IAB Sanger Sequencing

In order to complete our portfolio, IAB now offers Sanger sequencing – a reliable method long-term and widely used for analysis of primary structure of short DNA sequences, such as PCR amplicons. Fluorescent-labelled dideoxynucleotides (ddNTP) incorporated during DNA replication are read precisely one by one in sequencer using the principle of capillary electrophoresis. This method is cost-effective, readily available, and often preferred due to its well-established protocols.

Introduction

Sanger sequencing offers some notable advantages, including the capability to read sequences ranging from short (ones to tens of base pairs) to long DNA sequences of up to 1000 base pairs in a single sequencing reaction. It delivers highly accurate results with minimal errors. However, it is important to note that, unlike Next-Generation Sequencing (NGS), Sanger sequencing only sequences a single DNA fragment. While Sanger sequencing has its strengths, it also has limitations. Its throughput is relatively low, particularly when sequencing large amounts of DNA (such as whole genomes or multiple genes from a large number of organisms). It is also important to note that for short DNA fragments, non-specific binding can occur, leading to the generation of undesired replication products. Therefore, primer design is crucial, ensuring that the primers are located sufficiently upstream of the target region for sequencing.

To ensure the maintenance of sample quality from pick-up to data delivery, we offer free sample collection from your location to our facility.

IAB Expertise

The expertise developed by our laboratory team over the past years guarantees dependable results throughout the entire workflow, encompassing tasks such as isolation, primer design, PCR reactions, quality controls, and sequencing.

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Protocol

Sample

- Tissues or liquids (ready for isolation)
- Plasmid (with primers)
    - 500 ng/ µl in total volume 20 ul
- PCR product
    - 10 ng/ µl in total volume 20 µl
- Primers
    - 10 pmol/ µl in total volume 10 ul

Risk: *In case of short fragments non-specific binding can occur, therefore low amount of the desired product is produced. It is necessary to design primers far enough upstream of the sites targeted for sequencing.*

- Quality Control (DNA)
    - Purity
    - Quantity
    - Integrity
- Primers
    - Forward
    - Reverse
- Normalization of the DNA samples
    - Cleaning (removal of unwanted dNTPs and primers from PCR products)
- PCR
- Ethanol purification
- Sanger Sequencing (Fig 1)
- Data analysis

Figure 1

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Results

The results of our Sanger sequencing analysis are presented as ".ab1" chromatogram files (see Fig 2), which provide highly accurate determination of nucleotide positions in the sequenced region.

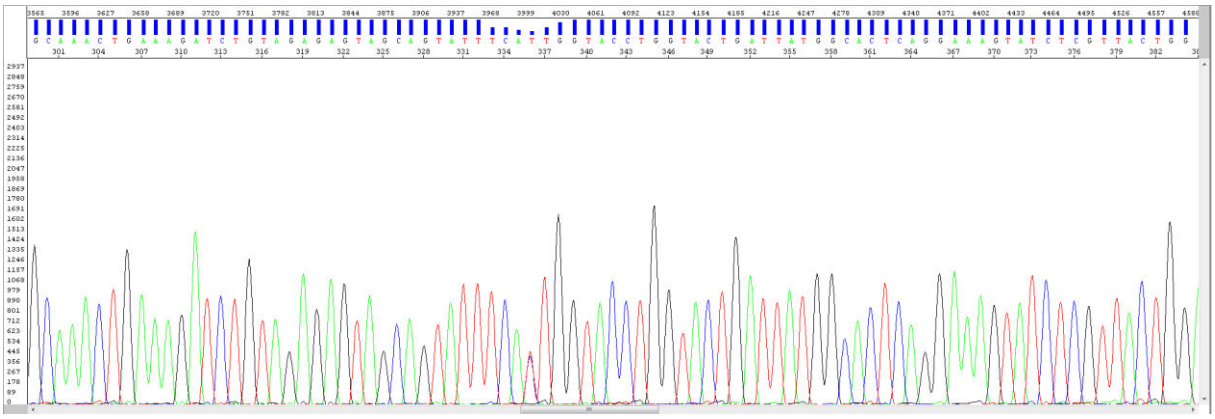

Figure 2

Conclusions

Both NGS and Sanger sequencing have wide applications in research and clinical disciplines. Sanger sequencing is primarily used when sequencing a small number of DNA fragments with a straightforward primer design along with high demands on data accuracy.

By offering Sanger sequencing as a complementary service to our NGS offerings, we aim to provide comprehensive solutions for diverse sequencing needs. Please reach out to our team for further information or to discuss your specific project requirements.

About IAB

The experienced team follows standardized IAB workflow with strict quality management to perform the essential QC steps on your samples and generate reliable and repeatable results.

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