SAWA UNIVERSITY

اسم المادة و الكورس

LAB. 8 HUMAN KARYOTYPING

Sawa University	جامعـــــــة ســـــــــاوة الاهلية
College of health and medical techniques	كليـــــة التقنيـــات الصحيـة والطبيـة
Department of Medical Laboratories	قسم تقنيات المختبرات الطبية
Third Stage	المرحلـــــــــــــة الثالثة

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الجانب العملي:الوراثة الطبية

Medical genetics-Practical

محاضرة رقم 8

Lecture No. 8

Objectives of karyotyping

By the end of this lab, students should be able to:

1. . Define human karyotype and understand its diagnostic value in medical genetics.
2. Describe the laboratory steps of preparing and analyzing a human karyotype.
3. Classify human chromosomes based on centromere position and length.
4. Identify normal and abnormal karyotypes using ISCN nomenclature.
5. Interpret clinical cases with chromosomal abnormalities (e.g., trisomy, monosomy, translocation).

Introduction

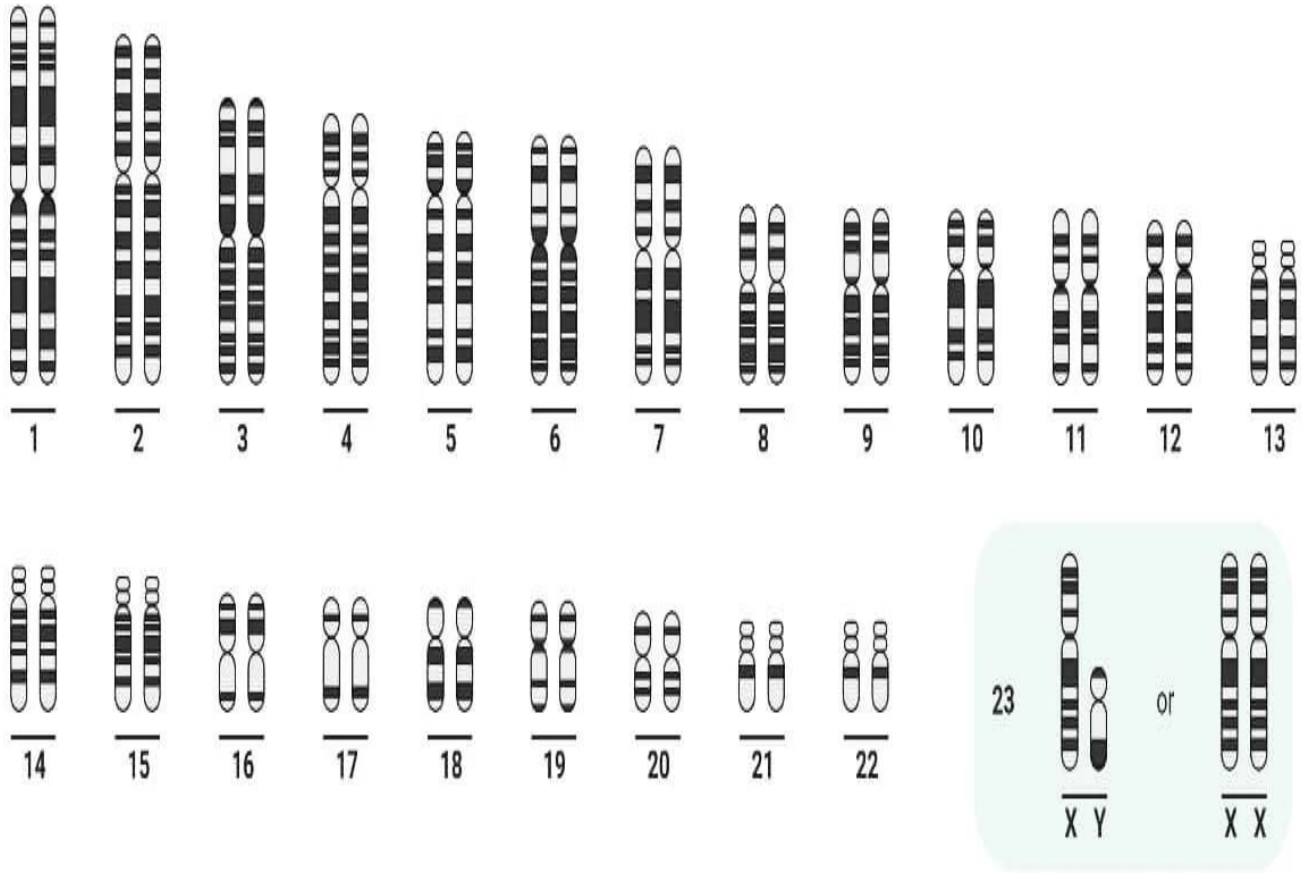
- A karyotype is the complete set of chromosomes in a cell, arranged in pairs according to size, centromere position, and banding pattern.
- It represents the chromosomal constitution of an individual and is used to detect numerical or structural abnormalities.

Typical human karyotypes:

- 46,XX → Normal female
- 46,XY → Normal male
- First human karyotype established in 1956 by Tjio and Levan → discovered that humans have 46 chromosomes, not 48 as previously thought.

Karyotype And Karyotyping

Definition, Procedure, Applications



Laboratory Procedure

1 Sample Collection

1. **Peripheral blood** (2 mL) in a **sodium heparin tube**.
2. Target: Lymphocytes (because they divide upon stimulation).

2 Cell Culture

1. Culture in **RPMI-1640 medium + FBS + PHA.**
2. Incubate 72 hours at 37°C with 5% CO_2.
3. (Stimulate mitosis → metaphase stage).

3 Mitotic Arrest

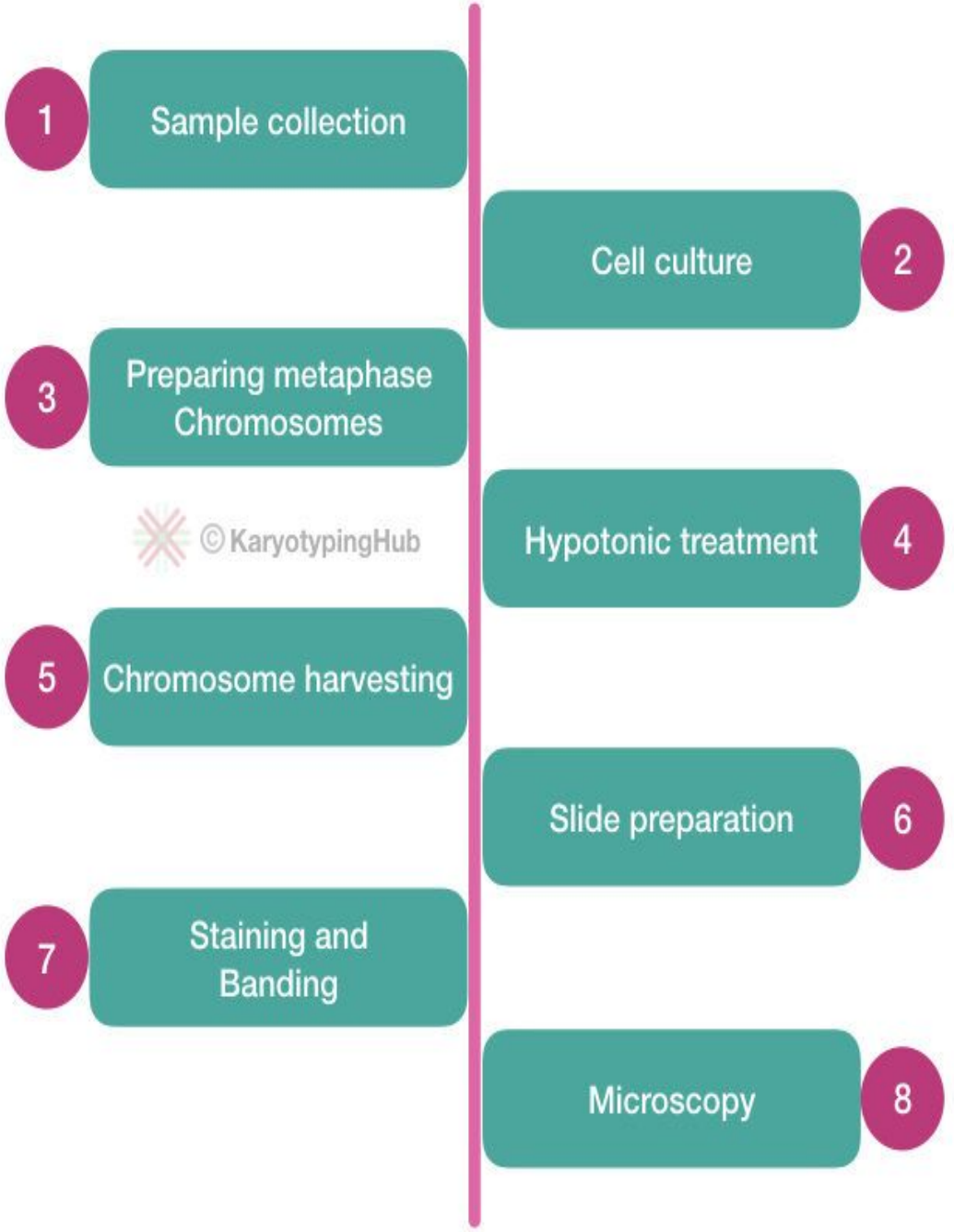
Add **Colchicine** or **Colcemid** for 30 min → stops spindle formation.

4 Hypotonic Treatment

Add 0.075 M **KCl** to swell cells → chromosomes spread apart.

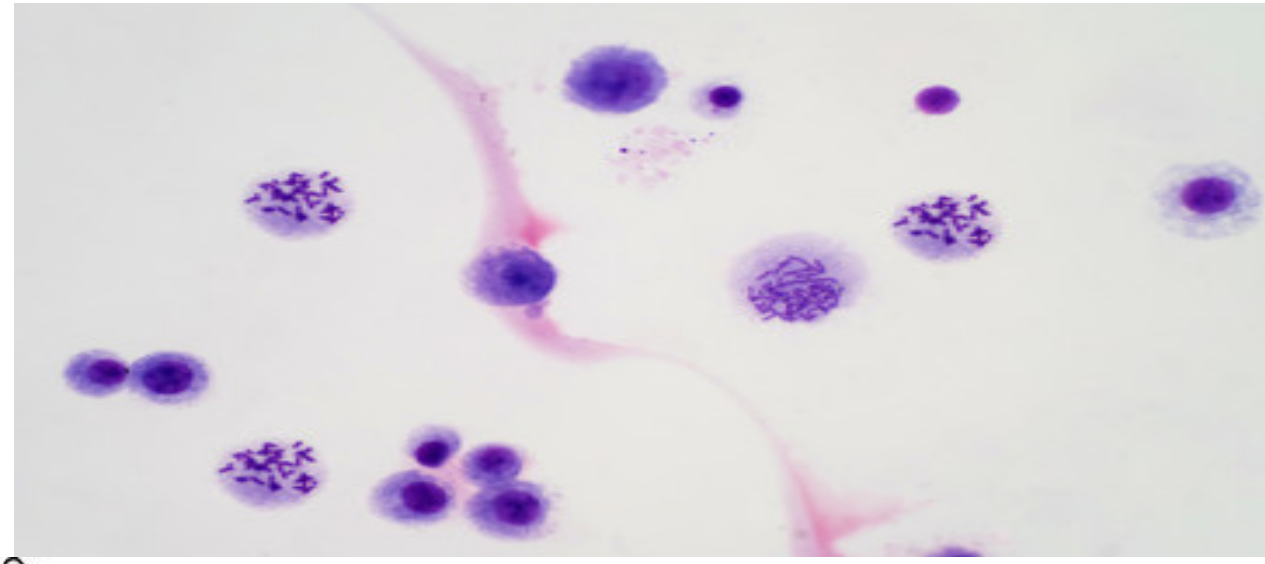
5 Fixation

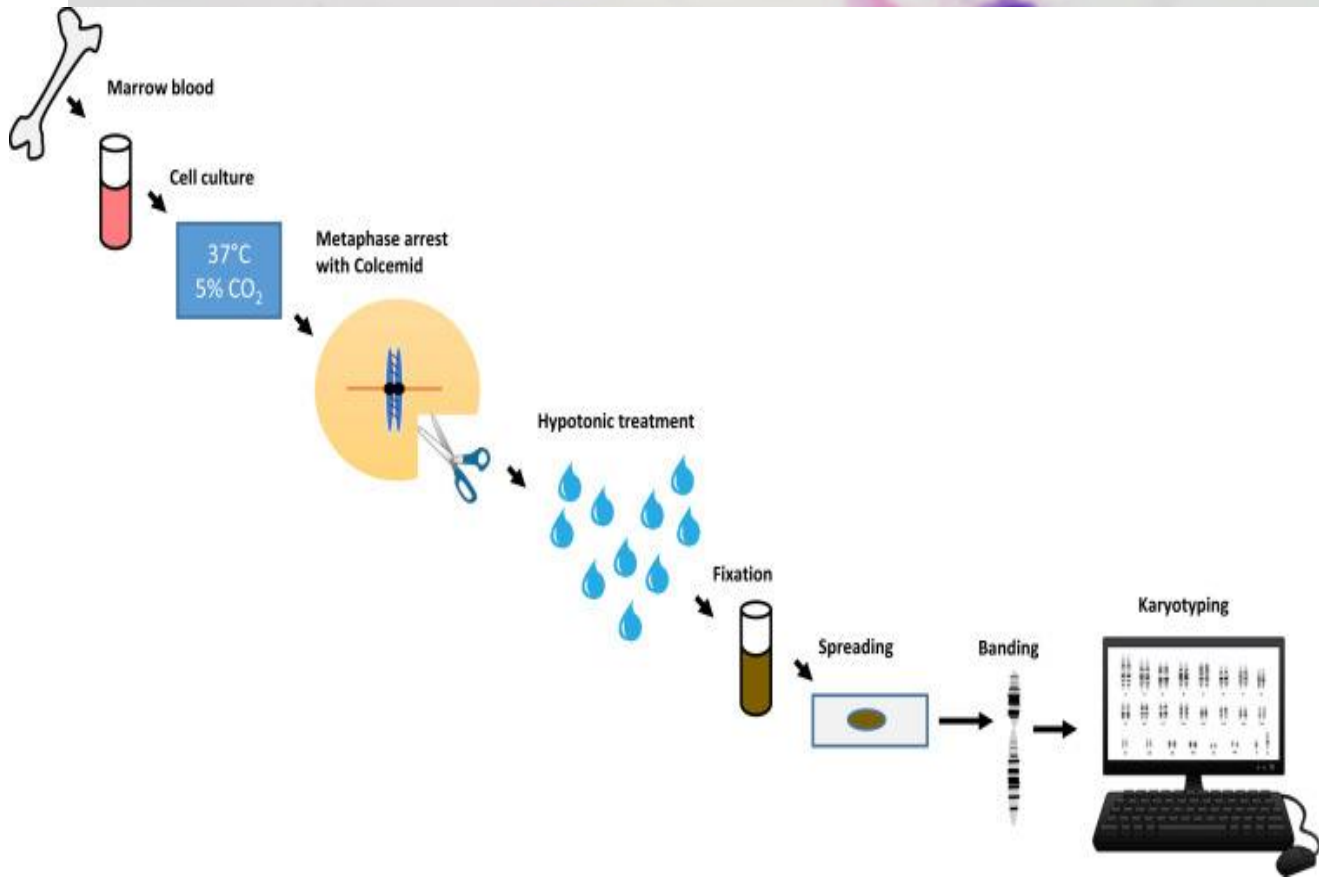
Methanol: Acetic acid (3:1) → fix cells.



Laboratory Procedure

- 6 Slide Preparation
- Drop fixed cells on a clean slide → air dry.
- 7 Staining
- Use G-banding (Trypsin + Giemsa) → banding pattern appears.
- 8 Microscopic Analysis
- Observe metaphase under microscope → capture image → analyze with software.
- 9 Arrangement
- Pair homologous chromosomes from 1 to 22 + XX/XY.
- Identify abnormalities using the ISCN standard.





Chromosome Classification

- Human chromosomes are classified into 7 groups (A–G) according to:

1 Size (length) 2 Centromere position

Group	Chromosomes	Type	Description
A	1–3	Metacentric(1,3) Submetacentric(2)	Large chromosomes with a central centromere
B	4–5	Submetacentric	Large with slightly off-center centromere
C	6–12 + X	Submetacentric	Medium-sized
D	13–15	Acrocentric	Centromere near one end(Medium)
E	16–18	Submetacentric(17,18) Metacentric(16)	Short, medium-sized
F	19–20	Metacentric	Short chromosomes
G	21–22 + Y	Acrocentric	Small, centromere near tip

Chromosome Classification

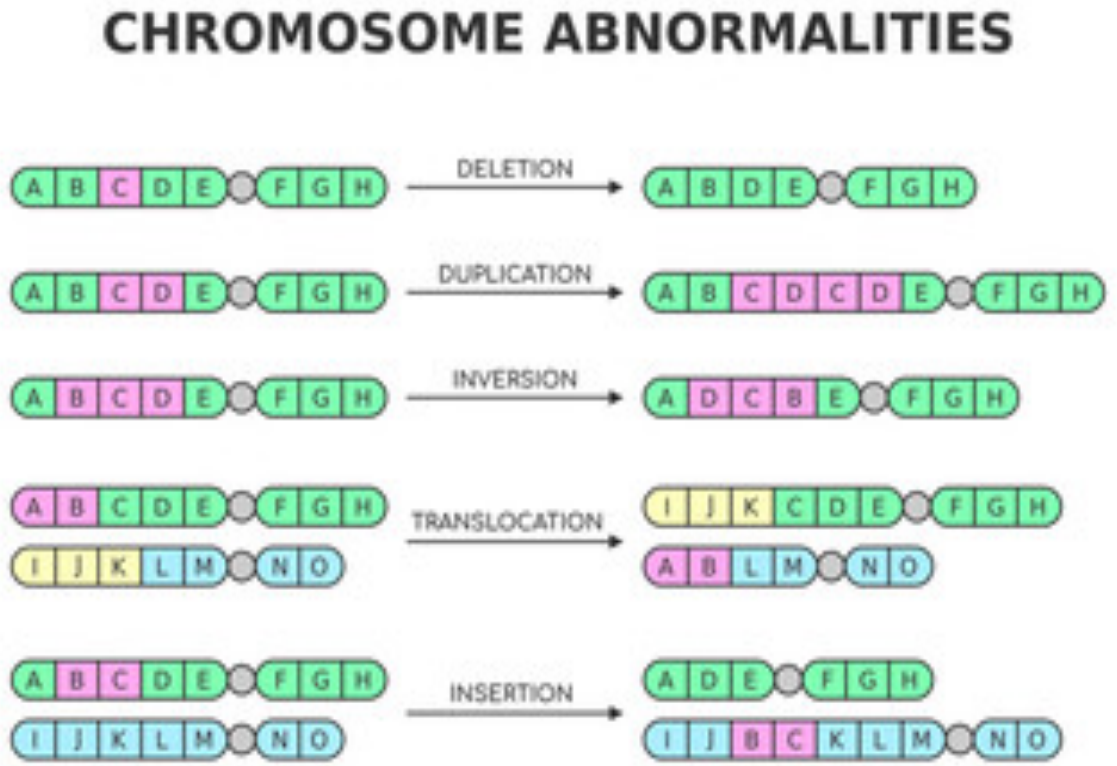
TABLE 2-4 Human Chromosomes

Group	Number	Diagrammatic Representation*	Relative Length†	Centromeric Index‡
Large chromosomes				
A	1		8.4	48 (M)
	2		8.0	39
	3		6.8	47 (M)
B	4		6.3	29
	5		6.1	29
Medium chromosomes				
C	6		5.9	39
	7		5.4	39
	8		4.9	34
	9		4.8	35
	10		4.6	34
	11		4.6	40
	12		4.7	30
D	13		3.7	17 (A)
	14		3.6	19 (A)
	15		3.5	20 (A)
Small chromosomes				
E	16		3.4	41
	17		3.3	34
	18		2.9	31
F	19		2.7	47 (M)
	20		2.6	45 (M)
G	21		1.9	31
	22		2.0	30
Sex chromosomes				
	X		5.1 (group C)	40
	Y		2.2 (group G)	27 (A)

*Black dot indicates position of centromere on length of chromosome.
†Percentage of the total combined length of a haploid set of 22 autosomes.
‡Percentage of chromosome's length spanned by its short arm. The four most metacentric chromosomes are indicated by an (M), the four most acrocentric by an (A).

Diagnostic Karyotyping

1. **Karyotyping is primarily used to:**
   - Detect numerical abnormalities (extra or missing chromosomes).
   - Identify structural abnormalities (translocation, inversion, deletion, duplication).
   - Confirm genetic syndromes and monitor cancer cytogenetics.
2. **Karyotyping is used to diagnose:**
   - Congenital anomalies
   - Recurrent miscarriage
   - Infertility
   - Prenatal diagnosis
   - Hematologic malignancies
   - Solid tumors



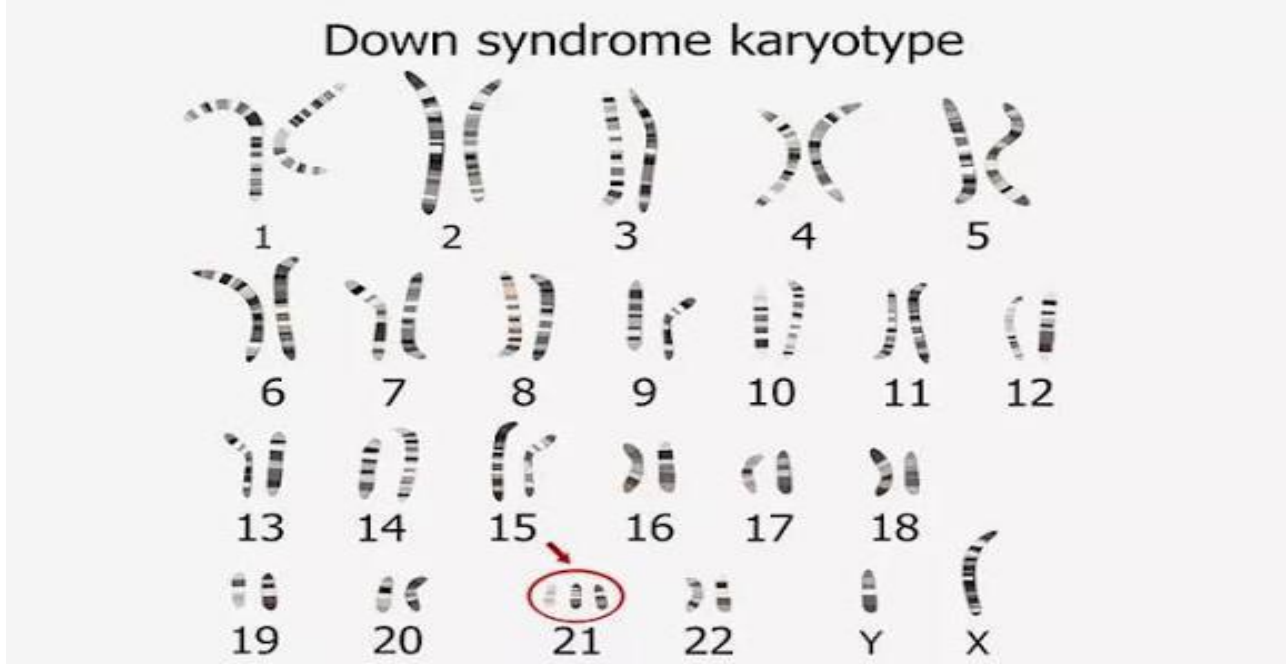
Types of Chromosomal Abnormalities

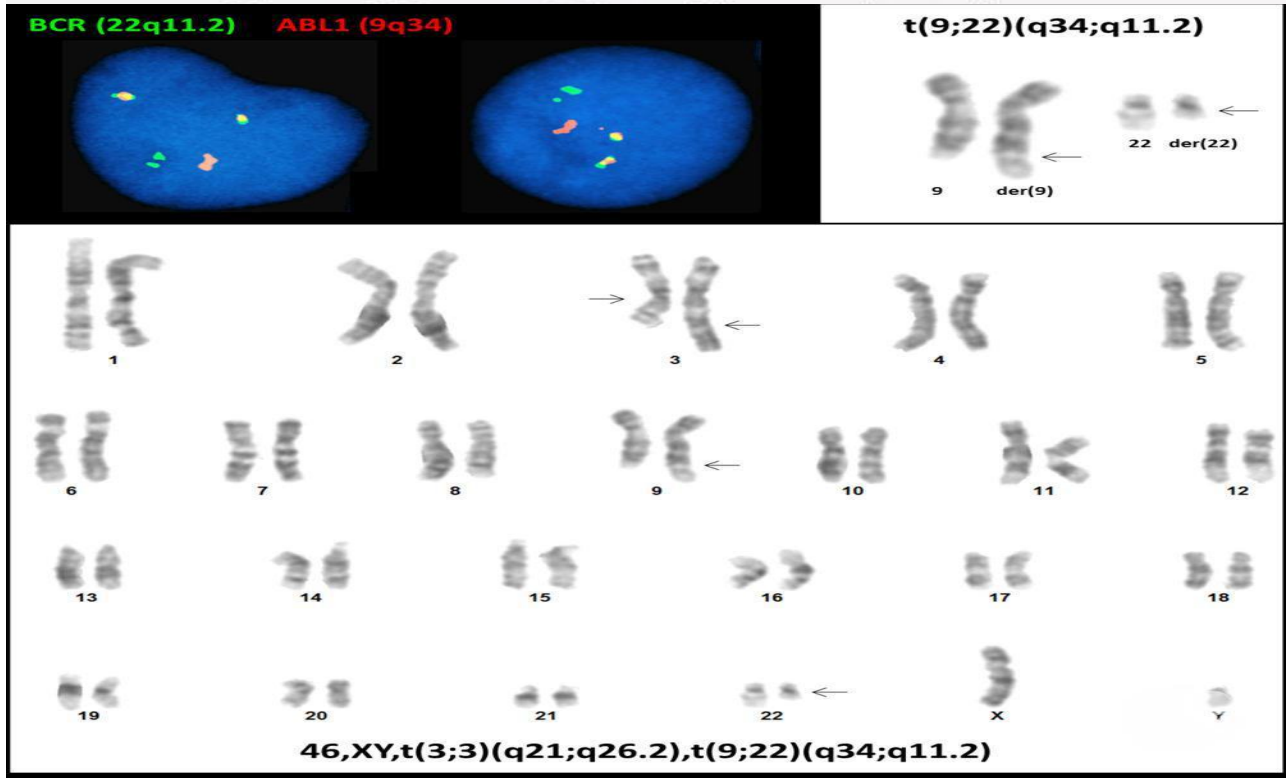
1. Numerical:

- Trisomy (e.g., Down syndrome, 47, XX,+21)
- Monosomy (e.g., Turner syndrome, 45, X)

2. Structural:

- Deletions (del)
- Duplications (dup)
- Translocations (t)
- Inversions (inv)
- Ring chromosomes (r)





ISCN Nomenclature

- **ISCN (International System for Human Cytogenomic Nomenclature) standardizes the reporting of chromosome findings.**
- **Examples:**

✔ **46, XX**

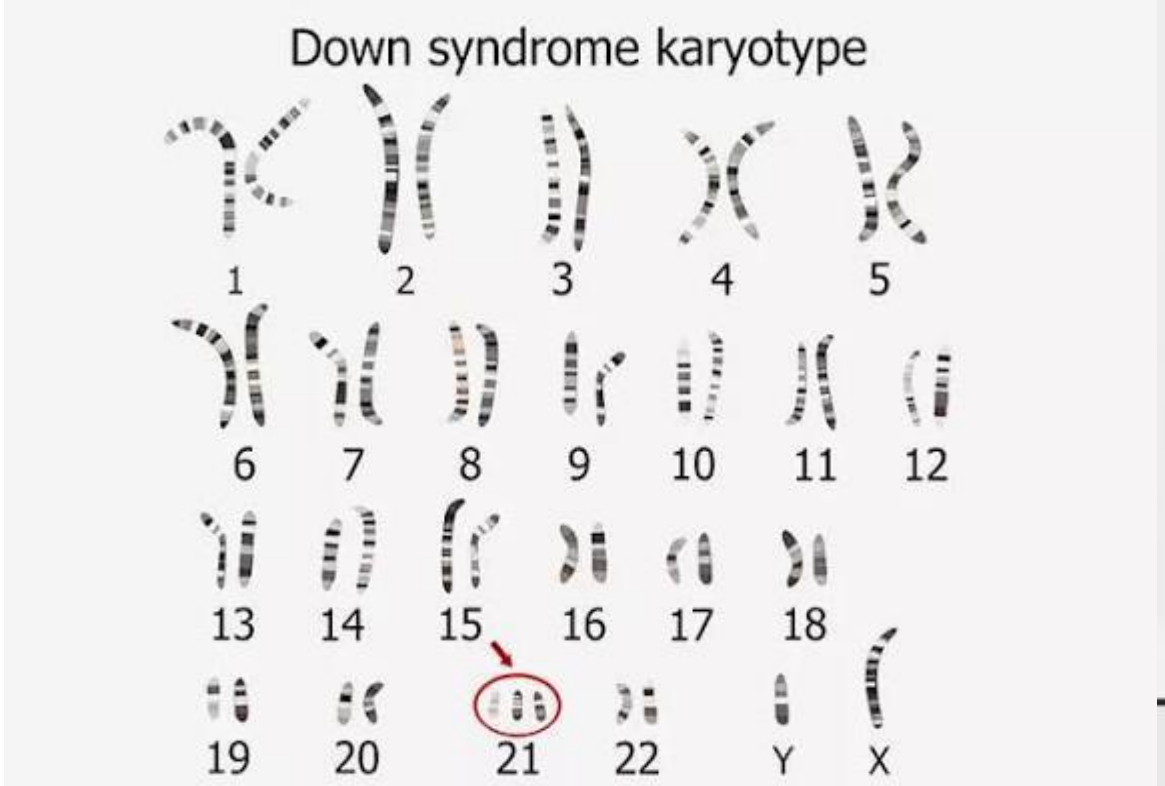
✔ **47, XY,+21**

✔ **46,XX,t(9;22)(q34;q11)**

✔ **46,XY,del(5q).**

Common Abnormal Karyotypes

Syndrome	Karyotype	Type	Clinical Features
Down Syndrome	47,XX,+21 or 47,XY,+21	Trisomy	Intellectual disability, flat face, single palmar crease
Turner Syndrome	45,X	Monosomy (sex chromosome)	Short stature, webbed neck, infertility
Klinefelter Syndrome	47,XXY	Trisomy (sex chromosome)	Tall male, gynecomastia, small testes
Patau Syndrome	47,XX,+13	Trisomy 13	Cleft palate, heart defects
Edward Syndrome	47,XX,+18	Trisomy 18	Micrognathia, overlapping fingers
Philadelphia Chromosome (CML)	t(9;22)(q34;q11)	Translocation	Chronic myeloid leukemia
Cri-du-chat Syndrome	del(5p)	Deletion	Cat-like cry, microcephaly


Down syndrome karyotype
1 2 3 4 5
6 7 8 9 10 11 12
13 14 15 16 17 18
19 20 21 22 Y X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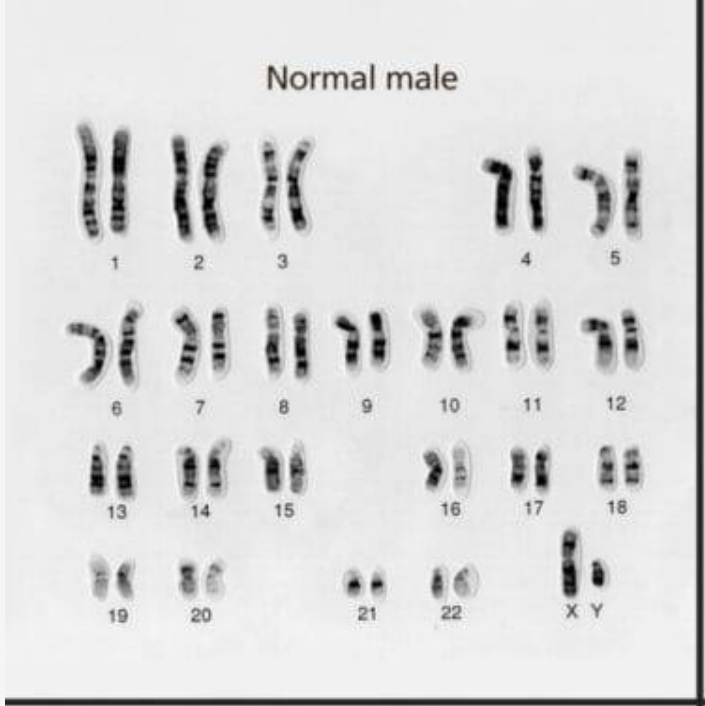
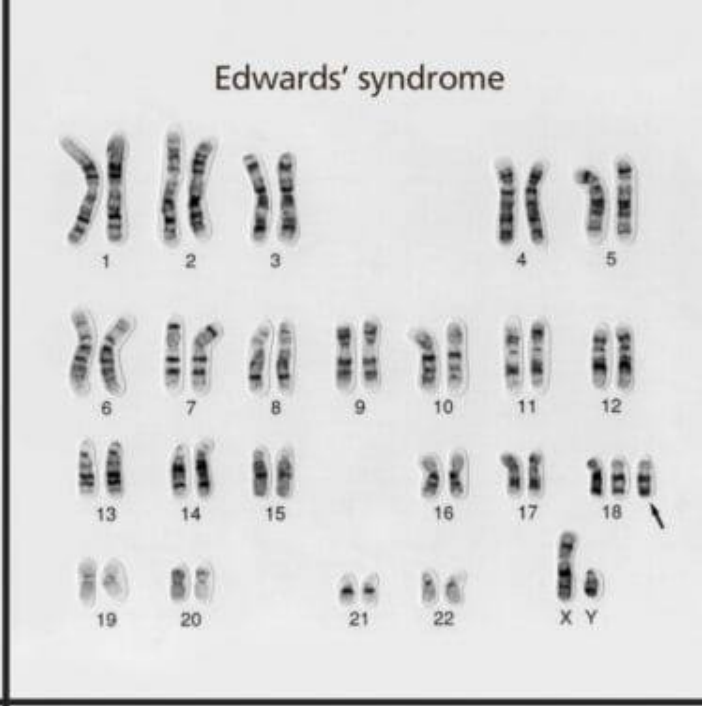
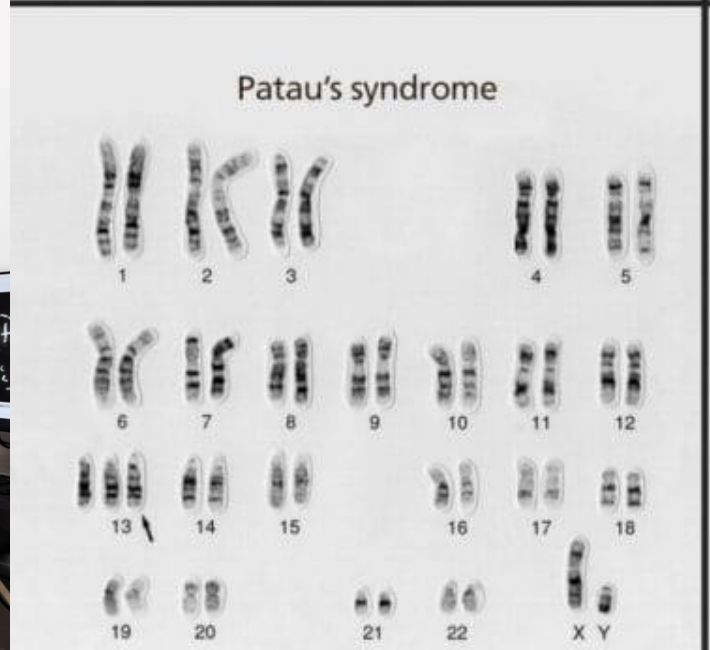
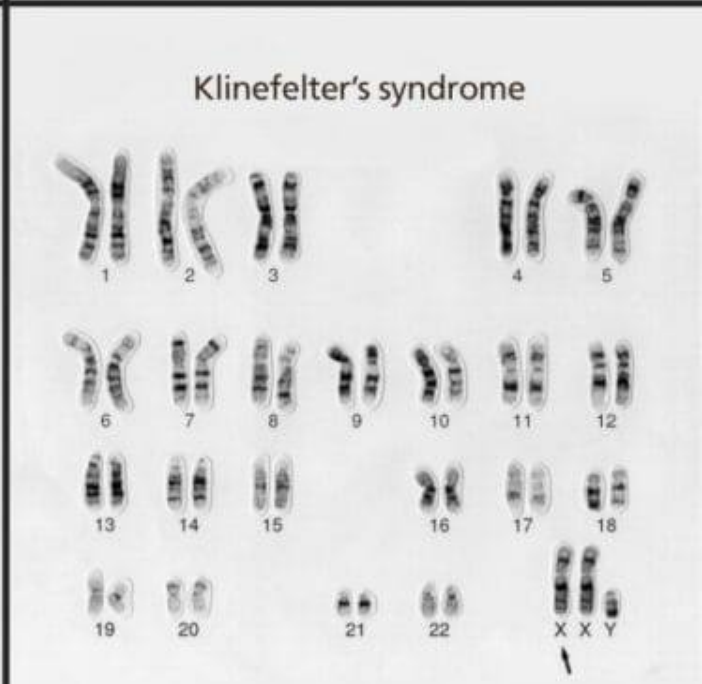

Normal male
1 2 3 4 5
6 7 8 9 10 11 12
13 14 15 16 17 18
19 20 21 22 X Y


Edwards' syndrome
1 2 3 4 5
6 7 8 9 10 11 12
13 14 15 16 17 18
19 20 21 22 X Y


Patau's syndrome
1 2 3 4 5
6 7 8 9 10 11 12
13 14 15 16 17 18
19 20 21 22 X Y


Klinefelter's syndrome
1 2 3 4 5
6 7 8 9 10 11 12
13 14 15 16 17 18
19 20 21 22 X X Y

ISCN Nomenclature

QUIZ

Q1/ What karyotype would you expect for a male with Down syndrome?

Q2/ If the karyotype shows 45, X, what is your diagnosis?

Any questions?