Principles of Cytogenetics
Categorical Course
Introduction to Cytogenetics 1

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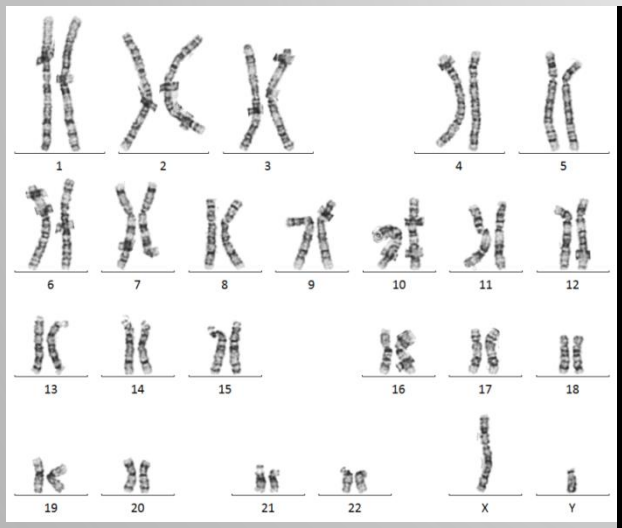
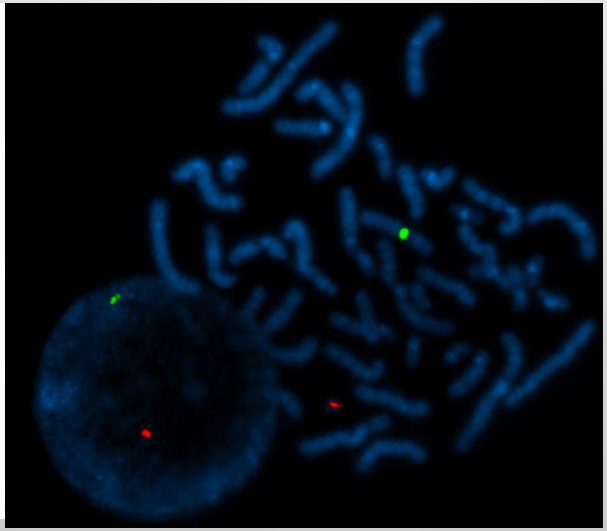
What is Cytogenetics?

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

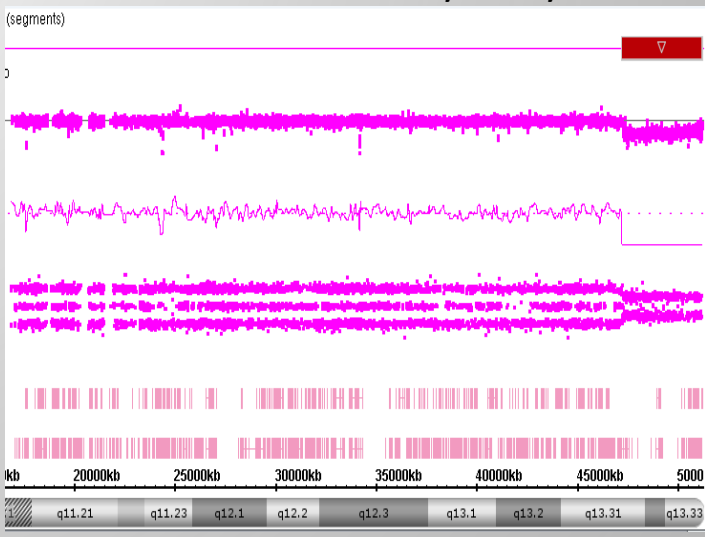
Chromosome analysis/ karyotyping



Fluorescence in situ hybridization



Genomic microarray analysis



Constitutional versus cancer cytogenetics

- Constitutional cytogenetics: diagnosis of heritable genetic abnormalities in children, adults, pregnancy, and fetal loss
  - Abnormalities may be inherited or *de novo*
- Cancer cytogenetics: detection of acquired or somatic (versus germline/constitutional) genetic abnormalities for the diagnosis, prognosis, therapy, and/or monitoring of many types of cancer, esp. hematologic

Indications for Cytogenetic Analysis (Constitutional)

- Postnatal, childhood growth and development
  - Perinatal/newborn: Birth defects, malformations, dysmorphisms, ambiguous genitalia
  - Growth: failure to thrive, growth delay, short stature
  - Developmental delay (fine and gross motor, speech)
  - Cognitive: intellectual disability, learning disability
  - Neurological: hypotonia, seizures, ataxia
  - Behavioral: autism, OCD, psychiatric illness

Tissues studied: Peripheral blood, buccal swab, skin biopsy

Indications for Cytogenetic Analysis (Constitutional)

- Adolescent, adult sexual development and fertility
  - Amenorrhea, primary or secondary ovarian failure, premature menopause
  - Azoospermia, oligospermia, hypogonadism
  - History of infertility or spontaneous abortions
  - Birth of a child with a chromosomal abnormality

Tissues studied: Peripheral blood

Indications for Cytogenetic Analysis (Constitutional)

- Prenatal
  - Abnormal maternal serum screening (first or second trimester)
  - Abnormal cell-free DNA testing (cfDNA), non-invasive prenatal testing (NIPT)/screening (NIPS)
  - Abnormal ultrasound findings: cystic hygromas/hydrops, cardiac defects, other malformations, IUGR, etc.
  - Advanced maternal age (AMA), generally ≥ 35 yrs
  - Parental or familial chromosome/genomic abnormality
- Fetal or neonatal demise (products of conception, POC)

Tissues studied: Amniotic fluid, chorionic villus sampling, fetal tissues

Indications for Cytogenetic Analysis (Cancer)

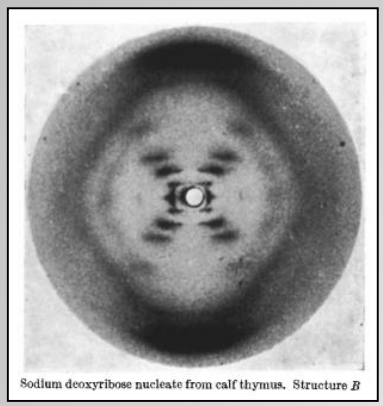
- Hematologic oncology
  - Myeloid diseases: AML, CML, MDS, MPNs
  - Lymphoid diseases: ALL, CLL, NHL, PCNs/MM
- Bone marrow transplant
- Other areas of oncology (solid tumors)

Tissues studied: Bone marrow, peripheral blood, lymph nodes, solid tumor, pleural fluid, spinal fluid

Introduction to Cytogenetics I

- DNA and Chromosomal Structure
- Cell Cycle, Mitosis and Meiosis
- Gametogenesis
- Nondisjunction and Aneuploidy
- Polyploidy and Errors at Fertilization
- Imprinting and Uniparental Disomy
- Sex Chromosomes
- Karyotyping and Nomenclature

DNA Structure and Organization



Sodium deoxyribose nucleate from calf thymus. Structure *B*

Franklin and Gosling (April 1953) Nature v171

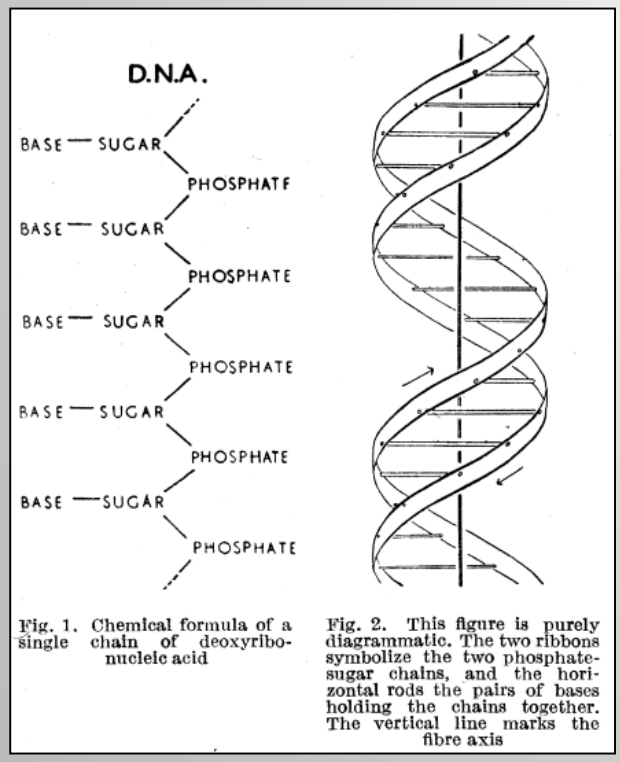
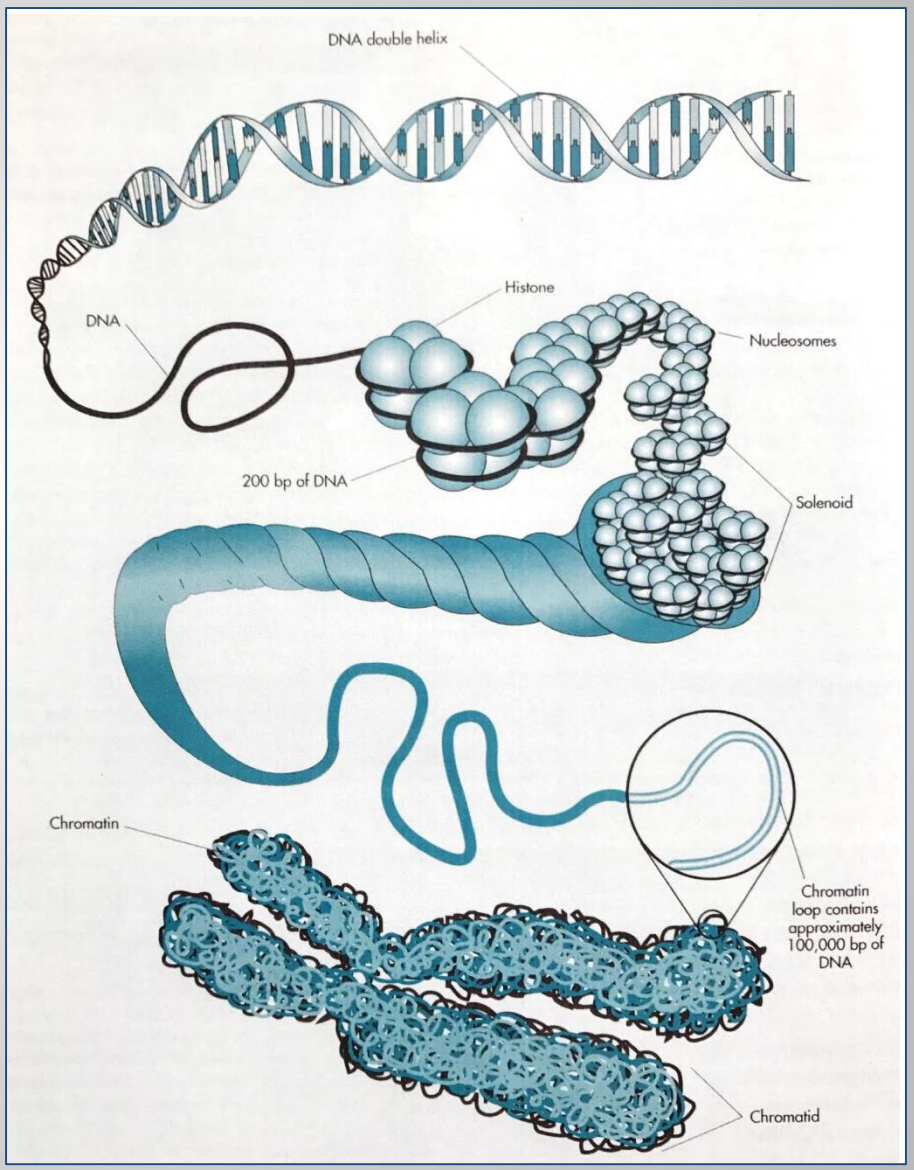


Fig. 1. Chemical formula of a single chain of deoxyribonucleic acid

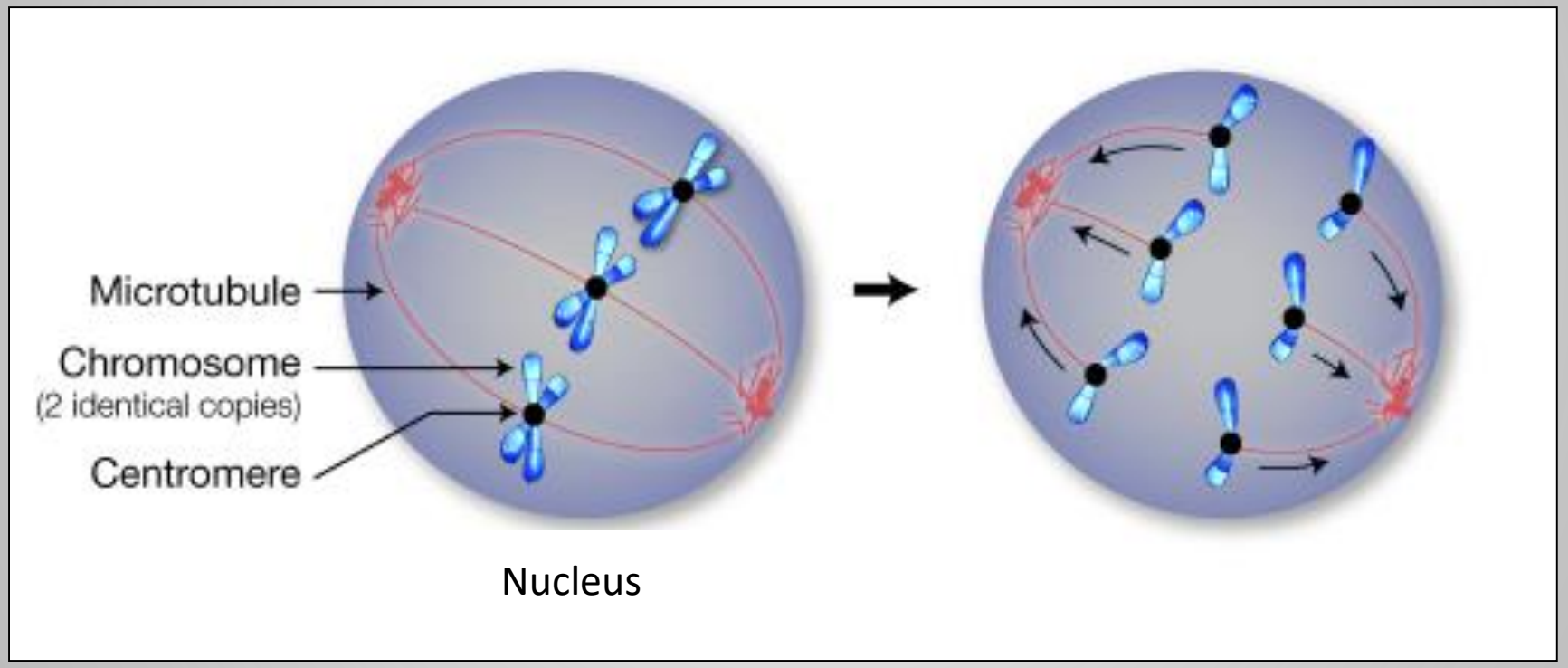
Fig. 2. This figure is purely diagrammatic. The two ribbons symbolize the two phosphate-sugar chains, and the horizontal rods the pairs of bases holding the chains together. The vertical line marks the fibre axis

Watson and Crick (May 1953) Nature v171



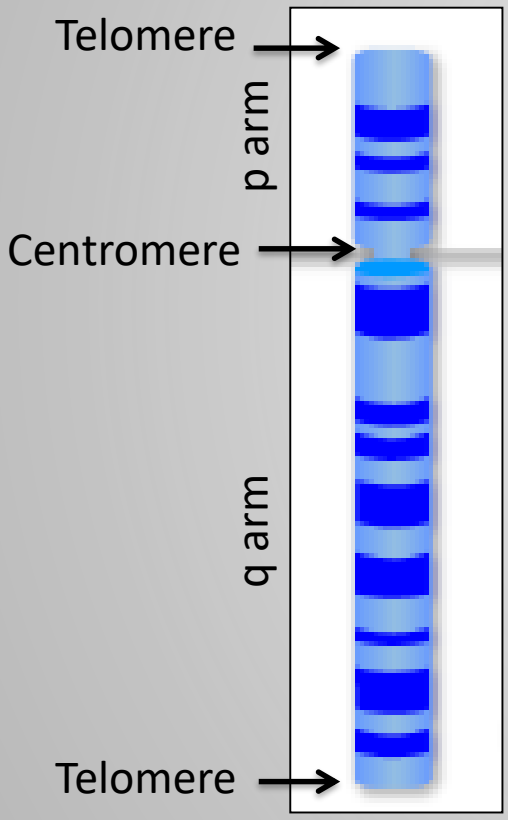
Gersen and Keagle, Principles of Cytogenetics, 3rd Ed 2013

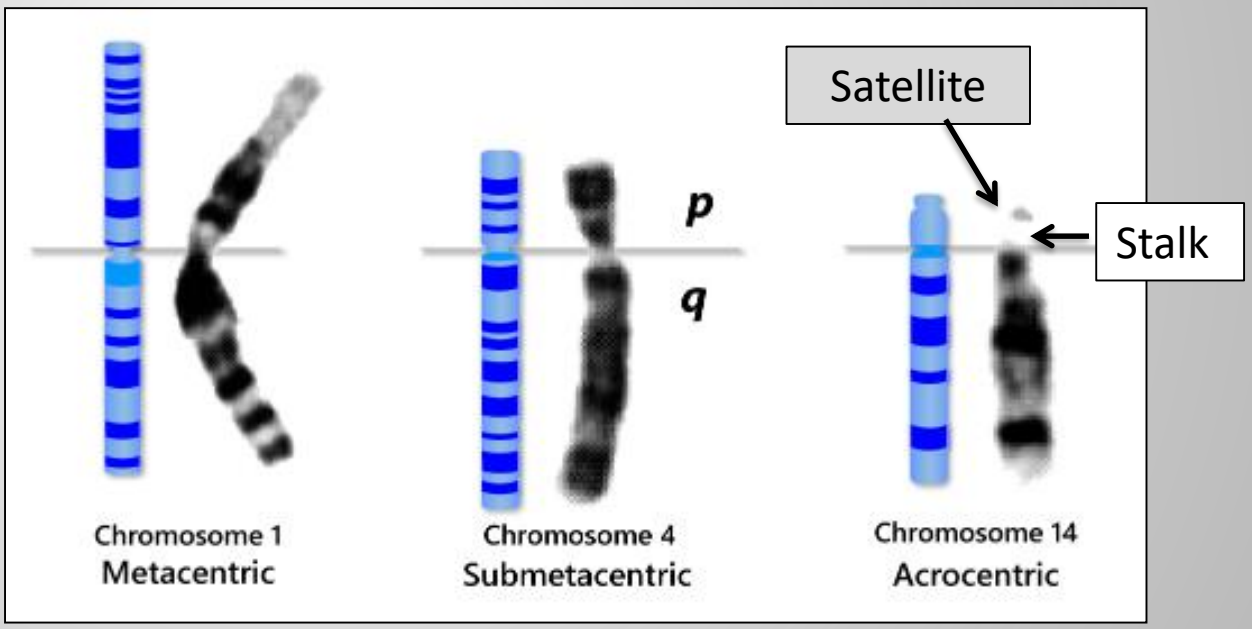
Role of the Centromere in Cell Division



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

Chromosome Structure and Classification

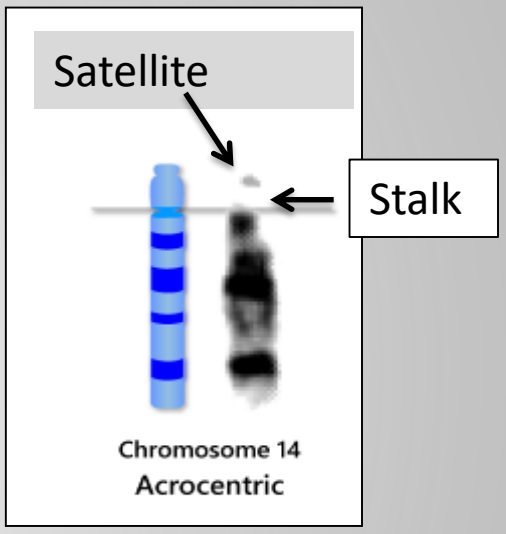




Images modified, source: http://learn.genetics.utah.edu/content/chromosomes/readchromosomes/

Acrocentric Chromosomes

- Chromosomes 13, 14, 15, 21, 22
- Small p arm composed of a stalk (pstk) and a satellite (ps)
- Stalk: contains multiple copies of ribosomal DNA genes
  - Stalks associate during interphase to form the nucleolus (also known as nucleolar organizing regions, NORs)
  - Nucleolus is the site of rRNA transcription and processing and ribosome assembly
- Satellite: a non-coding region
- Size of both regions is variable, or polymorphic



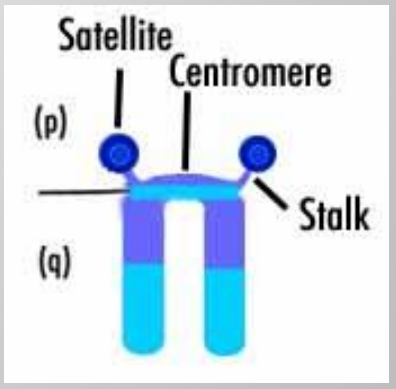
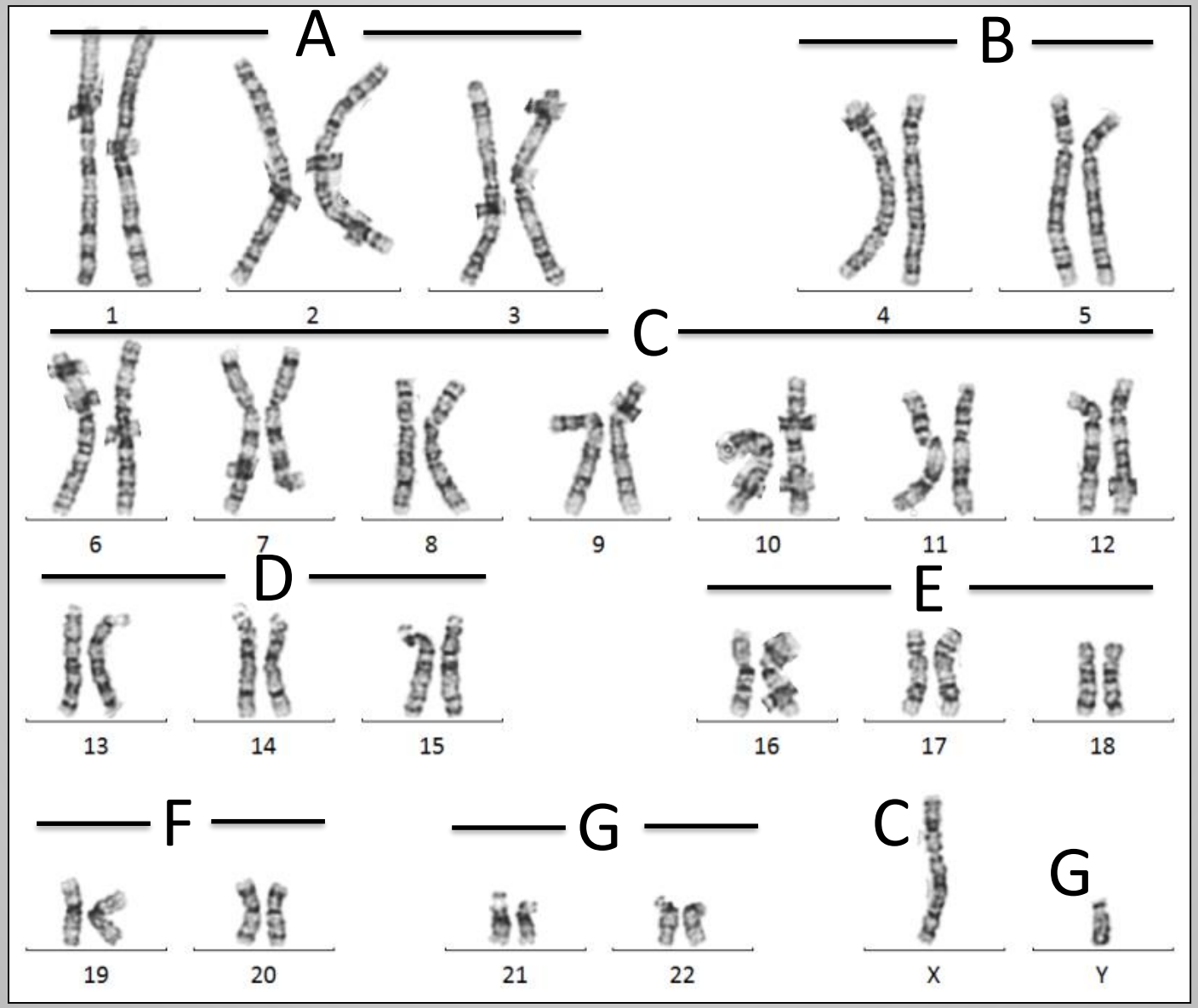


Image source: http://carolguze.com/text/442-4-chromosome_analysis.shtml

Chromosome Classification

Karyogram



DNA Classification

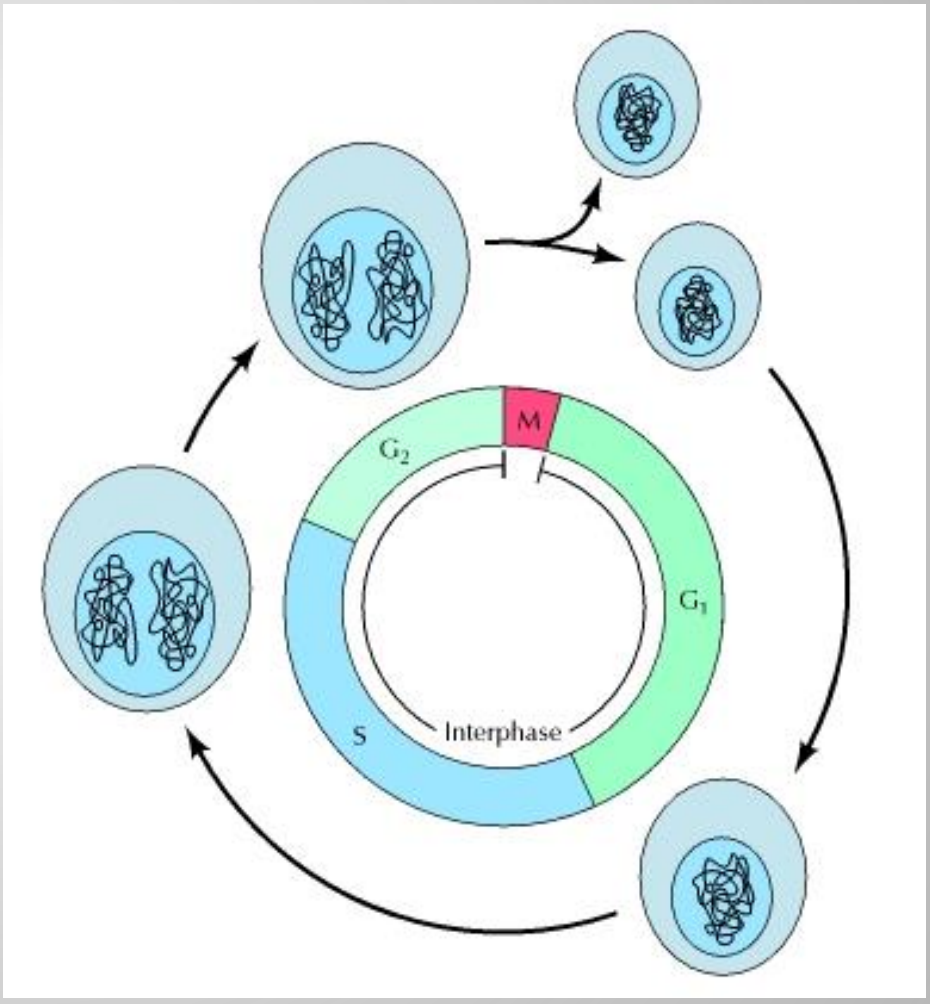
- Human genome comprises unique (75%) and repetitive (25%) DNA sequences
- Unique sequence occurs once per haploid (1n) set, includes genes and non-coding sequence
  - Genes: n= ~20,000, genome is 1.5% exonic, 26% intronic, ~8% regulatory, distribution is uneven
- Repetitive sequence includes tandemly arranged, satellite DNA: α-satellite, minisatellite, microsatellite and dispersed short or long elements (SINEs, LINEs)
  - α-satellite DNA: 171 bp, centromeres
  - Mini- (20-70 bp) and microsatellites (2-4 bp): variable across individuals, used for mapping and identity testing

Chromatin

- Euchromatin: loosely organized, contains active, early replicating genes
- Heterochromatin: highly contracted, contains late replicating genetically inactive sequence
  - Constitutive : located at centromeres and at the distal end of the Y chromosome long arm
  - Facultative: defines heterochromatin on the inactive X chromosome in females

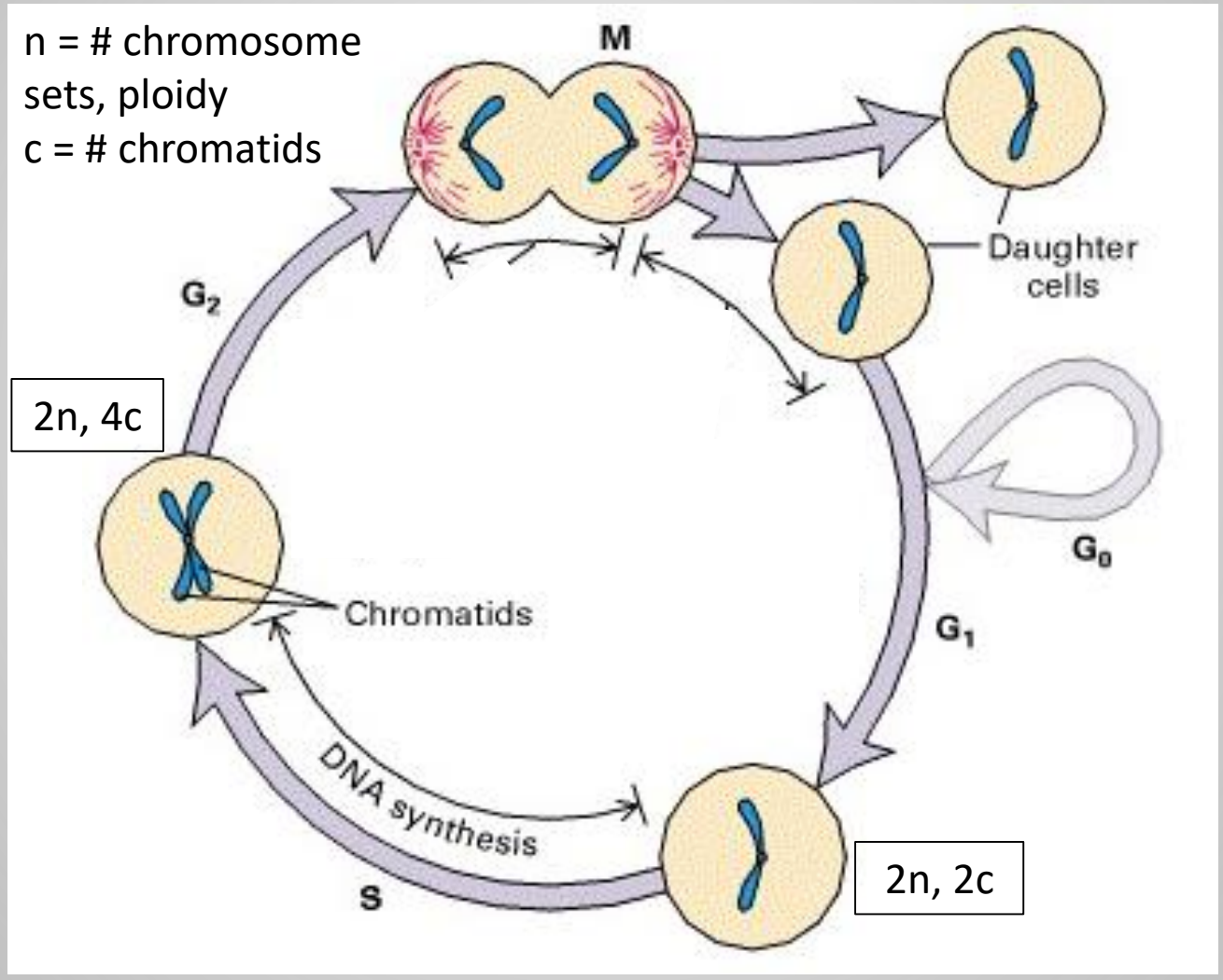
Cell Division and The Cell Cycle

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



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

Cell Division and The Cell Cycle

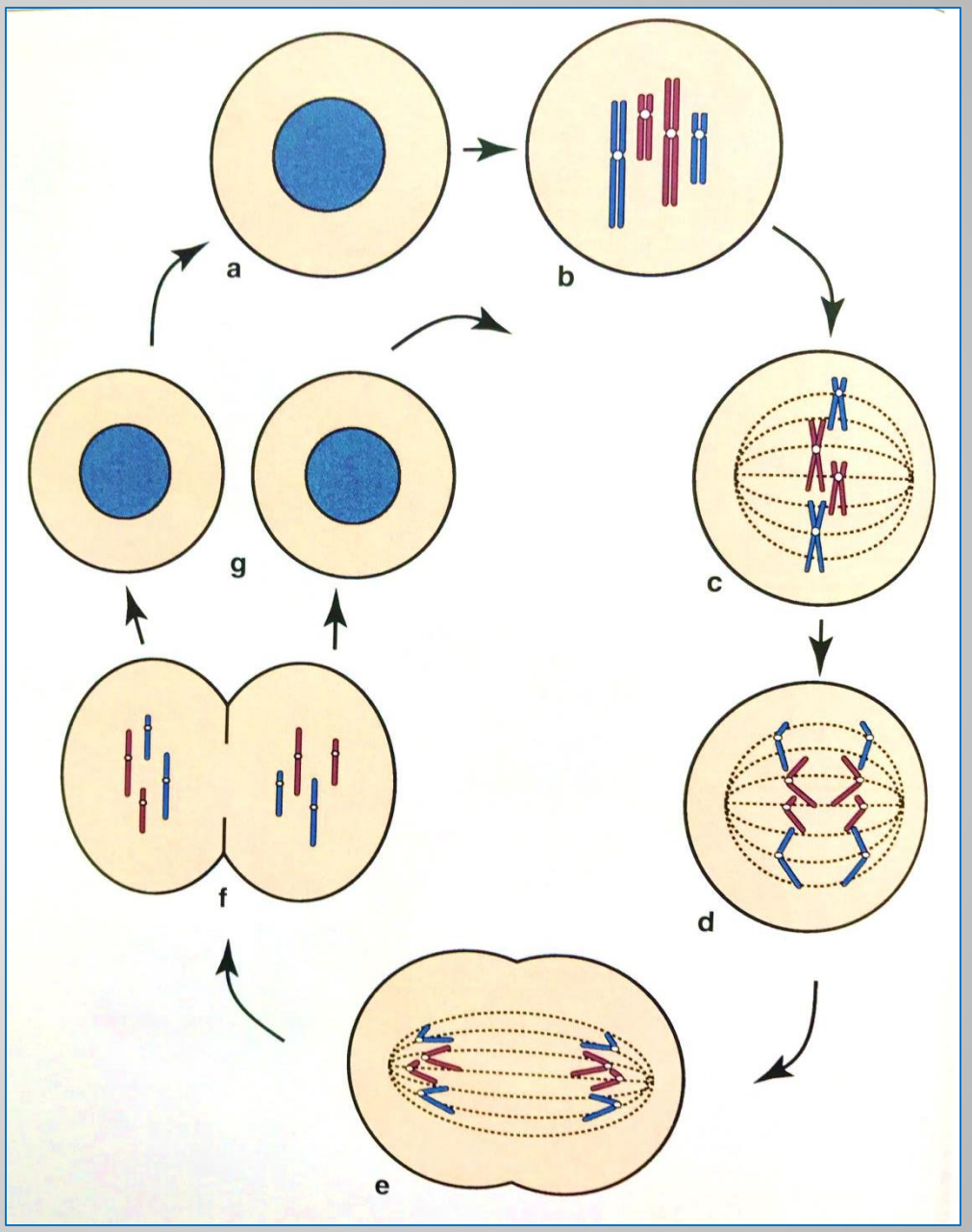


NCBI Bookshelf: Lodish, Berk, Zipursky, Molecular Cell Biology, 4th Ed 2000

Mitosis

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

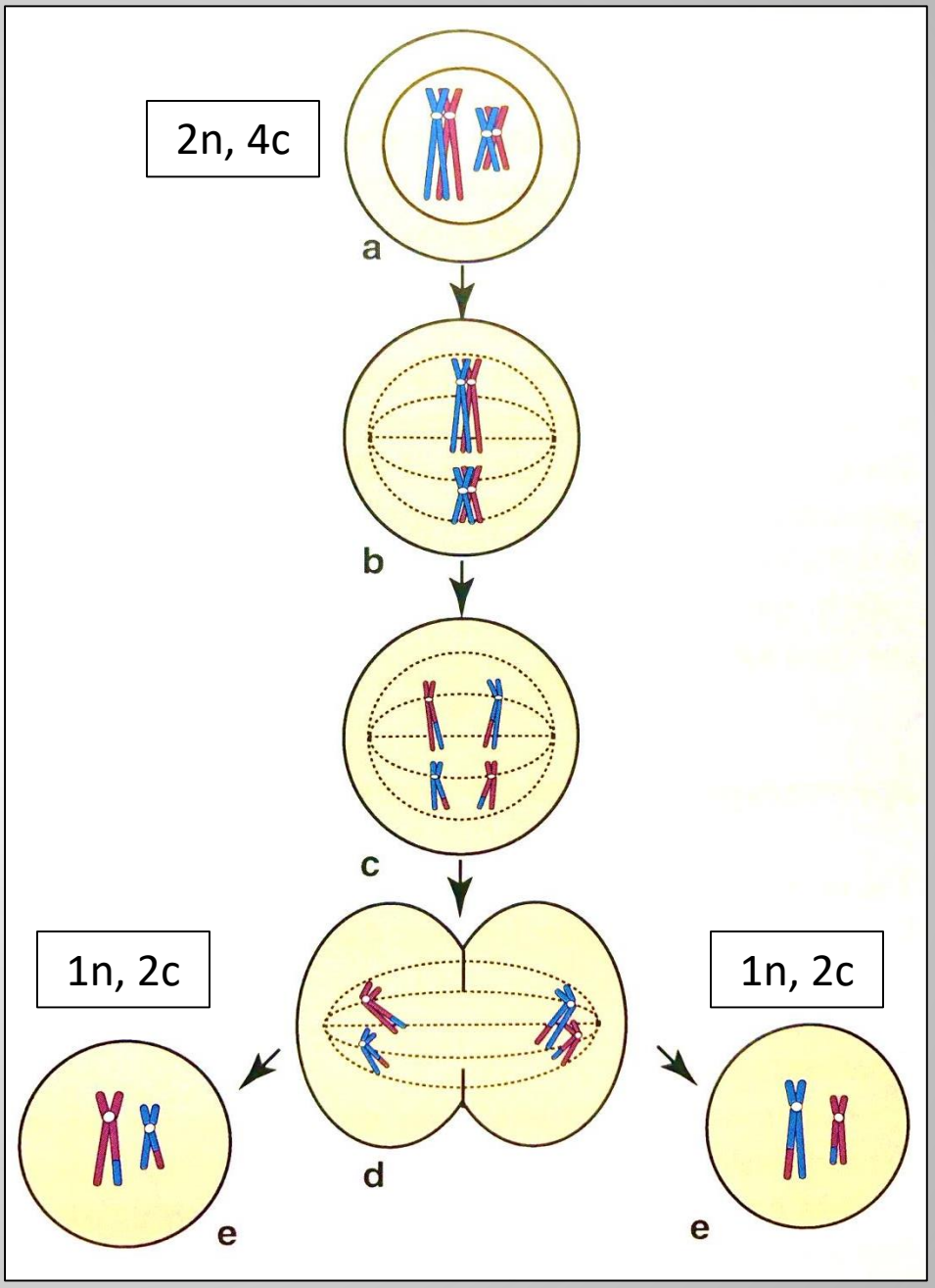
* Stage observed in chromosome analysis



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

Meiosis I

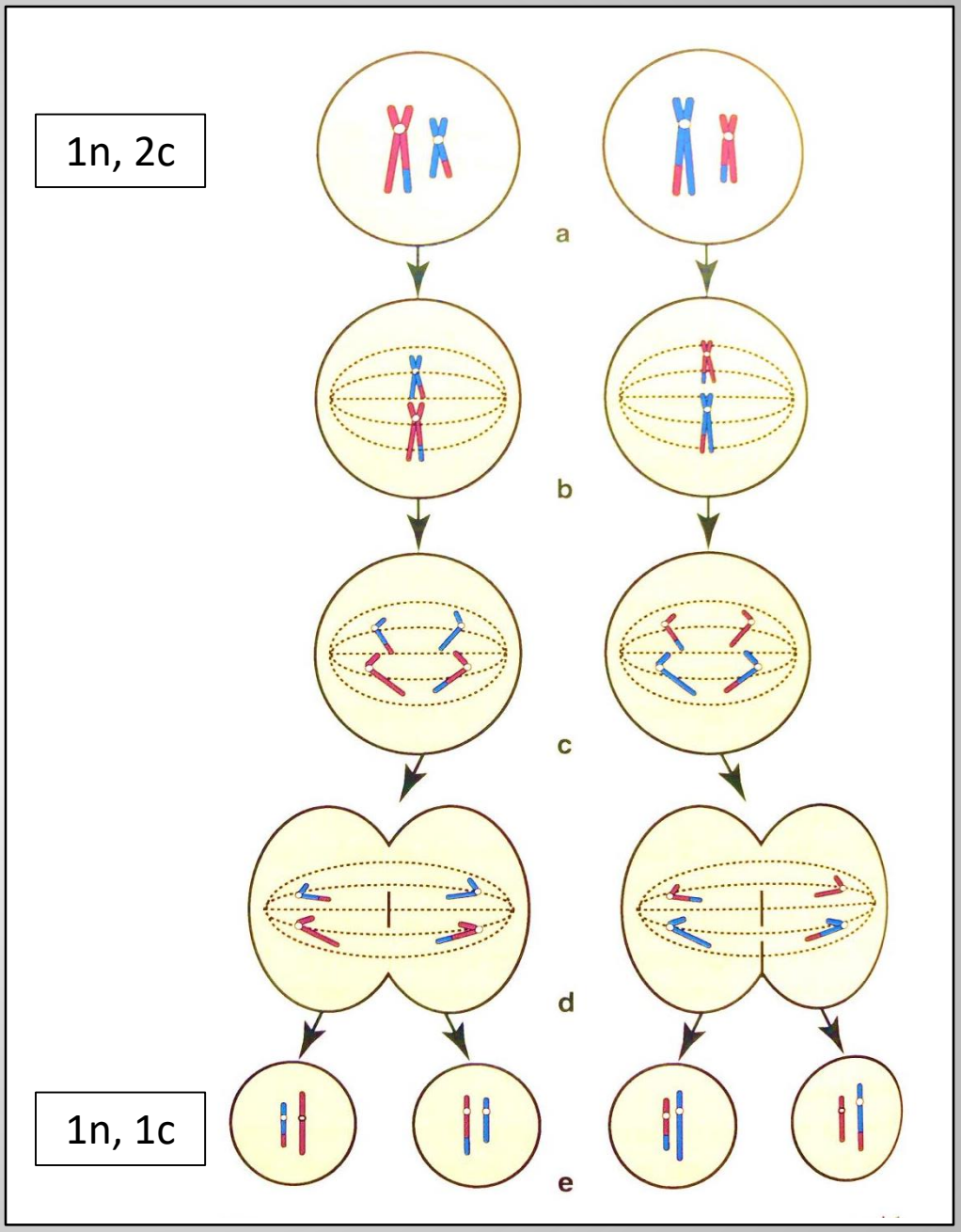
a) Prophase I: Homologs pair, cross-over
b) Metaphase I
c) Anaphase I
d) Telophase I
e) Daughter cells



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



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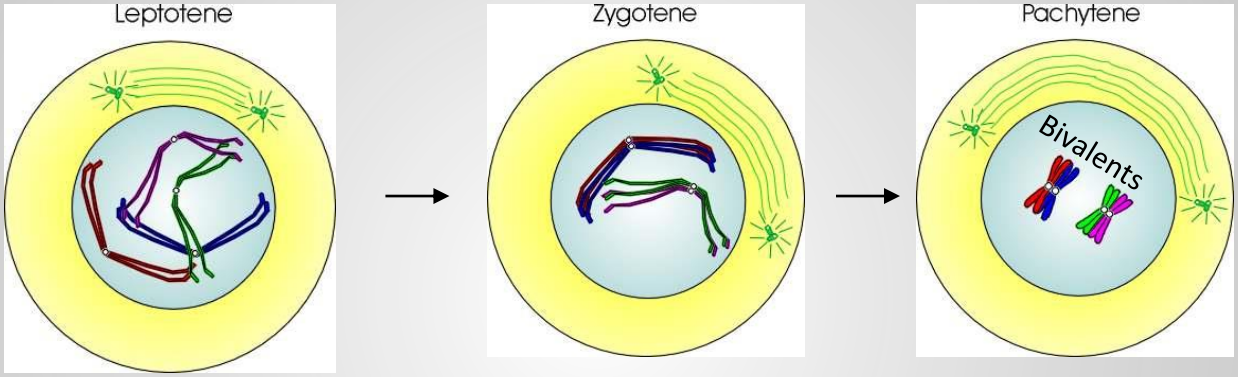
Prophase I

Interaction of homologous chromosomes

CHR 1 (Mat)
CHR 1 (Pat)
CHR 2 (Mat)
CHR 2 (Pat)



- Only one sister chromatid is involved in each crossover event

Leptotene:

- Chromosomes begin to condense but cannot yet be seen by light microscopy

Zygotene:

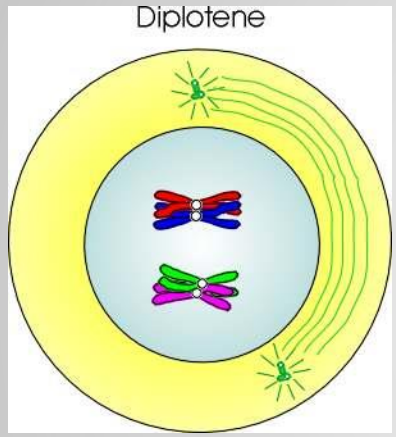
- Homologs appear as long thread-like structures and begin to pair (synapse)
- X and Y pair only at pseudoautosomal regions

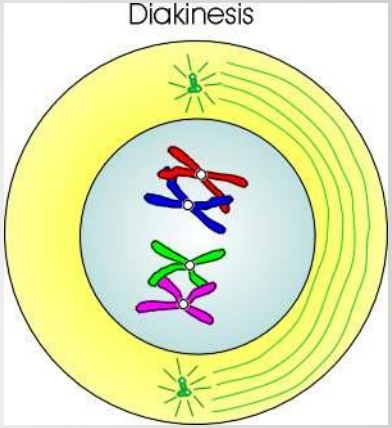
Pachytene:

- Chromosomes continue to condense, synapsis completes and homologous recombination (crossing over) occurs

Slide modified courtesy of Sarah South

Prophase I
Interaction of homologous chromosomes





Diplotene:

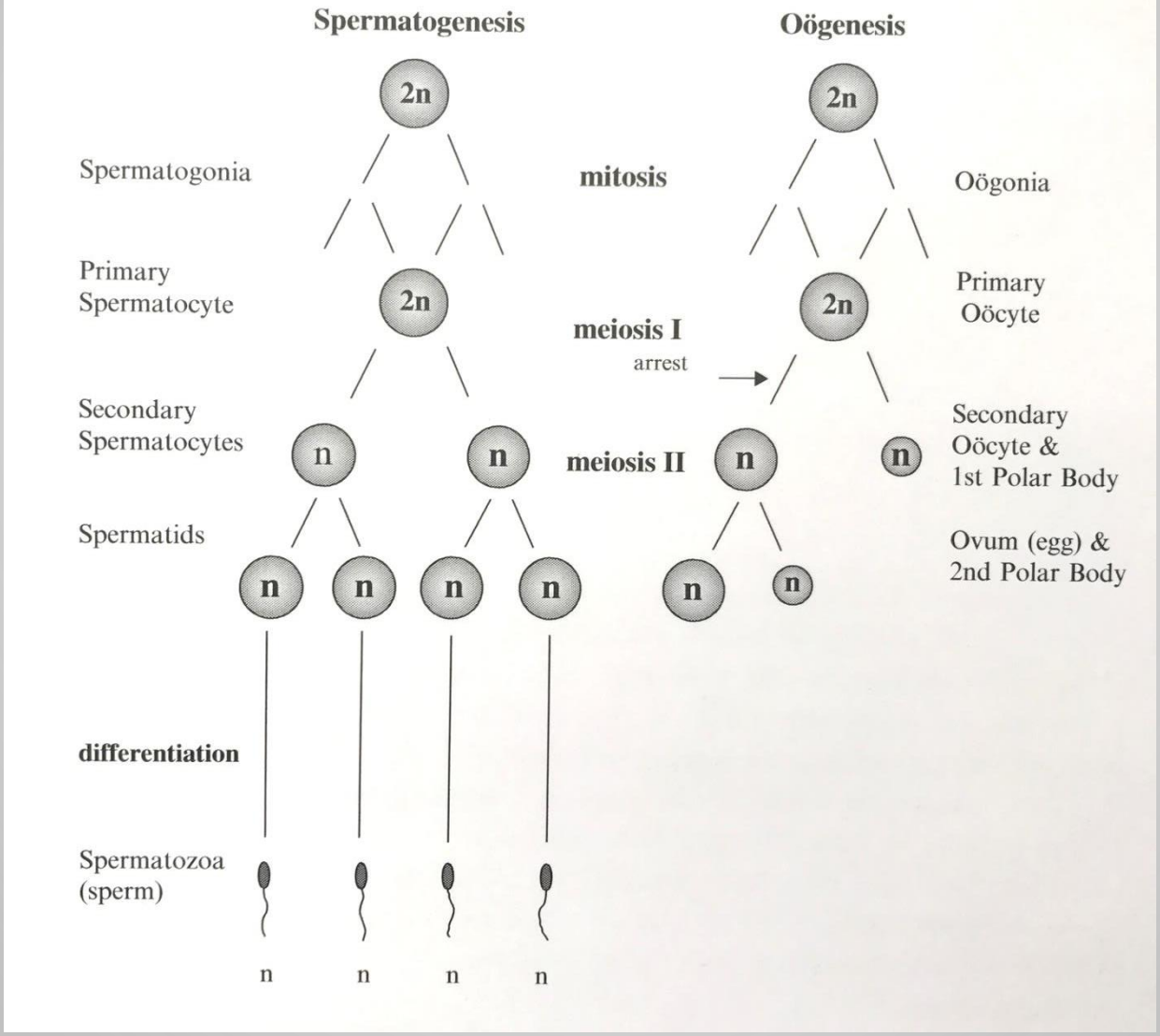
- Chromosomes continue to shorten and thicken, homologs begin to repel each other, only holding on at the regions of recombination (chiasmata)

Diakinesis:

- Chromosome completely condensed
- Centrioles have migrated to the poles and nuclear envelope begins to break down

Slide modified courtesy of Sarah South

Spermatogenesis vs Oogenesis



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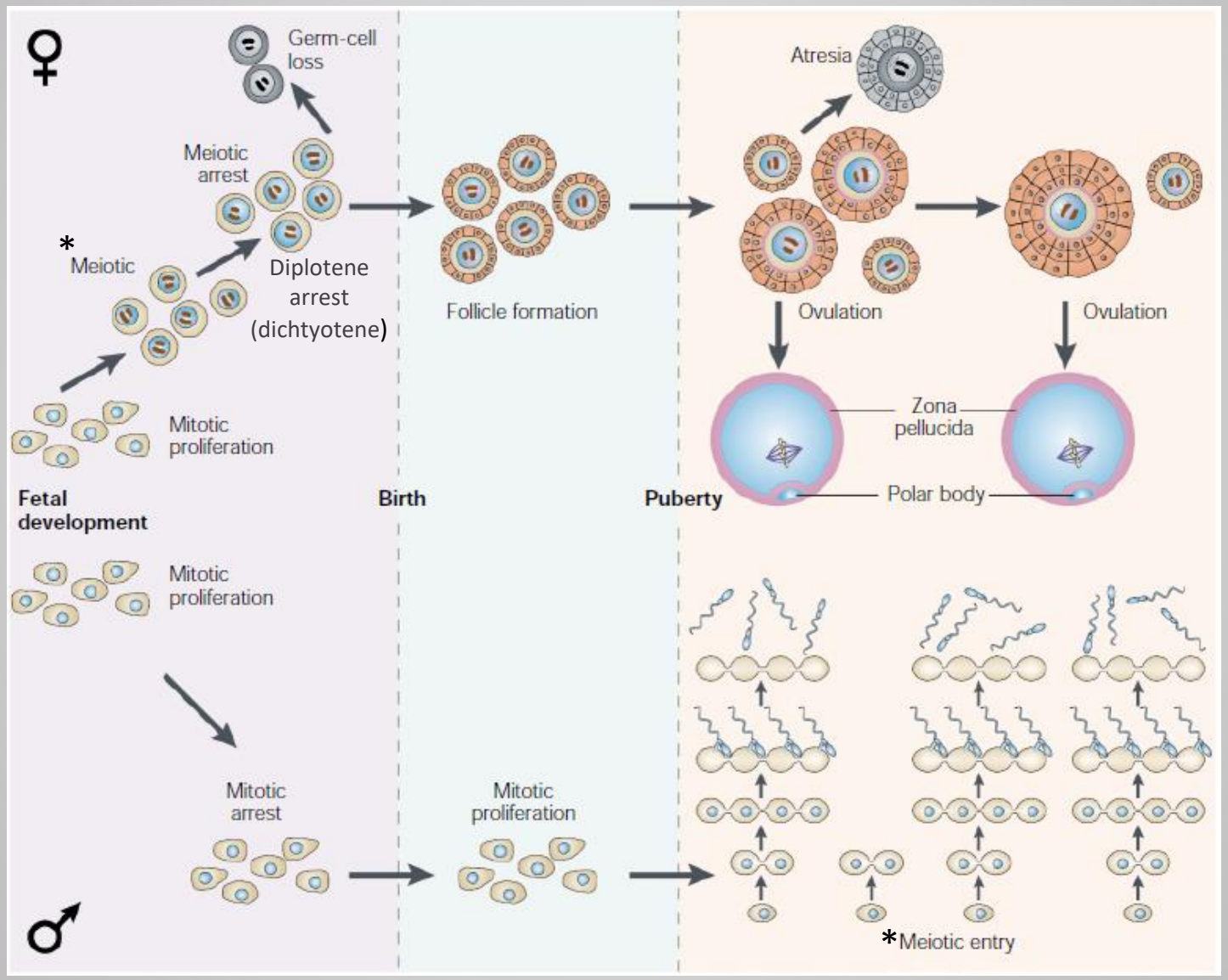
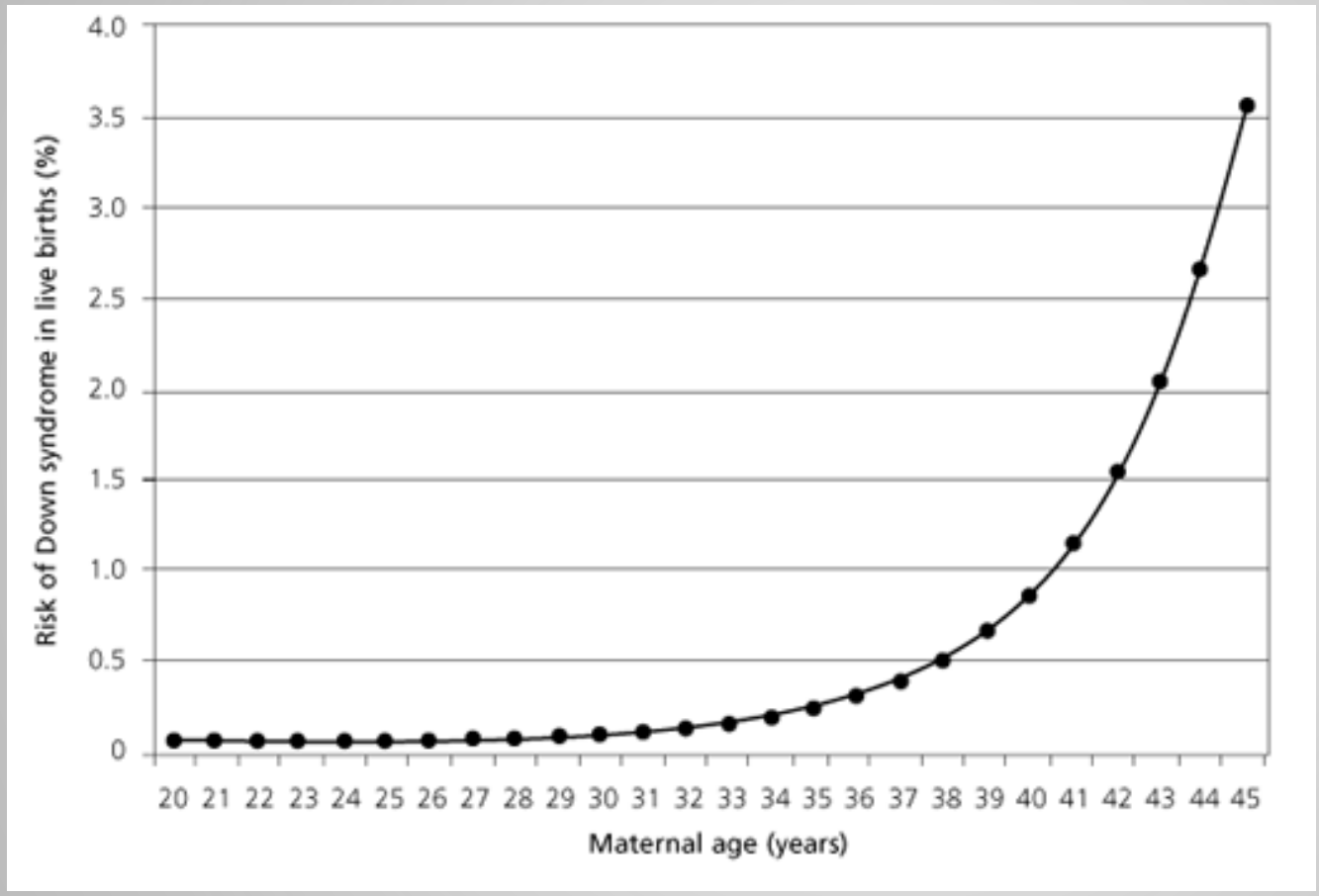
Spermatogenesis vs Oogenesis



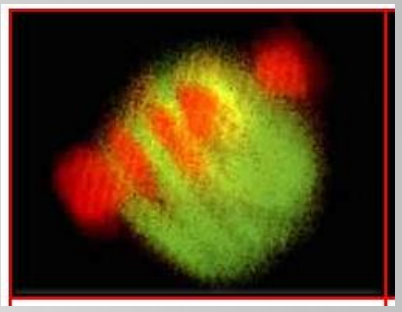
Hassold and Hunt (2001) Nat Rev Genet

Down Syndrome and Maternal Age

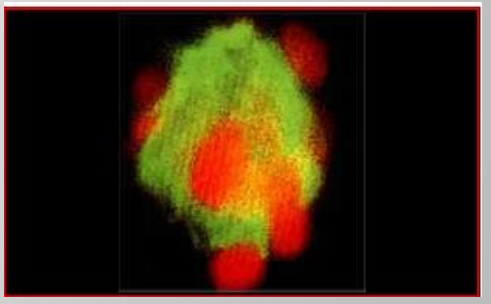


Newberger (2000) Am Fam Physician

Ovum from a woman in her 20's



Ovum from a woman in her 40's



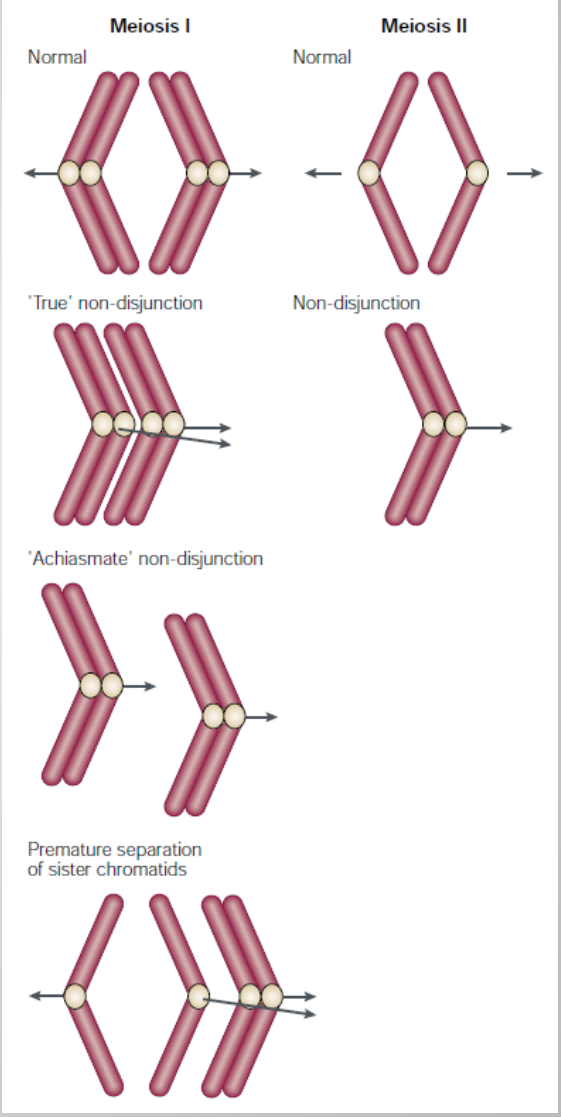
Battaglia et al., 1996

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

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

Mechanism Leading to Aneuploidy: Chromosomal Nondisjunction (ND)

- Definition: the failure of homologous chromosomes or sister chromatids to separate (segregate) properly
  - Meiotic or mitotic ND can occur
- Proposed mechanisms for MI ND
  - True: homologs travel together to same pole
  - Achiasmate: homologs failed to pair and travel independently to the same pole
  - Premature separation of sister chromatids: chromatids, rather than homologs, segregate



Hassold and Hunt, 2001, Nat Rev Genet

Modes of Nondisjunction

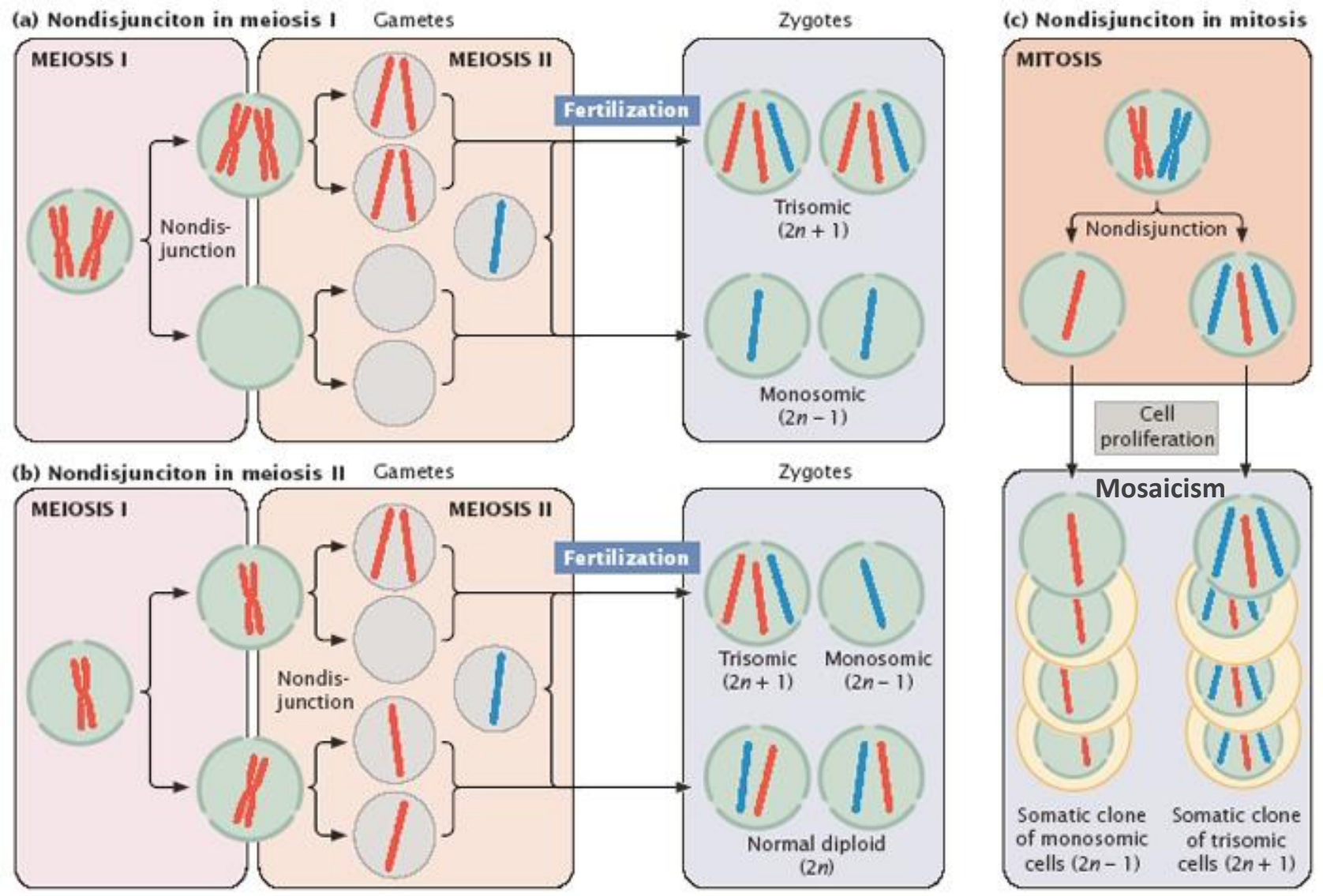
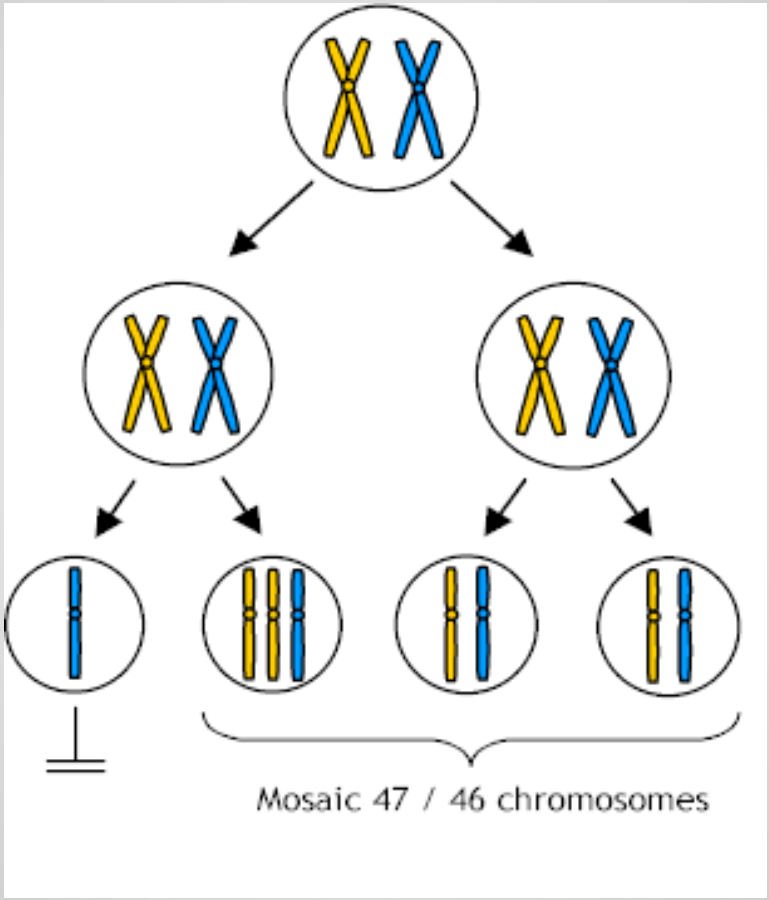


Image from: W. H. Freeman Pierce, Benjamin. Genetics: A Conceptual Approach, 2nd ed. 2005.

Mitotic ND in a normal zygote



e.g. trisomy 8 mosaicism

- Monosomic cell line often has a growth disadvantage and is lost

Image from: http://www.medgen.ubc.ca/robinsonlab/mosaic/

Parental Origins of Aneuploidy

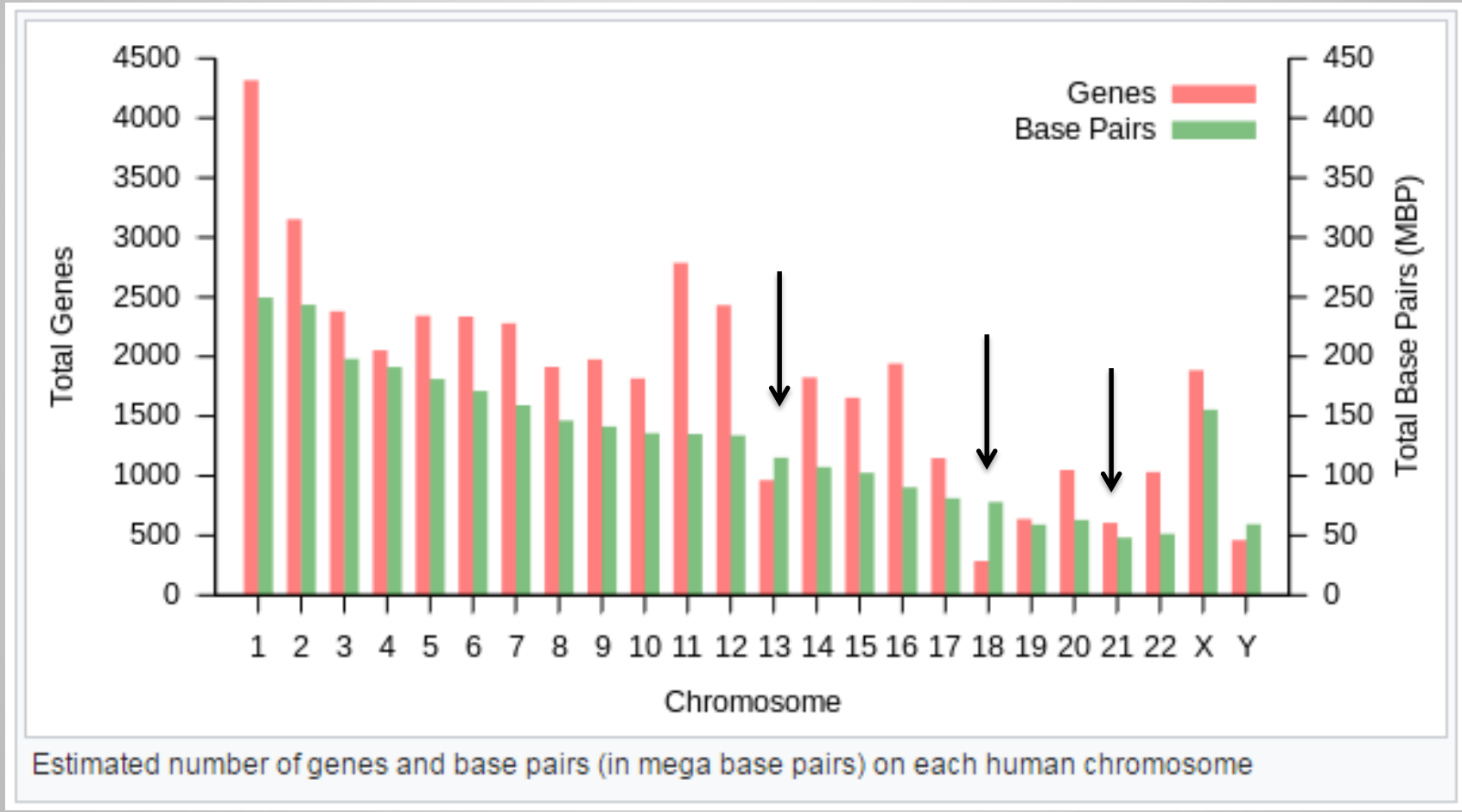
Table 1. Summary of studies of the origin of human trisomies[a]

Trisomy	*n*	Maternal		Paternal		PZM (%)
		MI (%)	MII (%)	MI (%)	MII (%)	
Acrocentrics						
13	74	56.6	33.9	2.7	5.4	1.4
14	26	36.5	36.5	0.0	19.2	7.7
15	34	76.3	9.0	0.0	14.7	0.0
21	782	69.6	23.6	1.7	2.3	2.7
22	130	86.4	10.0	1.8	0.0	1.8
Non-acrocentrics						
2	18	53.4	13.3	27.8	0.0	5.6
7	14	17.2	25.7	0.0	0.0	57.1
8	12	50.0	50.0	0.0	0.0	50.0
16	104	100	0.0	0.0	0.0	0.0
18	150	33.3	58.7	0.0	0.0	8.0
XXX	46	63.0	17.4	0.0	0.0	19.6
XXY	224	25.4	15.2	50.9	0.0	8.5
X		~30%		~70%		

[a]Adapted from Hall *et al*. (6). MI, meiosis I; MII, meiosis II; PZM, post-zygotic mitotic.

Hassold, Hall and Hunt, 2007, Hum Mol Genet

Chromosome size and gene content correlates with incidence of *postnatal* trisomy



Estimated number of genes and base pairs (in mega base pairs) on each human chromosome

Image source: wiki commons

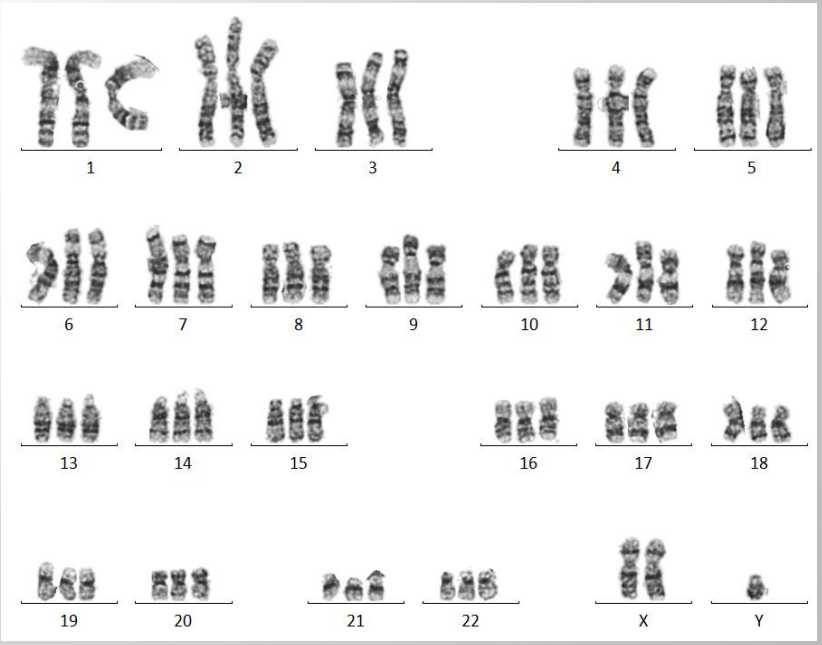
Common constitutional numerical abnormalities)

Aneuploidy

- 47,XXY (Klinefelter syndrome)
- 45,X (Turner syndrome)
- 47,XX,+21 (Down syndrome)
- 47,XY,+18 (Edwards syndrome)
- 47,XY,+13 (Patau syndrome)
- 47,XX,+16
- 45,XX,-21

Polyploidy

- Triploidy (e.g. 69,XXY)

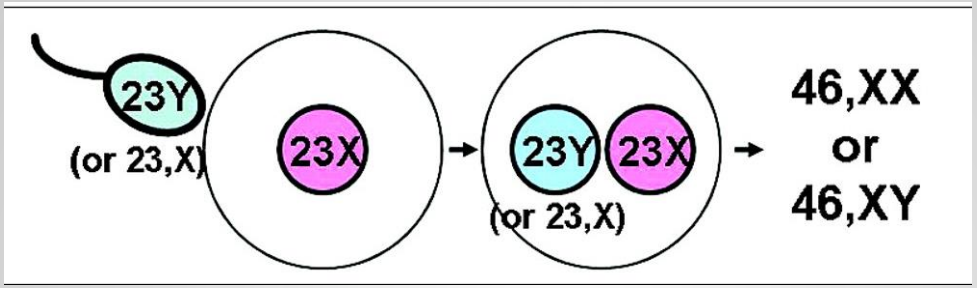


- Tetraploidy (e.g. 92,XXYY)

Mechanisms Leading to Aberrant Ploidy: Fertilization and Early Cell Division Errors

Mechanism

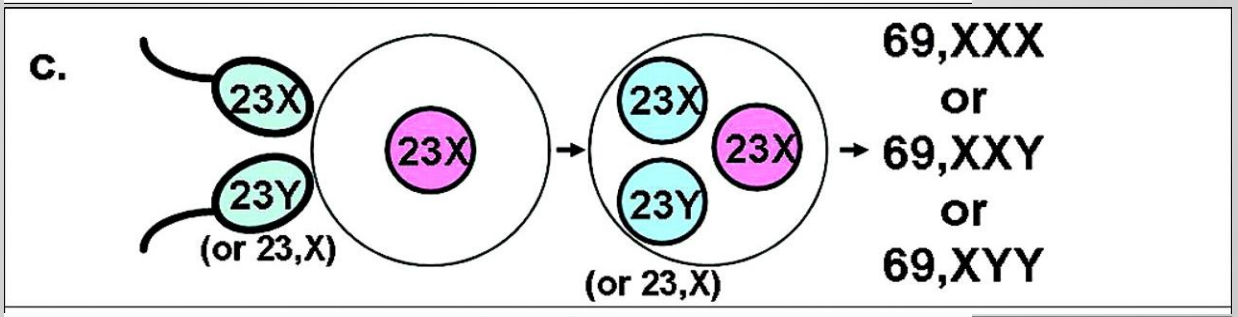
Normal fertilization



Phenotype

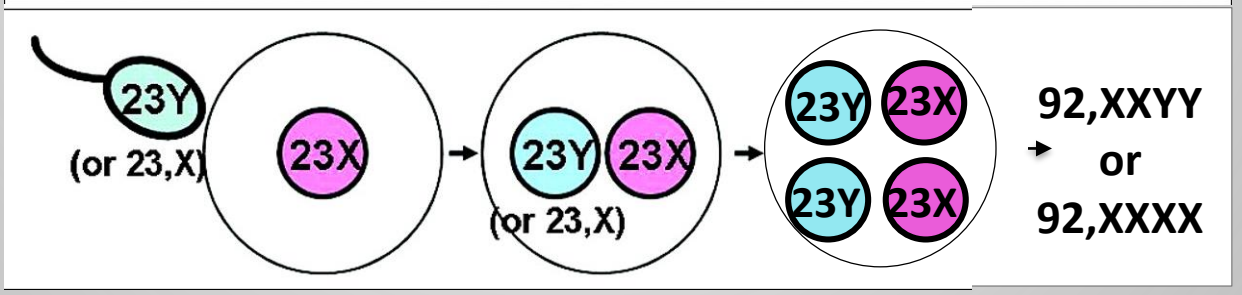
Normal, diploid

Dispermy of 23,X egg (not shown: failed polar body extrusion)



Triploidy

Failure of first cell division



Tetraploidy

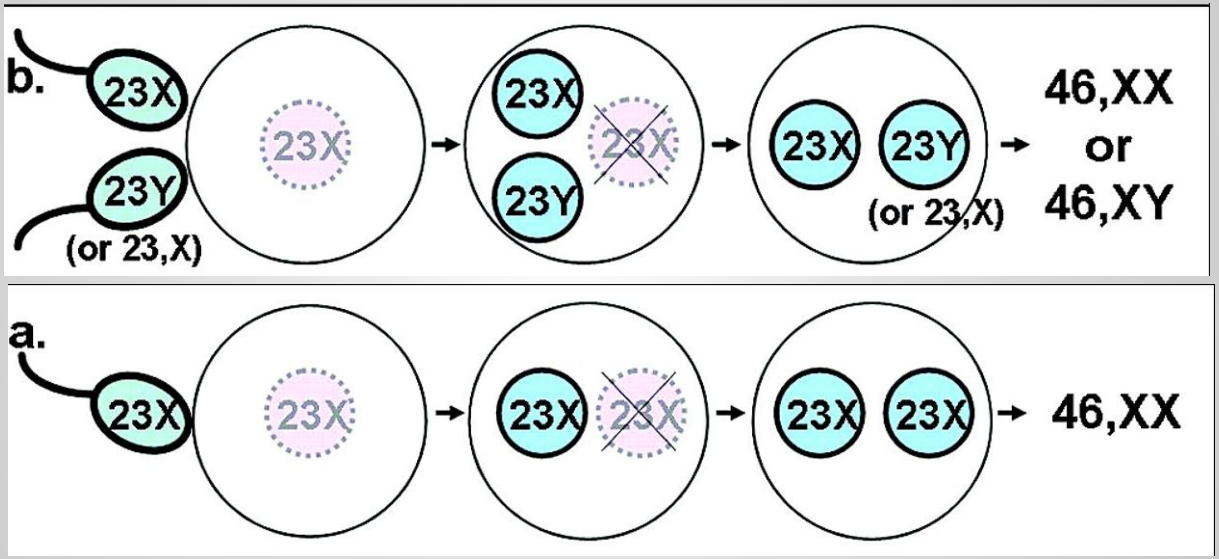
Image modified from: Van den Veyver I , and Al-Hussaini T Hum. Reprod. Update 2006;12:233-242

Other Fertilization and Early Cell Division Errors

Mechanism

Dispermy and loss or missing maternal nucleus

Endoreduplication of haploid genome

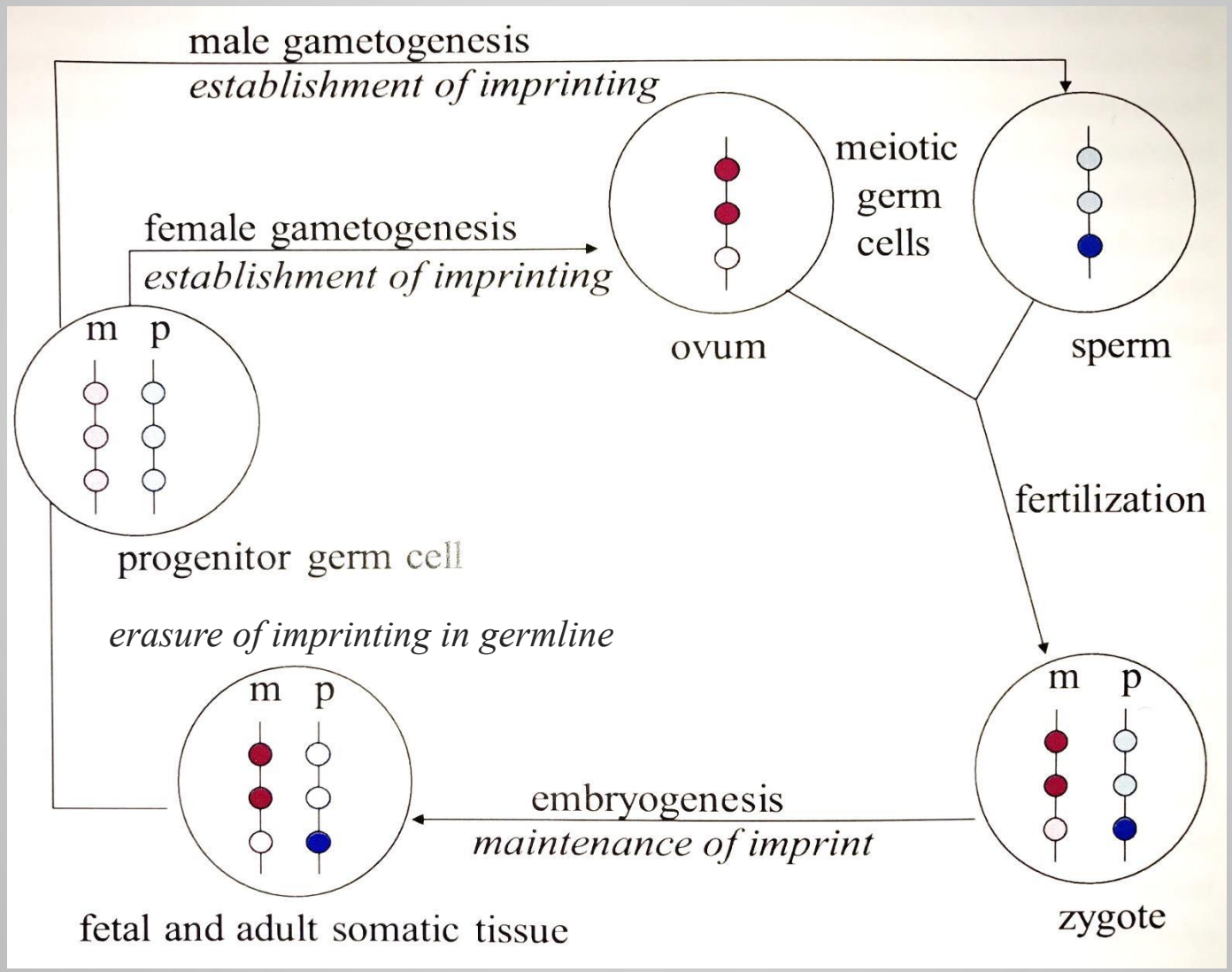


Phenotype

Molar Pregnancy (Uniparental disomy, genome-wide)

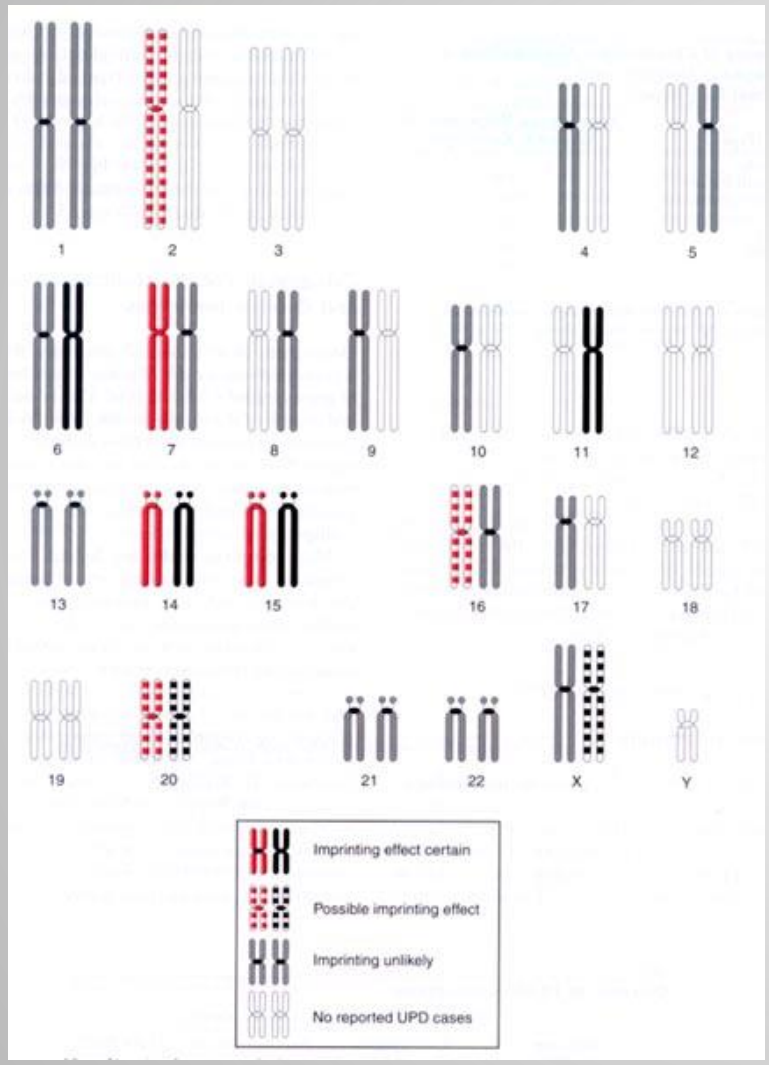
Image modified from: Van den Veyver I , and Al-Hussaini T Hum. Reprod. Update 2006;12:233-242

Imprinting



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

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



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	Hypotonia, motor development delay, mild dysmorphic facial features, low birth weight, growth abnormalities
Paternal UPD 14	Severe mental and muscoskeletal abnormalities
Maternal UPD 15	Prader-Willi syndrome
Paternal UPD 15	Angelman syndrome
Maternal UPD 16	Intrauterine growth retardation
Maternal UPD 20	Intrauterine growth retardation and/or postnatal growth retardation

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

Velissariou, Balkan J Med Gen

Uniparental disomy (UPD)

- Biparental: one copy derived from each parent, the normal chromosome complement

- Uniparental disomy: both chromosomes are derived from a single parent
  - Uniparental Heterodisomy: homologous, non-identical copies or regions
  - Uniparental Isodisomy: identical copies or regions
    - Carries risk for recessive disease if a recessive mutation resides in the chromosome/segment

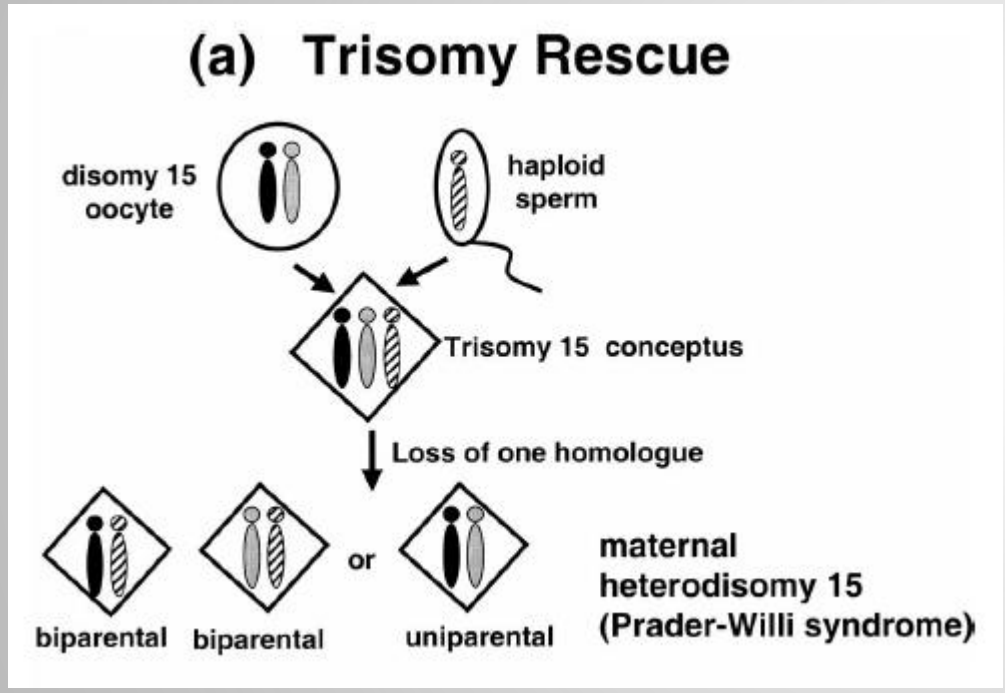
Biparental

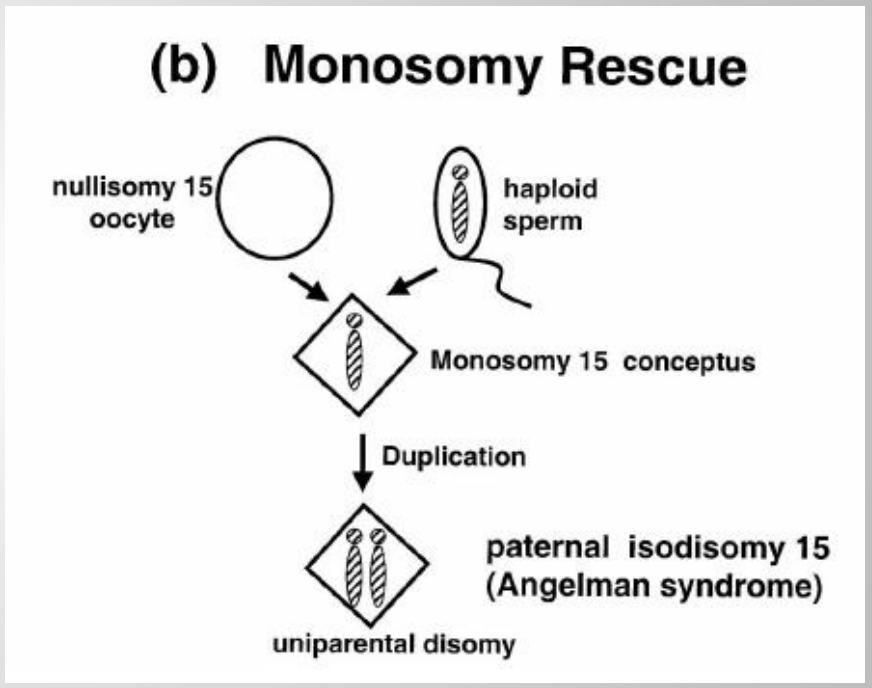
Heterodisomy

Isodisomy

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

Mechanisms Leading to UPD: Chromosomal Nondisjunction





Images from Shaffer et al., 2001, Genetics in Medicine

Incidence of aneuploidy detected in newborns

Abnormality	Rate/1000	Rate (1/n)
Autosomal Trisomy	**1.62**	**617**
+C (6,7,**8,9**,10,11,12)	0.01	120,290
+D (**13**,14,15)	0.04	24,058
+E (16,17,**18**)	0.21	4,812
+G (**21**,22)	1.37	730
Sex Chromosome Aneuploidies (All)	**2.70**	**375**
45,X and variants	0.29	3,509
47,XXX and 47,XXX/46,XX	0.50	2,000
47,XXY and variants	0.72	1,400
47,XYY and 46,XY/47,XYY	0.53	1,887

Data from: Milunsky and Milunsky, Genetic Disorders of the Fetus, 6th Ed. (2010). Benn, Chp. 6

- Incidence of sex chromosome aneuploidy is higher

- True rates are underestimated, especially for sex chromosome aneuploidies, which may be unrecognized at birth

Sex chromosome aneuploidy

Syndrome	Karyotype	Prevalence	Somatic features	Reproductive impact
Turner	XO[a]	0.04% (1/2500 females) *	Growth retardation Congenital heart disease Horseshoe kidney Visual impairment	Humans: sterile Mice: fertile but reduced oocyte pool and reproductive lifespan
Klinefelter	XXY[b]	0.1% (1/500-1000 males)	Variable Tall stature Gynecomastia Mild developmental and behavioral problems	Humans and mice sterile unless spontaneous X chromosome loss
Double Y	XYY	0.1% (1/1000 males)	Tall stature	Humans: commonly fertile because of high incidence of Y chromosome loss Mice: sterile because of low probability of Y chromosome loss

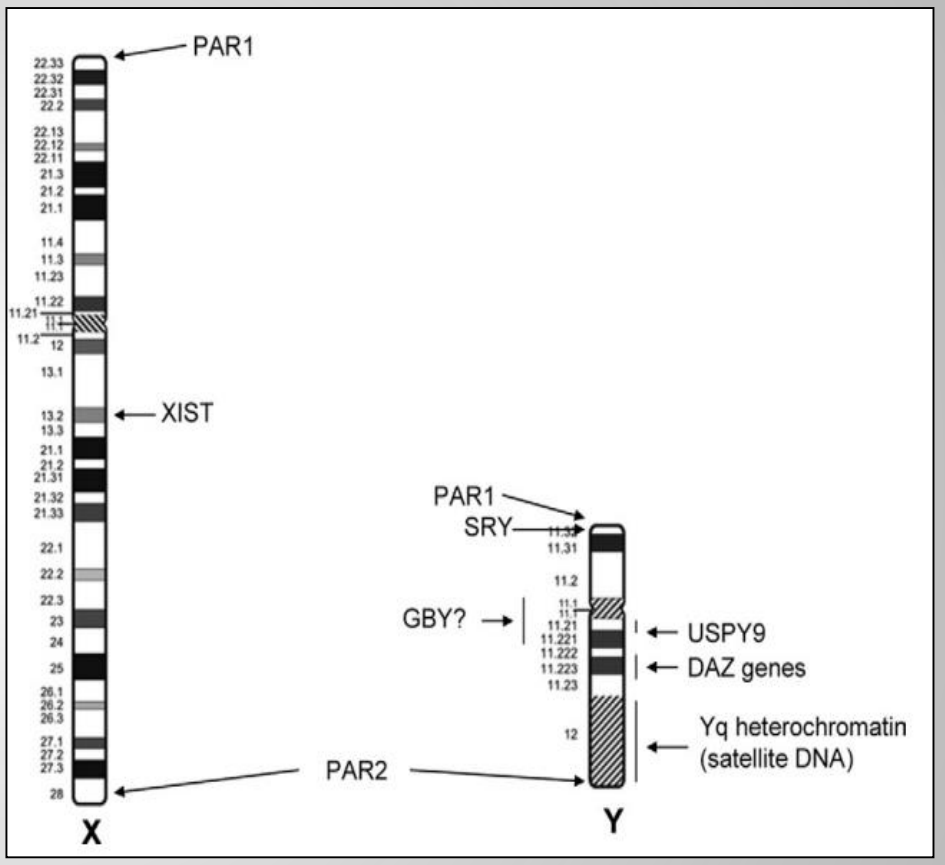
Do not use XO in karyotypes!

Modified from Heard and Turner, 2011

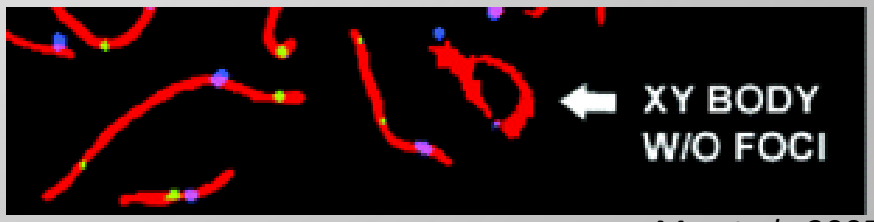
* 99% of 45,X conceptuses result in spontaneous abortion

Sex Chromosomes

- X chromosome: 1000's of genes, one X is inactive in females
  - XIST: dosage compensation
- Y chromosome: main function is in male sexual development
  - SRY determines male phenotype
  - Other genes regulate sexual development
  - Yqh is inactive
- Pseudoautosomal (PAR) regions are required for pairing and recombination between the X and Y in males
  - Obligatory crossing-over occurs in PAR1
  - Errors in XY pairing lead to increased incidence of XY nondisjunction, higher rates sex chromosome aneuploidy



Li, Clin Lab Med 31 (2011)



Ma *et al.*, 2005

Dosage compensation: X-inactivation

- X-inactivation rescues the potential damaging effect of increased X gene dosage in 47,XXX and 47,XXY
- In female somatic cells, only one X is active, the second is condensed/inactive (appears as Barr body in interphase cells)
- Number of Barr bodies = n(X)-1 *
  - *Applies to diploid cells only
  - 46,XX (1 Barr body)
  - 47,XXY (1 Barr body)
  - 47,XXX (2 Barr bodies)
  - 45,X (0 Barr bodies)

Barr body (Xi) in a normal female cell

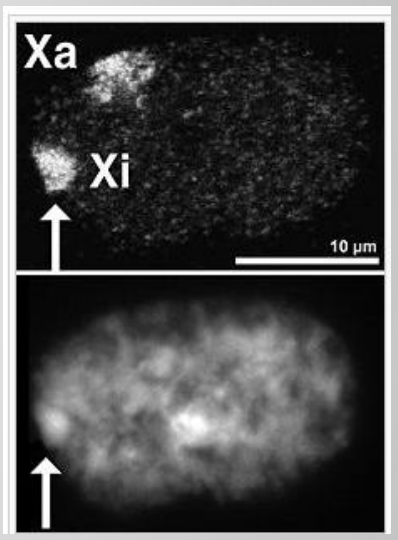


Image source: Wikipedia

Dosage compensation: X-inactivation

Lyon hypothesis (now Lyon Law) to explain X chromosome dosage:

- One X is inactivated in females
- X-inactivation occurs in early development
  - ~2 weeks after fertilization, ~100's cell stage/blastocyst
  - Must be re-activated in the germline
- X-inactivation is random
- X inactivation is clonal (females are essentially mosaics for X-linked genes)

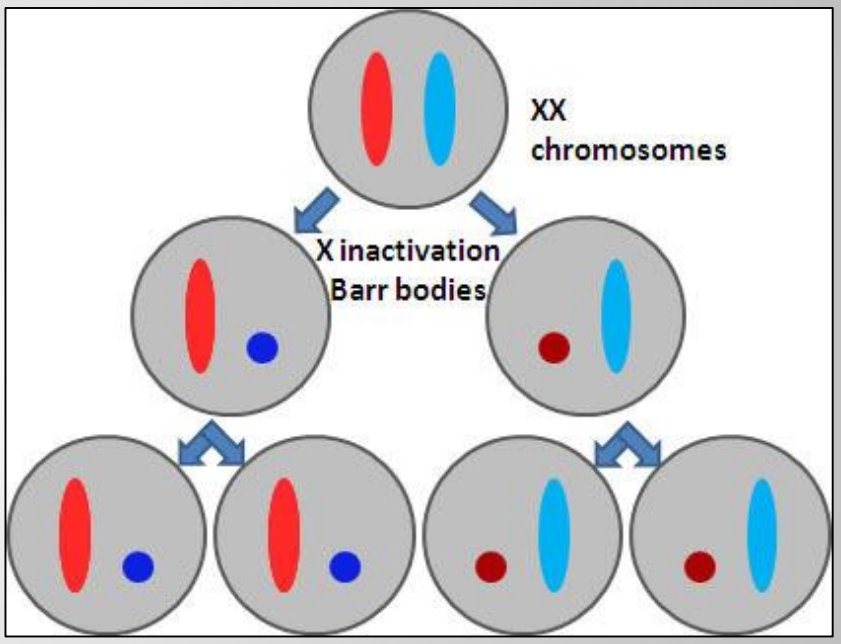
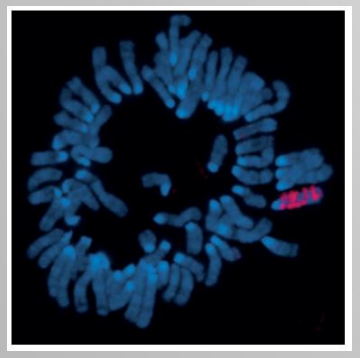


Image source:
http://www.scoop.it/t/molcyt/p/293460152/2011/07/14/50-years-of-the-lyon-hypothesis-and-x-inactivation-at-the-european-cytogeneticists-association-eca-conference-porto

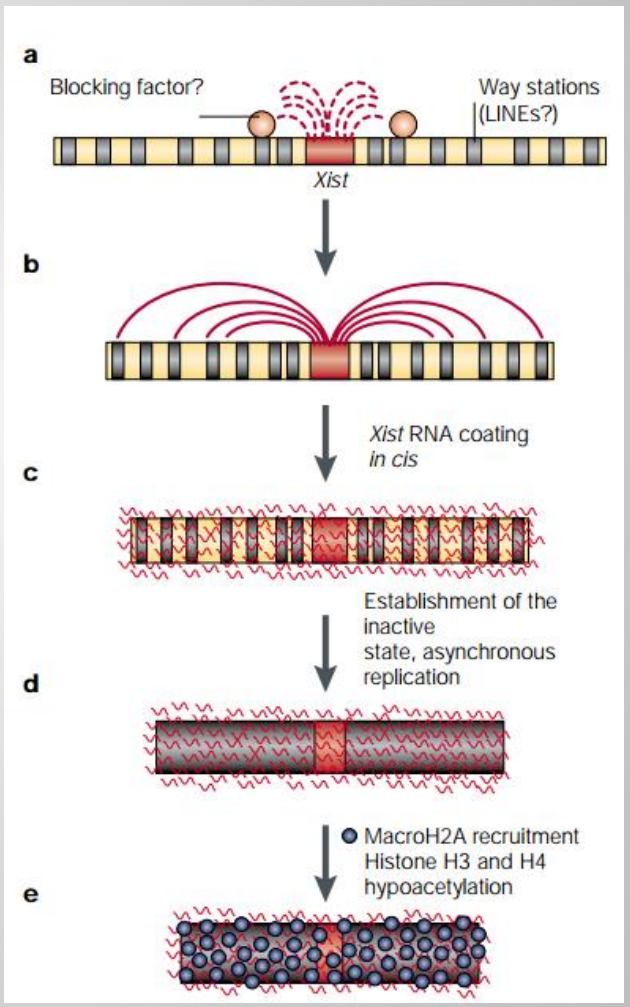
Mechanism of X-inactivation

- X-inactivation specific transcript (XIST) at Xq13 is transcribed only from the inactive X
- XIST mRNA acts *in cis*, coating the inactive X, which triggers condensation, affecting replication
- Histone modification leads to inactivation



Xist RNA-FISH in a mouse metaphase spread

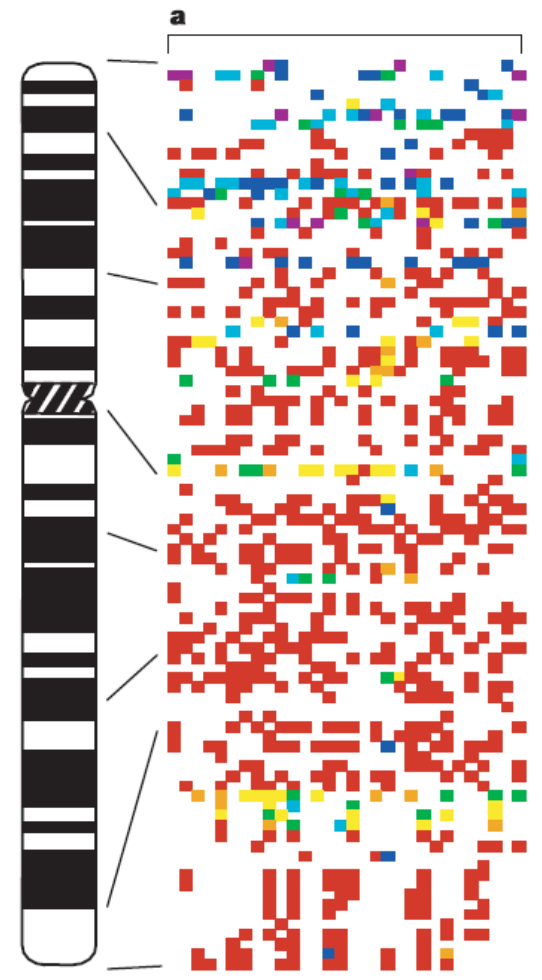
Ng et al., 2007, EMBO reports

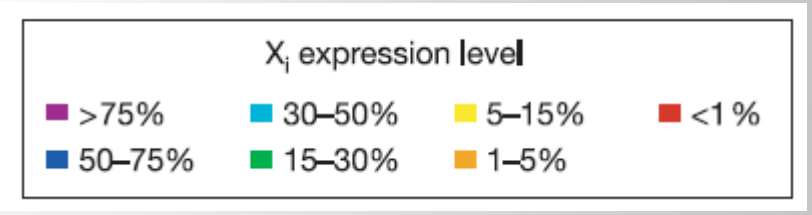


Avner and Heard, 2001, Nat Rev Genet

Not all X-chromosome genes are inactivated

- Explains why 45,X is associated with an abnormal phenotype

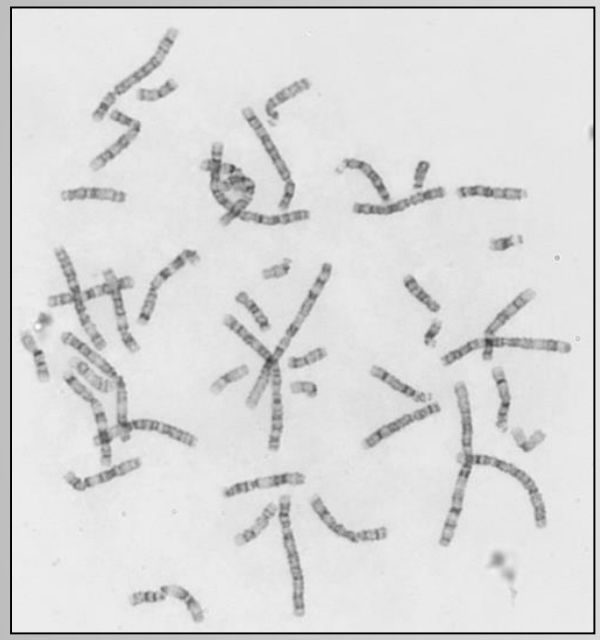




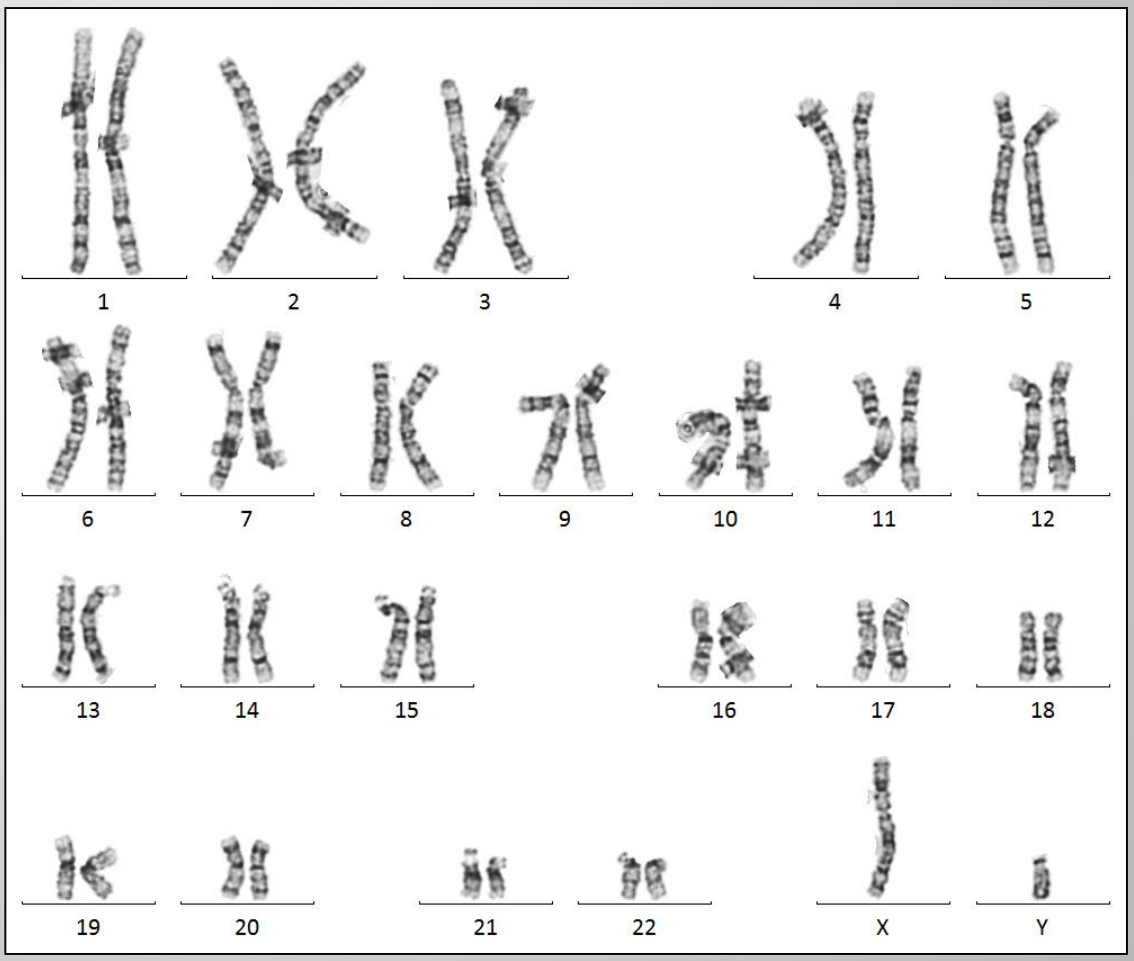
Carrel and Willard 2005

Karyotyping

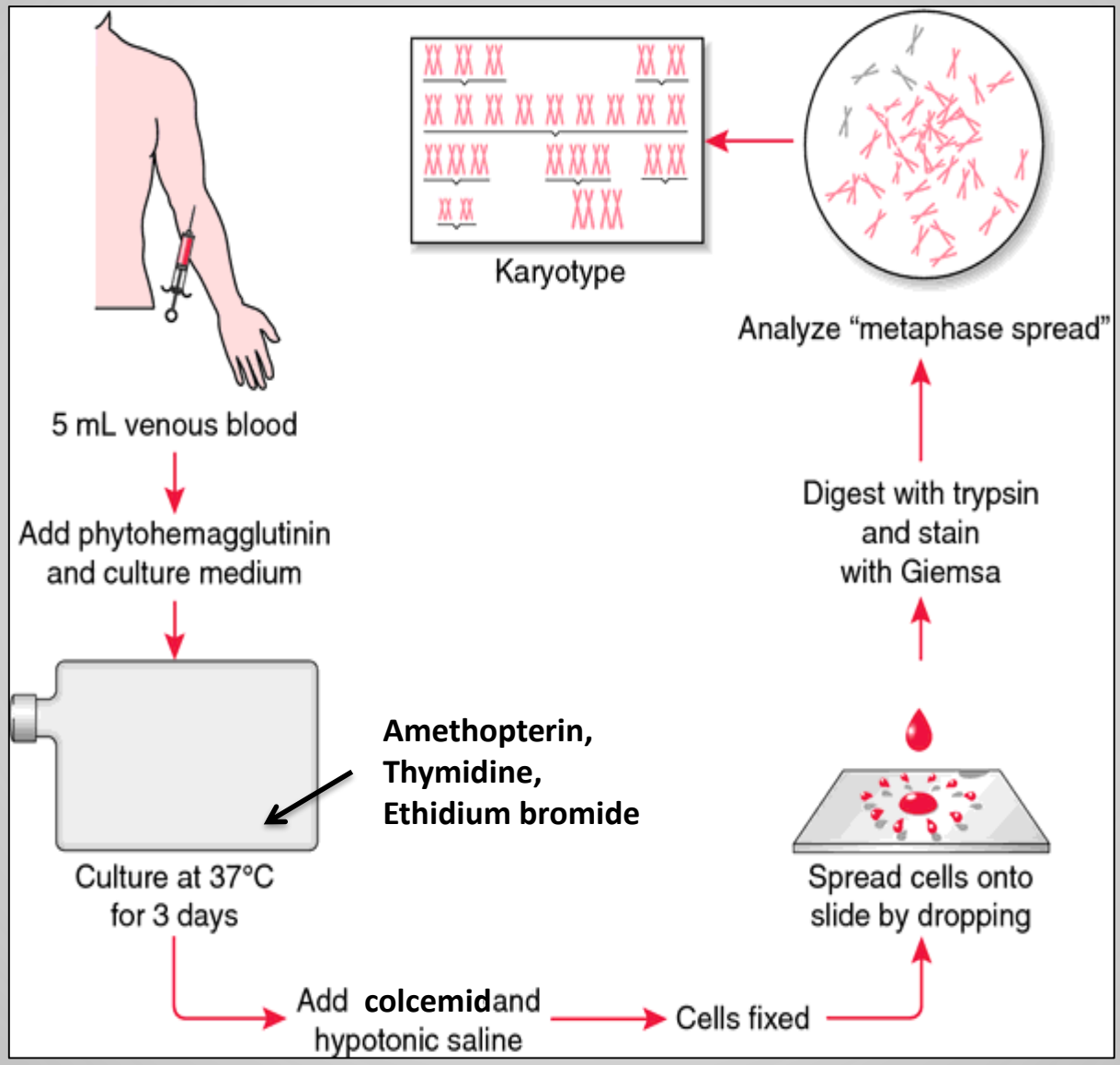
Metaphase spread



Karyogram



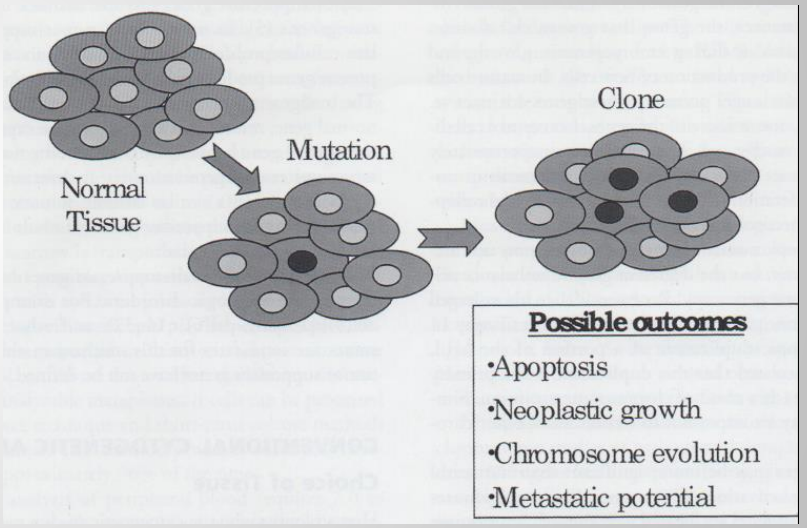
Preparation of metaphase chromosomes



Preparation of a karyotype. From Mueller and Young, 2001.

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 must be observed in two independent cultures (r/o in vitro artifacts)



Dewald *et al.*, Cytogenetic Studies in Neoplastic Hematologic Disorders 2nd Ed.

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:
  - Somatic and/or germline mosaicism
  - 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 variable or a milder clinical phenotype, may see skin pigmentation anomalies, may be suggestive by recurrent cytogenetic abnormality in offspring and normal constitutional result (i.e. gonadal mosaicism)

NOTE: there are special considerations when mosaicism is observed in a prenatal study

Standard Nomenclature for Karyotype Designation

General designation includes:

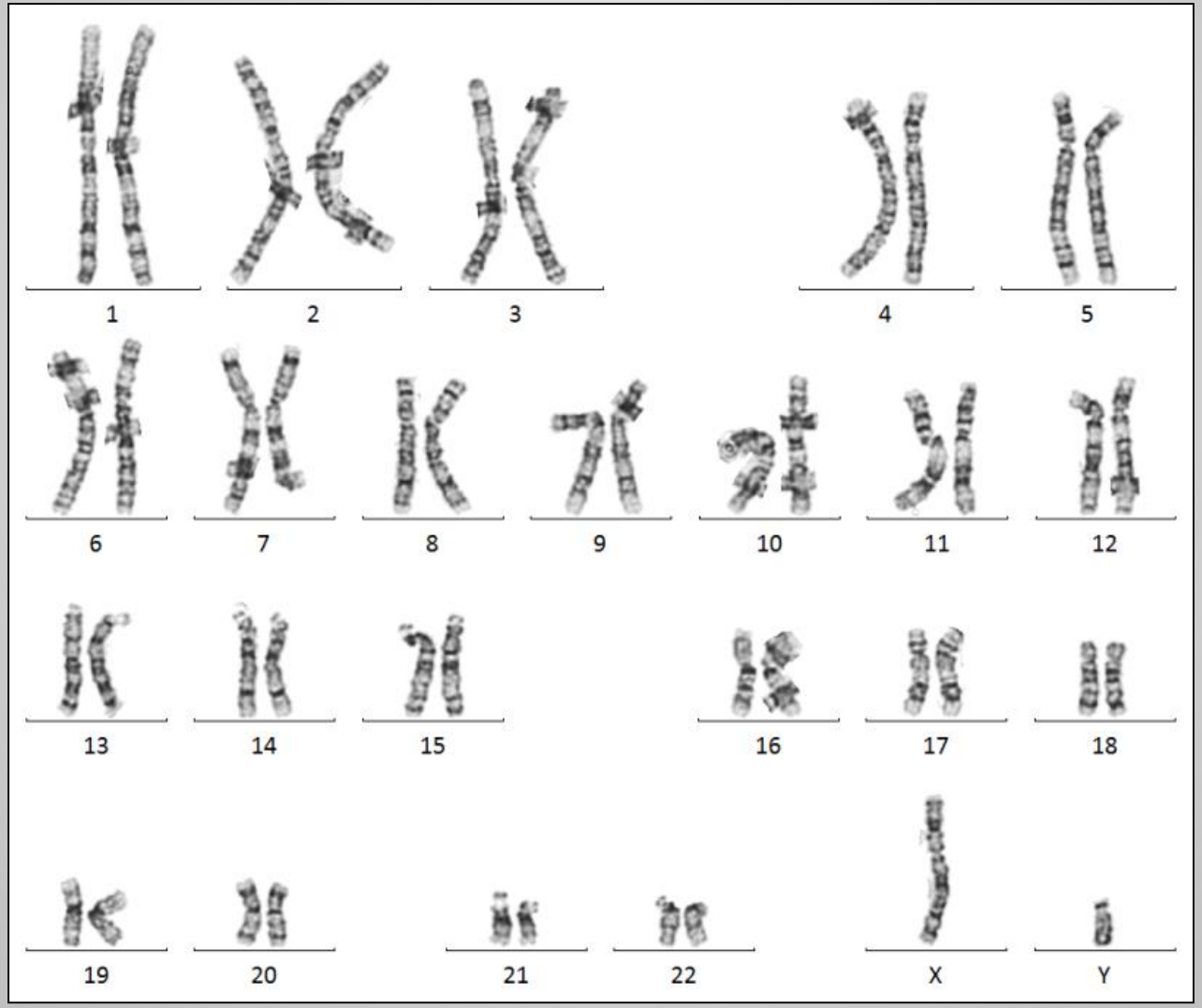
- Chromosome number (count)-based on #centromeres
  - Expressed relative to the ploidy level
- Sex chromosome constitution
  - Use +/- for acquired sex chromosome aneuploidy only
- List of abnormalities present
  - Ordered by chromosome number (sex chromosomes, then autosomes 1-22) and abnormality type (numerical abnormalities/aneuploidies, then structural abnormalities, listed alphabetically and by location/band, low to high)
- Multiple cell lines
  - Mosaicism: List abnormal clone(s) first, list multiple abnormal clones from largest to smallest in size
  - Chimerism: List recipient (individual's karyotype) first

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
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	mosaic (multiple cell lines/clones present)
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)

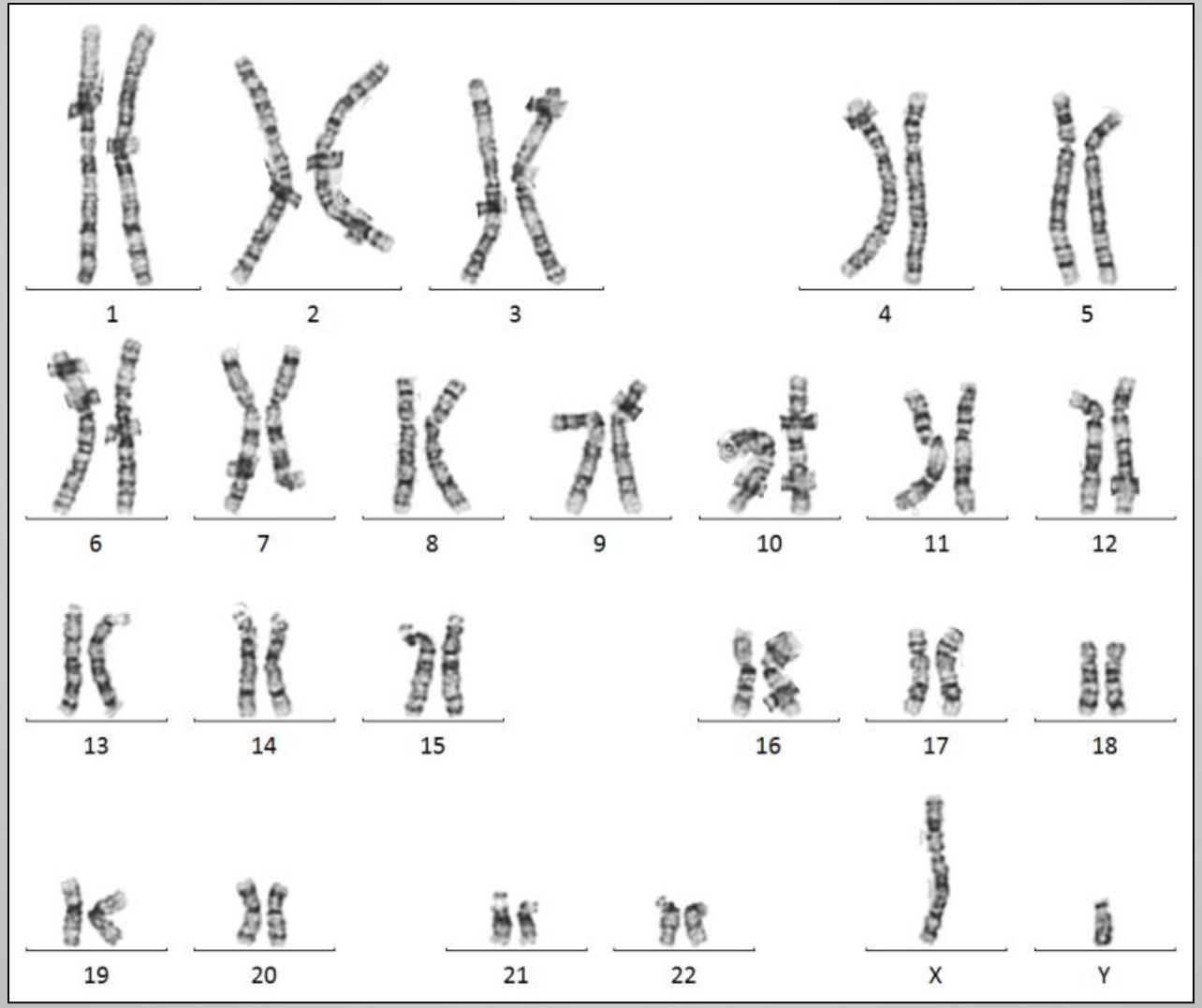
Nomenclature Practice:
Numerical Abnormalities

Normal, constitutional

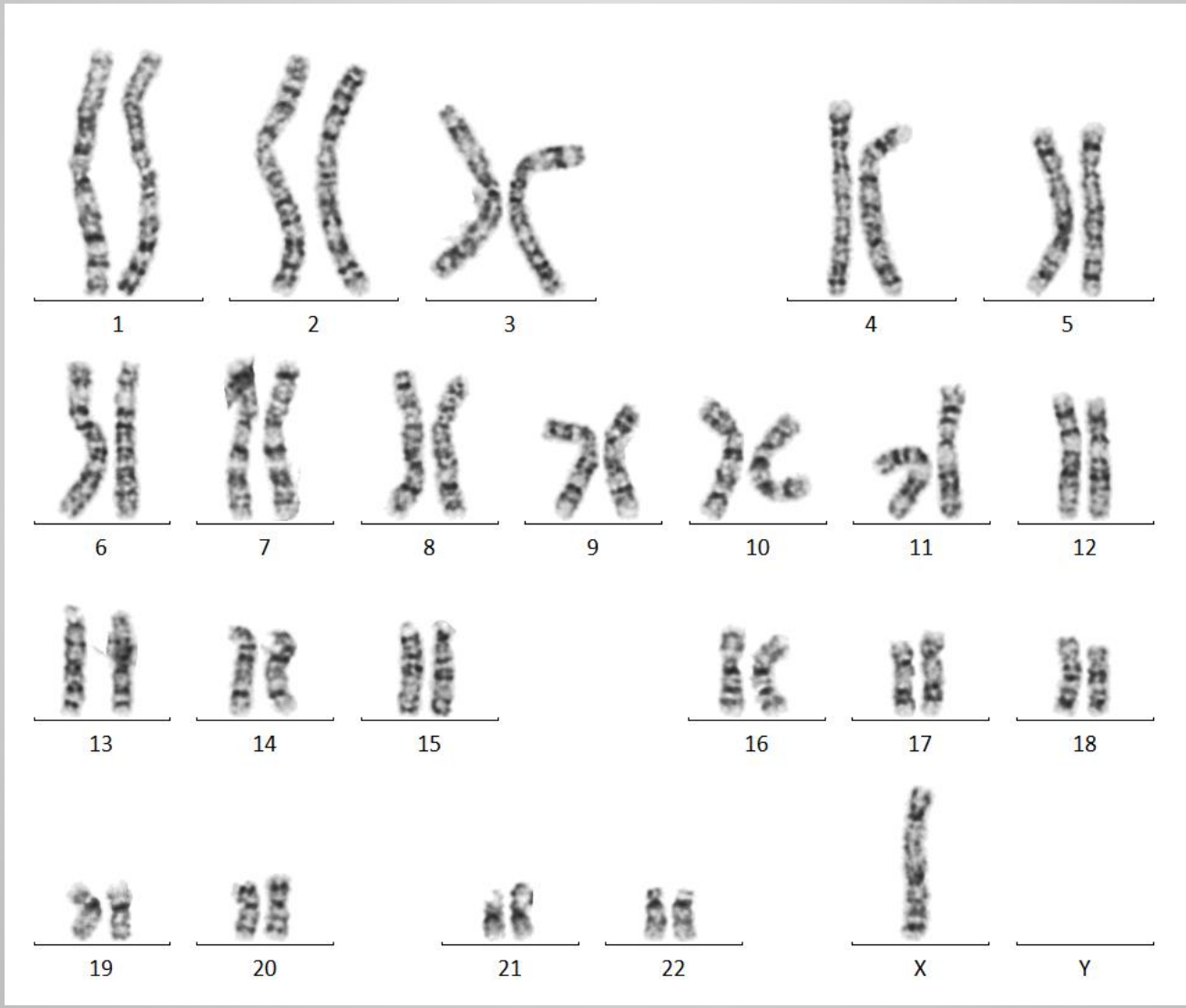


Normal, constitutional

Karyotype: 46,XY

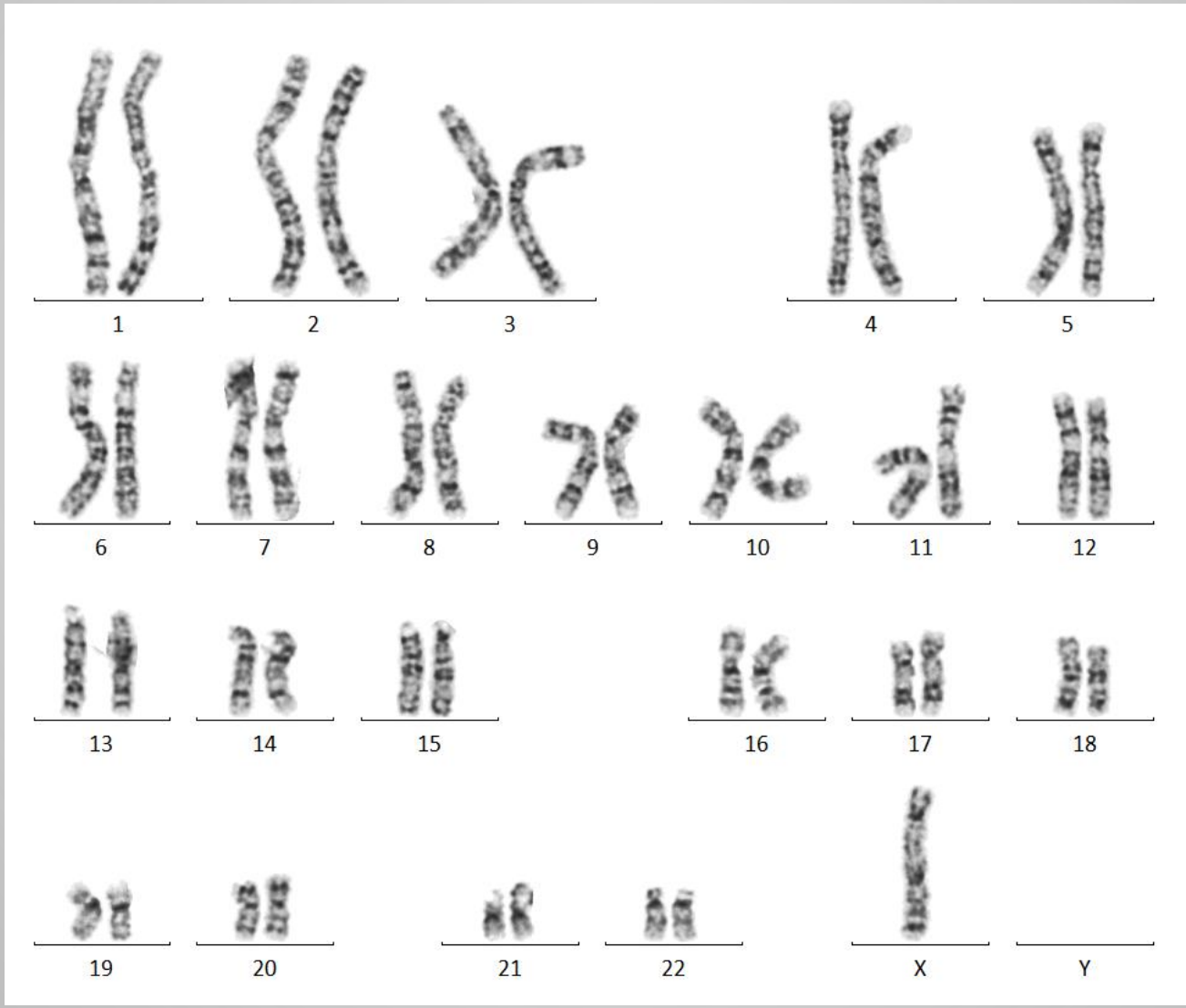


Abnormal, constitutional



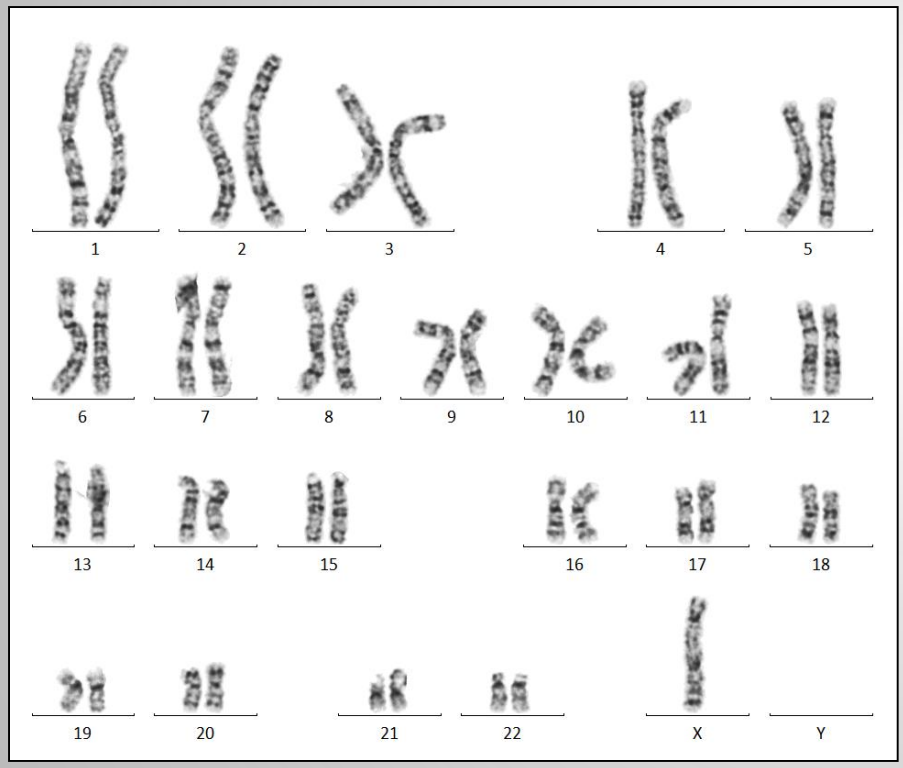
Abnormal, constitutional

Karyotype: 45,X

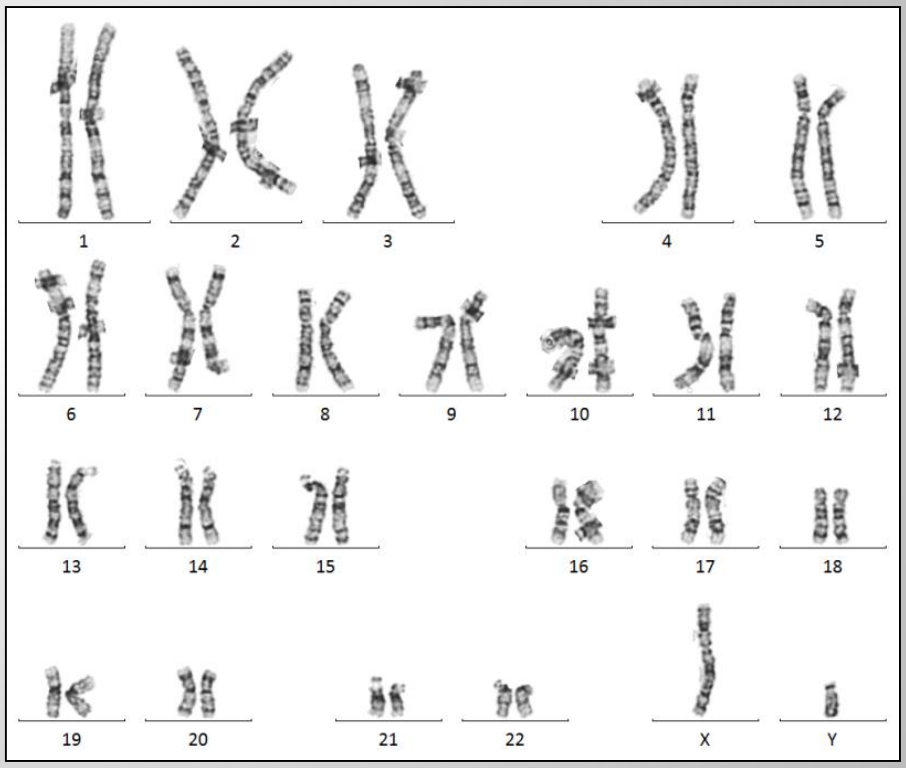


Abnormal, constitutional

12 cells



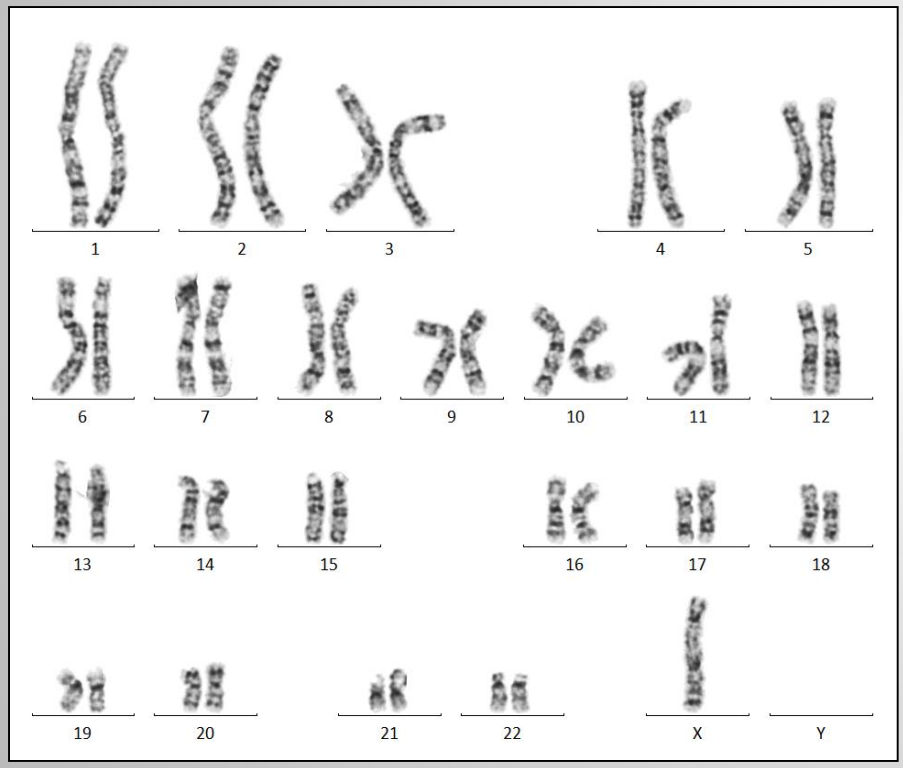
8 cells



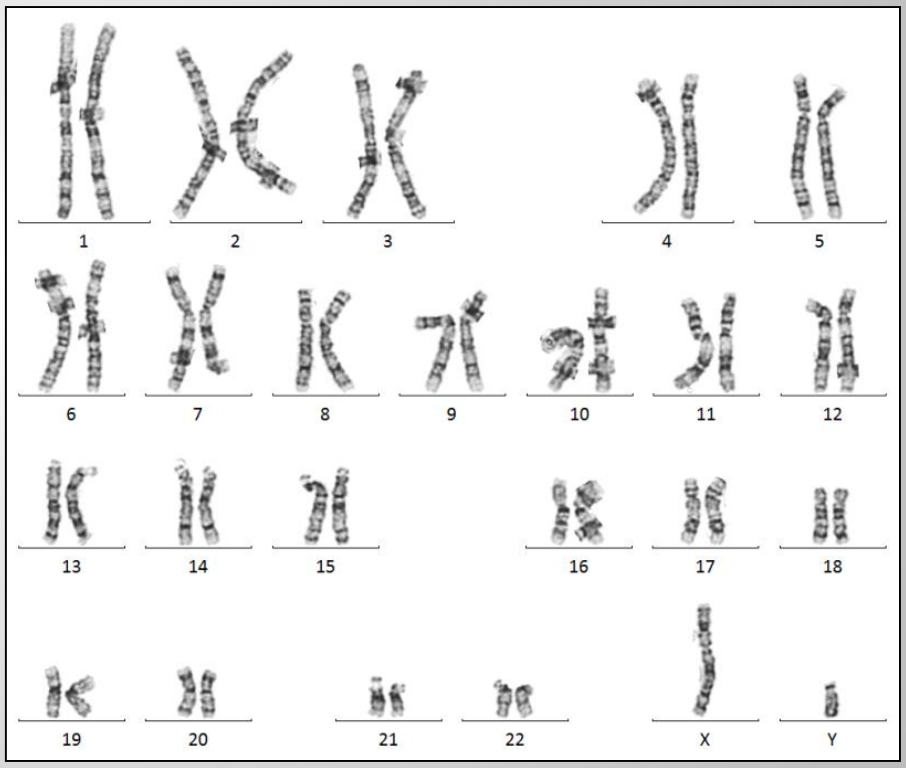
Abnormal, constitutional

Karyotype: 45,X[12]/46,XY[8]

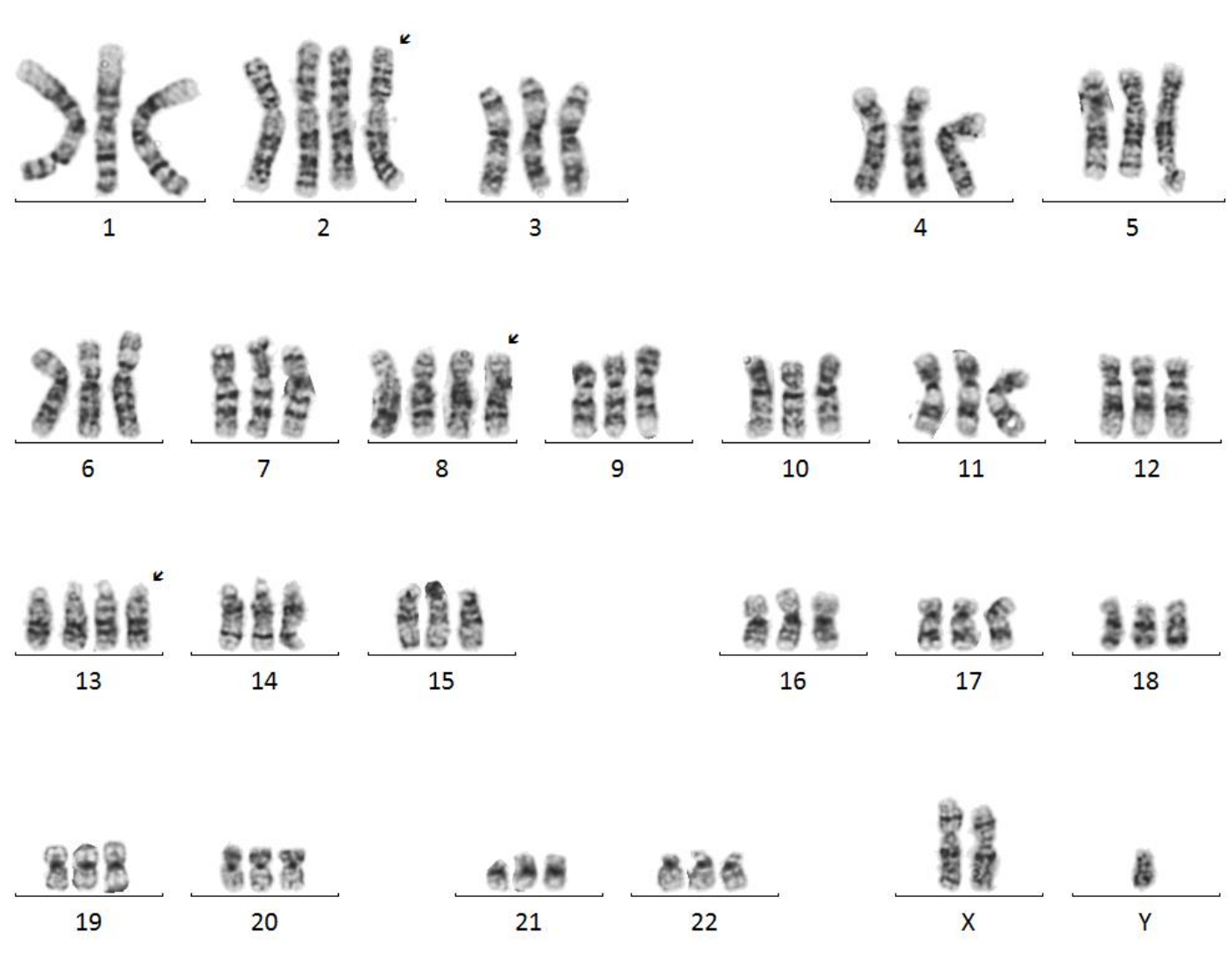
12 cells



8 cells

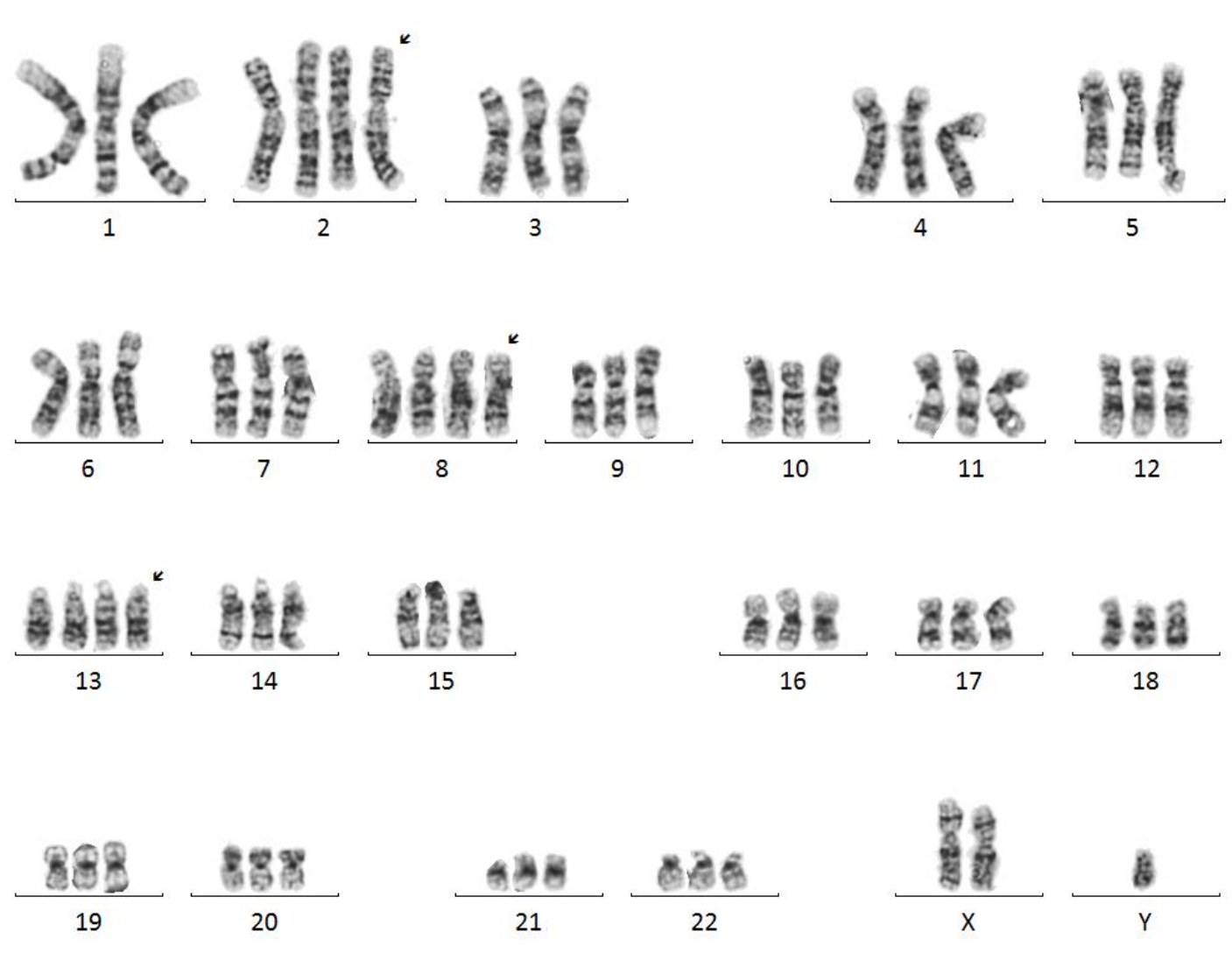


Abnormal, constitutional

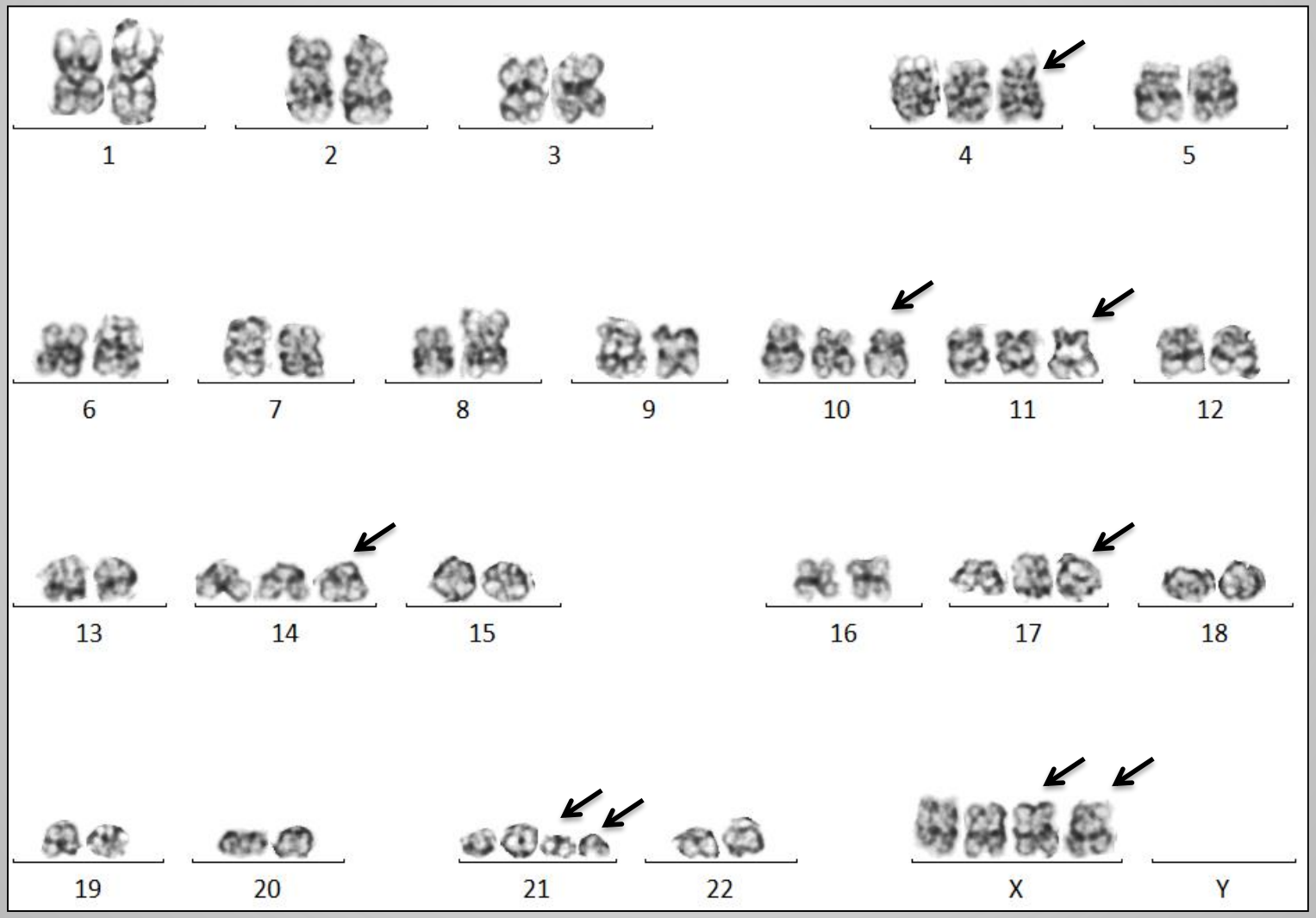


Abnormal, constitutional

Karyotype: 72,XXY,+2,+8,+13



Abnormal, oncology, 9 extra chromosomes, 20 cell study, 3 normal male (BM donor) cells present



Abnormal, oncology, 9 extra chromosomes, 20 cell study, 3 normal male (BM donor) cells present

Karyotype: 55,XX,+X,+X,+4,+10,+11,+14,+17,+21,+21[17]//46,XY[3]