

Evolution of Omics and its Clinical Applications

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<http://www.ncdsgenomics.org>

Gregor Johann Mendel theory of inheritance, 1866 - pea plants

**More on the discovery of DNA;
1900, 1920-1950, 1953, 1958**

DNA proofed to be the hereditary material - Griffith, Avery, Hershey, and Chase. Watson and Crick - DNA structure. Meselson and Stahl - Genetic coding

Molecular Technology - 1970 -1986

Arber and Smith - restriction enzymes
DNA seq. and Genomic seq. of E.coli by Frederick Sanger. Mullis PCR techn. and automated by Hood et al. Brown Stanford microarray Technology

Bacterial Genome - Celera, 1995
Yeast Consortium Genome - 1996
Arabidopsis thaliana Initiative - 2000
Human Genome published - 2001
(100 trillion cells & 3.1 billion NUs.)

The Genomics Revolution

How did it begin?

- DNA structure and the genetic code

1950's



Basic Sciences

- Recombinant DNA technology
- Polymerase Chain Reaction
- Automated DNA sequencing

1970's/1980's



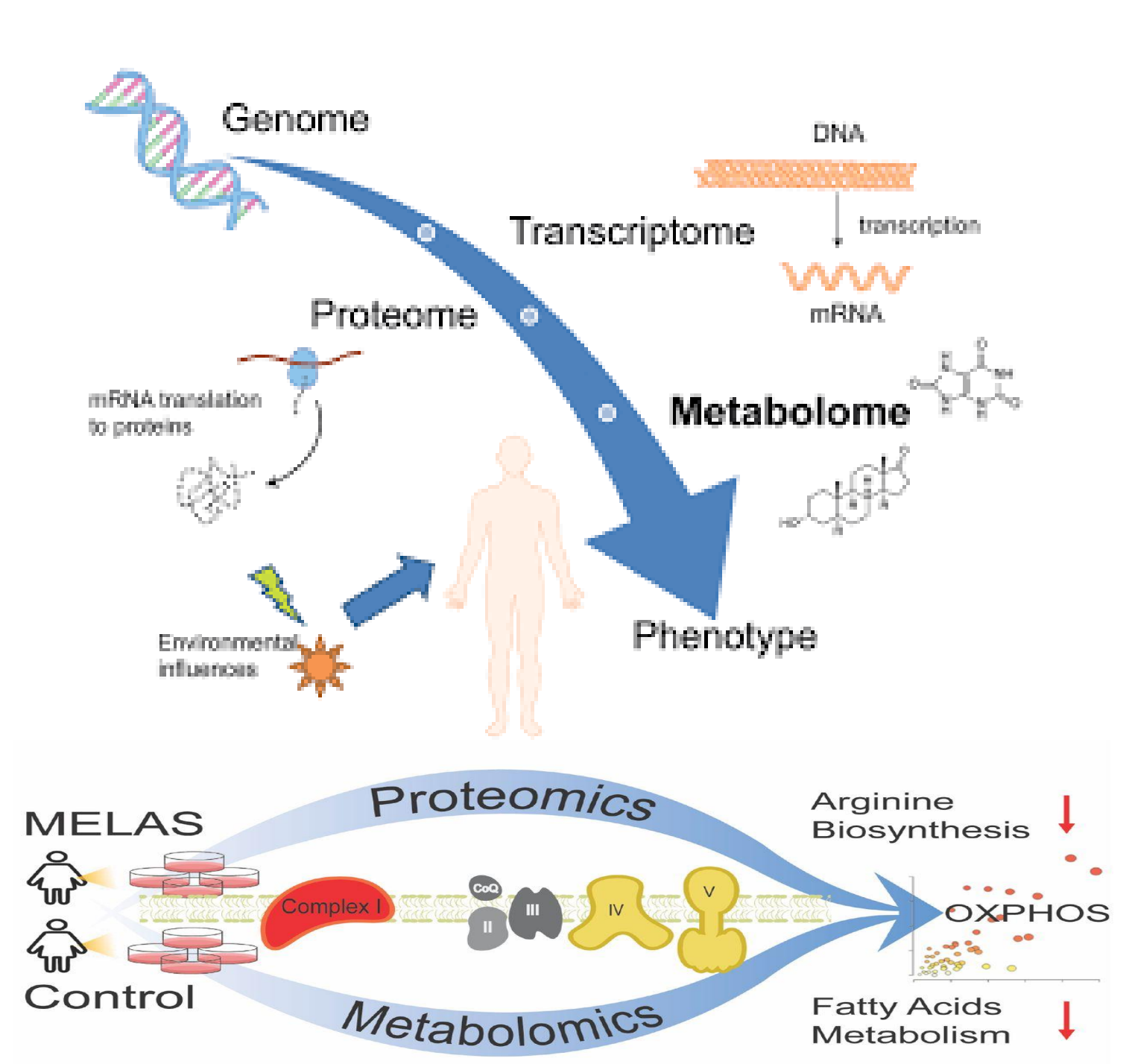
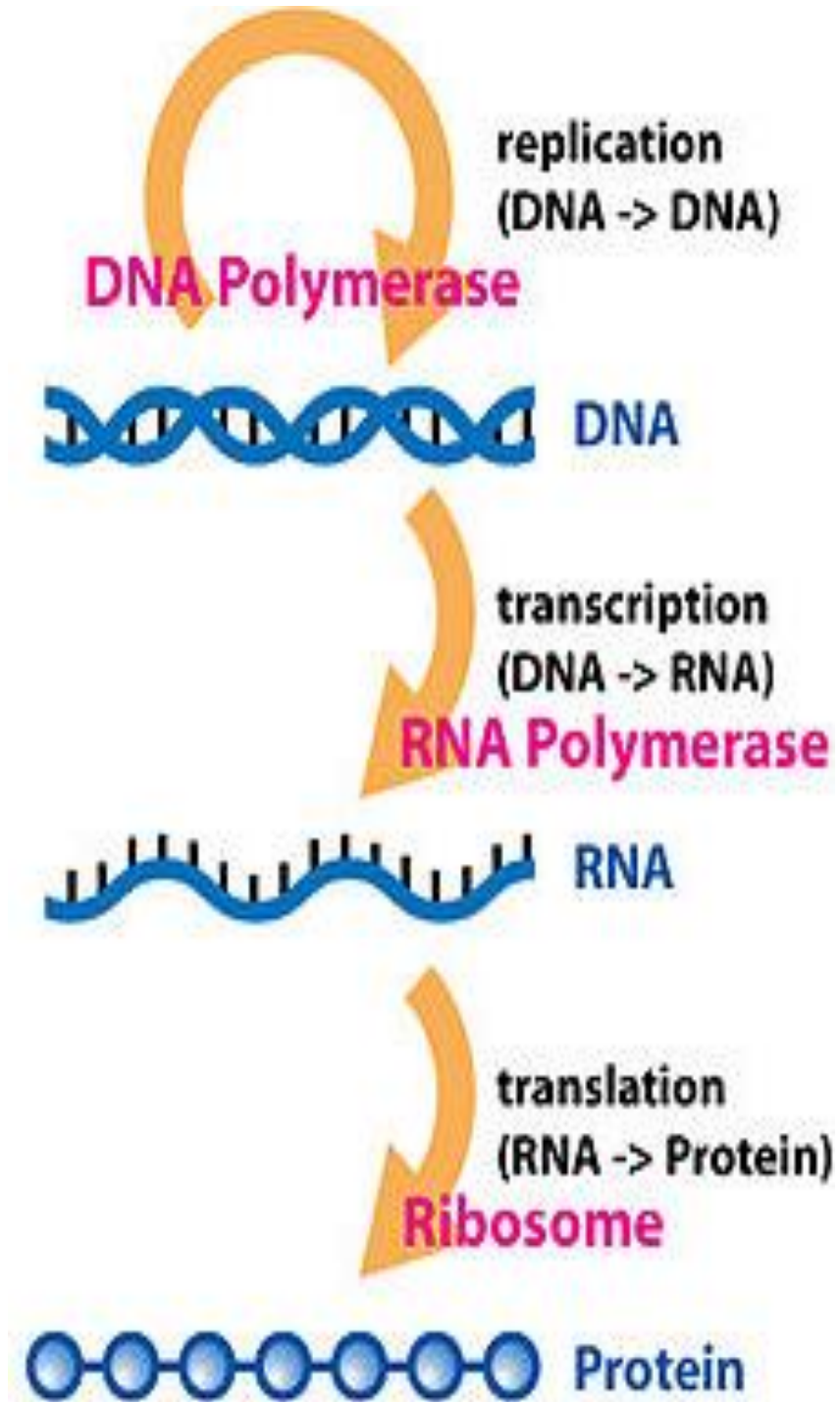
Technology

- The Human Genome Project

1990's

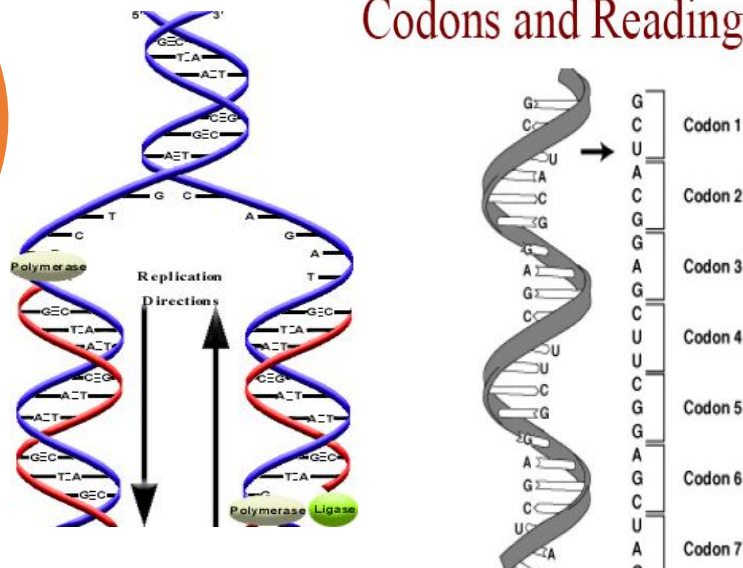


Applications



Basic Molecular Biology - Genomics and Proteomics (Functional Annotation)

Codons and Reading Frames

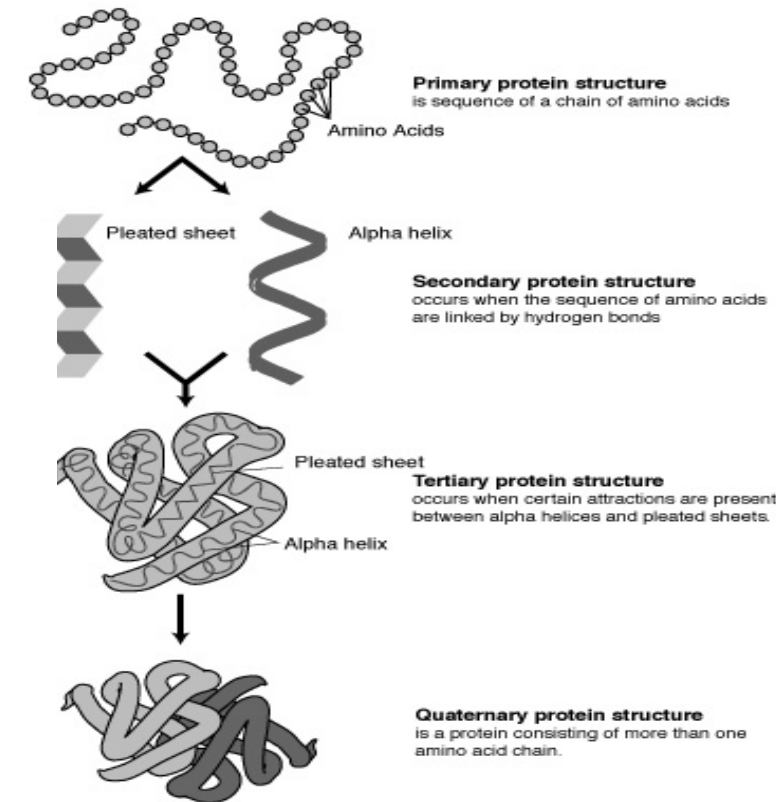


The Genetic Code

		Second letter				
		U	C	A	G	
First letter	U	UUU Phenylalanine UUC UUA Leucine UUG	UCU Serine UCC UCA UCG	UAU Tyrosine UAC UAA Stop codon UAG Stop codon	UGU Cysteine UGC UGA Stop codon UGG Tryptophan	U C A G
	C	CUU Leucine CUC CUA CUG	CCU Proline CCC CCA CCG	CAU Histidine CAC CAA Glutamine CAG	CGU Arginine CGC CGA CGG	U C A G
	A	AUU Isoleucine AUC AUA Methionine, initiation codon AUG	ACU Threonine ACC ACA ACG	AAU Asparagine AAC AAA Lysine AAG	AGU Serine AGC AGA Arginine AGG	U C A G
	G	GUU Valine GUC GUA GUG	GCU Alanine GCC GCA GCG	GAU Aspartic acid GAC GAA Glutamic acid GAG	GGU Glycine GGC GGA GGG	U C A G

Protein Functions

- structural support
- storage of amino acids
- transport of other substances
- coordination of an organism's activities
- response of cell to chemical stimuli
- movement
- protection against disease
- selective acceleration of chemical reactions



Summary

Cell – Prokaryotic and Eukaryotic cells

Nucleus – Functional unit of cell

Chromosomes – 22 pair somatic and 1 pair of sex

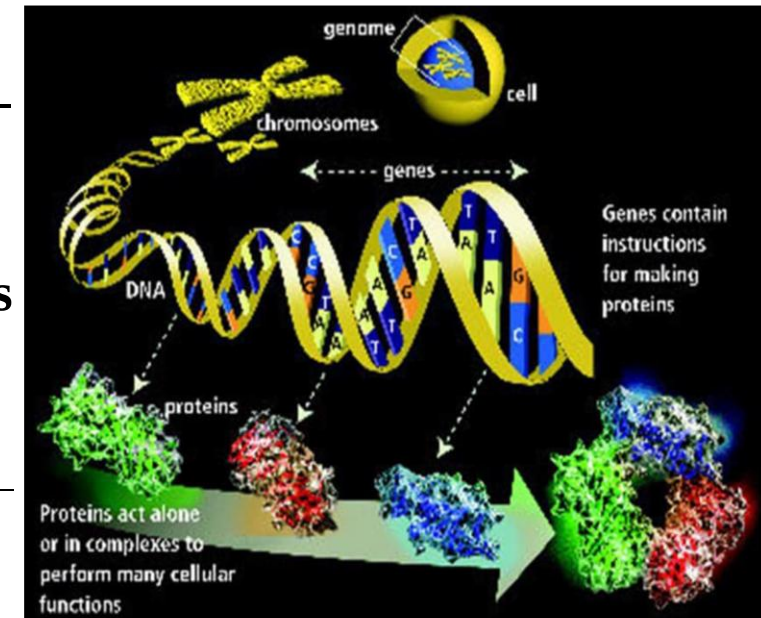
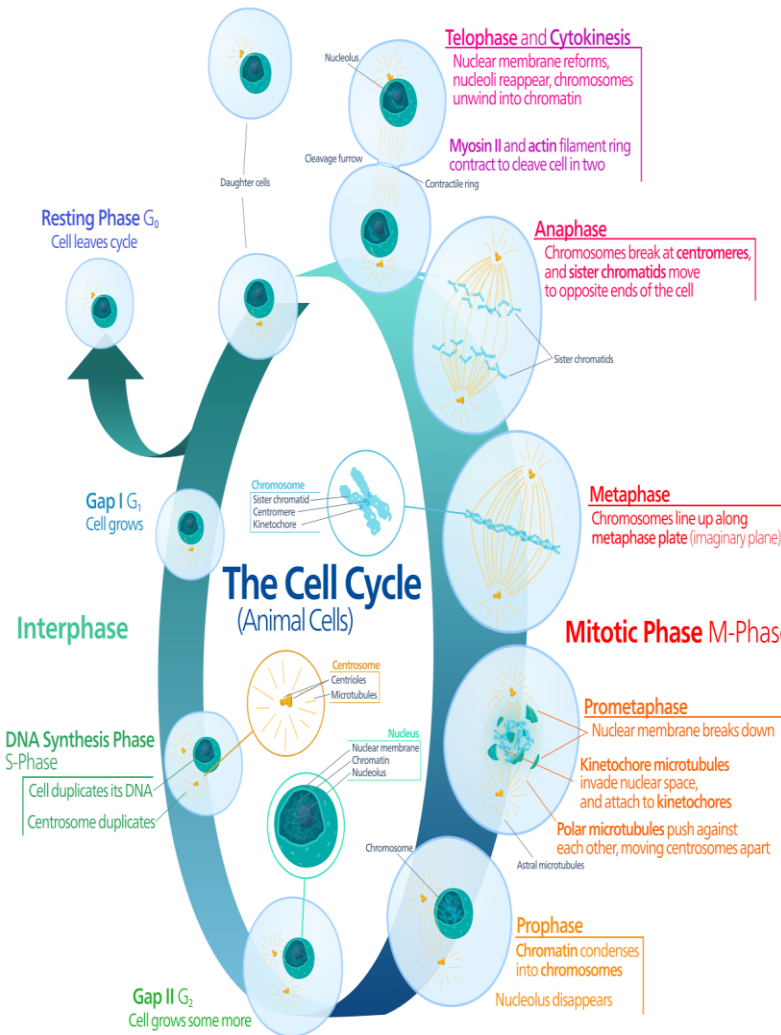
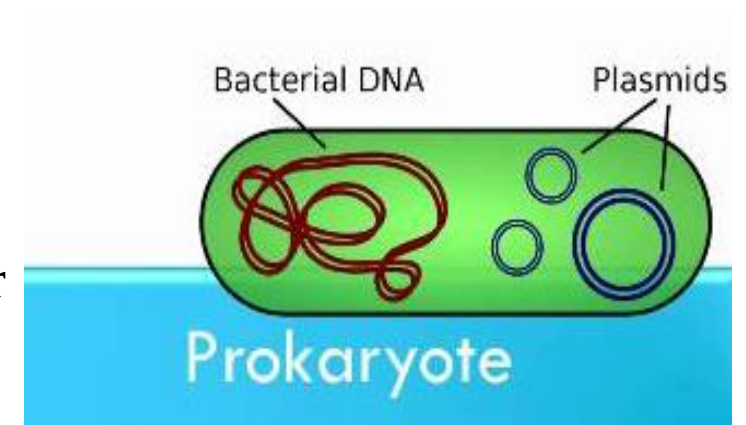
Gene – Functional unit of DNA sequences – intron and Extron

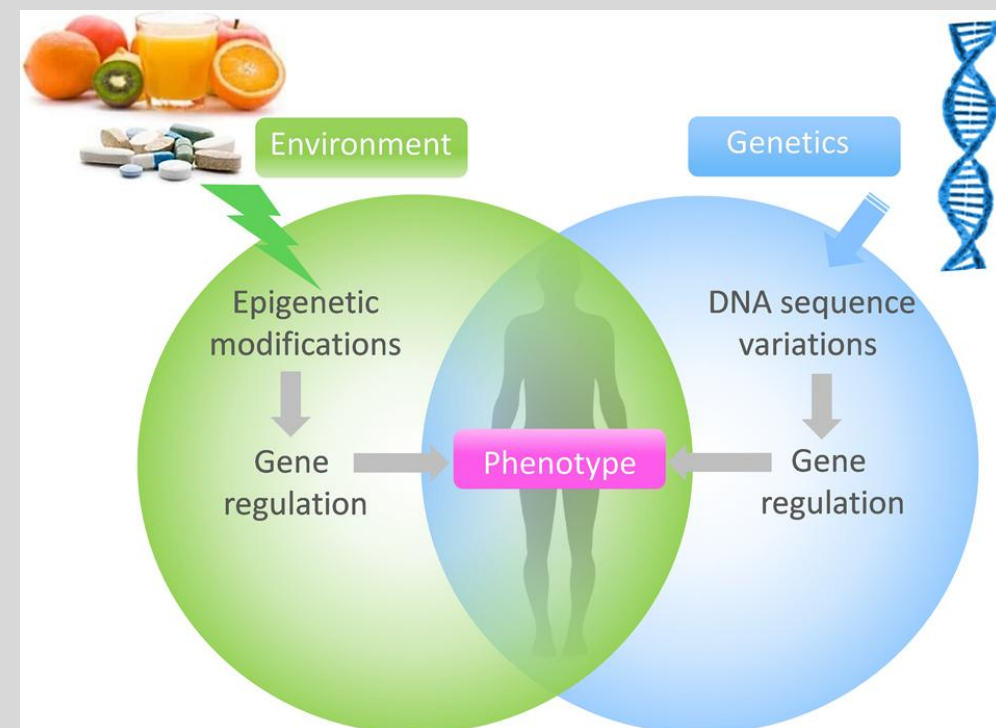
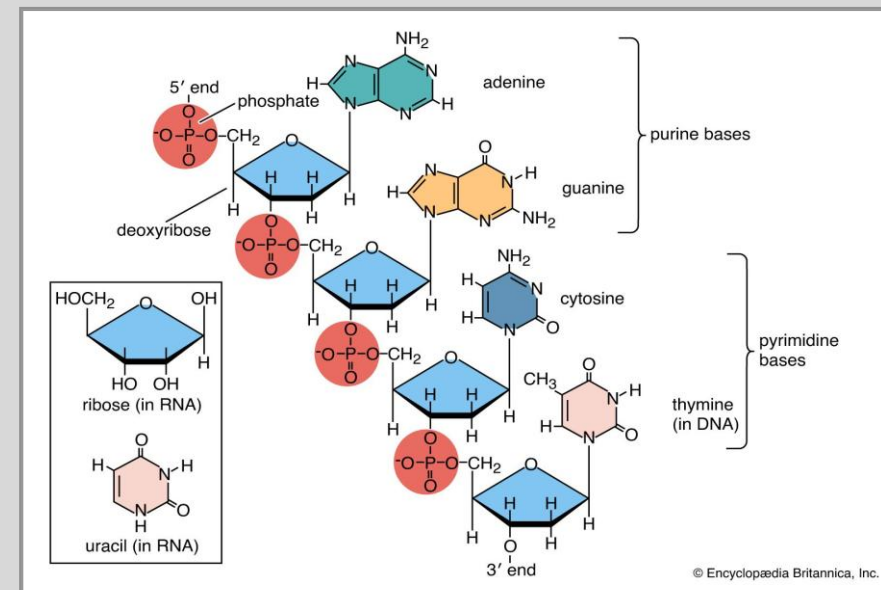
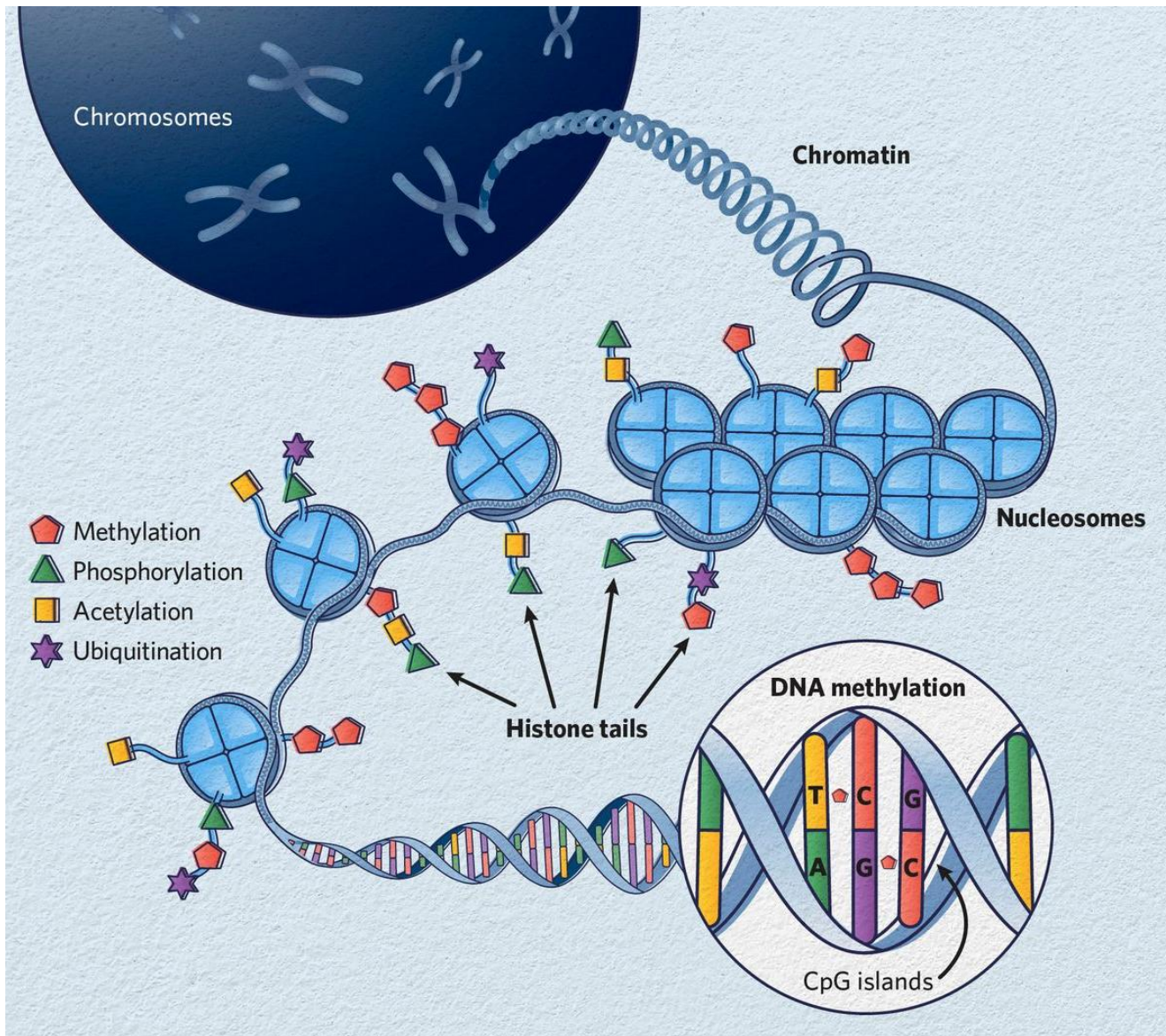
Nucleotides (DNA and RNA) sequences - **Genomics**

Transcription (mRNA) - **Transcriptomics**
and Translation to Protein – **Proteomics**

Structural and Functional Annotations –
Metabolism & Metabolites - **Metabolomics**

Health and Diseases – Surveillance,
Epidemiology, Public Health.





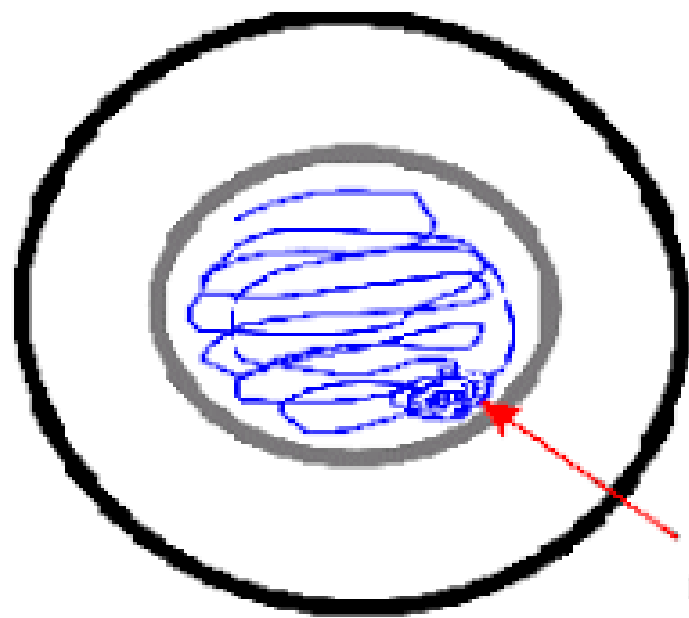
Molecular Technology

- ✓ Advance in molecular technology allow development of thorough put methods for clinical application
- Cytogenesis - Chromosomal pattern and modes of inheritance
- Genomics - DNA sequence and genetic expression
- Proteomics - AAs sequence and functional notation of the genes
- Metabolomics - Metabolites and end products of macromolecules metabolism
- ✓ An amazing understanding of the fate of cells in health and diseases

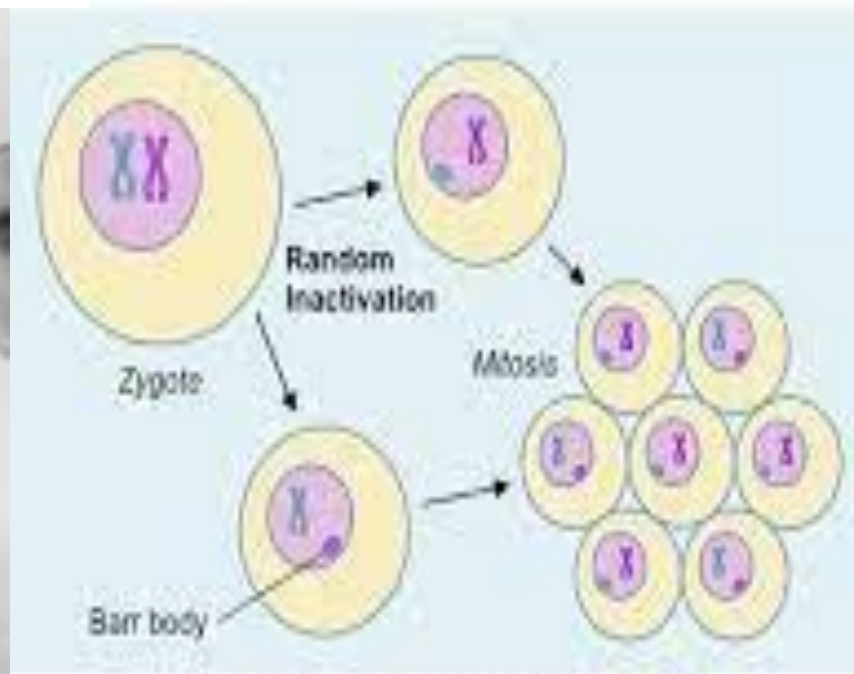
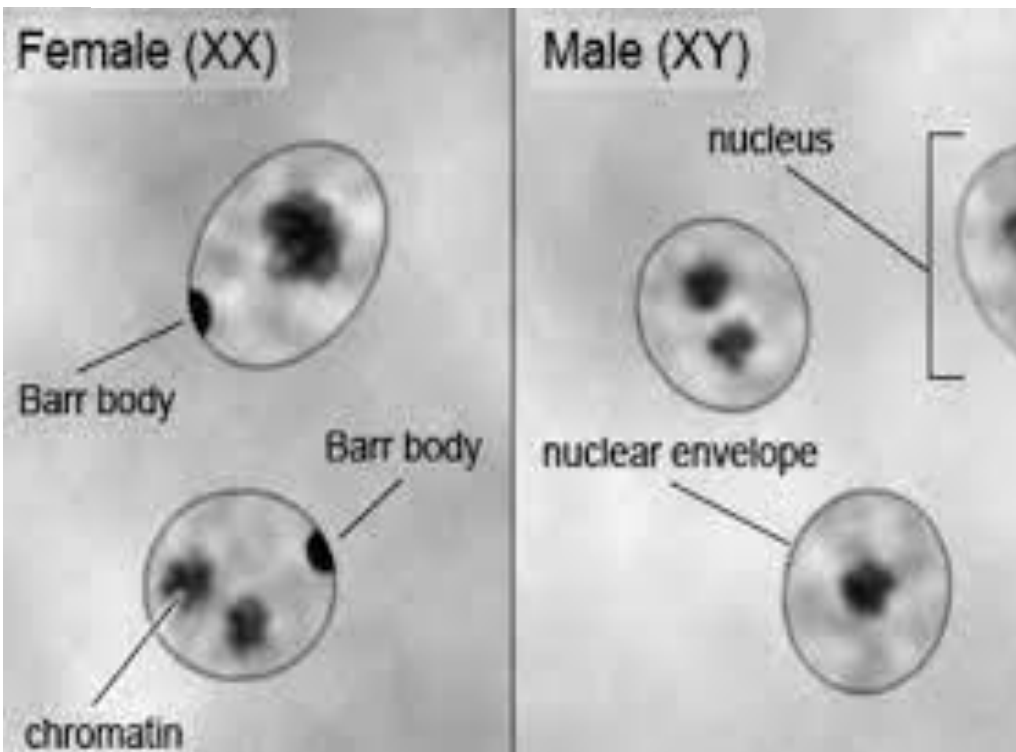
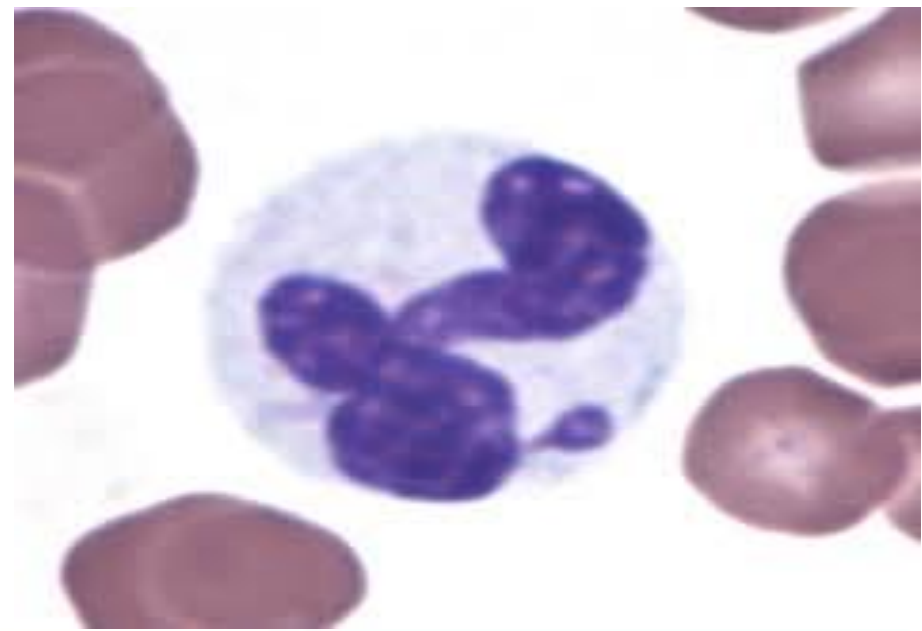
Techniques and Methodology in Genetics - Cytogenesis

- ☐ Chromosomal Pattern – Numbers and Structures: Electron microscopy
- ☐ Conventional Staining – Modified Giemsa Stain.
- ☐ Immunohistochemistry – Fluorescence Staining: Advance Microscopy.
- ☐ Single and Life Cells Imaging Techniques – 3D scanning microscopy

Gender Identification



Barr Body
(Inactive X)



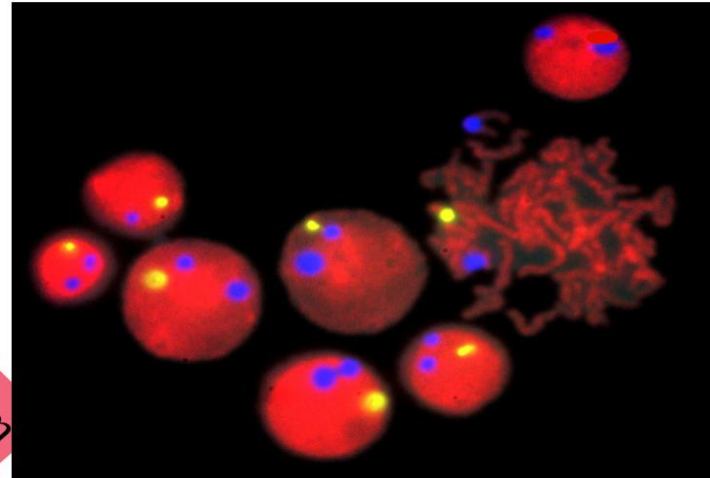
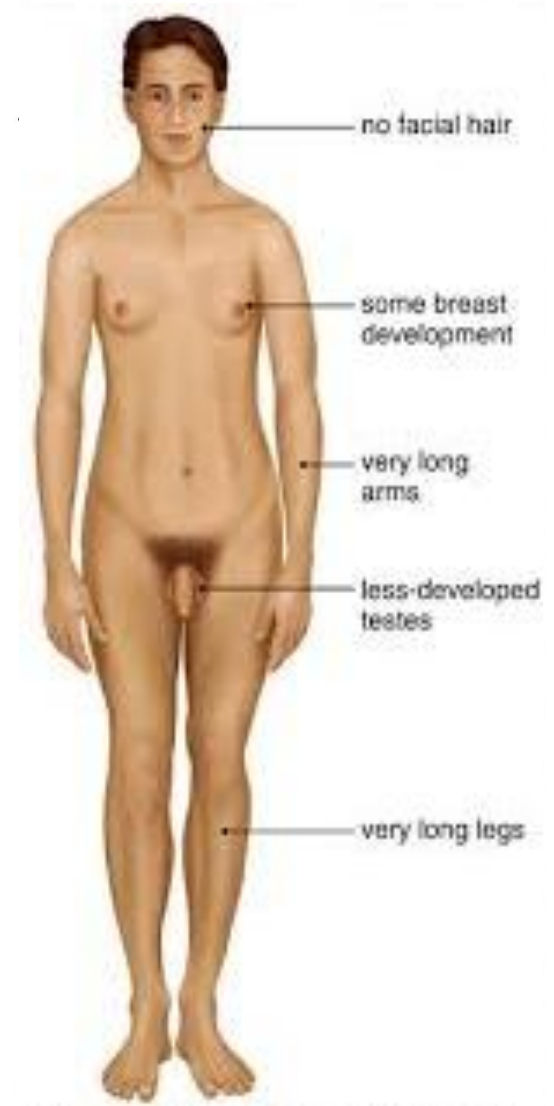
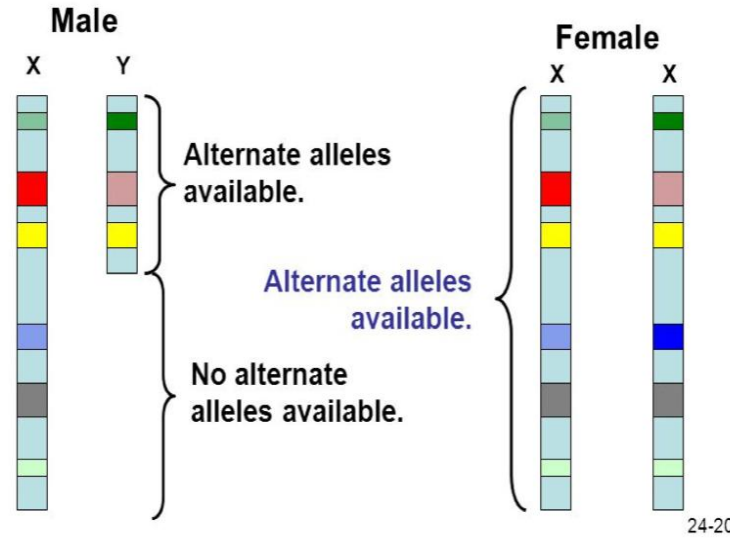
Summary of the Process of X-Inactivation



Barr Body in a Human Nucleus

Molecular Diagnosis - Cytogenesis

Comparison of the sex chromosomes



FISH - Y = yellow, X = blue Klinefelter Syndrome (47,XXY)

1.67% KS in Nigeria infertile males.
Compared to 5% reported in Europe.
Akinloye et al, 2011

KS - hypergonadotropic hypogonadism
spermatogenic failure usually with NOA.
Gynecomastia, poorly developed small
testis and penis, facial uniqueness and
lack of body hair - NG ????

Karyotyping and Phenotyping

❑ Chromosomal Aberrations:

❑ Two major categories:

- ❖ Constitutional
- ❖ Acquired

- Trisomy 21
- Klinefelter's syndrome
- Turner's syndrome
- Edward Syndrome
- DiGeorge Syndrome
- XYY

A) Constitutional aberrations:

- ❖ A cytogenetic abnormality which occurs from birth.
- ❖ Include *aneuploidy* (extra or missing chromosomes) and *structural aberrations* (deletions, duplications, translocations, inversions, marker chromosomes).

B) Acquired aberrations:

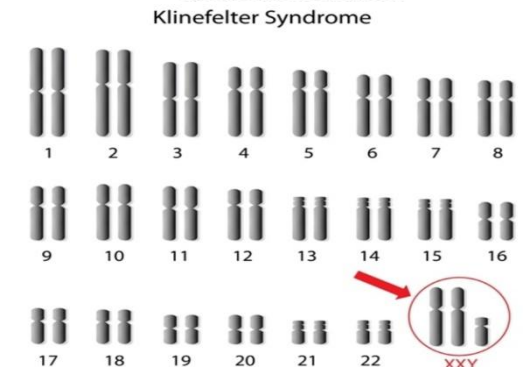
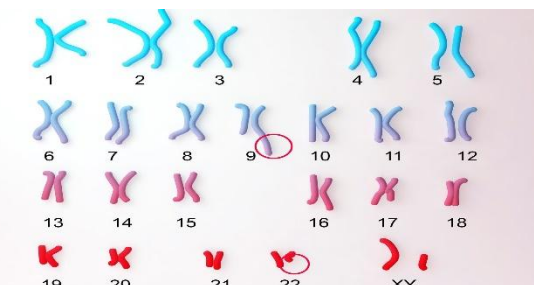
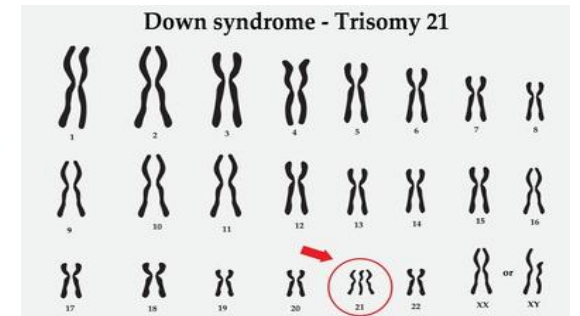
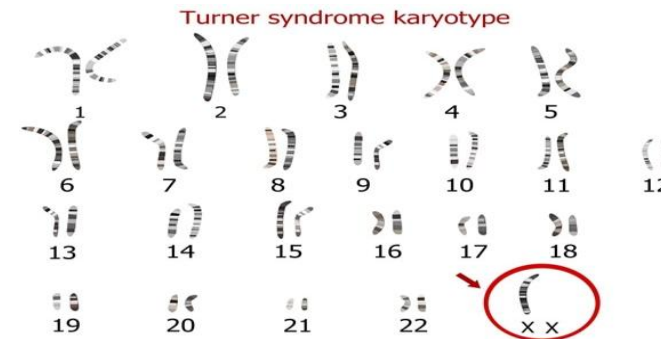
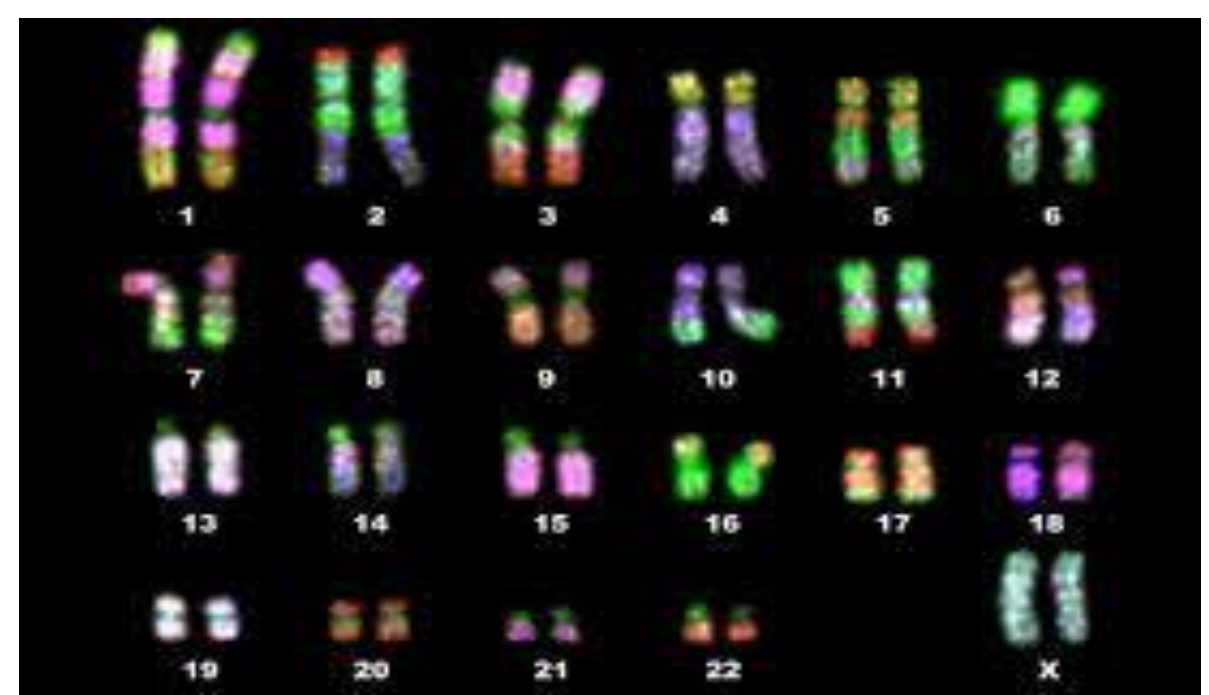
An *acquired* cytogenetic abnormality is a genetic change associated with a neoplastic or cancer disease process.

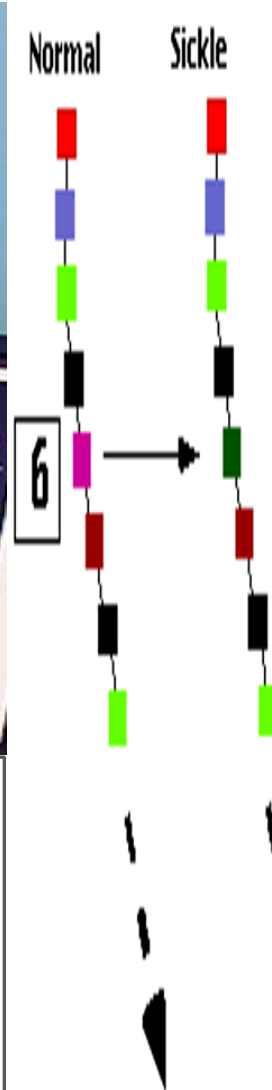
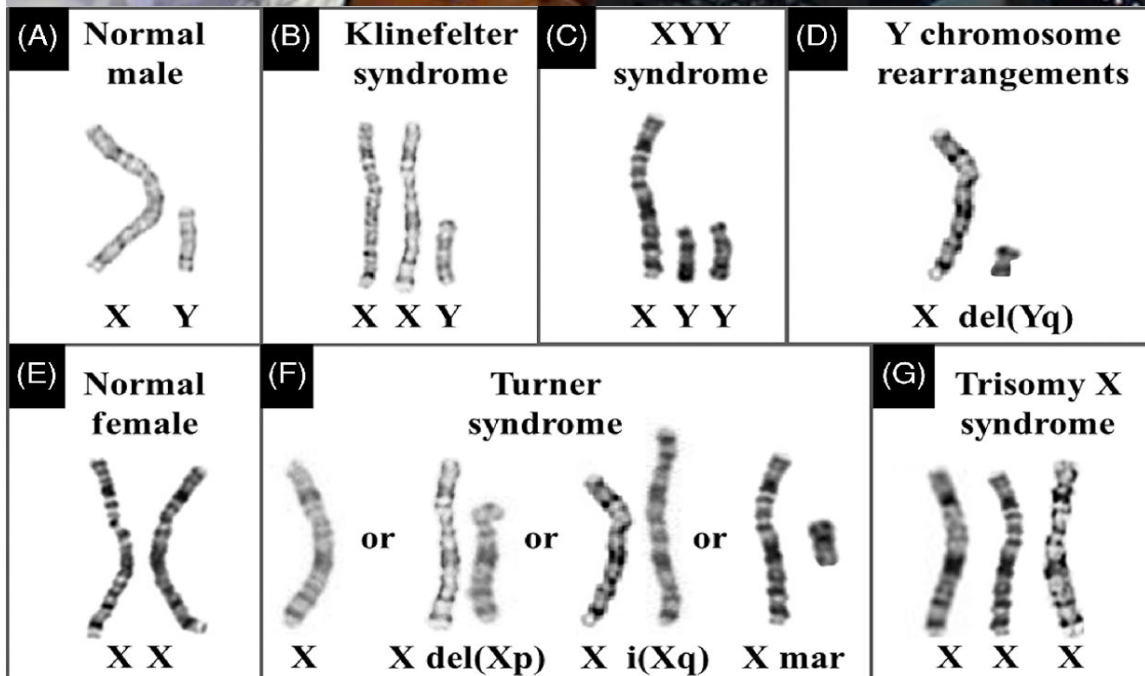
Example:

- Philadelphia Chromosome

- Inversion

Examples:

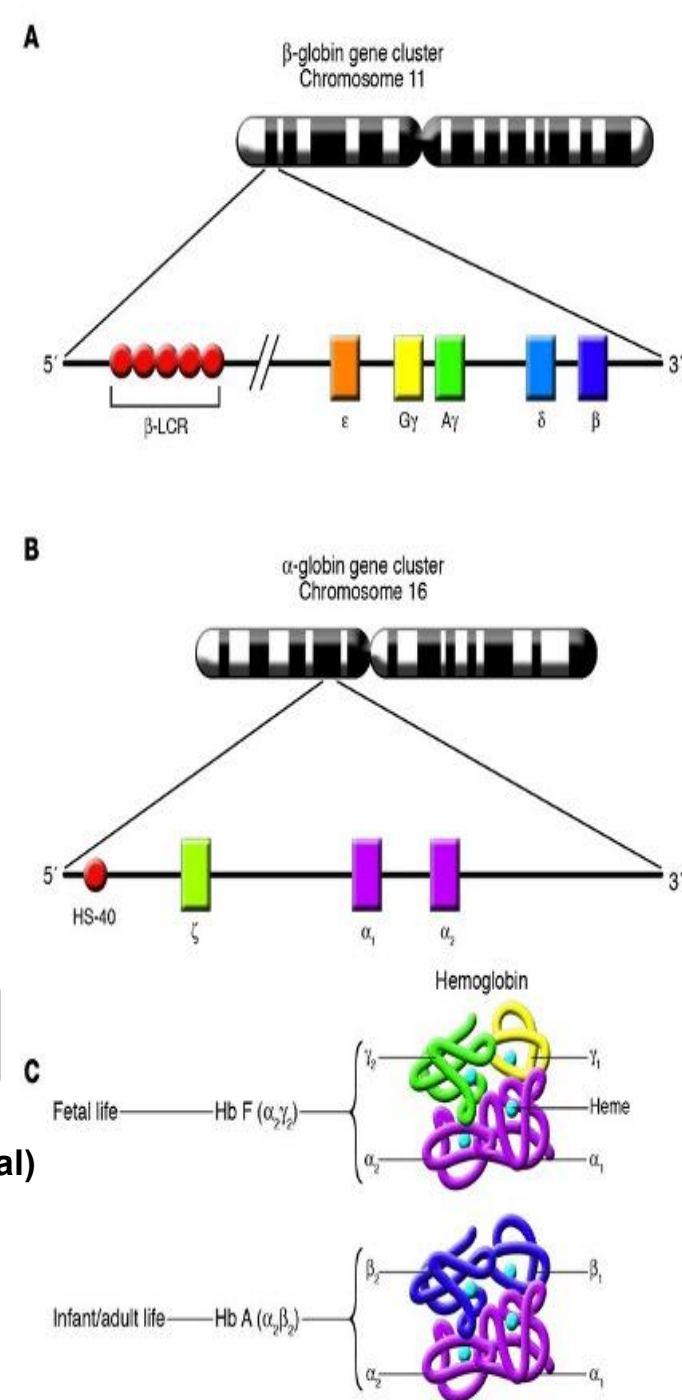




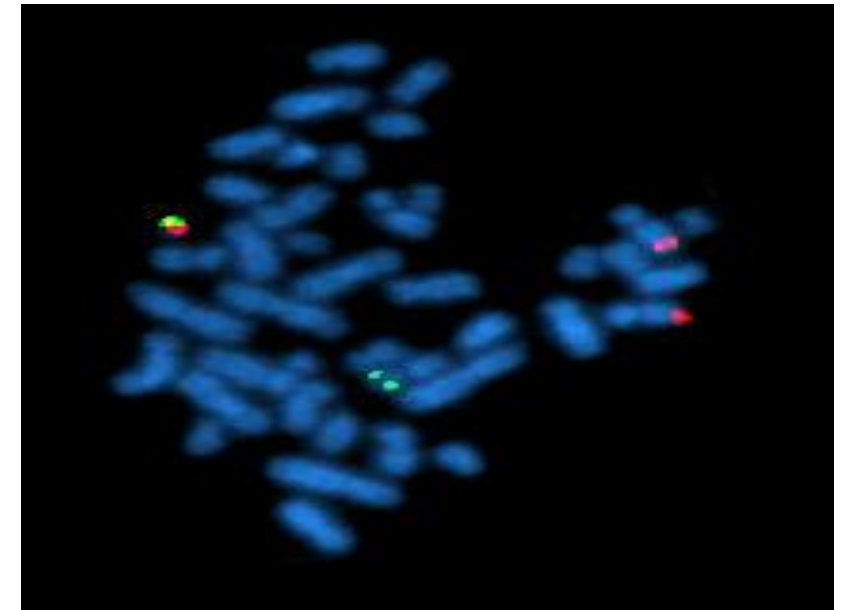
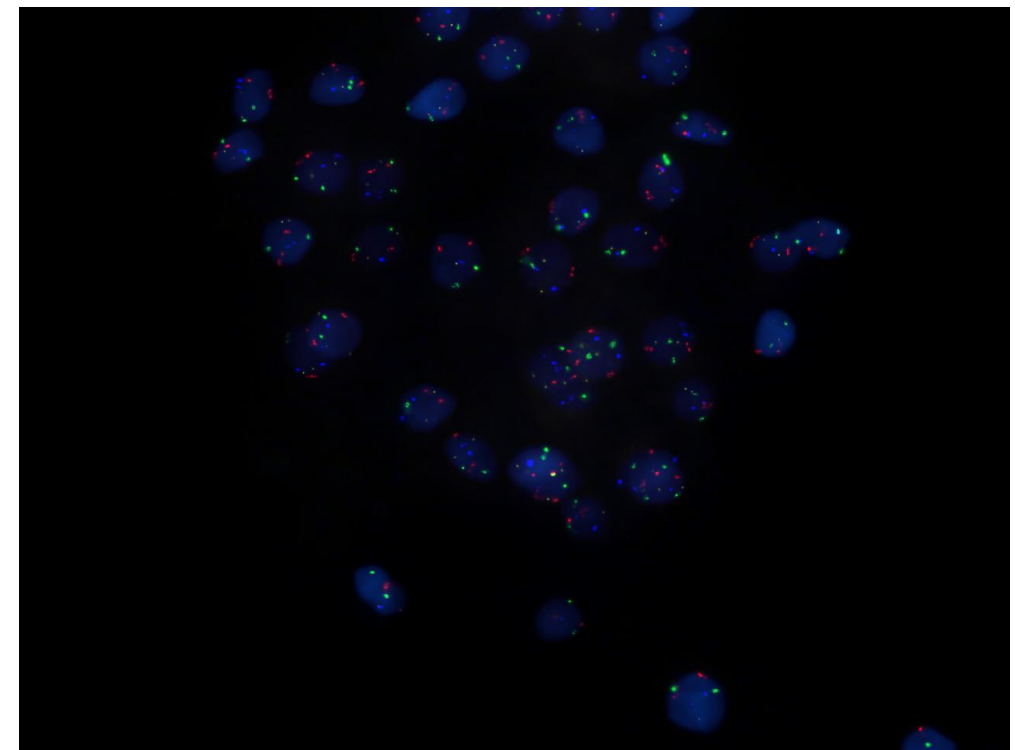
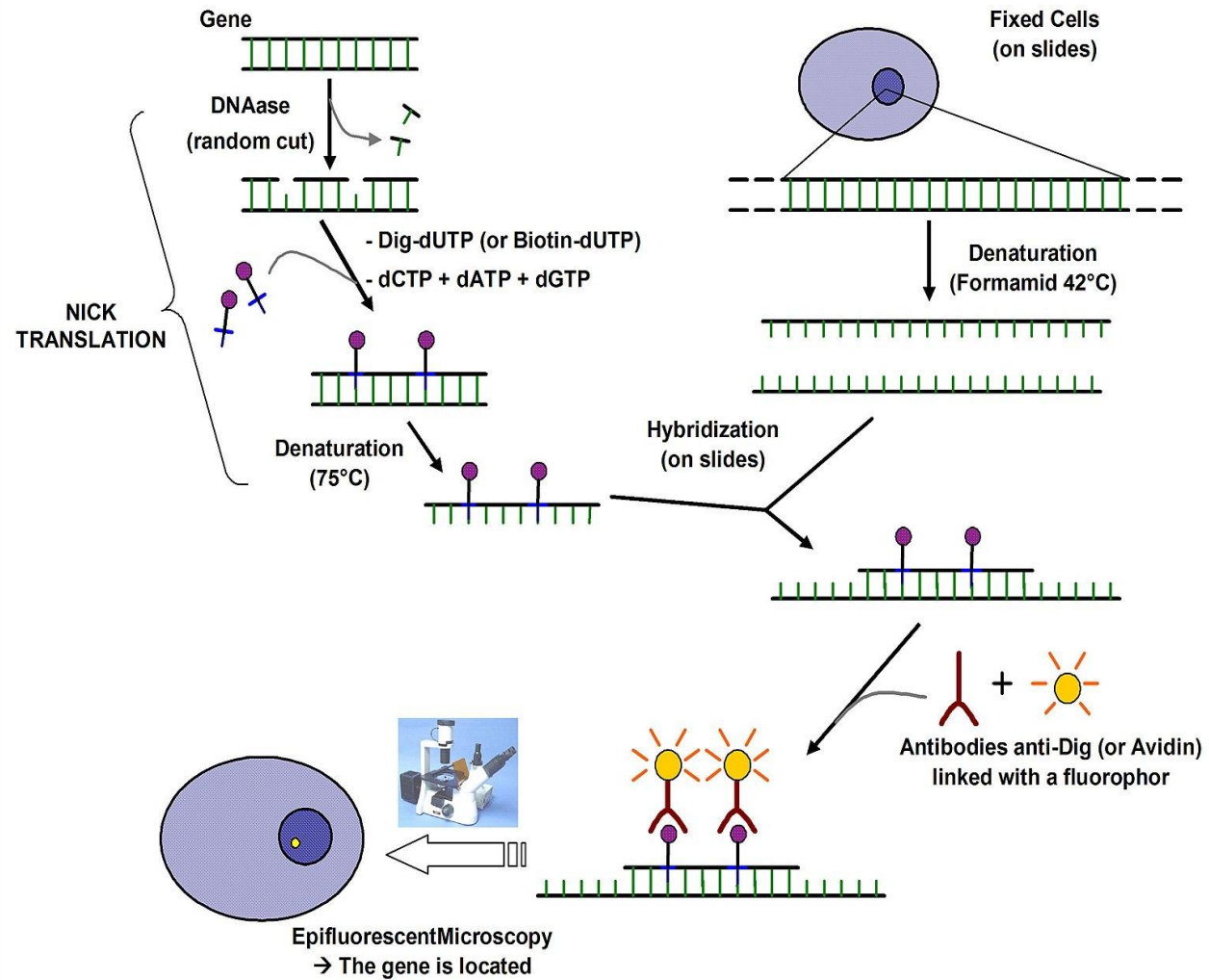
($\beta 6(A3)Glu \rightarrow Val$)

[GAA] = [GTA]

(A = T $\rightarrow$ A = T)



FISH (Fluorescent In Situ Hybridization)

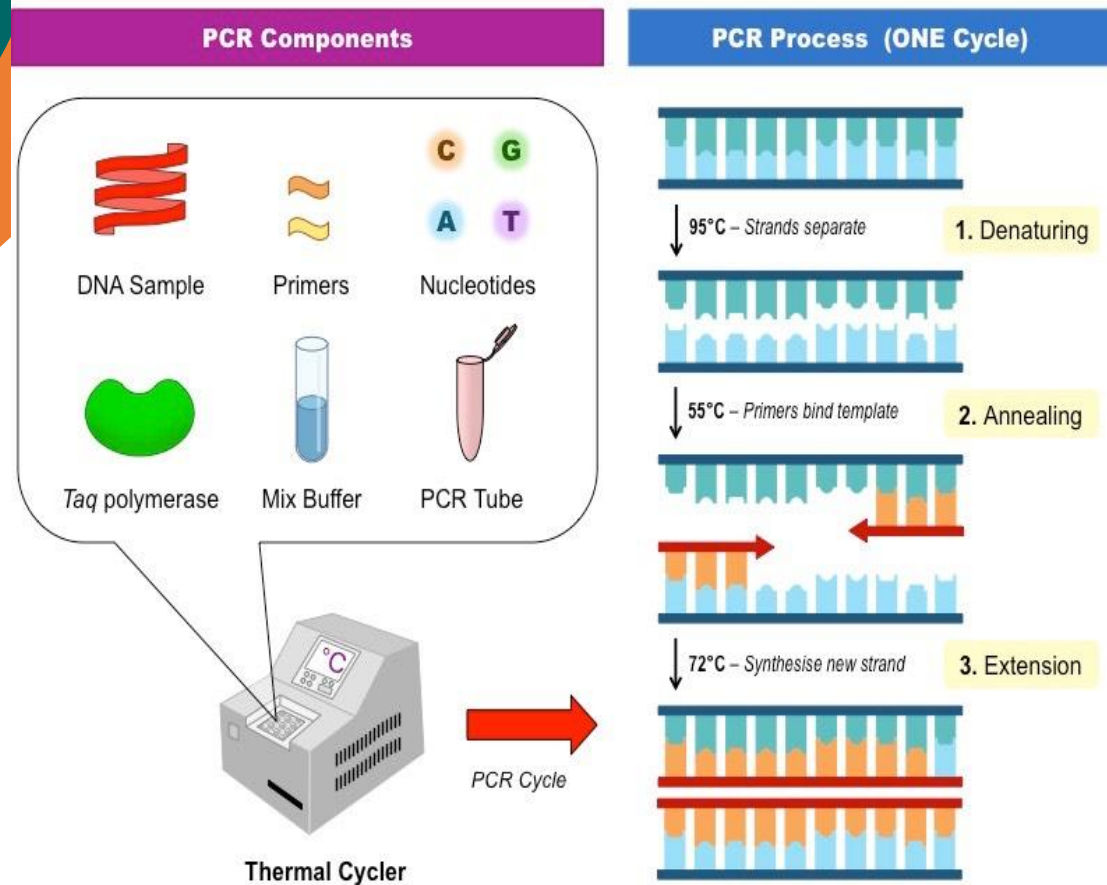


Techniques and Methodology in Genomics

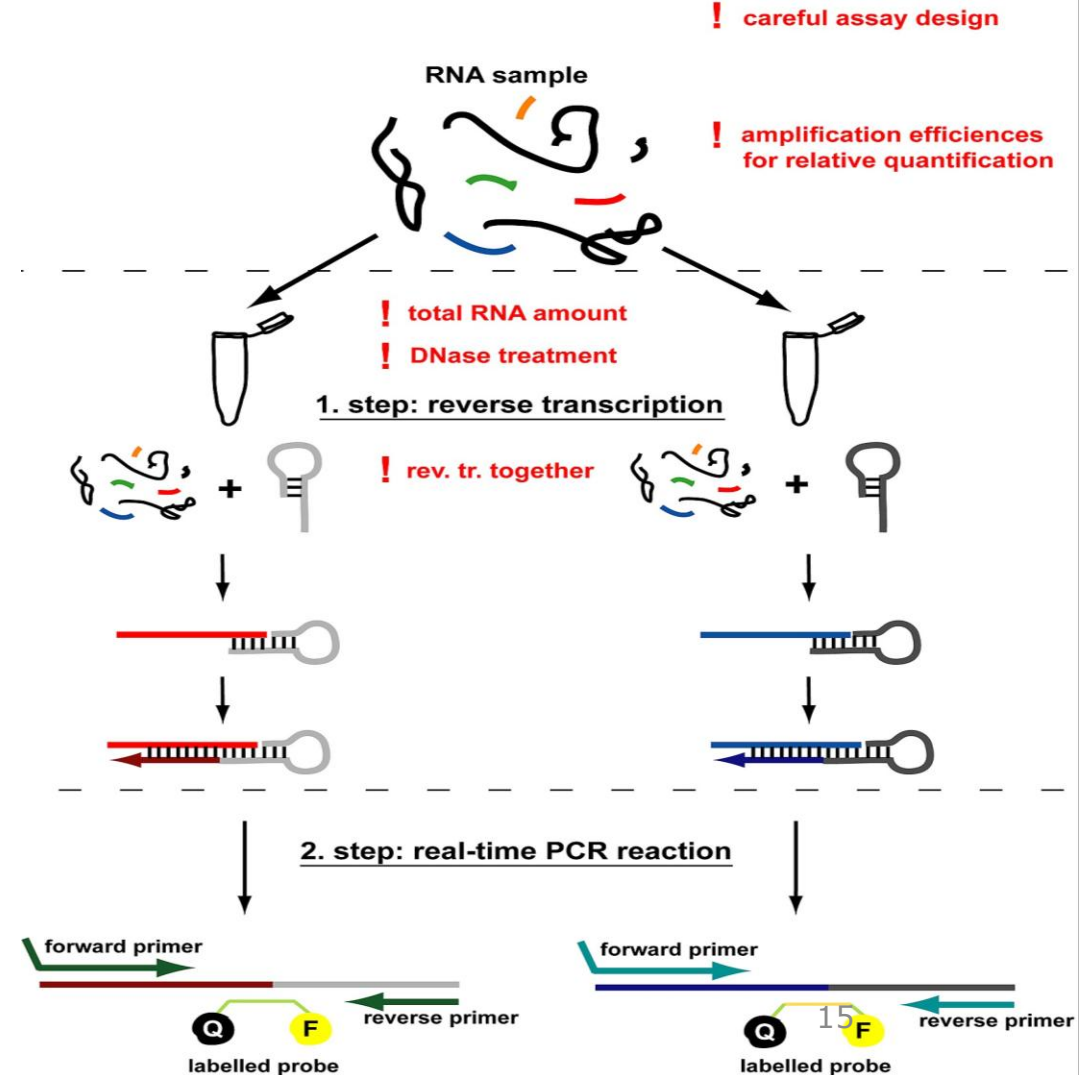
- ❑ Nano Imaging Technology – Live examination of DNA and cell divisions :high resolution microscopy
- ❑ Gene Amplification – Thermocycler – First Generation PCR
- ❑ Gene Expression – RT-PCR and dPCR
- ❑ Gene Expression – Immunofluorescence
- ❑ Nucleotides Sequencing (Sanger, NGS, GWAS) – Genomics or Exons

GENE AMPLIFICATION, DETECTION & EXPRESION

Polymerase Chain Reaction (PCR)



Real Time PCR - Polymerase Chain Reaction (PCR)

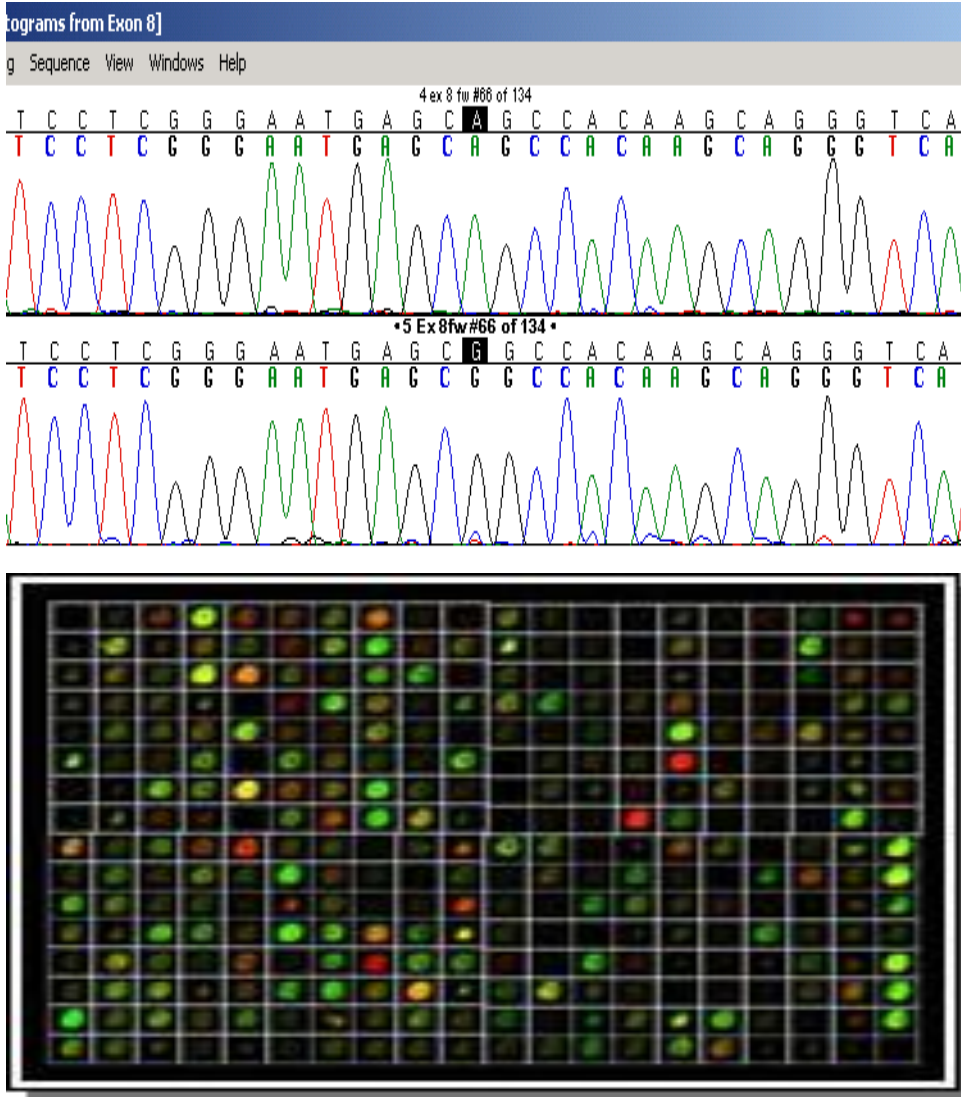


Use of PCR, RT-PCR and dPCR Technology

- **Application of Gene (DNA/RNA) allow Analysis:**
 - Mutation, polymorphism etc.
- **Identification of DNA/RNA sequence - detection**
 - HBV/HCV, SARS-COV 2 etc
- **Observation of Gene expression -up/down regulation**
 - P53 tumour suppression gene in cancer
- **Development of biomarkers for diagnosis & prognosis**
 - BRCA1 and BRCA2 mutation
- **Identification of candidate gene for drug target**
 - Dopamine D2R Gene for Schizophrenia
- **Variant tracing for diseases spread - public health/epid.**
 - SARS COV 2 - Delta, Omicron, etc
- **Management of Diseases and Therapeutic Monitoring**
 - AR-gene polymorphism and A replacement



Advance Genomics Methods

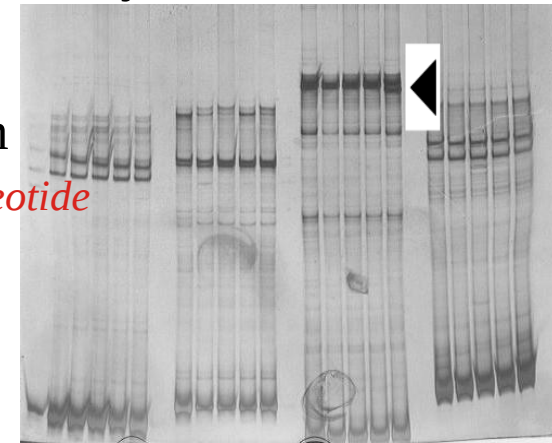


- Sequencing -*Detecting and monitoring changes in Genomic DNA -Sanger/NGS/GWAS*

- Genetic mutation
- Gene deletions
- Slicing and Hybridization

- Polymorphism

Repetition in nucleotide arrangement



- Microarray-*Analysis of gene expression*

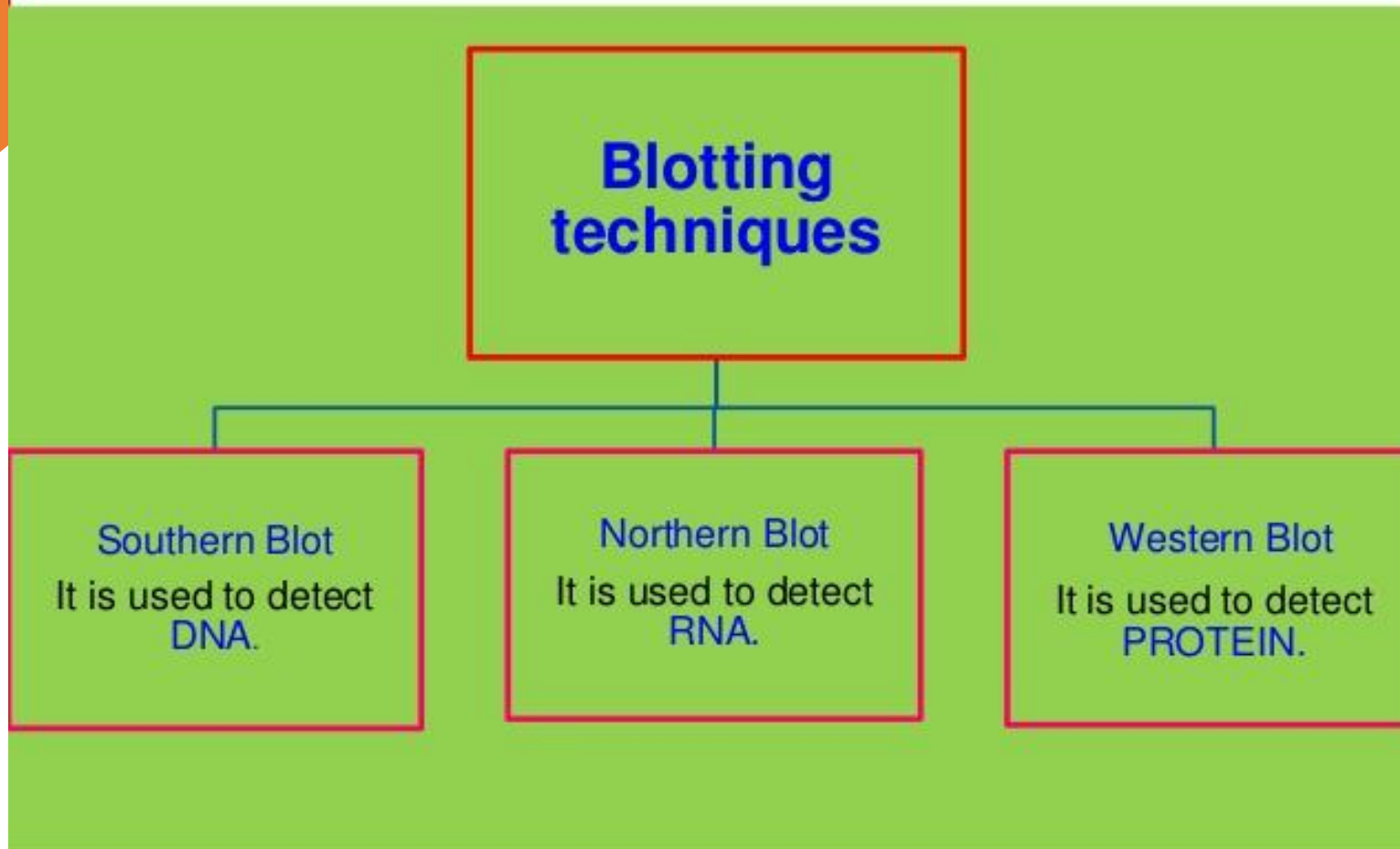
- Comparative Genomics
- Evaluation of Gene Expression (up/down regulation of genes)

Techniques and Methodology in Proteomics

- ❑ Blotting Technology – Western, Southern and Northern Blotting
- ❑ Evaluation of Proteins – ELISA and Immunochemistry
- ❑ Advance Microscopy
- ❑ Gene Expression – Immunofluorescence
- ❑ Advance Proteiomics

Basic Genomics and Proteomics Methods

Types of blotting techniques



Blotting is the **molecular biology technique** that allows transfer of products of a PCR reaction from a gel strip onto a matrix or specialized chemically reactive paper (cellulose or xray film) for further separation and/or studies.

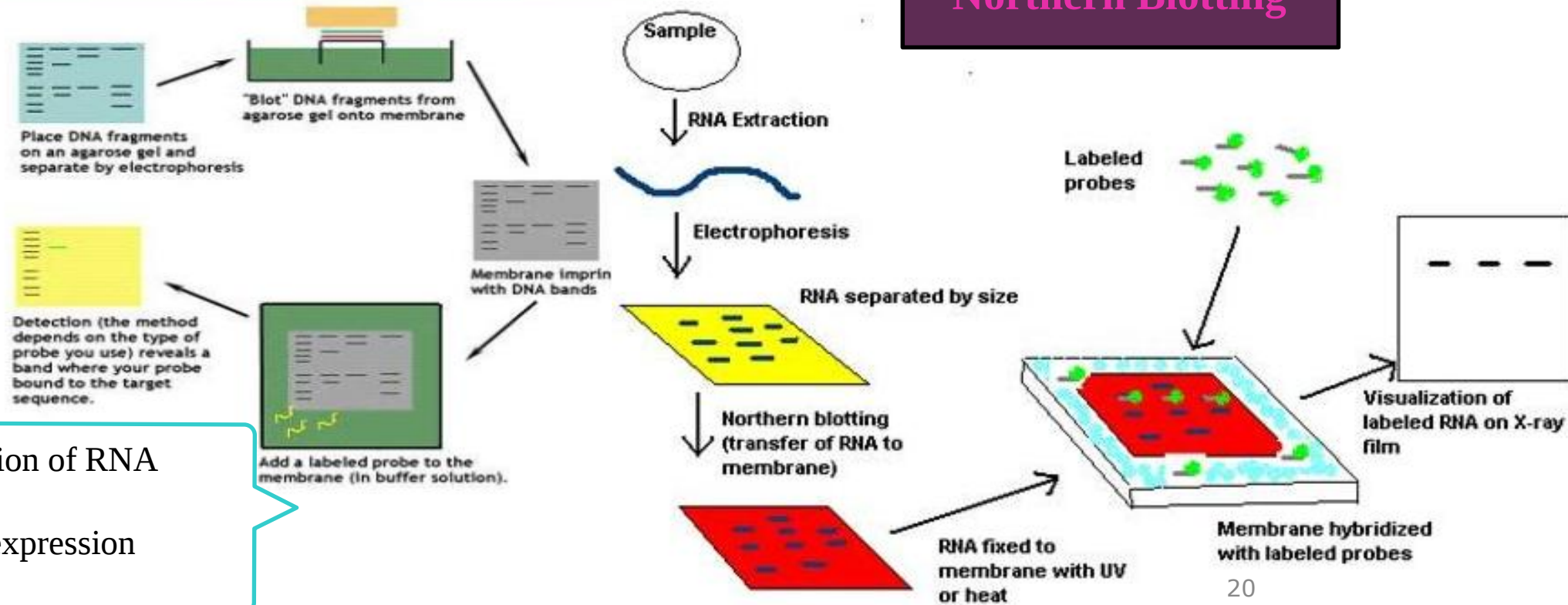
Detection of DNA and RNA

Southern blotting

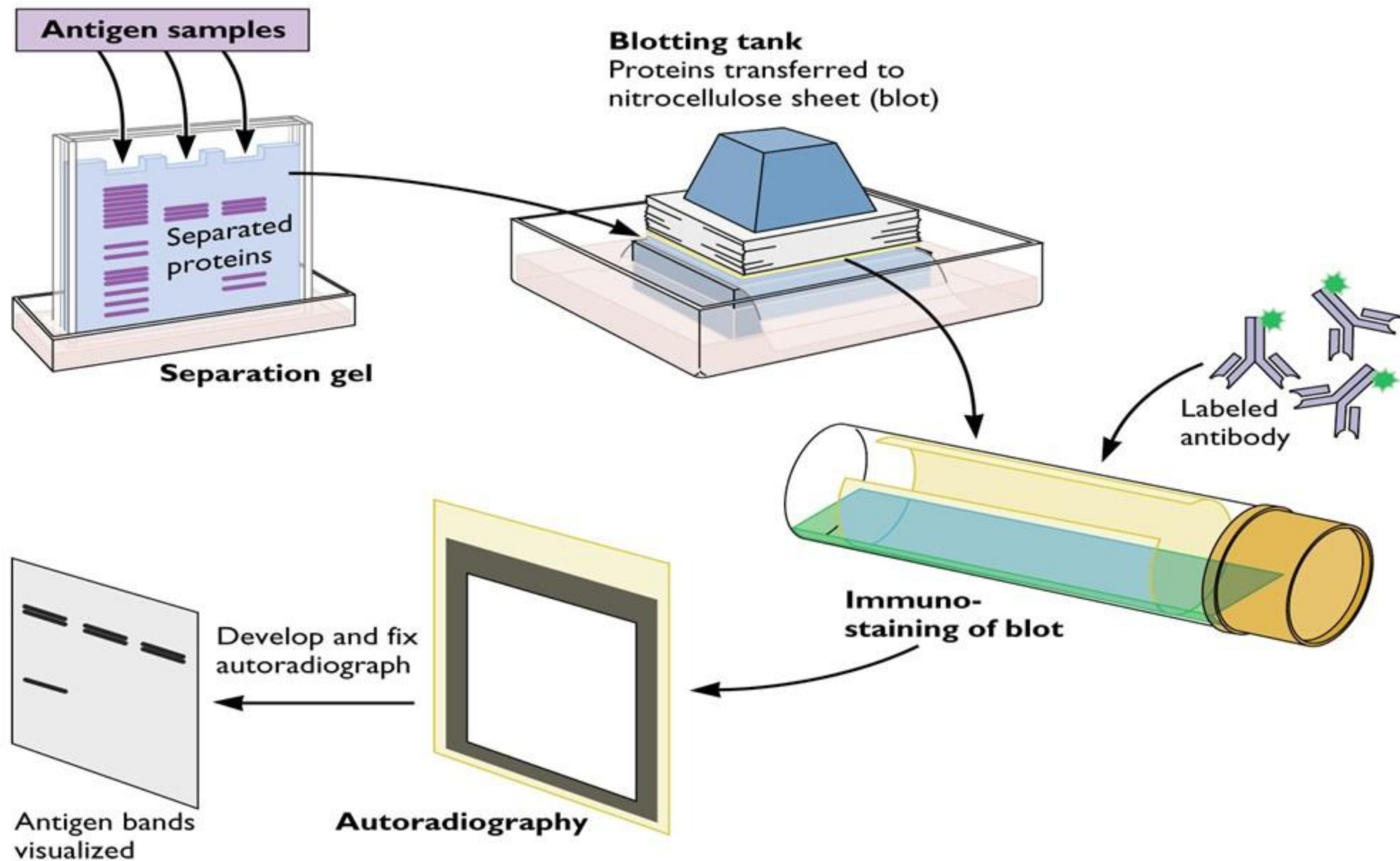
- Detection and identification of specific DNA sequence (Gene)

Northern Blotting

- Detection and identification of RNA
- Demonstration of Gene expression



Western Blotting Technique



Detection and Expression of Protein



Western Blot Detection Reagents

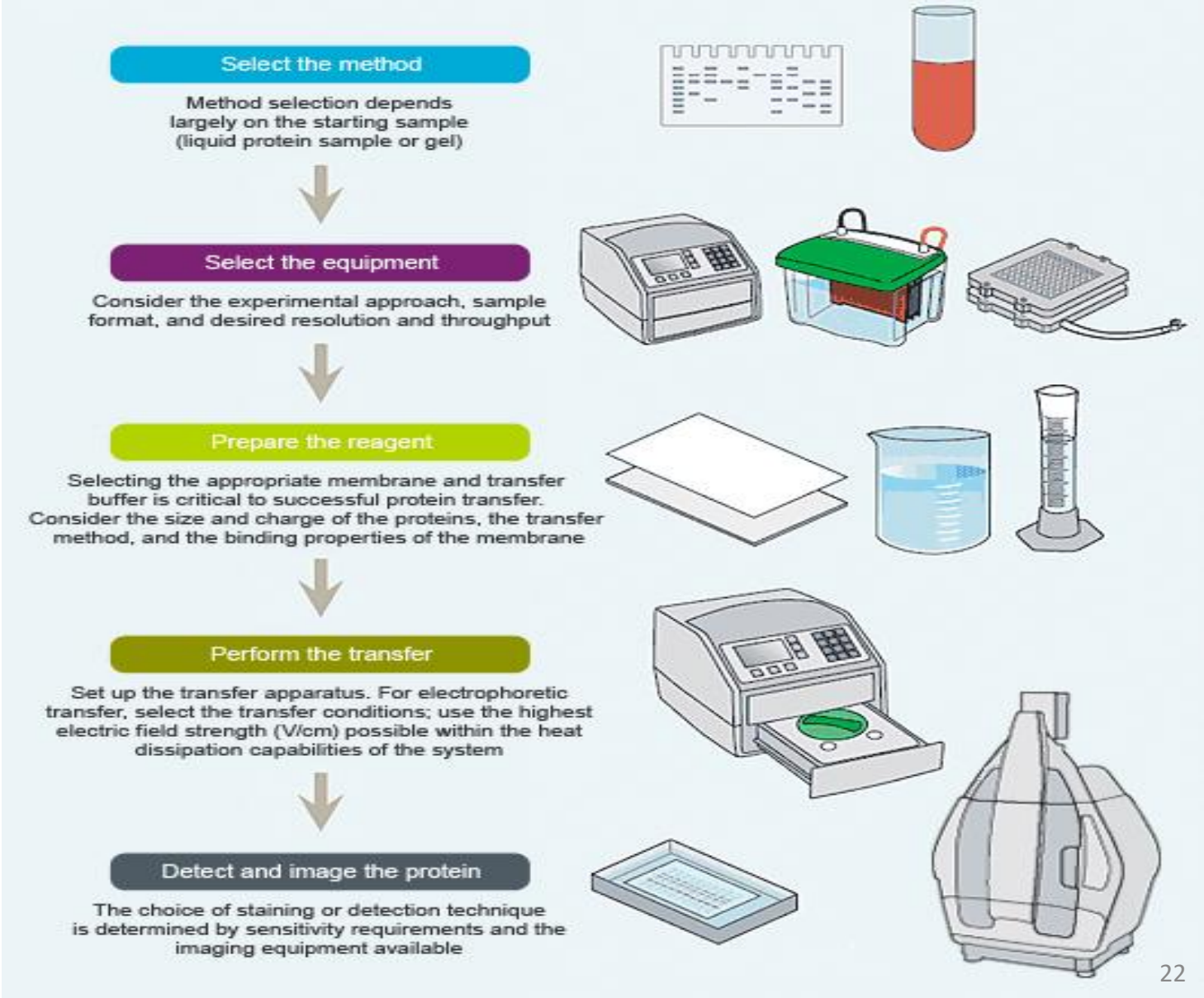


Western Blot Automated Processing



Western Blot Imaging and Analysis

Western Blotting Workflow



Advance Proteomics and Metabolomics

- **Protein Separation Technique**

- 1D Slab Gel Electrophoresis
- 2D Gel Electrophoresis (SDS-PAGE/IEF)
- Capillary Electrophoresis
- Chromatography (HPLC ,SEC, IEC, RP, Affinity, etc.)

- **Protein Chips (Protein microarray)**

allows quick and easily survey of the entire proteome of a cell within an organism

- **Applications:**

- identifying biomarkers for diseases,
- investigating protein-protein interactions,
- testing for the presence of a protein e.g Hb

- **Protein Identification Techniques**

- Edman AAs sequencing
- Micro-sequencing
- Mass spectroscopy

NMR - Spectroscopy



Crystallography

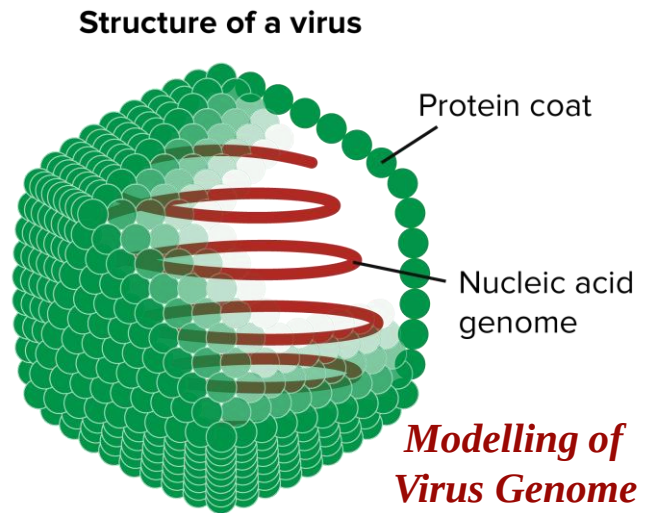
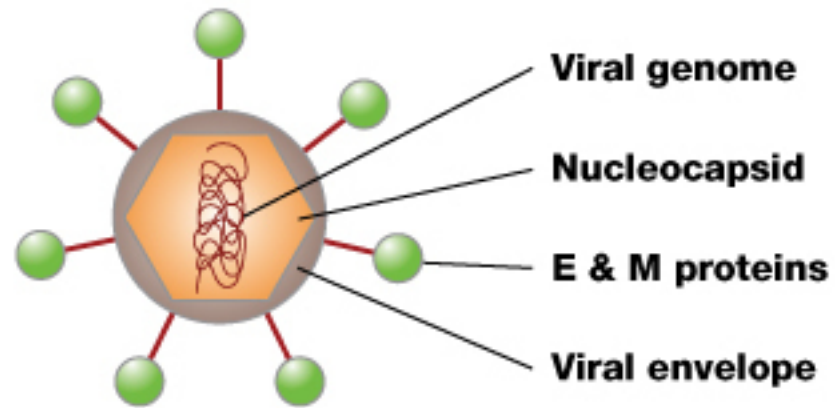


Mutation leading to AAs changes responsible for most inherited diseases

Three-dimension structure (3D) - AAs structural annotation

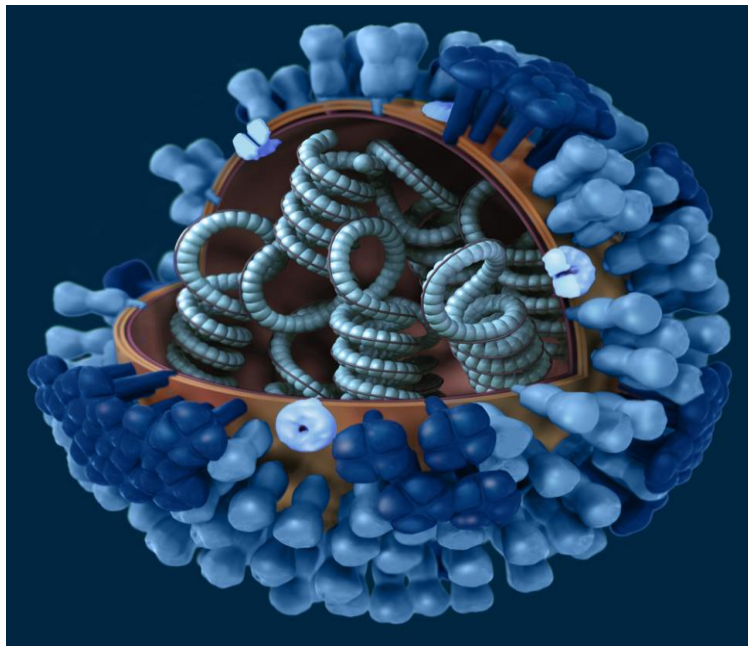
Clinical Application of Multiomics Technology

Molecular Diagnosis of Infectious Diseases

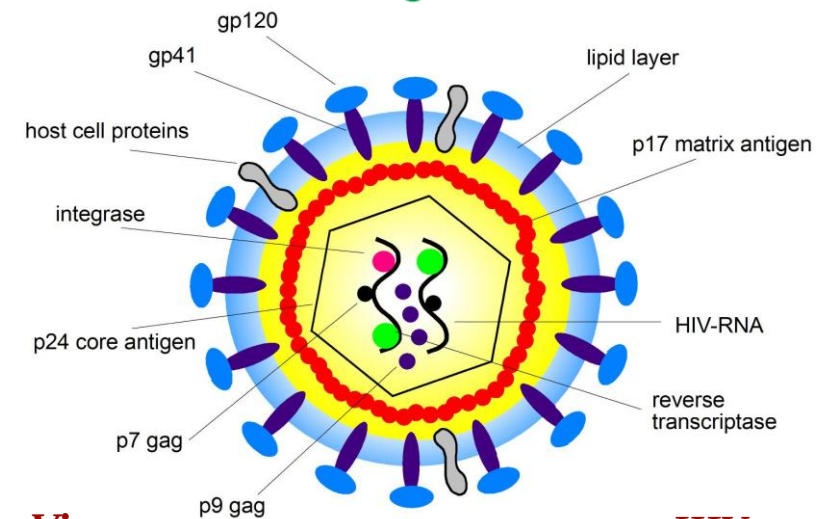


Dengue Virus

Modelling of Virus Genome

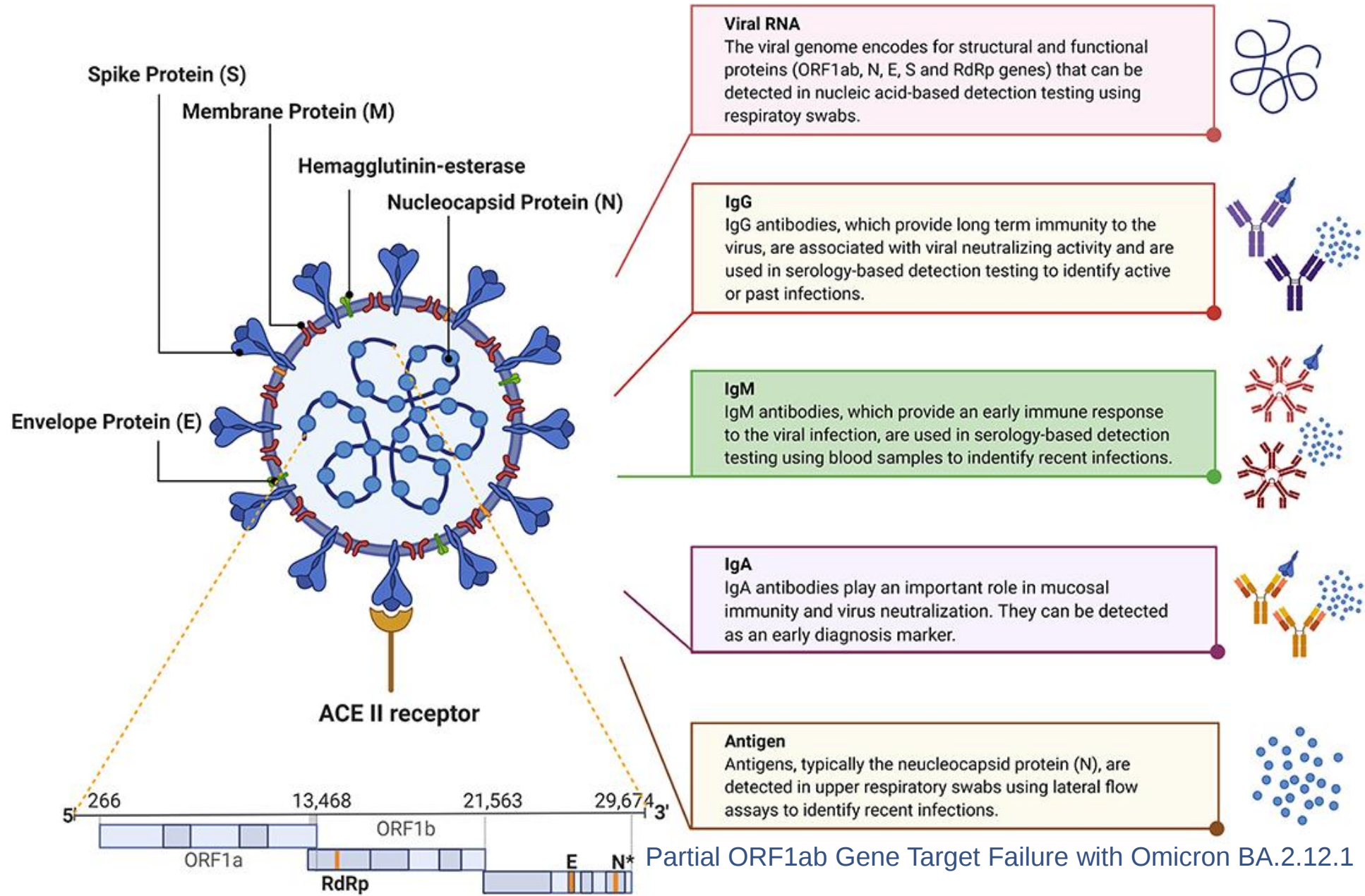


Influenza Virus



HIV

SARS COV 2 GENOME - DIAGNOSTIC MARKERS



Genomic Diversity and Phylogenetic Analysis of SARS-CoV-2 Circulating in Africa and Other Continents: Implications for Diagnosis, Transmission, and Prevention

SARS COV-2 Genome

Idowu A. Taiwo^{1,2}, Bamidele A. Iwalokun^{1,3}, Titilola A. Samuel^{1,4}, Adesola Olalekan^{1,5}, Khalid O. Adekoya^{1,2}, Oluwabukola M. Akinloye⁶, Gloria Amegatcher⁷, Eyitayo Adenipekun^{1,5}, Daniel Adewuni^{1,5}, Fatimah O. Anwoju^{1,2}, Olayiwola A. Popoola^{1,5}, Oluwatoyin P. Popoola^{1,8}, Bolanle Iranloye^{1,9}, Olubunmi A. Magbagbeola^{1,4} and Oluyemi Akinloye^{1,3, 5*}

¹Centre for Genomics of Non-communicable Diseases and Personalized Healthcare (CGNPH). ²Department of Cell Biology and Genetics, University of Lagos. ³Department of Molecular Biology and Biotechnology, Nigerian Institute of Medical Research (NIMR), Yaba, Lagos. ⁴Department of Biochemistry and University of Lagos. ⁵Department of Medical Laboratory Sciences, University of Lagos. ⁶Department of Medical Laboratory Sciences, Oulton College, Moncton, New Brunswick, Canada. ⁷Department of Medical Laboratory Sciences and West African Centre for Cell Biology of Infectious Pathogen (WACCBIP), University of Ghana. ⁸Department of Biomedical Engineering, University of Lagos. ⁹Department of Physiology, University of Lagos

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Received 18th November 2020; Revised 22nd December 2020; Accepted 23rd December 2020



Advantages:

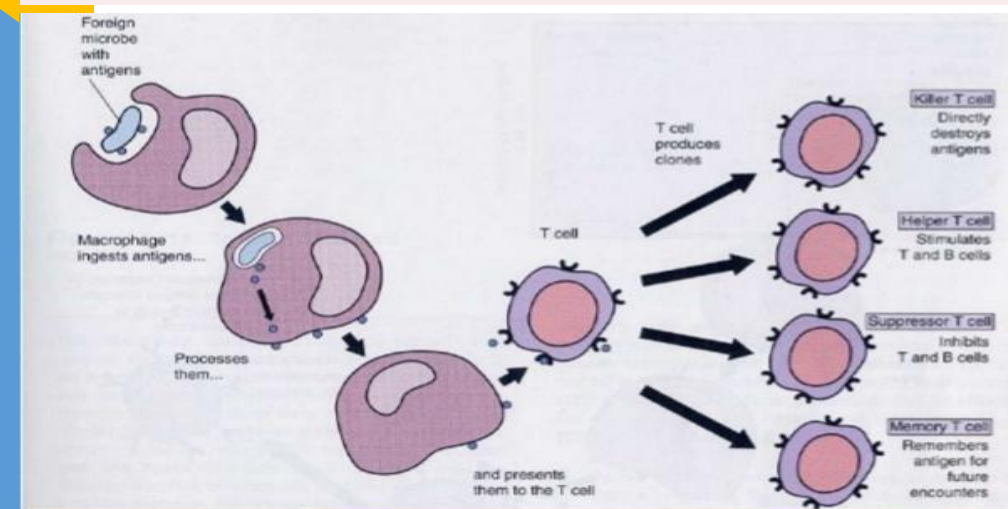
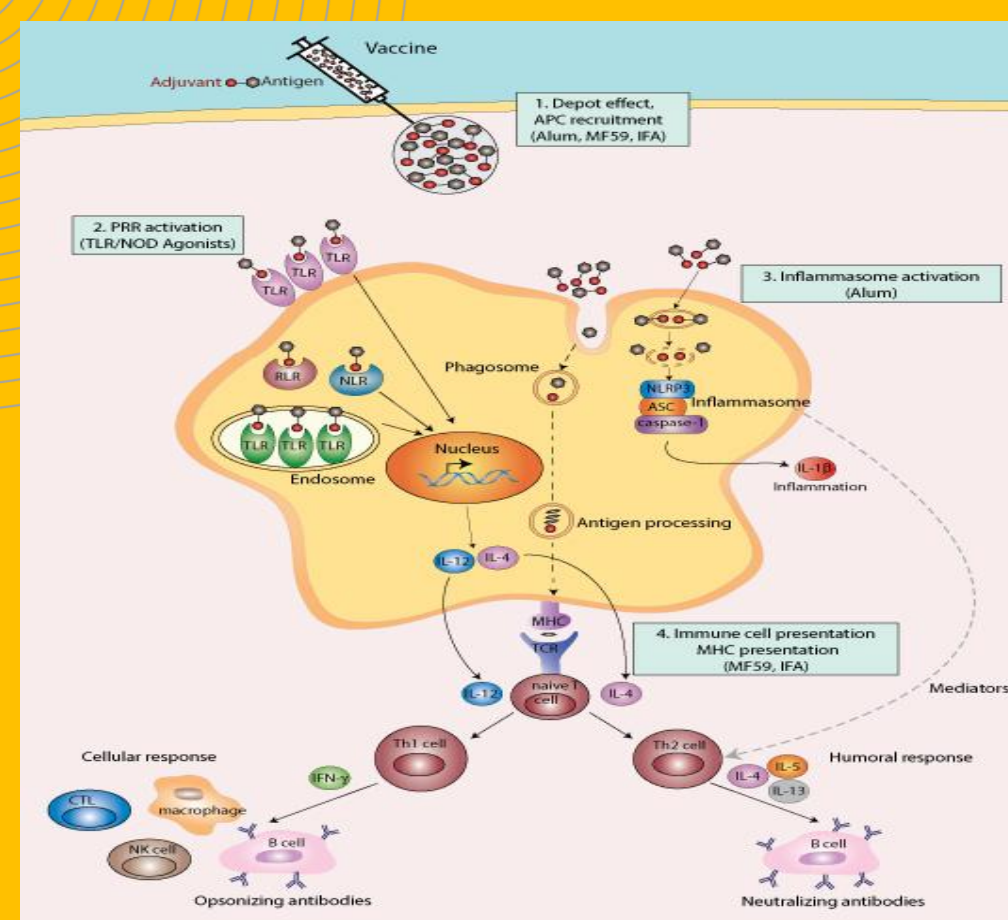
Almost eradicated polio and prevent diseases like measles, mumps and rubella

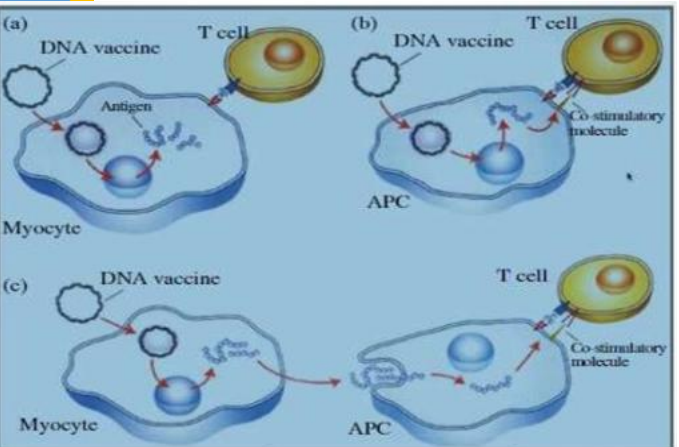
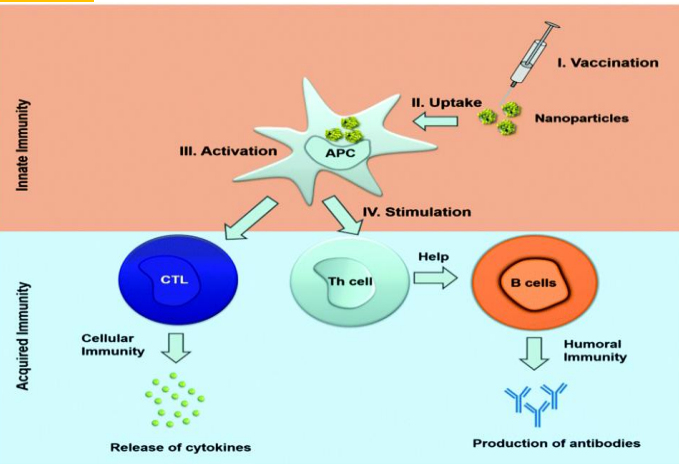
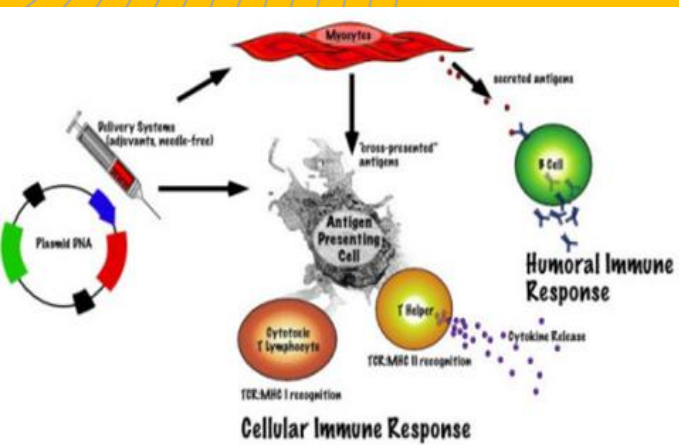
Disadvantages:

(1). Injection of weakened pathogens is associated with risk of developing the disease; For example, in 1955 some people given the new polio vaccine contracted polio from the immunization.

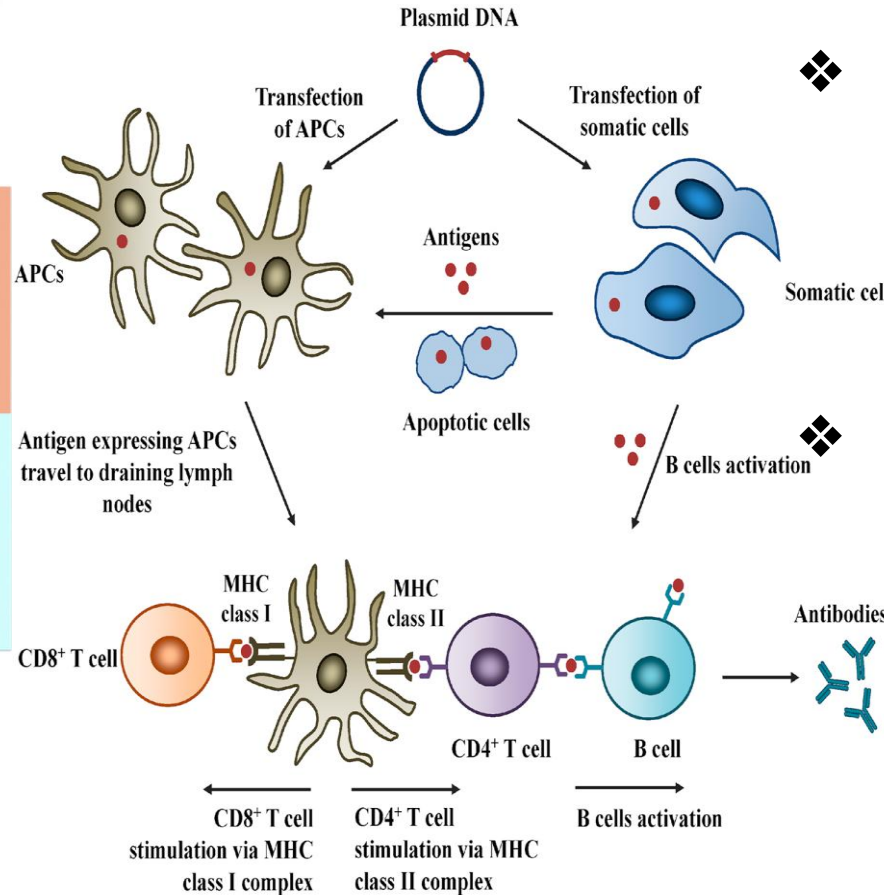
(2). Conventional vaccines are also fairly costly to produce.

(3). Require cold storage - hence affected by cold chain and making them unavailable to developing countries where they are often needed most.





GENOMICS VACCINE



❖ Genomic vaccine is DNA sequence code for antigenic protein of pathogen.

❖ As DNA is inserted into cells it is translated to form antigenic protein which is foreign to cells, hence immune response are raised against this proteins.

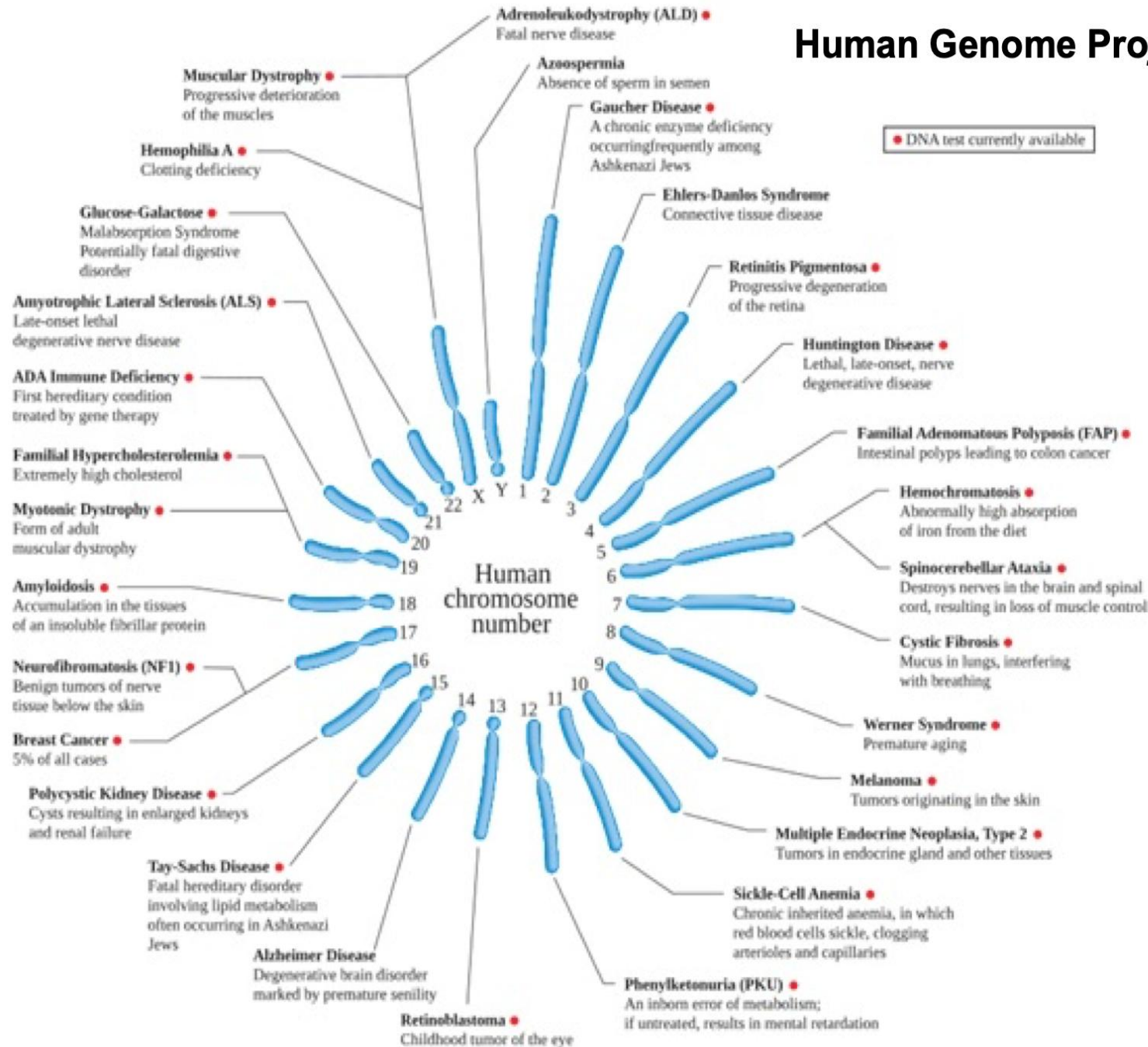
❖ "This genomic vaccine fools the immune system into thinking it has been infected by the real pathogen. Protection is produced without risk of infection, and it may be more effective than conventional vaccines," said Johnston.

❖ In this way, DNA vaccine provide immunity against that pathogen.

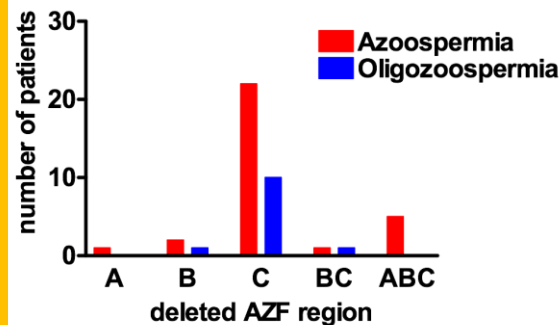
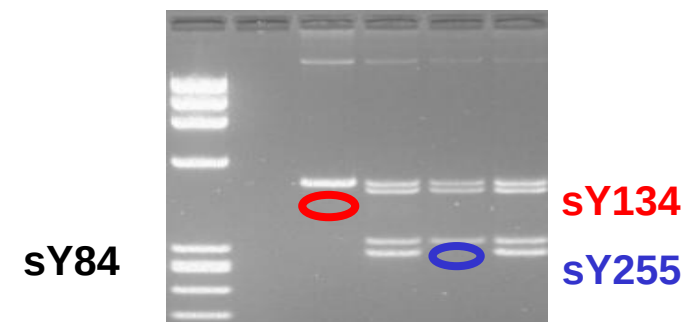
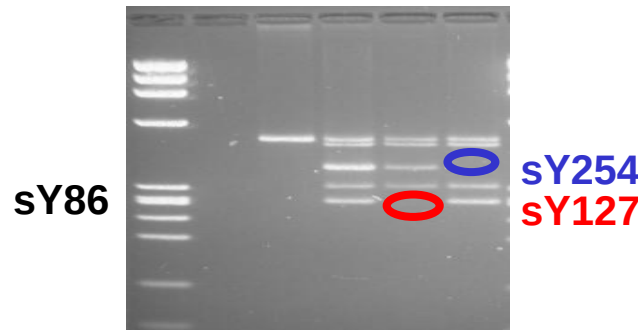
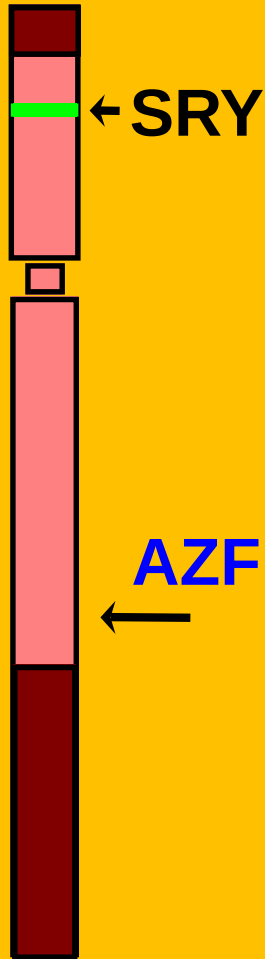
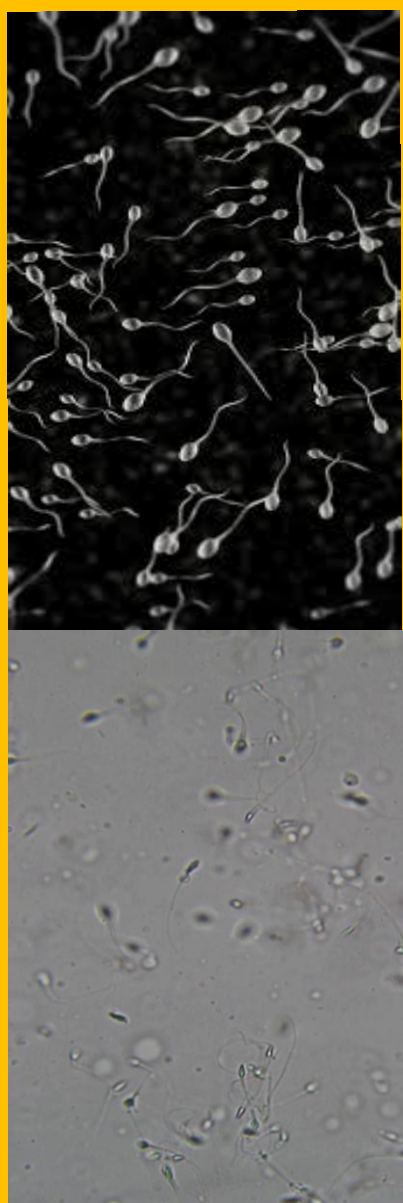
Human Genome Project

Evolution of Genomics Medicine

- ✓ Advances in Genomics allows genetics mapping of diseases.
- ✓ Genetics variation of this diseases influence Individual phenotypic variation and tracing.
- ✓ This Genetics variation influence responses to treatments
- ✓ Individual Genotype variation allow for precision diagnosis and annotated phenotype personalized



Y-CHROMOSOMES AND SPERMATOGENIC FAILURE



26 / 770 patients (3.4 %)

Odds ratio: 1.91 (0.63-6.54)

26 / 770 patients (3.4 %)

Odds ratio: 1.91 (0.63-6.54)

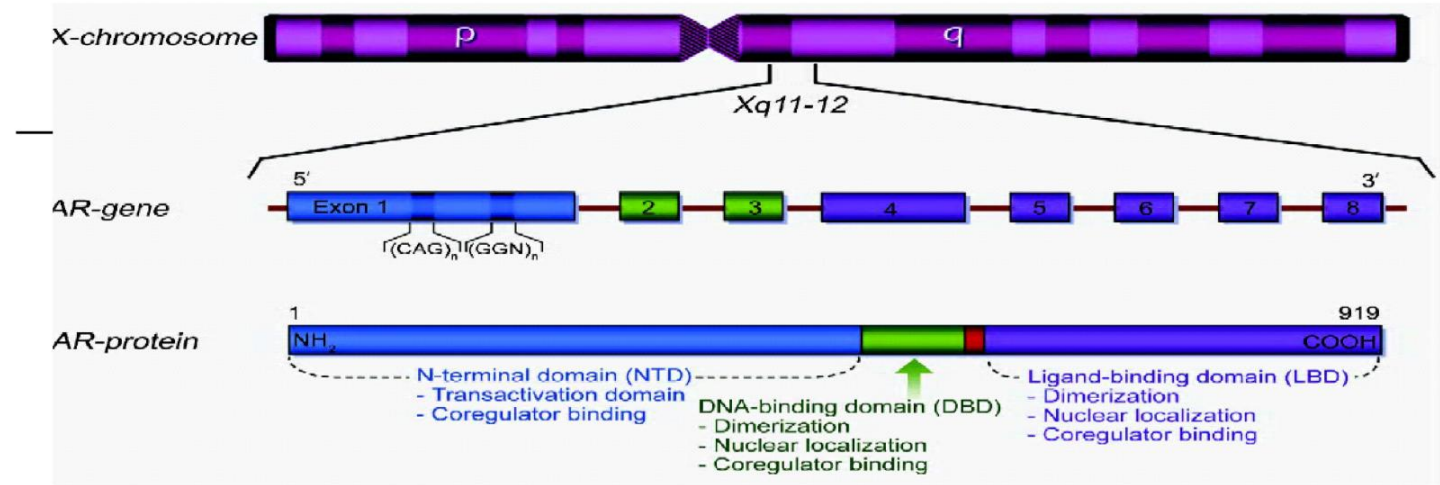
Akinloye et al, 2011



Microdeletions of
the Y chromosome

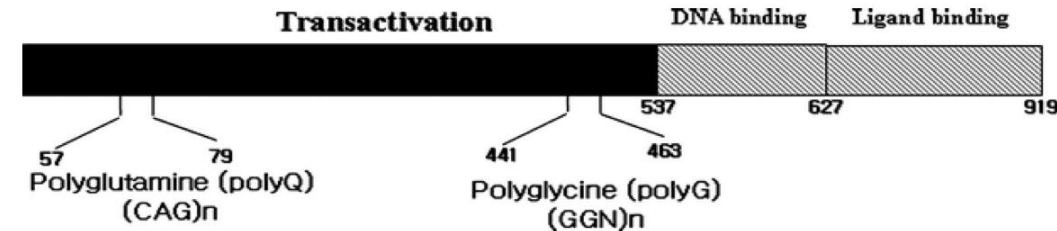


Androgen Gene and Androgen Receptor (AR) Mutation and Polymorphisms



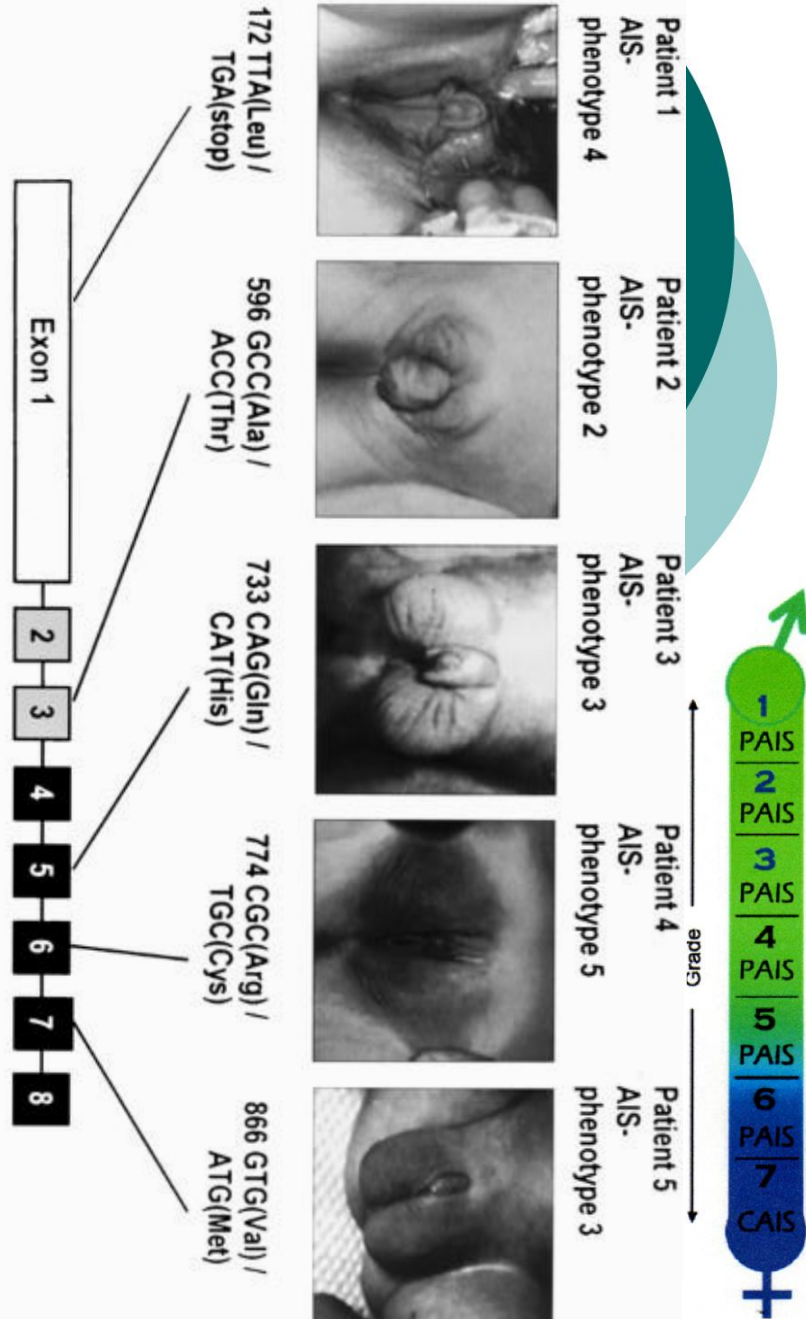
1. Genetic organization of the *AR* gene on the X-chromosome and major functional domains of the encoded protein.

<http://www.asiaandro.com>; aja@sibs.ac.cn



Androgen - Testosterone (M), Estrogen (F)
Androgenicity - Male reproduction; physical dev. of sex organs, spermatogenesis etc
Cell growth, cell cycles etc

androgen receptor gene



CAG-GGN Haplotype (GGN repeats)

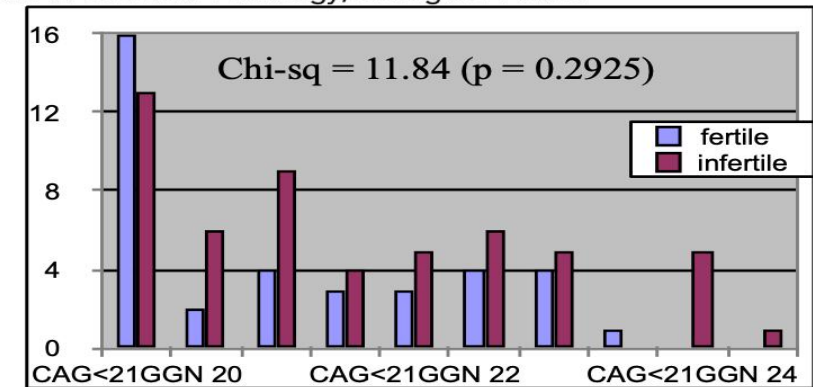
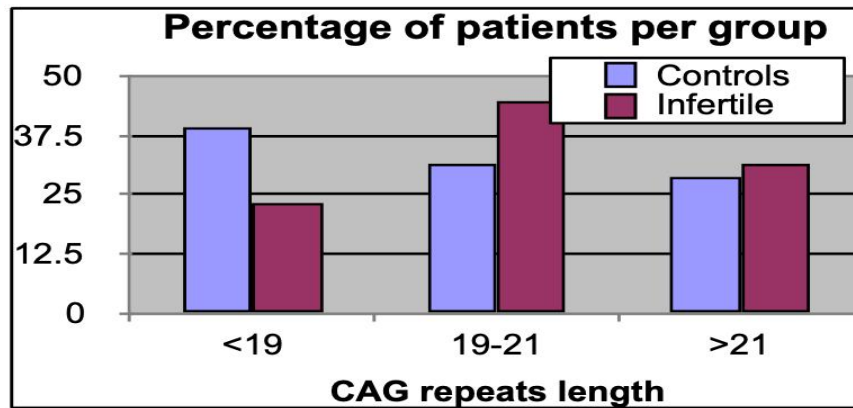
Examples of Clinical Applications of Genomics and Proteomics - **Infertility and Prostate Cancer**

J. Endocrinol. Invest. 32: 797-804, 2009

Androgen receptor gene CAG and GGN polymorphisms in infertile Nigerian men

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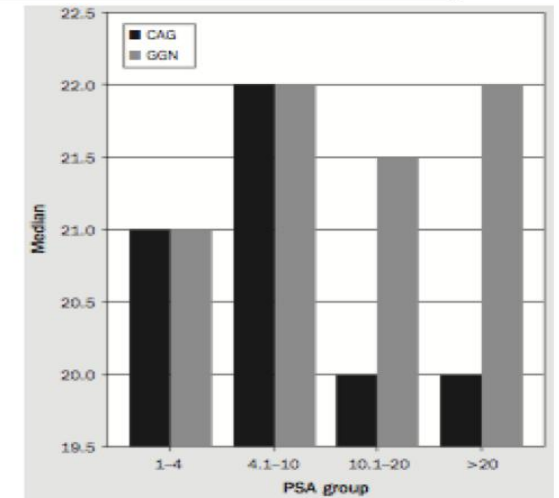
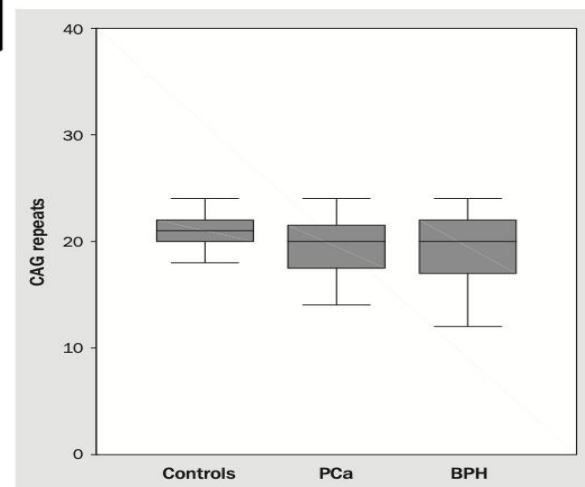
ORIGINAL ARTICLE

Variation in CAG and GGN repeat lengths and CAG/GGN haplotype in androgen receptor gene polymorphism and prostate carcinoma in Nigerians

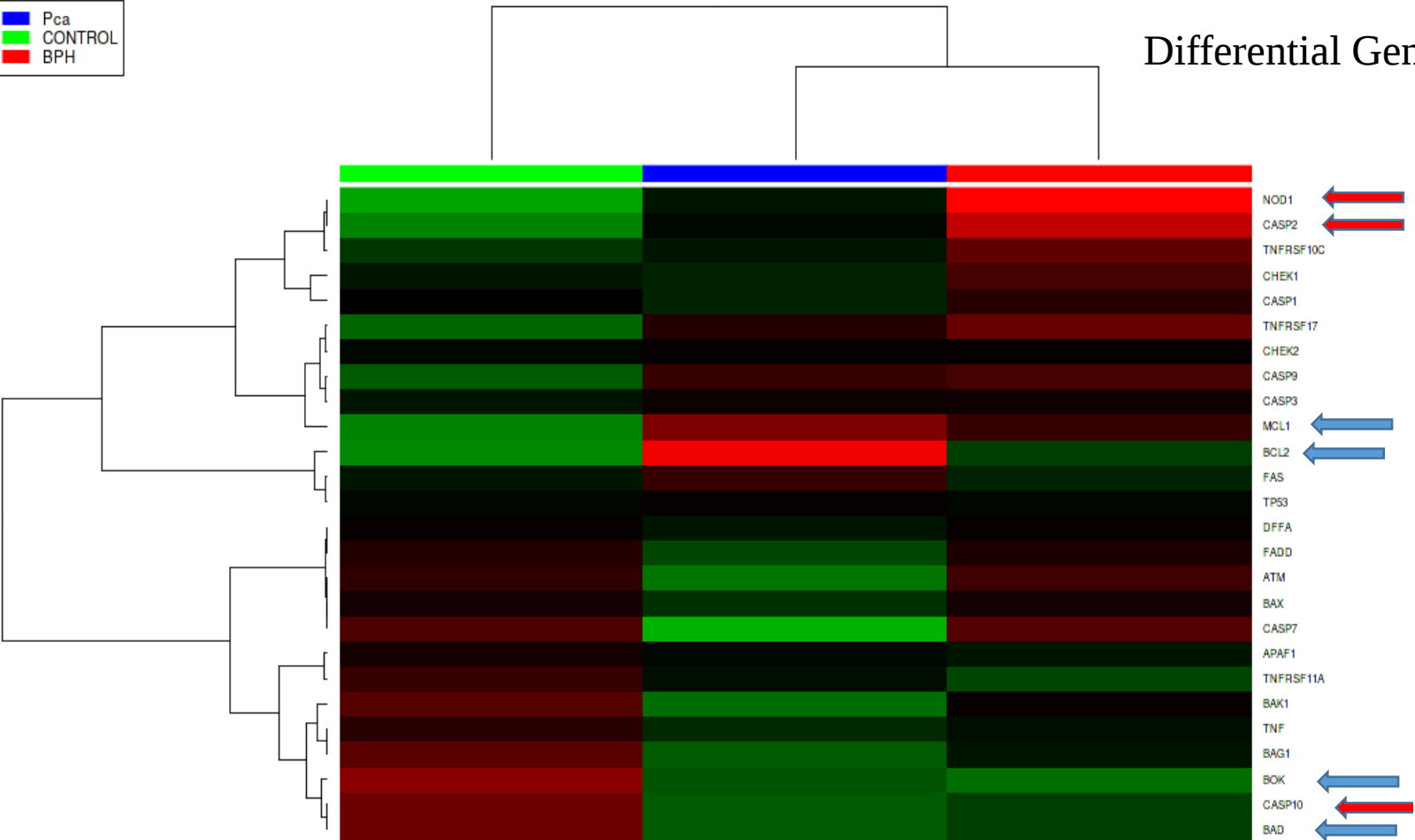
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ABSTRACT

Prostate cancer has become the most common cancer in Nigerian men. The growth of the prostate gland depends



Differential Gene Expression in PCa and BPH



Genes	Fold change	p-value
BCL2	2.71	0.000**
BOK	-2.38	0.000*
MCL1	1.73	0.023*
BAD	-1.56	0.035*
NOD1	-2.04	0.000**
CASP2	-1.81	0.035*
CASP10	1.64	0.043*

