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Protocol

DEL NGS Analysis Site: Usage Instructions

Summary

This document provides basic instructions for operating the MilliporeSigma DEL NGS analysis web portal.

Definitions

The terms defined here are used throughout this document.

Term	Definition
Ligated Analysis	The analysis of a DEL assay for paired compound binding, using a ligation step to join the fragments for paired compounds.
Unpaired Analysis	The analysis of a DEL assay for single compound binding.

Analysis

To analyze the results of your next-generation sequencing, you can securely upload your data to the MilliporeSigma DEL NGS Analysis Portal (https://www.sigmaaldrich.com/DELanalysis) and perform an automated analysis to find the most likely candidates for drug development.

Registration

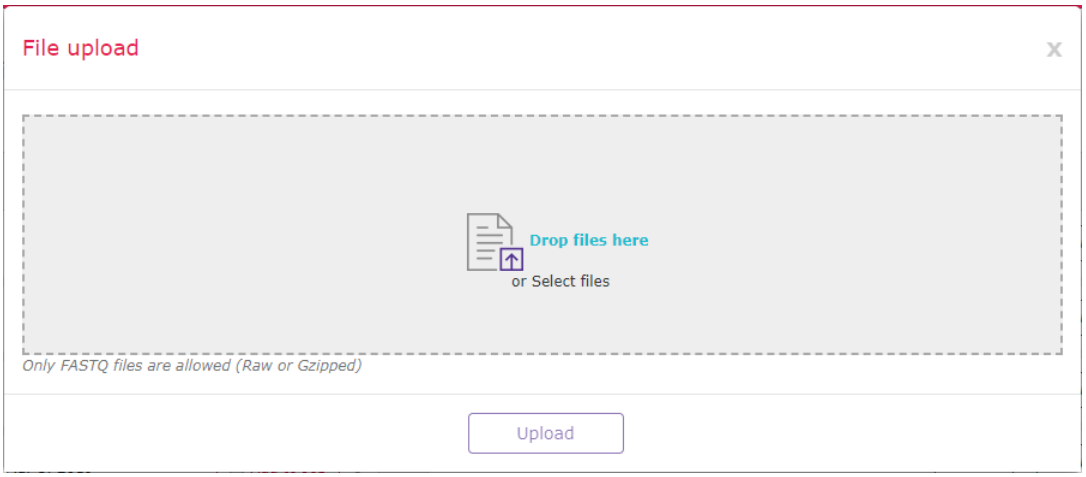
Before uploading any data to the server, you will need to register on the site. Go to the DEL NGS Analysis Portal (https://www.sigmaaldrich.com/DELanalysis) and click the **Register Now** link. For organizational registration, simply use a single user's account for the organization.

Note: Because of the high storage requirements for NGS data, each user is limited to **10 GB** of FASTQ storage.

Uploading Data

To upload your data in the Analysis Portal:

1. Click **Upload FASTQ Files**.
2. In the resulting dialog (screenshot below), perform one of the following actions:



   a. Drop your file onto the **Drop files here** section, OR
   b. Click **Drop files here** and select your file(s) from the file browser dialog that appears.
3. Click the **Upload** button.
4. Wait for the file to finish uploading (as shown by the progress bar next to its name).
5. Repeat the process for any other files you wish to upload.

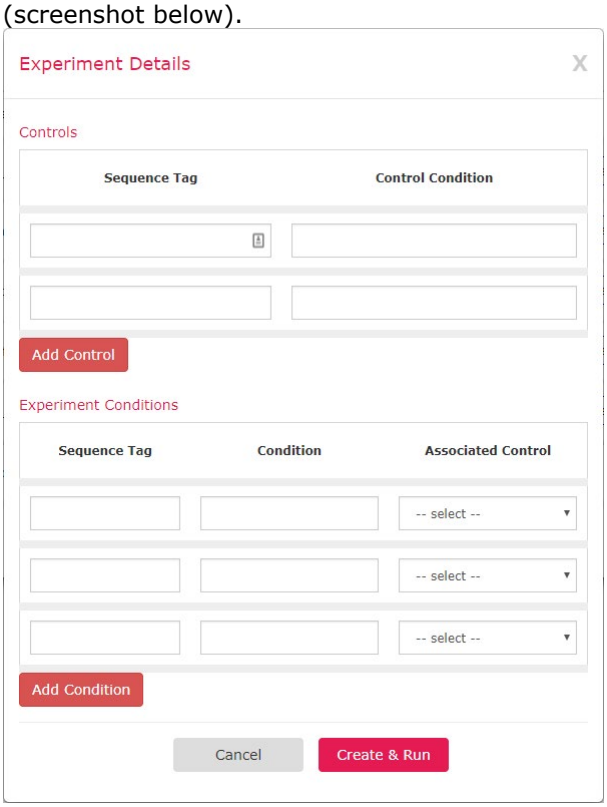
Analyzing Data

To analyze data, a new Job must be created on the site. Note that even though single- and dual-fragment assays can be performed simultaneously in the same tube, two separate jobs must be created on the analysis site—one for the single-fragment assay and one for the dual-fragment assay.

To create a job:

1. Click **Create Job For Analysis**.
2. In the resulting **New Job** dialog, enter a name for the job. This will be used to label the job when you view the completed results.
3. **IF** your assay is a Ligated Analysis, ensure that the **Two-Fragment Ligation Experiment** checkbox is selected. Otherwise, ensure that it is NOT selected.
4. Select "DyNAbind Library" from the **Encoded Library** dropdown and click **Next**.
5. Use the **Select** checkboxes to pick the file(s) that will be analyzed.
   a. If one or more files are not listed, click **Upload New File** to open the **File Upload** dialog and upload the necessary file(s).

6. Once all of the necessary files have been selected, click **Next** to bring up the **Experiment Details** dialog (screenshot below).



7. Enter each of the experiment controls in the **Controls** section of the **Experiment Details** dialog as follows:
    a. Enter the 12-bp DNA barcode associated with the control in the **Sequence Tag** column.
    b. Enter a unique name in the corresponding **Control Condition** column.
8. Enter each of the treated conditions in the **Experiment Conditions** section as follows:
    a. Enter the condition's 12-bp DNA barcode in the **Sequence Tag** column.
    b. Enter a unique name for the condition in the **Condition** column.
    c. **IF** a control is associated with this treated condition, select the control from the **Associated Control** dropdown menu.
        Note: This is required EXCEPT when running a Ligated Analysis. Because Ligated Analyses involve an exponentially greater number of potential combinations than Unpaired Analyses, a much greater sequencing depth would be required for any Ligation Analysis controls to be useful.
9. Click **Create & Run**. When the analysis is complete, it will produce a report if it completed successfully.

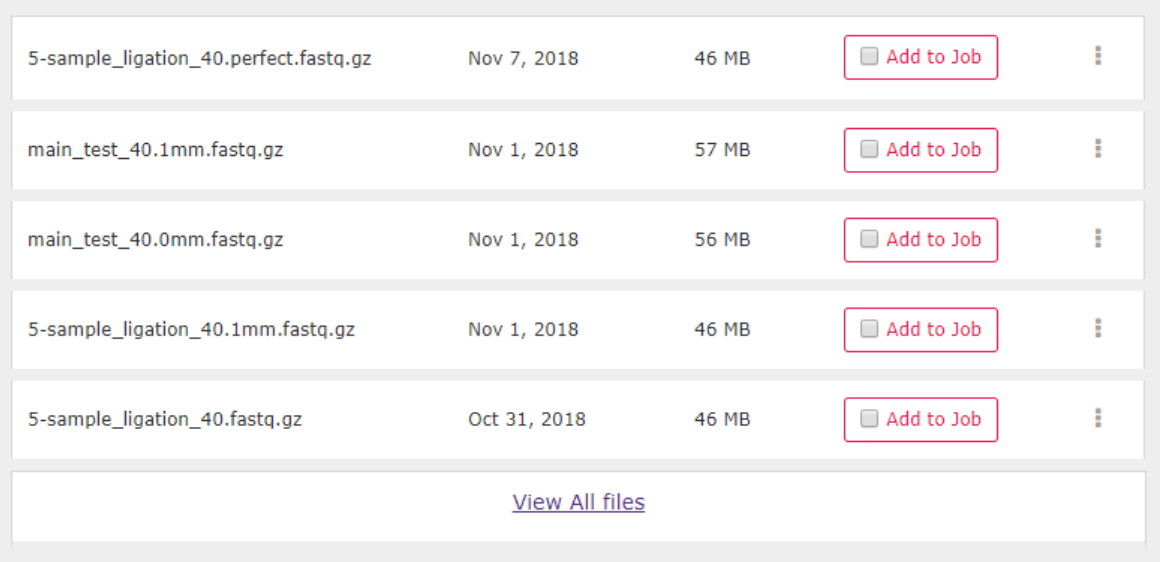
Viewing the Report

To view a report in the Analysis Portal:

- If the job you wish to view is in the **Recent Jobs** section, click the **View Report** button to show the report.
- Otherwise, click **View All Jobs** and scroll to the job of interest, then click **View Report**.

Running Files with Different Settings

If your files have already been uploaded, you can skip the file selection step during job creation by using the **Add to Job** checkboxes for the desired files on the portal main page (see screenshot below).



Troubleshooting Guide

Observation	Cause	Recommended Solution
File does not upload for several minutes	Network error	Refresh the page and try re-uploading the file
	Browser error	Refresh the page. If the file does not appear, or is not the right size, try re-uploading the file.
File upload is rejected	Not enough space remaining	Compress the FASTQ file with gzip. If the file upload is still rejected because there is not enough space, delete any unneeded FASTQ files from the account before trying again.
	Incorrect file extension	The file extension must be .fastq or .fastq.gz.
Barcode rejected by Experiment Details dialog	Incorrect barcode	Ensure that the barcode is a valid 12-bp DyNAbind barcode, as listed in your kit's documentation.
	Barcode not unique	Each condition must have a unique barcode. For example, you cannot use the same barcode for two different experimental conditions or for an experimental condition and its control. If you have more conditions than unique barcodes (including any control conditions), you will need to run multiple assays and analyses.
Failed analysis	No barcode matches for one or more samples	Ensure that the correct barcodes were entered.
		Ensure that the uploaded FASTQ file corresponds to the experiment setup.
	Invalid file format	Ensure that the contents of the uploaded sequencing file are in FASTQ format.

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