AUCTORES
Globalize your Research

New Medical Innovations and Research
Humaira Zafar *

Open Access

Letter to editor

Strategic Steps for Efficacious Primers Crafting to Accomplish Anticipated Analytical Upshots for Molecular Level Studies

Humaira Zafar [1]*, Irfan Ali Mirza [2], Muhammad Fayyaz [3]

[1] Professor of Pathology, Microbiologist, Al Nafees Medical College & Hospital, Islamabad, PhD Scholar-NUMS, Pakistan

[2] Professor of Pathology, Consultant Microbiologist, National University of Medical Sciences, Rawalpindi, Pakistan

[3] Assist Prof of Microbiology, Consultant Microbiologist, National University of Medical Sciences, Rawalpindi, Pakistan

***Corresponding Author:** Humaira Zafar, Professor of Pathology, Microbiologist, Al Nafees Medical College & Hospital, Islamabad, PhD Scholar-NUMS, Pakistan.

Received date: November 11, 2023; Accepted date: November 20, 2023; Published date: November 27, 2023

Citation: Humaira Zafar, Irfan Ali Mirza, Muhammad Fayyaz (2023), Strategic Steps for Efficacious Primers Crafting to Accomplish Anticipated Analytical Upshots for Molecular Level Studies, *J New Medical Innovations and Research*, 4(8); **DOI:10.31579/2767-7370/068**.

Summary:

Sir, the revolution in science and technology had brought the opening of many new horizons for accurate laboratory diagnosis. Ranking top amongst them are currently the molecular diagnosis. The identification of exact genomic characterization to reach the root cause of diagnosis is the reason imparting to their great significance in the field of medicine. As we know that field of medicine is almost the half deficient, without good pathological and radiological diagnosis. Therefore, the researchers around the Globe, besides paying attention to identify new vistas for management, are equally devoted to find accurate and rapid diagnostic modalities. Thus, simultaneous efforts are becoming fruitful to strengthen diagnostic and accurate management of patients.

The primers or genetic markers are the synthetic oligonucleotide sequences used for polymerase chain reaction (PCR). The selection, designing and application of primers is the main critical step in molecular diagnosis. The whole procedure and test is dependent upon these three steps. Therefore, it will never be wrong to stay that accurately designed primers will the one ensuring high sensitivity and specificity for amplification reaction, to get desired amplicon. [1]

The primer designing focuses the formulation of forward primers and reverse primers. The highlighted quality checkpoints for effective primer synthesis involves many significant points. [1,2] First one focusses the exact genomic target selection i.e whether deoxyribonucleic acid (DNA) or ribo nucleic acid (RNA) or proteins. Second one includes primer length. The literature supports that it should range between 18 – 24 nucleotide sequences. Reason being is that too short or even too long primers will result in dimerization. Thus, their effectiveness will be reduced to get exact amplification. Thirdly comes guanine: cytosine (G:C) bases content. To get the balanced and stable primer binding GC content must range between 40% to 60%. Fourthly comes identification of melting temperature for optimization. A careful eye watch should be there for adjusting to adjust within 2°C temperature either a case of forward or reverse primers. This fine adjustment will again support good specificity of primer binding during PCR. [1]

Next in sequence come the usage of data available on National center for bioinformatics (NCBI) regarding Fast all sequences (FASTA). This involves proteins and nucleotides i.e FAST-P and FAST-N. After getting the desired FASTA sequences for forward or reverse primers pertaining to relevant gene, primers generation can be running by using blast feature on NCBI. [3] The other primer designing software to strengthened their validation can be by using oligo calc and in-silico primer designing softwares. [4-5]

Either of these bio informatics tools, gathered information should address the primer properties like specificity, G:C content, dimerization, annealing and melting temperature. In view to avoid non-specific amplification, repetitive runs for same sequences should be avoided. Last not the least involves validation of primers along with positive and negative controls prior their usage in laboratory diagnosis. [1]

Beside considering all above points, due importance should be given to primer storage because that will safeguard their integrity, stability and specificity. Adding up to warrant effectiveness, high quality reagents and enzymes should be used to reduce chances of false positive or false negative results.[2]

The key to success for molecular level diagnostics i.e., various types of PCR is only and only dependent upon the accurate synthesis of intended primers. Considering in account all the above-mentioned details will be helpful in revitalizing accurate and reliable diagnostic outcomes. Therefore, on should be very meticulous in calculating and purifying the intended primer design and their usage

References

1. Delghandi M, Delghandi MP, Goddard S. (2022) The Significance of PCR Primer Design in Genetic Diversity Studies: Exemplified by Recent Research into the Genetic

Structure of Marine Species. *Methods Mol Biol*.;2392: 3-15.
2. Bustin SA, Mueller R, Nolan T. (2020) Parameters for Successful PCR Primer Design Methods *Mol Biol*.;2065:5-22.
3. National center for bioinformatics (NCBI).
4. Oligo calc primer designing tool.
5. Insilico primer designing software.

DOI:10.31579/2767-7370/068