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Primer Server

A web application to design primers for the amplification of unique DNA targets in complex genomes

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Abstract

Polymerase Chain Reaction (PCR) is one of the most important inventions of the 20th century in molecular biology. PCR is a technique to amplify or make in a test tube many copies of a specific DNA region. Miniscule amounts of the genetic material from any organism can now be amplified to identify individuals, manipulate DNA, detect infectious organisms including the viruses that cause AIDS, hepatitis, and tuberculosis, detect genetic variations including mutations in genes, and numerous other tasks.

PCR primers are short, single-stranded DNAs that define the section of DNA to be amplified. Two primers are used in each PCR reaction, designed so that they bind at flanking locations surrounding the target region. Critically, off-target binding may lead to experimental failure or worse, to misleading results. Thus, potential primers of approximately 20 DNA bases in length, must be examined for off-target binding among, for example, the 3.2 billion DNA bases from all human chromosomes, the human genome.

The purpose of our study is to make a user-friendly tool (**Primer Server**) that can design PCR primers efficiently and accurately as well as visualize the designed primers. Our web-based bioinformatics tool selects optimal primer sequences within the starting material by using a C module called primer3 and then prioritizing and/or eliminating potential primers based on comparison of the primer bases against all bases in the genome using an algorithm called BLAST. This tool has an easy-to-use interface which was designed using Angular2, and an efficient server-side code written in Python. While similar tools exist, our tool is more user-friendly, efficient and uses extensive form validation to minimize errors in the user input. Our tool can be used to design primers that will be used in laboratory experiments to amplify DNA from various organisms, including large, complex genomes such as humans, other animals and plants.

Goals

Develop a user-friendly tool that can design PCR primers, filter non-specific primers, and visualize the specific primers

Why develop new one?

	Primer3 Plus	Primer-BLAST	Primer Server
Is it open source?	☑	☑	☑
Is a web tool available?	☑	☑	☑
Does it have an easy-to-use user interface?	☒	☒	☑
Does the web tool use latest technologies? CGI/plain HTML vs. Python/Angular	☒	☒	☑
Can the user run their own instance of server?	☒	☒	☑
Can it filter for good primers?	☒	☑	☑
Can the user use their own genome to filter primers?	☒	☒	☑
Is this tool available to use in terminal?	☑	☒	☑

How to accurately find primers (primerDAFT)

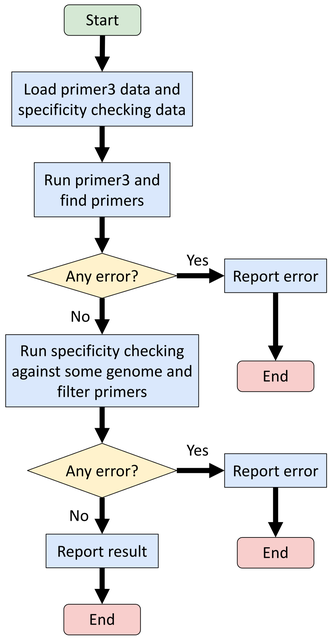


Figure 1: *Flow chart showing how the logic works*

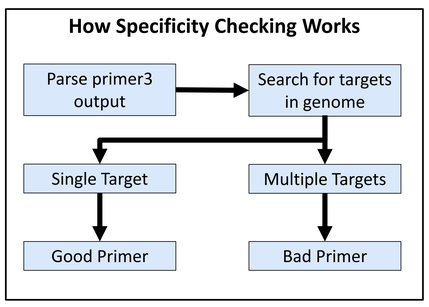
There are two main steps

1) Primer Design Step

The program runs primer3, C module for finding primers.

2) Specificity Checking Step

The program filters the primers into good or bad ones.



How the tool looks

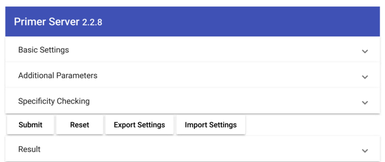


Figure 1: *Overview*

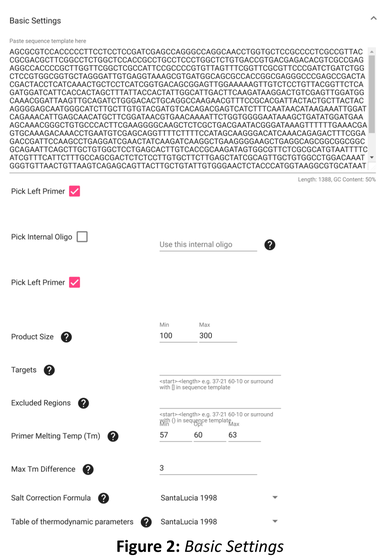


Figure 2: *Basic Settings*

There are 4 main parts in the tool.

1. Basic settings
2. Additional Parameters
3. Specificity Checking
4. Result

Basic Settings and Additional Parameters are settings for primer3. We picked up most commonly used parameters in the Basic Settings and minor options in Additional Parameters. The parameters for primer3 will have the default value from primer3. Specificity checking is for filtering primers more accurately.

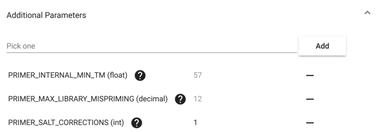


Figure 3: *Additional Parameters*

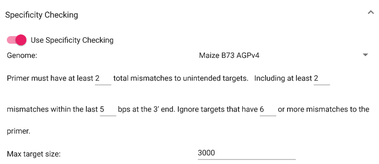


Figure 4: *Specificity Checking*

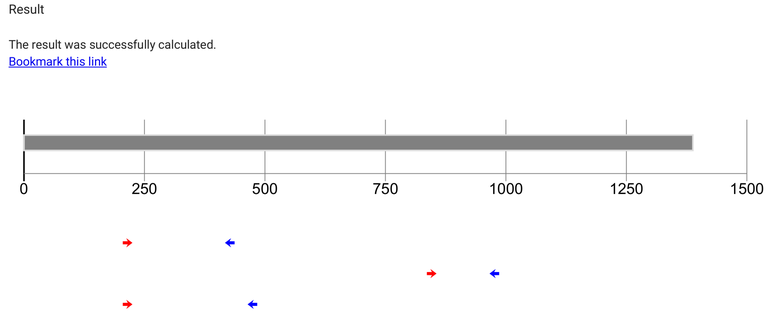


Figure 5: *Result Page*

The result page visualizes the primers by red and blue arrows. It also shows the details of each primer pair at the end. The User can see the result again by visiting the bookmarked link.

Technical Implementation of Primer Server

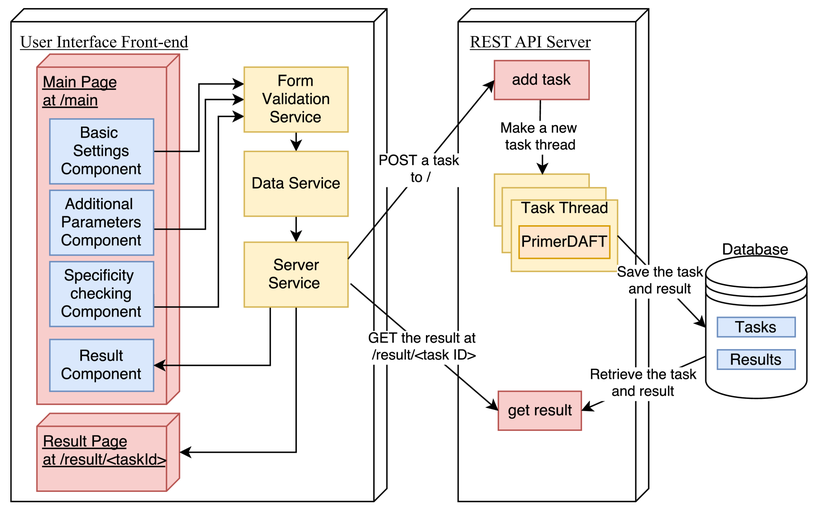


Figure 6: *Block Diagram showing how the tool works*

The user input from the main page goes through the form validation service and is stored in the data service. The form validation service filters any error in the input form. Once the submit button is clicked the new task data is posted to the REST server. The server will make a thread which uses primerDAFT (primer design and filtering tool package we separately developed) for finding specific primers. The thread stores the task and result in the database. After the task is submitted, the result component in the client will keep sending GET request to get the result. Once the result status is ok, the result component displays the result. The result page can be bookmarked to that the user can go back and look at the result again.

Tools and Technologies used

Primer3

Links

Primer Server Demo!

http://18.219.153.20/primer-server

Our GitHub Repo

https://github.com/vollbrechtlab

Conclusions

We have developed a user-friendly web-application that can design PCR primers, check for specificity against a user given genome, as well as visualize the results

Future Work

Publish the package on PyPi

Containerize the package

References

[1] Andreas Untergasser, Harm Nijveen, Xiangyu Rao, Ton Bisseling, René Geurts, and Jack A.M. Leunissen: Primer3Plus, an enhanced web interface to Primer3 Nucleic Acids Research 2007 35: W71-W74; doi:10.1093/nar/gkm306

[2] Ye J, Coulouris G, Zaretskaya I, Cutcutache I, Rozen S, Madden T (2012). Primer-BLAST: A tool to design target-specific primers for polymerase chain reaction. BMC Bioinformatics. 13:134.

Acknowledgements

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