# University Pompeu Fabra Master in Bioinformatics for Health Sciences DBW, 2019



# User manual

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## 1 Summary

PrimerSTOCK is a web application created by a group of four students from Pompeu Fabra University (UPF) of Barcelona during their Master's degree subject Databases and Web Development. The project came up as a way of digitalising and improving everyday laboratory tasks, helping not only to organize and share all primers information and details within a lab, but also to have a precise control of primers' storage position, available, stocked and ordered primers.

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## 3 Web application description

PrimerSTOCK provides a wide range of primer related services to research laboratories and industries. Being a small firm, we are able to give our clients personalized quality service. Below, we have listed the services that we offer to our clients along with a brief description.

- Primer storage: We provide an user-friendly database where to store the most relevant and useful primer information, which will be shared among all laboratory members.
- Primer search: It is possible to perform different searches depending on the users' needs: a quick search, an advanced search by selecting several fields or a BLAST to the database, which checks if there are any primers in his/her database that could amplify the target sequence.
- Primer resources: We facilitate several links to useful primer tools, which vary among primer designing, primer evaluating or external primer databases, and links to providers, to directly order new primers.

## 4 Before logging in



Figure 1: PrimerSTOCK main page

#### 4.1 Accounts

In the image below you can take a look at the main page of PrimerSTOCK. In it you will find an overview of this web application, plus links to sign-up and log-in. All the information is available in this page, structured in sections and accessible via the nav bar located in the top right of the page. The sections included in the main page of PrimerSTOCK are: Registration / Log in, Services, Statistics, About and Contact us.



Figure 2: Signing up and logging in process

#### 4.1.1 Laboratory registration

In order for you to start using PrimerStock your first need to register your laboratory. To do that, just click on the Registration/Log in button on the upper left-hand-side. Then, choose the option Laboratory registration by clicking on the button Register your lab. You will be redirected to a form that you will have to compile with the following data: Institution, Group name, Country and Email of reference. Also, in order for our application to function correctly, you will have to read and accept the privacy policy. You can do so by clicking on the link Privacy Policy given below the form. After you check that you filled the form with the correct data and you accepted the privacy policy, you can click the Submit button. You will get a message that the registration was successful and receive a default email on the email address listed in the form. In that email there will be an information about your Lab Code. This is a unique code that you will give to your lab members. They will use it when doing the sign up. That way, nobody that does not form part of your lab can access your lab data.

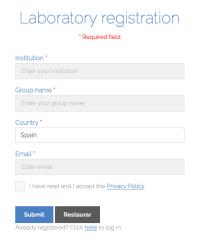


Figure 3: Laboratory registration form

#### 4.1.2 User sign up

Once your lab has registered and has obtained the Lab Code you can register as a user of that lab. To do that, just click on the Registration/Log in button on the upper left-hand-side. Then, choose the option User sign up by clicking on the Sign up button. You will be redirected to a form that you will have to compile with the following data: Name, Surname, E-mail, Password, Repeated Password and Lab Code. Also, in order for our application to function correctly, you will have to read and accept the privacy policy. You can do so by clicking on the link Privacy Policy given below the form. After you check that you filled the form with the correct data and you accepted the privacy policy, you can click the Submit button. You will get a message that the registration was successful and receive a default email on the email address listed in the form where there is a summary of your registration data. Once you did all of the above, you can now move to the next section where we explain the log in procedure.

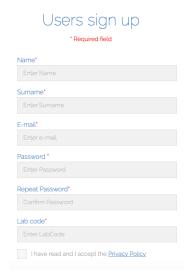


Figure 4: User sign up form

#### 4.1.3 Log in and log out

Once you signed up you are ready to Log in and start using PrimerSTOCK. To do that, just click on the Registration/Log in button on the upper left-hand-side. Then, choose the option Login by clicking on the Login button. You will be redirected to a form that you will have to compile with the following data: E-mail and Password. You will get a message that the registration was successful. If you want to log out, you can do so by clicking on the log out button in the upper right corner.

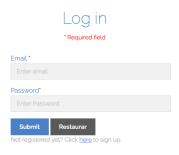


Figure 5: Log in form

#### 4.1.4 Users sign out

There is a possibility to delete a user in case you need it. For more information about this feature, please check the section 8.1.

#### 4.1.5 Deleting lab

there is no option to manually delete a lab. In case you want to delete your Lab account and all your lab data please contact us on the e-mail address: primerstock.customer@gmail.com.

#### 4.2 Services

In this section you will find an overview of PrimerSTOCK services. There is a brief explanation of the services and a video with a tour of PrimerSTOCK. Besides, below the video there is a link to PrimerSTOCK user guide.

#### 4.3 Statistics

This section is dedicated to display some statistics about PrimerSTOCK. As you can see, there is dynamic mapamundi and a Europe map were you can consult how many visits do we have from each country. You can also check other parameters like the total number of visits, the average session duration and the number of registered labs, current users and primers stored.

#### 4.4 About

You can take a look at the *About us* section to learn more about who we are and how PrimerSTOCK was born. Below it, you will find all the collaborations we have had until now.

#### 4.5 Contact us

You are welcome to contact us any time and you can easily do it through the contact us section. You have two options:

- You can send us an email by clicking in our email account (primerstock.customer@gmail.com). This action will open a window to send the email directly.
- You can fill the form and send us a message. Then, you will receive a confirmation mail with a copy
  of your request.

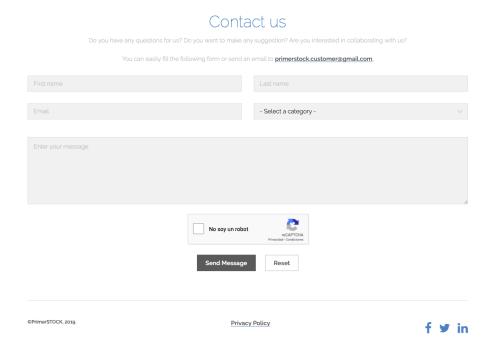


Figure 6: Contact us form

## 5 After logging in

#### 5.1 Database settings

After successfully logging into PrimerSTOCK, every user will only have available his/her own laboratory data and all the searches he could perform will be against his/her own laboratory database. But, as to be able to benefit from all our services it is needed to have, at least, one primer entry in the database, we will

see how the users can set up their laboratory database. To do so, just click on the 'Database settings' button on the navigation bar, or scroll down the screen until reach the 'Database settings' section.



Figure 7: Navigation bar

#### 5.2 Importing primers

If nobody has yet used the application, the primer database is going to be empty, so the first step to start using all the facilities of PrimerSTOCK is to upload your primers' information. Once in the 'Database settings' section, you will be able to choose between 'import primers from .csv' (more than one primer can be uploaded from a file with .csv extension), or manually import a single primer (only one primer can be uploaded after filling a form).

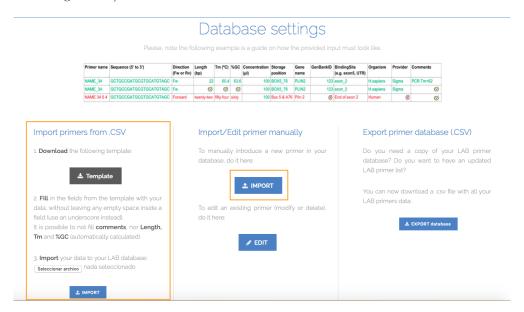


Figure 8: Importing primers

#### 5.2.1 Import primers from .csv

If you want to upload several primers at the same time, we recommend you to use the 'import primers from .csv' option, which is located on the left-hand-side of that section. To do so, you will need to download the template, which has a .csv extension that must be preserved, otherwise it will not be possible to upload it later on. Then, you should open the file and fill in the different fields with your primer data taking into account some considerations (see below). Finally, you must use the 'browse' button to select your modified template (.csv extension) from your computer, and immediately click 'Import' in order to upload all the file data into your laboratory database. Once all the .csv file would have been processed, you will be automatically redirected to a new window where we will inform you about the result of the uploading process, which will be explained below.



Figure 9: Importing primers from .csv

#### 5.2.2 Import a single primer manually

If you only want to upload a single primer, we recommend you to use the 'manual import primer' option, which is located on the middle-hand-side of that section. To do so, you will need to follow the 'Import' link, which will redirect you to an import primer form. You will need to fill all the required fields in order to be able to submit all the information into your laboratory database. Once the information is submitted, you will be automatically redirected to a new window where we will inform you about the result of the uploading process, which will be explained below.

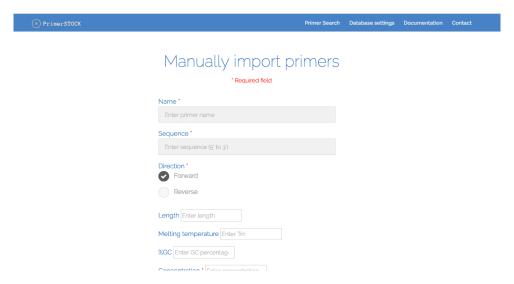


Figure 10: Manually import primers

#### Primers format considerations

- Almost all the fields are compulsory (Primer name, sequence, direction, concentration, storage position, Gene name, GenBankID, BindingSite, Organism, Provider), but there are some optional field (length, Tm, GC percetage, Comments).
- It is important to recall that leaving empty the Length, Tm and GC perentage fields will not cause any error, but will be automatically calculated by using the following website link: http://www.biophp.org/minitools/melting\_temperature/demo.php.
- When creating a new primer entity, the fields Stock and Ordered will be automatically filled, being the default stock value *Yes* and the ordered value *No*.

- It is advisable to strictly follow the instructions for each field to avoid getting back errors due to format mistakes. Moreover, we provide you some tips:
  - Do NOT leave empty spaces inside a field, use an underscore instead(except in comments).
  - Be aware of the type of character each field is expecting, numbers or letters.
  - Avoid writing special characters, (e.g. '=','?', '¿', '!', 'i', 'a'', 'o'', among other).
  - To indicate the primer direction, only 'Fw' and 'Rv' will be accepted.

Primer name		Direction (Fw or Rv)		Tm (°C)	%GC	Concentration (µl)		Gene	GenBankID	BindingSite (e.g. exon5, UTR)	Organism	Provider	Comments
NAME_34	GCTGCCGATGCGTGCATGTAGC	Fw	22	60.4	63.6	100	BOX5_76	PLIN2	123	exon_2	H.sapiens	Sigma	PCR Tm=62
NAME_34	GCTGCCGATGCGTGCATGTAGC	Fw	Ø	Ø	Ø	100	BOX5_76	PLIN2	123	exon_2	H.sapiens	Sigma	
NAME 34 5 4	GCTGCCGATGCGTGCATGTAGC	Forward	twenty-two	fifty-four	sixty	100	Box 5 & A76	Plin 2	<b>Ø</b>	End of exon 2	Human	⊗	

Figure 11: Primer format - example table

#### Primers uploading process results

After uploading any primer(s) information to your own database, we will inform you about how did the process go. It means, we will inform you of the primer(s) that have been successfully uploaded to the database and we will print you a table with the provided information. Your user will be considered the 'author' of all the successfully uploaded primers, and that will be saved in your lab 'History' (see section 8.1.) But, in case any error has occurred during the upload process, we will inform you about the primer(s) that have been affected (thus, not uploaded to the database) by indicating not only which problem has occurred, but also in which .csv file row we found that error (in case you were using the 'import data from .csv'). The errors you could face with are:

- Error due to an incorrect primer data format: We have found certain discrepancies between the data you have provide us and the data we expected to receive. Thus, we cannot create a primer instance with that information. We encourage you to check your data format and retry the same process. But, if you have already corrected the mistakes and still get the same error, or if you cannot find any mistakes, do not hesitate to contact us.
- Error due to an already existing primer in your database: This error can be generated when trying to upload a primer sequence that is already stored in the database, or when trying to upload a primer with the same name or storage position than an already existing primer. In that case, we suggest you to check whether you want to modify that existing primer, or you prefer to upload a new primer but changing the conflictive information.

#### 5.3 Exporting primers

When your laboratory primer database contains certain information, it will be display at the top of the users page. But, in case you are interested in downloading all the primers information for different personal reasons, PrimerSTOCK has thought about it. Being in the 'Database settings' section, you will need to move to the 'Export primer database (.csv)', which is located on the right-hand-side of that section, and click the 'Download Database' button. It will automatically display a download window from where you will be able to save a file containing all the existing information of primers in the database of your lab. The downloaded file is in .csv format, and it is called 'primerDB\_LabId\_DDMMYYYY', which is quite useful to differentiate among different database copies, or even to perform regular personal back ups.

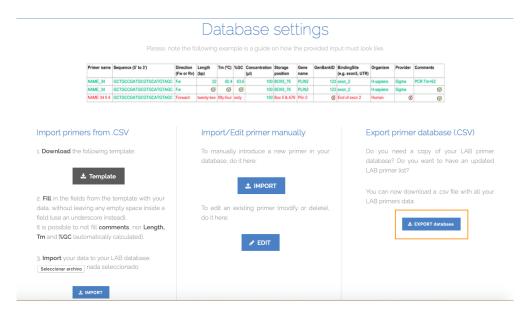


Figure 12: Export database primers

### 5.4 Editing primers

To edit your primers, go to Database settings  $\rightarrow$  Import/Export primer manually  $\rightarrow$  Edit.



Figure 13: Navigation bar

By clicking that button, you will access a page like the one below. In it, you can select a primer by its name and load it. After loading it, all the information about the selected primer will appear in the form below. Now, you can either delete it or modify it. There is also a reset button that will reload the selected primer with its initial values.



Figure 14: Edit primers

## Modify or Delete primers

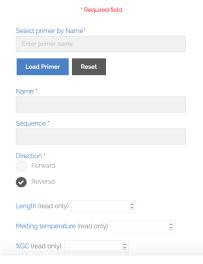


Figure 15: Modify or delete primer form: part - 1

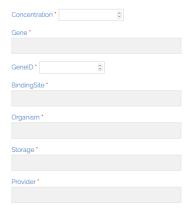


Figure 16: Modify or delete primer form: part - 2

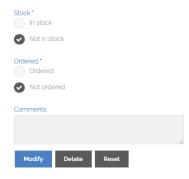


Figure 17: Modify or delete primer form: part - 3

#### 5.4.1 Modifying primers

If you want to modify the primer, change what you want and click modify. Please, mind that the fields length, melting temperature and GC content cannot be manually modified since they will be calculated based on the sequence. (Read the Import Pimers seccion to learn more about format considerations)

#### 5.4.2 Deleting primers

To delete the primer, just click the delete button (you will be asked to confirm this process).

#### 6 Primer searches

#### 6.1 Quick search

Once logged in, a table with all the primers of your lab databases will be displayed. Using the search bar at the top right, the user can search for any specific data in the table.

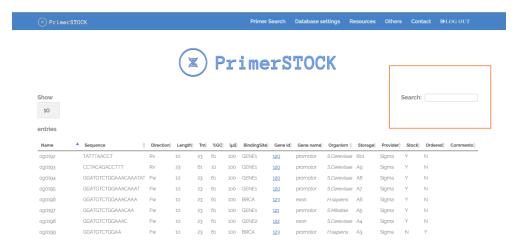


Figure 18: Screenshot of the initial table displayed when the user logs in. At the bottom right there is a search bar that allows users to quickly search primers by words.

#### 6.2 Advanced search – Search by field

Quick search can sometimes lead to high amount of information, making it difficult to find a specific primer (for example when searching for a provider). Thus, in Primerstock we have implemented the advanced search, which allows the user to search for more than one field. In the advanced search form, the user can fill any of the information fields of the primer (Name, direction, melting temperature, GC content, Storage, Position, Stock, Provider, Gene or GeneID, Binding site, Organism or comments). The result of the search is displayed in a search table, allowing the user to quickly filter information among this table.

#### Advanced search

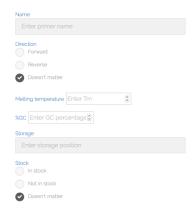


Figure 19: Advanced search form.

## 6.3 BLAST against your database - Search by sequence

As stated, this search method allows the user to search primers of their own lab database using a query sequence. The user can upload a FASTA file with the query sequence or directly introduce the sequence in the text area.

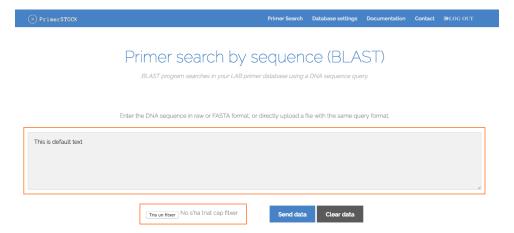


Figure 20: BLAST input website. Allows the user to insert the sequence directly or upload a file.

Then, the complement of the sequence is going to be internally calculated in order to perform two BLASTs: one of the initial string against a BLAST database of the reverse primers and another one of the complement against the database of the forward primers.

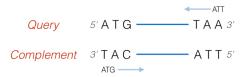


Figure 21: Schematic view of the procedure used to search primers by sequence. BLASTs of the query sequence against the forward primer database and of the complement sequence against the reverse primer database are performed.

The BLAST output is going to be displayed in two different tables with the corresponding hits for the forward and reverse primers. The user can then sort the result using the e-value or 100% identity. Additionally, PrimerSTOCK allows the user to view the overall BLAST output. When pressing the "View BLAST output" button, this will redirect the user to a new tab in which BLAST hits distribution in the query sequence are displayed, as well as the singly alignment of each hit. This tool allows the user to properly choose their primers, not only using local similarity between sequences but also its position among the query.

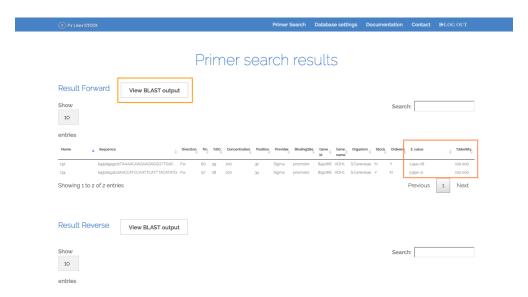


Figure 22: BLAST output overview.

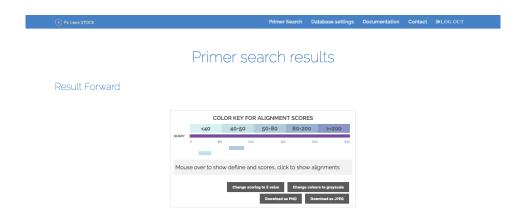


Figure 23: Overall BLAST output. Allows users to see hits distribution in the query as well as single alignments.Part 1.

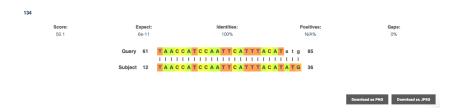


Figure 24: Overall BLAST output. Allows users to see hits distribution in the query as well as single alignments.Part 2.

#### 7 Resources

The resources section facilitates several links that might help the users to extend their service possibilities inside PrimerSTOCK, by using useful primer related tools and links to providers.

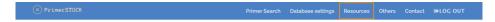


Figure 25: Navigation bar.

#### 7.1 Tools

We can find links to interesting tools to use when working with primers such as:

- Primer Design Tool, Eurofins Genomics
  - Helps to design new primers
- In-Silico PCR, University of California, Santa Cruz (UCSC)
  - Helps to accurately check which part of the genes will be amplified with the selelected primers as well as detect possible off-target amplifications.
- PrimerBank, The Massachusetts General Hospital
  - Helps to find possible already design primers in an important primer database.
- OligoEvaluator, Sigma-Aldrich (is now Merck)
  - Helps to evaluate the new designed primer by checking if it will dimerize, generate secondary structure, the percentage of GC, the approximate Tm, etc.

#### 7.2 Primer providers

We can find links to the most known providers websites to directly order primers:

- ThermoFisher Scientist
- SIgma-Aldrich, now is Merck
- Integrated DNA Technologies (IDT)
- BIO-RAD

#### 8 Other



Figure 26: Others section.

#### 8.1 History

PrimerSTOCK keeps track of all the changes of every lab's database performed by the users. Changes include modifications, insertions and deletions of primers. In the history, the name of the user and the nature modification are saved in a descendant chronological manner.



Figure 27: Others section.

#### 8.2 Users deregistration

The option of signing out from PrimerSTOCK has been implemented in the main page once logged in. The main information about the user is shown and when pressing the "Delete User" button, the information is going to be deleted from the database and no further data is going to be saved of this user.

## 9 Privacy policy

You can find the details about our Privacy Policy on the link:  $\text{http://mmb.irbbarcelona.org/formacio/} \sim \ dbw10/project/users/privacy\_policy.html.$