Interpret

NGS Analysis Software

A Sysmex Group Company

OGT Handbook

Interpret MRD Quick-Start Guide

Table of contents

The following guide briefly explains how to process FASTQ files generated from the SureSeq Myeloid MRD Panel, and monitor changes in variant allele frequencies in these samples over time, using OGT's Interpret NGS analysis software.

Accessing Interpret

Following deployment of an installation of the Interpret software, OGT support will provide users with:

1. The URL of the Interpret deployment (**https://web-app.*.interpret-ogt.com,** where "*" is a name specific to the deployment. E.g. **https://web-app.mylab.interpret-ogt.com**).
2. A user name (e.g. "admin")
3. A password for the user name.

To access Interpret:

1. Open a web browser and navigate to the URL provided. A screen like the following may appear, indicating that the software is loading. This may take a few minutes.

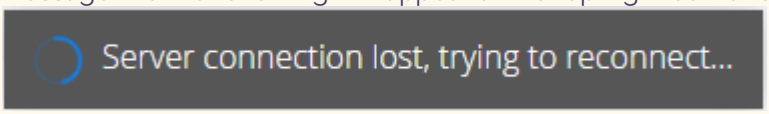
Figure 1: The Interpret start page, indicating that the software is loading

2. Once the software has loaded, a login screen like the following will be displayed. Enter the user name and password provided, and click **Log In**.

Figure 2: The Interpret login page, displayed when the software has loaded

Access Restrictions
Please note that it is possible to configure Interpret such that it is only accessible from specific IP addresses. If the message "You do not have permission to access this resource" is displayed instead of the loading or login screens, please contact OGT for support.

Automatic shutdown
In order to optimise use of computing resources, the Interpret web interface will shut down automatically after a period of user inactivity (between 10 and 20 minutes). When this occurs, a message like the following will appear at the top-right corner of the screen:



To access Interpret, simply refresh the page and login again when prompted. Please note that processing of samples by the analysis pipeline is unaffected by this shutdown.

Uploading FASTQ Files

There are currently 2 methods for providing FASTQ files to the system:

1. Via the AWS web "console".
2. Via the "Upload FASTQs" page in the Interpret software.

UMIs
If the intention is to run UMI processing on the FASTQ files, they must have beeen generated with UMIs included. If UMIs are not included then the analysis will not complete.

Uploading via the AWS web console

To access the AWS web console, which provides the most reliable upload mechanism, the following information will be provided:

1. A URL to the upload page (similar to **https://s3.console.aws.amazon.com/s3/upload/ogt-data-mylab?region=eu-west-2&prefix=incoming/**)
2. An AWS account ID (a 12-digit number)
3. A user name
4. A password

To upload FASTQ files to the system:

1. Navigate to the upload page URL in a web browser.
2. Select **IAM user**.
3. Enter the Account ID provided and click **Next**.
4. Enter the user name and password provided and click **Sign in**.
5. Click either **Add Files** (to select FASTQ files) or **Add Folder** (to select a folder containing FASTQ files), and select the appropriate file/folder from the file system.
6. Scroll down to the bottom of the page and click **Upload**.
7. Upload progress will be displayed in the next page. Do not navigate away from this page until the upload is complete, otherwise it may fail.
8. When the upload is complete, confirmation will be displayed on the page.
9. To verify that all FASTQ files have been added to the system, in Interpret, select **Batches -> Run Batch**, and check that they have appeared in the samples table.

Automatic Sample ID
In order to monitor changes in Variant Allele Frequency between samples from the same source in different batches, all samples must be assigned the same Sample ID when uploaded into the system. When FASTQ files are uploaded via the AWS console, the system will automatically extract a Sample ID from the name of the FASTQ file using the **standard naming convention**. If necessary, rename the FASTQ files before upload to ensure they are assigned the correct Sample ID.

Uploading via the Interpret software

Once logged in to Interpret, to upload FASTQ files to be processed by the system:

1. Click on the **Batches** button in the toolbar and select **Upload FASTQs**.
2. Click **Select FASTQ Files**, select the FASTQ files from your file system and click **Open**. The FASTQ files should be automatically paired and listed in the **Paired FASTQs** table.
3. If necessary, modify the Sample ID assigned to each pair of FASTQs by clicking on the button in the **Sample ID** column, typing the correct name of the sample in the input field, pressing Enter and clicking **Done**.

Note that, in order to monitor changes in Variant Allele Frequency between samples from the same source in different batches, all samples must be assigned the same Sample ID when uploaded into the system

4. Click **Upload Paired FASTQ Files**.
5. Click **Ok**.
6. Click **No**.

Progress of the upload can be monitored in the **Upload FASTQs** page, and an **Estimated Time Remaining** for all selected files to be uploaded is provided, which will be dependent on the total size of the FASTQ files and the upload speed.

While the upload is in progress, please note the following:

1. It is essential that the user does not navigate away from the page before upload is complete.
2. If using the Chrome web browser, ensure that Upload FASTQs tab is the one selected in the browser window, as selecting another tab will result in the upload being paused.

Processing samples in "Discovery Mode"

In order to identify candidate variants for use in "Monitoring Mode", where specific variants are interrogated by the pipeline in order to determine their allele frequency at very low depth, it may be necessary to process samples in "Discovery Mode". Discovery Mode uses the standard SNV, Indel and ITD detection algorithms built into OGT's NGS analysis pipeline to report all variants present in a sample above a specific allele frequency and according to other quality-related criteria. To process samples in Discovery Mode:

1. Click on the **Batches** button in the toolbar and select **Run Batch**.
2. Enter a name for the batch in the **Batch Name** field.
3. Select the **SureSeq Myeloid MRD Panel** from the **Panel** drop-down list.
4. Select **Discovery Mode** from the **Protocol** drop-down list.
5. Select the samples to be processed from the list of available samples such that they are displayed in the **Selected Samples** table.
6. Click **Run Analysis**.
7. Click **OK**.

Once the batch has been started, the **Batch** page will be displayed showing the current status of the processing of the samples in the batch. The status of each sample will be updated automatically (unless the web interface is shut down automatically due to inactivity - see **Automatic Shutdown** above), and, on completion, the **Completed Samples** table, displaying a summary of the results and relevant QC metrics, will appear.

Minimum Allele Frequency
By default, Discovery Mode is configured to detect variants at a minimum allele frequency of 1%. To reduce this value in order to increase the sensitivity, modify the Discovery Mode protocol as follows:

1. In the top-right corner of the screen, click on the user icon and select **Admin Controls**.
2. In the menu on the left-hand side, select **Analysis -> Protocols**.
3. In the **Protocols** list, select **Discovery Mode**.
4. Click the **Edit** button at the bottom of the screen.
5. Scroll down and select the **Advance Pipeline Configuration** tab.
6. In the **SNV Detection** section, modify the value of **Minimum Alt Fraction** as required.
7. Click **Save**.

Selecting Hotspots

To select hotspots for use in Monitoring Mode, they must first be selected from variants identified in Discovery Mode:

1. In the **Batch** page for the Discovery Mode batch, click on the **SNVs** button in the **Completed Samples** table for a sample that may contain potential hotspots.
2. If necessary, filter the list of variants in the **Variants** page in order to identify potential hotspots more quickly.
3. For each variant to be monitored in Monitoring Mode:
    a. Right-click on the variant in the table.
    b. Select **Add to...**
        i. If no Variant List has been created yet:
            1. Click **New List**
            2. Enter a **Name** for the list (e.g. "Hotspots")
            3. Click **Create**
        ii. Otherwise, click on the name of the Variant List.
4. Once all required hotspots have been added to the Variant List, modify the "Monitoring Mode" protocol to use those hotspots:
    a. In the top-right corner of the screen, click on the user icon and select **Admin Controls**.
    b. In the menu on the left-hand side, select **Analysis -> Protocols**.
    c. In the **Protocols** list, select **Monitoring Mode**.
    d. Click the **Edit** button at the bottom of the screen.
    e. Scroll down until the **Hotspots** table is displayed, and click on the name of the Variant List created in step 3 in the **List** column.
    f. In the **Variant** column, select all variants whose allele frequencies should be monitored, and click on the > button to add them to the **Selected Variants** table.

g. When all variants have been added to the **Selected Variants** table, click the **Save** button.

Batch hotspot selection
The list of variants included in the Hotspots list for the Monitoring Mode protocol should cover all variants to be monitored in all samples in a batch. If different variants are relevant to different samples, (preferably) create the super-set of these variants in the protocol, or create separate protocols for each set of variants, and run the samples in different batches using the appropriate protocol.

Hotspots not detected in Discovery Mode
If the variant required for monitoring has not been detected in Discovery Mode, contact OGT for assistance to add the variant to a variant list.

Processing Samples in "Monitoring Mode"

To determine the allele frequency of hotspots in a batch of samples at very low depth, the samples should be processed using the "Monitoring Mode" protocol:

1. Upload the FASTQ files for the batch using the method described in the **Uploading FASTQ Files** section above.
2. Click on the **Batches** button in the toolbar and select **Run Batch**.
3. Enter a name for the batch in the **Batch Name** field.
4. Select the **SureSeq Myeloid MRD Panel** from the **Panel** drop-down list.
5. Select **Monitoring Mode** from the **Protocol** drop-down list.
6. Select the samples to be processed from the list of available samples such that they are displayed in the **Selected Samples** table.
7. Click **Run Analysis**.
8. Click **OK**.

Once the batch is complete, allele frequencies of hotspots selected in the Monitoring Mode protocol for a specific sample may be viewed by clicking on the **SNVs** button in the **Completed Samples.**

Hotspot Monitoring

In order to visualise the results of hotspot monitoring:

1. Select**Tools -> Hotspot Monitoring Report**, and select the sample/source to be reported.

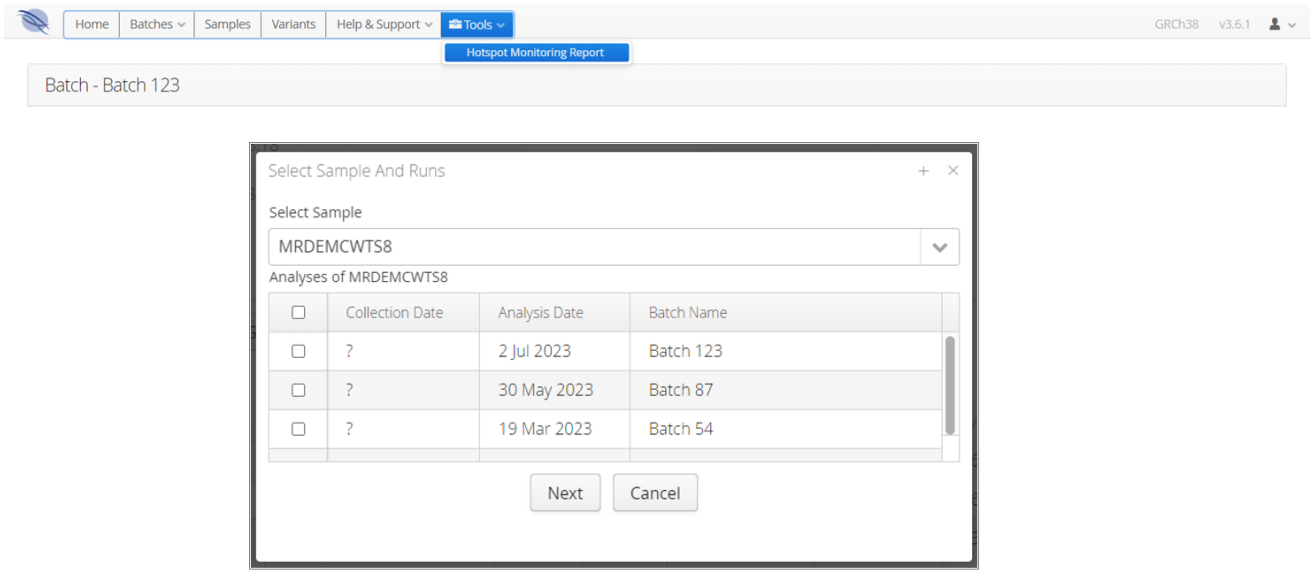


Figure 3: Selecting the sample(s) to be reported

2. If necessary, enter the **Collection Date** of the sample(s). This only needs to be carried out once for each sample and will be remembered for future reports. Click **Next**.

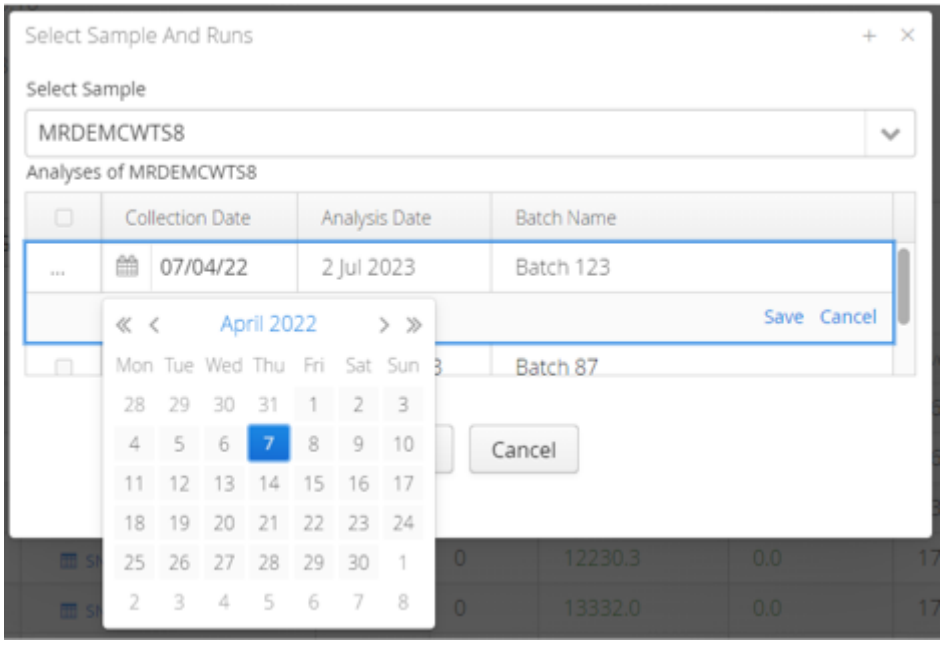


Figure 4: Entering the collection date of the sample

3. Select the hotspots to be reported using the same method described in **step 4e-f in the Selecting Hotspots** section and click **View**.

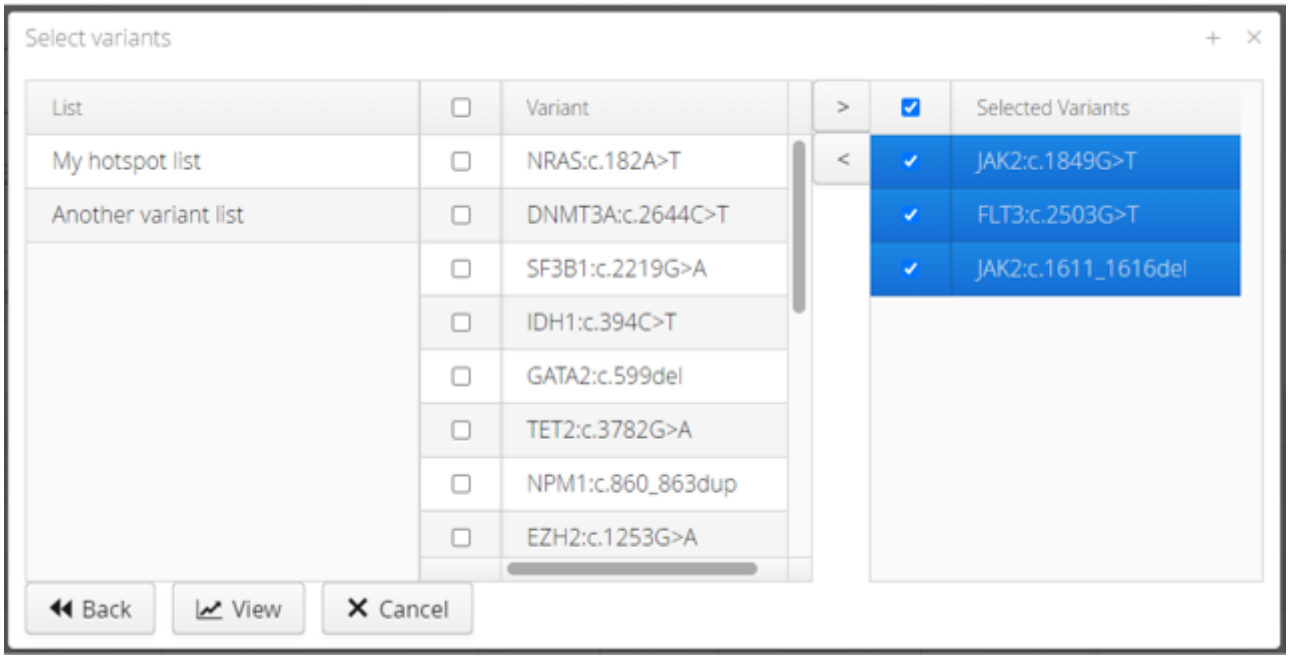


Figure 5: Selecting Hotspots to include in the report

4. A graph containing the allele frequencies of all selected hotspots in all selected sample runs will be displayed, along with tabs allowing the user to view the results for individual hotspots. Graph images may be exported by right-clicking of the graph and selecting "Save Image". The table containing the data underlying the graph may be exported as a CSV via the**Export Data**button.

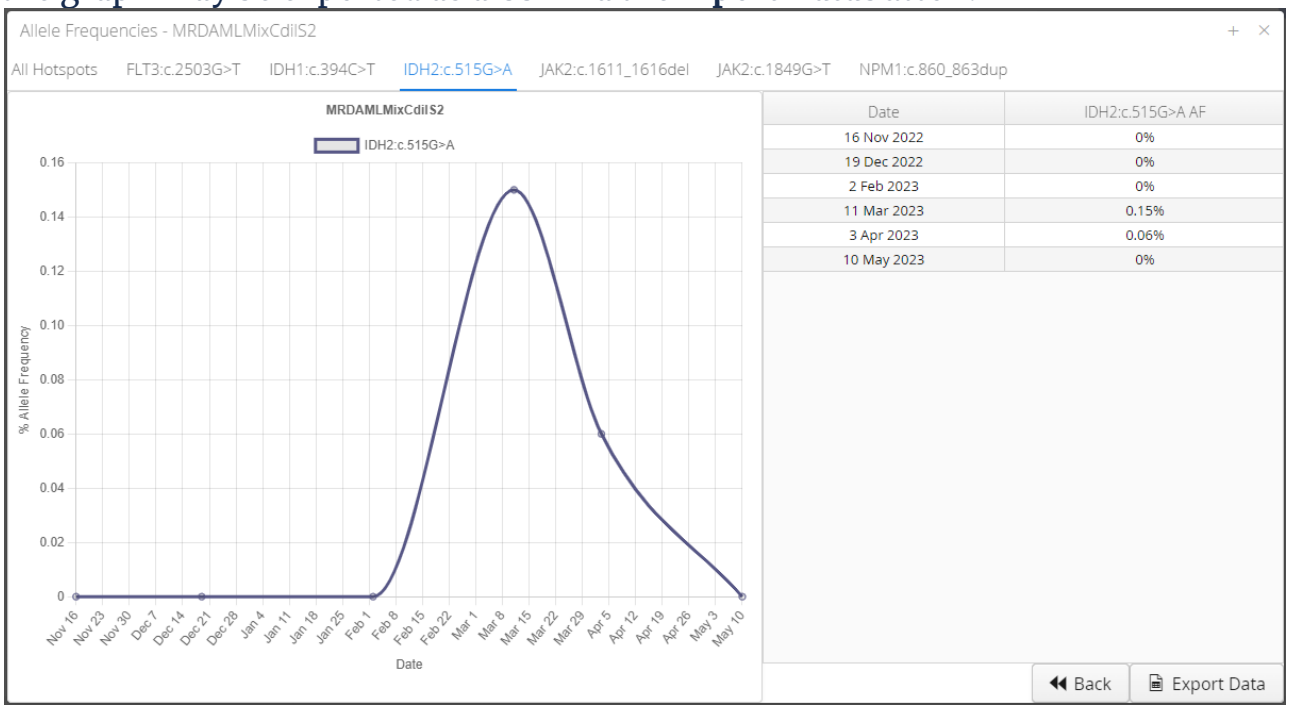


Figure 6: An example of a report

Legal Information

Interpret Software

Interpret software was developed by Oxford Gene Technology. The purchaser has the non-transferable right to use the product for RESEARCH USE ONLY AND NOT FOR DIAGNOSTICS PROCEDURES. It is not intended for use, and should not be used, for the diagnosis, prevention, monitoring, treatment or alleviation of any disease or condition, or for the investigation of any physiological process, in any identifiable human, or for any other medical purpose.

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