MEDICOVER
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SIRIUS
Genetic Data Management

User Guide

Proprietary Document: WI-30
Version 2.2

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Revision History

Date (YYYY-MM-DD)	Version	Description of Change (Initial Version/Revision)
2021-07-29	1.0	Initial Document Issue
2022-12-21	1.1	Edits for Release
2023-05-08	2	Additions in Indexing Methods, Clarifications on Workflow Steps and Rebranding
2024-02-28	2.1	Compliance with IVDR
2024-11-29	2.2	Edits to introduce sequencing on Element Biosciences platforms

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Trademarks and Disclaimers

Use of the genetic data management web application SIRIUS signifies the agreement of any user with the following terms:

1. It may be used solely with the products manufactured by Medicover Genetics Ltd.

2. Medicover Genetics Ltd with registration number HE 418406 (ex NIPD Genetics Molecular Laboratories Ltd) (the Manufacturer) grants no license to use or incorporate the benclosed component of SIRIUS except as described in the IFU.

3. SIRIUS is a proprietary web application for genetic data management and is licensed for one-time use and may not be modified, or resold.

4. The sale and the use of SIRIUS is conditioned on not using this product outside such prescribed fields of use or any other purpose not expressly authorized by the Manufacturer.

5. It may include limited, non-transferable, licenses under the relevant legislation of patents owned by Medicover Biotech Ltd with registration number HE 418372 (ex NIPD Genetics Biotech Ltd) or Medicover Public Co Ltd with registration number HE 275644 (ex NIPD Genetics Public Company Ltd).

Introduction

SIRIUS is a genetic data management web application that enables users to manage information generated by Next Generation Sequencing analysis after samples have been processed using the medical devices manufactured by Medicover Genetics Ltd. In general, the system facilitates the creation and modification of sample batches corresponding to sequencing runs and interfaces with the Medicover Genetics analysis engine, VEGA, to provide information for the analysis runs and retrieve the analysis data for visualization in graphical and tabular format.

SIRIUS, as an information management tool, is intended, but not restricted, to:

- Manage account and user creation requests
- Create batches of samples and perform sample validations
- Calculate estimated sequencing output for the run when using TarCET KIT assays, TarCET PGT assay, or in combination with VERACITY assays
- Create and maintain samples under each batch

- Collect and store sample information parameters for the analysis
- Communicate to cloud-based storage and analysis engine to exchange data
- Provide the means for intuitive visual analysis of data for fast interpretation
- Enforce strict security rules for data access based on user roles

System Requirements

SIRIUS is provided to users under a Software as a Service (SaaS) model. It is a web-based application and can be easily accessed through an internet browser.

Supported Internet Browsers

- Chrome: Version 95.0.4638.69 (Windows) or later, Version 95.0.4638.69 (MacOS) or later
- Safari: Version 15.0 (MacOS) or later
- Firefox: Version 94.0.1 (Windows and MacOS) or later
- MS Edge: Version 95.0.1020.44 (Windows) or later, Version 94.0.992.31 (MacOS) or later

NOTE: Observe the hardware and software requirements for the browser of choice as specified by the software provider

This document refers to a third-party tool for uploading raw sequencing data to cloud storage services, as part of the data flow. Installation of this tool should be performed on a computer, adhering to the following requirements:

Supported Operating System (OS)

- Windows 7/8/10

Minimum Hardware Requirements

- 1.4 GHz 64-bit processor
- 512 MB RAM, 100 MB minimum disk space
- Gigabit (10/100/1000baseT) Ethernet adapter

Software Prerequisites

- Microsoft .NET Framework 4.5.2

System Functionality

SIRIUS provides the following functionality to the user:

- Lab account self-registration and user maintenance
- Creation of new or modification of existing sample batches. Each batch corresponds to a sequencing/analysis run of samples processed using the Medicover Genetics line of products
- Sample processing workflow execution with respect to data management
- Interface with analysis engine (VEGA)
- Repository of processed runs, with the ability to retrieve data from individual samples generated from previous batches (runs)
- Visualization of the analysis results for each sample
- Export of analysis files to be used with third party tools for tertiary analysis

Access to the System

Users can access the system by using the URL https://sirius.medicover.com and providing their username and password. If users forget their password, they can reset it by following the "Forgot Password" link below the login box. First time users should follow the Account and User Registration process.

Account and User Registration

Users must first create a lab account and user accounts on SIRIUS in order to manage their sequencing runs and view or download analysis results.

Lab Account

The lab account is created during the initial registration, along with the Lab Director user account. Additional users can be registered, under the Lab Account at any time.

Users can register by using the URL https://sirius.medicover.com and clicking on the Register option (top right corner). The same URL can be used for subsequent logins to the system.

During registration, the user is requested to provide the following information for an account:

- Name of laboratory which will be processing the samples
- Telephone number of the laboratory
- Street address, postal code and country of the laboratory
- First and Last name of the user
- Email address of the user
- Mobile phone of the user
- Password

Each request for account creation will be subject to approval by Medicover Genetics and the user will be notified via email after approval.

Following the approval of the account, sequencing equipment used in the laboratory is required to set-up the account. More specifically the:

- Make
- Model
- Serial Number

User Accounts

The Laboratory account includes by default a Lab Director account with the credentials of the person who requested the lab account. Additional users can be created, by the Lab Director by providing the following information:

- Username (email address)
- First and Last Name
- Access level to be provided (Role)
- Mobile phone of the user

New users are subject to approval by Medicover Genetics and approved users are notified by email.

Dashboard and Menu

When users log in to the system they are presented with a dashboard and a left-side menu.

Menu

The menu is visible in all screens and can be used for accessing the system's functionality via links. There, the user can also select one of the available languages of the system.

The following items are visible on the dashboard:

Create New

This button is used for creating new batches. See Batch Creation section below for details.

Batches

This button is used for accessing the repository of batches previously entered the system. When users press the button, they are presented with a table listing all the previous batches created in the system. The following features are also available:

Filtering

Use this feature to filter the list of batches based on the following criteria:

- RUN ID
- Date Created
- Status Description
- Sequencing Kit Type
- Sequencer
- Flowcell Serial No

Export

Use this feature to export the contents of the batches table in a spreadsheet format.

Calendar View

Use this feature to view batches in calendar representation. When selecting the tab Calendar, the users are presented with a calendar form on which dates that contain batches are marked with a blue line. When clicking on the calendar date, the Sequencing Run IDs of the batch(es) appear on the left. The user can click on the Sequencing Run ID and navigate to the respective batch record.

Search

Used for accessing the general search functionality. See section Search Functionality.

Recent Batches

This is a table that lists the last five batches entered into the system for easy access. Click on a row of table to go to the respective batch details.

Sample Processing Workflow

SIRIUS is primarily used for the data management of the sample processing workflow. The sample processing workflow for the TarCET and TarCET PGT assays is described in Figure 1.

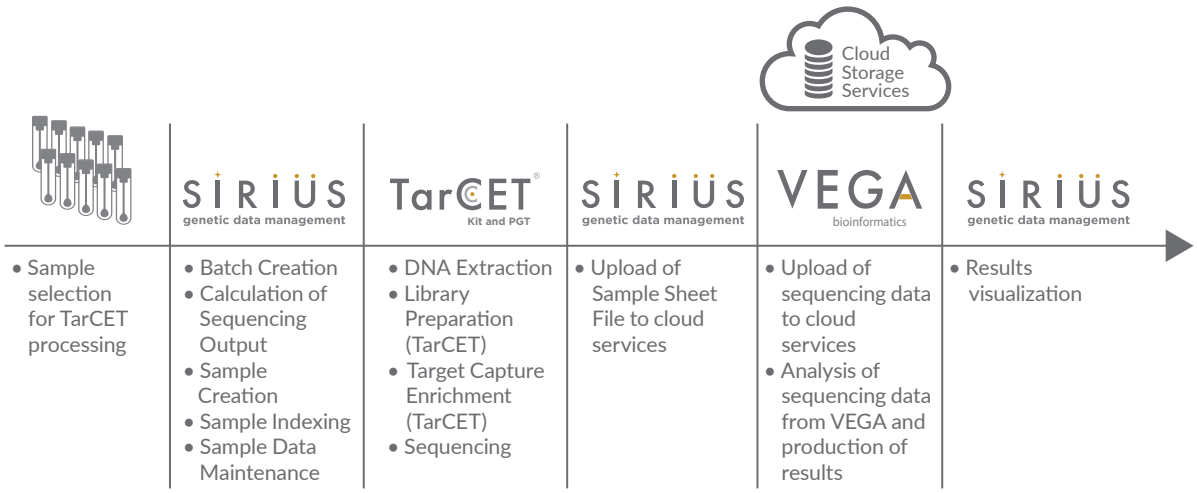


Figure 1. The Workflow of TarCET and TarCET PGT assays workflow

Sample Selection

Prior to the creation of a batch in SIRIUS the user should select the samples to process. Having the information of the samples, the user can execute the workflow steps related to SIRIUS.

Batch Creation

The steps below describe how a new batch is created:

- Log in
- Click on Create New icon
- Fill out the following parameters:

Parameter	Description	Mandatory
Sequencing Run ID	8-digit unique number	Yes
Flowcell Serial No.	9-digit Serial No. found on the flowcell of the sequencing kit to be used (e.g. HG7VWBGXJ)	Yes
Sequencer	(e.g. NEXTSEQ 550)	Yes
Sequencing Kit Type	(e.g. NextSeq 150 cycles High Output Kit)	Yes
TarCET and TarCET PGT Assays Kit LOT Number(s)	Scan the LOT number of the kit(s) used to process the batch. This is useful for traceability purposes.	Yes

Table 1. Batch parameters

CAUTION: Based on the selected type of Sequencing kit, the system provides information about the number of cycles in brackets. Please note this number for later use as an input parameter to the instrument during sequencing run loading.

Analysis Resolution Selection

For PGT assays and MiSeq users, there is an option for selecting from a drop-menu either the low (20Mb) or the high (10Mb) structural rearrangement analysis resolution.

Calculation of Reserved Sequencing Capacity

SIRIUS provides an especially useful functionality for sequencing capacity calculation. The purpose of this functionality is to inform the user on the percentage of the selected sequencing kit's capacity that will be consumed by the assays they intend to include in their batch.

The sequencing capacity calculation functionality is available at batch level for newly created batches (see Batch Creation section) or for existing batches (See Batch and Sample Data Maintenance section). To use this functionality, first the user selects the Kits to be used and declares the number of samples they intend to process with each type of assay. Based on the input parameters SIRIUS performs calculations to inform the user whether the sequencing run is valid with respect to the consumed sequencing capacity. The user is prompted to state the number and assay type of the samples to process. The system displays a percentage (in the form of a gauge) which indicates an approximation of the occupied sequencing kit's capacity. Apart from TarCET and TarCET PGT assays, the system provides the option to select VERACITY assay for sequencing in the same run. In such cases the system displays a "Polaris Transferred" value that, once is locked (see Batch and Sample Data Maintenance section), needs to be copied and pasted to the Polaris software under the following destination: Polaris sequencing run, Create, Available capacity.

A new batch is initiated in the system with the status **Created**.

Sample Creation

Once all the information has been introduced into SIRIUS and the user is ready to proceed to the Sample Creation and Indexing, the batch will change to the status **Open**.

If not already logged in to the batch's screen:

1) Log in
2) Click on Batches icon
3) Select the batch to process

There you will observe that SIRIUS automatically generated a number of sample records, per assay type, which equals to the number of samples defined in the calculation tool. The system also assigned a unique serial number to each sample as part of the system's internal traceability schema.

Subsequently, the user needs to define the following laboratory related parameters:

- Reference number originating from the Laboratory's own traceability schema
- Reference 1 and Reference 2 which are not mandatory for SIRIUS but can be used by the user for further traceability between samples
- The Panel requested for each sample, by clicking on the yellow flag (only for assays with multiple panel options)

Sample Indexing

Indices are used for uniquely identifying the samples of a batch in a sequencing run, thus enabling the analysis engine to provide results based on this identifier. TarCET compatible indexing primers are provided by Medicover Genetics under the UltraVerse line of products. Refer to the corresponding instructions of use documents WI-03-102-TT Instructions for Use – IFU – UltraVerse, WI-03-100-TT Instructions for Use – IFU – TarCET KIT, and WI-03-101-TT TarCET PGT for more details.

CAUTION: Before completing the indexing step in SIRIUS, the user must first determine the physical availability of the index plate(s) to be used for the execution of the lab workflow. There are four options for index plates (types A, B, C and D). Depending on which plate type is available in the lab's stock and its available indices (wells), the user must decide on which sample to assign each index. It is extremely important to assign the correct index to each sample in the correct order. Each index assigned in SIRIUS should correspond to the one used from the UltraVerse Indexing Oligos plate provided by Medicover Genetics. An error at this stage will result in incorrect traceability of the samples by the analysis engine and to sample mix up. Users must be extremely cautious of this fact.

Index assignment

To proceed with sample indexing follow the below steps:

If not already logged in to the batch's detail screen:

1) Log in
2) Click on Batches icon
3) Select the batch to process

There are two modes for selecting which index to use for each sample, both featuring a visual representation of the indexing plate. The manual mode does not have any restrictions on the order of assignment, but the automated mode assumes that the physically available indices are sequential:

A) Manual selection of individual indices

The manual indexing is the default mode under sample indexing functionality. With manual indexing the user individually selects which plate and index they want to assign to each single sample.

On the batch details list select the corresponding Plate and Index from the respective dropdown list for each sample in the batch. The user is free to select the indices in any plate or well combination, without restrictions.

To save the assigned indices to the samples the user must click the Save button.

B) Automatic selection of indices

Automatic indexing is used to assist the user by eliminating the need to individually select the index for each sample. This is especially useful in batches with many samples. The functionality allows only sequential indexing by choosing the plate type and the starting position (first Index) on that plate. Multiple plates can also be used if needed.

To assign the indices the user must first turn the "Manual Indexing" switch to "OFF". The system clears any previously assigned indices and the Plate and Indices columns on the list become inactive for selection.

Next, the user must select from the UltraVerse Plate dropdown menu the starting plate and click on the AUTO INDEXING button.

A pop-up window appears, and the user must select the starting index on the plate and click the AUTO ASSIGN button which places the indices on the plate sequentially in ascending order.

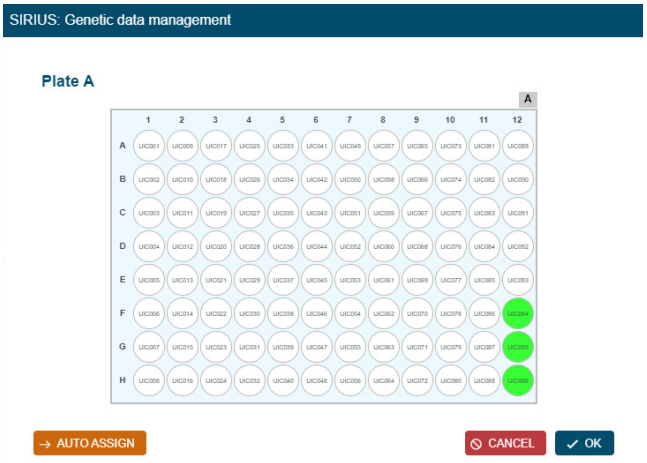


Figure 2. Selection of indexing starting position

By clicking the OK button, the system automatically assigns the indices to the list of samples and closes the pop-up window.

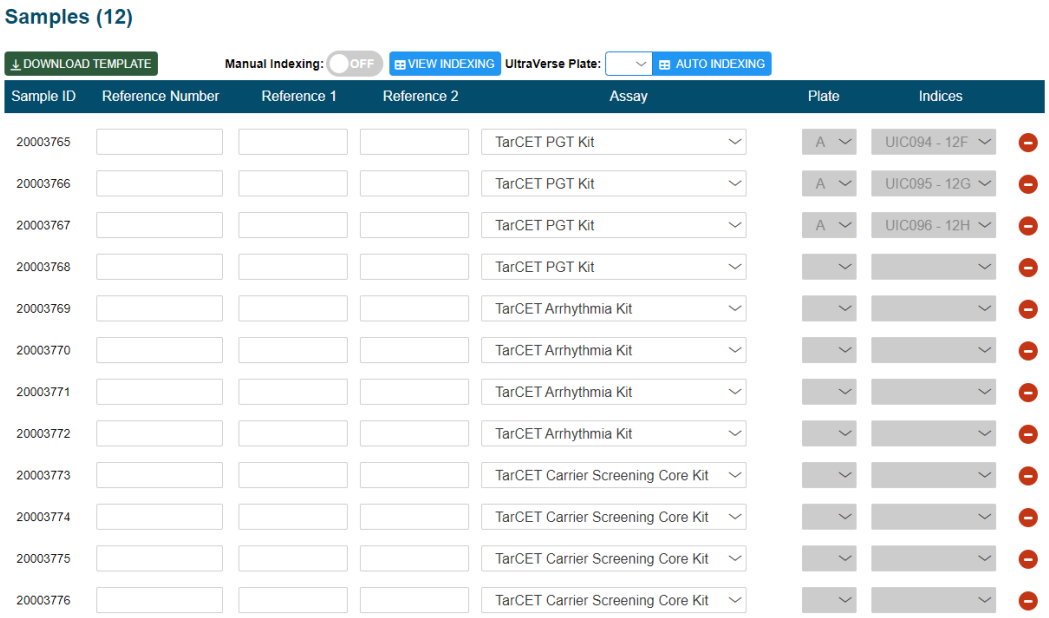


Figure 3. Automatically assigned indices

In cases where more than one index plate must be used, the user selects again from the UltraVerse Plate dropdown list, the next plate and repeats the process. In this case, it is not necessary to select a starting position since the auto assignment continues from position A1 of the subsequent selected plate.

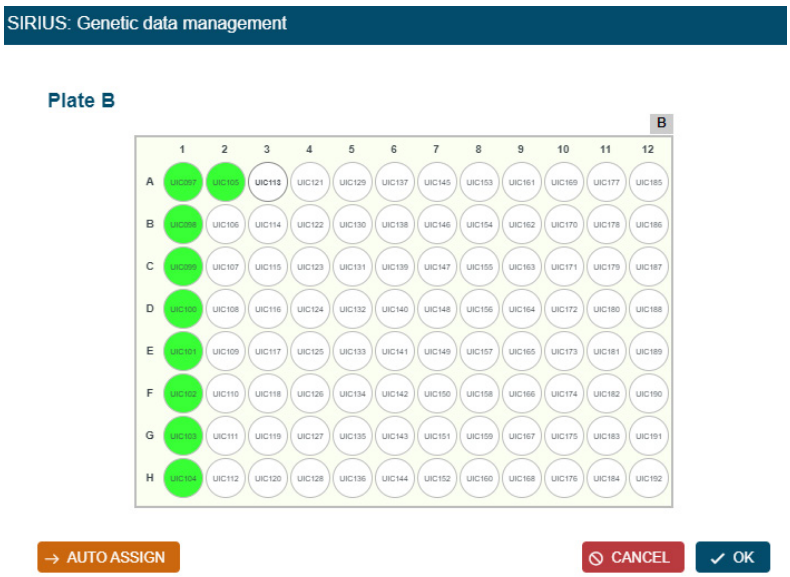


Figure 4. Assign indices to a subsequent plate

By clicking the OK button, the system automatically assigns the indices to the list of samples and closes the pop-up window.

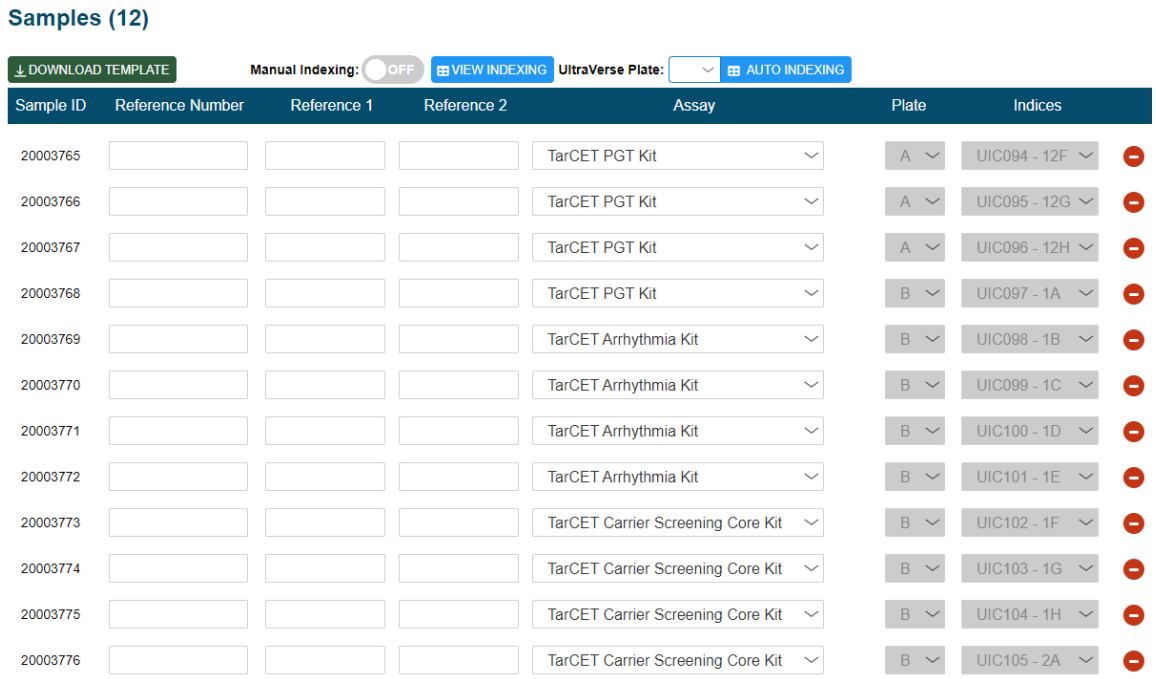


Figure 5. Automatically assigned indices (subsequent plate)

To save the assigned indices to the samples the user must click the Save button.

Resetting automatic indexing

In case the user needs to reset all automatic indexing, they can switch Manual Indexing to “ON” and then back to “OFF”. This clears the already assigned indices and the user can start Automatic Indexing from the beginning as described above.

Viewing assigned Indices

The system provides the option to view the assigned indices before proceeding to the next step. Clicking on VIEW INDEXING button, a pop-up window opens and contains the available plates. The assigned indices on each plate are highlighted with the corresponding sample ID.

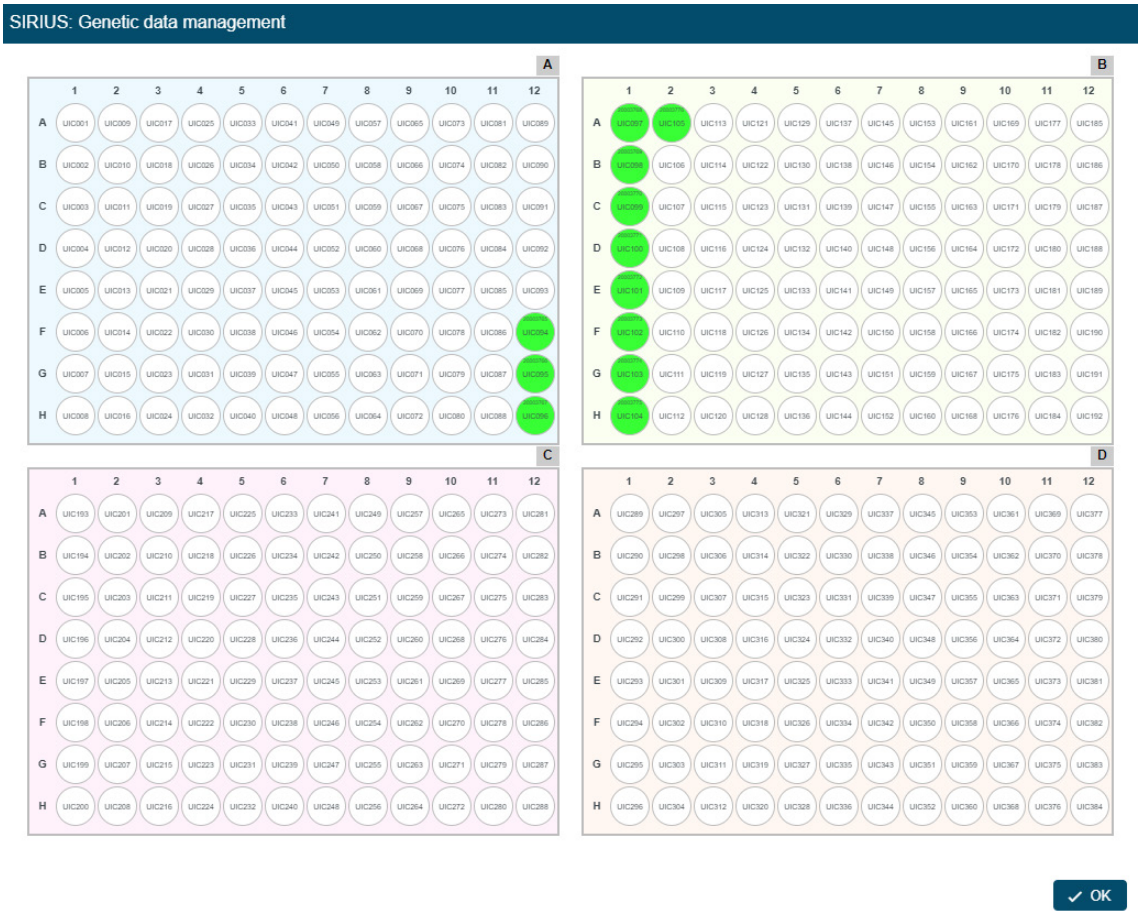


Figure 6. Assigned indices view

INFO: In both automatic and manual modes, before moving the batch to the next ("Locked") workflow status, SIRIUS validates whether the same index has been assigned to more than one sample in the same batch.

CAUTION: Each index assigned in SIRIUS should correspond to the one used from the UltraVerse Indexing Oligos plate provided by Medicover Genetics. An error at this stage will result in incorrect traceability of the samples by the analysis engine and to sample mix up.

Batch and Sample Data Maintenance

While the batch is at status **Open** the user can still add, remove, or edit any of the samples. The steps described above can be done either individually for each sample or collectively, via the use of interface files which can be imported into SIRIUS.

If not already logged in to the batch's detail screen:

1) Log in
2) Click on Batches icon
3) Select the batch to process

There are two different types of files that can be used:

1. The "SIRIUS Samples Import Template" file
   This file is a template that can be used to create new samples in the batch. The user downloads the template and fills in the following information:

Field	Description	Editable
S/N	An incrementing serial number, unique within the file. The system will assign another internal unique Sample ID at sample creation	Yes
Reference Number	The reference number for the sample under the Laboratory's traceability schema	Yes
Reference 1	Additional references for the sample under the Laboratory's traceability schema	Yes
Reference 2	Additional references for the sample under the Laboratory's traceability schema	Yes
Product	Product ordered i.e. TarCET PGT	Yes

Table 2. SIRIUS Samples Template file fields

2. The "Samples List" file
   This file can be exported (includes all the existing samples of the batch) modified by the user and imported back to the system. It is used to enter or update the following information:

Field	Description	Editable
Sample ID	The sample ID as assigned by SIRIUS upon creation	No
Reference Number	The reference number for the sample under the Laboratory's traceability schema	Yes
Reference 1	Additional references for the sample under the Laboratory's traceability schema	Yes
Reference 2	Additional references for the sample under the Laboratory's traceability schema	Yes
Product	Product ordered i.e. TarCET PGT	No
Indices	The Index code	No
Loading Quantity (ng)	The recommended DNA quantity to obtain the desired sequencing output	No

Table 3. SIRIUS Samples List File fields

NOTE: Updating sample details, introducing new or deleting existing samples, causes SIRIUS to re-calculate the sequencing capacity for the sequencing run.

Once all requirements are met the batch can proceed to the **Locked** status. At this status, no further editing can be done.

NOTE: Under special circumstances, a **Locked** batch can be unlocked for editing only by a user with Lab Director access level.

Lab workflow

At this stage (**Locked** status) the "Samples" file can also be used as a batch worksheet when processing the samples during the DNA Extraction, Fragmentation, Library Preparation and Target Capture Enrichment.

DNA Extraction, Fragmentation, Library Preparation and Target Capture Enrichment

Refer to WI-03-100-TT Instruction for Use – IFU - TarCET KIT and WI-03-101-TT Instructions for Use – IFU – TarCET PGT.

Sequencing

At the end of the workflow, the user needs to load the pool of samples into the sequencer and initiate the sequencing run.

Sample Loading Quantity for Pooling

Sample Loading quantity for pooling is the amount required to load on sequencer per sample depending on the assay type used. To determine the value of the loading quantity follow the steps below:

If not already logged in to the batch's detail screen:

1) Log in
2) Click on Batches icon
3) Select the batch to process

The loading quantity is listed in the samples screen as a column with header "Loading Quantity (ng)".

Samples (15)

Sample ID	Reference Number	Reference1	Reference2	Assay		Loading Quantity (ng)	Indices
10543	1			Cardiovascular Diseases - TarCET Cardiac Comprehensive Kit	Panel	0.40	UIC249 - 8A
10544	2			Cardiovascular Diseases - TarCET Cardiac Comprehensive Kit	Panel	0.40	UIC250 - 8B
10545	3			Cardiovascular Diseases - TarCET Cardiac Comprehensive Kit	Panel	0.40	UIC251 - 8C
10546	4			Cardiovascular Diseases - TarCET Cardiac Comprehensive Kit	Panel	0.40	UIC252 - 8D
10547	5			Cardiovascular Diseases - TarCET Cardiac Comprehensive Kit	Panel	0.40	UIC253 - 8E
10548	6			Carrier Screening - TarCET Carrier Screening Comprehensive Kit		0.55	UIC254 - 8F
10549	7			Carrier Screening - TarCET Carrier Screening Comprehensive Kit		0.55	UIC255 - 8G
10550	8			Carrier Screening - TarCET Carrier Screening Comprehensive Kit		0.55	UIC256 - 8H

Figure 7. Loading Quantity

Reagents and Samples Preparation for Sequencing

To perform a sequencing run, prepare the sequencing reagents according to the corresponding manufacturer's User Guide.

Prior to sequencing setup, denature and dilute the library pool and phiX according to the DNA Denaturation Guidelines protocol (document #WI-46).

Sequencer Setup

Instructions on how to set up the sequencer and which information needs to be entered can be found in the corresponding manufacturer's user guide. The Manual Option Setup is required for the Illumina platforms. Depending on the sequencing platform used, a set of parameters needs to be entered onto the sequencer's interface to correctly sequence the pool and identify the sequence run. (Table 4)

Manufacturer	Sequencing Kit	Read Type	Read Length	Index Read
Illumina Platforms	Miseq v3 150 cycles	Paired End	(2 x 75bp)	10bp (dual-indexing)
	Nextseq High-Output 150 cycles Or Nextseq Mid-Output 150 cycles	Paired End	(2 x 75bp)	10bp (dual-indexing)
	Novaseq SP 200 cycles	Paired End	(2 x 100bp)	10bp (dual-indexing)
	Novaseq SP 100 cycles	Paired End	(2 x 50bp)	10bp (dual-indexing)
Element Biosciences Platforms	AVITI Cloudbreak FS Medium Output	Paired End	(2 x 75bp)	10bp (dual-indexing)
	AVITI Cloudbreak FS High Output	Paired End	(2 x 75bp)	10bp (dual-indexing)

Table 4. Sequencer Setup Parameters

CAUTION: It is critical to correctly enter those parameters, during the loading of the run to the sequencer, as they are used for matching the sequencer produced data with the samples in SIRIUS.

Once the sequencer has been loaded and the sequencing run has been initiated the VEGA analysis engine must be provided with the appropriate data for the analysis as described in the following section.

Data Flow Between Systems

Medicover Genetics utilizes best in class cloud storage services for the purpose of data exchange and storage. Figure 8 depicts the architecture of data communication between SIRIUS, Sequencer, Cloud Storage Services (CSS) and VEGA.

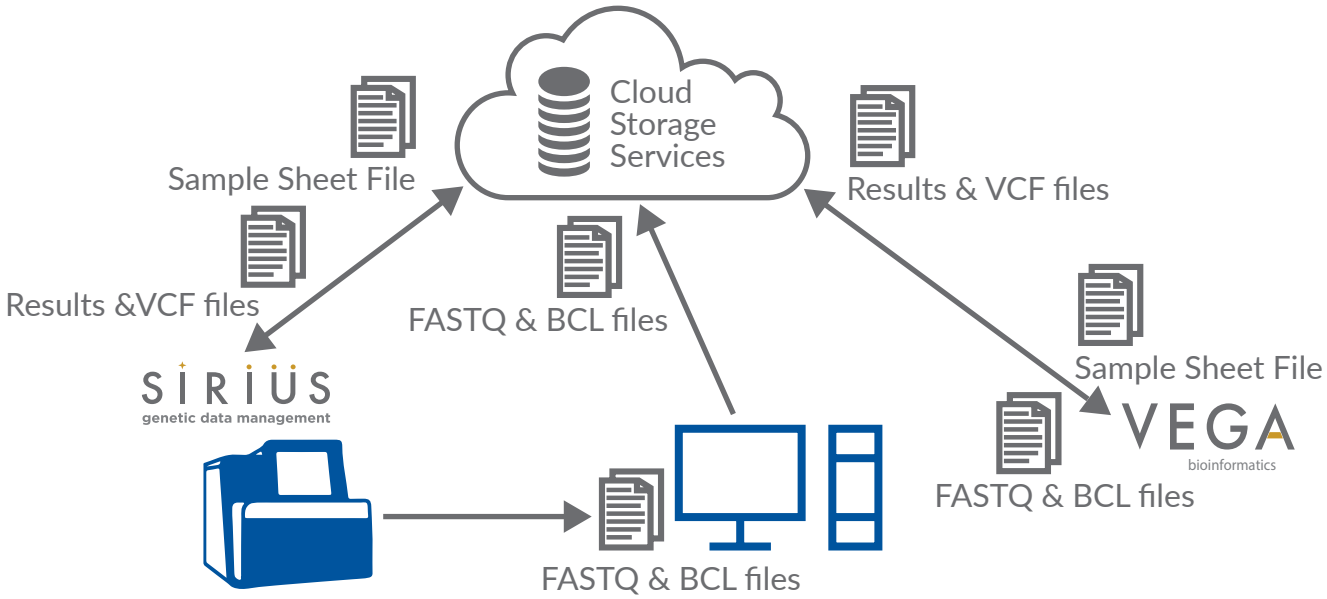


Figure 8. Data exchange between SIRIUS, Sequencer, CSS and VEGA

Sample Sheet File Export

The VEGA analysis engine requires two sets of data in order to analyze and provide results for the sequencing run. The first set is the Sample Sheet File which includes all the information maintained in SIRIUS that is necessary for the analysis of the samples such as indexing, type of kit/ panel etc. After the laboratory workflow is completed and the samples are ready to be introduced for sequencing the user must:

- Log in
- Click on Batches icon
- Select the batch to process
- Click on the "Upload Sample Sheet" button

This action creates the file and automatically uploads it to VEGA. After completing this step, the batch is forwarded to the Pending status where it awaits the import of the analysis results.

Uploading Raw Sequencing Data

The second set of data that the VEGA analysis engine needs for performing the analysis are the raw sequencing data. Following the completion of the sequencing run, the user must upload the raw sequencing data to the account's cloud storage and make them available to VEGA. Each account (laboratory) has a designated secured and private cloud storage space for uploading the raw sequencing data. A third-party application is utilized for the most efficient upload of the raw sequencing data to the cloud storage services. Medicover Genetics provides the credentials, Access key and Secret Key, for the laboratory's account and the necessary third-party tool license, upon request and subject to approval.

The third-party tool uses a letter drive (e.g. Z:) in the user's Windows Operating System that is mapped to the corresponding storage location dedicated to the lab's account. Instructions on how to map the drive (once-off) are provided during the installation of the tool by the manufacturer.

The sequencer produces the raw sequencing data in a standard format under a specific folder structure. To locate and transfer the data follow the steps below:

- Log on to the windows machine where the third-party tool is installed.
- Navigate to the network drive where the sequencer created the files. This location was defined by the user during the loading of the sequencer as per manufacturer's user manual.
- Copy the complete folder that corresponds to the flowcell number used during the specific run and the run date.
- Open the mapped drive corresponding to the lab's storage location and paste the folder.

CAUTION: The complete folder of the sequence run, produced by the sequencer, must be copied AS IS. No editing of the folder's structure, file contents or file names should be done at any point by the user. Such alterations will result to a failed analysis batch.

Sequencing Run Data Analysis

Upon the completion of raw sequencing data transfer, VEGA automatically commences the analysis. The duration of the analysis depends on several factors, for example the sequencing kit used, the type of panel, etc. During analysis, VEGA processes the available data and concludes by producing a set of files for use in SIRIUS.

Those files include qualitative and quantitative metrics for the sequencing run, Copy Number Variant (CNV) results, Single Nucleotide Variants (SNVs) and indels for each sample.

Results Import

When results data files are ready, the user will be notified via email and be prompted to update the samples in the batch with their results.

- Log in
- Click on Batches icon
- Select the batch to process
- Click on the "Import Results" button

When the batch has been updated with the sample results, its status changes to **Completed**.
For result interpretation see section Results visualization and Interpretation in SIRIUS.

For **Completed** batches the system lists the sequencing run metrics as provided by the instrument, for the user's reference:

Metric	Description
Sequencing Output	The amount of Gigabases produced by the Sequencing Kit
Passing Filter Percent	The percentage of sequence fragments which pass the internal quality FILTERING
Above Q30 Percent	The proportion of sequence bases with quality greater or equal than 30
Cluster Density	The density of the clusters produced

Table 5. Sequencing run metrics

Re-analysis of Samples

In some cases, there is a need to re-analyse one or more samples using the same raw sequencing data but modified parameters such as a different panel selection etc. For this purpose, SIRIUS provides the Re-Analyse functionality where the user can select an already analysed batch and duplicate it, in order to modify the batch samples and re submit the batch for analysis. This is achieved by:

- Log in
- Click on Batches icon
- Select the Completed batch to process
- Click on the “Duplicate” button

The new batch will include copies of all the original samples, with all their information, and it will be in the Open status. The user can perform sample maintenance as described under Sample Data Maintenance, to modify the desired analysis parameters (e.g., panel selection).

Modification of the duplicated batch is limited to samples that have been processed with TarCET or TarCET PGT assays and for which selection of multiple panels is permitted. In addition, the user can remove any samples they do not wish to re-analyse. As soon as the desired modifications are completed the user must follow the workflow as described in the section Data flow between systems.

SIRIUS creates a link between the original and the duplicate batch and the user can easily navigate between them. Re-analysis can only be performed twice for a given batch, within 30 days from the batch’s completion date.

Search Functionality

SIRIUS, through search functionality, provides the user with the ability to extract information easily and quickly about single samples, batches of samples or specific product-panels.

To access this functionality:

- Log in
- Click on Search icon

There are various filters that can be used to restrict the data returned from the search query:

- Assay-Panel
- Batch ID
- Sample ID
- Run ID
- Date of Batch Creation or Completion

When returning results from a search query, SIRIUS lists the hits in a table with the following columns:

- Batch ID
- Status
- Sample ID
- Reference Number
- Reference 1
- Reference 2
- Assay
- Indices

Results Visualization and Interpretation

All samples using any TarCET Kit have their analysis results visualized in a tabular format. In addition to tabular format, all samples processed using the TarCET PGT have their analysis results visualized in a graphical format.

Once the run and the analysis are completed, the results are automatically uploaded onto SIRIUS for results visualization. The user will need to go under Batches and select the run.

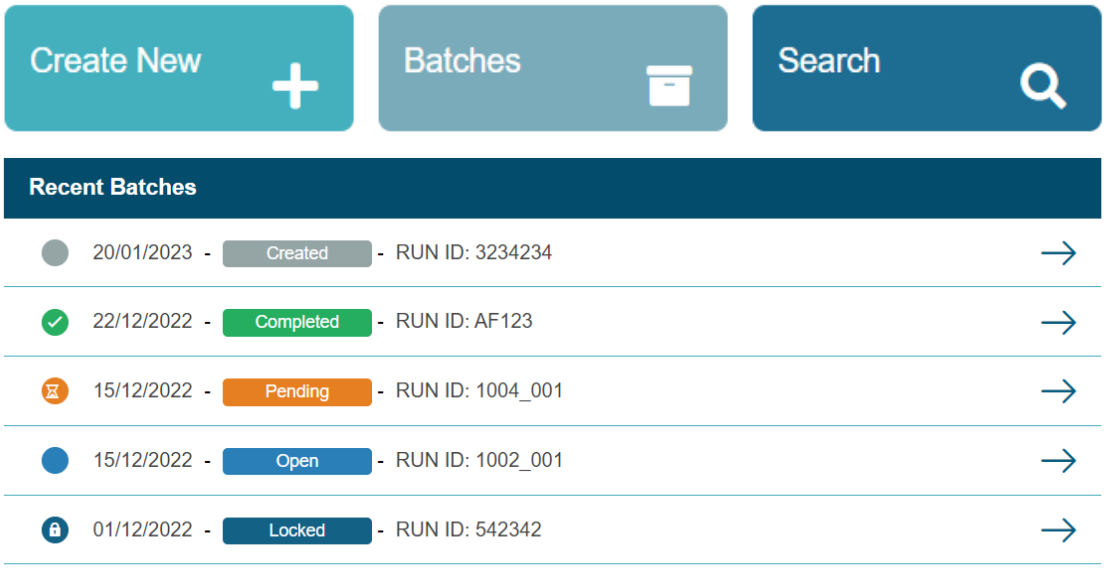
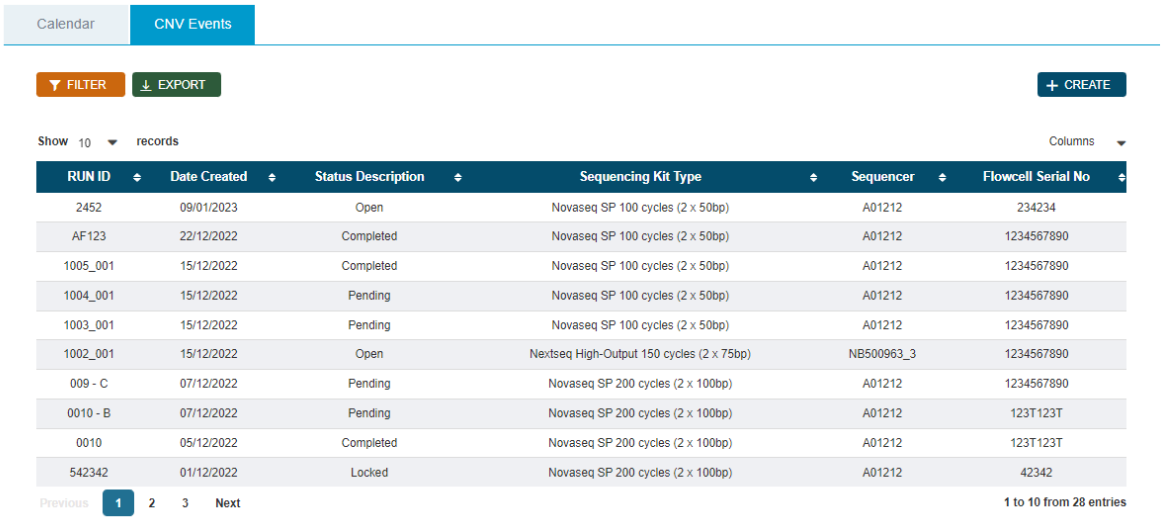


Figure 9. Dashboard screen



RUN ID	Date Created	Status Description	Sequencing Kit Type	Sequencer	Flowcell Serial No
2452	09/01/2023	Open	Novaseq SP 100 cycles (2 x 50bp)	A01212	234234
AF123	22/12/2022	Completed	Novaseq SP 100 cycles (2 x 50bp)	A01212	1234567890
1005_001	15/12/2022	Completed	Novaseq SP 100 cycles (2 x 50bp)	A01212	1234567890
1004_001	15/12/2022	Pending	Novaseq SP 100 cycles (2 x 50bp)	A01212	1234567890
1003_001	15/12/2022	Pending	Novaseq SP 100 cycles (2 x 50bp)	A01212	1234567890
1002_001	15/12/2022	Open	Nextseq High-Output 150 cycles (2 x 75bp)	NB500963_3	1234567890
009 - C	07/12/2022	Pending	Novaseq SP 200 cycles (2 x 100bp)	A01212	1234567890
0010 - B	07/12/2022	Pending	Novaseq SP 200 cycles (2 x 100bp)	A01212	123T123T
0010	05/12/2022	Completed	Novaseq SP 200 cycles (2 x 100bp)	A01212	123T123T
542342	01/12/2022	Locked	Novaseq SP 200 cycles (2 x 100bp)	A01212	42342

Figure 10. Batches listing screen

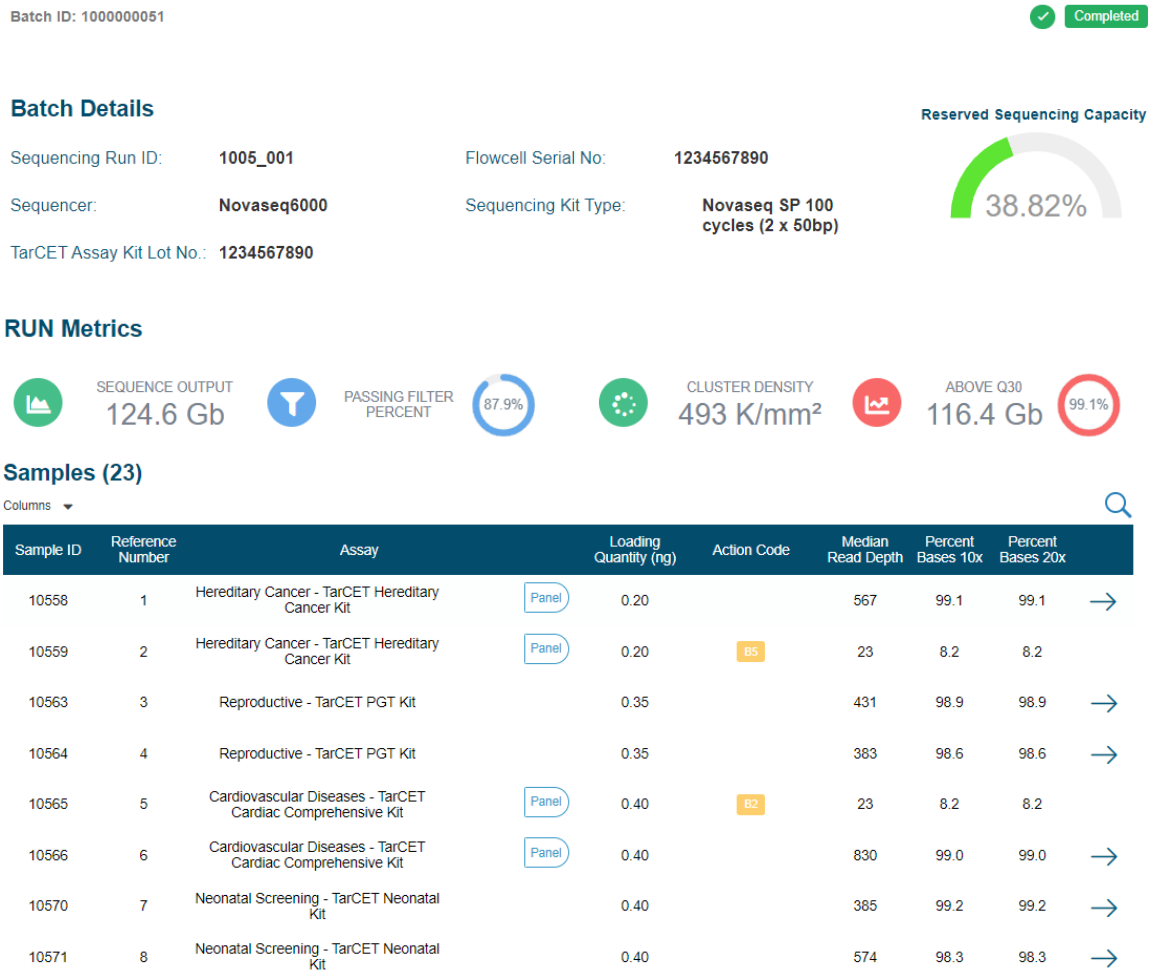


Figure 11. Batch details screen
By pressing on the arrow, the sample results will appear on the next screen.

Results for All TarCET Kits (except TarCET PGT)

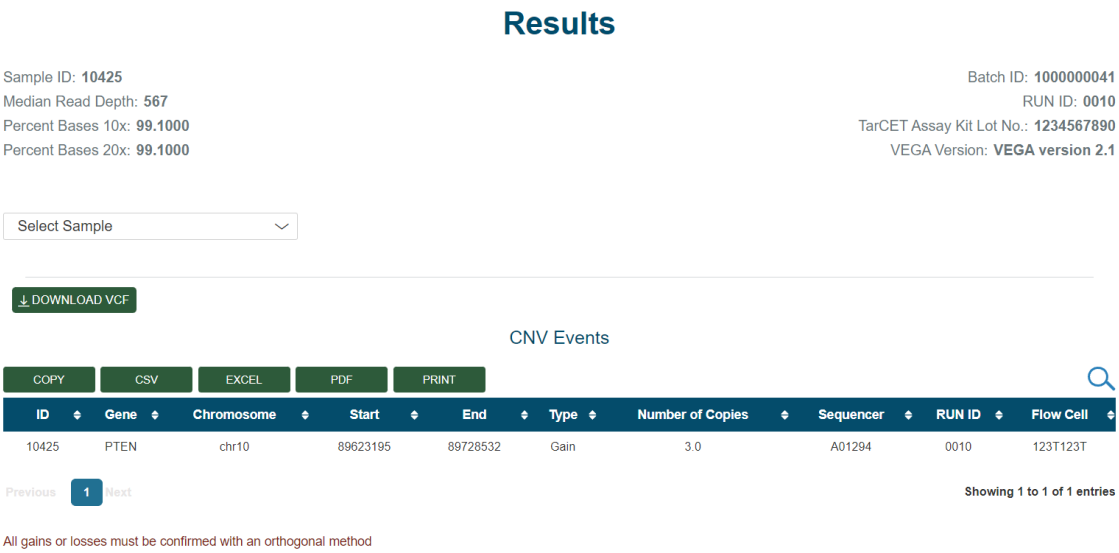


Figure 12. Example of CNVs results table for all TarCet kits (except Tarcet PGT)

ID	Sample ID
Gene	Name of the gene with detectable abnormality
Chromosome	Chromosome number
Start	The start site of the genomic site with the deletion/duplication
End	The end site of the genomic site with the deletion/duplication
Type	The type of the genetic alteration
Number of Copies	The number of copies of the genomic site
Sequencer	The sequencer machine used during the run
RUN ID	Run ID
Flow Cell	Flow Cell used during the Run

Table 6. Description of each column of CNVs results

The example given in Figure 12 is interpreted as follows: A heterozygous duplication within PTEN gene has been detected located on chromosome 10 starting from 89623195 and ending at 89728532 genomic positions.

Additional Quality Metrics are provided per sample, regarding Coverage and Coverage Uniformity. Coverage indicates the read depth achieved per sample during sequencing and Coverage Uniformity indicates the percentage of bases sequenced across the target region at a given depth (e.g., 97% of bases covered with a minimum 20x coverage).

Results for TarCET PGT

For TarCET PGT, SIRIUS provides a simple visualization platform for rapid interpretation and fast reporting time. In the graphical form, clear, intuitive plots provide a visual representation of the copy number state of the tested embryos (Figure 13). Green circles represent genomic regions (bins) at 1Mb resolution (virtual distance from one bin to the next is 1Mb) based on the normalized read counts for every bin. Solid green line depicts the copy number state (Y-axis) of the underlying genomic region (X-axis), following bioinformatics analysis. If the green line is shifted away from the expected copy number state (2 copies) there is an indication of a gain or loss. In case 3 copies of a whole/partial chromosome are detected, the green line will shift upward towards the solid blue line. If a deletion is detected the green line will move downward towards the red solid line. All alterations are also provided in a reports table (Figure 14) along with additional details regarding their respective size, chromosome location and copy number state.

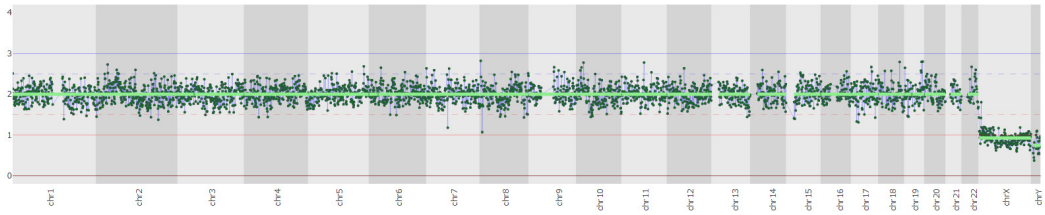


Figure 13. Graphical representation of the TarCET PGT test results

Events Table

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ID	Chromosome	Start	End	Copies	Confidence	Mosaicism	Size(Mb)	Cytogenetic Location
10590	chrX	59000	154309000	1.00	0.9973		154.250	X.p22.33-X.q28
10590	chrY	6209000	27809000	0.85	0.9993		21.600	Y.p11.2-Y.q11.23

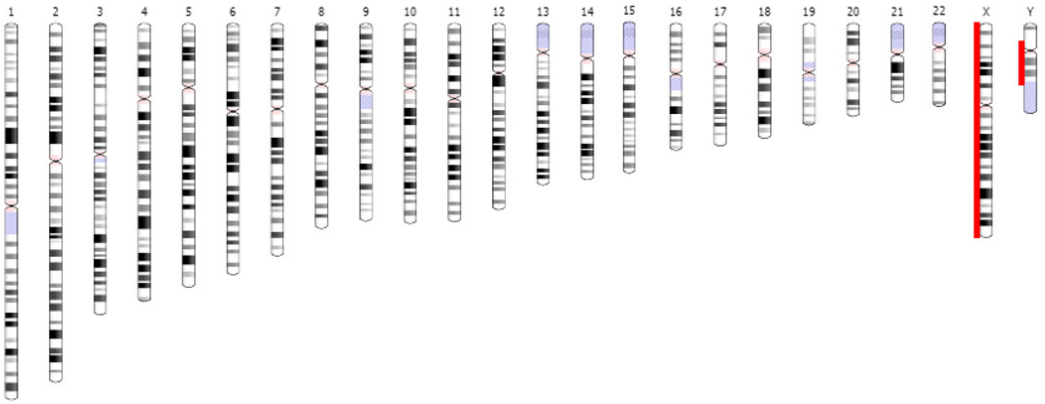

Figure 14. Table generated from TarCET PGT test results

The example given in Figures 13 and 14 indicates a normal male embryo.

Additional Results for TarCET Kit Infertility Panel

The Infertility Panel results include additional information in relation to specific tests performed by the kit:

- Chromosomal aneuploidies, structural rearrangements, and mosaicism detected on sex chromosomes (Table 7). The sample ID will be presented in the first column. In case of a positive result, the numerical or structural abnormality or mosaicism will be displayed in the respective columns.

ID	Aneuploidies	Structural rearrangements	Mosaicism
10010000	47,XXX detected		
10010001	47,XXX detected		
10010002	45,X detected		
10010003	47,XXY detected		
10010004			Loss of chrX detected 24%
10010005		Loss chrX: 113409000-114409000	
10010006		Gain chrY: 6109000-8609000	

Table 7. Results interpretation of TarCET Infertility test

CFTR 5T variant results. The sample ID will be presented in the first column. The number of thymidine's (T) on each allele will be displayed in the second column separated by '/ '.

ID	allele 1/allele 2
10101933	5/7
10101934	7/7

Table 8. CFTR 5T variant results

Potential Run Failures

In case of run failures, please follow the action codes below and the proposed actions on how to proceed.

Action Code	Sample Flag	Interpretation	Action Details
B2	High Sequencing Output	Sequencing output is out of range. Analysis not initiated	Repeat sequencing targeting lower output
B3	Low Sequencing Output	Sequencing output is out of range. Analysis not initiated	Repeat sequencing targeting higher output

Table 9. Potential flags and proposed actions

Technical Support

For assistance, contact the Medicover Genetics IVD Support team.

Email: ivdsupport.genetics@medicover.com

Terms and Conditions

The Terms and Conditions can be found online at SIRIUS genetic management application.