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ISSN: 2153-0602
Journal of
Data Mining in
Genomics & Proteomics

Perspective

The Principles and New Approaches in Nested Polymerase Chain Reactions

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DESCRIPTION

Polymerase Chain Reaction (PCR) is a technique that allows the amplification of specific regions of DNA (Deoxyribonucleic Acid) using a temperature-mediated DNA polymerase enzyme. PCR is widely used in genetics research, DNA fingerprinting, molecular diagnostics, and other applications that require the analysis or manipulation of DNA. However, PCR can also produce unwanted products due to the non-specific binding of primers to unintended regions of the DNA template. This can result in false-positive results, reduced sensitivity and specificity, and interference with downstream analysis. To overcome this problem, nested PCR was developed as a modification of PCR that aims to reduce non-specific binding and increase the yield of the desired product.

Nested PCR involves two sets of primers and two successive rounds of PCR. The first set of primers is designed to anneal to sequences flanking the target region of interest. The first round of PCR is performed for a low number of cycles (usually 15 to 30) to generate a primary product that contains the target region and some additional sequences on both ends. The primary product is then used as a template for a second round of PCR using a second set of primers that anneal to sequences within the target region. The second round of PCR is performed for a higher number of cycles (usually 25 to 35) to amplify the secondary product that corresponds exactly to the target region.

The advantages of nested PCR

- It increases the sensitivity of PCR by allowing more total cycles without increasing non-specific binding.
- It increases the specificity of PCR by verifying the identity of the primary product with the second set of primers.
- It reduces the background noise and interference from contaminants or inhibitors in the sample or reagents.

The disadvantages of nested PCR

- It requires more time, labor, and reagents than conventional PCR.
- It increases the risk of contamination and cross-contamination during the transfer of primary products to the second round tubes.
- It may introduce mutations or errors in the secondary products due to errors in DNA replication or primer annealing.
- To minimize these disadvantages, several variations and modifications of nested PCR have been developed, such as:
- **Hemi-nested PCR:** In this method, one primer in the second round is identical to one primer in the first round. This reduces the number of primers required and simplifies the design and optimization process.
- **Semi-nested PCR:** In this method, one primer in the second round overlaps with one primer in the first round. This increases the specificity and efficiency of amplification by reducing primer-dimer formation and extending primer annealing time.
- **Multiplex nested PCR:** In this method, two or more target regions are amplified simultaneously in a single reaction using multiple sets of primers. This allows simultaneous detection or identification of multiple pathogens or genes in a single sample.
- **Real-time nested PCR:** In this method, fluorescent probes or dyes are used to monitor the amplification process in real time using a specialized instrument. This allows quantification and validation of amplification products without post-PCR analysis.

Nested PCR is a powerful tool for molecular biology that can enhance the performance and reliability of conventional PCR. However, it also requires careful design, optimization, validation, and quality control to ensure accurate and reproducible results. Semi-nested PCR works on the same premise as nested PCR, except that one of the primers used in the second round of PCR is the primer used in the first round of PCR. Semi-nested PCR refers to the process of repeating PCR amplification with three templates twice. Reverse transcription nested PCR (RT-nested PCR): Based on RT PCR, RT-nested PCR was produced. The target gene is amplified using nested PCR using cDNA acquired through reverse transcription. It is similar to basic reverse

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Received: 03-Mar-2023, Manuscript No. JDMGP-23-20247; **Editor assigned:** 06-Mar-2023, JDMGP-23-20247 (PQ); **Reviewed:** 20-Mar-2023, QC No. JDMGP-23-20247; **Revised:** 27-Mar-2023, Manuscript No. JDMGP-23-20247 (R); **Published:** 03-Apr-2023, DOI: 10.4172/2153-0602.23.14.285

Citation: Bello K (2023) The Principles and New Approaches in Nested Polymerase Chain Reactions. J Data Mining Genomics Proteomics. 14:285.

transcription PCR in terms of determining whether or not a particular RNA is expressed or its proportional expression level, but it is more precise and dependable.