Takara Bio USA

Embgenix™ Analysis Software (RUO) User Manual

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(120624)

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I. Introduction

Embgenix Analysis Software (RUO) (also referred to in this document as 'analyzer') is cloud-based bioinformatics software for analysis of sequencing data as part of preimplantation genetic testing for aneuploidies (PGT-A) using the Embgenix PGT-A Kit (RUO) (Takara Bio, Cat. No. 634760).

After accessing the software via a web browser, sequencing output generated by the Illumina® MiSeq® or NextSeq® Systems is uploaded to it. The software then provides analysis information on copy number variants (CNVs) as a part of the Embgenix PGT-A (RUO) workflow. For technical support of this software, please contact takara-support@basepairtech.com. For assistance with the Embgenix PGT-A (RUO) reagent workflow, please contact Takara Bio technical support.

II. Before You Begin

Embgenix Analysis Software (RUO) is hosted on cloud servers and is designed to be used via a graphics-based web browser from any desktop or laptop computer with good internet connectivity. The requirements for using the software are listed below.

A. Supported Operating Systems

- Windows OS: Version 7 and higher
- Mac OS: Sierra (Version 10.12) or higher

B. Hardware requirements

Any standard desktop or laptop with the following specifications are recommended:

- Memory: 8 GB RAM or higher
- Free disk space: 100 GB or higher hard drive space
- Connectivity: Connection to a high-speed and reliable internet network for upload of input data

C. Additional software dependencies

- Web browser: Google Chrome (preferred), Safari, or Microsoft Edge
- Access to Illumina MiSeq or NextSeq sequencing data, stored either on the user's computer or a mapped network drive.

D. Required input files

- Single- or paired-end FASTQ files generated by an Illumina MiSeq or NextSeq System as part of the Embgenix PGT-A Kit (RUO) workflow.

Details on how to set up the sample sheet for the sequencing run are documented in the Embgenix PGT-A Kit (RUO) User Manual for Illumina MiSeq System or the Embgenix PGT-A Kit (RUO) User Manual for Illumina NextSeq 500/550 System, depending on which model of sequencer you use.

At the end of a sequencing run, the bcl2fastq command is triggered to automatically generate these files, based on the sample sheet, and stores them in an output folder. This output folder serves as the input to the analyzer and usually contains paired-end FASTQ files.

In general, any folder containing FASTQ files, as paired-end or single-end, and in compressed (`.fastq.gz`) or uncompressed (`.fastq`) format can be used as input for the analyzer; all other file types (e.g., with extensions like `.txt`, `.bam`, `.csv`, etc.) will be ignored. Additionally, if the files contain paired-end data, the analyzer expects the files to follow the same file-naming convention used by the Illumina bcl2fastq software.

In the rare event that the FASTQ files are not generated automatically, please refer to the Appendix, Section B for troubleshooting steps.

III. Software Overview

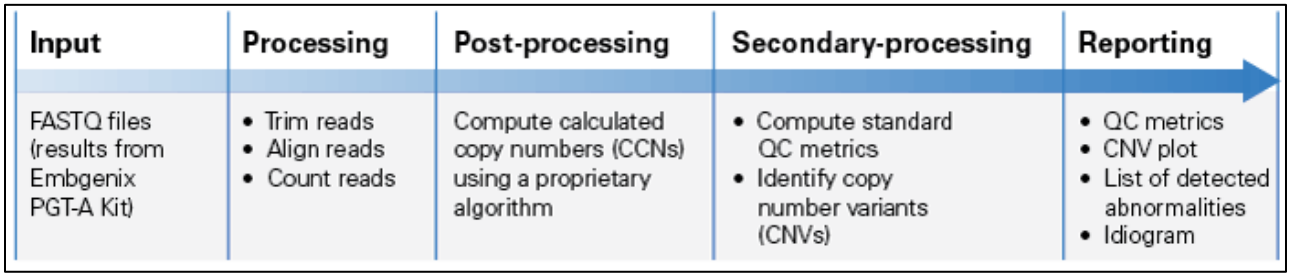


Figure 1. Embgenix Analysis Software (RUO) processing workflow.

The Embgenix Analysis Software (RUO) is cloud-based software to analyze the sequencing output generated by the Embgenix PGT-A workflow. It has a web-based graphical user interface (GUI) to import FASTQ files and return reports on identified CNVs, including chromosomal and segmental aneuploidies and mosaicisms.

- Input—FASTQ files containing sequencing data generated from your chosen Illumina sequencing platform (MiSeq or NextSeq Systems) are the required input into the analyzer.
- Trimming—The software performs trimming to remove adapter sequences and retain only the target sequence.
- Alignment—The target sequences are aligned to a human reference genome, hg38.
- Counting—The counts of reads mapping within pre-determined bins (fixed-length segments across the genome) are generated.
- Computing Calculated Copy Numbers (CCNs)—A proprietary algorithm computes CCNs using the bin counts from the previous step.
- Reporting Stats—The CCNs are then used to generate QC metrics that are reported by the software and identify CNVs.
- Reporting CNVs—The detected CNVs are reported as a genome-wide plot typical of such analysis, a companion list of detected abnormalities, and an idiogram.

IV. Accessing the Software

The Embgenix Analysis Software (RUO) can be accessed through sign-up on our website at takarabio.com. After signing up, an account will be created for you, and email sent to the email address provided in the form that will include the link to the software, a username, and temporary password to access to the software. An example login screen is shown in Figure 2.

Figure 2. Login screen of the Embgenix Analysis Software (RUO).

V. Importing and Analyzing Sequencing Data

The goal of this section is to provide instructions for setting up subjects in the database, importing FASTQ files into the database, associating them with a subject, and running the analysis program on the data.

After logging in, the Dashboard screen will display (Figure 3). For more information on the Dashboard page, refer to Section VII.A.

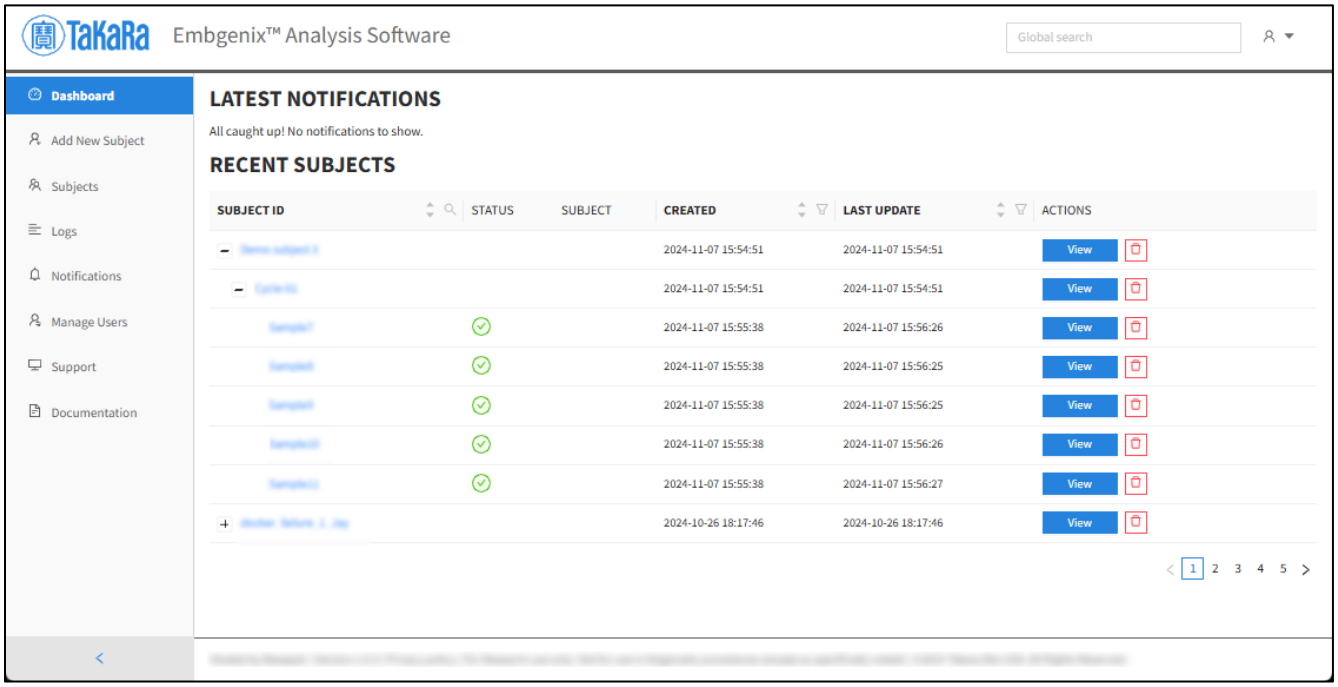


Figure 3. Dashboard. This is the default screen seen after login.

Figure 3 also illustrates the overall layout of the software.

- The items on the left of the frame list the Embgenix Analysis Software (RUO) menu (**Dashboard**, **Add New Subject**, etc.).
- The content corresponding to the selected menu option is displayed on the right-side pane.
- The person icon in the upper-right corner is a drop-down menu to access account and group-wide settings and the Logout option.
- The box to the left of the person icon is a global search against the database of subjects. Refer to Section VII.C for more information on this function.

A. Add New Subject

NOTE: For more details about the Add New Subject page, refer to Section VII.B.

To begin an analysis run, determine if:

- the subject whose samples are being analyzed already exists in the database or
- if the subject will need to be newly added.

Use the Subjects page (Section VII.C) to see the list of subjects previously set up in the database.

The instructions in this section assume the subject has not been added to the database. If the subject does exist in the database, skip to Section V.B ("Import FASTQ Files")

1. Add the new subject to the database. This can be done by one of two ways:
   - Click on the **Add New Subject** menu option in the left-nav bar

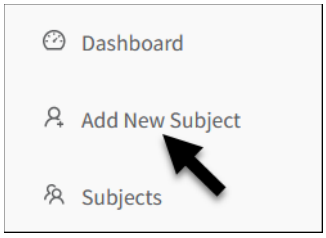


Figure 4. Add New Subject menu option.

   - From the Subjects page, click the [New] button

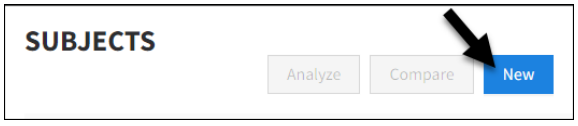


Figure 5. The [New] button in the Subjects view

   Either option will bring up the Accessioning page (see Figure 45 in Section VII.B).

2. Populate the two fields required to add a new subject:
   - Subject ID—This is a unique identifier to help you recognize the subject when listed in the Subjects page and on reports.
   - Cycle ID—the IVF cycle associated with the samples. This ID is used to group samples for reporting purposes.

   The remaining fields are optional but allow you to associate more information with the subject and store it in the database.

3. Once all desired fields are filled out, click the [Save] button on the bottom of the page. A "Data was saved successfully" message will pop up on the top center of the screen. The subject is ready to have sample files uploaded to it.

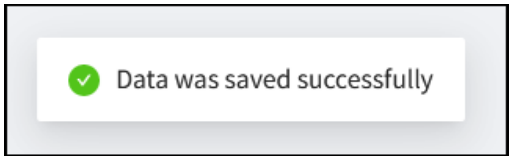


Figure 6. Success message after saving subject information.

B. Import FASTQ Files

The following steps explain how to upload the FASTQ files generated by sequencing the results of experiments run using the Embgenix PGT-A Kit (RUO) workflow.

1. Navigate to the Accessioning page of a previously configured subject; this can be done either immediately after creating a new subject (Section V.A, above), by clicking on the subject's ID number from the Subjects page (Figure 7), or by typing the subject ID into the Subject ID field on the Accessioning page (Section VII.B, Figure 47).

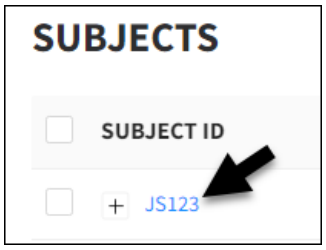


Figure 7. Navigating to the Accessioning page from an existing Subject ID.

2. At the bottom of the page, a view similar to Figure 8 should be seen.

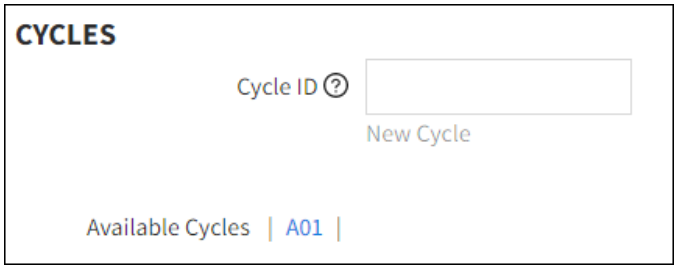


Figure 8. The list of "Available Cycles" on the Accessioning page.

Click on the Cycle ID in the "Available Cycles" list that you want to upload FASTQ files for. If no samples have been uploaded against this subject, a table similar to the image on the left of Figure 9 will display; if FASTQ files have previously been uploaded for the Subject+Cycle combination, it will resemble the image on the right of Figure 9, showing previous run information.

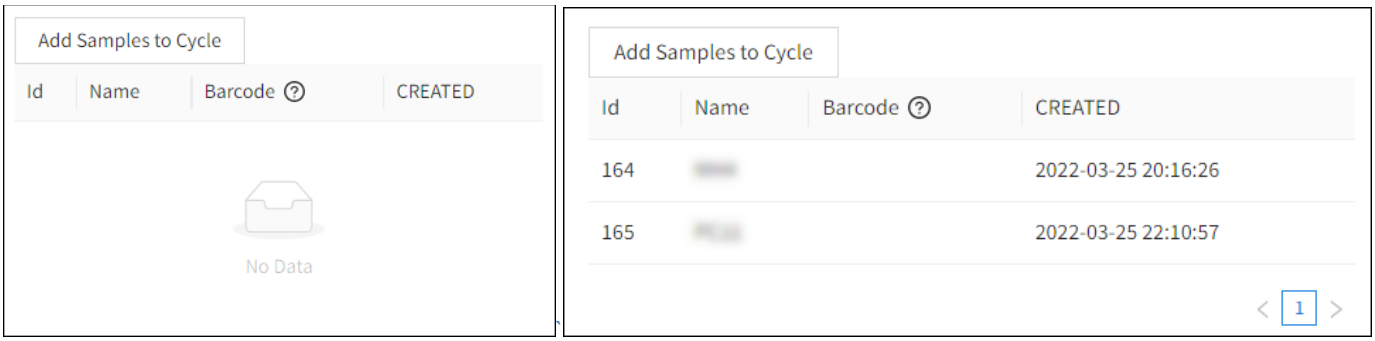


Figure 9. The samples table on the Accessioning screen. **(Left)** The table before any FASTQ files have been uploaded. **(Right)** The table displaying a record of previous FASTQ file uploads to the selected Subject ID+Cycle ID combination.

3. Click on the [Add Samples to Cycle] button above the table. The *New sample* window will pop up (Figure 10).

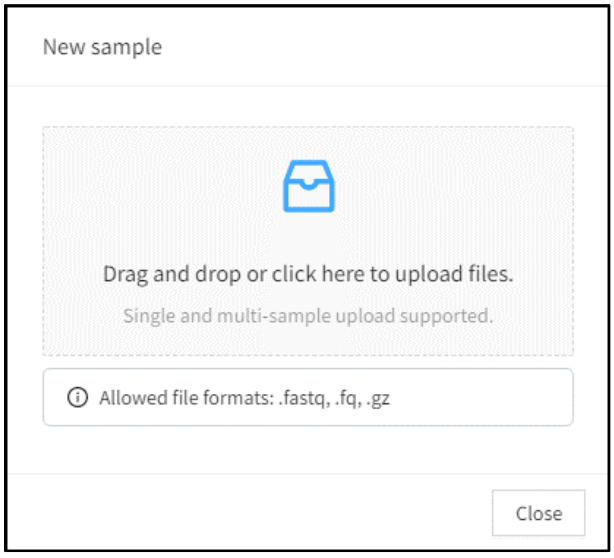


Figure 10. The default *New sample* window.

4. Follow the directions on the window to drag-and-drop a file or set of files onto the window or click on the icon to upload the zipped or unzipped FASTQ files.

   After the files are selected, the window will change to resemble the example in Figure 11.

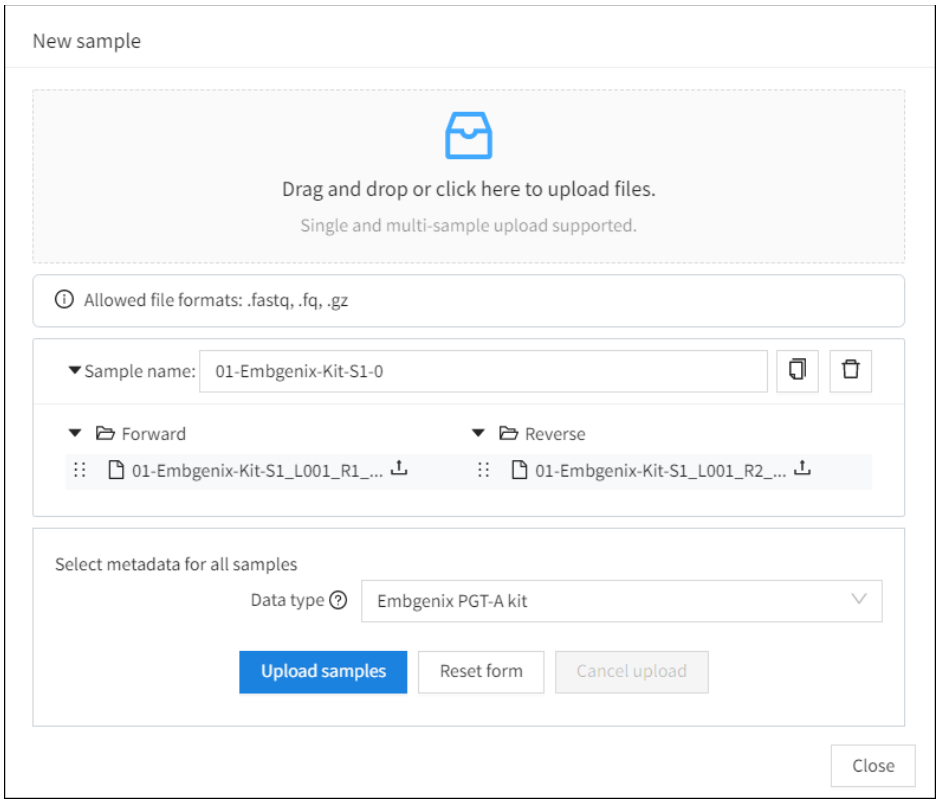


Figure 11. The *New sample* window, after forward and reverse index FASTQ files are uploaded.

The list below contains a brief description of the fields seen after uploading the files:

- "Sample name"—derived from the prefix of the FASTQ filename.
- "Forward"—the name of the FASTQ file for the forward indexes. A forward index is required for each sample.
- "Reverse"—the name of the FASTQ file for the reverse indexes. The sample name (base of the FASTQ filename) must be identical to be grouped together, if the files have different base names, they will be uploaded as multiple samples (Figure 12).

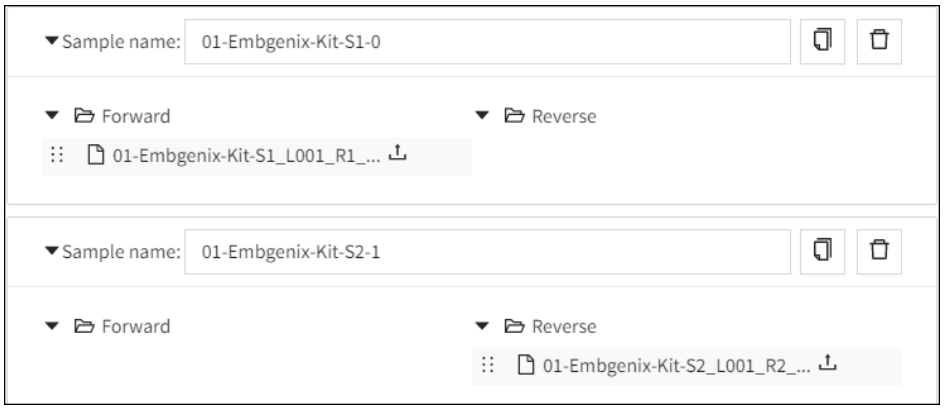


Figure 12. The *New sample* window if the FASTQ filenames have differing prefix text.

NOTES:

– For the "Data type" field in the "Select metadata for all samples section", do NOT change from the default 'Embgenix PGT-A kit' value.

– For more details about elements of the *New sample* window interface, refer to Section VII.B.

5. Once all desired FASTQ files are configured in the *New sample* window for the Subject+Cycle, click [Upload samples] to send the files to the software. Depending on the speed of your internet connection, this may take some time. A progress bar will display the status of the upload.

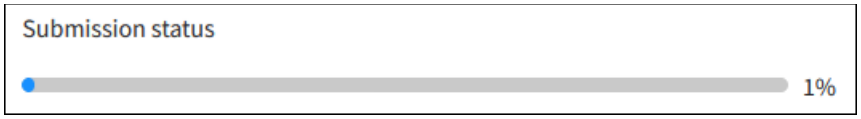


Figure 13. The "Submission status" upload progress for FASTQ files.

6. After the upload is complete, the sample table will update with the name of the sample(s) added and the timestamp of when they were uploaded.

Figure 14. The sample table on the Accessioning page after a FASTQ file upload.

C. Analyze Data

The following steps explain how to initiate a data analysis on the uploaded FASTQ files.

1. Click on **Subjects** in the left side menu.

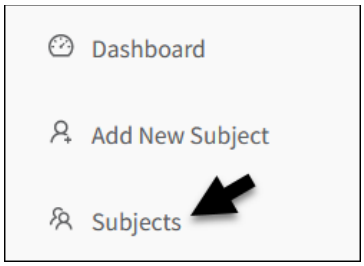


Figure 15. Subjects menu option.

2. In the Subjects table, click on the [+] icon next to the Subject ID then the second [+] icon next to the Cycle ID for the Subject+Cycle combination you want to run the analysis on.

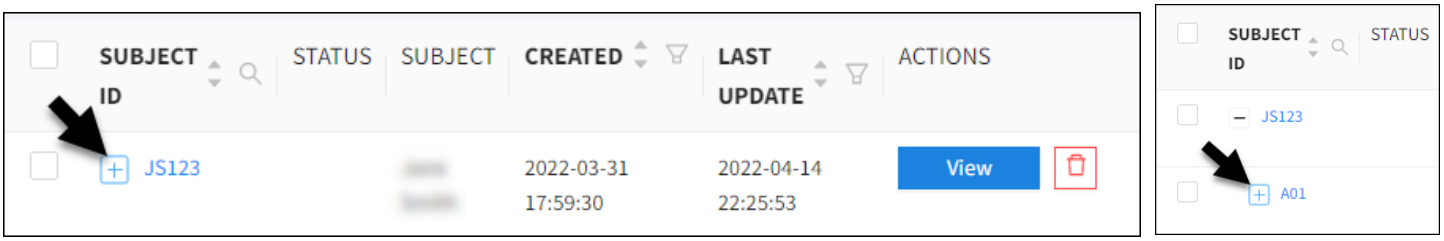


Figure 16. The Subject ID and Cycle ID selection hierarchy in the Subjects table.

The list of uploads executed for the combination will list below the Cycle ID, shown in rows 3–4 in Figure 17.

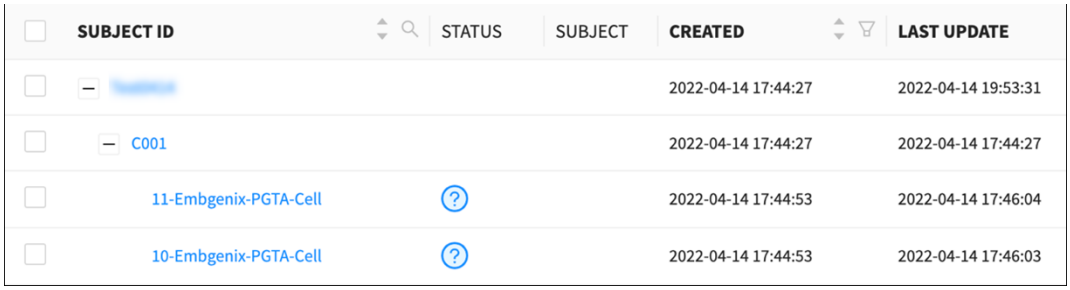


Figure 17. The record of FASTQ uploads for the Subject ID+Cycle ID in the Subjects table.

3. Analysis of sequencing data for a given sample can be initiated individually by clicking the corresponding [Analyze] button in the right-hand column of the row occupied by the sample as shown in Figure 18.

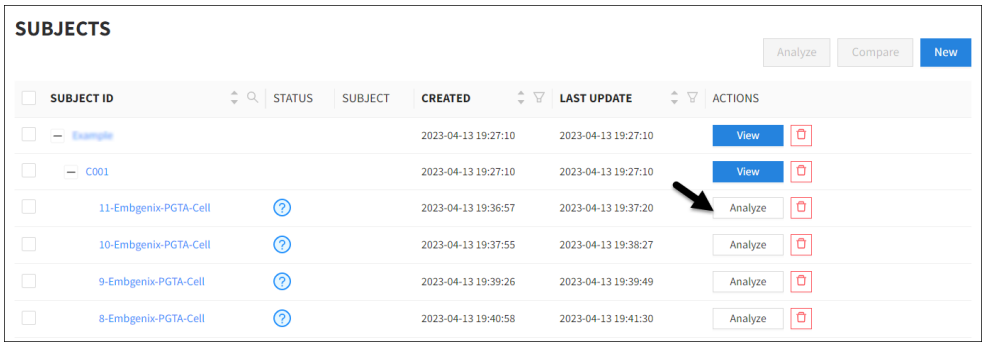


Figure 18. Initiating analysis of an individual sample.

Analysis of multiple samples can be initiated in parallel by selecting the check box(es) in the left-hand column for a given cycle or subset of samples and clicking the [Analyze] button in the top-right corner of the window (Figure 19).

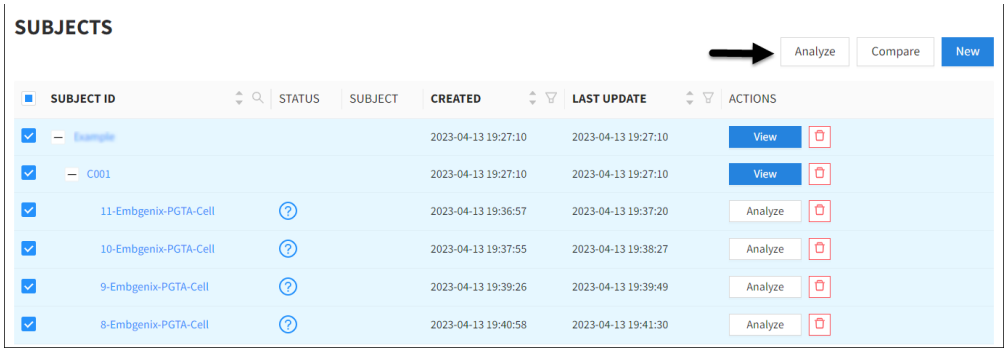


Figure 19. Initiating analysis of multiple samples in parallel.

4. When the analysis completes successfully, the data row will display a green check mark in the "STATUS" column and a [View] button in the "ACTIONS" column (Figure 20).

NOTE: For a list of potential status icons, refer to Table 1 in Section VII.C.

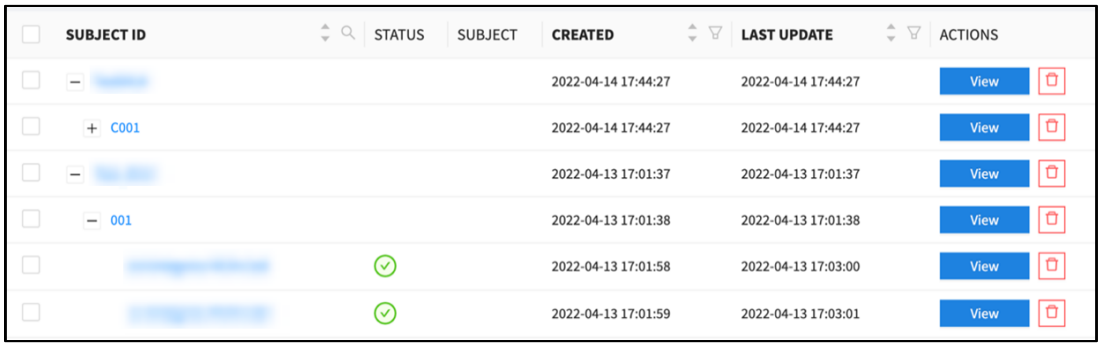


Figure 20. The sample row in the Subjects table, post-analysis

D. Processing time

In general, the time taken to process a sample is dependent on two factors: the time taken to upload the data when adding an experiment, and the time taken to complete the CNV analysis of the samples. The size of the input FASTQ files can have a small impact on the time taken for uploading and processing, although we generally do not recommend sacrificing sequencing depth (and thus potentially impacting the resolution of CNV detection) for improved analysis time.

The time taken to upload data for analysis is generally based on the available internet bandwidth and any related slowdowns by firewalls, VPNs, etc. It is recommended to use a stable, reliable, and reasonably fast network for this purpose.

It typically takes 10–15 minutes to process a sample on the cloud servers; this timeline can be used as a general guideline for tailoring expectations of analysis time across individual use cases.

VI. Analysis Results

There are two ways the analyzed data can be viewed in a report.

- The data for an individual set of sequencing results can be accessed by clicking the [View] button for the data row in the Subjects table (Figure 21). More information about this report type can be found in Section VI.A.

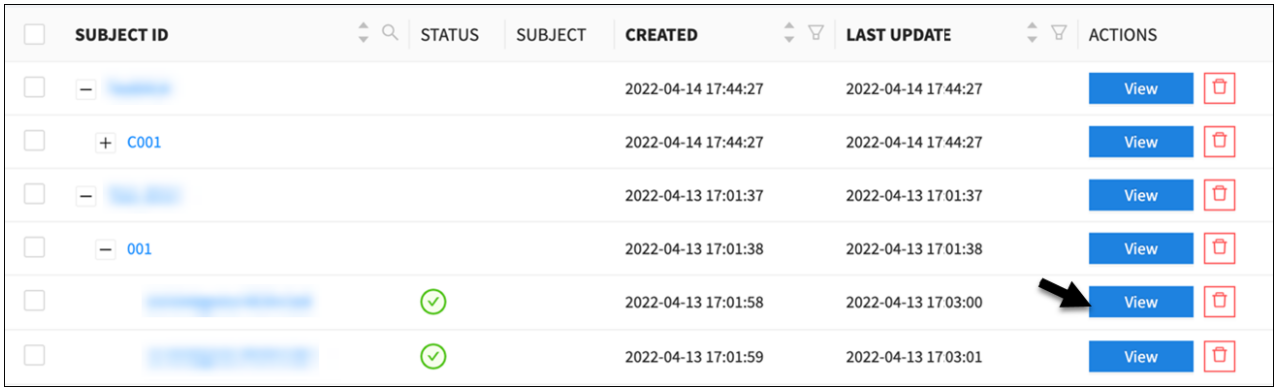


Figure 21. The report [View] button for an individual set of sample FASTQ files, post-analysis.

- The data for an aggregate set of samples collated in a cycle can be accessed by clicking the [View] button for Cycle ID row (Figure 22). Information about this report type can be found in Section VI.B.

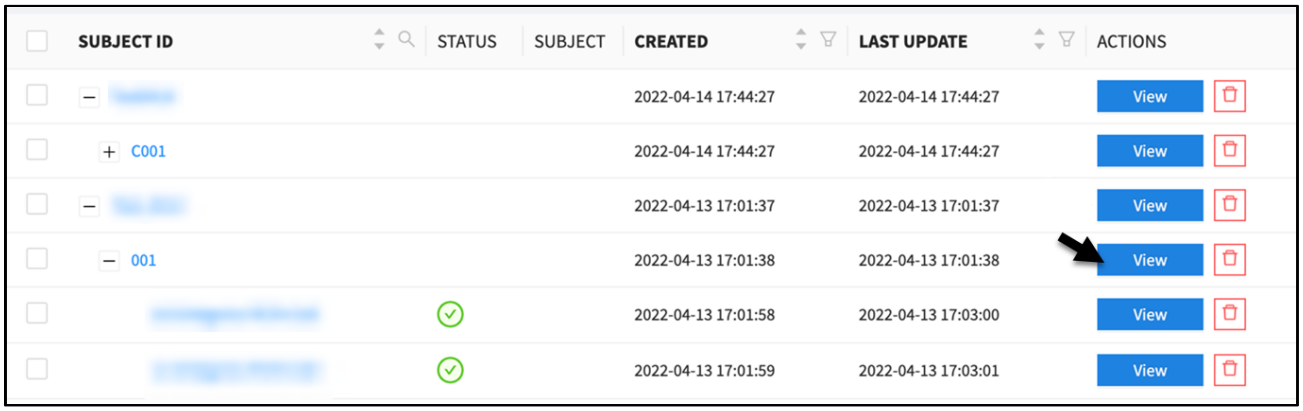


Figure 22. The report [View] button for a Cycle ID in the Subjects table.

A. Reports for Individual Samples

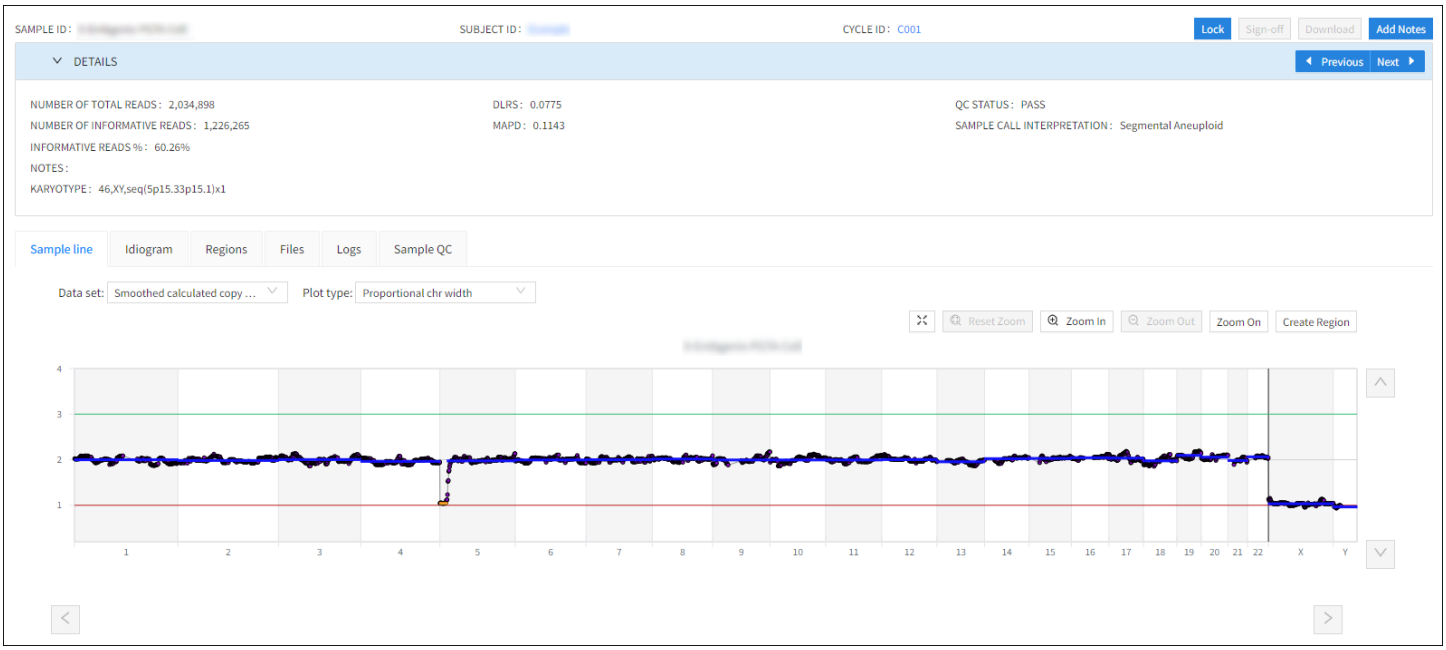


Figure 23. The analysis report page for a sample.

Figure 23 shows the default sample results page. The list below details a short description of the parts of the page:

- Header—Lists identifying information for the report, correlating to information on the Subjects page (i.e., Sample ID, Subject ID, etc.) and buttons for additional actions (see the [Lock] and [Sign-off] section, below)
- Details pane—displays several quality metrics including:
  - alignment metrics;
  - sample-level noise metrics;
  - predicted karyotype of the sample;
  - notes, which can be used by the user to add notes, such as ‘Pass’, ‘Low Quality’, etc. to summarize the QC metrics. Notes can be added to this field by clicking on the [Add Notes] button in the top, right corner of the page; and
  - sample-level aneuploidy call interpretation ("Sample Call Interpretation"), that broadly describes the predicted aneuploidy status of the sample. The options for this value are listed below in order

of priority from high to low (i.e., a sample predicted to contain multiple abnormalities will be classified according to the abnormality with the highest priority):

- Aneuploid—the sample is predicted to contain at least one full whole chromosomal gain or loss
- Segmental Aneuploid—the sample is predicted to contain at least one full segmental gain or loss
- Mosaic High—the sample is predicted to contain at least one whole chromosomal mosaicism at ≥50% frequency
- Mosaic Low—the sample is predicted to contain at least one whole chromosomal mosaicism at ≥30% frequency
- Mosaic Segmental High—the sample is predicted to contain at least one segmental mosaicism at ≥50% frequency
- Mosaic Segmental Low—the sample is predicted to contain at least one segmental mosaicism at ≥30% frequency
- Euploid—the sample is predicted to contain no abnormalities

IMPORTANT: The "Sample Call Interpretation" is provided to assist in identification but should not be used as a substitute for careful review of the sample results by a licensed clinician.

- Report tabs—below the Details pane are six tabs that cover different reports from this analysis. A description of each report is summarized below.

1. *Sample line*

The Sample line plot provides a genome-wide view of the CCNs and CNVs identified using them. A breakdown of the elements of the report follows.

a) "Data set" and "Plot type" options

Figure 24. The "Data set" and "Plot type" options.

The "Data set" and "Plot type" drop-down menu options can be used to view the data in different formats.

Three Data set options are available:

- Smoothed calculated copy number
- Calculated copy number
- Read bin counts

Two plot types are available; 'chr' is used as an abbreviation for 'chromosome':

- Proportional chr width
- Equal chr width

b) Navigation tools

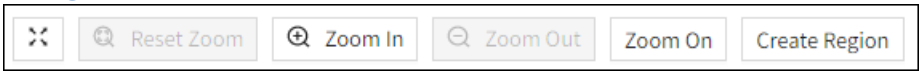


Figure 25. The navigation tools for the *Sample line* plot.

Using the navigation tools, located above the top-left corner of the plot, users can zoom in to view more details on areas of the chart, zoom out for a wider view of the chart, reset zoom back to the default, and define a custom region (described below under Manual identification of CNVs). Clicking on a chromosome number in the X-axis labeling will also zoom in to a more granular view of the particular chromosome.

NOTE: Clicking on the plot or the [Zoom On] button will toggle the ability to zoom; the [Zoom In] and [Zoom Out] buttons must be used to perform those actions. [Reset Zoom] will return the plot to the default view.

c) Plot

Figure 26 shows the important parts of the CNV sample line plot:

- Title of the plot—centered above the chart (blurred out in the figure)
- Navigation arrows—located to the right side of the chart along the Y-axis (up and down) and below the chart along the X-axis (left and right). These can be useful to scroll within the chart while zoomed in for more detail.

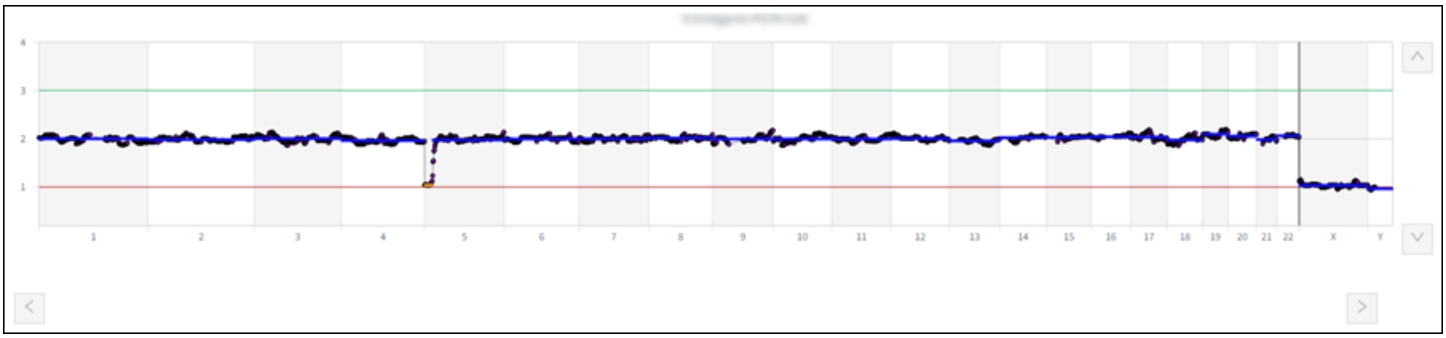


Figure 26. Detailed view of the *Sample line* plot.

d) Manual identification of CNVs

The analyzer is designed to automatically identify chromosomal and segmental aneuploidies and mosaicisms. However, this can be challenging in samples that have a low signal-to-noise ratio or hard-to-detect CNVs. The software has additional tools for expert users to evaluate indeterminate results then manually indicate and add CNVs to the reports, as described below.

NOTE: The calculated copy number obtained for a manually selected region will not always exactly match the calculated copy number obtained for an automatic call involving the same region (i.e., the results may differ on the order of hundredths). This is because the automatic and manual calling approaches handle noise in a slightly different way, with the latter employing an approach that more closely aligns with the presentation of results on the CNV plot.

1. Click on the [Create Region] button in the set of Navigation tools, shown in Figure 27 (left). The label will change to [Review Region] after being selected, highlighted by the red box (right), and display a [Cancel] button that can be used to quit out of the region creation process without making any changes.

Figure 27. The region button location in the Sample line report page. **(Left)** Click on the [Create Region] button. **(Right)** The text will change to [Review Region] and a [Cancel] button is introduced.

2. Click-and-drag the cursor on the CNV plot to make a rectangular selection on the chart (Figure 28). In general, it is recommended to select a region contained within a single chromosome, even for calls that span multiple chromosomes.

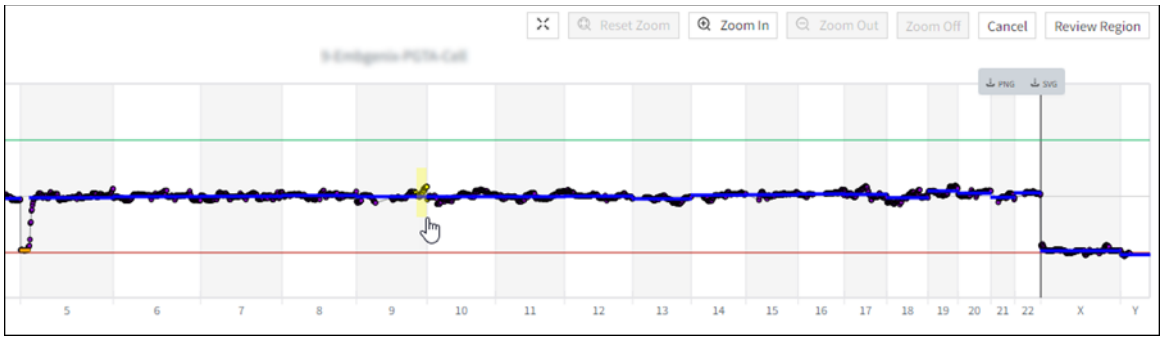


Figure 28. Selecting a region on the Sample line plot. The area selected is highlighted by the yellow box and only includes part of the line located in part of only one chromosome (9).

Click on the [Review Region] button. Once this selection is made, the *Create Region* pop-up (shown in Figure 29) appears.

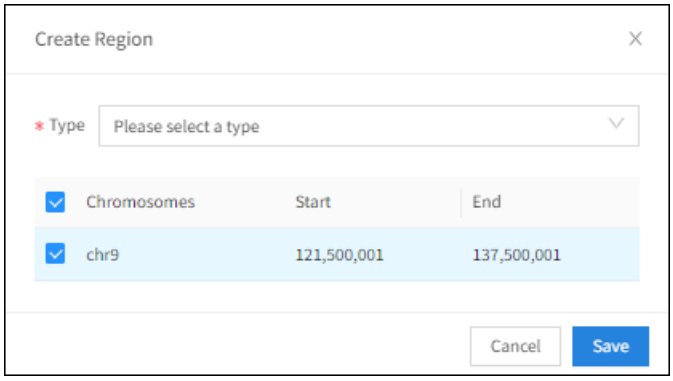


Figure 29. An example of the *Create Region* pop-up window displayed during manual identification of CNVs.

3. Use the "Type" drop-down menu to identify the CNV with one of the listed options (Figure 30).

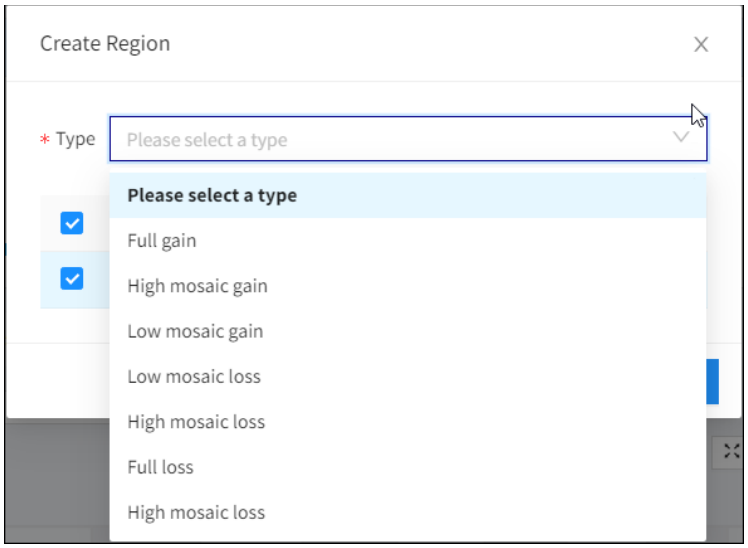


Figure 30. The drop-down menu options for the *Create Region* "Type" field.

4. After selecting the type, click on [Save]. A success message will briefly display (Figure 31), and the CNV is added to the plot (Figure 32) as well as under the corresponding *Regions* tab (Regions, below).

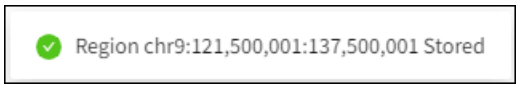


Figure 31. Example message pop-up of a successful manual region designation.

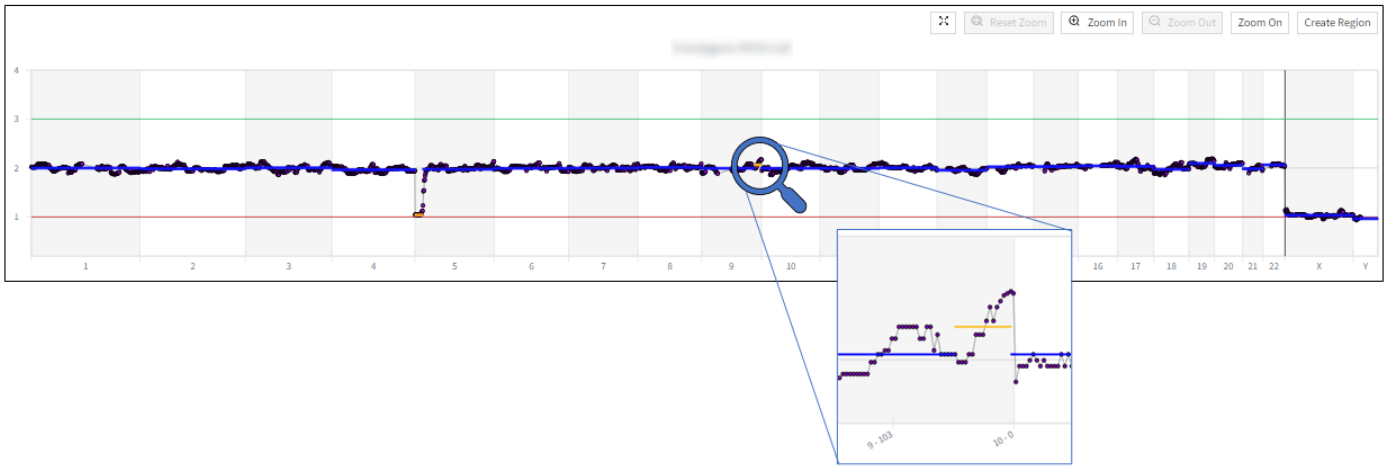


Figure 32. The Sample line plot, after manually defining a region. The yellow line in the range of chromosome 9 indicates the newly defined region.

e) *Downloading the chart*

The chart can be downloaded by hovering the mouse cursor over the plot image. A [PNG | SVG] button will pop up (Figure 33).

Click on the file type you want to save the image as. The image will save in the method configured in your browser for file downloads.

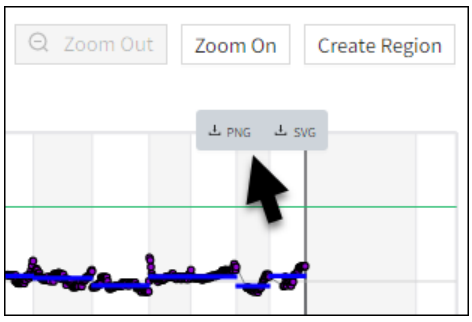


Figure 33. The cursor hover pop-up window to save a sample line chart as an image.

2. *Idiogram*

The tab view shown in Figure 34 represents the CNV calls in an idiogram format. The table below the idiogram marks the CNV calls in the corresponding chromosome column.

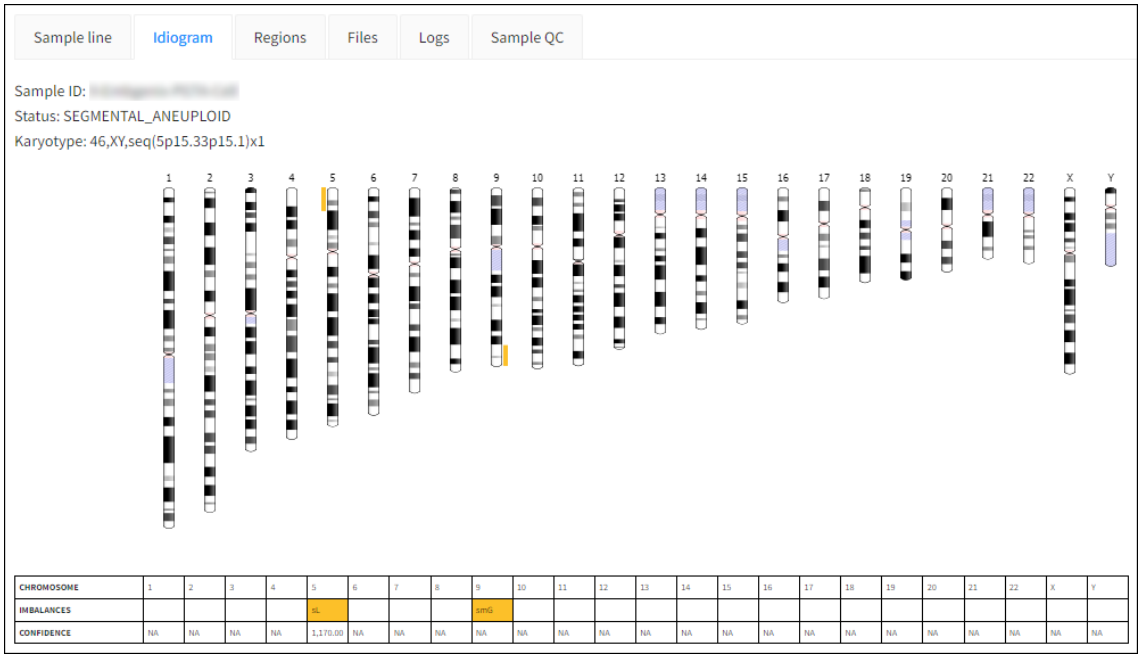


Figure 34. An example idiogram plot. Any region highlighted in the CNV sample line will also display highlighted here.

3. *Regions*

The *Regions* tab view lists the CNV calls with additional descriptions including:

- the approximate chromosome start and end positions,
- the type of call ("automatic", called by the software or "manual", called by user) the row represents, and
- an adjusted log probability score that may provide guidance to the user regarding the significance of a given call.

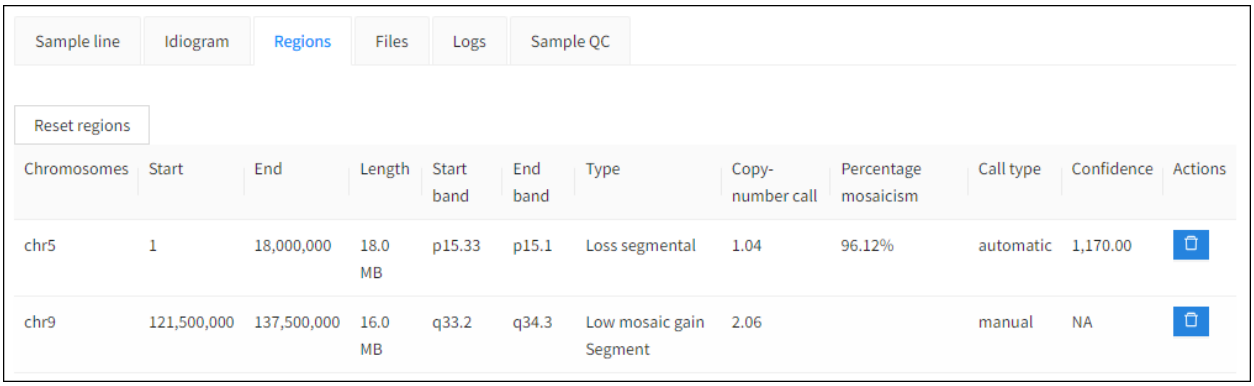
An example of a manually defined region update to the table (see: Manual identification of CNVs) is shown in Figure 35.

Automatic or manual calls can be deleted individually by clicking on the delete icon in the "Actions" column at the end of the row. Manual calls or deletion of automatic calls can be universally undone using the [Reset regions] button above the table.

NOTES:

– When an automatic CNV call involving a sex chromosome is deleted, an automatic copy number fallback will be applied consistent with the called sex of the embryo (e.g., the chrY copy number will fall back to 1 when a CNV involving chrY is deleted; the chrX copy number will fall back to 1 for an embryo called as male and to 2 for an embryo called as female)

– There is no mechanism to undo deletion of manual calls, so be careful when using these functions.



Chromosomes	Start	End	Length	Start band	End band	Type	Copy-number call	Percentage mosaicism	Call type	Confidence	Actions
chr5	1	18,000,000	18.0 MB	p15.33	p15.1	Loss segmental	1.04	96.12%	automatic	1,170.00	
chr9	121,500,000	137,500,000	16.0 MB	q33.2	q34.3	Low mosaic gain Segment	2.06		manual	NA	

Figure 35. An example table under the *Regions* report tab. The table illustrates a CNV region call made automatically by the analyzer and a manual call from a user.

4. *Files*

The *Files* tab lists the FASTQ files uploaded for the analysis, with high-level information.

5. *Logs*

The *Logs* tab lists additional details regarding the analysis run, including any potential error messages.

6. *Sample QC*

The *Sample QC* tab displays sample-level information for quality control (QC), including CNV plot noise metrics and alignment metrics, which can provide assistance in qualifying a given sample, provide insights to general sample issues, or provide assistance in troubleshooting suboptimal applications of the Embgenix PGT-A kit and/or sequencing runs (Figure 36).

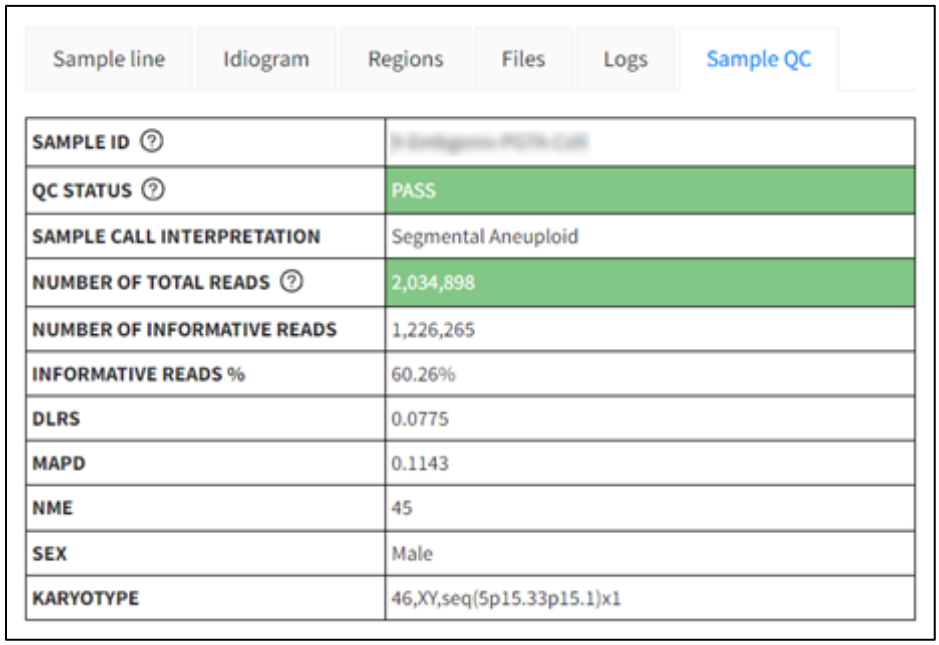


Figure 36. An example *Sample QC* table.

Many of the values in this table are common industry concepts or described elsewhere in this manual, but three that may not be familiar—DLRS, MAPD, and NME—are described below:

- The Derivative Log Ratio Spread (DLRS) and Median Absolute Pair-wise Difference (MAPD) are two metrics that can provide useful criteria for assessing noise in CNV detection and are widely used for this purpose. The analyzer implements a DLRS score and MAPD score closely derived from existing formulations of DLRS and MAPD.

  NOTE: The DLRS and MAPD values calculated by the Embgenix Analysis Software (RUO) are from proprietary formulas and should not be compared directly with metrics with similar names from other CNV detection platforms. Variance in how the DLRS and MAPD metrics are calculated between software will often lead to differences in the reported values.

- A third noise metric called Noise Metric Euploid (NME) is also provided and can additionally be considered for purposes of QC.

  NOTE: NME is a calculated value from a proprietary algorithm and is applicable only for comparisons of plot noise across euploid samples analyzed by the Embgenix analysis software.

7. Locking a Sample Report

The [Lock] can be found in the top right corner of the Report page (Figure 37).

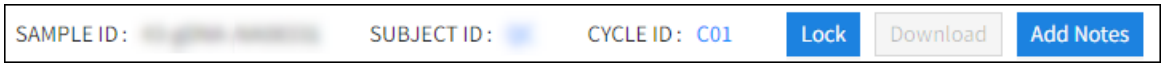


Figure 37. The header of the Report screen and location of the [Lock] button.

This button should be to mark as completed a review of the sample results by a licensed clinician

1. In a typical scenario, the clinician would review the report results and could add notes on any CNVs that the software has identified (using the [Add Notes] button) and could also include addition of manually identified CNVs (refer to Manual identification of CNVs).
2. Once the review of the sample is completed, click the [Lock] button to freeze the changes and prevent other users from modifying the report. The [Lock] button will change to an [Unlock Sample] button (Figure 38).

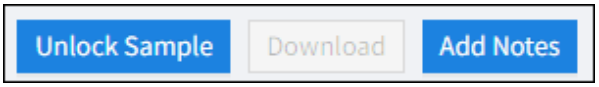


Figure 38. The configuration of the header after the [Lock] button is selected.

To remove the lock and to continue analyzing/editing the reports, click the [Unlock Sample] button. Notes can continue to be added even after the sample is locked.

3. Once a sample is locked, notify a user with an administrative access account, who can sign off on the sample. Refer to Section VIII.B for more information on that process.

Once an admin has signed off, the [Download] button becomes active (Figure 39). Limited access users will no longer be able to unlock or change the report but can still add notes.

Figure 39. The configuration of the report header buttons after being signed off by an admin account.

B. Cycle ID Reports

Multiple samples analyzed in a cycle can be viewed together by clicking either on the Cycle ID hyperlink for a given subject or the [View] button in the Cycle ID row. The page view that returns is determined by the "Cycle view mode" profile setting for the account. For more information about this setting, see Section VII.H.

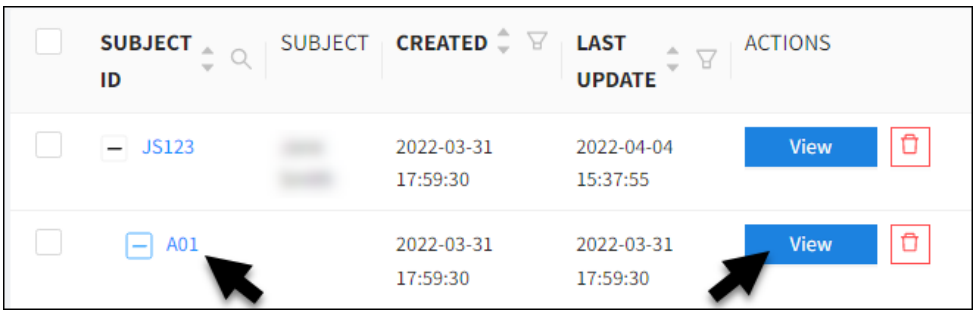


Figure 40. Links to access the aggregate report for a subject's Cycle ID.

After accessing the Cycle ID report page, there are two options:

- On the default plot view (Figure 41), a collected view of the reports for all samples in the cycle can be viewed

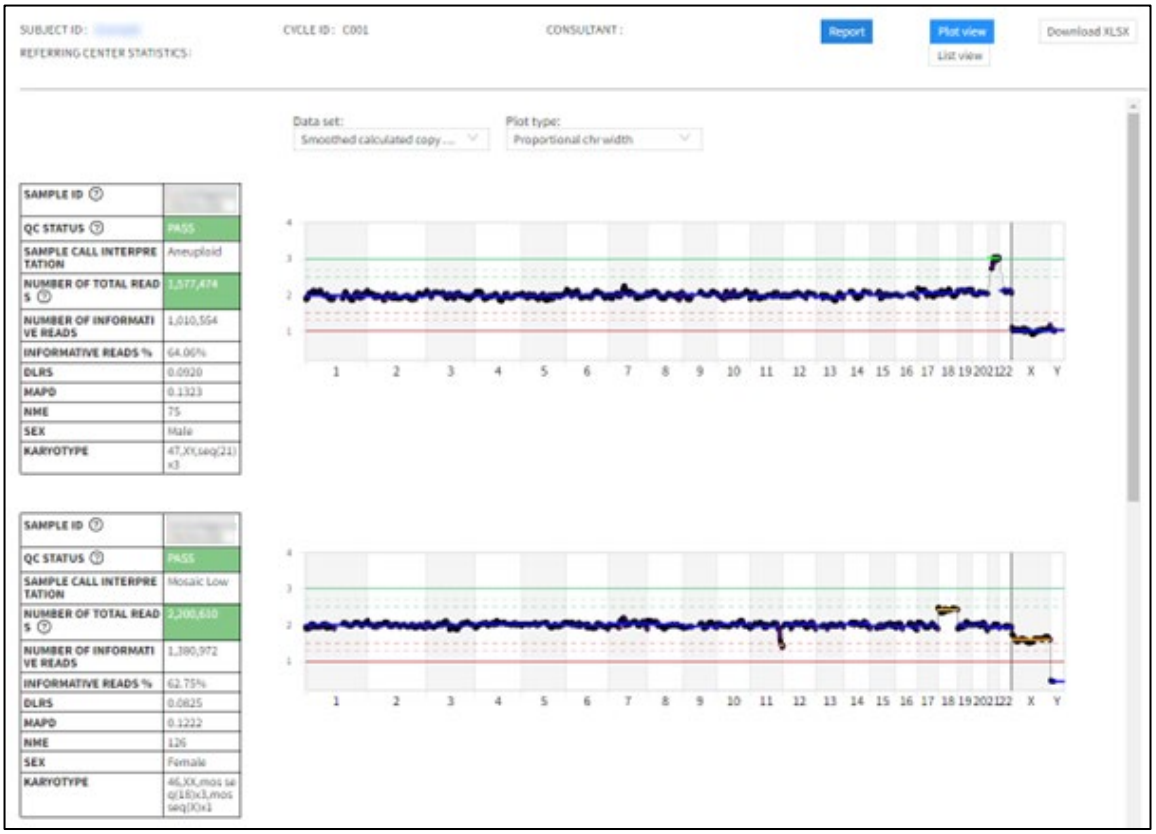


Figure 41. The Cycle ID plot view. The report shows multiple samples in a single cycle showing QC metrics and CNV plots.

The tables to the left of the plots summarize the sample's QC information, while the right panel displays the CNV plots for each sample. This allows users to view a summary of the CNV calls and QC metrics of all the samples in the cycle and compare one or more samples in the cycle.

- On the list view (Figure 42), reports for individual samples categorized into the Cycle ID can be accessed by clicking on the Sample ID name in the table.

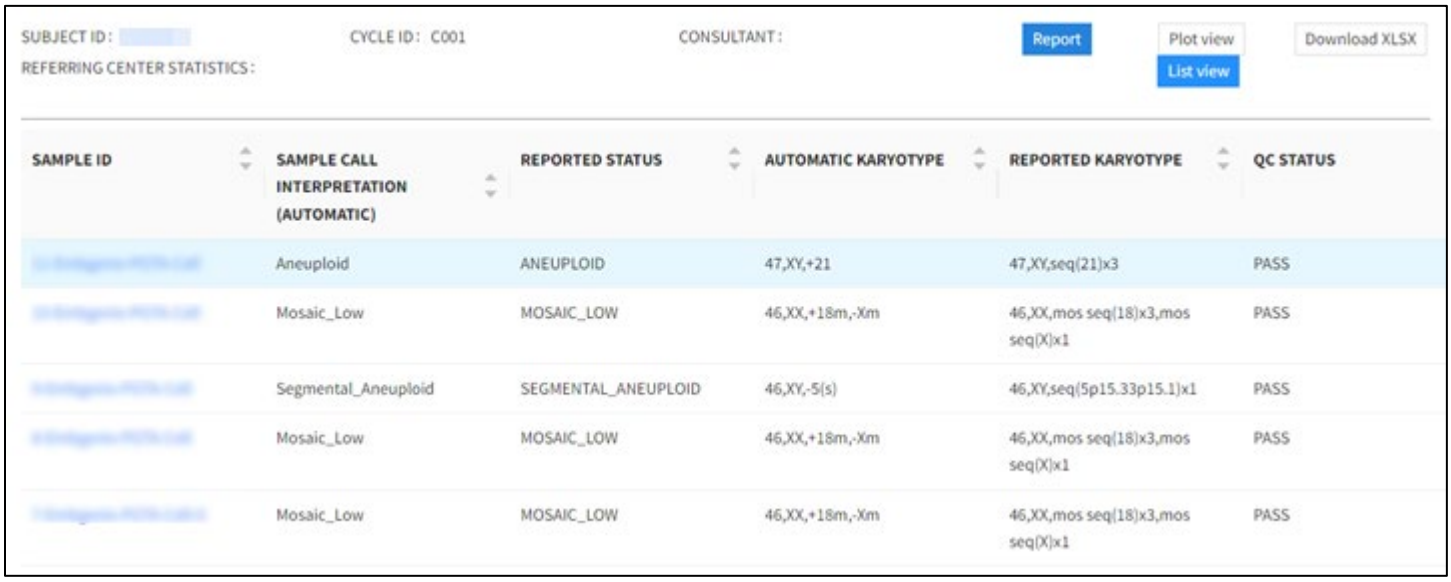


Figure 42. The Cycle ID list view report.

Clicking on the [Plot view] and [List view] buttons on the right-hand side of the page header allows you to switch back and forth between the two views.

C. Downloading Reports

1. Once a report has been signed off by an administrator (Section VIII.B), the report can be downloaded from the tool by clicking the [Download] button in the header (Figure 43).

Figure 43. The initial report download interface.

This will take you to the page shown in Figure 44.

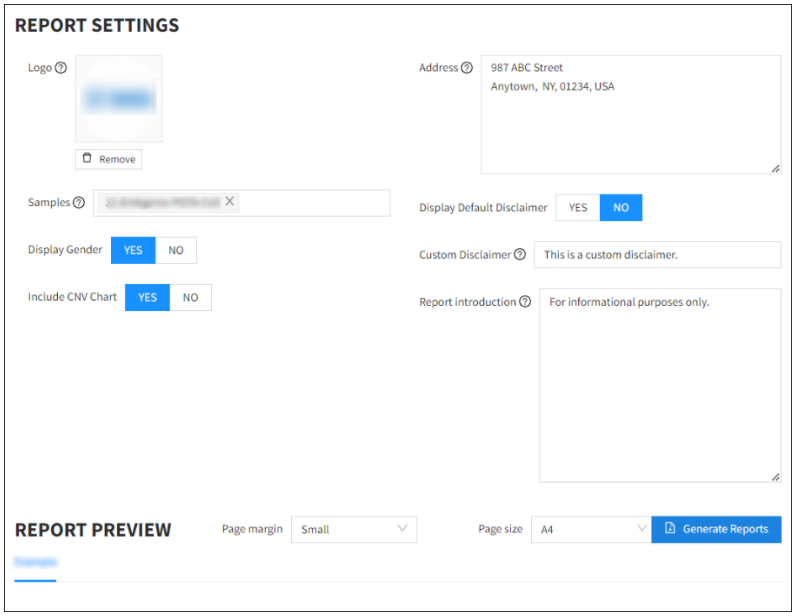


Figure 44. The initial report download interface.

The settings for the Logo, Display Gender, Include CNV Chart, Address, Display Default Disclaimer, Custom Disclaimer, and Report introduction are all imported from the definitions configured in the Settings page (users: Section VII.H; administrators: Section VIII.C).

- Display Gender—Selecting 'Yes' will include the gender of the embryo in the report, any abnormalities in the X or Y chromosomes, and display the data points in the X and Y chromosomes in the CNV plot. Selecting 'No' will hide the embryo's gender and delete the CNV plot points in the X and Y chromosomes.
- Include CNV Chart—Selecting 'Yes' will append the sample line plot on the final page of the report; selecting 'No' omits the chart from the report.

2. To generate the report, either select an option from one of the Report Preview options ("Page margin" or "Page size") or click the [Generate Reports] button.

   A PDF preview will display under the Report Preview header (Figure 45); where the settings information is populated in the report is also indicated in the figure.

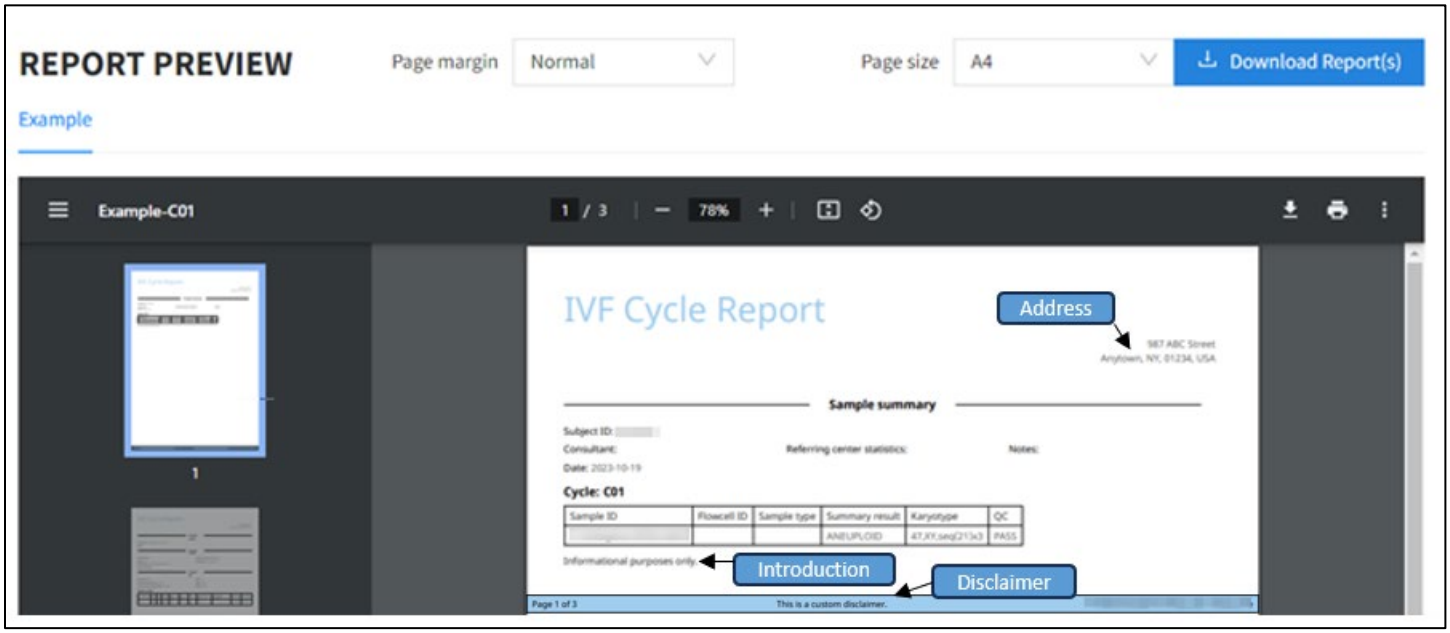


Figure 45. The report preview.

3. Click the [Download Report(s)] button to save the report to your local drive as a PDF. The default name given the file is `<SubjectID>-<CycleID>.pdf` but can be customized when being saved.

D. Downloading Results in Bulk

1. Results for samples that have been successfully analyzed can be downloaded in bulk as an .xlsx file via the Subjects page (Section VII.C).
2. Results can be selected for download at the Subject, Cycle, and/or Sample levels by checking the corresponding boxes in the leftmost column, as shown in Figure 46. Results from different subjects or cycles can be downloaded together in the same .xlsx file.

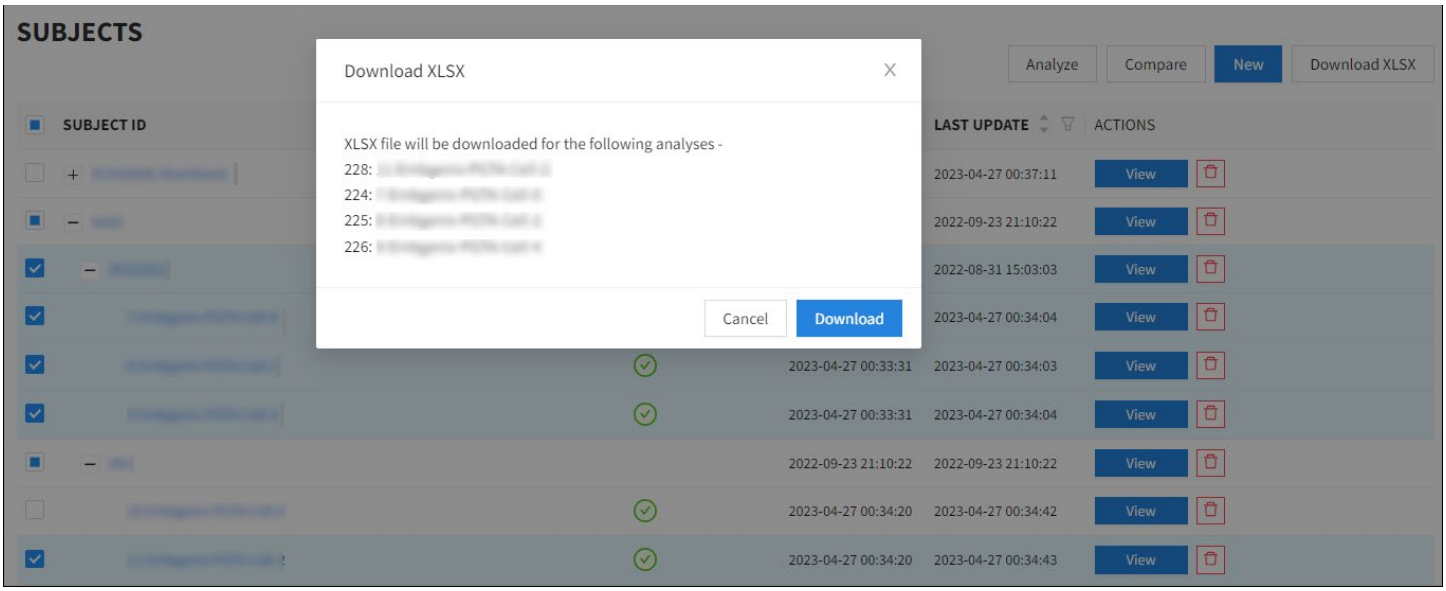


Figure 46. Selection of sample results for bulk download to an .xlsx file.

3. The downloaded .xlsx file will include all the results provided in the *Sample QC* table for each sample (Section VI.A.6).

VII. Interface Overview

The following section provides an overview of the user interface independent of workflow usage.

A. Dashboard

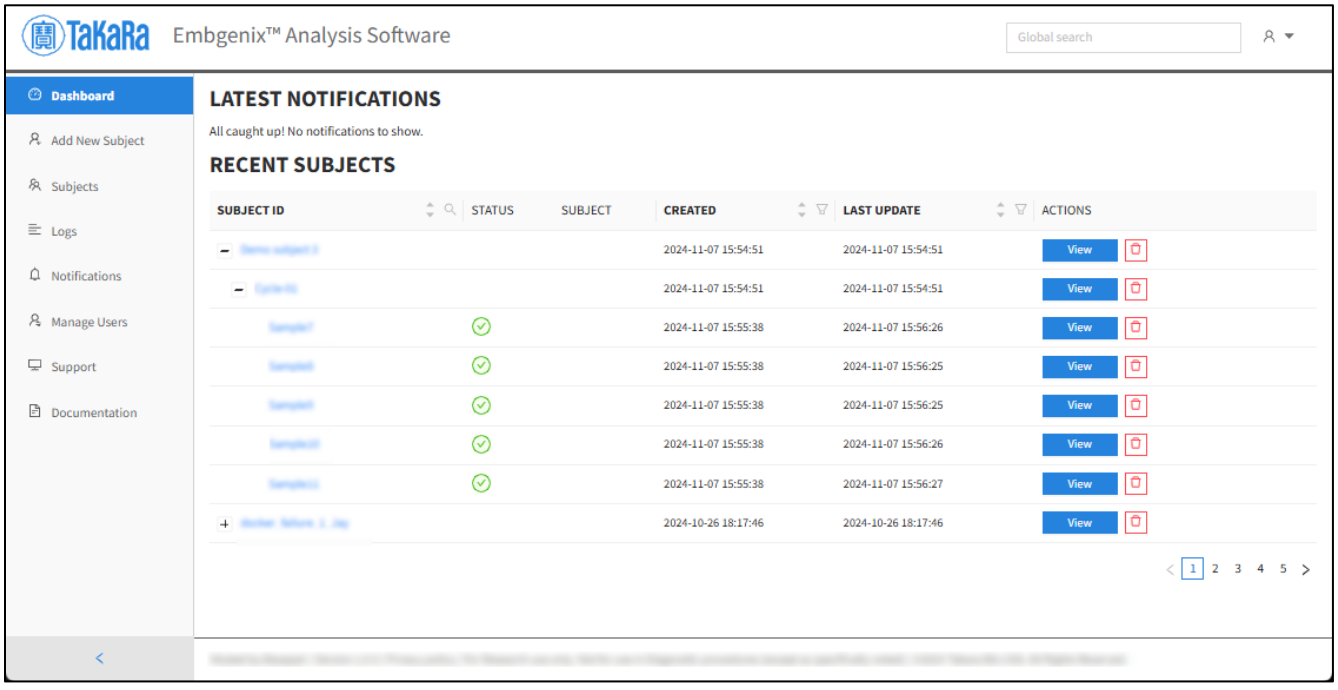


Figure 47. The Dashboard page for users.

The Dashboard page displays a table (Recent Subjects) of the subjects configured in the database, listing them from the most recently modified descending by the "Last Update" value to further in the past

(Figure 47). This allows users to see at a glance any activity that might have occurred within the lab since the last time they logged in.

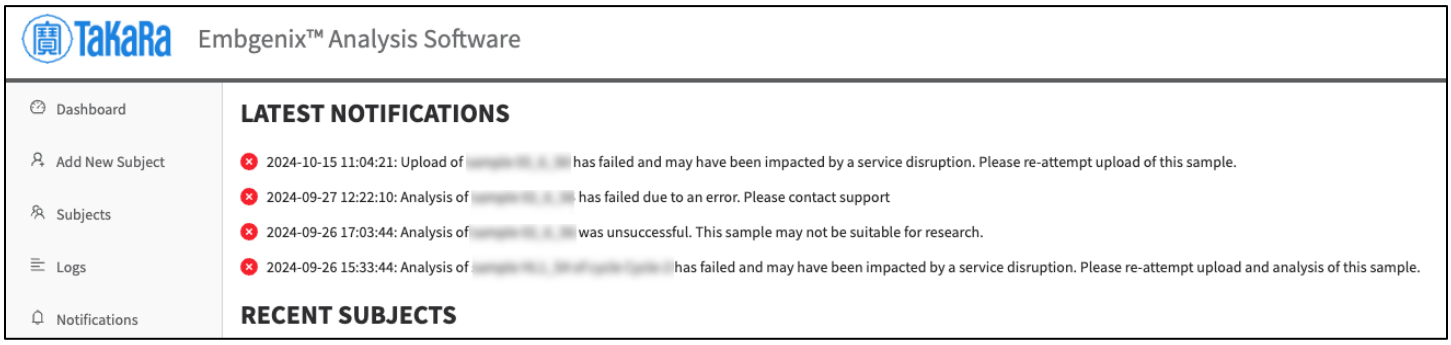


Figure 48. Latest Notifications, displayed on the Dashboard page.

The Latest Notifications pane at the top of the Dashboard page lists samples that were impacted by an upload or analysis failure (Figure 48). Up to four notifications can be displayed in the section at one time and the most recent notifications are displayed by default. Notifications can be removed from the list by hovering over them and selecting the Acknowledge Notification popup (eye icon) next to each notification (Figure 49).

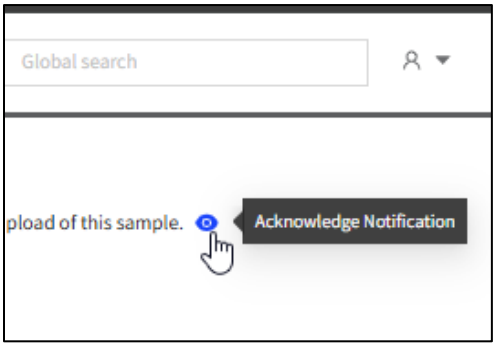


Figure 49. The Acknowledge Notification popup.

All notifications, acknowledged or unacknowledged, can be viewed on the Notifications page, accessible via the left-hand menu option. For an in-depth discussion of the various notification types, please refer to Section VII.E below.

B. Add New Subjects

There are two purposes for using the Add New Subject (Accessioning) page:

1. Adding a new subject to the Subjects database.
2. Adding or editing information for an existing subject, including uploading sample sequencing files (Section V.B)

These two purposes have slightly different interfaces, covered below.

1. Default Accessioning Page

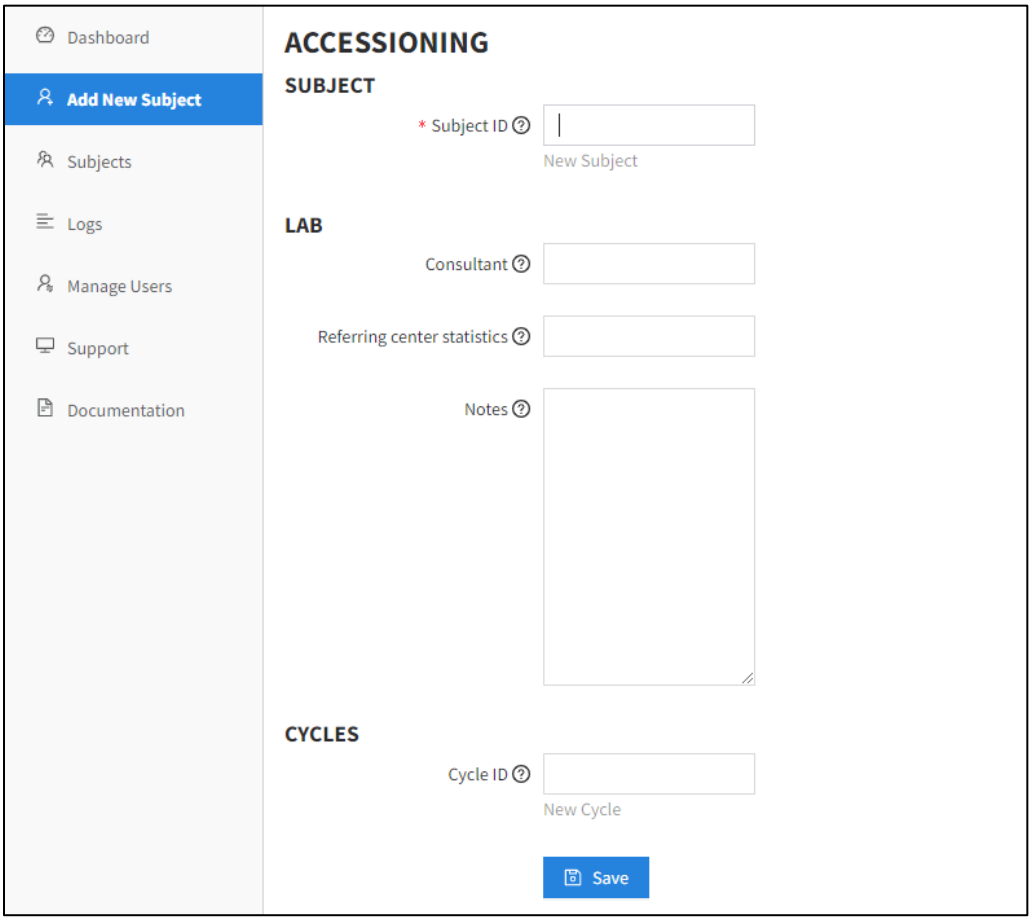


Figure 50. Default Add New Subject (Accessioning) page.

The default version of the Accessioning page contains fields to associate information with the top-level subject in the database. "Subject ID" and "Cycle ID" are required fields, while the remaining fields are optional. More information about each field can be accessed through the (?) icon next to each field name (Figure 50).

For more information about creating a new subject, refer to Section V.A.

2. Editing an Existing Subject

There are two methods for initiating an edit or the addition of information to a previously defined subject.

1. Through the Subjects page by clicking on the "Subject ID" hyperlink or the [View] button in its row (Figure 51).

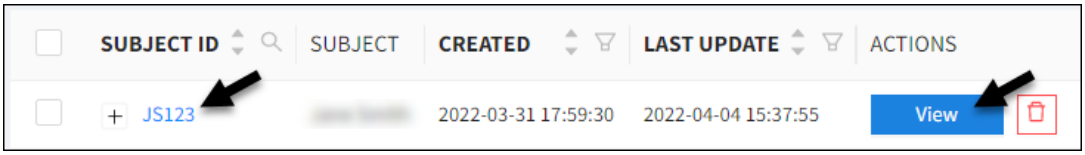


Figure 51. Accessing the page to add or edit information for an existing subject through the Subjects view.

2. In the Add New Subjects page itself, if you type in the name of an existing subject, it will recognize the subject already exists and provide a clickable "Edit" string. Click on it to edit any of the fields in Figure 52.

Figure 52. Accessing add or edit subject information for an existing subject through the Add New Subjects page. **(Top)** The default for the "Subject ID" field. **(Bottom)** The "Subject ID" field when an existing subject ID is typed into the field. The text under the field changes to "Existing Subject" and a clickable "Edit" string.

In addition to being able to edit the top-level information about a subject (the default fields), editing the subject information is also how to:

- upload FASTQ files against an existing cycle ID (Section V.B), or
- create additional cycle IDs for an existing subject.

Example:

Figure 53 (below) shows the Cycles section of the page. The Cycle ID field is editable, while the previously defined cycle (A01) is hyperlinked below the field.

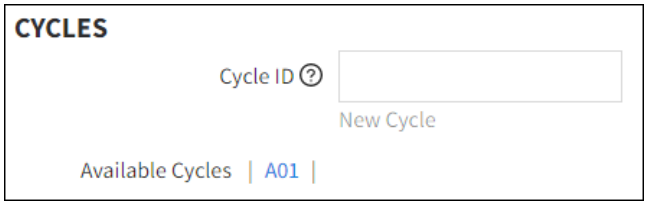


Figure 53. Accessing existing subject cycle IDs or adding new ones in the Add New Subjects page.

A new Cycle ID can be typed into the field (Figure 54). Once input, click [Save].

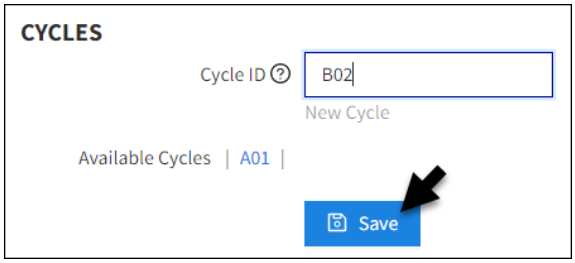


Figure 54. Adding a new Cycle ID to an existing subject.

Once saved, the new cycle ID will be appended to the list of Available Cycles (Figure 55).

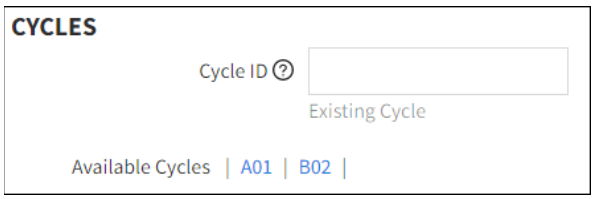


Figure 55. Cycles section, after a new cycle is added.

3. Elements of the *New sample* Window

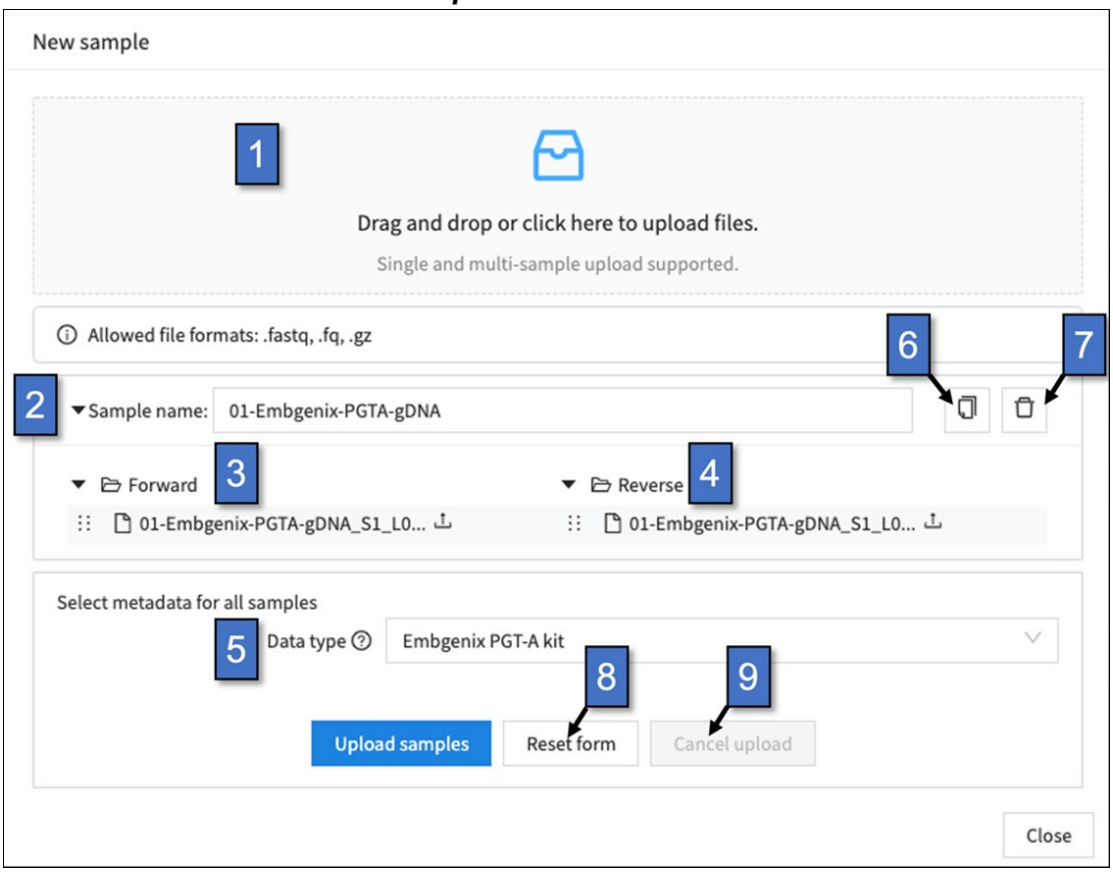


Figure 56. *New sample* window: identifying parts of interest on the interface.

Figure 56 labels parts of the *New sample* window described in the list below.

(1) File upload—this works similarly to file uploads in many common software packages. Uploads can be initiated by dragging and dropping files from desktops or folders (e.g., Windows File Explorer), or by clicking on the area to bring up an *Open* window standard in most web browsers.

(2) Sample name—this is initially derived from the beginning of the FASTQ file name (i.e., everything before the first underscore in the file's name) but can be customized by typing into the box. The down-pointing arrow to the left of "Sample name" can be used to collapse the information box, which may be useful if processing multiple samples and files at one time (Figure 57).

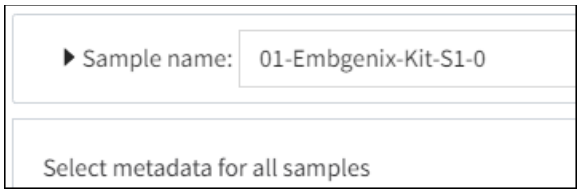


Figure 57. *New sample* window: collapsing the sample information.

(3) Forward—the text below the folder icon is the first 27 characters of the FASTQ file name identified as the forward index sequences (R1). Forward and Reverse share additional characteristics:

a. Users are unlikely to need to do this, but the rectangle of six dots to the left of the filename can be used to move the file from the Forward to the Reverse folder or vice versa. Figure 58 shows how this might look in action.

Figure 58. *New sample* window: using the move file icon.

b. The curved line and up arrow icon immediately to the right of the FASTQ file name is a status message indicating the file is ready to be uploaded.

c. If the mouse is hovered over the area to the right of the status icon, a second icon (shown in Figure 59) appears. This is a delete icon, if you want to individually remove a designated FASTQ file from the upload being configured.

Figure 59. *New sample* window: location of the [Delete FASTQ from upload] icon.

NOTE: A forward index FASTQ file is REQUIRED for file upload. If the forward file is removed, an error will display.

(4) Reverse—similar to the forward index file section (#3), this folder displays the reverse index sequences (R2) FASTQ file. Because the software accepts single- or paired-end FASTQ files, this file upload is optional.

(5) Data type—the value of this drop-down menu tells the software what type of data is represented by the FASTQ files being uploaded. It will default to 'Embgenix PGT-A kit' and should not be modified.

(6) Copy sample—this icon can be used to duplicate the existing sample and create a second copy of it (Figure 60).

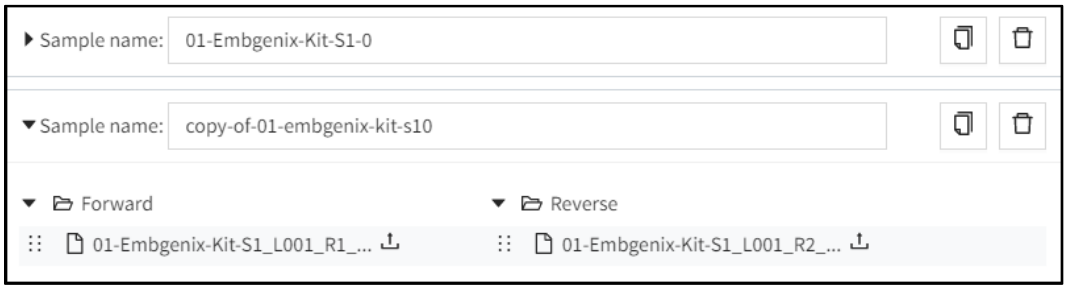


Figure 60. ***New sample*** **window: effect of the [Copy sample] icon.**

(7) Delete sample—this icon can be used to delete the sample from the upload queue.
(8) [Reset form]—clicking this button will clear all samples and their associated files from the window and return it to its default state (Section V.B, Figure 10)
(9) [Cancel upload]—this button is grayed out (inactive) until after the [Upload samples] button is clicked to initiate the file upload. Once the upload has started, the three buttons will change to what is shown in Figure 61.

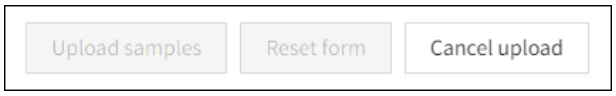


Figure 61. ***New sample*** **window: buttons while file upload is in progress.**

Clicking [Cancel upload] at this time will terminate the upload process and return to the Accessioning page with the message in Figure 62 displayed at the top of the page.

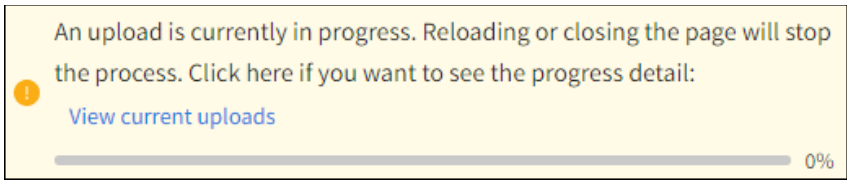


Figure 62. ***New sample*** **window: warning message if a file upload is cancelled.**

It is recommended that you reload (refresh) the browser page to stop the process.

NOTE: Cancelled uploads will be displayed in the record table of FASTQ files uploaded against the cycle at the bottom of the page (Section V.B, Figure 9 (right)) and under the list of samples for the Cycle ID in the Subjects page (Section VII.C, below).

Cancelled uploads can be deleted by clicking on the red trash can (delete) icon to the right of the sample row on the Subjects page (see Figure 63); this action will also delete the entry from the Accessioning table row.

C. Subjects

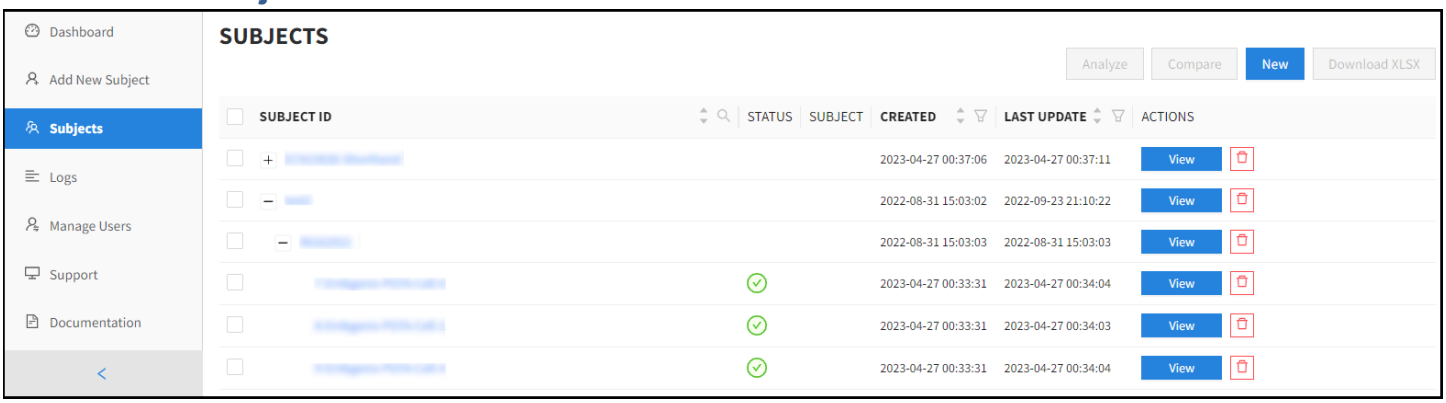


Figure 63. Subjects page.

The Subjects page displays a list of subjects previously configured in the software database. The columns are explained below.

- "Subject ID" corresponds to the value input on the Add New Subjects page and is a unique identifier within the database.
- "Status" indicates what stage of the upload process the given sample is at. Possible icon values are shown in Table 1.

Table 1. Status icons on the Subjects page.

Icon	Description
	Sample has not been analyzed
	Analysis in progress (animated icon)
	Analysis completed successfully
	Incomplete file upload
	Analysis failed

- "Subject" is the "First name" + "Last name" field from the Add New Subjects page.
- "Created" is when the subject was initially configured in the database.
- "Last update" is the date when the subject was last edited (see "Editing an Existing Subject")

- "Actions" are the activities that can be performed against the data row.
  - [View]–What this button returns is determined by what level of data the button is for

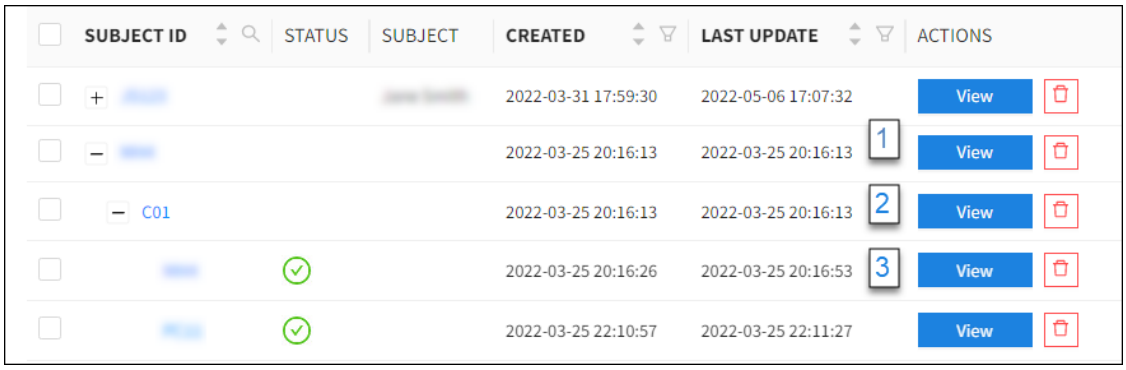


Figure 64. Understanding the Subjects levels of data.

1. Subject ID–brings up the Add New Subjects page.
2. Cycle ID–brings up the Cycle ID report page (Section VI.B)
3. Sample ID–brings up the individual sample report (Section VI.A)

  - The red icon (trash can) will delete the record and data for the row.

IMPORTANT: Deleting a Subject ID or Cycle ID record row will also delete the child-level information below it, i.e.:

- deleting a Cycle ID will also delete the sample information attributed to it
- deleting a Subject ID will delete all cycles and sample information

USE THIS FUNCTION CAREFULLY.

1. Additional Subjects Page Features

- Any column name in bold text (e.g., Subject ID) can be sorted using the up and down arrows next to the column name text. Columns in normal (unbolded) font are non-sortable.
- Sortable fields may also have additional actions that can be taken on them.
  - Subject ID–click on the magnifying glass to search the column for matching text in either the Subject ID or Sample ID fields.
    NOTE: Cycle ID strings are not a valid search parameter (e.g., C01 in Figure 63).
  - Created and Last Update–click on the funnel icon to filter by a specific time frame using a start and/or end date
- Multiple samples that have been uploaded can be analyzed at the same time by:
  - Checking the box next to the Subject in the subject table and clicking the "Analyze" button to the top right; this would analyze all the samples that are yet to be analyzed under this Subject ID
  - Checking the box next to the Cycle in the subject table and clicking the "Analyze" button to the top right; this would analyze all the samples that are yet to be analyzed under this Cycle ID
  - Checking the box next to individual samples that are yet to be analyzed and clicking the "Analyze" button to the top right; the samples selected need not be in the same cycle/subject

An example of multiple sample analysis can be seen in Figure 65.

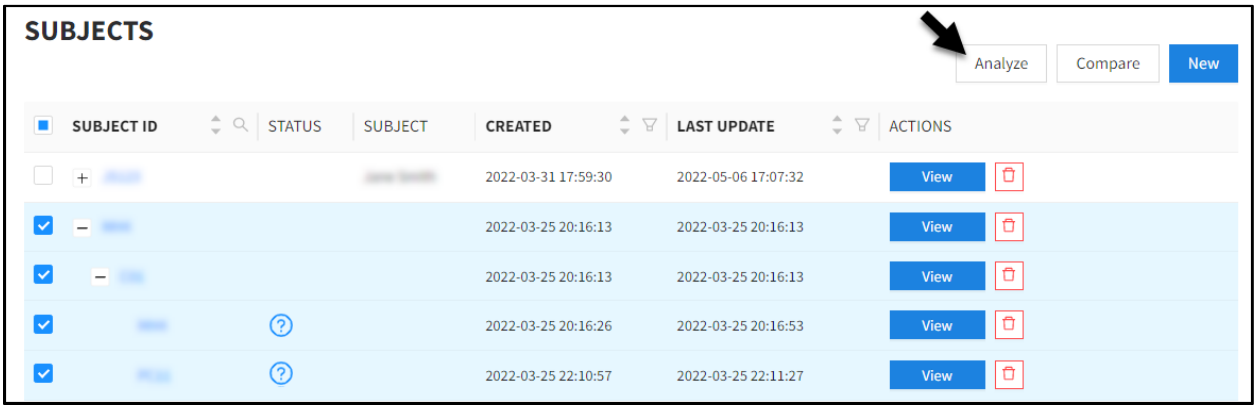


Figure 65. An example use of the [Analyze] button on the Subjects page.

- The boxes to the left of each table row can be used to compare one or more groups of data. The level of data which is selected determines what samples are included in the comparison and applies to all levels below it.
  - Checking the box next to a Sample ID will include that individual sample in the comparison.
  - Checking the box for Cycle ID will include all samples categorized in the cycle.
  - Checking the box for Subject ID will include all samples imported against the subject.

  The levels of data selected do not need to be equivalent: a single sample ID from one subject (Sample ID) can be compared against a set (Subject ID) or subset (Cycle ID) of samples from another subject, as shown in Figure 66.

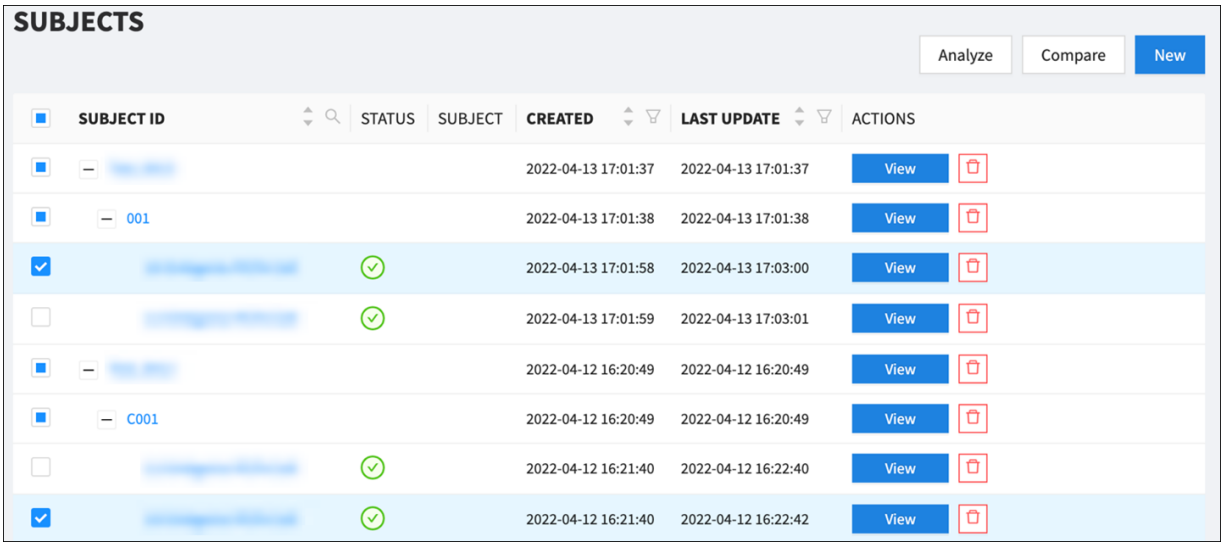


Figure 66. An example use of the [Compare] button on the Subjects page.

Click the [Compare] button to bring up the comparison report. Figure 67 depicts the compare results for the selection shown in Figure 66.

Click the [Compare] button again to return to the default Subjects table page.

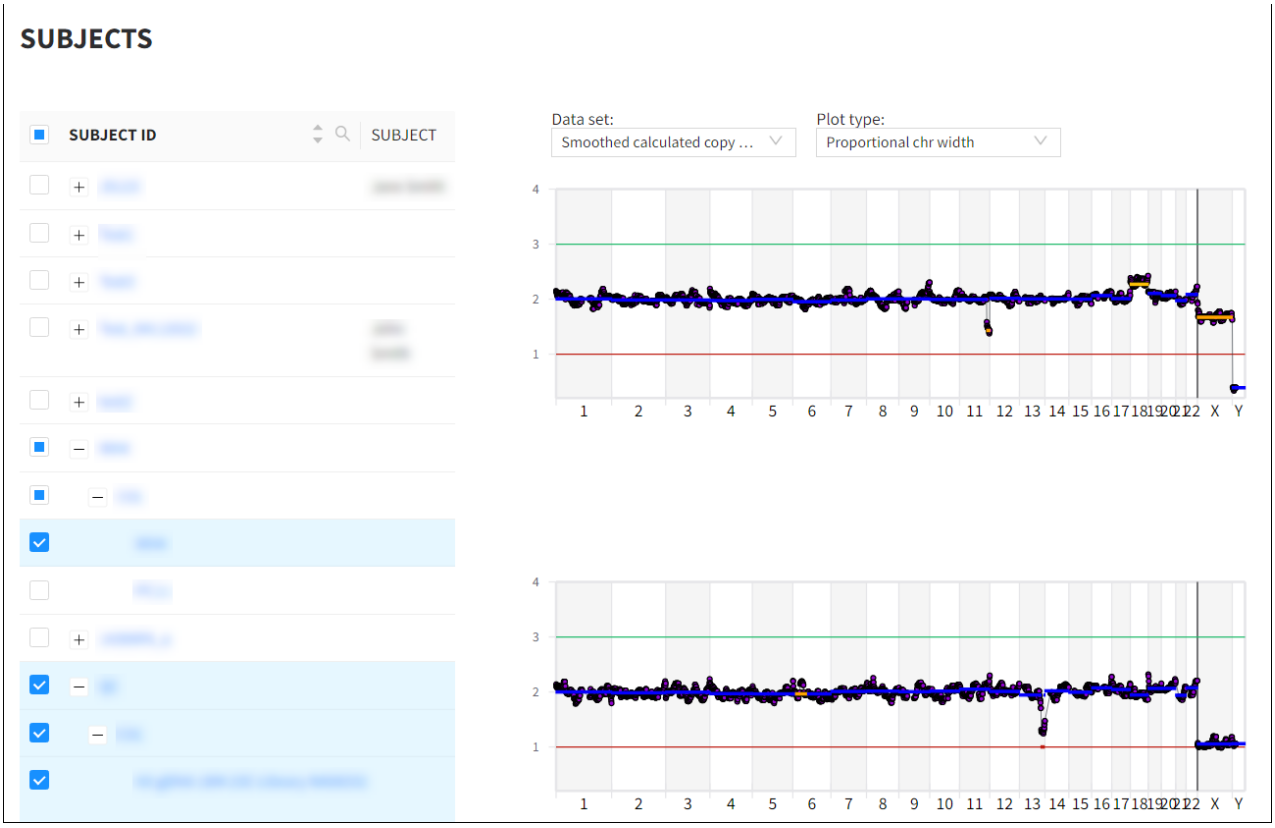


Figure 67. The report results for using the [Compare] button on the Subjects page.

- The boxes to the left of each table row can also be used for bulk download of results for selected samples that have been successfully analyzed as an .xlsx file. The level of data which is selected determines which sample results are included in the download and applies to all levels below it.
    - Checking the box next to a Sample ID will include the result for that individual sample in the download.
    - Checking the box for Cycle ID will include results for all analyzed samples categorized in the cycle.
    - Checking the box for Subject ID will include results for all analyzed samples imported against the subject.
- The levels of data selected do not need to be equivalent: the result for a single sample ID from one subject (Sample ID) can be downloaded along with results for a set (Subject ID) or subset (Cycle ID) of samples from another subject, as shown in Figure 68.

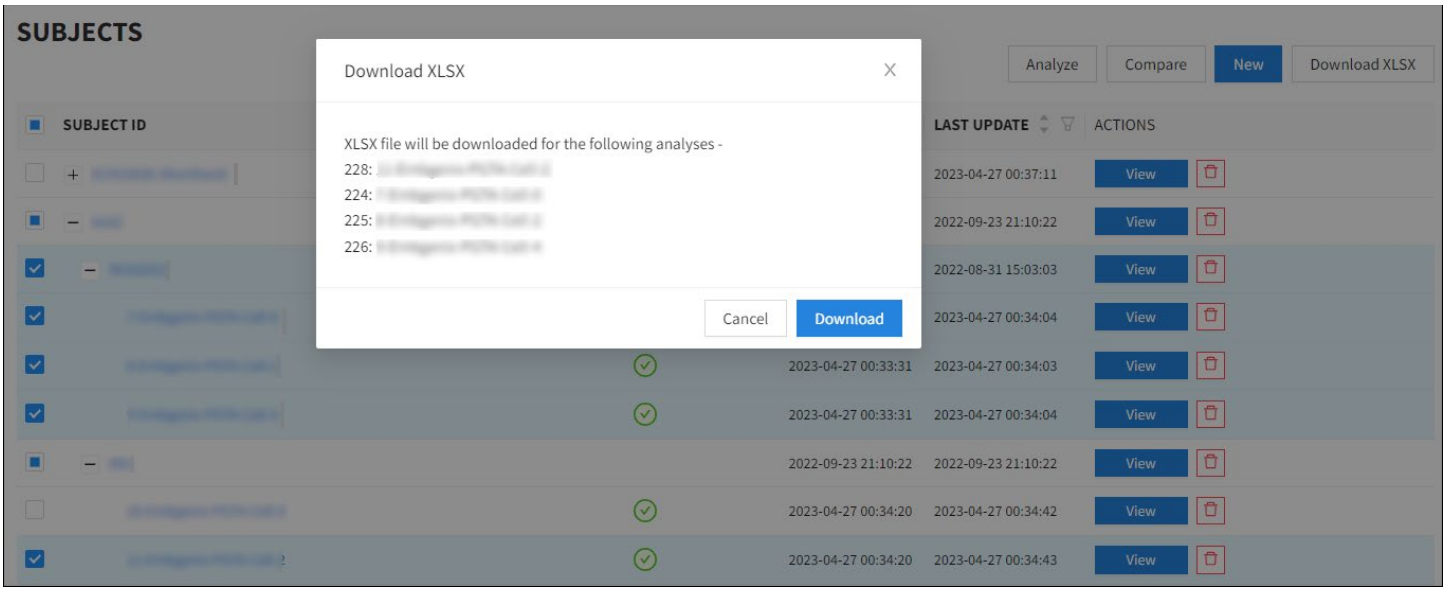


Figure 68. Selection of sample results for bulk download in an .xlsx file.

Click the [Download XLSX] button on the Subjects page and then the [Download] button in the resulting popup window to download an .xlsx file containing results for the selected samples.

2. Global Search

The "Global search" field in the web page header can also be used to search for matching text strings in any field of the table. Type in the text you wish to search for, and the main page below it will display all matches to the text string with the matching text highlighted, as in Figure 69. In addition to the Subject ID and Sample IDs (searchable by the magnifying glass icon in the table), this search can also be used to find specific Cycle IDs, as shown in the figure, or specific numerical strings representing dates or times.

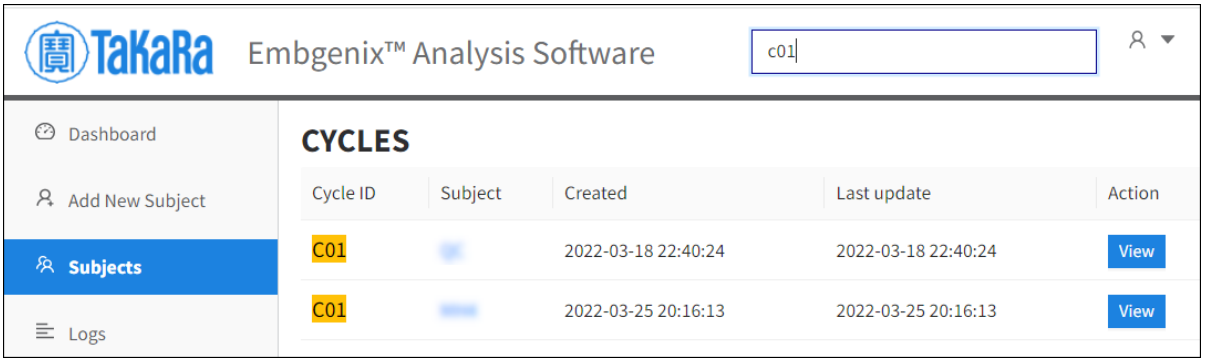


Figure 69. Example output display of the Global search function.

D. Logs

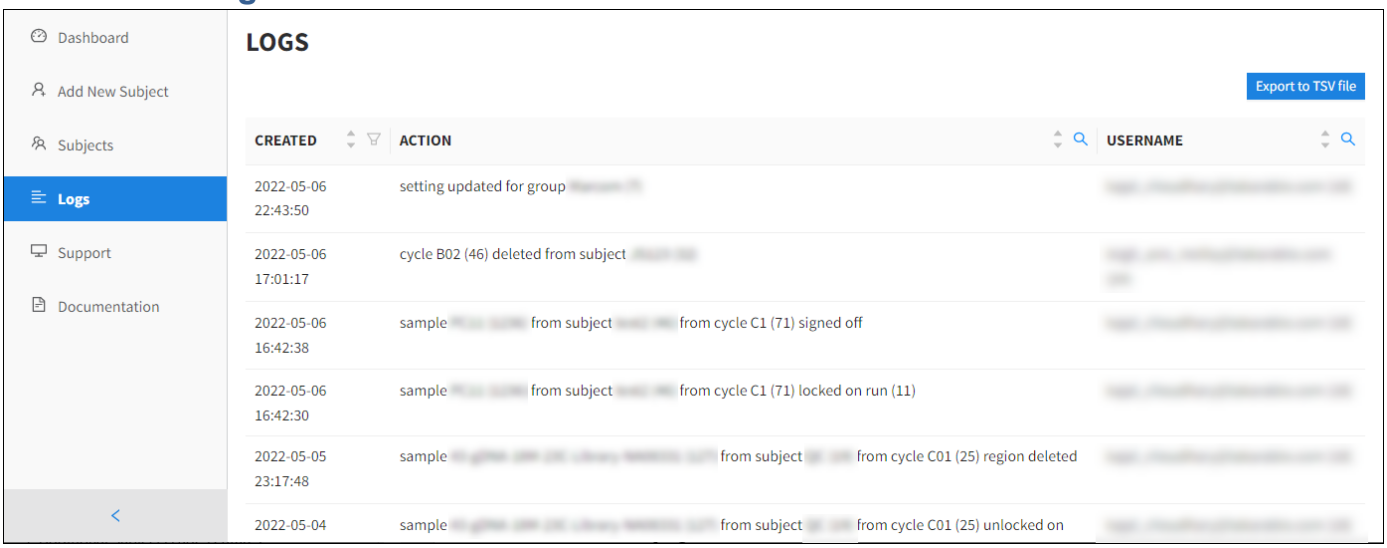


Figure 70. Example of the information available for view on the Logs page.

The **Logs** menu option from the sidebar displays system-level information about the actions performed by the users. Figure 70 shows a sample of what the log might look like. Each row is an action, with data recorded such as when the action was performed (against the server time), the action taken, and the username of the account that acted.

- The log entries can be sorted by any of the three fields by clicking the up and down arrow icons to the right of the field header.
- Entries can be filtered by the icons in the header rows:
  - For a given date, using the funnel icon in the "CREATED" column. E.g., if wanting to search for an action performed on 05-May-2022, a 'Start Date' of 05-May-2022 and an 'End Date' of 06-May-2022 should be used.
  - By specific string values, using the magnifying glass search icon in the "ACTION" and/or "USERNAME" columns.

  NOTE: These search parameters follow AND/intersection logic, i.e., if multiple parameters are specified, results do not represent a union of all entries matching any search parameter.

- Additional pages of the log file can be accessed using the navigation tools in the bottom right-hand corner, and the log file can be exported in TSV (tab-separated value) format to a file, with the button in the top right-hand corner.

Information about the *Logs* tab in the sample reports can be found in Section VI.A.

E. Notifications

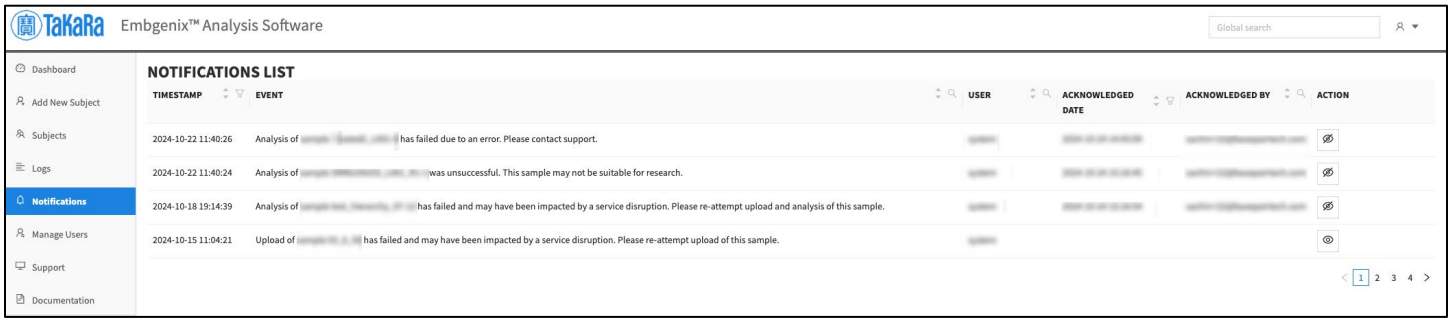


Figure 71. Example of notifications provided for various sample upload or analysis failure scenarios.

The **Notifications** menu option displays a list of all samples that have been impacted by an upload or analysis failure with accompanying timestamps, notification messages, and acknowledgement information. Selecting the eye icon in the "ACTION" column will acknowledge or cancel acknowledgement of a notification, determining whether the notification can be displayed in the corresponding section of the Dashboard window (the four most recent unacknowledged notifications are displayed in the Dashboard window). Either of four different notification messages (listed below) will display for a given sample depending on the nature of the error:

- "Analysis of sample [Sample ID] has failed due to an error. Please contact support."—Embgenix software is designed to prevent analyses from proceeding and return this notification message if it detects an imbalance in the quantities of R1 and R2 reads for a given sample, which may be indicative of a data processing error. Please contact takara-support@basepairtech.com if you encounter this notification.
- "Analysis of sample [Sample ID] was unsuccessful. This sample may not be suitable for research."—The software returns this notification when a sample is inherently flawed such that it cannot be successfully processed via the Embgenix analysis pipeline. The most common cause for this notification is insufficient sequencing reads. Re-assessment/re-sequencing of the corresponding NGS library and/or troubleshooting of the wet lab workflow may be required to generate sufficient sequencing data for analysis.
- "Analysis of sample [Sample ID] has failed and may have been impacted by a service disruption. Please re-attempt upload and analysis of this sample." —This notification is returned when sample analysis is disrupted by a lapse or error involving the cloud-based infrastructure supporting Embgenix software. Samples impacted by such errors are typically analyzed successfully upon reattempt.
- "Upload of sample [Sample ID] has failed and may have been impacted by a service disruption. Please re-attempt upload and analysis of this sample." —This notification is returned when sample upload is impacted by a service disruption involving the user's network or the cloud-based infrastructure supporting Embgenix software.

F. Support

The **Support** menu option is a shortcut link to contact technical support via email. The email address is configured by a group administrator account in Settings but can also be viewed by all users on that page.

For more information about troubleshooting and contacting Support, please see Section A of the Appendix.

For more information for administrators wanting to configure the target support email, please refer to Section VIII.D.

G. Documentation

The **Documentation** menu option displays links to the current version of this document, the Embgenix Analysis Software Agreement, and Takara Bio Privacy Policy.

H. User Configuration Menu

The user configuration menu is accessed through the icon in the upper right corner of the screen (Figure 72). This section describes the options available there: Profile, Settings, and Logout.

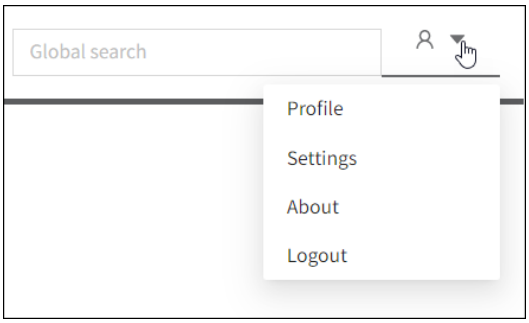


Figure 72. The user configuration menu drop-down.

1. Profile

The Profile page can be accessed either by selecting Profile from the user configuration drop-down menu (Figure 72) or by clicking on the person icon of the menu. Either option will bring up the Profile page (Figure 73).

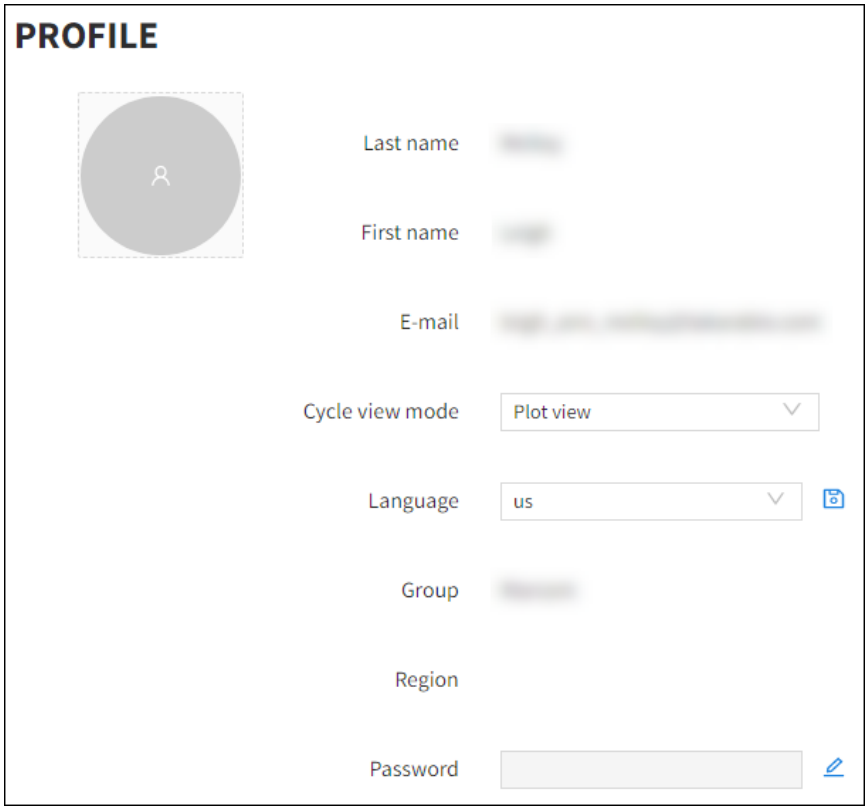


Figure 73. The user account profile page.

The profile page contains high level information relating to the user's login account. Some information fields (blurred out in Figure 73) are static, defined at the time of the account setup or when edited by an administrator account.

The fields which can be modified by the user are described briefly below:

- Cycle view mode–the drop-down menu has two options, 'Plot view' (the default setting) or 'List view'. This relates to which view a user would see upon first accessing a Cycle ID report, described in Section VI.B.
- Password–The password for the account can be updated by clicking the blue pencil icon below the grayed-out input box. The interface will change to allow for input. Follow the directions on the screen to enter your password twice, then click on the [Save] icon, indicated by the black arrow in Figure 74. The blue X icon can be used to cancel the password update (no change).

Figure 74. The password change interface.

2. Settings

The Settings page includes several pieces of account settings information and configuration options that affect the user account and web page behavior. For a Limited access (user) account, the settings displayed here are informational only; configuration of the settings are determined by Admin access account for the "Group", displayed at the top of the page. Refer to Section VIII.D for more information about these fields or within the website by hovering your mouse cursor over the (?) tooltip icon for the fields, if available.

Figure 75. Where to find the Group information in the Settings page.

3. Logout

Selecting this option will log a user out of the account and return them to the login page (Section IV, Figure 2). This is recommended after completing each working session in the application to securely exit the software and prevent unauthorized access.

VIII. Administrator Accounts

A. Signing Off On a Sample Report

In addition to the option of locking a report for an individual sample (Section VI.A), accounts with admin access also have the power to sign off on a sample report, adding additional restrictions to the ability to edit elements of the report.

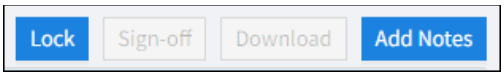


Figure 76. The header of the Report screen and location of the [Lock] and [Sign-off] buttons. Note the [Sign-off] button is inactive (grayed out) if the report page is in an unlocked state.

When a clinician reviews and locks a report, the expected workflow would be to then have the clinician notify a user with admin access to sign off on the report. In this scenario, an admin would see the header buttons as shown in Figure 77.

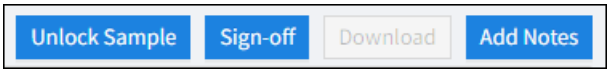


Figure 77. The configuration of the header when a sample report is locked.

1. After reviewing the report, if the admin user is satisfied with its setup, they would click the [Sign-off] button. A window will pop up, as shown in Figure 78.

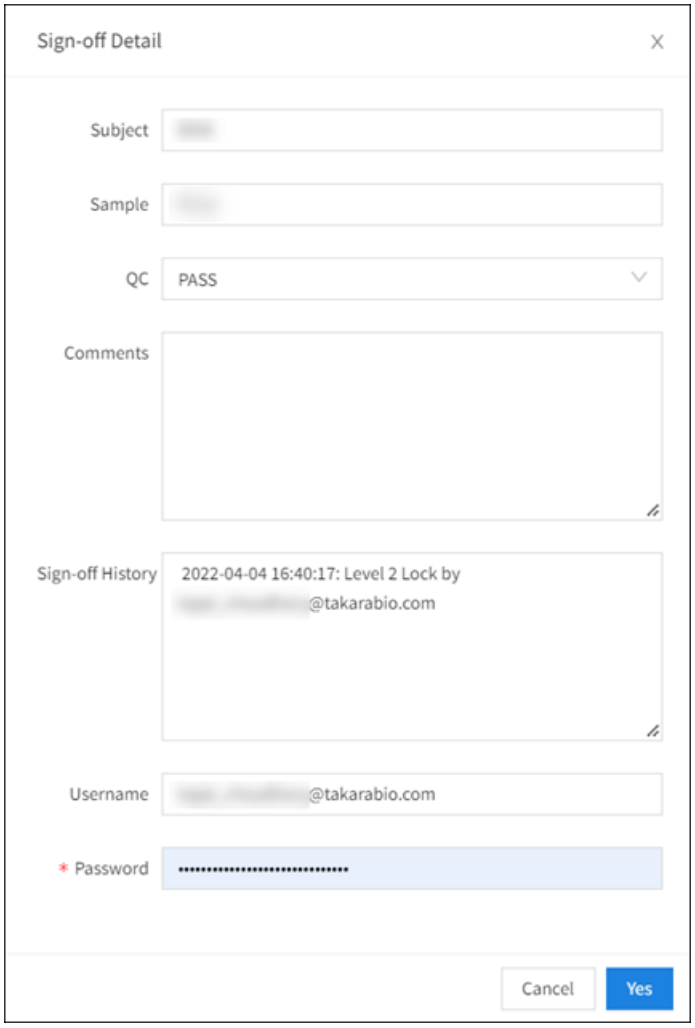


Figure 78. The *Sign-off Detail* window.

2. Enter the fields of information which summarize the review of the given sample. These fields include:
   - “QC” which can be selected as 'Pass' or 'Fail' from the drop-down menu,
   - “Comments” to add comments about the sample, and
   - "Username", to type in the name of the user signing-off on the sample.
3. Fill in the password for your login, then click [Yes].

The message "Sample has been signed-off" will appear briefly on the screen and the header buttons will change to what's shown in Figure 79.

Figure 79. The configuration of the header after the [Sign-off] button is selected.

At this point, no changes can be made within the report and limited access users no longer have the power to unlock the report.

4. If changes need to be made to the report, click the [Cancel Sign-off] button, which will reactivate the [Unlock Sample] button. The report can then either be unlocked now or by a limited access user account.

B. Managing Users

Administrator accounts have the ability to add, manage, or delete accounts for their Group in the analyzer website. The **Manage Users** menu option on the left sidebar, as seen in Figure 80, is only visible to admin users.

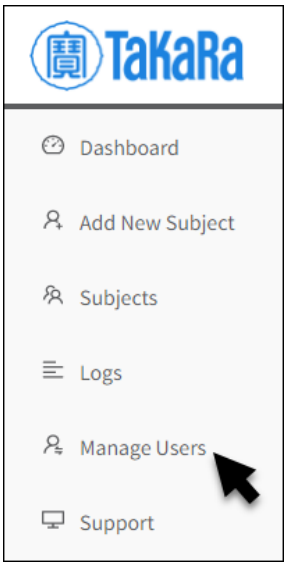


Figure 80. The Manage Users menu option for administrator accounts.

Clicking the option brings up the Users page (Figure 81).

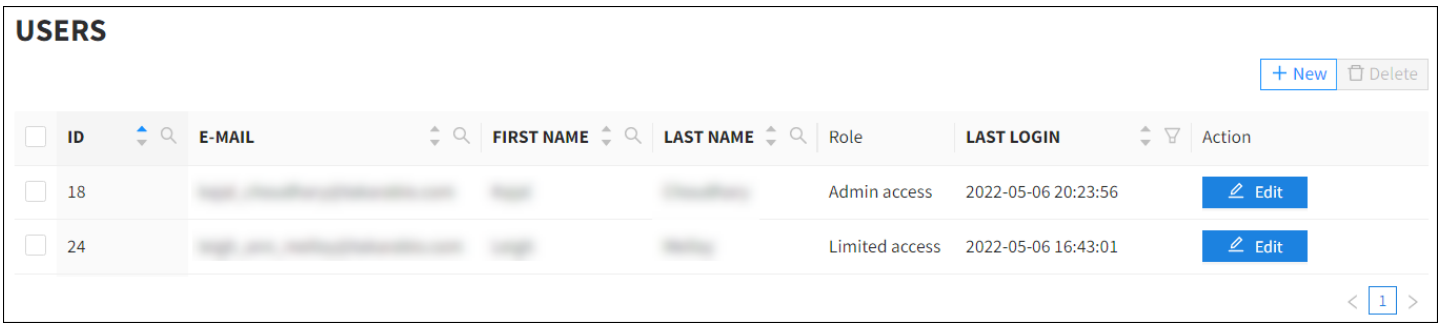


Figure 81. Example Users page.

The following information is displayed in the table:

- ID–a unique numerical identifier associated with the user account. This number will be displayed in parentheses after the user account email in the system logs page (Section VII.D).
- E-mail–the email address associated with the account; also the account's username.
- First name–the first name of the account user
- Last name–last name of the account user
- Role–this shows the type of account the user has, which in turn defines the level of access and actions the user can do on the website.
  - Admin access
  - Limited access
- Last Login–the time (on the server) for the most recent time the user logged into the account.

Administrators can take the following actions on the page:

- Add users by clicking the [New] button. This will bring up the New User page (Figure 82).

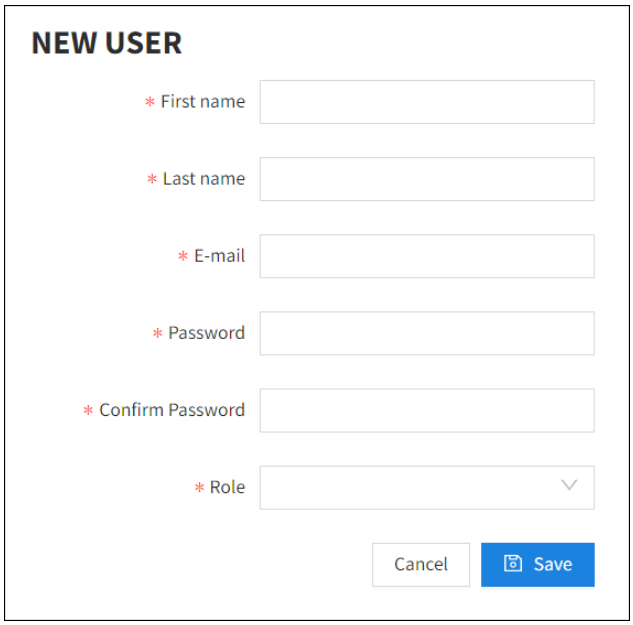


Figure 82. New User page.

Fill out the information for the new user account and select the Role (described above) for the account. Click [Save] to create the account, or [Cancel] to return to the Users page with no changes made.

- Edit existing users by clicking on the [Edit] button in the "Actions" column of the table. This will bring up the Editing page as seen in Figure 83.

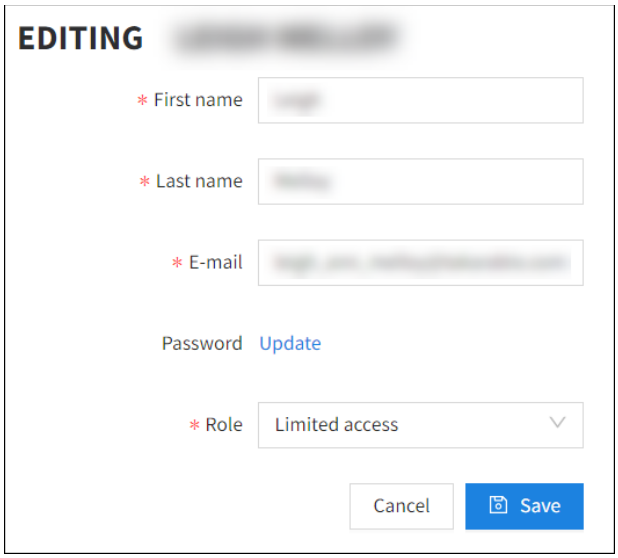


Figure 83. User editing page.

Administrator can update any of the fields on Editing page for the user account. Clicking on 'Update' for the "Password" field will display two fields to set and confirm a new password for the account (Figure 84). This can be useful if a user forgets their password or to lock a user out of the account, if necessary.

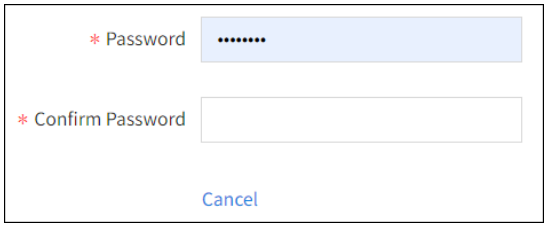


Figure 84. Setting a new password for a user account.

Click [Save] to retain the changes or [Cancel] to quit out without applying any changes.

- Delete users by checking the box next to their account ID, then clicking [Delete]. A confirmation prompt will pop up above the [Delete] button. Click [Yes] to delete the account or [No] to cancel out (Figure 85).

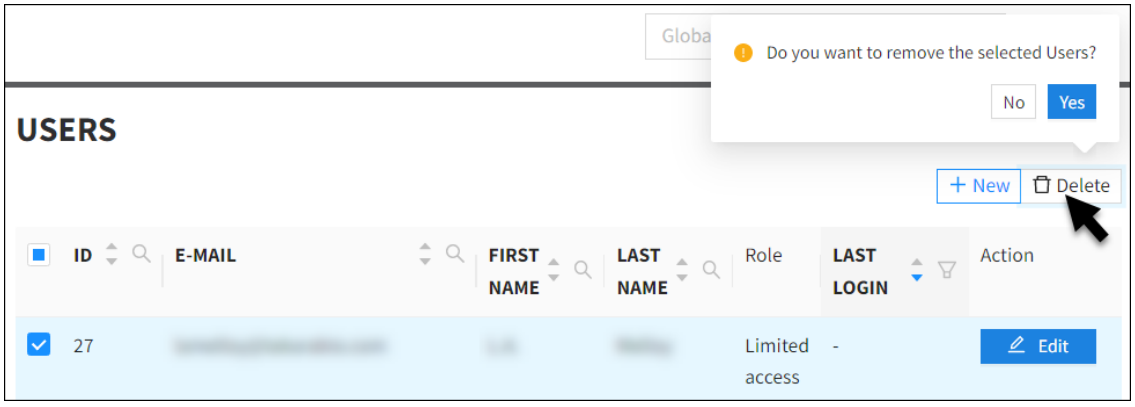


Figure 85. Deleting a user account.

C. Settings

The Settings page includes several pieces of account settings information and configuration options, which are described by section below and shown in Figures 86, 87, and 88. Information can also be accessed within the website by hovering your mouse cursor over the (?) tooltip icon.

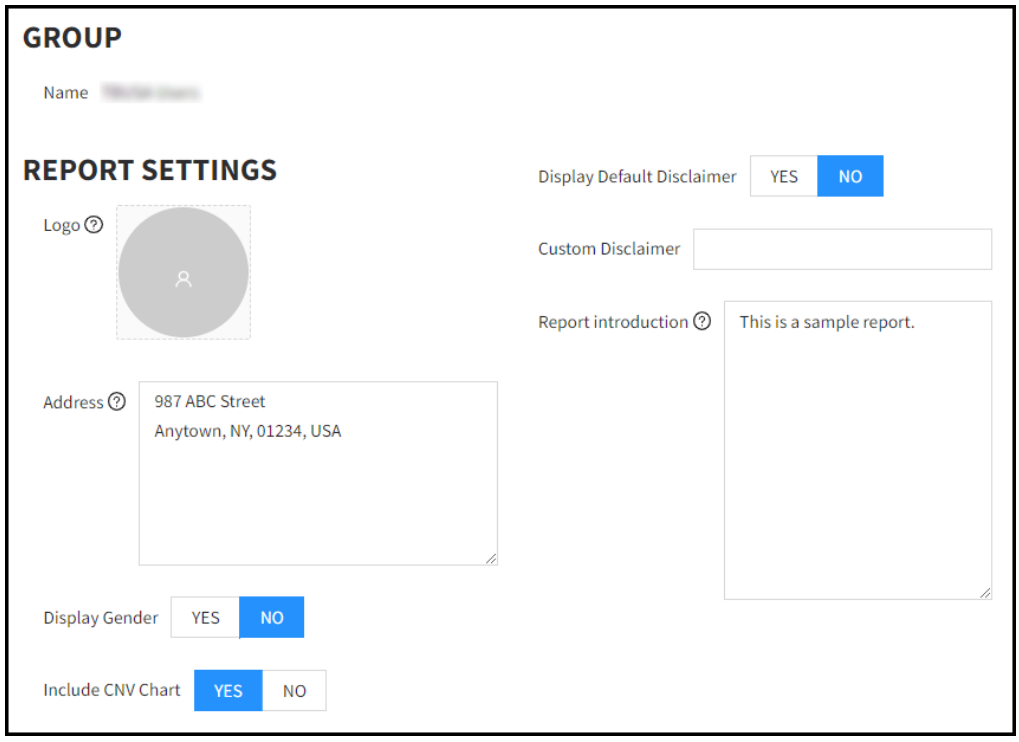


Figure 86. Settings page: the Group and Report Settings sections.

- Group
  The group is typically defined as the laboratory processing samples sequenced using the Embgenix PGT-A kit. This will be configured during initial admin access account set-up by Takara Bio.

- Report Settings
  - Logo–a small image that will display in the header of PDF reports
  - Address–if configured, this information will be added to the PDF reports
  - Display Default Disclaimer–if ‘yes’, a default disclaimer will be included in the report footer; ‘no’ will trigger display of the ‘Custom Disclaimer’ field
  - Custom Disclaimer–text entered into this field will display in the report footer in lieu of the default disclaimer text
  - Report introduction–if configured, this text will display in the first page of the report
  - Display Gender–if 'yes', the report will display gender information derived from the sample; 'no' will omit the gender information from the report
  - Include CNV Chart–if 'yes', the CNV chart will be appended to the downloaded reports; 'no' will omit the chart from the report

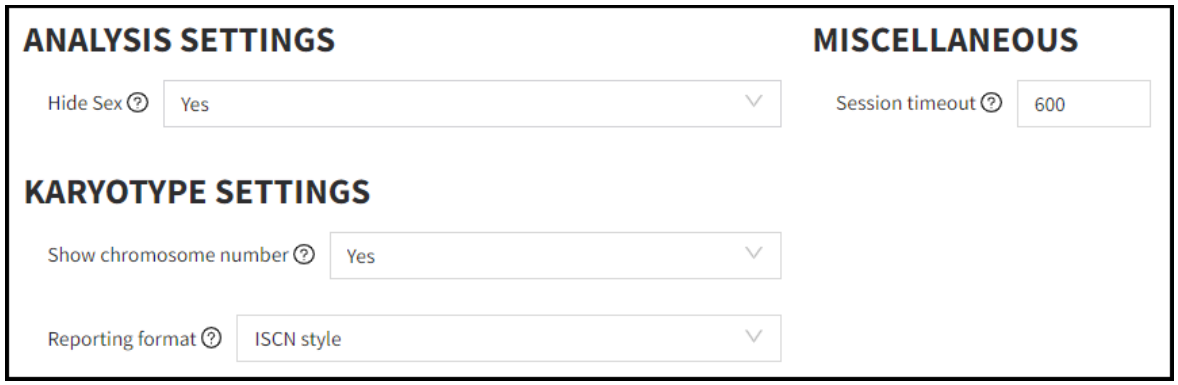


Figure 87. Settings page: the Analysis, Karyotype, and Miscellaneous sections.

- Analysis Settings
  - Hide Sex–a simple binary setting, a 'Yes' will mask the sex assignment and sex chromosome from the default view (unless an abnormality is detected on it). 'No' will display sex assignment determinations and sex chromosomes in the reports.
- Karyotype Settings
  - Show chromosome number–'Yes' will append the whole-chromosome number as a prefix to the reported karyotype; 'No' will omit the prefix.
  - Reporting format–options are 'Shorthand', ‘Longhand’, or 'ISCN style'
- Miscellaneous
  - Session timeout–defines the inactivity timeout value, in seconds, for the user accounts administered by the admin. In Figure 87, this is set to 600 sec (5 min).

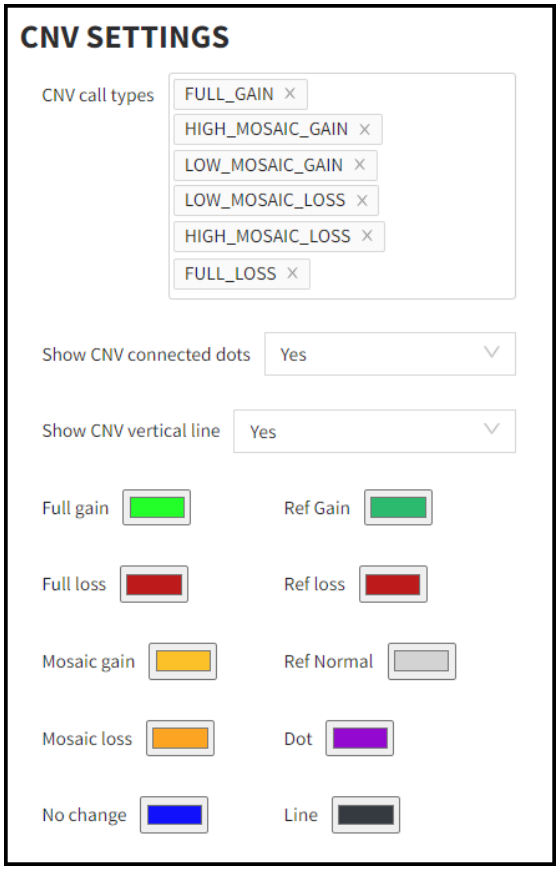


Figure 88. Settings page: the CNV Settings section.

- CNV Settings
  This section includes the customizable options for the CNV charts displayed throughout the user interface of the tool.
  - CNV call types–these options display the types of calls that might display on the CNV chart. Options can be removed by clicking on the X icon on the right side of the box or, if previously deleted, by typing the type name into the box and hitting [Enter] (Figure 89).

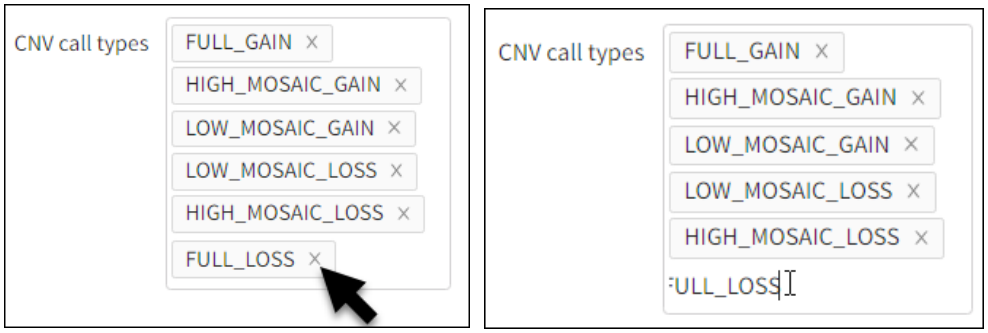


Figure 89. Settings page: removing or restoring CNV call type options. **(Left)** Deleting an option by clicking the X icon. **(Right)** Restoring the deleted call type by typing the name into the box, just prior to hitting [Enter].

If a type is deleted and later needs to be restored, refer to the full list of available CNV in Table 2.

Table 2. CNV call type options on the administrator Settings page.

CNV call types
FULL_GAIN
FULL_LOSS
HIGH_MOSAIC_GAIN
LOW_MOSAIC_GAIN
HIGH_MOSAIC_LOSS
LOW_MOSAIC_LOSS

NOTE: There is no error checking performed on manual additions to the call type settings list. For best performance, if adding a call type, ensure the text exactly matches the text for it listed in Table 2.

  - Show CNV connected dots–'Yes' includes dots within the line charts to distinguish data points; 'No' will display only the line
  - Show CNV vertical line–'Yes' adds a link between chromosome 22 and the X chromosome; 'No' omits the line.

The two columns under the text field options define the color representation for the events of interest in the charts. Administrators can modify the colors of the events by clicking on the color box beside the event of interest to bring up the color selection window.

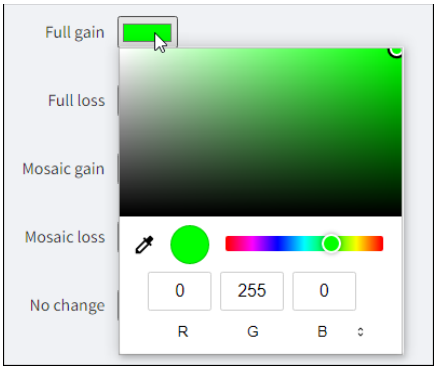


Figure 90. Customizing CNV chart colors in the Settings page (RGB).

There are several approaches administrators can take to modify the event colors:

- By clicking and dragging the black circle (shown in part in the top, right corner in Figure 90) through the color field
- The color field can be changed by:
  - Dragging the circle horizontally along the rainbow slider.
  - Clicking on the eyedropper icon, which will bring up a magnifying circle attached to the mouse cursor (Figure 91). Focus the circle on a color to copy and left click on the mouse to capture it.

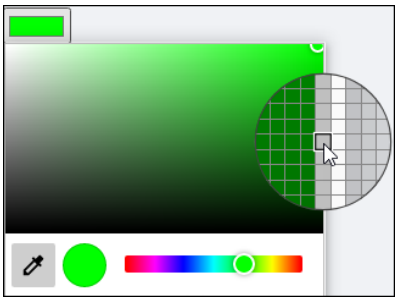


Figure 91. Customizing CNV chart colors in the Settings page (eyedropper).

  - By adjusting the settings in the color code boxes below the rainbow slider.

    By default, the color code option is set to RGB, but HSL or Hex can be selected by clicking on the up-down arrow icon to the left of the field legends (Figure 92).

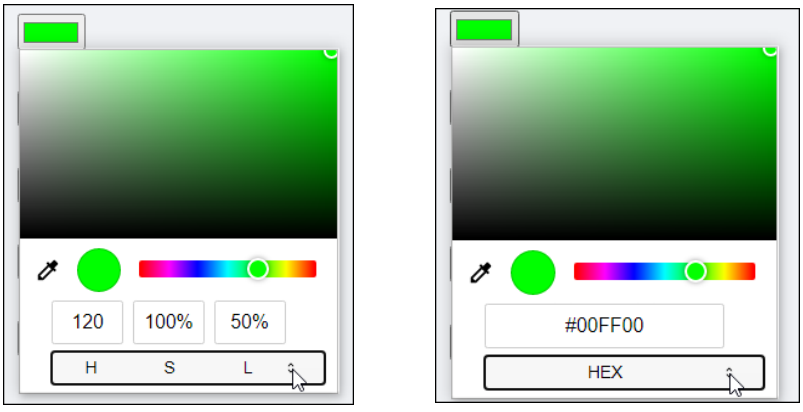


Figure 92. Customizing CNV chart colors in the Settings page (HSL and Hex).

- Action buttons

On the bottom right corner of the page is a set of three buttons (Figure 93) that affect the entire Settings page.

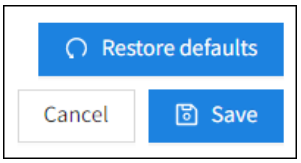


Figure 93. Settings page: action buttons

- Restore defaults–removes all customized settings on the page and reverts to the original website settings
- Cancel–quits out from the page without saving any changes that may have been made
- Save–saves any changes to the options to become customized settings for all users in the group

Appendix. Troubleshooting and Support

A. Contacting Support

If you encounter an error message or unexpected behavior in the analyzer, if possible, take a screenshot of the problem, then send an email to takara-support@basepairtech.com.

In the email, please include:

- a brief description of the issue,
- what steps you may have taken just prior to seeing the problem, to help us with recreating it, and
- screenshot(s) (if available) of the error message or behavior.

B. FASTQ Files Are Not Being Generated by the Sequencer

Typically, the Illumina MiSeq and NextSeq platforms will automatically generate FASTQ files when provided with the sample sheet set up according to the Embgenix workflow documentation.

In the case when the FASTQs are not generated, we recommend following the troubleshooting steps below, going down in the order listed, until the problem is resolved:

1. Troubleshoot the configuration of the sequencing run referring to the Illumina documentation.
2. Verify that the sample sheet has been configured correctly, according to the guidelines in the appropriate Embgenix PGT-A Kit instructions for use document.
   Information on how to edit a Sample Sheet and requeue analysis are available on the Illumina webpage for MiSeq and NextSeq platforms.
3. If neither of the previous steps resolves the problem, the files can be manually generated by the following instructions. The steps should be executed on a Linux server where bcl2fastq is installed (refer to the bcl2fastq manual for details) that also has access to the sequencing run folder.
   a. Log into the server.
   b. Move to a working folder where you want to generate the FASTQ files.
   c. Determine if the sample sheet is located in the sequencing run folder.
      – If it is, run `bcl2fastq` with the following syntax:

```
bcl2fastq -R <RUN_FOLDER> -o <OUTPUT_FOLDER> > stdout 2>
  stderr
```

– If the sample sheet is not located in the sequencing run folder, use the following syntax:

```
bcl2fastq -R <RUN_FOLDER> -o <OUTPUT_FOLDER> --sample-sheet
  <SAMPLE-SHEET> > stdout 2> stderr
```

where:

- `<RUN_FOLDER>` is the path to the sequencing run folder,
- `<OUTPUT_FOLDER>` is the ID automatically generated by Illumina sequencer, and
- `<SAMPLE-SHEET>` is the path to the sample sheet created for the Embgenix data.

NOTE: As shown in the two instances above, the `--sample-sheet` parameter is optional if the sample sheet is located in the sequencing run folder.

Examples:

– If it is, run `bcl2fastq` with the following syntax:

```
bcl2fastq -R MyRun1 -o MyOutput1 > stdout 2> stderr
```

– If the sample sheet is not located in the sequencing run folder, use the following syntax:

```
bcl2fastq -R MyRun2 -o MyOutput2 --sample-sheet
  /usr/home/myacct/Embgenix_MiSeq96_SampleSheet.csv >
  stdout 2> stderr
```

C. Sample Upload or Analysis Failures

Embgenix software can identify and notify users of scenarios where sample upload or analysis were impacted by lapses involving network connectivity or the cloud-based data processing infrastructure, or instances of failed analyses due to inadequate sample quality. Please refer to Section VII.E for further discussion of the various sample failure modes and associated notifications.

Contact Us	
Customer Service/Ordering	**Technical Support**
tel: 800.662.2566 (toll-free)	tel: 800.662.2566 (toll-free)
fax: 800.424.1350 (toll-free)	fax: 800.424.1350 (toll-free)
web: takarabio.com/service	web: takarabio.com/support
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This document has been reviewed and approved by the Quality Department.