Nevada Genomics Center

These are general instructions on how to use dnaTools to submit next generation sequencing samples to be run on either the Ion Torrent Proton or the Illumina NextSeq500. We here at the Nevada Genomics Center feel that dnaTools is user friendly and fairly intuitive; but we still recommend taking a moment to read through these instructions as well as taking time to investigate dnaTools and read the “Learn More” options that can be found throughout. Please share this PDF file with the rest of your lab.

If you have any difficulties using dnaTools, please phone (775-784-1657) or email us (genomics@cabnr.unr.edu) and we will assist you.

How to use dnaTools for Next Gen Sequencing Submissions

Table of Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creating an Account</td>
<td>2</td>
</tr>
<tr>
<td>Entering a Next Gen Sequencing Request</td>
<td>3</td>
</tr>
<tr>
<td>Viewing your Orders</td>
<td>6</td>
</tr>
<tr>
<td>Deleting an Order</td>
<td>7</td>
</tr>
<tr>
<td>Ion Torrent &amp; Illumina Data</td>
<td>7</td>
</tr>
</tbody>
</table>
Creating an Account

You will find a link to the dnaTools server on the NGC homepage at http://www.ag.unr.edu/genomics/

If you do not have an account, choose the Create Login Account for dnaLIMS option and fill out the form with a login name, password, your first and last name, email address and phone number. Then select the name of your PI from the drop-down list of PI's and this will auto fill the Billing and PI Information. If your PI does not yet have an account then please email NGC to have an account opened for your PI. When creating your login information, do not use the following characters: < > / ; " =. Login Name and Passwords must have at least 6 characters. The Password must have a numeric character (0-9) AND one of the following special characters: ! @ & ? : +

**Step #1**
Fill-in Login Info

**Step #2**
Select your PI from drop-down list

**Step #3**
Select “Submit” at the bottom of the form.

Once you have set up your account then you can choose the Login to dnaLIMS to enter the dnaTools server.
Entering a Next Gen Sequencing Request

Begin on the main page, upper left-hand corner, and select the NextGen tab:

This will take you to the Next Gen Sample submission page. Once you are done on this page, you may return to the dnaTools main page or logout by selecting either tab in the upper left-hand corner.

To submit an order, begin by selecting NextGen Order Form.

That will take you to the first page (shown below) where you enter how many samples/libraries you will be working with and where you enter a title for the project. Then “submit”.

On the next page we request more information about your next gen sequencing project.
Beginning at the top of the form we request a payment type. The payment options are Purchase Order, Account Name, or credit card. When you select your payment option a dialog box will come up so that you can enter the PO, Acct Name, or credit card. If the dialog box does not appear, check to make sure you do not have pop-ups blocked while working in dnaTools. If entering credit card information note that must be handwritten on the printed form – never type that information into the server. UNR campus users should choose Acct Name and enter the PI’s last name. If your UNR lab uses more than one PCard then please also add either the last 4 digits of the PCard number or enter the name of the account that you have established with us in the comments field.

We need information about your library/libraries.

- For Library Preparation you have the options of submitting a library your lab has prepared (Client Prepared Library) or requesting that we prepare the library for you (NGC Library Prep Requested). If you request we run the library prep we will email you so that we can learn the specifics of what library prep you are requesting.

- We then ask what the starting material was for the library: DNA, RNA, or Amplicons.

The next six questions focus on the sequencing run.

- Choice of sequencing platform: Ion Torrent Proton or Illumina NextSeq500.
- Sequencing flow-cell or chip: There is only one choice for the Proton – the P1 chip. There are several flow-cell and chemistry options for the NextSeq. If you have questions about which is the correct option for an Illumina run please email us (genomics@cabnr.unr.edu).
- Number of runs: How many runs (flow cells or chips) do you want run.
- Barcoding or Indexing. Ion Torrent samples may have Ion Torrent barcodes, one barcode per library. Illumina libraries may have one OR two indices per library. For indexed reads on the NextSeq, you cannot have 2 Gs as the first two bases of the index IF it is single plex. The Illumina index that starts with 2 Gs is Index 1 (705) from the Nextera kits. If you have questions about barcoding or indexing please email us (genomics@cabnr.unr.edu). If your libraries have barcodes or indices, how many do you want pooled for each chip or flow cell?
- Bioanalyzer Data. Every library must be checked for size and concentration with a High Sensitivity Agilent run. If you ran the High Sensitivity in your lab you should email the data to NGC.
- Reference Sequence. If you are providing a reference sequence for a Proton run, please select “yes”.

NextGen Order Form

<table>
<thead>
<tr>
<th>Payment Type: dropdown box</th>
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<tbody>
<tr>
<td>Principal Investigator: auto filled upon login into dnaTools</td>
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<th>Library Preparation: dropdown box</th>
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<td>Sample Type: dropdown box</td>
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<th>Sequencing Platform: dropdown box</th>
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<tr>
<td>Sequencing Kit or Chip: dropdown box</td>
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<tr>
<td>Number of Runs (Flowcells or Chips): fill-in box</td>
</tr>
<tr>
<td>Barcoding or Indexing: dropdown: yes or no</td>
</tr>
<tr>
<td>Bioanalyzer Data emailed to NGC: dropdown: yes or no</td>
</tr>
<tr>
<td>Reference sequences provided: dropdown: yes or no</td>
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Comments:

Illumina BaseSpace PI account login email for data transfer:

- Use the comments for any comments you wish to add. If you are pooling barcoded/indexed libraries and running several chips/flow-cells, you can list here how you want the libraries pooled.

- For all Illumina NextSeq sequencing runs the data will be transferred to the Illumina BaseSpace cloud. In order to transfer the data NGC needs the email BaseSpace login for lab PI. Please write that in here.

<table>
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<tr>
<th>Sample Name</th>
<th>Amount ng</th>
<th>Conc. Ng/uL</th>
<th>Quantification</th>
<th>Barcode / Index 1</th>
<th>Index 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>fill-in box</td>
<td>fill-in box</td>
<td>fill-in box</td>
<td>dropdown box</td>
<td>fill-in box</td>
<td>fill-in box</td>
</tr>
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</table>

We ask for a number of items in this table.

- Please enter the sample/library name. The sample name has specific restrictions: the sample name must be more than one character but cannot longer than 20 characters. Use only legal characters: a-z, A-Z, 0-9, and - Illegal characters are any other characters. Do not use underscore.
- How many nanograms of sample/library are you submitting?
- What is the concentration of the sample/library in ng/uL?
- How did you quantify the sample/library?
- If the library was barcoded or indexed, what is the barcode or index for each sample? Please note: Ion Torrent libraries can have one barcode per sample while Illumina can have one OR two indices per sample.

Once you have filled in the boxes, click the ‘submit’ key at the bottom of the page. After you click ‘submit’ and the request is accepted, a summary table is displayed. The first field of the table is the Order Number.

Please print out 2 copies of this summary page. Send one copy with your samples and keep the other copy for your records. Please label your tubes with the Requisition number for each sample and place the tubes in a bag labelled with the job number. It is vital that the number on the sample tube match the number on job request in order for the proper sample name be assigned to the data.
Viewing Your Orders

To view your job requests, go to Export and View Order Summary. This will take you to a page where you may select to either view current orders or completed orders. Make your selection and select “submit”.

Current Orders will show you the orders waiting for processing. Completed Orders will show all the orders which have been processed. To see the order summary you select “View”. When viewing the order summary you have the option of downloading the summary in an Excel format.

You may select the View Your Requests, in the upper left-hand Sequencing box, to display a summary table of all requests. The request queue can be searched by Request or Order Number. This form also allows you to delete requests and orders.
Deleting an Order

To delete an order, begin by selecting the order in the Queue Viewer box. Simply click on the red job number. This will take you to a page where you have the options of displaying the order summary or deleting the order. If you select “Delete Order” a pop-up box will appear telling you that you are about to delete an order. Select “OK” if you do wish to delete the order. If the pop-up box does not appear, check to make sure you do not have pop-ups blocked while working in dnaTools. Finally, you click “submit” and the order will be deleted.

Ion Torrent Proton Data

The data files generated in Proton sequencing are large and thus not accessible through the dnaTools server. Unlike the Sanger 3730 sequencing data that we back up and store on the server, the data from a Proton sequencing run cannot be backed up here at NGC. The data from a Proton sequencing run will be held for only two weeks. The data for each run/project will be copied to the hard drive you provide at the time of sample submission and it is the responsibility of your lab to make sure the data is accessible and to back up that data if desired.

Illumina NextSeq Data

The data files generated in Illumina sequencing are large and thus not accessible through the dnaTools server. Unlike the Sanger 3730 sequencing data that we back up and store on the server, the data from an Illumina sequencing run cannot be backed up here at NGC. Before submitting a job request, your lab PI must generate an account with Illumina BaseSpace (https://accounts.illumina.com/). The data ownership will be transferred to the PI’s account. The data from an Illumina sequencing run is too large for NGC to store. Your lab is responsible for downloading and backing up the data. Once the data is received into the PI’s account, it may be transfer or shared with other lab or collaborator accounts.
The next generation sequencing data generated will require further analysis and it is the responsibility of your lab to arrange for bioinformatics services. We highly recommend that the first step in considering running Ion Torrent sequencing is to meet with a bioinformatics specialist.