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Short Communication

# **DNA Sequencing**

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# **INTRODUCTION**

Finding the precise nucleotide order of a particular DNA molecule is the process of sequencing. It is used to establish the sequence of a DNA strand's four bases, adenine (A), guanine (G), cytosine (C), and thymine (T).

The term "DNA sequencing" refers to a common laboratory procedure for figuring out the precise order of bases, or nucleotides, in a DNA molecule (1-5). The biological information that cells require to develop and function is encoded in the sequences of the bases, which are frequently referred to by the initial letters of their chemical names: A, T, C, and G (Chmielecki J et al., 2014).

# Description about the study

#### What are the steps in DNA sequencing?

- Sample preparation (DNA extraction)
- PCR amplification of target sequence
- Amplicons purification
- Sequencing pre-prep
- DNA Sequencing
- Data analysis

**Sample preparation (DNA extraction):** Since DNA is our starting point in this situation, we must first extract DNA. For it, DNA from plants, animals, microbes, plasmids, or the environment can be utilized.

We advise utilizing procedures that offer a high yield, such as proteinase K or spin-column DNA extraction, as the purity of DNA is the main issue in sequencing (Clarke J et al., 2009).

**PCR amplification of target sequence:** The most important stage in sequencing is amplification. Here, just the study-relevant DNA sequence or gene is amplified at first; all other DNA is eliminated. However, whole-genome sequencing

allows for the sequencing of an organism's whole genome (Onaga LA, 2014).

**Amplicon purification:** The words "PCR product" and "amplicons", which are DNA fragments produced by a PCR reaction, are frequently used interchangeably. Amplicons provide for greater signals during sequencing, which in turn allows for more confidence sequencing findings. Amplicons increase the number of copies of a particular DNA region of interest.

**Sequencing pre-preparation:** During DNA sequencing, sample pre-preparation is a very important step. The adaptor DNA sequences are ligated on both ends of the DNA during this stage. The primer anneals to the adapter in order to do amplification. Sequencing uses an amplification method similar to PCR, except the nucleotides are either radioactively or fluorescently tagged (Anger F et al., 1977).

High fidelity DNA polymerase and other essential components are introduced to the process along with it.

**DNA sequencing:** The sequencing machine is filled with the prepared reaction tubes. Denaturation, annealing, and extension happen all at once during the reaction in the sequencer. The signals generated during the reaction are captured in this case since we are utilizing labelled nucleotides.

The machine records the signals of each complementary nucleotide addition and sends the information to the computer (Xu M et al., 2009).

**Data analysis:** After the entire DNA has been sequenced, the data is recorded in a single, distinctive file format. The manufacturer's built-in software analyzed the data and compared it to the data that was already accessible.

The programmer compares the sequencing data with other information to identify variants and other mutations in a gene.

#### **Different methods of DNA sequencing**

- Maxam-Gilbert method
- Chain termination method
- Semi-automated method
- Automated method
- Pyro sequencing
- The whole-genome shotgun sequencing method
- Clone by the clone sequencing method
- Next-generation sequencing method

### CONCLUSION

The applications of DNA sequencing span a wide range of disciplines. Its use in the diagnosis of multigenic genetic diseases is the area of greatest hope. A valuable tool for prenatal and preimplantation genetic investigations is the current methodology.

According to current scientific thinking, NGS-like robust approaches are required for the creation of gene therapies and the notion of personalized medicine. To decrease the costs, time-duration, and mistake rate in the current genetic technology, greater optimization and innovations are needed.

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Not applicable.

## **CONFLICT OF INTEREST**

Author declares no conflict of interest.

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