Olink®

NPX™ Map

Software User Manual

1532, v1.0.2, 2025-02-07

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Part 1: Introduction

Olink high-multiplex immunoassay panels provide an efficient and innovative tool for targeted human protein biomarker discovery, development and validation.

1. About this manual

This user manual provides you with the instructions needed for data processing when running Olink® Reveal, Olink® Explore HT, or Olink® Explore 3072/384 panels with a locally installed set up of software.

NOTE: *The information in the NPX™ Map Software User Manual is believed to be accurate. However, the displayed screenshots may differ from actual user interface and should be considered as examples.*

1.1 Intended use

NPX™ Map is a data analysis software that is designed for Olink Reveal, Olink Explore HT, and Olink Explore 3072/384 platforms. It allows for importing data, validating data quality, and normalizing Olink data for subsequent statistical analysis.

NPX Map is intended for Research Use Only. Not for use in diagnostic procedures.

 For questions, guidance, and support, contact Olink Support at *support@olink.com*.

1.2 Intended target group

NPX Map is intended to be used by staff certified to run the Olink Reveal, Olink Explore HT or Olink Explore 3072/384 platform. Quality control should be performed by trained users that determine whether data from a run can be approved for further analysis.

2. List of abbreviations

%CV	Coefficient of Variance
NGS	Next Generation Sequencing
PCR	Polymerase Chain Reaction
PEA	Proximity Extension Assay
QC	Quality Control
SD	Standard Deviation

3. Associated documentation

3.1 Olink documentation

- Olink® Map Preprocessing Technical information, vx.x or later
- NPX™ Map CLI Technical information, vx.x or later

Olink Reveal manuals

- Olink® Reveal Overview User Manual
- Olink® Reveal Laboratory Instructions

Olink® Explore HT manuals

- Olink® Explore HT Overview User Manual
- Olink® Explore HT F.A.S.T. lab instructions
- Olink® Explore HT Mosquito lab instructions
- Olink® Explore HT Sequencing using NovaSeq 6000 S4 User Manual
- Olink® Explore HT Sequencing using NovaSeq X User Manual

Olink® Explore 384/3072 manuals

- Olink® Explore Overview User Manual
- Olink® Explore 384 User Manual
- Olink® Explore 4 x 384 User Manual
- Olink® Explore 3072 User Manual
- Olink® Explore Sequencing using NextSeq 550 User Manual
- Olink® Explore Sequencing using NextSeq 2000 User Manual
- Olink® Explore Sequencing using NovaSeq 6000 User Manual
- Olink® Explore 384/3072 Sequencing using NovaSeq X Plus User Manual

All relevant Olink documentation is available from the Olink website *www.olink.com/downloads*.

3.2 Other documentation

- Article & lot configuration sheet - delivered together with the analysis kit.

4. Technical support

For questions, guidance and support, please contact Olink Proteomics at *support@olink.com.*

5.Process

5.1 Hardware and software requirements

5.1.1 System requirements for NPX™ Map

Components	Minimum	Recommended
Operating System	Windows® 10 or Windows® 11	
Processor	Intel® Core™ i5	Intel® Core™ i7 or higher
Memory	16 GB RAM	32 GB RAM or more
Disk Space	500 GB	500 GB
Display scale of computer screen		Scale 100 % * 14" screen

* The scale resolution might affect different functions and features in the NPX Map software, and different scale resolutions might be needed for different displays. Please, go to the computer settings to change to your preferred settings.

5.1.2 System requirements for Preprocessing

Components	Minimum	Recommended
Operating System	Linux Ubuntu 20.04 LTS or Linux Ubuntu 22.04 LTS	
Other	Linux administrative knowledge	

5.1.3 Requirements for analysis

Files and information needed for analysis:

- Count files in CSV format and run metadata in JSON format (NGS raw data preprocessed using ngs2counts)
- Plate layout with sample names
- Data analysis reference ID number of reagents used (provided on the Lot configuration insert delivered with the kit)

5.2 Preprocessing runs

Preprocessing is a necessary step in NPX Map analysis. The Next Generation Sequencing (NGS) output from the sequencing instrument is converted to counts files containing the number of reads for each Olink sequence and a run metadata file containing additional information which is necessary for importing the counts files into the analysis software NPX Map or NPX™ Map CLI.

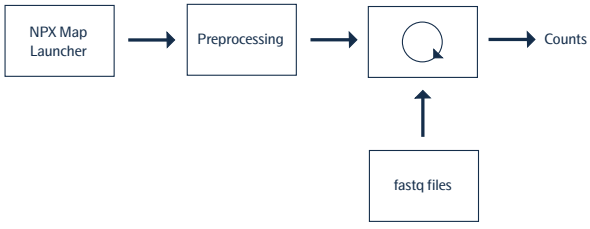
The preprocessing can be performed in two different ways:

Alternative A

In this alternative, the preprocessing is done in the preprocessing software.

Alternative B

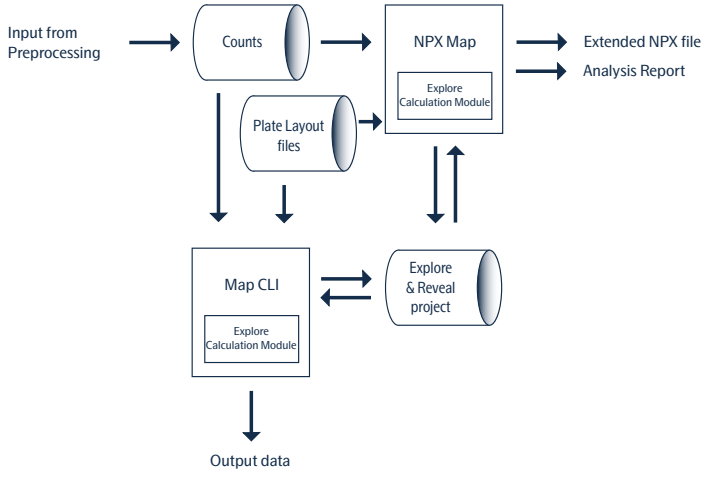
In this alternative, the fastq files are imported directly to the NPX Map.



For more information about the different preprocessing versions, refer to the NPX™ Map Preprocessing Technical information.

5.3 CLI

QC and normalization of Olink Reveal, Olink Explore HT, and Olink Explore 3072/384 data can be performed either using the NPX Map CLI or the NPX Map Software, or using a combination of the two.



NPX Map is a command-line interface (cli). The application is capable of performing normalization, Quality Control (QC) and CV computations on NGS data, and exporting the results on several supported formats.

For more information, refer to the NPX™ Map CLI Technical Information.

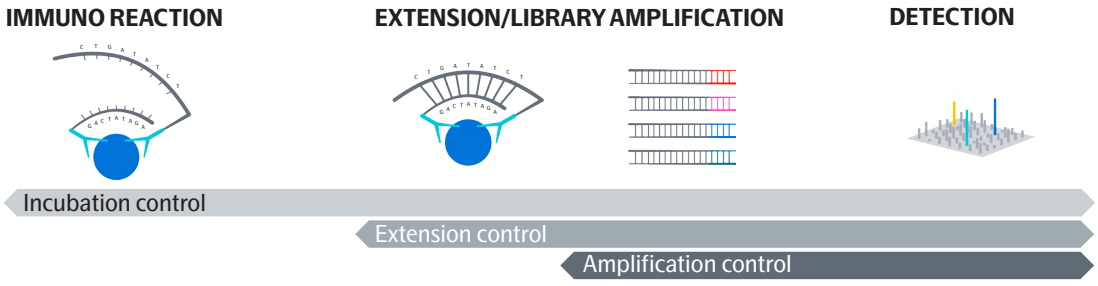
Part 2: Technology description

6. Overview

Olink Reveal, Olink Explore HT, and Olink Explore contains a built-in quality control system using internal and external controls, which enables full control over the technical performance of assays and samples.

6.1 Internal controls

Three internal controls are spiked into every sample for each panel and dilution. The internal controls are designed to monitor the quality of the assay's performance, as well as the quality of individual samples:



Incubation Control (Immuno Control)
The Immuno Control is a non-human antigen measured with PEA. This control is included in the immuno reaction and monitors potential technical variation in all three steps of the reaction.

Extension Control
The Extension Control is an antibody coupled to a unique pair of DNA-tags. These DNA-tags are always in proximity so this control is expected to give a constant signal independent of the immuno reaction. This control monitors variation in the extension and amplification step.

Amplification Control
The Amplification Control is a complete double-stranded DNA amplicon that does not require any proximity binding or extension step to generate a signal. This control monitors the amplification/sample indexing step.

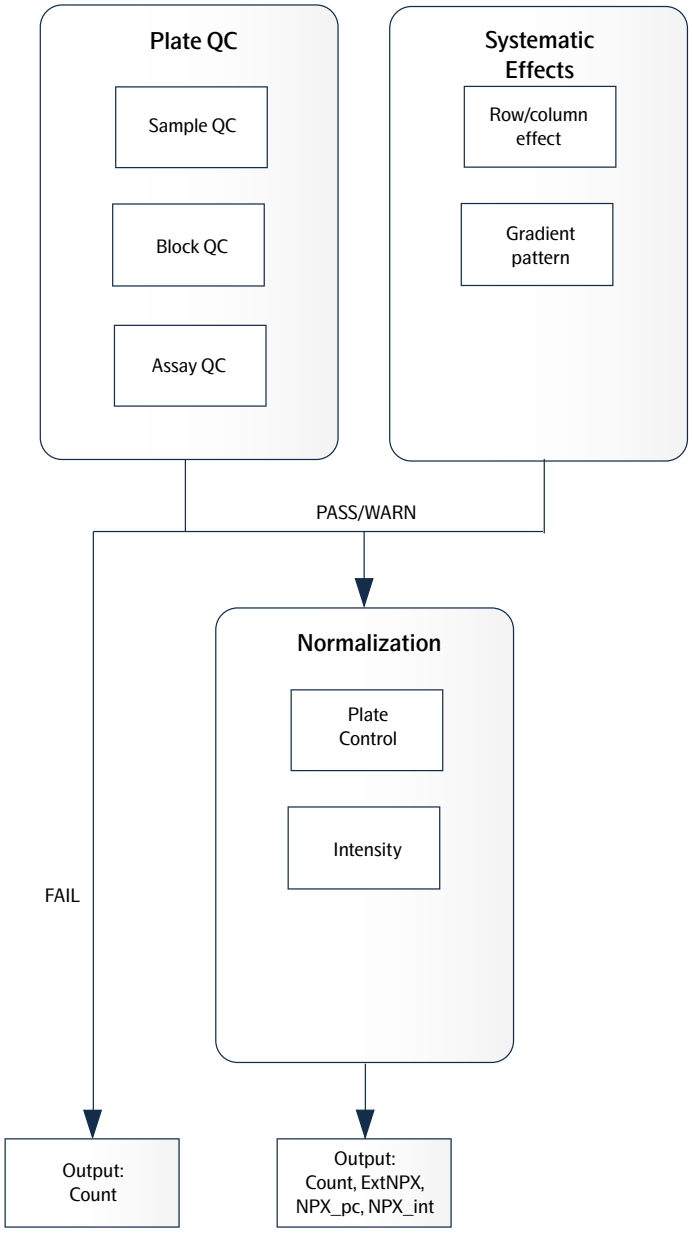
The Extension Control is used to calculate the NPX, refer to *7.4 Normalization*, and the other two are used in the Quality Control.

7.The QC workflow

Three internal controls are added to each sample to monitor the quality of assay performance, as well as the quality of individual samples:

- Incubation Control
- Extension Control
- Amplification Control

The Extension Control is used to calculate the NPX, refer to *7.4 Normalization*, and the other two are used in Quality Control (described below).



7.1 Plate QC

The QC is performed directly on Counts, on one block per 96 samples. The Plate QC are divided into three parts: Sample QC, Block QC, and Assay QC. For acceptance criteria, refer to *8. QC criteria*.

7.1.1 Sample QC

Samples and external controls that fail Sample QC will not be considered for additional QC steps and not normalized. Only counts will be reported for those.

1. Low total number of counts per sample per block
   Too low total counts for a sample might indicate that the sample is missing or the index was not added to the sample. Each sample and external control should have a minimum number of counts in a block, otherwise they fail.
2. Low number of counts of internal controls per block
   Low counts in any of the internal controls might indicate a technical error in the workflow for the corresponding sample, for example a missing internal control.
   - Each external control should have a minimum number of counts for each of the internal controls, otherwise they fail.
   - Each sample should have a minimum number of counts for each of the internal controls, otherwise they fail or get a warning.
3. Deviation of counts in Plate Controls
   Deviation of internal control's counts from the expected ranges in Plate Controls might indicate different technical errors.
   - A Plate Control fails if the fraction of counts of all internal controls to total counts in logarithmic scale, deviates positively or negatively from the reference values. The reference ranges are block specific and kit lot related.
4. Unexpected signal in Negative Controls
   Detection of high number of counts for many assays, relative to the counts of internal controls, in any Negative Controls might indicate that signals are from other sample types or not from pure buffer.
   - A Negative Control fails if many assays get higher number of counts relative to the counts of internal controls.

5. Deviation of incubation control ratio per sample per block
The relative count levels of incubation control to other internal controls (ratio in logarithmic scale) should be consistent across the plate. Any sample where these ratios deviate from the expectation by an amount larger than a reference value indicates a technical error in the workflow.

- The expected incubation control ratios are given by the median of the plate controls and sample controls per plate per block.
- An external control fails if the incubation control ratio is much lower than the expectation.
- A sample gets a warning if the incubation control ratio is much lower than the expectation.

7.1.2 Block QC

Block QC is to quality control whether a block-plate is affected by technical errors. This step is based on the number of Plate and Negative Controls that pass Sample QC. A failed block is recommended to be rerun.

1. Based on Plate Controls:
   - A block-plate fails if <50% of Plate Controls pass the Sample QC (a minimum of 3 Plate Controls is required to pass the block-plate).
2. Based on Negative Controls:
   - A block-plate fails if none of the Negative Control pass the Sample QC.

7.1.3 Assay QC

Detection of high number of counts for any assay, relative to the median count of three internal controls in all the Negative control is considered as unexpected signal. This step is performed on Negative Controls that pass Sample QC. An assay gets a QC warning if it gets high number of counts relative to the median counts of three internal controls in all Negative controls,

7.2 Systematic effect detection

Systematic effects are defined as a systematic pattern on a plate caused by a deviation in the workflow such as instrument failure or human error. A pattern corresponds to a particular type of failure. Detection of systematic effects is complementary to the Plate QC and is performed on NPX. All samples and data-points (passed, failed, and warned) are included in the computation.

Systematic effects are categorized into patterns that impact the full plate or impacting a row or/and column. To detect the patterns, three main criteria are considered:

- The frequency of assays representing the effect
- The intensity of the effect on NPX deviation
- Number of samples showing the effect

A pattern is detected per assay/block/plate unit. If enough assays (internal controls are not included) are affected, the block will get a systematic effects warning corresponding to that effect in NPX Map, refer to *15.3 Quality Control Summary*. Systematic effects may be due to either non-randomized plate design or technical errors and should be investigated further when warned.

7.2.1 Types of systematic effects

- Row
- Column
- Four_column
- Alternating_column
- Row_gradient
- Column_Gradient
- Diagonal_Gradient

7.3 Summary notes for exported data

- Data related to all assays, including the internal controls, will be presented in the output NPX file.
- Data related to all samples, including the external controls, will be presented in the output NPX file.
- Data related to any failed sample or block that do not pass QC criteria will not be normalized and therefore no NPX will be computed. Counts are reported for the failed data-points in the NPX file.
- Any data-points with a QC warning should be used with caution.

For any more information about Plate QC and systematic effects, please contact *support@olink.com*.

7.4 Normalization

The two between-plate-normalization methods are called Plate Control (PC) and Intensity normalization. They both adjust each assay per plate to a median, but they differ in how these medians are calculated. An important concept when selecting normalization procedure is randomization, which in this context applies to the sample placement across the plates. For details, refer to *Randomization FAQ* on the Olink website.

Plate Control normalization is performed by default. Intensity normalization should be selected for all Olink Reveal, Olink Explore HT, and Olink Explore 3072/384 projects where samples are randomized. Contact support for study design assistance and choosing the appropriate normalization type.

7.4.1 Converting counts to NPX

The Olink Reveal, Olink Explore HT, and Olink Explore 3072/384 system's raw data output is counts, where each combination of assay and sample is given an integer value based on number of copies detected. These raw data counts are converted into NPX values for use in the continued analysis.

NPX generation

The NPX values are calculated in two main steps. First, the assay counts of a sample are divided with the Extension Control for that sample and block, and a log2 transformation is applied (1). The resulting scale has increasing values with increasing concentration for each assay.

The second step is either Plate Control normalization (2a) or Intensity normalization (2b). Plate Control normalization subtracs the median of the Plate Controls. Intensity normalization subtracs the median of all samples (excluding the control strip).

Steps in the NPX generation described in equation form, where `i` refers to a specific assay, `j` refers to a sample, and ExtNPX defines an extension normalized NPX value.

1. `ExtNPX_i,j = log2(counts(sample_j,Assay_i)/counts(ExtCtrl_j))`
   - Relate counts to known standard (Extension Control).
   - For all assays and all samples, including Negative Controls, Plate Controls, and Sample Controls.
   - Log2 transformation gives more normally distributed data.

2a. `NPX_i,j = ExtNPX_i,j - median(ExtNPX(Plate Controls_i))`
   - Normalize by median of Plate Controls.
   - For each assay, per plate.

2b. `NPX_i,j = ExtNPX_i,j – median(ExtNPX(Samples_i))`
   - Normalize by median of samples (excluding control strip).
   - For each assay, per plate.

7.4.2 CV calculation

To evaluate the reproducibility and repeatability of data related to each plate in the project, and to assess the within- and between-plate variation in the project, two types of CV are computed for each of the pre-selected assays: within plate (intra CV) and between plates (inter CV) in Sample Controls. For the inter CV calculation, the mean of Sample Controls is used to represent the plate before CV calculation.

In each block, pre-selected assays with good performance in Olink Sample Controls are used so that any deviating CV actually indicates a potential technical issue within the workflow. The CV is calculated for NPX of those selected assays (i), using the assumption of a log-normal distribution, in Sample Controls using the formula below. The CV is part of the UI assessing run quality, and is presented in a CV Table showing average CV:s over the blocks

$$CV_i = 100 \sqrt{(e^{Sln_i^2} - 1)}, \text{ where } Sln_i = \ln(2) \times SDisk$$

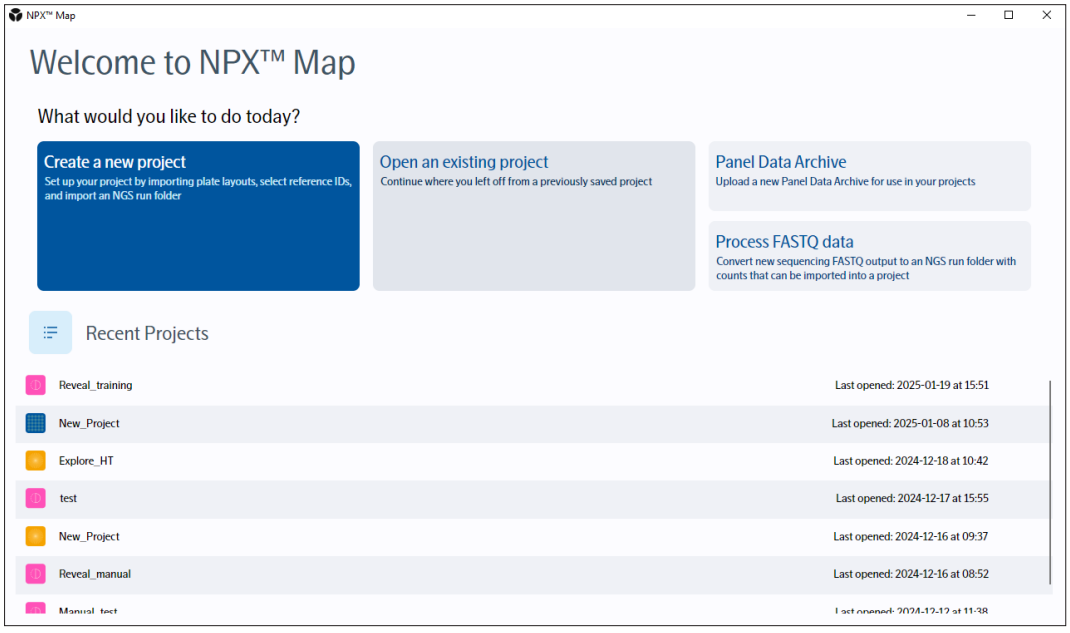
8.QC criteria

Criteria	FAIL *NPX is not calculated, exclude from statistical analysis*	WARN *NPX is calculated, assess further, use data with caution*
Sample QC		
Total counts per sample	< 10 000	N/A
Incubation control counts per sample	< 150	< 500
Extension control counts per sample	< 150	< 1000
Amplification control counts per sample	< 150	< 500
Internal control count fractions per sample	N/A	Log2 of incubation-to-amplification control count ration < -3.5 and,
	N/A	Log2 of incubation-to-extension control count ratio < -3.5 and,
	N/A	Absolute value of log2 of extension-to-amplification cotrol count ratio >3.5
External Control QC		
Total counts per sample	< 10 000	N/A
Incubation control counts per sample	< 500	N/A
Extension control counts per sample	< 1000	N/A
Amplification control counts per sample	< 500	N/A
Plate control internal control counts relative to assay counts	< or > internal control reference range to assay counts	N/A
Negative Control internal control counts relative to assay counts	Negative control fails	N/A
Internal control count fractions per sample	Log2 of incubation-to-amplification control count ratio < -3.5 and,	N/A
	Log2 of incubation-to-extension control count ratio < -3.5 and,	N/A
	Absolute value of log2 of extension-to-amplification control count ratio >3.5	N/A
Block QC		
Plate Controls passing external sample QC	For Olink Explore HT: < 50% (minimum 3 Plate Controls must pass QC)	N/A
	For Olink Explore 3072/384:: All 3 Plate Controls must pass QC.	N/A
Negative Controls passing external sample QC	< 1	N/A
Systematic effect (NPX)	N/A	>10% of assays Systematic effect identified
Assay QC		
Assay count relative to internal control count in negative control	N/A	Assay count ≥ median of all internal control count in all negative controls.

If a run fails to meet the acceptance criteria, or the QC is not accurate, please contact support@olink.com.

Part 3: Operation

9.Introduction



This section describes how you analyze data step-by-step in NPX Map. The following steps are included in the standard operating workflow:

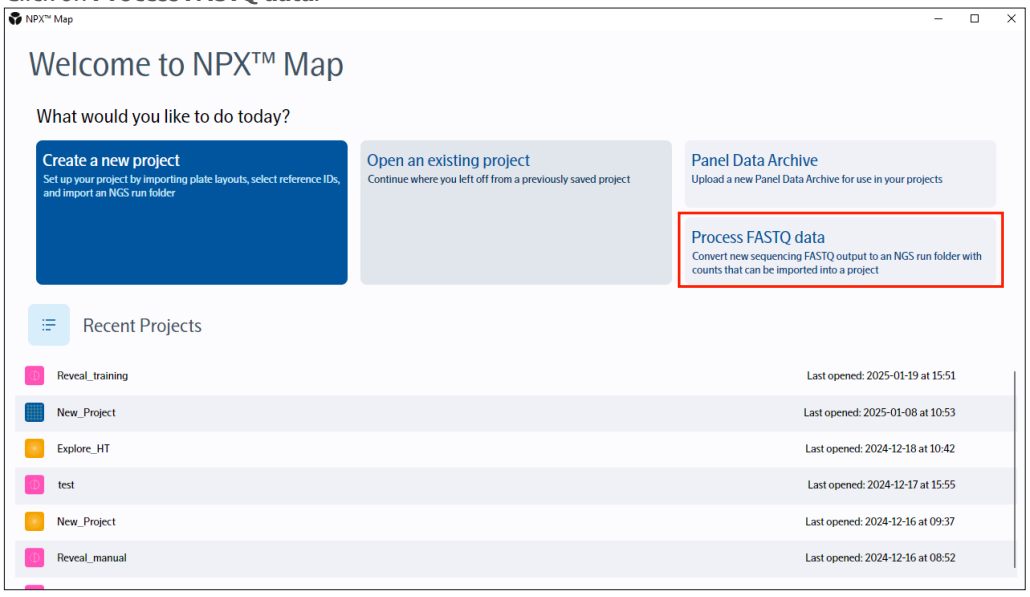
1. Process fastq data
2. Upload Panel Data Archive
3. Create a new project or open an existing project
4. Import NGS run data
5. Perform quality control
6. Export data files and Analysis Report
7. Finalize the project

10.Operating workflow

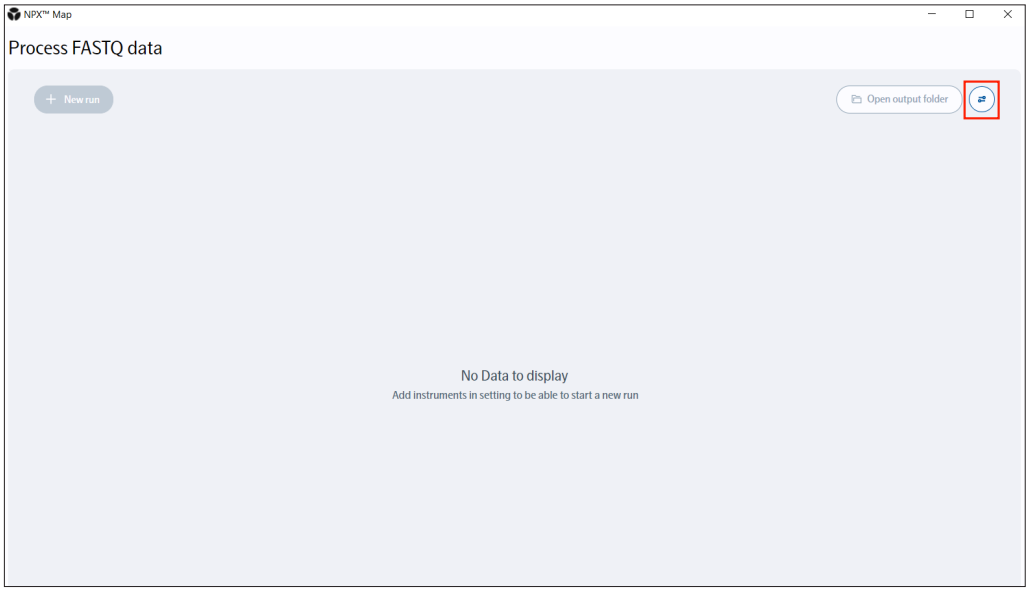
10.1 Process FASTQ data

It is possible to add one or more FASTQ runs in NPX Map. The FASTQ file is a text file that contains biological sequence and its corresponding quality scores.

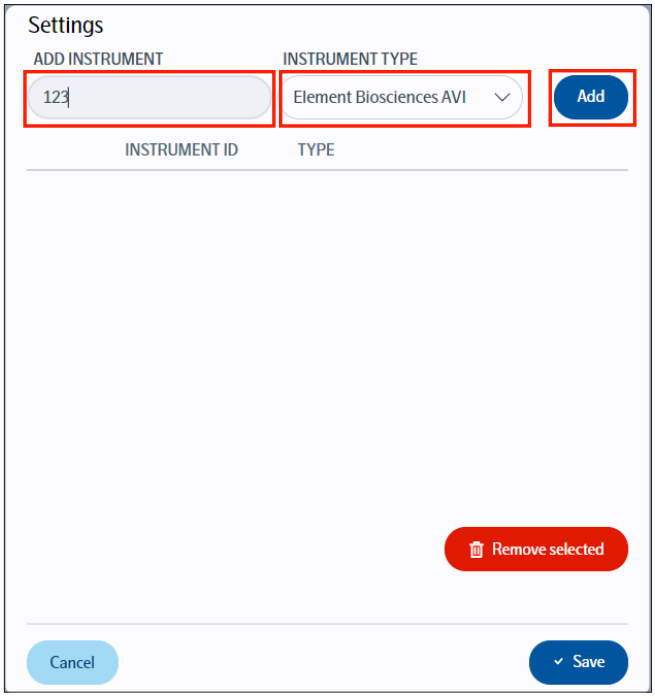
1. Click on **Process FASTQ data**.



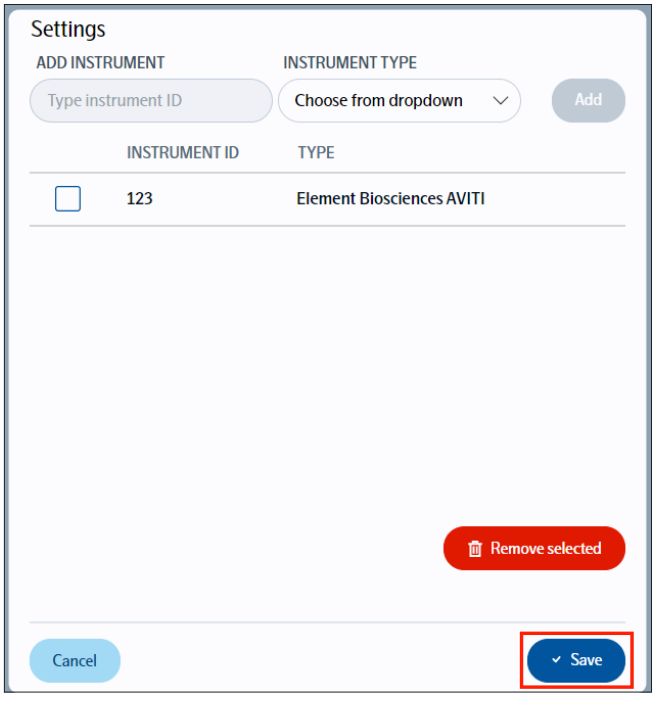
2. Click on the **Settings** menu in the upper right corner to configure a new sequencing instrument



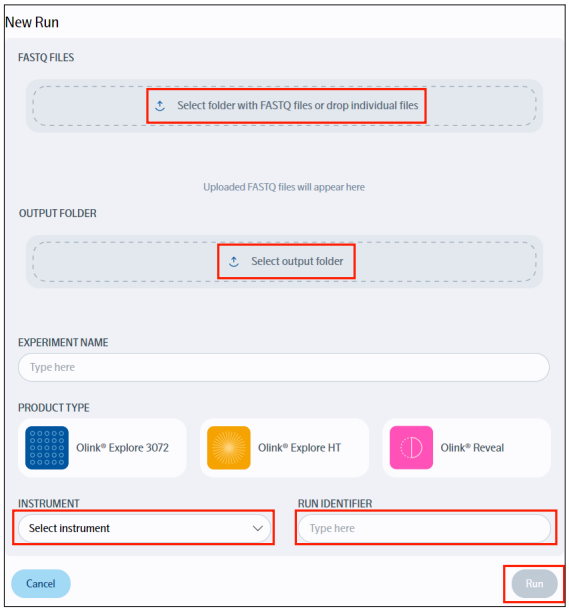
3. Select Instrument Type from the drop-down menu and fill in type instrument ID. Click **Add**.
   Instrument ID is used for traceability and will be present in downstream data, it doesn't necessarily have to be the correct ID if that information is not known.
   It's important to set the correct Instrument Type since it affects how the software process the FASTQ files in question.
   If uncertain please contact your service provider about what instrument was used in the sequencing.



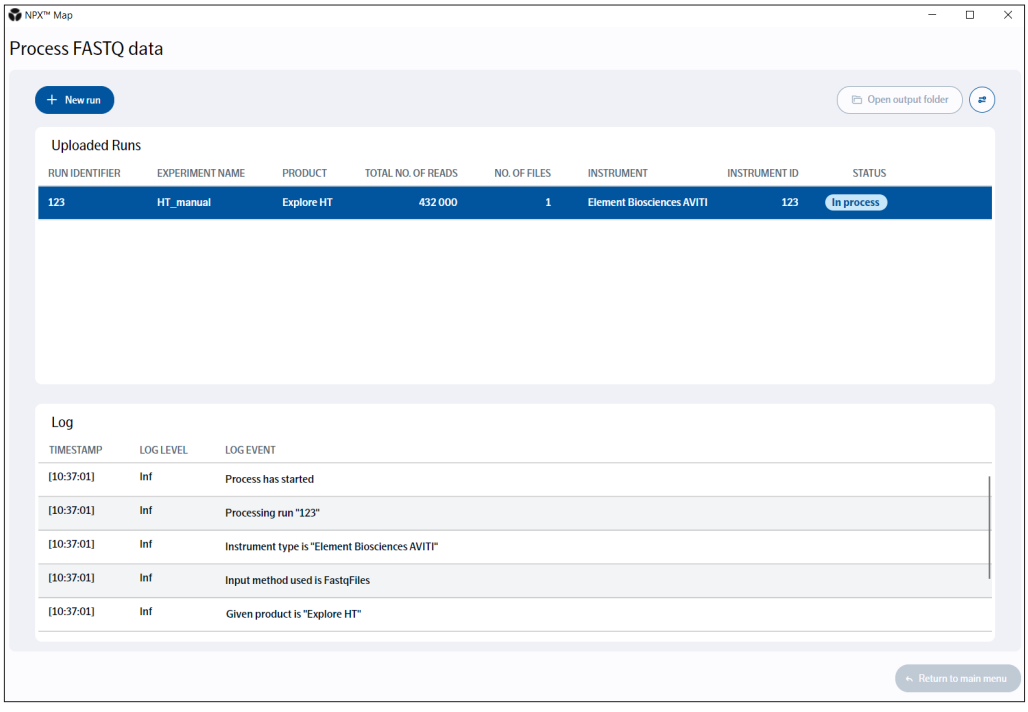
4. The added instrument(s) will be listed. When finnished, click **Save**.



5. Click **New run** , and import compressed FASTQ files and select an output folder to save the run.
   Individual compressed FASTQ files can only be imported by drag and dropping them onto the FASTQ FILES field. Folders containing compressed FASTQ files can be imported both by drag and drop or with the file picker.
   The experiment name field is optional but if set will appear as metadata for the processed run. Pick the product type used in the run, and also fill in the run identifier field. The run identifier field is mandatory to help identify the processed run, it does not have to be a correct ID if that information is not known.
   When all fields are filled, click **Run** and the processing of the data will start.



6. It is not possible to leave the Process FASTQ data view while the uploaded run is processed. The FASTQ batch jobs are listed in the Process FASTQ data main window.
   Completed batch jobs are marked as either success or failure. Click on a batch job to view detailed information.
   Click on the **Open output folder** button to open the output folder for the selected batch job.



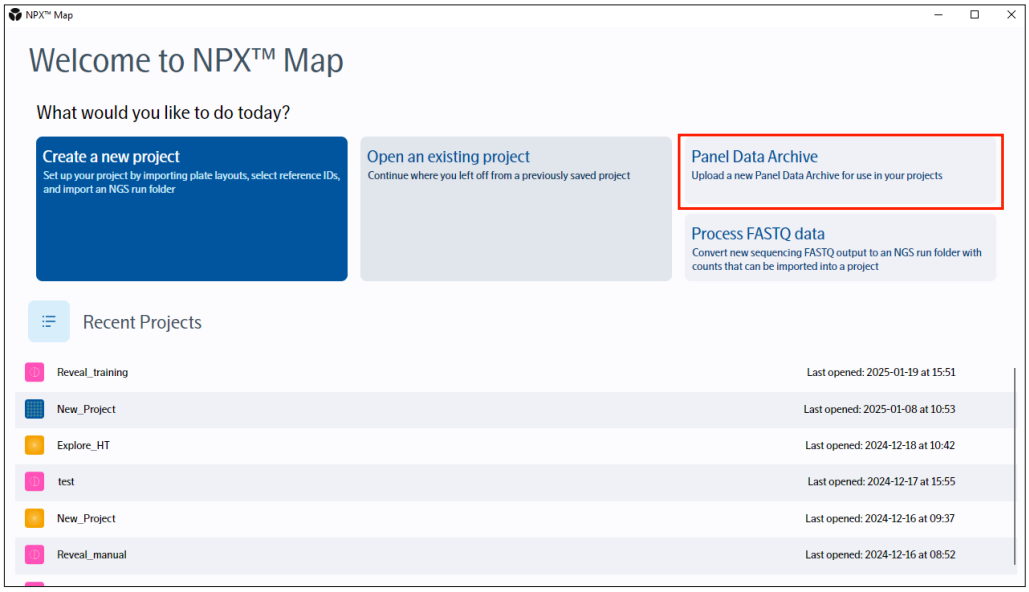
   Click **Return to main menu** to return to start page.

10.2 Upload Panel Data Archive

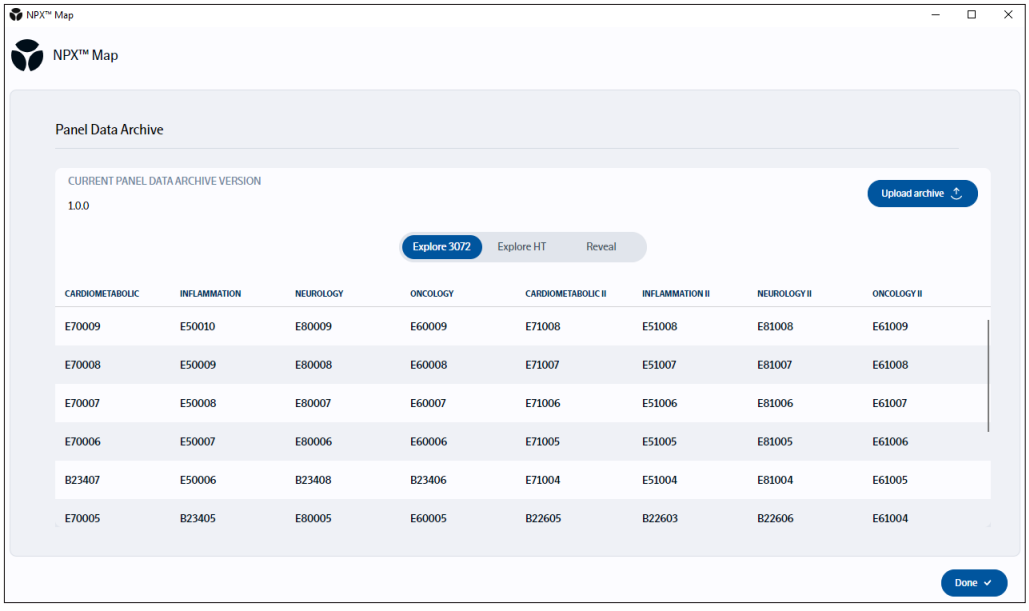
Before creating a project, a compatible panel archive must be imported into the NPX Map.

The following steps must be repeated when new panel versions are needed and been downloaded from the Olink web page or been provided by Olink.

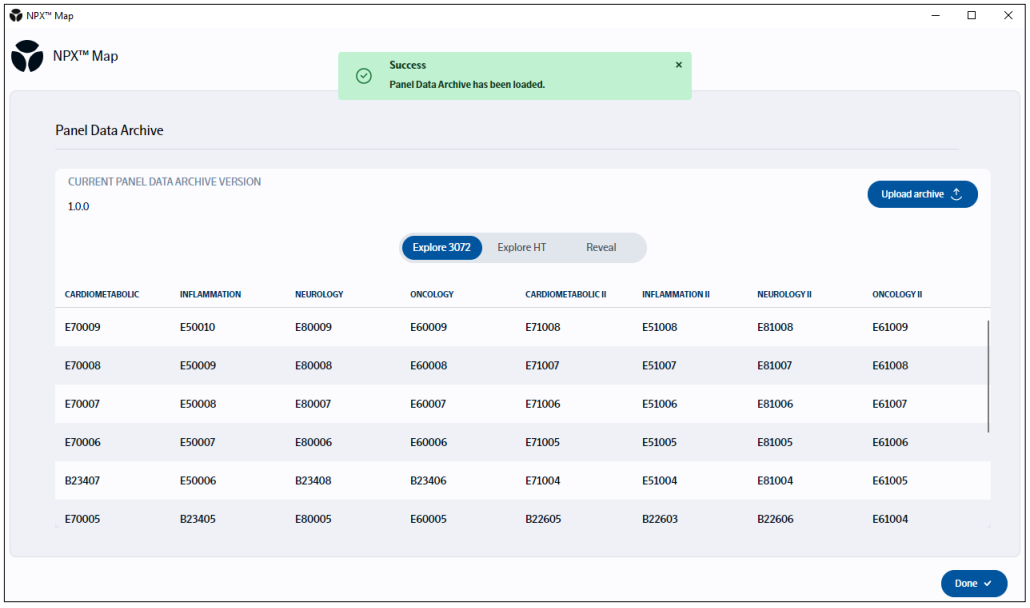
1. On the start page, click **Panel Data Archive.**



2. The Panel Data Archive view shows latest version and minimum compatible version. Click **Upload archive**.



3. Browse for files to upload. Click **Open**.
   The uploaded panel data files will be listed. Click **Done** to get back to the start page.

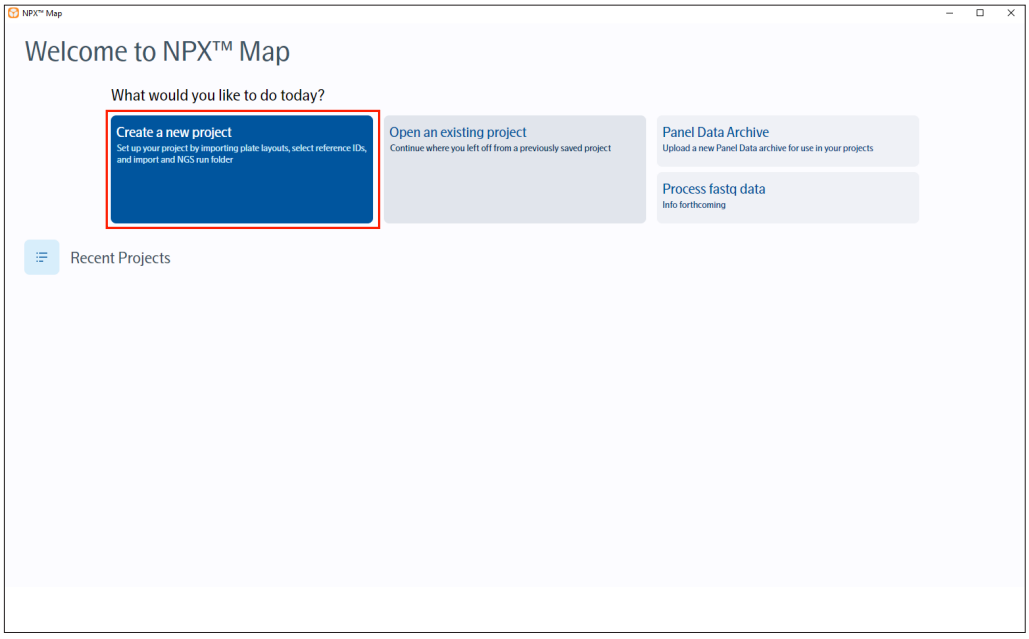


10.3 Create a new project or open an existing project

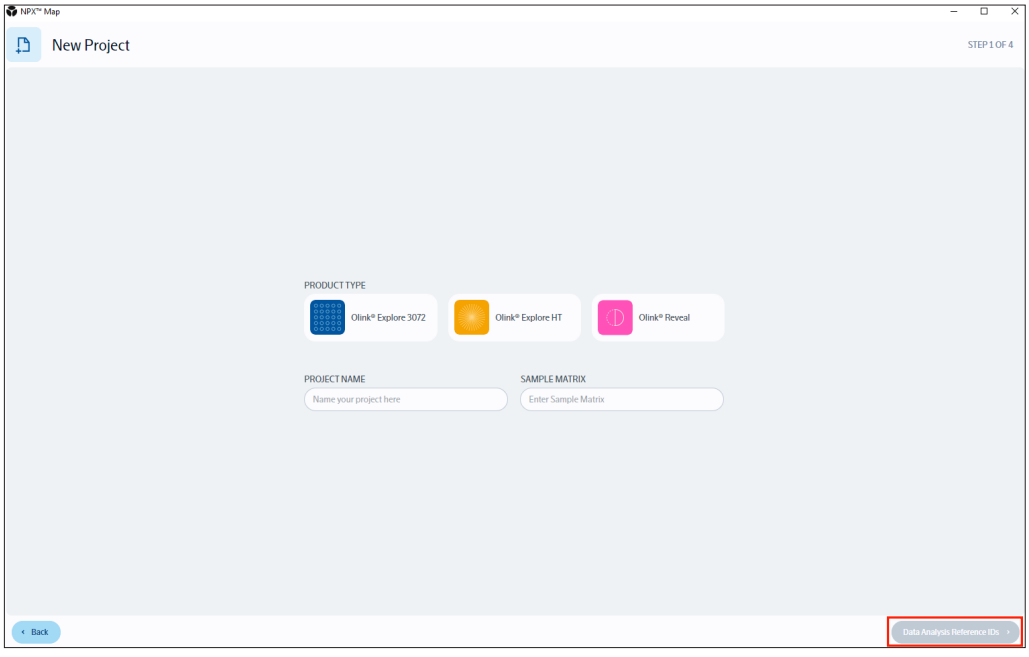
10.3.1 Create a new project

NOTE: *Some of the viws differs between the products.*

1. Start NPX Map and click on **Create a new project**.



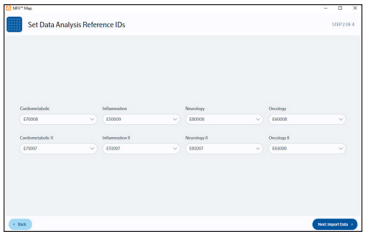
2. Select **Project Type** and enter **Project name** and **Sample matrix** (optional).



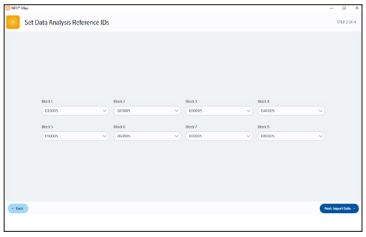
3. Click **Data Analysis Reference IDs.**

4. Select Data Analysis Reference IDs for the blocks/panels in the product from the drop-down menu.

Olink Explore 3072/384



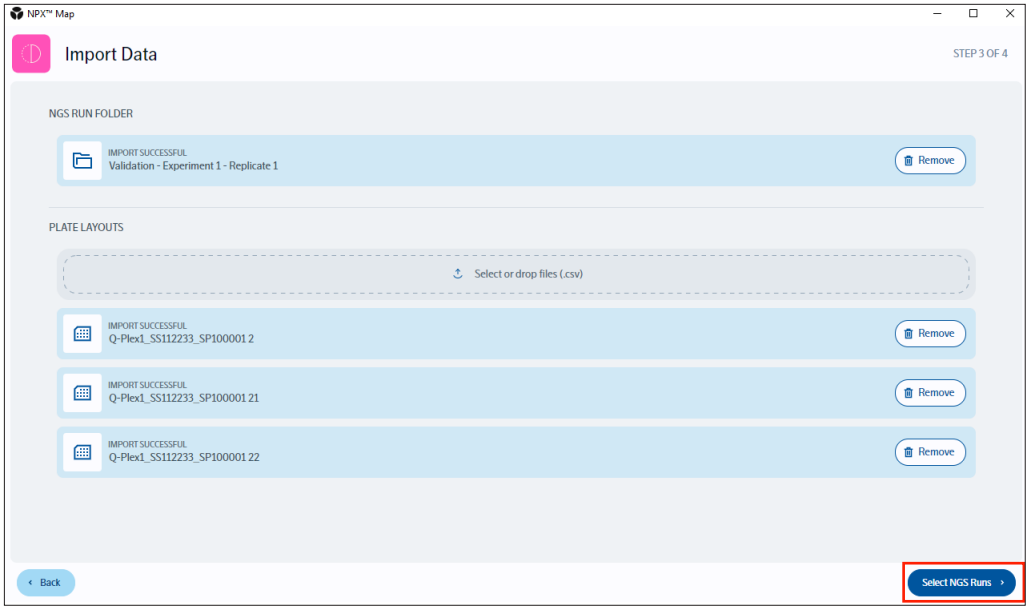
Olink Explore HT



Olink Reveal

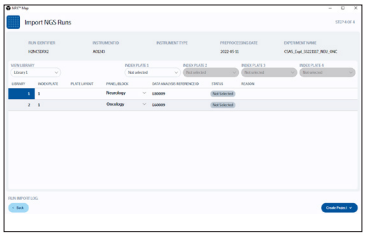
5. Click **Next: Import Data.**
   Clicking CreateProject will create a project without plate layoyts and NGS runs. They can be imported later, from the Modify Project Data menu, refer to *14.2 Modify Project Data menu.*
6. Import plate layouts and NGS run folder.
   It is not possible to import a zipped NGS run folder.
   It is also possible to create a project without plate layouts and NGS runs by clicking **Create project**.
   It is not possible to create a project when only either plate layout or NGS run folder is uploaded.

7. When the files are uploaded, click **Select NGS Runs.**

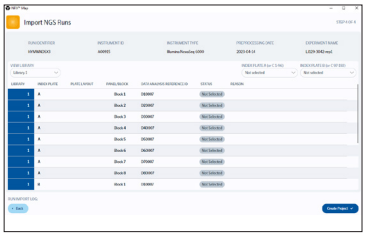


8. Import NGS run.

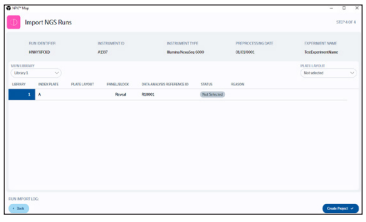
Olink Explore 3072/384



Olink Explore HT



Olink Reveal

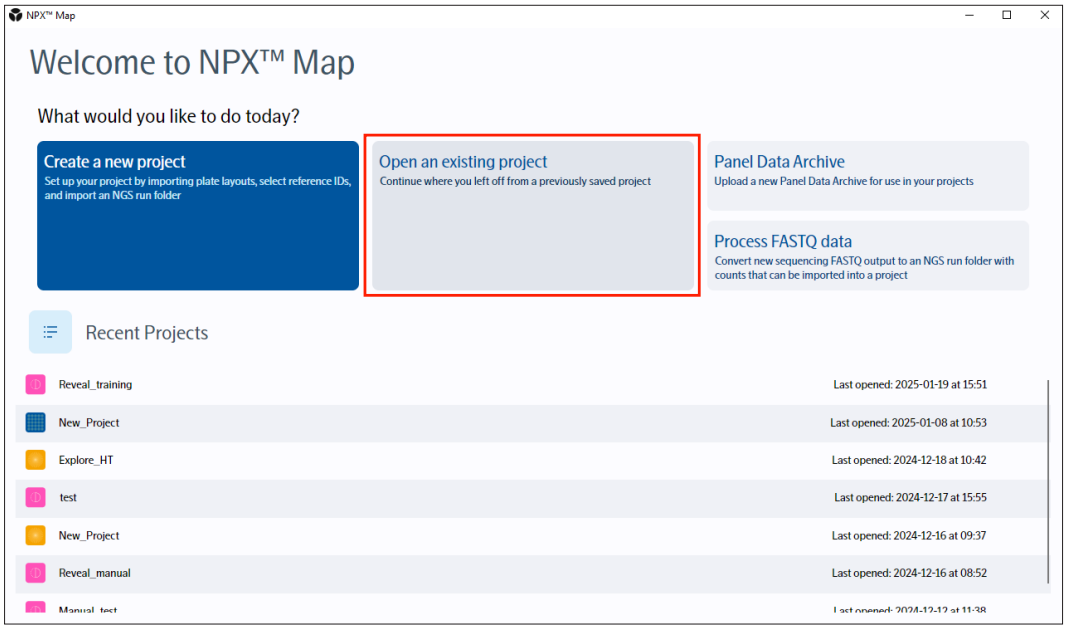


Click **Create Project.**

The project is created and the Project view is displayed.

10.3.2 Open an existing project

1. Click on **Open an existing project**.



2. Browse for project to open.
3. Click **Open**.

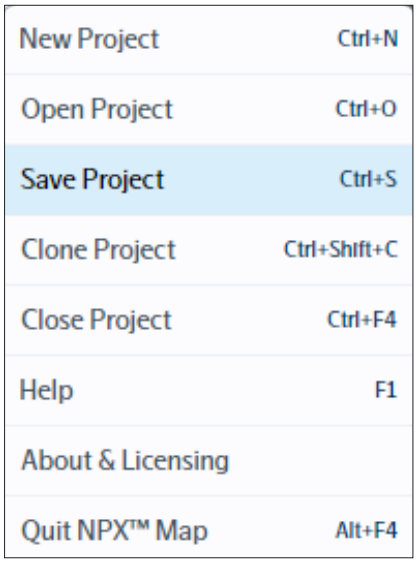
or:

Open an existing project from the **Recent Projects** list.

It is possible to migrate an old Olink Explore 3072/384 project using this function. Refer to *12. Migration of Olink® Explore 3072/384 projects* for more information.

10.4 Save project

1. Go to the **Project Actions menu -> Save Project**.



2. Browse for preferred folder to save the project.*
3. Click **Save**.

* In order to reduce the risk of file corruption due to potential limitations in network connectivity while using a VPN, it is recommended to save projects locally. By working directly from local storage, interruptions can be minimized and it can be ensured that the progress is consistently saved. Once the project work is complete, it can be uploaded or synced to a network location after disconnecting from the VPN or when network stability improves.

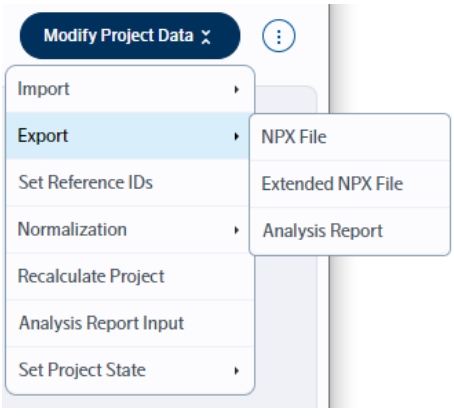
10.5 Perform quality control

Refer to *15. Views* for instructions on how to use the different views to perform quality control.

10.6 Export data and Analysis Report

Three different files can be exported from NPX Map: NPX file, Extended NPX file and Analysis Report. For more information about the files and file formats, refer to *11. Export result files.*

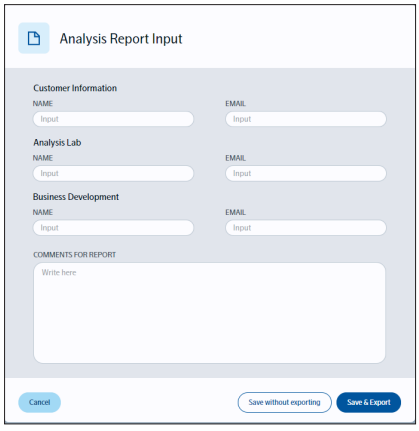
For smaller projects, CSV format is available.



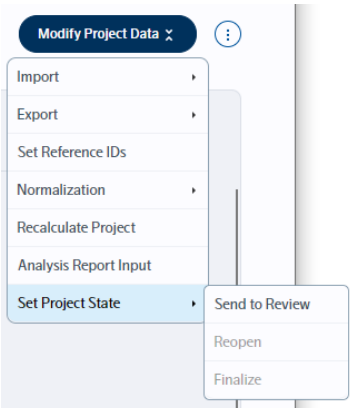
To export the file, go to the **Modify Project Data -> Export**, and select preferred file type to export.

Before an Analysis Report an be exported, the Analysis Report input form must be entered.

4. Go to **Modify Project Data -> Analysis Report input**.
5. Fill in the fields and click either **Save without exporting** or **Save & Export**.



10.7 Finalize a project



Before a project can be finalized, it need to be sent for review.

1. Save the project.
2. Go to **Modify Project Data -> Set Project State -> Send to Review.**
   The project is set to status Review, and no changes can be done. To cancel the review, go to **Modify Project Data -> Set Project State -> Reopen.**
3. When the review is done, **Modify Project Data -> Set Project State ->Finalize.**
   The project cannot be reopened, and no more changes can be made to the project.

11. Export result files

11.1 NPX file

The NPX file is a Parquet or CSV-file that can be generated from NPX Map. It contains the following information about the project in table format:

Column	Description	Type	Typical value
SampleID	The annotated sample ID	String	
Sample Type	Type of sample	String	PLATE_CONTROL, NEGATIVE_CONTROL, CONTROL, SAMPLE
WellID	Id for well	String	Capital letter A–H followed by number 1–12
PlateID	Name of the plate the sample was run on	String	
DataAnalysisRefID	Reference ID for data analysis	String	
OlinkID	OlinkID for assay	String	
UniProt	UniProt ID for assay	String	
Assay	Gene name for assay	String	
AssayType	Type of assay	String	Amp_ctrl, inc_ctrl, ext_ctrl
Panel	Panel name	String	Reveal
Block	Name of the block the sample was run on	String	1, 2, 3, 4, 5, 6, 7, or 8
Count	The total number of counts	Integer	Greater than or equal to 1
ExtNPX	Intermediate value between count and NPX: log2 of the ratio between data-point Count value and the count for the Extension Control assay for the same sample.	Double	-1.94701
NPX	NPX value	Double	
Normalization	Type of normalization used in project	String	Plate control, Intensity or EXCLUDED
PCNormalizedNPX	NPX value displayed if plate control normalization has been chosen.	Double	1.735509
AssayQC	Overall QC status for an assay	String	NA, PASS, WARN
SampleQC	Overall QC status for a sample in a block	String	NA, PASS, WARN, FAIL
MapVersion	Software version of the module in NPX Map used for panel calculations and normalization	String	

11.2 Extended NPX file

The Extended NPX file is a Parquet or CSV-file generated by the NPX Map CLI and NPX Map. It contains all the columns in the NPX file, plus the set of additional columns listed below.

- IntraCV
- InterCV
- SampleBlockQCWarn
- SampleBlockQCFail
- BlockQCFail
- AssayQCWarn

For more information, refer to the NPX™ Map CLI Technical Information.

11.3 Analysis Report

The analysis report is a pdf file generated by the NPX Map CLI and NPX Map. It summarizes the quality analysis of the project. It contains basic information and descriptions of the project. It also contains a summary of the Quality control including sample passed QC.

The Analysis Report contains the following:

- Project information
  - Sample matrix
  - Project specific comments
- Quality control
  - QC summary
- Data output
- Software version information
- Appendix - A table including the following columns
  - Olink Panel
  - Block
  - UniProt
  - Assay Olink ID

11.4 Parquet file format

NPX data is exported from NPX Map in either CSV or Parquet file format. Parquet is a free open source file format built to handle columnar storage data.

Parquet files provide several advantages compared to CSV, such as

- Much more efficient data compression with enhanced performance to handle complex data in bulk.
- Improved data integrity through binary encoding (files can't be opened and changed using a text editor).

The binary encoding also means the file cannot be opened in Microsoft® Excel. If required, Parquet files can be exported to CSV format. The CSV format is available for projects with approximately 1 million datapoints.

Parquet is available in multiple languages including Java, C++, Python, C# etc. You can find further details at these locations

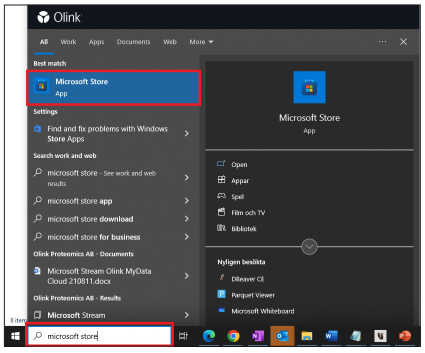
- https://parquet.apache.org/docs/
- https://en.wikipedia.org/wiki/Apache_Parquet

11.4.1 How to interact with a Parquet file

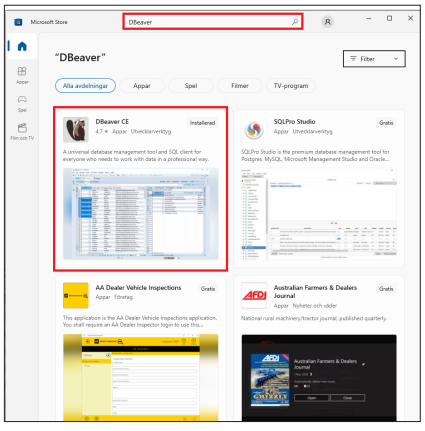
There are many different solutions to interact with a Parquet file, for example DBeaver. Olink do not recommend any specific solution.

Installing DBeaver

1. Download DBeaver for free from the Microsoft® Store by clicking on the **start menu** and type "Microsoft store".

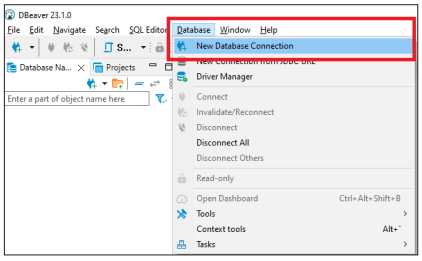


2. Type "DBeaver" in the search field, and press **Enter**.

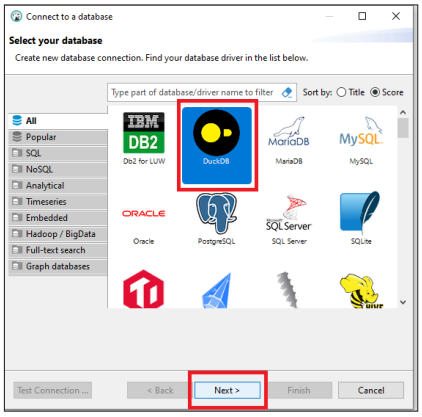


3.

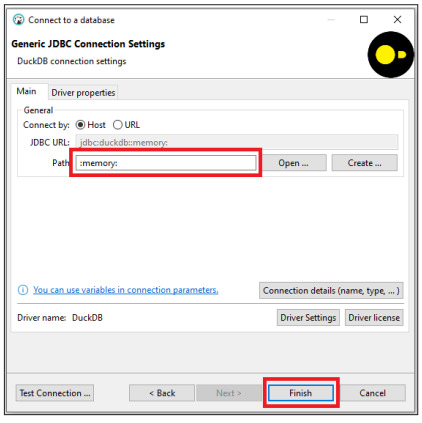
4. Once DBeaver is installed, setup a new database connection.



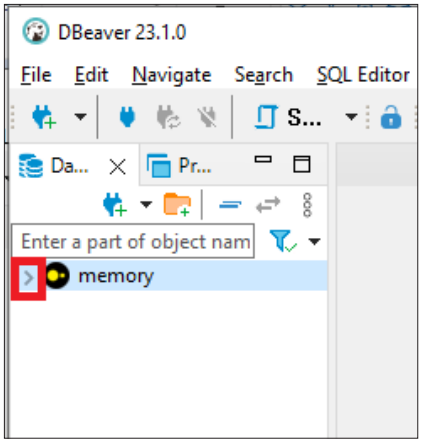
5. Choose **DuckDB** and hit the **Next** button.



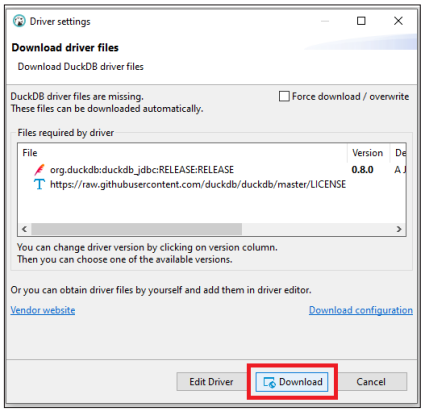
6. Type ":memory:" in the **Path** input field.



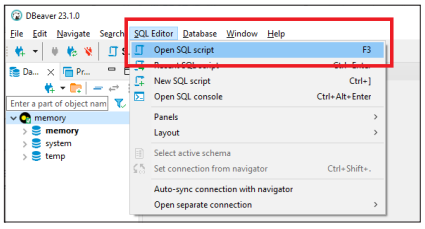
7. Click the gray arrow.



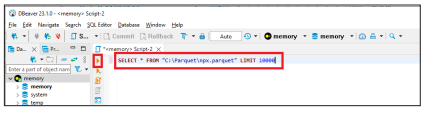
8. Download driver files.



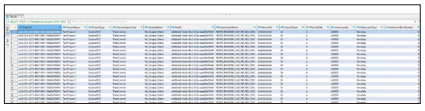
9. Open a new script window.



10. Type a PostgreSQL query such as
    `SELECT * FROM 'C:/npxfile.parquet'.`
    Make sure that the path in double quotes refers to the NPX file you want to query.
11. Click the "Execute SQL query" button

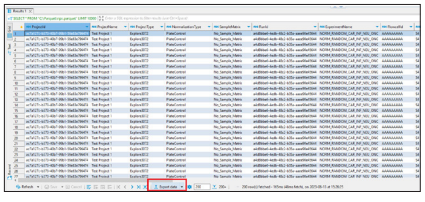


12. View the result.

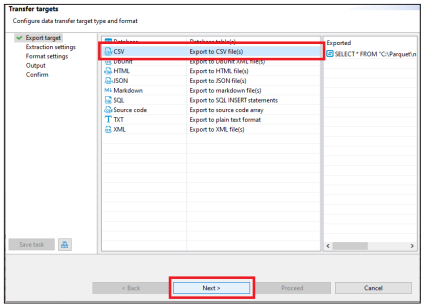


Export data to a CSV file

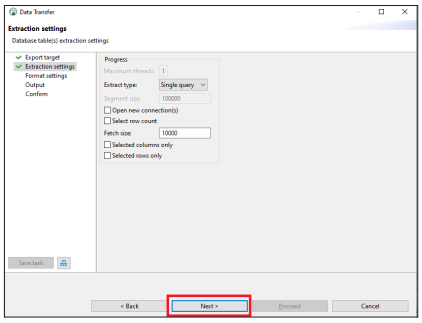
1. Click **Export data.**



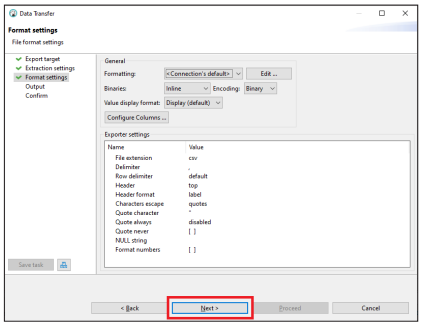
2. Highlight CSV and click **Next**.



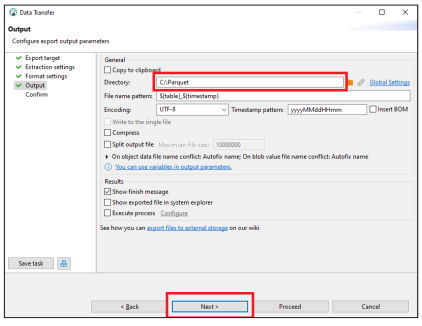
3. Click **Next**.



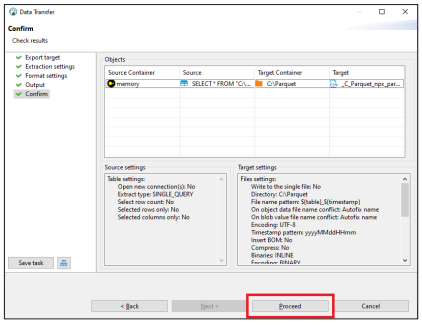
4. Click **Next.**



5. Set the "Directory" field to a location on your hard drive.
6. Click **Next.**



7. Click **Proceed**.



11.4.2 Quick-check parquet-viewer

To quick-check the parquet, perform the following steps:

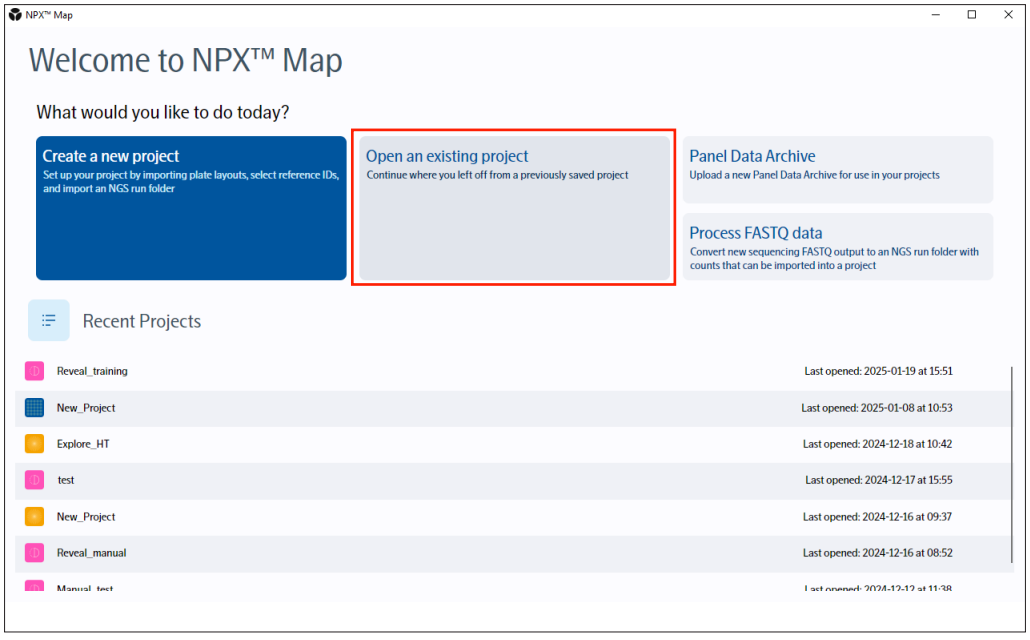
1. Download ParquetViewer from the GitHub wepage: *https://github.com/mukunku/ParquetViewer/releases.*
2. Install ParquetViewer and open it.
3. Drag and drop the parquet file to the application window.
4. Use the query input field to filter out desired datapoints, or use the Record Offset filed to jump in the file.
5. For more information or help, refer to the GitHub homepage: *https://github.com/mukunku/ParquetViewer/wiki/Basics.*

12.Migration of Olink® Explore 3072/384 projects

Olink Explore 3072/384 projects created in NPX Explore can be migrated into NPX Map to utilize the new QC procedure and features.

12.1 Migrate a project

1. Click on Open an existing project.



2. Browse for project to migrate
3. Click **Open**.

12.2 Migration information

Not all data will be copied over into the migrated project such as data specific to the old QC and normalization. Some project data fields may also be trimmed due to newer constraint requirements on length and content. Data such as counts, plate layouts and imported run units are imported as they were in the old project. The migration of the project leaves the old project and files intact and can be opened in the old software as usual.

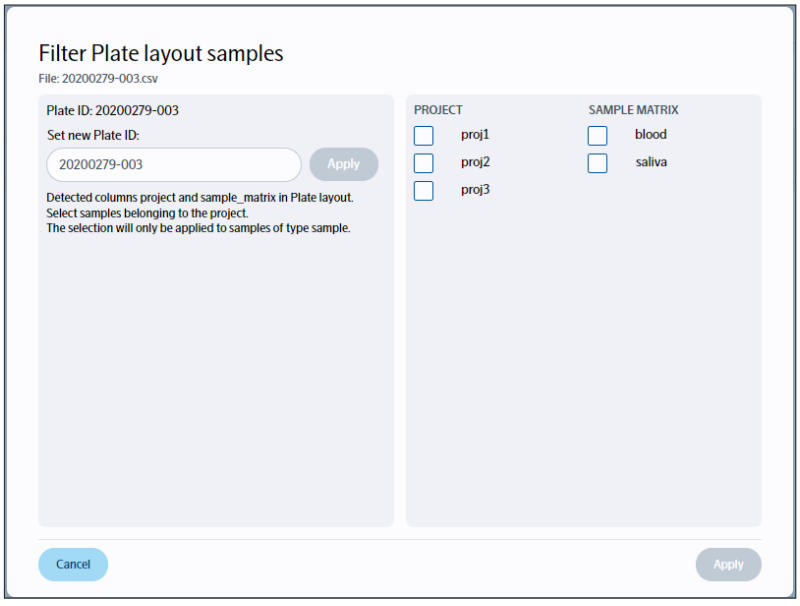
13. Different projects or sample matrices on the same plate

When running different projects or sample matrices on one plate, an extended plate layout must be created.

1. Manually create a single plate layout file with two extra columns: Project Name and Sample Matrix, according to the following order:

   ```
   well_ID; sample_ID; sample_type; sample_matrix; project
   ```

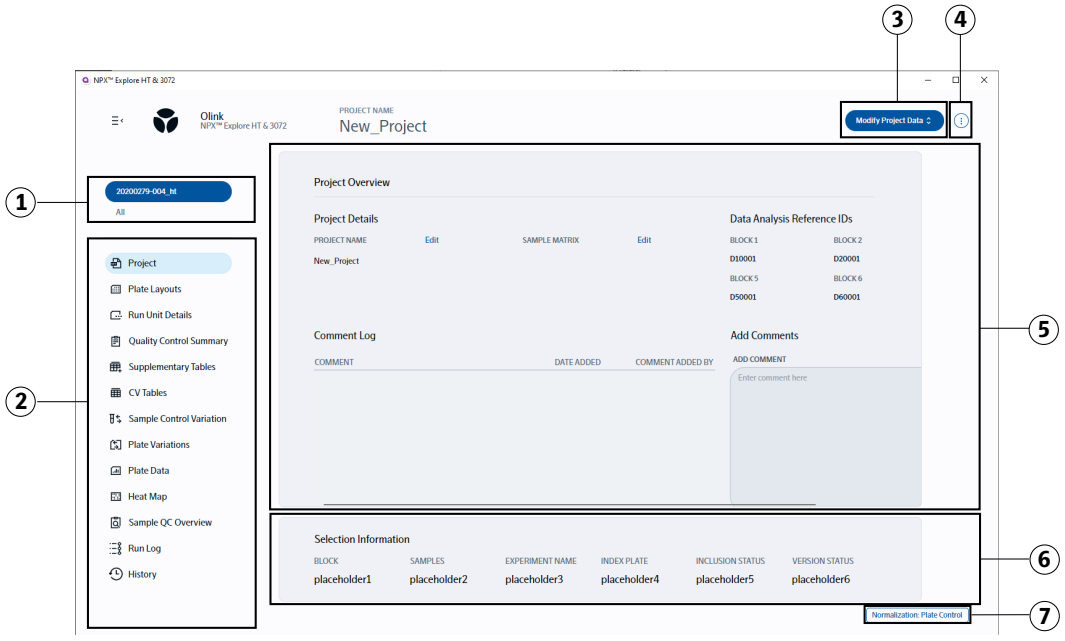
2. Create one project per project/sample matrix, according to *10.3 Create a new project or open an existing project.*
3. Upload the manually created plate layout in both projects. The following view will be displayed:



4. Fill in **new Plate ID** and select **Project** and **Sample Matrix**.
5. Click **Apply**.
6. Continue according to *10.3 Create a new project or open an existing project.*

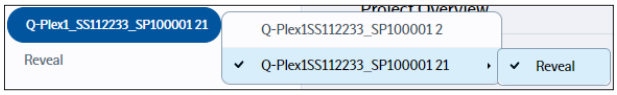
Part 4: User Interface

14.General



No	Part	Description
1	View menu	For information about the different views, refer to *15. Views*
2	Plate Selection	Select plate and panel to display (For Olink Reveal and Olink 3072/384) Select plate and block to display (For Olink Explore HT)
3	Minimize View menu	Minimaie or maximize the View menu
4	Modify Project Data menu	Menu for modifying the project data, such as Set Reference IDs, Normalization etc. Refer to *14.2 Modify Project Data menu*
5	Project Actions menu	Menu for handling the project, such as Open Project, Save Project, Clone Project etc. Refer to *14.3 Project Actions menu.*
6	Main view	Shows the selected view from the View menu.
7	Normalization	Shows selected normalization method. To change normalization method, refer to *14.2 Modify Project Data menu.*

14.1 Plate Selection



Use this menu to select which plate and panel/block to view.

NOTE: *Olink Reveal consists of only one panel, called Reveal.*

14.2 Modify Project Data menu

Menu	Expansion	Description
Import	Plate Layouts Import NGS Run Folder Import Zipped NGS Run File	Import Plate layouts and NGS run folders to the current project.
Export	NPX File Extended NPX File Analysis Report	Export the data files. Refer to *10.6 Export data and Analysis Report.*
Set Reference IDs	–	Set reference IDs for the current project.
Normalization	Plate Control Intensity	Change normalization method for the project. Refer to *7.4 Normalization.*
Recalculate Project	–	Recalculate the project.
Analysis Report Input	–	Enter information to the Analysis Report. Refer to *11.3 Analysis Report.*
Set Project State	Send to Review Reopen Finalize	Final stages for the project. Refer to *10.7 Finalize a project.*

14.3 Project Actions menu

Menu	Description
New Project	Create a new project. Refer to *10.3 Create a new project or open an existing project.*
Open Project	Open a saved project.
Save Project	Save the current project. Refer to *10.4 Save project.*
Clone Project	Create a clone of the current project.
Close Project	Close the current project.
Help	Open the NPX™ Map User Manual as a pdf file.
About & Licensing	Show information about the software and licensing information.
Quit NPX™ Map	Close the software.

15. Views

Project
Overview of the project, showing project details, comment log etc.

Run Unit Details
The Run Unit Details shows information about the selected plate and panel/block.

Quality Control Summary
The Quality Control Summary tab displays a summary of the QC criteria per block.

Supplementary Tables
Contains tables that display sample IDs and assays that failed or warned based on any of the QC criteria. It helps identify the possible root cause for sample and block failure.

CV Information
Assess the precision of the data based on a group of pre-selected assays across different blocks on the Sample Controls.

Plate Variation
Display differences in sample distributions within and between plates.

Plate Data
Shows the values of NPX and Counts for internal controls and assays for each sample per block. Assess if there are patterns in the plate representing a possible technical error.

Correalation Assays (Only for Olink Explore 3072/384)
Assess the correlations of each overlapping assay between blocks. Skewed data correlation in any of the plots can indicate the technical error in the lab workflow.

Heat Map
Search for extreme outlier samples and systematic patterns that reflect a possible technical error.

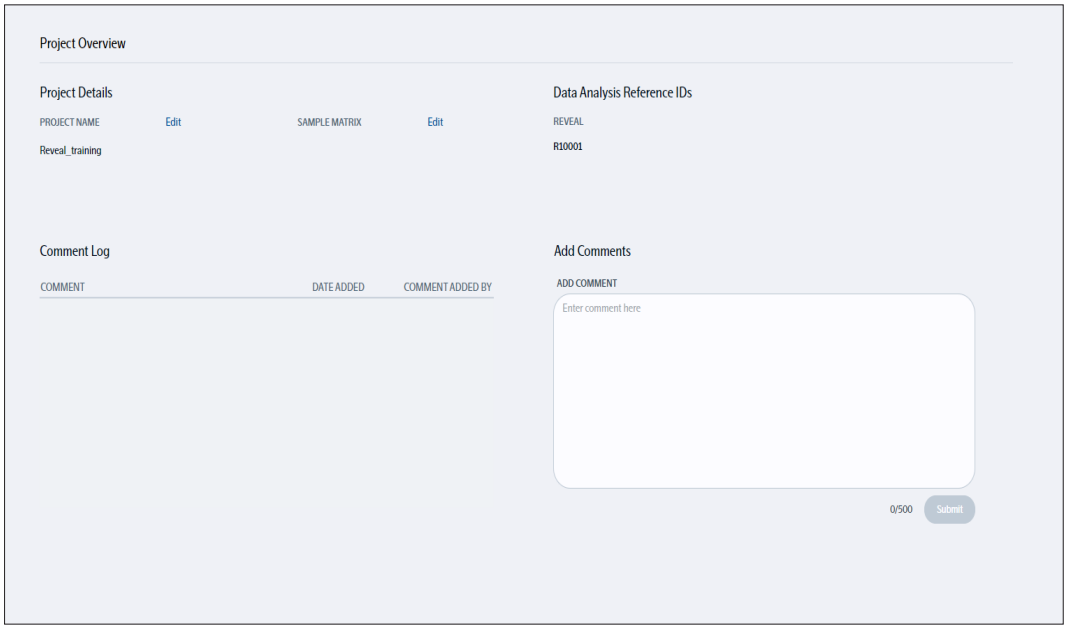
Sample QC Overview (Only for Olink Explore HT and Olink Explore 3072/384)
View and evaluate patterns of failed/warned samples within and across the blocks in each plate and view the criteria that have resulted in failure or warning status.

History
Shows the changes made in the project.

15.1 Project

General:
The Project view shows the following information about the project:

- Project Details
- Comment Log
- Data Analysis Reference IDs
- Add Comments



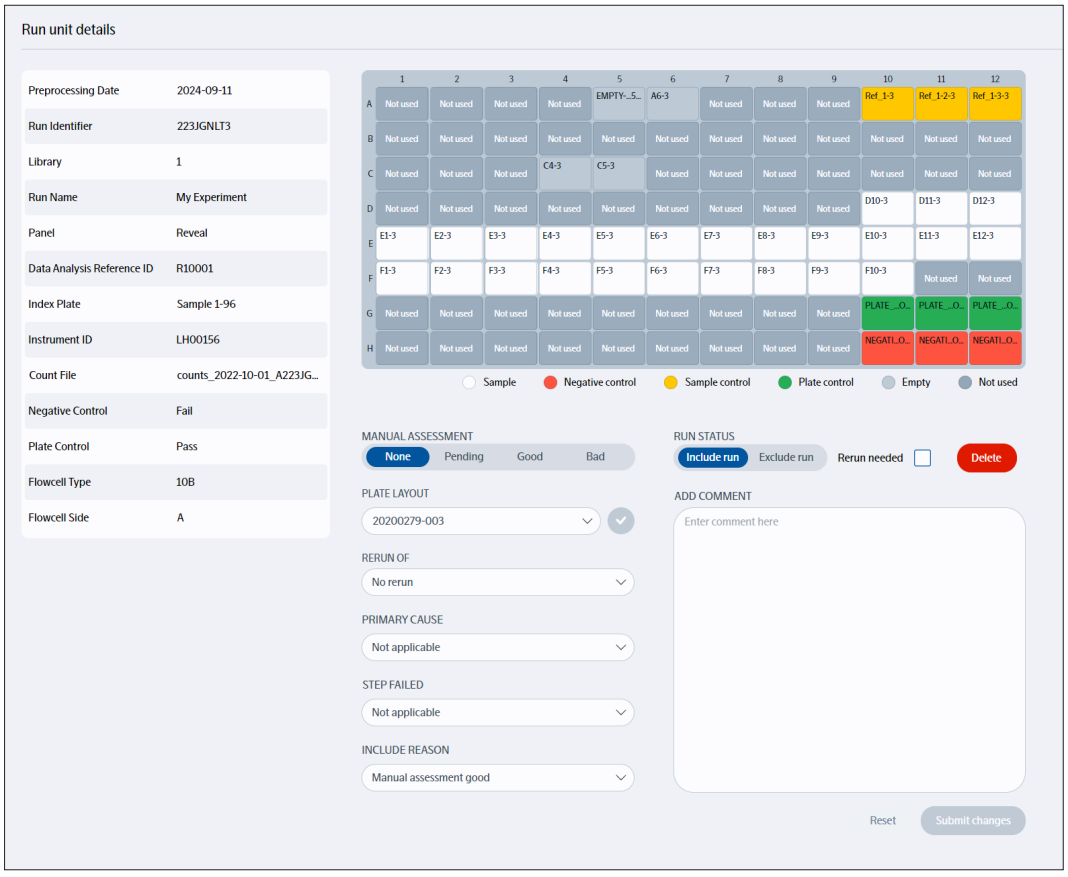
Useful for:
Add comments regarding the QC. The comments will not be included in the Analysis Report.

Functions:
- Change Project Name or Sample Matirix by clicking on **Edit** and write in the field. Click **Done**.
- Add comments for the project by writing in the **Add Comment** field. The comment will be shown in the Comment Log field. The comments will be included in the Analysis Report.

15.2 Run Unit Details

General:

The Run Unit Details shows information about the selected plate and panel.



Useful for:

- Reviewing failed blocks/samples.
- Finding systematic effects.
- Include, exclude, or delete plate.

Functions:

- For each plate, the following information is displayed:
  - Preprocessing Date
    The date when instrument data for this run unit was pre-processed.
  - Run Identifier
    The unique identifier of the instrument run this run unit belonged to.
  - Library
    The assay library of this run unit.
  - Run name
    The name of the instrument run containing this run unit.
  - Block (Only for Olink Explore HT)
    The block of this run unit.
  - Panel (Only for Olink Reveal and Olink Explore 3072/384)
    The panel of this run unit.
  - Data Analysis Reference ID
    The specific Data Analysis Reference ID this run unit was calculated with.

- Index Plate
  The index range of the samples in this run unit.
- Instrument ID
  The ID of the instrument that performed the run.
- Count File
  The name of the file that contained the counts data for this run unit.
- Negative Control
  The status of the negative controls in this unit; "Fail" if any negative control is failed, otherwise "Pass"
- Plate Control
  The status of the plate controls in this unit; "Fail" if any plate control is failed, otherwise "Pass"
- Flowcell Type
  The type of flow cell that was used in the corresponding run (only applicable to Illumina instruments).
- Flowcell Side
  The side of the instrument that was used in the corresponding run (only applicable to Illumina instruments)

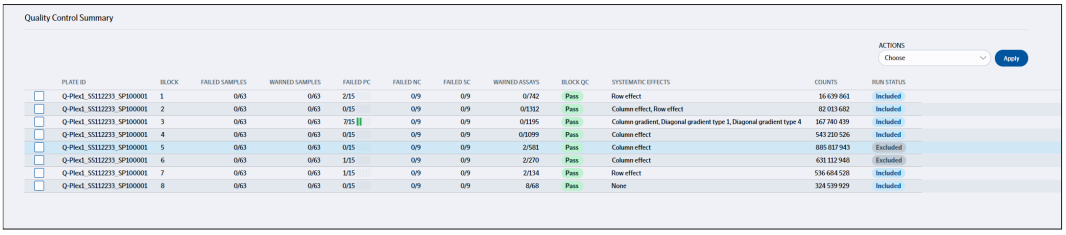
- Select **Manual Assessment:** None/Pending/Good/Bad.
- Set **Run Status** to Include run/Exclude run or Rerun needed.
- Use the drop-down menues to do the following changes:
  - Select **Plate**
  - Annotate a **Rerun of**
  - Set **Primary error cause**
  - Set **Step fail reason**
  - Set **Include reason**
- **Add comments** in the comment field. Comments will not be included in the Analysis Report.

NOTE: *Tthe Counts presented in this view is expected to differ from the sum of counts for individual data points presented in the NPX File. The counts presented in the NPX File is the value that shall be used in down stream analysis.*

15.3 Quality Control Summary

General:

The Quality Control Summary view displays a summary of the QC criteria per plate.



Useful for:

- Reviewing status of the selected block.
- Finding systematic effects.

Functions:

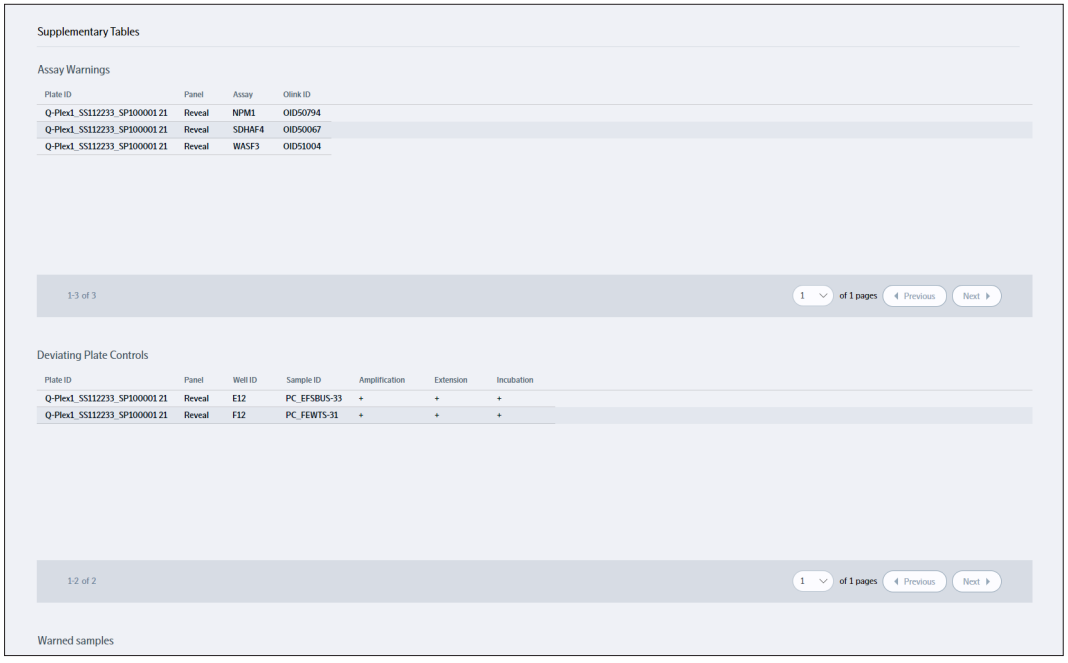
- For each plate, the following information is displayed:
  - Plate ID
  - Panel (Only for Olink Reveal and Olink Explore 3072/384)
  - Block (Only for Olink Explore HT)
  - Failed samples
  - Warned samples
  - Failed PC
  - Failed NC
  - Failed SC
  - Warned assays
  - Block QC
  - Systematic effects
  - Counts
  - Run status
- Use the actions drop-down menu to include, exclude or remove the selected items. Click **Apply**.

15.4 Supplementary Tables

General:

The supplementary Tables view contains five tables displaying samples that have failed or have warnings based on QC criteria. All data for one sample source plate is displayed at a time.

Only tables that contain data will be displayed.



Useful for:

- Reviewing flags of all samples and assays in tables, divided into five different flag reasons:
  - Assay warnings
  - Samples with low total counts
  - Samples with low internal control count
  - Deviating plate controls
  - Failed negative controls

Functions:

- The following information will be displayed in the different tables:

Assay warnings	Samples with low total Couns	Samples with low internal control counts	Deviating Plate Controls	Failed Negative Controls
• Plate ID • Panel • Block* • Assay • Olink ID	• Plate ID • Panel • Block* • Well ID • Sample ID • Sample Type	• Plate ID • Panel • Block* • Well ID • Sample ID • Sample Type • Total Count	• Plate ID • Panel • Block* • Well ID • Sample ID • Amplification • Extension • Incubation	• Plate ID • Panel • Block* • Well ID • Sample ID • Sample Type

* Block only for Olink Explore HT and Olink Explore 3072/384.

- The Failed Negative Controls table includes empty wells that show too high signal.

15.5 CV Information

General:

The CV Information view shows the CV information, one row per block. CV is calculated based on the data points of Sample Controls that passed the QC criteria.

NORMALIZATION: PLATE CONTROL	Block 1	Block 2	Block 3	Block 4	Block 5	Block 6	Block 7	Block 8
Intra-Assay % CV Mean	22,4	30,3	14,3	16,8	11,4	15,8	21,5	19,9
Inter-Assay % CV Mean	9,8	10,7	8,4	7,1	6,9	5,6	7,9	4,7
Number of plates with missing intra CV mean	0	0	0	0	0	0	0	0

Useful for:

- Getting an overview of the CV values per block, and which blocks that have missing CV values.

Functions:

- The following information will be displayed:
  - Panel (Only for Olink Reveal)
  - Block (Only for Olink Explore HT and Olink Explore 3072/384)
  - Plate
  - Mean CV value
- The CV Table shows the following information:
  - Intra-Assay %CV Mean
  - Inter-Assay % CV Mean
  - Number of plates with missing intra CV mean

Reference values:

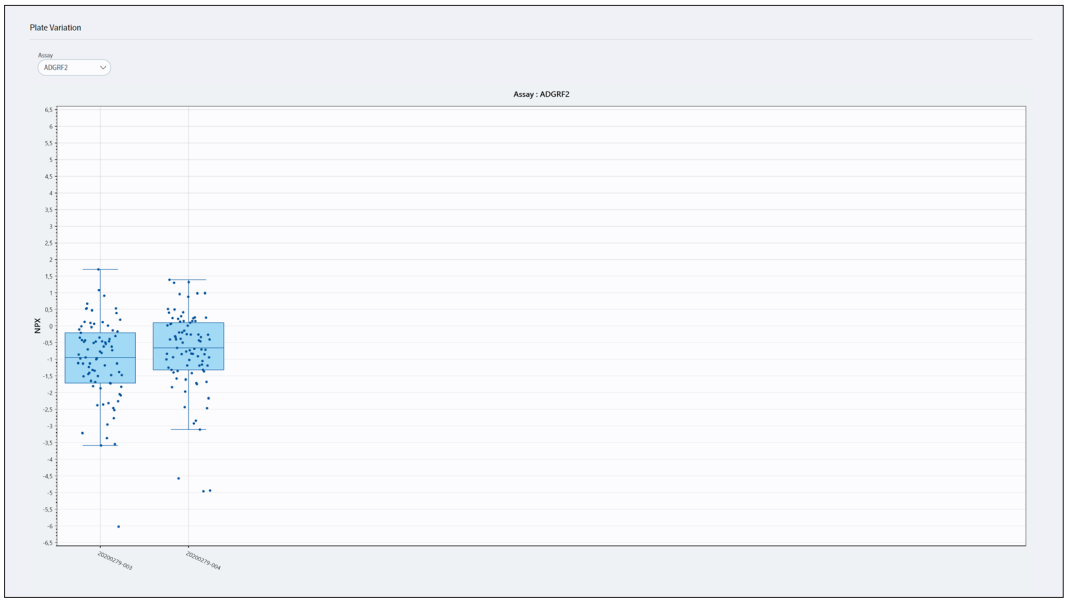
The reported %CV is the mean %CV over all pre-selected assays. A high %CV does not fail a run automatically but could indicate a technical issue or poor sample randomization and can be a cause for further investigation.

- Inter %CV: < 25%
- Intra %CV: < 15%

15.6 Plate Variation

General:

The Plate Variation tab displays a box plot with NPX values from the Sample wells. Failed run units will be left empty, refer to the left run in the picture below.



Useful for:

- Detecting systematic variance in total intensity between plates.

Functions:

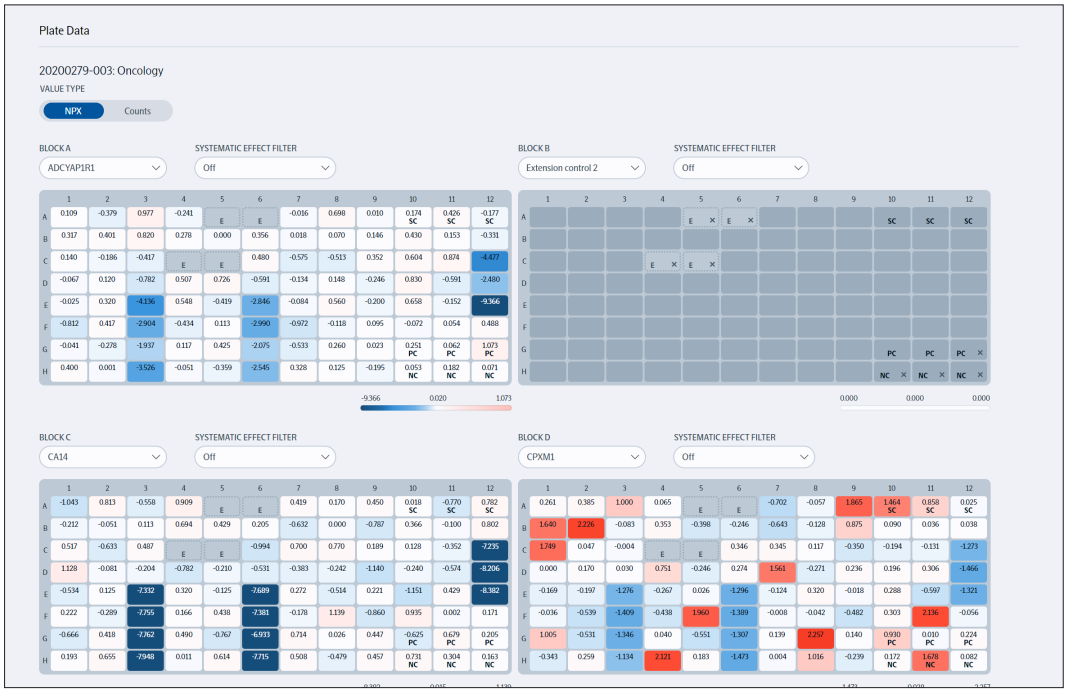
- Use the **Assay** drop-down list to show specific control or assay.
- Select All in the **Assay** drop-down list to view the summary of all assays and controls for each sample.
- Select **block** to view (Only for Olink Explore 3072/384)

15.7 Plate Data

General:

The Plate Data view shows heatmaps of one unit a time, including the internal controls: extension control, incubation control and amplification control. Empty wells show counts but not NPX.

For Olink Explore 3072/384, all blocks are shown at the same time.



Useful for:

- Inspecting counts and NPX across a plate for each assay.
- Discovering systematic patterns.
- Discovering plates swap based on data pattern across the plate (If the plate has any empty well).

Functions:

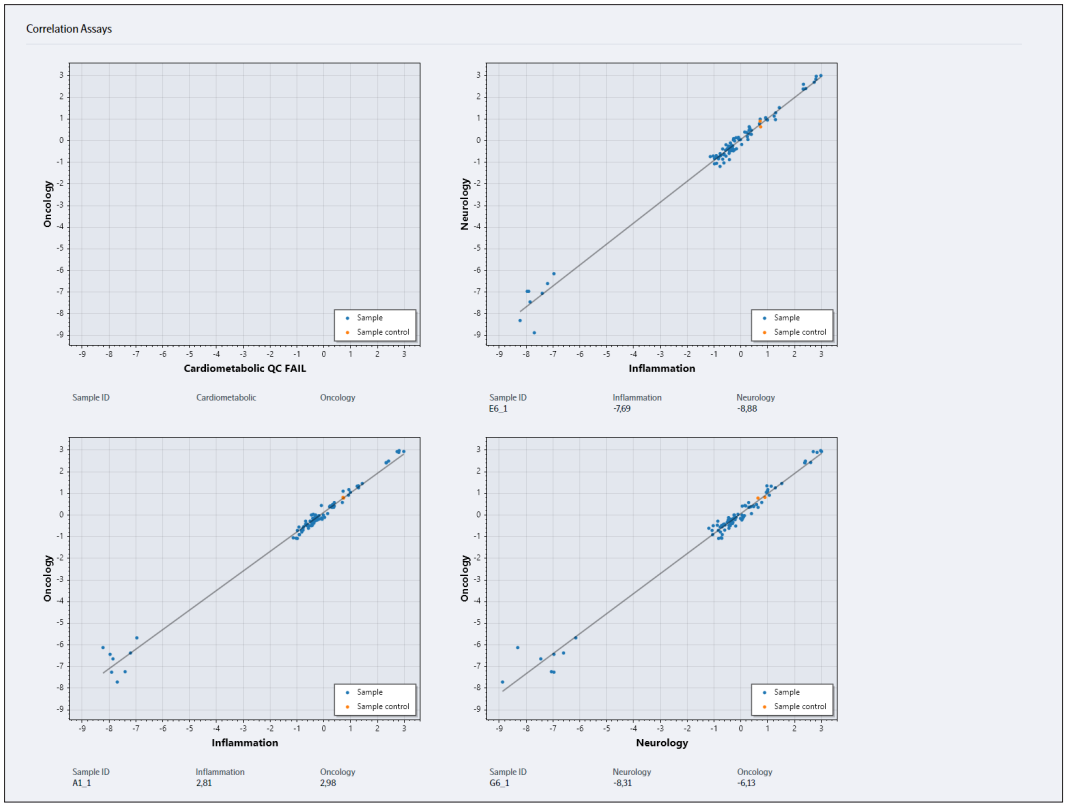
- Use the **assay** drop-down menu to change which assay to display.
- Change **value type** by selecting either NPX or Counts.
- Use the **Systematic effect filter** to select type of systematic effect, or swith it off. (Only available systematic effects types are shown in the drop-down menu)

15.8 Correlation Assays (Only for Olink® Explore 3072/384)

General:

The Correlation Assays tab shows correlation assays that overlaps across the blocks per sample plate.

- A plate is chosen: all included run units are shown.
- An excluded run unit is chosen: the text "No data display" is shown.
- Failed samples: no data points will be shown.
- Failed blocks: an empty graph with red text "Panel QC failed [NPX] "will be shown.



Useful for:

- Getting an overview of correlation assays.

Functions:

- Use the Assay drop-down list to switch between correlation assays.

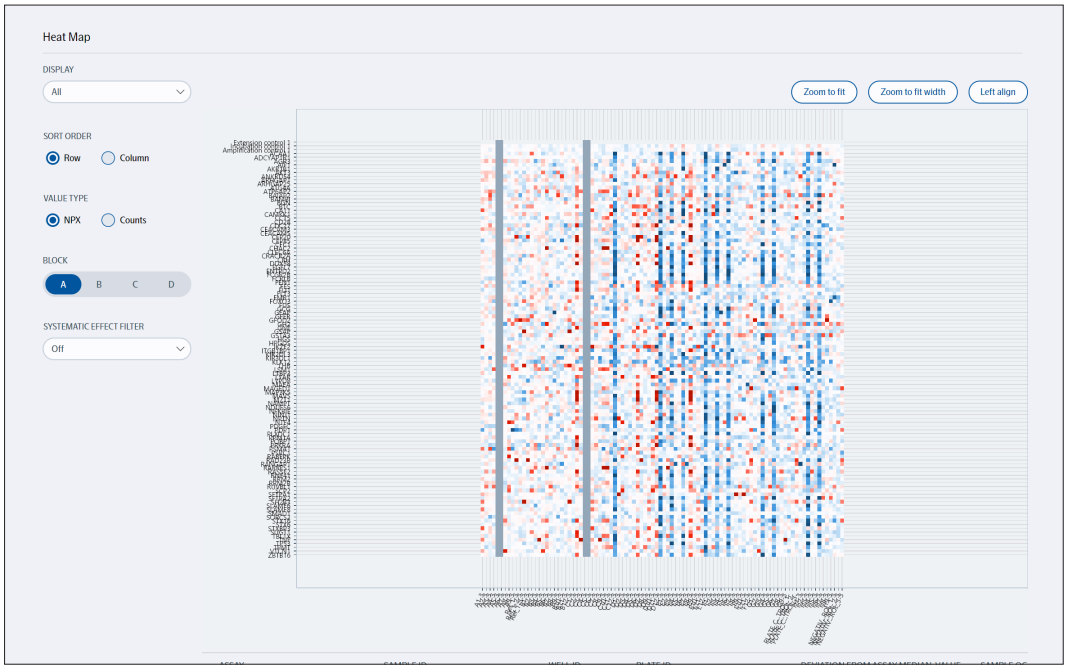
15.9 Heat Map

General:

The Heatmap view contains a color-coded view for all assays and samples in the chosen block. The assays are displayed in the horizontal direction and samples in vertical direction. Empty wells show counts but not NPX. A failed block is marked by a red banner.

The heatmap displays deviations from the plate median for the selected assay using the colors blue and red:

- Blue: values below median
- Red: values above median.



Useful for:

- Finding systematic patterns.
- Finding outlier samples for specific assays.

Functions:

- Select sample type to display. (All External Controls, Sample, Plate Control, Negative Control, Sample Control)
- Switch **Sort order** between row and column.
- Change **Value type** by selecting either NPX or Counts.
- Select **Block** to view (Only for Olink Explore 3072/384)
- Use the **Systematic effect filter** to select type of systematic effect, or swith it off. (Only available systematic effects types are shown in the drop-down menu)
- Hover over a well to show:
  - Assay
  - Sample ID
  - Well ID
  - Plate ID
  - Deviation from assay median
  - Value
  - Sample QC

- Zoom functions
  - Zoom in and out with scroll wheel.
  - **Zoom to fit**: will adjust the heat map to fit in the view.
  - **Zoom to fit width**: willl adjust the heat map to fit vertically.
  - **Left align**: will adjust the heaat map to align on the left of the view.
- Move the map to see different assays by clicking and holding the left mouse button and drag the heatmap.

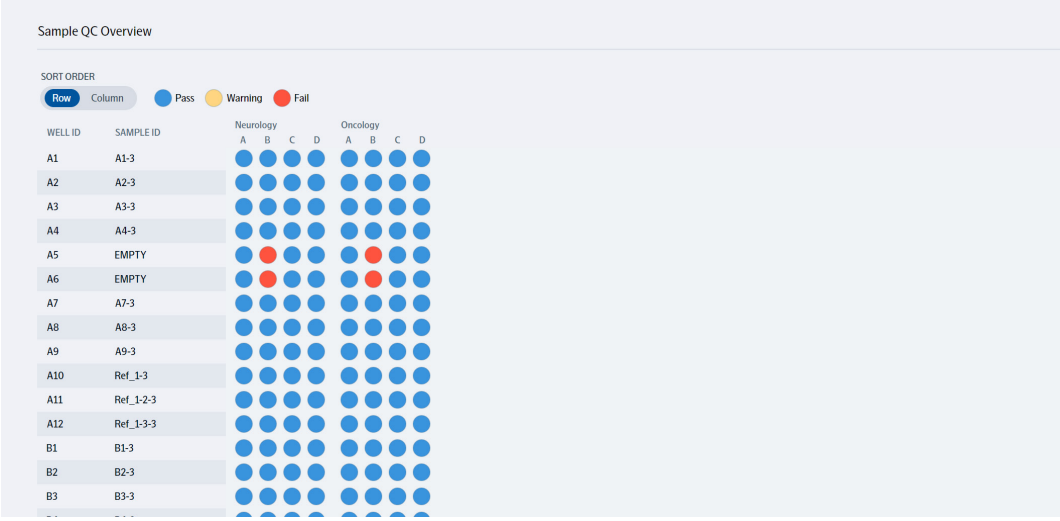
15.10 Sample QC Overview (Only for Olink® Explore HT)

General:

The Sample QC Overview displays a color-coded heatmap showing status for each sample in all blocks.

- Blue: Pass
- Red: Fail
- Yellow: Warning

If a sample has both a fail and a warning, it is shown as fail.



Useful for:

- Finding systematic errors across different blocks.

Functions:

- Switch **Sort order** between row and column.
- Hover over the cells provides information on:
  - Sample ID
  - Block
  - Well ID
  - For failed and warned samples: reason for failure or warning.

15.11 History

General:

The History view displays all events for the current project.

Useful for:

- Seeing what actions that has been done on the project.

Functions:

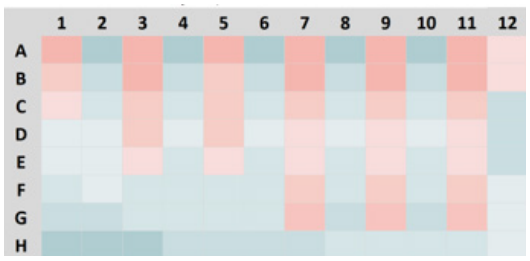
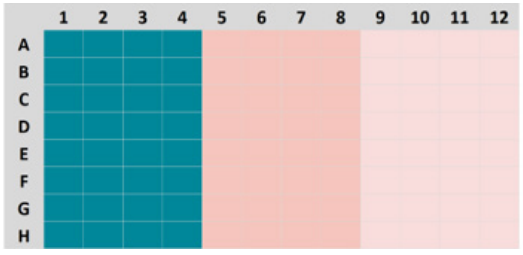
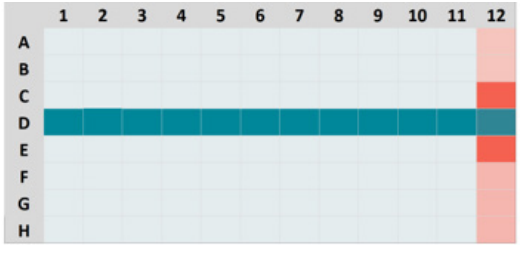
- The upper table shows changes not yet saved.
- The lower table shows changes that has been saved.

Part 5: Troubleshooting

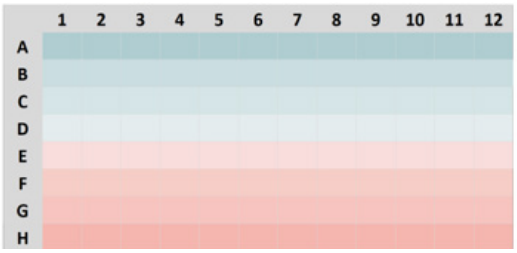
16. Troubleshooting

This chapter includes a trouble shooting guide, describing QC issues, affected level (sample, block, plate) and possible root causes for some potential non-expected results. Reruns are not recommended except for the projects that have failed to pass formal QC. Please contact *support@olink.com* for further investigations and receiving appropriate guidance for any of these cases.

QC issue	Affected samples/blocks/Sample plates	Possible cause
Plate Controls and/or samples fail		
Failed Plate Controls: Deviation from reference values (±)	Multiple blocks (1-4 or 5-8) Both sample plates plates	– Dilution plates or Sample Plate swapped positions on Mosquito – Wrong dilution protocol was used
Plate Controls fail: Deviation from reference values (Inc & Ext -, AMP +) (Inc, Ext & AMP -)	Multi blocks in both Sample plates	Poor PCR enzyme efficiency due to not adding enough Enzyme A or Enzyme B
Plate Controls fail: Deviation from reference values (Inc -, Amp & Ext +) – Low counts for Incubation Controls – Low counts for assays – High counts for Amplification and External Controls	The same single block in both Sample Plates (e.g. Block 2 in SP1 and SP2)	1A: Forward probe was not added to the incubation reagent mix 1B: Not enough incubation solution was added while preparing the reagent mix
	The same two blocks in both Sample Plates (e.g. Blocks 2 and 5 in SP1 and SP2)	2A: Forward probes were mixed up for the two blocks when preparing incubation mix (wrong pair of probes) 2B: Not enough incubation solution was added while preparing the reagent mix
Samples and/or external Controls fail: Too low counts	1. One or multi samples in a block One or both Sample Plates.	1. Incubation reagents mix or index not added properly due to Mosquito pipetting error
	2. All or multi samples in multi blocks (Likely blocks 1-4) Both Sample Plates.	2. Poor sequencing if %PF is low
Samples and Plate Controls fail: Too low counts – Low counts for assays – Low counts for all internal controls	1. One same block in both Sample plates (e.g. Block 2 in SP1 and SP2)	1. Reverse probe was not added into incubation reagent mix
	2. The same two blocks in both Sample plates (e.g. Blocks 2 and 5 in SP1 and SP2)	2. Reverse probes were mixed up for the two blocks when preparing incubation mix (wrong pair of probes). Amplification Control might have some counts.

Negative Control fail		
Negative Controls fail (Plate Controls might fail too)	1. Multi blocks in one Sample Plate (likely blocks 5-8)	1A. The plate layout does not match with the uploaded run. 1B. NCs related to one of the Sample Plates were pipette into wrong wells on Sample Source Plates. 1C. Negative Controls are contaminated
	2. The same multi blocks in both Sample Plates (blocks 1-4 or 5-8)	2A. Sample Source Plates was rotated in the workflow 2B. Samples added instead of Negative Controls in Sample Source Plates 2C. Index plate A and B were swapped positions on mosquito between the two stages of adding indexes 2D. Negative Controls are contaminated
Systematic effect		
Alternating column effect: NPX value deviates in every other column	One or multiple blocks in one or both Sample Plates	Mosquito pipetting error while dispensing reagent mix into incubation plate
		
Every 4 columns effect: NPX values deviate between every 4 columns	Two blocks in both Sample Plates	– Dragonfly faulty syringe during dispensation of PCR mix – Not well vortex of PCR mix
		
Row effect: NPX values deviate between samples in one row and samples in all other rows	Same row in the same block in both Sample Plate	– EpMotion pipetting error when pooling the samples – Human error missing one well during pooling step
		

Gradient patterns (row/column/diagonal wised): NPX Values deviate gradually in samples across the plate in row, column, or diagonal pattern	One or multi blocks in both Sample Plates	– Not well vortex of reagent mix/ plates – Not well adjusted/calibrated instruments



17.Revision history

Version	Software version	Date	Description
1.0.2	1.0.2	2025-02-07	*7.1.3* updated. *8* Assay QC in table updated.
1.0.1	1.0.1	2025-01-27	New

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1532, v1.0.2, 2025-02-07