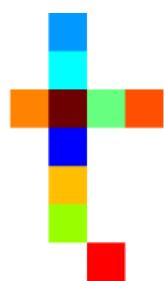


0401 - Tercen FlowJo synergy: the export route



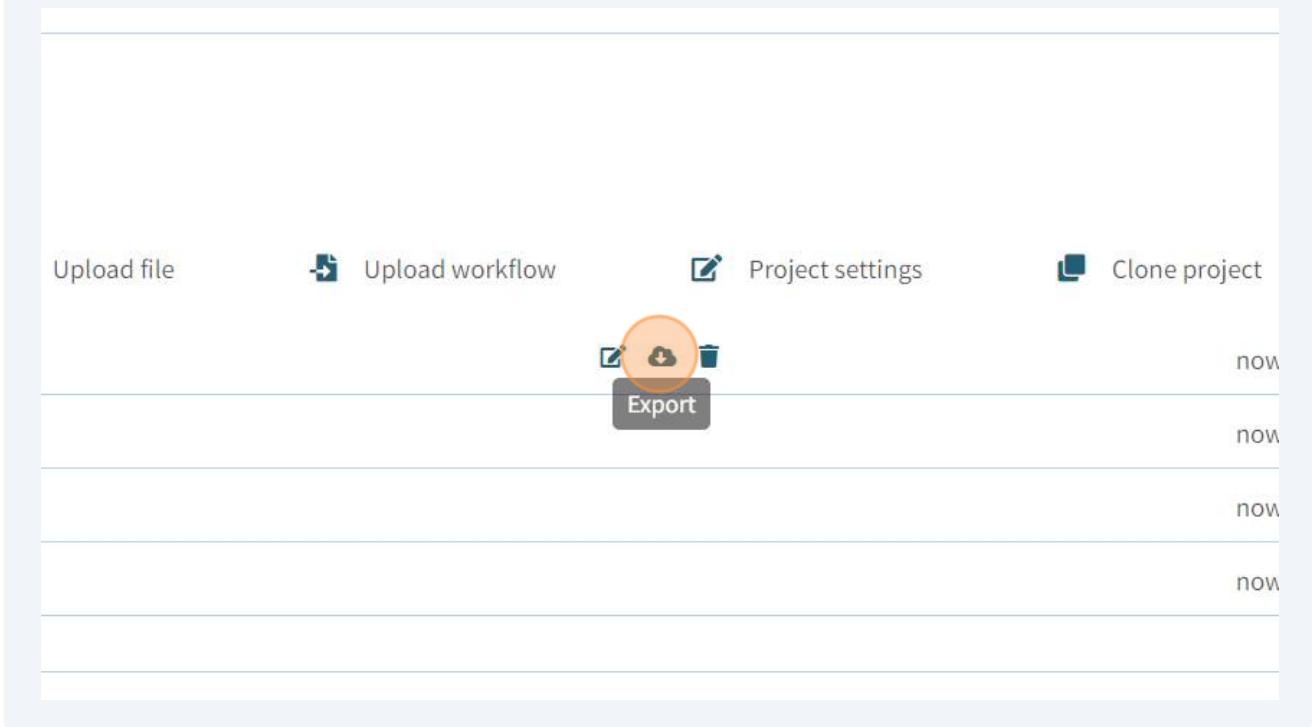
This guide provides step-by-step instructions on how to export FCS files from FlowJo for specific subpopulations/gates and then upload them to Tercen for further analysis.

- 1 Navigate to the 'LevelUpWorkshopsTeam'. In the *Projects* tab find **Workshop IV** project and clone it.

The screenshot shows the 'Projects' tab of a team interface. At the top, there are tabs for 'Projects', 'Members', 'Library', and 'Activities'. Below the tabs, there is a 'New project' button. The 'Projects' section displays three projects:

- Workshop IV**: This project is highlighted with a red circle around its 'Clone' button. It contains a folder named 'Tercen FlowJo synergy & Reporting'.
- Workshop III_SJ**: Contains a folder named 'Clustering and Dimension Reduction'.
- Workshop III - Inês**: Contains a folder named 'Inês'.

- 2 Click on the download icon.



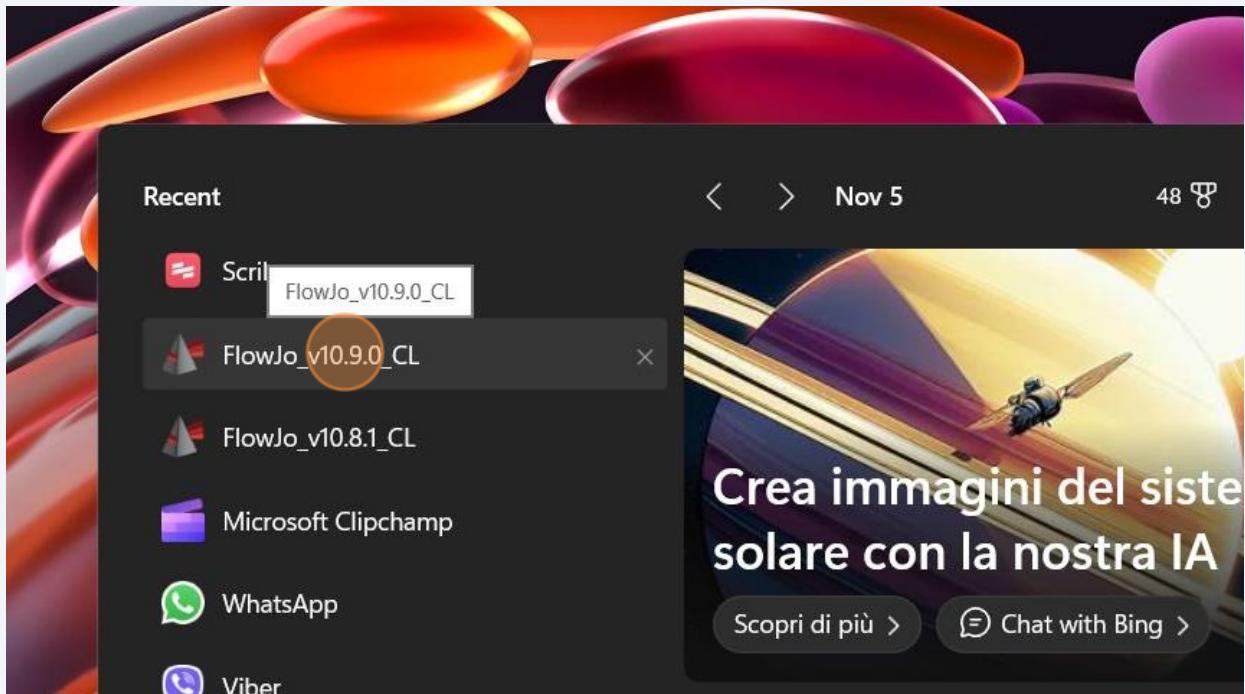
- 3 Export the .zip file named **Tercen_Roche_LevelUp_DataWorkshop.zip**

A screenshot of the Tercen platform. At the top, it shows 'opsTeam > Workshop IV - MAK'. The main header is 'LevelUpWorkshopsTeam'. Below the header, there are tabs for 'Project' (which is selected) and 'Activities'. Under the 'Project' tab, there's a section for 'Workshop IV - MAK' with the subtitle 'Tercen FlowJo synergy & Reporting'. A list of files is shown: 'Tercen_Roche_LevelUp_DataWorkshop_IV.zip' (10 seconds ago), 'README.md' (10 seconds ago), 'PDFs' (10 seconds ago), and 'Guides' (10 seconds ago). The 'Export' button from the previous screenshot is visible here, highlighted with an orange circle. Below this, there's a 'Workshop IV' section with a table:

Number	Agenda	Type	Time	Workshop link
Part A				
A0	Getting together, Workshop III quizzes results & Tercen FlowJo synergy	presentation	30	A0 - The Tercen FlowJo synergy
A1	Exporting FCS files from FlowJo	hands-on	15	A1 - Exporting FCS files from FlowJo

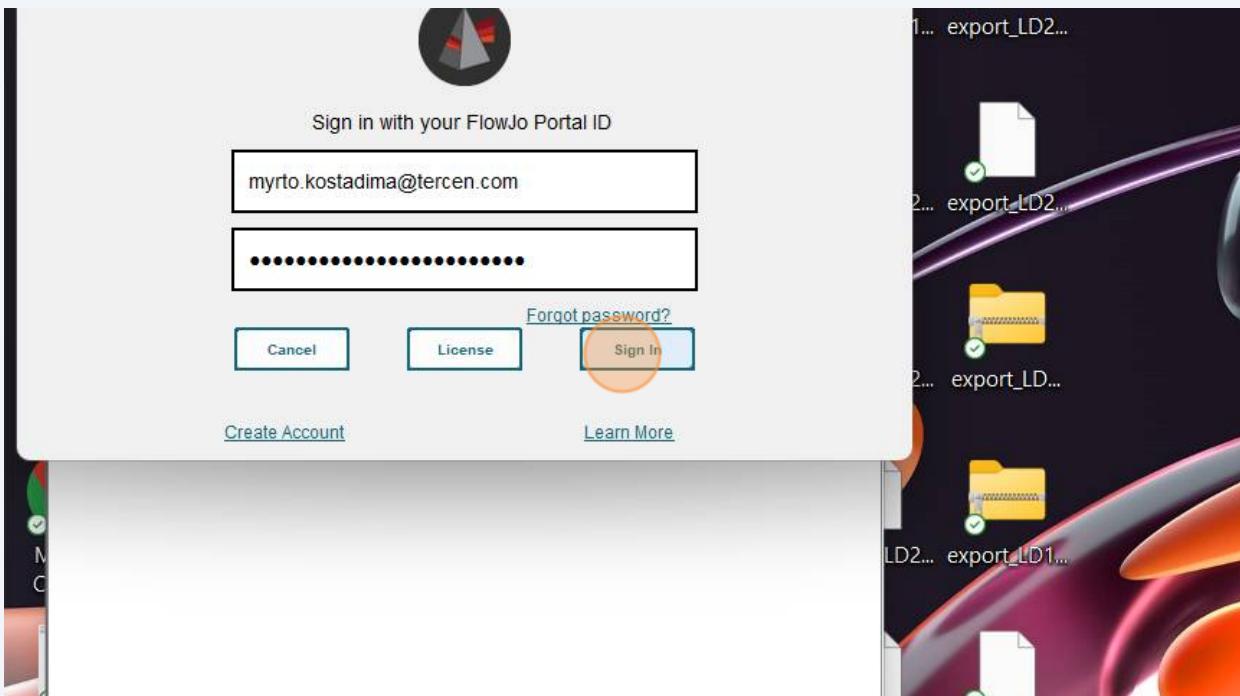
4

While the file is being downloaded, let's fire up FlowJo on your computer. If you have multiple versions installed, please choose the latest one available.

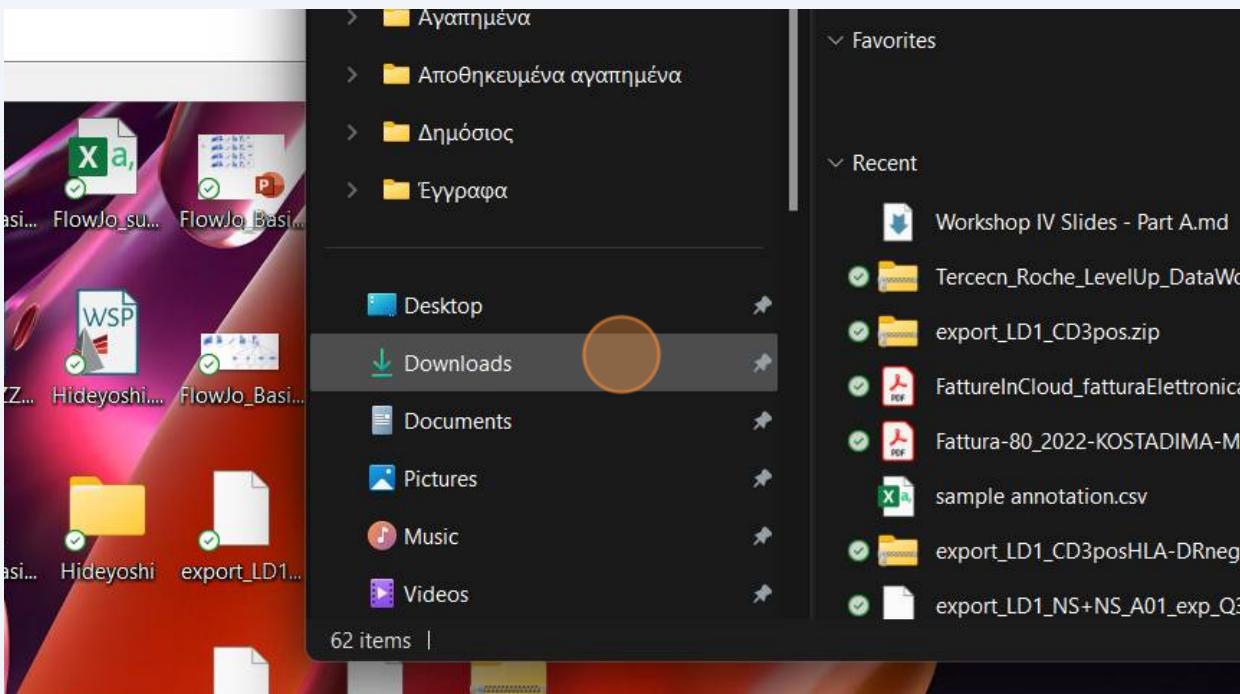


Don't panic, if you don't have FlowJo installed on your computer. Simply skim through this guide to get an idea of how it would be done in case you had FlowJo.

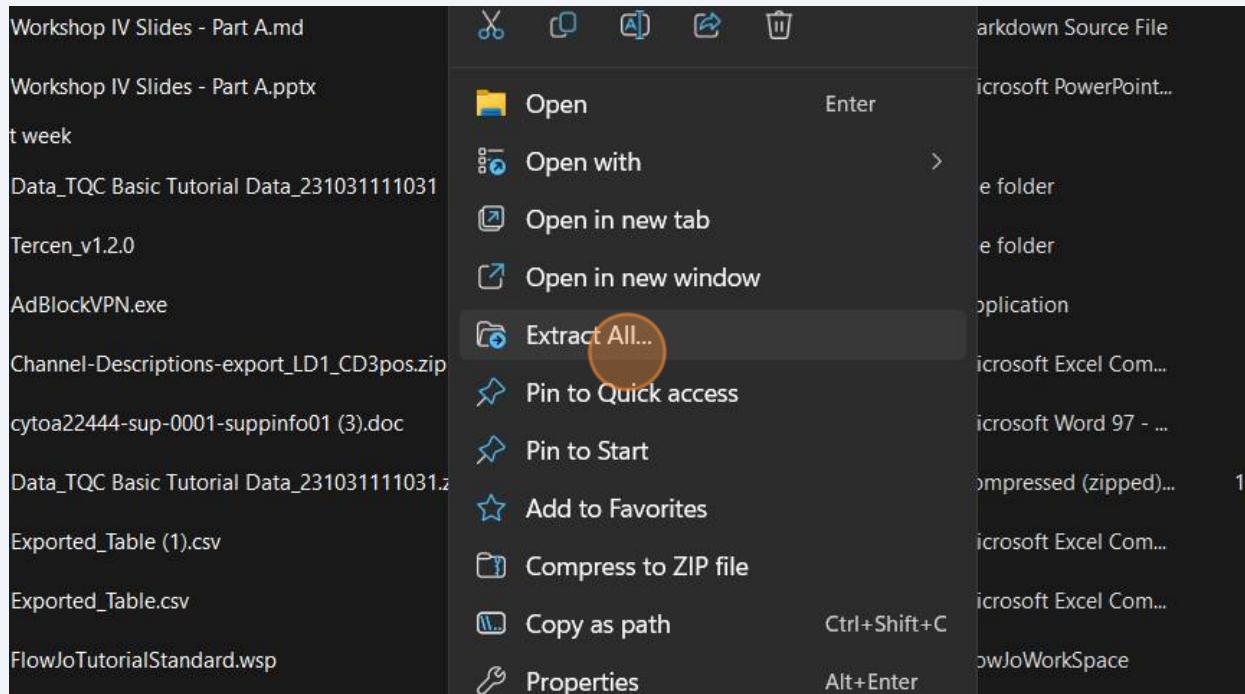
- 5 Make sure you sign in to your FlowJo account.



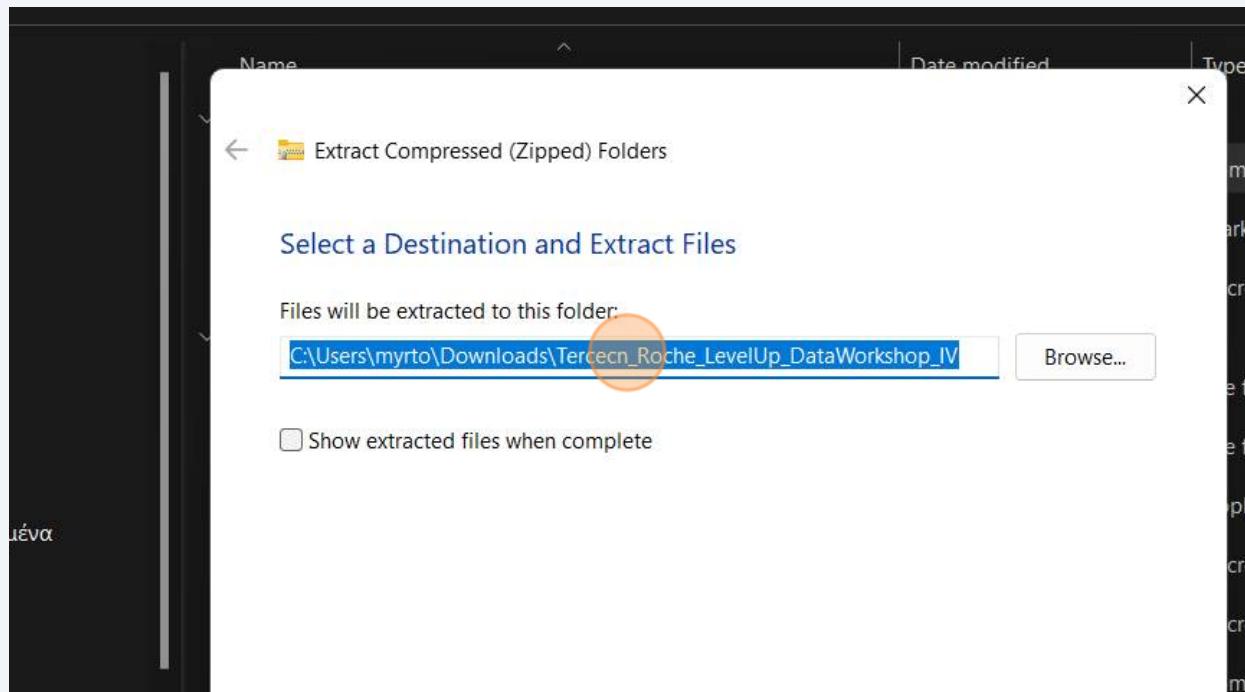
- 6 On your computer navigate to the folder where the .zip file has been exported. By default in most cases it will be in the **Downloads** folder.



- 7 Right-click on the .zip file and select "Extract All...". On the pop-up window select an appropriate destination file and click **Extract**.

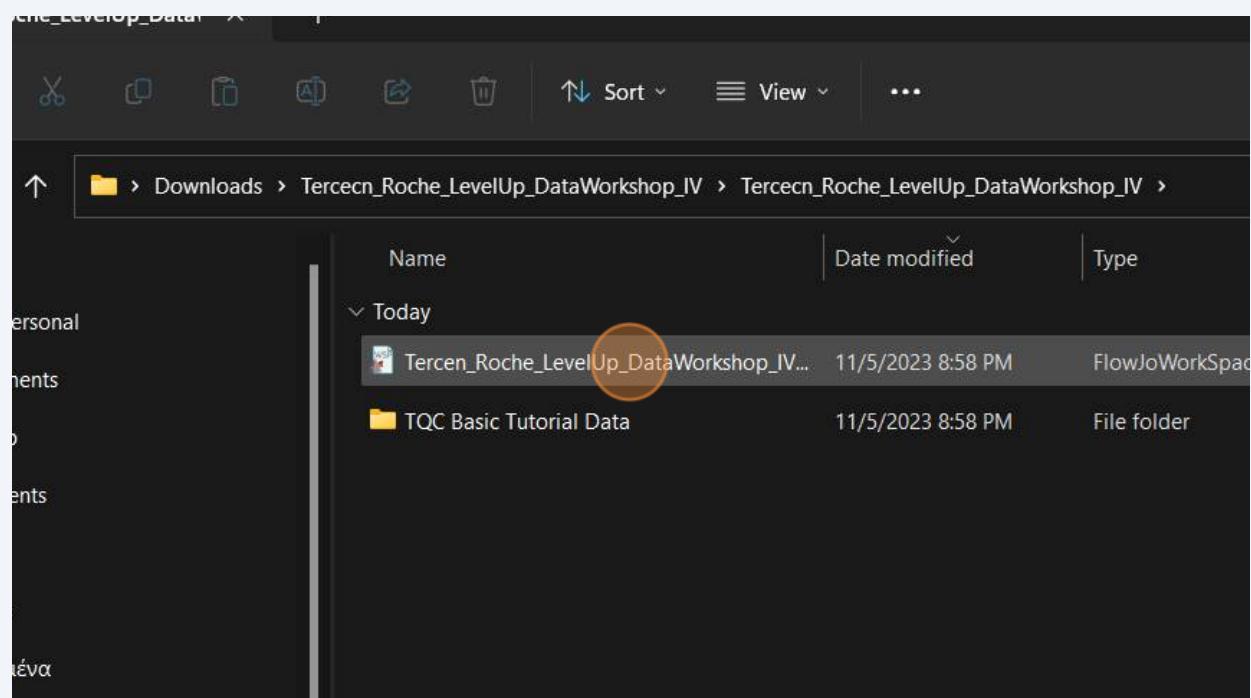


- 8 Click "Files will be extracted to this folder:"

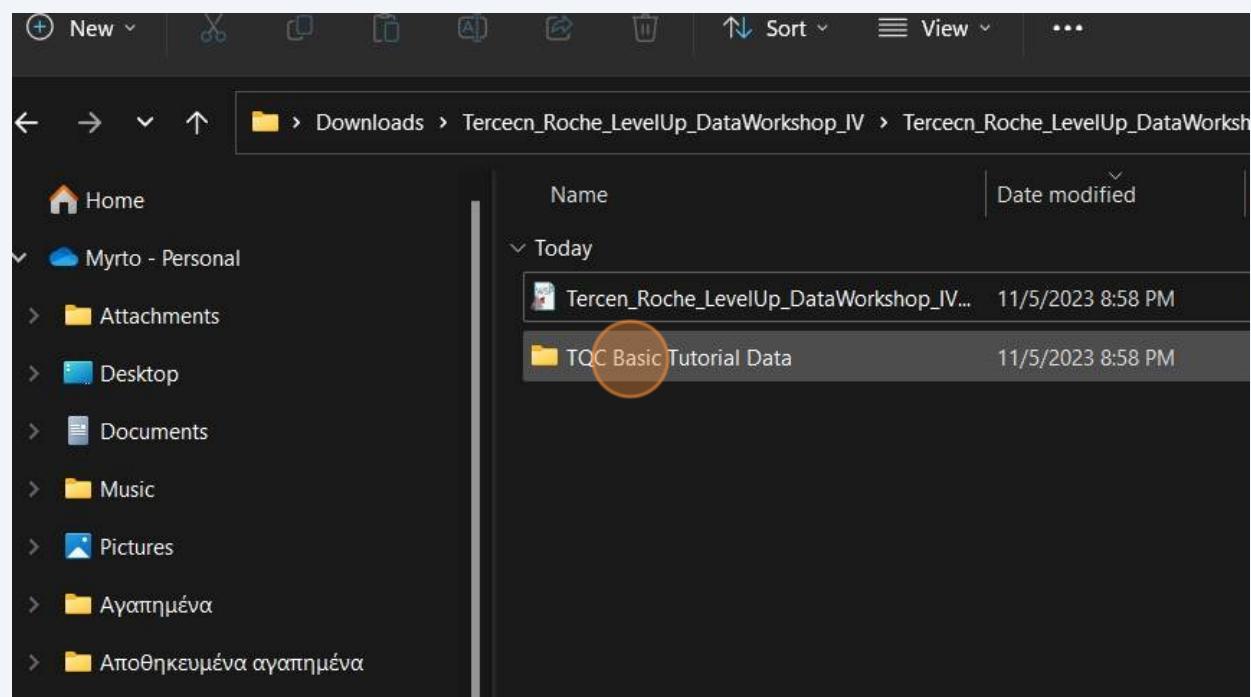


- 9 Navigate to the destination folder selected in the previous step and identify a FlowJoWorkspace file named **Tercen_Roche_LevelUp_DataWorkshop_IV.wsp**

Drag and drop it into FlowJo to open it.



- 10 If FlowJo complains about not locating the original .FCS files, click to *Search for FCS files...* and in the browser navigate to the folder **TQC Basic Tutorial Data** located in the same destination folder as the FlowJo workspace.



11

The FlowJo workspace contains four samples from two donors (LD1 and LD2) for two different stimulation conditions:

- NS + NS : initially with BFA+M (no stimulation, NS) followed by a further BFA+M stimulation, and
- PI + PI : for 2 h with PMA+I and BFA+M followed by 20 min with PMA+I and BFA+M (PI)

Expand one of the samples.

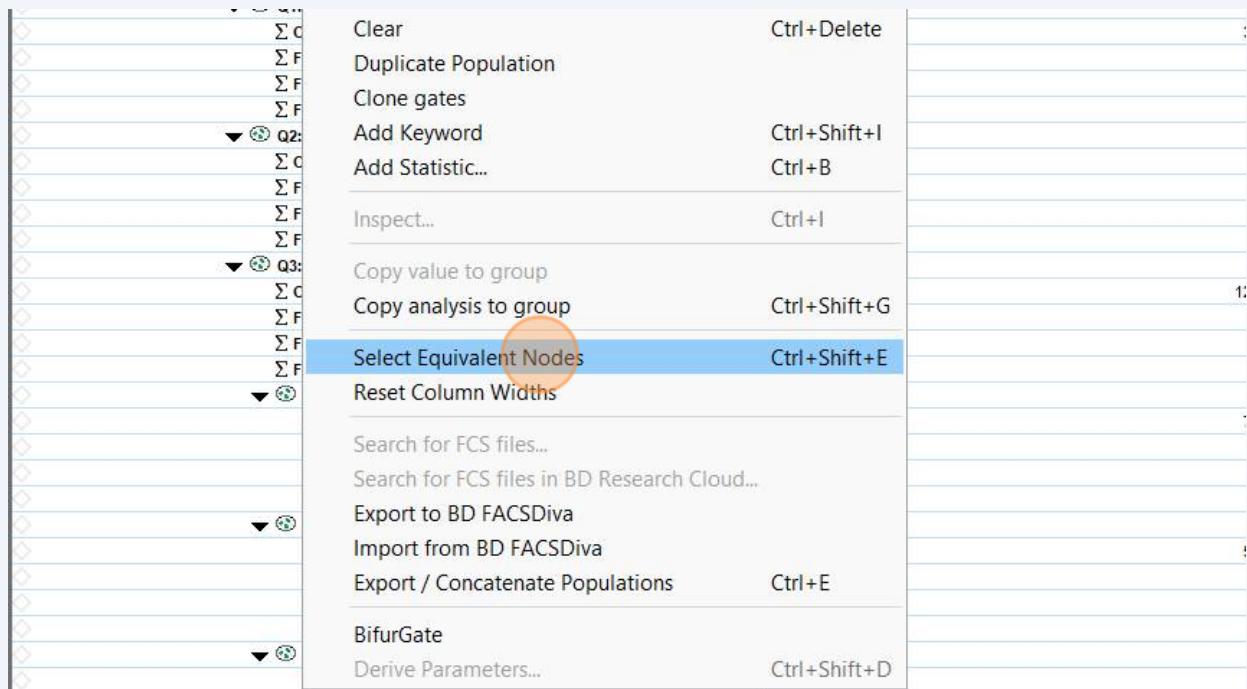
The screenshot shows the FlowJo software interface. The top menu bar includes 'New Workspace', 'Add Samples...', 'Create Group...', 'Table Editor', 'Layout Editor', 'Preferences...', 'Add Keyword', 'Annotate Experiment...', 'Plate Editor', 'Cell Cycle...', 'Kinetics...', 'Compare Populations...', and 'Proliferation Modeling'. The main window has a 'Navigate' tab selected. The left sidebar shows a hierarchical tree view under 'Group': 'All Samples' is expanded, showing 'Lymphocytes' and 'Live' branches. 'Lymphocytes' has a child node 'Σ Freq. of Total'. 'Live' has two children: 'Σ Freq. of Parent' and 'Σ Freq. of Total'. Below this tree is a table with a single column 'Name' containing four entries, each preceded by a circular icon with a blue dot and a small orange square. The first entry, 'LD1_NS+NS_A01_exp.fcs', is highlighted with a red oval circle. The table rows are as follows:

Name
LD1_NS+NS_A01_exp.fcs
LD1_PI+PI_D01_exp.fcs
LD2_NS+NS_A02_exp.fcs
LD2_PI+PI_D02_exp.fcs

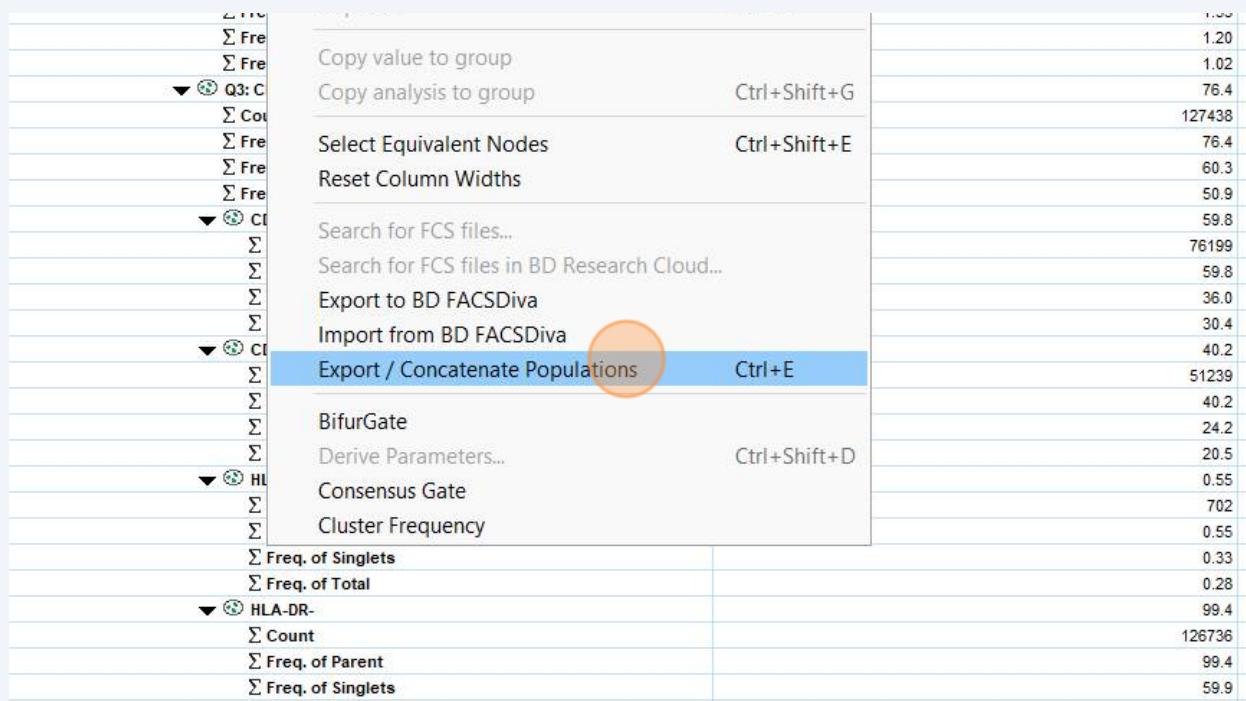
12 Navigate down to the subpopulation named **CD3+**

Name	Statistic
Σ Count	21
Σ Freq. of Total	
Live	
Σ Freq. of Parent	
Σ Freq. of Total	
Singlets	
Σ Count	21
Σ Freq. of Parent	
Σ Freq. of Total	
CD3+	11
Σ Count	
Σ Freq. of Parent	
Σ Freq. of Total	
Q1: CD4-, CD8+	
Σ Count	
Σ Freq. of Parent	
Σ Freq. of Singlets	
Σ Freq. of Total	
Q2: CD4+, CD8+	
Σ Count	
Σ Freq. of Parent	
Σ Freq. of Singlets	
Σ Freq. of Total	

13 Right-click on it and click **Select Equivalent Nodes**



14 Right-click on it and click **Export / Concatenate Populations**



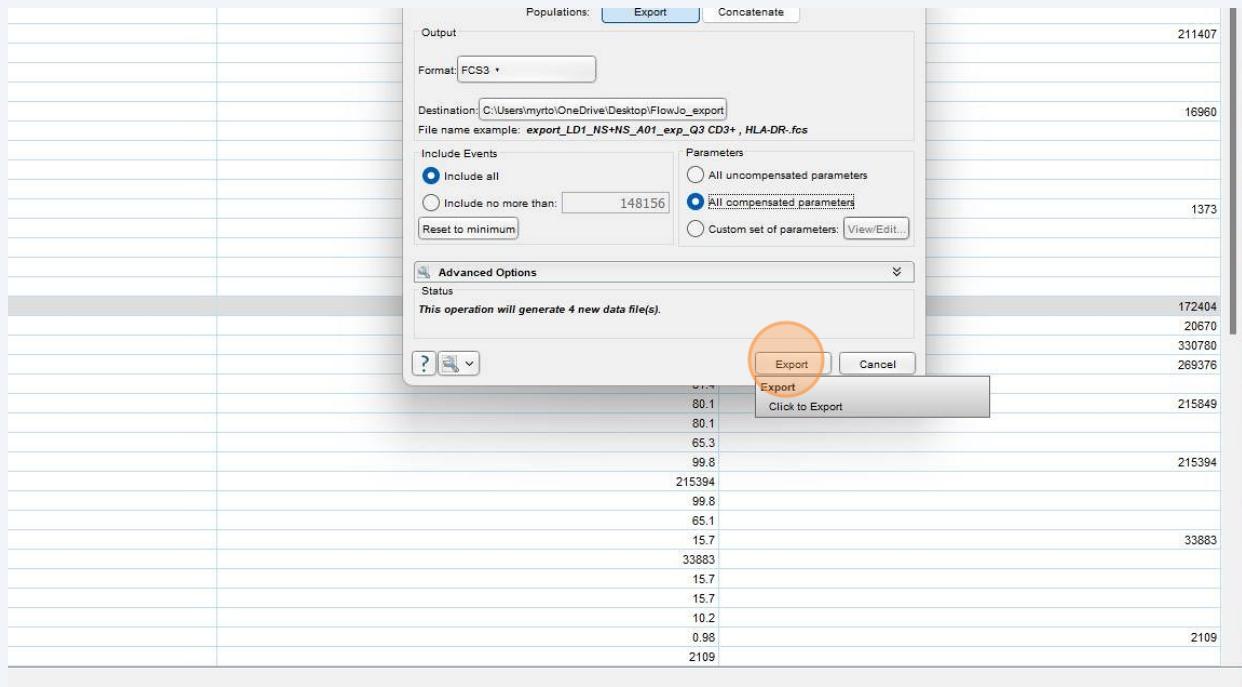
15 On the popup window select

Format: **FCS3 ***

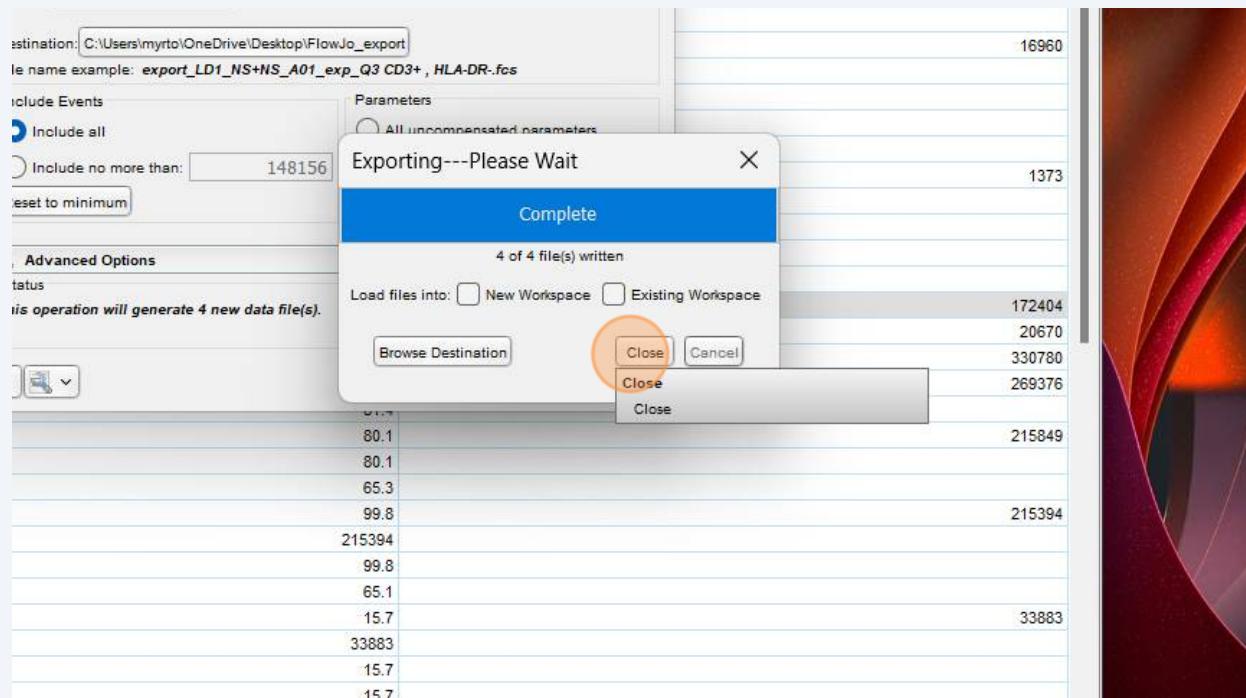
Destination: <select any folder you want these FCS files exported>

Parameters: **All compensated parameters**

and then click *Export*.

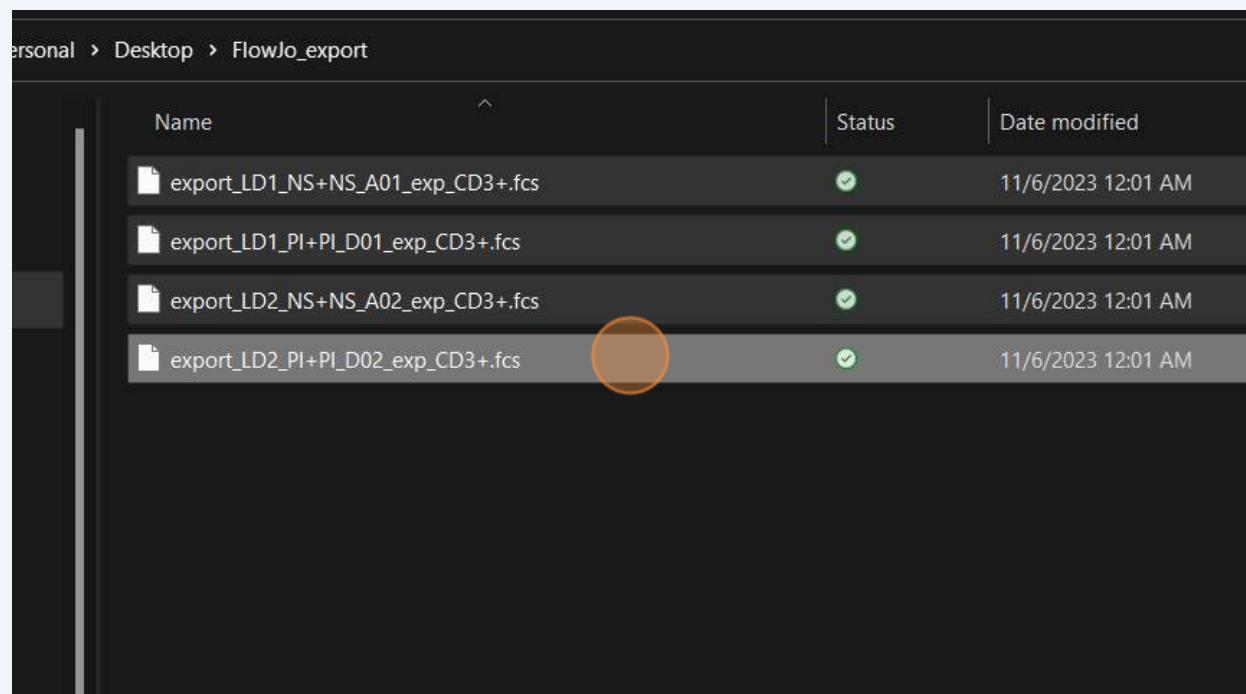


16 Once the exporting is complete click **Close** and then **Cancel**.

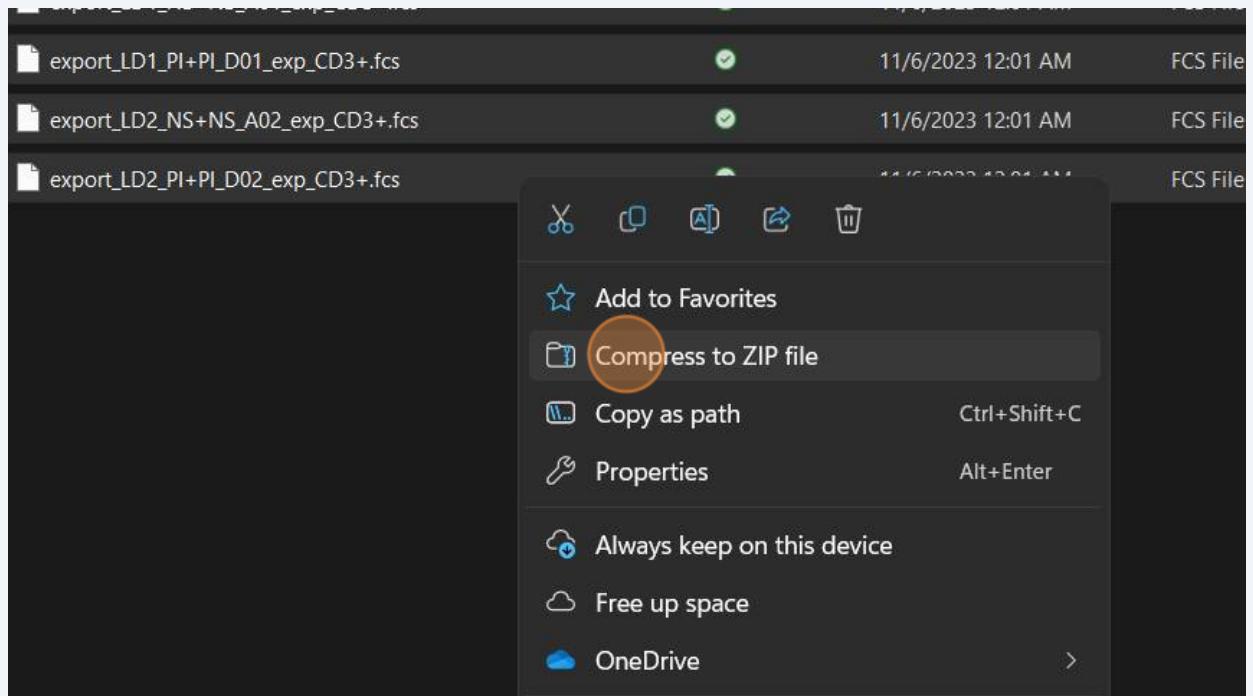


17 On the computer's File Browser navigate to the folder where you selected the FCS files to be exported in.

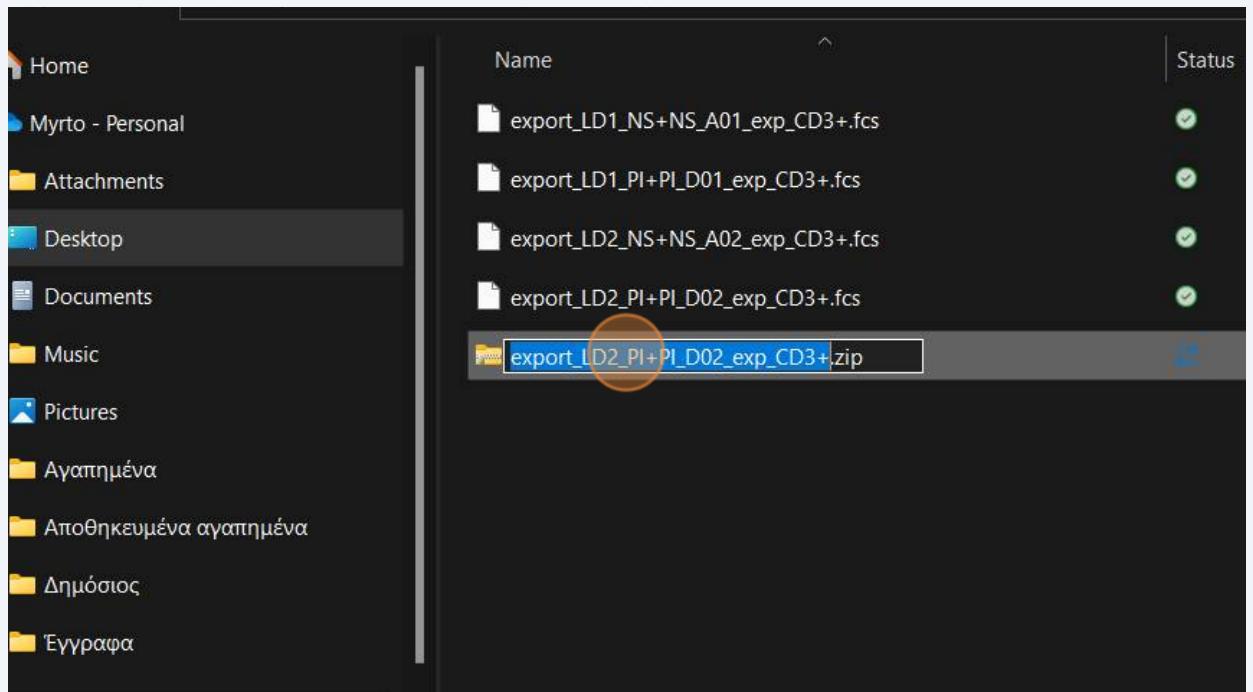
Select all four .FCS files and right-click.



18 Click Compress to ZIP file

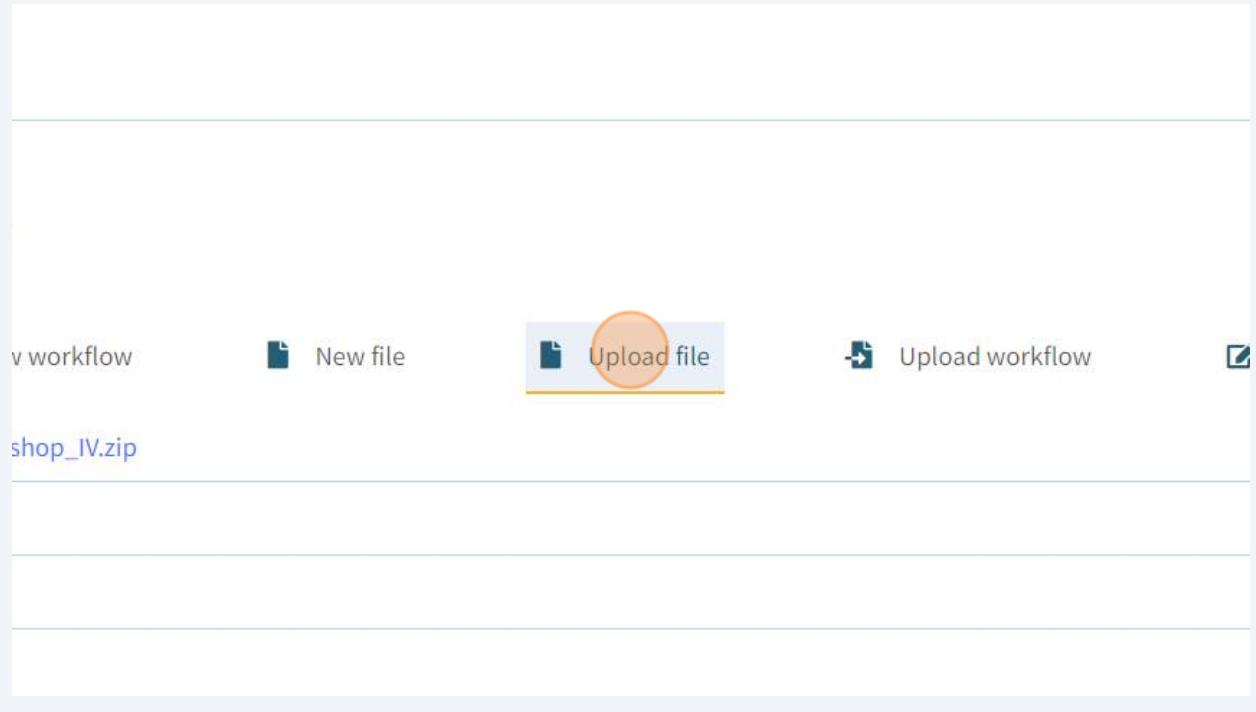


19 Modify the name of the .zip file to **export_LD1_LD2_CD3pos.zip**



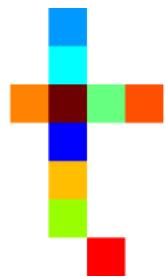
20

Back on the Tercen project click **Upload file** to upload the .zip file you just created onto Tercen.



Congratulations! You just uploaded on Tercen four .FCS files (compressed into one .zip file) you had exported from FlowJo, containing a subset of the original samples.

0402 - How to run a Tercen template workflow



This guide provides step-by-step instructions on how to run a Tercen template workflow. You can easily navigate through the process of setting up and running a workflow, saving time and streamlining your data analysis tasks.

- Now that we have uploaded our .FCS files onto Tercen we would like to run a preliminary analysis that includes clustering & dimension reduction.

Click **New workflow**.

Project Activities

Workshop IV - MAK
Tercen FlowJo synergy & Reporting

New data set New workflow New file Upload

makostadima created file [export_LD1_LD2_CD3pos.zip](#)

[Tercen_Roche_LevelUp_DataWorkshop_IV.zip](#)
[README.md](#)
[PDFs](#)

- 2 Under the *Installed* tab select **Preliminary Flow Template**

The screenshot shows a software interface with a sidebar on the left and a main content area. In the top right corner of the content area, there is a search bar labeled 'Search' and a toggle switch labeled 'Display latest version only'. Below these are three workflow templates listed in a grid:

- Preliminary Flow Template 0.0.1**: A template to perform clustering (Phenograph) and dimension reduction (UMAP). It has a blue icon of three squares connected by lines. An orange circle highlights the 'Play' button next to the template name.
- Simple 1.0.1**: A basic Table and Data Step workflow. Press Play to get started. It has a blue icon of three squares connected by lines.
- Cytokine Analysis Template**: This template is partially visible at the bottom of the list.

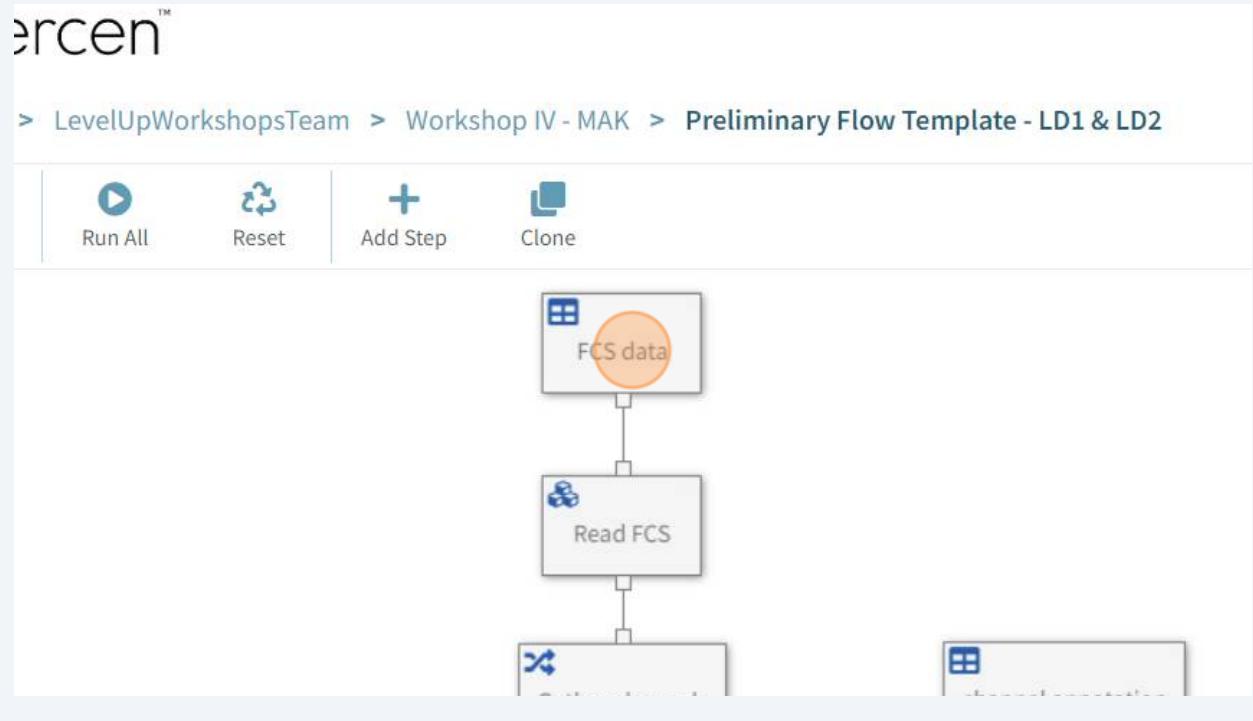
- 3 Before proceeding let us also modify the default name of the workflow. Add - **LD1 & LD2** at the end of the workflow name on the textbox on the top center of the popup window.

Then Click **Ok**.

The screenshot shows the same software interface as the previous one, but with a modified workflow name. The title bar now displays 'Preliminary Flow Template - LD1 & LD2'. The rest of the interface remains the same, showing the 'Installed' tab with the 'Simple' template and the 'Cytokine Analysis Template'.

- 4 You will be automatically directed to the template workflow. We need to go through a few setting up steps. First let us attach the .zip file with the FCS files we exported from FlowJo to the workflow.

Click on the **FCS data** table and then click **Run**.

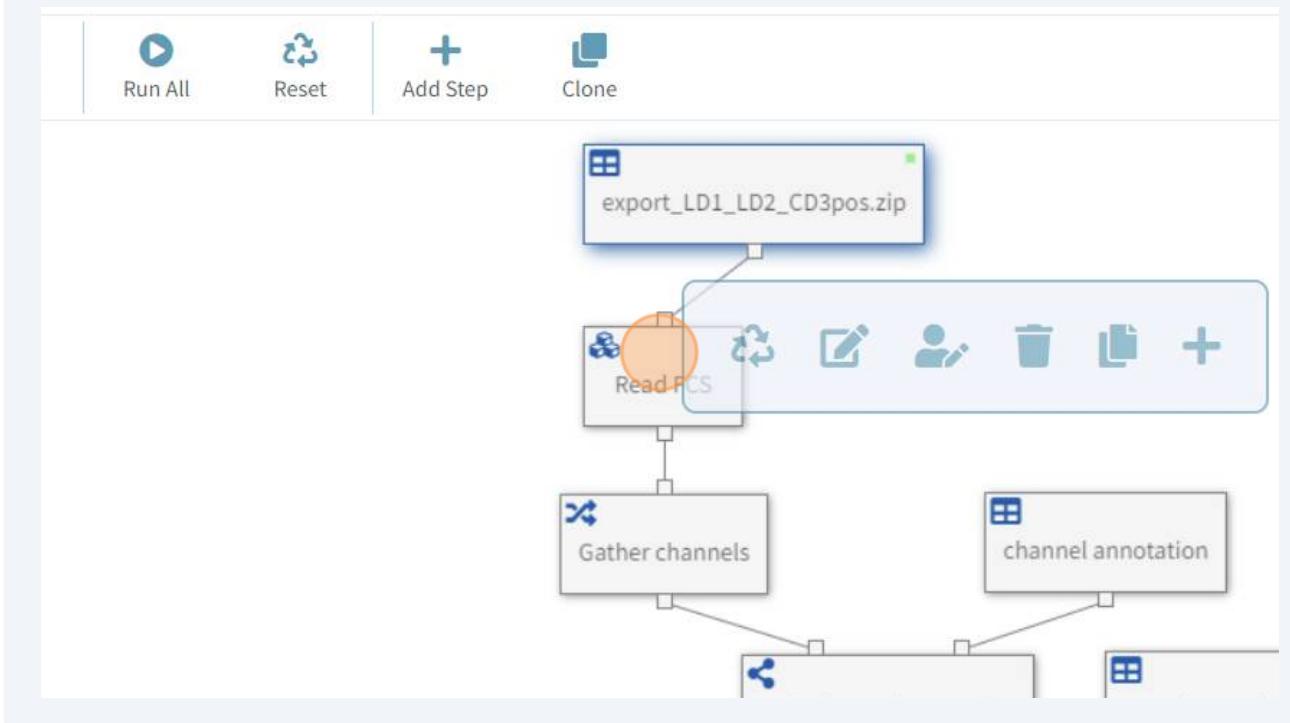


- 5 Under the *Object* tab select **export_LD1_LD2_CD3pos.zip** and click **OK**

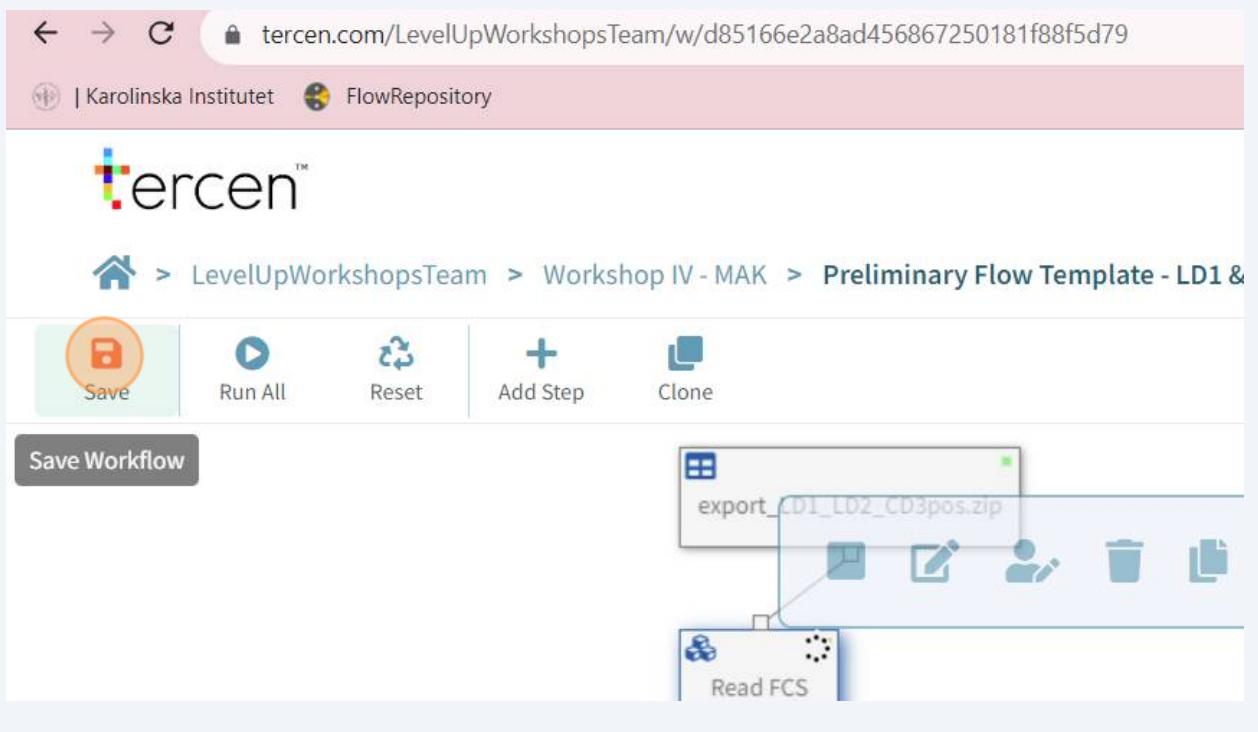
Table	Installed	Library	Object	External Folder
			Preliminary Flow Template - LD1 & LD2 0.0.1	A template to perform clustering (Phenograph) and dimension reduction
			export_LD1_LD2_CD3pos.zip	
			export_LD1_LD2_CD3pos_sample annotation.csv	
			README.md	
			Tercen_Roche_LevelUp_DataWorkshop_IV.zip	
			WorkshopIII_PartA.pdf	

- 6 Then click on the **Read FCS** data step and click **Run**.

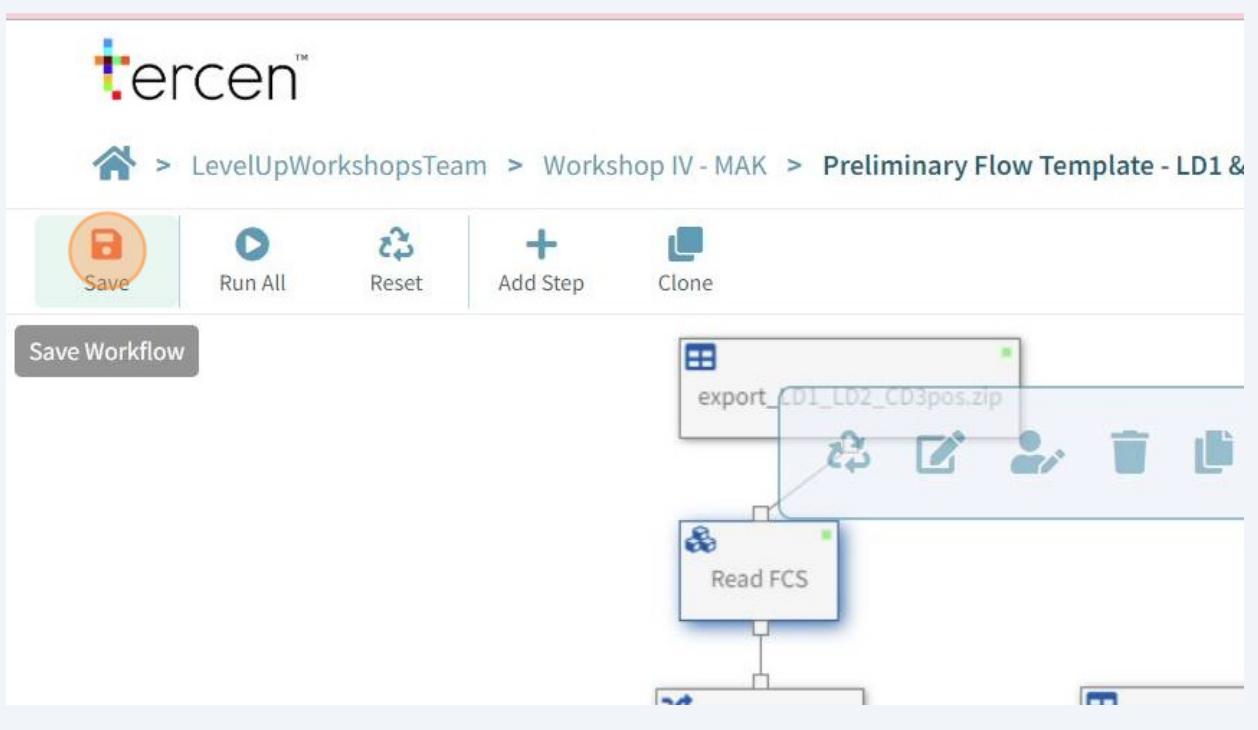
Wait until this step is completed successfully. You will notice a green square on the top right of the data step once it is successfully run.



- 7 Click "Save Save Workflow"



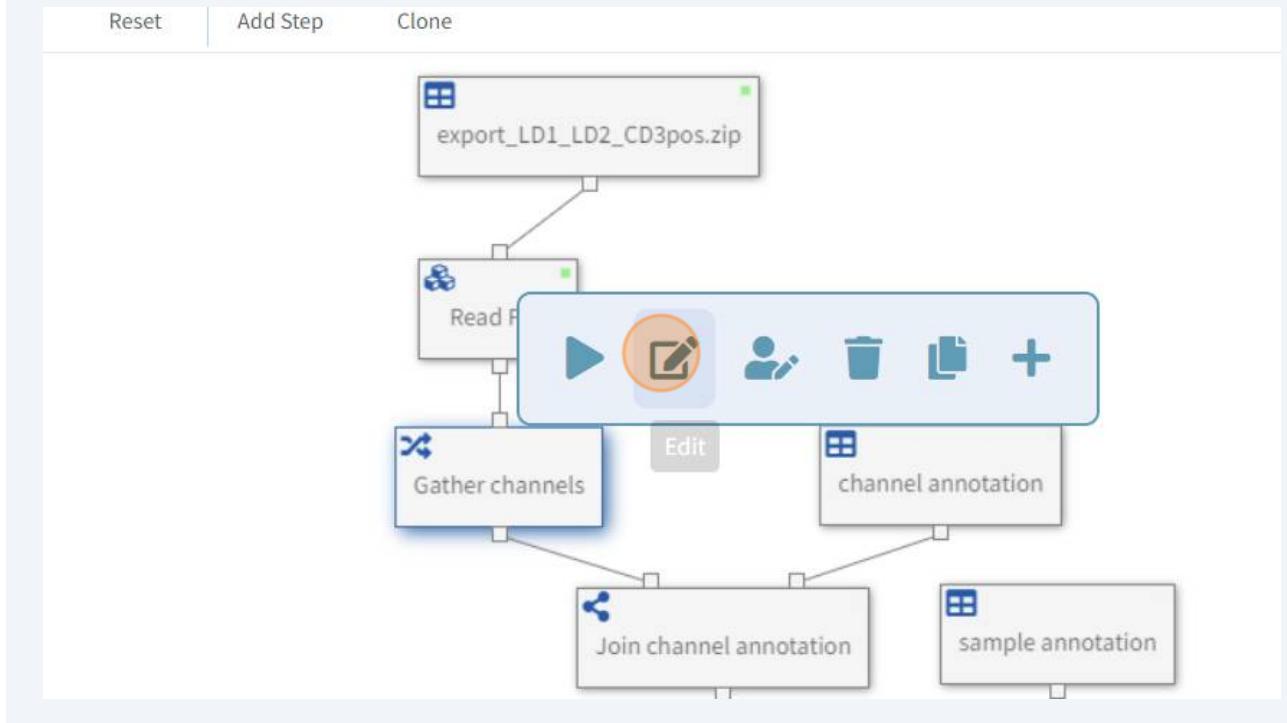
- 8 Don't forget to Save the workflow periodically.



- 9 Click Edit on the **Gather channels** step to observe which channels have been selected by default.

Notice that we have not selected the parameters FSC-A, FSC-H, SSC-A, SSC-H and Time, as we will not be using them for the clustering and dimension reduction analysis.

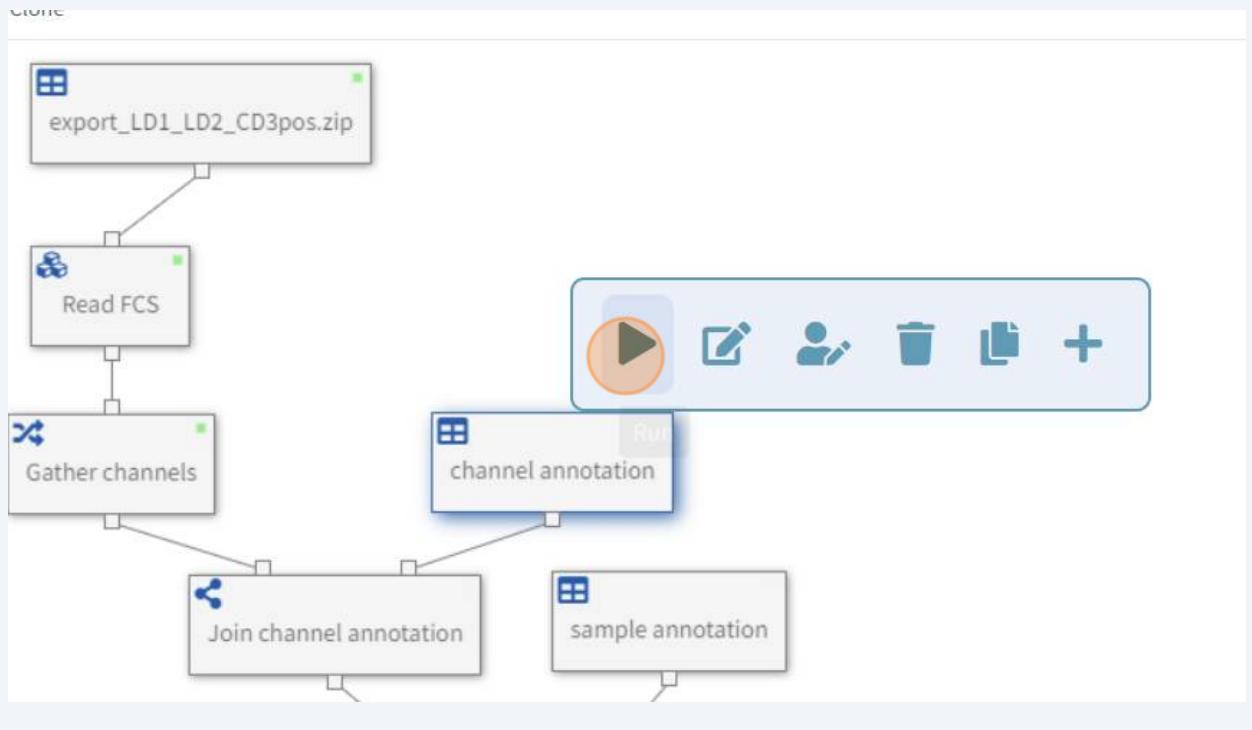
Without applying any changes click **Save & Run Step**



10

To the **channel annotation** table step we will attach the channel annotation table that was generated by the Read FCS step. It can be found under the *Table* tab and is called **Channel-Descriptions-export_LD1_LD2_CD3pos.zip** followed by the timestamp of when it was created.

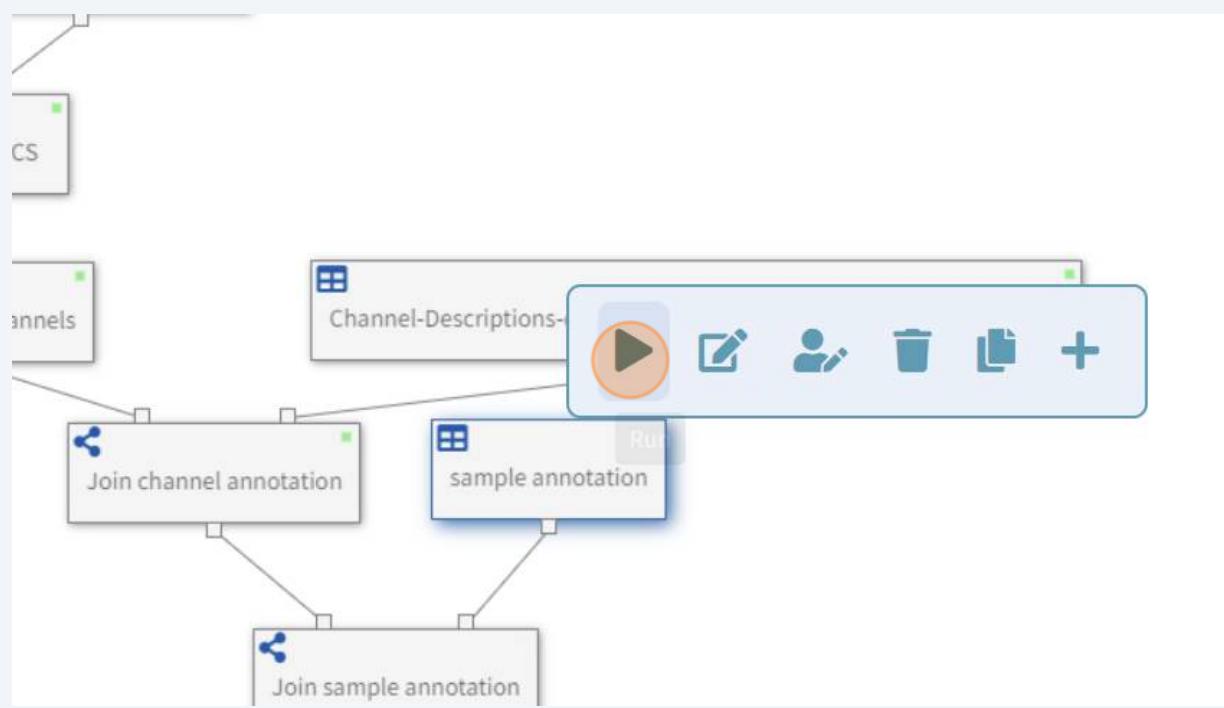
Click **OK**



11

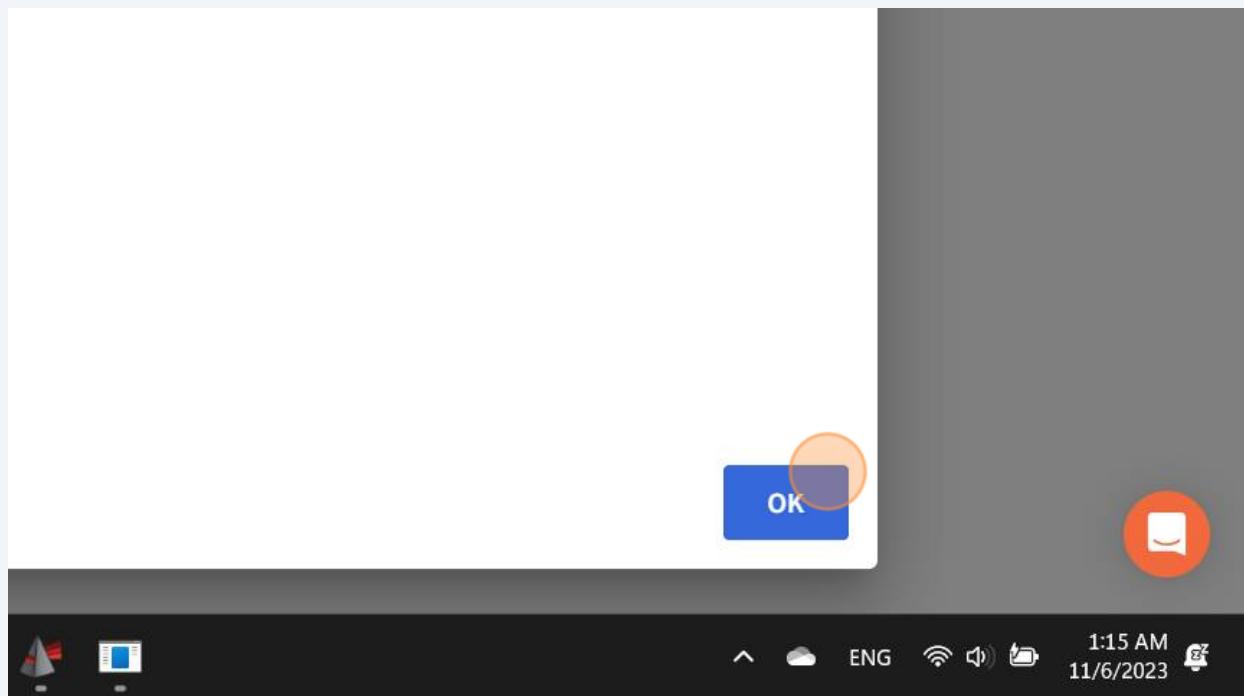
To the **sample annotation** table step we will attach the sample annotation table that is called **export_LD1_LD2_CD3pos_sample annotation.csv**.

Click OK



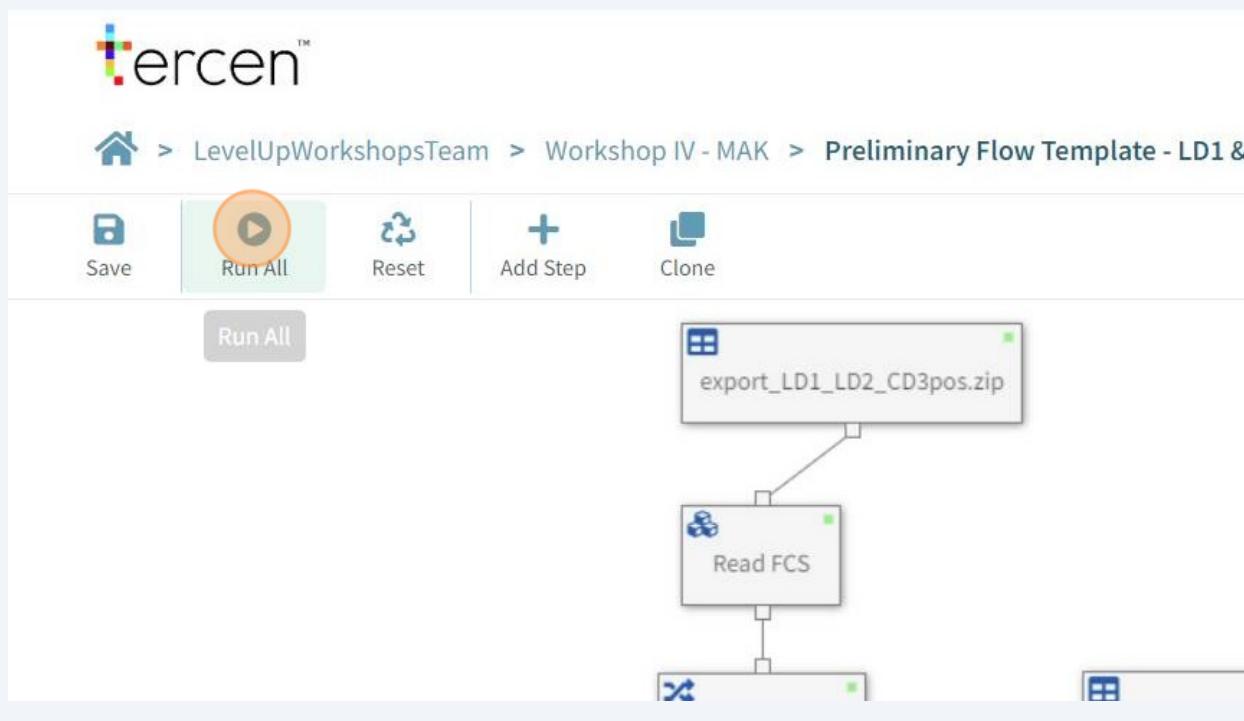
The sample annotation was already included in the Project when you cloned it.

12 Click "OK"



13 Click **Save** and then **Run All**.

The latter command ensures that all data steps in the template workflow will be run (as long as you leave the web page open).





Congratulations! That's how easy it is to run clustering & dimension reduction (or any other template workflow) on Tercen.

Don't worry about the results! We will have a thorough look at the results together in Part B of this workshop.