Certified Specialist Programme in Next-Generation Sequencing

Sequencing Chemistry and Platforms

Sequencing Chemistry and Platforms are fundamental concepts in the field of Next-Generation Sequencing (NGS). Understanding these concepts is crucial for anyone looking to gain expertise in NGS technology. In this explanation, we will explore key terms and vocabulary related to Sequencing Chemistry and Platforms in the context of the Certified Specialist Programme in Next-Generation Sequencing.

Sequencing Chemistry refers to the chemical reactions that occur during the sequencing process, which enable the detection and decoding of nucleotides in a DNA or RNA sample. The two most commonly used sequencing chemistries are Sanger sequencing and Next-Generation Sequencing (NGS) chemistry.

Sanger sequencing is a method of DNA sequencing that was developed in the late 1970s by Frederick Sanger. It is a chain-termination method that involves the synthesis of a DNA strand using a DNA polymerase enzyme, dNTPs, and a primer. The process is repeated multiple times with different ddNTPs (dideoxynucleotides) added to the reaction mixture, which terminate the synthesis of the DNA strand at different points. The resulting fragments are then separated by size using electrophoresis, and the sequence of nucleotides is determined by reading the fluorescent labels on the ddNTPs.

Next-Generation Sequencing (NGS) chemistry, on the other hand, is a more recent development that allows for the sequencing of large numbers of DNA or RNA molecules in parallel. There are several different NGS chemistries available, including Illumina, Ion Torrent, and Pacific Biosciences (PacBio) chemistries.

Illumina chemistry is based on the sequencing-by-synthesis (SBS) principle, which involves the detection of single nucleotides as they are added to a growing DNA strand. The process involves the sequential addition of four fluorescently labeled nucleotides to the reaction mixture, which are then detected using a laser and a camera. The sequence of nucleotides is determined by reading the fluorescent signals emitted by the labeled nucleotides.

Ion Torrent chemistry is based on the detection of hydrogen ions that are released during the polymerization of nucleotides. The process involves the sequential addition of nucleotides to a growing DNA strand, which is attached to a semiconductor chip. The hydrogen ions released during polymerization are detected using a ion-sensitive field-effect transistor (ISFET), which converts the hydrogen ion signal into an electrical signal. The sequence of nucleotides is determined by reading the electrical signals generated by the ISFET.

Pacific Biosciences (PacBio) chemistry is based on the detection of fluorescence emitted by nucleotides as they are incorporated into a growing DNA strand. The process involves the use of a polymerase enzyme that is attached to the bottom of a zero-mode waveguide (ZMW), which is a small optical device that confines light to a tiny volume. The ZMW allows for the detection of individual nucleotides as they are added to the growing DNA strand. The sequence of nucleotides is determined by reading the fluorescent signals emitted by the labeled nucleotides.

Sequencing Platforms are the physical devices used to perform sequencing reactions. The two main types of sequencing platforms are Sanger sequencing platforms and Next-Generation Sequencing (NGS) platforms.

Sanger sequencing platforms include capillary electrophoresis instruments such as the Applied Biosystems 3730xl DNA Analyzer. These instruments use capillary tubes filled with a gel matrix to separate DNA fragments by size. The DNA fragments are labeled with fluorescent dyes, and the sequence of nucleotides is determined by reading the fluorescent signals emitted by the labeled fragments as they migrate through the gel matrix.

Next-Generation Sequencing (NGS) platforms include instruments such as the Illumina HiSeq, Ion Torrent Personal Genome Machine (PGM), and Pacific Biosciences Sequel II. These instruments use different sequencing chemistries and detection methods to sequence large numbers of DNA or RNA molecules in parallel.

Illumina sequencing platforms use sequencing-by-synthesis (SBS) chemistry and a laser and camera to detect the fluorescent signals emitted by labeled nucleotides. The Illumina HiSeq is a high-throughput instrument that can generate terabases of data in a single run.

Ion Torrent sequencing platforms use semiconductor technology to detect hydrogen ions released during polymerization. The Ion Torrent Personal Genome Machine (PGM) is a compact and portable instrument that can generate megabases of data in a single run.

Pacific Biosciences sequencing platforms use a polymerase enzyme attached to a zero-mode waveguide (ZMW) to detect fluorescence emitted by labeled nucleotides. The Pacific Biosciences Sequel II is a high-throughput instrument that can generate terabases of data in a single run.

In summary, Sequencing Chemistry and Platforms are critical concepts in the field of Next-Generation Sequencing. Understanding the different sequencing chemistries and platforms available is essential for anyone looking to gain expertise in NGS technology. Sanger sequencing chemistry is a chain-termination method used for DNA sequencing, while Next-Generation Sequencing (NGS) chemistry involves the sequencing of large numbers of DNA or RNA molecules in parallel. Sequencing Platforms include Sanger sequencing platforms such as capillary electrophoresis instruments, and Next-Generation Sequencing (NGS) platforms such as the Illumina HiSeq, Ion Torrent Personal Genome Machine (PGM), and Pacific Biosciences Sequel II. Each platform has its strengths and limitations, and choosing the right platform for a particular application is essential for achieving accurate and reliable sequencing results.