

Molecular Biology Techniques & HIV

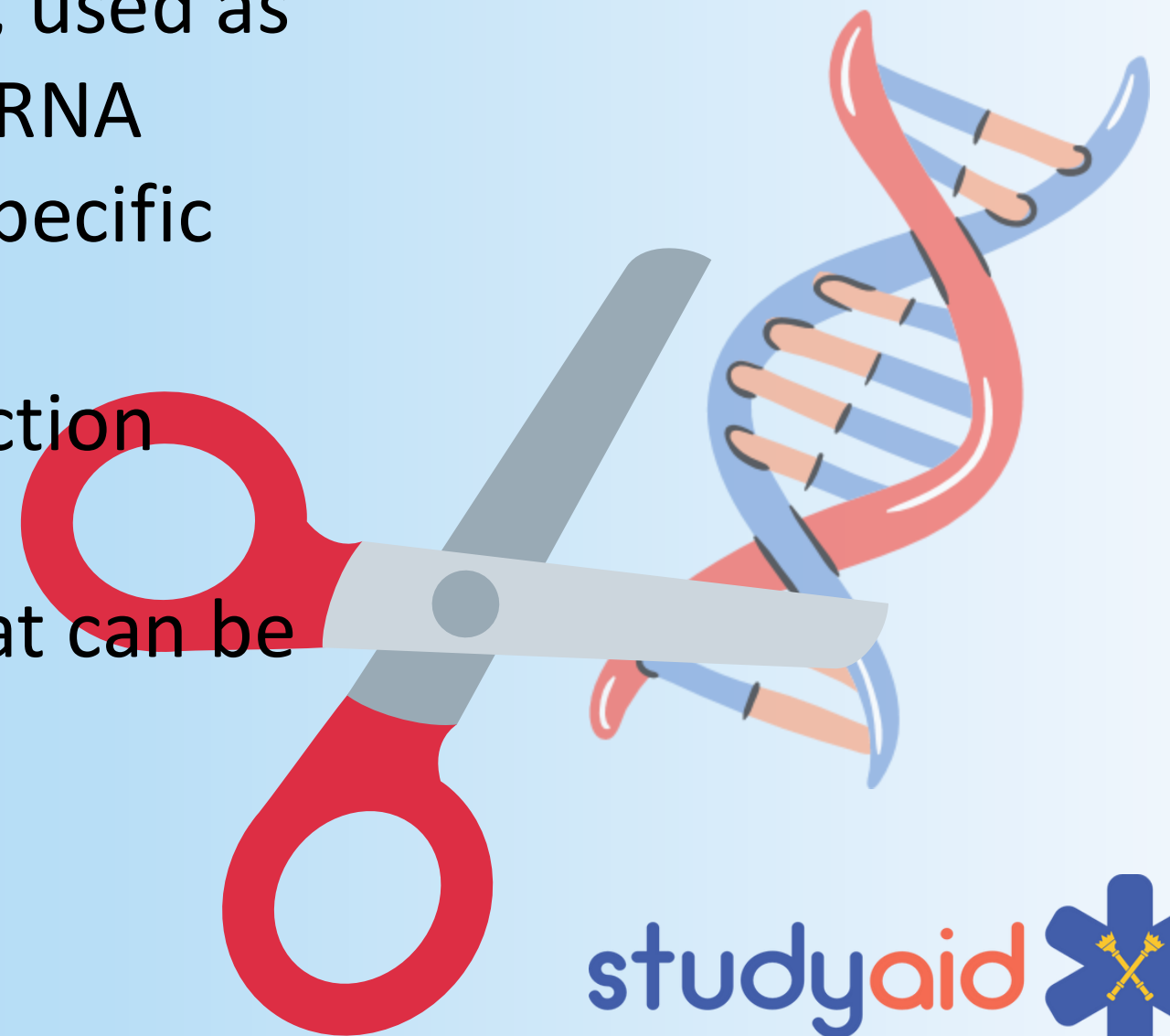
By Zofia Gowin

What are Molecular Biology Techniques?

Methods used in molecular biology to manipulate and analyse DNA, RNA, proteins and lipids

Key Terms:

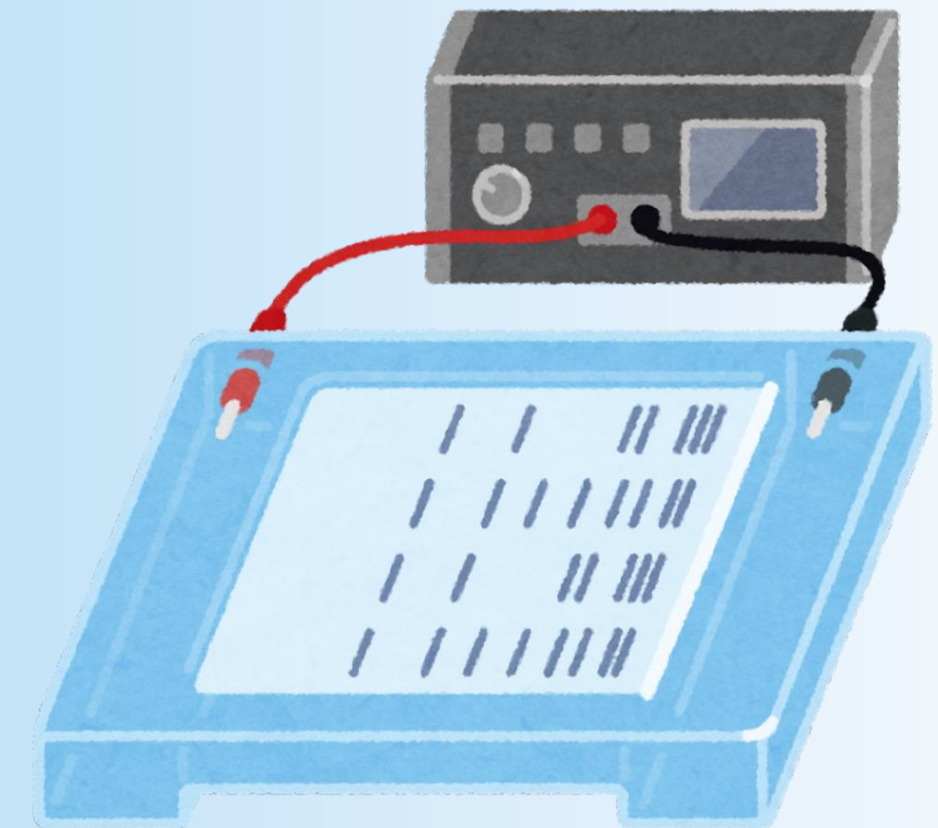
- **Oligonucleotides** – short DNA sequences synthesised in vitro, used as primers for DNA synthesis or as probes to detect DNA or RNA
- **Restriction endonucleases** – enzymes that cleave DNA at specific sequences
 - **Palindromes** – short DNA sequences recognised by restriction endonucleases
- **DNA fragments** – DNA segments containing specific genes that can be isolated using restriction enzymes



Identifying DNA Sequences



- 1. Probes**
- 2. Gel Electrophoresis**
- 3. Sanger Sequencing**



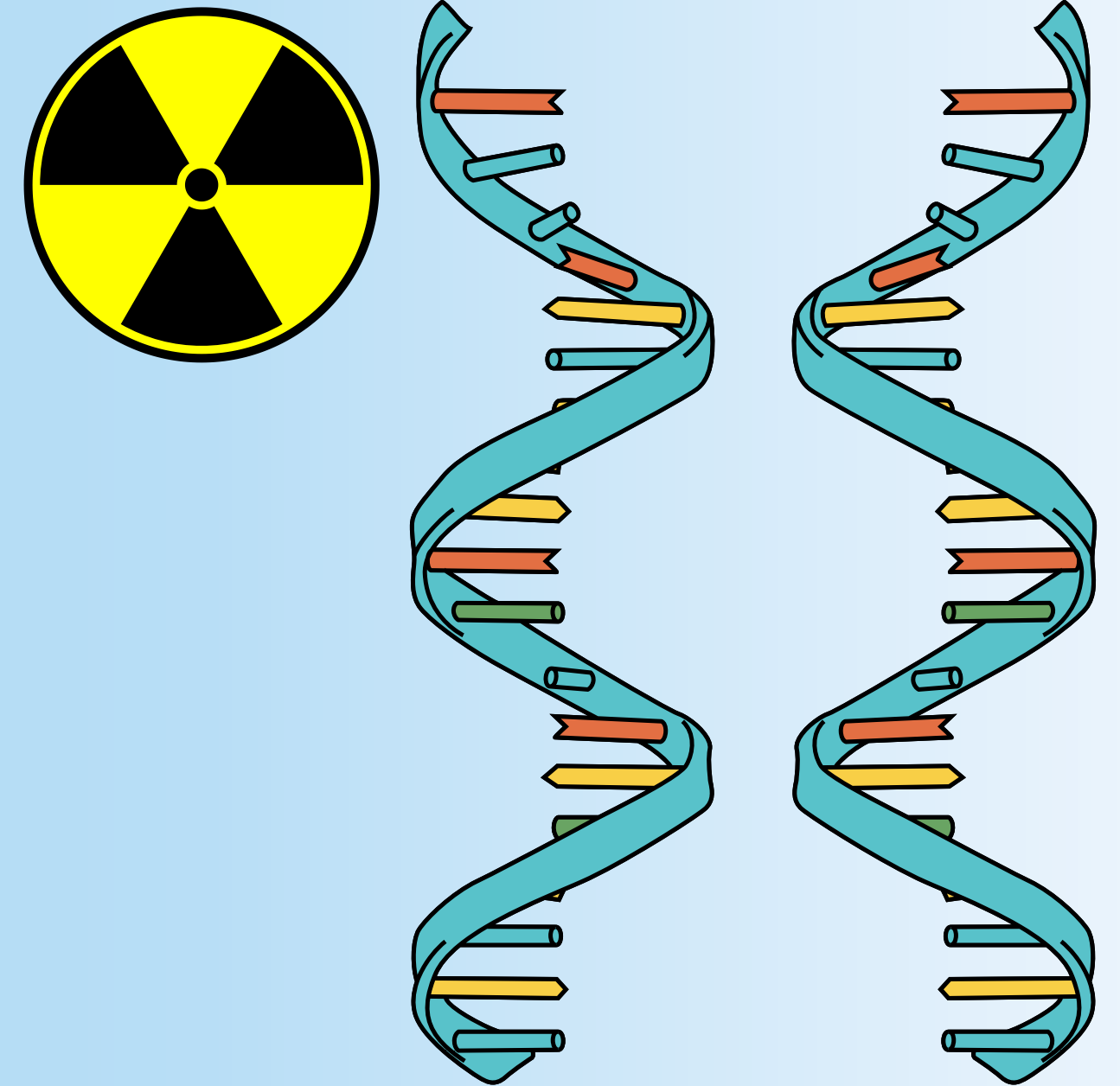
Identifying DNA Sequences

1. Probes

Single strand of DNA that creates a **base pair** with a complementary sequence on another single-stranded polynucleotide composed of DNA or RNA

MUST contain a label!

Label can detect complementary DNA or RNA
It may be radioactive or fluorescent



Identifying DNA Sequences

2. Gel Electrophoresis

Separates DNA chains of varying length

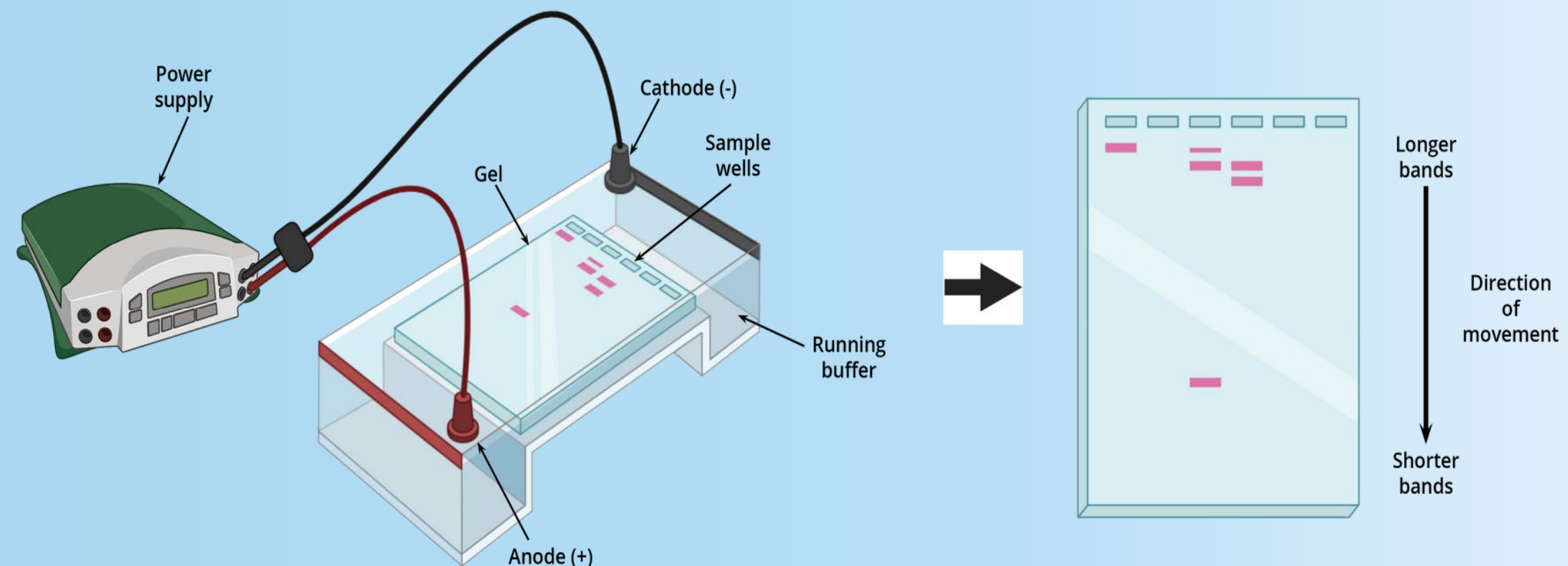
Polyacrylamide gel separate short DNA chains

Agarose gels separate longer DNA chains

Separation depends on length, **shorter** chains migrate more **rapidly**

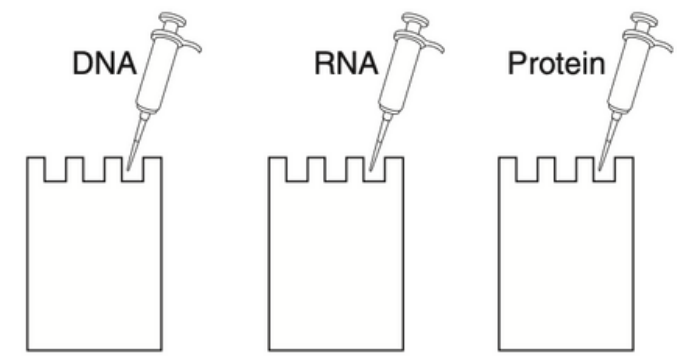
Remember!
DNA is **negatively** charged, and so it will migrate towards **positive** electrode!

DNA migrates toward the positive electrode because of its negatively charged phosphate backbone.

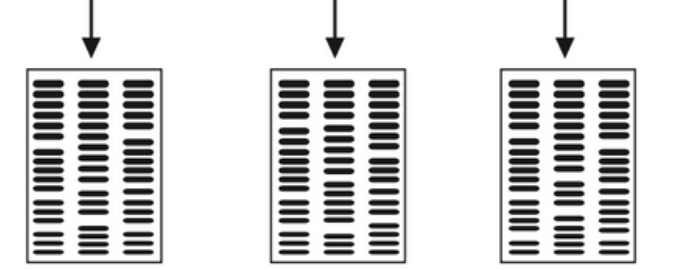


Southern Northern Western

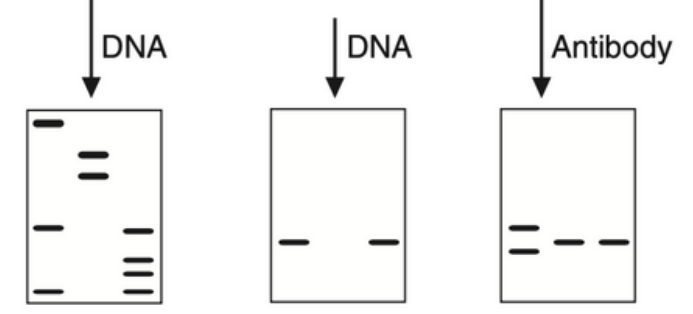
Gel electrophoresis



**Transfer to paper
(bands not visible)**



**Add probe to
visualize bands**



Autoradiograph

Mnemonic!

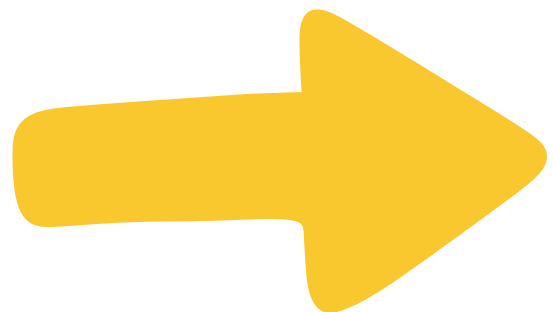
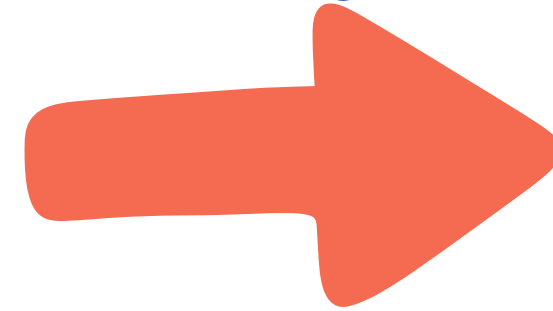
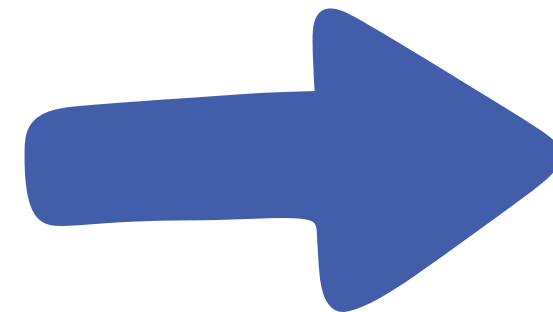
Types of Electrophoresis - SNOW DROP

SOUTHERN

NORTHERN

O

WESTERN



DNNA

RRNA

O

PROTEIN

Identifying DNA Sequences

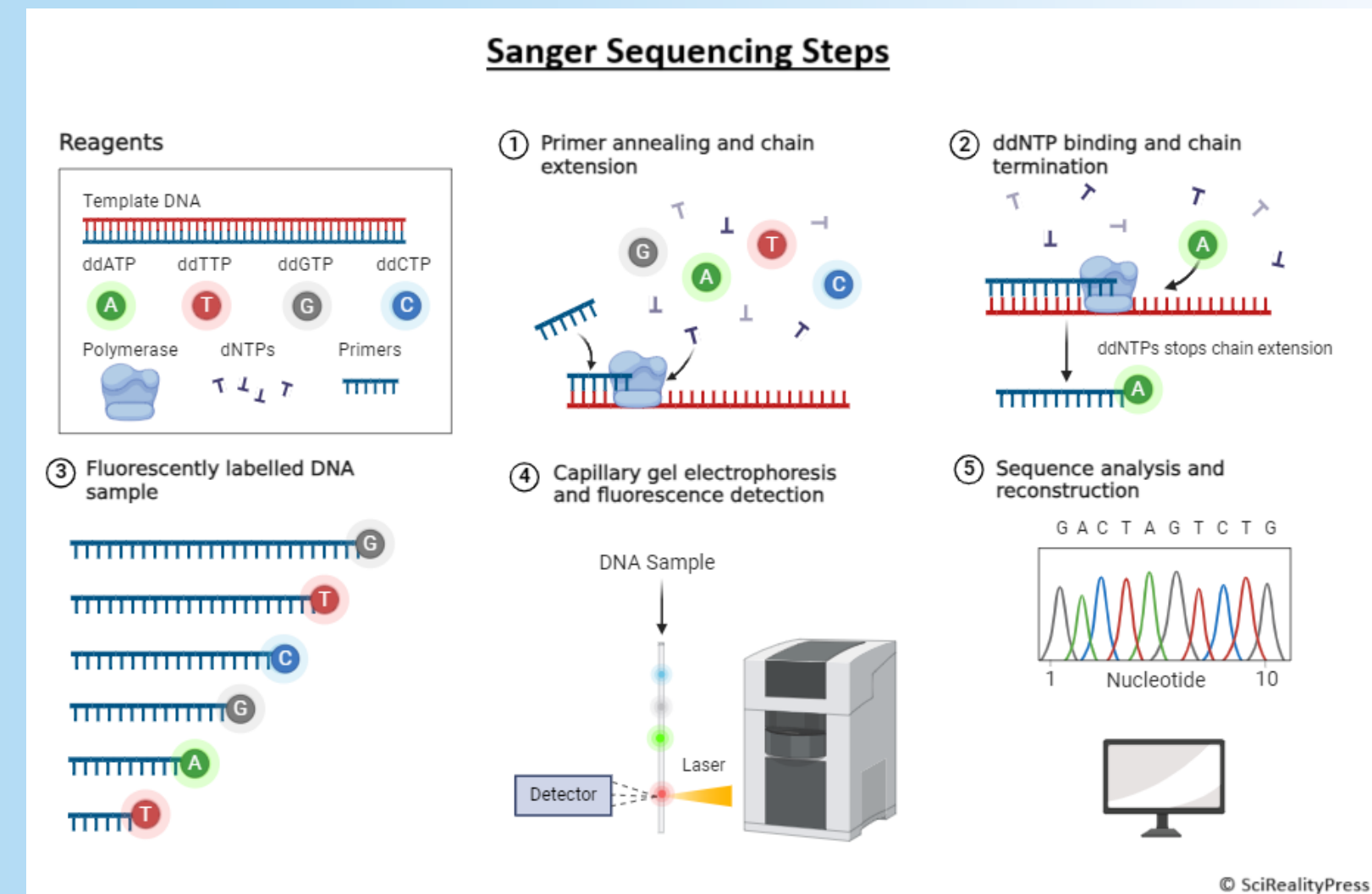
3. Sanger Sequencing

Sanger sequencing determines DNA sequence by incorporating chain-terminating dideoxynucleotides (ddNTPs) during in vitro DNA synthesis using DNA polymerase.

ddNTPs lack a 3'-hydroxyl group, causing termination of DNA chain elongation.

Shorter fragments migrate faster and are found near the 5' end of the sequence.

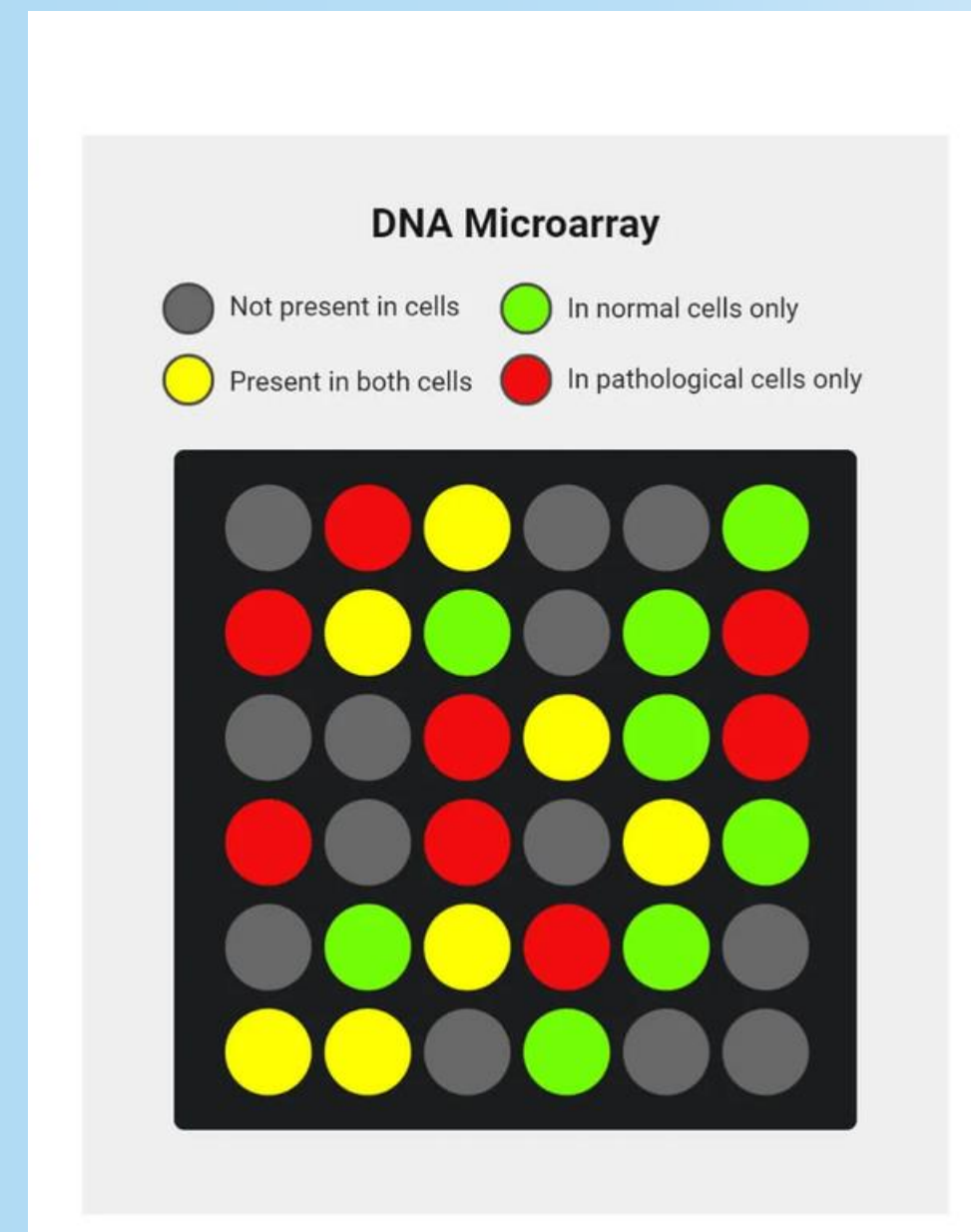
The DNA sequence is read 5' → 3' from the bottom to the top of the gel.



DNA Microarrays

Produces a genetic portrait by screening for thousands of genes simultaneously. cDNAs or oligonucleotide probes for different genes are arrayed individually on a glass slide or nitrocellulose paper

- a. **Genotyping** - detects mutations or polymorphisms
- b. **Gene Expression** - uses mRNA from two different sources for a comparative analysis. mRNA is converted into cDNA, and each population of cDNA is labelled with a different fluorescent dye, mixed together and hybridised to examine the relative abundance of genes

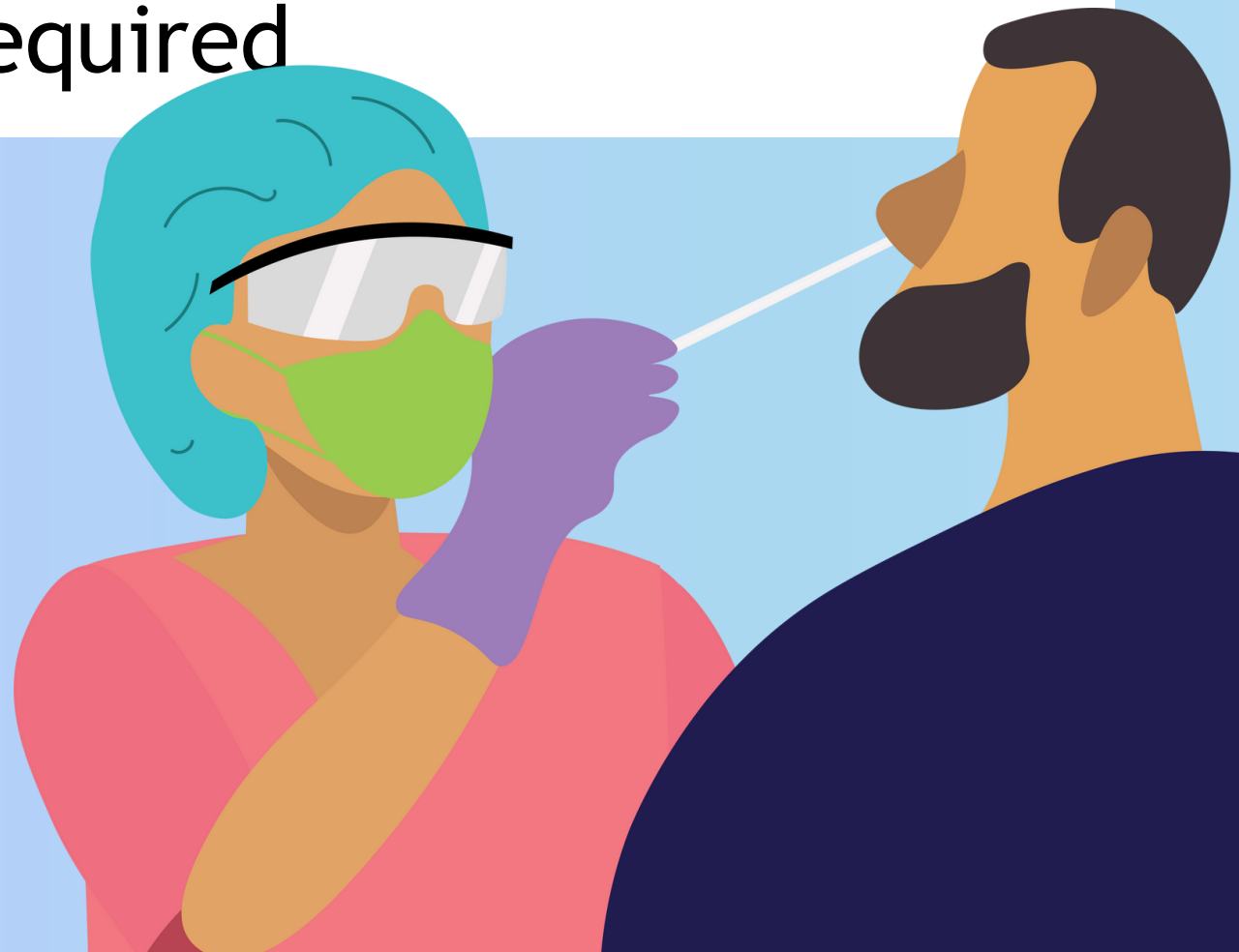


Clinically, they can be used to predict response to cancer treatment by identifying genes associated with pathogenesis and sensitivity or resistance to treatment

Amplification

DNA Amplification is the production of multiple copies of a sequence of DNA
i.e. it is the repeated copying of a piece of DNA

Polymerase Chain Reaction (PCR)
Rapidly produces large amounts of DNA.
Suitable for clinical or forensic testing as only **small** quantities of DNA are required



Cloning of DNA

Foreign DNA is obtained and can be then inserted into a DNA **vector**

It is then used to transform cells from another organism (e.g. bacteria), replicating the foreign DNA, as well as it's own

Large quantities of DNA can be isolated or the DNA expressed and it's protein product can be obtained in large quantities

Amplification

PCR steps:

- Denaturation ($\approx 95\text{ }^{\circ}\text{C}$)
- Annealing ($\approx 50\text{--}60\text{ }^{\circ}\text{C}$)
- Extension ($72\text{ }^{\circ}\text{C}$)

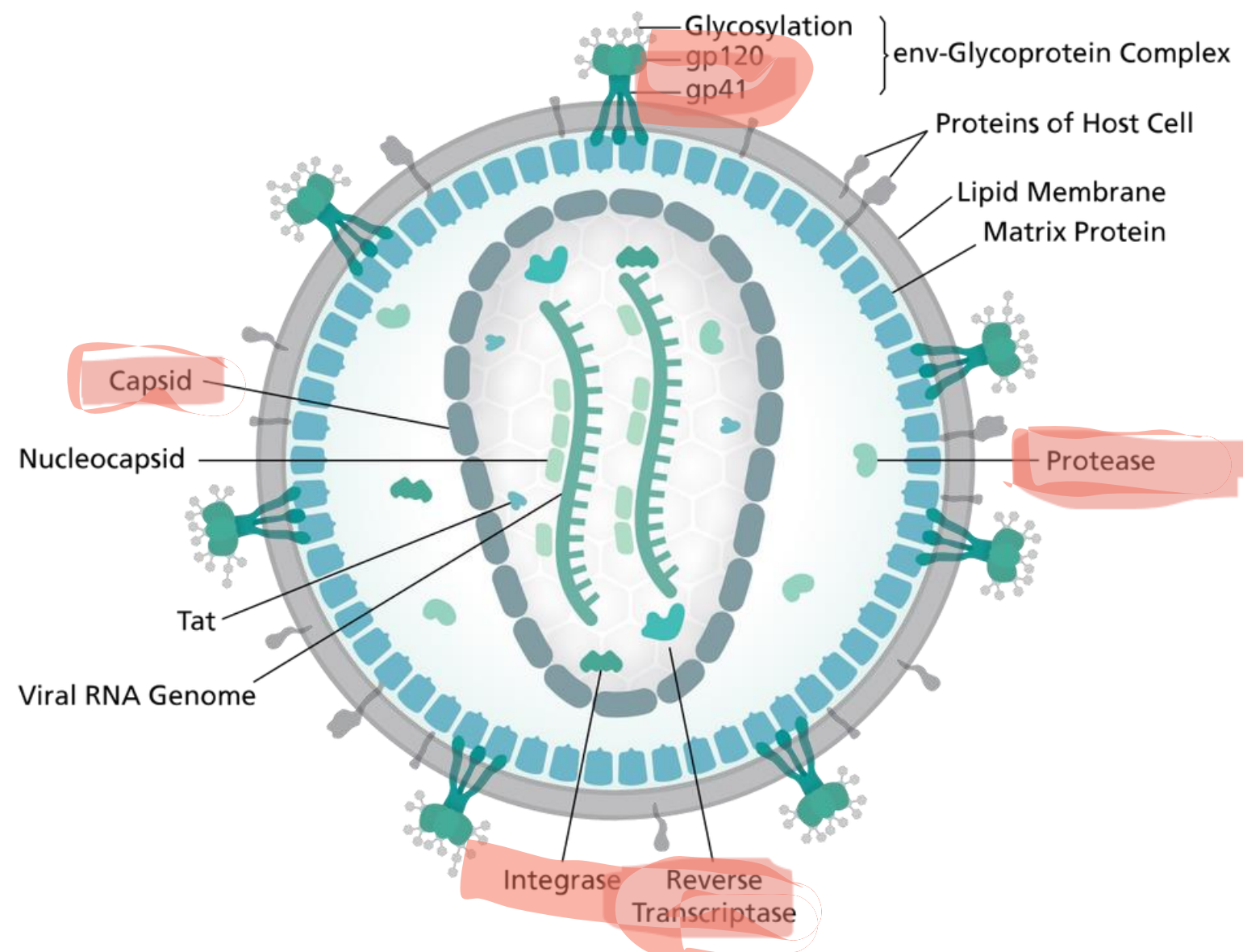
PCR requires:

- DNA template
- Forward and reverse primers (oligonucleotides)
- Thermostable DNA polymerase (e.g. Taq polymerase)
- dNTPs
- Mg^{2+} ions (cofactor)



Human Immunodeficiency Virus HIV

Structure of HIV



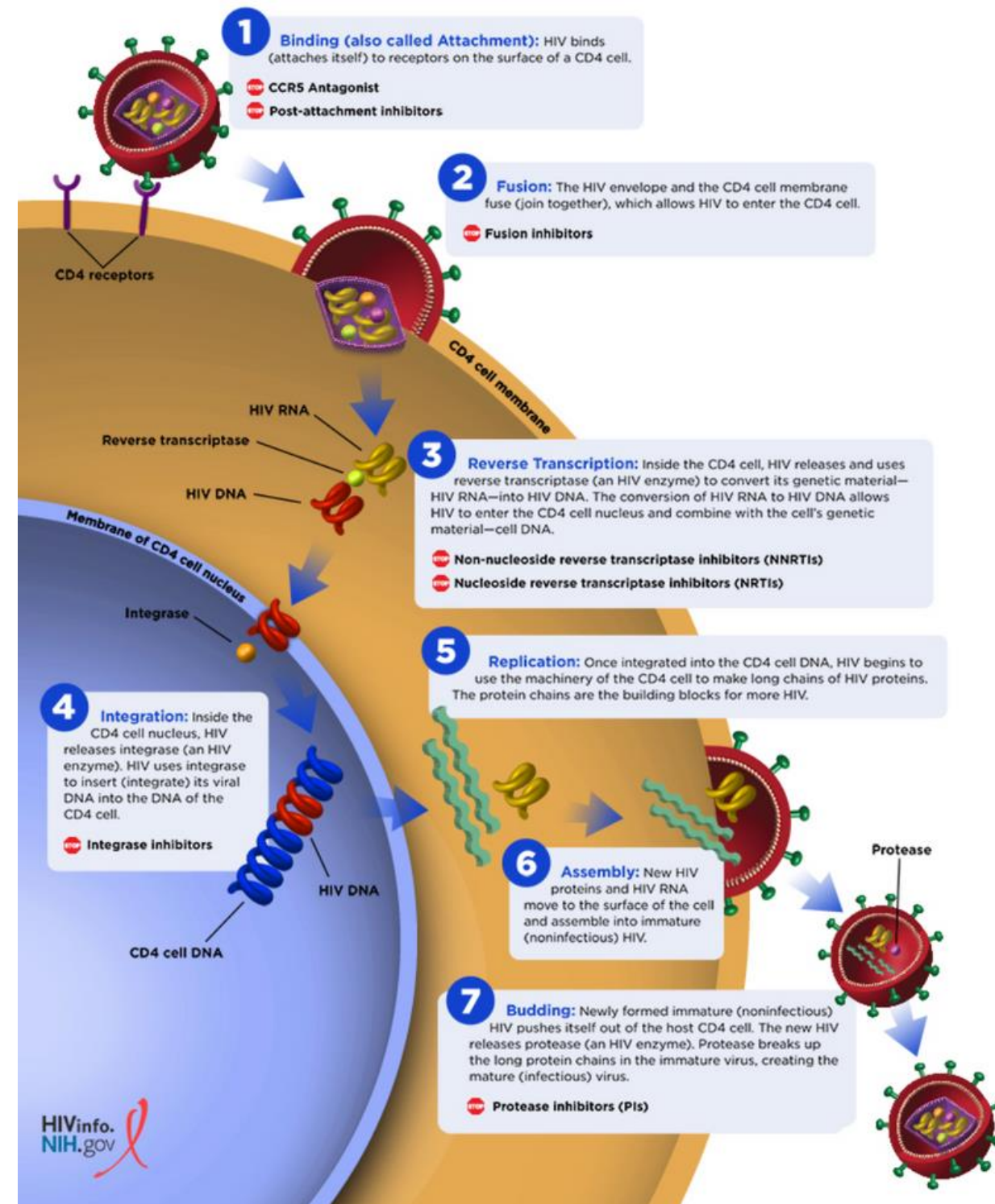
Functions:

- **gp120** – envelope glycoprotein that binds the CD4 receptor and CCR5/CXCR4 co-receptors on the host cell
- **gp41** – envelope glycoprotein that mediates fusion of the viral and host cell membranes during entry
- **p24** – capsid protein responsible for capsid formation and structural stability
- **Integrase** – enzyme that inserts viral DNA into the host cell genome
- **Reverse transcriptase** – enzyme that converts viral RNA into DNA (reverse transcription)
- **Protease** – enzyme that cleaves viral polyproteins into functional proteins required for viral maturation

Life Cycle of HIV

The HIV Life Cycle

HIV medicines in seven drug classes stop (🛑) HIV at different stages in the HIV life cycle.



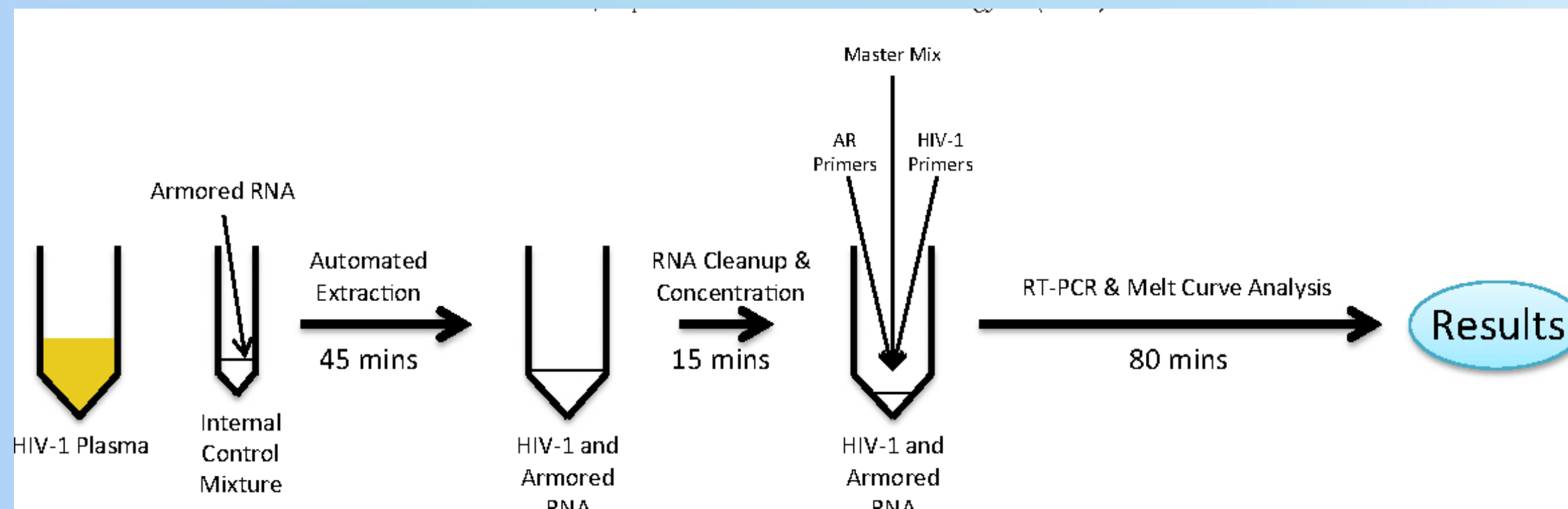
RT-PCR in HIV

One of the most commonly used methods to detect HIV nucleic acid

PCR is coupled with reverse transcription, whereby the RNA is reverse transcribed to cDNA and then amplified in one reaction

With HIV's long latency period of infection, it is difficult to test early after infection but can be detected using this method

Can detect 10 days after exposure to virus

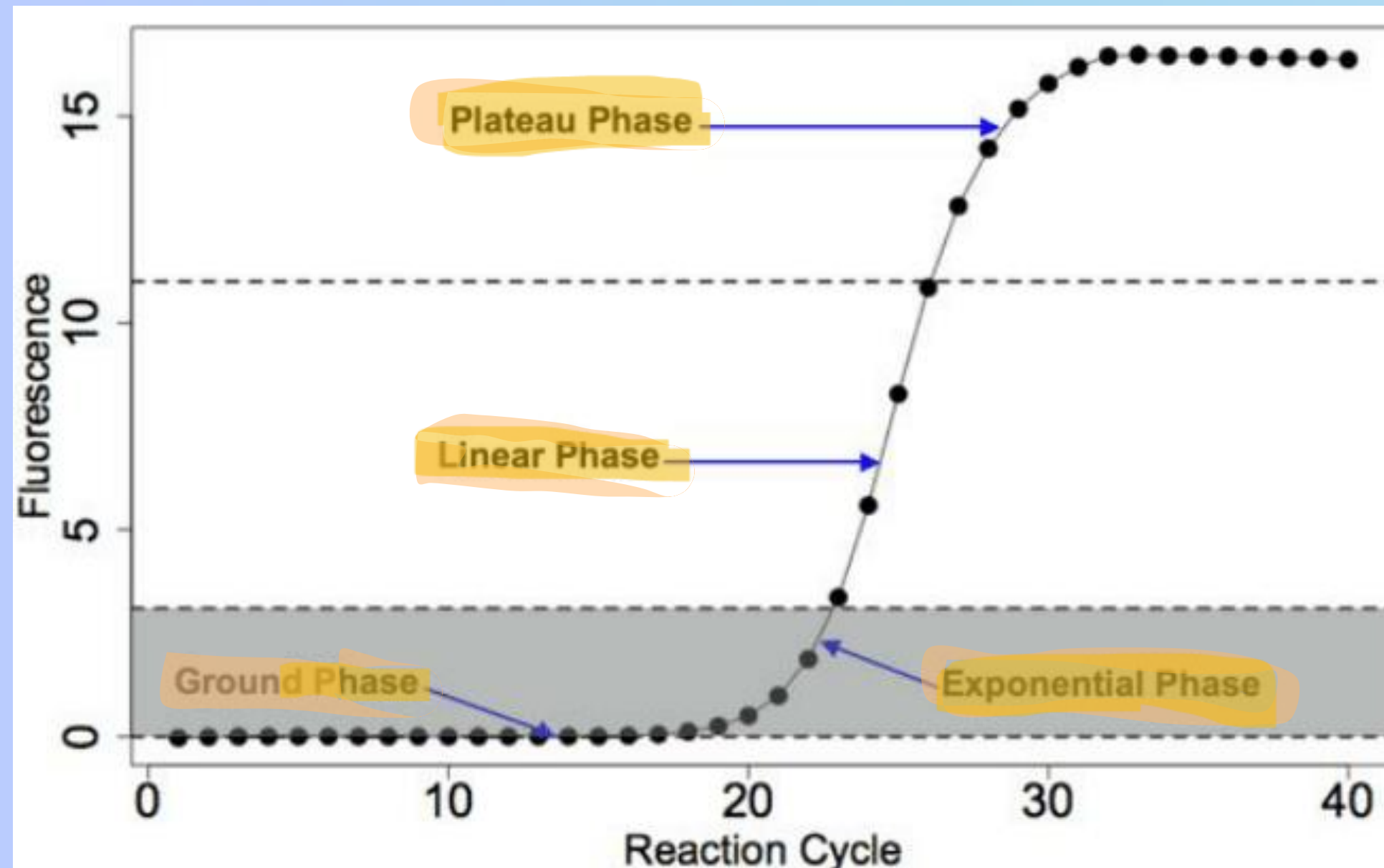


RT-qPCR in HIV

It used to **detect and quantify** RNA

Total RNA or mRNA is first transcribed into complementary DNA (cDNA). The cDNA is then used as the template for the quantitative PCR or real-time PCR reaction (qPCR).

Ct measure is the measurement taken during the linear phase whereby the reaction crosses the **threshold** limit



PCR and RT-PCR = endpoint reactions

RT-qPCR = rate of product synthesis during the PCR reaction to quantitate the amount of template present.

ELISA (Enzyme-linked Immunosorbent Assay)

1. **Coating/capture**—direct or indirect immobilisation of antigens to the surface of polystyrene microplate wells
2. **Plate blocking**—addition of irrelevant protein or other molecule to cover all unsaturated surface-binding sites of the microplate wells
3. **Probing/detection**—incubation with antigen-specific antibodies that affinity-bind to the antigens

Commonly
used to
screen for
HIV



Not sensitive
during initial 3 to 4
weeks of
infection. Positive
occurs **22 to 26**
days after
infection

ELISA Generations

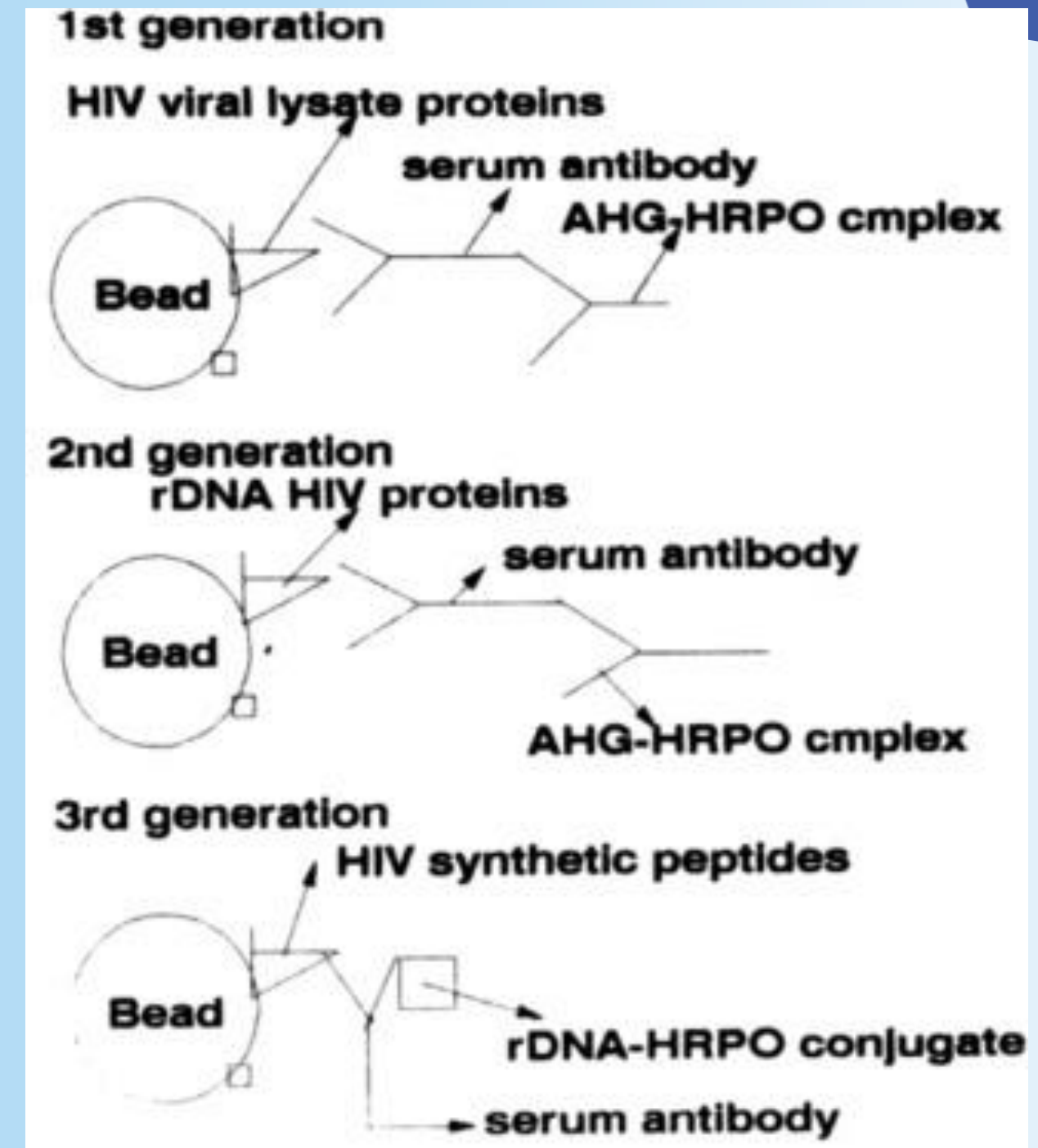
1st Generation - viral lysate antigens that frequently had non-specific reactions between the antibodies and the cell antigens

2nd Generation - incorporated recombinant and/or synthetic peptide antigens

***3rd Generation** - detects HIV antibodies

***4th Generation** - detects both p24 and HIV antibodies

*In the present day we mostly use 3rd and 4th generation ELISA

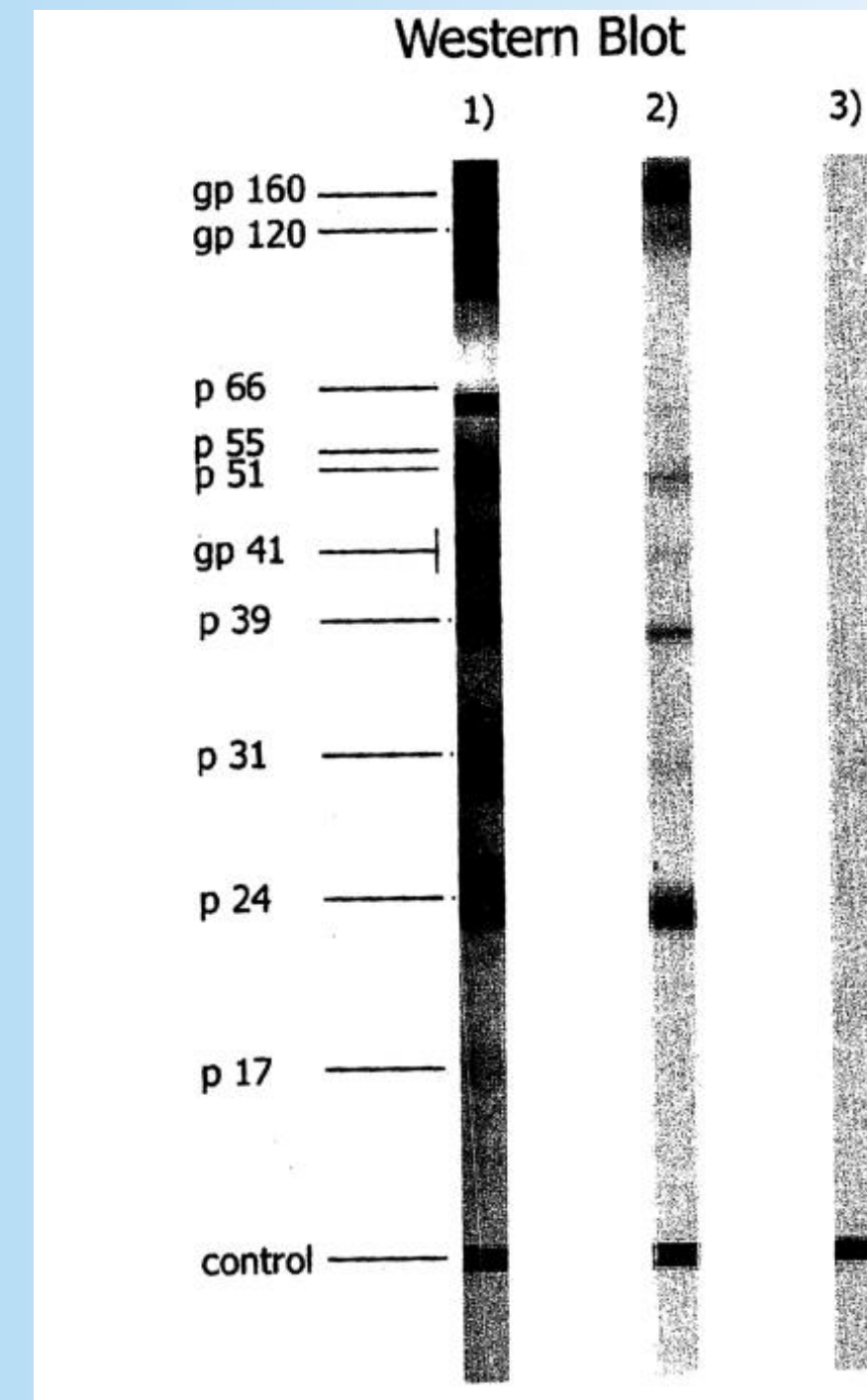


Western Immunoblotting

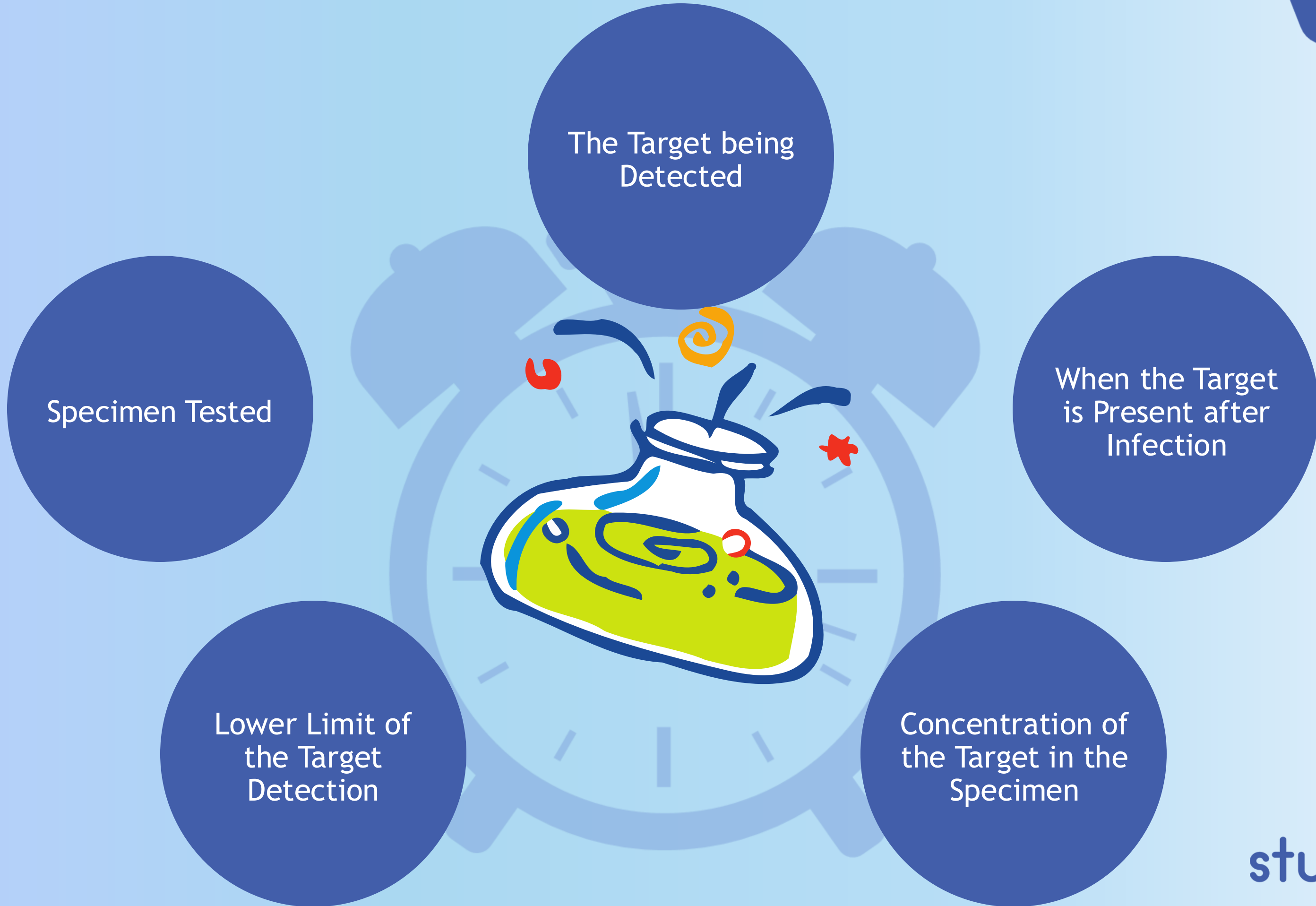
1. 'Blotted' protein antigen
2. Patient samples for antibodies is directed against the antigens
3. if antibodies are present, they will bind to the antigen
4. To detect if antibodies are bound, anti-IgG is coupled to a reporter, and after excess secondary antibody is washed free
5. substrate added with conjugate, resulting in visible band where the primary antibody is bound to the antigen

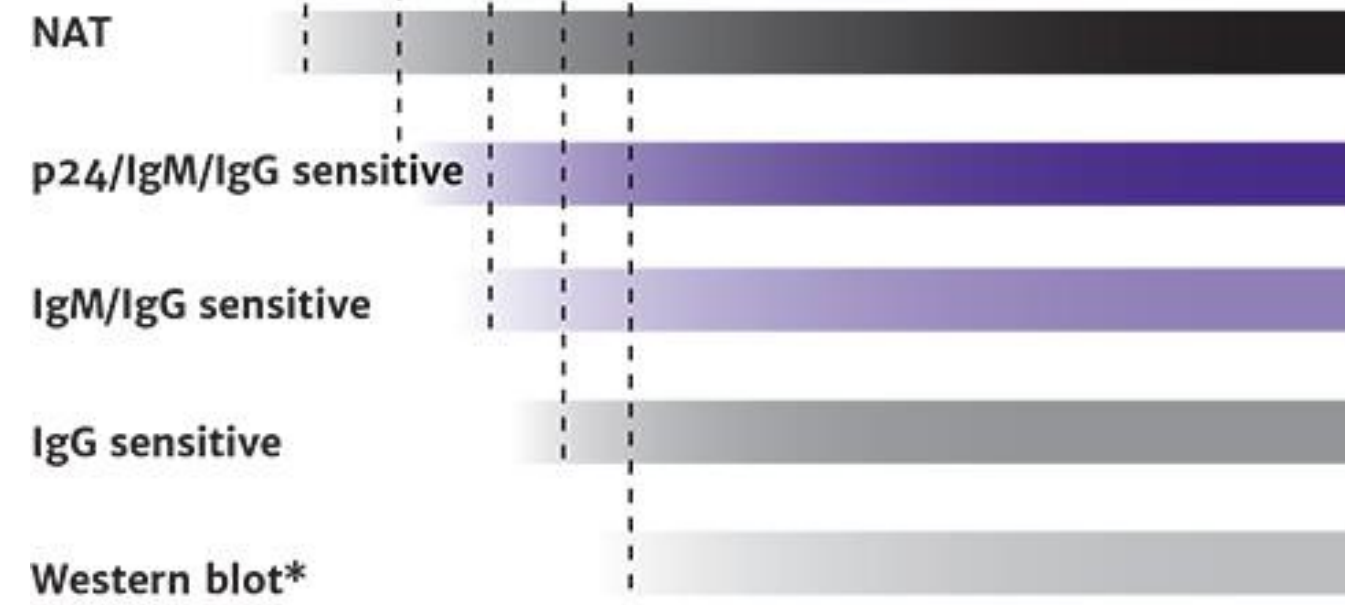
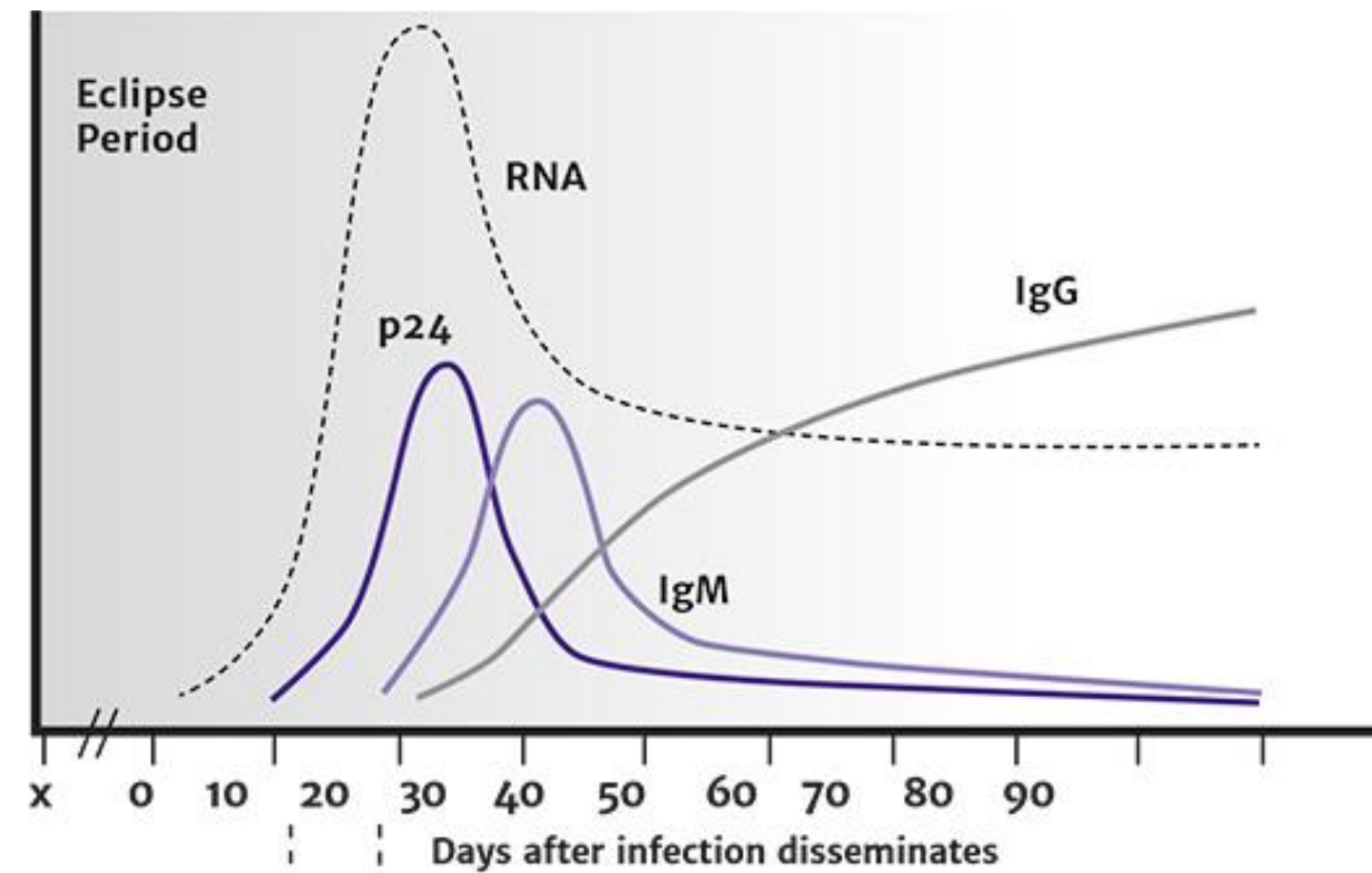
If positive, it is confirmed by the presence of at least two HIV viral envelope proteins (gp160, gp120 and gp41) and p24

Western Blotting confirms HIV infection, it is more specific than ELISA



Time to Reactivity





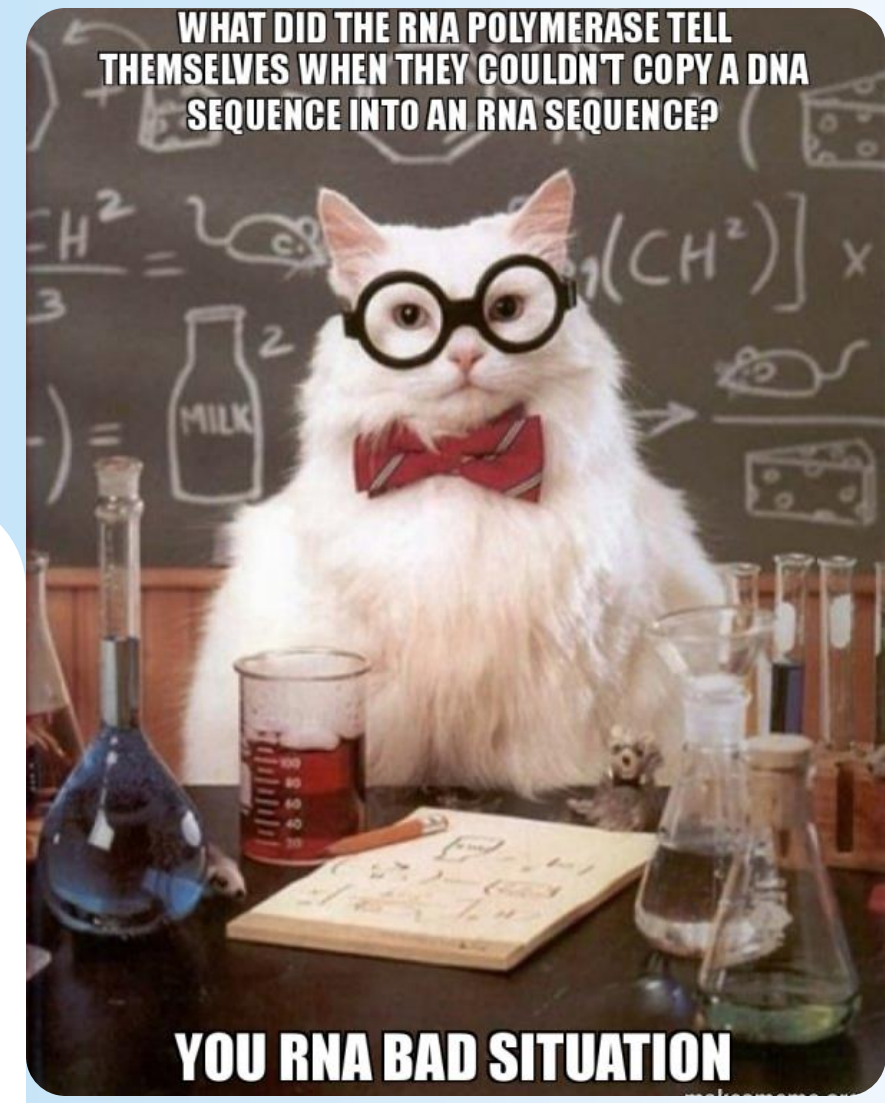
QUIZ TIME!

- 1 Go to wooclap.com
- 2 Enter the event code in the top banner







Event code
GDFHDM



Thank you and Good Luck!



Western Blot Protocol

 <p>Sample extraction</p>	 <p>Run Gel</p>	 <p>Transfer proteins to membrane</p>
 <p>Antibodies incubations and washes</p>	 <p>Fail to visualize protein bands</p>	 <p>Cry</p>