**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 many 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.

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**How the tools look**

There are 4 main parts in the tool.
1. Basic settings
2. Additional Parameters
3. Specificity checking
4. Result

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**Technical Implementation of Primer Server**

**Tools and Technologies used**

- Primer3
- Flask
- Node
- PyPI
- Docker

**Links**

- Primer Server Demo!
- Our GitHub Repo
  - [https://github.com/vollbrechtlab](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**


**Acknowledgements**

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**Goals**

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

**Why develop new one?**

<table>
<thead>
<tr>
<th>Primer3</th>
<th>Primer Server</th>
</tr>
</thead>
<tbody>
<tr>
<td>Is it open source?</td>
<td>Yes</td>
</tr>
<tr>
<td>Is a web tool available?</td>
<td>Yes</td>
</tr>
<tr>
<td>Does it have an easy-to-use user interface?</td>
<td>Yes</td>
</tr>
<tr>
<td>Does the web tool use latest technologies?</td>
<td>Yes</td>
</tr>
<tr>
<td>Can the user run their own instance of server?</td>
<td>Yes</td>
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<tr>
<td>Can it filter for good primers?</td>
<td>Yes</td>
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<tr>
<td>Can the user use their own genome to filter primers?</td>
<td>Yes</td>
</tr>
<tr>
<td>Is this tool available to use in terminal?</td>
<td>Yes</td>
</tr>
</tbody>
</table>

**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.

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**Figure 2: Basic Settings**

**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.