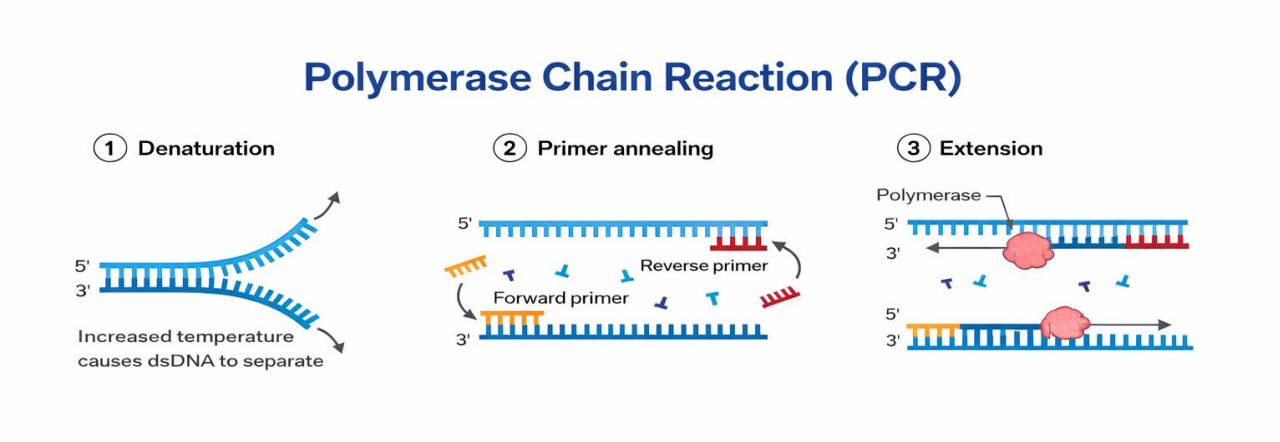
Molecular In My Pocket™

Molecular Techniques

In recent years, many molecular techniques have been widely accepted in the field of clinical diagnostics. These techniques include Polymerase Chain Reaction (PCR), Sanger Sequencing, and Next-Generation Sequencing (NGS).

Polymerase Chain Reaction (PCR) is a widely used method in the field of molecular biology with a wide variety of applications. During this process, a heat stable enzyme called Taq DNA polymerase and short nucleotide sequences called primers, that bind to specific DNA sequences, help amplify the DNA. The process is divided into 3 stages: Denaturation, Annealing, and Extension. Denaturation is a high-temperature environment that splits double-stranded DNA, annealing allows binding of primers to single-stranded DNA, and extension is the step where specific nucleotides are added, and a new double-stranded DNA is created. Each of these steps is repeated between 25-40 cycles to generate detectable amounts of amplified DNA. More evolved versions of PCR like Real-Time PCR (RT-PCR), Quantitative PCR (qPCR), nested PCR, multiplex PCR, and digital PCR (dPCR) have wider applications in clinical diagnostics.



Real-Time Polymerase Chain Reaction: This technique allows real-time visualization of amplification using different molecular chemistries like fluorescent labeled oligonucleotides and DNA Binding Dyes. Real-Time PCR is used to detect specific targets and has applications to detect the presence or absence of any genetic mutation or pathogen.

Quantitative PCR (qPCR): As the name suggests, this variation of PCR is used to not only detect a certain target but also provide quantitation. A standard curve is generated using serial dilution of known concentration of the specific target, followed by using cycle threshold (Ct) value for the unknown sample in comparison to the standard curve to quantify the sample. Ct value is defined as the number of cycles required to generate detectable levels of fluorescence and amplified products. qPCR is widely utilized in infectious disease diagnosis allowing viral load detection for various sample types. Other applications include SNP analysis, genotyping, and gene expression studies.

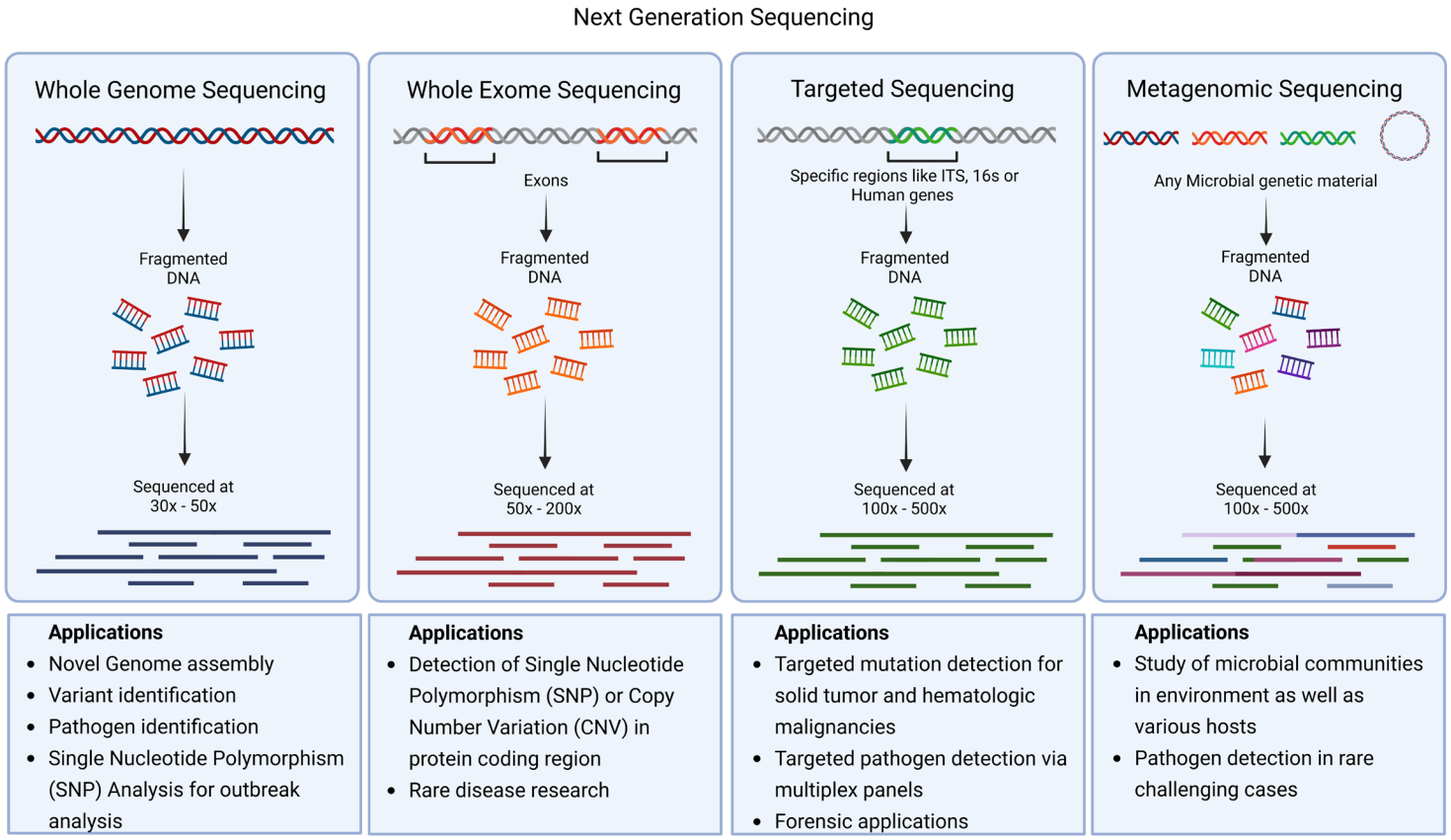
Nested PCR: This subtype of PCR uses 2 sets of primers along with an increased number of cycles for higher sensitivity as well as to identify low levels of any target. The first set of primers (outer primers) binds to a targeted sequence and amplifies a region which functions as a template usually located on the inner side for the second set of primers (nested primers). Using 2 sets of primers reduces the chances of mis-priming resulting in increased specificity. Nested PCR has applications in pathogen detection as well as identification of mutations related to genetic diseases.

Multiplex PCR: This is an advanced version of Real-Time PCR where multiple sets of primers (tagged with different dyes) are used within the same assay to detect presence or absence of various targets at the same time. This is a widely used technique in clinical laboratories for the detection of more than one target in one assay, reducing turnaround time as well as cost of testing. Many commercial kits are available for infectious disease diagnostics as well as oncology studies.

Digital PCR (dPCR): dPCR is a PCR variation for absolute quantification of nucleic acid concentrations through division of input sample and reagents to generate micro reactions that theoretically only contain one copy of DNA. These micro reactions are read individually to detect the presence or absence of a specific target. Applications of dPCR include low level pathogen detection, detection of rare mutations (cancer diagnostics), as well as copy number variation analysis.

Sanger Sequencing: Sanger sequencing is a first-generation sequencing technique and is also known as the "chain termination method". This chemistry includes fluorescent ddNTPs (Dideoxyribonucleoside triphosphates) insertion during PCR reaction to generate millions of copies of random length sequences that can no longer add nucleotides to the sequence. Generated oligonucleotides with various length sequences are separated using gel electrophoresis through capillaries set in the genetic analyzer instrument. The genetic analyzer instrument detects the end nucleotides using excitation and fluorescence detection to provide the nucleotide sequence.

Next-Generation Sequencing (NGS): NGS is also referred to as Massively Parallel Sequencing as it allows many strands of DNA to be read and sequenced simultaneously. There are few chemistries that have been utilized till now, like Sequencing by Synthesis, Ion Semiconductor, and Nanopore. Different types of chemistry can be used for different applications. Types of NGS and their applications are explained in the figure below.



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