BHARATHIDASAN UNIVERSITY

Tiruchirappalli- 620024,
Tamil Nadu, India

Programme: M.Sc., Biomedical science

Course Title : Molecular medicine

Course Code : BM48C16M

Unit - II

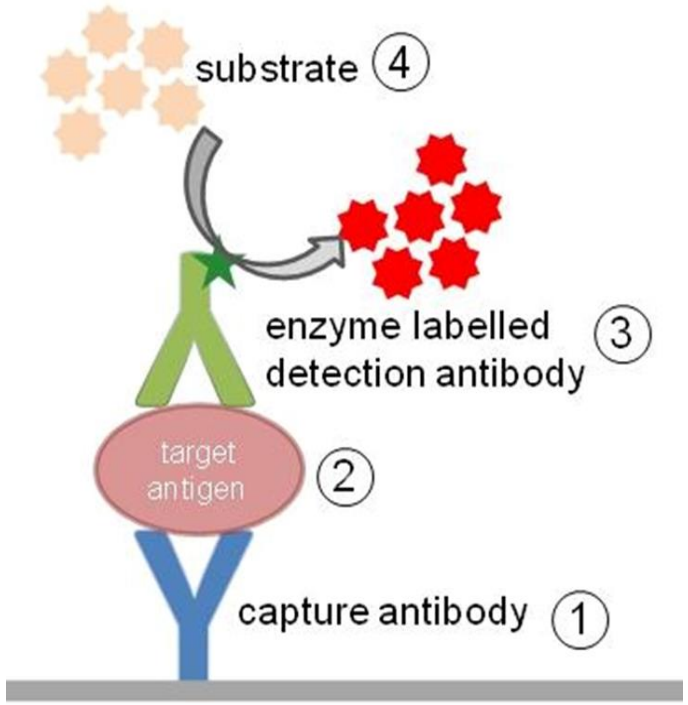
TOPIC: ELISA AND NEXT GENERATION SEQUENCING

Dr. A. S. VIJAYAKUMAR
Guest lecturer
Department of Biomedical Science

ELISA

- ELISA (enzyme-linked immunosorbent assay) is a plate-based assay technique designed for detecting and quantifying peptides, proteins, antibodies and hormones. In an ELISA, an antigen must be immobilized to a solid surface and then complexed with an antibody that is linked to an enzyme. Detection is accomplished by assessing the conjugated enzyme activity via incubation with a substrate to produce a measurable product.
- The most crucial element of the detection strategy is a highly specific antibody-antigen interaction.
- ELISAs are typically performed in 96-well (or 384-well) polystyrene plates, which will passively bind antibodies and proteins. It is this binding and immobilization of reagents that makes ELISAs so easy to design and perform.

- Having the reactants of the ELISA immobilized to the microplate surface makes it easy to separate bound from non-bound material during the assay. This ability to wash away nonspecifically bound materials makes the ELISA a powerful tool for measuring specific analytes within a crude preparation.
- Unless you are using a kit with a plate that is pre-coated with antibody, an ELISA begins with a coating step, in Which the first layer, consisting of a target antigen or antibody, is adsorbed onto a 96-well polystyrene plate. This is Followed by a blocking step in which all unbound sites are coated with a blocking agent.
- Following a series of Washes, the plate is incubated with enzyme-conjugated antibody. Another series of washes removes all Unbound antibody. A substrate is then added, producing a colorimetric signal. Finally, the plate is read .



PRINCIPLE:

In ELISA various antigen-antibody combinations are used always including at enzyme labeled antigen or antibody and enzyme activity is measured colorimetrically the enzyme activity is measured using a substrate that changes color when modified by the enzyme. Light absorption of the product formed after substrate addition is measured and converted to numeric values .

DEFINITION :

ANTIBODY : gamma globulin proteins that are found in blood and are used by the immune system to identify and neutralize foreign objects such as bacteria and viruses.

ANTIGENS : a substance that when introduced into the body stimulates the production of an antibody

IMMUNOASSAY : A laboratory technique that makes use of the binding between the antigen and its homologous antibody in order to identify and quantify the specific antigen or antibody in the sample

TYPES :

- DIRECT ASSAY
- INDIRECT ASSAY
- SANDWICH ASSAY
- COMPETITIVE ASSAY

DIRECT ELISA :

- For direct detection, an antigen coated to a multi-well plate is detected by an antibody that has been directly conjugated to an enzyme.
- This detection method is a good option if there is no commercially .

Advantages

- Quick because only one antibody and fewer steps are used . Cross-reactivity of secondary antibody is eliminated.

Disadvantages

- Immunoreactivity of the primary antibody might be adversely affected by labeling with enzymes.

Indirect ELISA :

- For indirect detection, the antigen coated to a multi-well plate is detected in two stages or layers. First an
- Unlabeled primary antibody, which is specific for the antigen, is applied. Next, an enzyme-labeled Secondary antibody is bound to the first antibody. The secondary antibody is usually an anti-species

SANDWICH ELISA :

- Sandwich ELISAs typically require the use of matched antibody pairs, where each antibody is specific for a different, non-overlapping part (epitope) of the antigen molecule. A first antibody (known as capture antibody) is coated to the wells. The sample solution is then added to the well. A second antibody (known as detection antibody) follows this step in order to measure the concentration of the sample. This type of ELISA has the following advantages:

This type of ELISA has the following advantages :

High specificity: the antigen/analyte is specifically captured and detected

• Suitable for complex (or crude/impure) samples: the antigen does not require purification prior to measurement

• Flexibility and sensitivity: both direct or indirect detection methods can be used .

COMPETITIVE ELISA :

- The key event of competitive ELISA (also known as inhibition ELISA) is the process of competitive reaction between the sample antigen and antigen bound to the wells of a microtiter plate with the primary antibody.
- First, the primary antibody is incubated with the sample antigen and the resulting antibody–antigen complexes are added to wells that have been coated with the same antigen

- ✓ After an incubation period, any unbound antibody is washed off. The more antigen in the sample, the more primary antibody will be bound to the sample antigen.
- ✓ Therefore, there will be a smaller amount of primary antibody available to bind to the antigen coated on the well, resulting in a signal reduction.

Advantage

- A wide variety of labeled secondary antibodies are available commercially.
- Versatile because many primary antibodies can be made in one species and the same labeled

Disadvantages

- Cross-reactivity might occur with the secondary antibody, resulting in nonspecific signal.
- An extra incubation step is required in the procedure.

APPLICATIONS :

ELISA (Enzyme-Linked Immunosorbent Assay) is a widely used laboratory technique primarily applied in immunology to detect the presence of antibodies, antigens, proteins, and glycoproteins in biological samples. Here are some common applications of ELISA:

- **Medical Diagnostics**
  - Infectious Diseases
  - Autoimmune Disorders
  - Allergy Testing
- **Vaccine Development**
- **Research Applications**
  - protein quantification
  - biomarker detection
  - gene expression studies
- **Food Industry**
  - food safety
  - meat speciation
- **Environmental Monitoring**
  - pollutant detection
  - pathogen detection in water
- **Drug Testing**

NEXT GENERATION SEQUENCING (NGS) :

- Next-generation sequencing (NGS) is a new technology used for DNA and RNA sequencing and variant/mutation detection.
- NGS can sequence hundreds and thousands of genes or whole genome in a short period of time.
- The sequence variants/mutations detected by NGS have been widely used for disease diagnosis, prognosis, therapeutic decision, and follow up of patients.
- The capacity of its massive parallel sequencing offers new opportunities for personalized precision medicine

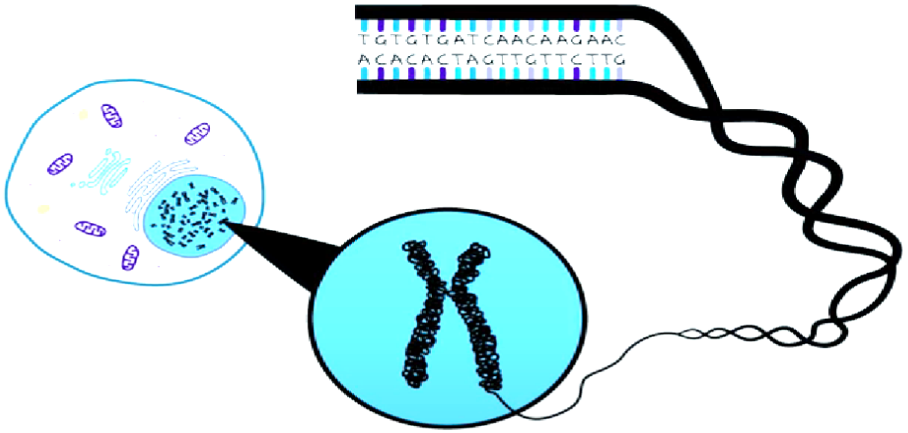
- NGS1-4 is a new technology for DNA and RNA sequencing and variant/mutation detection. This technology combines the advantages of unique sequencing chemistries, different sequencing matrices, and bioinformatics technology. Such a combination allows a massive parallel sequencing of various lengths of DNA or RNA sequences or even whole genome within a relatively short period of time. It is a revolutionary sequencing technology after Sanger sequencing.

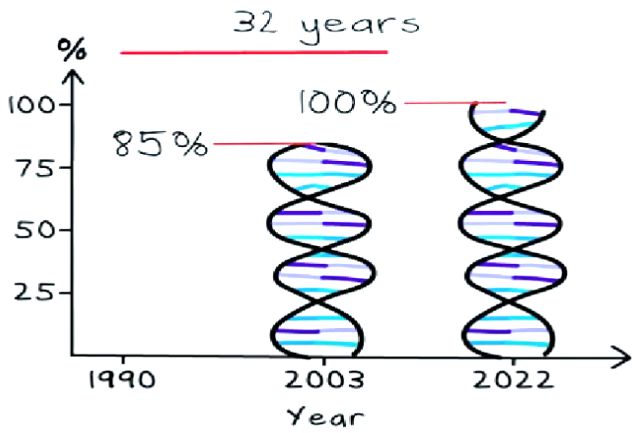
- NGS involves several major steps in sequencing. For example, DNA NGS involves DNA fragmentation, library preparation, massive parallel sequencing, bioinformatics analysis, and variant/mutation annotation and interpretation.

Next Generation Sequencing (NGS)

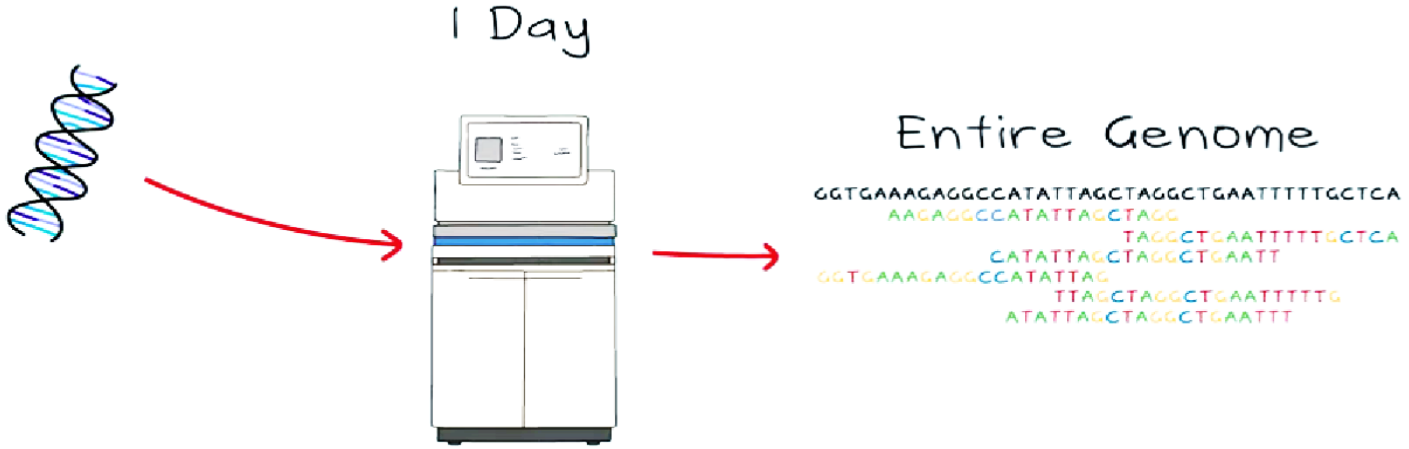
The Human Genome Project

3.2 Billion Bases





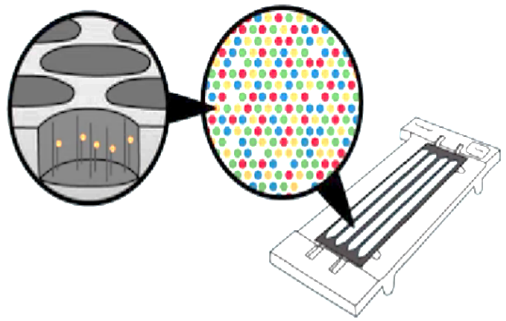
Subtitles/closed captions (c)



Number of DNA Strands Sequenced

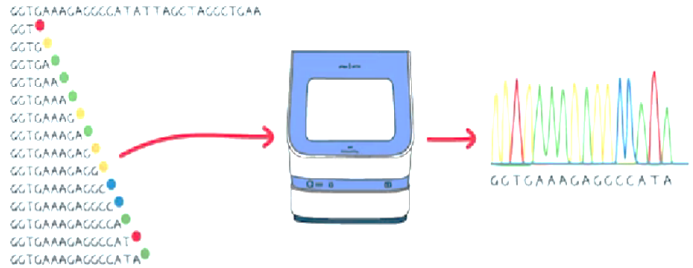
NGS

Billions of Strands



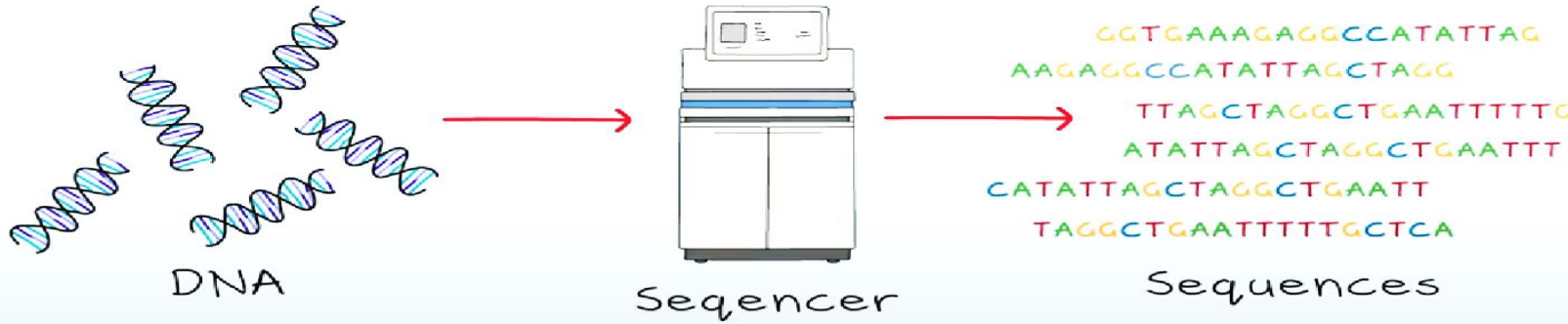
Sanger Sequencing

One Strand

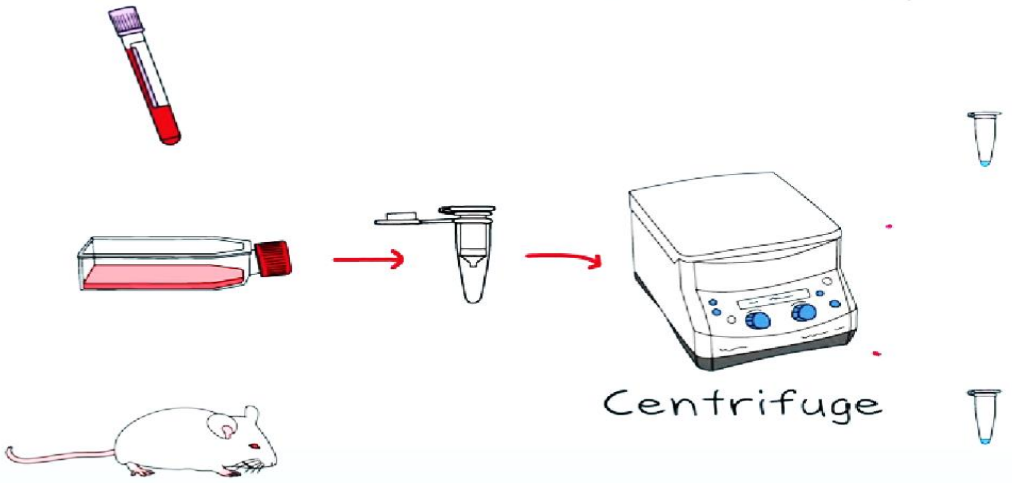


Human Genome Project → Human Reference DNA

..... GGTGAAAGAGGCCATATTAGCTAGGCTGAATTTTTGCTCA .....

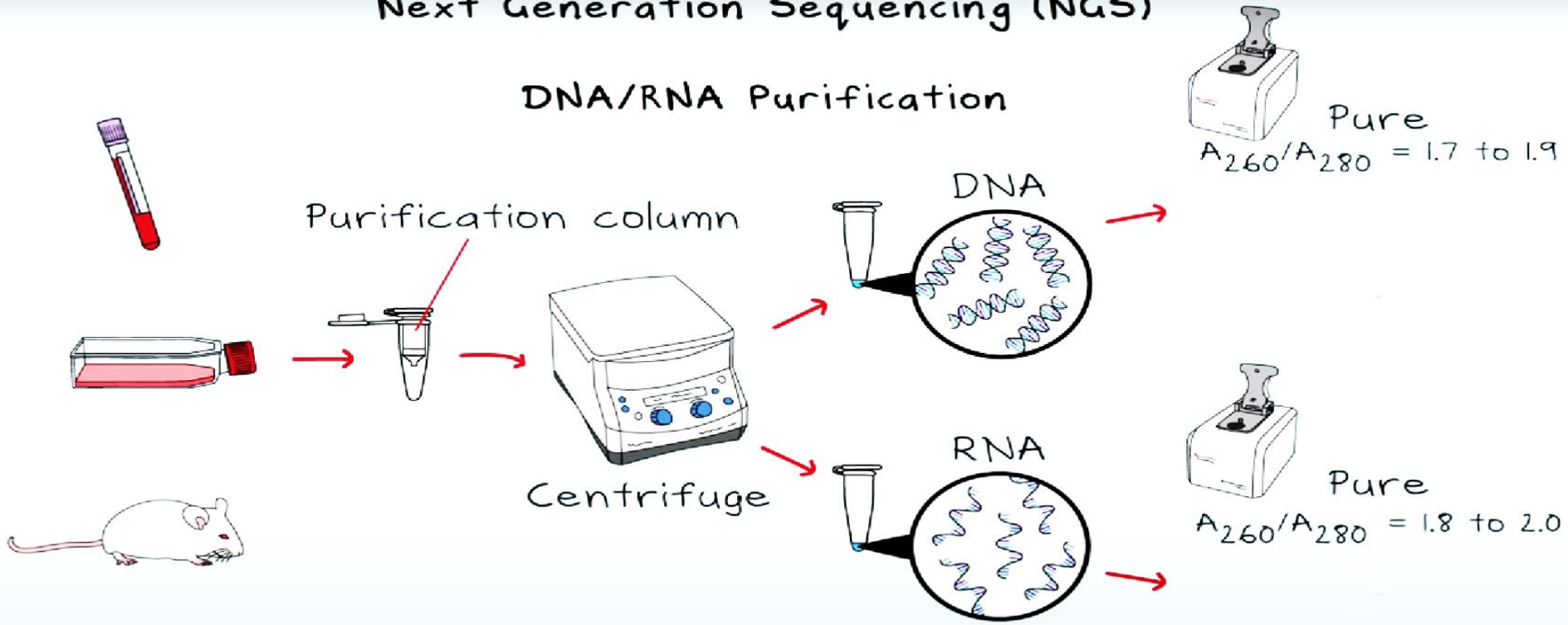


DNA/RNA Purification



Next Generation Sequencing (NGS)

DNA/RNA Purification



- Sample preparation
- Amplification
- Sequencing
- Data analysis
- Interpretation

1. Sample Preparation

•**DNA/RNA Extraction:** The first step is extracting DNA or RNA from the biological sample.

•**Library Preparation:** The extracted DNA/RNA is fragmented into smaller pieces and adapters (short DNA sequences) are added to both ends of these fragments.

2. Amplification (Optional)

•**PCR Amplification:** In some platforms, the DNA fragments are amplified using PCR to create multiple copies of the fragments, which enhances detection sensitivity.

3. Sequencing

•**Cluster Generation:** The DNA fragments are attached to a solid surface, such as a flow cell, and amplified to form clusters of identical DNA strands.

•**Sequencing by Synthesis (SBS):** In this step, nucleotides (A, T, C, G) are incorporated one by one, and the sequence is determined by detecting the fluorescent signal emitted when each nucleotide is added.

4. Data Analysis

- **Raw Data Generation:** The sequencing instrument generates raw data in the form of short DNA sequences, known as reads.
- **Alignment:** The reads are aligned to a reference genome or assembled de novo (without a reference).
- **Variant Calling:** Differences between the sequenced data and the reference genome are identified.

5. Interpretation

- **Data Interpretation:** The final step is to interpret the identified variants in the context of the biological question being asked.

1.SAMPLE PREPARATION

DNA collection → DNA extraction

2.LIBRARY PREPARATION

DNA fragmentation → End Repair → Adaptor Ligation → Size selection → Amplification

3.SEQUENCING

4.DATA ANALYSIS

5. INTERPRETATION AND VALIDATION

Next Generation Sequencing Techniques

1. Second generation sequencing techniques
2. Third generation sequencing techniques
3. Fourth generation sequencing techniques

SECOND GENERATION SEQUENCING TECHNIQUES

- 454 sequencing or pyrosequencing
- Illumina (Solexa) HiSeq and MiSeq sequencing
- SOLID Sequencing
- Polony sequencing
- Massively parallel signature sequencing (MPSS)

THIRD GENERATION SEQUENCING TECHNIQUES

- Single molecule real time sequencing biosciences
- Heliscope sequencing

FOURTH GENERATION SEQUENCING TECHNIQUES

- DNA nanoball sequencing
- Nano pore DNA sequencing

APPLICATION :

Next generation sequencing has enabled researchers to collect vast quantities of genomic sequencing data. This technology has a plethora of applications, such as:

- diagnosing and understanding complex diseases
- whole-genome sequencing
- analysis of epigenetic modifications
- mitochondrial sequencing
- transcriptome sequencing understanding how altered expression of genetic variants affects an organism
- exome sequencing mutations in the exome are thought to contain up to 90% of mutations in the human genome, which leads to disease.
- DNA techniques have been used to identify and isolate genes responsible for certain diseases, and provide the correct copy of the defective gene known as 'gene therapy'.

Reference

- Neil Boonham, Jan Kreuze, Stephan Winter, René van der Vlugt, Jan Bergervoet, Jenny Tomlinson, Rick Mumford. (2014). “Methods in virus diagnostics: From ELISA to next generation sequencing”. Virus research, ELSEVIER, 186, 20-31.
- https://www.thermofisher.com/in/en/home/life-science/sequencing/sequencing-learning-center/next-generation-sequencing-information/ngs-basics/what-is-next-generation-sequencing.html
- Boonham, N.; Kreuze, J.; Winter, S.; Vlugt, R. van der.; Bergervoet, J.; Tomlinson, J.; Mumford, R. (2014). Methods in virus diagnostics: From ELISA to next generation sequencing. Virus Research. (Netherlands). https://doi.org/10.1016/j.virusres.2013.12.007

Thank you