EMBRYOMAP™
EMAP SOFTWARE GUIDE

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Revision history

Version	Date	Description of Change
1.0	07-Jan 2022	Initial release.
2.0	24-Jun 2024	Minor language updates, updated page and table references. Addition of user instructions for new features released with eMap Software v0.3.4, in the following sections: • Introduction: added links to terms & conditions for available eMap servers. • Table 4, Table 5, Table 7. • Laboratory Planner – Pool and Sequence • 3 Uploading Data – Automatic Upload • Appendix 4.
3.0	16-Sep 2024	Minor language updates, updated page and table references. Addition of user instructions for new features released with eMap Software v1.0.0, in the following sections: • Getting started with eMap – Search • eMap Account – User Profile • Account Security – Brute Force Protection, Multi-Factor Authentication • Laboratory Planner – Create a New Plate, Pool and Sequence • Analysis – Results Overview, Reviewing Sample Results • Reporting – Summary Report, Reporting sample failures • Export Data – Files Menu • Appendix 2 and Appendix 3.
4.0	18-Aug 2025	Minor language updates, updated page and table references. Addition of user instructions for new features released with eMap Software v2.0.0 to enable the EmbryoMap SNP workflow, in the following sections: • Introduction: EmbryoMap SNP workflow • eMap Account – Analysis Settings, QC Settings, and Miscellaneous settings. • Laboratory Planner – Create a New Plate, Create a New Plate – Fast Track. • Pooling and Sequence – SampleSheet and Normalisation reference Selection, Generate SampleSheet. • Uploading data – Table 9. • Analysis – Sequencing Run QC (Figure 12), Data Lists (Table 12), Run view, Export data by Run. • Reviewing Sample Results – Sample Navigation (Table 13), Download Sample Data, Logs, Sample QC, Experimental Details, Sign-off a Sample, • Reporting – Summary Report format (Table 17), Full Report format (Tables 19 & 20), Reporting Sample Failures (Tables 21 & 22) • Export Data – Analysis Result Files. • Appendix 2 – Fast Track Import • Appendix 3 – Export Sample Metadata

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1 INTRODUCTION

The EmbryoMap portfolio offers two streamlined workflows, EmbryoMap for assessing chromosome copy-number (CNV) and EmbryoMap SNP for combined CNV and SNP analysis. Both workflows are designed for use with multi-cell samples (e.g. trophectoderm biopsy) using next-generation sequencing. The eMap analysis software enables run planning, sample tracking, data analysis and reporting in a single software solution. This guide describes the features and functions of the eMap software. **Training is highly recommended prior to use.** Refer to the **EmbryoMap Calling Guide** (v2.0 or later) for guidance on interpreting challenging data.

Chromosome copy-numbers are assigned according to the number of sequencing reads detected for a given region of the genome. eMap internally cleans and normalises the data to remove the reproducible, workflow-specific biases, and then re-scales the counts to correspond with copy-number (2n). If sub-chromosomal imbalance detection is enabled, the software inspects the identified chromosome segments before assigning the chromosome and sample status.
Single Nucleotide Polymorphisms (SNP) are genetic variations and specific loci in the genome. The EmbryoMap SNP workflow uses CNV and SNP data to reads are identified separated from the reads used for CNVs and are used to assess a samples ploidy state and

The eMap software user interface has been designed to guide the User through Laboratory Planning and Analysis, allowing faster assessment of results. A high-level overview of the EmbryoMap workflow to prepare samples for sequencing is shown in Figure 1.

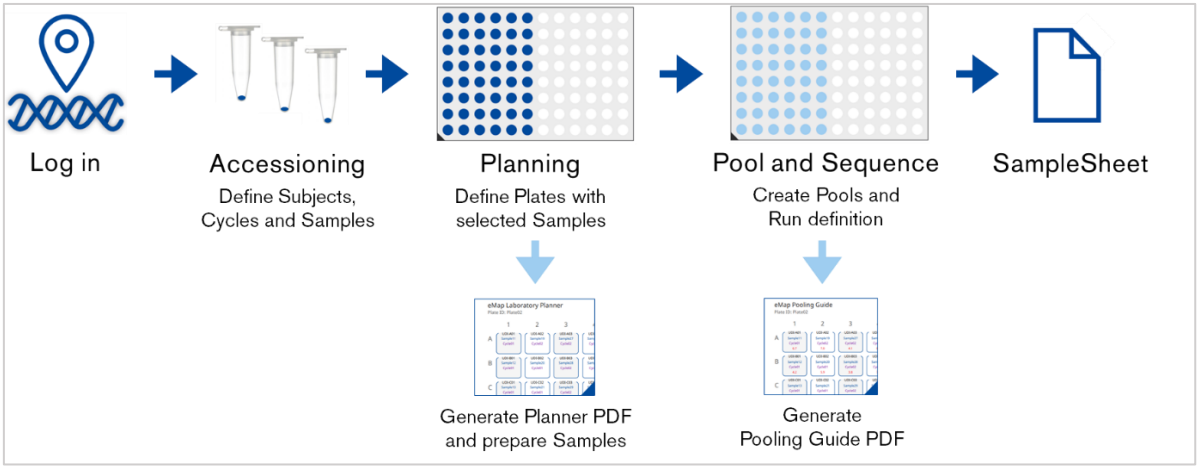


Figure 1: Run planning in the eMap software

The sequencing reads are automatically aligned to the human genome and saved as BAM files on the sequencing instrument. Data is then automatically or manually uploaded to the User's eMap account and matched with the planned run definition. Data analysis begins immediately, notifying the User by e-mail when complete. Data can be reviewed in drill-down levels, with each level providing more detailed information about the samples (Figure 2).

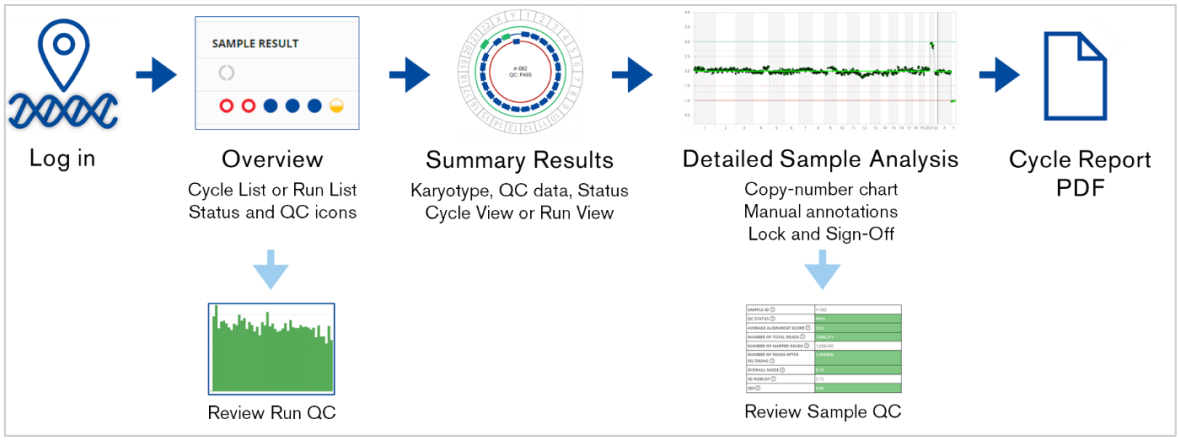


Figure 2: Data review and reporting in the eMap software

Getting Started with eMap

eMap Components

eMap Uploader Software: The Uploader Software is a set of Microsoft Windows™ executables that must be installed on the sequencing instrument or a computer with access to the EmbryoMap sequencing data. Installation and usage are described in *Appendix 4*.

eMap Analysis Software: The eMap analysis software is a cloud-based solution for the management and analysis of EmbryoMap data. eMap software can be securely accessed from any networked computer using an internet browser and a personal login.
Use of both software components are regulated by the Terms and Conditions outlined at the following links, or by clicking *Terms* in the eMap webpage footer:

Europe 1	https://eu-central-1.emap.vitrolife.com/terms
North America 1	https://us-west-2.emap.vitrolife.com/terms
APAC 1	https://ap-southeast-1.emap.vitrolife.com/terms
APAC 2	https://ap-northeast-1.emap.vitrolife.com/terms
APAC 3	https://ap-southeast-2.emap.vitrolife.com/terms

Accessing eMap

The eMap software is available and free-to-use by all EmbryoMap customers. Each customer will be provided with an eMap account for their Laboratory or Group, which they can self-administer to add additional Users or adjust software Settings. Two different levels of User account can be configured, to control access to specific software features between Users. Further details on the User and Group options are explained in the *eMap Account* section. Once each User has their own login details, they can access their eMap Group account from https://emap.vitrolife.com. We strongly recommend Users to keep their password secure and log out from the system after completing a session.

The menu links ensure easy navigation through the software and are shown in the top right-hand side of the browser on all pages (Table 1).

Table 1: eMap navigation menus

Menu	Navigation Links
HOME	View the eMap Dashboard
PLANNING	Accessioning, Laboratory Planner, Pool and Sequence
DATA	Subjects, Cycles, Sequencing Runs, Custom Imports, Report Builder, Logs
ACCOUNT	Profile, Settings, Manage Users [Level 2 Users only], Support, Logout

Search

The text box on the top right-hand corner of every page offers global search functionality for Accessioning and Result data within the specific Group's database.

1. Enter text and press enter to search.
2. The result page is split by the result type (e.g. Subjects, Samples, Plates, Pools).
3. The search term is highlighted, and links provide access to the source pages of the data.

Subject, Cycle and Sequencing Run lists have search functionality built-in for specific fields. These are indicated by the search , sort and filter tools in the column headers.

eMap Account

User Management

Each Group account can be self-administered to create or modify User profiles for team members in eMap. To allow traceability of changes to the data as part of GxP compliance, each User should always log in with their own credentials. The account levels are:

- **Level 1:** For standard Users performing sample preparation and initial data analysis
- **Level 2:** Same as Level 1, with additional privileges to:
    - Administer Users
    - Modify system Settings
    - Sign-off samples for reporting

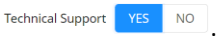
1. Navigate to *ACCOUNT* > *Manage Users* (only available to Level 2 Users)
2. Review the list of Users. Search , sort and filter options are available in the column headers.
3. Click + New to create a new User or click Edit to edit an existing User account.
4. Select a User with the checkbox and select Delete to remove a User profile.

User Profile Configuration

1. Creating or editing a User profile allows the input of:
    a. First name
    b. Last name
    c. E-mail address used for login and receiving system notifications
    d. Set or Update a password
    e. Select profile Role privileges, Level 1 (Restricted) or Level 2 (Admin).
    f. Apply Technical Support Access restrictions (see below).
2. Click Save to apply changes.

Technical Support Access

A special type of User profile can be created by a Level 2 User (account admin), for allowing external support (including Vitrolife representatives) or for the (temporary) sharing of data. Technical Support User profiles are configured such that privacy-sensitive data is obfuscated (made un-readable) when this User logs in.

1. Technical Support accounts are controlled by the database owner (account Admins) and should only be created when Technical Support is required. In most cases support can be given without needing to share access to the account.
2. The account Admin is responsible for sharing the Technical Support login information and deleting the User profile or updating the password once the required support has been provided.
3. When creating a new User, or editing and existing one:
    a. Use the selector to enable Technical Support restrictions 

.
    b. Click 

to apply changes.
4. The data fields in **Table 2** will not be readable to the Technical Support User profile, which also applies to the system Logs visible to the User:

Table 2: Obfuscated data fields are applied when Technical Support restrictions are enabled for a User profile.

Parameter	Obfuscated data fields
Software User	First name, last name, email address
Subject	First name, last name, date of birth, partner first name, partner last name, notes
Data	BAM sequence file (not available for download)

Note: User profiles with Level 2 permissions and the *Technical Support* option **enabled** will not be able to Manage Users or disable (self-administer) the *Technical Support* restrictions.

5. System IDs (Subject, Cycle, Sample, Plate and Pool IDs) are **not obfuscated** and therefore should be chosen to not contain any personal, sensitive information in routine use of eMap.
6. When the support case is resolved, the account can be deleted, or the password changed until required again.

User Profile

All Users (Level 1 and Level 2) can set or update the following options on their own profile page (Figure 3).

1. Log into eMap.
2. Navigate to *ACCOUNT* > *Profile.*
3. Click the area under “Profile” to upload a profile image (Maximum size 2 Mb).
4. Select to enable e-mail notifications for failed login attempts (See: *Account Security*), analysis status and system errors, using the address used to log into eMap.
5. Select the default sample order when creating new Plate IDs. Samples shall be ordered by UDI name in the SampleSheet file and User Interface sample lists, either by Row or by Column order.
6. Select the default format for the *Cycle View* page (Circos view or List view). See section *4 Analysis*.
7. Select system language (when available).
8. Click  to change the password, re-enter, and then click  to Save.
9. Click **Access Keys** to view the unique access credentials for this database for performing data uploads. Never share the Access Key with anyone outside your laboratory. See *Appendix 4* for installation instructions.

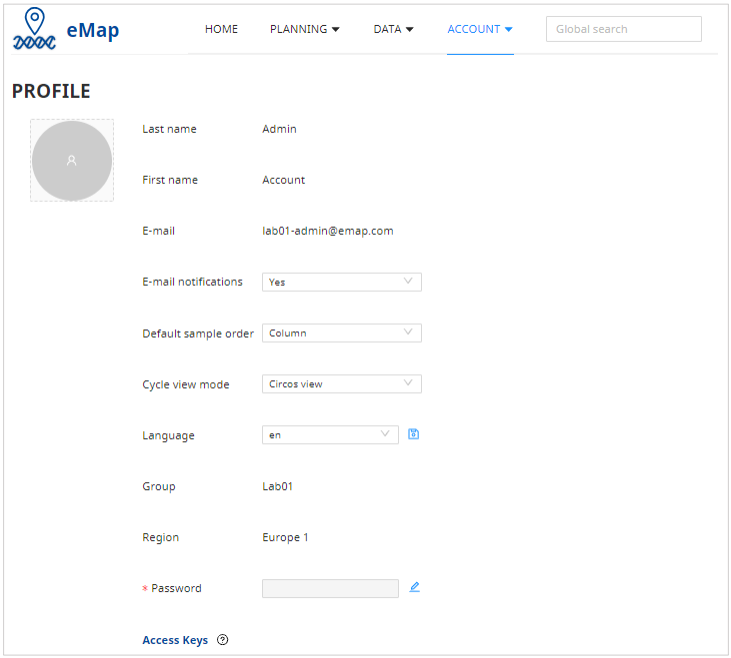


Figure 3: eMap User Profile page

System Logs

For traceability, actions performed within eMap are recorded in the system log. This log can be reviewed and downloaded from the *DATA* > *Logs* menu.

1. The time of the event in universal time, the action and the Username are recorded.
2. Use the column header tools to search, sort and filter results.
3. Click Export to TSV file to export and archive the system logs in tab-delimited .tsv format.

Detailed logs for the analysis of specific samples are displayed separately on the *Sample View* page in the *Logs* tab. Critical errors and QC failures will also be displayed as *Alerts* on the *HOME* dashboard page with Links to the relevant items, allowing quick review of any issues.

Settings

Navigate to the system *Settings* page by clicking *ACCOUNT* > *Settings*. The *Settings* page allows Users to customise the PDF Report and adjust the copy-number analysis algorithm parameters as required.

Please review the settings before using the eMap software. Changes made on the *Settings* page via the *ACCOUNT* menu will apply to all Users in the Group, and all EmbryoMap datasets analysed thereafter. Previously analysed data will not be affected by changes to the copy-number algorithm parameters until the sample(s) are re-analysed.

The symbol provides a short description for all available settings; hover the cursor over the icon to reveal the text. Modification of system settings can only be performed by Level 2 Users.

1. Press Save after modifying any values.
2. Modified analysis settings will be applied to all analyses initiated after saving new values.

3. Click Restore defaults to revert parameters to Vitrolife default values except for Report Settings, Brute Force and MFA protection settings, Instruments. The Default Analysis Software, Default Normalisation and Default SampleSheet Type selections shall be removed and will need to be manually selected prior to saving.
4. Click Cancel to discard any changes and return to the previous page.

Report Settings

The settings described in Table 3 will be applied as default values when using the *Report Builder* tool, although Users have the option to modify the values during the generation of individual reports.

Table 3: Report Settings

Parameter	Description	Default Values	Allowed Values
Logo	Upload an image file to be visible at the top of the PDF Report. Maximum size 2 Mb.	None	png, gif or jpeg files
Address	The laboratory/group address to be visible at the top of the PDF Report.	None	Any text
Disclaimer	A line of text visible in the footer of the PDF Report, e.g. a specific disclaimer.	None	Any short text
Report introduction	A block of text visible on the first page (cycle summary) of the PDF Report, e.g. description of the test and reporting criteria.	None	Any text
Include CNV chart	Use the selector to include the copy-number (CNV) chart for each sample as a full-page image.	Yes	Yes/No

Analysis Settings

Table **4** describes the settings relating to the copy-number algorithm and display of the copy-number chart. eMap software includes the option to automatically detect sub-chromosomal and/or intermediate (putative mosaic) copy-number changes. It is recommended to establish the settings suitable for your Group, especially concerning copy-number calling options, as part of a local validation.

To re-analyse a single sample with different settings, click Re-Analyse from the *Sample View* page. This will open the settings dialog applicable for analysing that sample only; the general system settings will remain unchanged. See the *Re-analyse a Sample* section for detailed instructions.

Table 4: Analysis Settings

Parameter	Description	Default Values	Allowed Values
Smoothing	Number of bins on either side used for smoothing the copy-number chart (sliding window).	3	0-10
Segmental	Enable or disable automatic calling of sub-chromosomal copy-number changes ≥10 bins (approx. 10 Mb).	Yes	Yes / No
Custom copy-number threshold	Enable or disable automatic assessment of intermediate copy-number changes (putative mosaic calls). The thresholds can also be used to override the standard calling algorithm with the fixed cut-offs provided. The default settings disable low-level mosaic calling by setting identical threshold values for low- and high-levels for gains and losses, respectively.	Yes	Yes / No
Gain	Minimum copy-number to assign a copy-number gain	2.7	2.1-4.0
High mosaic gain	Minimum copy-number to assign a high-level mosaic copy-number gain.	2.5	2.1-3.0
Low mosaic gain	Minimum copy-number to assign a low-level mosaic copy-number gain.	2.5	2.1-3.0
Low mosaic loss	Maximum copy-number to assign a low-level mosaic copy-number loss.	1.5	1.0-1.9
High mosaic loss	Maximum copy-number to assign a high-level copy-number loss.	1.5	1.0-1.9
Loss	Maximum copy-number to assign a copy-number loss.	1.3	0.0-1.9
Minimum SNP depth	Number of heterozygous SNPs with the minimum read depth. (EmbryoMap SNP Only)	10	10-100
Hide ploidy status	Option to show or hide the detected ploidy status. (EmbryoMap SNP Only)	No	Yes / No
Hide Allelic consistency status	Option to show or hide the detected Allelic consistency status. (EmbryoMap SNP Only)	No	Yes / No
Hide Sex	Option to show or hide the detected sample sex, reported by the sex field and the sex chromosomes. Automatic sex / sex chromosomes will always be displayed if an abnormality is detected on either of the sex chromosomes.	No	Yes / No
Telomeric drop correction	Experimental feature to detect and automatically adjust the copy-numbers of bins at chromosome telomeres that exhibit a falling slope. These events are considered as artefacts because they affect fewer bins than the limit for *automatic* segmental imbalance detection (10 bins). Therefore, using this feature may mask putative telomeric imbalances affecting <10 consecutive bins.	No	Yes / No

Quality Control

Table 5 describes the settings and values used by the copy-number algorithm to set the automatic QC status of the sample. The quality control parameters are set by default to the Vitrolife recommended values. In some cases, the Group may wish to modify the parameters in line with local validation and applications.

Table 5: CNV Quality Control Settings

Parameter	Description	Default Values	Allowed Values
Minimum total reads	Minimum total sequencing reads (not pairs) expected per sample.	300,000	200,000-2,000,000
Minimum filtered reads	Minimum filtered sequencing reads used for the analysis expected per sample.	200,000	150,000-2,000,000
Minimum average quality score	Minimum mean read alignment score, which also considers read quality.	50	25-60
Maximum noise	Maximum allowed sample noise, comparable to the DLR of microarray results.	0.4	0.1-0.9
Maximum SDI	Maximum allowed step-wise sample noise.	4.5	0.0-6.0
Default pooling mass	Default pooling mass per sample (ng).	30	1-1000
Minimum confidence score	Region confidence scores below this value will be highlighted in the *Regions* tab of the *Sample View* page, to assist manual review of reported abnormalities.	0.7	0.0-1.0
Log level	Level of detail to include in the analysis Log. “Debug” will generate the most additional processing messages.	Debug	Info / Error / Debug
Default analysis software	Default version of the copy-number algorithm used for incoming sample data.		By account
Default Normalisation	Default normalisation reference dataset to use for incoming sample data.		By account
Recentering	Option to select specific autosomes to use for centering the copy-number chart, instead of all autosomes. This can be useful for defining the baseline when re-analysing specific samples with multiple imbalances (but should usually be set directly in the corresponding dialog when re-analysing individual samples).	None	Comma-separated list with numbers 1-22

Table 6 describes the settings and values used by the SNP algorithm to set the QC status of the sample. **These quality control parameters are not visible in the user settings; they are fixed at threshold values and cannot be modified by the user.**

Table 6: Internal SNP Quality Control Parameters (EmbryoMap SNP Only)

Parameter	Description	Value range	Threshold Values
Number of valid SNP reads	Minimum number of reads available for SNP-calling.	Not applicable	180.000
Number of heterozygous SNP calls	Minimum number of heterozygous SNP with the minimum read depth.	Not applicable	100
Ploidy confidence	The level of confidence in the predicted ploidy status.	0-1	0.7
Allelic consistency	How closely the pattern of alleles matches the predicted ploidy status.	0-1	0.7

Karyotype Settings

Table 7 describes the karyotype settings used to display the sample results on the Summary Report and Full Report PDFs, as well as in the eMap interface. Modifying the following settings will take effect immediately and does not require the samples to be re-analysed.

Table 7: Karyotype Settings

Parameter	Description	Default Values	Allowed Values
Show chromosome number	Option to show or hide the count of whole-chromosome copy-numbers.	Yes	Yes / No
Reporting format	Options for how the Reported Karyotype is constructed. See the *Karyotype* section of this guide.	Shorthand	Shorthand / Cytogenetic bands / Cytogenetic bands and molecular position

Miscellaneous

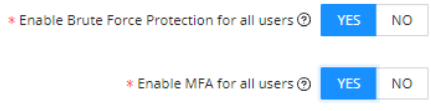
8 describes additional settings relating to the eMap user interface and may be configured according to the Group needs.

Table 8: Miscellaneous Settings

Parameter	Description	Default Values	Allowed Values
Enable Brute Force Protection for all users	Enable protections to limit the number of unsuccessful log in attempts before applying a temporary block of the username and notifying the Group's Level 2 users.	No	Yes / No
Enable MFA for all users	Enable protections requiring all Group users to setup Multi-Factor Authentication for access to eMap.	No	Yes / No
Languages	Additional interface languages may be available. Users will be able to choose their preferred language from this list.	en	Contact Vitrolife
Session timeout	Number of seconds of inactivity before the browser session will expire, requiring password re-entry.	600	60-36000
Instruments	Enter your Sequencer serial IDs that are configured for use with EmbryoMap.	None	List of IDs
PDF guide key 1	Choose an appropriate ID to be displayed on the Laboratory Planner PDF and the Pooling Guide PDF. The available options of the keys are updated depending on the selection of key 1, 2, and 3.	Subject-ID	Subject-ID, Cycle-ID, Sample-ID, Requisition, Tube-ID
PDF guide key 2	Choose an appropriate ID to be displayed on the Laboratory Planner PDF and the Pooling Guide PDF.	Cycle-ID	Subject-ID, Cycle-ID, Sample-ID, Requisition, Tube-ID
PDF guide key 3	Choose an appropriate ID to be displayed on the Laboratory Planner PDF only. *On the Pooling Guide PDF this value will be the Pooling Volume and cannot be changed.*	Tube-ID	Subject-ID, Cycle-ID, Sample-ID, Requisition, Tube-ID
Default SampleSheet Type	Select the required SampleSheet for your Sequencing platform and Windows version.		By account
Default Workflow	Select the default workflow that will be used for the majority of sample preparations.	EmbryoMap	EmbryoMap, EmbryoMap WGA, EmbryoMap SNP

Account Security

The eMap software includes optional login security features that can be enabled for all users of the Group Account. The features can be enabled by any Level 2 user, via the *ACCOUNT* > *Settings* page.



Brute Force Protection

When enabled, the Brute Force Protection will:

1. Warn the user when incorrect login credentials have been submitted.
2. Display the number of login attempts remaining before the user is locked-out.
3. Lock-out the user account for 5 minutes after three failed login attempts.
    a. A countdown will show the lock-out time remaining.
    b. Repeated login attempts during a lock-out period (including with correct credentials) will restart the lock-out period. Please do not refresh the login page during this time.
4. Display failed login attempts by username in System Logs.
   Notify Group Account Level 2 users by e-mail of the failed login resulting in a lock-out, and the user's IP address.
   Note: E-mail notifications are dependent on the Level 2 user settings in *ACCOUNT > Profile*.

Brute Force Protection is applied at the Group Account level (for all users), but enforced at the username level. Therefore, if one user is temporarily locked-out, a different user of the same Group Account will still be able to log in with correct credentials.

Multi-Factor Authentication

When enabled for the Group Account, each user will be directed to their *ACCOUNT > Profile* page immediately or upon next login. Each user is required to setup the Multi-Factor Authentication (MFA) before access is granted to eMap (this includes access to the *Settings* page). There are two options for MFA setup. One or both can be configured for each user:

1. MFA Authenticator App
    - The user will need to download and setup an MFA Authenticator App (e.g. Microsoft Authenticator, Google Authenticator, or other) on a smartphone or tablet with camera access.
    a. In eMap, the *MFA Authenticator App* option will show Not Enabled by default, indicating verification setup is required.
    b. Click the configure icon to display a unique QR code and verification entry:

    c. Generate a verification code using your Authenticator App:
        i. If using e.g. Microsoft Authenticator on a smartphone, select the "Verified IDs" tab and then "Scan QR code". Scan the eMap on-screen QC code with the device camera, then select the Authenticator tab to view the current verification code. Enter the code into eMap and click Verify.
        ii. If using e.g. Google Authenticator on a smartphone, select "Add a code" and then "Scan a QR code". Scan the eMap on-screen QC code with the device camera to generate a response code. Enter the response code in eMap and click Verify.
    d. In eMap, the *MFA Authenticator App* option will show Enabled to confirm setup is complete.
    e. Clicking the delete icon will remove this MFA setup. If the user has no enabled MFA options and MFA is enabled at the group Account level, the user will be prompted to complete setup again before eMap access is granted.
2. MFA e-mail
    a. Click the configure icon to send an authentication code to the e-mail address that associated with the eMap user account.

    b. Enter the MFA code contained in the e-mail and click Verify.

c. In eMap, the *MFA e-mail* option will show Enabled to confirm setup is complete.

d. Clicking the delete icon will remove this MFA setup. If the user has no enabled MFA options and MFA is enabled at the group Account level, the user will be prompted to complete setup again before eMap access is granted.

3. Logging into eMap:
   a. Upon login the user will be presented with the MFA approval screen.
   b. If multiple MFA methods have been configured, select [Authenticator App] or [E-mail] from a list.
   - **Authenticator App:** Enter the MFA code currently displayed in the app on your smartphone or tablet, then click Verify MFA Code.
      i. **E-mail:** Click Send MFA Code, enter the code received and click Verify MFA Code.
   c. Selecting the Trust device checkbox will add the current device (computer) to a trusted list for 30 days. During this time, MFA will not be required for this User-Device combination when logging in to eMap.
4. MFA management
   a. If a user cannot use an existing MFA configuration to log in to eMap, another Level 2 user of the same Group Account can reset the affected user's MFA setup though the *ACCOUNT > Manage Users* menu.
   b. From the *Manage Users* table, click Edit on the affected user.
   c. Click Reset MFA, then read and confirm a warning message.
   d. The affected user will be prompted to setup MFA again, before access to the Group Account is granted.

Home Dashboard

Upon logging into eMap, Users will be presented with a dashboard (Figure 4) providing an overview of the following:

- **Alerts**: Important system messages.
- **Recent sequencing runs**: Expandable view of Sequencing Runs with Cycle IDs, Subject IDs and a quick view of individual sample status.
- **Recent cycles**: Expandable view of Cycles with Subject details and a quick view of individual sample status.
- **Referring centre statistics**: Summary plots of Signed-Off samples over time.

Return to the dashboard at any time by clicking on *HOME* in the top right-hand side of the browser.

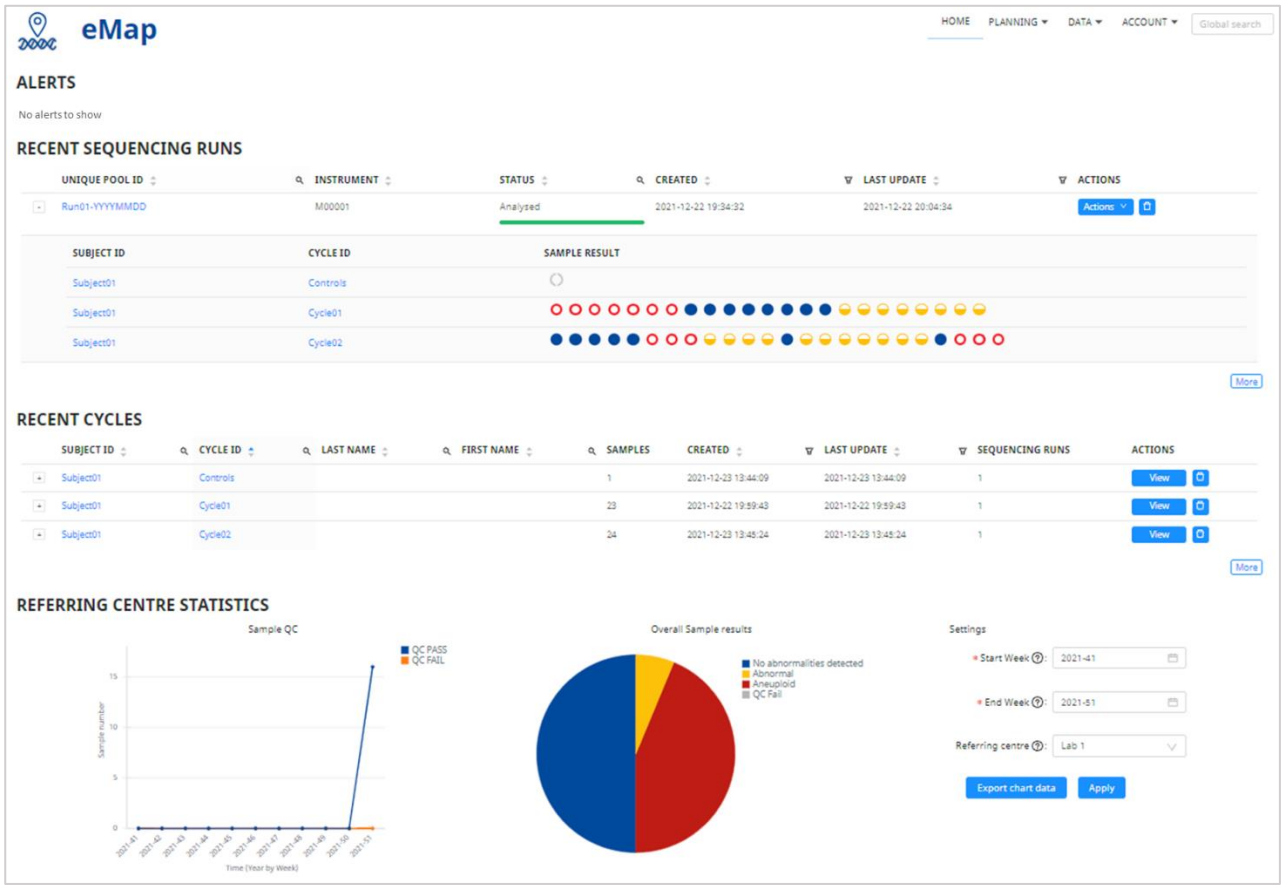


Figure 4: HOME Dashboard view

Recent Sequencing Run and Cycle Data

The *HOME* dashboard will display the five most recent *Sequencing runs* and *Cycles*.

1. Click on More underneath the tables to view the full lists of *Cycles* or *Sequencing Runs* respectively.
2. Use the column header tools to search, sort and filter results.
3. Expand a specific *Cycle* or *Sequencing Run* by clicking on the + icon, to show status icons for all samples associated with that Cycle ID or Pool ID.
4. See section *3 Uploading* Data and section *4 Analysis* for descriptions of data lists, status updates, icons and Actions.

Referring Centre Statistics

Two statistics charts are presented to show the sample throughput by CNV QC status per week (left) and the proportion of samples by copy-number status (right).

1. The charts show data for samples that have been Signed-Off for reporting. See *Sample Sign-off*.
2. Select a timeframe Start and End using the calendar tool. The week is displayed as <Year>-<Week Number>.
3. **Optional**. Select a Referring Centre to filter results. The list of Referring Centres is populated using entities made during Subject *Accessioning*.
4. Click Apply to save the settings and update the charts.
5. Click Export chart data to save a tab-delimited .tsv file containing the data required to re-draw the charts in other applications.

2 PLANNING

Planning for an EmbryoMap run is performed in three stages.

1. **Accessioning**: Add subject and sample details to the eMap system.
2. **Laboratory Planner**: Select a workflow and assign sequencing indexes for up to 96 samples, grouped by a unique Plate ID.
3. **Pool and Sequence**: Import library quantification values, then generate Pooling Guide(s) and SampleSheet(s) for sequencing.

Accessioning

Enter Sample Data Manually

The following sections describe actions on the *Accessioning* page, under the eMap *PLANNING* menu. A full list of existing Subjects can also be viewed under *Subjects* in the *DATA* menu; click View on a Subject row to navigate to the *Accessioning* page. For data fields, hover over the ⓘ icons to see a description for each field.

Add a New Subject

1. Enter a unique identifier in the Subject ID field.
2. **Optional**. Add Subject name, date of birth, Partner's name, consultant, referring centre and relevant notes.
   Note: *Data privacy obligations applicable to the account owner's organisation should be followed.*
3. Click Save at the bottom of the page to add the new Subject to the system.

Edit an Existing Subject

1. Begin typing the existing Subject ID into the Subject ID field, and the eMap software will suggest matching records. Complete the entry or select an ID from the list to load the Subject data.
2. Click on Edit beneath the Subject ID to enable editing of Subject information.
3. Click Save to save changes.

Add a New Cycle

1. Type a new identifier into the Cycle ID field and click Save.
   Saved Cycles are shown as clickable links under the heading *Available Cycles* e.g.:
   Available Cycles | Cycle01 | Cycle02 |

Add Samples to an Existing Cycle

1. Select the Cycle where samples are to be added.
2. Click Add Sample to add new rows to the Sample Information Table.
3. The default Sample Type for new sample rows can be pre-selected using the drop-down list.
4. Data fields that can be defined for each sample are shown in
5. Table **6**.
6. Click Save at the bottom of the page when all the information has been entered.

Table 6: Sample Information Table values

Value	Description
Tube ID	**Optional, recommended.** The short ID written on the sample tube. This value is displayed by default on the Laboratory Planner PDF to assist sample-to-index tracking during sample preparation.
Sample ID	**Required.** A unique identifier of the Sample, containing only alphanumeric characters or dashes [-]. Duplicate Sample IDs cannot be added to the same Plate during Laboratory Planning and must be unique within a Sequencing Run.
Barcode	**Optional.** A secondary unique identifier of the Sample. Barcodes must be unique within the eMap database.
Sample Type	**Required.** Input must match one of the following values: Trophectoderm, Genomic DNA, Other, Negative. Selecting 'Negative' will set the appropriate QC flags for this Sample as a control sample, where very few sequencing reads are expected.
Requisition	**Optional.** An identifier for the sample batch, such as a job requisition number or work order number.
Description	**Optional.** Free text field to add additional notes about a specific sample, e.g. embryo grade, biopsy notes or expected karyotype. **Note:** Use of the comma character [,] is not permitted.

Edit Sample Data

1. Click  under *Actions* in the corresponding sample row to modify sample data.
2. Sample records can be duplicated by clicking  (Clone).
   Cloning a Sample allows re-sequencing, by creating a new Sample entity that can be added to a new Plate and Pool. This ensures that the analysis result does not overwrite the original sample data. The cloned entry will be created with the suffix text "-RP*x*" (where *x* is the repeat number) added to Tube ID, Sample ID and Barcode fields. The repeat number will increment with additional cloning. The IDs can also be overridden manually by editing the Sample Information Table.
3. Click 

to save changes.

Delete Individual Sample Data

1. Click the  button under *Actions* to delete a sample row.

Note: Samples already selected for sample preparation or sequencing cannot be modified or deleted, without first deleting the Pool ID and Plate ID associated with that Sample.

Import Sample Data Using an Accessioning File

Subject, Cycle and Sample information can alternatively be entered into the system by importing from a predefined tab-delimited .txt or .tsv file. The import function is convenient for data already stored in a LIMS or similar system. See *Appendix 2* for rules concerning valid characters and required fields.

Import Data for a Single Subject

1. Use the template file *eMap_Accessioning_A.txt.*
2. Enter File and Subject information in the [Header] section.
3. Enter Cycle and Sample information in the [SampleTable] section.

4. Click Import Sample Data on the *Accessioning* or *Subjects* page and browse to the import file.

Import Data for Multiple Subjects

1. To import data from multiple Subjects, use the template file *eMap_Accessioning_B.txt*.
2. Enter File information in the [Header] section.
3. Enter Subject, Cycle and Sample information in the [SampleTable] section.
4. Ensure the Subject-level information is identical on all sample rows for each Subject. Data clashes will result in an import failure.
5. Click Import Sample Data on the *Accessioning* or *Subjects* page and browse to the import file.

Import Summary

1. Imported data is validated against existing Subject and Cycle records and the format requirements. A summary table is shown confirming if any Subject or Cycle matches have been found, and the number of samples to be added to each Subject-Cycle (Figure 5).
2. If no matches are found, a new Subject and Cycle(s) will be created using the information provided.
3. If importing data to existing Subjects (e.g. adding a new Cycle), the Subject data (names, D.O.B., consultant, referring centre and notes) must match the existing entries in the eMap software exactly, or be left blank.
4. Click Accept to confirm the import and add data to eMap. Click Cancel to stop the import and exit without adding data to eMap.

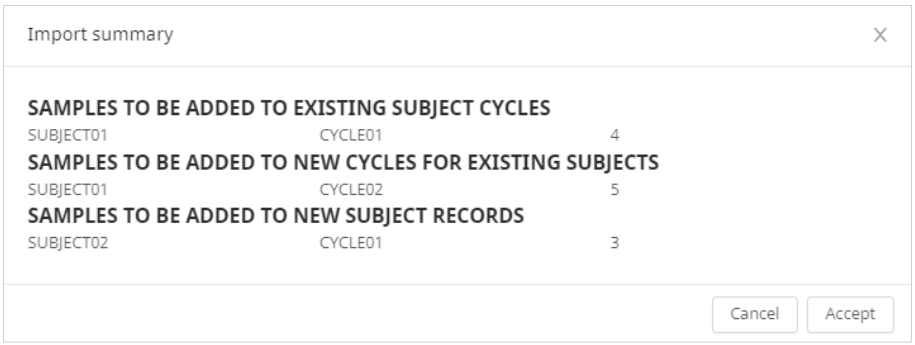


Figure 5: Sample Accessioning import dialogue

Laboratory Planner

To plan a Sample Preparation batch, accessioned samples must be assigned to a Plate ID using the Laboratory Planner tools. The assigned amplification-plate-well corresponds to the specific sequencing index name and maps to the reagent well of the Library Index Plate. For example, the sample added to amplification plate well A1, shall be configured to receive index reagent *UDI-A01* from well A1 of the Library Index Plate.
The output is a Laboratory Planner PDF which can be used for sample-to-index tracking during the Library Amplification step of the EmbryoMap protocol.

Select *Laboratory Planner* under the *PLANNING* menu to get started.

The *Available Plates* section displays a list of saved Plates in the system with the Plate ID, Preparation Date, EmbryoMap Sample Prep Kit Lot number and the User who created the Plate (Figure 6).

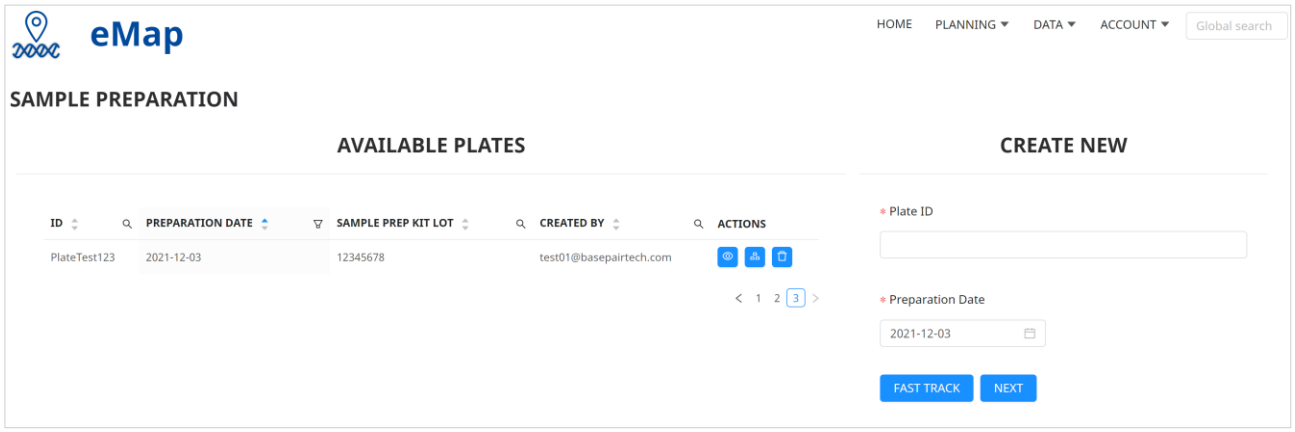


Figure 6: Laboratory Planning - Create new Plate

Create a New Plate - Plate Builder

1. Under *Create New,* enter a unique Plate ID and the Preparation Date as shown in Figure 6.
   The Plate ID must only contain alphanumeric characters or dashes [-].
2. Click NEXT to proceed.

On the *Plate Definition* page, under the section of *Available Samples* the data is arranged by Subject ID, Cycle ID and Sample ID. The list displays samples from accessioned Subject-Cycles that have not yet been assigned to a Plate (Figure **7**). Clicking on a Subject ID will expand the view to display the associated Cycles and then the Samples. The most recently accessioned samples are listed first. Alternatively, the search bar can be used to locate a specific Subject. If no samples are 'available' for a Subject, then the Subject-Cycle entry will no longer appear in the list.

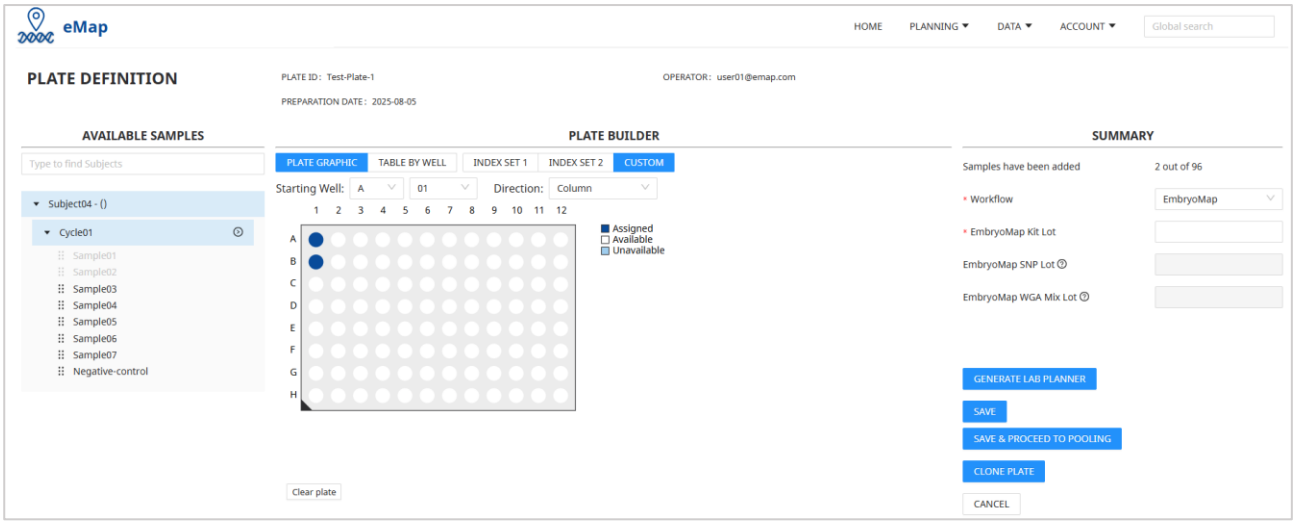
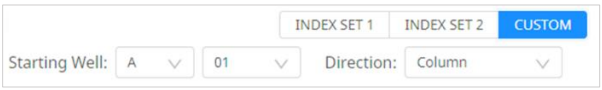


Figure 7: Plate Builder

3. Add each sample from the list of *Available Samples* to an empty well of the library plate diagram in the *Plate Builder* section. A minimum of 8 and a maximum of 96 samples can be added to each Plate. Assigned wells are shown with solid fill; hover the cursor over the well to reveal the Sample information.
    a. Individual samples can be added to specific wells using the drag-and-drop feature with the left-mouse button.
    b. The Cycle autofill button ⓘ will add all samples of the Cycle to the next available well positions in the Plate.
4. Use the Index Set selector to configure the function of the Autofill ⓘ feature:



    a. The default selection shall be *Custom* with the *Direction* (**Row or Column**) set according to the default setting in *ACCOUNT > Profile*.
    Note: The sample *Direction* will be saved to the Plate ID and used to configure the sample order in the sequencing SampleSheet file and user-interface table lists.
    b. *Custom* will also allow customisation of the autofill starting well and direction.
    c. *Index Set 1* will set the autofill to begin from well A1 in a **column-wise** order.
    d. *Index Set 2* will set the autofill to begin from well A7 in a **column-wise** order.
5. Click Clear plate to remove all sample assignments from the Plate.
6. Use *Table by Well* from the view selector PLATE GRAPHIC TABLE BY WELL to view additional sample accessioning information, or to specify a sample index assignment using the *Index ID* drop-down list. Directly changing an index selection in the table will automatically update the *Plate Graphic*.
7. When the required samples have been added to the plate, select the workflow for the plate from the **Workflow** dropdown.
8. Enter the EmbryoMap Sample Prep Kit Lot number and any additional Lot numbers required for the selected workflow.
    a. EmbryoMap SNP and EmbryoMap WGA Lot numbers are required if either of these workflows are selected.

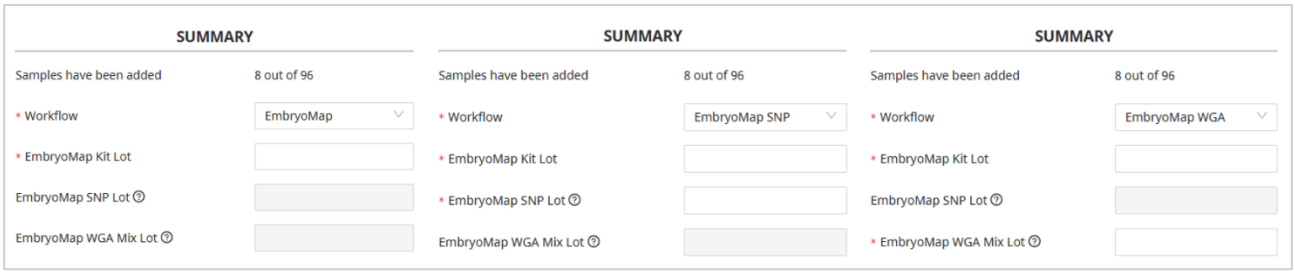
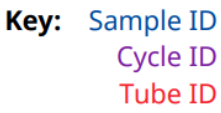
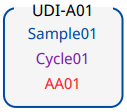


Figure 8: Workflow Selection

9. Select SAVE to save the index assignment for sample preparation.
10. Select GENERATE LAB PLANNER to produce a Laboratory Planner PDF for sample-to-index tracking during the Library Amplification step of the EmbryoMap protocol.
    In the wells of the plate graphic of the PDF guide, three rows of IDs can be displayed. Choose the types of IDs most appropriate for your laboratory on the *Settings* page.



Example Key selection defined in Settings



Example Laboratory Planner PDF Plate Graphic well

Changing a Plate Workflow - Plate Builder

The workflow assigned to a specific plate may be changed immediately after the plate has been created and saved, providing that the user has not exited the Plate Builder page.

1. Select a new workflow from the dropdown, add any additional Lot number information and save the plate again to update the assigned workflow.
    a. This will update the list of SampleSheet and Normalisation reference options available on the Pooling page.

If the user has exited the Plate Builder page (e.g., Proceeded to the Pooling Page) they will not be able to change the workflow assigned to a plate. Instead they will need to delete the existing plate and create a new one with the correct workflow.

1. Click the delete icon to open the plate deletion pop-up window.
2. If the new plate will created manually, follow the steps below:
    a. **Do not** check the box to delete all samples linked to the plate. This would require the samples to be re-accessioned.
    b. Click **Yes** to delete the plate.
3. If the new plate will created via Fast Track follow the steps below:
    a. **Do** check the box the delete all samples linked to the plate.
    b. **Click** Yes **to delete the plate.**
    c. Add the Subject and Sample information to the correct Fast Track file version for the desired workflow (see Create a New Plate – Fast Track).
    d. Import the new Fast Track file and confirm the correct workflow is selected before saving the plate.

Create a New Plate - Fast Track

The Fast Track import function allows sample *Accessioning* and *Laboratory Planning* steps to be performed using a **single import file**. This method is well suited to high-throughput Users or where data is already stored in a LIMS or similar

system. It may also be used as a tool to instruct eMap if a different system is configured to perform Laboratory Planning. See *Appendix 2* for rules concerning valid characters and required fields.

1. Prepare the import file using the tab-delimited *eMap_Fast_Track.txt* template.
    a. Enter File information in the [Header] section, including:
        i. File Version (EmbryoMap = 1, EmbryoMap WGA = 2, EmbryoMap SNP =3)
        ii. EmbryoMap Kit Lot
        iii. WGA Mix or SNP Kit Lot (Only for file version 2 or 3)
        iv. Unique Plate ID
        v. File Date (date that the Fast Track file was generated; YYYY-MM-DD)
        vi. Preparation Date (Date of sample preparation; YYYY-MM-DD)
    b. Enter the following required fields in the [SampleTable] section:
        i. Index ID with the format as it appears in the SampleSheet e.g. *UDI-A01*
        ii. Unique Subject ID
        iii. Cycle ID
        iv. Unique Sample ID
        v. **Recommended.** Sample Tube ID (ID written on the sample [biopsy] tube cap).
        vi. Sample Type (Trophectoderm, Genomic DNA, Other, Negative).
        vii. **Recommended**. Referring Centre (to allow reporting of centre statistics).
    c. Enter the following **optional** Accessioning fields.
        i. Unique sample barcode
        ii. Sample requisition / work order number
        iii. Sample description
        iv. Subject's First name and Last name
        v. Subject date of birth (YYYY-MM-DD)
        vi. Consultant
        vii. Notes
        viii. Subject Partner's First name
        ix. Subject Partner's Last name
    d. Depending on the Fast Track workflow, the final library quantification values (ng/µL) can also be entered at the time of data import – i.e. if run planning was performed using another application (e.g. LIMS).
    If the *Lib_Quant_ng_ul* field is left <empty>, the quantification values can be imported separately on the *Pool and Sequence* page in eMap.

    Note: Ensure all date fields are stored as text with the format YYYY-MM-DD. Opening a .txt file in Microsoft Excel may automatically update the date fields to an unsupported format to e.g. DD/MM/YYYY.

2. Ensure the Subject-level information is identical on all sample rows for each Subject. Data clashes will result in an import failure.
3. Click FAST TRACK and browse to the import file location.
4. Imported data is validated against existing Subject and Cycle records. A summary table is shown confirming if any Subject or Cycle matches have been found, and the number of samples to be added to each Subject-Cycle (Figure 8).

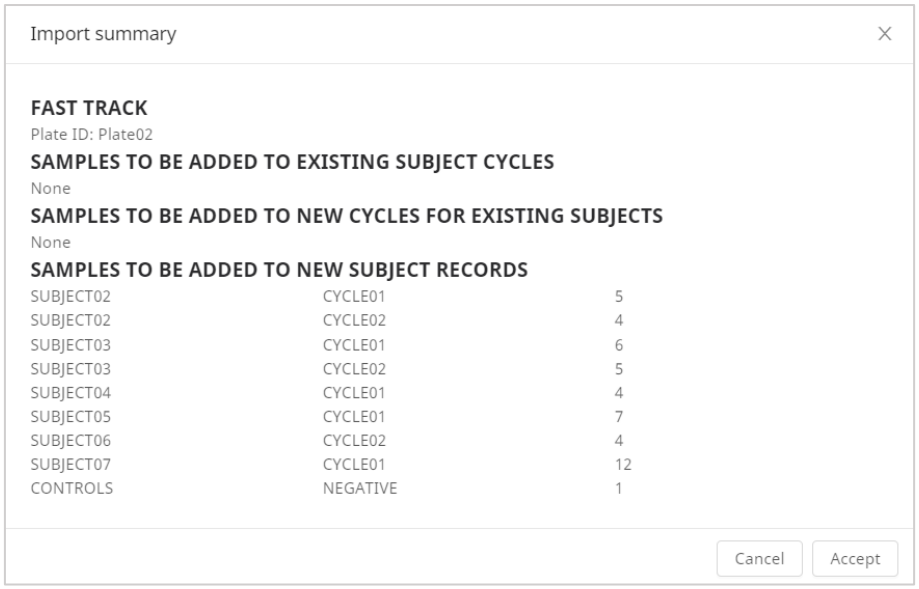


Figure 8: Fast Track import summary

5. Click Accept to add data to eMap and save the Plate definition. Click Cancel to stop the import and exit without adding data to eMap.
6. Review the *Plate Definition* page as described previously.
7. Add more samples using the *Plate Builder* method if required, then click SAVE to save new changes.
8. Select GENERATE LAB PLANNER to produce a Laboratory Planner PDF for sample-to-index tracking during the Library Amplification step of the EmbryoMap protocol.
9. Click SAVE & PROCEED TO POOLING to proceed directly to Pooling. If the quantification values were included in the Fast Track import file, they will be populated automatically.

Modify an Existing Plate

1. The Laboratory Planner PDF can be accessed by clicking the button under *Actions,* for *Available Plates* (Figure 6).
2. Click on an available plate to return to the Plate Builder and review planning information.
3. To delete a plate, click the button.
   A dialogue box will be displayed with a checkbox option to also delete all samples associated with the Plate. By selecting to delete Samples, any Cycle ID that no longer contains samples will also be deleted; and any subsequent Subject ID that no longer contains a Cycle ID will also be deleted.

Add Samples to an Existing Plate

When performing sample amplifications in smaller batches prior to sequencing, it is possible to add samples to a single Plate ID over time, which may represent multiple low-throughput sample preparation events. When a sufficient number of total samples is reached, the single Plate ID can be used to make sequencing Pool(s).

1. Select *Laboratory Planner* under the *PLANNING* menu.
2. Find the Plate in the *Available Plates* list and click the *Plate Builder* button .
3. Review the plate information and select ADD TO PLATE to make changes.

4. The software will display the *Plate Builder* page. The wells/indexes that already have samples assigned will be shown as 'unavailable' and cannot be edited.
5. The remaining available wells can be assigned to new samples using the methods described in the *Create a New Plate - Plate Builder* section.
6. Select SAVE and GENERATE LAB PLANNER to produce an updated Laboratory Planner PDF for use during sample preparation.

Proceed to Pooling

Once the *Plate Definition* has been finalised, select SAVE & PROCEED TO POOLING.

Re-Pool from an Existing Plate

To create new pool configurations for an existing Plate ID (e.g. to use a different pooling mass specification, or to select a different combination of samples from the same plate), use the *Clone Plate* feature.

1. Select *Laboratory Planner* under the *PLANNING* menu.
2. Find the Plate in the *Available Plates* list and click the *Plate Builder* button .
3. Click CLONE THIS PLATE to duplicate the Plate and Sample definitions in the system.
   The cloned ID values for Plate ID, Sample ID (including additional Tube ID, Sample ID and Barcode fields) will be created with the suffix text "-RPx" (where x is the repeat number). The repeat number will increment with additional cloning.
4. Review the *Plate Definition* and then select SAVE & PROCEED TO POOLING.
5. Follow the steps in the following section to create new Pool(s) for sequencing.

Pool and Sequence

There are two navigation routes to the *Run Definition* page in eMap:

1. Select SAVE & PROCEED TO POOLING after creating a new plate, on the *Plate Definition* page.
2. Select *Pool and Sequence* under the *PLANNING* menu, double click on the Plate ID in the *Available Plates* list, and then select PROCEED TO POOLING.

The page will display a sample summary, allowing review of sample details as a *Plate Graphic* or *Table by Well* using the toggle selector PLATE GRAPHIC | TABLE BY WELL (

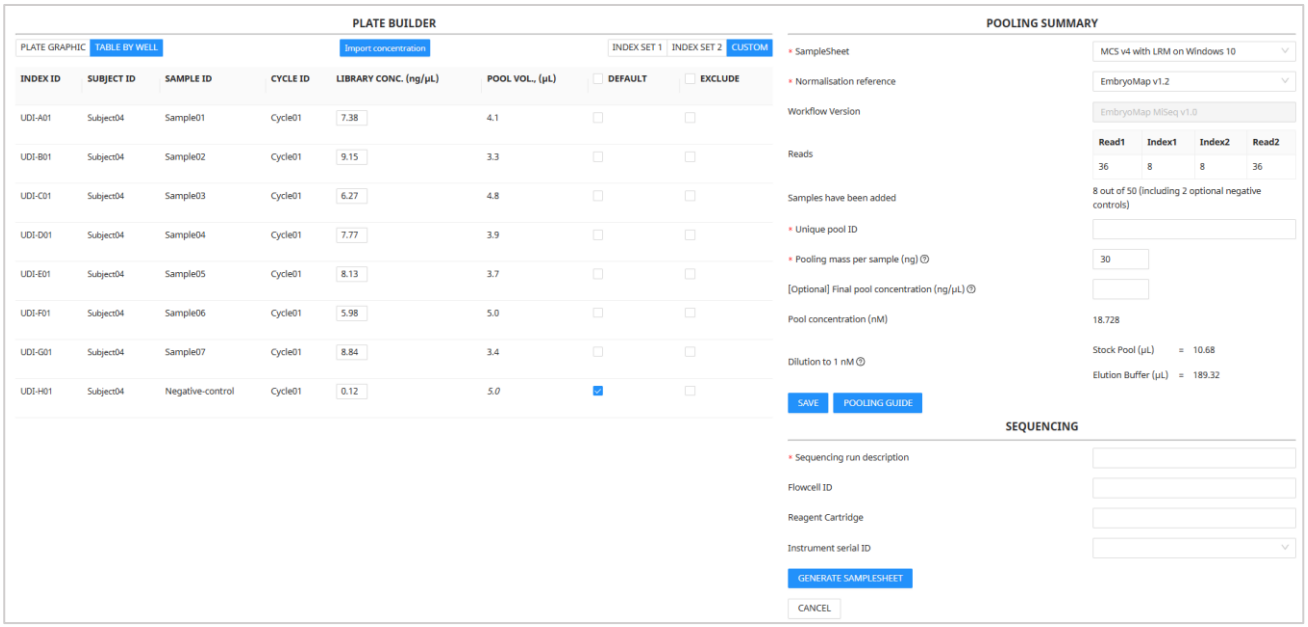


Figure **9**).

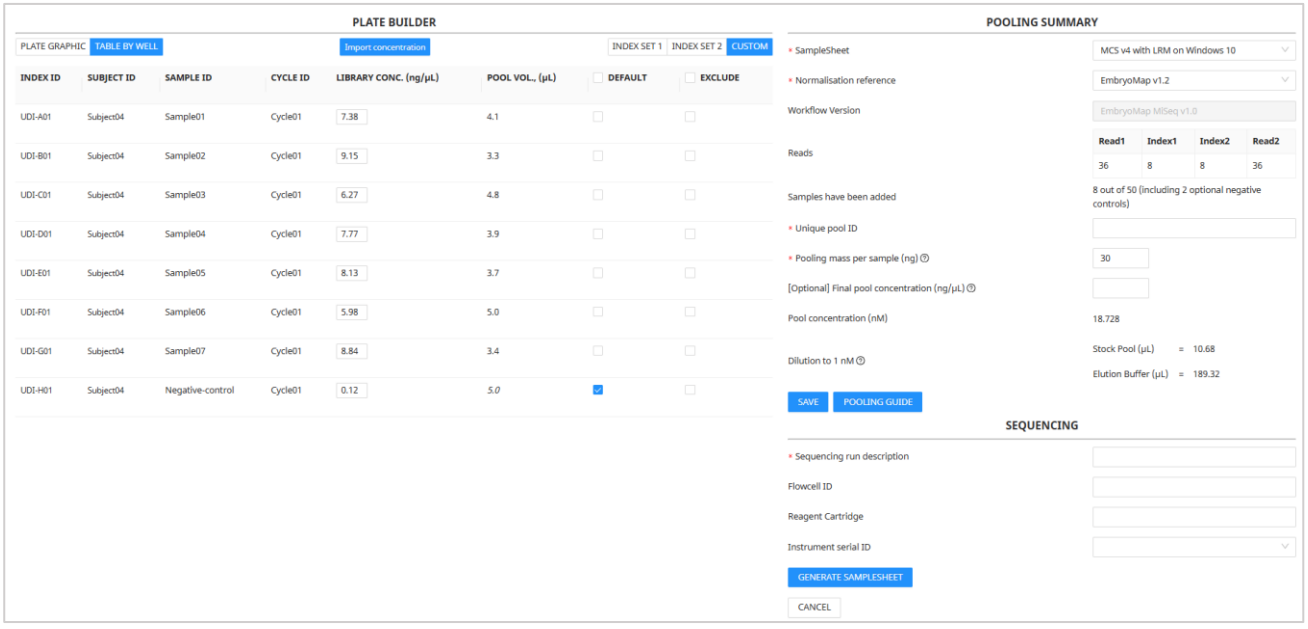


Figure 9: Run Definition page

Add Sample Quantification Data

There are three options to add sample quantification data to eMap:

1. Manually enter the concentration values in the *Library Conc. (ng/µL)* column on the *Table by Well* view.
2. Use the tab-delimited *eMap_QuantData.txt* text file to import concentrations (ng/µL) by index name using the Import concentration button. See the Vitrolife support material for the *eMap_QuantData.txt* template.
3. Specify the concentration values in the *eMap_FastTrack.txt* import file at the time of Laboratory Planning. This option is recommended for Users that use an alternate application (e.g. a LIMS) for planning the sample preparation steps, and only import data via *Fast Track* once they are ready to define a sequencing Pool.

SampleSheet and Normalisation reference selection

1. In the *Pooling Summary* section, select the appropriate *SampleSheet* for your Sequencing instrument and Windows OS version, using the SampleSheet dropdown menu. The selection is pre-set to the "Default SampleSheet Type" selection on the *Settings* page.
    a. Only SampleSheets compatible with the selected workflow will be available.
    b. The *Workflow Version* and *Reads* sections are read-only fields that will be populated automatically based on the Workflow and *SampleSheet* options selected. These capture details regarding the *Workflow, SampleSheet,* and *Sequencing Run Type* in a single field.
        i. Standard EmbryoMap sequencing runs are categorised by v1.0 while EmbryoMap SNP runs are categorised as v2.0
2. Select a *Normalisation reference* from the dropdown from the list available. This will instruct the normalisation reference to be used for analysis when the data is uploaded. The selection of normalisations available is filtered based on the selected *Workflow* and *SampleSheet*.

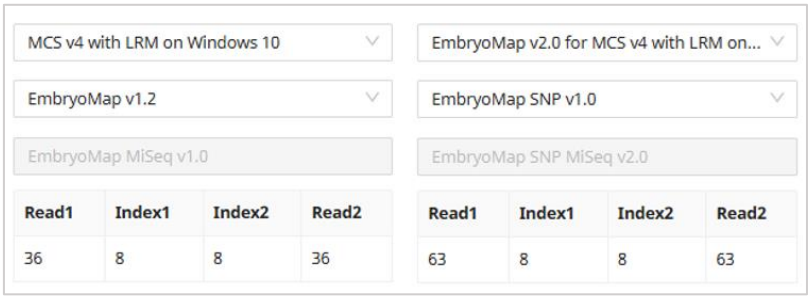


Figure 10: Example SampleSheet and Normalisation selections

Pooling with normalisation

Users are required to pool a minimum of 8 positively amplified library samples for a single run. Run multiplexing limits are determined by the number of positively amplified libraries in the pool. However, additional negative control samples can be included beyond the multiplexing limit if the UDI barcodes are unique to the pool. This is because negative (no template) control samples are expected to have very few or no sequencing reads. Follow on-screen instructions for guidance.

Pooling with library normalisation by DNA mass is the most effective way to ensure that each sample receives a sufficient number of sequencing reads for copy-number analysis, particularly when running at the maximum flowcell throughput.

1. Enter a *Unique Pool ID* that will be used in eMap to group the analysis results by Run.
2. Enter the *Pooling mass per sample (ng)*. The default value is defined in the eMap *Settings* page.
3. The selected pooling mass value is used to automatically calculate the volume of each sample to add to the pool to achieve sample normalisation.

INDEX ID	SUBJECT ID	SAMPLE ID	CYCLE ID	LIBRARY CONC. (ng/µL)	POOL VOL., (µL)	DEFAULT	EXCLUDE
UDI-A01	Subject01	Subject01-01A-1	01A	4.76	6.3		

Note: Select a pooling mass value to ensure a pooling volume of 2-20 µL per sample. A minimum of 2 µL per sample reduces normalisation variability when pipetting small volumes, and a maximum of 20 µL ensures each library may be pooled twice (out of a total final library volume of 45 µL), in case of sample repeats.

4. Sample rows with pooling volumes outside the 2-20 µL range will be flagged with orange shading as a warning, however the User may still proceed.

INDEX ID	SUBJECT ID	SAMPLE ID	CYCLE ID	LIBRARY CONC. (ng/µL)	POOL VOL., (µL)	DEFAULT	EXCLUDE
UDI-A01	Subject01	Subject01-01A-1	01A	2.2	ⓘ22.7	☐	☐

5. Sample rows with pooling volume >40 µL will be flagged with red shading, where there is insufficient volume in the sample well to achieve the pooling mass; it will not be possible to save the pool definition in this state. Either change the pooling mass to a lower value or select the *Default* or *Exclude* checkbox for the sample row.

INDEX ID	SUBJECT ID	SAMPLE ID	CYCLE ID	LIBRARY CONC. (ng/µL)	POOL VOL., (µL)	DEFAULT	EXCLUDE
UDI-A01	Subject01	Subject01-01A-1	01A	0.2	ⓘ250.0	☐	☐

The *Default* ☑ checkbox will flag the sample for inclusion in the Pool but will set the pooling volume to a nominal value of 5.0 µL. Select the *Default* checkbox to include negative controls or failed amplification samples in the pool for QC purposes. The pool concentration will be updated automatically.

INDEX ID	SUBJECT ID	SAMPLE ID	CYCLE ID	LIBRARY CONC. (ng/µL)	POOL VOL., (µL)	DEFAULT	EXCLUDE
UDI-A01	Subject01	Subject01-01A-1	01A	0.2	*5.0*	☑	☐

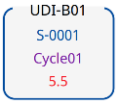
6. Alternatively, select the *Exclude* ☑ checkbox to remove the sample from the Pool. The sample will not be listed in the SampleSheet and no pooling volume will be calculated for the pooling event.

INDEX ID	SUBJECT ID	SAMPLE ID	CYCLE ID	LIBRARY CONC. (ng/µL)	POOL VOL., (µL)	DEFAULT	EXCLUDE
UDI-A01	Subject01	Subject01-01A-1	01A	0.2		☐	☑

7. Once samples have been selected for pooling, and an appropriate pooling mass has been entered, the stock library pool concentration (nM) and dilution instructions of the stock pool to 1 nM are calculated automatically.
8. Select SAVE to confirm the Pool definition.
9. Click POOLING GUIDE to download a PDF table and plate graphic containing the sample volumes for pooling, the final pool concentration, and the instructions to make the 1 nM dilution.
   In the wells of the PDF plate graphic, two rows of IDs can be displayed in addition to the pooling volume (µL). Choose the most appropriate IDs from the *Settings* page. Key 1 and 2 are shared with the Laboratory Planner PDF as described previously.

Key: Sample ID
Cycle ID
Pool Vol. (µL)

Example Key selection defined in Settings



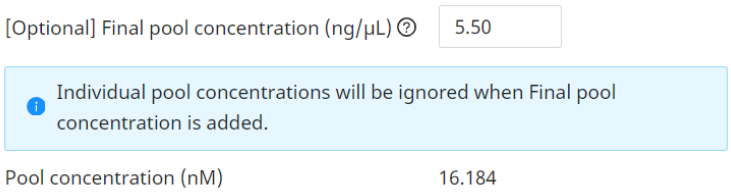
Example Pooling Guide PDF Plate Graphic well

Pooling without normalisation

Pooling by combining equal volumes of each sample, without normalisation, is also supported by eMap Software. Caution should be taken to ensure that individual sample concentrations have an acceptable variance, in accordance with the planned sequencing throughput to achieve a sufficient number of reads per sample for analysis. Contact Vitrolife Genomics Support for guidance.

To inform library dilution and denaturing, the final pool should be quantified and the *Final pool concentration (ng/ul)*, entered directly into the eMap Software.

Alternatively this feature can be used for any occasion when the Final Pool is re-quantified (e.g. after extended storage), to inform accurate loading of the sequencing instrument.



1. In eMap, select the *Default* checkbox for all samples to be included in the pool.
2. Pool 5ul of each selected sample and quantify the final pool concentration (ng/ul) according to the EmbryoMap Sample Prep Guide.
3. Enter the concentration of the pool in the *[Optional] Final pool concentration (ng/ul)* box. eMap will now use this value to calculate the *Pool concentration (nM)*, and the subsequent dilution steps to 1nM.

   Note: If an *[Optional] Final pool concentration (ng/ul)* value is entered, the pooling mass (ng) setting shall be greyed-out, and eMap will ignore any individual library concentrations (ng/ul) when calculating the Pool concentration (nM)

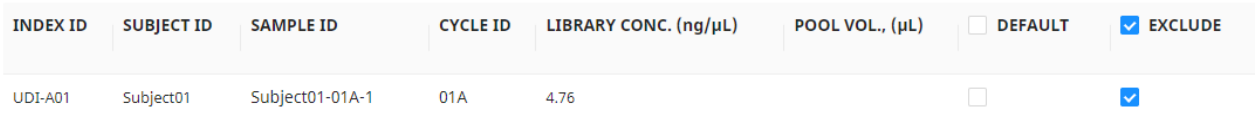
4. The *POOL VOL., (uL)* column in the *Plate Builder* will reflect the 5uL pooling volume per sample.
5. Samples can still be excluded from the Final Pool by checking the *Exclude* checkbox.

Index Set Selection

For Plate IDs containing 96 prepared Final Libraries, two separate 48-plex pools for index set 1 (samples in wells A01 to H06) and Index set 2 (samples from wells A07 to H12) can be defined for sequencing. When defining the first Pool, the *Exclude* checkbox is used to exclude the samples that will constitute the subsequent pool(s).

There are two methods to exclude samples from a *Pool Definition,* using the selector toggle:

1. In *Custom* mode, click the *Exclude* checkbox for each sample as required.
   Alternatively, click the *Exclude* in the table header to exclude all samples, then deselect the samples to include in the pool.



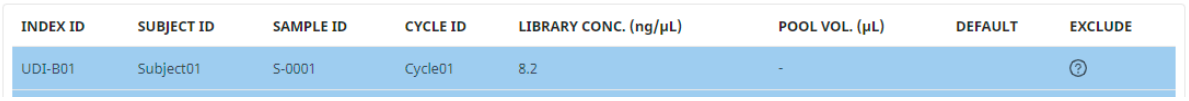
2. Use either of the two *Index Set* buttons to quickly select 48 samples for pooling in high-throughput workflows.
   a. Select *Index Set 1* to automatically exclude any sample with an index from Plate column 7-12.
   b. Select *Index Set 2* to automatically exclude any sample with an index from Plate column 1- 6.

Create Multiple Pools from a Single Plate ID

A plate may contain up to 96 prepared Final Libraries. Users will have the option to create multiple pools which can be defined from a single Plate ID.

1. Prepare the first Pool definition as per the previous instructions.
2. After clicking SAVE, any samples that are assigned to a Pool ID will become locked.
3. To create a new pool, select *Pool and Sequence* under the eMap *PLANNING* menu, double click on the Plate ID in the *Available Plates* list, and then select PROCEED TO POOLING.

4. Locked samples that are already assigned to another Pool or Sequencing run, will be displayed as read-only. Hover the cursor over the ⓘ for a description.

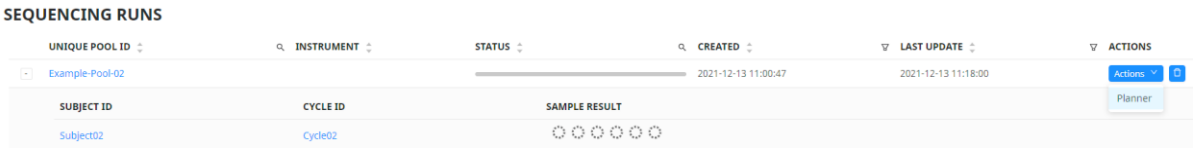


INDEX ID	SUBJECT ID	SAMPLE ID	CYCLE ID	LIBRARY CONC. (ng/µL)	POOL VOL. (µL)	DEFAULT	EXCLUDE
UDI-B01	Subject01	S-0001	Cycle01	8.2	-		ⓘ

5. Define a new Pool using the available samples in the table.
6. Deleting a pool definition or planned sequencing run from the *Sequencing Runs* list under the eMap *DATA* menu will unlock samples for assignment to a new Pool definition.
7. See sections for *Edit Sample Data* and *Re-Pool from an Existing Plate* for details on cloning a Sample entity in eMap for re-sequencing. Briefly, a unique Sample ID in eMap can only be assigned to one Plate ID and one Pool ID to ensure data integrity and prevent overwriting of result files.

Generate SampleSheet

1. To start sequencing, continue directly after defining a Pool, or navigate back to a saved Pool by clicking *Planner* under the Actions menu in the Sequencing Runs list:



2. Enter a sequencing run description and select the sequencing instrument serial number that will be used for sequencing.
   Serial numbers of new sequencing instruments can be added through the *Settings* page.
   The run information can be reviewed on the instrument screen prior to sequencing as a final check, to aid run planning.
3. **Optional.** The *Flow Cell ID* and *Reagent Cartridge ID* can also be entered for planning purposes.
   These values are automatically updated upon data import from the sequencing result files, as a record of the sequencing event.
4. Click GENERATE SAMPLESHEET to open a *Workflow Confirmation* window. This will display the *Plate ID, SampleSheet*, *Workflow Version,* and *Normalisation* information in a read-only display.
5. Click GENERATE SAMPLESHEET in the *Workflow Confirmation* window to download the <Pool ID>_SampleSheet.csv and finalise the sequencing run set-up within the eMap software.
6. Transfer the SampleSheet file to the Sequencer computer or download it directly by accessing eMap from the Sequencer computer itself and using the Actions menu from the Sequencing Runs list.
7. Do not modify the SampleSheet file. The SampleSheet contains a checksum that ensures the data entered into the system is not changed during processing. Editing any part of the SampleSheet contents will invalidate the data import into eMap.
8. The sequencing run shall now be displayed on the HOME page and under *DATA* > *Sequencing Runs* with the status “Waiting”.

3 UPLOADING DATA

Once sequencing and secondary analysis are completed on-board the sequencing instrument, the data output files required for analysis are automatically uploaded to the Group's eMap account. However, manual data upload can be initiated if required. The *eMap-upload* software is a Microsoft Windows™ application designed to operate on the Sequencer computer, however any Windows machine connected to the internet and with access to the sequencing run folder may be configured for uploading. Installation instructions are provided in *Appendix 4*.

Automatic Upload

If automatic upload is configured, the following steps are performed automatically to upload your data to eMap:

1. The *emap-monitor.xml* will monitor the designated sequencer output folder for completed EmbryoMap runs.
2. Once a completed run is detected, the *emap-upload.exe* software will upload the data to eMap using the account Access Key found in the *upload-config.json*.

Manual Upload

If automatic upload is not configured for the sequencing instrument, or the automatic upload did not complete successfully then a manual upload will be required.

1. Open the *eMap-upload-interface.exe* software and use the Browse button select the Run Folder to be manually uploaded to eMap for analysis (Figure 10).
2. For the default use no further setup is required; press Upload and monitor the *Log* area for success or failure messages. An *emap_upload.log* file will also be saved in the same location as the eMap-upload-interface.exe software.
3. Further settings are defined in the *Advanced Configurations* section, which can be expanded by clicking ▲. Note that the absolute paths will require updating if not using system defaults. Details of each setting are described in Table 7.

Table 7: eMap-upload-interface.exe Advanced Configurations

Action	Description
Alignment Folder	Relative path to the sub-folder containing the BAM files.
eMap Configuration	Absolute path to the JSON file with connection credentials.
eMap Upload Software	Absolute path to the eMap-upload.exe software.
Overwrite	Force the upload of already uploaded files.
Ext. import	Flag the data as an import if the samples are not yet defined in the eMap system. The upload software will create the Run and the Sample entries, but manual linking of the Samples to specific (new or existing) Subjects and Cycles in eMap will be required.
Retry count	The number of times the upload software should try to upload the files in case there are problems (e.g. the internet connection being lost).
Retry interval [sec]	In case the upload software must retry the upload multiple times, it can wait this number of seconds between each re-try.
Clear cache	Select this option when using a new *upload-config.json* file for the first time.

4. During the upload, the window may be unresponsive, show a spinning wheel or display “Not Responding” status. The speed of the upload is dependent on the internet connection; a typical 48-plex run should complete in < 30 minutes. The *Log* window will populate as the data upload progresses.
5. Click Quit to close the application.
   Any changes to the *Advanced Configuration* settings will be saved to *emap_upload.json* in the same location, and restored upon next use.
   Clicking the Windows close button in the top right-hand corner will not save changes and settings will revert to the default values upon next use.

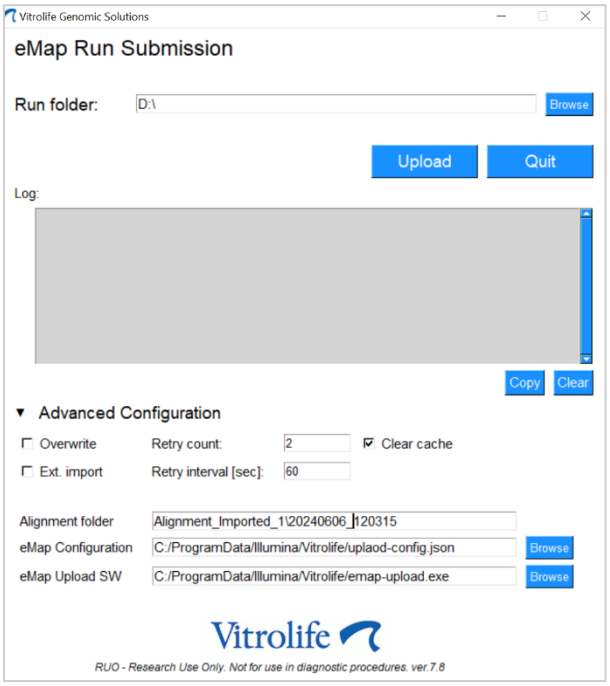


Figure 10: eMap-upload-interface.exe User Interface

Automatic Data Processing

For runs that have been planned in eMap Software, data processing is triggered automatically following a successful automatic or manual upload. For runs that were not planned in eMap Software, see the section on *Custom Import* for guidance. Automatic data processing uses the following steps:

1. Upon successful upload, the analysis is automatically initiated using the parameters defined in the current Group *Settings* and the Normalisation selected on the *Pooling Page*.
2. The sample data is automatically linked to the *Run Definition* that was created in eMap software during the Laboratory Planning steps. Data integrity is maintained by the checksum within the SampleSheet file.
3. The run status will be displayed and updated accordingly (as in **Error! Reference source not found.**).
4. **Optional**. E-mail notifications will be sent to Users of the group indicating the status of the analysis. See *ACCOUNT* > *Profile* to enable this feature.

Table 8: Sequencing Run status types.

Sequencing run status	Identifier	Description
Waiting	Waiting	The run has been set up and can be sequenced using the generated SampleSheet. eMap is waiting for sequencing data to be uploaded.
Importing	Importing	The sequencing data files are uploading.
Processing	Processing	Copy-number analysis in progress.
Analysed	Analysed	Copy-number analysis completed for all samples and data is ready for review.
Analysed with failures	Analysed with failures	≥1 sample did not complete analysis, check for missing data.
Error	Error	Processing not initiated. See also, *Custom Imports.*
Incorrect workflow detected	Incorrect workflow detected	Processing not initiated due to a mismatch between the data uploaded to eMap and the default normalisation during a *Custom Import*. This analysis will need to be re-queued with a compatible normalisation. See also, *Re-analysis of multiple samples in a run.*

Custom Import

To analyse and review data not planned in eMap, the eMap upload software allows runs to be imported and the samples manually linked to Subject-Cycles afterwards.

This path can be used in unlikely cases when eMap cannot be accessed to generate a SampleSheet to start a sequencing run (e.g. due to a network error). A SampleSheet may be prepared offline, which does not contain a valid checksum, and therefore uploaded samples must be manually linked to Subject-Cycle(s) in eMap prior to data processing. The fully automated path should always be preferred, because it requires no User interaction to upload and mitigates the risk of sample mix-up once data is received by eMap.

1. Contact Vitrolife Support if you cannot generate a SampleSheet to start a sequencing run. Vitrolife can assist in generating an offline SampleSheet that is compatible with eMap software.
2. Once the run has completed, use the *eMap-upload-interface.exe* software to manually upload the data. Under *Advanced Configurations*, select “Ext. import”.
3. A full list of Custom Import runs can be viewed by navigating to *DATA > Custom Imports*.
4. In eMap, custom import runs are initially shown with the red Error status. If this is the expected state of your run data, press the Bypass button on the right side of the run list.
5. A summary message is shown. Click Proceed to bypass the error, this will trigger an the analysis using the default settings in the account. Send action details to Vitrolife Support for assistance. Only the information displayed on screen is shared (Figure 11). Alternatively, click Cancel to exit. Data will not be visible until the run import error is bypassed.

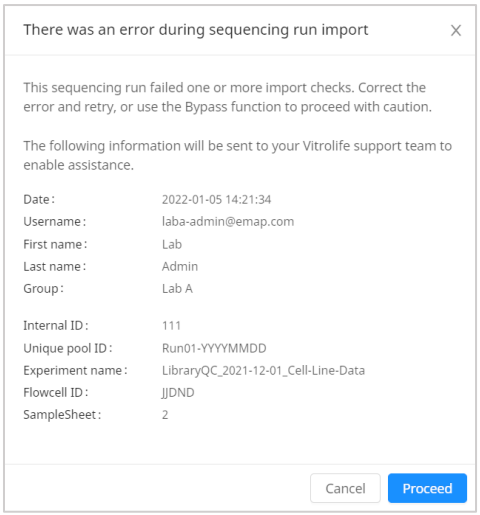


Figure 11: Custom Import bypass summary

6. Ensure the required Subject IDs have already been accessioned into the eMap software. Cycle ID information can be created later.
7. To review the imported samples, click on *Link Samples to Subjects*, in the Actions menu of the Run list. Note that this menu item is only available if the run is a Custom Import.
8. All the samples in the run are summarised in a table. Use the checkbox to select individual samples to be assigned to a specific Subject-Cycle. Clicking the checkbox in the table header will select/deselect all the samples.

SAMPLE ID	INDEX	WELL	CREATED	LAST UPDATE	SUBJECT ID	CYCLE ID
A-082	UDI-B01	B01	2021-12-14 09:14:02	2021-12-14 09:14:02	No Subject	No Cycle

9. Click Link to Subject and follow the on-screen prompts:
    a. Search and select an existing Subject
    b. Select an existing Cycle in the dop-down list, or;
    c. Type in a new Cycle ID and press enter/return to create a new Cycle for the selected Subject.
10. Click SAVE to create the links, then repeat the process for the remaining Subject-Cycles in the run.
11. The run status will display as Processing, and then update to reflect the status of the analysis once completed.

4 ANALYSIS

Results Overview

Sequencing Run QC

Basic run metrics generated on the sequencing instrument can be assessed on the *QC Metrics* page (

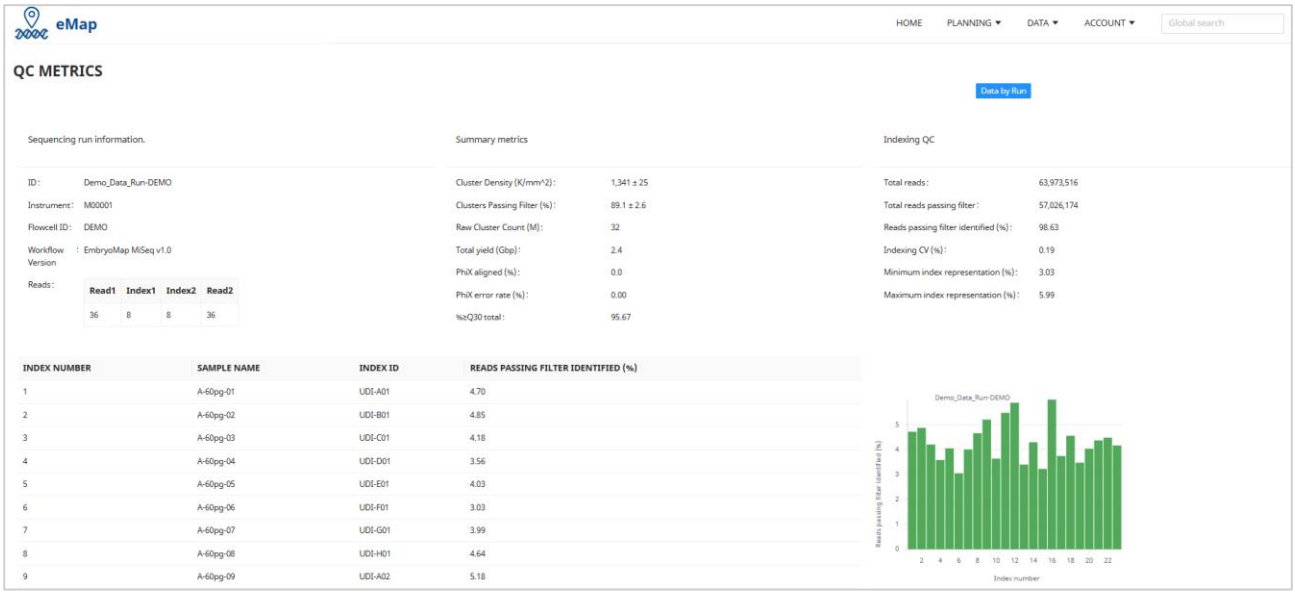


Figure **12**).

1. Click *Primary Run QC* under the Actions menu on any Run list (See below), or;
2. Click QC Metrics on the *Run View* page (See below).

The data is read from the sequencing result files upon import and provides summary information in a format similar to the *Sequencing Analysis Viewer* application (Illumina Inc.). If the metrics data was unavailable during the upload, then this page will remain empty.

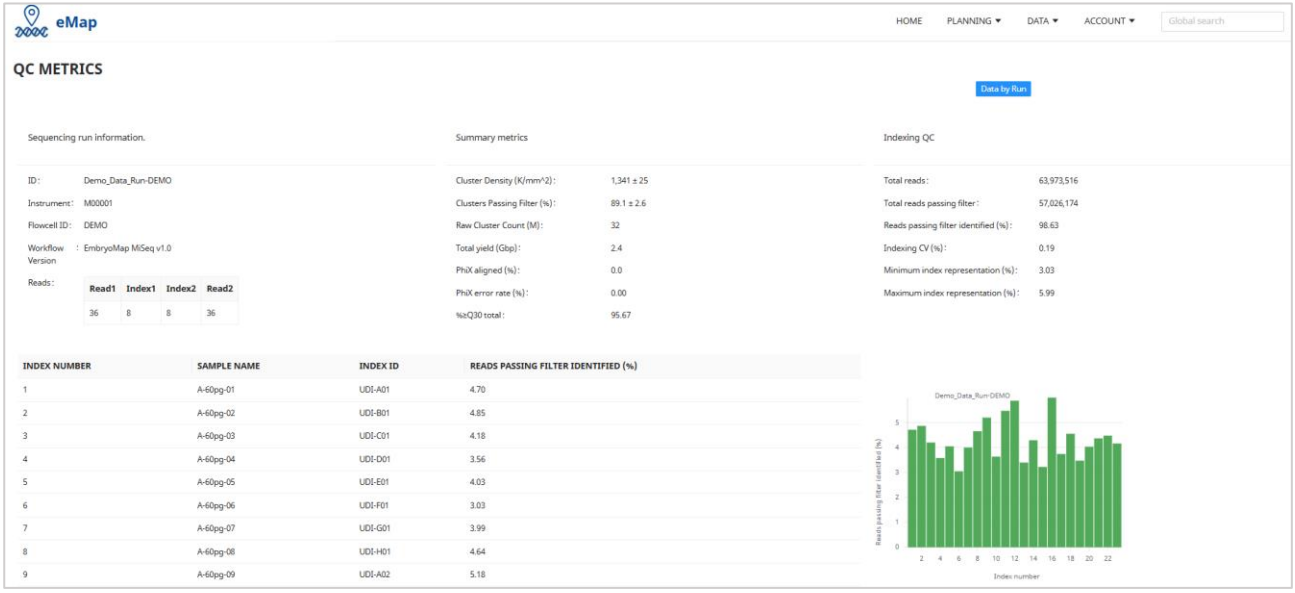


Figure 12: Sequencing Run QC metrics page

Data Lists

Results can be reviewed Pool ID or by Cycle ID. Results from the last five datasets can be seen on the *HOME* dashboard page or as complete lists from the *DATA* > *Cycles*, and *DATA* > *Sequencing Runs* menus.

1. On the *HOME* page, click More underneath the list to see more entries, then navigate with < 1 2 >.
2. Use the column header tools to search, sort and filter results.
3. Expand a list using the + button next to the respective Cycle ID or Pool ID.
4. The icons representing the samples highlight the outcome of the data analysis.
    a. In the Sequencing Runs list, Samples are divided by Subject-Cycle.
    b. In the Cycles list, Samples are viewed in the context of “This Cycle” and “Other Cycles”, so that the most relevant data is shown.

Sample icons are described in Table 9 for EmbryoMap and in Table 12 for EmbryoMap SNP workflows.

Table 9: eMap sample status icons

Icon	CNV Status	Description
	Waiting	Data for the sample has not yet been received.
	No abnormalities detected	The sample was analysed and the chromosome copy-numbers were found to be as expected.
	Abnormal, unknown significance	The sample was analysed and only intermediate copy-number and/or sub-chromosomal imbalance(s) were detected.
	Aneuploid	The sample was analysed and a whole-chromosome, full copy-number imbalance was detected.
	Negative control sample	The sample type was defined as *Negative*. The QC status will be "PASS" if the read number is low.
	No abnormalities detected with QC FAIL	The sample was analysed, but at least one QC criteria was not met.
	Abnormal, unknown significance with QC FAIL	The sample was analysed, but at least one QC criteria was not met.
	Aneuploid with QC FAIL	The sample was analysed, but at least one QC criteria was not met.
	Negative control sample with QC FAIL	The sample was analysed, but at least one QC criteria was not met.
	Sample analysis failure	The sample could not be analysed for copy-number. If the Run status is "Analysed", check for sample amplification failure or pooling errors. If the Run status is "Analysed with failures", check for BAM file data integrity.

Table 102: eMap SNP sample status icon

Icon	CNV Result	SNP Result	Description
	No abnormalities detected	Diploid	The sample was analysed, the chromosome copy-numbers were found to be as expected, and diploid is the predicted ploidy status.
	No abnormalities detected	NA (Hide Ploidy Status Enabled)	The sample was analysed, the chromosome copy-numbers were found to be as expected, and the sample ploidy status has been hidden.
	Abnormal, unknown significance	Diploid	The sample was analysed and only intermediate copy-number and/or sub-chromosomal imbalance(s) were detected, and diploid is the predicted ploidy status.
	Abnormal, unknown significance	NA (Hide Ploidy Status Enabled)	The sample was analysed and only intermediate copy-number and/or sub-chromosomal imbalance(s) were detected, and the sample ploidy status has been hidden.
	Aneuploid	Diploid	The sample was analysed and a whole-chromosome, full copy-number imbalance was detected, and diploid is the predicted ploidy state.
	Aneuploid	NA (Hide Ploidy Status Enabled)	The sample was analysed and a whole-chromosome, full copy-number imbalance was detected, and the sample ploidy status has been hidden.
	Any CNV result with CNV QC PASS	Triploid/Haploid	The sample was analysed, the CNV QC is “PASS” and has returned a result, and the predicted ploidy state is either triploid or haploid.
	Negative control sample		The sample type was defined as *Negative*. The QC status will be “PASS” if the read number is low.
	No abnormalities detected with CNV QC FAIL	SNP QC FAIL	The sample was analysed, the chromosome copy-numbers were found to be as expected, but the CNV QC and/or SNP QC results were “FAIL”.
	CNV QC FAIL	Diploid	The sample was analysed, diploid was the predicted ploidy status, but the CNV QC result was “FAIL.
	Abnormal, unknown significance with CNV QC FAIL	SNP QC FAIL	The sample was analysed and only intermediate copy-number and/or sub-chromosomal imbalance(s) were detected, but the CNV QC and/or SNP QC results were “FAIL”.
	Aneuploid with CNV QC FAIL	SNP QC FAIL	The sample was analysed and a whole-chromosome, full copy-number imbalance was detected, but the CNV QC and/or SNP QC results were “FAIL”.
	CNV QC FAIL	Triploid/Haploid	The sample was analysed, triploid/haploid was the predicted ploidy status, but the CNV QC result was “FAIL”.
	Negative control sample with QC FAIL		The sample was analysed, but the CNV QC and/or SNP QC result was “FAIL”.
	Sample analysis failure		The sample could not be analysed. If the Run status is “Analysed”, check for sample amplification failure or pooling errors. If the Run status is “Analysed with failures”, check for BAM file data integrity.

5. Hover the cursor over the sample icon to display the Sample ID and written status.
6. Click on the sample icon to open the *Sample View* page showing the copy-number chart for this Sample.
7. Click on the Pool ID to open the *Run View*.

8. Click on the Cycle ID to open the *Cycle View*.
9. Click on the Subject ID to open the *Accessioning* page and load information for this Subject.

Sequencing Run Lists

Each Sequencing Run has an Actions menu and a button (Figure 13). Click and confirm to delete a Pool ID or Sequencing Run and all associated result data from the system. An additional checkbox option allows further deletion of the Plate and all sample definitions associated with the Run.

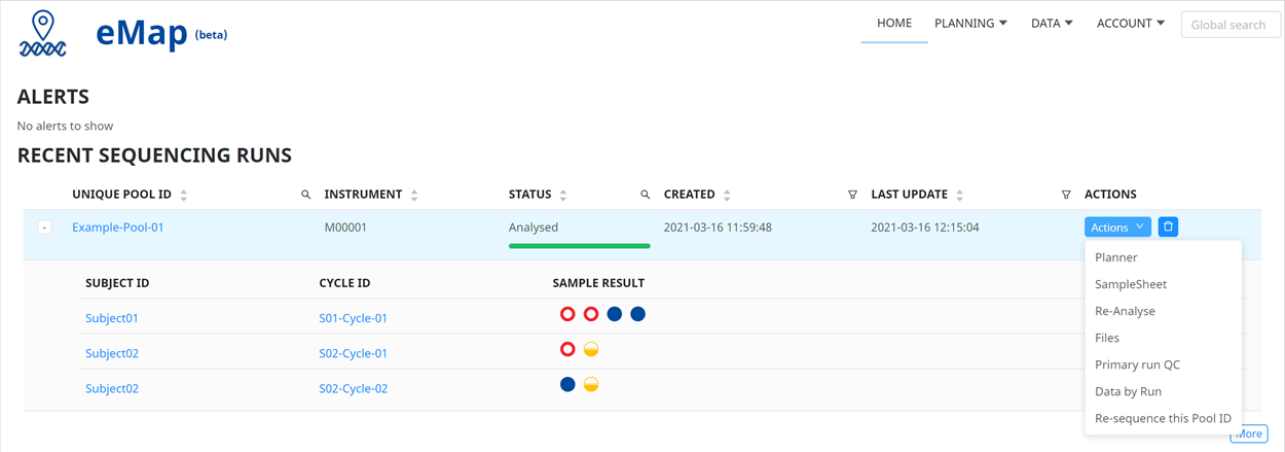


Figure 13: Recent Sequencing Runs list with expanded sample list and expanded action menu.

The Actions menu gives access to the run-specific details and the quick-access actions are listed in Table 11.

Table 11: Sequencing Run actions menu

Action	Description
Planner	Open the *Pool and Sequence* page, showing the Pooling and Run Definition data for this run.
SampleSheet	Download the SampleSheet.csv file to initiate sequencing on the instrument.
Re-Analyse	Repeat the copy-number analysis for a selection of samples in this run and select the required analysis settings for those samples.
Files	Open page listing all data files available to download for this run.
Link Samples to a Subject (when applicable)	Data from runs that are not planned within eMap and custom imports must be manually linked to Subjects and Cycles through this action before review.
Primary run QC	Display flow cell quality control values for this run.
Data by Run	Open the *Run View* page, displaying all samples and results in a compact and run-centric list.
Re-Sequence Pool ID	Create a clone of the Pool, Plate and Sample definitions for re-sequencing. All new IDs will have the suffix "-RP*x*" where *x* is the repeat number.

Re-analysis of multiple samples in a run.

1. From *Recent Sequencing Runs* or *DATA > Sequencing Runs*, click Actions.

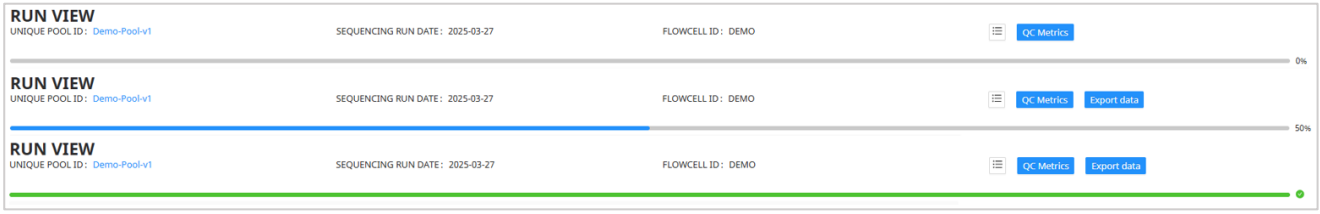
2. Click Re-Analyse and a pop-up will appear displaying all the samples in this run. All samples that have not been locked or signed off will be selected by default.
3. Select the samples you would like to re-analyse from that run by clicking the checkbox next to the Subject ID and select Next.
   Note: Selecting samples with Locked or Signed-Off status will display the following warning message:
   "*WARNING: Locked or Signed-Off sample selected! Re-analysis will remove protections and update results!"*

   Proceeding to re-analyse Locked or Signed-Off samples will automatically perform the "Cancel Sign-Off" and "Unlock" actions on these samples prior to re-analysis. The Lock and/or Sign-Off status will not be restored once analysis has completed. Level 1 users are permitted to re-analyse Unlocked and Locked samples only. Level 2 users will be able to re-analyse Unlocked, Locked and Signed-Off samples.
4. A pop up will then appear displaying the default analysis settings. Choose your desired analysis settings and click Re-Analyse.
5. The run status linked to this sample will change to *Processing,* and the sample icon shall change to *Waiting* . The run status shall change to *Analysed* and the sample results shall be updated once the data is returned.
6. Triggering a re-analysis will remove any manual annotations made to these samples.
   To restore a sample back to the automatic calls and remove all manual annotations, it is not necessary to trigger a re-analysis. Instead, see the *Reset Regions* section for a quicker method.

Run View

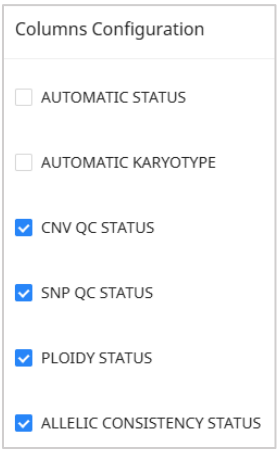
The Run View page shows sample results for an entire Run (Pool ID) in a compact format.

1. Samples on the Run View page will be loaded in batches of 12 with a progress bar to indicate the percentage of the samples in the run that have loaded



2. The header section shows the Pool ID, Sequencing run date and Flow cell ID extracted from the sequencing result files.
3. Click QC Metrics to review the flow cell quality control values for this run.
4. Use the links on each sample row to navigate away from the Run View page:
   a. Click on the Subject ID to open the *Accessioning* page and load information for this Subject.
   b. Click on the Cycle ID to open the *Cycle View*, showing only the results for samples in this Subject-Cycle.
   c. Click on the Sample ID to open the *Sample View* page showing the copy-number chart.
5. The sample information table shows the *Automatic* and *Reported* sample status, karyotype, Ploidy status, and Allelic Consistency status; where the *Reported* result is adjusted for any manual annotations made to the sample on the Sample View page. Only the *Reported* results are used to construct the Report PDF.
6. The columns displayed in the sample information table can be changed by selecting the icon, which will open a window called *Columns Configuration*.
7. Selecting the box next to a particular field will display that column on the Run View page.

a. The most recently saved columns configuration is applied to the Run view pages of all runs.



8. Clicking on a sample row will highlight that row with light blue shading. The following data can then be reviewed for the selected sample using the panel on the right-hand side of the page. See the *Reviewing Sample Results – Tabs* section for detailed information on these data tabs.
    a. Idiogram
    b. Sample QC
    c. Experimental details
9. The *QC Status* for each sample is set automatically to "PASS" or "FAIL" according to the sample-level QC metrics and the criteria defined in the eMap system *Settings.* The QC status can be manually overridden during the sample Sign-off procedure if necessary.
10. The Sample Sign Off column shows the Sign-off status of that sample.
    a. Locked samples will display an icon.
    b. Signed Off Samples will display an icon.
    c. Samples that have not been Locked or Signed off will not display an icon.

Export Data by Run

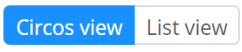
1. Click Export data to download a tab-delimited .tsv file containing sample-level information and results for all Samples in this Pool ID.
2. The file header contains:
    a. Timestamp of export in the format YYYY-MM-DD HH:MM:SS
    b. Username of the User profile that generated the file.
3. The machine-readable file reflects the current status of all Samples in a single Pool ID at the time of export.
4. A summary of exported data fields is shown in *Appendix 3*.

Please note: The Export data will be disabled until all of the samples in the run have loaded onto the run view page.

Cycle View

The Run View page shows sample results for an entire Cycle ID in a similar compact format to the Run View page described previously.

1. The header section shows the Subject ID, Cycle ID and the following optional Subject-level data fields added during Accessioning: Last name, First name, Date of birth, Consultant and Referring Centre.
2. Use the toggle selector to change the way that samples are presented:



a. **List view:** Presents samples in a table as described for the **Run View**, with the addition of a link to navigate directly to the Pool ID to view data for all samples in a single run.
b. **Circos view:** Presents a graphical, circular representation of each sample status, with chromosome-specific abnormalities highlighted using concentric rings representing relative gains and losses. A full description of how to read a Circos plot is provided in the *Reviewing Sample Results* section of this guide.
c. The default view setting (List or Circos) can be selected by the User on the *ACCOUNT* > *Profile* page for each User.

3. Use the links to navigate away from the Cycle View page:
    a. Click on the Subject ID in the header section to open the *Accessioning* page and load information for this Subject.
    b. If List presentation is selected, click on the Pool ID to view data for all samples in a single run.
    c. If List presentation is selected, click on the Sample ID to open the *Sample View* page showing the copy-number chart.
    d. If Circos presentation is selected, double-click on a Circos plot to open the *Sample View* page showing the copy-number chart.
11. Single-click on a Circos plot or sample row to review sample-specific data in the panel on the right-hand side of the page, as described in the *Run View* section. The selected Circos plot shall be highlighted with a square border and light shading. The selected sample row shall have light blue shading. See the *Reviewing Sample Results - Tabs* section for detailed information on these data tabs:
    a. Idiogram
    b. Sample QC
    c. Experimental details
12. For Circos plots, samples with a QC Status “FAIL” will also be shown with a red square border.
13. For Circos plots, the Sample *Sign Off* icon will be displayed in the plot under the *QC Status*

Generate a Cycle Summary Report

1. From the Cycle View page, click the Summary Report button to generate a Summary report forall samples in the current Cycle.
2. See section *5 Reporting* for detailed instructions on generating eMap Report PDF documents.

Please note: The Summary Report will be disabled until all of the samples in the cycle have loaded onto the cycle view page.

Generate a Cycle Full Report

3. From the Cycle View page, click the Full Report button to launch the *Report Builder* for all Signed-Off samples in the current Cycle.
4. See section *5 Reporting* for detailed instructions on generating eMap Report PDF documents.

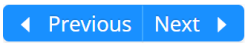
Reviewing Sample Results

The following sections describe features of the *Sample View* page, containing the copy-number chart. The *Sample View* page can be reached by clicking on a Sample ID, sample icon or Circos plot from elsewhere in the eMap system.

Sample Navigation

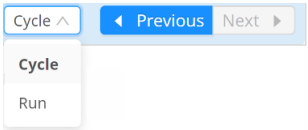
The sample header allows for quick identification of the Sample ID and navigation:

1. Click on the Subject ID to open the *Accessioning* page to load and edit information for this Subject.
2. Click on the Cycle ID to return to the *Cycle View*, showing only the samples in this Subject-Cycle.
3. Click on the Pool ID to return to the *Run View*, showing all samples in this sequencing run.
4. The Details panel provides important sample-level metrics from the analysis. See also, *Tabs - Sample QC*.
5. The Details panel can be collapsed and expanded by clicking anywhere on the

header (Table 12).
6. Use the 

buttons to navigate between samples at the *Sample View* level.
   a. If one button is greyed-out, you have reached the end of the dataset: 

.
   b. The navigation setting is selected automatically, depending on whether the User navigates to the *Sample View* page from either a *Cycle View* or a *Run View* page.



   c. Use the dropdown selection to change the navigation function manually. The selection determines whether to navigate within a Cycle ID dataset in the order of Accessioning, or a Run (Pool ID) dataset in the order of the SampleSheet file.

Table 12: Parameters in the Sample View - Details panel

Parameter	Description
Total reads	Number of reads found in BAM file. Both ends of a read pair are reported.
Filtered Reads	Number of reads that can be used for the copy-number analysis.
CNV QC Status	Automatically assigned PASS or FAIL status based on the QC criteria of the *Settings*.
Overall Noise	Quality of the data based on the variation within regions analysed.
SDI	Summed Deviation to nearest Integer describes the level of step-wise noise in a sample.
SNP QC Status	Automatically assigned PASS or FAIL status based on the fixed SNP criteria.
Notes	Any notes about the sample results added with the Add Notes button on this page.
Ploidy Status	Classification of the Sample based on the predicted ploidy result.
CNV Status	Classification of the Sample based on the copy-number result.
Karyotype	Encoding of the copy-number analysis result including the chromosome numbers, sex and any abnormalities. The reported format can be adjusted on the *Settings* page and is either Shorthand only, with Cytogenetic bands, or with Cytogenetic bands and molecular position included.
Allelic Consistency Status	Automatically assigned status based on how the pattern of alleles matches the expected distribution of the predicted ploidy state.

Add Sample Notes

1. Click Add Notes to add comments to the sample during the review.
2. Saved notes are displayed in the *Details* panel on the *Sample View* page. The first 45 characters will appear on the sample report followed by "…" if the note entry is longer.
3. Click Add Notes to read and edit existing notes.
4. Click Save to save changed or Cancel to exit without saving.

Re-analyse a Sample

To re-analyse multiple samples within a run simultaneously, see the *Re-analysis of Multiple Samples within a Run* section under *Data Lists*. To re-analyse individual samples, follow the steps below:

1. Click Re-Analyse from the *Sample View* to view a Settings window displaying the parameters used for the most recent analysis of this sample.
2. Use this window to configure the settings for the re-analysis. e.g. to hide the sex, select a custom normalisation according to the sample type, or to select chromosomes for re-centering.
3. Any changes made here will apply to this sample only when re-analysing. Making changes will not affect the Group Settings saved in *ACCOUNT > Settings*.
4. Click Restore defaults to restore the settings to the current Group Settings.
5. Click Cancel to discard any changes and return to the *Sample View*.
6. Click Save to trigger the copy-number analysis for this sample only, using the settings shown on screen.
7. The run status linked to this sample will change to *Processing,* and the sample icon shall change to *Waiting* . The run status shall change to *Analysed* and the sample results shall be updated once the data is returned.
8. Triggering a re-analysis will remove any manual annotations made to this sample.
   To restore a sample back to the automatic calls and remove all manual annotations, it is not necessary to trigger a re-analysis. Instead, see the *Reset Regions* section for a quicker method.

Download Sample Data

1. Click Download to save the following files to the User's computer.
   a. Sample-level results including sample details, analysis and QC outcomes (*.stats.tsv).
   b. Positions and copy-number assessments of all genomic regions with confidence and noise scores (*.chr_stats.tsv). The regions will be chromosome-level results or sub-chromosome-level results in the event that *Segmental* calling was turned on or regions were manually annotated on the copy-number chart.
   c. Bin-level results with genomic positions, raw count data, scaled and smoothed copy-number data, GC content (%) and cytogenetic band information (*.bin_stats.tsv).
   d. SNP results for EmbryoMap SNP samples (*snp_stats.tsv).
2. All files are tab-delimited .tsv files that are suitable for archiving or to process the analysis results in other systems (e.g. LIMS).
3. Note that the files will reflect the status of the sample at the time of downloading, including any manual annotations. Be sure to use the download feature in line with Locking and Sign-off procedures.

Karyotype

The copy-number analysis result is encoded in the reported karyotype. The karyotype format and display functions can be defined in *ACCOUNT* > *Settings.*

Always review the copy-number chart and the displayed karyotype to ensure the intended result is shown before Signing-Off and Reporting. See the *Manual Annotation* section for details on updating the karyotype by annotating the copy-number chart.

The different karyotype formats are intended to provide various levels of details to meet different reporting requirements. They do not comply with official ISCN notation but should allow the user to extract all information required.

Karyotype Format

1. **Shorthand** notation – **Example:** 47,XX,+5,-13(sM)
    a. The notation shows the chromosome count, the sex chromosomes and any abnormalities in a comma-separated list.
    b. Abnormalities are displayed by chromosome number with the following shorthand annotation(s):
        + Copy-number gain
        - Copy-number loss
        s Segmental copy-number change (affecting part of a chromosome only)
        m Low-level intermediate copy-number change (putative mosaic change)
        M High-level intermediate copy-number change (putative mosaic change)
    c. The intermediate copy-number thresholds can be defined in *ACCOUNT* > *Settings*.

2. **Cytogenetic bands** notation – **Example:** 47,XX,sseq[hg38](5)x3,mos sseq[hg38](13q12.11q13.1)x1
    a. As with Shorthand, but abnormalities are prepended by "sseq" to indicate *shallow sequencing*, and the genome build used for sequence alignment in square brackets e.g. "[hg38]".
    b. Abnormalities are provided with chromosome number and the nearest *abnormal* copy number integer, e.g. "×1" for a loss (copy-number one).
    c. If a sub-chromosome region is affected (segmental change) the start and end cytogenetic bands are provided in parentheses.
    d. If copy-numbers are not full level changes (intermediate copy-number) "mos" is prepended to the abnormality to indicate the region is *mosaic*. There is no distinction between a high- or low-level putative mosaic change.
    e. If a chromosome has multiple regions the comma "," separator, "sseq" and genome build are not repeated for subsequent regions on the same chromosome.
    e.g. 46,XY,sseq[hg38](18p11.32p11.1)x1(18q11.1q23)x3.

3. **Cytogenetic bands and molecular position** notation –
    Example: 47,XX,sseq[hg38](5)x3,mos sseq[hg38]13q12.11q13.1(18900004_32602848)x1
    a. As with Cytogenetic bands, but segmental abnormalities are described by the start and end cytogenetic bands, followed by the start and end molecular positions, which are separated by an underscore "_" and enclosed in parentheses.
    b. If a chromosome has multiple regions the comma "," separator, "sseq" and genome build are repeated for subsequent regions on the same chromosome.
    e.g. 46,XY,sseq[hg38]

If there are more than six abnormalities recorded, the karyotype will show "multiple".

Show Chromosome Number

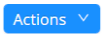
The option to prepend the number of chromosomes (e.g. “46”) to any karyotype format can be selected in *ACCOUNT > Settings.* The default chromosome number is 46 (diploid), and adjusted according to the number of whole-chromosome aneuploid gains and losses that are reported in the karyotype. Note that changes listed as sub-chromosome or intermediate copy-number are not represented in the displayed chromosome number.

Circos Plot

EmbryoMap data is displayed as a circular map (Circos plot) to provide a high-level overview of the results and provide additional navigation features for the linear copy-number chart (Figure 14). To download the Circos plot image as a *.png or *.svg, hover the cursor over the chart and click the

buttons. The downloaded Circos plot image will also contain a footer listing the following information if applicable:

1. Subject ID
2. Run (Pool ID)
3. Sample (<Internal ID>_<Cycle ID>_<Sample ID>)
4. Date (of download, timestamp of analysis server)
5. Lock (username performing the action)
6. Sign-off (username performing the action)

Alternatively, to export Circos plot files for all samples in the same Run, select *Files* in the 

menu for the sequencing run (*DATA > Sequencing Runs* or *HOME > Recent Sequencing Runs*).

The Circos plot displays the chromosomes arranged in a circular orientation, represented by individual boxes. The chromosomes a positioned in clockwise order with labels on the outer edge.

The chromosome box position in the circular map indicates the copy-number count:

1. **Central ring (blue)** – Two chromosome copies (autosomal normal)
2. **Outer ring (green)** – More than two chromosome copies
3. **Inner ring (red)** – Less than two chromosome copies

The chromosome box colour will also change according to the following rules:

1. **Blue** – Copy-number is as expected.
2. **Green** – Whole-chromosome copy-number gain, relative to expected normal state.
3. **Red** – Whole-chromosome copy-number loss, relative to expected normal state.
4. **Yellow** – Chromosome abnormality with unknown significance (putative mosaic and/or segmental imbalance). In cases where multiple sub-chromosomal gains and losses are present within one chromosome, the larger region will determine the ring position (gain or loss).

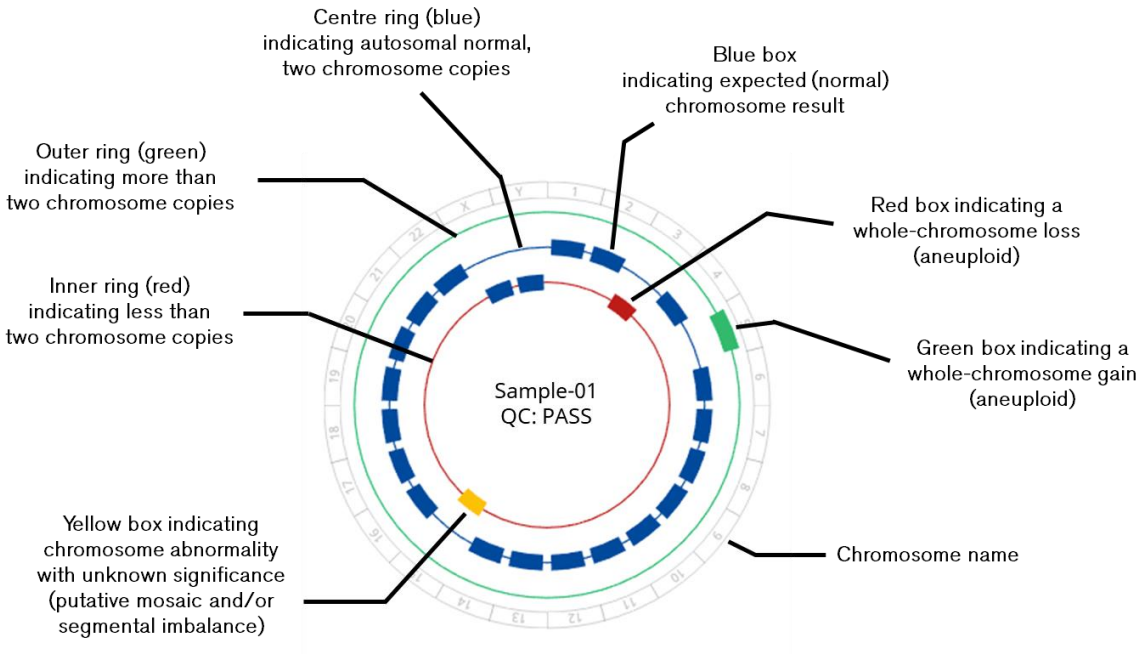


Figure 14: Circos plot

Reading Sample Sex from the Circos Plot

1. For a euploid XY (male) sample, both the X and Y chromosome boxes will be represented on the innermost ring showing a relative loss (copy-number 1) to normal autosomes (copy-number 2). The chromosome box colour for X and Y will be blue, indicating a normal or expected chromosome copy-number for this sample.

2. For a euploid XX (female) sample, the X chromosome box will be represented on the central ring showing equivalent copy-number to normal autosomes (copy-number 2), and the Y chromosome box will be absent. The chromosome box colour for X will be blue, indicating a normal or expected chromosome copy-number for this sample.

3. If manual annotations are made to the sex chromosomes, the colour and position of the chromosome box will be updated in the context of the re-calculated sex. The manual annotation icon will appear in the centre of the Circos plot.

Circos Plot Icons

The icons described here relate to actions for *Manual annotation* and s*ample locking.*

The following icons will be displayed in the centre of the Circos plot when:

1. The sample contains manual annotations.
2. The sample is locked for editing (Level 1)
3. The sample is Signed-Off (Level 2).

Please note that when downloading the Circos plot images, these icons will **not** be shown.

Using the Circos Plot for Copy-number Chart Navigation

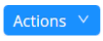
1. Click on a chromosome box or chromosome label to automatically zoom the copy-number chart to the full length of that chromosome.

Copy-Number Chart

The Copy-number chart (CNV chart or linear map) is the main element for region assessment and manual annotation of sample results. The chart shows the normalised sequencing read counts per bin (green dots) plotted against the genomic position (Figure 15). To download the Copy-number chart image as a *.png or *.svg, hover the cursor over the chart and click the

buttons. The downloaded CNV chart image will also contain a footer listing the following information if applicable:

1. Subject ID
2. Sample (<Internal ID>_<Cycle ID>_<Sample ID>)
3. Run (Pool ID)
4. Date (of download, timestamp of analysis server)
5. Lock (username performing the action)
6. Sign-off (username performing the action)

Alternatively, to export CNV chart files for all samples in the same Run, select *Files* in the 

menu for the sequencing run (*DATA > Sequencing Runs* or *HOME > Recent Sequencing Runs*).

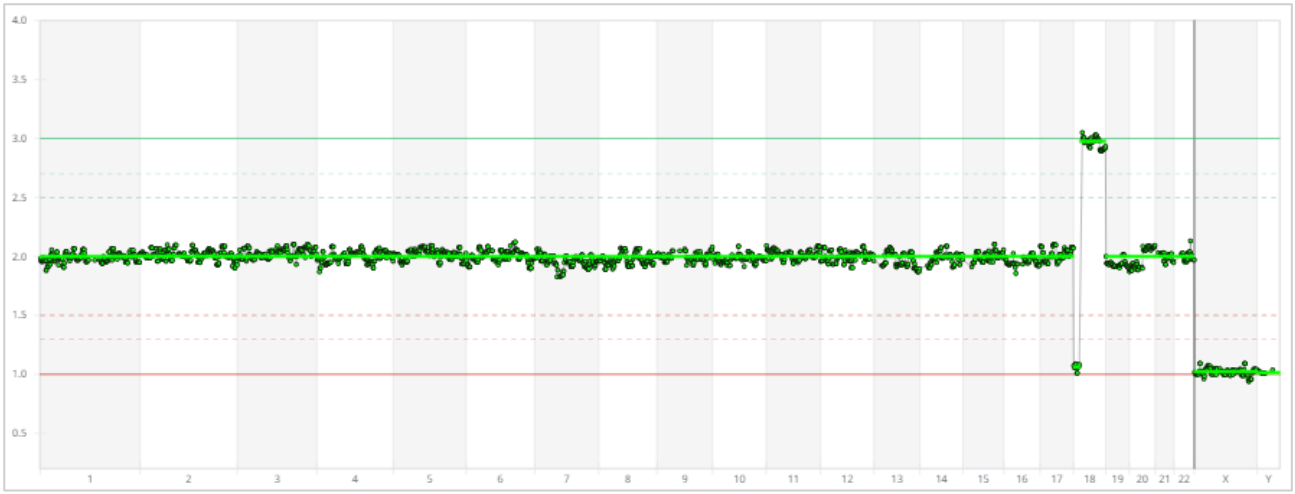
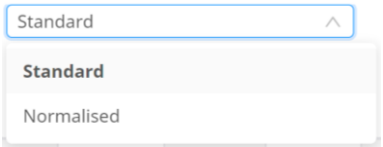


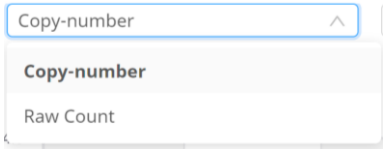
Figure 15: Copy-number chart showing karyotype 46,XY,sseq[hg38](18p11.32p11.1)x1(18q11.1q23)x3

1. Chromosomes are represented on the X-axis, and distinguishable by alternating vertical grey and white shading. Each chromosome width is scaled by default to the relative bp length (*Standard*). To switch the view for *Normalised* (i.e. equal) chromosome widths, use the drop-down selector above the chart:

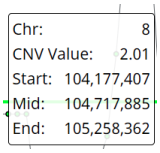


2. The bright, thick green horizontal line indicates the detected copy-number. For autosomes with the expected normal copy-number state, it will be drawn at 2.0. Where sex chromosomes have a normal copy number of 2.0, 1.0 or 0.0 the line will be drawn at the integer value if the chromosome is determined to have a normal copy-number state. Where any region copy-number deviates from the expected normal state, the line will be drawn at the median level measured across the regions' bins.

3. The Y-axis scale has a high dynamic range from copy-number 0 to 4, allowing for clear identification of gains or losses. If any bins have copy-numbers greater than 4, the Y-axis is automatically adjusted, and a warning message is added above the chart to indicate the different scale.
4. Two further solid horizontal lines are shown to represent an autosomal full copy number gain (green: 3.0) and loss (red: 1.0), respectively.
5. If intermediate copy-number thresholds are defined in *ACCOUNT* > *Settings,* these will be displayed on the chart as horizontal dashed lines for gain (green) and loss (red), respectively.
6. The copy-number chart is displayed with smoothed *Copy-number* values by default. To switch to the *Raw Count* view, use the drop-down selector above the chart:



7. Hover the cursor over a bin data point to reveal the Chromosome, CNV value, Start, Midpoint, and End hg38 molecular position data:



8. For a maximized view, click the button above the chart to quickly collapse the *Details* panel, hide the *Circos plot* and expand the copy-number chart to fill the available screen space. To return to the default view, click the button.

CNV Chart Settings

Specific settings can be applied to each CNV chart by selecting the *Settings* icon (Figure 16).

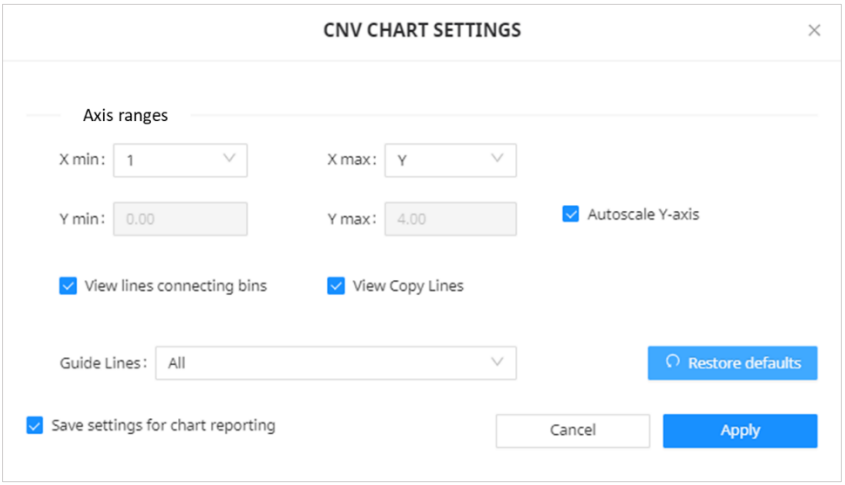


Figure 16: CNV chart settings window

1. *X min* and *X max* will define a continuous range of whole chromosomes on the X-axis. All chromosomes are displayed by default.
2. *Y min* and *Y max* will define a continuous copy-number range on the Y-axis. The default range is 0.00 to 4.00.
3. When the *Autoscale Y-axis* checkbox is selected, the *Y max* value will be automatically adjusted if any sample bin values are > 4.00. This ensures that all bin data is displayed in the CNV chart image, in the event of high copy-number values.
4. The following reference lines can be enabled/disabled. All are enabled by default:
    a. **View lines connecting bins** will draw lines between adjacent bins.

b. **View Copy Lines** will display the copy-number value lines for each chromosome or abnormal region.
c. The **Guide Lines** selection will control the display of solid guidelines indicating the copy-number gain (3.00) and loss (1.00), and dashed guidelines for intermediate copy-number thresholds.

5. To apply the changes to the CNV chart, click Apply.
   a. If the *Save settings for chart reporting* checkbox is selected, the currently selected settings will be saved and applied to the chart image when generating the **Summary Report** and **Full Report** PDFs, or downloading the chart image from the *Sequencing Runs* > *Files* menu.
   b. When a sample is **Locked** by the User, the CNV chart view shall be updated to display the chart settings that shall be used for generating reports for this sample.
   c. CNV chart downloads from the Sample view page will always show the current chart settings, irrespective of the *Save settings for chart reporting* selection.
6. Clicking Restore defaults will cancel any changes to the CNV chart settings and return to the defaults.
   a. If custom settings were previously saved for reporting, the User must ensure the *Save settings for chart reporting* checkbox remains selected, in order to update the saved settings for this sample in the eMap system.
   b. The *Save settings for chart reporting* checkbox value is not modified by the Restore defaults action, and its current value takes effect when the user clicks Apply.

Chart Navigation

1. Click the Zoom In and Zoom Out buttons to make the central part of the visible chart larger and smaller, respectively.
2. To zoom in with the cursor (left-click):
   a. Click the Zoom On button or click once in the chart area to activate the zoom cursor tool.
   b. Click again and hold to draw a box covering the area to zoom.
   c. Release the cursor click to zoom to the selected area.
   d. To cancel a zoom, click the Zoom Off button.

   Note: If the zoom cursor tool is activated (Zoom On), hovering over a bin will no longer reveal the position data.
3. To reset the zoom:
   a. Click the Reset Zoom button, or;
   b. Double-click anywhere in the chart area
4. If the chart is zoomed, use the < and > buttons underneath the chart to scroll horizontally across the bins.
5. Click a chromosome number on the X-axis to quickly zoom the copy-number chart to the full length of that chromosome.

Manual Annotation

It will not be possible to make manual annotations to a sample that is in Locked or Signed-Off status.

Remove a Region

1. Click on the *Regions* tab to see a list of sample abnormalities.
2. Click the button under *Actions* to remove the region.
3. The sample karyotype, Circos plot, status and copy-number chart shall be updated automatically.

4. If the removed region was an *automatic* annotation, the manual annotation  icon will be added to the centre of the Circos plot.

Create a New Region

To begin, all bins in the region of interest must be at 'no change' status. In some cases, this may mean removing an automatic region before creating a new region in its place.

1. Zoom to the region of interest.
2. Click Create Region to activate the region selector cursor.
   Click again and hold to draw a box to select bins (green markers). Include the first and last bin in the region of interest, bins in between will automatically be included. You must select at least 2 bins.
3. Release the cursor click to select the bins. Selected bins will change to yellow.
4. Click Cancel to exit or click Review Region to proceed.
5. Follow the on-screen prompts to select an abnormality type for the selected region. e.g. "Full gain".
6. Click Cancel to exit or click Save to finalise the new region.
7. The sample karyotype, Circos plot, status and copy-number chart shall be updated automatically.
8. The manual annotation  icon will be added to the centre of the Circos plot.
9. The new region can be reviewed on the *Regions* tab. The region Start and End positions, and therefore Size (Mb), shall be determined by the first bp of the first selected bin, and the last bp of the last selected bin of the manual region. Note that the selected bins may not cover the entire hg38 reference where copy number detection is less reliable e.g. in acrocentric chromosomes, regions of heterochromatin or repeat elements.

Reset Regions

The following action will restore the sample to the automatic calls from the last copy-number analysis. This will remove any manual annotations that have been made to the sample. Using this method is much faster than using the Re-Analyse button.

1. Click on the *Regions* tab to see a list of chromosome abnormalities.
2. Click Reset regions.
3. Click Yes to acknowledge the removal of any manual annotations.
4. The sample karyotype, Circos plot, status and copy-number chart shall be updated automatically.
5. If applicable the  icon will be removed from the centre of the Circos plot

Annotating Sex Chromosomes

Applying manual annotations to the sex chromosomes may require the sample sex to be recalculated. If a presence of Y is annotated (automatic or manual), the sample sex will be reported as Male. When removing whole-chromosome abnormalities in either X or Y, the User is required to provide the new 'no change/normal' copy-number value for the chromosome using an on-screen dialogue. The horizontal green line will be fixed to the selected value and interpreted by the software as 'normal' for the purpose of calculating the sample sex and chromosome number.

Tabs

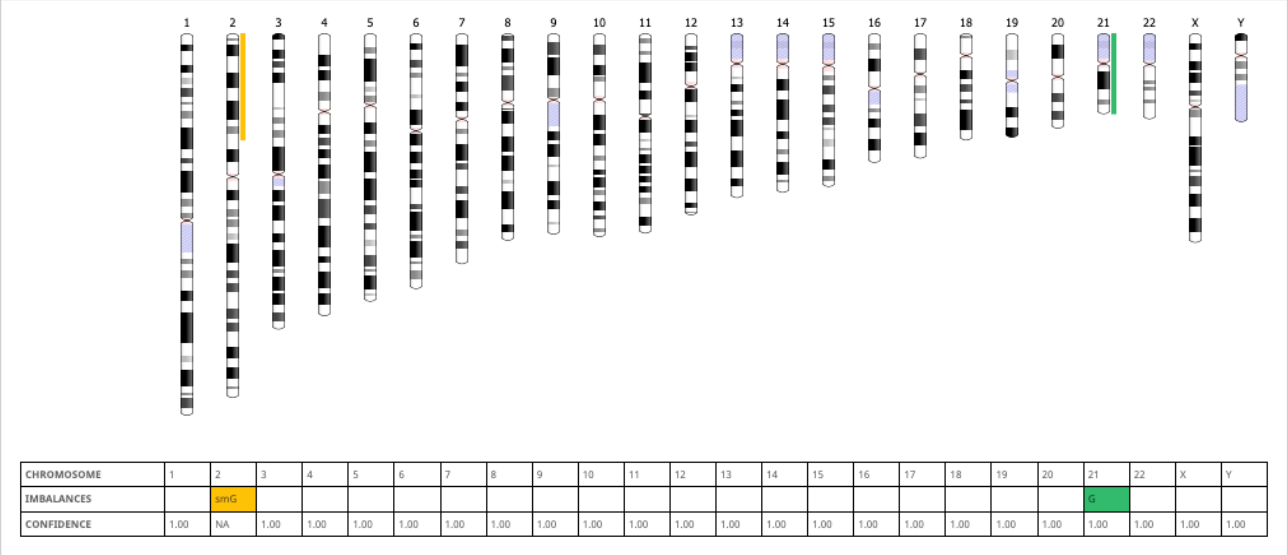
In addition to the copy-number chart, the flowing Tabs provide additional sample information.

Sample line | Idiogram | Regions | Files | Logs | Sample QC | Experimental details

The *Ideogram*, *Sample QC* and *Experimental Details* tabs are also available from the *Cycle View* and *Run View* pages.

Idiogram

The idiogram provides a visual representation of all human chromosomes assessed in the analysis with their typical banding pattern and sizes (Figure 17).



CHROMOSOME	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Y
IMBALANCES		smG																			G			
CONFIDENCE	1.00	NA	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

Figure 17: Idiogram and imbalance table

1. Any copy-number changes are shown at the affected region of the chromosome as coloured bars:
   a. **Whole-chromosome loss:** Red bar, left of chromosome.
   b. **Whole-chromosome gain:** Green bar, right of chromosome.
   c. **Intermediate copy-number or segment loss:** Yellow bar, left of chromosome.
   d. **Intermediate copy-number or segment gain** Yellow bar, right of chromosome.

2. The **table** underneath the idiogram provides a summary of changes for each chromosome.

   Copy-number changes are listed as *imbalances* using the colour system described previously, and using a combination of the following shorthand letter notations, where applicable:

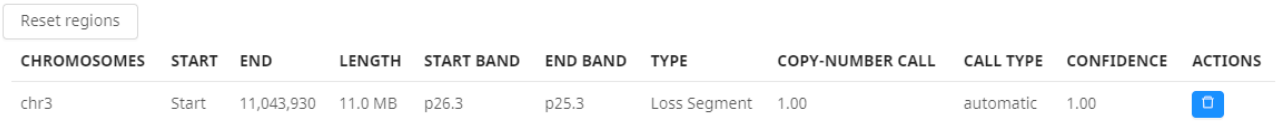
   L Copy-number loss
   G Copy-number gain
   s Segmental copy-number change (affecting part of a chromosome only)
   m Low-level intermediate copy-number change (putative mosaic change)
   M High-level intermediate copy-number change (putative mosaic change)

   User-defined thresholds for low- and high-level intermediate copy-number changes are configured in *ACCOUNT > Settings*.

A confidence value is provided for automatic calls ranging from 0.0–1.0. A value above >0.9 can be considered a high confidence copy number assignment. A high level of noise across the sample or an un-annotated segmental copy number change in the chromosome can cause lower confidence values.
When thresholds are set for intermediate (putative mosaic) copy-number calling, the confidence score will be low for "abnormal" calls that have a copy-number median value close to the "normal" level, i.e., "low-level mosaic" calls. These calls should be carefully examined.
A value of 0.0 indicates that the analysis failed for this experiment.
Manual annotations will not receive a confidence score and will display "NA", indicating a manual change was applied.

Regions

The regions table provides a list of all region annotations (automatic and manual) that will be reported for the current sample, for example:



1. Click the button under *Actions* to remove a region.
2. Click Reset regions then Yes to restore the sample back to **automatic** calls only.
3. The region *Start* and *End* hg38 bp position will be shown for segmental changes. If the segment begins or ends at the first or last assessed base, then the word "Start" or "End" will be shown. Likewise, whole-chromosome changes will have the positions "Start" and "End" instead of a numerical position. The numerical positions are relative to each chromosome.
4. The segment *Length* is calculated in megabase pairs (Mb). If the region is whole-chromosome, the relative length according to the reference bin matrix will be shown.
5. The region cytogenetic *Start Band* and *End Band* positions are shown for all imbalance types (See *Idiogram*, for a visual representation).
6. The region *Type* describes the copy-number classification, according to the system *Settings* at the time of copy-number analysis and any manual annotations:
   Full gain
   High mosaic gain
   Low mosaic gain
   Low mosaic loss
   High mosaic loss
   Full loss
   If the region affects a sub-chromosome region, the word "Segment" will be appended to the *Type* name e.g. "Full gain Segment".
7. The *Call Type* will be listed as either:
   automatic The region was automatically annotated according to the system *Settings* used during copy-number analysis.
   manual The region was created as a manual annotation, and therefore will not have a confidence score.
8. A *Confidence* score is reported for all *automatic* regions. The score describes the confidence that the region copy-number belongs to the *Call Type* classification (normal or abnormal), based on integer copy-number states whilst also considering sample noise. For example:

a. An autosomal Full gain shall have a score reflecting classification to the nearest abnormal integer (e.g. copy-number 3.0). The score is expected to be high.
b. An autosomal Low-level putative mosaic gain (e.g. copy-number 2.35), shall have a score reflecting classification to the nearest *abnormal* integer (e.g. copy-number 3.0). Therefore, the score is expected to be low because 2.35 is closer to "autosomal normal" (copy-number 2.0) than "autosomal abnormal" (copy-number 3.0 or higher). If the User configures these parameters as abnormal using the system *Settings,* the confidence is displayed in the context of being "abnormal".
c. Regions with low confidence can be flagged by setting a custom threshold under *ACCOUNT* > *Settings*. Regions that fail to meet the threshold will be shaded in the *Regions* table (Figure 18).

Note: This is a visual queue only. Failure to meet the confidence threshold will not affect the overall Sample QC status.

CHROMOSOMES	START	END	LENGTH	START BAND	END BAND	TYPE	COPY-NUMBER CALL	CALL TYPE	CONFIDENCE	ACTIONS
chr3	53,581,830	74,439,334	20.9 MB	p21.1	p12.3	High mosaic gain Segment	2.59	automatic	0.74	
chr4	Start	60,496,289	60.5 MB	p16.3	q13.1	High mosaic gain Segment	2.57	automatic	0.58	
chr21	Start	31,733,889	16.3 MB	q11.2	q22.11	Gain Segment	2.99	automatic	0.98	

Figure 18: Region with a confidence score less than the threshold settings will be displayed with red shading.

Files

The aligned read data (BAM file) uploaded from the Sequencer is shown in the Files tab. Click the file name to download. The raw data is stored for 30 days after upload. For longer term data storage options, contact your Vitrolife representative.

Ensure that the raw data files are archived in a secure storage location independent of eMap for the time period and of the data type (e.g. bcl, fastq, or BAM files) required by the regulations applicable for your institution, region and/or country.

Logs

The command used to analyse the sample and any processing messages are provided here to assist troubleshooting. It's good practice to briefly review this tab to confirm that there are no unexpected "ERRROR" or "WARNING" messages. If there are unexpected failures, text from this log can be exported and shared with your Vitrolife representative for support, after removing any sensitive information about the samples.

Sample QC

Table 13 summarises the key quality control metrics of the sample and its analysis results.

Table 13: Sample QC parameters

Parameter	Description	Automatic QC
Sample ID	User-provided ID	N/A
CNV QC Status	Automatically assigned PASS or FAIL status based on the QC criteria of the *Settings*.	Yes
Average Alignment Score	Mean score of filtered reads based on the alignment software	Yes
Number of Total Reads	Number of reads found in BAM file. Both ends of a read pair are reported.	Yes
Number of Mapped Reads	Aligned sequencing reads found in the BAM file	No
Number of Reads After Filtering	Number of reads that can be used for the copy-number analysis.	Yes
Overall Noise	Quality of the data based on the variation within regions analysed.	Yes
SD Robust	Estimation of data quality using the robust standard deviation.	No
SDI	Summed Deviation to nearest Integer describes the level of step-wise noise in a sample.	Yes
SNP QC Status	Automatically assigned PASS or FAIL status based on the fixed QC criteria.	Yes
Number of Valid SNP Reads	Number of reads that can be used for the SNP analysis.	No
Number of Heterozygous SNP Calls	Number of heterozygous SNPs with the minimum read depth.	Yes
Ploidy Confidence	The level of confidence in the predicted ploidy status	Yes
Allelic Consistency	How closely the pattern of alleles matches the predicted ploidy status. A reduction in score can be an indication of sample noise or external contamination (see EmbryoMap calling guide v2.0 or later).	Yes

1. Selected metrics are associated with Automatic QC. User-defined thresholds can be configured under *ACCOUNT* > *Settings*. The data row will be highlighted according to status:

Green	The metric passes the criteria defined in system *Settings*.
Yellow	The metric does not pass the criteria defined in system *Settings* and should be investigated further.
Red (*QC Status* only)	One or more metrics do not pass the criteria, and the Sample *QC Status* is set to "FAIL". Following manual data review, the *QC Status* may be changed to "PASS" during the Sign-off procedure.

2. If the *Sample Type* is set to *Negative* and the Sample *QC Status* = "PASS", only the *Total Reads* metric will be displayed and coloured green.
3. If the *Sample Type* is set to *Negative* and the Sample *QC Status* = "FAIL", then the remaining sample-level metrics shall be revealed, but no automatic QC colours shall be set.

Experimental Details

Table 14 describes key parameters of the sequencing run and high-level result data for the sample.

Table 14: Experimental Details

Parameter	Description
Sequencing Run Date	Extracted from sequencing result files
Experiment Name	Provided during Laboratory Planning
Unique Pool ID	Pool ID provided during Laboratory Planning
Sequencing Run Description	Description provided during Laboratory Planning
Sample ID	Sample ID provided during Accessioning
Internal ID	eMap-internal reference number
Workflow	Sequencing workflow
BAM File	Raw data file name
Sample Type	Sample Type provided during Accessioning
Flow cell ID	Extracted from sequencing result files
Reagent Cartridge ID	Extracted from sequencing result files.
Genome Build	Human reference genome used for read alignment
Plate ID	Plate ID that the current sample was assigned to during Laboratory Planning.
Index ID	UDI index name and Final Library Plate well position e.g. “UDI-A01”
Normalisation	The reference database used to correct protocol bias during copy-number analysis
Analysis Version	Copy-number analysis algorithm version
Automatic Sex	Sample sex determined by the copy-number algorithm
Sex	Sample sex to be reported (accounting for manual annotations)
Automatic CNV Status	Sample status determined by the copy-number algorithm
CNV Status	Sample status to be reported (accounting for manual annotations)
Automatic Karyotype	Sample karyotype determined by the copy-number algorithm
Karyotype	Sample karyotype to be reported (accounting for manual annotations)
Ploidy Status	Sample predicted ploidy status determined automatically by the SNP algorithm
Allelic Consistency Status	Sample status determined automatically by the SNP algorithm. If there is evidence of external contamination, it will be reported here.

1. The *Automatic Status* and *Status* rows will be highlighted according to the following:

Blue	No abnormalities detected
Yellow	Abnormal, unknown significance (Putative mosaic and/or segmental changes only)
Red	Aneuploid (Whole-chromosome, full copy-number change)

2. If the *Sample Type* is set to *Negative*, and the Sample *QC Status* = “PASS”, then the *Automatic Sex*, *Status* and *Karyotype* shall display “NA”.
3. If the *Sample Type* is set to *Negative*, and the Sample *QC Status* = “FAIL”, then the *Automatic Sex*, *Status* and *Karyotype* shall display the result of the copy-number analysis.

Sample Sign-off

There are two levels to the Sign-Off procedure, Lock and Sign-off. Locking a sample prevents further changes being made to the annotations and protects the sample pending final review. A sample must be Unlocked to allow editing again. Signing off a sample can only be performed by a Level 2 User and allows the Sample to be added to a Full Report.

Sign-off History

1. From the *Sample View* page, click Sign-off History to display a pop-up detailing the complete sign-off history of the current sample. This includes the locking and unlocking of samples, and the signing-off of samples. It also includes a timestamp of the action performed, and the username of the account that performed the action.
2. The dialogue can be expanded by dragging the bottom right corner or navigated using the scroll bar.
3. This log will contain a local timestamp, action performed, and User performing the action.
4. For Sign-off actions, the entry will also contain the selected QC Status, and if applicable, the Sign-off comment to be included on the Full Report or *<Pool ID>_export.tsv file*.
5. The Sign-off History will capture Locking and Unlocking, Sign-off and Cancel Sign-off actions.

Lock a Sample

6. Level 1 and Level 2 Users can Lock and Unlock samples.
7. Click Lock on the *Sample View* page.
8. The 🔒 icon will appear in the centre of the Circos plot.
9. The User's email address will be recorded as "Level 1 Sign-off" in the result export file.
10. To Unlock the sample, click Unlock Sample.
11. All Locking and Unlocking actions are recorded in the system *Logs* and *Sign-off History* with the User's email address.

Sign-off a Sample

1. Only Level 2 Users can Sign-off samples.
2. A sample must already be Locked, before it can be Signed-Off.
3. Click Sign-off to open the Sign-off dialogue window.
    a. Review the Subject and Sample ID.
    b. The automatic CNV QC Status and SNP QC Status are shown. Use the drop-down tool to select and override the CNV QC Status, the SNP QC Status cannot be overridden.
    c. Use the free-text box to enter Comments.
    Note: The last Comment entered will appear on the Full Report PDF.
    d. Review the Sign-off History, showing details of sample Locking, Unlocking and Sign-off actions.
    e. Enter Username and password (Level 2 only).
    f. Click Yes to confirm the Sign-Off or click Cancel to exit without saving.
4. The 🔒 icon will appear in the centre of the Circos plot.
5. Signed-Off samples are available for Reporting.
6. To cancel a Sign-off, click Cancel Sign-off then Yes to confirm. The action will be recorded in system *Logs* and *Sign-off History* with the User's email address. The sample will return to Locked status.

5 REPORTING

The copy-number analysis results can be reported in two PDF formats for archiving and distribution. Reports are generated with samples grouped by Cycle ID. Start by clicking on a Cycle ID to view the following report options:

Summary Report
Includes a table of all samples that have completed analysis at the time of report generation, with results displayed per chromosome. The table is followed by the sample CNV charts in a compact format.

Full Report
Detailed report for user-selected samples that have been **Signed-Off** only, including a summary karyotype table, detailed analysis results per sample and an optional large-format CNV chart per sample. The Full report also includes additional options for customisation, see *Report Settings*.

This section describes the format and customisation of eMap Cycle Reports and how to build Reports for individual or multiple Subject-Cycles.

Report Settings

The Report settings can be pre-configured under *ACCOUNT* > *Settings*. The saved values will be automatically applied upon report generation. See the Table 15 below for guidance on each setting by report type. When creating a Full Report, the settings can be previewed together with the page margin/size selection.

Table 15: PDF Report settings by format

	Summary Report	Full Report
Logo	-	Will appear in the page header.
Address	-	Will appear in the page header.
Include CNV Chart	Compact charts will be shown for all samples with an analysis result.	Optional: <Yes> / <No> to display a large format CNV chart per sample.
Disclaimer	Will appear in the page footer.	Will appear in the page footer.
Report introduction	-	Will appear on the Summary page only.
Page margin	Small	Optional: <Small> / <Normal>
Paper size	US Letter	Optional: <A4> / <US Legal> / <US Letter>

Summary Report

Create a Summary Report

The Summary Report can be generated directly from any *Cycle View* page, for the current Cycle.

1. Click Summary Report to generate the report and in a PDF preview window.
2. Click Download PDF to save the report with the filename *<Pool ID>_<Cycle ID>_Summary.pdf*

   Note: Clicking the download button within the preview window  will not include the filename format described above.

Summary Report format

Each page of the *eMap* Full Report contains the following elements in the footer section:

1. Custom disclaimer statement defined in *Report Settings*
2. Page number "*n* of *N*"
3. RUO statement
4. Printed (generated) date
5. Username (first part of the User's email address used for login)

The Summary report represents the Cycle overview at the time of report generation, including a **Subject information** header (included on every page of the report) and a summary table detailing chromosome-specific region abnormalities by sample. Following the summary table, the corresponding CNV charts will be displayed in a compact format with up to 6 charts per page. Each CNV chart includes footer information, as described in Section *Reviewing Sample Results: Copy-Number Chart* (Table 16).

Table 16: Summary Report sections

Report Section	Description	
Subject information	Subject ID, Cycle ID and optional fields completed at the time of *Accessioning*: First name, Last name, Date (last update), Date of Birth, Consultant, Subject Notes, Referring Centre. The Subject information section will be repeated on every page of the PDF Report.	
Summary table sample header	Sample ID	-
	Sample Type	Trophectoderm, Genomic DNA, Negative or Other
	Pool ID	-
	Index adapter	-
	CNV QC Status	Current or reported value after Sign-off
	CNV Status	Reported value, considering any manual annotations
	Karyotype	Reported value with format (*Settings*), considering any manual annotations.
	SNP QC Status	Automatic SNP QC Status
	Ploidy Status	Automatic Ploidy Status
	Allelic Consistency Status	Automatic Allelic Consistency Status
	Locked	User that locked the sample. "N/A" if sample is not locked.
	Signed-Off	User that signed-off the sample. "N/A" if sample is not signed-off.
Summary table sample results by chromosome	Abnormal (CNV) regions listed by chromosome 1-22, X and Y. Each region is reported with an abnormality code using a combination of the following notations, followed by the region copy-number median value. **L** Copy-number loss **G** Copy-number gain **s** Segmental copy-number change (affecting part of a chromosome only) **m** Low-level intermediate copy-number change (putative mosaic change) **M** High-level intermediate copy-number change (putative mosaic change) If the chromosome has multiple regions, these shall be separated by a line break. Intermediate copy-number thresholds can be configured in *Account > Settings* prior to running an analysis. Chromosome result fields shall be colour-coded according to the abnormality type for whole chromosome gains (green), whole-chromosome losses (red) and mosaic and/or segmental imbalances (yellow).	
Sample CNV Chart	See Section *Reviewing Sample Results: Copy-Number Chart* for details of image format and image footer information. If a sample has been analysed but contains no CNV result (e.g. Negative), the report shall display placeholder text "<Sample ID>. No data to be displayed".	

If the sample information does not fit on a single page each additional page will repeat the Subject information to ensure sample tracking.

Full Report

Add Samples to a Full Report

The *Report Builder* tool allows selection of samples for inclusion on a Full Report. Only Signed-Off samples will be available for selection.

1. Navigate to *DATA* > *Report Builder*.
2. Using the *Samples by Subject* list on the left-hand side, select a Subject-Cycle, then:
    a. Drag-and-drop or click on individual samples to add to the *Samples* bar, or;
    b. Use the Cycle autofill button to add all samples for that Cycle to the *Samples* bar.
3. Click the "×" next to the Sample ID to remove a sample from the *Samples* bar and the Report PDF.

4. Addition of samples from different Subjects will result in the creation of one Report PDF document for each Subject ID found.
5. Once the required samples are selected, click Create Report to proceed.
6. Alternatively, click Report from any *Cycle View* page, to generate a single Report for that specific Subject-Cycle only.

Create a Full Report

The *Report Preview* page is shown after clicking Create Report on the *Report Builder* page, or by clicking Report from an individual *Cycle View* page. The *Report Preview* page is divided into two sections, Report Settings and a Preview Window.

1. Click Generate Reports to open the PDF preview window.
2. A list of reported Subjects is shown under the heading *Report Preview* as tabs.
3. Click on a Subject ID tab to preview the PDF document for that Subject.
4. Multiple Cycles for a single Subject will be included in the same PDF report. See *Report Format*.
5. One PDF document shall be created for each included Subject.
6. Click Download Report(s) to download all Subject reports (Recommended).
7. The PDF file name format is dependent on how many Cycles are included in the report, and includes either a single Cycle ID or the timestamp of report generation, with the format:

Single Cycle	<SubjectID>-<CycleID>.pdf
Multiple Cycles	<SubjectID>-YYYYMMDDHHMMSS.pdf

8. Clicking the download button within the preview window will only download the selected Subject Report and will not include the filename format described above.

Full Report format

Each page of the Report contains the following elements in the footer section:

1. Custom disclaimer statement defined in *Report Settings*
2. Page number "*n* of *N*"
3. RUO statement
4. Printed (generated) date
5. Username (first part of the User's email address used for login)

The first page of the report represents the reported Cycle(s) overview, providing Subject information from the *Accessioning* page and a summary table of each reported sample. There will be one summary table per Cycle, with the headings described in Table 17.

Table 17: Full Report: Cycle summary table

Parameter	Description
Sample ID	Sample identifier assigned during Accessioning
CNV QC Status	CNV QC Status (PASS or FAIL), according to automatic QC thresholds in *Settings* and manual override during the Sign-off dialogue.
CNV Status	CNV Status of the sample, including any manual annotations
Karyotype	Chromosome status of the sample including any manual annotations, with the format selected in *Settings* (± Chromosome number and Sex).
SNP QC Status	SNP QC Status (PASS or FAIL), according to fixed QC thresholds.
Ploidy Status	Predicted Ploidy status of the sample.
Allelic Consistency Status	Summary status of how the allele balance matches to the predicted Ploidy status.
Result	eMap icon of the summary result for a quick overview

The individual sample result pages that follow shall be presented in sample order, with the detailed information sections described in

Table **18**.

Table 18: Full Report: Sample detail report sections

Report Section	Description
Subject information	Subject ID and optional fields completed at the time of Accessioning: Last name, First name, D.O.B, Consultant, Referring Centre, Subject Notes, Date (last update). The Subject information section will be repeated on every page of the PDF Report.
Sample detail	Sample ID, Sample Type, BAM file name, eMap Internal ID, Pool ID, Flow cell ID, Sequencing instrument, Sample Notes.
Sample result	The reported sample result includes all manual annotations: eMap icon, Sample sex, Summary result status, Karyotype, Ploidy Status, Allelic Consistency Status.
CNV QC	CNV QC Status, Overall noise (DLR), Total reads, Filtered reads, Step-wise noise (SDI), SD Robust, Sign-off Comment, Sign-off User. The Sign-off comment is the last entered comment during the Sign-off dialogue.
SNP QC	SNP QC Status, Filtered SNP Reads, Het SNP Calls, Ploidy Confidence, Allelic Consistency.
Circos plot	Circos image as displayed in the *Sample View* page, with the Sample ID written in the centre and the phrase “Manually annotated” if manual annotations were applied.
Regions called	The Regions table as appears in the tab on the *Sample View* page, including: Chromosome, Start bp, End bp, Length Mb, Start band, End band, Type, Copy-number, Call type, Confidence.
Analysis settings	The settings used for the copy-number analysis for this sample, including: Software version (eMap user interface), Analysis version (copy-number algorithm), Reference version (normalisation), Smoothing, Segmental calling (Yes/No), Custom copy-number thresholds (Yes/No), Thresholds (Gain) (Full gain / High-level mosaic / Low-level mosaic), Thresholds (Loss) (Low-level mosaic / High-level mosaic / Full loss), Hide sex (Yes/No), Telomeric drop correction (Yes/No), Recentered chromosomes (if applicable), Workflow version, Minimum SNP depth, Hide ploidy status, Hide Allelic consistency status, SNP Panel.

If the sample information does not fit on a single page each additional page will repeat the Subject information to ensure sample tracking.

If the option to include the copy-number (CNV) chart was selected, the following page will be presented in landscape orientation with the Subject information and the fully annotated copy-number chart. The Sample ID is included as the chart title.

Reporting Sample Failures

If a sample does not meet the configured CNV QC criteria in *ACCOUNT > Settings*, or the CNV QC status was updated to "FAIL" during the Sign-off dialogue, the following shall apply to *Reported* result values in data export files and PDF Reports (where applicable).

Note: The *Automatic* result values displayed in the User Interface shall still display the outcome of the analysis algorithm, prior to manual annotation. This will include predicted karyotype and sample status, but these will not appear on PDF reports.

Table 21: Reported Results for CNV QC Fails

Reported field	Value when CNV QC Status = FAIL
CNV Status	No Result
Karyotype	NA
CNV QC Status	FAIL
Result icon (Full Report only)	NA (Unless SNP QC "PASS")
Sex	NA
Regions Table (Full Report only)	One line entry with "NA" for each field.
Summary table (Summary Report only)	<Empty> for each chromosome field.

If a sample does not meet the fixed SNP QC criteria, the following shall apply to result values in data export files and PDF Reports (where applicable).

Table 22: Reported Results for SNP QC Fails

Reported field	Value when SNP QC Status = FAIL
SNP QC Status	FAIL
Ploidy Status	NA
Allelic Consistency Status	NA/Possible Contamination detected
Result icon (Full Report only)	NA (Unless CNV QC "PASS")

The PDF reports will still display the Circos plot and CNV chart (where applicable) for samples with FAIL status.
The complete sample result can still be exported in the <Pool ID>_export.tsv file (see Section *Export Data*), including the automatic karyotype, regions table (regions_called) and individual results by chromosome.

6 EXPORT DATA

Sequencing Run Data

Files Menu

The *Files* menu gives quick access to the SampleSheet, BAM files, flow cell Metrics, Copy-number result files, Circos plot and CNV chart images for all samples in a *Sequencing Run.*

It is recommended to back-up the sequencing BAM files locally for long-term storage, directly from the sequencing instrument. Note that by default, BAM files are available in eMap for 30 days only.

1. Navigate to *DATA* > *Sequencing Runs* or find the run in the *Recent Sequencing Runs* list on the *HOME* page.
2. Select *Files* in the Actions menu.
3. Click the download buttons next to the target data:
    a. Clicking to the right of a section header (SampleSheet, BAM Files, Result Files, CNV Charts, Circos Plots, Metrics) will download the files for all samples in a single .zip folder.
    b. Clicking to the left of a specific file name will download only that file.
4. Files for individual samples can also be downloaded directly from the *Sample View* page.

Export SampleSheet

1. The SampleSheet is automatically downloaded during run planning.
2. Download the SampleSheet again by:
    a. Selecting *SampleSheet* in the Actions menu for a Sequencing Run.
    b. Select *Files* in the Actions menu, then as described above.

Metrics

The *Metrics* files, together with the SampleSheet are required for viewing run-level QC data in eMap and other applications such as *Sequencing Analysis Viewer* (Illumina Inc.). It may be necessary to share the Metrics files with your Service or Support representative for troubleshooting instrument-related issues.

Metrics files are accessed at the run-level using the *Files* menu described above. If the Metrics files are missing, check that they were included during the upload. It may be possible that Metrics files were not included if the data was uploaded from a different location than the Sequencer computer itself.

Clicking on the Metrics header will download a single .zip folder containing the *runinfo.xml*, *runparameters.xml* and the *InterOp* subfolder containing the metrics files.

Analysis Result Files

The copy-number analysis result files may be used to configure other applications to re-draw elements of the copy-number chart, plot b-allele frequency histograms, or generate alternate Report formats.

1. The *.stats.tsv, *chr_stats.tsv, *.bin_stats.tsv, and *.snp_stats.tsv files can be exported for individual samples by clicking Download release notes on the *Sample View* page. See the *Download Sample Data* section under *Reviewing Sample Results.*
2. Alternatively, to export files for multiple samples in the same Run, select *Files* in the Actions menu, then click ⭳, as described above.

Export Metadata by Sequencing Run

The sample metadata file may be used to configure other applications to generate alternate Report formats. The exported file for a Sequencing Run contains the complete sample data including Accessioning, Lab Planning, QC Data, Results, Sign-off status and Analysis Setting entries.

1. See the *Export Data by Run* section under *Run View* for instructions on downloading sample metadata.
2. The tab-delimited .tsv file contains a timestamp for traceability.
3. See *Appendix 3* for a list of data fields that are exported.

7 TECHNICAL SUPPORT

Release Notes

Release notes for the Software Version (eMap web application and user interface) and Analysis Version (algorithm and data processing) can be accessed from the webpage footer, displayed throughout eMap.

1. Log in to your eMap account.
2. Click on the current Software Version of the webpage footer *Release vN.N.N*.

3. A new page will open with Release Notes listed according to Software or Analysis Version.
4. Click on a Release Note to view and download via your web browser.

Contact Information

For technical assistance, contact Vitrolife Genomics Support:

Email	support.genomics@vitrolife.com
Website	www.vitrolife.com

APPENDIX 1: FREQUENTLY ASKED QUESTIONS

1. Is my data safe?

eMap has been designed to ensure data security. Data protection is provided at multiple levels:

- The hardware and software infrastructure is set up by Amazon Web Services (AWS) to a level that reaches HIPAA and HITECH compliance and industry-recognised certifications and audits such as ISO 27001, FedRAMP, and the Service Organization Control Reports (SOC1, SOC2, and SOC3).
- All data is encrypted during transfer and at rest. Ask your representative about further protection mechanisms.
- By using upload keys and passwords, only the specified Group of Users have access to the data. Vitrolife personnel or the third-party provider managing the system, cannot log into your account without the User granting them access.
- If identifiable information is a concern, the eMap system can be used with non-identifiable IDs and without providing personal information such as Subject names.
- The sequencing data BAM files will be automatically deleted after 30 days.
- By default, Vitrolife does not have access to the data entered into the system.
- Please refer to the terms and conditions of the software and the contracts you have with Vitrolife for additional information.

2. Which browser should I use?

The eMap analysis software has been tested to be accessible using the following systems and browsers (earliest version tested):

Operating System	Browser
Microsoft Windows 7	Chrome 89.0.4389.90 Microsoft Edge 89.0.774.54
Microsoft Windows 10	Chrome v88.4324.150 Microsoft Edge 88.0.705.63 Firefox Browser 85.0.2 Opera 74.0.3911.107
Apple OSX	Chrome 88.0.4324.182 Safari 14.0.3 Firefox 84.0.2

*The Microsoft ”Internet Explorer” browser is outdated and not supported by eMap.

3. Why does the view not show the state of the Run or Sample I expect?

Refresh your browser page to make sure you are viewing the most recent data.

4. My Sequencer had problems with secondary analysis, how can I generate BAM files and analyse them in eMap?

You will need to re-queue the run on the sequencing instrument to re-initiate analysis. For instruments operating on Windows 10, this can be performed from the LRM dashboard. New BAM files will be created in a new alignment folder (e.g. Alignment_2) of the run output directory. Once finished, you can upload the data with the eMap manual uploader (*eMap-upload-interface.exe*), by setting the path to the new BAM files in the run output directory (e.g. “Alignment_2\YYYYMMDD_HHMMSS”).

Alternatively, a new run can be created in LRM and existing run data can be imported to re-queue the analysis. In this case, the new BAM file directory will appear within the latest alignment folder, but with a new timestamped sub-folder for the new analysis, e.g. “Alignment_1\YYYYMMDD_HHMMSS”.

Ensure that the correct path to BAM files is entered in the eMap-upload-interface.exe for upload. If part of the run was previously uploaded to eMap, also select the “Overwrite” checkbox in the eMap-upload-interface.exe under *Advanced Configuration* settings.

5. I’ve lost internet connection, but need to start a sequencing run, what do I do?

You can create an “offline” SampleSheet with the Microsoft Excel template provided by Vitrolife and initiate your run with this file. After the manual data import (see section *3 Uploading Data - Custom Import*), you will need to link the Samples to Subjects and Cycles manually as they are not yet known to eMap.

6. Is there a LIMS integration?

As there are many different laboratory data systems on the market and no standardised interfaces defined, eMap has the option to import Subject and Sample data from simple tab-separated text files (Accessioning and Fast Track, see section *2 Planning*) and export all results of a run to a tab-separated text file format (see section *6 Export Data – Export Metadata by Sequencing Run*). This way the integration with a LIMS can be made with simple custom scripts.

7. Why does eMap report different genomic coordinates when using segmental calling?

Segmental changes are reported with the exact start and end coordinates of the region interrogated. If the telomeric start of a chromosome contains only “N” bases in the reference genome, a copy-number cannot be measured for this part of the chromosome and the first region might start at position e.g. *chr6:6384*. Chromosome-wide results reported simply use the official start and end coordinates of the chromosome and would show e.g. *chr6:1* as the start.

8. Why is my run in the “Waiting” or “Processing” state for a long time?

The system is designed to scale with the processing demand and should process your data with a speed of a few minutes per sample at the most. Upload speed from your location will influence the total time. Please check the log file of the upload software to make sure the data has been transferred into your eMap account.
Refresh your browser window to make sure you are viewing the latest data. If no progress is made after 2h, please contact your Vitrolife representative.

APPENDIX 2: DATA IMPORT FORMATS

Note for all import files, the **date format** must be stored as text with the format *YYYY-MM-DD.* When opening template files in Microsoft Excel or similar, the date format may be automatically updated and will require converting to text before saving the file as a tab-delimited TXT or TSV file.

Accessioning

Two file formats may be used to accession sample data.

1. Format A (*eMAP_Accessioning_A.txt*) is suitable for single Subject records, and;
2. Format B (*eMAP_Accessioning_B.txt*) is suitable for records combining data from multiple Subjects.

If importing data to existing Subjects (e.g. adding a new Cycle), the Subject data (names, DOB, consultant, referring centre and notes) must match the existing entries in the eMap software exactly, or be left blank.

Table 19: eMap_Accessioning_A.txt requirements

Parameter	Description
[Header]	
File_Date	Date the file was created, stored as text with the format YYYY-MM-DD
File_Type	A
File_Version	1
Subject_ID	Unique identifier for the Subject; alphanumeric characters and dashes only [-]
Last_Name	Optional. 60 character limit.
First_Name	Optional. 60 character limit.
DOB	Optional. Date of Birth of the Subject, stored as text with the format YYYY-MM-DD
Consultant	Optional. 60 character limit.
Referring_Centre	Recommended. Used to filter sample statistics on the HOME page. 60 character limit.
Notes	Optional.
Partner_Last_Name	Optional. 60 character limit.
Partner_First_Name	Optional. 60 character limit.
[SampleTable]	
Cycle_ID	Required. Identifier of the Cycle containing Samples
Sample_Tube_ID	Recommended. Used on Laboratory Planner PDF to identify Sample tubes
Sample_ID	Unique identifier for the Sample; alphanumeric characters and dashes only [-]
Sample_Barcode	Optional. Must be unique to eMap database
Sample_Type	Required. Match exact text [Trophectoderm / Genomic DNA / Other / Negative]
Sample_Requisition	Optional.
Sample_Description	Optional.

For Format B files, ensure the Subject-level information is identical on all sample rows for each Subject. Data clashes will result in an import failure.

Table 20: eMap_Accessioning_B.txt requirements

Parameter	Description
[Header]	
File_Date	Date the file was created, stored as text with the format YYYY-MM-DD
File_Type	B
File_Version	1
[SampleTable]	
Subject_ID	Unique identifier for the Subject; alphanumeric characters and dashes only [-]
Cycle_ID	Required. Identifier of the Cycle containing Samples
Sample_Tube_ID	Recommended. Used on Laboratory Planner PDF to identify Sample tubes
Sample_ID	Unique identifier for the Sample; alphanumeric characters and dashes only [-]
Sample_Barcode	Optional. Must be unique to eMap database
Sample_Type	Required. Match exact text [Trophectoderm / Genomic DNA / Other / Negative]
Sample_Requisition	Optional.
Sample_Description	Optional.
Last_Name	Optional. 60 character limit.
First_Name	Optional. 60 character limit.
DOB	Optional. Date of Birth of the Subject, stored as text with the format YYYY-MM-DD
Consultant	Optional. 60 character limit.
Referring_Centre	Recommended. Used to filter sample statistics on the HOME page. 60 character limit.
Notes	Optional.
Partner_Last_Name	Optional. 60 character limit.
Partner_First_Name	Optional. 60 character limit.

Quantification Data

Quantification data for the final prepared and size-selected libraries may be imported to the Pool and Sequence page of eMap software. Quantification values (ng/µL) must be arranged according to Index Name, e.g. “UDI-A01” using the template *eMAP_QuantData.txt*. Save as a tab-delimited TXT file.

Table 21: eMap_QuantData.txt requirements

Parameter	Description
[Header]	
File_Type	QT
File_Version	1
[Data]	
Index_ID	Required. The index name as it appears on the SampleSheet. e.g. UDI-A01
Lib_Conc_ng_ul	Required. Enter the concentration value for the respective Index name.

Fast Track Import

If Laboratory Planning and index tracking are to be performed using an alternate software or system (e.g. LIMS), sample Accessioning and Laboratory Planning data can be imported together using a single file per Plate ID, prior to pooling. The import file *eMap_Fast_Track.txt* contains all relevant fields for Accessioning, index assignment and quantification values. Upon importing a Fast Track file, the User can continue with the EmbryoMap protocol from the Pooling stage.

For Fast Track import files, ensure the Subject-level information is identical on all sample rows for each Subject. Data clashes will result in an import failure.

Table 22: eMap_Fast_Track.txt requirements

Parameter	Description
[Header]	
File_Date	Date the file was created, stored as text with the format YYYY-MM-DD
File_Type	FA
File_Version	1 = EmbryoMap, 2 = EmbryoMap WGA, 3 = EmbryoMap SNP
Kit_Lot	Required. Lot number of the EmbryoMap Sample Prep Kit
WGA_Mix_Lot	Required for file version 2 only. Lot number of the EmbryoMap WGA Kit
SNP_Lot	Required for file version 3 only. Lot number of the EmbryoMap SNP kit
Plate_ID	Required. Must be unique in eMap database
Preparation_Date	Date the samples were prepared, stored as text with the format YYYY-MM-DD
User_Initials	Username of the operator
[SampleTable]	
Index_ID	Required. The index name as it appears on the SampleSheet. e.g. UDI-A01
Subject_ID	Unique identifier for the Subject; alphanumeric characters and dashes only [-]
Cycle_ID	Required. Identifier of the Cycle containing Samples
Sample_Tube_ID	Recommended. Used on Laboratory Planner PDF to identify Sample tubes
Sample_ID	Unique identifier for the Sample; alphanumeric characters and dashes only [-]
Sample_Barcode	Optional. Must be unique to eMap database
Sample_Type	Required. Match exact text [Trophectoderm / Genomic DNA / Other / Negative]
Sample_Requisition	Optional.
Sample_Description	Optional.
Last_Name	Optional. 60 character limit.
First_Name	Optional. 60 character limit.
DOB	Optional. Date of Birth of the Subject, stored as text with the format YYYY-MM-DD
Consultant	Optional. 60 character limit.
Referring_Centre	Recommended. Used to filter sample statistics on the HOME page. 60 character limit.
Notes	Optional.
Partner_Last_Name	Optional. 60 character limit.
Partner_First_Name	Optional. 60 character limit.

APPENDIX 3: EXPORT SAMPLE METADATA

Parameter	Description
Sample_ID	The accessioned Sample ID
Index	Sequencing index assigned during Laboratory Planning
Sample_Type	The accessioned Sample Type [Trophectoderm / Genomic DNA / Other / Negative]
Internal_ID	Internal eMap reference number for the Sample
Sample_Name	Concatenation of <Cycle ID>-<Sample ID>
BAM_File	Name of the imported sequencing result file containing aligned reads
Subject_ID	The accessioned Subject ID
Last_Name	The accessioned Subject last name
First_Name	The accessioned Subject first name
Date_of_Birth	The accessioned Subject date of birth
Partner_Last_Name	The accessioned Subject partner's last name
Partner_First_Name	The accessioned Subject partner's first name
Consultant	The accessioned Consultant
Referring_Centre	The accessioned Referring Centre
Notes	The accessioned Notes for the Subject
Cycle_ID	The accessioned Cycle ID
Tube_ID	The accessioned Tube ID
Barcode	The accessioned Sample Barcode
Requisition	The accessioned Sample Requisition ID
Sample_Description	The accessioned Sample Description
Accessioning_Date	The date the Sample was accessioned to eMap
Plate_ID	The Plate ID the Sample is assigned to after Laboratory Planning
Preparation_Date	The date the samples were prepared, as defined during Plate ID creation.
Plate_Operator	The eMap User who created the Plate ID
Kit_Lot	The EmbryoMap Sample Prep Kit lot entered on the Plate Builder page
Pool_ID	The Pool ID the Sample is assigned to after Run Definition
Lib_Conc_ng_uL	The imported/entered final library concentration ng/µL
Pooling_Mass_ng	The selected pooling mass (ng) used for Pooling
Pooling_Vol_uL	The volume µL of sample final library added to the Pool
Default_Vol	Selection of the *Default* checkbox during Pooling. <Yes> or <No>
Measured_Pool_Conc_ng_uL	The [Optional] Final pool concentration (ng/µL) value entered on the Run Definition page, if applicable.
Pool_Conc_nM	Calculated concentration (nM) of the Pool according to the Pooling Guide PDF
Run_description	The description entered during Run Definition
Flowcell_ID	The Flow Cell ID used for sequencing, read from imported sequencing result files
Cartridge_ID	The Reagent Cartridge ID used for sequencing, read from imported sequencing result files
Sequencing_instrument	The serial number of the sequencing instrument, read from imported sequencing result files
Run_Folder	Name of the Run Folder containing sequencing result files
Sequencing_Date	Date of sequencing, read from imported sequencing result files
Sex_Automatic	Sex of the Sample predicted by the eMap algorithm according to the Settings selected.
Sex_Reported	Sex of the Sample to be reported, following any manual annotations made to the copy-number chart.
CNV_Status_Automatic	Written copy-number imbalance status of the Sample predicted by the eMap algorithm according to the Settings selected.

CNV_Status_Reported	Written copy-number imbalance status of the Sample to be reported, following any manual annotations made to the copy-number chart.
Karyotype_Automatic	Karyotype of the Sample predicted by the eMap algorithm according to the Settings selected.
Karyotype_Reported	Karyotype of the Sample to be reported, following any manual annotations made to the copy-number chart.
Regions_called	A single string containing abnormal region information separated by a colon [:] Chromosome Start Position (hg38), where "Start" refers to the 0-based start of the chromosome reference. End Position (hg38), where "End" refers to the last bp of the chromosome reference. Size (Mb) Region Type Copy-number median Region Confidence Annotation Type <automatic> or <manual> Where multiple regions are included in the same string, and separated by a semi-colon [;]
CNV_QC_Status_Automatic	The QC Status of the Sample set by the eMap algorithm according to the Settings selected.
CNV_QC_Status_Reported	The QC Status of the Sample to be reported, following manual selection of the status during sample Sign-off.
Analysis_Notes	Sample-level notes entered on the Sample View page
Av_Align_Score	Mean alignment quality for the Sample reads, which also considers read quality.
Total_Reads	Number of total reads for the Sample
Mapped_Reads	Number of aligned reads for the Sample
Filtered_Reads	Number of reads after filtering for the Sample
Overall_Noise	Overall noise (DLR) value for the Sample
SD_Robust	Robust standard deviation value for the Sample
SDI	SDI noise value for the Sample
Manual_Annotation	Flag if manual annotations were made to the copy-number chart <Yes> or <No>
Level_1_signoff	Username of the User who 'Locked' the sample, where <empty> means the sample is unlocked.
Level_2_signoff	Username of the User who 'Signed-Off' the Sample, where <empty> means the Sample is not Signed-off.
Sign_Off_Comment	If the sample is Signed-Off, show the most recent comment entered in the Sign-off dialogue. This is the same value that would be displayed on the Full Report.
Sign_Off_History	A single string containing Sign-off history entries information separated by a comma [,] Timestamp <YYYY-MM-DD HH:MM:SS> Action Username Sign-off Comment (where applicable, and Action is "Level 2 Sign-off"). Sign-off Status (See also *QC_Status_Reported*) Where multiple Sign-off history entries are included in the same string, and separated by a semi-colon [;]
Date_Sample_Last_Update	Timestamp of the last change made to the Sample
Software_Version	Version number for the eMap User Interface
Analysis_Version	Version number of the eMap copy-number algorithm used for analysing this Sample.
Reference_Version	Name of the reference dataset used for normalisation by the eMap copy-number algorithm when analysing this Sample.
Smoothing	The smoothing setting used by the eMap copy-number algorithm when analysing this Sample.
Minimum_Manual_Region	Minimum permitted number of consecutive bins to create a manual annotation. Default = 2
Minimum_Automatic_Region	Number of bins required for automatic sub-chromosomal region annotation. Default = 10
Custom_CN_Thresholds	Custom copy-number thresholds enabled when analysing this Sample <Yes> or <No>
Segmental_Calling	Sub-chromosomal detection enabled when analysing this Sample <Yes> or <No>

Telomere_Correction	Putative artefact correction enabled when analysing this Sample <Yes> or <No>
Sex_Masking	Masking of sample sex enabled when analysing this Sample <Yes> or <No>
Recentering	Chromosome(s) selected for re-entering the copy-number chart where <empty> = feature disabled
Thresholds_Gain	A single string containing absolute copy-number thresholds separated by a colon [:] (e.g. "2.7:2.5:2.3") Full gain High-level mosaic gain Low-level mosaic gain
Threshold_Loss	A single string containing absolute copy-number thresholds separated by a colon [:] (e.g. "1.7:1.5:1.3") Low-level mosaic loss High-level mosaic loss Full loss
Chromosome_Number	The sum of whole-chromosome copies reported in the sample karyotype. Displayed if Group Setting *Show chromosome number* = "Yes", else "NA"
Individual result columns for chromosomes 1-22, X and Y.	Copy-number changes are displayed by chromosome in a single string, using a combination of the following shorthand letter notations and the region copy-number median value, separated by a colon [:] L Copy-number loss G Copy-number gain s Segmental copy-number change (affecting part of a chromosome only) m Low-level intermediate copy-number change (putative mosaic change) M High-level intermediate copy-number change (putative mosaic change) Where multiple chromosome abnormalities are included in the same string, and separated by a semi-colon [;]
Karyotype_Reported_Format	The applied Group Setting for karyotype reporting format
Show_Chromosome_Number	The applied Group Setting for including the chromosome number in the reported karyotype <Yes> or <No>.
SNP_Lot	The EmbryoMap SNP Kit lot entered on the Plate Builder page
WGA_Mix_Lot	The EmbryoMap WGA Kit lot entered on the Plate Builder page
Workflow_Version	The EmbryoMap workflow version displayed on the pooling page based on the selected Workflow and SampleSheet.
Reads_length	The read length in the SampleSheet based on the workflow version.
SNP_Analysis	Whether the SNP analysis has been applied for a sample based on the Workflow and sequencing run <Yes> or <No>.
SNP_Ploidy_Status	Ploidy Status automatically predicted by the SNP algorithm for each sample.
Allelic_Consistency_Status	Status automatically generated by the SNP algorithm to summarise the Allelic Consistency result.
Allelic_Consistency	The score assigned to each sample to reflect how closely the allele balance reflects the predicted ploidy status.
SNP_QC_Status	The SNP QC Status of the Sample set by the eMap algorithm according to fixed settings.
SNP_Filtered_Reads	Number of reads available for SNP analysis for the sample.
Heterozygous_SNP_Calls	Number of SNPs with the minimum read depth available for analysis.
Ploidy_Confidence	The level of confidence in the predicted Ploidy Status
SNP_Reference_Version	The EmbryoMap SNP Panel version.
Minimum_SNP_Depth	The minimum read depth for SNPs to be utilised by the SNP analysis. Default = 10.
Hide_Ploidy_Status	The applied Group Setting to enable hiding of the sample Ploidy Status from the SNP results <Yes> or <No>.
Hide_Allelic_Consistency_Status	The applied Group Setting to enable hiding of the Sample Allelic Consistency Status from the SNP results <Yes> or <No>.

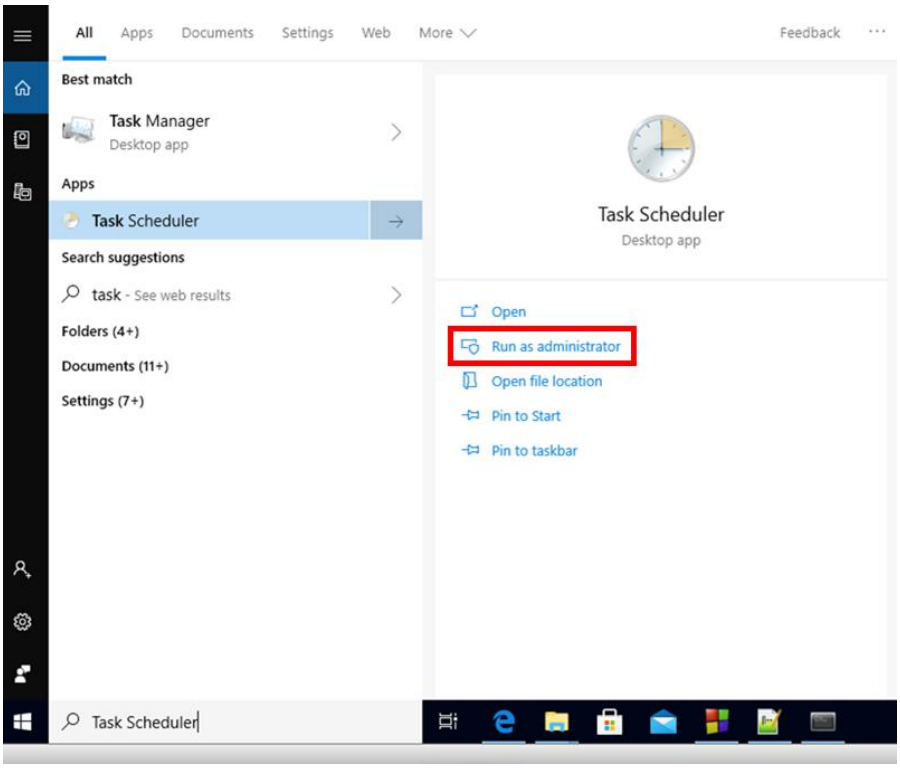
APPENDIX 4: INSTALLING THE EMAP UPLOAD SOFTWARE

Once the sequencing and initial processing of EmbryoMap samples on the Sequencer is completed, the files required for analysis are automatically uploaded to the Vitrolife eMap software platform set up for every customer account.
To allow this to happen, a software monitors the Sequencer output folder and triggers the upload once all data for a new run has arrived in this folder. The data upload is performed by a second piece of software connecting to the eMap platform, validating the files found and securely transferring the data into the customer's account.
For occasions where a manual upload is required or desired, an additional User-friendly software interface is provided to initiate the transfer of a specific sample set.
To install all three components on your Sequencer computer follow the steps below. To install the manual upload software on additional computers, you can ignore the "*monitor*" software component and stop after completing Step 4 below.
Check LRM for the default path for the sequencing output folder.

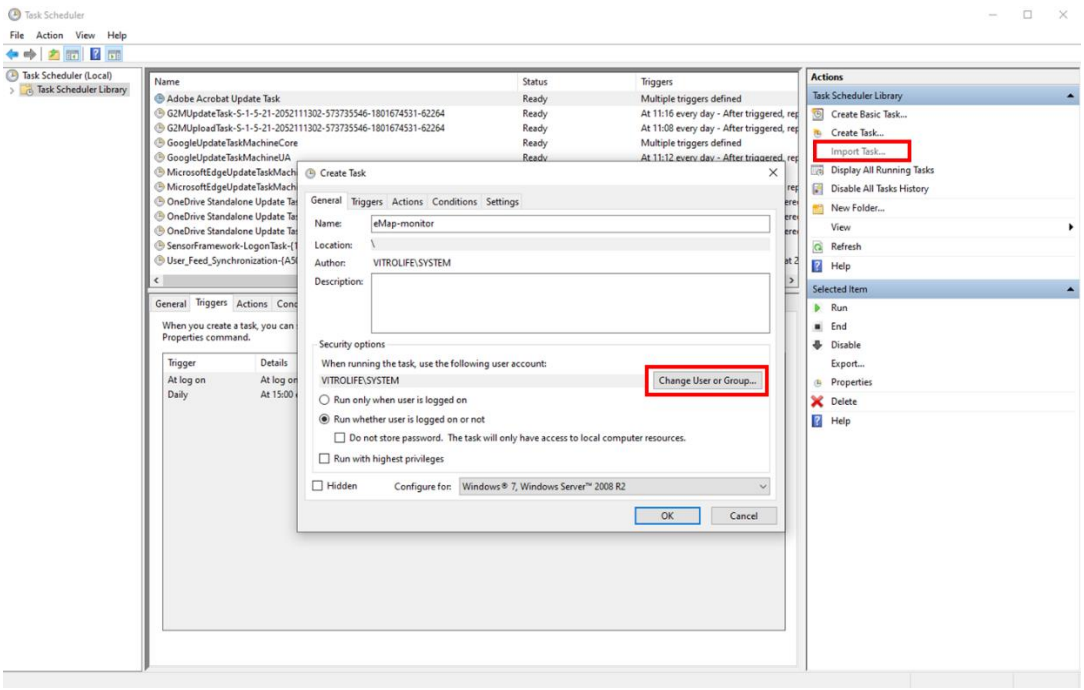
1. Download and extract the software .zip package provided by your Vitrolife Support representative.
   The .zip folder contains:

Program Name *config file name*	Description
emap-upload.exe *upload-config.json* (User-supplied)	Connect to eMap User account, validate input, transfer, and validate files.
emap-monitor.exe *monitor-config.txt*	Run in the background to detect new EmbryoMap run data and initiate upload automatically.
emap-upload-interface.exe	User interface to perform data uploads manually.
emap-Monitor.xml	Definition file for Windows *Task Scheduler* for automatic monitoring of the Sequencing analysis folder (using the emap-monitor.exe).

2. Copy the entire *Vitrolife* folder containing the software in the location "*C:\ProgramData\Illumina*" on the sequencing instrument. Ensure the location is protected from unauthorised access (Administrator permission may be required).
   Custom locations may be used. Update the file paths accordingly in the *monitor-config.txt* file and manual uploader (*eMap-upload-interface.exe*) advanced configuration settings. Contact Vitrolife Support for assistance.
3. **Optional**. Add a shortcut to the *eMap-upload-interface.exe* software on the Desktop.
4. Prepare the unique Access Key:
   a. Copy and paste the entire personal **Access Keys** text from the eMap *ACCOUNT* > *Profile* page, into a text editor (e.g. Notepad++).
   b. Save with the file name *upload-config.json.*
   c. Place the *upload-config.json* file in the *Vitrolife* folder together with the other eMap upload software.
5. Set up the automatic task:
   a. Open the Windows *Task Scheduler* by searching for it and selecting *Run as administrator.* Ensure you have the computer's administrator name and password at hand.

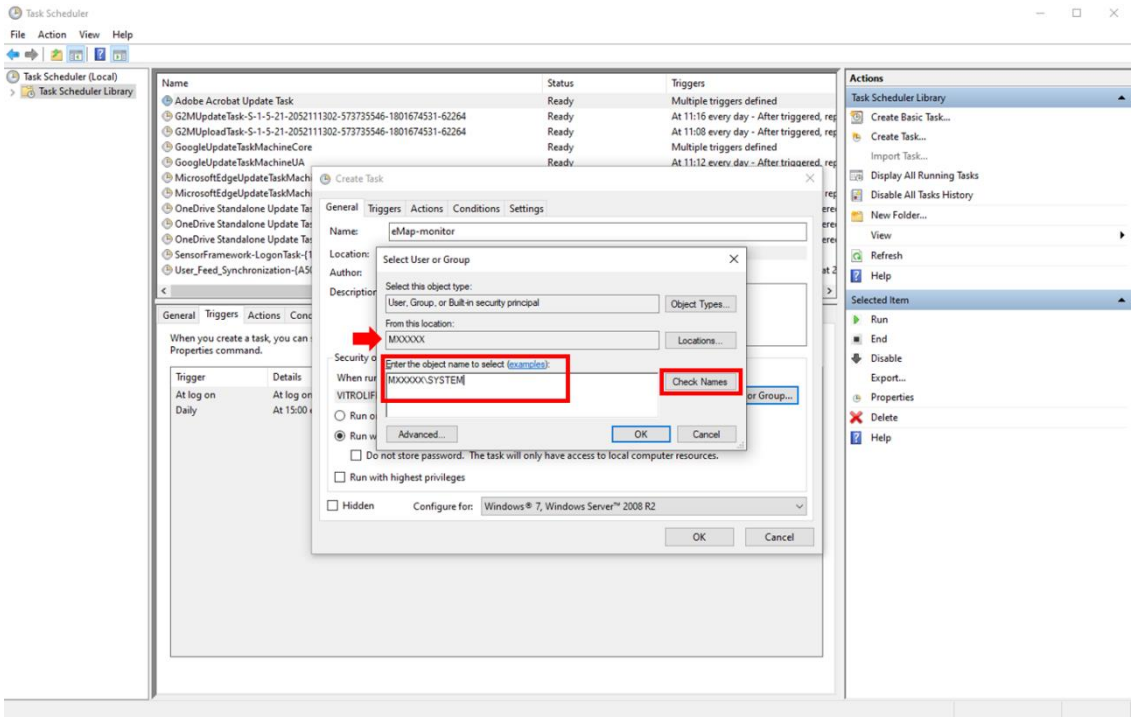


b. Select *Import Task* from the Actions menu on the right-hand side of the screen and open the *MiSeq-monitor.xml* file. Most required fields will be automatically filled.
Select the *Change User or Group* button.

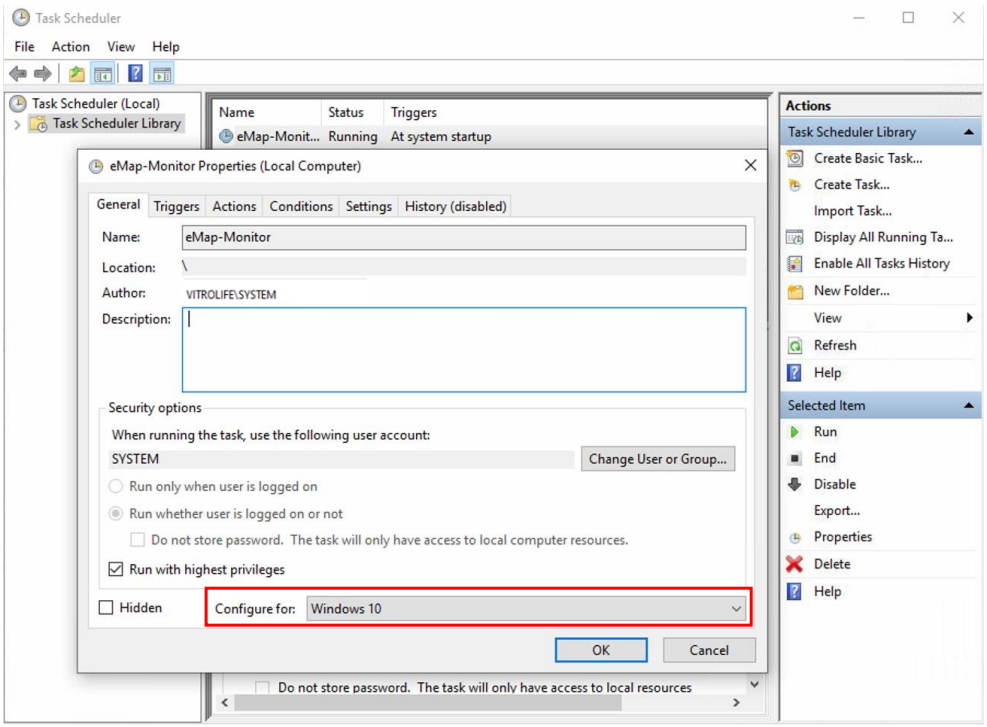


c. Modify the User by typing *<ComputerName>\SYSTEM* into the object name text box. The <ComputerName> is shown under "From this location" (red arrow). e.g. for a MiSeq with computer name "MXXXXX", type

MXXXXX\SYSTEM. Click the *Check Names* button to confirm the User account. If correct, you will see the User account update to "SYSTEM". Click *OK.*



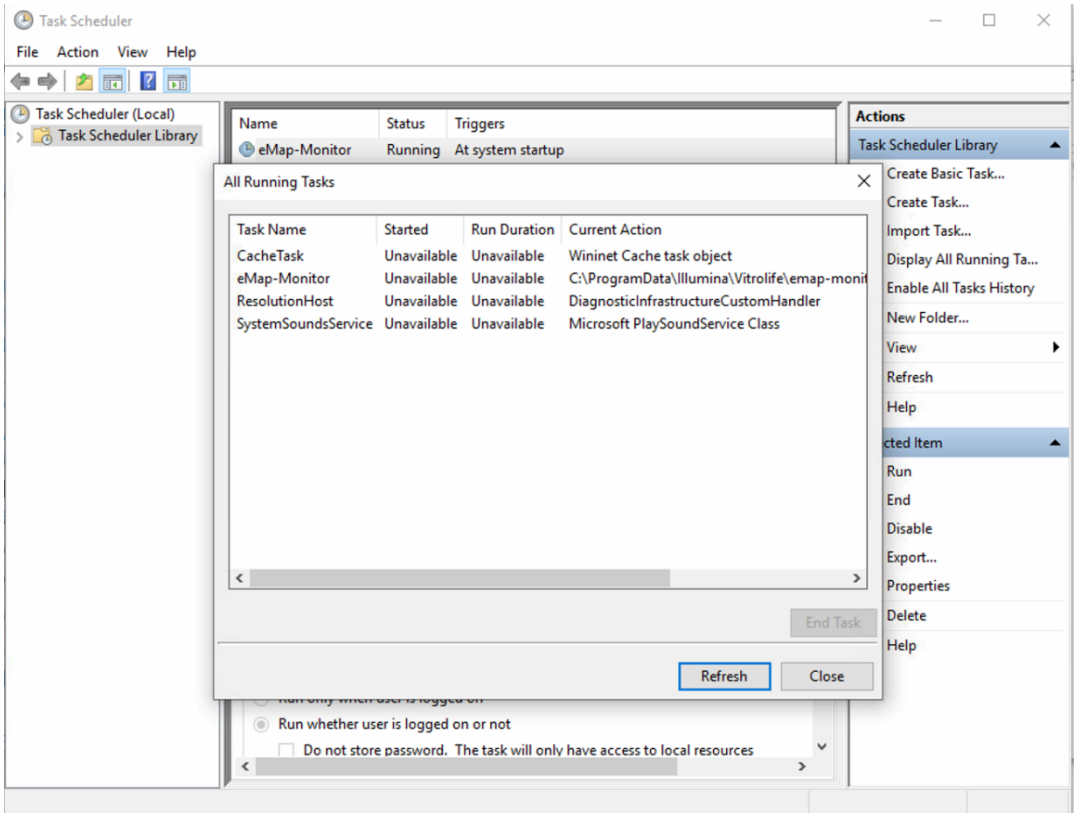
d. Select the current version of Windows for the computer from the menu, then click *OK* to complete the setup.



e. Click *Task Scheduler Library* (on the left) to confirm the new task is listed, select the new task (in the centre) and click *Run* (on the right) to start it. Verify that the new task is running by clicking on *Display all running tasks* (on the right).

f. Close the *Task Scheduler.*

- A log file called “emap-monitor.log” is created when the monitor software is started. The default location of the log file is in the Sequencer output folder in "*D:\Illumina”*. Open the log file with a text editor to verify correct operation.



6. After a new run folder is detected in the output directory, the monitor software will:
   a. Identify if the run is an EmbryoMap run. The run folder will be *ignored* if the parameters do not match those expected for an EmbryoMap run.
   b. Wait for sequencing and secondary analysis (alignment) to complete.
   c. Wait an additional 15 minutes to allow all copy operations to complete.
   d. Trigger the data upload software *emap-upload.exe* using the unique *upload-config.json* credentials present in the same directory.
7. Review the emap-monitor.log file from the Sequencer output folder in "*D:\Illumina”*, or share it with Vitrolife Support to troubleshoot any issues.