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## **Instructions for Entering and Retrieving Your Sequencing Requests**

Here at Retrogen we are switching to a new method of delivering sequencing data to our customers. We believe this new method will be an improvement and should be easy to use.

In order to log in first go to the website: <http://sequencing.retrogen.com/>

Login using -

Login name: «login»

Password: «password»

These are both case sensitive so check that if you're having trouble logging in.

Once you are logged in you should see 3 columns: Sequencing Requests, User Profile, and Sequencing Resources.

The first thing you should do is change your default password to ensure the security of your data. It's the first option under User Profile.

### **Change User Profile**

Please make sure your User Profile information is up to date. This will insure we are able to quickly notify you of any issues with your sequencing orders.

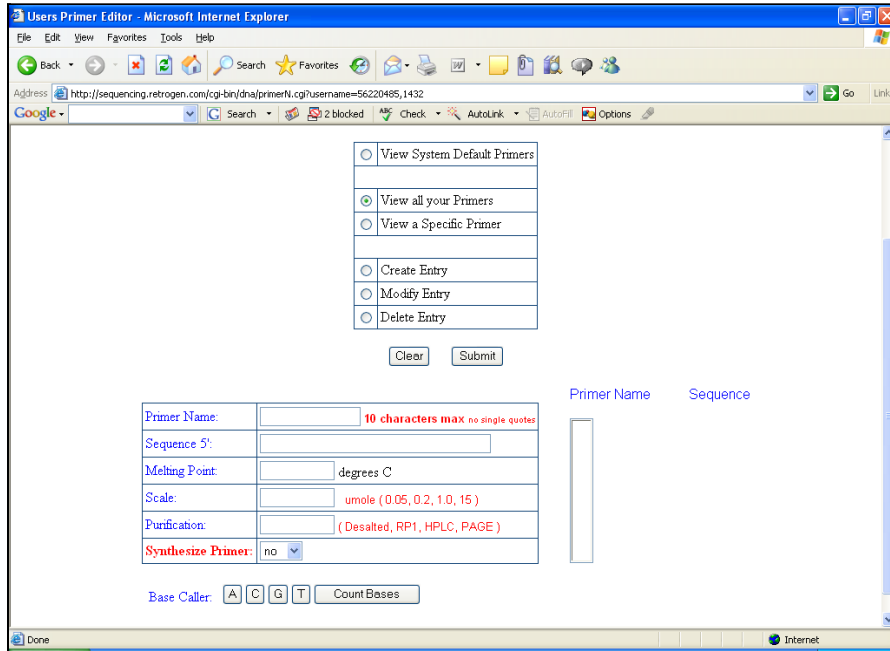
### **Request a Sample Pickup**

In order to keep orders best organized under the new system the preferred method for ordering sample pickups is through your LIMS account. Under the sequencing request section there is an option for San Diego based customers to request sample pickups. Select the time period the sample will be ready, enter time when prompted, and click the "submit" button.

Make sure that you first have entered you contact information in your user profile so we will know where to pickup your order.

### **Enter your Sequencing Primers**

The Custom Primer Editor form allows the user to create, modify, or delete custom primers. These primers are used with the Sequencing Request form. This can be to have them synthesized or just to add the names of primers you will be using repeatedly to the system. This section can also be used to see the sequence of all the universal primers we can provide free of charge for your sequencing orders.



If you select “yes” to have the primer synthesized it won’t be made until a sequencing order has been placed that makes use of it as the charge for the synthesis will be added to that order. Only use this system to orders primers you want Retrogen to keep for the purpose of performing sequencing reactions. Otherwise place the order directly with our Oligo department.

## Enter DNA Sequencing Requests

Using your account you can now enter your sequencing orders directly into our system. This will ensure your samples are named correctly and will allow us to begin sequencing your samples as soon as we receive them.

The form prompts the user for how many sequencing requests will be entered and based upon that number dnaLIMS dynamically creates a table of requests with that number of rows.

Choose the appropriate selections from the drop down boxes. Any condition that is not available as an option in the drop down boxes should be entered in the comments section.

### Select Sequencing Request Type:

**Tube:** Choose this option if you are sending samples in individual tubes.

**Plate:** Choose this option if you are sending samples in 96 well plates.

**Request type “File Upload”:** Choose this option if you would like to import your request from a spreadsheet. The spreadsheet will need to be saved in a “text tab delimited” format. You can download a blank copy of the template from the “Support Tools” page. If you use this option be aware that every field must be filled out.

**Sample Pickup:** If you use this to request a pickup of your sequencing samples make sure that you first have entered you contact information in your user profile.

### Enter a Sample Name

Retrogen DNA Sequencing Request Form - Microsoft Internet Explorer

Address: http://sequencing.retrogen.com/cgi-bin/dna/seqTableretrogen.cgi

Payment Type:

DNA Type: Plasmid

DNA Provided: Purified

Sequencing Type: 2000-01\_Primer\_Extension

Purification: Select

Sample Pickup: Shipping

Comments:

Clear Validate Table Submit

No Validation response means all required fields are filled in.

CLONE				PRIMER	Difficulties
Name	Index	Insert Size bp	Amount ng	Name	Special Request
1				- Select -	None_Known

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The form requires the user to enter a Sample Name then select attributes associated with the sample.

After the Sequencing Request Form is filled in, the user selects the Validate button to test if all values within the form are filled in.

The Submit button is selected to enter the request into the sequencing request queue. After the requests are accepted, a summary table of the requests is displayed. Please print two copies of this form and send one of the forms to Retrogen with your samples.

## Sample Name

Each sequencing request is required to have a sample name. The sample name has specific restrictions; the name cannot be longer than 15 characters and can only contain legal characters.

Legal characters are: a-z, A-Z, 0-9, and -  
 Illegal characters are any other characters.  
 Do not use underscore or period.

## Index Samples

This feature allows the user to append a range of numbers to the sample name:

sample1, sample2, sample3.... or sample7, sample8, sample9....

The user can create up to 96 individual sample requests with this feature.

To use this feature:

1. Enter a Sample Name in the first row of the table.
2. Select the Index icon within the Sample Name column.
3. Enter the beginning number within the dialog box.

### **Alternate Primers**

This feature allows the user to toggle/alternate between two primers, i.e.

sample1 primer1  
sample1 primer2  
sample2 primer1  
sample2 primer2

The Alternate Primers feature is used in conjunction with the Index Sample Name feature to provide a range of sample names with two different primers.

The user can create up to 48 unique sample names with two different primers per sample name. This creates a total of 96 sample requests.

To use this feature:

1. Enter a Sample Name in the first row of the table.
2. Select the Fill 2X icon and enter the beginning number within the dialog box
3. Select the first primer with the Primer select list in the first row.
4. Select the second primer with the Primer select list in the second row.
5. Select the Alternate icon within the Primer column.

### **Primer (select list and text box)**

Each sequencing request is required to have a primer. A system primer or a custom primer (see above) can be selected from the list. Create the custom primer before using the custom primer name. The primer length is 10 characters.

Do not manually edit the name in the Primer Name text box if you are using a primer from the primer list.

Legal characters for primer names are: a-z, A-Z, 0-9 and -  
Do not use underscore or period.

### **Summary Table**

After the request is accepted into the system, a summary table listing each value of the request is displayed. If multiple requests were created, an abbreviated summary table is displayed.

Print two copies of the summary table and bring one with the samples to the core facility. Keep the other for your records.

### **Retrieve Your Sequencing Results**

Every order under your name should be listed with an order #, the number of reactions, and the date they were processed. The system will automatically email you a notification with the order number when it is ready.

Once you select the order you wish to view and click "**submit**" you should see your results. At the top there are options for downloading the entire order as a ".zip" file with instructions for both Windows and Mac computers. On the list of reactions below you have the option of downloading them individually or viewing them within you web browser.

At the right end of each reaction is a Phred Q20 score - meaning the number of bases read with a Q value > 20. If this number is low that is a quick indication that something when wrong with the sequencing reaction. We've attached a copy of our troubleshooting guide to help you identify the cause of any poor results you may see and feel free to contact us if you need help with the quality of your sequencing reactions.

## **View Your Sequencing Results**

To view your sequencing results on Mac.

You need to download Resource Fork Tool <http://www.dnertools.com/download.html> to change the file type so you can view with the following free software:

1. EditView  
<http://www.appliedbiosystems.com/support/software/dnaseq/installs.cfm>
2. Xplorer <http://www.dnertools.com/download.html>
3. Sequencher Demo <http://www.genecodes.com/demos/>
4. 4Peaks <http://mekentosj.com/4peaks/>
5. TraceViewer <http://www.codoncode.com/TraceViewer/download>

To view your sequencing results on PC.

1. Sequence Scanner  
[http://marketing.appliedbiosystems.com/mk/get/SSS\\_login?isource=fr\\_E\\_Pg\\_Prod\\_AB\\_Gbl\\_SeqScan\\_20050920](http://marketing.appliedbiosystems.com/mk/get/SSS_login?isource=fr_E_Pg_Prod_AB_Gbl_SeqScan_20050920)
2. Xplorer <http://www.dnertools.com/download.html>
3. TraceViewer <http://www.codoncode.com/TraceViewer/download>
4. Sequencher Demo <http://www.genecodes.com/demos/>
5. Chromas <http://www.technelysium.com.au/chromas.html>

6. BioEdit <http://www.mbio.ncsu.edu/BioEdit/bioedit.html>

For more details see User Help <http://sequencing.retrogen.com/dnaManual/help.html>