USER GUIDE

Proteograph™ Analysis Suite

FOR USE WITH
Proteograph Product Suite

seer™

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Revision history

DOCUMENT	DATE	DESCRIPTION OF CHANGE
CF-1003 C (PAS version 2.0)	August 2022	Per *Proteograph Analysis Suite Release Notes* (CF-1034 A) • **PAS Proteogenomics Workflow to identify and explore peptides with protein sequence altering genetic variants** – **Build a custom peptide database** . New feature to build a customized peptide sequence database using user-uploaded custom or sample-specific variant call files (VCF). . VCF files are used to annotate genetic variants that may result in amino acid variants (i.e., variant peptides) not captured in the canonical reference database. . Variant peptide sequences are combined with the reference database to generate customized peptide sequence databases. – **Search for variant peptides** . Using the customized peptide sequence database, now users can search their DDA data for variant peptides using MSFragger. – **Variant Peptide Browser and Proteogenomics Data Explorer** . *Variant Peptide Browser* — Identified variant peptides are summarized in interactive plots and table. . Summary table shows all identified variant peptides and can be sorted/filtered by sample, nanoparticle, protein, variant, and allele frequency. Each variant (row) links to a view within the Proteogenomics Data Explorer. . The number of variant peptides found across samples, conditions, and nanoparticles are shown. The distribution of variant peptide intensity is stratified by reference and alternative alleles and homozygous/heterozygous alleles across samples, conditions, and nanoparticles. . *Proteogenomics Data Explorer* — Interactive tool to explore peptide and variant peptide data maps in genomic space for entire proteins and at nucleic acid/amino acid resolution . Gene structure, protein structure, domain information, and functional region information are displayed. . Amino acid variants within variant peptides are highlighted, including both reference and alternative alleles. • **Improvements to analysis protocols** – Integration of MSFragger database search engine. . Pre-installed MSFragger-based Proteogenomics protocol (DDA, VCF required). – Enabled DIA-NN MBR feature[1]. • **Dashboard to monitor PAS account usage** – Track the total number of plates added, projects added, completed analyses, and data storage. – Added links to most recently added plates, projects, completed analyses, and data storage. • **Expansion and improvement of analysis visualizations** – Added additional background datasets and overlay for multiple samples in

DOCUMENT	DATE	DESCRIPTION OF CHANGE
		waterfall plots. – Added ability to interact with sample correlation and similarity plots and visualize plots showing underlying data for each comparison. – Added option to view and compare all metrics from plate map grid across all wells and across all plates analyzed. – Added option to select order of comparison for differential abundance group analysis. – Added new interactivity with differential abundance group analysis volcano plot display. • **Changes to file and project management** – Added feature to multi-select projects when launching analyses. – Added feature to collapse Analyses folders by project. – Added project filtering capabilities. • **Improvements to analysis stability and plot visualizations for large-scale study sizes** – Stability and browser performance upgrades. – Analysis visualization and plot rendering speed improvements. – Streamlined analysis summary menu improves navigation between visualizations. • **Added PAS software end user license agreement (EULA)** • **Changes to help content** – Updated help system and user guide. – Updated tooltips. • **Other general improvements** – Sample Description File is now stored in Data Files. – Minor bug fixes. [1]Demichev, V., et al. DIA-NN: Neural Networks and interference correction enable deep proteome coverage in high throughput. *Nature Methods* 17, 41–44 (2019).
CF-1003 B (PAS version 1.5)	October 2021	Per *Proteograph Analysis Suite Release Notes* (CF-1018 A) **New analyses to visualize differences in peptide/protein group intensities between samples and groups** • New interactive table containing peptide/protein group intensities across samples/groups • New heatmap plot for visualizing peptide/protein group intensities across samples/groups **New analysis to identify peptide/protein groups with significantly different intensities between groups (e.g., healthy vs. disease samples)** • Added new "Group analysis" sections to 'Analysis' section • Guided comparison setup providing data filtering, normalization, imputation, and statistical test options • Toggle for viewing only 'Significant Peptides/Proteins' in dataset • Interactive plots and tables for exploratory expression analysis of significantly regulated targets with dynamic fold-change and p-value options • New group analysis plots to visualize peptides/protein groups with statistically

DOCUMENT	DATE	DESCRIPTION OF CHANGE
		different intensities – Volcano plot – fold-change vs. statistical significance with coloring options to highlight proteins-of-interest – Coverage viewer – Amino acid sequence coverage by peptides display, including PTM detection – Clustered heatmap – visualizing peptide/protein group intensities across samples/groups with rows (peptide/protein groups) and columns (samples) ordered using hierarchical clustering – Protein-protein interaction – Visualization of PPIs (STRING db) of significant proteins – Intensity box plots – Compare significant peptide/protein group intensities between groups **Changes to plot visualizations** • Added ability to export plot source data to .csv files • Improved plot rendering speeds • Added user preferences to modify plots **New analysis protocols** • Integration of DIA-NN database search engine – Pre-installed DIA-NN library-based protocol (DIANN - System Provided DIA Protocol)[1] – Expanded Seer human plasma spectral library file (4,011 protein groups; 62,687 precursors) – Optional in silico predicted library-free protocol **Changes to results summary** • Simplified peptide and protein group results tables – Panel level summary – NP level summary **Changes to help content** • Updated user guide • Indexed online help • Tooltips **New data file management** • New AutoUploader application allows automatic data transfer from LC-MS system to PAS account [1]Demichev, V., et al. DIA-NN: Neural Networks and interference correction enable deep proteome coverage in high throughput. *Nature Methods* 17, 41–44 (2019).
CF-1003 A (PAS version 1.0)	May 2021	Initial release

Contents

Chapter 1

Welcome to PAS

This chapter introduces you to the Seer Proteograph Analysis Suite (PAS) software application, part of the Seer Proteograph™ Product Suite.

With this dedicated software solution, you can process, analyze, and visualize proteomics data sets generated by liquid chromatography–mass spectrometry (LC-MS). PAS includes an integrated search engine for identification and annotation of LC-MS data.

Access the PAS application

To access the PAS application, point your web browser to pas.seer.software. Compatible browsers include Chrome, Chromium, Edge, Firefox, and Safari.

When prompted to log in, enter your username and password. In the future, if you configure two-factor authentication, you will also need to enter a code generated by an authenticator app on your mobile device. (For details for setting up two-factor authentication, see *Configure two-factor authentication for yourself* (page 25).)

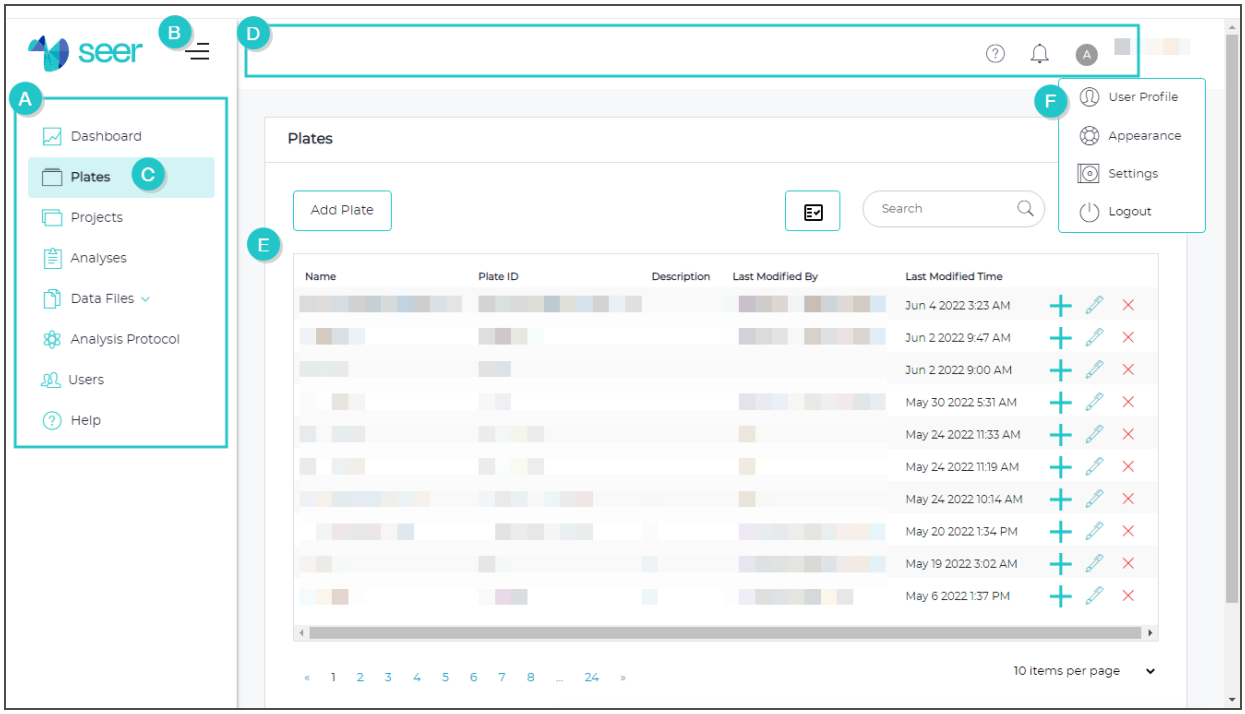
Tour of the PAS application

The following sections offer a tour of the PAS application window and describe common ways to navigate among and to work with its pages.

Parts of the PAS application window

The PAS application window is organized into navigational, informational, and user functions. A page you'll work on often is the Plates page, illustrated here.

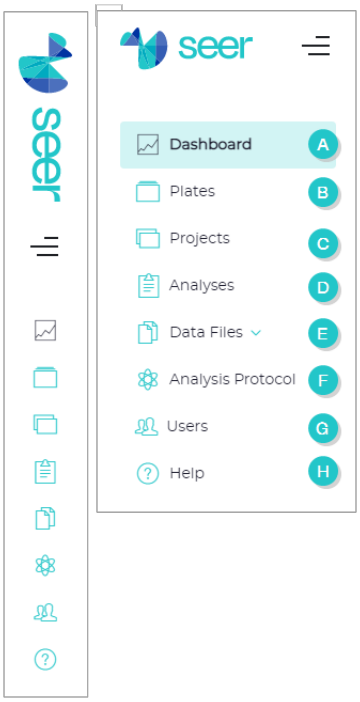
Main parts of the application window



- **A** Sidebar menu
- **B** Button to expand and collapse the sidebar menu
- **C** Active page
- **D** Top bar
- **E** Table items. For general techniques for working with tables, see *Work with PAS tables* (page 16).
- **F** User Account menu, accessible from your username

Sidebar menu

Use the sidebar menu to access PAS functionality and data.

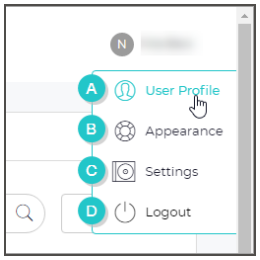


- **A** **Dashboard** — Summarizes the total number of completed analyses, plates, and projects in PAS, as well as offering links to the most recent of each. Also shows the amount of storage space used. See *Dashboard page* (next page).
- **B** **Plates** — For importing sample metadata from the Proteograph Assay method and associated MS data files. See *Plates and samples* (page 89).
- **C** **Projects** — For grouping Proteograph Assay plates at the experimental project level and submitting data analysis jobs. See *Projects* (page 96).
- **D** **Analyses** — For accessing data analysis jobs, data analysis results, and data visualizations. See *Analyses* (page 99).
- **E** **Data Files** — For accessing and managing the repositories of MS data files and VCF files. See *Data files* (page 104).
- **F** **Analysis Protocol** — For accessing a collection of database search analysis protocols. See *Analysis protocols* (page 110).
- **G** **Users** — (Admin only) For viewing, adding, editing, and deleting users. See *User management* (page 26).
- **H** **Help** — For accessing PAS documentation including the user guide (PDF), web-based Help system, and videos. See *Get help with PAS* (page 15).

Top bar and User Account menu

The top bar of the PAS application window has just one component, the **User Account** menu, at the far right. To open the menu, select your username.

NOTE
When the sidebar menu is collapsed, the top bar — including your username — is hidden.



A User Profile — For managing your user information, such as your telephone number. See *Manage your user profile* (page 22).

B Appearance — For selecting a light or dark theme for the PAS user interface. See *Change the appearance of the user interface* (page 24).

C Settings — For configuring various application settings, including which DDA and DIA protocols will be used by default for data analysis. See *Configure application settings* (page 25).

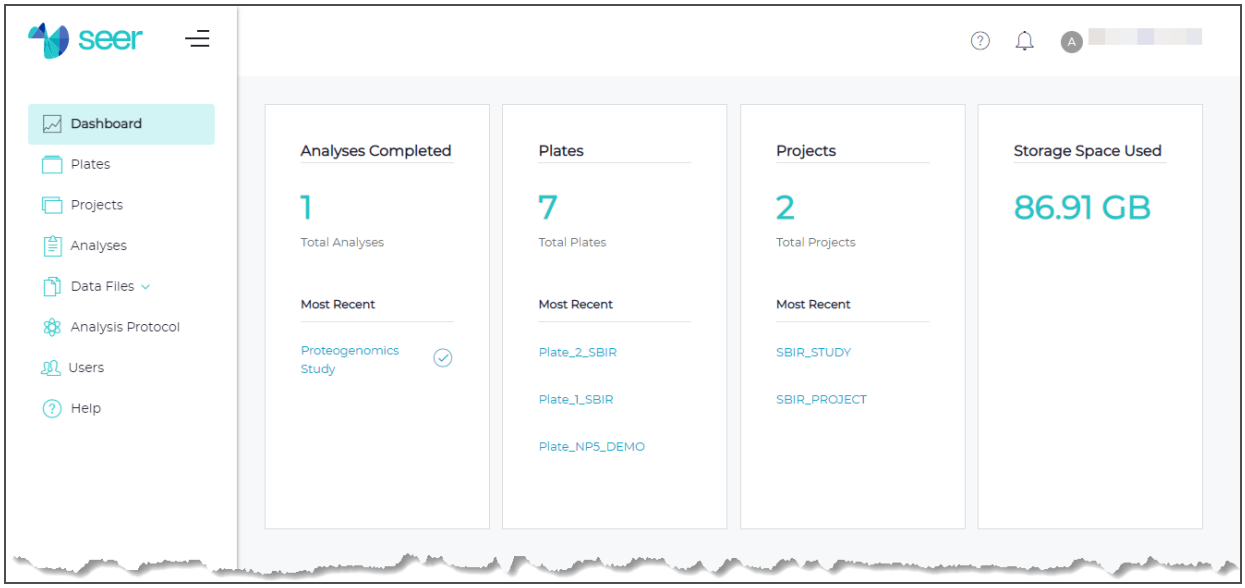
D Logout — Logs you out of PAS.

Dashboard page

The **Dashboard** page summarizes the total number of analyses in progress (not illustrated below), completed analyses, plates, and projects in PAS, as well as offering quick links to the most recent of each. For the analyses, you can see their status:

- **Analysis Started**
- **Analysis Finished Successfully**
- **Analysis Failed**

The **Dashboard** page also shows the total amount of file storage space used in your account.

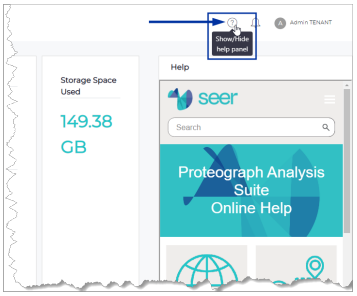


Get help with PAS

Help for PAS is always just a click or two away. You can view the online help within in the application itself or in a separate browser window. It's the same information, just in a different window.

To open the online help within the application:

- Near the right end of PAS's top bar, select **Show/Hide help panel** to open the **Help** panel. The panel is positioned on the right side of the application window. To close the **Help** panel, select that button again.



To open the online help in a browser window:

1. On the sidebar menu, select **Help** to open the **Help** page.
2. Under **Online Help**, select **View Help**, which launches the online help in your default browser.

> **TIP**
>
> If you prefer a traditional manual, download the *PAS User Guide* (PDF) from the **Help** page. You'll find the same information as in the online help.

To use the online help:

Unless otherwise stated, you can use the following techniques with either the in-app online help or browser online help.

- **Select a section tile on the home page** — Each of the major sections of the online help has a tile for quick access.
- **Return to the home page** — Click the Seer logo in the upper left.
- **Use Search** — A prominent Search bar appears above each help topic. Just enter your search terms and press **Enter**. (To look for an exact phrase, frame it in quotation marks.) Then select a topic from among those returned. The search terms will be highlighted, which you can remove with the **Remove search highlighting** button.
- **Use the menu of topics** — In the in-app online help, click the **Help menu** button to slide out the menu of topics. In the browser help, the menu is already open at the left, unless you resize the browser window narrow enough to show the **Help menu**. Expand and collapse sections of the menu, and then select the topic you want to view.
- **Follow links** — Ample links are provided within topics for navigating to related topics. Also use "You are here" links above a topic to go back to a topic higher in the menu of topics.

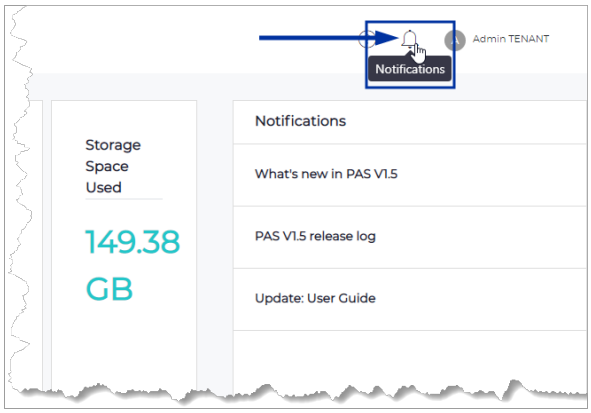
- **Navigate with buttons** — Use **Back** and **Forward** to step through the history of topics you've visited. Use **Previous topic** and **Next topic** to go to the previous or next topic relative to the current topic.
- **Expand and collapse content** — To open and close a specific section, use **Expand** and **Collapse** beside its heading. To expand and collapse all sections in a topic, use the toolbar buttons **Expand all** and **Collapse all**.
- **Print a topic** — Use the **Print topic** button.

Get notifications

Use the **Notifications** panel to find out what's new in PAS, read the release log, and view other important messages from Seer.

To open the Notifications panel:

- Near the right end of the top bar, select **Notifications** to open the **Notifications** panel. The panel is positioned on the right side of the application window. To close the **Notifications** panel, select that button again.



To view a specific notification:

- Click the notification you want to view. Depending on your selection, you might view a guided tour of what's new in PAS, view a PDF, or jump to a specific place in the PAS application itself.

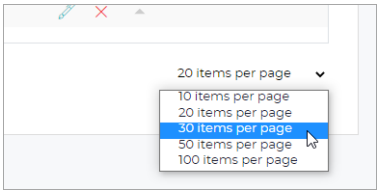
Work with PAS tables

Information in PAS is organized into tables. For example, the *Projects page* (page 96) offers a table of all projects, while the *Users and Permissions page* (page 26) offers a table of all PAS users.

Use the following common techniques to work with PAS tables. (Some tables offer additional features, discussed in detail in *Data Management* (page 88).)

Set the number of items per table page

To set how many table items PAS shows per page, select an option from the **Items per page** list, located at the lower right below the table.

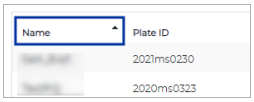


Navigate among table pages

Depending on how many items are shows per page, some tables may be broken into several pages. To navigate among the pages, use the left and right arrows located below the table. You can also select a specific page to navigate to.

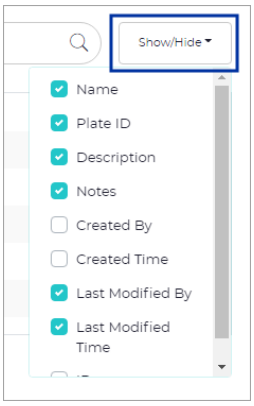
Sort a table

Sort table items by selecting the heading of the column you want to sort by. PAS sorts in descending order by default. To reverse the order, select the heading again. To turn off sorting entirely, select the heading one more time.



Show or hide table columns

Some tables offer a **Show/Hide** list from which you can show and hide columns by selecting and clearing checkboxes.



Add custom table columns

You can add custom columns to the *Plate Samples section* (page 91) and *Sample List section* (page 98). Any custom column you add to one of these tables is available for the other table. You can show and hide custom columns like any other column.

1. Select **Show/Hide**, scroll to the bottom of the list, and select **Add Custom** to open the **Add Custom Field** dialog.
2. Complete the fields.

- **Custom Name** — A unique name for this custom field, within this table and with no spaces.
- **Description** — The description of the custom field.
- **Notes** — Additional information about the custom field.

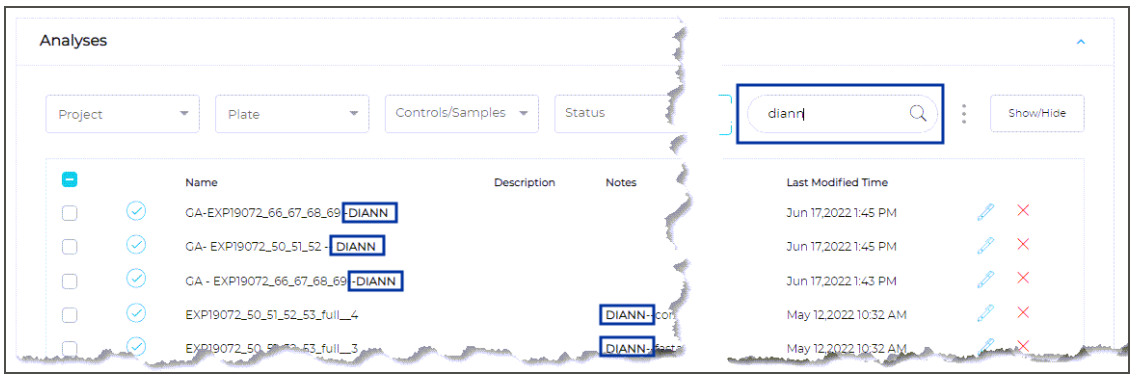
3. Select **Create**.

   The custom field (column) appears at the bottom of the **Show/Hide** column with the prefix `custom_`.

Find table items

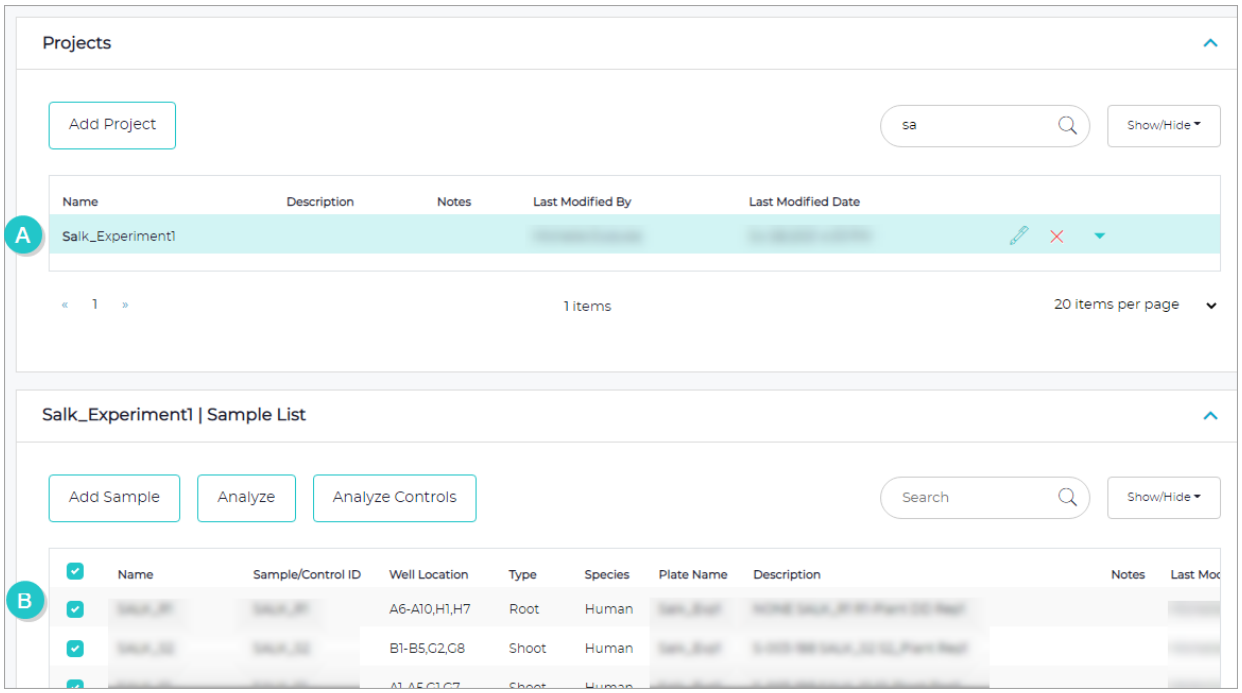
Use a table's **Search** field to find a specific table item.

As you type in this field, PAS refreshes the table to show only matching table items, with the matching character string in bold in the column in which it was found. To show all table items again, clear the **Search** field.



Access table item details and/or sub-items

Some tables offer access to item details and/or related sub-items. To access an item's details or its sub-items, select that item's row. For example, on the **Projects** page, selecting a project row to access its related samples.

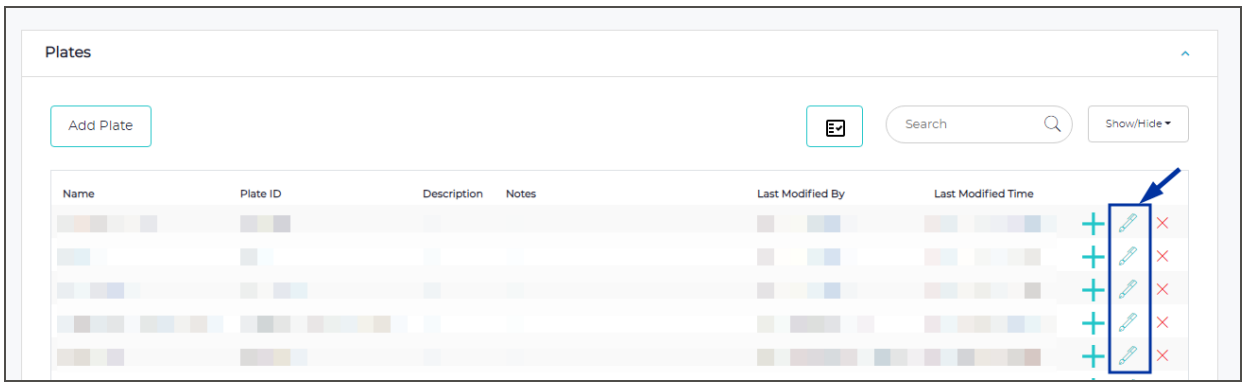


A Selected item

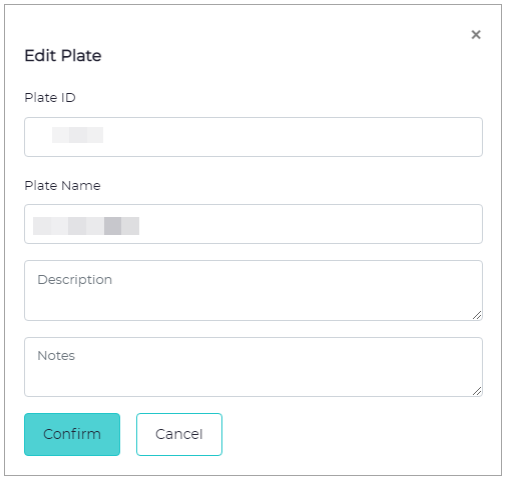
B Table of sub-items associated with the selected item

Edit table items

On many PAS tables, you can edit an individual table item by selecting its **Edit** button. (You may need to scroll to the right to see this button.)

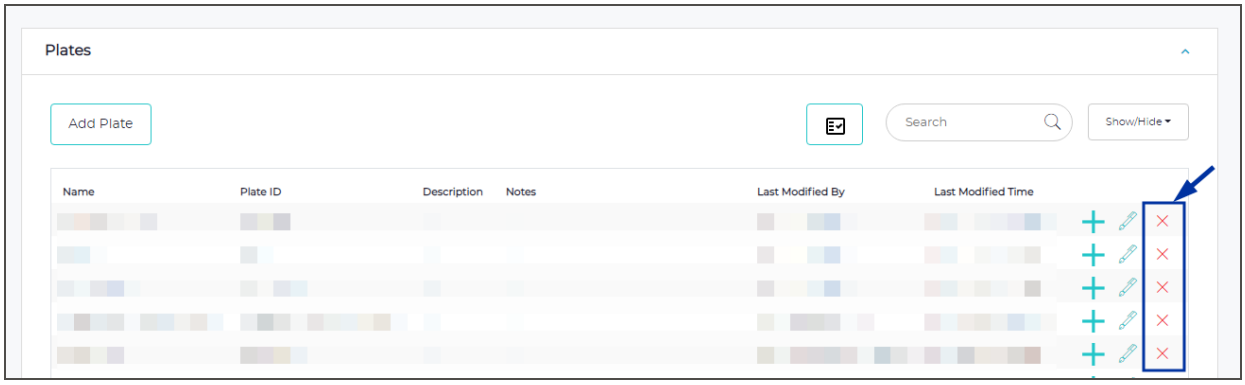


Once you select **Edit**, a dialog box opens where you can edit information such as the ID or name of the item, its description, and notes.



Delete table items individually

On all PAS tables, you can delete an individual table item by selecting its **Delete** button. (You may need to scroll all the way to the right to see this button.)



Once you select **Delete**, a dialog box opens for you to confirm the deletion.

Delete multiple table items at the same time

On many PAS pages, you can select and delete multiple table items at the same time.

NOTE

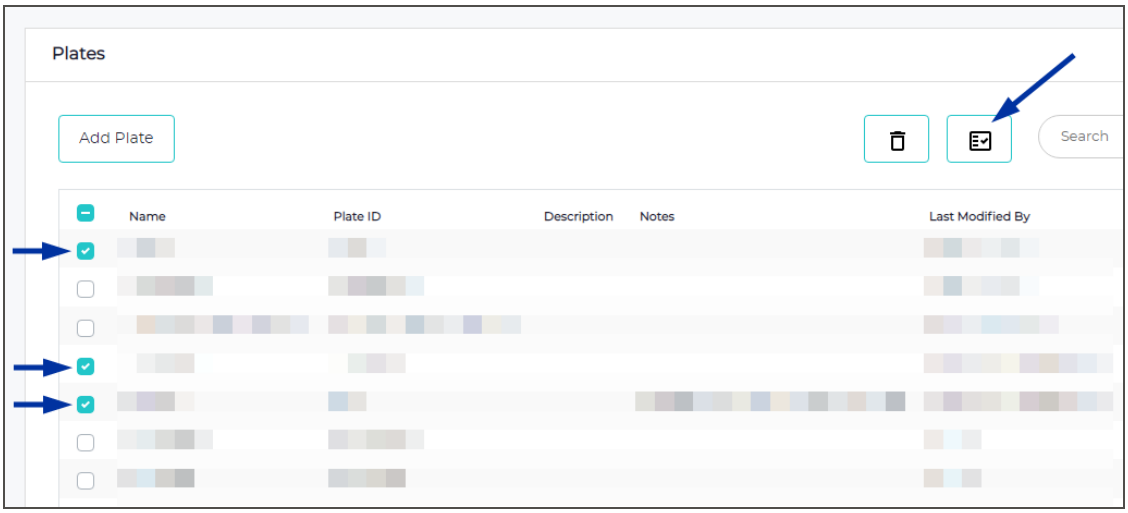
The exceptions are the **Users and Permissions** page and **VCF Files** page, which only allow individual deletions with the **Delete** button. See *Delete table items individually* (previous page).

Depending on the page, PAS offers different ways to delete multiple table items. Each way is described below.

Delete multiple table items with Batch Delete

On pages that show the **Multi-selection** button, you can select multiple table items and then use the **Batch delete** button. Pages with these buttons include the *Plates page* (page 89), *Projects page* (page 96), and *Protocols page* (page 110).

1. Select **Multi-selection** > **Show checkboxes**.
2. Use the following techniques to select one or more table items.
   - Select individual table items' checkboxes. The table items don't need to be on the same page.



- To select all table items on the current page, either select the checkbox in the table's header row or select **Multi-selection** > **Select files on this page**.
- To deselect all table items on the current page, either clear the checkbox in the table's header row or select **Multi-selection** > **Deselect files on this page**.
- To select all table items across all pages, select **Multi-selection** > **Select files on all pages**.
- To deselect all table items across all pages, select **Multi-selection** > **Deselect files on all pages**.

> **TIP**
> For most tables, as you select items, PAS updates the `x of xxx selected` message below the table.

3. When ready to delete the selected table items, select **Batch delete**.
4. Select **Delete** to confirm.

Delete multiple table items on with ⋮ ***Menu*** *> Delete*

On pages on which table items' checkboxes are always shown, you just need to select the ones to delete and then use the ⋮ **Menu** button to select **Delete**. Pages with the ⋮ **Menu** button include the *Analyses page* (page 99) and *MS Files page* (page 104).

1. Use the following techniques to select one or more table items.
   - Select individual table items' checkboxes. The table items don't need to be on the same page.
   - To select all table items on the current page, select the checkbox in the table's header row.
   - To deselect all table items on the current page, clear the checkbox in the table's header row.

> **TIP**
> For most tables, as you select items, PAS updates the `x of xxx selected` message below the table.

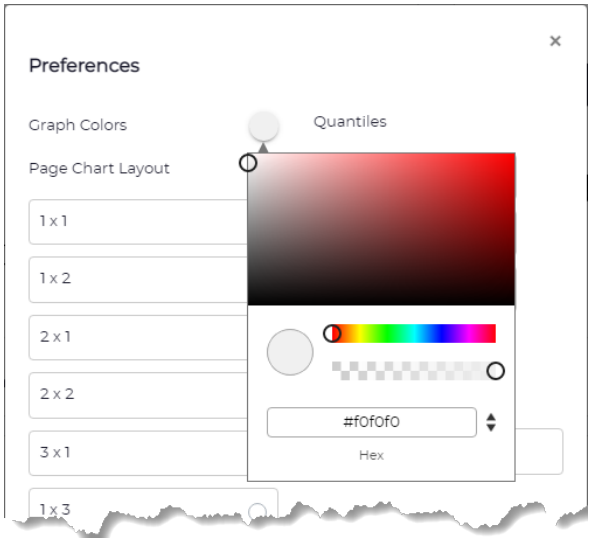
2. When ready to delete the selected table items, select ⋮ **Menu > Delete**.
3. Select **OK** to confirm.

Set color preferences in PAS graphs

Many PAS graphs offer a **Preferences** dialog with which you can set colors to suit your preferences.

To set a color preference:

1. Select the color control, such as **Graph Colors** as shown here, to open the color picker.
2. Select a color on the color spectrum or enter a color's hexadecimal value.



3. Click outside color picker to close it.

Manage your user profile

1. On the **User Account** menu, select **User Profile** to open the **User Profile** page.
2. Edit the fields as needed. (Depending on your role and organization, the fields may vary.)
   - **User Profile** — Edit your first name, last name, and phone number.
   - **Tenant Info** — Edit information about your organization and your address.
3. Select **Save**.

Input files

PAS accepts the following files as input for analysis.

- **Analysis protocol** (.xml, .json) — (Optional) The parameters for an MS database search. PAS includes pre-installed analysis protocols for data-dependent acquisition (DDA), data-independent acquisition (DIA), and Proteogenomics workflows. See *Analysis protocols* (page 110).
- **MS data files** (.raw, .wiff, .wiff.scan) — The results of MS analysis for each sample or control in a plate. Each sample or control is associated with one MS data file. See *MS data files* (page 104).
- **VCF files** (.vcf, .vcf.gz) — Custom or sample-specific variant call files that contain gene sequence variants. A standard VCF file contains a header line with information describing the data, followed by data lines containing information about a position in the human genome. See *VCF Files page* (page 107).
- **Plate map file** (.csv) — The location of each sample in a plate with information on each sample, control, nanoparticle, and peptide. See *Plate map file format* (next page).
- **Sample description file** (.csv) — Metadata for each sample in a plate, such as sample name and IDs, type, species, and condition. See *Sample description file format* (next page).

Plate map file format

The Proteograph Instrument Control Software (ICS) generates the plate map file (.csv). All columns are prefilled except tor MS file name, which you must define.

Table 1. *Columns in a plate map file*

COLUMN	DESCRIPTION	NOTES
MS file name	The name of the MS data file, including extension (for example, `EXP20101_2020ms0321X83_A.raw`)	
Sample name	A unique identifier for the sample, typically the biological sample name	a
Sample ID	A unique identifier for the sample	a
Well location	The column and row of the well the sample occupies	
Nanoparticle	The nanoparticle name (nanoparticle 1–5)	
Nanoparticle ID	The nanoparticle lot (kit ID)	
Control	The control type: Process Control, Digestion Control, MPE Control, or Mass Spec Control	
Control ID	The control lot	
Instrument name	The name of the liquid handler that converted the samples into peptides	
Date sample preparation	The date the method ended	
Sample volume	The volume of sample in the indicated well	b
Peptide concentration	The concentration of peptide in the well	b
Peptide mass sample	The mass of peptide in the well	b
Recon volume	The volume of Peptide Reconstitution Buffer added to the well	b
Kit ID	The ID of the assay kit used to convert the samples into peptides	
Plate ID	A unique identifier for the plate	
Plate Name	A descriptive name for the plate	

a. The sample name links the plate map and sample description files, while the sample ID groups the nanoparticles in an assay.
b. The file shows the numeric value only, without unit of measure.

Sample description file format

The sample description file (.csv) contains the following information about each sample in a plate. The file can also include custom (user-defined) columns. These columns must include the prefix `custom_`.

Table 2. Columns in a sample description file

COLUMN	DESCRIPTION
Sample Name	A unique identifier for the sample, typically the biological sample name
Sample Type	The type of sample, such as plasma or tissue lysate
Species	The species (human or mouse) from which the sample was collected
Description	A description of the sample
Sample Receipt Date	The date your laboratory received the sample
Sample Collection Date	The date the sample was collected
Condition	The categorical group the sample belongs to
Biological Replicate	The biological replicate number
Technical Replicate	The technical replicate number

Output files

An analysis generates several results files, which record search results for each sample in .txt and .xml formats.

Quality control

A plate includes prepared samples and the following controls for quality control (QC) purposes. The control results provide insight into the variation and reproducibility of experiments and LC-MS analysis.

- **Process Control** — A reference sample added before protein corona formation.
- **Digestion Control** — A reference sample added before trypsin digestion.
- **MPE Control** — Reference peptides added before desalting cleanup.
- **Mass Spec Control** — Reference peptides added before LC-MS analysis.

Application settings

You can configure several PAS application settings, including the default analysis protocols for controls and samples. You can also change the appearance of the PAS user interface. These settings are global and apply to all analyses and pages. Finally, you can configure two-factor authentication for yourself.

Change the appearance of the user interface

1. On the **User Account** menu, select **Appearance** to open the **Appearance** page.
2. Select the theme you want to use:
   - **Light** (default) — Makes the background of the user interface white.
   - **Dark** — Makes the background of the user interface black.
3. Select **Save**.

Configure application settings

Follow these steps to configure PAS application settings, except two-factor authentication. (For those instructions, see *Configure two-factor authentication for yourself* (below).)

1. On the **User Account** menu, select  **Settings** to open the **Settings** page.
2. In the **Settings** section, configure settings as needed.
   - **Default Analysis Protocol** — Select the DDA-based and DIA-based analysis protocols you want to use for controls and for samples.
   - **Remove MS data files when plate is deleted** — Select either:
     - *No (default)* to have PAS retain the MS data files linked to the plate.
     - *Yes* to have PAS delete the MS data files linked to the plate.
   - **Receive email notifications on analysis updates** — Select either:
     - *Yes (default)* to have PAS send you an email whenever an analysis status changes.
     - *No* to have PAS not send emails about analysis updates.
3. Select **Save**.

Configure two-factor authentication for yourself

You can configure PAS to require two-factor authentication when you log in. That means that PAS will prompt not only for your username and password, but also for a generated authentication code.

NOTE

Before continuing, you will need to have already installed an authentication app on your mobile device. PAS is compatible with many such apps, including Google Authenticator, Duo Mobile, 1Password, Auth, and Microsoft Authenticator.

1. On the **User Account** menu, select  **Settings** to open the **Settings** page.
2. In the **Two-factor Authentication** section, select **Use Authenticator App** to open the **Authenticator verification** dialog.
3. Using the authenticator app on your mobile device, either scan the QR code or enter the key shown on-screen into the authenticator app.
4. In PAS, enter the generated code.
5. Select **Submit** and then select **OK** to close the success message.

NOTE

Notice that the **Use Authenticator App** button now reads **Disable Authenticator App Security**. To turn two-factor authentication off, follow the same steps as above, select that button, and follow the on-screen prompts.

User management

If you are a PAS administrator, you can add, edit, and delete users.

Users and Permissions page

If you are a PAS administrator, use the **Users and Permissions** page to manage PAS users. To open this page, select **Users** on the sidebar menu.

> **TIP**
> Use this page's **Collapse** and **Expand** buttons to selectively collapse and expand sections.

Toolbar items

- **Add User** — Select to add a new user. See *Add a user* (below).
- **Search** — Use to find a specific item. See *Find table items* (page 18).

Table columns

- **Name** — The user's name.
- **Email** — The user's email address.
- **Role** — The user's role, which grants access to specific PAS functionality.
  - *User* — Can add plates, create projects, create analysis protocols, view MS data files, and view results files.
  - *Admin* — In addition to *User* functionality, can also manage users.
- **Group** — The group or groups the user is assigned to.

  > **NOTE**
  > - Users are not automatically assigned to groups. You must explicitly assign them.
  > - Use group assignments to limit user access to specific plates, projects, and analyses.
  > - Members of a group can access and view the plates, projects, and analyses of everyone in the group.

- **Status** — The user's status (e.g., *Confirmed*, meaning acceptance of the invitation to join PAS).
  - **Reinvite User** — Select to resend the invitation to a user to join PAS. See *Reinvite a user to join PAS* (next page).
- **Edit** — Select to edit the selected user. See *Edit a user* (next page).
- **Delete** — Select to delete the selected user. See *Delete a user* (next page).

Add a user

1. On the sidebar menu, select **Users** to open the **Users and Permissions** page.
2. Select **Add User** to open the **Invite User** dialog.
3. Complete the fields. Required fields are marked on-screen with an asterisk.

- **Username** — Enter a unique identifier for the user.
- **Email** — Enter the user's email address.
- **Role** — From the list, select the user's role: *User* (the default) or *Admin*.
- **User Groups** — (For a user of *User* role) From the list, select one or more existing user groups to which to assign the new user. To add a new group, enter its name.

NOTE

- Users are not automatically assigned to groups. You must explicitly assign them.
- Use group assignments to limit user access to specific plates, projects, and analyses.
- Members of a group can access and view the plates, projects, and analyses of everyone in the group.

4. Select **Send**.

   The user will be sent an email invitation with the assigned username, a temporary password, and a link to PAS.

Reinvite a user to join PAS

1. On the sidebar menu, select **Users** to open the **Users and Permissions** page.
2. Find the user to whom you want to resend the invitation to join PAS.
3. Select **Reinvite User**.
4. Select **OK** to confirm.

   The user is sent an email invitation with the assigned username, a temporary password, and a link to PAS.

Edit a user

1. On the sidebar menu, select **Users** to open the **Users and Permissions** page.
2. Find the user you want to edit and select **Edit** to open the **Edit User** dialog.
3. Edit the fields as needed.
4. Select **Save**.

Delete a user

1. On the sidebar menu, select **Users** to open the **Users and Permissions** page.
2. Find the user you want to delete and select **Delete**.
3. Select **OK** to confirm.

Chapter 2

Analysis Setup

This chapter offers step-by-step guidance for different setup workflows for preparing your data for analysis.

Analysis setup workflows

PAS offers several workflows for setting up a data analysis.

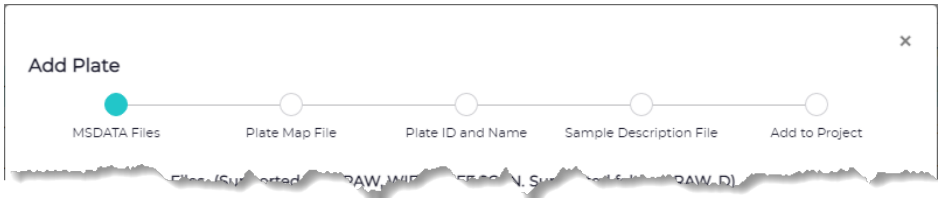
WORKFLOW	DESCRIPTION	ANALYSIS PROTOCOL
Add a plate (below)	This is the most straightforward workflow. You begin on the **Plates** page and add a plate. You can elect to start the analysis immediately or defer it for later.	DDA, DIA
Link to a plate (page 32)	You begin on the **MS Files** page and link the files to a plate. You can start the analysis immediately or defer it for later. (For this workflow, you must have already uploaded MS data files. See *Upload MS data files* (page 105).)	DDA, DIA
Select samples or controls for analysis (page 35)	You begin on the **Projects** page and select and analyze samples. Choose this workflow: • To analyze samples in more than one plate in a single analysis. • To reanalyze samples. • To analyze samples with the Proteogenomics analysis protocol. (For this workflow, you must have already defined associations between samples and VCF files. See *VCF files* (page 107).)	DDA, DIA, Proteogenomics

Add a plate

When you set up analysis by adding a plate, PAS guides you through the workflow with the **Add Plate** dialog. In this workflow, you add a new plate to PAS and then add samples to a new or existing project. Depending on your choices during the workflow, the analysis will start immediately or will be deferred for later.

To begin this workflow:

1. On the sidebar menu, select **Plates** to open the **Plates** page.
2. Select **Add Plate** to open the **Add Plate** dialog.



Add one or more MS data files

In the **MSData Files** section, add one or more MS data files.

1. Select one or more MS data files.
   - To add a single file, select **Files** to open the **Add Files** dialog.
   - To add multiple files, select **Folder** to open the **Add Folder** dialog.

   NOTE

   Supported file formats are .raw, wiff, or .wiff.scan. Supported folders are RAW and D.
2. Either drag the file or folder into the drag-and-drop area or use **Browse** to navigate to and select it.
3. Select **Add**.

4. Review the list of selected files.
   - To remove a file, select **Delete**.
   - To remove all files, select **Clear**.
5. Select **Next** to advance.

Add the plate map file

In the **Plate Map File** section, add a plate map file, which specifies the locations of samples.

1. If you don't have a plate map file, create one before continuing.
   a. Select the on-screen link from which you can download an example plate map file (.csv).
   b. Open the file and edit it as needed.

      The **MS file name**, **Sample ID**, and **Plate ID** columns are required. (See *Plate map file format (page 23)* for detailed descriptions of the columns in the file.)
   c. Save as a .csv file.
2. Select **Add File** or **Add** to open the **Add File** dialog.
3. Either drag the file into the drag-and-drop area or use **Browse** to navigate to and select it.
4. Select **Add**.
5. Select **Next** to advance.

Specify plate information

In the **Plate ID and Name** section, link the MS data files to a new or existing plate.

1. Do either of the following:
   - To link to an existing plate, select **Use Existing Plate**, and then select a plate from the **Select Existing Plate** list.
   - To set up a new plate, complete the fields.
     - **Plate ID** — A unique identifier for the plate.
     - **Plate Name** — A descriptive name for the plate.
2. Select **Next** to advance.

Add a sample description file (optional)

In the **Sample Description File** section, upload metadata for each sample in the plate.

NOTE
To skip this optional part of the workflow, select **Next** to advance.

1. Select **Add** or **Add File** to open the **Add Files** dialog.
2. Either drag the file into the drag-and-drop area or use **Browse** to navigate to and select it.
3. Select **Add**.
4. Select **Next** to advance.

Add the samples to a project

In the **Add to Project** section, add samples to a new or existing project.

1. Create or select a project:
   - To create a new project, select **New Project**, enter a project name, and select **Add**.
   - To use an existing project, select it from the **Select Project** list.
2. Select or clear the applicable checkboxes. (To defer analysis, clear both checkboxes.)
   - **Analyze samples after addition** — Select to analyze samples.
   - **Analyze controls after addition** — Select to analyze controls.
3. Select the MS method:
   - *DDA* — Derives an MS/MS spectra from selection, isolation, and fragmentation of an individual precursor ion.
   - *DIA* — Derives an MS/MS spectra from selection, isolation, and fragmentation of all precursor ions in a defined m/z range.
4. From the **Analysis Protocol** list (which shows only protocols compatible with the selected MS method), select a protocol.
5. Select **Add Plate**, and then select **Close**.

   Depending on prior choices, the analysis either begins immediately or you must start it manually. See *Start the analysis manually* (below).

Start the analysis manually

If you chose to defer the analysis during the setup workflow, follow these steps to start it manually.

1. On the sidebar menu, select **Projects** to open the **Projects** page.
2. Select the applicable project.
3. Select one of the following options:
   - **Analyze** — Opens the **Analysis** dialog from which to analyze all selected samples in the project.
   - **Analyze Controls** — Opens the **Analyze Controls** dialog from which to analyze the controls only.
4. If you are analyzing all selected samples, in the **Analysis Name** field, enter a name for the analysis.
5. Select the MS method:
   - *DDA* — Derives an MS/MS spectra from selection, isolation, and fragmentation of an individual precursor ion.
   - *DIA* — Derives an MS/MS spectra from selection, isolation, and fragmentation of all precursor ions in a defined m/z range.
   - *PROTEOGENOMICS* — (Appears only for samples, not controls.) Identifies variant peptides arising from single nucleotide variants or short insertions and deletions.
6. From the **Analysis Protocol** list (which shows only protocols compatible with the selected MS method), select a protocol.
7. In the **Description** field, enter a description of the analysis.
8. In the **Notes** field, optionally enter any additional information about the analysis.

9. If you are analyzing all selected samples and want to exclude the controls, select the **Exclude controls** checkbox.
10. If you are analyzing only controls, under **Analysis Name Pattern**, review the MS data file name format and list of controls to analyze.
11. Select **Start**, and then select **OK**.

    The samples or controls are queued for analysis.
12. On the sidebar menu, select **Analyses** to open the **Analyses** page.
13. Confirm that the new analysis appears on the **Analyses** page.

Link to a plate

When you set up analysis by linking to a plate, PAS guides you through the workflow with the **Link to Plate** dialog. In this workflow, you link MS data files and associated sample information to a new or existing plate. Depending on your choices during the workflow, the analysis will start immediately or will be deferred for later.

> **NOTE**
>
> If you have not already uploaded the MS data files you want to work with, follow the steps in *Upload MS data files* (page 105) before starting this workflow.

To begin this workflow:

1. On the sidebar menu, expand **Data Files** and select **MS Files** to open the **MS Files** page.
2. Select the folder that holds the files you want to work with.
3. Select the checkbox of each MS data file to include in the analysis. Each MS data file represents one sample.
4. Select **Link to Plate** to open the **Link to Plate** dialog.

Review the MS data files to be linked a plate

In the **MSData Files** section, review the selected MS data files to be linked to a plate.

1. Review the list of selected files.
   - To remove a file, select **Delete**.
   - To remove all files, select **Clear**.
2. Select **Next** to advance.

Add the plate map file

In the **Plate Map File** section, add a plate map file, which specifies the locations of samples.

1. If you don't have a plate map file, create one before continuing.
    a. Select the on-screen link from which you can download an example plate map file (.csv).
    b. Open the file and edit it as needed.

       The **MS file name**, **Sample ID**, and **Plate ID** columns are required. (See *Plate map file format (page 23)* for detailed descriptions of the columns in the file.)
    c. Save as a .csv file.
2. Select **Add File** or **Add** to open the **Add File** dialog.
3. Either drag the file into the drag-and-drop area or use **Browse** to navigate to and select it.
4. Select **Add**.
5. Select **Next** to advance.

Specify plate information

In the **Plate ID and Name** section, link the MS data files to a new or existing plate.

1. Do either of the following:
    - To link to an existing plate, select **Use Existing Plate**, and then select a plate from the **Select Existing Plate** list.
    - To set up a new plate, complete the fields.
        - **Plate ID** — A unique identifier for the plate.
        - **Plate Name** — A descriptive name for the plate.
2. Select **Next** to advance.

Add a sample description file (optional)

In the **Sample Description File** section, upload metadata for each sample in the plate.

NOTE
To skip this optional part of the workflow, select **Next** to advance.

1. Select **Add** or **Add File** to open the **Add Files** dialog.
2. Either drag the file into the drag-and-drop area or use **Browse** to navigate to and select it.
3. Select **Add**.
4. Select **Next** to advance.

Add the samples to a project

In the **Add to Project** section, add the samples to a new or existing project. Depending on your choices, the analysis will start immediately or will be deferred for later.

1. Create or select a project:
    - To create a new project, select **New Project**, enter a project name, and select **Add**.
    - To use an existing project, select it from the **Select Project** list.

2. Select or clear the applicable checkboxes. (To defer analysis, clear both checkboxes.)
   - **Analyze samples after addition** — Select to analyze samples.
   - **Analyze controls after addition** — Select to analyze controls.
3. Select the MS method:
   - *DDA* — Derives an MS/MS spectra from selection, isolation, and fragmentation of an individual precursor ion.
   - *DIA* — Derives an MS/MS spectra from selection, isolation, and fragmentation of all precursor ions in a defined m/z range.
4. From the **Analysis Protocol** list (which shows only protocols compatible with the selected MS method), select a protocol.
5. Select **Add Plate**, and then select **Close**.

   Depending on prior choices, the analysis either begins immediately or you must start it manually. See *Start the analysis manually* (below).

Start the analysis manually

If you chose to defer the analysis during the setup workflow, follow these steps to start it manually.

1. On the sidebar menu, select **Projects** to open the **Projects** page.
2. Select the applicable project.
3. Select one of the following options:
   - **Analyze** — Opens the **Analysis** dialog from which to analyze all selected samples in the project.
   - **Analyze Controls** — Opens the **Analyze Controls** dialog from which to analyze the controls only.
4. If you are analyzing all selected samples, in the **Analysis Name** field, enter a name for the analysis.
5. Select the MS method:
   - *DDA* — Derives an MS/MS spectra from selection, isolation, and fragmentation of an individual precursor ion.
   - *DIA* — Derives an MS/MS spectra from selection, isolation, and fragmentation of all precursor ions in a defined m/z range.
   - *PROTEOGENOMICS* — (Appears only for samples, not controls.) Identifies variant peptides arising from single nucleotide variants or short insertions and deletions.
6. From the **Analysis Protocol** list (which shows only protocols compatible with the selected MS method), select a protocol.
7. In the **Description** field, enter a description of the analysis.
8. In the **Notes** field, optionally enter any additional information about the analysis.
9. If you are analyzing all selected samples and want to exclude the controls, select the **Exclude controls** checkbox.
10. If you are analyzing only controls, under **Analysis Name Pattern**, review the MS data file name format and list of controls to analyze.
11. Select **Start**, and then select **OK**.

    The samples or controls are queued for analysis.

12. On the sidebar menu, select **Analyses** to open the **Analyses** page.
13. Confirm that the new analysis appears on the **Analyses** page.

Select samples or controls for analysis

The **Projects** page is the starting point for the sample selection method of analysis setup.

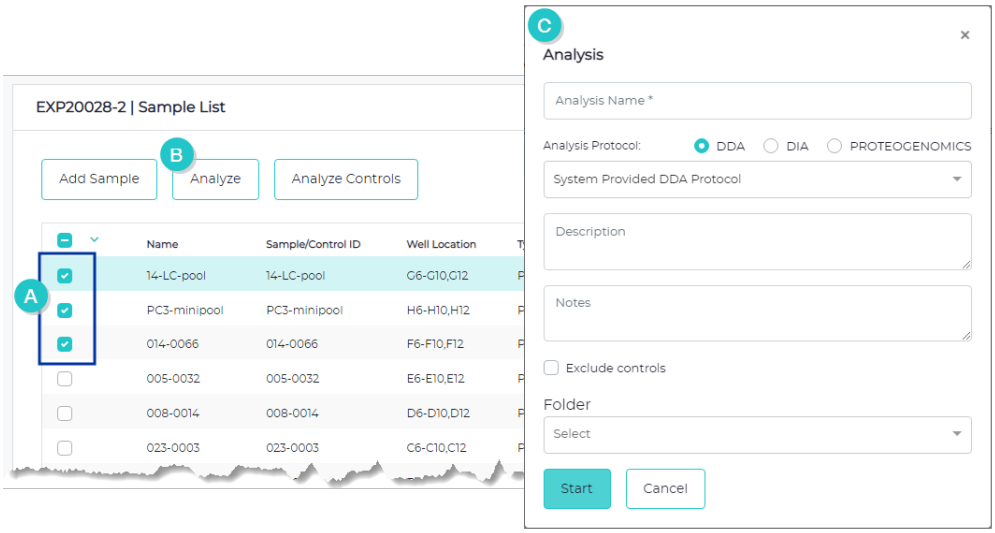
Choose this workflow when you have more than one plate whose samples you want to analyze in a single analysis. You can also use this workflow to reanalyze samples. See *Analyze selected samples* (below).

Use this workflow also to analyze controls only. See *Analyze controls only* (next page).

NOTE

For this workflow, PAS must already contain the applicable plate and MS data files in a project. Additionally, if you plan to use the Proteogenomics analysis protocol to analyze samples, you must have already defined associations between the samples and VCF files. See *VCF files* (page 107).

Figure 1. *Projects page's Sample list and Analysis dialog*



A Selected samples

B Analyze button

C Analysis dialog

Analyze selected samples

1. On the sidebar menu, select **Projects** to open the **Projects** page.
2. Select the applicable project.
3. Select the checkbox of each sample to analyze.
4. Select **Analyze** to open the **Analysis** dialog.

a. In the **Analysis Name** field, enter a name for the analysis.

b. From the **Analysis Protocol** list (which shows only protocols compatible with the selected MS method), select a protocol.

   - If you selected the *Proteogenomics* protocol, also select from the list a protocol that is specific to your instrument. Then, for each sample you will analyze, select its VCF file.

c. In the **Description** field, enter a description of the analysis.

d. (Optional) In the **Notes** field, enter any additional information about the analysis.

e. If you want to exclude the controls, select the **Exclude controls** checkbox.

f. Select **Start**, and then select **OK**.

   The samples are queued for analysis.

5. On the sidebar menu, select **Analyses** to open the **Analyses** page.
6. Confirm the new analysis is listed on the **Analyses** page.

Analyze controls only

1. On the sidebar menu, select **Projects** to open the **Projects** page.
2. Select the applicable project.
3. Select samples, and then select **Analyze Controls** to open the **Analyze Controls** dialog.

   a. Select the MS method:

      - *DDA* — Derives an MS/MS spectra from selection, isolation, and fragmentation of an individual precursor ion.
      - *DIA* — Derives an MS/MS spectra from selection, isolation, and fragmentation of all precursor ions in a defined m/z range.

   b. From the **Analysis Protocol** list (which shows only protocols compatible with the selected MS method), select a protocol.

   c. In the **Description** field, enter a description of the analysis.

   d. (Optional) In the **Notes** field, enter any additional information about the analysis.

   e. Under **Analysis Name Pattern**, review the MS data file name format and the list of controls to analyze.

   f. Select **Start**, and then select **OK**.

      The samples are queued for analysis.

4. On the sidebar menu, select **Analyses** to open the **Analyses** page.
5. Confirm the new analysis is listed on the **Analyses** page.

Chapter 3

Analysis Results

This chapter describes analysis results (exclusive of Group Analysis) and offers step-by-step guidance for working with them.

Access analysis results

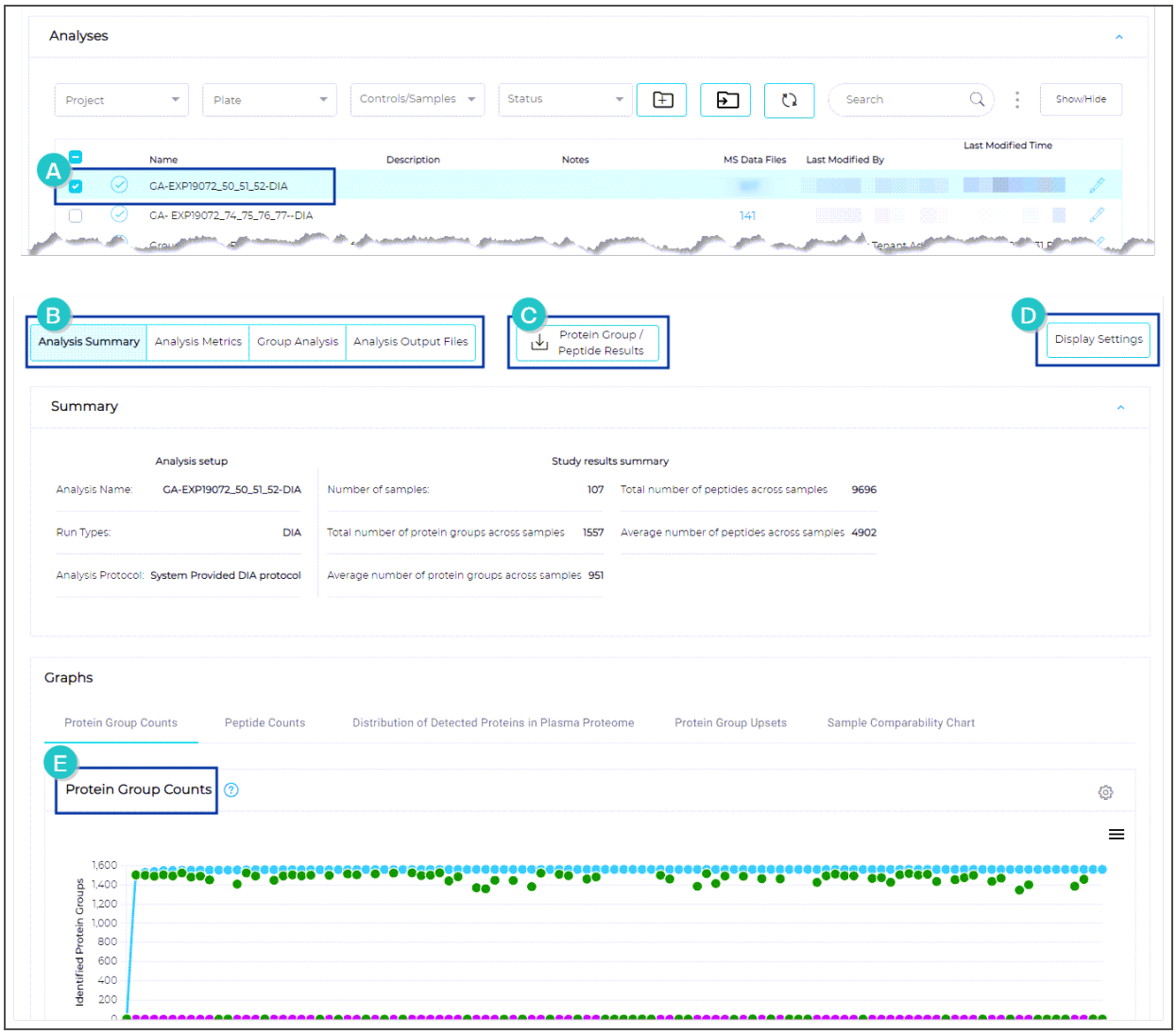
Access analysis results on the *Analyses page* (page 99), where you can review data in a variety of graphs and download results files.

When you select a DDA- or DIA-based analysis, up to five tabs are shown (illustrated below): **Analysis Summary**, **Analysis Metrics**, **QC Charts**, **Group Analysis**, and **Analysis Output Files**.

NOTE
Depending on the selected analysis, the **QC Charts** tab might not be shown.

When you select a Proteogenomics-based analysis, only two tabs are shown (not illustrated): **Proteogenomics** and **Analysis Output Files**.

Figure 2. Analyses page, with a selected analysis, its summary, and one of its graphs shown



A Select an analysis

B Select tabs to view subsets of the analysis's results

C Download a DDA or DIA method analysis's protein groups and peptide results; not shown for a Proteogenomics method analysis

D Set data visualization preferences for this analysis's graphs

E View a results graph

Review analysis results

1. On the sidebar menu, select **Analyses** to open the **Analyses** page.
2. (Optional) From the **Status** list, select *Completed* to show only completed analyses in the table.
3. To apply additional filters, select options from any of the following lists:
   - **Project** — Shows analyses for specified projects.
   - **Plate** — Shows analyses for specified plates.
   - **Controls/Samples** — Shows analyses for controls only or samples only.
4. Select the analysis whose results you want to view.

   NOTE

   If you select multiple DDA- or DIA-based analyses, PAS does not show an analysis summary or results files and, instead, groups all data in the graphs on the remaining tabs.

   Tabs and buttons now appear below the table. They vary depending on the selected analysis.

   For any analysis:

   - **Analysis Output Files** tab — Use to download any of the outputs of the search engine results for the analysis. See *Analysis Output Files tab* (page 65).

   For a DDA or DIA method analysis:

   - **Analysis Summary** tab — Use to compare samples and review a summary of the analysis, counts of peptides and protein groups, the dynamic range distribution of detected proteins, sample overlap, and sample correlation. See *Analysis Summary tab* (next page).
   - **Analysis Metrics** tab — Use to review the intensities, sample clusters, nanoparticle counts, and other QC metrics. See *Analysis Metrics tab* (page 49).
   - **QC Charts** tab — (Appears if the analysis included controls.) Use to select the **QC Charts** tab to review the control results. See *QC Charts tab* (page 60).
   - **Protein Group / Peptide Results** — Use to download the analysis's protein groups and peptide results. See *Download an analysis's protein groups and peptides results* (page 101).

   For Proteogenomics method analysis:

   - **Proteogenomics** tab — Use its **Variant Peptide Browser** to view the number of variant peptides found across samples, conditions, and nanoparticles. Use its **Proteogenomics Data Explorer** to examine how proteogenomic peptide and variant peptide data map in genomic space for both entire proteins and at the nucleic acid/amino acid scale resolution. See *Proteogenomics tab* (page 66).

Change data visualization preferences for analysis results

For an analysis that uses the DDA or DIA method, you can change data visualization preferences for analysis results. These display settings determine which analysis results are shown and how they are grouped and sorted. Most of the settings are global and will apply to all analyses. (The exception is the list of samples and controls, which vary depending on the selected analysis.)

1. On the sidebar menu, select **Analyses** to open the **Analyses** page.
2. Select any analysis.
3. Below the **Analyses** table, locate and select **Display Settings** to open the **Display Settings** dialog.
4. Configure settings as needed.
   - **Group by** — Select an option for grouping protein group and peptide counts. To leave data ungrouped, select *Disabled*.
   - **Sort by** — Select an option for sorting data on the **Analysis Summary** tab.
   - **<list of samples and controls>** — Select the samples and/or controls to include in graphs.

     NOTE

     The listed samples and controls vary depending on the selected analysis, and all are selected by default. To show fewer samples and/or controls in graphs, clear the **Select All** checkbox and then select individual checkboxes.
   - **Analysis** — (Available when a control is selected.) — Select an analysis.
   - **<list of wells>** — (Available when a control is not selected.) Select or clear checkboxes to show or hide wells. Scroll to see all wells.
   - **Hide Controls** — Set the toggle key to *ON* to hide controls.
   - **Visualization graphs** — Select each toggle key to *ON* or *OFF* to show or hide the corresponding graph.
5. Select **Continue** to apply the settings.

Analysis Summary tab

The **Analysis Summary** tab on the *Analyses page* (page 99) offers an overall view of analysis results, organized into graphs.

For some graphs, you can modify labels, reorganize layouts, and otherwise adjust display preferences. These modifications are temporary and apply only to the select analysis. Once you leave the **Analyses** page, each graph returns to its default view. To change data visualization preferences for all analyses, see *Change data visualization preferences for analysis results* (above).

Open an analysis's Analysis Summary tab

1. On the sidebar menu, select **Analyses** to open the **Analyses** page.
2. (Optional) From the **Status** list, select *Completed* to show only completed analyses in the table.
3. Select an analysis to open its analysis results.
4. Select the **Analysis Summary** tab.

Summary section

The **Summary** section shows the following information.

- **Analysis setup**
  - *Analysis name* — The name of the analysis.
  - *Run Types* — The analysis protocol type, either *DDA* or *DIA*.
  - *Analysis Protocol* — The name of the analysis protocol applied to the analysis.
- **Study results summary**
  - *Number of samples* — The number of samples analyzed.
  - *Total number of protein groups across samples*
  - *Average number of protein groups across samples*
  - *Total number of peptides across samples*
  - *Average number of peptides across samples*

Graphs section

The **Graphs** section gives you access to the various graphs with which you can visualize results data. Select a graph's tab to view it.

- *Protein Group Counts graph* (below)
- *Peptide Counts graph* (page 43)
- *Distribution of Detected Proteins in Plasma Proteome graph* (page 44)
- *Protein Group Overlap Sets graph* (page 45)
- *Sample Comparability graph* (page 47)

Protein Group Counts graph

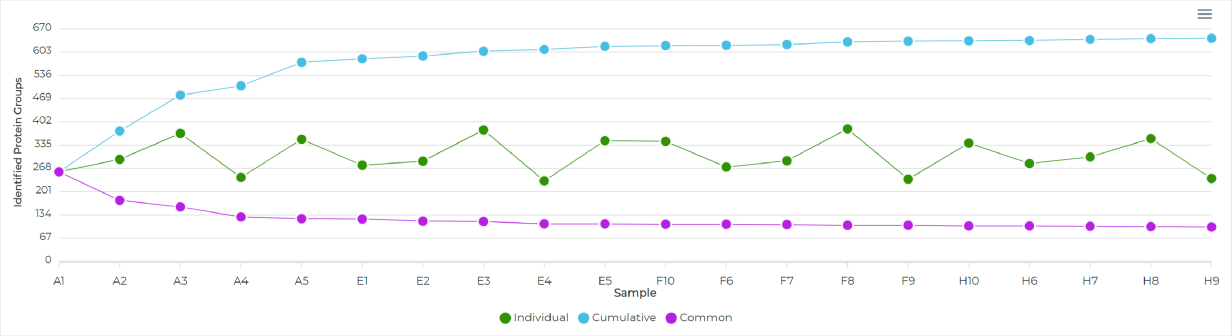
The **Protein Group Counts** graph shows the number of proteins in each sample.

The y-axis plots the number of identified protein groups and the x-axis plots each well. Three lines compare protein group trends for the plate:

- The individual line traces the total number of unique protein groups observed in each sample.
- The cumulative line traces the cumulative number of protein groups observed with each successive sample. For example, the A3 value reflects the number of protein groups detected at least one time among A1, A2, and A3.
- The common line indicates the intersection of the protein groups observed with successive samples. For example, the A3 value reflects the number of proteins in common with A1, then A2.

Dots along each line identify values for each well.

Figure 3. Line graph of peptide group counts



- To open this graph, select **Analyses**, select an analysis, select the **Analysis Summary** tab, and scroll down to the **Graphs** section. This graph is shown by default.
- Hover over a dot on the line graph to view an exact value.
- Hide a line by selecting its dot in the legend.
- Select **Settings** to customize the graph. See *Set preferences for the Protein Group Counts graph (below)*.

  NOTE

  You might want to change graph appearance in preparation for downloading the graph to an image file. The next time you view the graph, it will have reverted to its default settings.

- Download graph data as a .csv file by selecting the CSV option from the "hamburger menu".
- Download a graph as an image file by selecting an image option from the "hamburger menu".

Set preferences for the Protein Group Counts graph

You can temporarily customize the appearance of the *Protein Group Counts graph* (previous page).

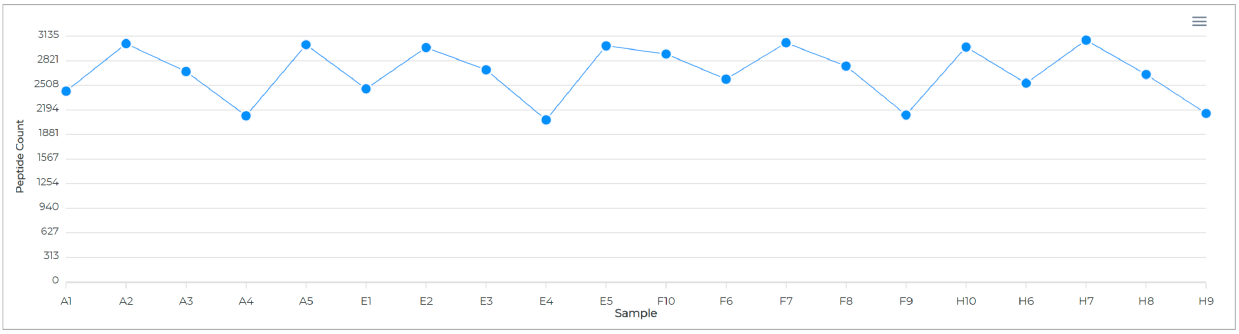
1. Open the **Analysis Summary** tab. (See *Open an analysis's Analysis Summary tab* (page 40).)
2. If needed, scroll down to the **Graphs** section. The **Protein Group Counts** graph is already shown.
3. Select **Settings** on the graph to open the **Preferences** dialog.
4. Adjust the settings as you prefer.
   - **X Tick Label** — Select the label to show along the x-axis, e.g., *Sample Name*, *Plate Name*, *Sample ID*.
   - **Y Axis Range** — Select the range for the y-axis.
     - *Min is 0 and Max Data Dependent* — The minimum value is set at 0 and the maximum value depends on data.
     - *Min and Max Data Dependent* — Both the minimum and maximum values depend on data.
     - *User Defined* — Enter the minimum and maximum values.
5. Select **Continue** to apply the settings.

Peptide Counts graph

The **Peptide Counts** graph shows the number of peptides in each sample.

The y-axis plots the peptide count, and the x-axis plots each sample.

Figure 4. Line graph of peptide counts



- To open this graph, select **Analyses**, select an analysis, select the **Analysis Summary** tab, and scroll down to the **Graphs** section. Then select the **Peptide Counts** tab.
- Hover over its dot on the line graph to view an exact value.
- Hide a line by selecting its dot in the legend.
- Select **Settings** to customize the graph. See *Set preferences for the Peptide Counts graph* (below).

  NOTE

  You might want to change graph appearance in preparation for downloading the graph to an image file. The next time you view the graph, it will have reverted to its default settings.

- Download graph data as a .csv file by selecting the CSV option from the "hamburger menu".
- Download a graph as an image file by selecting an image option from the "hamburger menu".

Set preferences for the Peptide Counts graph

You can temporarily customize the appearance of the *Peptide Counts graph* (above).

1. Open the **Analysis Summary** tab. (See *Open an analysis's Analysis Summary tab* (page 40).)
2. If needed, scroll down to the **Graphs** section. Then select the **Peptide Counts** tab.
3. Select **Settings** on the graph to open the **Preferences** dialog.
4. Adjust the settings as you prefer.
   - **X Tick Label** — Select the label to show along the x-axis, e.g., *Sample Name*, *Plate Name*, *Sample ID*.
   - **Y Axis Range** — Select the range for the y-axis.
     - *Min is 0 and Max Data Dependent* — The minimum value is set at 0 and the maximum value depends on data.
     - *Min and Max Data Dependent* — Both the minimum and maximum values depend on data.
     - *User Defined* — Enter the minimum and maximum values.
5. Select **Continue** to apply the settings.

Distribution of Detected Proteins in Plasma Proteome graph

The **Distribution of Detected Proteins in Plasma Proteome** graph shows the dynamic range of proteins identified in each sample compared to a deep reported human plasma proteome index.[1, 2]

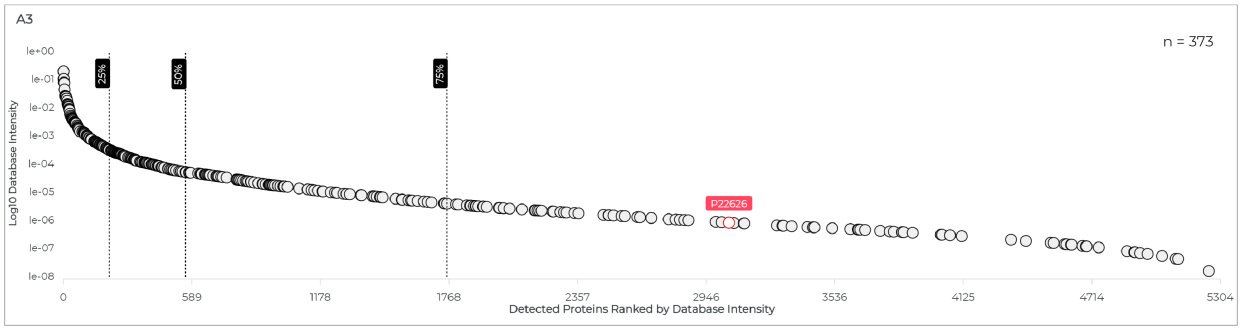
[1]Keshishian, Hasmik, Michael W. Burgess, Michael A. Gillette, Philipp Mertins, Karl R. Clauser, D. R. Mani, Eric W. Kuhn, et al., "Multiplexed, Quantitative Workflow for Sensitive Biomarker Discovery in Plasma Yields Novel Candidates for Early Myocardial Injury," 14, no. 9 *Molecular & Cellular Proteomics* (September 2015): 2375–2393, https://doi.org/10.1074/mcp.M114.046813.

[2]Deutsch, Eric W., Gilbert S. Omenn, Zhi Sun, Michal Maes, Maria Pernemalm, Krishnan K. Palaniappan, Natasha Letunica, Yves Vandenbrouck, Virginie Brun, Sheng-ce Tao, Xiaobo Yu, Philipp E. Geyer, Vera Ignjatovic, Robert L. Moritz, and Jochen M. Schwenk, "Advances and Utility of the Human Plasma Proteome," 20, no. 12 *Journal of Proteome Research* (December 2021): 5241–5263, https://pubs.acs.org/doi/10.1021/acs.jproteome.1c00657.

The n-value equals the number of proteins detected in the sample that were also detected in the index.

The x-axis plots the number of identified proteins ranked by database intensity. The y-axis plots the intensity calculated in the database using the logarithm base 10 (log10) function. Each protein appears as a circle on the plot.

Figure 5. Graph comparing the proteins detected in sample A3 to the plasma proteome



- Hover over a circle to view the name of the protein and the exact intensity values.
- Use the scroll arrows and page numbers to move through all the graphs.
- Annotate proteins you want to highlight, as shown in red in the figure above.
- Select or clear wells in the **Samples** list to show or hide the corresponding graphs.
- Find specific graphs by entering a keyword or term in the Search field.
- Switch to a different data set by selecting an option from the **Data Set** list.
- Use the **Graph Type** list to switch the graph type.
  - *Single* — Shows a graph for a single sample.
  - *Overlay* — Shows the ranked intensities (x-axis) only for all samples, enabling comparison across multiple samples.
  - For an overlay graph, group data by selecting an option from the **Group By** list.
- Select **Settings** to customize the graph. See *Set preferences for the Distribution of Detected Proteins in Plasma Proteome graph* (next page).

NOTE

You might want to change graph appearance in preparation for downloading the graph to an image file. The next time you view the graph, it will have reverted to its default settings.

Set preferences for the Distribution of Detected Proteins in Plasma Proteome graph

You can temporarily customize the appearance of the *Distribution of Detected Proteins in Plasma Proteome graph* (previous page).

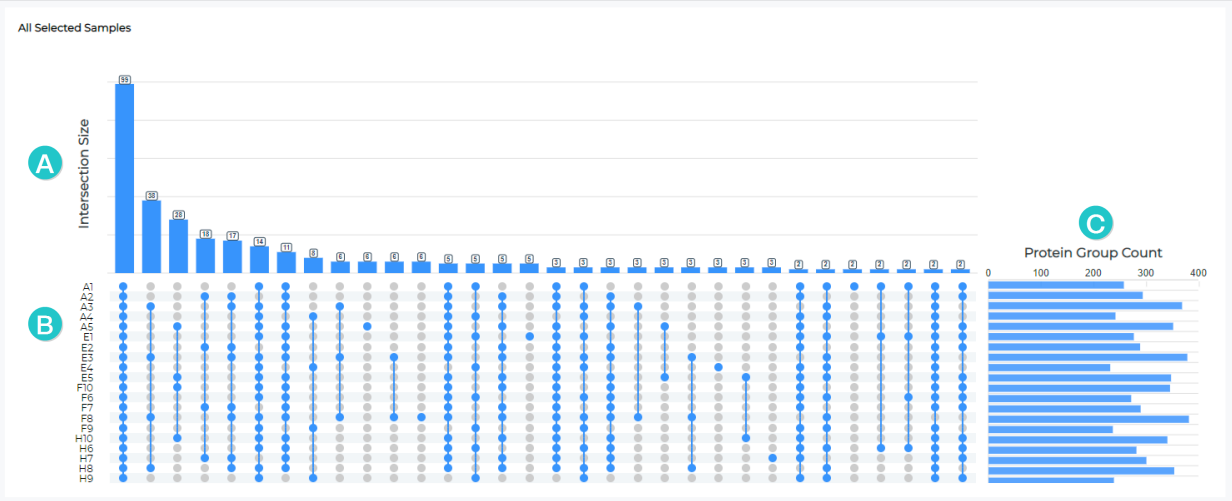
1. Open the **Analysis Summary** tab. (See *Open an analysis's Analysis Summary tab* (page 40).)
2. If needed, scroll down to the **Graphs** section. Then select the **Distribution of Detected Proteins in Plasma Proteome** tab.
3. Select **Settings** on the graph to open the **Preferences** dialog.
4. Adjust the settings as you prefer.
   - **Point Color** and **Point Highlight Color** — Use the color picker to select these colors. (See *Set color preferences in PAS graphs* (page 21).)
   - **Point Size** — Set the size of the point. The default is 8.
   - **Page Chart Layout** — Select an option for how many graphs appear on a page and the layout. For example, the 2 × 2 option shows four graphs per page, two down and two across.
   - **Quantiles** — Adjust the frequency distribution. The default quantiles are 0.25, 0.5, and 0.75.
     - To add a quantile, select **Add** and enter its value.
     - To modify existing quantiles, update the values in each field.
     - To delete a quantile, select **Delete**.
   - **Proteins** — Annotate proteins of interest by entering their names, separated by commas (e.g., `P35579,F5H7Y6,Q5VZ73`). The dots representing the annotated proteins will appear on each graph in red with the protein names above.
5. Select **Continue** to apply the settings.

Protein Group Overlap Sets graph

The **Protein Group Overlap Sets** graph is divided into two bar graphs and a matrix that together show protein group intersections.

The **Within Samples** checkbox shows intersection size and protein group count as standalone graphs for each sample with a dot representing the MS data file.

Figure 6. Graphs and a matrix show protein group overlaps



A Intersection Size bar graph

B Matrix

C Protein Group Count bar graph

- To open this graph, select **Analyses**, select an analysis, select the **Analysis Summary** tab, and scroll down to the **Graphs** section. Then select the **Protein Group Upsets** tab.
- Hover over a vertical bar to view the intersection size for the group of samples as an integer and the percentage of the total it represents.
- Hover over a horizontal bar to view the sample and the exact number of protein groups it contains.
- Select or clear wells in the **Samples** list to show or hide their corresponding bars.
- Find specific graphs by entering a keyword or term in the Search field.
- Use the scroll arrows and page numbers to move through all the graphs.
- Select **Settings** to customize the graph. See *Set preferences for the Protein Group Overlap Sets graph* (below).

  NOTE

  You might want to change graph appearance in preparation for downloading the graph to an image file. The next time you view the graph, it will have reverted to its default settings.

- Download graph data as a .csv file by selecting the CSV option from **Menu**.
- Download the graph as an image file by selecting an image option from **Menu**.

Set preferences for the Protein Group Overlap Sets graph

You can temporarily customize the appearance of the *Protein Group Overlap Sets graph* (previous page).

1. Open the **Analysis Summary** tab. (See *Open an analysis's Analysis Summary tab* (page 40).)
2. If needed, scroll down to the **Graphs** section. Then select the **Protein Group Upset** tab.

3. Select **Settings** on the graph to open the **Preferences** dialog.
4. Adjust the settings as you prefer.
   - **Horizontal Bar Position** — Select an orientation for the graph.
     - *Left* — The graph is positioned left.
     - *Right* — The graph is positioned right.
     - *None* — The graph is hidden.
   - **Vertical Height Rate** — Enter a value for the relative bar height in the graph.
   - **MS file's name length** — Enter the character limit for the MS data file name.
   - **Display Top N Intersection** — Select which bars appear on the graph.
     - *All* — Shows intersection sizes for all samples.
     - *4* — Shows the intersection sizes of the top four largest samples only.
     - *8* — Shows the intersection sizes of the top eight largest samples only.
     - *16* — Shows the intersection sizes of the top 16 largest samples only.
     - *32* — Shows the intersection sizes of the top 32 largest samples only.
   - **Display Percentage** — Select to show percentages on top of each bar in the Intersection Size graph.
   - **Page Chart Layout** — Select an option for how many graphs appear on a page and the layout. For example, the 2 × 2 option shows four graphs per page, two down and two across.

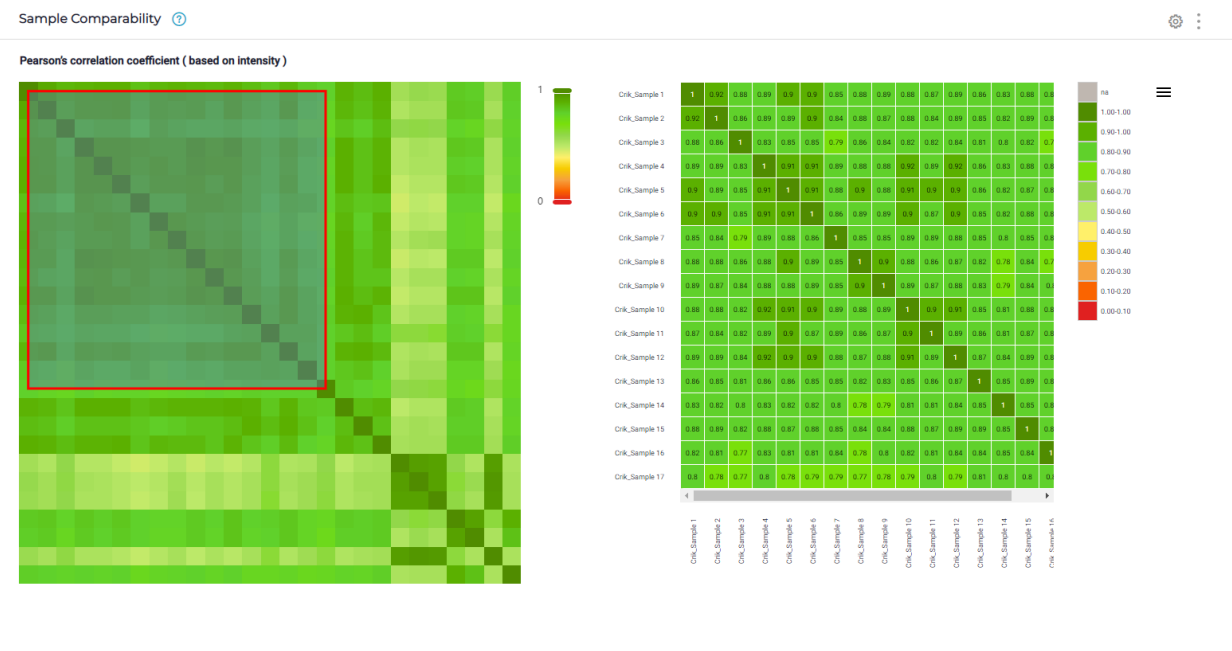
     By default, this section consolidates information for all samples into two bar graphs and a matrix for easy comparison. You can divide these elements into graphs for individual samples and change the page layout accordingly.
   - **<colors>** — Set colors for the various parts of the graph, such as for the vertical and horizontal bars. Use the techniques in *Set color preferences in PAS graphs* (page 21).
5. Select **Continue** to apply the settings.

Sample Comparability graph

The **Sample Comparability** graph shows the degree of statistical correlation between samples based on the Pearson's correlation coefficient (PCC) and the similarity in protein groups between samples based on the Jaccard Index, which measures the linear correlation of data. You can switch between the PCC and Jaccard index.

Each matrix is color-coded for easy reference. Samples on the green end of the spectrum have high correlation. Samples on the red end of the spectrum have low correlation.

Figure 7. A color-coded matrix shows sample comparability data using PCC (left) and the Jaccard index (right)



- To open this graph, select **Analyses**, select an analysis, select the **Analysis Summary** tab, and scroll down to the **Graphs** section. Then select the **Sample Comparability Chart** tab.
- Select a cell on the matrix to view a scatter plot that compares two samples.
- Hover over a dot on the scatter plot to view the intensity of a single protein.
- Select **Settings** to customize the graph. See *Set preferences for the Sample Comparability graph* (below).

  NOTE

  You might want to change graph appearance in preparation for downloading the graph to an image file. The next time you view the graph, it will have reverted to its default settings.

- Download graph data as a .csv file by selecting the CSV option from **Menu**.
- Download a graph as an image file by selecting an image option from the "hamburger menu".

Set preferences for the Sample Comparability graph

You can temporarily customize the appearance of the *Sample Comparability graph* (previous page).

1. Open the **Analysis Summary** tab. (See *Open an analysis's Analysis Summary tab* (page 40).)
2. If needed, scroll down to the **Graphs** section. Then select the **Sample Compatibility** tab.
3. Select **Settings** on the graph to open the **Preferences** dialog.
4. Adjust the settings as you prefer.

- **Similarity Score** — To change how sample intensity data are compared, select either:
  - *Pearson's correlation coefficient* — Shows data calculated by normalizing the covariance measurement.
  - *Jaccard index* — Shows data calculated by dividing the intersection size by the union size.
- **Color Labels By** — To change the matrix label colors, select an option, e.g., *Sample Name*, *Sample ID*, *Plate Name*. To not use colors for labels, select *Disabled*.
- **Label Font Size** — Select the point size text of the label.

5. Select **Continue** to apply the settings.

Analysis Metrics tab

The **Analysis Metrics** tab on the *Analyses page* (page 99) shows the QC metrics for an analysis result, organized into graphs. The metrics indicate how well an analysis method performed.

For some graphs, you can modify labels, reorganize layouts, and otherwise adjust display preferences. These modifications are temporary and apply only to the select analysis. Once you leave the **Analyses** page, each graph returns to its default view. To change data visualization preferences for all analyses, see *Change data visualization preferences for analysis results* (page 40).

Open an analysis's Analysis Metrics tab

1. On the sidebar menu, select **Analyses** to open the **Analyses** page.
2. (Optional) From the **Status** list, select *Completed* to show only completed analyses in the table.
3. Select an analysis to open its analysis results.
4. Select the **Analysis Metrics** tab.

Graphs section

The **Graphs** section gives you access to the various QC metrics graphs, which metrics indicate how well an analysis method performed. Select a graph's tab to view it.

- *Intensities graphs* (below)
- *Plate Map Grid graphs* (page 51)
- *Lamppost Proteins' Concentration graph* (page 52)
- *PCA Analysis graph* (page 54)
- *Peptide Counts Distribution and Protein Group Counts Distribution graphs* (page 55)
- *Peptide Counts of Nanoparticles and Protein Group Counts of Nanoparticles graphs* (page 57)
- *Hierarchical Clustering graph* (page 58)

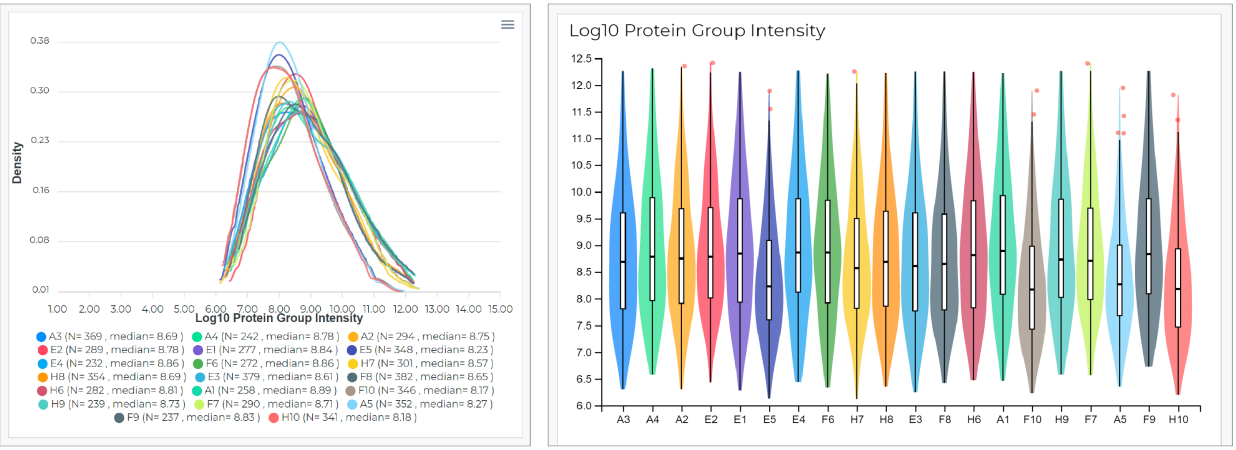
Intensities graphs

The **Intensities** graphs show the protein and peptide intensities and the distribution of protein sequence coverage for each sample, including the coefficient of variation (CV).

The default chart type is a density plot, which you can change to a violin plot.

- On a density plot, the full distribution of data for each sample is shown as a continuous curve. The x-axis plots intensity and the y-axis plots density.
- On a violin plot, data for each sample is shown as quartiles displaying the full distribution of data. The sample appears along the x-axis and the y-axis plots intensity. Box plots represent quantile ranges with box plots with density on either end. Red dots represent outliers.

Figure 8. Density plot (left) and violin plot (right) showing the intensity distribution for a sample



- To open this graph, select **Analyses**, select an analysis, and select the **Analysis Metrics** tab. This graph is shown by default.
- Use the scroll arrows and page numbers to move through all the graphs.
- For the density plot:
  - Move the mouse pointer along the curve to view the exact intensity value at each point in a curve.
  - Hide a line by selecting its label in the legend.
- For the violin plot:
  - Hover over a box plot to view the quantile for a sample.
  - Hover over a dot to view an exact outlier value.
- Select **Settings** to customize the graph. See *Set preferences for Intensities graphs* (below).

  NOTE

  You might want to change graph appearance in preparation for downloading the graph to an image file. The next time you view the graph, it will have reverted to its default settings.

- Download graph data as a .csv file by selecting the CSV option from **Menu**.
- Download a graph as an image file by selecting an image option from the "hamburger menu".

Set preferences for Intensities graphs

You can temporarily customize the appearance of the *Intensities graphs* (previous page).

1. Open the **Analysis Metrics** tab. (See *Open an analysis's Analysis Metrics tab* (previous page).)
2. If needed, scroll down to the **Graphs** section. The **Intensities** graph is already shown.

3. Select **Settings** on the graph to open the **Preferences** dialog.
4. Adjust the settings as you prefer.
   - **Page Chart Layout** — Select an option for how many graphs appear on a page and the layout. For example, the 2 × 2 option shows four graphs per page, two down and two across.
   - **Chart Type** — Select an option.
     - *Density plot* — Shows the full distribution of data for each sample as a continuous curve.
     - *Violin plot* — Shows data for each sample as quartiles displaying the full distribution of data.
5. Select **Continue** to apply the settings.

Plate Map Grid graphs

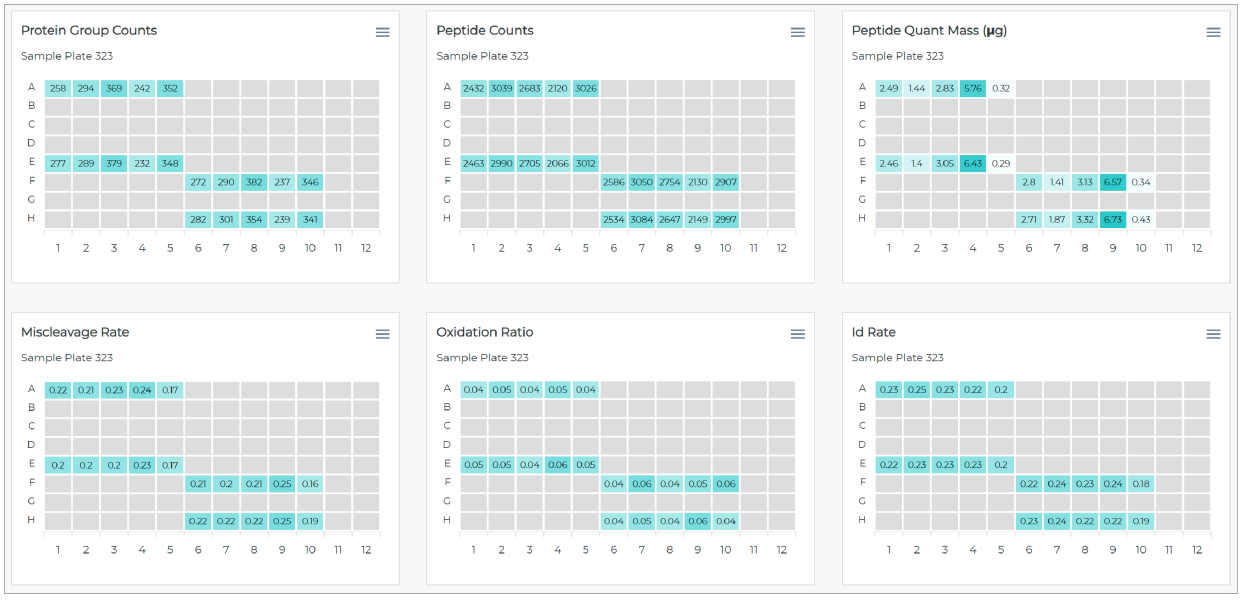
The **Plate Map Grid** graphs show metrics for the individual samples in the format of 96-well plates.

The default chart type is a plate grid, which you can change to a scatter plot.

Each plate represents one of the following metrics. Hover over a well to view the value. Alternatively, you can consolidate these data into a single scatter plot.

- **Protein group counts** — The number of protein groups identified per well.
- **Peptide counts** — The number of peptides identified per well.
- **Peptide quant mass** — The mass of peptide in the well as calculated by the peptide quantification assay.
- **Miscleavage rate** — The fraction of peptides identified as having missed cleavages based on the maximum allowable missed cleavages set in the analysis protocol.
- **Oxidation ratio** — The fraction of peptides identified with methionine oxidation.
- **ID rate** — The rate at which an MS/MS scan is converted into a database peptide identification.

Figure 9. Grids for each metric showing results for each sample



- To open this graph, select **Analyses**, select an analysis, and select the **Analysis Metrics** tab. Then select the **Plate Map Grid** tab.
- Use the scroll arrows and page numbers to move through all the graphs.
- For a plate grid, hover over a well to see its value.
- For a scatter plot:
  - Hover over a dot to view an exact value.
  - Hide a line by selecting its label in the legend.
  - Hide all lines except one by hovering over that line's label in the legend.
- Select **Settings** to customize the graph. See *Set preferences for Plate Map Grid graphs* (below).

  NOTE

  You might want to change graph appearance in preparation for downloading the graph to an image file. The next time you view the graph, it will have reverted to its default settings.
- Download graph data as a .csv file by selecting the CSV option from the "hamburger menu".
- Download a graph as an image file by selecting an image option from the "hamburger menu".

Set preferences for Plate Map Grid graphs

You can temporarily customize the appearance of the *Plate Map Grid graphs* (previous page).

1. Open the **Analysis Metrics** tab. (See *Open an analysis's Analysis Metrics tab* (page 49).)
2. If needed, scroll down to the **Graphs** section. Then select the **Plate Map Grid** tab.
3. Select **Settings** on the graph to open the **Preferences** dialog.
4. Adjust the settings as you prefer.
   - **Graph Types** — Select each toggle key to show or hide a graph or table column (depending on the **Display Type**).
   - **Scaling** — Select *Each graph* or *Each type* scaling for reviewing multiple plates.
   - **Display Type** — Select an option:
     - *Plate Map Grid* — Shows each metric in a separate grid.
     - *Table* — Shows the protein group counts, peptide counts, and other metrics in one table.
   - **Page Chart Layout** — Select an option for how many graphs appear on a page and the layout. For example, the 2 × 2 option shows four graphs per page, two down and two across.
5. Select **Continue** to apply the settings.

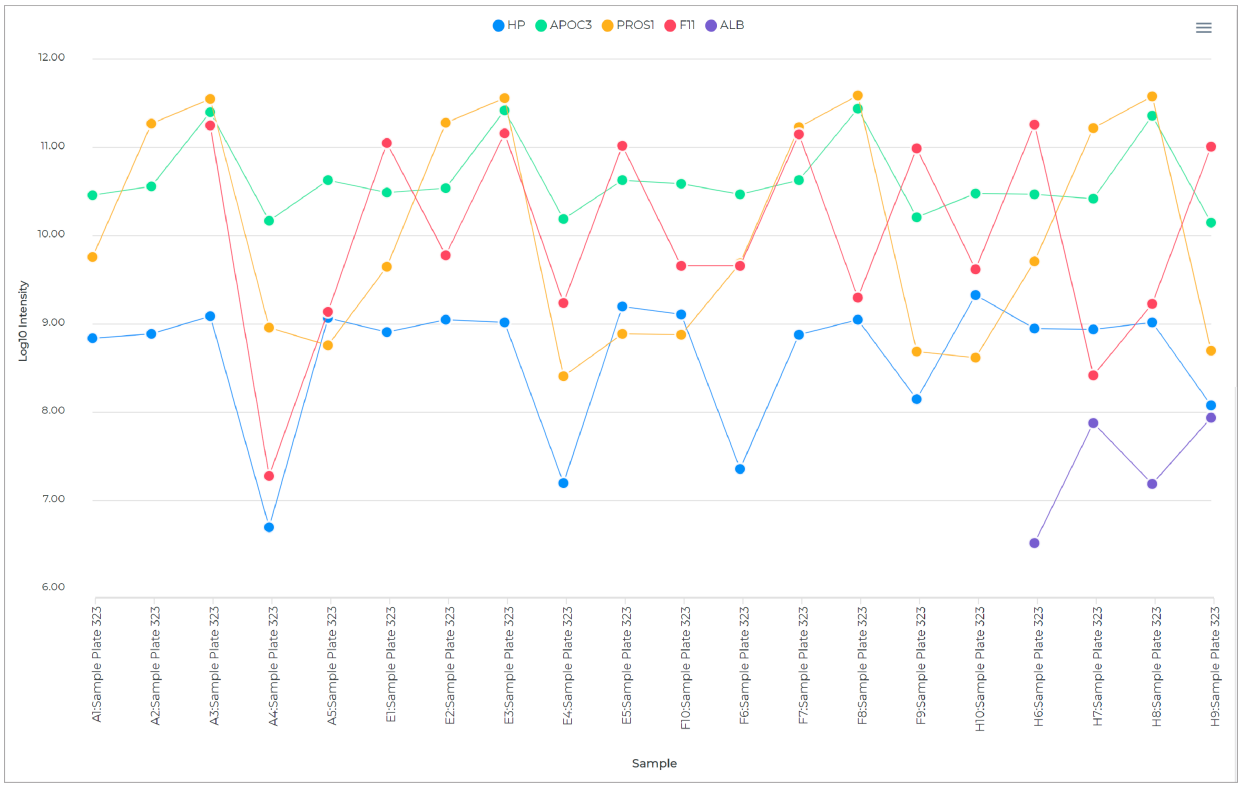
Lamppost Proteins' Concentration graph

The **Lamppost Proteins' Concentration** graph shows the intensity of lamppost proteins in each sample.

The x-axis plots each well and plate and the y-axis plots log10 intensity.

Five lines compare intensities for the proteins HP, APOC3, PROS1, F11, and ALB. Dots along each line identify the intensity value for each protein in the sample.

Figure 10. Line graph showing intensities for lamppost proteins



- To open this graph, select **Analyses**, select an analysis, and select the **Analysis Metrics** tab. Then select the **Lamppost Proteins' Concentration** tab.
- Hover over a dot to view the exact values for all five proteins.
- Hide a line by selecting its label in the legend. (You many need to scroll right to see the label, which appears above the graph.)
- Hide all lines except one by hovering over that line's label in the legend.
- Select **Settings** to customize the graph. See *Set preferences for the Lamppost Proteins' Concentration graph* (below).
- Download graph data as a .csv file by selecting the CSV option from the "hamburger menu". (You may need to scroll all the way to the right to see the button.)
- Download a graph as an image file by selecting an image option from the "hamburger menu".

Set preferences for the Lamppost Proteins' Concentration graph

You can temporarily customize the appearance of the *Lamppost Proteins' Concentration graph* (previous page). Specifically, you can add, edit, and delete proteins to use instead of the default QC lamppost proteins.

1. Open the **Analysis Metrics** tab. (See *Open an analysis's Analysis Metrics tab* (page 49).)
2. If needed, scroll down to the **Graphs** section. Then select the **Lamppost Proteins' Concentration** tab.
3. Select **Settings** on the graph to open the **Lamppost Proteins** dialog.
4. To add a protein or group of proteins:

a. Select **Add Proteins** to open the **Add Proteins** dialog.
b. In the **Name** field, enter a label for a detected protein or group of proteins.
c. In the **Proteins** field, enter a protein name or enter multiple names separated by commas.
d. Select **Add**.

Notice that the label you entered for the protein or group of proteins is now selected.

> **TIP**
> To revert to using the default QC lamppost proteins, select **Abundance monitoring**.

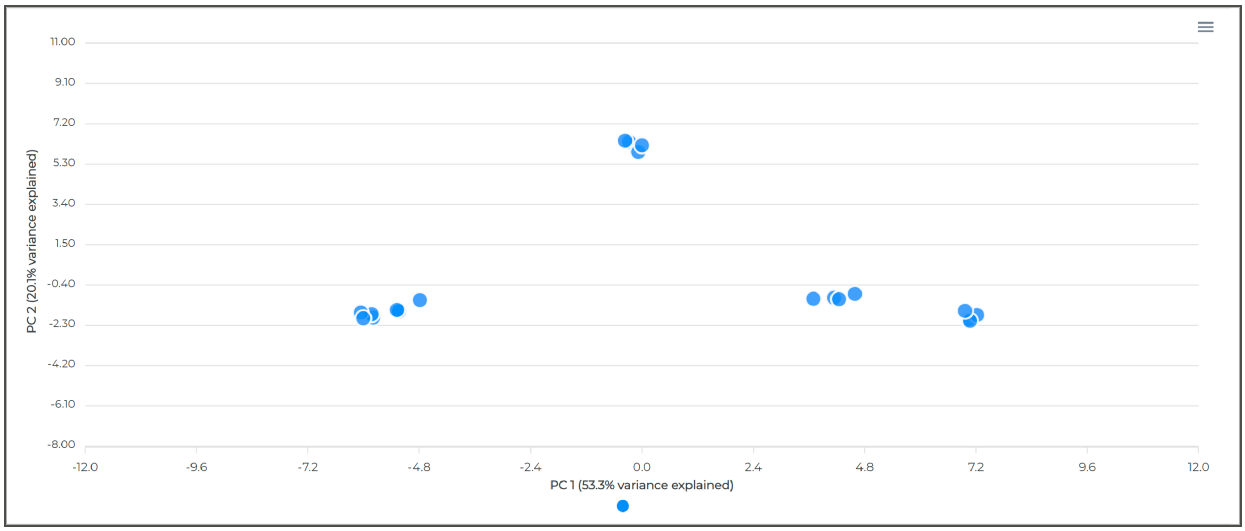
5. To edit a protein or group of proteins:
   a. Select its **Edit** button to open the **Edit Proteins** dialog.
   b. Change the fields as needed.
   c. Select **Edit** to save the changes.
6. To delete a protein or group of proteins:
   a. Select its **Delete** button.
   b. Select **OK** to confirm.
7. Select **Continue** to apply the settings.

PCA Analysis graph

The **PCA Analysis** graph clusters similar samples in a scatter plot.

The x-axis plots principal component 1 (PC1) analysis scores, and the y-axis plots principal component 2 (PC2) analysis scores. Each dot represents one sample.

Figure 11. Scatter plot showing clusters of similar samples



- To open this graph, select **Analyses**, select an analysis, and select the **Analysis Metrics** tab. Then select the **PCA Analysis** tab.
- Hide a sample by selecting its dot under the x-axis.

- Hover over dots to view sample and plate names.
- Change the color scheme of the graph, such as by *Condition* or *Sample Name*. See *Change the color scheme of the PCA Analysis graph* (below).

> NOTE
>
> You might want to change graph appearance in preparation for downloading the graph to an image file. The next time you view the graph, it will have reverted to its default settings.

- Download graph data as a .csv file by selecting the CSV option from ⋮ **Menu**.
- Download a graph as an image file by selecting an image option from the ☰ "hamburger menu".

Change the color scheme of the PCA Analysis graph

You can temporarily change the color scheme of the *PCA Analysis graph* (previous page).

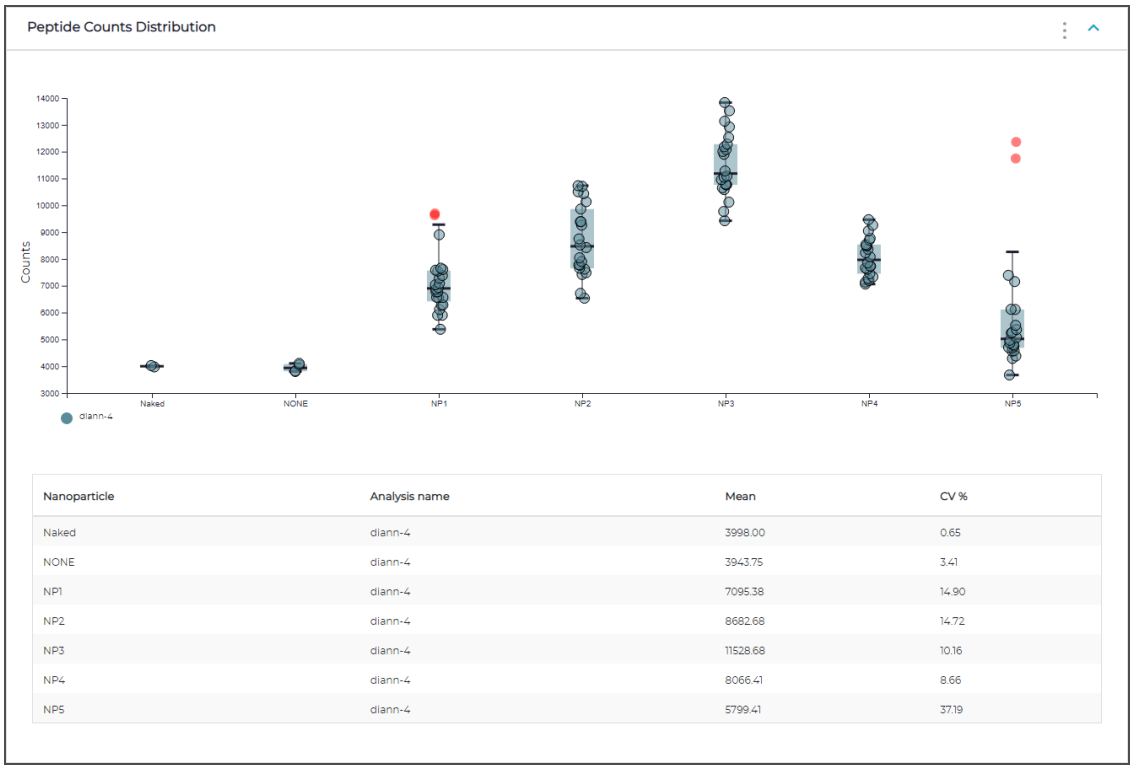
1. Open the **Analysis Metrics** tab. (See *Open an analysis's Analysis Metrics tab* (page 49).)
2. If needed, scroll down to the **Graphs** section. Then select the **PCA Analysis** tab.
3. To change the color scheme, select options from the two lists.
   - From the first list, select:
     - *Condition* — Applies the same color to all data points. The color represents sample quality.
     - *Sample Name* — Applies a unique color to each sample.
     - *Plate Name* — Applies the same color to all data points. The color represents the sample plate.
     - *Sample ID* — Applies a unique color to each sample.
   - From the second list, select either *Protein* or *Peptide*.
4. Set colors as you prefer.
   a. Select a label box below or to the right of the **Color By** lists. For example, if you selected *Sample ID* above, you can adjust the color for each sample.
   b. Use the color picker to select a color. (See *Set color preferences in PAS graphs* (page 21).)
   c. Repeat for other label boxes.

Peptide Counts Distribution and Protein Group Counts Distribution graphs

The **Peptide Counts Distribution** and **Protein Group Counts Distribution** graphs show the peptide and protein counts for the five nanoparticles as box plots.

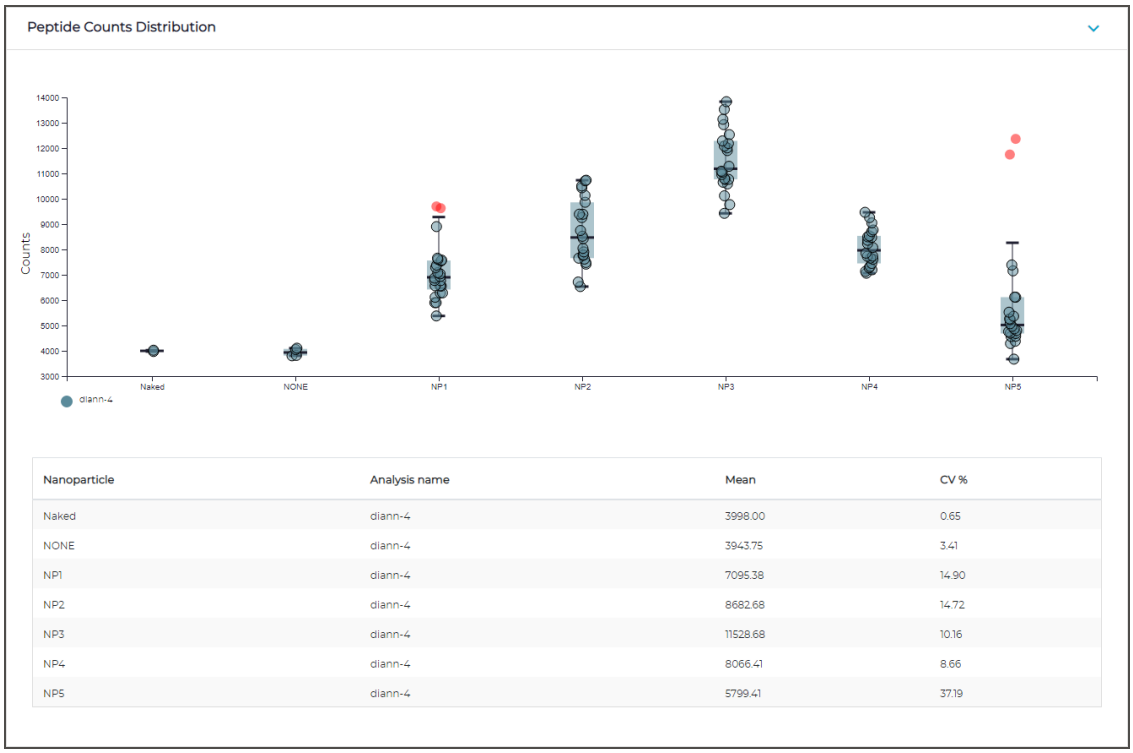
A table below each plot shows the analysis name, mean value, and CV percentage for each nanoparticle. Each box plot and table represent one plate.

Figure 12. Box plots showing peptide counts distribution



Nanoparticle	Analysis name	Mean	CV %
Naked	diann-4	3998.00	0.65
NONE	diann-4	3943.75	3.41
NP1	diann-4	7095.38	14.90
NP2	diann-4	8682.68	14.72
NP3	diann-4	11528.68	10.16
NP4	diann-4	8066.41	8.66
NP5	diann-4	5799.41	37.19

Figure 13. Box plots showing protein group counts distribution



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NONE	diann-4	3943.75	3.41
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NP2	diann-4	8682.68	14.72
NP3	diann-4	11528.68	10.16
NP4	diann-4	8066.41	8.66
NP5	diann-4	5799.41	37.19

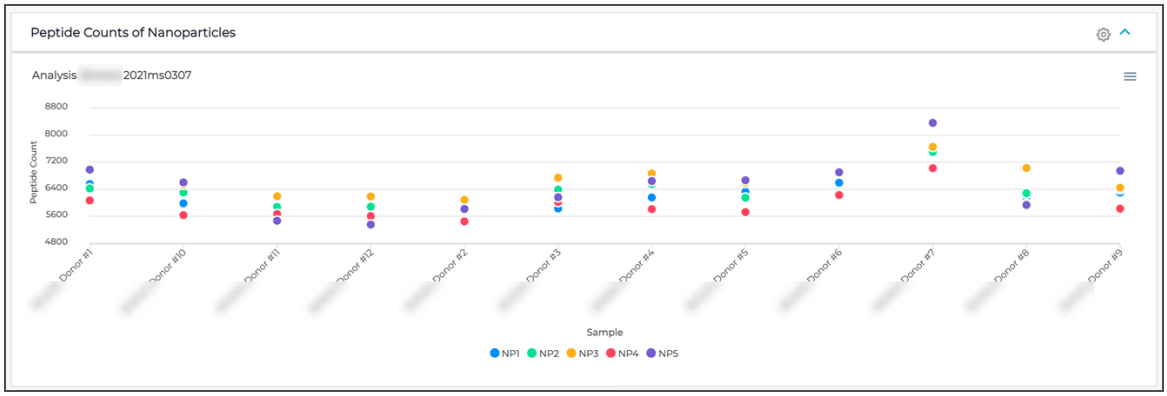
- To open these graphs, select **Analyses**, select an analysis, and select the **Analysis Metrics** tab. Then select the **Peptide/Protein Group Counts Distribution** tab.
- Hover over a dot to view the peptide or protein count, file, and sample name.
- Hover over a box to view the quantile for the nanoparticle.
- Download graph data as a .csv file by selecting the CSV option from **Menu**.
- Download a graph as an image file by selecting an image option from the "hamburger menu".

Peptide Counts of Nanoparticles and Protein Group Counts of Nanoparticles graphs

The **Peptide Counts of Nanoparticles** and **Protein Group Counts of Nanoparticles** graphs offer show the number of peptides or protein groups counted for each nanoparticle per sample.

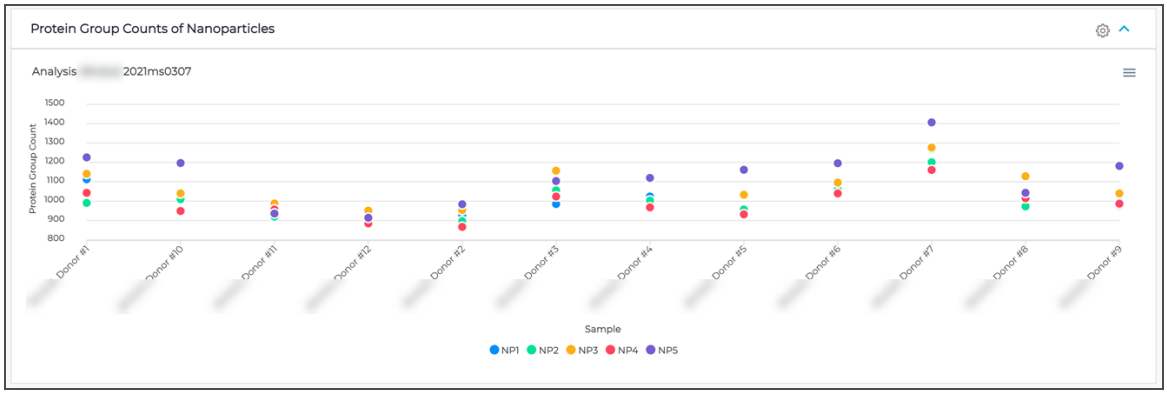
- **Peptide Counts of Nanoparticles** — Shows the sample protein group counts identified by each nanoparticle. Each dot represents a sample. The x-axis plots samples and the y-axis plots the number of peptides.

Figure 14. Scatter plot showing peptide counts of nanoparticles



- **Protein Group Counts of Nanoparticles** — Shows the sample peptide counts identified by each nanoparticle. Each dot represents a sample. The x-axis plots samples and the y-axis plots the number of peptides.

Figure 15. Scatter plot showing protein group counts of nanoparticles



- To open these graphs, select **Analyses**, select an analysis, and select the **Analysis Metrics** tab. Then select the **Peptide/Protein Group Counts of Nanoparticles** tab.
- Hover over a dot to view a well, the nanoparticle that occupies the well, and the peptide count.
- Remove a well from the plot by selecting its dot under the x-axis.
- Select **Settings** to customize the graph. See *Set preferences for the Peptide Counts of Nanoparticles and Protein Group Counts of Nanoparticles graphs* (below).
- Download graph data as a .csv file by selecting the CSV option from the "hamburger menu". (You may need to scroll all the way to the right to see the button.)
- Download a graph as an image file by selecting an image option from the "hamburger menu".

Set preferences for the Peptide Counts of Nanoparticles and Protein Group Counts of Nanoparticles graphs

You can temporarily customize the appearance of the *Peptide Counts of Nanoparticles and Protein Group Counts of Nanoparticles graphs* (previous page).

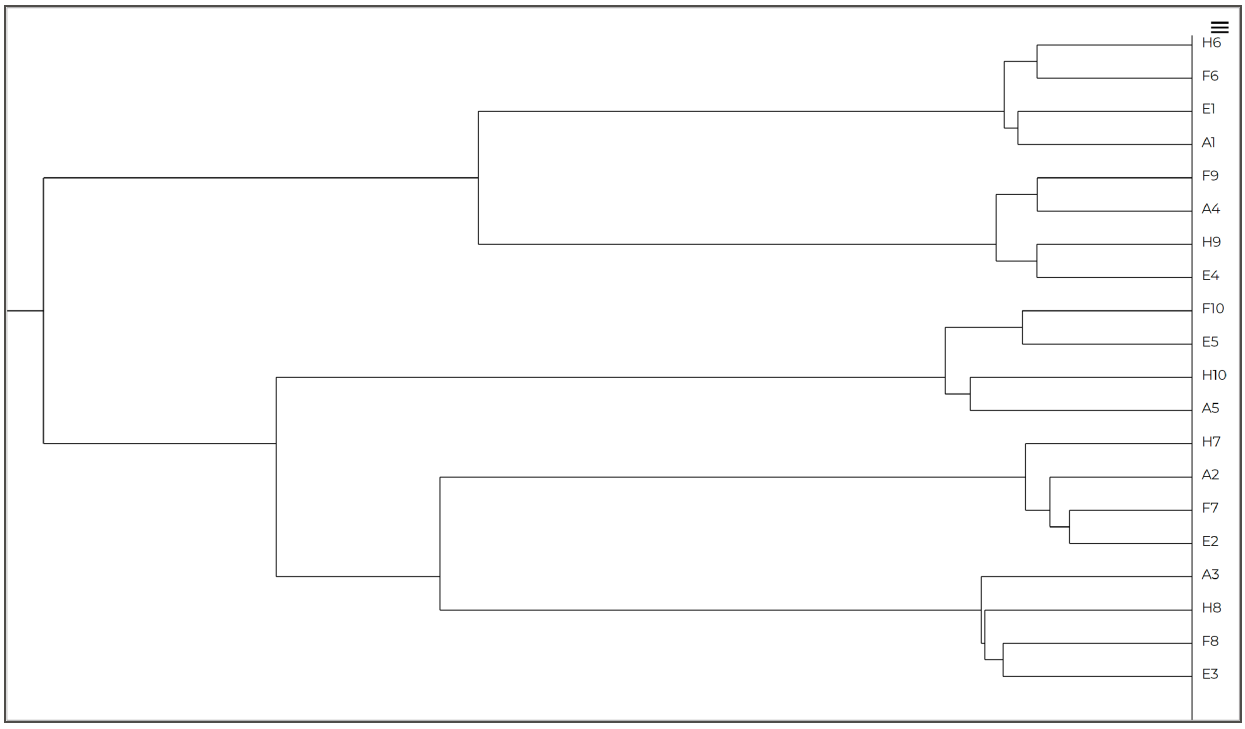
1. Open the **Analysis Metrics** tab. (See *Open an analysis's Analysis Metrics tab* (page 49).)
2. If needed, scroll down to the **Graphs** section. Then select the **Peptide/Protein Group Counts of Nanoparticles** tab.
3. For the **Peptide Counts of Nanoparticles** graph, do the following:
    a. Select **Settings** on the graph to open the **Preferences** dialog.
    b. Adjust the settings as you prefer.
        - **Y Axis Range** — Select the range for the y-axis.
            - *Min is 0 and Max Data Dependent* — The minimum value is set at 0 and the maximum value depends on data.
            - *Min and Max Data Dependent* — Both the minimum and maximum values depend on data.
            - *User Defined* — Enter the minimum and maximum values.
    c. Select **Save**.
4. Scroll down to the **Protein Group Counts of Nanoparticles** graph and use the same techniques to adjust its settings.

Hierarchical Clustering graph

The **Hierarchical Clustering** graph shows a cluster analysis based on agglomerative nesting, which groups samples in clusters based on similarity.

Each sample starts a single cluster. Pairs of clusters are then successively merged until all clusters are merged into a dendrogram.

Figure 16. Dendrogram successively grouping similar samples



- To open this graph, select **Analyses**, select an analysis, and select the **Analysis Metrics** tab. Then select the **Hierarchical Clustering** tab.
- Select **Settings** to customize the graph. See *Set preferences for the Hierarchical Clustering graph* (below).

  NOTE

  You might want to change graph appearance in preparation for downloading the graph to an image file. The next time you view the graph, it will have reverted to its default settings.

- Download a graph as an image file by selecting an image option from the "hamburger menu".

Set preferences for the Hierarchical Clustering graph

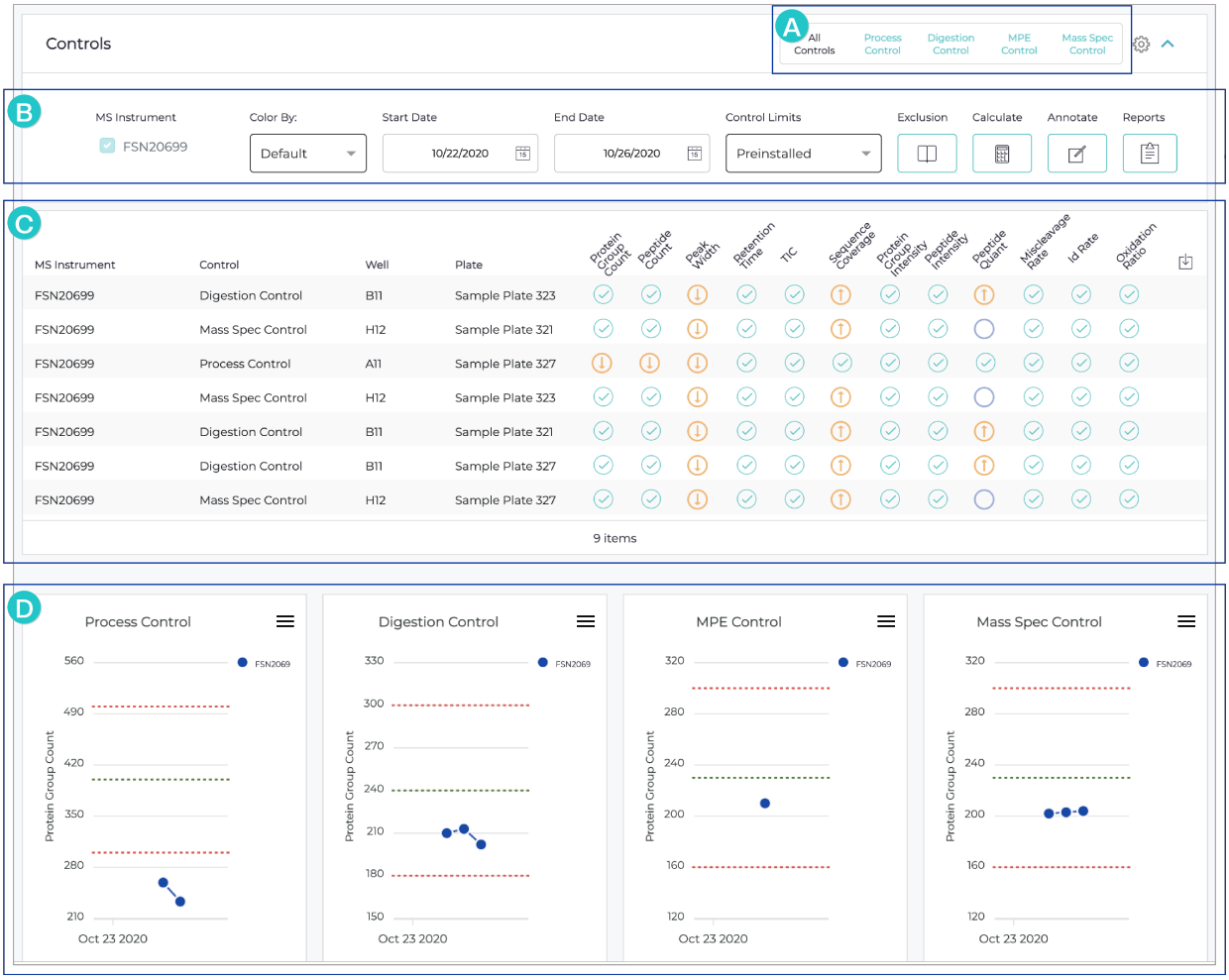
You can temporarily customize the appearance of the *Hierarchical Clustering graph* (previous page).

1. Open the **Analysis Metrics** tab. (See *Open an analysis's Analysis Metrics tab* (page 49).)
2. If needed, scroll down to the **Graphs** section. Then select the **Hierarchical Clustering** tab.
3. Select **Settings** on the graph to open the **Preferences** dialog.
4. From the **Color Labels By** list, select an option to color labels by, e.g., *Condition*, *Sample Name*. To not show colors for labels, select *Disabled*.
5. Select **Continue** to apply the settings.

QC Charts tab

The **QC Charts** tab on the **Analyses** page shows assay control data for an analysis. Each chart plots one metric for one assay control category. Charts are organized in a grid, with the same metric across each row and the same control type down each column. The x-axis is labeled with the date of analysis and the y-axis depends on the metric.

Figure 17. Results for controls



A Filters for viewing charts for all controls or a selected control type

B Toolbar with additional filters and functions

C Summary of control data for the selected analysis time frame

D QC charts with metrics of each control

Toolbar items

- **<control filters>** — By default, all controls are included. Select another option to filter by a specific control type.
  - *Process Control* — Results for the process controls only.
  - *Digestion Control* — Results for the digestion controls only.

  - *MPE Control* — Results for the MPE controls only.
  - *Mass Spec Control* — Results for the mass spec controls only.
- **MS Instrument** — The instruments listed vary depending on your organization. Select up to six instruments to aggregate their data.
- **Color By** — (Available when only one MS instrumented is selected.) Select your preferred coloration for the graphs, e.g., *Gradient*.
- **Start Date** — Enter or select the earliest date of data you want to see.
- **End Date** — Enter or select the latest date of data you want to see
- **Control Limits** — Select how you want control limits to be defined.
  - *Preinstalled* — Use the pre-installed control limits.
  - *User Defined* — Define your own control limits. See *Manage user-defined and calculated control limits* (page 63).
  - *Calculated* — Calculate control limits from data within one or more date ranges, which you define with the **Calculate** button (see below).
- **Limits** — (Available when *Calculated* is selected in the **Control Limits** list.) Select to view the calculated control limit date ranges. From here, you can also delete a range. See *Manage user-defined and calculated control limits* (page 63).
- **Exclusion** — Select to exclude an outlier or other data point from the control limit calculation. Selecting one data point (sample) in a plot excludes the corresponding sample from the other plots. A well containing the mass spec control has 10 reported QC metrics. All other control wells have 11 reported QC metrics. See *Manage user-defined and calculated control limits* (page 63).
- **Calculate** — Select to define a calculated control limit based on a range of dates. See *Manage user-defined and calculated control limits* (page 63).
- **Annotate** — Select to annotate all control charts for a specific date. See *Annotate control charts* (page 64).
- **Reports** — Select to generate a PDF report of control results, with specific criteria. See *Generate a detailed PDF report of control results* (page 65).

How to use

For additional usage instructions, see *Open and filter an analysis's QC Charts tab* (next page).

- To jump to a specific control chart, select its status icon (e.g.,) in the summary table above the control charts.
- Hover over a dot on a control chart to view general information about a control, such as well and plate.
- Select **Settings** to customize the graph. See *Set QC Charts preferences* (next page).

  NOTE

  You might want to change graph appearance in preparation for downloading the graph to an image file. The next time you view the graph, it will have reverted to its default settings.
- Download a graph as an image file by selecting an image option from the "hamburger menu".

Open and filter an analysis's QC Charts tab

1. On the sidebar menu, select **Analyses** to open the **Analyses** page.
2. (Optional) From the **Status** list, select *Completed* to show only completed analyses in the table.
3. From the **Controls/Samples** list, select *Samples included*.
4. Select the checkbox of each applicable analysis whose QC charts you want to work with.
5. Select the **QC Charts** tab.

   The *QC Charts tab* (page 60) presents each control with metrics for assay and MS performance.
6. Under **MS Instrument**, select up to six instruments.

   The tab aggregates data from the selected instruments and hides the **Color By** menu.
7. To filter the results for a specific control type, select an option:
   - *Process Control* — Results for the process controls only.
   - *Digestion Control* — Results for the digestion controls only.
   - *MPE Control* — Results for the MPE controls only.
   - *Mass Spec Control* — Results for the mass spec controls only.
8. To filter results for a specific time frame, enter the MS injection time frame in the **Start Date** and **End Date** fields.

Set QC Charts preferences

1. Open and filter the **QC charts** tab for an analysis. (See *Open and filter an analysis's QC Charts tab* (above).)
2. Select **Settings** to open the **Preferences** dialog.
3. Adjust the settings as you prefer.
   - **Show Annotations** — Select to show or hide annotation. See *Annotate control charts* (page 64).
   - **Show Historical Data** — Select to show or hide historical data. By default, each control chart shows values from historical data. Although independent of the selected analyses, these data points are sourced from previous analyses that exist in the user group.
   - **Show Variables** — Select the checkbox of each variable of interest, e.g., *Protein Group Count*, *Peak Width (seconds)*. Clear the checkbox of any you're not interested in.
   - **Process Variables Y Axis Range** — Select the range for the y-axis for processed variables.
     - *Min and Max Data Dependent* — Both the minimum and maximum values depend on data.
     - *Min is 0 and Max Data Dependent* — The minimum value is set at 0 and the maximum value depends on data.
     - *User Defined* — Enter the minimum and maximum values.
4. Select **Save**.

Manage user-defined and calculated control limits

Depending on the MS injection time frame, filtering results on the **QC Charts** tab by time frame shows a summary that presents each control with metrics for assay and MS performance. Each metric is color-coded to indicate whether it is within the control limits (teal) or outside the control limits (orange).

Control limits help determine whether results are expected. The mean provides a historical average, while the upper and lower control limits indicate normal variation. PAS includes default control limits calculated using two standard deviations above and below the mean. Calculated control limits are generated from user data.

To define and use user-defined control limits:

1. On the **Controls** section's toolbar, select the **Control Limits** list and select *User Defined* to open the **Control Limits** dialog.
2. Along the top of the dialog, select the tab of the control whose limits you want to define, e.g., *Digestion Control*. Use the scroll arrows to view all tabs.
3. For each control limit, enter a value.
   - *Mean* — The value to serve as the historical mean.
   - *UCL* — The value to serve as the upper control limit.
   - *LCL* — The value to serve as the lower control limit.
4. Select **Save**.

To define and use calculated control limits:

1. On the **Controls** section's toolbar, select  **Calculate** to open the **Calculate Control Limits** dialog.
2. In the **Start Date** and **End Date** fields, enter or select the applicable dates.
3. Select **Save**.

   A teal background appears on the plots, indicating that data are shown with modified control limits.

   NOTE

   The new control limit date range has been added to the **Calculate Control Limits Range** dialog, which you can open with the  **Limits** button.
4. On the toolbar, select the **Control Limits** list and select *Calculated* to use the calculated control limits.

To delete a calculated control limit date range:

1. On the **Controls** section's toolbar, select  **Limits** to open the **Calculate Control Limits Range** dialog.
2. Find the date range you want to delete and select its  **Delete** button.
3. Select **Yes** to confirm.
4. Select **Save**.

To exclude an outlier or other data point

Exclude an outlier or other data point from the control limit calculation. Selecting one data point (sample) in a plot excludes the corresponding sample from the other plots. A well containing the mass spec control has 10 reported QC metrics. All other control wells have 11 reported QC metrics.

1. Select **Exclusion** to open the **Control Limit Exclusion** dialog and display a list of samples that are being excluded.
2. Find the data point (sample) you want to remove and select its **Delete** button.
3. Select **Yes** to confirm.
4. Select **Close**.

Annotate control charts

You can add an annotation that will be applied to all control charts for a specific date. An annotation is indicated by a dot above a control chart with the full annotation below the chart. Select an annotation dot to view its annotation type, date, and accompanying message.

To add an annotation:

1. Open and filter the **QC charts** tab for an analysis. (See *Open and filter an analysis's QC Charts tab (page 62)*.)
2. Select **Annotate** to open the **Annotate** dialog.
3. Complete the fields.
   - **Annotation Type** — Do either of the following:
     - To select an existing annotation type, select it from the list.
     - To add a new annotation type, select **Add Annotation Type**, enter the annotation, and select **Add**.
   - **Date** — Enter or select the date the event occurred.
   - **Message** — Enter more information about the annotation.
4. Select **Save**.

To edit an annotation:

1. Select the annotation dot for the annotation you want to edit.
2. In the annotation pop-up, select **Edit** to open the **Annotate** dialog.
3. Edit the annotation as needed.
4. Select **Save**.

To delete an annotation:

1. Select the annotation dot for the annotation you want to delete.
2. In the annotation pop-up, select **Delete**.
3. Select **OK** to confirm.

Generate reports of control results

Summarize and output control results in a .csv file or generate a more detailed report as a .pdf file. The summary lists each QC metric with the control, well, plate, value, and result. The report summarizes results and shows a table for each control. Each table includes the well and plate and lists values for each QC metric with optional notes.

Download summarized control results

1. Open and filter the **QC charts** tab for an analysis. (See *Open and filter an analysis's QC Charts tab (page 62)*.)
2. In the summary table above the control charts, select **Download CSV** to open the **Download CSV** dialog.
3. Select the checkbox of each field to add to the summary:
   - **File name** — The full name of the MS data file, including extension.
   - **Assay instrument name** — The name of the liquid handler that prepared the assay.
   - **Mass spec instrument name** — The name of the MS instrument that analyzed the assay output.
   - **Injection time** — The month and day of MS injection.
   - **Gradient** — The duration of the LC gradient.
4. Select a format:
   - **One line per metric** — Organizes the summary by metric.
   - **One line per control** — Organizes the summary by control.
5. Select **Download** to generate the summary.

Generate a detailed PDF report of control results

1. Open and filter the **QC charts** tab for an analysis. (See *Open and filter an analysis's QC Charts tab (page 62)*.)
2. Select **Reports** to open the **Controls Report** dialog.
3. Complete the fields.
   - **Assay Equipment ID** — Select the name of the liquid handler used to prepare the assay.
   - **Mass Spec ID** — Select the name of the MS instrument that analyzed the assay output.
   - **Report By** — (Read-only) Shows your username.
   - **Report Date** — (Read-only) Shows the current date and time.
4. Select **Create** to generate the report.

Analysis Output Files tab

The **Analysis Output Files** tab on the **Analyses** page lists all outputs of the search engine results for the analysis. You can download an output file simply by selecting it.

To open this tab:

1. On the sidebar menu, select **Analyses** to open the **Analyses** page.
2. (Optional) From the **Status** list, select *Completed* to show only completed analyses in the table.
3. Select an analysis to open its analysis results.
4. Select the **Analysis Output Files** tab.

Proteogenomics tab

The **Proteogenomics** tab on the **Analyses** page appears for analyses that used a Proteogenomics workflow.

- Use the **Variant Peptide Browser** to view the number of variant peptides found across samples, conditions, and nanoparticles.
- Use the **Proteogenomics Data Explorer** to examine how proteogenomic peptide and variant peptide data map with respect to genomic coordinates for both entire proteins and at the nucleic acid/amino acid scale resolution.

Open the Proteogenomics tab

1. On the sidebar menu, select **Analyses** to open the **Analyses** page.
2. (Optional) From the **Status** list, select *Completed* to show only completed analyses in the table.
3. Select an analysis for which a Proteogenomics workflow was used.

   The analysis results are loaded. By default, the **Proteogenomics** tab is open, where you can browse the variant peptides and explore the proteogenomics data.

Summary section

The **Summary** section shows all variant peptides identified in the selected analysis.

- **Analysis setup**
  - *Analysis Name* — The name of the analysis.
  - *Run Types* — The analysis protocol type, specifically *Proteogenomics*.
  - *Analysis Protocol* — The name of the analysis protocol applied to the analysis.
- **Study results summary**
  - *Number of samples* — The number of samples analyzed.
  - *Total number of peptides across samples*
  - *Average number of peptides across samples*
- **Proteogenomics summary**
  - *Total variant peptides across all analyzed samples*
  - *Total variant peptides average*

Variant Peptide Browser

Use the **Variant Peptide Browser** to view the number of variant peptides found across samples, conditions, and nanoparticles. The distribution of variant peptide intensity is stratified by reference and by alternative alleles across samples, conditions, and nanoparticles.

See also *Samples graph* (page 68).

Toolbar items

- **Group by** — Select a category (e.g., *Condition*) by which to group data. To not group, select *None*.

  NOTE

  The categories offered vary depending on the sample description information.

- **Protein/Gene Search** — Use to find data for a specific protein or gene.
- **Filter Data** — Use to exclude individual samples, groups, and/or nanoparticles from the browser. To remove the exclusion for an item, select its button.
- **Summarize by Variant** — (Selected by default.) Use this checkbox to summarize the data by variant.

Table columns

TIP

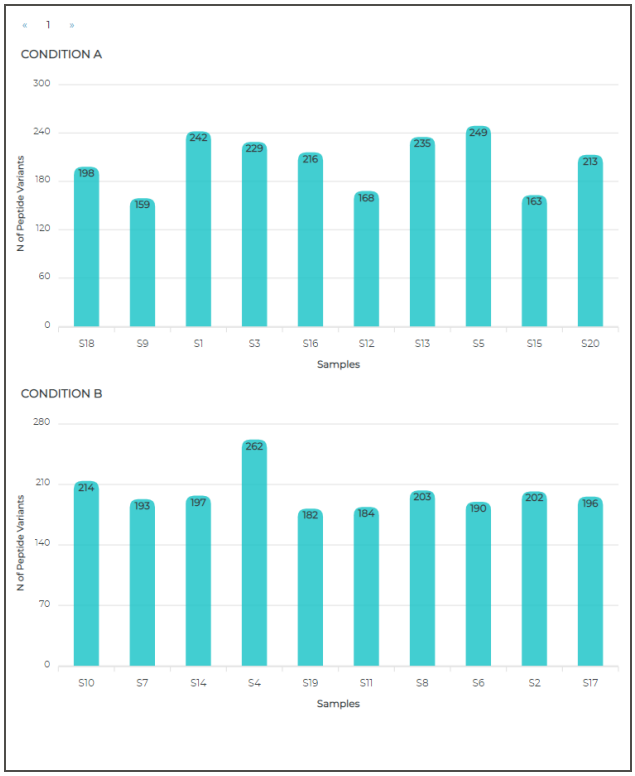
You may need to scroll the table all the way to the right to see all the columns.

- When the **Summarize by Variant** checkbox is selected, the following columns are shown:
  - / buttons — Use to open and close data about individual variant peptides. For each variant, the group, sample name, nanoparticle, and peptide are shown.
  - **Number of Measurements** — The number of measurements in an individual variant peptide.
  - **Protein** — The name of the protein corresponding to a variant peptide.
  - **Gene** — The gene where a variant peptide is located.
  - **Variant** — The name of a variant peptide. Select a variant link to load protein data and view it in the *Proteogenomics Data Explorer* (next page).
  - **AF** — The allele frequency of a variant peptide. Use this column's *Filter* option to show only values that are greater than, less than, greater than or equal to, or less than or equal to the value you specify or are between two values you specify. To apply the filter, select . To clear it, select .
- When the **Summarize by Variant** checkbox is cleared, the following columns are shown.
  - **Group By** — The category (e.g., *Condition*) by which data is grouped.
  - **Sample Name** — The name of a sample.
  - **NP** — The name of a nanoparticle.
  - **Protein** — The name of the protein corresponding to a variant peptide.
  - **Peptide** — The name of a peptide.
  - **Intensity** — The raw MS intensity values of a variant peptide.
  - **Variant** — The name of a variant peptide.
  - **AF** — The allele frequency of a variant peptide. See above.

Samples graph

The **Samples** graph shows the number of variant peptides by samples. If you group samples (e.g., by *Condition*), there is one graph for each group.

Figure 18. Samples graph



- To open this graph, select **Analyses**, select an analysis for which the Proteogenomics workflow was used, and select the **Proteogenomics** tab. The graph is shown by default.
- Hover over a bar to view details about a sample.
- Select **Settings** to customize the graph.

Proteogenomics Data Explorer

Use the interactive **Proteogenomics Data Explorer** to examine how proteogenomic peptide and variant peptide data map with respect to genomic coordinates for both entire proteins and at the nucleic acid/amino acid scale resolution.

To load protein data into the explorer, either use the **Protein/Gene** field (see below) to find and select a protein or gene or select the protein's variant link in the *Variant Peptide Browser* (page 66).

Toolbar items

- **Protein/Gene** — The name of the protein or gene selected in the Variant Peptide Browser. Or, if none has been selected, enter the name of the protein or gene whose data you want to explore.

> **TIP**
>
> As you type characters into this field, a list of matching items appears. You can then select from among the matches.

- **Gene** — The name of a gene.
- **Chromosome** — The name of a chromosome.
- **Genome Version** — The reference genome.
- **Group By** — Select the category (e.g., *Condition*) by which to group data. To leave data ungrouped, select *None*.
- **Panel** — The values of the five nanoparticles rolled up into one value.
- **Display Introns** — (Selected by default.) Select or clear this checkbox ☐ to show or hide introns.
- **Move < >** — Use these buttons to move left and right within the explorer.
- **Zoom** — Use these buttons to show data at a higher or lower level. For illustrations, see *Proteogenomics Data Explorer graph - Protein view* (page 71).
  - **Protein** — Shows view of the entire protein coding region.
  - **Base** — Shows data at the nucleotide/amino acid level.

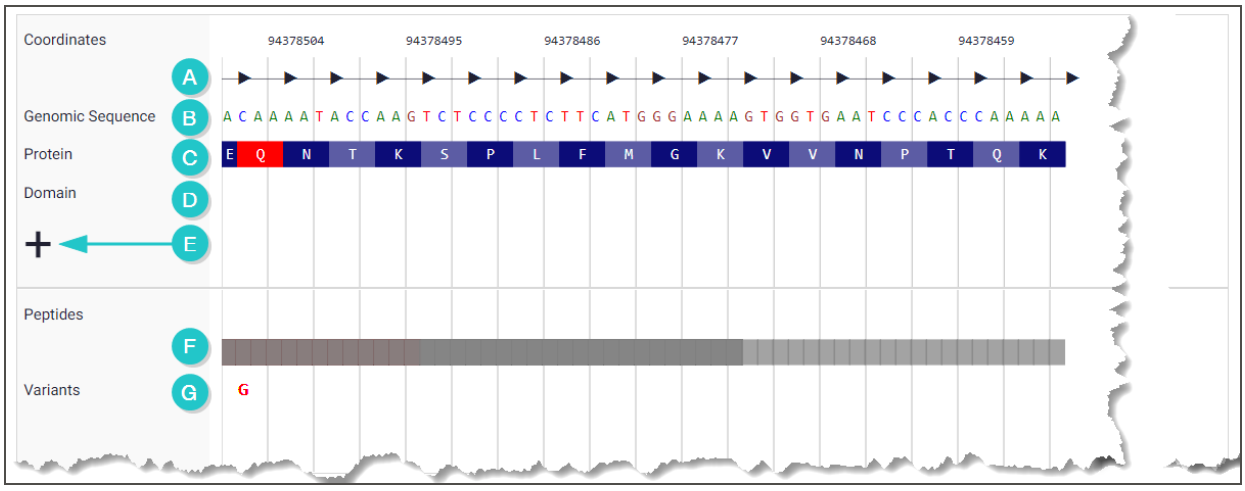
    When you set the explorer's zoom level to **Base**, you are exploring the data at the nucleotide/amino acid level.
- **Export** — Select to export the proteogenomics data to a .csv file.

Proteogenomics Data Explorer graph - Base view

The *base view* of the Proteogenomics Data Explorer's graph shows DNA sequence, amino acid sequence, domain, and region. Amino acid variants within variant peptides are highlighted, including both reference and alternative alleles.

When you set the Proteogenomics Data Explorer's zoom level to **Base**, data is visualized at the nucleotide/amino acid level.

Figure 19. Proteogenomics data visualized at the base level



A Strand direction

B Nucleic acid or DNA sequence

C Amino acid sequence

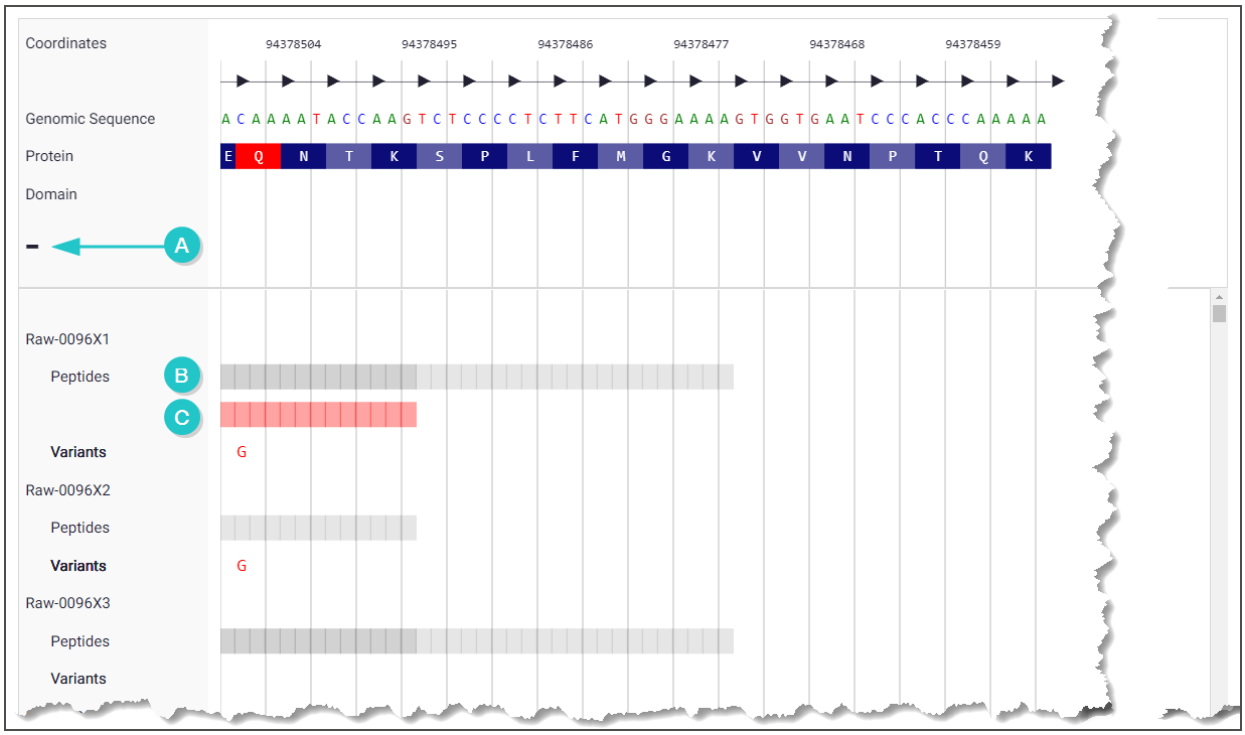
D Functional protein domains / regions

E Button to show data for individual reference peptides and variant peptides

F Identified reference peptides and variant peptides across all samples

G Location of the variant peptide

Figure 20. Proteogenomics data visualized for individual peptides and variant peptides



A Button to hide data for individual reference peptides and variant peptides

B The identified reference peptide for this particular sample

C The identified variant peptide for this particular sample

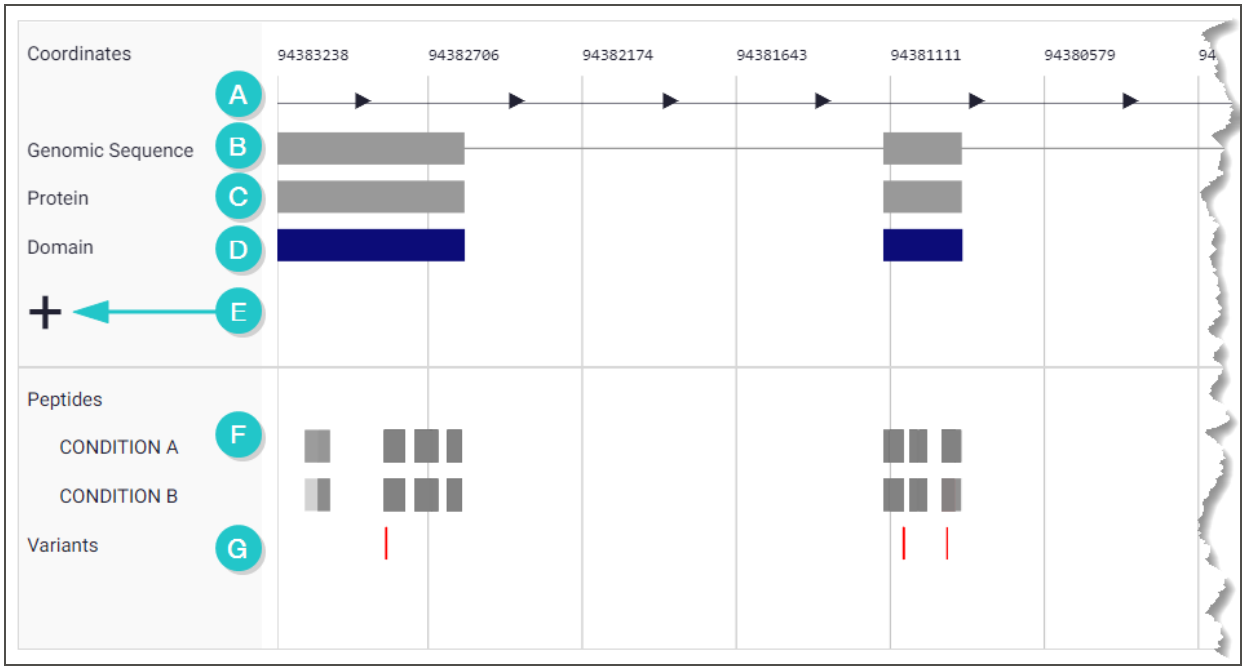
- Load protein data in the Proteogenomics Data Explorer either by using the **Protein/Gene** field to find and select a protein or gene or by selecting the protein's variant link in the *Variant Peptide Browser* (page 66).
- Filter and group the data, as needed.
- Select the **Base** zoom level.
- When viewing for individual reference peptide and variant peptide data, hover over a bar to view that peptide's amino acid sequence and intensity and the filename of the raw MS data file in which the peptide was identified.

Proteogenomics Data Explorer graph – Protein view

The *protein view* of the Proteogenomics Data Explorer's graph shows gene structure, protein structure, domain information, and region information. Amino acid variants within variant peptides are highlighted, including both reference and alternative alleles.

When you set the Proteogenomics Data Explorer's zoom level to **Protein**, the entire protein coding region is visualized.

Figure 21. Proteogenomics data visualized at the protein level



- **A** Strand direction
- **B** Protein coding DNA sequence
- **C** Protein (translated protein coding DNA sequence)
- **D** Functional protein domains / regions
- **E** Button to show data for individual reference peptides and variant peptides
- **F** Identified reference peptides across all samples
- **G** Identified variant peptides across all samples

Figure 22. Proteogenomics data visualized for individual peptides and variant peptides

A Button to hide data for individual reference peptides and variant peptides

B The identified reference peptide for this particular sample

C The identified variant peptide for this particular sample

- To load protein data in the Proteogenomics Data Explorer, either use the **Protein/Gene** field to find and select a protein or gene or select the protein's variant link in the *Variant Peptide Browser* (page 66).
- Filter and group the data, as needed.
- Select the **Protein** zoom level.
- Hover over a domain bar to view the amino acid coordinates of the domain / region.
- When viewing for individual reference peptide and variant peptide data, hover over a bar to view that peptide's amino acid sequence and intensity and the filename of the raw MS data file in which the peptide was identified.

Export proteogenomics data

1. Open the **Proteogenomics** tab for an analysis for which the Proteogenomics workflow was used. (See *Open the Proteogenomics tab* (page 66).)
2. Load protein data by doing either of the following:
   - In the *Variant Peptide Browser* (page 66), select a protein's variant link to the genomic coordinates.
   - In the *Proteogenomics Data Explorer* (page 68), use the **Protein/Gene** field to find and select a protein or gene.

3. Select **Export** to export the proteogenomics data.

   Depending on your browser or browser preferences, the download may begin immediately, or you may be prompted where to save the file. Follow any on-screen prompts.

Chapter 4

Analysis Interpretation

This chapter describes techniques for interpreting analysis results, specifically for group analyses, performed from the **Group Analysis** tab of the **Analyses** page.

Open an analysis's Group Analysis tab

1. On the sidebar menu, select **Analyses** to open the **Analyses** page.
2. (Optional) From the **Status** list, select *Completed* to show only completed analyses in the table.
3. Select an analysis to open its analysis results.
4. Below the table, select the **Group Analysis** tab. (See *Group Analysis tab* (page 77).)

   PAS prepares the data. This may take a few moments.

Export raw data to a file

Prior running a group analysis, you can export raw protein or peptide group data to a .csv file.

1. Open the **Group Analysis** tab. (See *Open an analysis's Group Analysis tab* (above).)
2. Select **Export**.
3. Depending on your browser or browser preferences, the download may begin immediately, or you may be prompted where to save the file. Follow any on-screen prompts.

View a raw data heatmap

Prior to doing a group analysis, you can visualize a heatmap of raw protein or peptide group intensity.

1. Open the **Group Analysis** tab. (See *Open an analysis's Group Analysis tab* (above).)

   The *Raw Data section* (page 79) is the only section open at this point.
2. From the **Feature** list, select *Protein* or *Peptide* to filter raw data.
3. Select **Heatmap**.

   PAS prepares the data and then displays the heatmap. This may take a few moments.

Set up and start a group analysis

With a group analysis, you can then evaluate and visualize differences in peptide or proteins detected between groups.

To begin this workflow:

1. Open the **Group Analysis** tab. (See *Open an analysis's Group Analysis tab* (above).)
2. Select **Setup Group Analysis** to open the **Setup Group Analysis** dialog.

Continue to the next section.

Specify the quantitation level and grouping of samples

In the **Feature and Grouping** section, you specify the parameters for the differential expression analysis between groups of samples.

1. For **Feature**, select the quantitation level, either *Protein* or *Peptide*.
2. For **Group By**, select the category by which you want to group samples, e.g., by a condition or custom field. The options vary based on the metadata imported from the sample description file.
3. Select **Next** to advance.

Filter out (exclude) samples and groups

In the **Filter Samples and Group** section, you specify the individual samples or whole groups of samples to be filtered out of (excluded from) the final comparison.

1. (Optional) From **Select Sample**, select one or more samples.
   - If you change your mind about an excluded sample, select its ✕ **Delete** button.
2. (Optional) From **Select Groups**, select one or more groups of samples.
   - If you change your mind about an excluded group, select its ✕ **Delete** button.
3. Select **Next** to advance.

Filter out (exclude) proteins or peptides

In the **Filter Peptides / Proteins** section, you specify measurement completeness thresholds for the final comparison.

1. Complete the fields.

   NOTE

   PAS will use whichever minimum (percentage or specific value) yields the larger number of samples.

   - **Minimum % of valid values in at least one group** — Enter the minimum percentage of samples to consider. For example, if there are 24 samples and you enter 50, a minimum of 12 samples will be considered. The default is *0*.
   - **Minimum number of valid values in at least one group** — Enter the exact minimum number of samples to consider. The default is *2*.
   - **Remove peptides / proteins with complete missing values in at least one group** — Select to exclude peptides / proteins for which data is entirely missing (i.e., all table cells that show –).
   - **Remove peptides / proteins with missing values** — Select to exclude peptides / proteins with one or more missing values (i.e., some table cells that show –).
2. Select **Next** to advance.

Normalize values and impute sparse or missing values

In the **Normalization and Imputation** section, you specify the processes by which raw measurement values are normalized and sparse and missing values are handled.

1. For **Normalization**, select either:
   - *Median* — Raw MS intensity values are normalized on a run-by-run basis. This will account for potential measurement bias and make samples more comparable.
   - *None* — No normalization of raw measurements will occur.
2. For **Imputation**, select either:
   - *Minimal Probability* — Substitute missing values with random values from a normal distribution using a mean that is down-shifted from the sample mean and a standard deviation (SD) that is a fraction of the SD of the sample distribution.
   - *None* — No imputation of raw measurements will occur.
3. Select **Next** to advance.

Select the statistical test and the groups to compare

In the **Statistical Test and Group Comparison** section, you select the statistical test to use for comparison analysis and then select the groups of samples you want to compare.

1. For **Statistical Test**, select the appropriate test to use for comparison analysis.
   - *T Test* — A parametric test. Select this if you previously elected to normalize data.
   - *Wilcoxon* — A non-parametric text rank-based test. Select this if you previously elected not to normalize data.
2. Under **Groups for Comparison**, select a group from the left list and a different group from the right list. The groups offered vary, based on selections you made earlier in the setup workflow.
3. Select **Start**.

   The group analysis begins immediately. When it is finished, the analysis results are shown on at *Group Analysis tab* (below), organized into graphs, with the*Volcano graph* (page 80) shown.

Export group analysis data

After running a group analysis, you can export the results table or processed data as .csv files.

1. Run a group analysis. (Begin with *Set up and start a group analysis* (page 75).)
2. On the **Group Analysis** toolbar, select **Export** and then select either *Result table* or *Processed data*.
3. Depending on your browser or browser preferences, the download may begin immediately, or you may be prompted where to save the file. Follow any on-screen prompts.

Group Analysis tab

An analysis's **Group Analysis** tab (on the **Analyses** page) shows the analysis and results from comparing two user-defined groups, organized into sections. You can visualize differences in proteins detected between the groups and explore initial biological insights into the data.

NOTE

Samples must be annotated with at least two conditions. You can add or modify conditions in your sample description file or by editing samples in the relevant plates on the **Plates** page.

TIP

Use this page's **Collapse** and **Expand** buttons to selectively collapse and expand sections.

Toolbar items — before group analysis

- **Search** — Use to find a specific item. See *Find table items* (page 18).
- **Feature** — Select *Protein* or *Peptide* to filter the **Raw Data** table.
- **Group By** — Select the categorical grouping (e.g., *Condition*) by which you want to group raw data.
- **Heatmap** — Select to visualize a heatmap of raw protein group data. See *View a raw data heatmap* (page 75).
- **Export** — Select to export raw data to a .csv file. See *Export raw data to a file* (page 75).
- **Setup Group Analysis** — Select to set up groups for and start a comparative analysis. See *Set up and start a group analysis* (page 75).

Toolbar items — after group analysis

- **Setup Group Analysis**— Select to rerun the group analysis with different settings, without having to go back to raw data.
- **Raw Data** / **Results** — Use to switch back and forth between the raw data and group analysis results.
- **Significant Proteins** — Select this checkbox to show only significant proteins.
- **Search** — Use to find a specific item. See *Find table items* (page 18).
- **Volcano Plot** — Select to view the *Volcano graph* (page 80).
- **Coverage** — (Available when at least two proteins are selected.) Select to view the *Coverage graph* (page 81).
- **Clustered Heatmap** — Select to view the *Clustered Heatmap graph* (page 83).
- **PPI Network** — (Available only for significant proteins.) — Select to view the *Filtered PPI Network graph* (page 84).
- **Enrichment** — (Available only for significant proteins.) — Select to view the *Enrichment graph* (page 85).
- **Box Plots** — (Available only for significant proteins.) — Select to view the *Box Plots graph* (page 86).
- **Export** — Select to export the group analysis results table or processed data. See *Export group analysis data* (previous page).
- **Threshold Preferences** — Select to set threshold preferences. e.g., *High Regulation Threshold*. The high and medium threshold values indicate when a value will be flagged as high (three up arrows) or medium (two up arrows). The graph values will re-draw the dashed lines on the graph according to these values.

Raw Data section

The **Raw Data** section shows a table consisting of protein or peptide groups (rows) identified across the analyzed samples (columns) and their corresponding intensity levels (values).

Samples for which a protein or peptide group was detected show a numerical value. However, samples for which a protein or peptide group was not detected show a hyphen – character.

Figure 23. Raw Data table listing proteins and Intensity Plot for the selected protein

Figure 24. Raw data table listing proteins and heatmap for all proteins

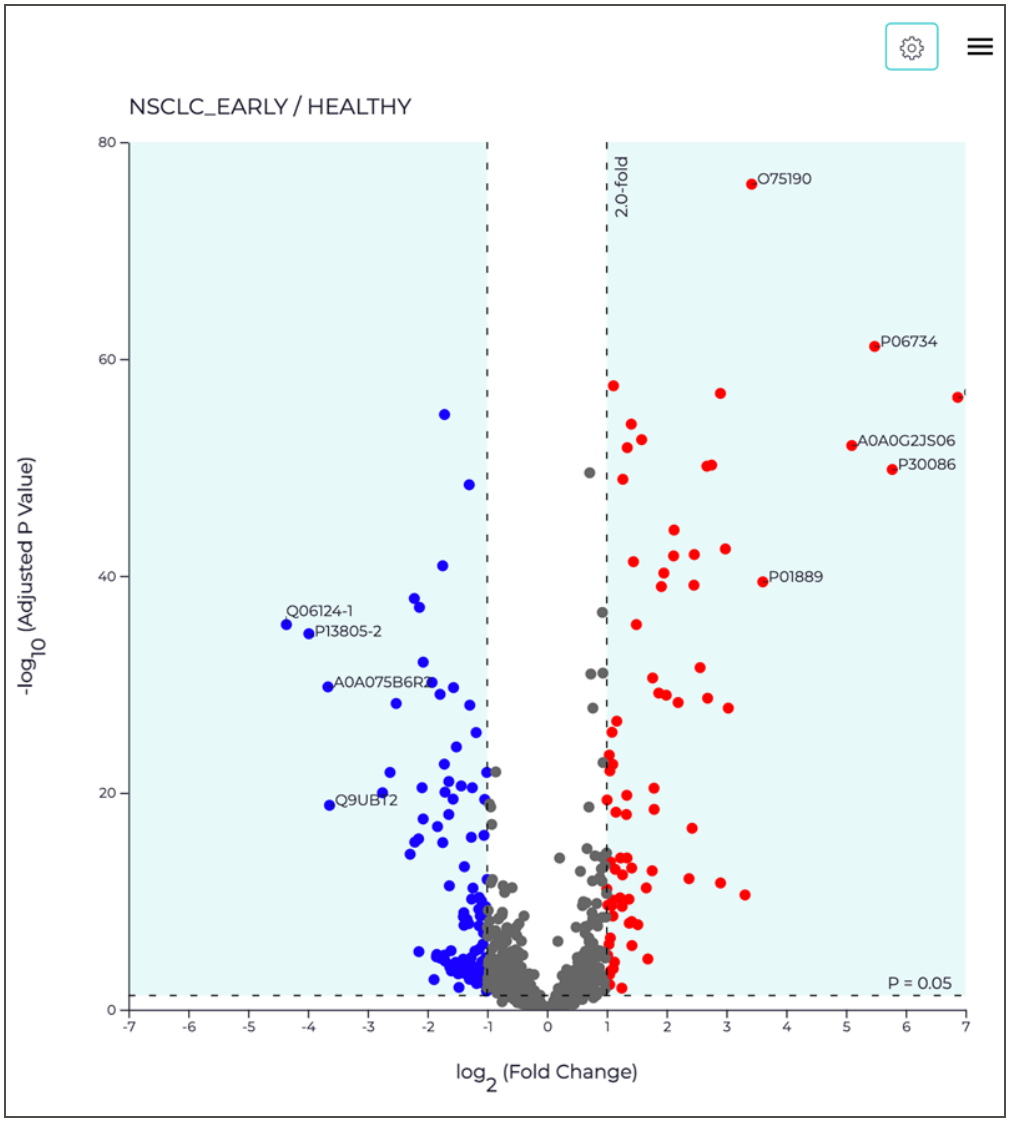
- Select **Heatmap** to visualize a heatmap of raw protein or peptide group data. (See also *View a raw data heatmap* (page 75).)
- Use the + / – buttons to zoom in and out of the graph.
- Hover over a cell to view the protein or peptide name, sample name, and intensity.
- Download a graph as an image file by selecting an image option from the "hamburger menu".

Volcano graph

After running a group analysis, you can view the **Volcano** graph.

Here you can visualize a scatter plot showing the statistical significance (p-value) in abundance differences between your compared groups versus the magnitude of the change (Fold Change). Each point in the plot represents a protein or peptide. Highlighted in the teal quadrants are proteins with large fold changes (default: ±2) and statistically significant (default: $p < 0.05$). Data displayed in the plot are shown in detail in the table on the left of the plot.

Figure 25. Volcano graph



- To open after running a group analysis, select **Volcano Plot** on the **Group Analysis** toolbar.
- Hover over a dot to view protein or peptide group, fold change, and p-value.
- Clear the **Significant Proteins** checkbox on the toolbar to view both significant and non-significant proteins.

- Select **Settings** to customize the graph.
- Use the mouse wheel to zoom into and out of the graph. To reset to the original size, select **Reset Zoom** on the graph.
- Download a graph as an image file by selecting an image option from the "hamburger menu".
- In the table at the left of the graph, use columns' *Filter* option to show only values that are greater than, less than, greater than or equal to, or less than or equal to the value you specify or are between two values you specify. To apply the filter, select . To clear it, select .

> **TIP**
>
> Filtering data this way offers you more control over what appears in the graph, particularly if you want to export different versions of it.

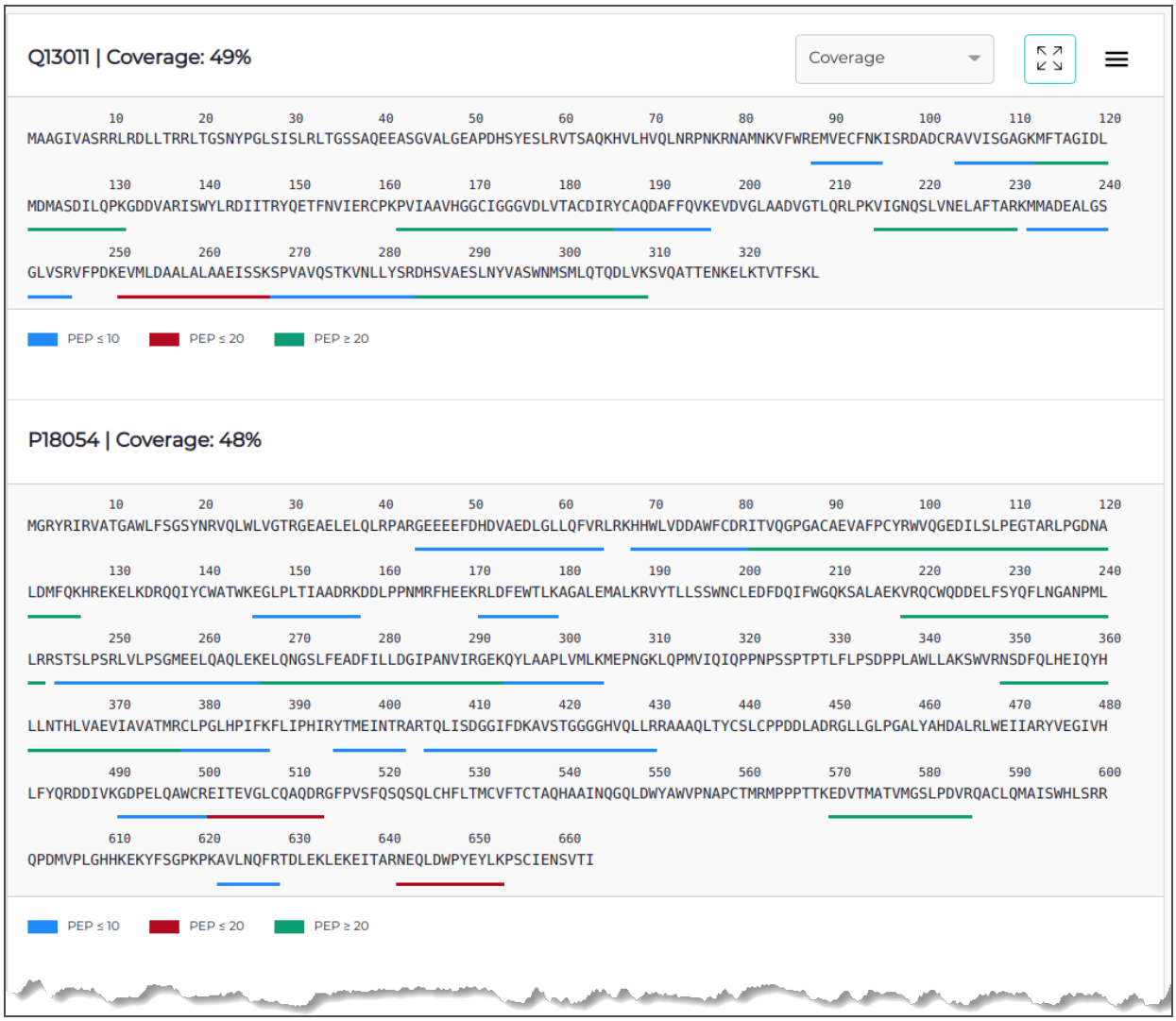
Coverage graph

After running a group analysis, you can view the **Coverage** graph.

Here you can visualize the amino acid sequence of your selected protein and the regions where measured peptides map. You must select at least one protein in the table to the left of the graph.

Coverage is represented as percent (%) of sequence observed by measured peptides.

Figure 26. Coverage graph, shown in the default collapsed (summarized) view



- To open after running a group analysis, select at least one protein in the table at the left and then select **Coverage** on the **Group Analysis** toolbar.
- Use the table columns' *Filter* options to show only values that are greater than, less than, greater than or equal to, or less than or equal to the value you specify or are between two values you specify. To apply the filter, select . To clear it, select .

  TIP

  Filtering data this way offers you more control over what appears in the graph, particularly if you want to export different versions of it.

- In cases where a PTM is detected for the selected proteins, select *PTM* from the **Coverage** list to view where the PTM occurs.
- Select **Expanded View** to view individual peptides from different sample-nanoparticle measurements.

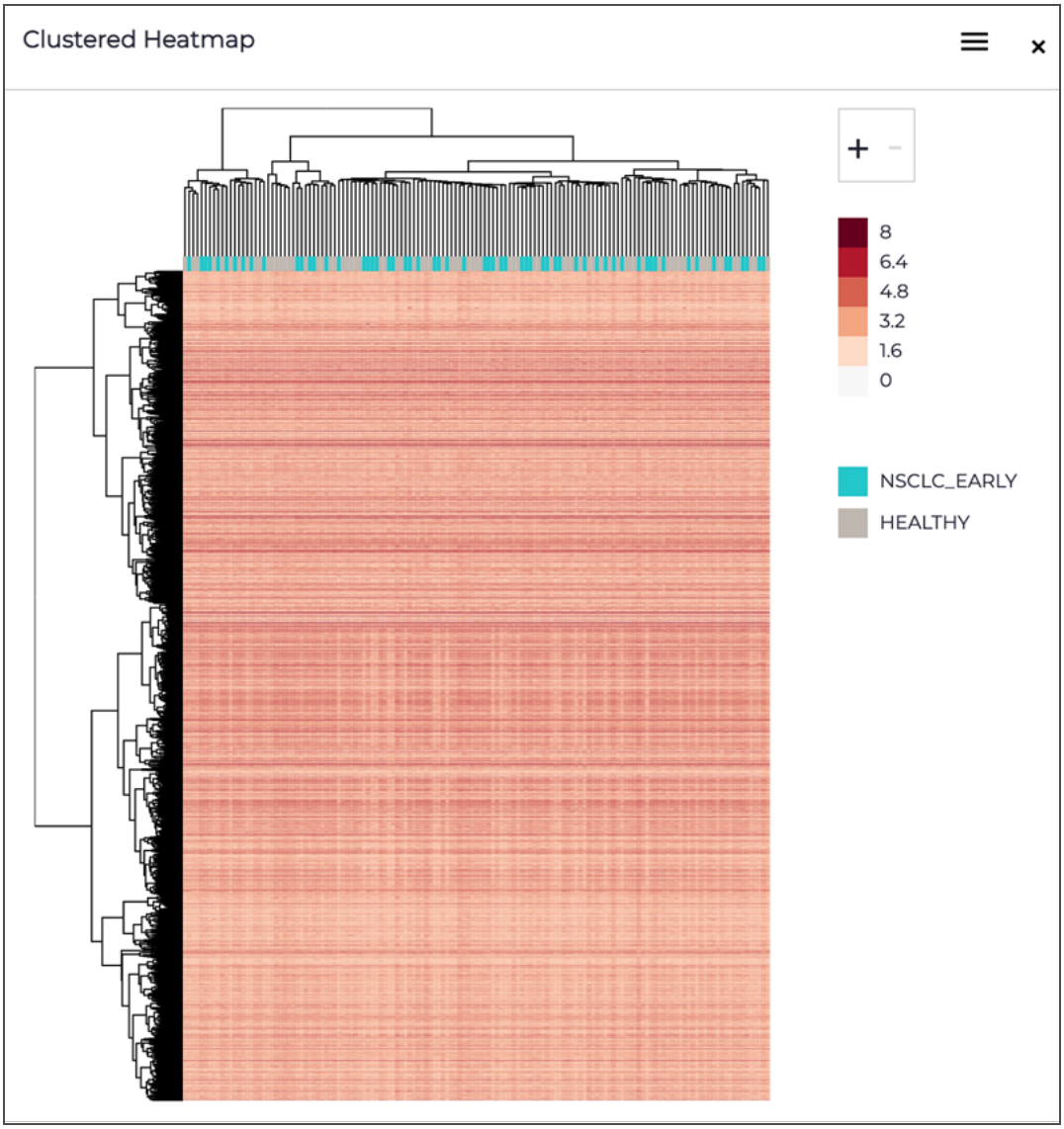
  - In this view, hover over a horizontal bar to view a PEP value.
- Select **Collapsed View** to return to the summarized view.
- Download a graph as an image file by selecting an image option from the "hamburger menu".

Clustered Heatmap graph

After running a group analysis, you can view the **Clustered Heatmap** graph.

Here you can visualize protein abundances across samples, with both proteins (rows) and samples (columns) clustered based on agglomerative nesting. Cell colors range from blue (low abundance) to red (high abundance).

Figure 27. Clustered heatmap graph



- To open after running a group analysis, select **Clustered Heatmap** on the **Group Analysis** toolbar.
- Use the + / - buttons to zoom in and out of the graph.
- Hover over a cell to view the protein or peptide name, sample name, and intensity.

- Download a graph as an image file by selecting an image option from the "hamburger menu".
- In the table at the left of the graph, use columns' *Filter* option to show only values that are greater than, less than, greater than or equal to, or less than or equal to the value you specify or are between two values you specify. To apply the filter, select . To clear it, select .

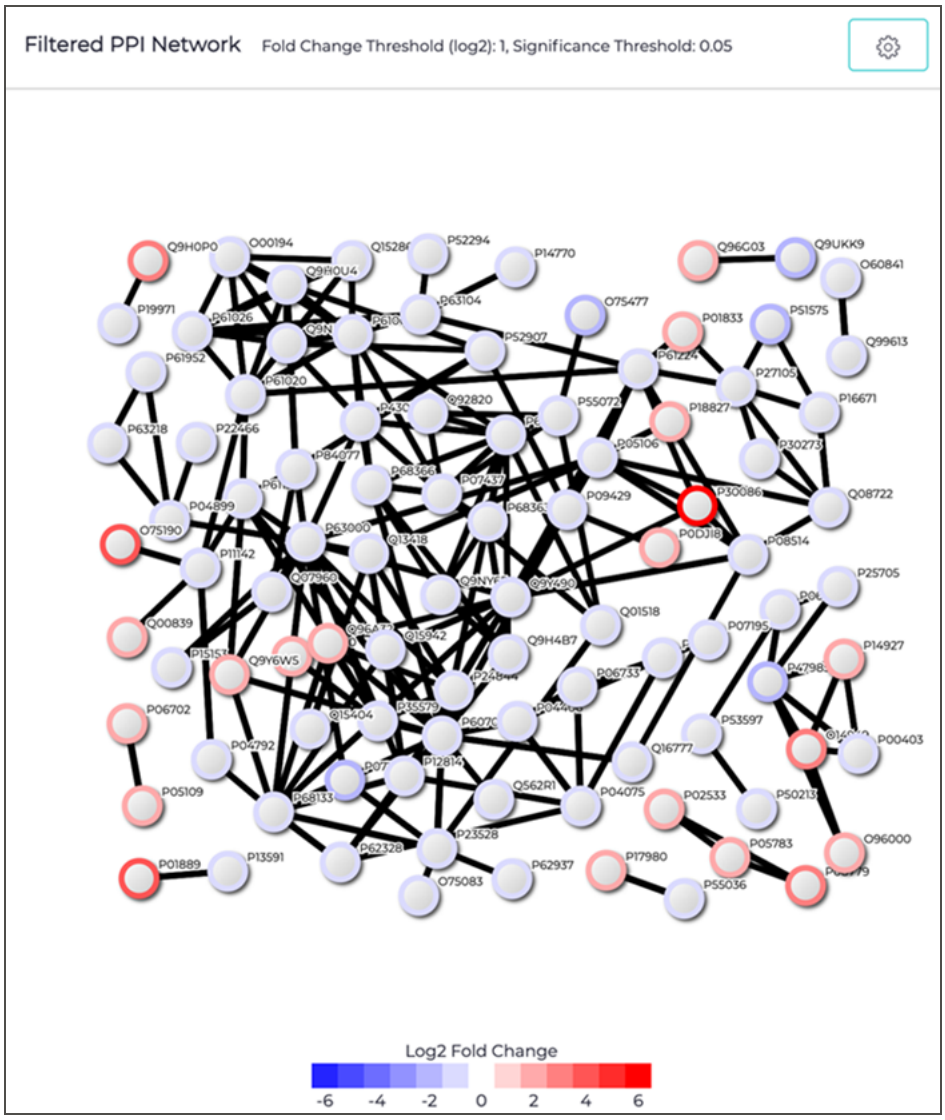
> **TIP**
>
> Filtering data this way offers you more control over what appears in the graph, particularly if you want to export different versions of it.

Filtered PPI Network graph

After running a group analysis and with the **Significant Proteins** checkbox selected, you can view the **Filtered PPI Network** graph.

Here you can visualize a network plot showing protein-protein interactions between proteins with significant differences between groups compared.

Figure 28. Filtered PPI Network graph



- To open after running a group analysis, select **PPI Network** on the **Group Analysis** toolbar.
- To adjust the confidence of the Minimum Interaction Score, select **Settings** on the graph.
- Nodes in the graph are selectable to view protein description and can be moved around.
- In the table at the left of the graph, use columns' *Filter* option to show only values that are greater than, less than, greater than or equal to, or less than or equal to the value you specify or are between two values you specify. To apply the filter, select . To clear it, select .

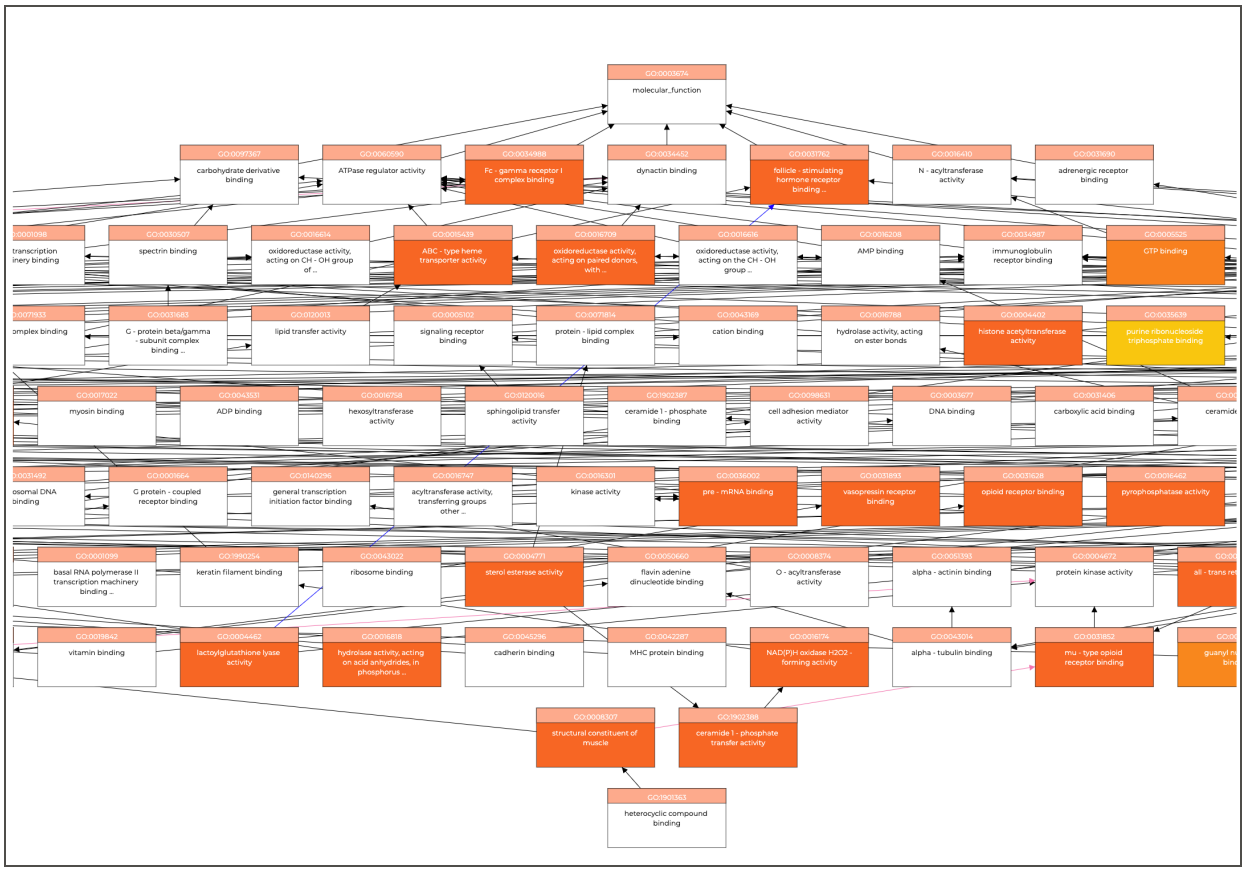
> **TIP**
>
> Filtering data this way offers you more control over what appears in the graph, particularly if you want to export different versions of it.

Enrichment graph

After running a group analysis and with the **Significant Proteins** checkbox selected, you can view the **Enrichment** graph.

Here you can functionally characterize proteins showing abundance difference between groups compared by performing gene ontology (GO) enrichment.

Figure 29. Enrichment plot, shown as an ontology plot (hierarchical tree)



- To open after running a group analysis, select **Significant Proteins** and then select **Enrichment** on the **Group Analysis** toolbar.

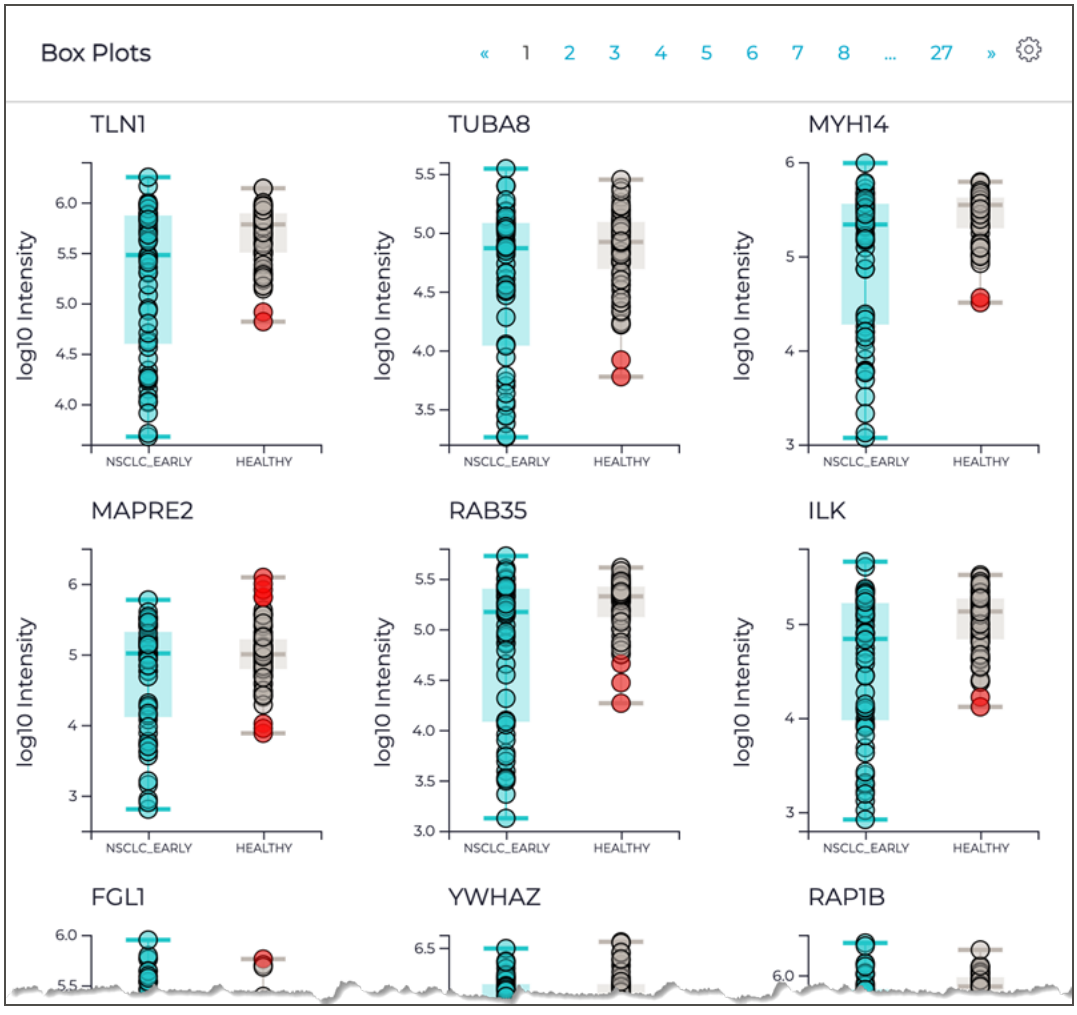
- Use the list to switch between GO categories: *Molecular Function*, *Biological Process*, and *Cellular Compartment*.
- To set the cutoff (adjusted p-value upper limit), select **Chart Settings** on the graph.
- Display the GO term enrichment as either:
  - **Dot Plot** — To "condense" similar terms by family (i.e., condense all children from each superfamily), select the **Summarize Terms** checkbox.
  - **Ontology Plot** (hierarchical tree)
    - Hover over a box to view GO information.
    - Use the mouse wheel to zoom into and out of the plot.
    - Drag the plot to reposition it so that you can examine different areas.

Box Plots graph

After running a group analysis and with the **Significant Proteins** checkbox selected, you can view the **Box Plots** graph.

Here you can visualize the intensity differences between groups of significantly different proteins.

Figure 30. Box plots



- To open after running a group analysis, select **Significant Proteins** and then select **Box Plots** on the **Group Analysis** toolbar.
- To change the layout for the charts, select **Settings** on the graph.
- Hover over a dot to view a sample's count, name, and statistics.
- In the table at the left of the graph, use columns' *Filter* option to show only values that are greater than, less than, greater than or equal to, or less than or equal to the value you specify or are between two values you specify. To apply the filter, select . To clear it, select .

> **TIP**
>
> Filtering data this way offers you more control over what appears in the graph, particularly if you want to export different versions of it.

Chapter 5

Data Management

This chapter covers the components of PAS's main pages and how to use them.

For common techniques for working with the tables on these pages, see *Work with PAS tables* (page 16).

Plates and samples

A plate in PAS represents a Peptide Collection Plate, which is the output of the Proteograph Assay method analyzed with MS. A plate contains the samples, controls, and nanoparticles from a method run with the corresponding metadata.

Plates page

Use the **Plates** page to manage plates and sample information and to upload custom files. To open this page, select **Plates** on the sidebar menu.

TIP

Use this page's **Collapse** and **Expand** buttons to selectively collapse and expand sections.

Toolbar items

- **Add Plate** — Select to add a plate to PAS when setting up an analysis. See *Add a plate* (page 29).
- **Batch delete** — (Appears when you select table items with **Multi-selection**, below.) Use to delete the selected table items as a batch. See *Delete multiple table items at the same time* (page 20).
- **Multi-selection** — Use to show or hide checkboxes in the table and to select or deselect table items on the current table page or all table pages.
- **Search** — Use to find a specific item. See *Find table items* (page 18).
- **Show/Hide** — Select or clear checkboxes to show or hide table columns. See *Show or hide table columns* (page 17).

Table columns

- **<checkboxes>** — Use to select or deselect individual table items. To select all items in the current page of the table, select the column header's checkbox.

  NOTE

  Checkboxes are hidden by default on this page. To show the checkboxes, click **Multi-selection** > **Show checkboxes**.

- **Name** — The name of the plate.
- **Plate ID** — The unique identifier of the plate.
- **Description** — The description of the plate.
- **Notes** — Additional information about the plate.
- **Created By** — The user who created the plate.
- **Created Time** — The date and time the plate was created.
- **Last Modified By** — The user who last modified the plate.
- **Last Modified Time** — The date and time the plate was last modified.
- **ID** — The unique, internal identifier of the plate.

- **Add Sample** — Select to add samples to the selected plate. See *Add samples to an existing plate* (page 92).
- **Edit** — Select to edit the selected plate. See *Edit a plate* (below).

Other page sections

- **Plate Samples** — Shows a table of samples associated with the plate selected in the **Plates** section. For detailed information about this section, see *Plate Samples section* (next page).
- **Plate Grid** — Shows a grid or table of wells associated with one or more samples selected in the Plate Samples section. For detailed information about this section, see *Plate Grid section* (page 94).

Review samples in a plate

1. On the sidebar menu, select **Plates** to open the **Plates** page.
2. Select a plate whose samples you want to review.

   The **Plate Samples** section appears below the **Plates** table, listing the samples in the selected plate.

   > TIP
   >
   > Adjust, sort, and filter the table as needed, using the techniques covered in *Work with PAS tables* (page 16).

3. Select samples for review:
   - To review all samples, select the column header's checkbox.
   - To review selected samples, select each sample's checkbox.

   The **Plate Grid** section appears below the **Plate Samples**, showing the plate with yellow wells indicating the selected samples.
4. Review sample details:
   - To individually review sample details, select a yellow well.
   - To simultaneously review details for all samples, select **Table View**.
   - To modify sample details, see *Edit or delete samples in wells* (page 94).

Edit a plate

1. On the sidebar menu, select **Plates** to open the **Plates** page.
2. Find the plate you want to edit and select its **Edit** button to open the **Edit Plate** dialog.
3. Edit the fields as needed.
   - **Plate ID** — Enter a unique identifier for the plate.
   - **Plate Name** — Enter a descriptive name for the plate.
   - **Description** — (Optional) Enter a description of the plate.
   - **Notes** — (Optional) Enter additional information about the plate.
4. Select **Confirm**.

Delete a plate

1. On the sidebar menu, select **Plates** to open the **Plates** page.
2. Find the plate you want to delete and select its **Delete** button.
3. Select **Yes** to confirm.

Plate Samples section

The **Plate Samples** section of the *Plates page* (page 89), appears below the table of plates. It shows the samples associated with the selected plate. To open this section, select **Plates** on the sidebar menu and then select a plate. You may need to scroll down to see the **Plates Samples** section.

Toolbar items

- **Link to Sample Description File** — Select to link a sample description file to the selected plate. See *Add sample descriptions* (page 94).
- **Search** — Use to find a specific item. See *Find table items* (page 18).
- **Show/Hide** — Select or clear checkboxes to show or hide table columns. See *Show or hide table columns* (page 17).

> **TIP**
> You can add custom columns to this table. See *Add custom table columns* (page 17).

Table columns

- **<checkboxes>** — Use to select or deselect individual table items. To select all items in the current page of the table, select the column header's checkbox.
- **Name** — The descriptive name of a sample.
- **Sample/Control ID** — The unique identifier of a sample or control.
- **Well Location** — The location of a sample or control in the selected plate.
- **Type** — The type of sample: *Plasma* or *Peptide*.
- **Species** — The species a sample was collected from: *Human* or *Mouse*.
- **Sample Collection Date** —The date the sample was collected.
- **Sample Receipt Date** — The date your laboratory received the sample.
- **Condition** — The categorical group the sample belongs to.
- **Biological Replicate** — The biological replicate number.
- **Technical Replicate** — The technical replicate number.
- **Description** — The description of the sample.
- **Notes** — Additional information about the sample.
- **Created By** — The user who created the sample.
- **Created Time** — The date and time of the sample was created.
- **Last Modified By** — The user who last modified the sample.
- **Last Modified Time** — The date and time the sample was last modified.

- **ID** — The unique, internal identifier of the sample.
- **Plate ID** — The unique identifier of the plate.
- **Plate Name** — The descriptive name of the plate.
- **Edit** — Select to edit information about the selected sample. See *Edit sample information* (page 94).
- **Delete** — Select to delete the selected sample. See *Delete a sample from a plate* (page 94).

Add samples to an existing plate

> **NOTE**
>
> Adding samples to an existing plate follows a similar workflow to the add-a-plate workflow of analysis setup (described in *Add a plate* (page 29)). The difference is that in the add-a-plate workflow, you add a new plate and its samples to PAS, whereas you can also add samples to an existing plate, as described below.

1. On the sidebar menu, select **Plates** to open the **Plates** page.
2. Find the plate to which you want to add sample and select its **Add Sample** button to open the **Add Sample** dialog.
3. In the **MSDATA Files** section, you add one or more MS data files.
    a. Select one or more MS data files.
        - To add a single file, select **Files** to open the **Add Files** dialog.
        - To add multiple files, select **Folder** to open the **Add Folder** dialog.

        > **NOTE**
        >
        > Supported file formats are .raw, wiff, or .wiff.scan. Supported folders are RAW and D.

    b. Either drag the file or folder into the drag-and-drop area or use **Browse** to navigate to and select it.
    c. Select **Add**.
    d. Review the list of selected files.
        - To remove a file, select **Delete**.
        - To remove all files, select **Clear**.
    e. Select **Next** to advance.
4. In the **Plate Map File** section, you add a plate map file, which specifies the locations of samples.
    a. If you don't have a plate map file, create one before continuing.
        i. Select the on-screen link from which you can download an example plate map file (.csv).
        ii. Open the file and edit it as needed.

        The **MS file name**, **Sample ID**, and **Plate ID** columns are required. (See *Plate map file format* (page 23) for detailed descriptions of the columns in the file.)

        iii. Save as a .csv file.
    b. Select **Add File** or **Add** to open the **Add File** dialog.
    c. Either drag the file into the drag-and-drop area or use **Browse** to navigate to and select it.

d. Select **Add**.

e. Select **Next** to advance.

5. In the **Plate ID and Name** section, you link the MS data files to an existing plate.

   a. Select **Use Existing Plate**, and then select the applicable plate.

   b. Select **Next** to advance.

6. (Optional) In the **Sample Description File** section, upload metadata for each sample in the plate.

   NOTE

   To skip this optional part of the workflow, select **Next** to advance.

   a. Select **Add** or **Add File** to open the **Add Files** dialog.

   b. Either drag the file into the drag-and-drop area or use **Browse** to navigate to and select it.

   c. Select **Add**.

   d. Select **Next** to advance.

7. In the **Add to Project** section, you add the samples to a new or existing project.

   a. Create or select a project:

      - To create a new project, select **New Project**, enter a project name, and select **Add**.
      - To use an existing project, select it from the **Select Project** list.

   b. Select or clear the applicable checkboxes ☐. (To defer analysis, clear both checkboxes.)

      - **Analyze samples after addition** — Select to analyze samples.
      - **Analyze controls after addition** — Select to analyze controls.

   c. Select the MS method:

      - *DDA* — Derives an MS/MS spectra from selection, isolation, and fragmentation of an individual precursor ion.
      - *DIA* — Derives an MS/MS spectra from selection, isolation, and fragmentation of all precursor ions in a defined m/z range.
      - *PROTEOGENOMICS* — (Appears only for samples, not controls.) Identifies variant peptides arising from single nucleotide variants or short insertions and deletions.

   d. From the **Analysis Protocol** list (which shows only protocols compatible with the selected MS method), select a protocol.

   e. Select **Add Plate**, and then select **Close**.

      - If you had selected either or both **Analyze...** checkboxes earlier, the analysis starts immediately.
      - If you deferred analysis, you must start it manually. See *Start the analysis manually* (page 31).

   f. Select **Add Plate**, and then select **Close**.

      Depending on prior choices, the analysis begins immediately or you must start it manually. See *Start the analysis manually* (page 31).

Add sample descriptions

1. On the sidebar menu, select **Plates** to open the **Plates** page.
2. In the **Plates** table, select a plate to add sample descriptions to.
3. On the **Plate Samples** section's toolbar, select **Link to Sample Descriptions File** to open the **Upload File** dialog.
4. Either drag the file into the drag-and-drop area or use **Browse** to navigate to and select it.
5. Select **Upload**.

   The information from the uploaded file appears in the **Plate Samples** section of the **Plates** page.

Edit sample information

1. On the sidebar menu, select **Plates** to open the **Plates** page.
2. Select the plate for which you want to edit information about a sample.
3. In the **Plate Samples** table, find the sample you want to edit and select its **Edit** button to open the **Edit** window.
4. Edit the fields as needed.
5. Select **Confirm**.

Delete a sample from a plate

1. On the sidebar menu, select **Plates** to open the **Plates** page.
2. Select the plate from which you want to delete a sample.
3. In the **Plate Samples** table, find the sample you want to delete and select its **Delete** button.
4. Select **Yes** to confirm.

Plate Grid section

The **Plate Grid** section of the **Plates** page appears below the **Plate Samples** section. It shows the selected plate with yellow wells indicating the samples selected in the **Plate Samples** list.

The section offers two views of the same information. **Grid View** facilitates editing and deleting samples one well at a time, while **Table View** offers a more efficient means to work with samples in multiple wells. For detailed instructions, see *Edit or delete samples in wells* (below).

If you have created a custom .csv, .tsv, .xls, or .xlsx file containing sample information, you can upload it to the **Plates** page from the **Plate Grid** section. See *Upload a custom file containing sample information* (next page).

Edit or delete samples in wells

The **Plate Grid** section of the **Plates** page offers two views for editing samples in and deleting samples from wells. **Grid View** facilitates editing and deleting samples one well at a time, while **Table View** offers a more efficient means to work with samples in multiple wells.

To open the Plate Grid section:

1. On the sidebar menu, select **Plates** to open the **Plates** page.
2. Select the plate whose samples you want to edit or delete.
3. In the **Plate Samples** table, select the checkbox of each sample you want to work with.

   The **Plate Grid** appears below the **Plate Samples** table and shows the selected samples as yellow wells in a 96-well plate.
4. Edit or delete samples as described below.

Grid view

1. Select **Grid View** if it is not already selected.
2. To edit a sample in a well:
   a. Select a yellow well to open its details and then select its **Edit** button.
   b. Edit values in the editable fields. PAS saves each change as you work.
   c. When finished editing, select the **Edit** button again to save all the changes.
3. To delete a sample from a well:
   a. Select a yellow well to open its details and then select its **Delete** button.
   b. Select **Yes** to confirm.

Table view

1. Select **Table View**.
2. To edit samples in a well:
   - To edit an individual cell, enter a value, and then press **Enter**.
   - To apply the same value to all cells in a column, select the column heading's **Update** button, enter a value, and then select **Apply**.
   - To copy and paste a row, scroll all the way to the right and select **Copy**. Edit the copied row as needed and then select **Save Copy**.
3. To delete a sample from a well, select the well's **Delete** button. Select **Yes** to confirm.

Upload a custom file containing sample information

You can create a custom .csv, .tsv, .xls, or .xlsx file containing sample information and upload it from the **Plates** page. To create the file, match the columns in the file to the columns in the table view.

1. On the sidebar menu, select **Plates** to open the **Plates** page.
2. Select the applicable plate.
3. In the **Plate Samples** table, select the checkbox of at least one sample to open the **Plate Grid** sections.
4. Select **Upload File** to open the **Upload File** dialog.
5. Either drag the file into the drag-and-drop area or use **Browse** to navigate to and select it.

6. Select **Upload**.

   PAS populates the plate grid with imported sample information.

Projects

When you set up an analysis, PAS creates a corresponding project and adds it to the **Projects** page. The project lists all samples in an analysis with sample information. On the **Projects** page, you can add samples to the analysis and then start the analysis.

Projects page

Use the **Projects** page to manage projects, to manage the samples associated with a project, and to view the MS data files associated with a specific sample. To open this page, select **Projects** on the sidebar menu.

> TIP
>
> Use this page's **Collapse** and **Expand** buttons to selectively collapse and expand sections.

Toolbar items

- **Add Project** — Select to add a new project. See *Add a project* (next page).
- **<filters>** — Use these filters individually or together to filter the table to show only certain table items.
  - **Plate** — Show table items only for the selected plate.
  - **User** — Show table items for the only the selected user.
  - **Sample Type** — Show table items for only the selected sample type.
  - **Start Date - End Date** — Use to show table items whose dates fall within a specific period of time.
- **Batch delete** — (Appears when you select table items with **Multi-selection**, below.) Use to delete the selected table items as a batch. See *Delete multiple table items at the same time* (page 20).
- **Multi-selection** — Use to show or hide checkboxes in the table and to select or deselect table items on the current table page or all table pages.
- **Search** — Use to find a specific item. See *Find table items* (page 18).
- **Show/Hide** — Select or clear checkboxes to show or hide table columns. See *Show or hide table columns* (page 17).

  > TIP
  >
  > You can add custom columns to this table. See *Add custom table columns* (page 17).

Table columns

- **<checkboxes>** — Use to select or deselect individual table items. To select all items in the current page of the table, select the column header's checkbox.

  > NOTE
  >
  > Checkboxes are hidden by default on this page. To show the checkboxes, click **Multi-selection** > **Show checkboxes**.

- **Tenant ID** — The user identification.
- **Name** — The name of the project.

- **Description** — The description of the project.
- **Notes** — Additional information about the project.
- **Created By** — The user who created the project.
- **Created Date** — The date and time of project was created.
- **Last Modified By** — The user who last modified the project.
- **Last Modified Date** — The date and time the project was last modified.
- **ID** — The unique, internal identifier of the project.
- **Edit** — Select to edit the selected project's name, description, and/or notes.
- **Delete** — Select to delete the selected project (one that you originally added). See *Delete a project* (below). You cannot delete someone else's project (unless you are an Admin).

Other page sections

- **Sample List** — Shows a table of samples associated with the project selected in the **Projects** section. For detailed information about this section, see *Sample List section* (next page).
- **MS Data files** — Shows a read-only table of MS data files associated with the sample selected in the **Samples List** section. You can show or hide columns and can search for a specific data file.

Add a project

1. On the sidebar menu, select **Projects** to open the **Projects** page.
2. Select **Add Project** to open the **Create Project** dialog.
3. Complete the fields.
   - **Project Name** — Enter a unique name for the project.
   - **Select Plate** — Select a plate to add to the project.
   - **Add** — Select to add another plate to the project. Repeat as needed.
   - **Description** — Enter a description of the project.
   - **Notes** — Enter any additional information about the project.
4. Select **Save**.

Delete a project

You can delete any project that you originally created. Unless you are a PAS administrator, you cannot delete someone else's project.

1. On the sidebar menu, select **Projects** to open the **Projects** page.
2. Find the project you want to delete and select its **Delete** button.
3. Select **Yes** to confirm.

Sample List section

The **Sample List** section of the *Projects page* (page 96) appears below the project table. It shows a table of samples associated with the selected project. To open this section, select **Projects** on the sidebar menu and then select the project whose samples you want to manage.

Toolbar items

- **Add Sample** — Select to add samples to the project. See *Add samples to a project* (next page).
- **Analyze** — Select to select samples for analysis, as described in *Select samples or controls for analysis* (page 35).
- **Analyze Controls** — Select to select controls only for analysis. See *Analyze controls only* (page 36).
- **Search** — Use to find a specific item. See *Find table items* (page 18).
- **Show/Hide** — Select or clear checkboxes to show or hide table columns. See *Show or hide table columns* (page 17).

  TIP

  You can add custom columns to this table. See *Add custom table columns* (page 17).

Table columns

- **Tenant ID** — The user identification.
- **Name** — The descriptive name of a sample.
- **Sample/Control ID** — The unique identifier of a sample or control.
- **Well Location** — The location of a sample or control in the selected plate.
- **Type** — The type of sample: *Plasma* or *Peptide*.
- **Species** — The species a sample was collected from: *Human* or *Mouse*.
- **Sample Collection Date** —The date the sample was collected.
- **Sample Receipt Date** — The date your laboratory received the sample.
- **Condition** — The categorical group the sample belongs to.
- **Biological Replicate** — The biological replicate number.
- **Technical Replicate** — The technical replicate number.
- **Plate Name** — The descriptive name of the plate.
- **Description** — The description of the sample.
- **Notes** — Additional information about the sample.
- **Created By** — The user who created the sample.
- **Created Date** — The date and time of the sample was created.
- **Last Modified By** — The user who last modified the sample.
- **Last Modified Date** — The date and time a sample was last modified.
- **ID** — The unique, internal identifier of the sample.

Add samples to a project

1. On the sidebar menu, select **Projects** to open the **Projects** page.
2. Select a project to add samples to.
3. Above the **Sample List** table, select **Add Sample** to open the **Add Sample** dialog.
4. In the **Select Plate** field, select the plate to which you want to add samples.
5. Do either of the following:
    a. To add all samples to the selected plate, select **All Samples**.
    b. To add specific samples, select **Specific Samples**, select a sample from the list, and then select **Add**. Repeat to add more samples.
6. Select **Save**.

Analyses

When you set up an analysis, PAS adds the analysis to the Analyses page with general information about the analysis: the number of MS data files in the analysis, who last modified the analysis and when, and optional notes and descriptions.

An icon to the left of the analysis name indicates the status: **Started**, **Succeeded**, or **Failed**. You can open the analysis log by selecting an icon.

Analyses page

Use the **Analyses** page to manage analyses and to view and download analysis results and logs. To open this page, select **Analyses** on the sidebar menu.

> **TIP**
> Use this page's **Collapse** and **Expand** buttons to selectively collapse and expand sections.

Toolbar items

- **Project** — Select the project whose analysis data you want to work with.
- **Plate** — Select the plate whose analysis data you want to work with.
- **Controls/Samples** — Select whether to show controls or samples on the list.
- **Status** — Select the status of analyses that you're interested in, e.g., *Completed*.
- **Create a new folder** — Select to create a new folder into which you can organize analyses for the selected project. See *Create a folder and add analyses to it* (page 102).
- **Add to folder** — Select to add the selected analyses to an existing folder. See *Add analyses to a folder* (page 103).
- **Remove from folder** — Select to remove the selected analyses from the open folder. See *Remove analyses from a folder* (page 103).
- **Refresh** — Select to refresh the table.
- **Search** — Use to find a specific item. See *Find table items* (page 18).

- **Menu** — Select to open a menu of options (e.g., *Delete*) to use when multiple analyses are selected. See *Delete one or more analyses* (page 102).
- **Show/Hide** — Select or clear checkboxes to show or hide table columns. See *Show or hide table columns* (page 17).

> **TIP**
> You can add custom columns to this table. See *Add custom table columns* (page 17).

Table columns

- **<checkboxes>** — Use to select or deselect individual table items. To select all items in the current page of the table, select the column header's checkbox.
- **<status>** — The status of an analysis: **Started**, **Succeeded**, **Failed**.
- **Name** — The name of an analysis.
- **Description** — The description of the analysis.
- **Notes** — Additional information about the analysis.
- **Protocol** — The protocol used for the analysis.
- **MS Data Files** — The number of MS data files associated with the analysis.
- **Analyzed By** — The user who created the analysis.
- **Analysis Start** — The date and time the analysis started.
- **Analysis End** — The date and time the analysis ended.
- **Last Modified By** — The user who last modified the analysis or folder.
- **Last Modified Time** — The date and time the analysis or folder was last modified.
- **ID** — The unique, internal identifier of the analysis.
- **Edit** — Select to edit the selected analysis or folder. See *Edit an analysis* (next page) or *Edit the name of a folder of analyses* (page 102).
- **Delete** — Select to delete the selected analysis or folder. See *Delete one or more analyses* (page 102) or *Delete a folder of analyses* (page 104).

Download an analysis log

1. On the sidebar menu, select **Analyses** to open the **Analyses** page.
2. Find the analysis whose log you want to download and select its status icon (e.g., **Succeeded**).

   The **Analysis Log** dialog opens, listing all analysis events from oldest to newest.

   > **TIP**
   > To peruse the log on-screen, use the mouse wheel rather than clicking the scroll bar, as clicking will close the dialog.

3. Select **Download Log**.
4. Depending on your browser or browser preferences, the download may begin immediately, or you may

be prompted where to save the file. Follow any on-screen prompts.

PAS saves the downloaded file as a .txt file.

Download an analysis's protein groups and peptides results

You can download results for protein groups and peptides for a selected analysis. The results are organized into several files, each offering simplified data tables containing summarized results.

- **Peptide_NP.tsv** — Peptides, abundances, and other metadata across nanoparticles.
- **Peptide_Panel.tsv** — Peptides, abundances, and other metadata with nanoparticle values rolled up.
- **Protein_Group_NP.tsv** — Protein groups, abundances, and other metadata across nanoparticles.
- **Protein_Group_Panel.tsv** — Protein groups, abundances, and other metadata with nanoparticle values rolled up.

NOTE

For data shown at the panel level, PAS summarizes all nanoparticle values for a single protein into a single value. Specifically, a single protein may have been measured up to five times, once for each nanoparticle (ie.g., the NP level). To derive the single measurement (i.e., the panel level), PAS uses a maximum representation approach. Maximum representation works by evaluating all NP measurements for a single protein and selecting the largest measurement.

To download analysis results:

1. On the sidebar menu, select **Analyses** to open the **Analyses** page.
2. Select the analysis whose results you want to download.
3. Select **Protein Group / Peptide Results**, located to the right of the of the analysis's tabs.
4. Depending on your browser or browser preferences, the download may begin immediately, or you may be prompted where to save the file. Follow any on-screen prompts.

Edit an analysis

1. On the sidebar menu, select **Analyses** to open the **Analyses** page.
2. Find the analysis you want to edit and select its **Edit** button to open the **Edit Analysis** dialog.
3. Edit the fields as needed.
   - **Analysis Name** — Enter a name of the analysis.
   - **Description** — (Optional) Enter a description of the analysis.
   - **Notes** — (Optional) Enter additional information about the analysis.
4. Select **Save**.

Delete one or more analyses

To delete a single analysis:

1. On the sidebar menu, select **Analyses** to open the **Analyses** page.
2. Find the analysis you want to delete and select its **Delete** button.
3. Select **OK** to confirm.

To delete multiple analyses:

1. On the sidebar menu, select **Analyses** to open the **Analyses** page.
2. Select the checkbox of each analysis you want to delete.
3. Select **Menu > Delete**.
4. Select **Yes** to confirm.

Working with folders of analyses

On the **Analyses** page, you can organize analyses into folders, just as you would files on your computer. And just as with files, an individual analysis can be in only one folder at a time — or in no folder.

Create a folder and add analyses to it

1. On the sidebar menu, select **Analyses** to open the **Analyses** page.
2. Select **Create a new folder** to open the **Create Folder** dialog.
3. Edit the fields as needed.
   - **Folder Name** — Enter a unique name for the folder.
   - **Analyses** — From the list (which shows only those analyses that are not already in folders), select an analysis.
4. Select **Create**.

Edit the name of a folder of analyses

1. On the sidebar menu, select **Analyses** to open the **Analyses** page.
2. Find the folder you want to rename and select its **Edit** button to open the **Update Folder** dialog.
3. In the **Folder Name** field (the first field), edit the name.

   TIP

   You can also add analyses to or remove analyses from the folder in this dialog. See *Add analyses to a folder* (next page) and *Remove analyses from a folder* (next page).

4. Select **Update**.

Add analyses to a folder

To add analyses to a folder directly on the Analyses page:

1. On the sidebar menu, select **Analyses** to open the **Analyses** page.
2. Select the checkbox for each analysis you want to add to an existing folder.
3. Select **Add to folder** to open the **Move to Folder** dialog.
4. Select a folder from the list.
5. Select **Save**.

To add analyses while editing a folder

1. On the sidebar menu, select **Analyses** to open the **Analyses** page.
2. Find the folder you want to add analyses to and select its **Edit** button to open the **Update Folder** dialog.
3. From the second field's list (which shows only those analyses that are not already in folders), select an analysis. Repeat to select more.
4. Select **Update**.

Remove analyses from a folder

NOTE

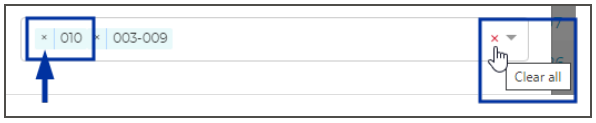
Removing an analysis from a folder does not delete it. It only takes it out of the folder. To delete an analysis, see *Delete one or more analyses* (previous page).

To remove analyses from a folder directly on the Analyses page:

1. On the sidebar menu, select **Analyses** to open the **Analyses** page.
2. Find and open the folder that contains the analyses you want to remove from the folder.
3. Select the checkbox for each analysis you want to remove from the folder.
4. Select **Remove from folder**.
5. Select **Confirm**.
6. To go back to the **Analyses** page, select ← **Back**.

To remove analyses while editing a folder:

1. On the sidebar menu, select **Analyses** to open the **Analyses** page.
2. Find the folder from which you want to remove analyses and select its **Edit** button to open the **Update Folder** dialog.
3. In the **Analyses** field (the second field):
   - Remove an individual analysis by selecting the x button to its left.
   - Remove all analyses by selecting the **Clear all** button to the left of the drop-down arrow.



4. Select **Update**.

Delete a folder of analyses

> NOTE
>
> Before you can delete a folder from the **Analyses** page, the folder must be empty. To remove analyses from it, see *Remove analyses from a folder* (previous page).

1. On the sidebar menu, select **Analyses** to open the **Analyses** page.
2. Find the folder that you want to delete and select its **Delete** button.
3. Select **OK** to confirm.

Data files

In the **Data Files** area of PAS, you can manage MS data files and VCF files.

MS data files

When you upload MS data files during analysis setup, PAS creates a folder to contain the files. On the *MS Files page* (below), you can customize any folder and manually create folders.

MS Files page

Use the **MS Files** page to create, move, and delete MS data files and folders and to download an application for automatic file updates. To open this page, expand **Data Files** on the sidebar menu and select **MS Files**.

Toolbar items

- **Link to Plate** — (Available when one or more files or folders are selected.) Select to link the selected files or folders to a plate when setting up an analysis. See *Link to a plate* (page 32).
- **New** — Select to add a new, empty folder.
- **Upload** — Select to upload data files. See *Upload MS data files* (next page).
- **Download data files** — Select to download one or more data file or folder. See *Download MS data files or folders* (page 106).
- **Refresh** — Select to refresh the table.
- **Start Date** — Set the earliest creation date for the files you want to view.
- **End Date** — Set the latest creation date for the files you want to view.
- **Last Uploaded** — Select a filter the list to show only those files created or uploaded within the last day (*1D*), last five days (*5D*), or last month (*1M*). To clear the active filter, select it again.
- **Menu** — Select to move, rename, or delete the selected files or folders. See *Move MS data files and folders* (page 106), *Rename MS data files and folders* (page 107), and *Delete MS data files and folders* (page 107).

- **AutoUploader** — Select to download the Seer AutoUploader application with which you can automatically transfer new MS data files to PAS. You may find it a more convenient means of uploading a large set of files rather than using the **Upload** button. See *Download and install the Seer AutoUploader application* (next page).

Table columns

- **<checkboxes>** — Use to select or deselect individual table items. To select all items in the current page of the table, select the column header's checkbox.
- **<folders/files>** — Initially shows the top level of folders into which data files are organized. Select a folder to view its contents. The column's heading shows the breadcrumb (path) to the folder whose contents are listed. To return to another folder in the path, select its link in the breadcrumb. To return to the top-level folders, select **Home**.
- **Filepath** — Shows the location where a file or folder is stored.
- **Date** — Shows the date the file or folder was added to PAS.
- **Time** — Shows the time the file or folder was added to PAS.
- **Size** — Shows the disk size of the file or folder.
- **Download** — (Not available for folders.) Select to download the selected file. See *Download MS data files or folders* (next page).
- **Delete** — Select to delete the selected file or folder. See *Delete MS data files and folders* (page 107).

Create a folder to hold MS data files

1. On the sidebar menu, expand **Data Files** and select **MS Files** to open the **MS Files** page.
2. Select **New** to open the **Create Folder** dialog.
3. Enter the name of the new folder.
4. Select **Create**.
5. To add MS data files to this new folder, follow the steps in *Move MS data files and folders* (next page).

Upload MS data files

TIP

You can also use the Seer AutoUploader to upload MS data files. See *Download and install the Seer AutoUploader application* (next page).

1. On the sidebar menu, expand **Data Files** and select **MS Files** to open the **MS Files** page.
2. Select **Upload** to open the **Add Raw MSDATA Files** dialog.
3. Select the MS data files.
   - To upload a single file, select **Add Files** to open the **Add Files** dialog.
   - To upload multiple files, select **Add Folder** to open the **Add Folder** dialog.
4. Either drag the file or folder into the drag-and-drop area or use **Browse** to navigate to and select it.
5. Select **Upload**.
6. Review the uploaded files.

- To remove a file, select its **Delete** button.
- To remove all files, select **Clear**.

Download and install the Seer AutoUploader application

The Seer AutoUploader is an application that runs on your MS computer to automatically upload new MS data files to your PAS account. You may find it a more convenient means of uploading a large set of files rather than using the **Upload** button on the **Data Files** page.

1. On the sidebar menu, expand **Data Files** and select **MS Files** to open the **MS Files** page.
2. Select **AutoUploader** to open the **Download AutoUploader** dialog.
   a. From the **Select file to download** list, select the version of the AutoUploader you want to download.
   b. In the **Save As** window, navigate to the folder in which you want to save the installation package and select **Save**.
   c. Select **Done**.
3. In your computer's file system, navigate to and double-click the installation package to launch it. Follow the prompts to install the software.

Download MS data files or folders

1. On the sidebar menu, expand **Data Files** and select **MS Files** to open the **MS Files** page.
2. If you want to download a folder that is listed at the top-level of the page, go to the next step. Otherwise, navigate into the folder that holds the files and/or folders you want to download.
3. Find the files and/or folders you want to download and do one of the following:
   - To download an individual file, select its **Download** button.
   - To download an individual folder, select its checkbox and then select **Download data files** on the toolbar.
   - To download multiple files and/or folders, select the checkbox of each and then select **Download data files** on the toolbar.
4. Depending on your browser or browser preferences, the download may begin immediately, or you may be prompted where to save the file. Follow any on-screen prompts.

Move MS data files and folders

1. On the sidebar menu, expand **Data Files** and select **MS Files** to open the **MS Files** page.
2. If the folder (or folders) you want to move is listed at the top-level of the page, go to the next step. Otherwise, navigate into the folder that holds the files and/or folders you want to move.
3. Select the checkbox of each file and/or folder you want to move.
4. Select **Menu > Move**.
5. Select the destination folder, and then select **Move Here**.
6. Select **OK** to confirm.

Rename MS data files and folders

1. On the sidebar menu, expand **Data Files** and select **MS Files** to open the **MS Files** page.
2. To rename a folder listed at the top-level of the page, go to the next step. Otherwise, navigate into the folder that holds the files and/or folders you want to rename.
3. Select the checkbox of each file and/or folder you want to rename.
4. Select **Menu > Rename** to open the **Rename** dialog.
5. For each file and/or folder you selected, enter its new name.
6. Select **Save.**
7. Select **OK** to confirm.

Delete MS data files and folders

1. On the sidebar menu, expand **Data Files** and select **MS Files** to open the **MS Files** page.
2. If you want to delete a folder that is listed at the top-level of the page, go to the next step. Otherwise, navigate into the folder that holds the files and/or folders you want to delete.
3. Find the files and/or folders you want to delete and do one of the following:
   - To delete an individual file or folder, select its **Delete** button.
   - To delete multiple files and/or folders, select the checkbox of each and then select **Menu > Delete**.
4. Select **OK** to confirm.

VCF files

You can upload custom or sample-specific variant call files (VCF) to PAS. These files are used to identify genetic variants that may result in amino acid variants (i.e., variant peptides) not captured in the canonical reference database. After uploading the VCF files, you can associate them with samples for which you plan to use the Proteogenomics workflow for analysis.

VCF Files page

Use the **VCF Files** page to manage the associations of samples with variant call files (VCF) that you upload to PAS. Each table item represents the association of one sample with one or more VCF files. When you use a Proteogenomics analysis protocol to analyze samples, PAS shows these associations in a list.

To open this page, expand **Data Files** on the sidebar menu and select **VCF Files**.

Toolbar items

- **Add Sample VCF** — Use to associate one or more VCF files with a sample. See *Associate one or more VCF files with a sample* (next page).
- **Batch Upload** — Use to upload multiple VCF files to PAS, making them available for association with samples. See *Upload a batch of VCF files to PAS* (next page).

Table columns

- **Add VCF** — Select to associate additional VCF files with a sample for which at least one VCF file is already associated. See *Associate one or more VCF files with a sample* (below).
- **Sample Name** — The name of a sample with which one or more VCF files are associated.
  - To remove the association of the VCF file(s) to this sample, select **Delete Sample** immediately to the right of the sample name. See *Remove the association of a sample to VCF files* (next page).
- **VCF File Name** — The file names of the .vcf or .vcf.gz files associated with the sample. The primary VCF file for the sample is indicated by the selected checkbox. See *Associate one or more VCF files with a sample* (below).
- **Uploaded By** — The name of the user who uploaded a VCF file.
- **Time** — The date and time a VCF file was uploaded.
- **Delete VCF Files** — Select to delete the selected VCF file from PAS. See *Delete VCF files from PAS* (next page).

Upload a batch of VCF files to PAS

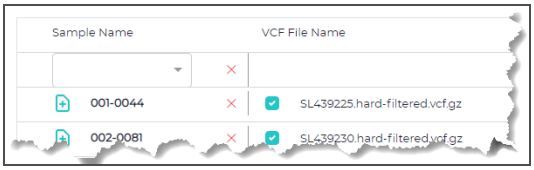
1. On the sidebar menu, expand **Data Files** and select **VCF Files** to open the **VCF Files** page.
2. Select **Batch Upload** to open the **VCF Batch Upload** dialog.
3. Click **Browse** to open the **Upload VCF File** dialog
4. Either drag the file or folder into the drag-and-drop area or use **Browse** to navigate to and select it.
5. Select **Upload**.
6. Select the option that describes where to get the relationship between the sample name and the VCF file path:
   - To use the name of each selected VCF file as the name of a sample (e.g., `sample1` will become `sample1.vcf`), select *Sample Name is part of VCF file path*.
   - To use a CVS file that contains the relationships (e.g., `Sample Name 1` will become `Example1.vcf`), select *A CVS file* and then browse to and select that file.
7. Select **Add**.

   PAS adds a new table row for each VCF file that you uploaded.

Associate one or more VCF files with a sample

1. On the sidebar menu, expand **Data Files** and select **VCF Files** to open the **VCF Files** page.
2. Do either of the following:
   - To add more VCF files to a sample already listed, locate the sample, and then skip ahead to step 3.
   - To add a new association of a sample with VCF files:
     a. Select **Add Sample VCF** on the toolbar.

        A new table item is added, with a field in the **Sample Name** column.



b. In the field, enter the name of the sample with which you want to associate VCF files.

> NOTE
> Technically, you can also select an existing sample here. Once you add VCF files for it, PAS will update the existing sample with the additional VCF files.

3. Select **Add VCF** to open the **Add VCF File** dialog.
   a. Either drag the file or folder into the drag-and-drop area or use **Browse** to navigate to and select it.
   b. Select **Upload**.
   c. Select **Add**.
4. If you associated multiple VCF files with the sample, select the checkbox of one to serve as the primary VCF file.

Remove the association of a sample to VCF files

1. On the sidebar menu, expand **Data Files** and select **VCF Files** to open the **VCF Files** page.
2. Find the sample for which you want to remove its association to VCF files.
3. Select **Delete Sample** to the right of the sample name.

> NOTE
> You will not be deleting the sample itself. Rather, you are removing the association of the sample to VCF files.

4. Select **OK** to confirm.

Delete VCF files from PAS

> NOTE
> VCF files that are associated with a sample being used by a Proteogenomics analysis protocol cannot be deleted from PAS.

1. On the sidebar menu, expand **Data Files** and select **VCF Files** to open the **VCF Files** page.
2. Find the sample with which the VCF file is associated.
3. To delete the VCF file from PAS, select its **Delete VCF File** (at the far right of its row).
4. Select **OK** to confirm.

Analysis protocols

An analysis protocol specifies search parameters for the MS database search engine. PAS includes several pre-installed analysis protocols, identified on the *Protocols page* (below) by the ☆ **star/pre-installed** icon.

You can create custom protocols by copying an existing protocol or by uploading a protocol.

PROTOCOL	DESCRIPTION
MSFragger[1, 2]	Used for Proteogenomics-based analyses. Includes Human Genome Reference build hg38 FASTA file and gene model/gene boundary coordinates BED file.
MaxQuant[3]	Used for DDA-based analyses.
EncyclopeDIA[4] or DIA-NN[5]	Used for DIA-based analyses.

[1]Kong, Andy T., Leprevost, Felipe V., Avtonomov, Dmitry M., Mellacheruyu Dattatreya, and Nesvishskii, Alexey I. MSFragger: ultrafast and comprehensive peptide identification in mass spectrometry–based proteomics. 14 *Nature Methods* (April 2017): 513-520, doi:10.1038/nmeth.4256.

[2]MSFragger ©2016 The Regents of the University of Michigan.

[3]Cox, Jürgen, and Matthias Mann, "MaxQuant enables high peptide identification rates, individualized p.p.b.-range mass accuracies and proteome-wide protein quantification," 26 *Nature Biotechnology* (November 2008): 1367–1372, doi.org/10.1038/nbt.1511.

[4]Searle, Brian C., Lindsay K. Pino, Jarrett D. Egertson, Ying S. Ting, Robert T. Lawrence, Brendan X. MacLean, Judit Villén, et al., "Chromatogram libraries improve peptide detection and quantification by data independent acquisition mass spectrometry," 9 *Nature Communications* (December 2018): 5218, doi.org/10.1038/s41467-018-07454-w.

[5]Demichev, V., Messner, C.B., Vernardis, S.I. et al., "DIA-NN: neural networks and interference correction enable deep proteome coverage in high throughput." 17 *Nat Methods* (January 2020): 41–44, doi.org/10.1038/s41592-019-0638-x.

Protocols page

Use the **Protocols** page to manage analysis protocols and to view analysis parameters. To open this page, select **Analysis Protocol** on the sidebar menu.

Toolbar items

- **Copy** — (Shown once you select a protocol.) Select to copy a custom (user-defined) or pre-installed protocol. See *Copy an analysis protocol* (page 112).
- **Upload** — Select to upload a protocol. See *Upload a custom analysis protocol* (next page).
- **Batch delete** — (Appears when you select table items with **Multi-selection**, below.) Use to delete the selected table items as a batch. See *Delete multiple table items at the same time* (page 20).
- **Multi-selection** — Use to show or hide checkboxes in the table and to select or deselect table items on the current table page or all table pages.
- **Search** — Use to find a specific item. See *Find table items* (page 18).
- **Show/Hide** — Select or clear checkboxes to show or hide table columns. See *Show or hide table columns* (page 17).

> **TIP**
> You can add custom columns to this table. See *Add custom table columns* (page 17).

Table columns

- **<checkboxes>** — Use to select or deselect individual table items. To select all items in the current page of the table, select the column header's checkbox.

  > **NOTE**
  > Checkboxes are hidden by default on this page. To show the checkboxes, click **Multi-selection > Show checkboxes**.

- **Name** — The name of the protocol.
- **Type** — The type of protocol, e.g., *DDA*, *Proteogenomics*.
- **Species** — Whether the analyzed sample is from a human or mouse.
- **Version** — The version of the protocol, useful if you want to keep track of different versions (updates) of protocols.
- **Description** — The description of the protocol.
- **Notes** — Additional information about the protocol.
- **Created By** — The user who created the protocol.
- **Created Time** — The date and time the protocol was created.
- **Parameters** — The name of the file in which the search engine parameters are defined. To view the search engine parameters for a protocol, select its row. See *View an analysis protocol's search engine parameters* (below).
- **Preinstalled** — Shows which protocols are pre-installed (indicated by **star/pre-installed**). You may copy but not delete pre-installed protocols.
- **ID** — The unique, internal identifier of the protocol.
- **Download** — Select to download a protocol. See *Download an analysis protocol* (next page).
- **Delete** — Select to delete selected protocol. See *Delete analysis protocols* (page 113).

View an analysis protocol's search engine parameters

1. On the sidebar menu, select **Analysis Protocol** to open the **Protocols** page.
2. Select the analysis protocol whose search engine parameters you want to view.

   A panel opens to the right of the table to display the selected protocol's parameters, which are read in from the protocol's parameters file.

Upload a custom analysis protocol

You can upload custom analysis protocols to PAS. For a DDA-based analysis, an .xml file is required. For a DIA- or Proteogenomics-based analysis, a .json file is required.

1. On the sidebar menu, select **Analysis Protocol** to open the **Protocols** page.
2. Select **Upload** to open the **Analysis Protocol** dialog.

3. Complete the fields.
   - **Name** — Enter a unique name for the protocol.
   - **Analysis Type** — Select the MS method.
     - *DDA* — Derives an MS/MS spectra from selection, isolation, and fragmentation of an individual precursor ion.
     - *DIA* — Derives an MS/MS spectra from selection, isolation, and fragmentation of all precursor ions in a defined m/z range.
     - *PROTEOGENOMICS* — Identifies variant peptides arising from single nucleotide variants or short insertions and deletions.
   - **Analysis Engine** — Select an analysis engine, e.g., *Max Quant*. For information about the analysis engines supported by PAS, see *Analysis protocols* (page 110).
   - **Species** — Select the species to analyze: *Human* or *Mouse*.
   - **Description** — Enter a description for the protocol.
   - **Notes** — Enter any additional information about the protocol.
4. When ready to upload the protocol:
   a. Select **Upload** to open the **Upload File** dialog.
   b. Drag-and-drop the file or select **Browse** to navigate to and select the file.
   c. Select **Upload**.
5. On the **Analysis Protocol** dialog, select **Save**.

Copy an analysis protocol

You may copy both custom (user-defined) and pre-installed protocols.

1. On the sidebar menu, select **Analysis Protocol** to open the **Protocols** page.
2. Select the protocol you want to copy and select **Copy**.

   TIP

   Be sure to copy a protocol of the correct type (e.g., *DDA*) because you cannot later change the analysis type in the copied protocol.

3. In the **Edit Protocol** dialog, edit fields for the new protocol.

   NOTE

   The search engine parameter fields vary, depending on which search engine is being used, e.g., *MaxQuant*.

4. Select **Save**.

Download an analysis protocol

You may download both custom (user-defined) and pre-installed protocols.

1. On the sidebar menu, select **Analysis Protocol** to open the **Protocols** page.
2. Find the protocol you want to download and select its **Download** button. (You may need to scroll to the far right of the table to see this button.
3. Depending on your browser or browser preferences, the download may begin immediately, or you may be prompted where to save the file. Follow any on-screen prompts.

Download FASTA files

1. On the sidebar menu, select **Analysis Protocol** to open the **Protocols** page.
2. Select the analysis protocol whose search engine parameters you want to view.
3. In the panel at the right, select **Download** beside *FASTA File*.
4. Depending on your browser or browser preferences, the download may begin immediately, or you may be prompted where to save the file. Follow any on-screen prompts.

Delete analysis protocols

You may not delete pre-installed protocols (indicated by the **star/pre-installed** icon).

To delete a single protocol:

1. On the sidebar menu, select **Analysis Protocol** to open the **Protocols** page.
2. Find the protocol you want to delete and select its **Delete** button.
3. Select **OK** to confirm.

To delete multiple protocols:

1. On the sidebar menu, select **Analysis Protocol** to open the **Protocols** page.
2. Select **Multi-selection**, show checkboxes, and select the protocols you want to delete. (Use the techniques described in *Delete multiple table items at the same time* (page 20).)
3. Select **Batch delete**
4. Select **Delete** to confirm.

Glossary

A

Admin
Role that allows adding plates, creating projects, creating analysis protocols, viewing MS data files, viewing results files, and adding and deleting users.

analysis
A search for identification and annotation of LC-MS data.

Analysis Metrics
Tab that provides assay, sample, and performance metrics for an analysis result.

analysis protocol
The parameters for an MS database search in a .xml or .json file.

Analysis Summary
Tab that provides an overall view of analysis results.

annotation
A highlight or explanatory note added to a chart.

C

control limits
Parameters that help determine whether results are expected. The mean provides a historical average, and the upper and lower control limits indicate normal variation.

custom file
Optional sample information in a .csv, .tsv, .xls, or .xlsx file.

CV
Coefficient of variation.

D

data-dependent acquisition
MS method that derives an MS/MS spectra from selection, isolation, and fragmentation of an individual precursor ion.

data-independent acquisition
MS method that derives an MS/MS spectra from selection, isolation, and fragmentation of all precursor ions in a defined m/z range.

DDA
Data-dependent acquisition.

DIA
Data-independent acquisition.

Digestion Control
A reference sample added before nanoparticle incubation.

distribution of detected proteins in plasma proteome
The dynamic range of proteins identified in each sample compared to a deep reported human plasma proteome index.

F

FAS
Field application scientist.

H

hierarchical clustering
Cluster analysis based on agglomerative nesting, which groups samples in clusters based on similarity.

I

ICS
Proteograph Instrument Control Software.

intensities
Protein and peptide intensities and the distribution of protein sequence coverage for each sample, including the coefficient of variation.

L

lamppost protein concentration
The intensity of landmark proteins in each sample.

LC
Liquid chromatography.

LC-MS
Liquid chromatography mass spectrometry.

LCL
Lower control limit.

M

Mass Spec Control
Reference peptides added before LC-MS analysis.

MS
Mass spectrometry.

MS data file
The results of MS analysis for each sample or control in a plate in a .raw, .wiff, .wiff.scan file.

N

NP
Nanoparticle.

P

PAS
Proteograph Analysis Suite.

PC1
Principal component 1.

PC2
Principal component 2.

PCA
Principal component analysis.

PCA analysis
Clusters of similar samples based on PC1 and PC scores.

PCC
Pearson correlation coefficient.

Pearson correlation coefficient
A measure of the linear correlation of data.

peptide counts
The number of peptides in each sample.

peptide counts distribution
Peptide counts for the five nanoparticles processed with the samples.

peptide counts of nanoparticles
Sample counts plotted by nanoparticle.

plate
The samples, controls, and nanoparticles from a Proteograph Assay method run.

plate map file
The location of each sample in a plate in a .csv file. Used when analyzing MS data in PAS and for automated peptide reconstitution on the SP100.

plate map grid
Metrics for each sample in the format of 96-well plates.

Plate Samples
Table listing all samples in a plate selected on the Plates page.

Plates
Table on the Plates page listing all plates in PAS.

Process Control
A reference sample added before nanoparticle incubation.

project
All samples in an analysis with sample information.

Projects
Table on the Projects page listing all projects in PAS.

protein group counts
The number of proteins in each sample.

protein group counts distribution
Protein group counts for the five nanoparticles processed with the samples.

protein group overlap sets
Protein group intersections, including intersection size and protein group counts.

proteogenomics
Incorporation of genomic information with proteomic data analysis to identify variant peptides not captured in canonical reference protein databases.

Proteograph Analysis Suite
Seer software used to process, analyze, and visualize LC-MS data.

Proteograph Assay Kit
A Seer kit containing the reagents and labware for preparing samples on the SP100.

Proteograph Product Suite
The bundle of Seer kits, instrument, and analysis software.

Protocols
Table on the Protocols page listing all analysis protocols in PAS.

Q

QC
Quality control.

QC charts
Charts presenting quality control data and metrics for an analysis.

QC metrics
Metrics indicating how well an analysis performed.

R

report
A summary of control results in a .pdf file.

results file
The output of an analysis in .txt or .xml file format.

S

sample comparability
The degree of statistical correlation between samples based on the Pearson correlation coefficient or Jaccard index.

sample description file
Metadata for each sample in plate in a .csv file.

Sample list
List of all samples in a project selected on the Projects page.

Seer AutoUploader
An application that runs on the MS computer and automatically transfers new MS data files to PAS.

SP100 Automation Instrument
The Seer liquid handling instrument.

summary
A list of each QC metric with the control, well, plate, value, and result in a .csv file.

U

UCL
Upper control limit.

User
Role that allows adding plates, creating projects, creating analysis protocols, viewing MS data files, and viewing results files.

user group
A group of users that can access and view the plates, projects, and analyses of all other users in the group.

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Y

Technical Support

For technical assistance, contact your field service representative or Seer support.

Contact Information

Seer, Inc.
3800 Bridge Pkwy #102
Redwood City, CA 94065
Email: support@seer.bio

seer.bio

Telephone

833.254.7337 (US only)

Seer, Inc.
3800 Bridge Pkwy #102
Redwood City, CA 94065
650.453.0000
support@seer.bio
seer.bio

seer™