Introduction to Clinical Cytogenetics: Lecture 1

- Cinthya J Zepeda Mendoza, PhD
- Medical Director, Cytogenetics and Genomic Microarray ARUP Laboratories
- Assistant Professor, Department of Pathology, University of Utah
- Email: Cinthya.Zepeda@aruplab.com

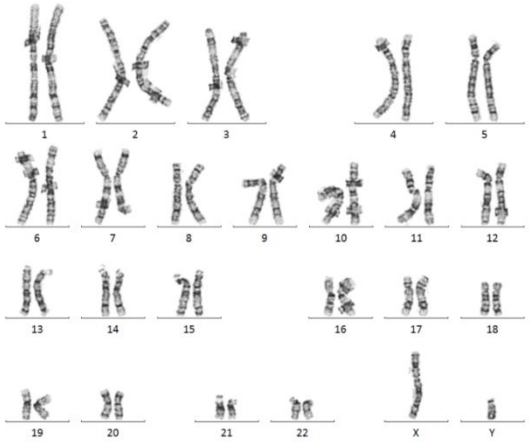
Cytogenetics Textbooks

- Gardner and Amor. (2018) Gardner and Sutherland's Chromosome Abnormalities and Genetic Counseling. 5th edition
- Gersen and Keagle. (2013) The Principles of Clinical Cytogenetics. 3rd edition

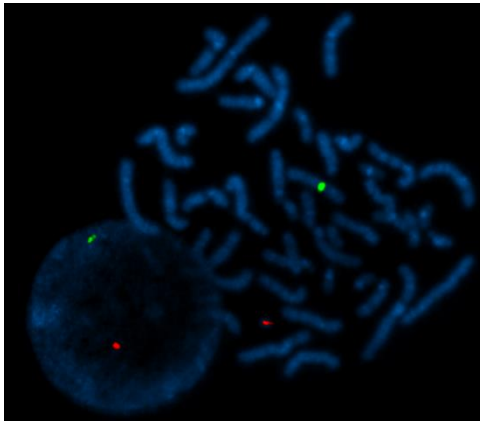
What is Cytogenetics?

- The study of chromosomes and genomic structure, function, and variation and their role in human disease and heredity

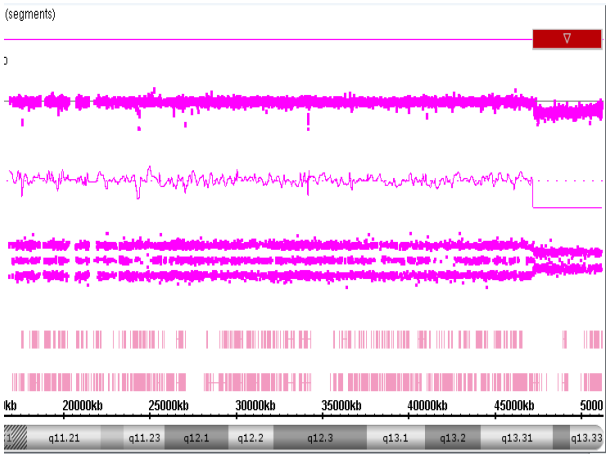
Chromosome analysis/ karyotyping



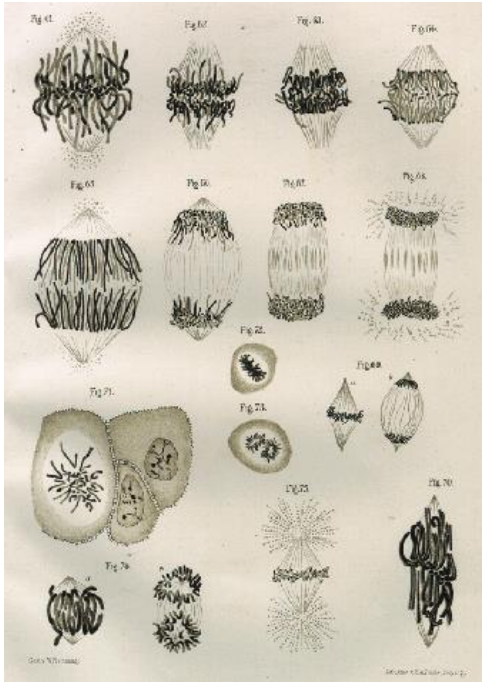
Fluorescence in situ hybridization



Genomic microarray



A brief history of cytogenetics



1882: W Flemming observed chromosomes and their movement during cell division.

1902: T Boveri and Walter Sutton noticed that that chromosomes obey Mendel's Laws during cell division

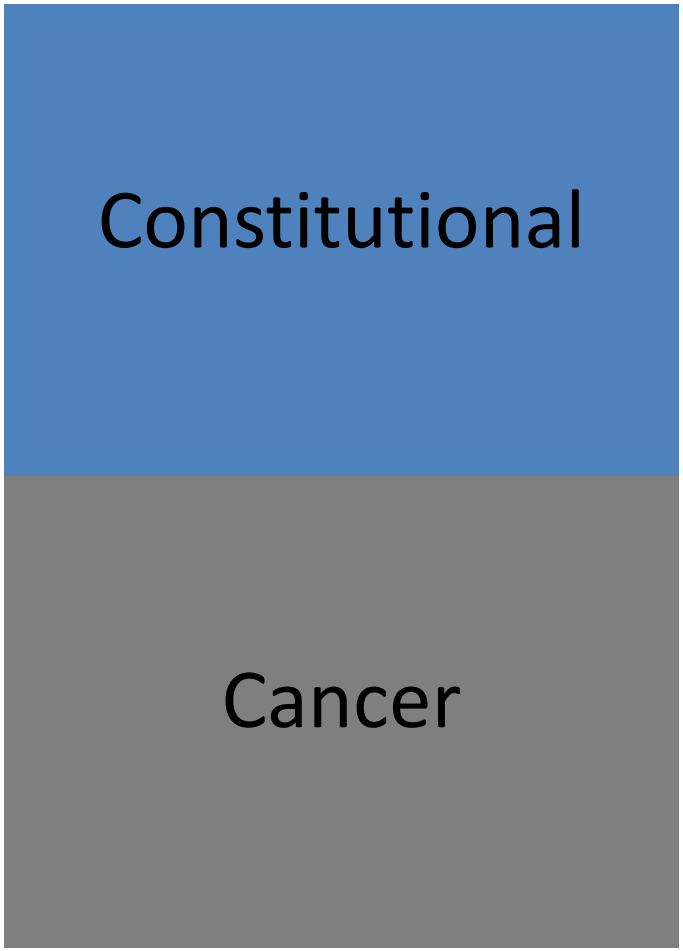
1912: T Morgan and C Bridges discovered linkage and established the chromosomal theory of inheritance

1921: Human chromosome count set at 48 chromosomes

1956: Human chromosome count set at 46 chromosomes by J Tjio and A Levan

1959: first successful diagnosis of a human chromosome aberration by J Lejeune - trisomy 21 in three cases of Down syndrome

Cytogenetics in the clinic



- Diagnosis of heritable genetic abnormalities in children, adults, pregnancy, and fetal loss
- Detection of acquired or somatic abnormalities for the diagnosis, prognosis, therapy, and/or monitoring of many types of cancer (especially leukemia and lymphoma)

Indications for Constitutional Cytogenomic Analysis

Prenatal

- Abnormal maternal serum screening
- Abnormal cfDNA/NIPT/NIPS
- Abnormal ultrasound
- AMA, ≥ 35 yrs
- Familial chromosome abnormality

Fetal or neonatal demise

Amniotic fluid, chorionic villus sampling, fetal tissues

Postnatal, childhood

- Perinatal: Birth defects, dysmorphisms, ambiguous genitalia
- Developmental delay/Intellectual disability
- Neurological & behavioral: seizures, autism, etc

Peripheral blood, buccal swab, skin biopsy

Adolescent & adult

- Amenorrhea, ovarian failure
- Infertility or spontaneous abortions
- Birth of a child with a chromosomal abnormality

Peripheral blood

Introduction to Cytogenetics I

- DNA and Chromosomal Structure
- Cell Cycle, Mitosis and Meiosis
- Gametogenesis
- Mosaicism vs Chimerism
- Imprinting and Uniparental Disomy
- Karyotyping and Nomenclature

DNA Structure and Organization

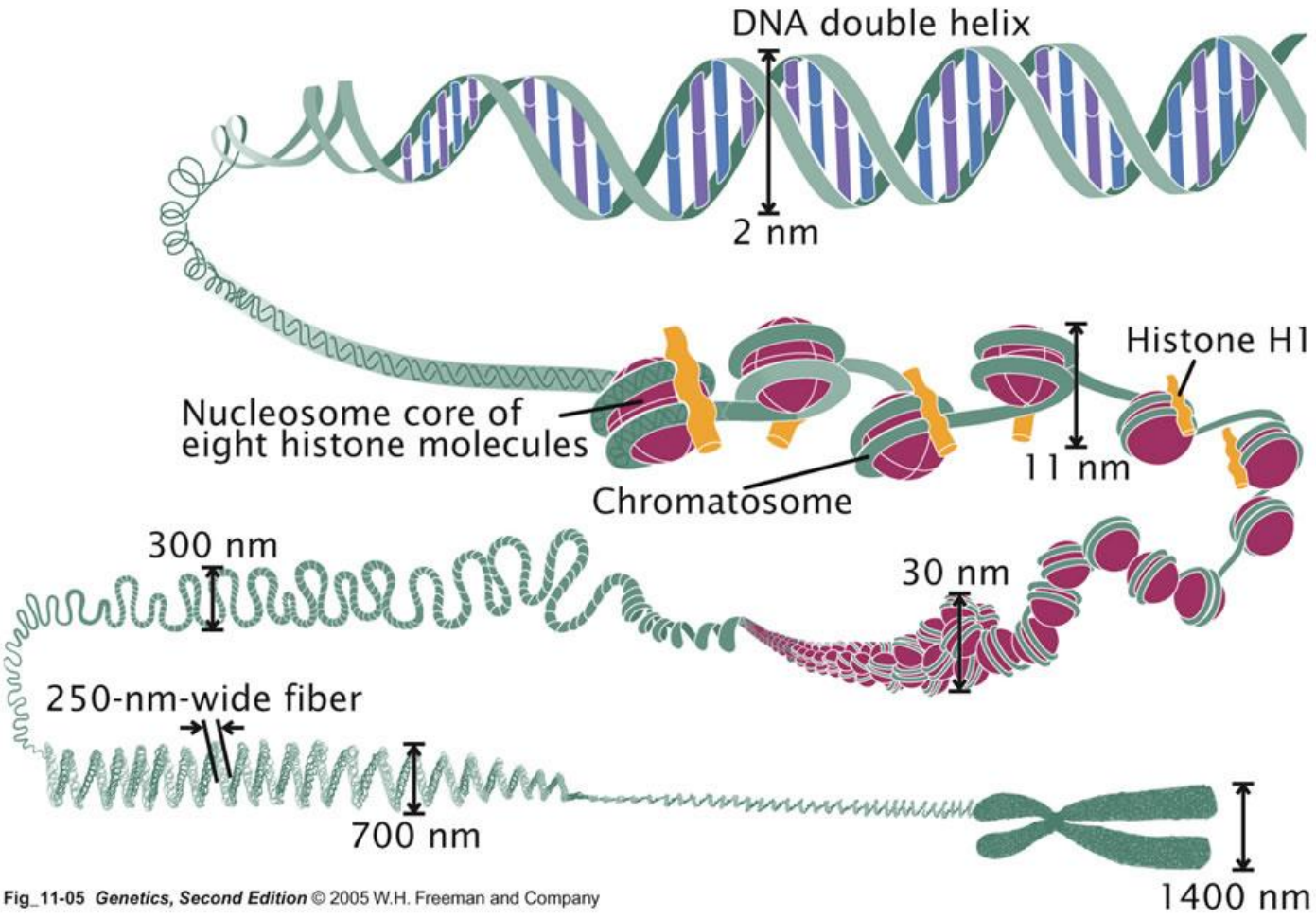
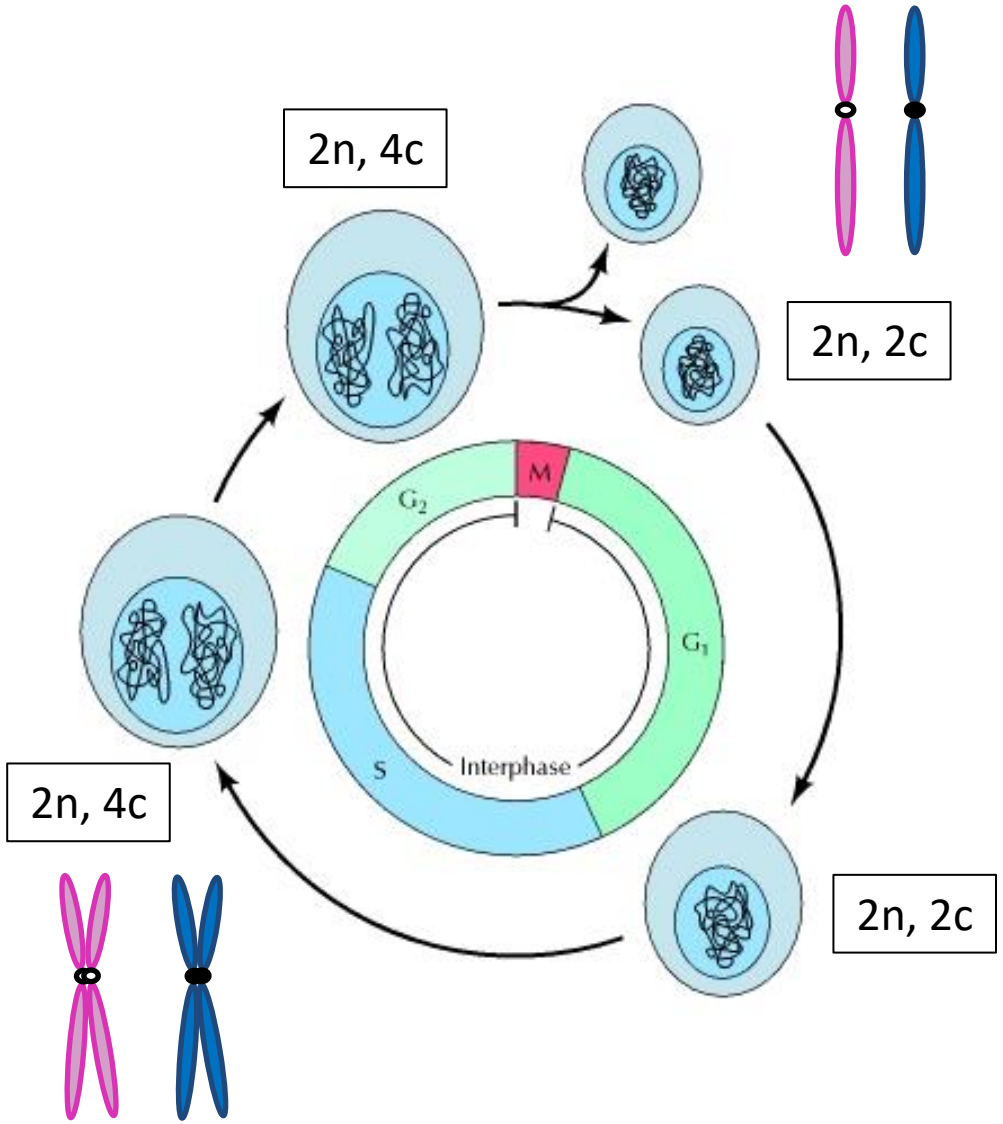


Fig_11-05 *Genetics, Second Edition* © 2005 W.H. Freeman and Company

Cell Division and The Cell Cycle

- Cell cycle: 4 phases: Mitosis (M), Gap1 (G1), Synthesis (S), Gap2 (G2)
- Mitosis: division of somatic cells

n = # chromosome sets, ploidy
c = # chromatids

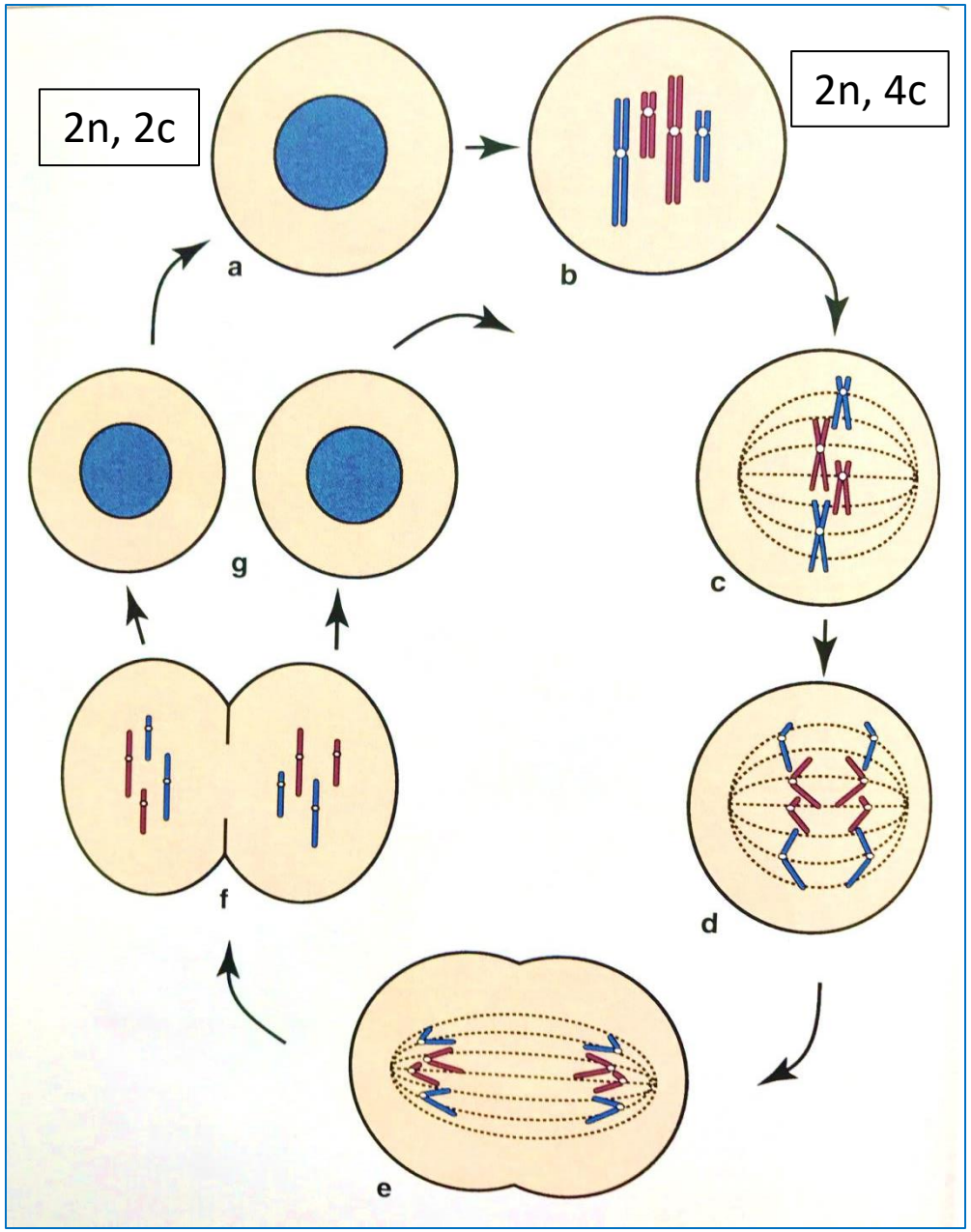


NCBI Bookshelf: Cooper, The Cell: A Molecular Approach, 2nd Ed 2000

Mitosis

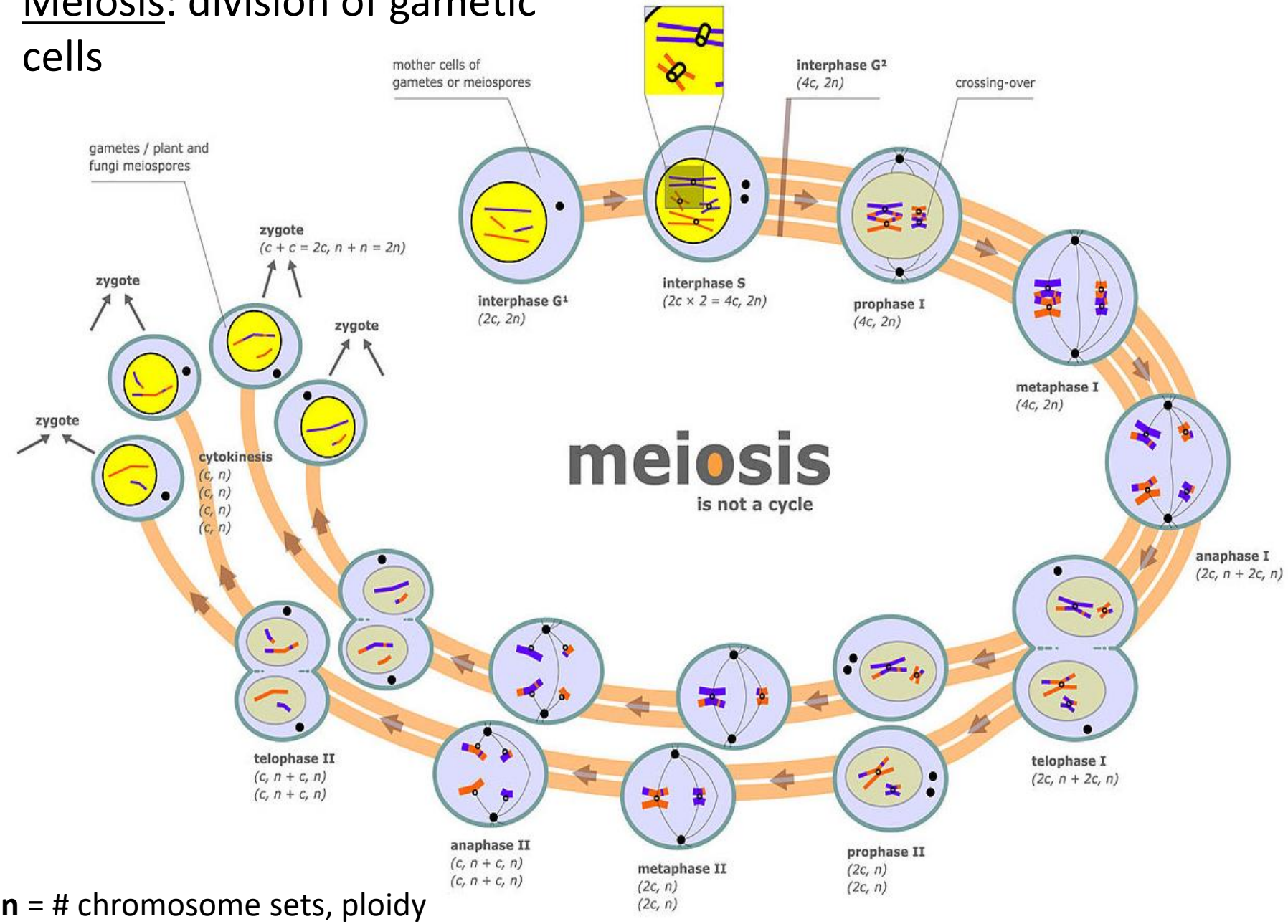
a) Interphase
b) Prophase
c) Metaphase*
d) Anaphase
e) Telophase
f) Cytokinesis
g) Interphase

* Stage observed in chromosome analysis



Modified from Gersen and Keagle, Principles of Cytogenetics, 3rd Ed 2013

Meiosis: division of gametic cells



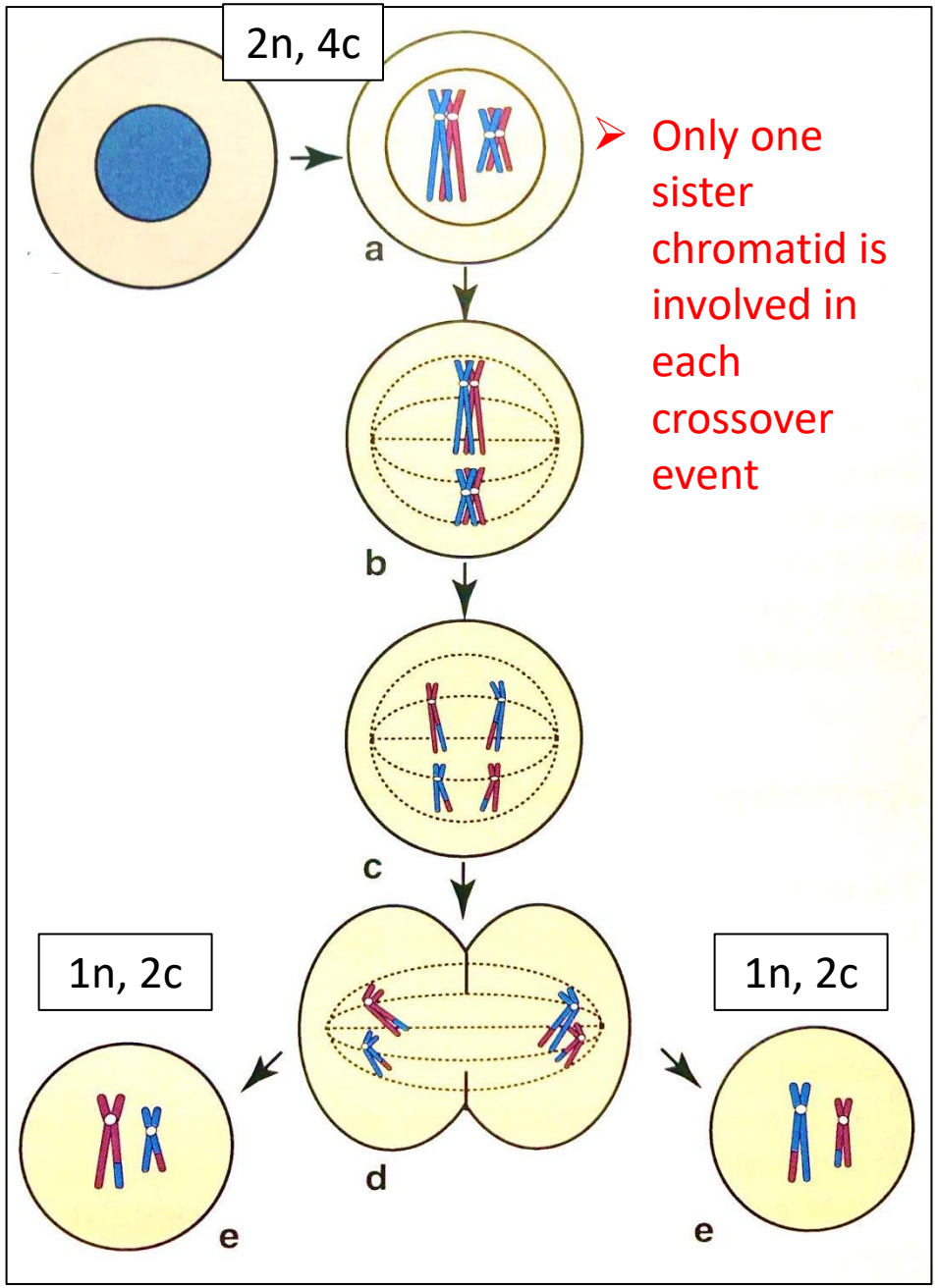
n = # chromosome sets, ploidy

c = # chromatids

Image source: http://www.macroevolution.net/reduction-division.html

Meiosis I

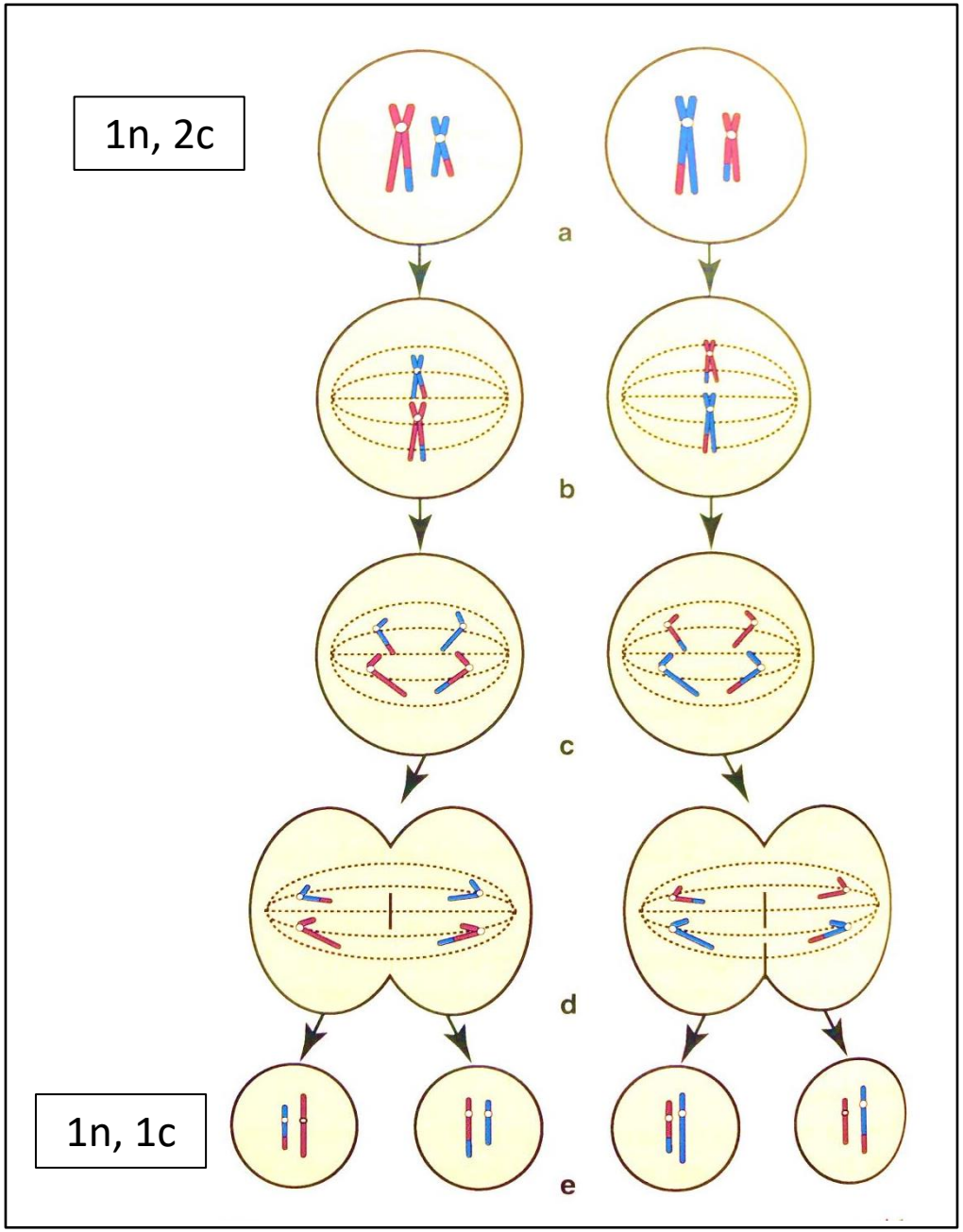
a) Prophase I: Homologs pair, cross-over
b) Metaphase I
c) Anaphase I
d) Telophase I
e) Daughter cells



Modified from Gersen and Keagle, Principles of Cytogenetics, 3rd Ed 2013

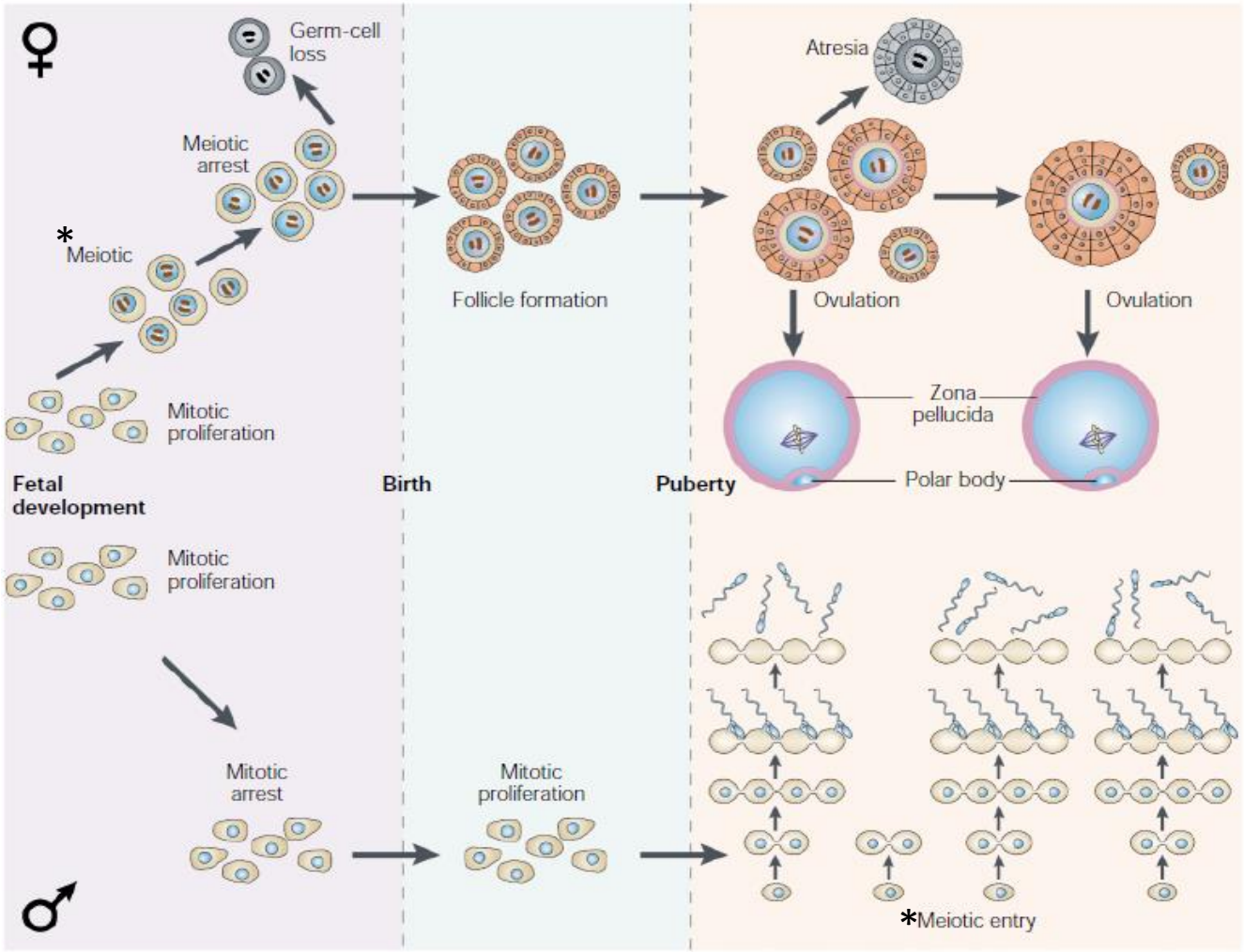
Meiosis II

a) Prophase II
b) Metaphase II
c) Anaphase II
d) Telophase II
e) Daughter cells



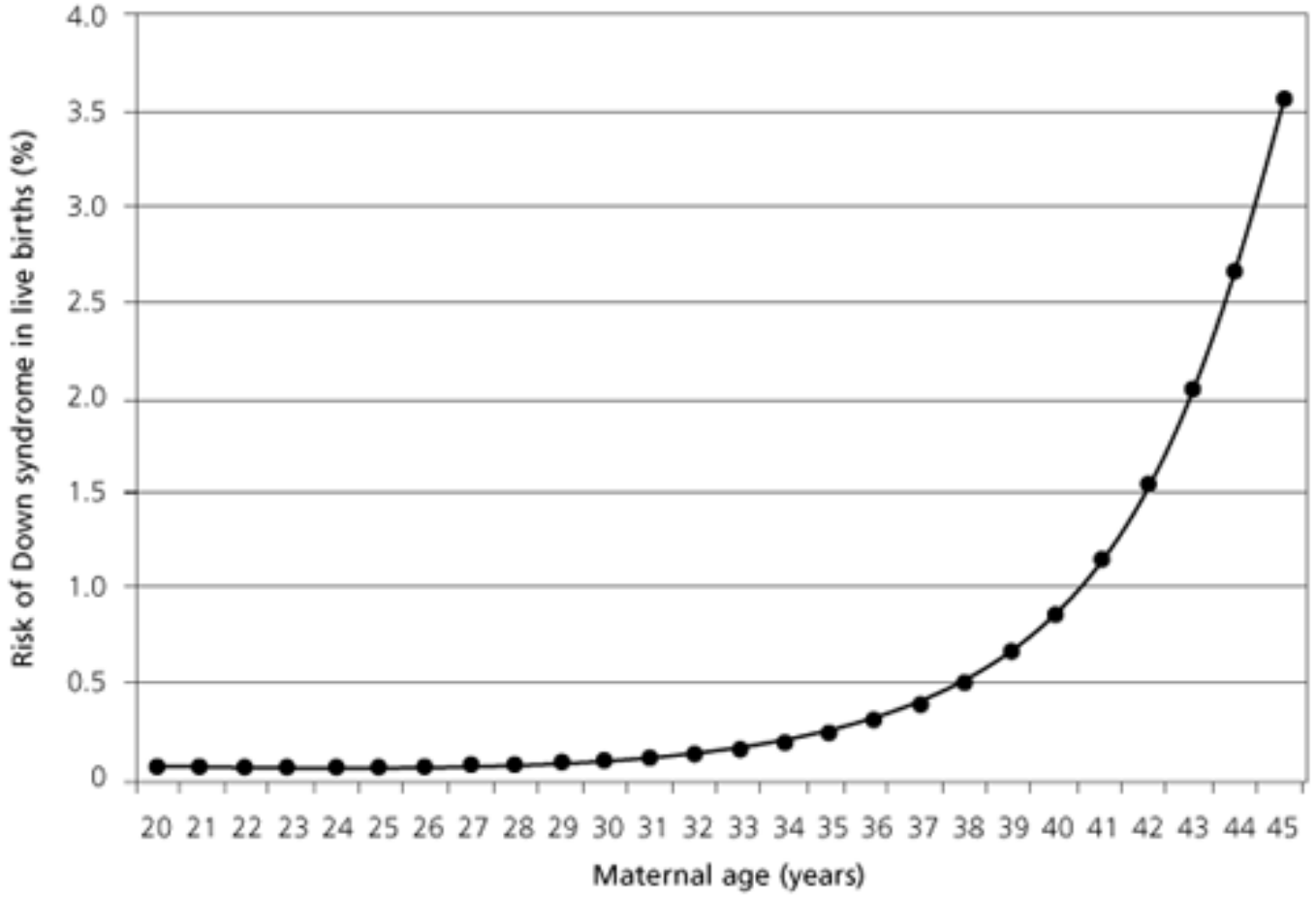
Gersen and Keagle, Principles of Cytogenetics, 3rd Ed 2013

Oogenesis vs Spermatogenesis



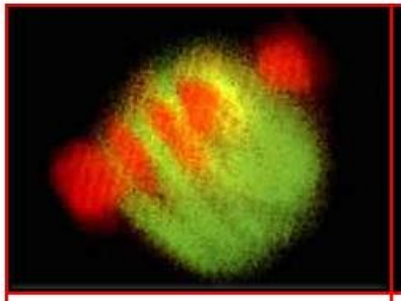
Hassold and Hunt (2001) Nat Rev Genet

Down Syndrome and Maternal Age

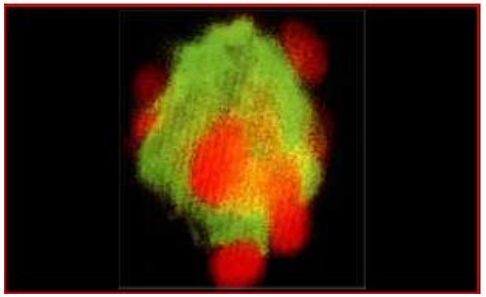


Newberger (2000) Am Fam Physician

Ovum from a woman in her 20's



Ovum from a woman in her 40's



Battaglia et al., 1996

Errors in Meiosis, Mitosis and Cell Division Lead to Genomic Imbalance

Euploid	Aneuploid	Polyploid
Normal chromosome complement	Abnormal chromosome complement	Abnormal number of chromosome sets
• Haploid: 1n, 23 chroms = 23 count • Diploid: 2n, 23 pairs of chroms = 46 count	• Monosomy: 2n-1, missing chrom = 45 count • Trisomy: 2n+1, additional chrom = 47 count	• Triploidy: 3n, 3 sets of chroms = 69 count • Tetraploidy: 4n, 4 sets of chroms = 92 count

Mixture of Cell Lines: Mosaicism versus Chimerism

Mosaicism: the presence of at least two genetically distinct, but related cell lines (clones) arising in the same individual

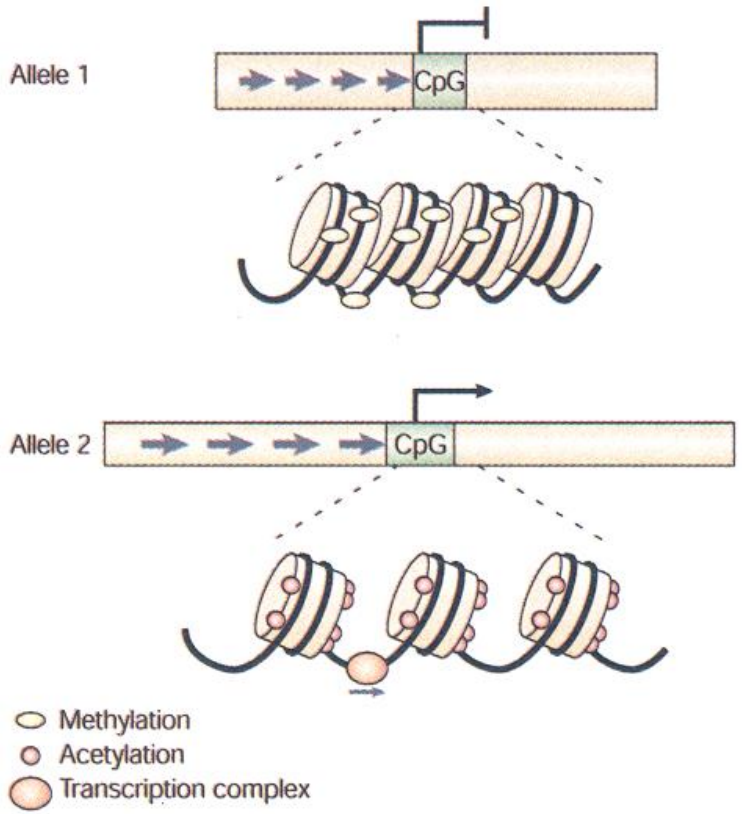
- e.g. constitutional, Turner syndrome: 45,X[15]/46,XX[5]
- e.g. acquired, CML: 46,XX,t(9;22)(q34;q11.2)[9]/46,XX[11]

Chimerism: the presence of at least two genetically distinct cell lines that are derived from different conceptions:

- e.g. twin-twin fusions or transfusions, tissue/organ transplants
- e.g. Bone marrow transplant patient: 46,XX[3]//46,XY[17]

Clinical presentation may be similar, variable, milder, or normal; may see skin pigmentation anomalies; may be identified by a recurrent cytogenetic abnormality in the children of parents who have normal karyotypes (i.e. gonadal mosaicism)

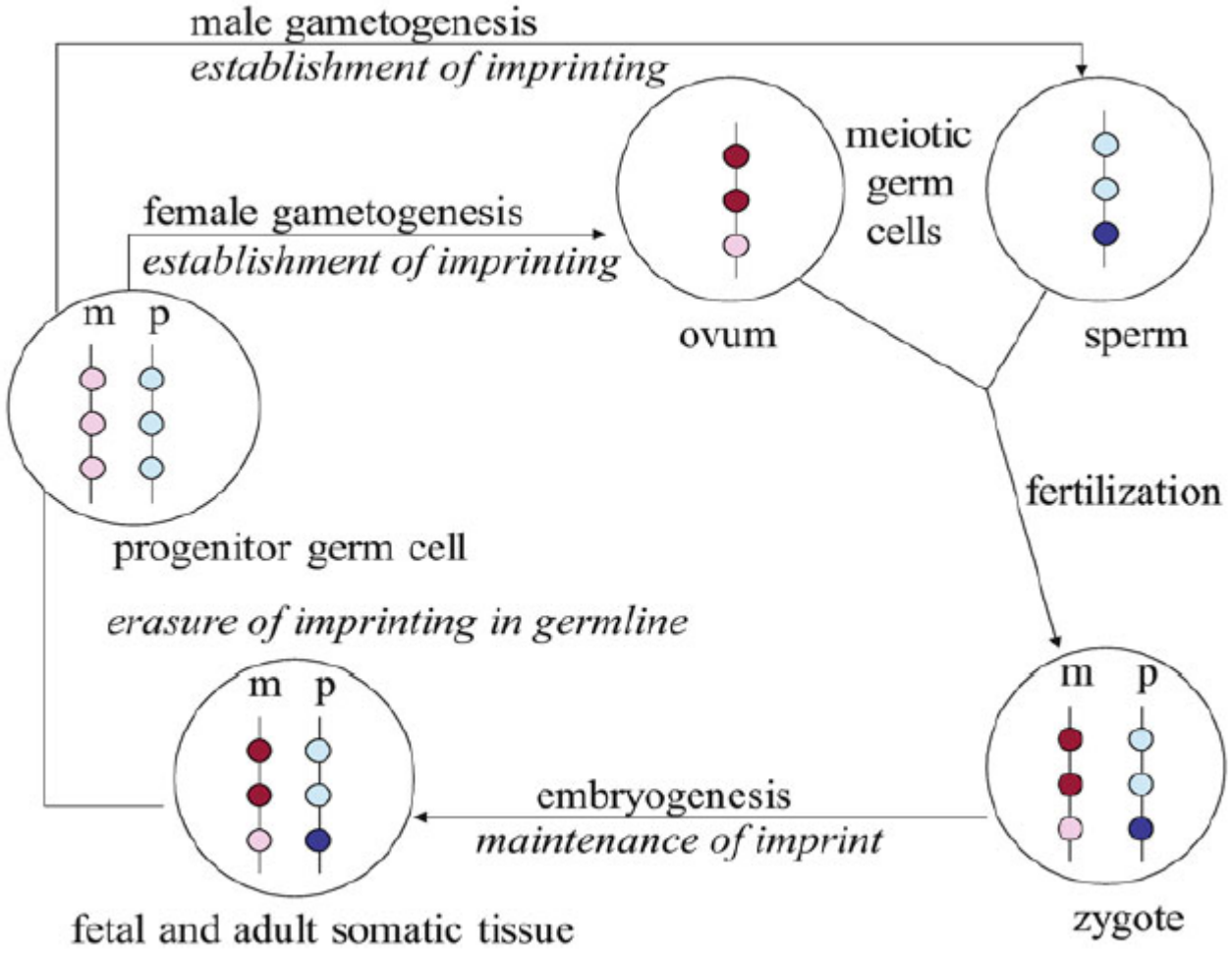
Genomic imprinting



- **Allele 1**
  - CpG island **<u>methylation</u>**
  - Histone deacetylation
  - Nucleosomal condensation
- **Allele 2**
  - Opening of the chromatin by acetylation and **<u>demethylation</u>**
  - Transcription complex can access genes

Reik and Walter, Nat Rev Genet 2,21-32(2001)

Genomic imprinting



Gersen and Keagle, Principles of Cytogenetics, 3rd Ed 2013

Uniparental disomy (UPD)

- Biparental inheritance: the normal situation; one chromosome is inherited from each parent

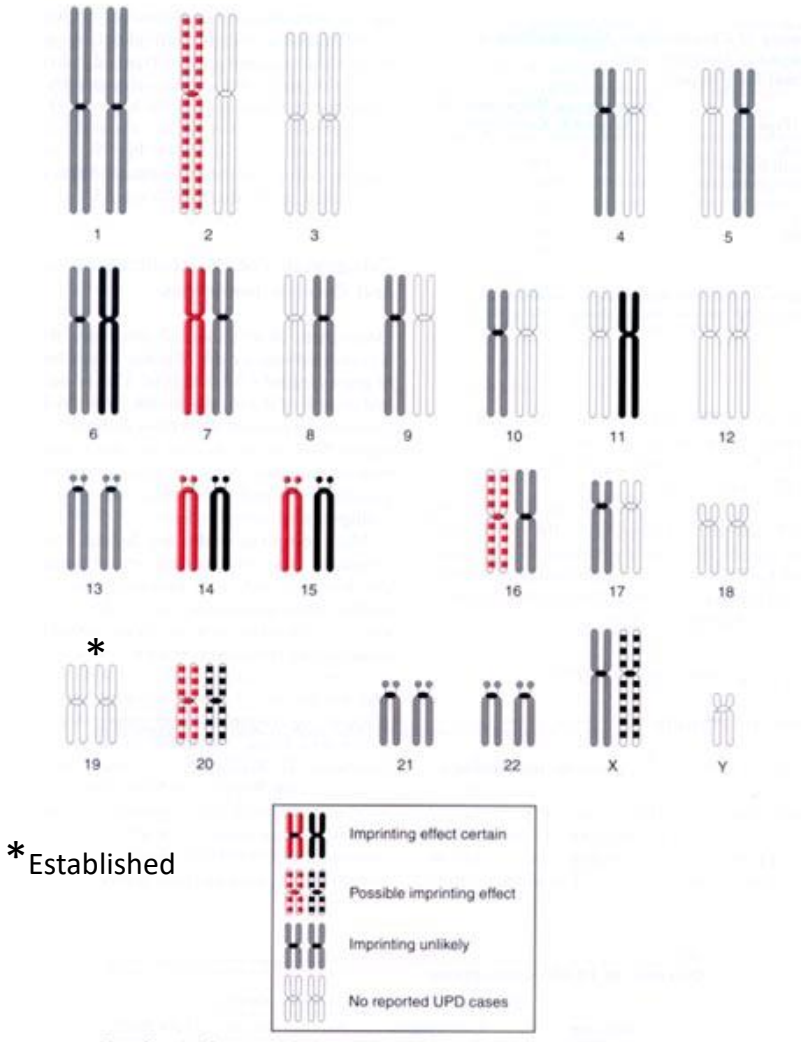
Biparental

- Uniparental disomy: both chromosome copies come from a single parent
  - Risk for recessive disease for genes in the homozygous chromosome segment
  - Risk for imprinting disorder if involving chromosomes that contain imprinted genes, differentially expressed dependent on parent of origin

Uniparental

Images modified from Yamazawa et al., 2010, Am J Med Gen C

Imprinted chromosomes and human disease due to uniparental disomy (UPD)



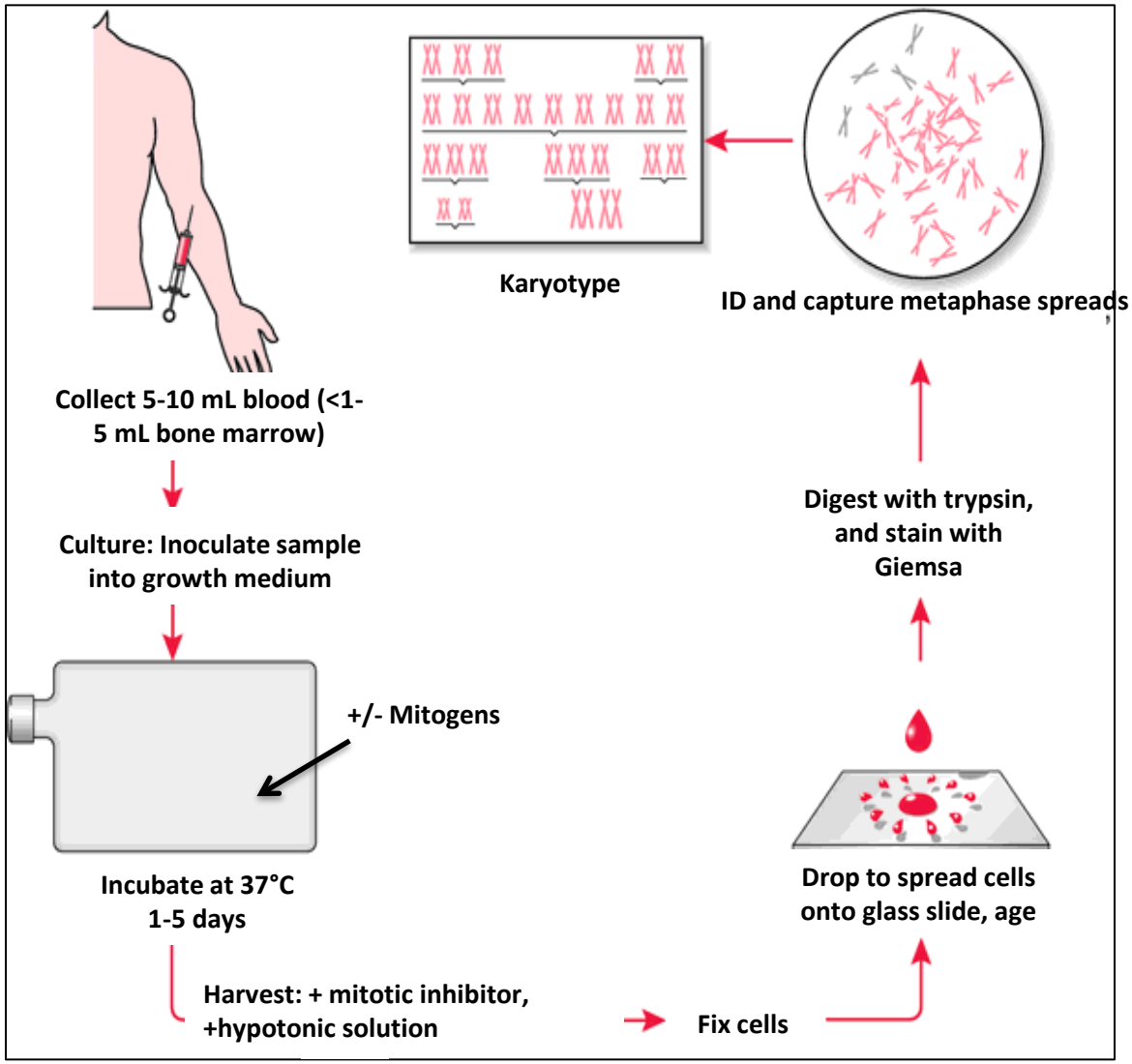
*Established

Chromosome UPD and Inheritance	Associated Genetic Disease or Abnormalities
Paternal UPD 6	Transient neonatal diabetes mellitus
Maternal UPD 7	Silver-Russell syndrome
Paternal UPD 11	Beckwith-Wiedemann syndrome
Maternal UPD 14	Temple syndrome Hypotonia, motor development delay, mild dysmorphic facial features, low birth weight, growth abnormalities
Paternal UPD 14	Kagami-Ogata syndrome Severe mental and muscoskeletal abnormalities
Maternal UPD 15	Prader-Willi syndrome
Paternal UPD 15	Angelman syndrome
Maternal UPD 20	Intrauterine growth retardation and/or postnatal growth retardation

Image from: http://carolguze.com/text/442-10-nontraditional_inheritance.shtml

Modified from Velissariou, Balkan J Med Gen

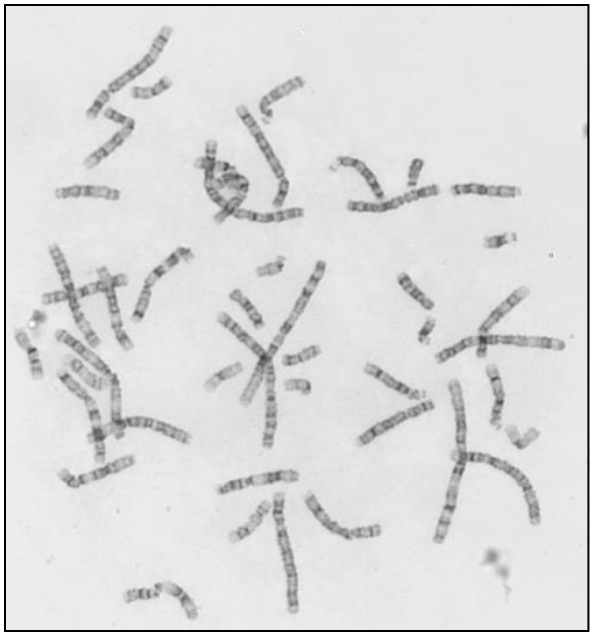
Preparation of metaphase chromosomes



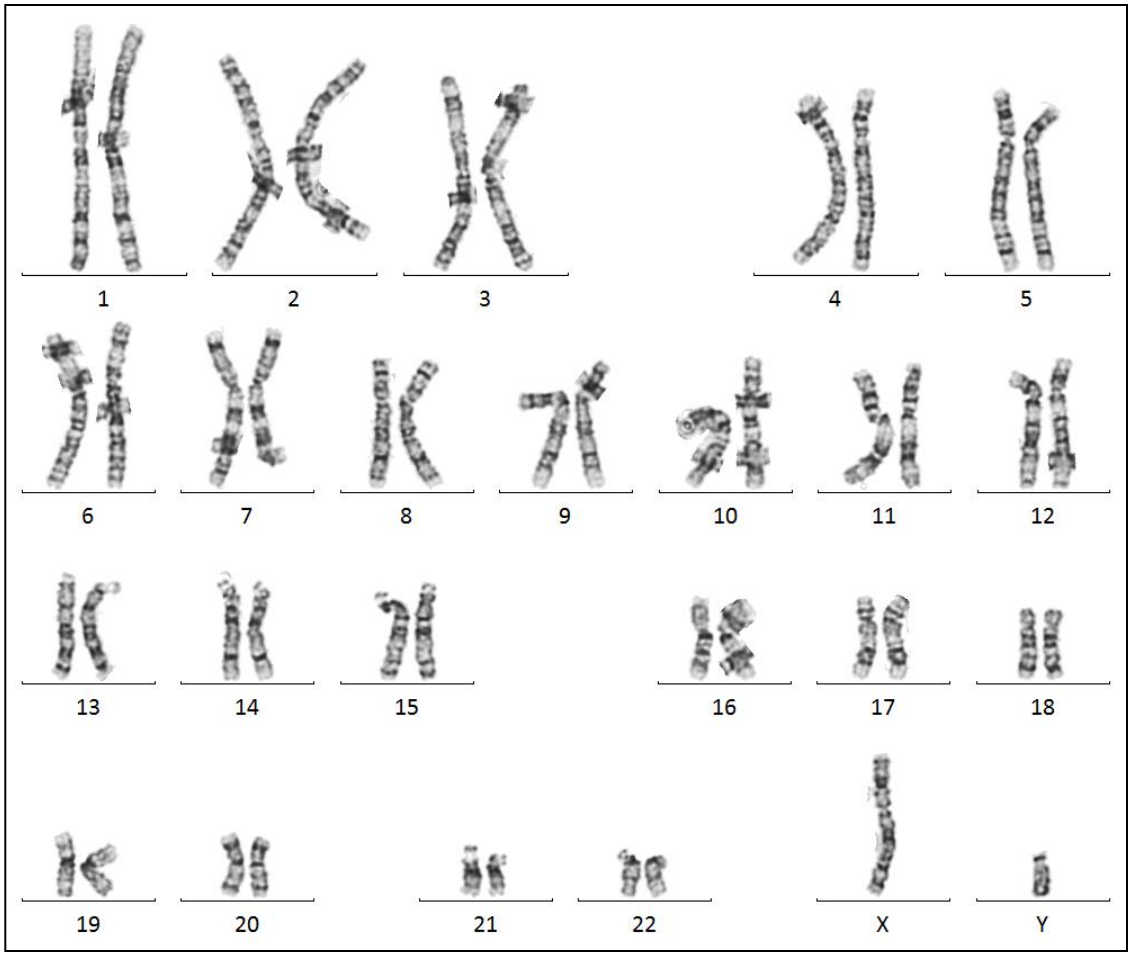
Modified from Preparation of a karyotype. Mueller and Young, 2001

Karyotyping

Metaphase spread



Karyogram



Overview of chromosome analysis

- Generally, 20 cells are analyzed from multiple cultures
- Definition of a clone:
  - At least two metaphase cells with the same extra chromosome or structural abnormality
  - At least three metaphase cells with the same chromosome loss
  - Abnormality observed in two independent cultures (r/o in vitro artifacts)

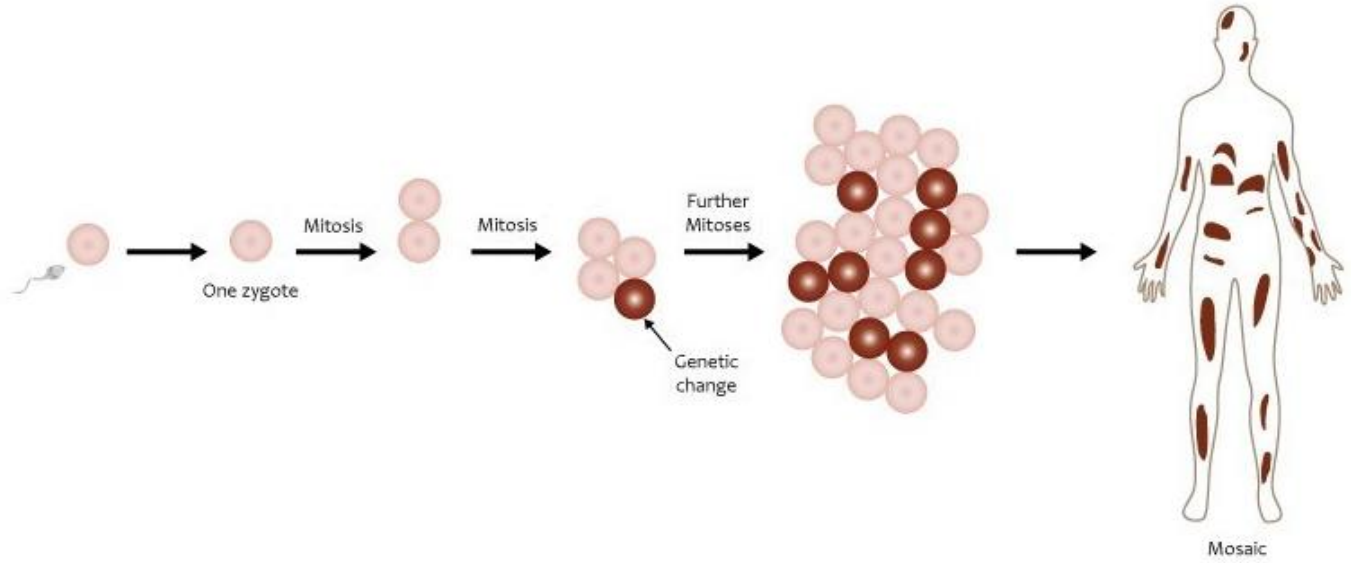
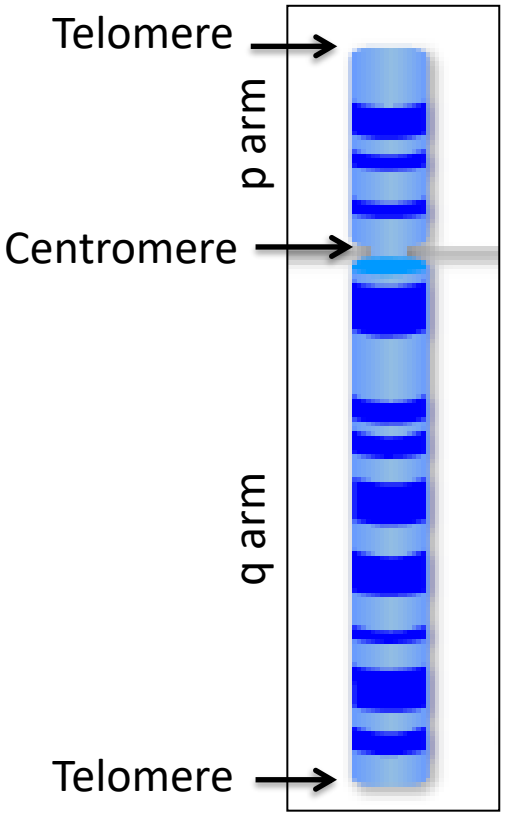
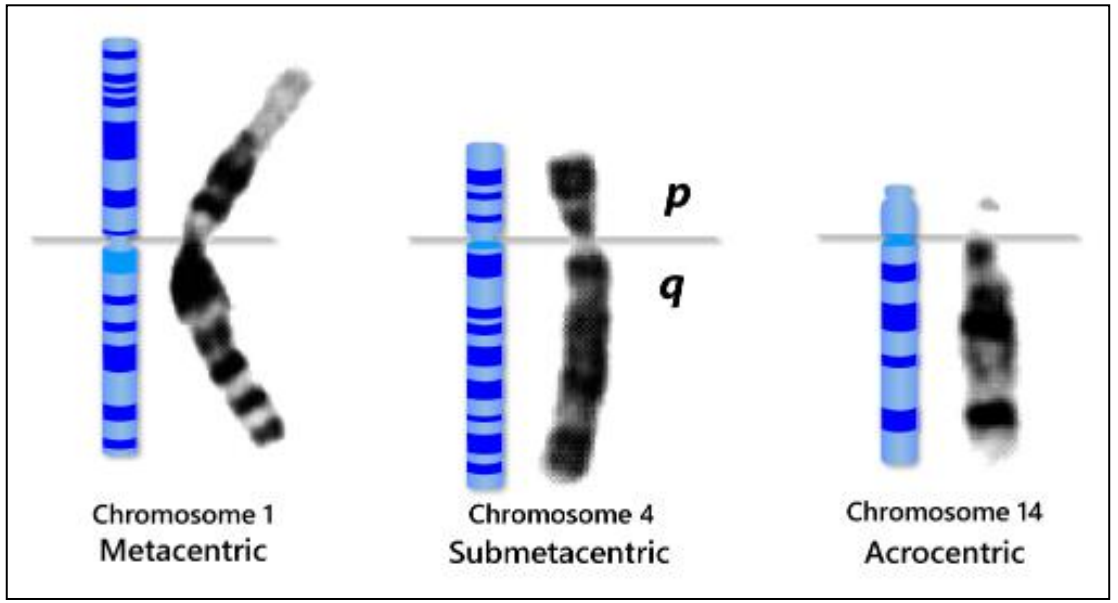


Image source: https://ugc.futurelearn.com/

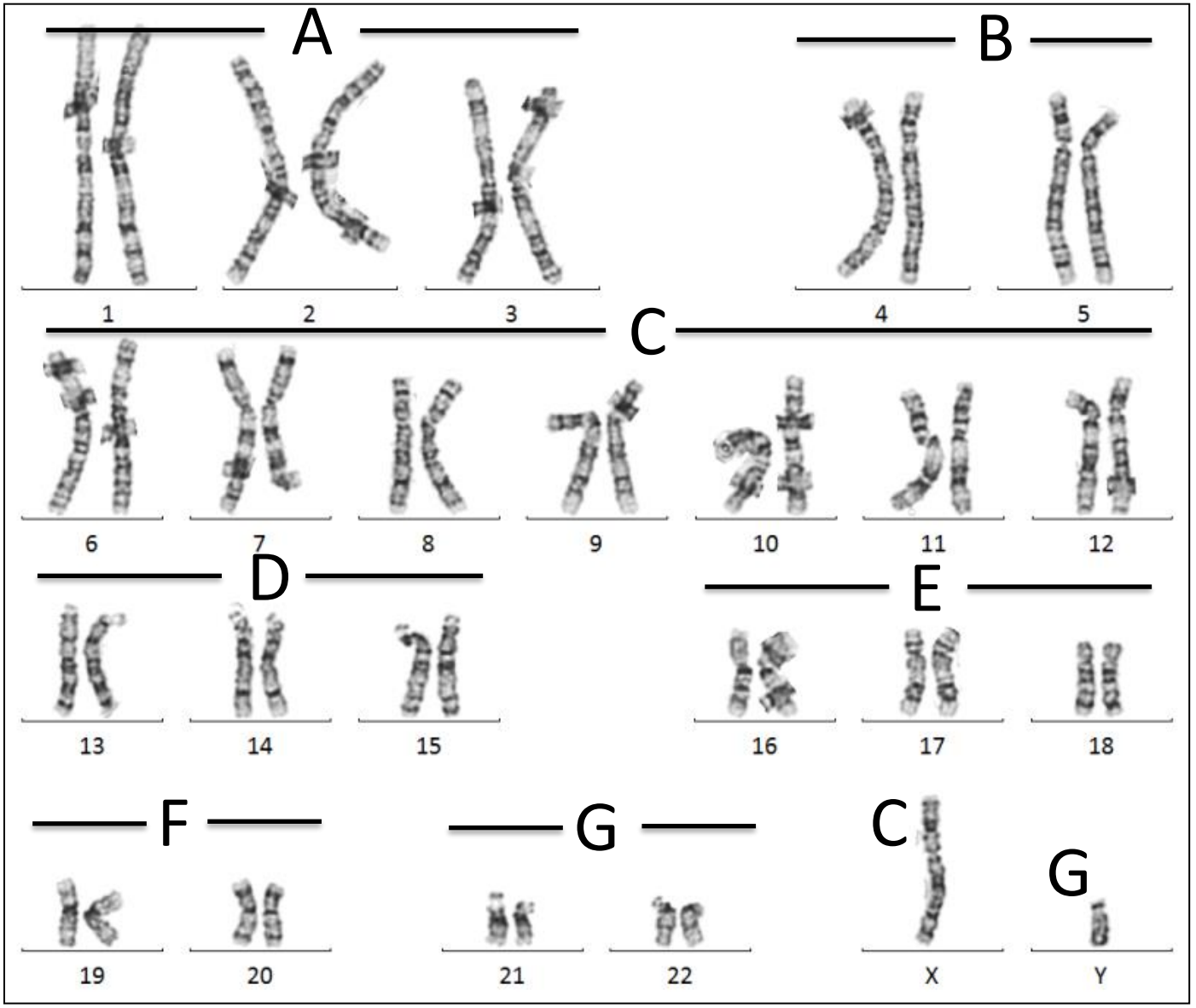
Chromosome Structure and Classification





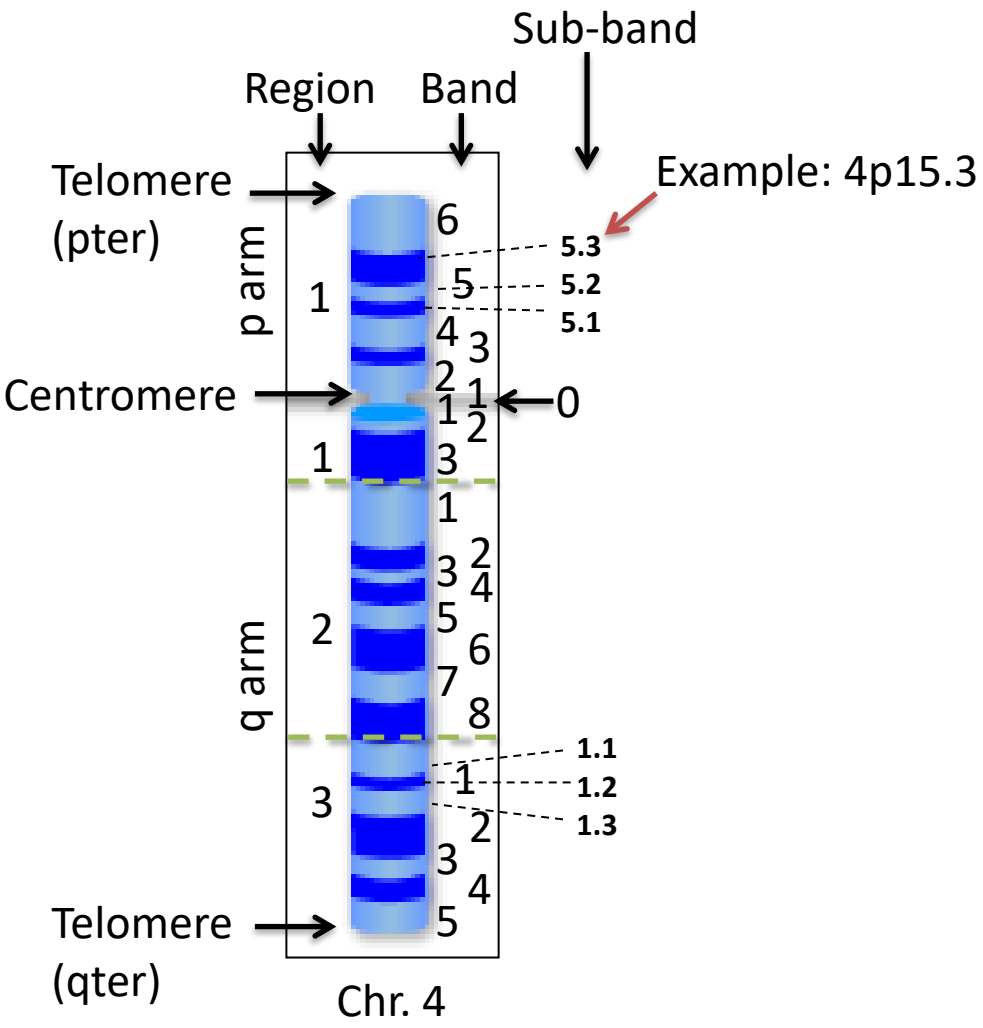
Images modified, source: http://learn.genetics.utah.edu/content/chromosomes/readchromosomes/

Chromosome Classification

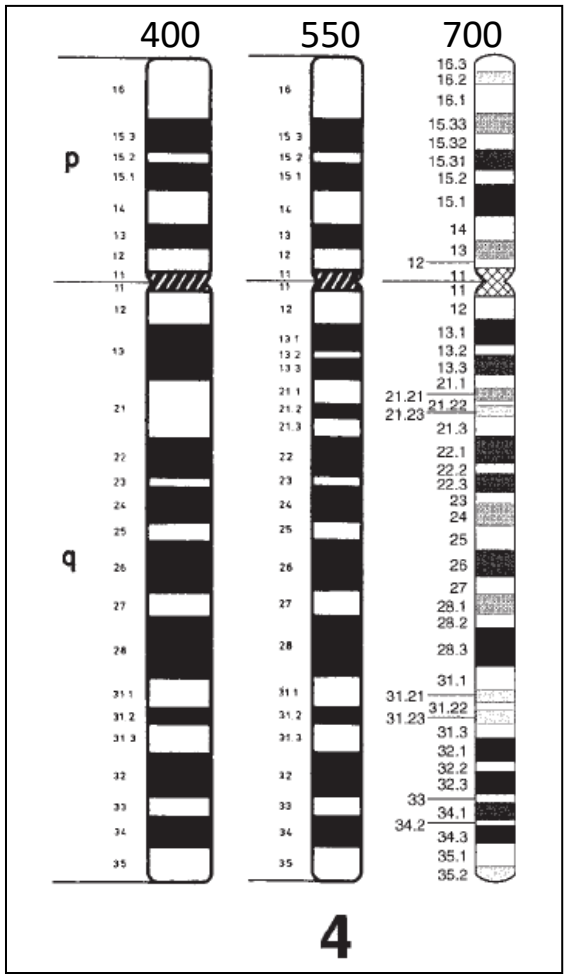


Karyogram

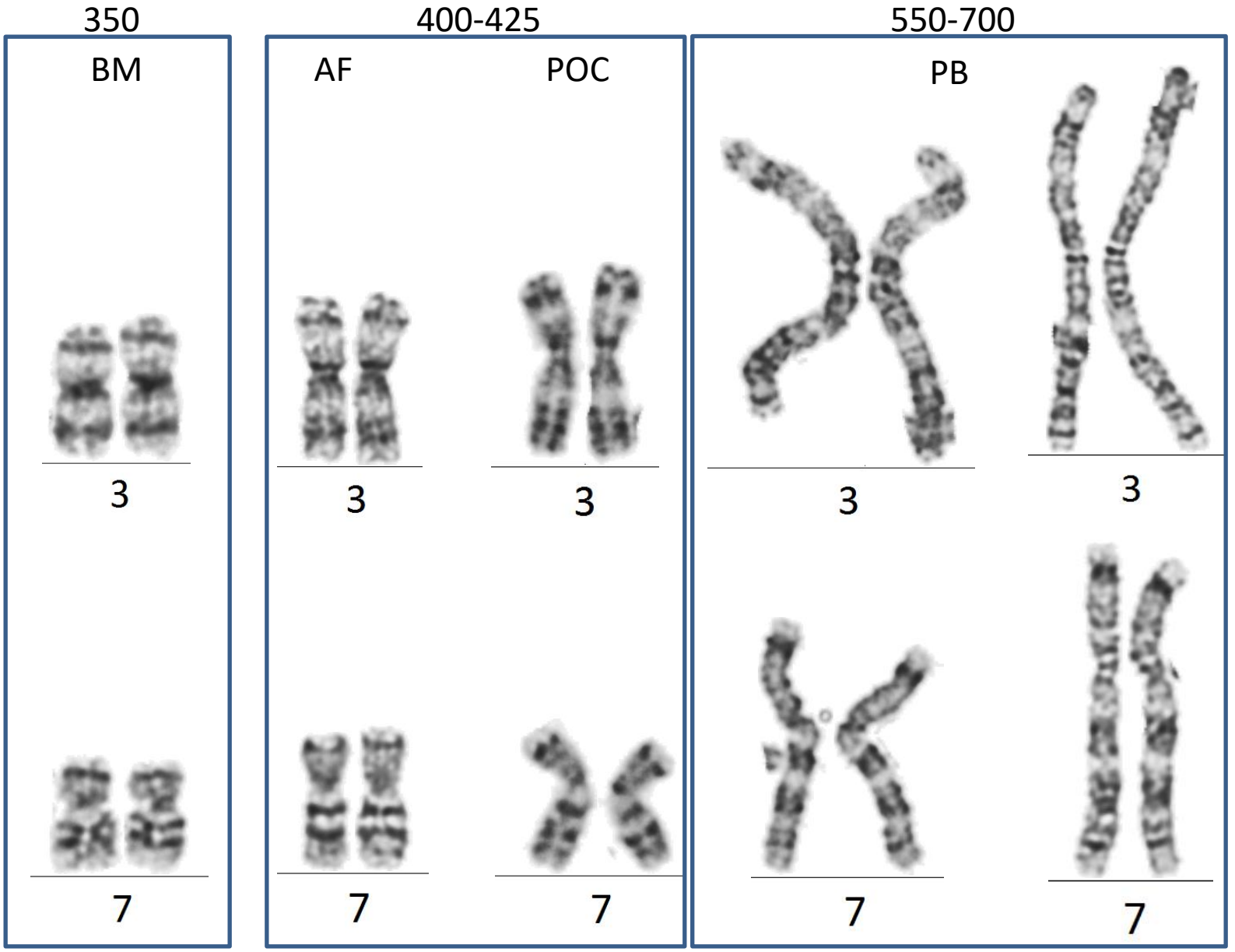
Designation of Regions, Bands, Sub-bands



Idiogram



Differences in level of resolution by sample type



Writing Karyotypes

Description o as):

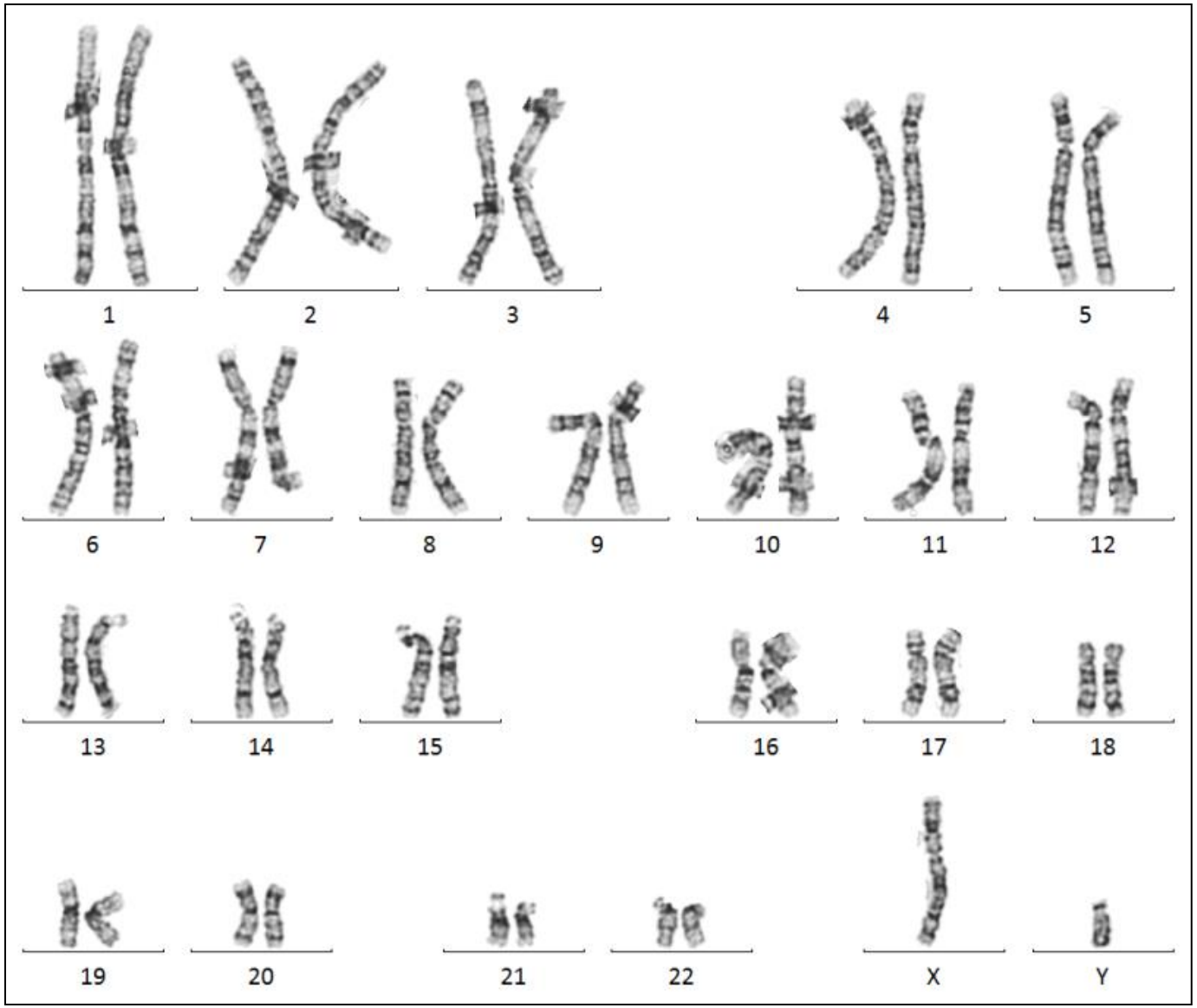
1) Chromoso tromeres
   – Numerica ploidy level
2) Sex chrom
   – Use +/- fo dy only
3) Abnormali
   – Ordered l omes, X then Y, then autc merical abnorma ormalities, listed alphabeti)
4) Multiple c
   – Mosaicisr iple abnormal cell lines from
   – Chimerisr) first

e.g. 48,XYY,+13; 68,XXY,-22; mos 47,XXY[10]/47,XYY[5]/46,XY[5]

Common symbols and abbreviated terms

+	additional normal or abnormal chromosome (trisomy)
-	loss of a chromosome (monosomy)
add	added material of unknown origin (typically resulting in a loss of material distal to breakpoint)
chi	chimerism (multiple cell lines deriving from the different individuals)
del	deletion
der	derivative chromosome, due to structural rearrangement(s)
dic	dicentric chromosome
dup	duplication
dn	de novo (not inherited)
i	isochromosome (composed of two identical chromosome arms)
idic	isodicentric chromosome (isochromosome w/ two centromeres)
ins	insertion
inv	inversion
mar	marker chromosome, unknown origin
mat	maternal origin
mos	mosaicism (multiple cell lines deriving from the same individual)
pat	paternal origin
r	ring chromosome
rob	Robertsonian translocation, a whole arm translocation between acrocentric chromosomes
t	translocation
/	separates clones (for mosaic karyotypes)
//	separates clones (for chimeric karyotypes)
[ ]	indicate number of cells (for mosaic or chimeric karyotypes)

Nomenclature Practice:
Numerical Abnormalities (Constitutional)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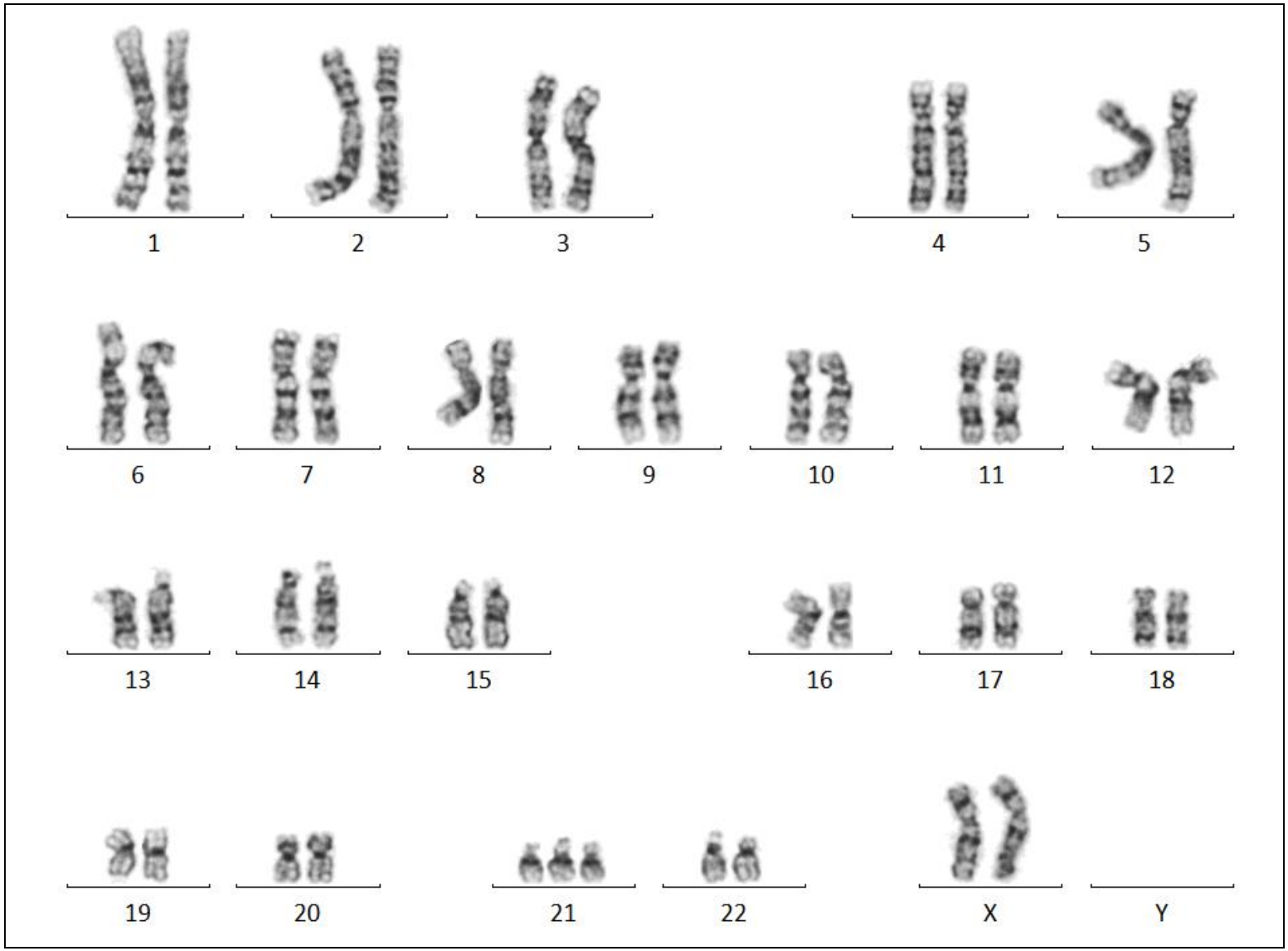
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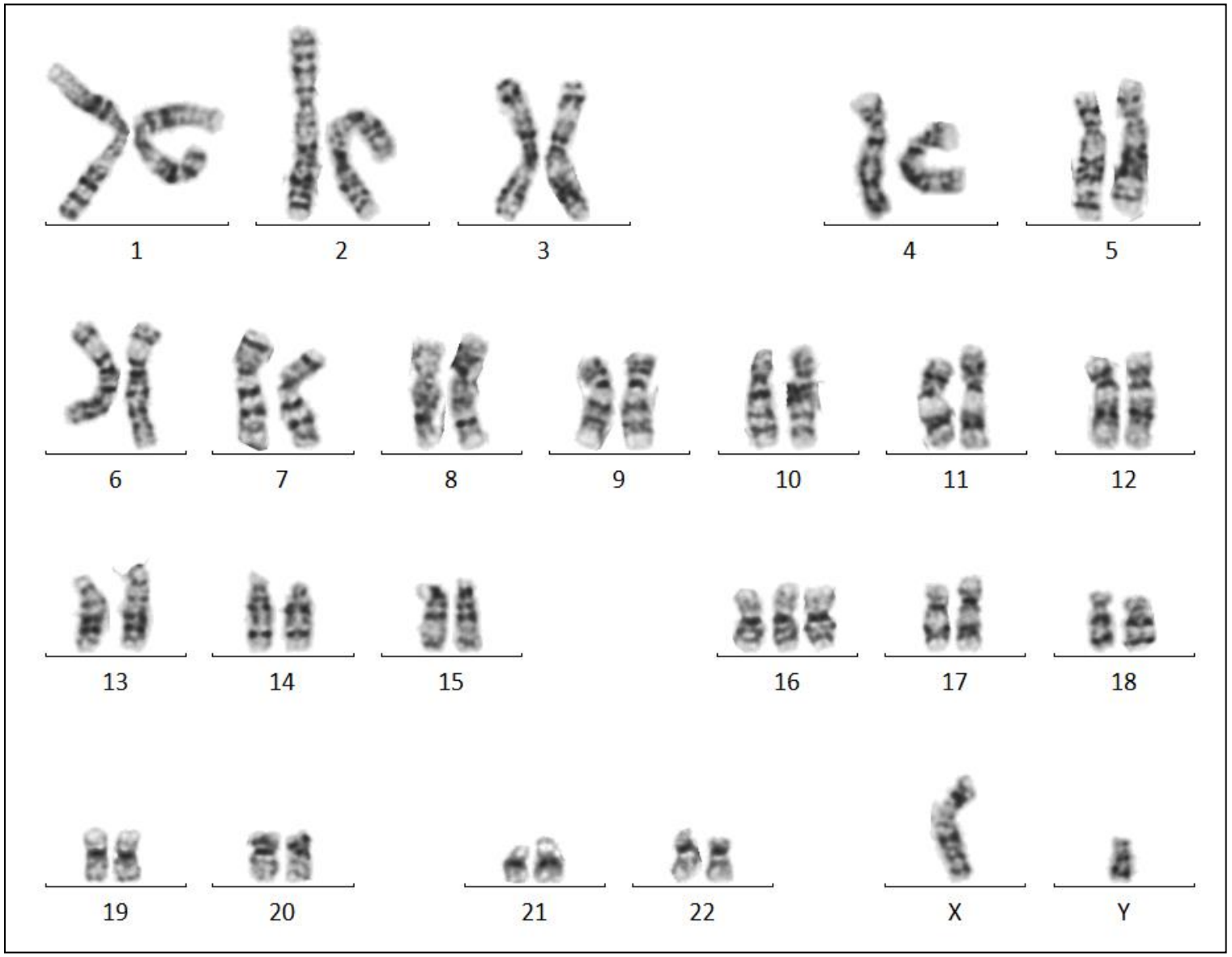
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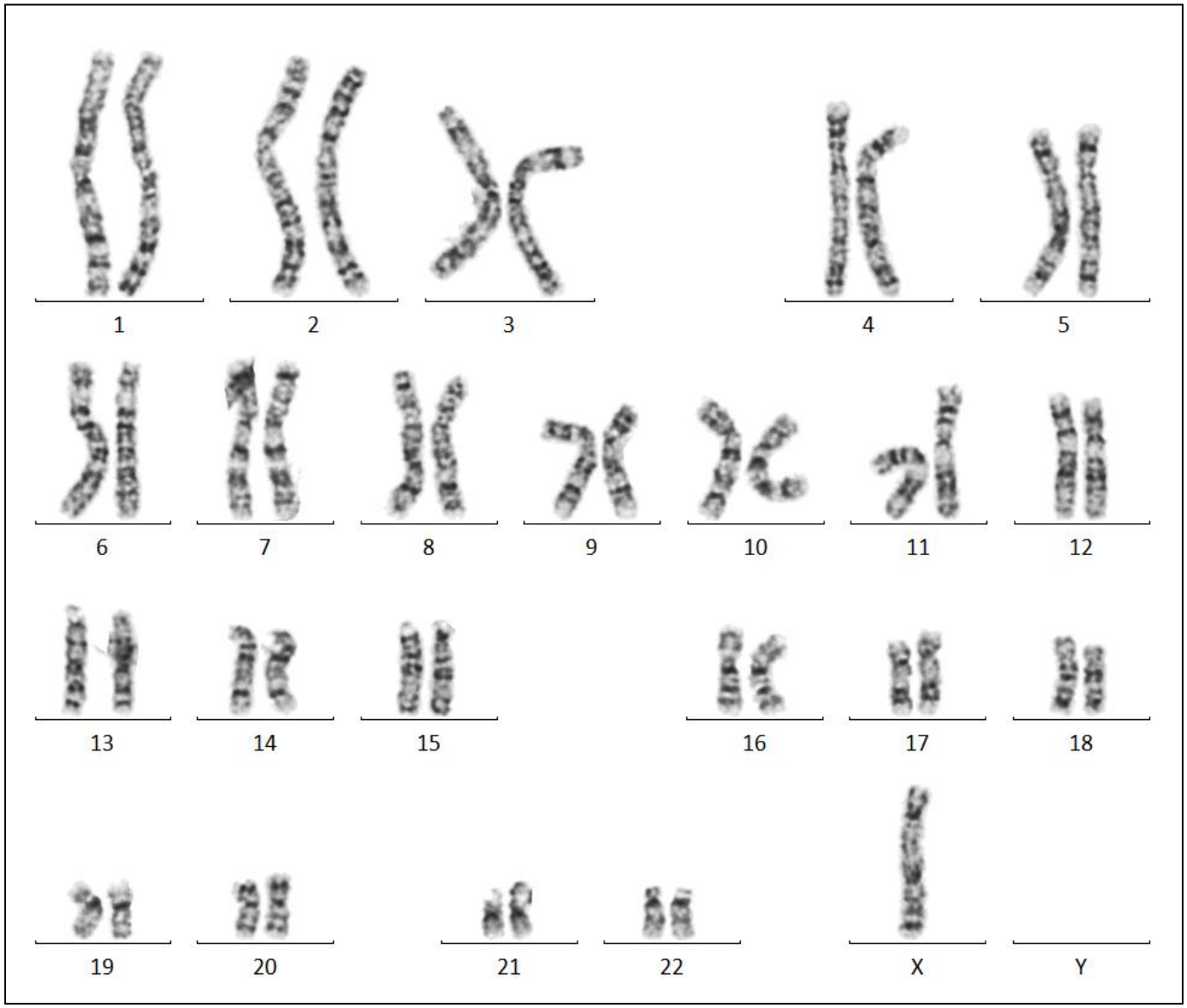
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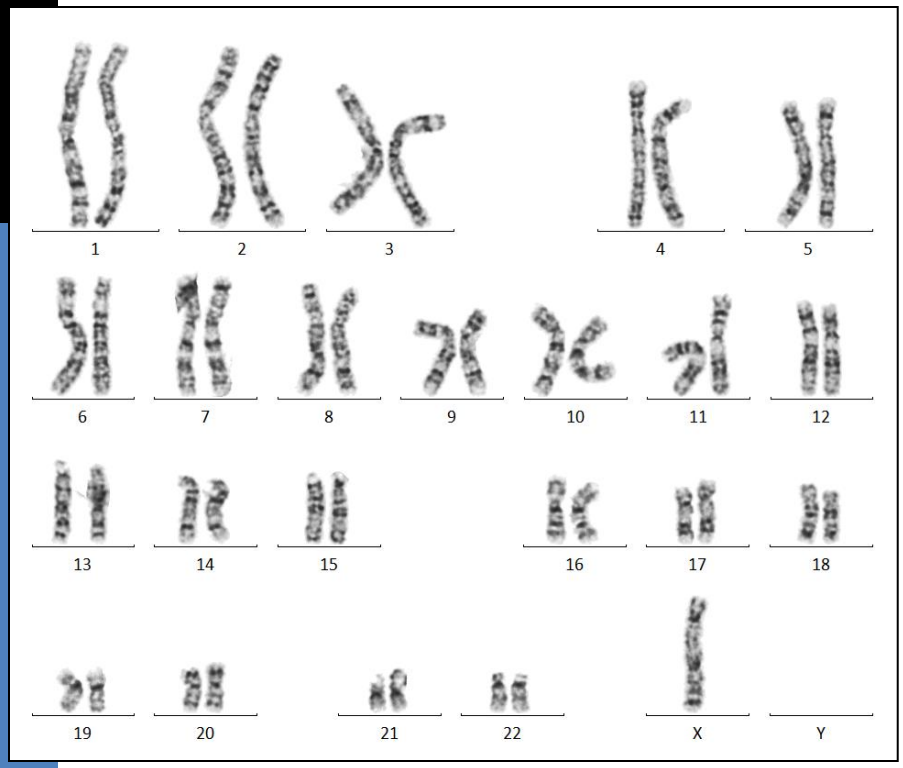
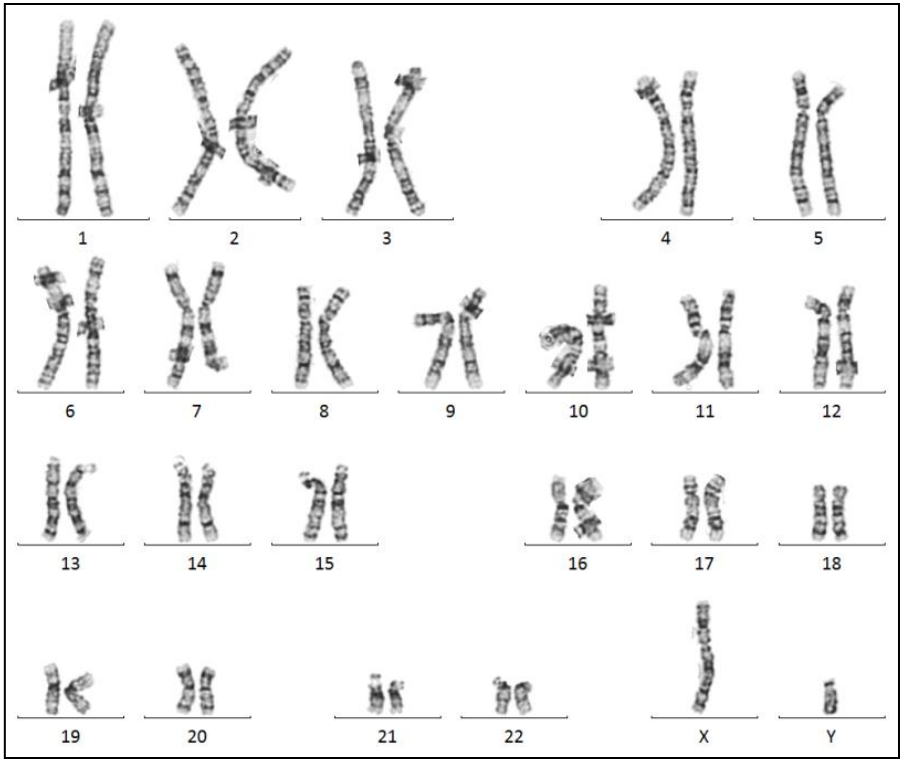
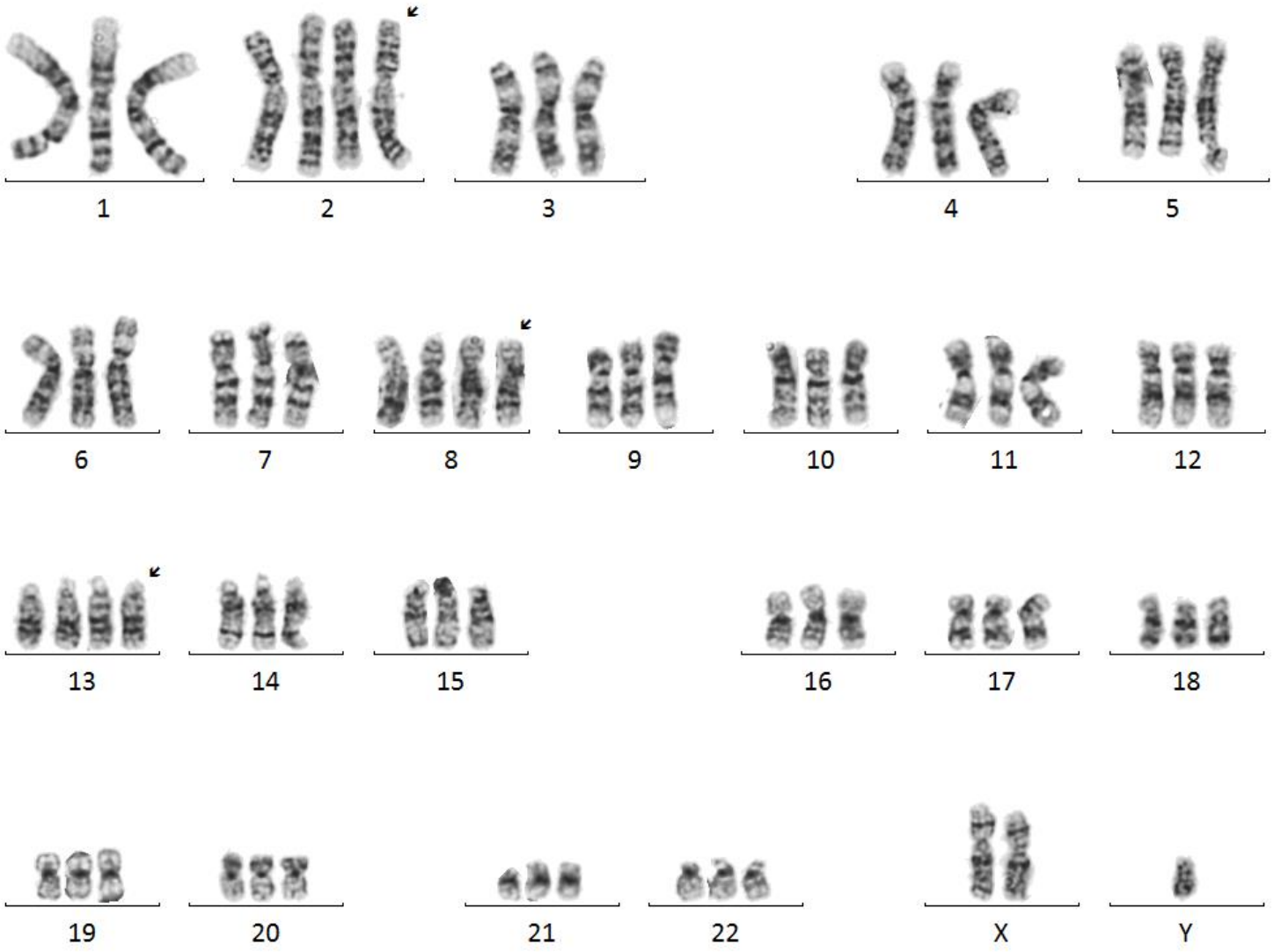
12 cells



8 cells

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