

ProteinLynx Global Server Tutorial

This tutorial will cover basic features available in the PLGS for creating a project, setting up workflow and processing parameters, creating a database, processing of raw data acquired using MassLynx, and protein identification.

Getting Started

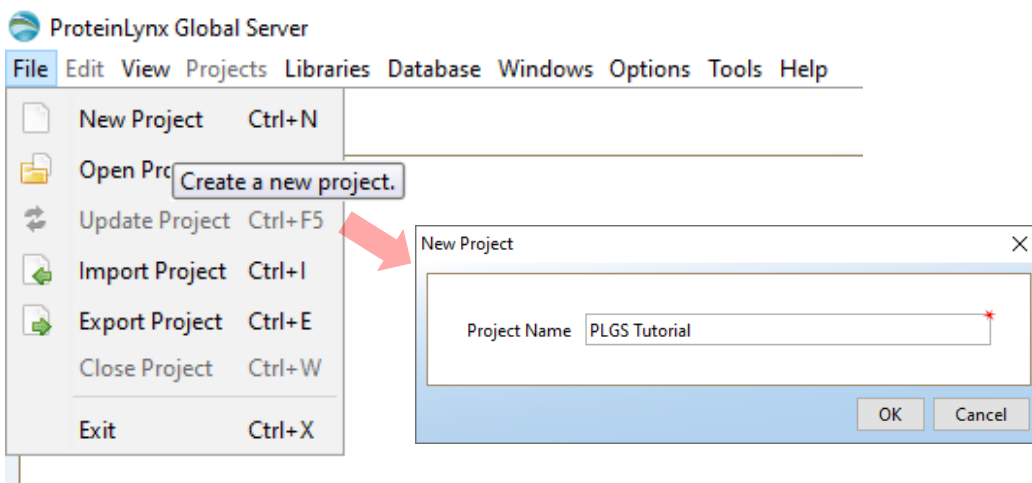
To start this tutorial, please download “PLGS Tutorial” folder and its content into your desktop.

Now start PLGS 3.0.3, and you will be presented with a new empty document. Before you import and process data, you must set up a project that is ready to accept raw data.

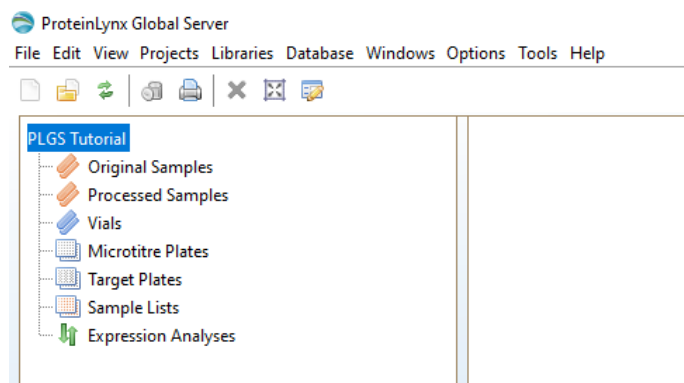
Setting up projects

You can build your project in PLGS by performing the following steps:

- On the File menu, click New Project.
- Give a name to your project.



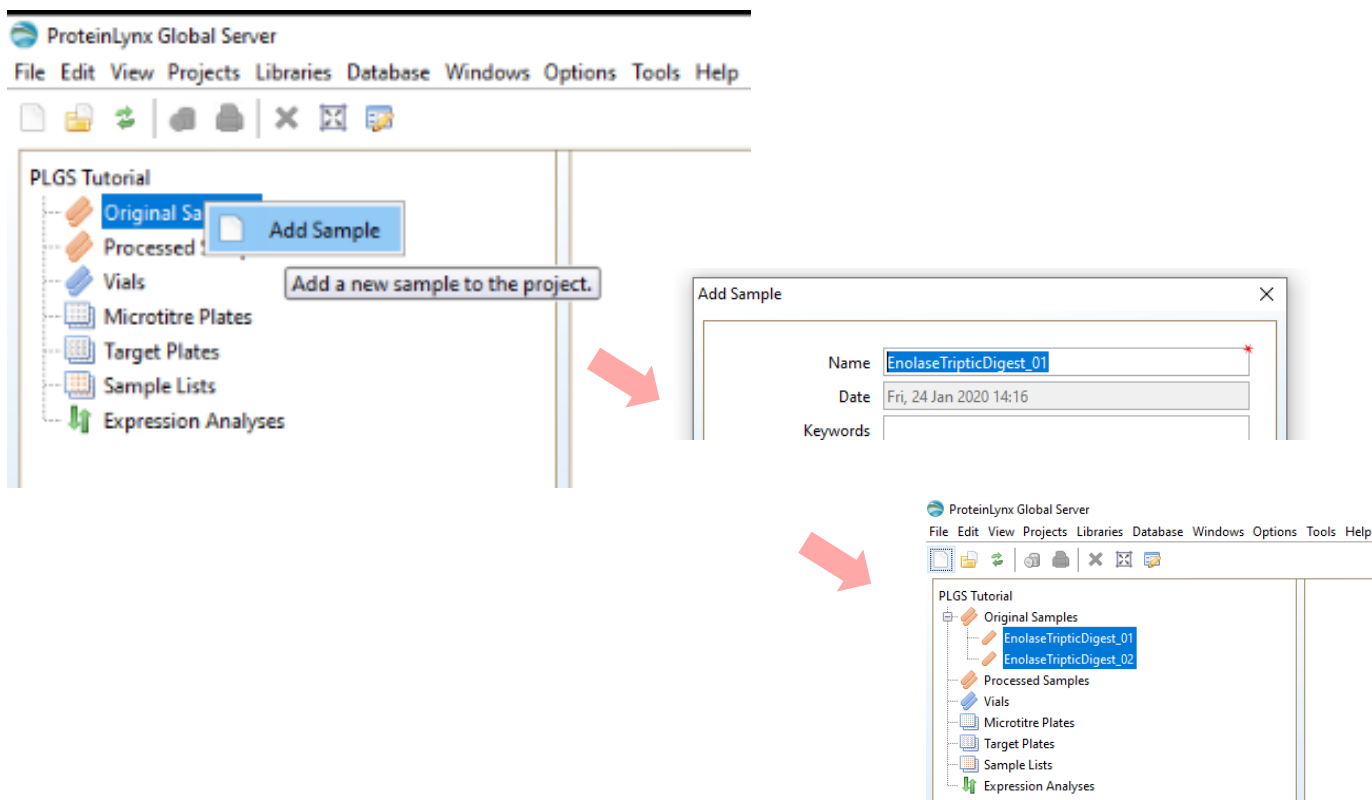
- In the Project Name field, enter “PLGS Tutorial”
- You should see that PLGS has added the newly created project to the document (top left corner)



Creating Samples

Samples must be added into the analysis before an expression analysis experiment can be performed.

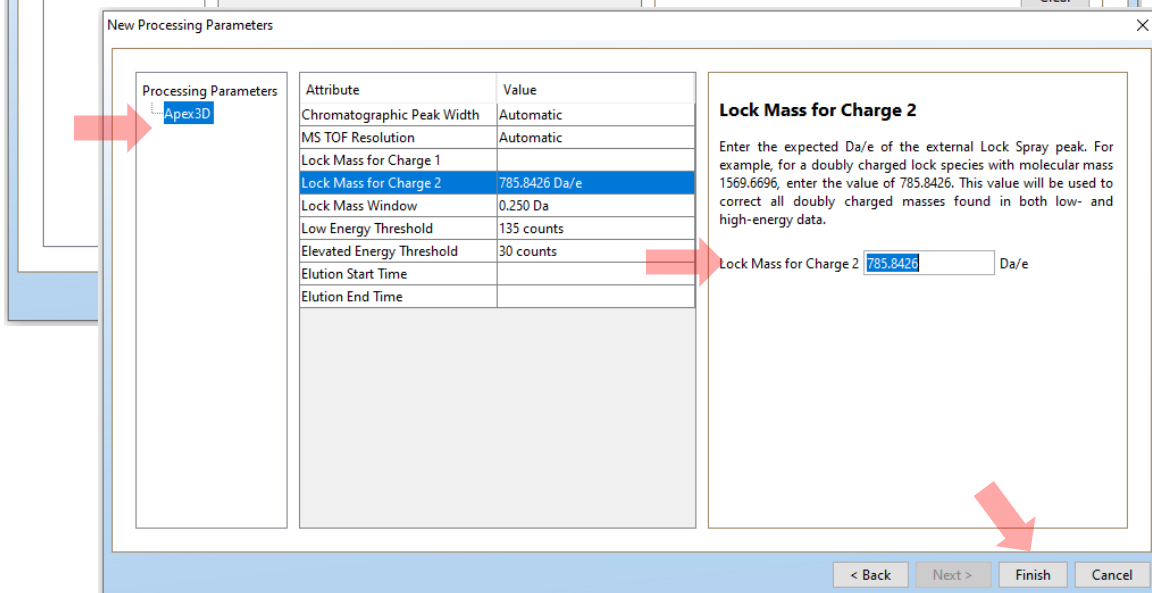
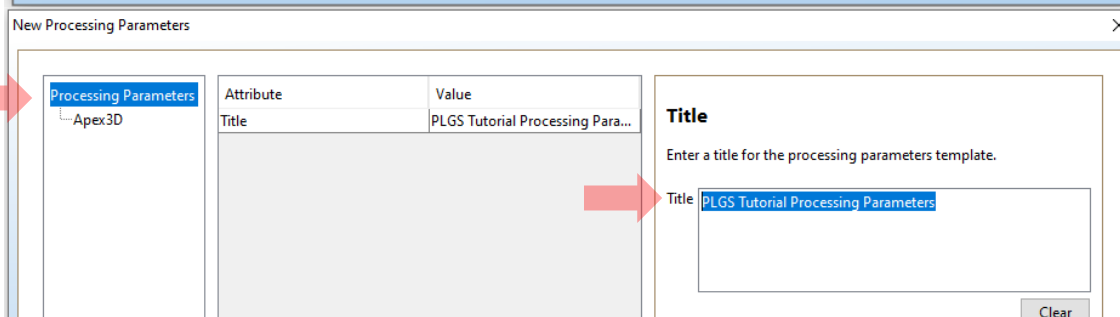
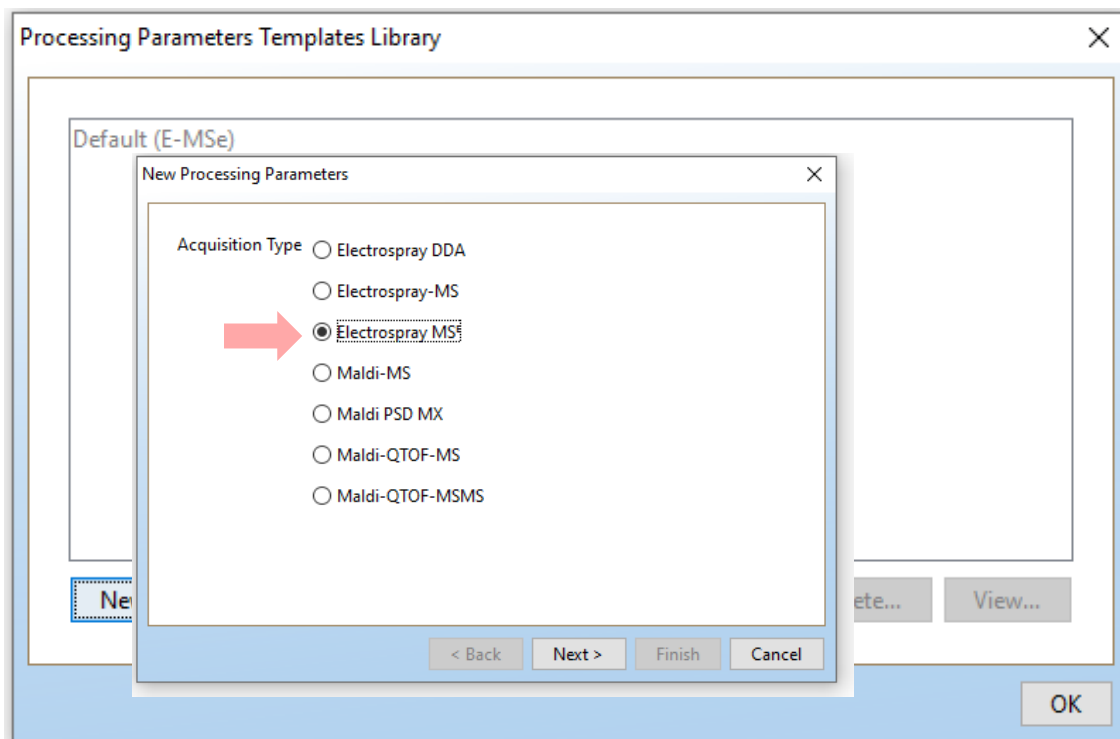
- In the tree, click Original Samples, and right click
- Click Add Sample
- Type a sample name in the Name Field and click OK.
- Repeat this process to specify a name/s for next sample/s



Once you create samples you need specify processing parameters for each sample. These parameters determine how the raw data are processed. In this tutorial we will be creating one processing parameter template for all the samples.

Creating Processing Parameters Template

- On the Libraries menu, click Processing Parameters.
- Processing Parameters > New
- Click Electrospray MS^E > Next
- Click Processing parameters in the dialog box > Type " PLGS Tutorial Processing Parameters" in the title field
- Click Apex 3D in the dialog box > Lock mass for Charge 2 and Type 785.8426 da/e in the lock mass charge field as can be seen in the following screen shots. (This is the +2 charge state of the lock mass, Glu Fibrinopeptide)
- Click Finish



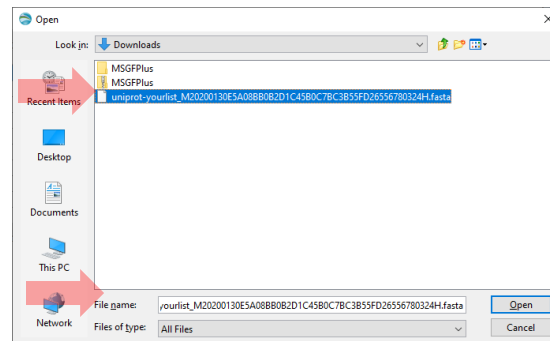
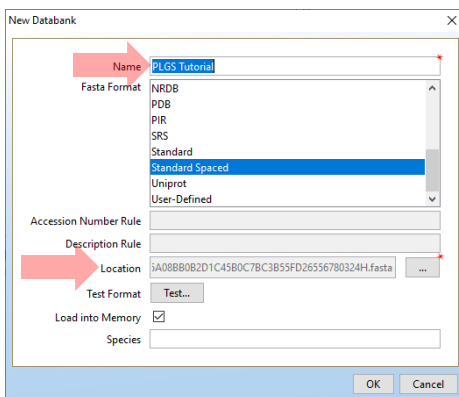
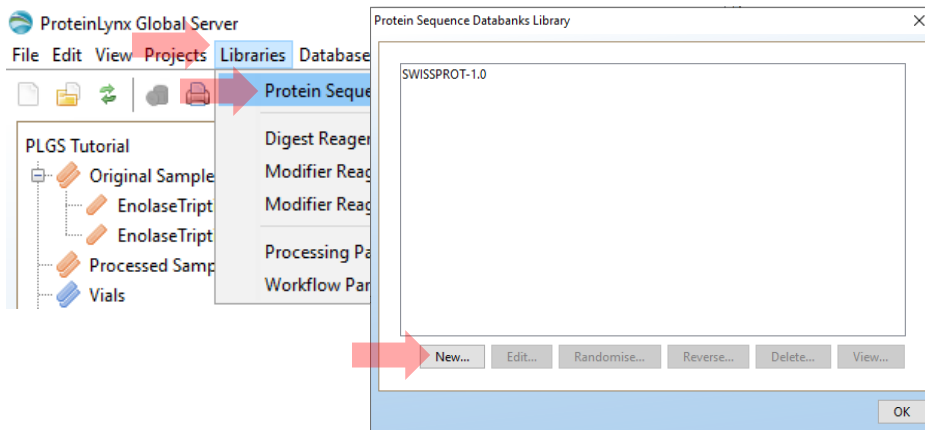
Hint: You may consider changing other settings in the processing parameter template to ensure that data processing meets your requirements.

Database Setup

For this tutorial, we will be using a trypsin digest of Enolase (P00924), ENO1_YEAST (Mass Prep Digest standard from Waters). Therefore we will have to download the appropriate FASTA file (i.e. amino acid sequence in FASTA file format) from UniProt.org.

Hint: You may enter the protein accession number if you know the protein/s that you are working with. If not you may have to download the entire proteome of the species.

- Go to UniProt.org
- Input P00924 in the search bar and click search
- Click add to basket
- Go to basket and download the file in FASTA (canonical) format into PLGS Tutorial folder.
- On the PLGS menu, open Libraries and then click "Protein Sequence Databanks"
- Click New> Type "PLGS_Tutorial" in the Name field and locate the Downloaded FASTA file
- Then Click OK.

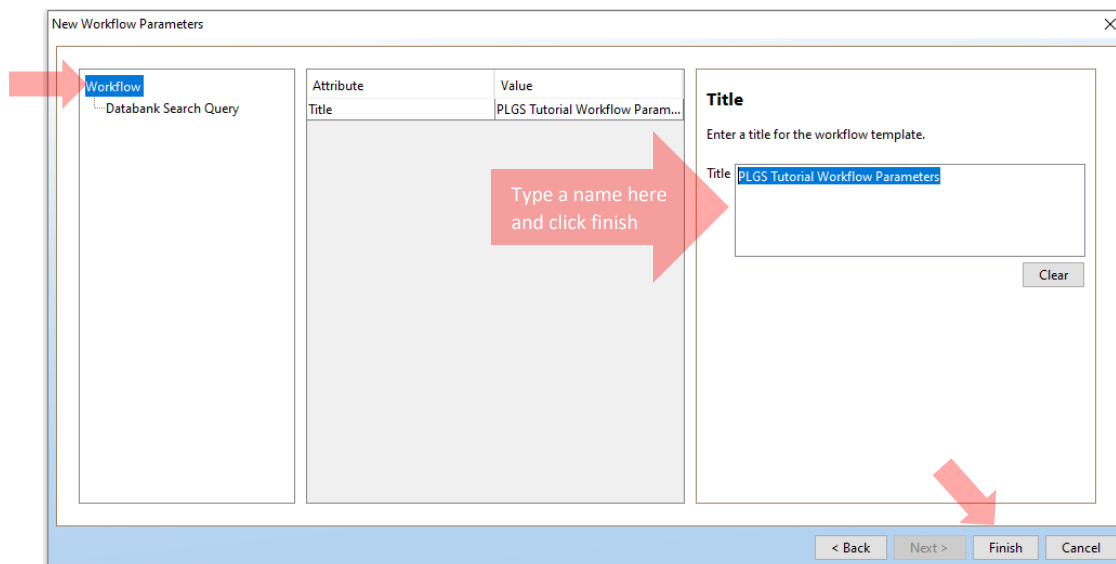
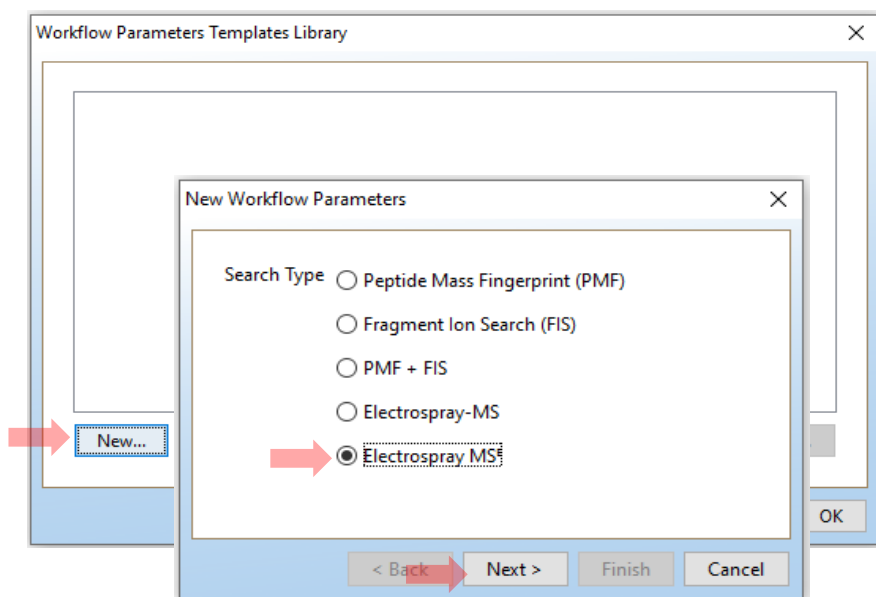


Creating workflow Template

The workflow defines the protein sequence database and defines the parameters (i.e., missed cleavages, modifications, FDR) for ion accounting algorithm.

- On the Libraries menu, click Workflow Parameters.
- Workflow parameters > New
- Click Electrospray MS^E > Next
- Click Workflow in the dialog box > Type “ PLGS Tutorial workflow Parameters” in the title field
- Click Finish
- In the navigator tree, click “Databank Search Query” > Databank > PLGS_Tutorial
- In this section you may consider changing parameters such as your modifiers, Primary digest reagent, FDR, and missed cleavages etc.
- Click “Finish” to close the dialog box and click “OK” to exit from workflow parameter setup.

Hint: In this tutorial we will be using a single FASTA file to create a database. These databases contain information about the protein sequences of amino acids. You may download multiple FASTA files and combine them into a single database depending your needs.



New Workflow Parameters

Workflow

Databank Search Query

Attribute	Value
Search Engine Type	PLGS
Databank	PLGS_Tutorial-1.0
Taxonomy	
Peptide Tolerance	Automatic
Fragment Tolerance	Automatic
Min Fragment Ion Matches p...	3
Min Fragment Ion Matches p...	7
Min Peptide Matches Per Pro...	1
Maximum Protein Groups to ...	20
Maximum Protein Mass	250000
Primary Digest Reagent	Trypsin
Secondary Digest Reagent	None
Missed Cleavages	1
Fixed Modifier Reagents	Carbamidomethyl C
Fixed Modifier Reagent Groups	
Variable Modifier Reagents	Oxidation M
Variable Modifier Reagent Gr...	
Enriched Variable Modification	
Variable Glycosylation Modifi...	

Databank

Select the databank to search from the list.

Databank

- SWISSPROT-1.0
- PLGS_Tutorial-1.0

< Back Next > Finish Cancel

Workflow Parameters Templates Library

- PLGS Tutorial Workflow Parameters

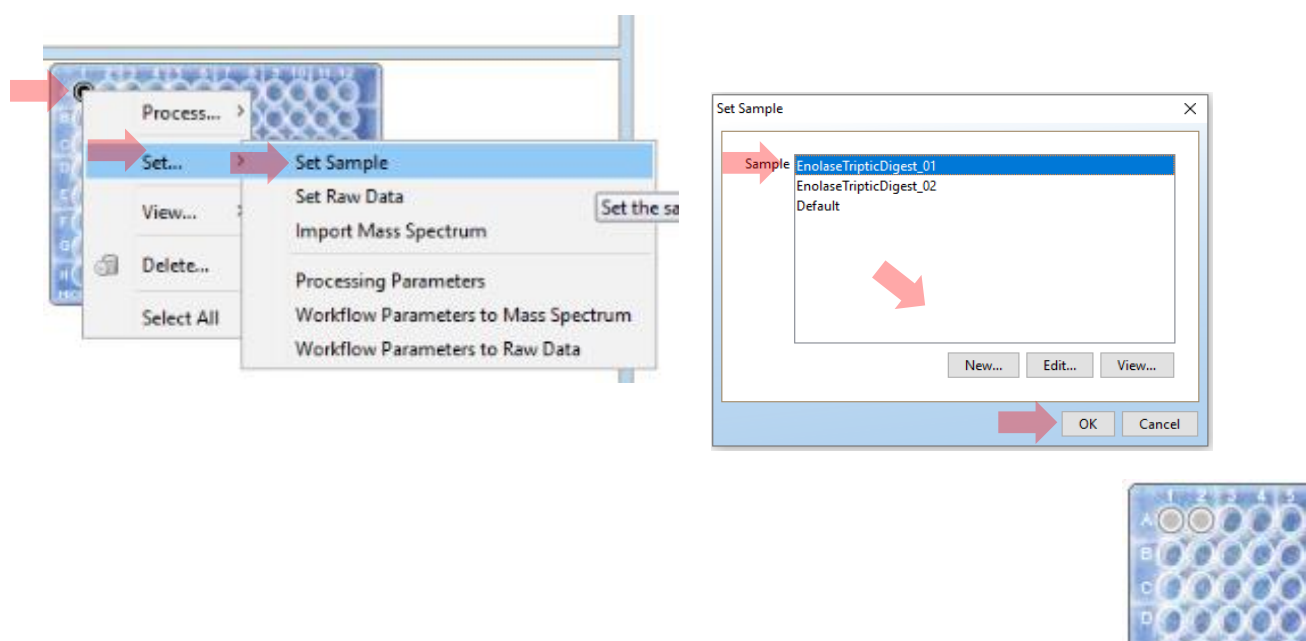
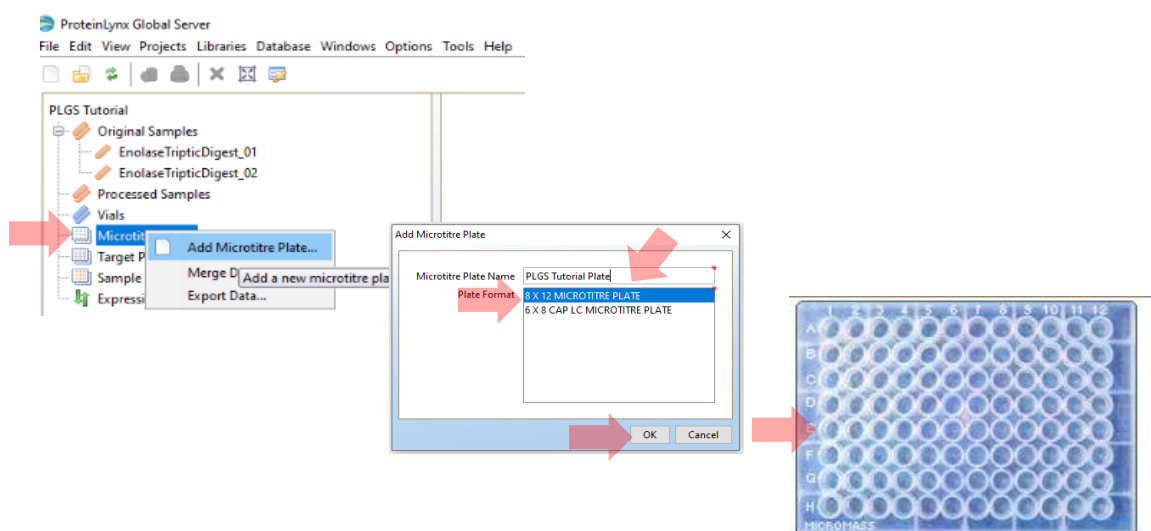
New... Edit... Copy... Import... Export... Delete... View...

OK

Specifying Samples into Plates, Vials, and targets

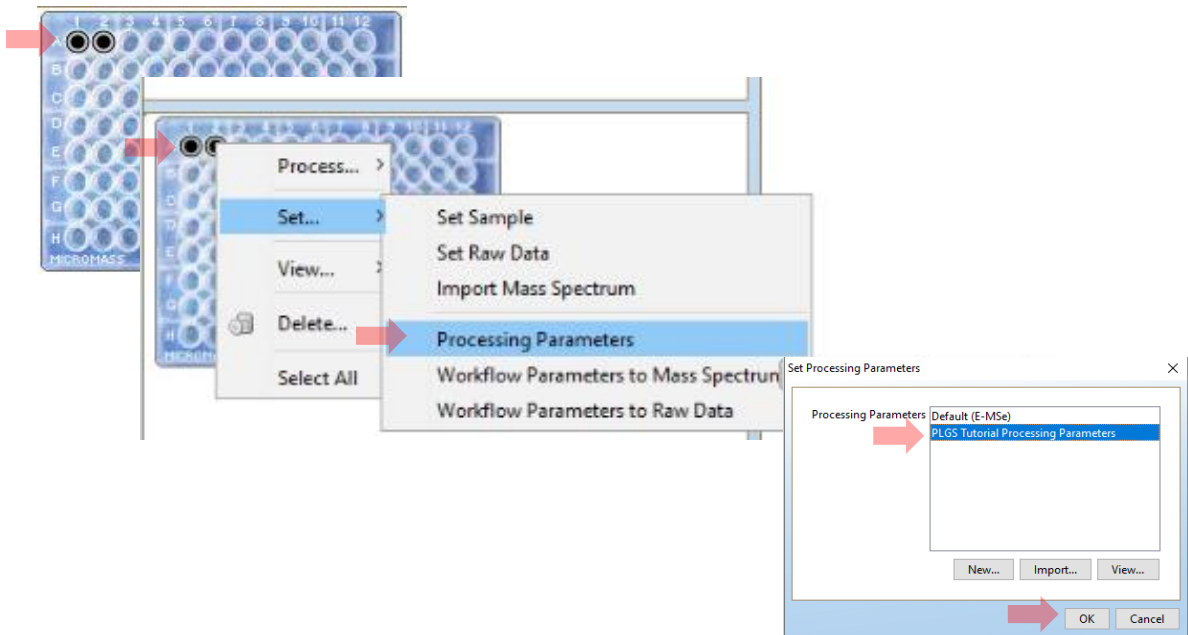
In this section, we will be creating a sample plate or target plate to assign the samples that we already created. This enables to specify the samples, attach templates that determine how data is processed, and access results.

1. In the main navigator tree, right click on “Microtitre plates” > “Add Microtitre plate”
2. Select 8 X 12 Plate and Type “**PLGS Tutorial Plate**” in the Name field, Then Click OK.
3. You should now see a Microtitre plate on the left bottom corner
4. Left Click on A1 position of the plate and then Right Click> Set > Set Sample
5. Select EnolaseTripticDigest_01 from the list. (Specifying a sample into the plate)
6. Repeat steps 4&5 to specify EnolaseTripticDigest_02 sample into A2 position.
7. You should now see the specified samples at A1 and A2 spots on the target plate in grey.

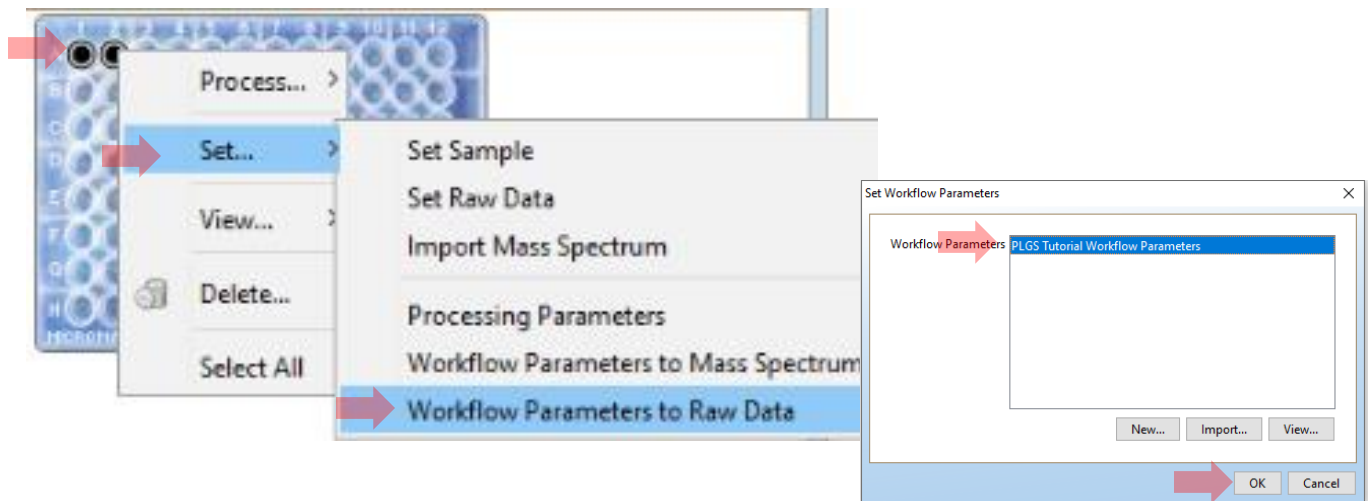


Attaching Processing Parameters and Workflow Parameters

- Select both samples on the Microtitre Plate by clicking on each sample while holding the CTRL Key. (Selected sample spots will turn into black spots)
- Right click on selected samples > Set > Processing parameters
- Select PLGS Tutorial Processing Parameters from the dialog box and Click “OK”



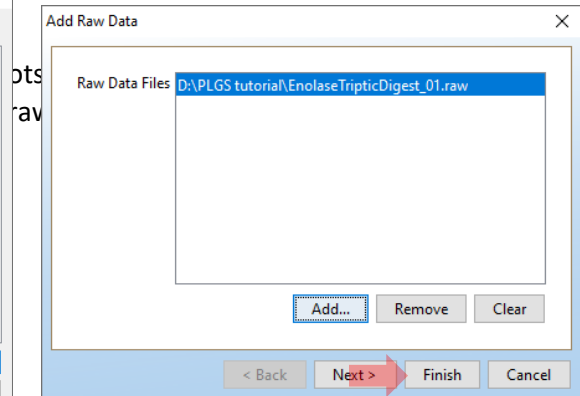
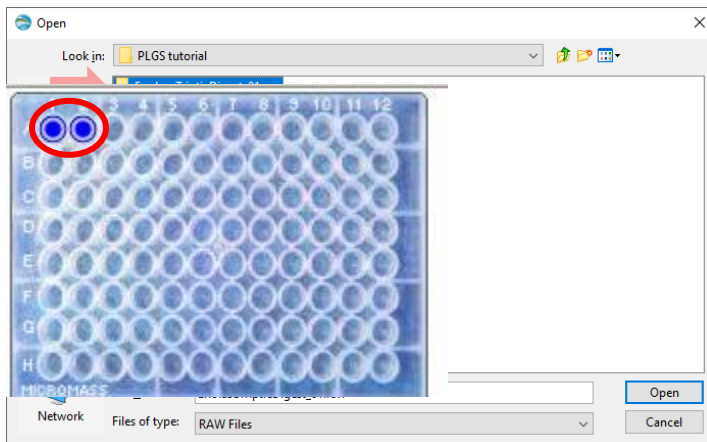
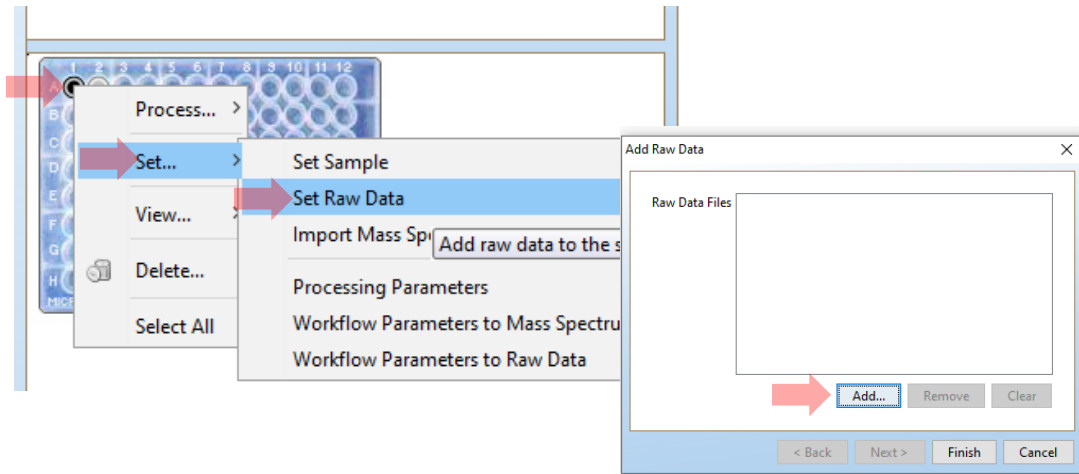
- Right click on selected samples > Set > Workflow Parameters to Raw Data.
- Select PLGS Tutorial Workflow Parameters from the dialog box and Click “OK”



Attaching Raw Data into specified samples:

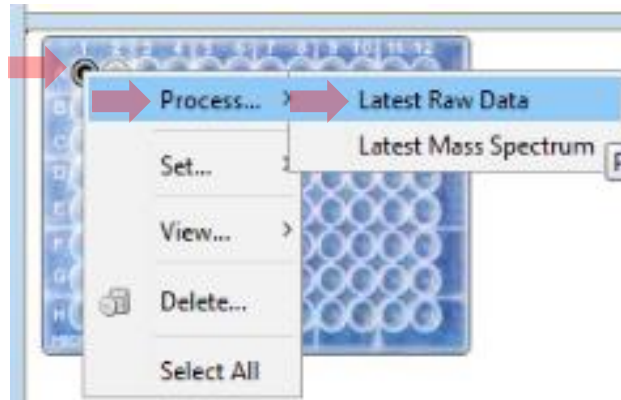
Tip: So far, the samples are set up and configured to meet your requirements. Now you can assign the raw data files that you downloaded at the beginning of the tutorial into corresponding sample on the Microtitre plate.

1. Click Spot A1 (EnolaseTrypticDigest_01 sample) and then right-click > Set > Raw Data File
2. Brows the folder you created and click "EnolaseTrypticDigest_01.raw" then Click OK.
3. Repeat steps 1 and 2 for EnolaseTrypticDigest_02 on the Spot A2, setting the sample as EnolaseTrypticDigest_02.



Processing sample raw data:

- In microtiter plate, click EnolaseTrypticDigest_01 sample, and then right click.
- Click process > Latest Raw Data
- Repeat these steps for the next sample to process raw data.
- Save the project by right-clicking on the Save icon



Hint: Once the process command is given, PLGS begins to process the data. This might take from minutes to hours depending on your computers processing power. Data processing progress is shown at the bottom right corner of the screen.

When PLGS has finished data processing, additional nodes will be added to the tree indicating that the samples are processed and Sample spots on the plate will turn into green.

