slide to jar 2. Incubate for optimal trypsinization time.
- Insufficient trypsinization results in evenly stained slides with no bands. Over-trypsinization results in pale “puffy” chromosomes with staining around the outside of the chromosome.

## GTG Technique for G-Banding

- 4. Place slide in jars 3 to 5, dipping slide 3 to 4 times in each jar. Air dry.
- 5. Place slide in jar 6 for 4 min.
- 6. Place slide in jar 7 for ~30 sec. Air dry.
- 7. View and photograph with bright-field microscope

# CENTROMERIC HETEROCHROMATIN STAINING (C-BANDING)

- Materials
- Air-dried slides of metaphase chromosomes aged 1 week at room temperature
- 0.2 M HCl
- 5% (w/v) barium hydroxide [Ba(OH)<sub>2</sub>], 50°C
- 2× SSC , 60°C
- 4% Giemsa staining solution in Gurrs buffer Gurrs buffer, pH 6.8: dissolve Gurrs pH 6.8 buffer tablets in water according to manufacturer's instructions
- Glass and polyethylene Coplin jars
- 50° and 60°C water baths

## CENTROMERIC HETEROCHROMATIN STAINING (C-BANDING)

- Air-dried slides of metaphase chromosomes aged 1 week at room temperature
- 0.2 M HCl
- 5% (w/v) barium hydroxide [ $\text{Ba}(\text{OH})_2$ ], 50°C
- 2× SSC , 60°C
- 4% Giemsa staining solution in Gurrs buffer Gurrs buffer, pH 6.8: dissolve Gurrs pH 6.8 buffer tablets in water according to manufacturer's instructions
- Glass and polyethylene Coplin jars
- 50° and 60°C water baths

## CENTROMERIC HETEROCHROMATIN STAINING (C-BANDING)

- 1. Place air-dried slide of metaphase chromosomes in glass Coplin jar containing 0.2M HCl. Let stand 1 hr at room temperature.
- 2. Rinse slide well by agitating it in a Coplin jar filled with distilled water. Air dry.
- 3. Immerse one slide at a time in polyethylene Coplin jar containing freshly prepared 5% Ba(OH)<sub>2</sub> prewarmed to 50°C. Incubate 5 to 15 min in a 50°C water bath.
- 4. Wash slide very thoroughly with water until no white film remains.
- 5. Place slide in 2× SSC prewarmed to 60°C in a polyethylene Coplin jar. Incubate 1 hr in a 60°C water bath.
- 6. Rinse well in water and air dry.
- 7. Stain 10 min in 4% Giemsa staining solution/Gurr buffer.
- 8. Rinse well in water and air dry.
- 9. View and photograph with bright-field microscope. It is not necessary to mount the slide.

## Reagents and Solutions for C-Banding

- ***Barium hydroxide [Ba(OH)<sub>2</sub>], 5% (w/v)***

Add 2.5 g Ba(OH)<sub>2</sub> to 50 ml H<sub>2</sub>O in a polyethylene Coplin jar. Mix frequently while heating to 50°C. Always prepare just before use.

***Giemsa staining solution in Gurrs buffer, 4% (v/v)***

2 ml Giemsa stain (Bio/medical Specialties or Fisher)

48 ml Gurr buffer, pH 6.8 (Bio/medical Specialties)

Prepare fresh daily

## NUCLEOLAR-ORGANIZER-REGION STAINING (NOR STAINING)

- Materials
- Air-dried slides of metaphase chromosomes (<7 days old, unheated)
- 2% gelatin solution 50% silver nitrate solution
- 3% (v/v) acetic acid (3 ml in 97 ml H<sub>2</sub>O; prepare fresh)
- 65°C slide warmer or drying oven

## NUCLEOLAR-ORGANIZER-REGION STAINING (NOR STAINING)

- 1. Add 3 drops of 2% gelatin solution and 4 drops of 50% silver nitrate solution to recently made, unheated, air-dried slide of metaphase chromosomes. Cover with coverslip.
- 2. Heat 2 to 4 min on 65°C slide warmer or in drying oven. Remove slide from heat when solution appears golden brown.
- 3. Rinse in beaker with ~100 ml of 3% acetic acid.
- 4. Rinse in water and air dry.
- 5. View and photograph with bright-field microscope

# Reagents and Solutions for NOR Staining

- 

## ***Gelatin solution, 2% (w/v)***

1 ml formic acid

2 g gelatin (Fisher)

99 ml H<sub>2</sub>O

Stir with heat to get gelatin into solution. Store in dark bottle at room temperature ≤1 year.

## ***Silver nitrate solution, 50% (w/v)***

5 g crystalline silver nitrate

10 ml H<sub>2</sub>O

Store in dark bottle ≤1 year at 4°C

*Silver nitrate crystals should appear white, not gray.*

## RHG Technique for R-Banding/REVERSE BANDING (R-BANDING)

- In some cases, R-banding is a useful complement to G-banding because some bands (e.g., small negative G-bands) can be more easily detected when they are stained in reverse. R-banding is also useful for visualization of telomeric ends of chromosomes; these ends stain intensely with R-banding and negatively with G-banding.
- Materials
- 1.25 M sodium phosphate buffer, pH 4.0
- Air-dried slides of metaphase chromosomes (prepare fresh)
- 10% Giemsa staining solution
- Coplin jars
- 88°C circulating water bath with cover

## RHG Technique for R-Banding/REVERSE BANDING (R-BANDING)

- 1. Preheat sodium phosphate buffer (pH 4.0) to 87° or 88°C in covered Coplin jars in covered circulating water bath.
- 2. Prewet each air-dried slide of metaphase chromosomes in water and place in 87° to 88°C sodium phosphate buffer (pH 4.0), one slide per jar, 5 to 15 min. Rinse in water.
- [Use freshly prepared slides (5 min to 8 hr old) for best results. For older slides (1 to 3 days) subtract 1 min heating time per day slide has aged. Addition of more than one slide per Coplin jar will cause a significant change in the temperature of the buffer and adversely affect staining].
- 3. Stain slide 5 to 10 min in 10% Giemsa staining solution. Rinse in water and air dry.
- 4. View and photograph with bright-field microscope