Karyotype

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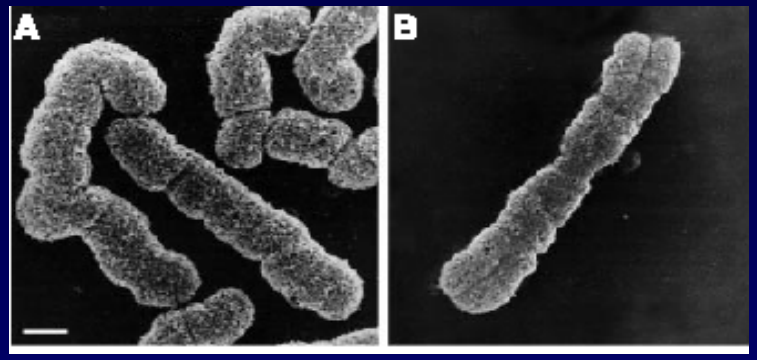
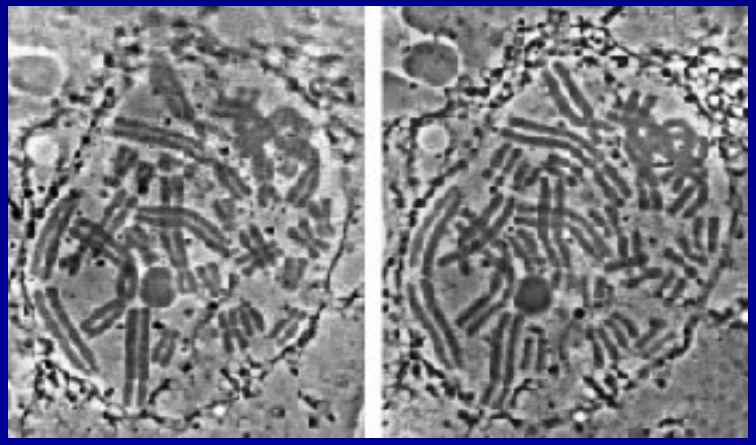


Karyotype



Definitions

- Cytogenetics
  - Visual study of chromosomes at microscopic level
- Karyotype
  - Chromosome complement
  - also applied to picture of chromosomes
- Idiogram
  - Stylised form of karyotype

Chromosomal abnormalities

- 1959 - Down syndrome (LeJeune)
- 1970 - banding techniques
  - identification of individual chromosomes
- Karyotype and FISH
  - types of abnormalities:
    - Extra copy of chromosome
    - Missing copy of chromosome
    - Structural abnormalities

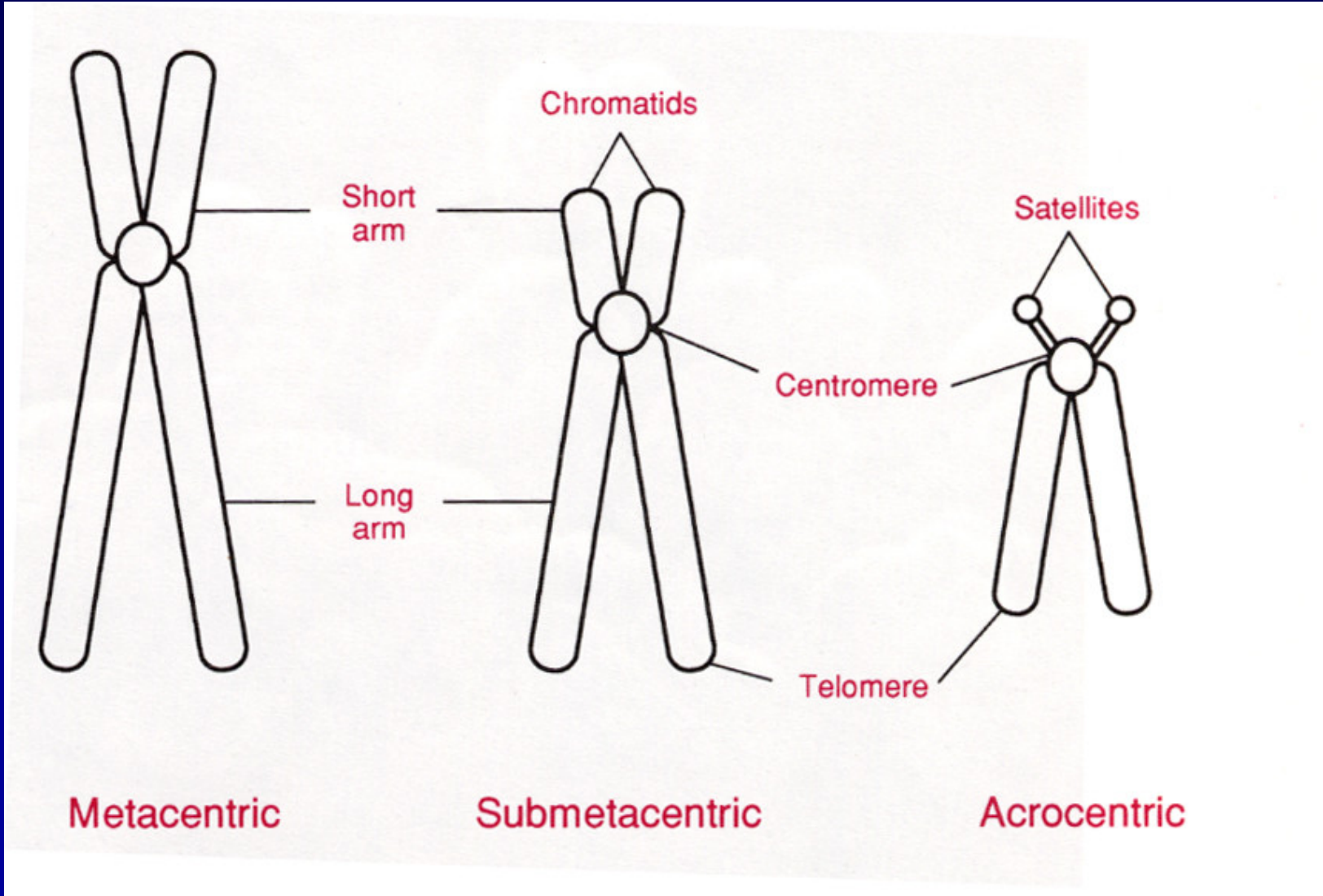
Chromosomes

- Centromere - movement during cell division
  - divides the chromosomes into short (p) and long (q) arms
- Telomere - tip of each chromosome
  - seal chromosomes and retain chromosome integrity
  - telomere consists of tandem repeats TTAAGGG
  - maintained by enzyme - telomerase
  - reduction in telomerase and decrease in number repeats important in ageing and cell death

Chromosomes

- Classified according to position of centromere
- Central centromere - metacentric
- Sub-terminal centromere - acrocentric
  - have satellites which contain multiple copies of genes for ribosomal RNA
- Intermediate centromere - submetacentric

Chromosomes



Chromosomes

- 22 autosomes and sex chromosomes in pairs
- Classified according to:
  - Length
  - position of centromere
  - presence or absence of satellites
- Chromosomes divided into groups labelled A-G

  - A 1-3
  - B 4-5
  - C 6-12 + X
  - D 13-15
  - E 16-18
  - F 19-20
  - G 21-22 +Y

Karyotyping

- Staining methods to identify chromosomes

- G banding - Giemsa
- Q banding - Quinacrine
- R banding - Reverse
- C banding - Centromeric (heterochromatin)

- Ag-NOR stain - Nucleolar Organizing Regions (active)

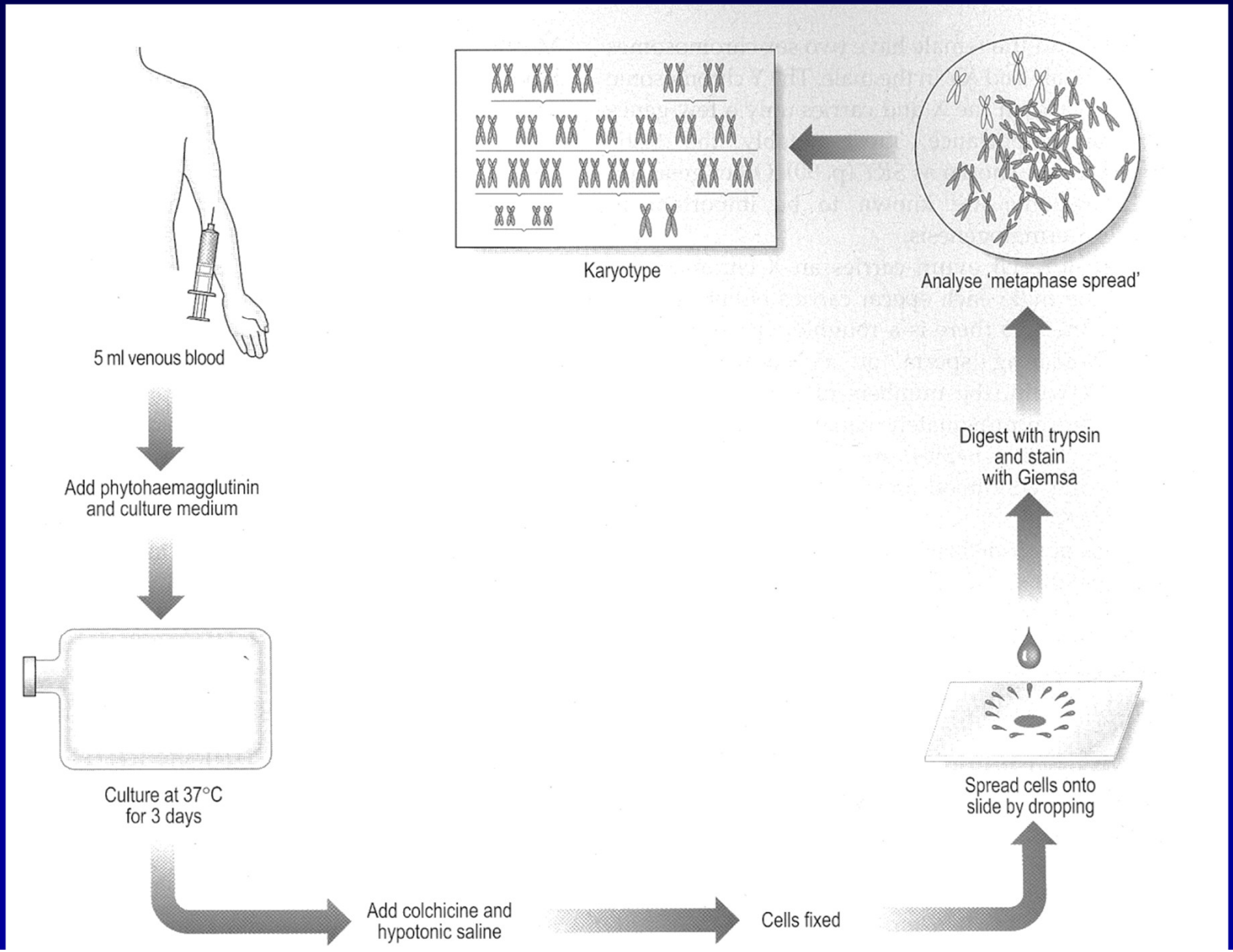
Karyotyping – cell preparation

- Need metaphases
- Culture cells until sufficient mitotic activity
- Add colchicine (or colcemid) to arrest in metaphase
  - prevents mitotic spindle fibres forming
- Add hypotonic salt solution to swell cells
- Fix with mix of methanol;acetic acid
- Want long chromosomes with none overlapping

G banding

- Most common method used
- Chromosomes treated with trypsin
  - denatures protein
- Giemsa stain
  - each chromosome characteristic light and dark bands
  - 400 bands per haploid genome
  - Each band corresponds to 5-10 megabases
  - High resolution (800 bands ; prometaphase chromosome)
    - use methotrexate and colchicine
- Dark bands are gene poor

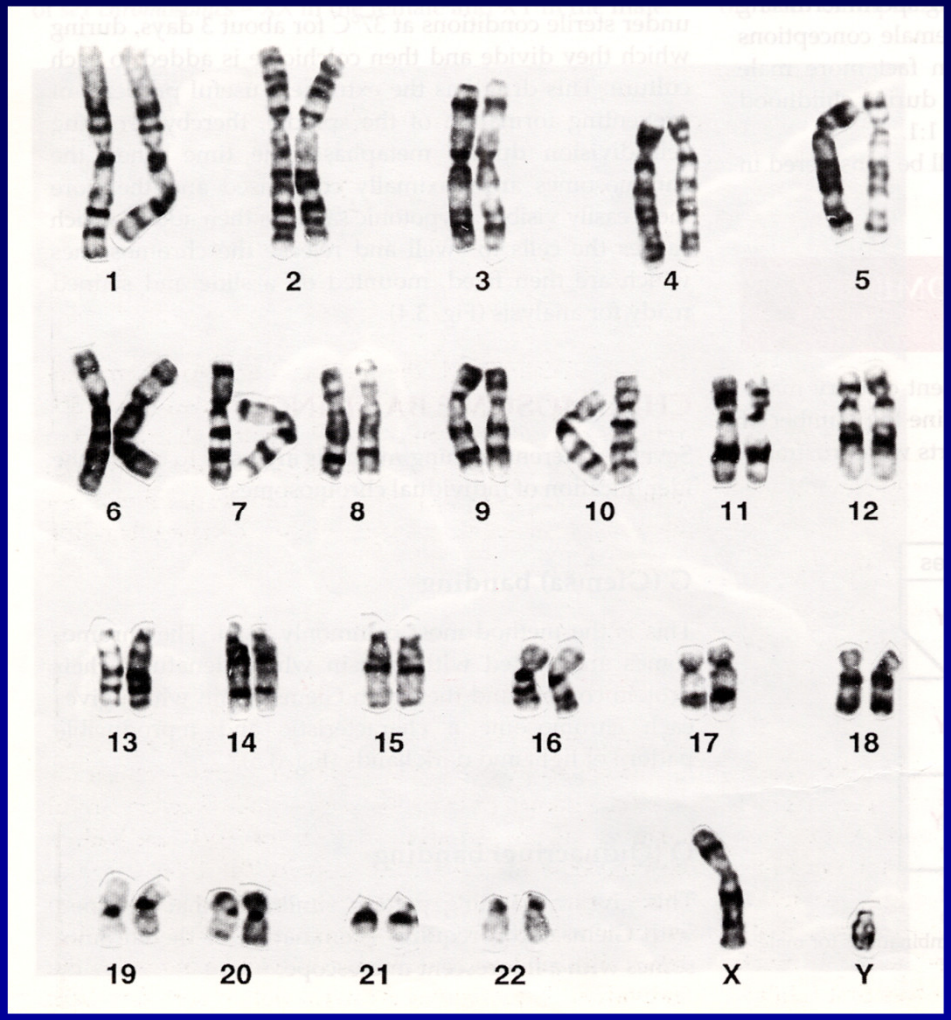
Preparation of G banded karyotype



G banding

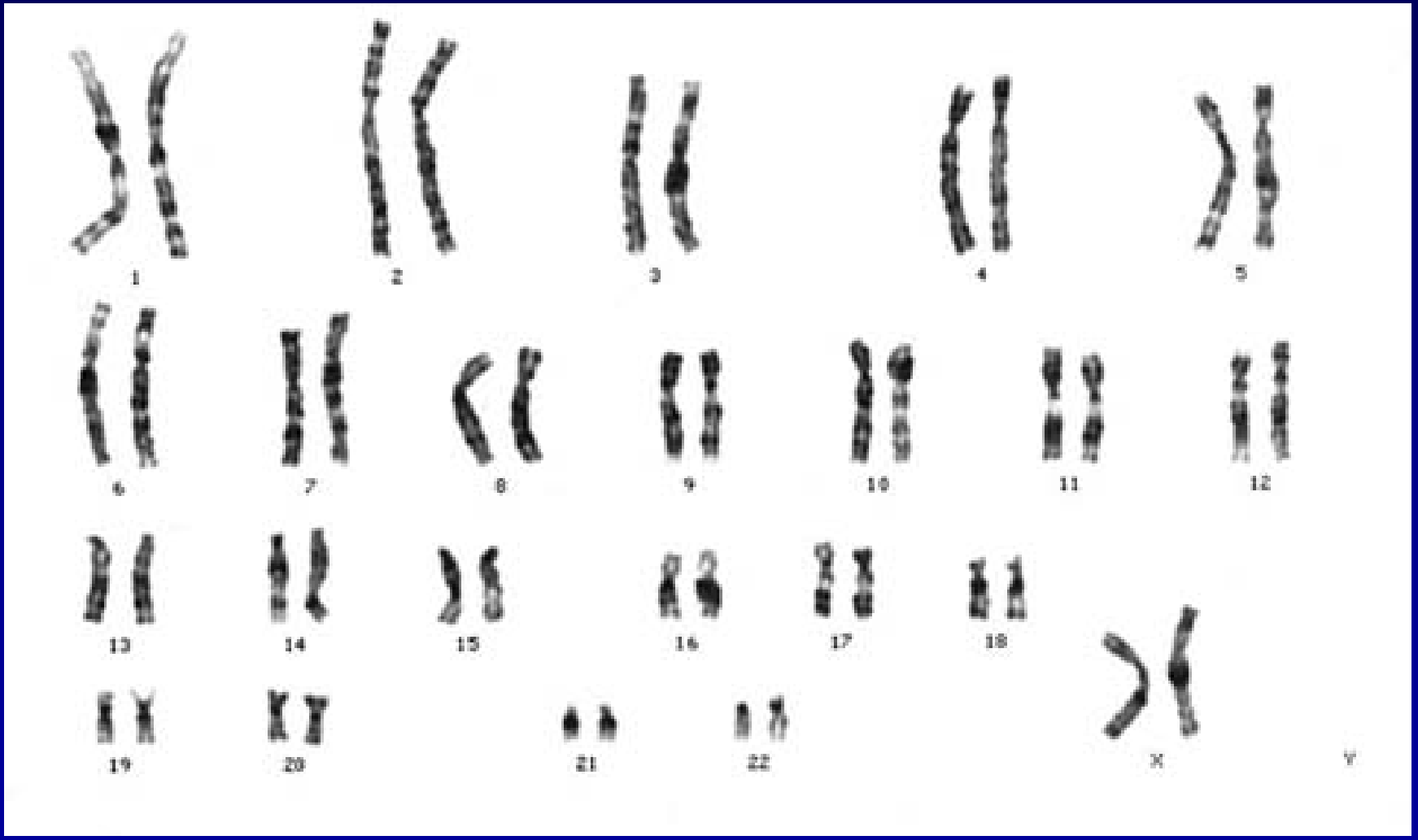
- Metaphase spreads
- Count chromosomes in 10-15 metaphases
- If mosaicism suspected, count 30
- Detailed analysis of 3-5 metaphases
- Used to photograph and cut out
- Now computer programmes

Normal male karyotype



- 13, 18, 21 gene poor
  - Very dark chromosomes
- 21 smaller than 22
  - Wrong way around
- 22 twice as many genes as 21
  - 200 on 21
  - 400 on 22

Normal female karyotype

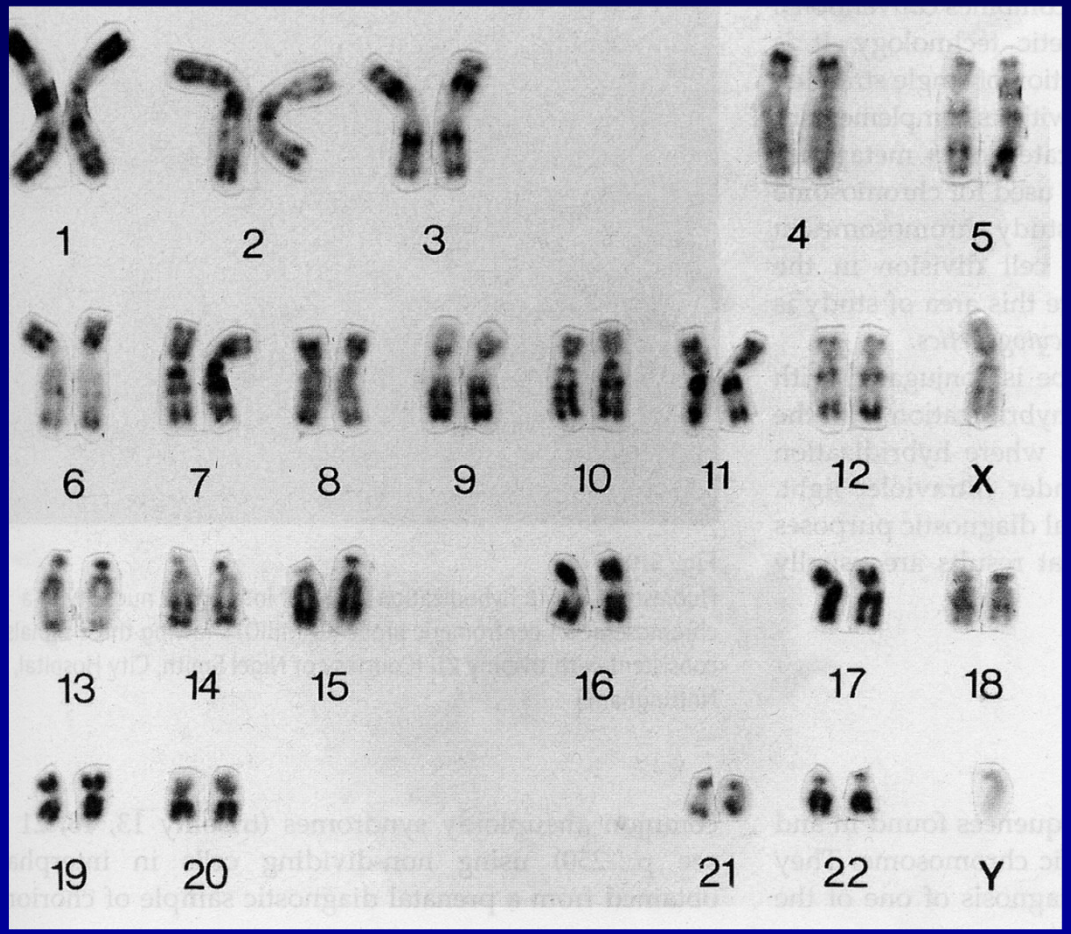


Q banding

- Used especially for Y chromosome abnormalities or mosaicism
- Similar pattern to G banding
  - But can detect polymorphisms
- Needs fluorescent microscope

R banding

- Used to identify X chromosome abnormalities
- Heat chromosomes before staining with Giemsa
- Light and dark bands are reversed

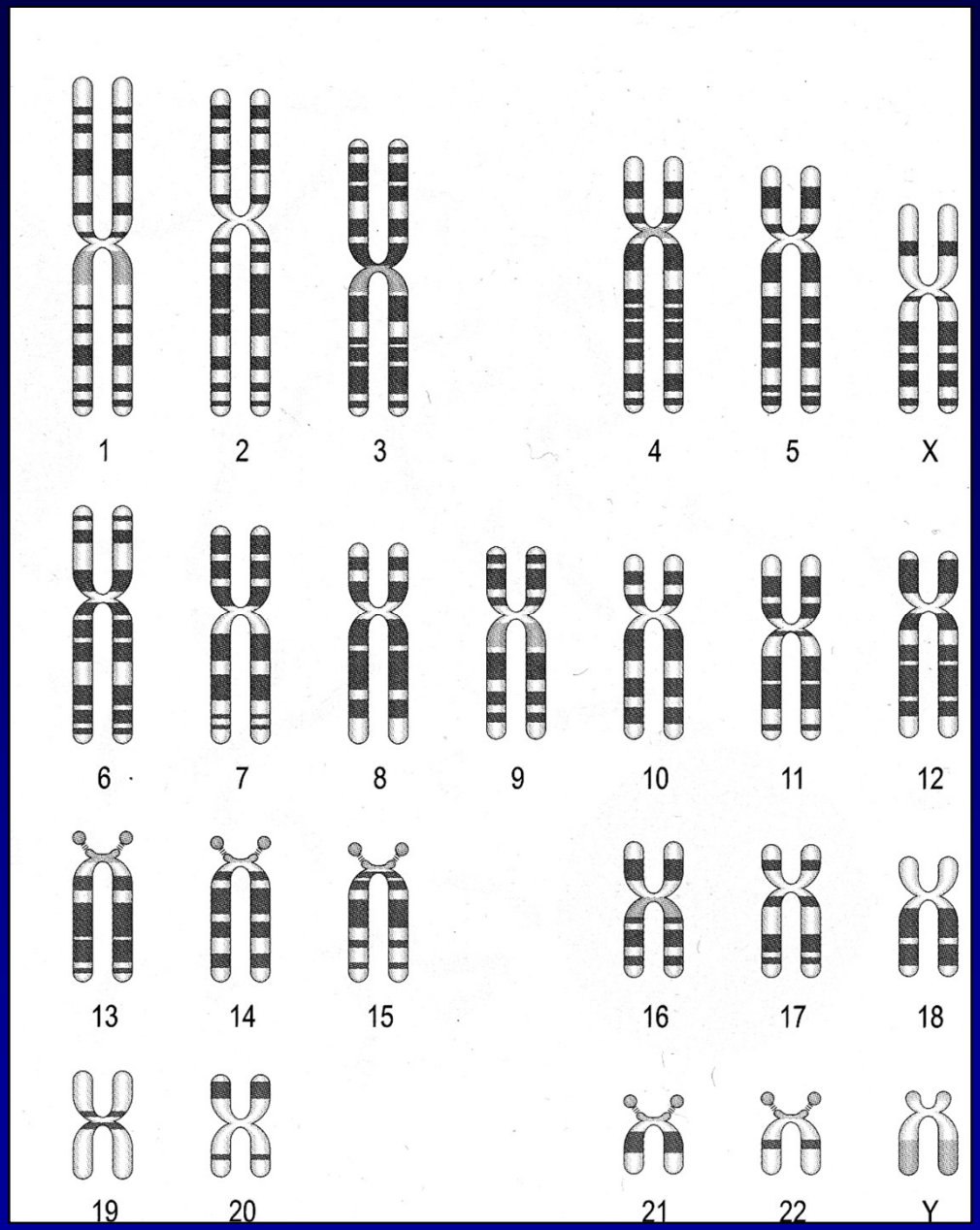


C banding

- Used to identify centromeres / heterochromatin
- Heterochromatic regions
  - contain repetitive sequences
  - highly condensed chromatin fibres

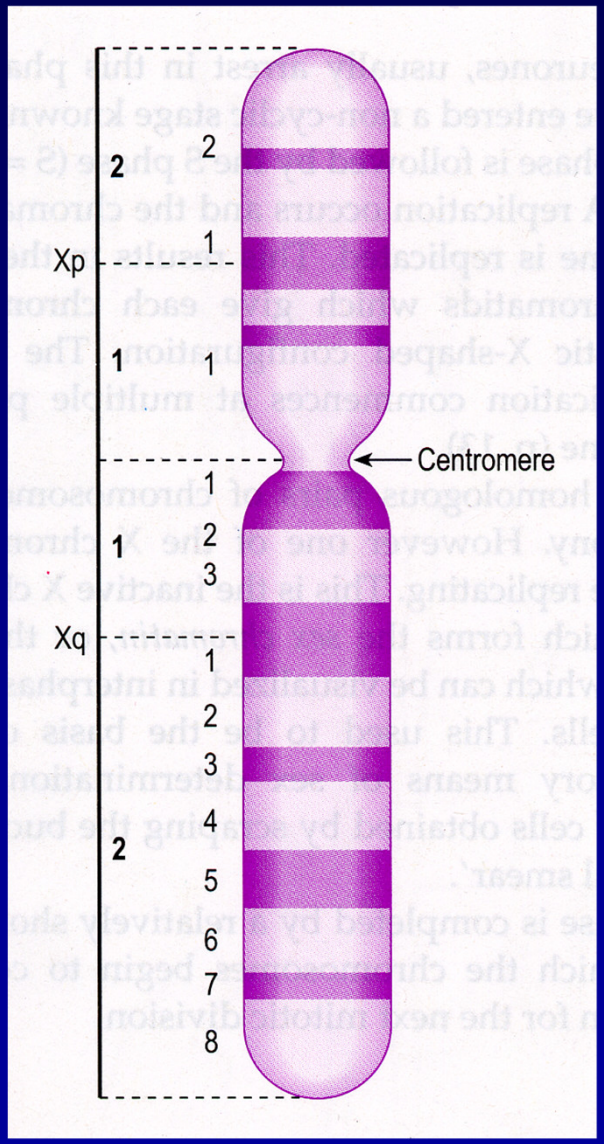
- Treat with chromosomes with
  1. Acid
  2. Alkali
  3. Then G band

Idiogram



ISCN

- International System for Cytogenetic Nomenclature
- Each area of chromosome given number
- Lowest number closest (proximal) to centromere
- Highest number at tips (distal) to centromere



ISCN

- del - deletion
- dic - dicentric
- fra - fragile site
- i - isochromosome
- inv - inversion
- p - short arm
- r - ring
- der - derivative
- dup - duplication
- h - heterochromatin
- ins - insertion
- mat - maternal origin
- q - long arm
- t - translocation

ISCN

, 46,XX,del(5p)

- separates
  - chromosome numbers
  - sex chromosomes
  - chromosome abnormalities

; 46,XX,t(2;4)(q21;q21)

- separates
  - altered chromosomes
  - break points in structural rearrangements involving more than 1 chromosome

ISCN

- Normal male
  - 46,XY
- Normal female
  - 46,XX

Types of chromosome abnormalities

- Numerical
  - Aneuploidy (monosomy, trisomy, tetrasomy)
  - Polyploidy (triploidy, tetraploidy)
- Structural
  - Translocations
  - Inversions
  - Insertions
  - Deletions
  - Rings
  - Isochromosomes
  - ESAC

Numerical

- Aneuploidy
  - Autosomal trisomy, 47
  - Sex chromosomes, 45, 47, 48, 49
- Polyploidy
  - Whole chromosome set
  - Triploidy, 69
  - Tetraploidy, 92

Aneuploidy

- Almost all been found in oocytes and early embryos, trisomies and monosomies
- Most lethal (miscarry)
- Do not see in pregnancy or live born
- Exceptions sex chromosomes and Down
- Some aneuploidy is age related

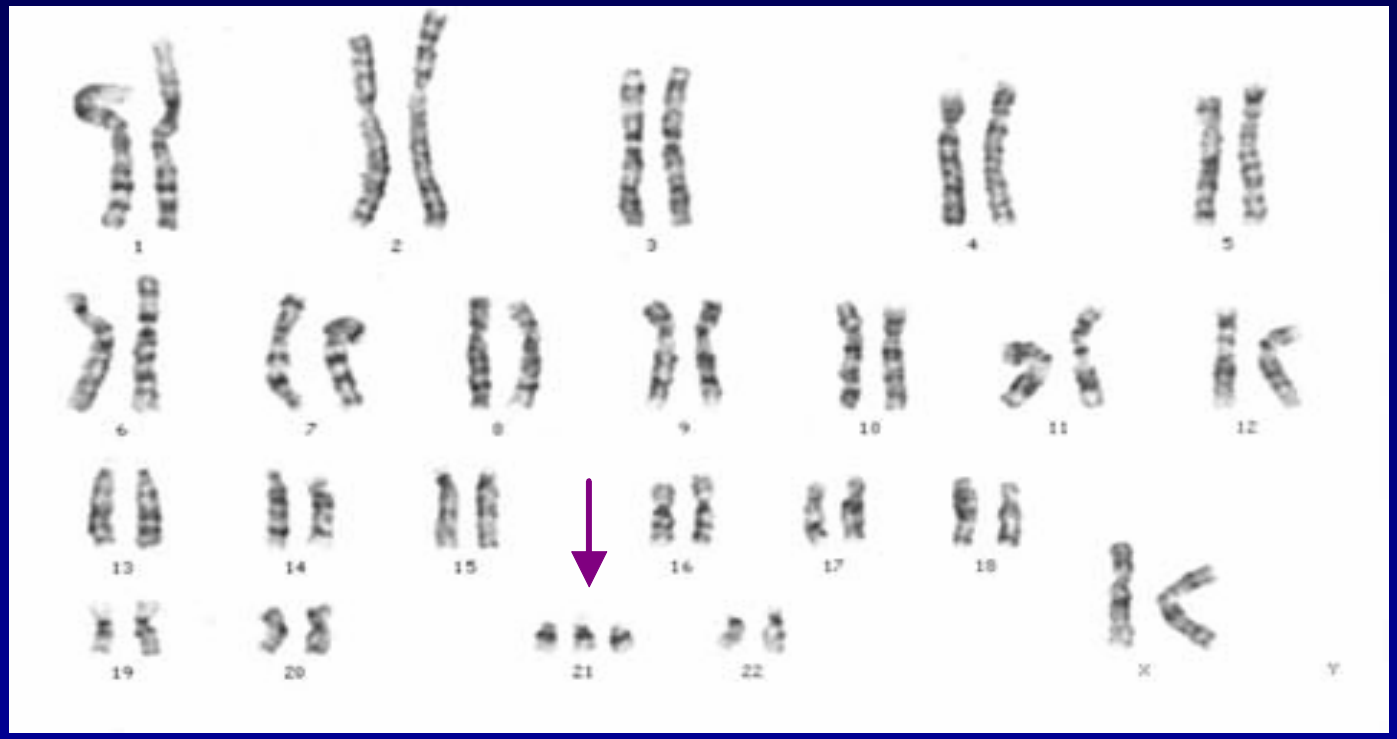
Sex chromosomes

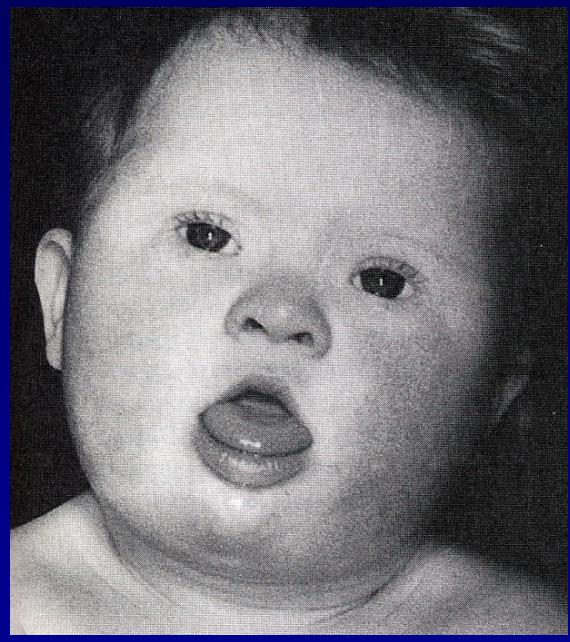
- Abnormalities more tolerated
- If have extra Y, few genes mainly for sex determination
- If have extra X, excess X is inactivated
- Monosomy X, Turners
  - Majority die during development
  - Only small proportion survive to birth
  - Short and infertile

Sex chromosome abnormalities

- Turner Syndrome 45,XO (female)
- Trisomy X 47, XXX (female)
- Klinefelter Syndrome 47,XXY (male)
- Extra "Y" chromosome 47,XYY (male)

Down syndrome, trisomy 21





47,XX,+21 or 47,XY,+21

Incidence at birth 1/700

Edwards syndrome, trisomy 18

47,XX,+18 or 47,XY,+18

Incidence at birth 1/5,000

Patau syndrome, trisomy 13

47,XX,+13 or 47,XY,+13

Incidence at birth 1/5,000

Structural

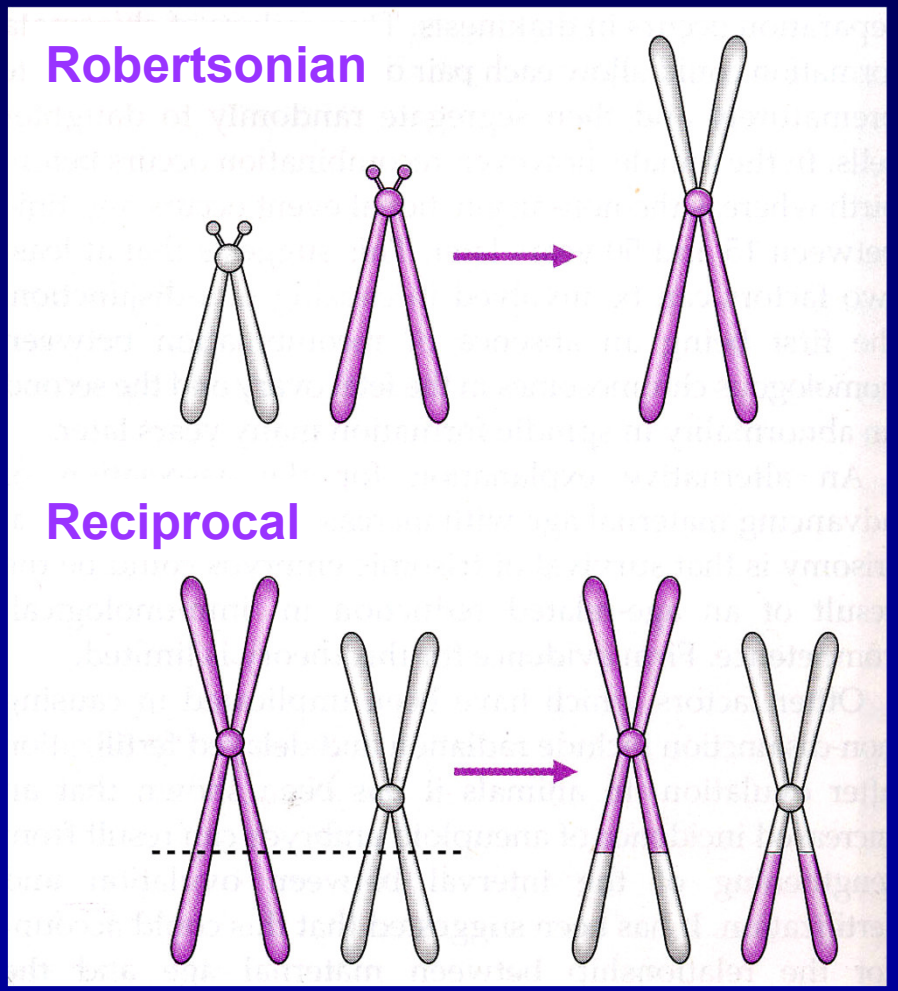
- Breakage in at least 1 chromosome
- Translocations
  - 2 different chromosomes break and rejoin incorrectly
- Inversions
  - 2 breaks in same chromosome
- Insertions
  - Piece of chromosome inserted
- Deletions
  - Piece of chromosome missing

Chromosome breaks

- Once chromosome broken by some means
- Unstable situation as telomeres not at end
- Usually join up to other piece

Translocations

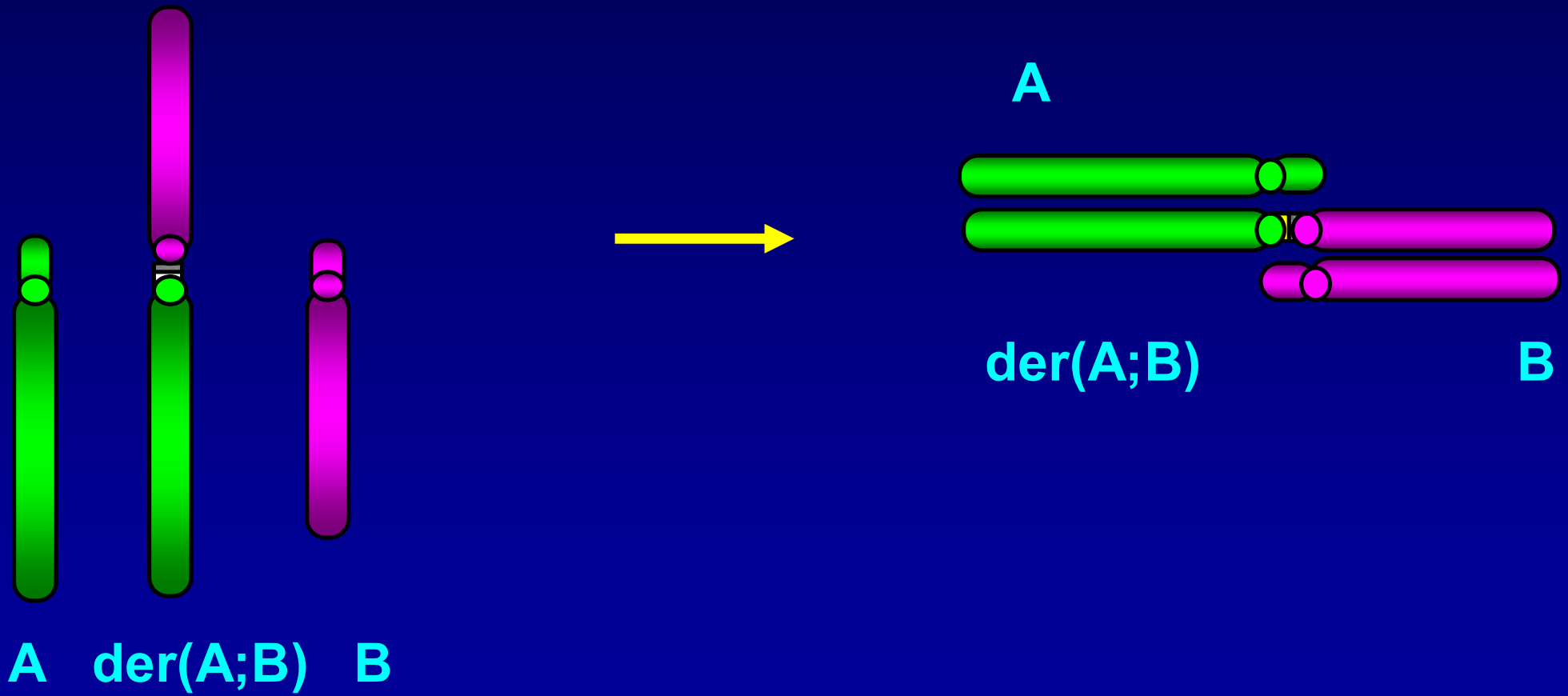
- Chromosome moves from normal position to abnormal position
- Robertsonian
  - Acrocentric chromosomes
  - D and G groups (13, 14, 15, 21, 22)
- Reciprocal
  - Any chromosome



Robertsonian translocations

- Lose satellite and short arms
  - Genes for rRNA
  - Repeated on other acrocentric chromosomes
- Reduce chromosome number by one (45)
  - but no loss of chromatin from long arms
- Phenotypically normal – problems at meiosis
- Involved in evolution

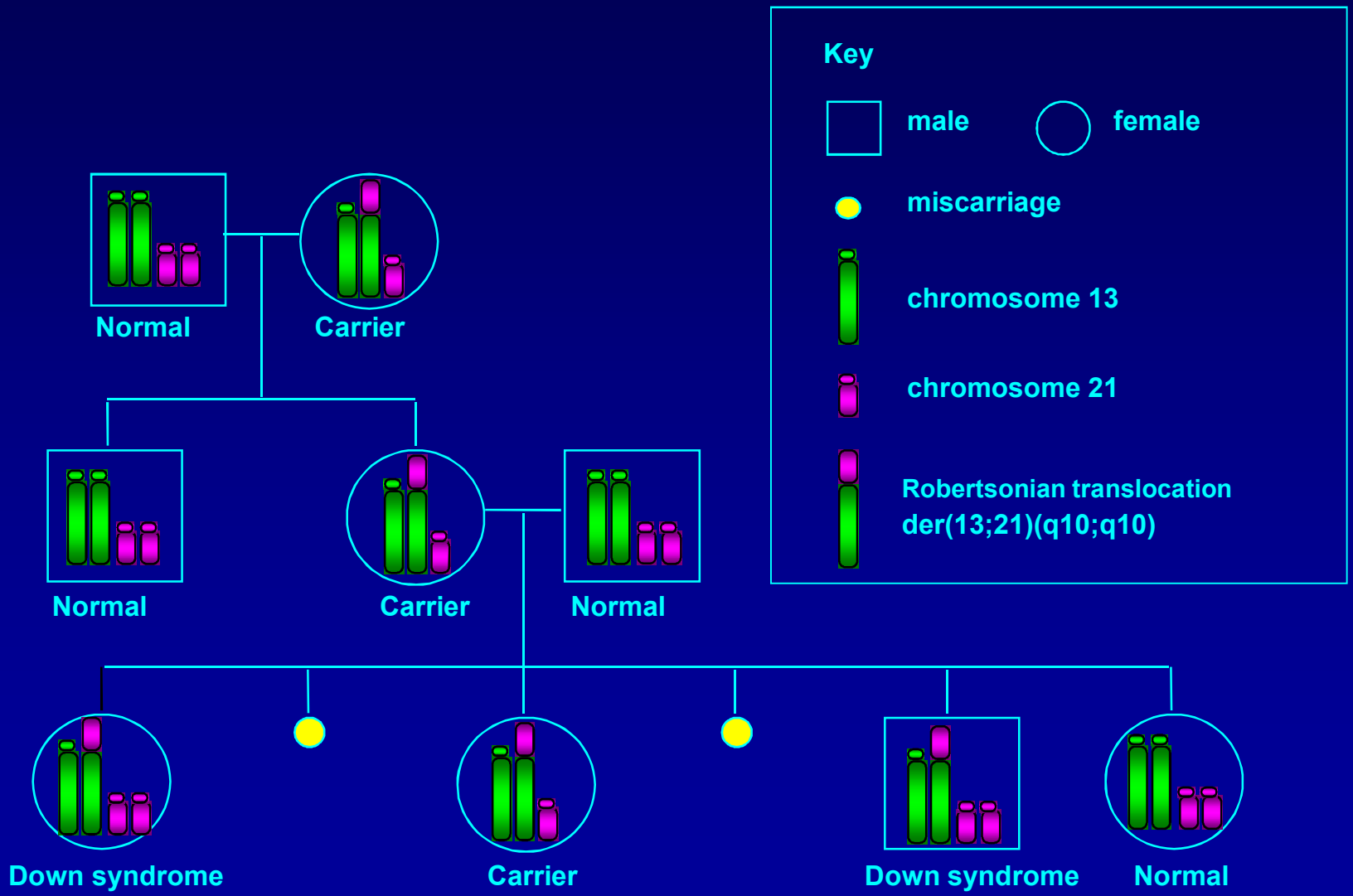
Robertsonian translocations



Robertsonian translocations

- D:G translocation
  - Often 14:21 joined
- G:G translocation
  - 21:22 joined
  - 21:21 joined
    - 21 smallest chromosome

Robertsonian translocation family pedigree

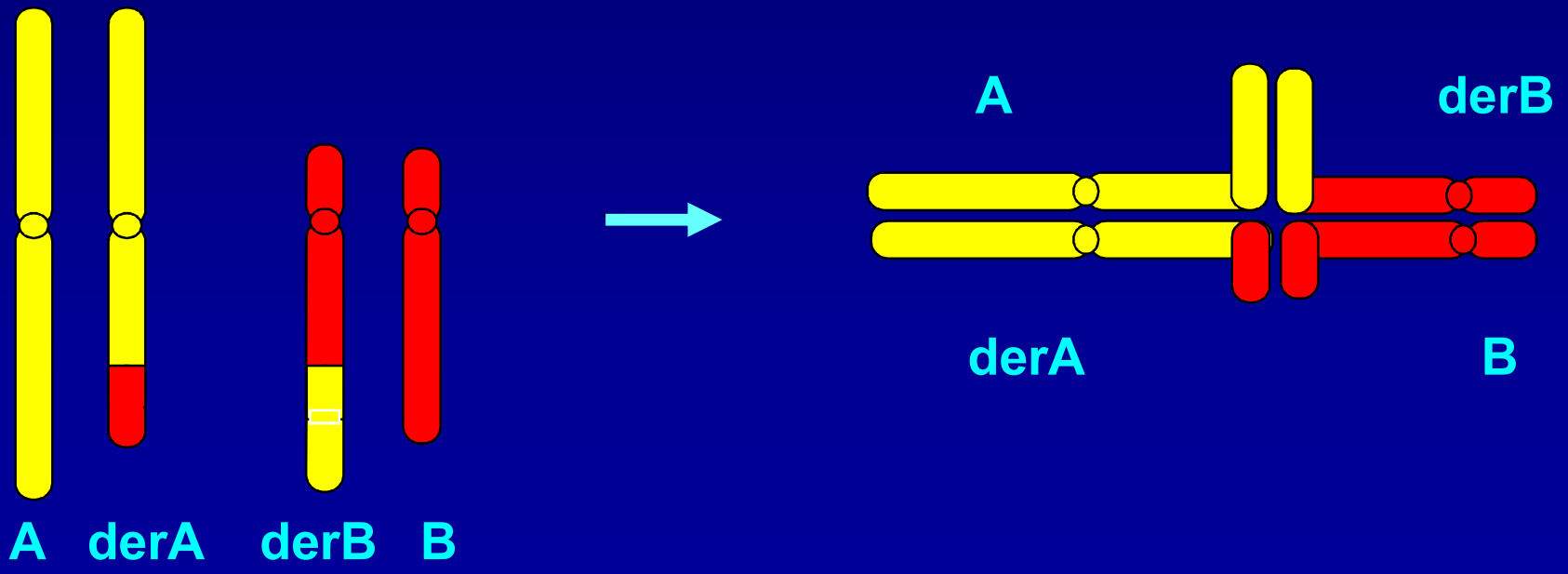


Robertsonian translocations

- 45,XY,der(13q;14q)(q10;q10)
- 45,XX,der(13q;21q)(q10;q10)

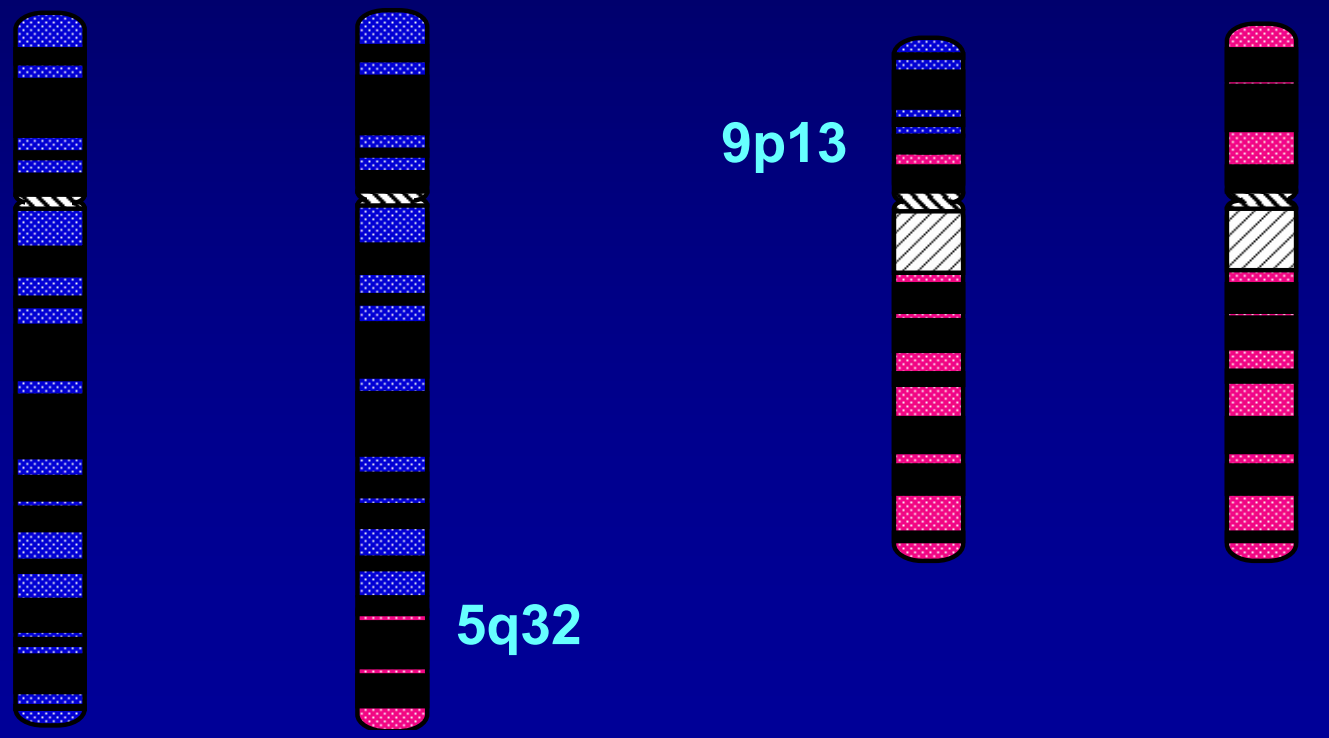
Reciprocal translocations

- More common than Robertsonian
- Break in any chromosome at any point
- Phenotypically normal – problems at meiosis



Reciprocal translocation

46,XX,t(5;9)(q32;p13)

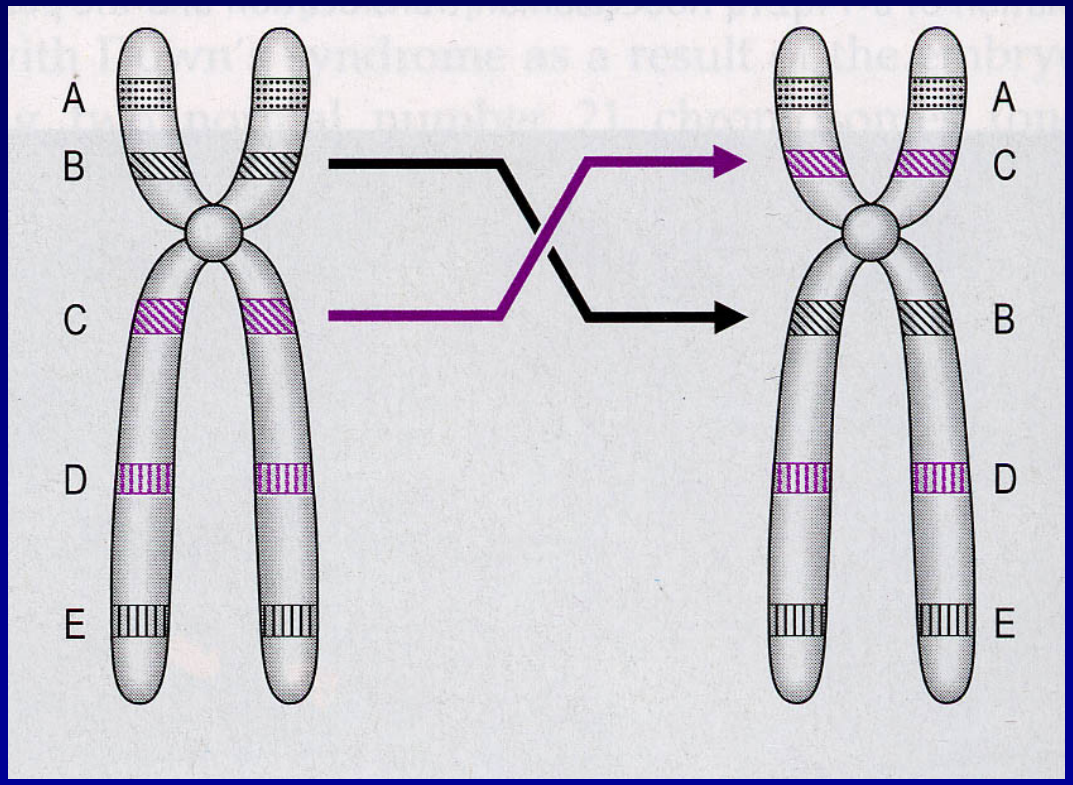


Inversions

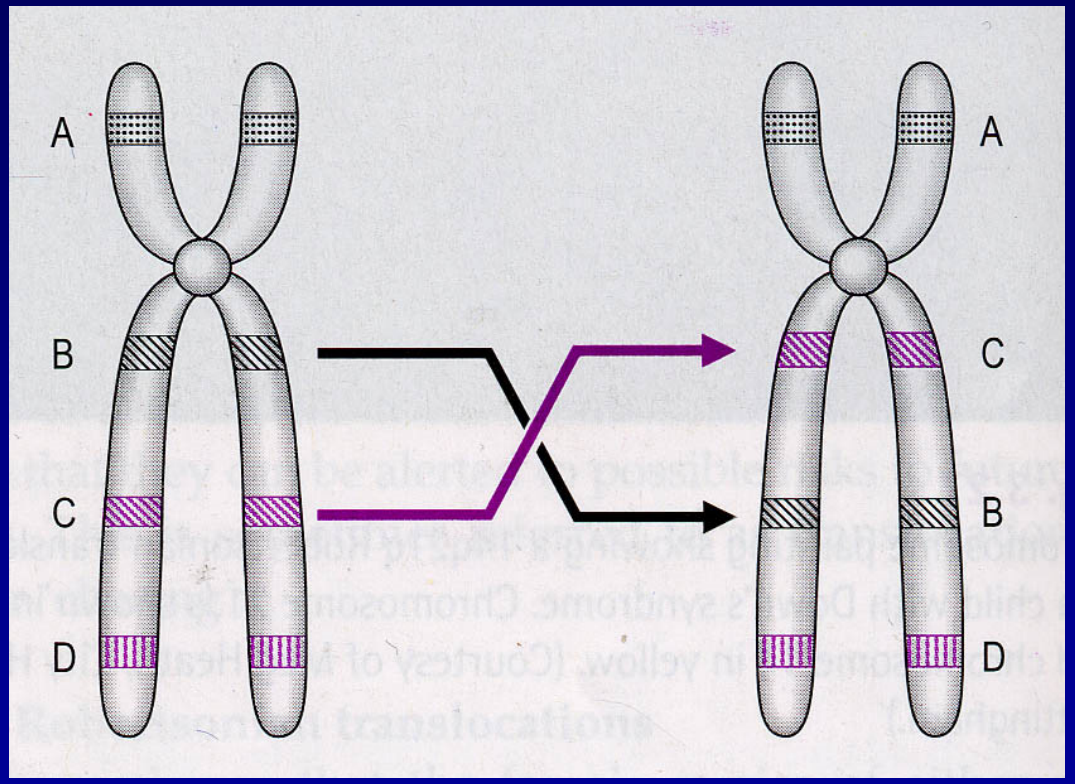
- Reversal of segment of chromosome
  - If too small cannot detect by karyotype
  - Very rare in humans
  - Selected against as would get reduced fertility
- Pericentric
  - reversed segment includes centromere
- Paracentric
  - within one chromosome arm

- Paracentric inversion
  - main difference in karyotypes of great apes and humans so important in evolution

Inversions



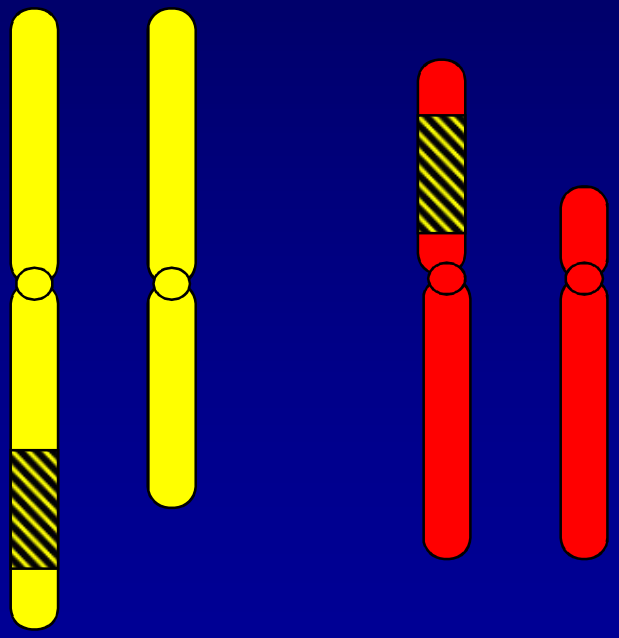
Pericentric



Paracentric

Insertions

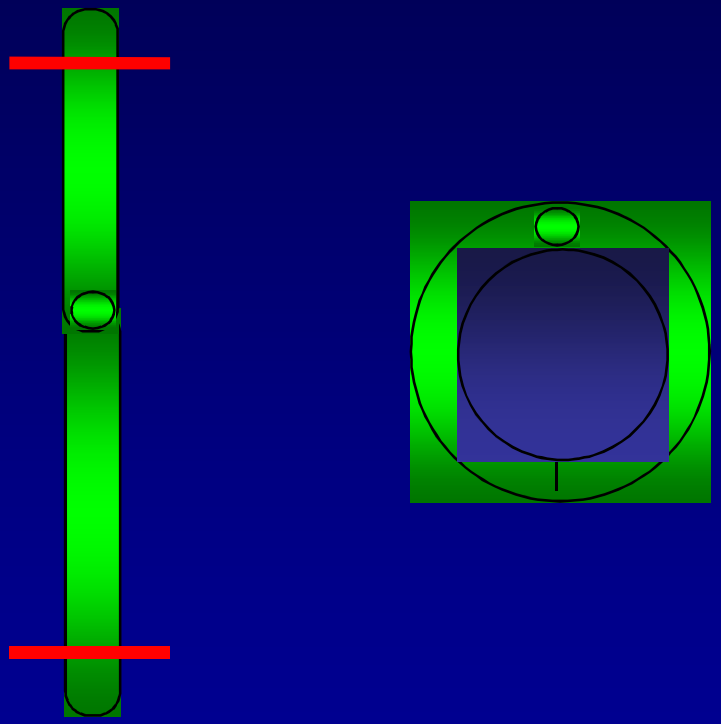
- Segment of 1 chromosome inserted into another



Deletions

- Terminal
  - loss of end of chromosome
  - 46,XY,del(10)(q26) missing long arm of 10
- Interstitial
  - loss of segment from within chromosome
  - 46,XY,del(10)(q24q26) missing segment of 10
- All result in unbalanced karyotype
- Partial monosomy
- Serious clinical effect

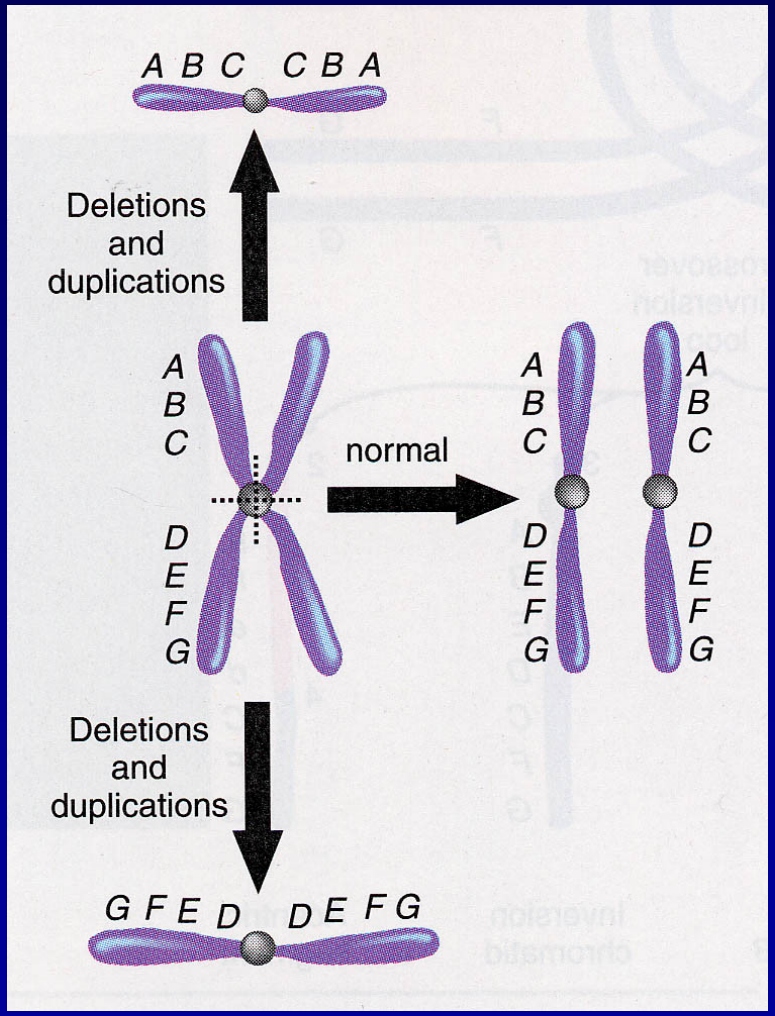
Ring chromosome



A derA

Isochromosome

- Two copies of the same arm
- Mirror image around centromere
- Centromeres part in wrong plane
  - Monosomy for 1 chromosome arm
  - Trisomy for the other arm



ESAC

- Extra Structurally Abnormal Chromosome
- Abnormal chromosome in addition to 46
- Small and difficult to identify
- Sometimes called marker chromosomes
- Difficult to work out effect on person
- May be benign or cause serious mental handicap

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Applications

1) See if there are extra chormosomes or less chromosomes.
   2) See if there are extensions or deletions.
   3) To study chromosomal aberrations.
   4) To study cellular functions.
   5) To study taxonomic relationships.
   6) To gather informations about past evolutionary events.
2) Spectral Karyotyping (SKY) can detect1. Chromosomal material of unknown origin2.