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BASIC CONCEPTS OF PRIMER DESIGNING: A MINIREVIEW

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Abstract- Primer design is an important pre-requisite for many of the molecular biology experiments. It certainly requires using multiple databases and bioinformatics tools to identify sequence of interest. The essential features of 'good' primers need to be identified first before proceeding further for any molecular technique. Amplification of the target of interest is based on the fact that the primer matches specifically to the target and there is no non-specific amplification. The process of primer designing is not an easy process and essentially completes in two parts. Initially primers flanking the regions of interest are generated and then using tools such as BLAST these regions are searched to analyze the potential targets. The analysis is complex and time-consuming as number of technical observations is to be made and the problem becomes harder when large numbers of the primer hits are observed. Therefore, understanding the principles of primer designing are essential to tackle the possible troubleshoots. This review highlights thebasic fundamentals of primer designing.

Keywords: Primer, Blast, PCR

I. INTRODUCTION

Polymerase chain reaction (PCR) has become the preferred approach in the era of molecular biology. Primers are one of the constituents of reaction mix. Primers play an essential role in selecting the region of interest in the complex genome [1]. Primers or oligonucleotidesare short sequences of DNA or RNA complementary to a given DNA sequence at which replication can proceed as in a PCR cycle. Primers can also be used in variety of methods such as gene sequencing,amplified fragment length polymorphisms,single strand confirmation polymorphisms etc.[2]. The most critical step in any of these techniques is designing primers as poorquality primers give little or no PCR product. Sometimes it canalso yield unwanted amplified DNA fragments. This may interfere in subsequent applications such as gene sequencing etc. Primer designing requires extensive computer-based analysis of gene sequence and therefore use of various platforms such as NCBI etc. is recommended.

II.PRIMER DESIGNING

Primer designing is a critical step for successful PCR reaction. Designing of primers can be performed by using NCBI "Primer-Blast" tool apart from various other online tools such as Gene fisher, DoPrimer, primer 3, web primer etc [3]. Primer-Blast is an easily available user-friendly tool for designing primers. The complete program comprises of instructional modules guiding how to generate primer pairs and for assessing target specificity of generated primer pairs. In Primer-Blast, Primer3 is used to generate candidate primer pairs for a given sequence. The specificity check is done in default by Blast search that looks for primers-targets matches. New primer pairs are designed by entering DNA template that can be the raw DNA template in FASTA format or a NCBI refseq accession number. The steps followed for designing primers are depicted in figure 1. At the NCBI website, the "gene" is selected from the resources tool and name of the gene is entered. From the search results, gene (homo sapiens/other species)" is selected. The next page shows the details of the target gene such as summary, genomic region, transcripts and products. In the heading of "genomic sequence", the "RefSeqGene" is clicked in the dropdown. Next, gene sequence is given with locations of exons/introns. The exon/intron of interest is selected and "Primer Blast" option is selected by right clicking on selected region. The selection opens the Primer-Blast

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submission form that shows the RefSeq accession number. Next, in section of primer parameters, PCR product size, numbers of primers with melting temperatures are entered. In the section of primer pair specificity checking parameters, appropriate data base ("Primary Genome Assembly") is selected. Finally, "Get Primer" button is clicked to submit search. Next page gives the graphical view and detailed primer reports from where the appropriate primers are selected based on guidelines.

There is another method for primer designing in the same tool. After getting the gene summary as described earlier, same page can be used to click the option "NCBI Reference Sequence" on the right side. Next page shows the NCBI Reference Sequence (RefSeq) details. In the section genomics, NG_012840.1 "RefSeqGene-sequence viewer (Graphics)" is clicked. Subsequent page gives gene sequence on the corresponding chromosome. On the right side of the same page, the option "Highlight Sequence Features" is clicked. The details of gene sequencesuch as exons, mRNA, (non-coding) ncRNA, CDS (coding sequence), STS (sequence tagged sites) etc. can be scanned. Left lower corner shows the dropdown options for the highlighting the same. Region of interest such as "exon/intron" is clicked. Next, target regions are highlighted on the gene sequence. One or more target sequences (FASTA FORMAT) are chosen from each sequence. Primer-Blast submission form is opened by clicking the "Primer-Blast" on the NCBI home page. Now, the chosen target sequences are pasted in the section "PCR Template". Other details are filled as described earlier. Next "Get Primer" button is clicked to submit the search and to receive template and specificity information. Finally, the designed primer pairs are shown and are chosen based on the selection criteria that are discussed in the subsequent section.

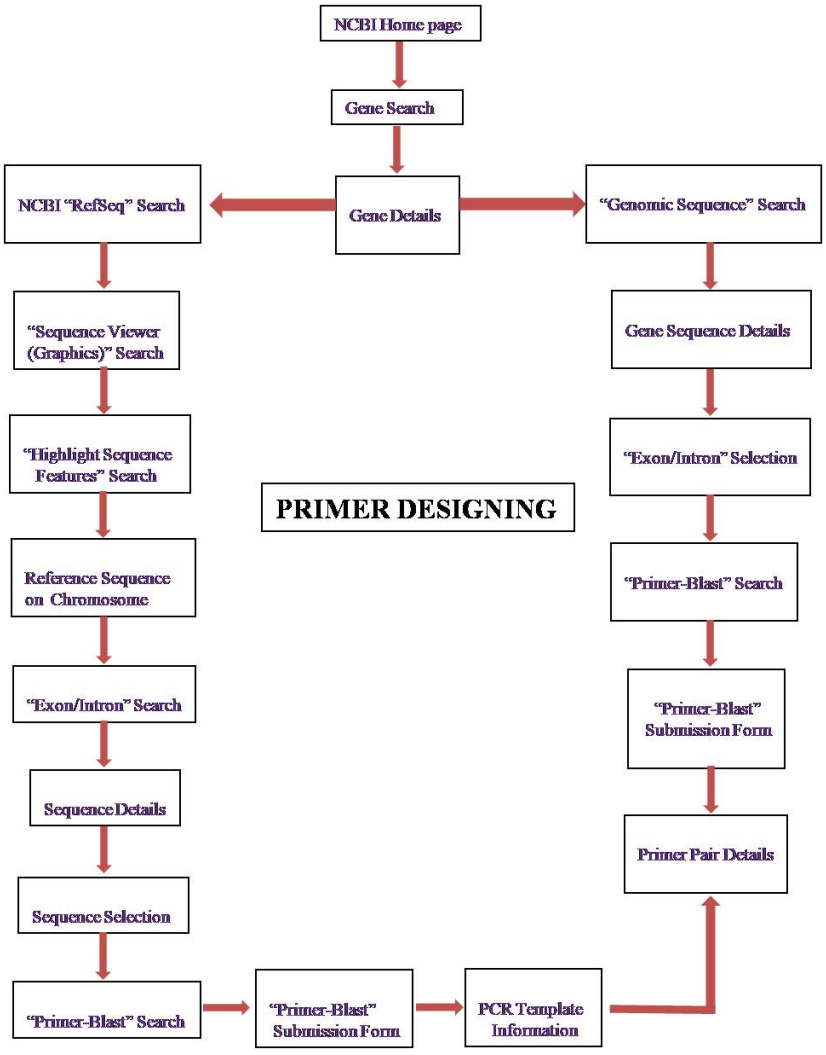

Figure 1: Flow Chart Showing Steps of Primer Designing

III.SELECTING A GOOD PRIMER DESIGN

A good primer amplifies the selective region of interest in the complex genome. There are certain basic criterias to examine the primer quality. **Specificity** of primers enables a PCR cycle to amplify region of interest out of a complex mixture of DNA. A primer is said to be specific if it is complementary to the sequence of region of interest and not to many non-target regions of the genome. BLAST is a common tool to check for non-specific binding using primers sequences as query. For a standard PCR, **primer length** of 18-24 nucleotides is ideal. This size is specific to the target region and most commonly annealing at 50 - 65°Cis done efficiently. Annealing temperature (Ta) is the temperature at which the maximum amount of primer is bound to its target sequence. Ta depends on the primer length and if it comes below 50°C then it is better to exclude that primer. **Melting temperature (**Tm) is the temperature at which dsDNA becomes ssDNA. Primers with Tm 52-60°C bring better results. The ideal and most acceptable Tm difference between primer pair should be within 5°C [4]. Ta is generally set at 2-5°C below the Tm. Designing of too short primers (of 10 nucleotides) should also be avoided as their Ta is lower (36 - 40°C) that compromises specificity of the PCR product [5]. **Primer Dimers** are the products of hybridization of complimentary bases in the primers. **G/C Content** between 40-60% is essential for stable binding between the primer and its template DNA. It is the percentage ratio of number of G and C nucleotides and the total nucleotides. Primer should (preferably) terminate with either a 'G' or 'C' residue preceeded by a pyrimidine base, termination with double purines ('GG'; 'GC' or 'CC') is acceptable but avoid terminating with 'T' residues and > 2 G/C residues in last 3' bases and If primer must end in 2-3 GC residues then terminate with an 'A' residue [4].

IV.CONCLUSIONS

Primer designing is the single most critical step of any PCR assay as the specificity and sensitivity of a good primer makes this method the most powerful. A weak primer design will cause reduced technical precision with false positive or negative detection of amplified targets. There are numerous publications and information available still chances of designing low yield primer do exist. More the time and intelligent efforts given for primer designing, lesser will be the post-assay troubleshoots. Primers are to be identified based on certain parameters such as specificity, primer length, extent of dimerization, Tm, G/C content etc. The ideal sets of primers should have optimal length, GC content between 40-60%. The primers that show undesirable self-hybridization are not selected as such primers will be unavailable for hybridization to the template. The difference in Tm of reverse and forward primers should not be more than 5°C. Finally,selected good primer designs only are sent to the laboratories forsynthesis. Thus, awareness regarding primer designing will subsequently increase the competence and output of the molecular labs.

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