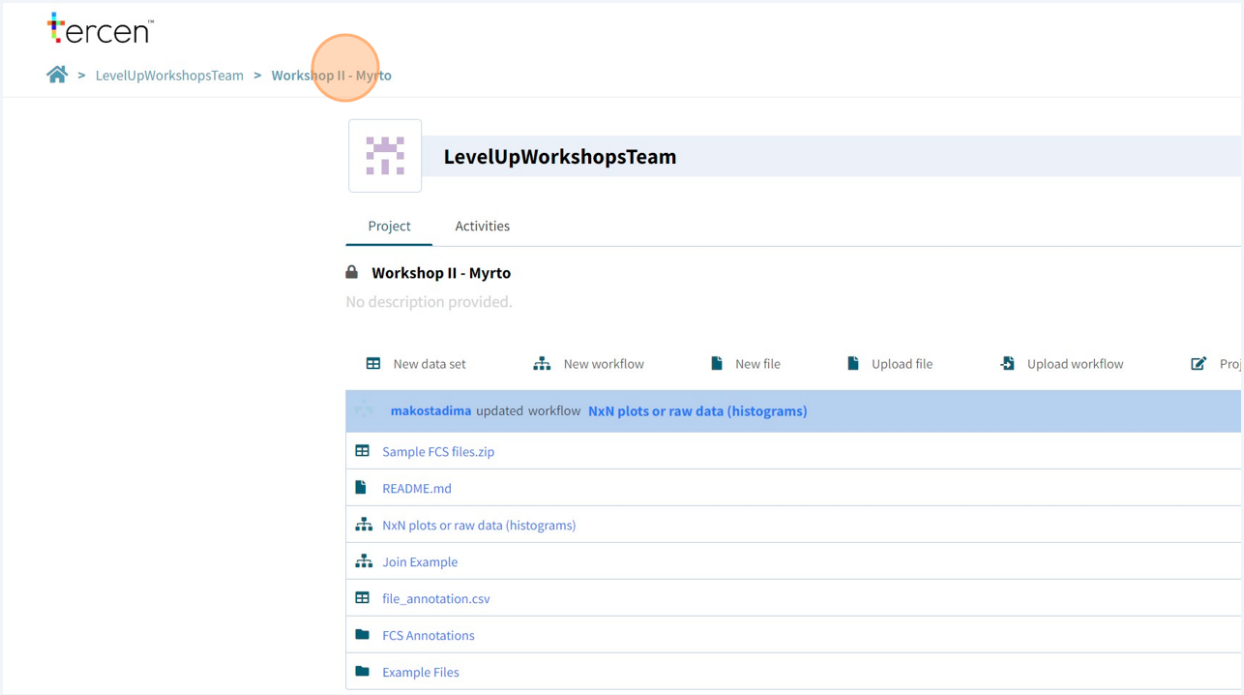
LEVEL UP WORKSHOP II
Part B

0205 - Plot Channels vs. Channels (NxN)

In this lesson, we will create Channel vs. Channel (i.e., NxN) plots based on raw FCS data and on-the-fly data transformations (histogram) on Tercen.

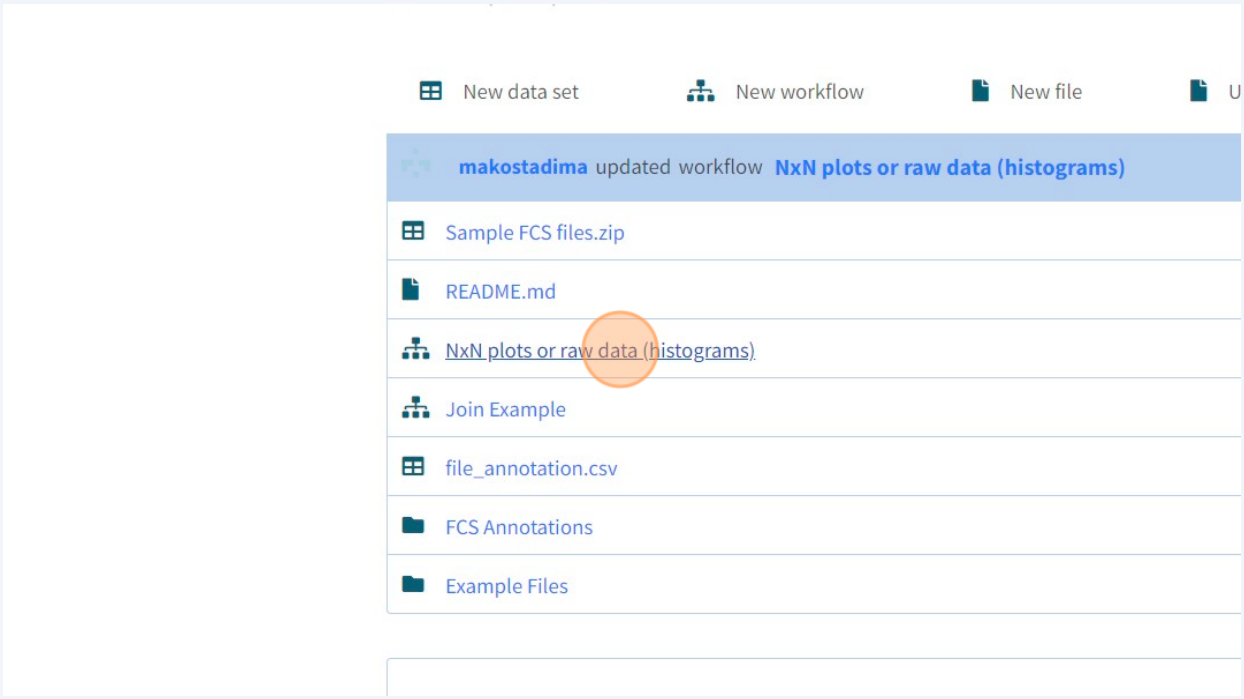
1 Click on your copy of the Workshop II Tercen project.



2 For the purposes of this lesson, we will use an already created workflow called "NxN plots on raw data (histograms)".

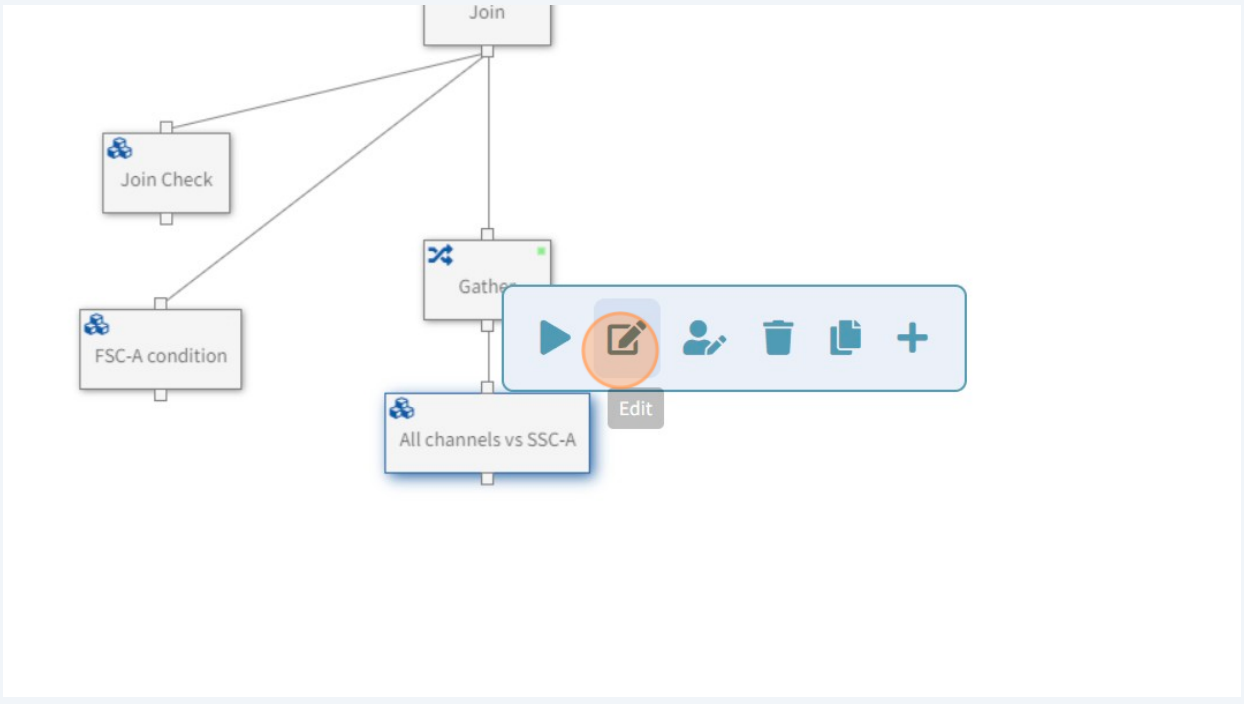
This workflow contains all the steps you've already seen and created in the previous lessons of the day.

3 Click "NxN plots on raw data (histograms)"

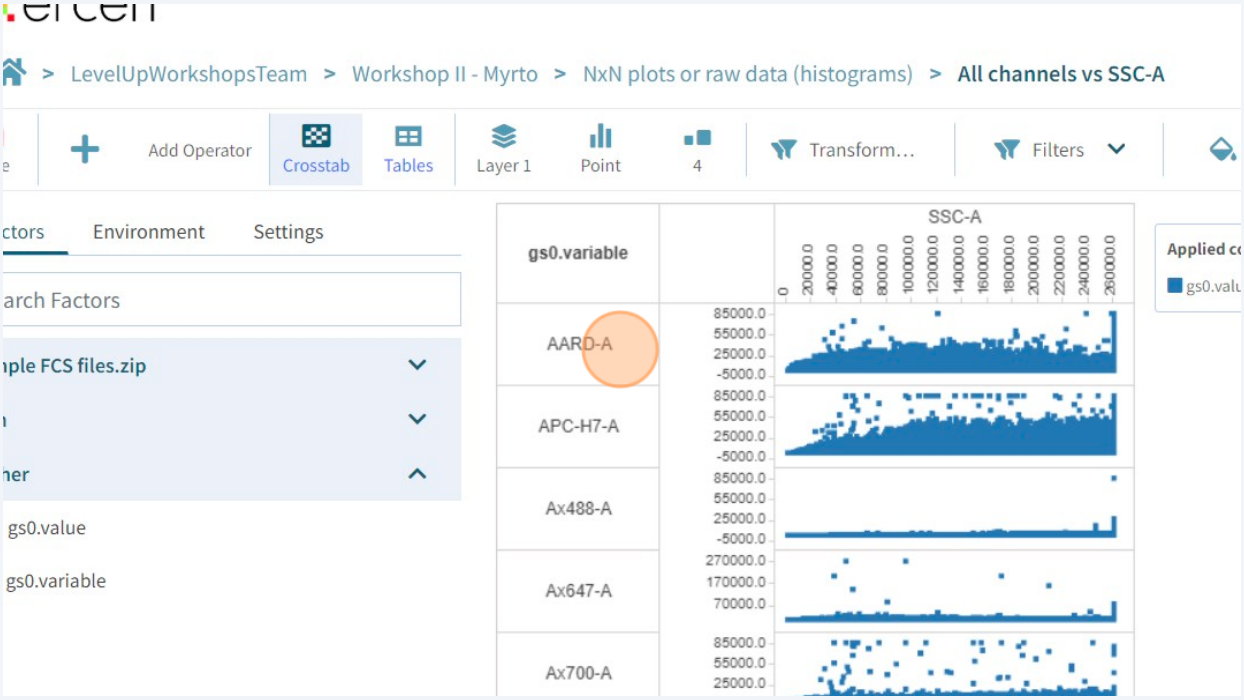


4 Now, you will review the last plot you generated in the guide '0203 - Gather'.

Click the 'All channels vs SSC-A' step and select **Edit**.



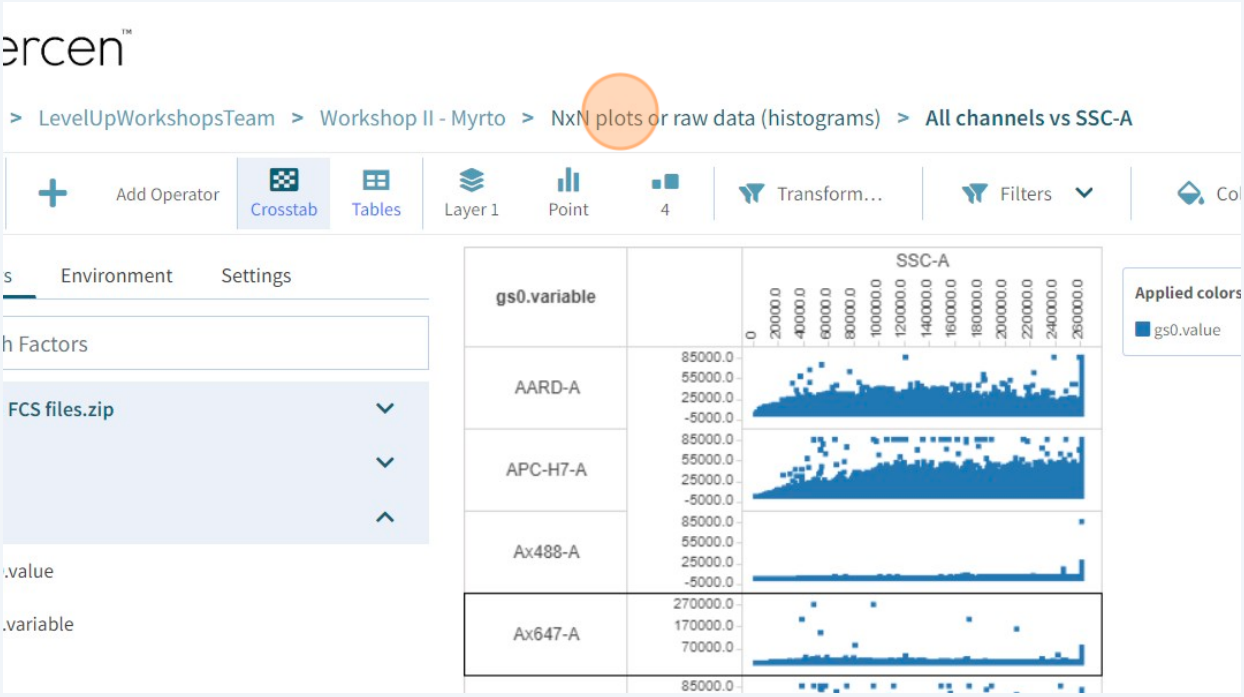
5 The marker names on the rows represent the colors used but are not very informative (unless one remembers the colors per marker used).



6 Go back to the workflow to fix this.

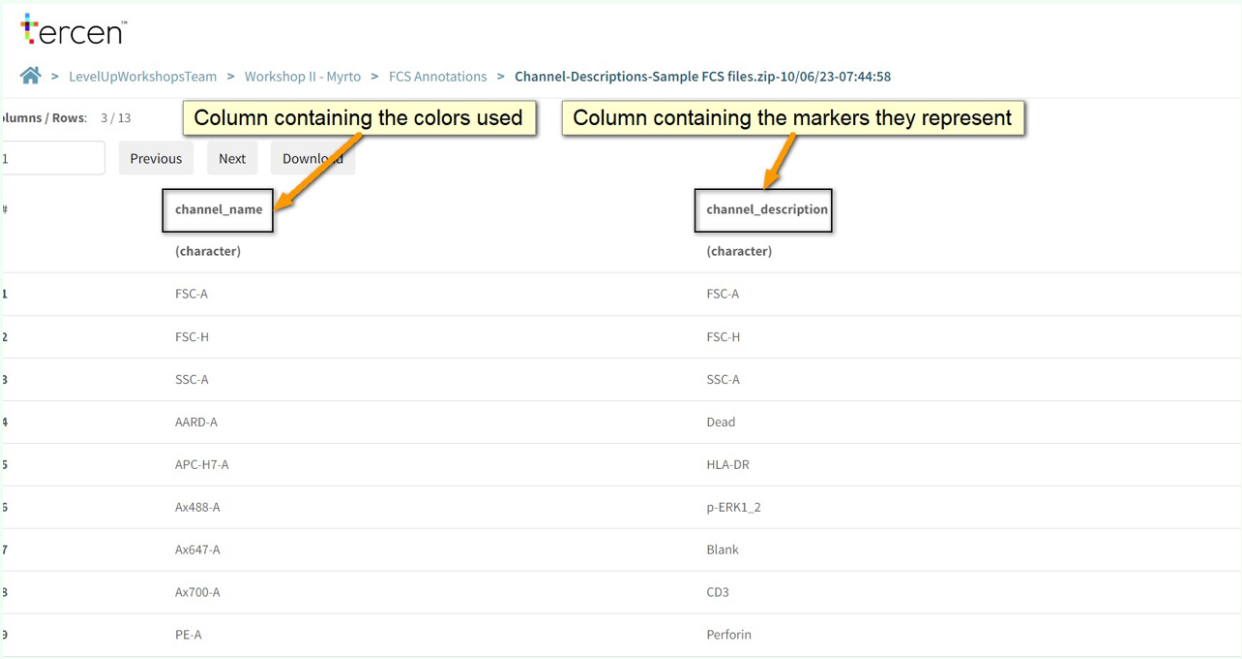
Save your workflow if needed. Is the disk icon orange? Then click on it to save your workflow.

Click "NxN plots on raw data (histograms)" on the breadcrumb to go back to the workflow.

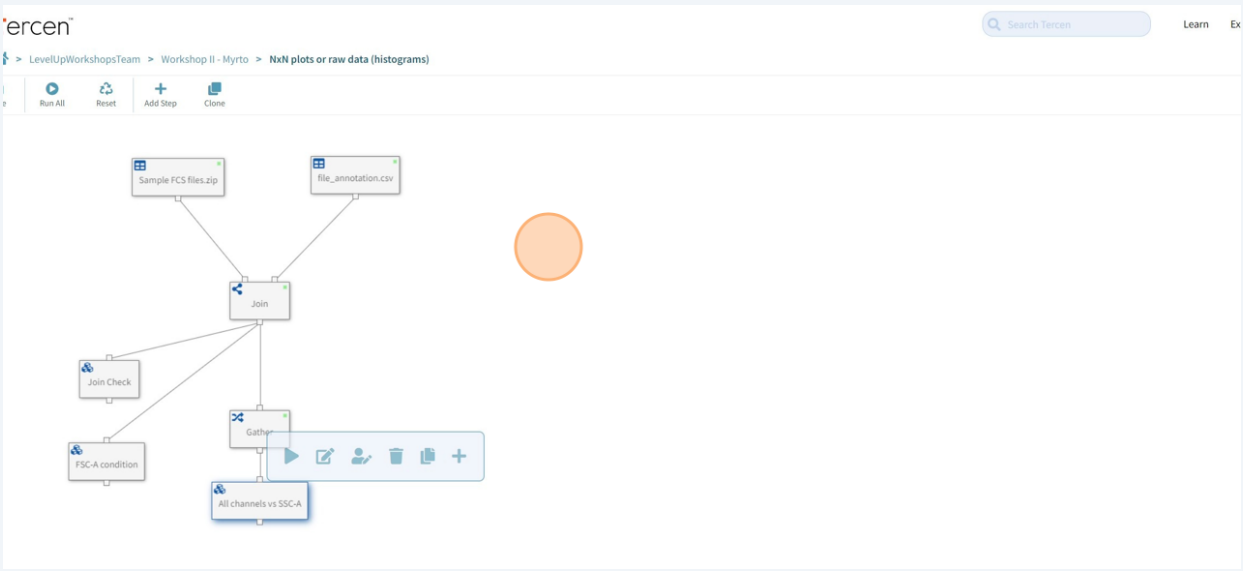


The data table we will add is automatically generated by the FCS operator when we read our data onto Tercen and is stored under the folder 'FCS Annotations.' It contains two columns, **channel_name** and **channel_description**.

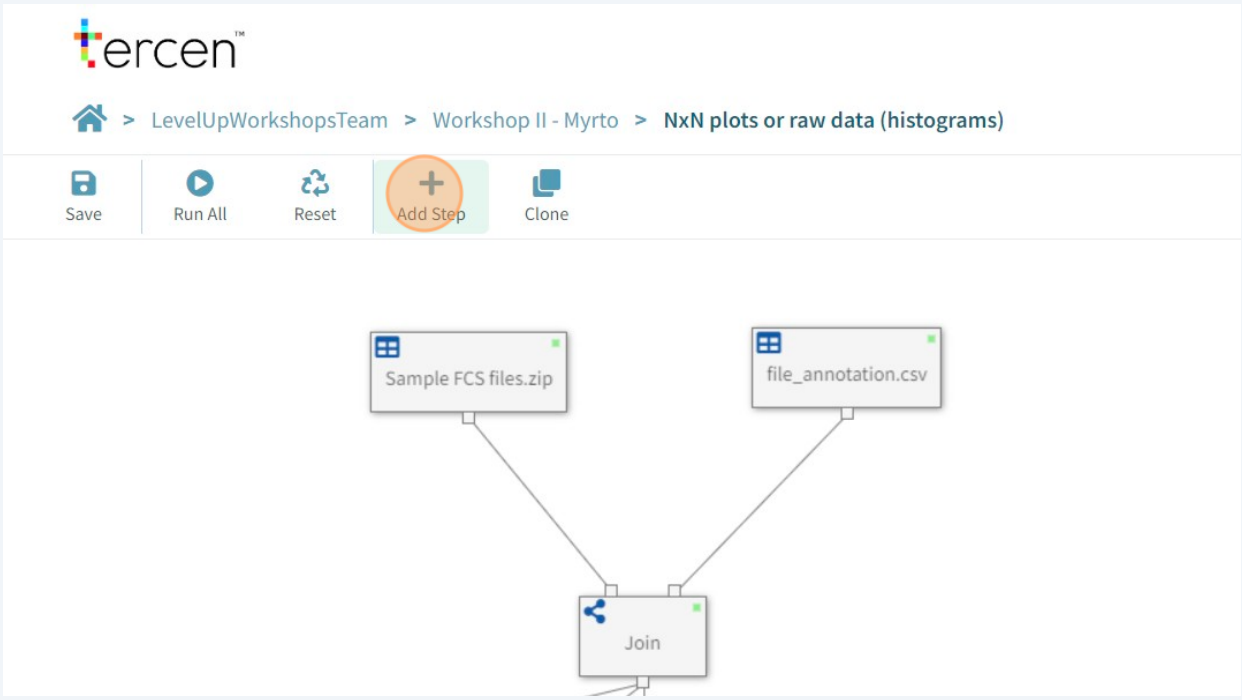
We will use the channel_name column to join the names of the channels to their description, which represent the names of the markers assayed.



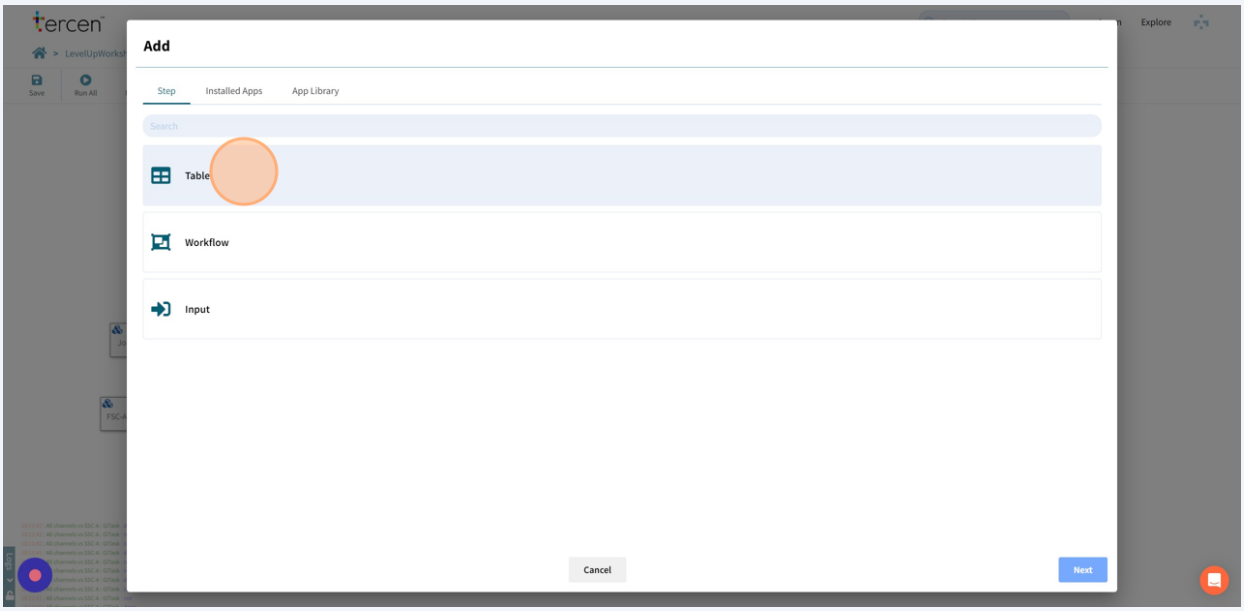
7 Click anywhere on the white area to remove any local toolbars.



8 Click on 'Add Step' from the global toolbar to add a new data table.

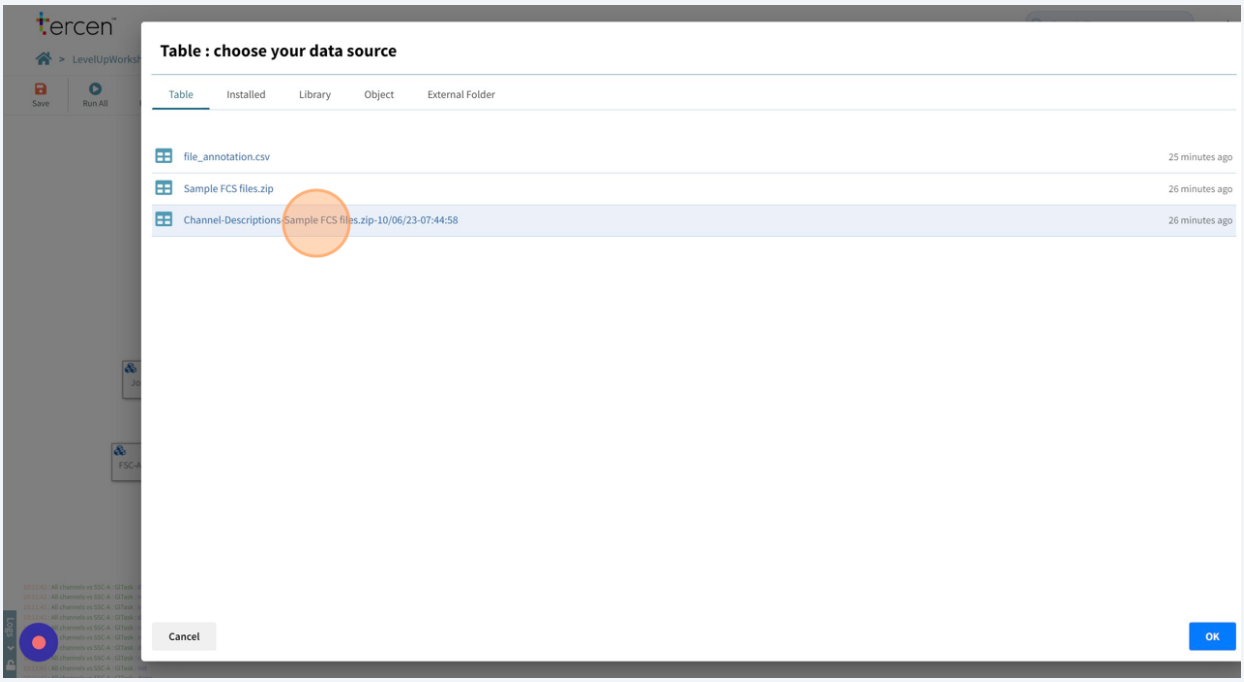


9 Click "Table"

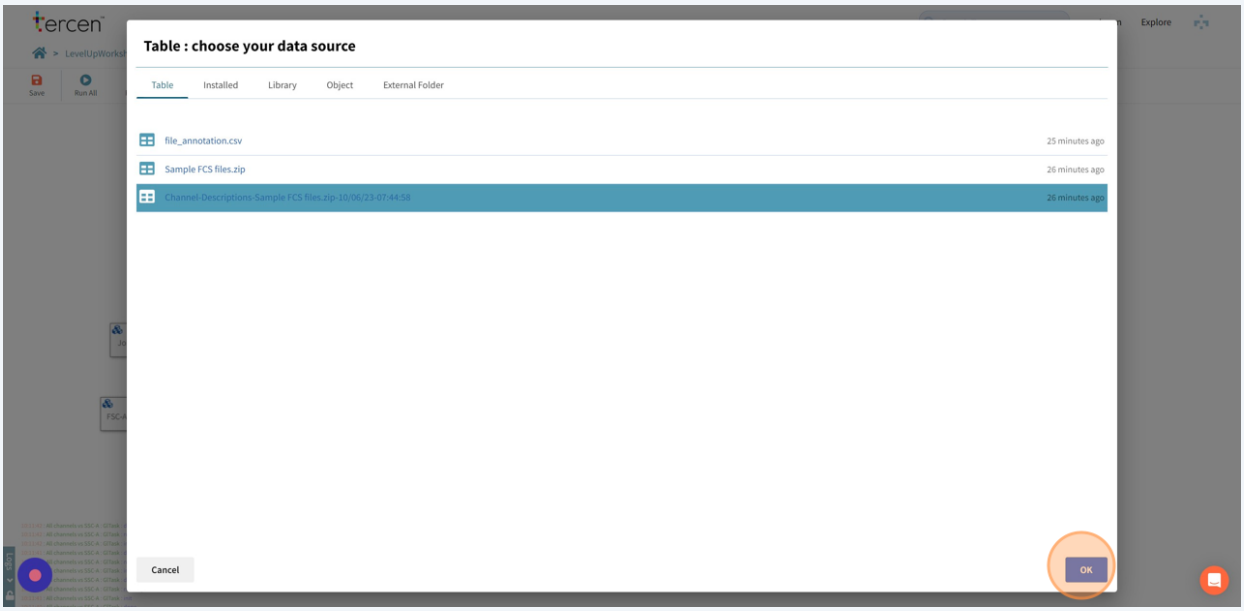


10 Click the file with the prefix 'Channel-Descriptions-Sample FCS files.zip'.

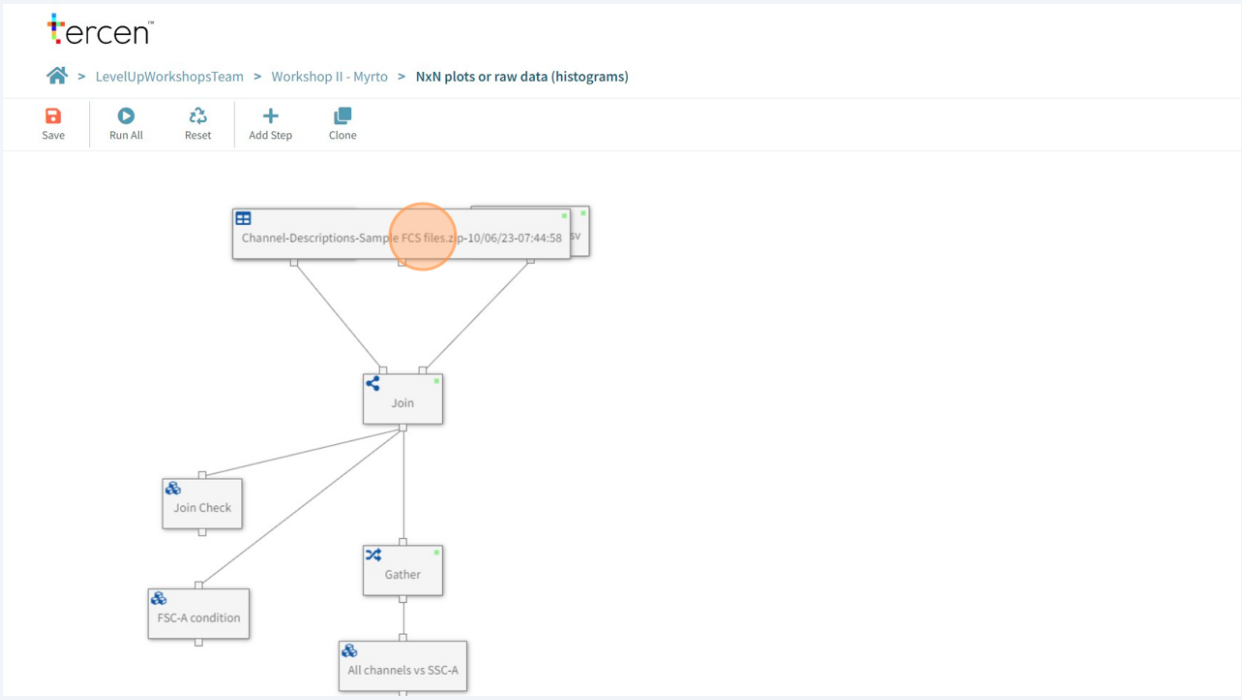
Note that the timestamp suffix will be different for each one of you.



11 Click "OK"

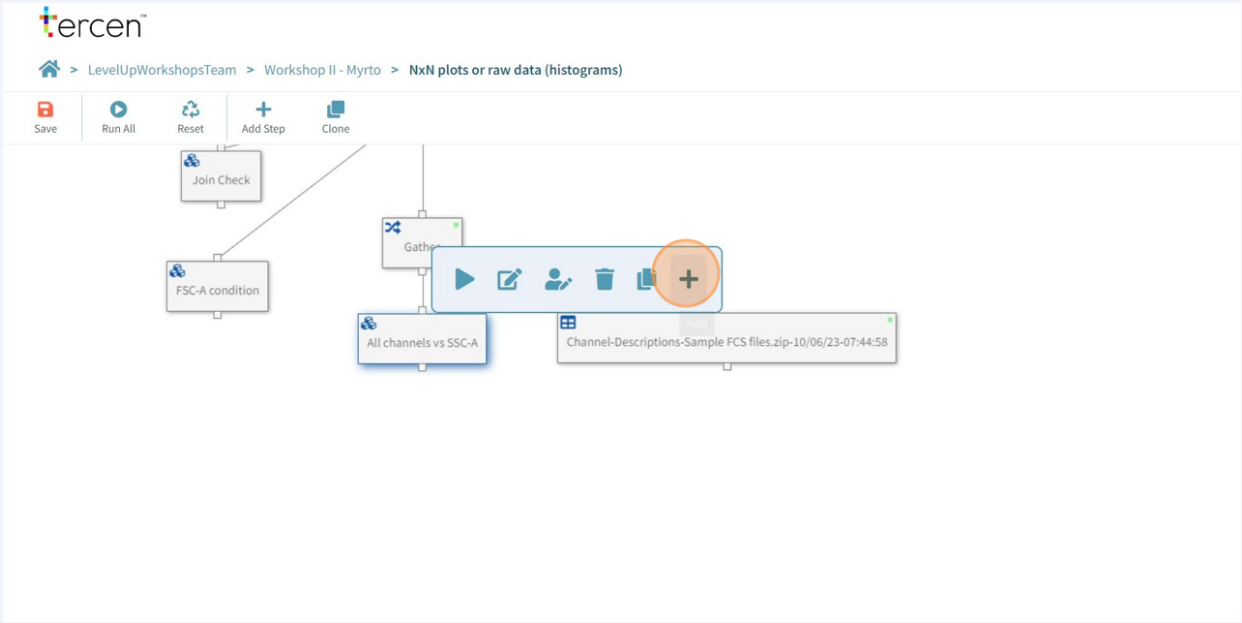


12 Click and drag the table to place it in an empty area of the workflow.

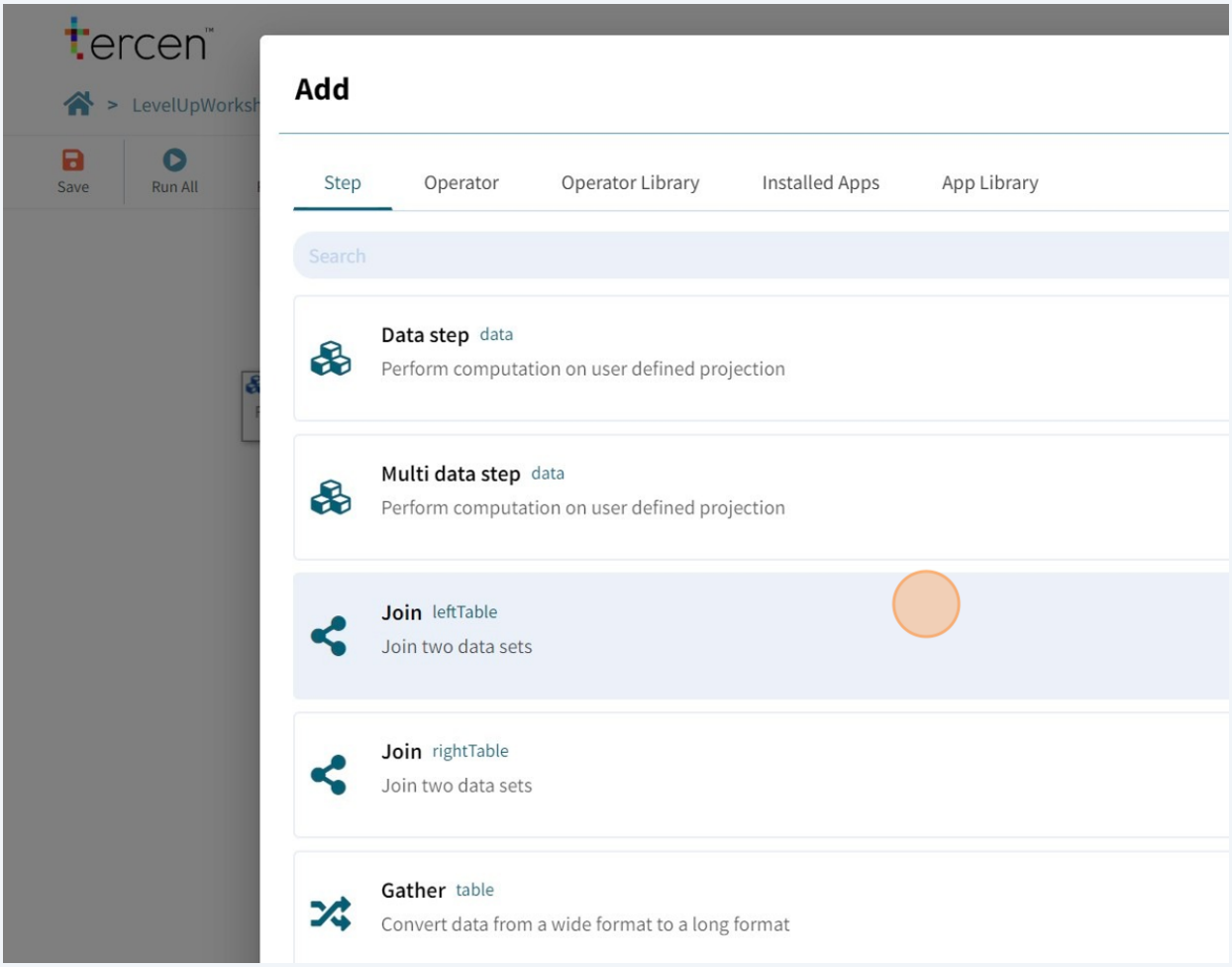


13 Let's do the join.

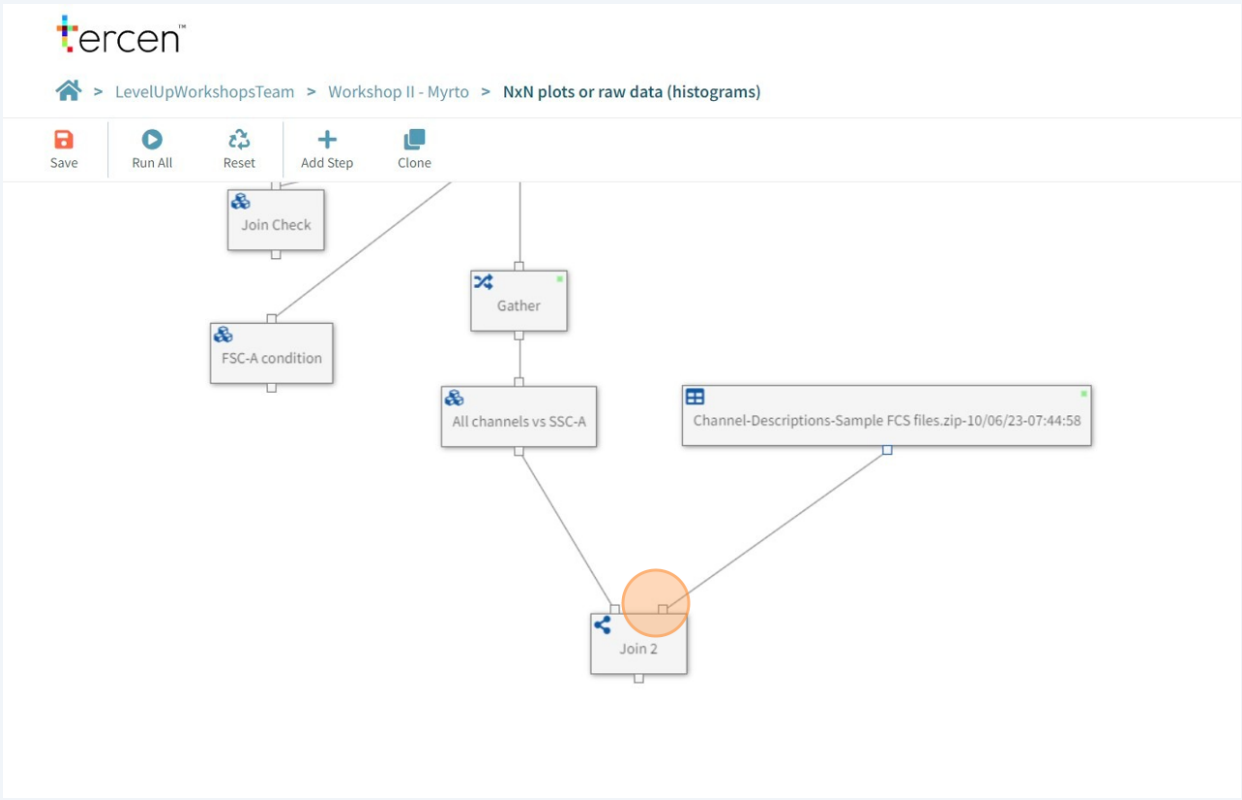
Click the **All channels vs SSC-A** data step to bring up the local toolbar and click "Add".



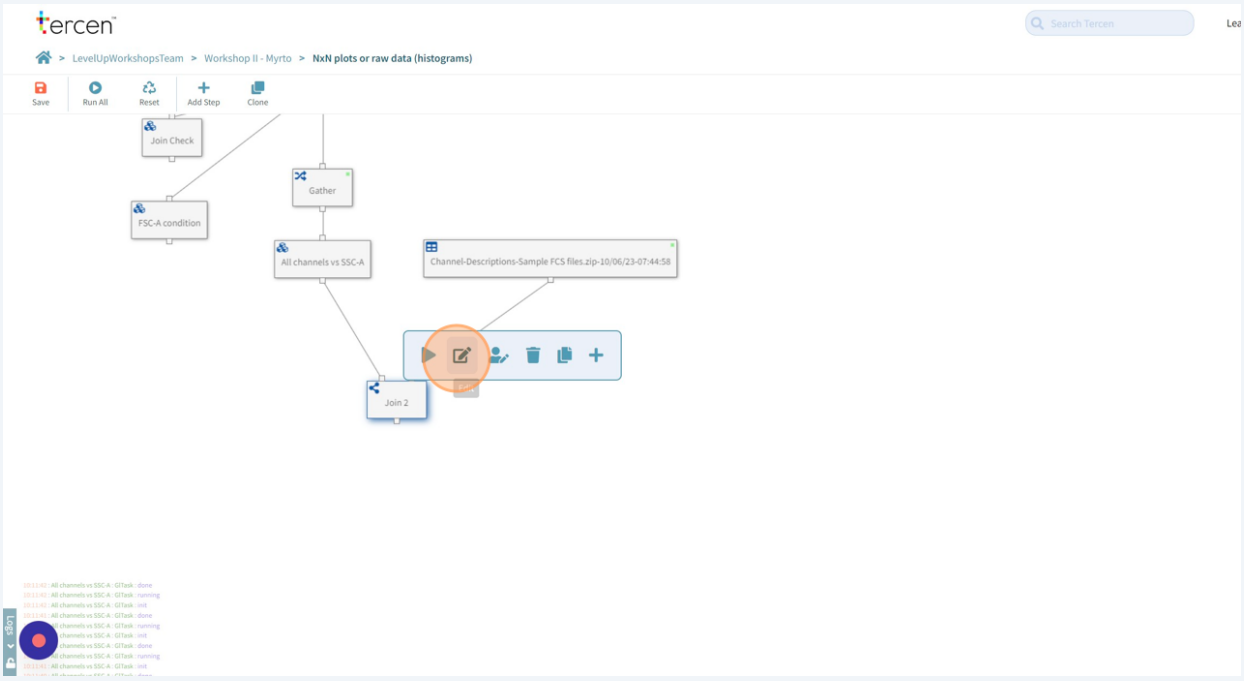
14 Select "Join (left table)".



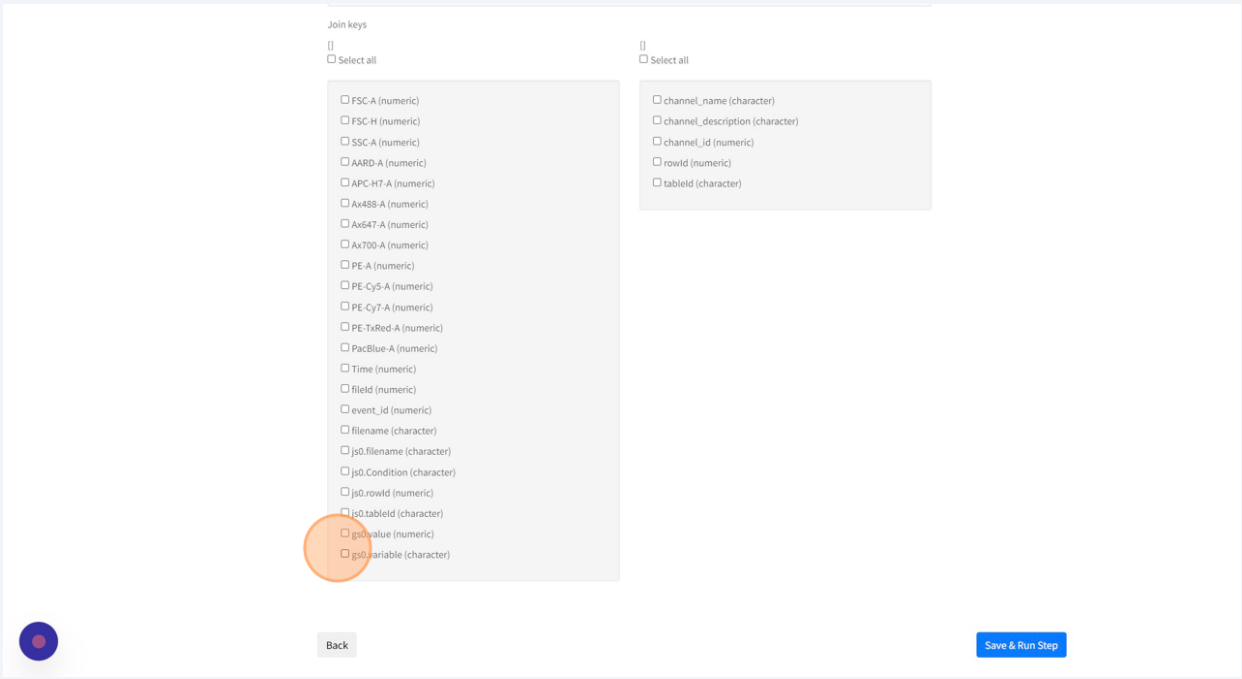
15 Link the **Join 2** step with the table using the bottom node of the table and the free top node of the Join 2 step.



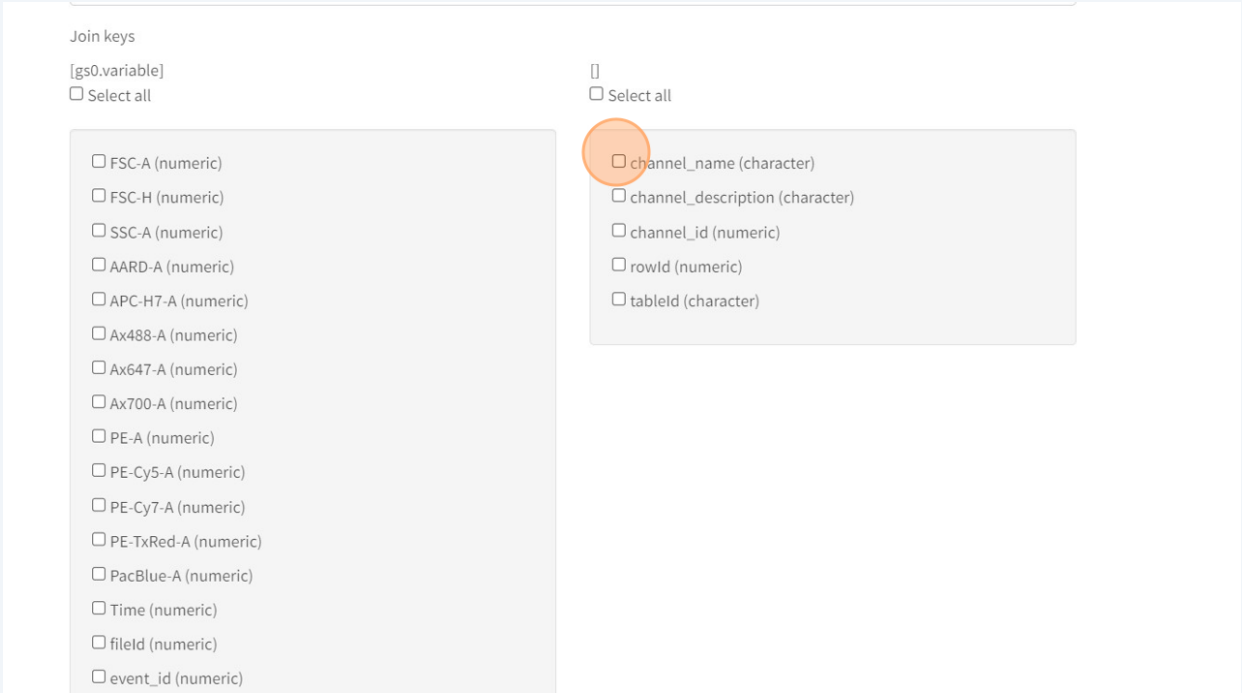
16 Click on the **Join 2** step and from the local toolbar select **Edit**.



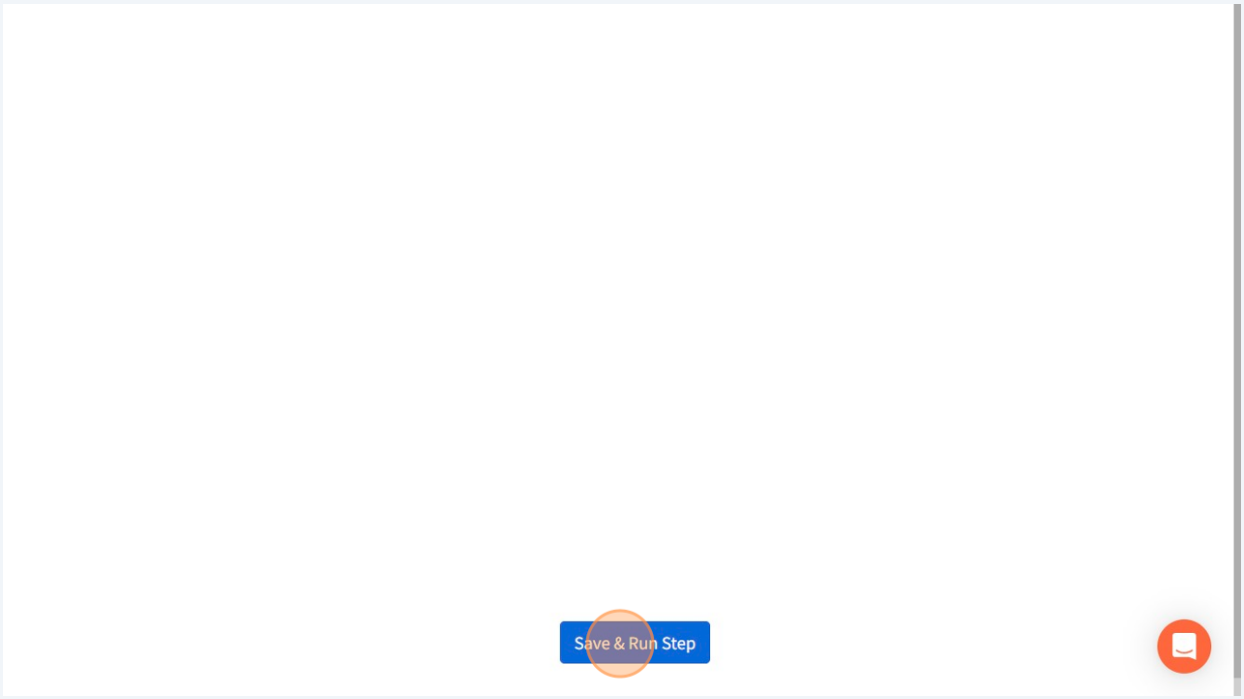
17 Select **gs0.variable (character)** from the left hand side table



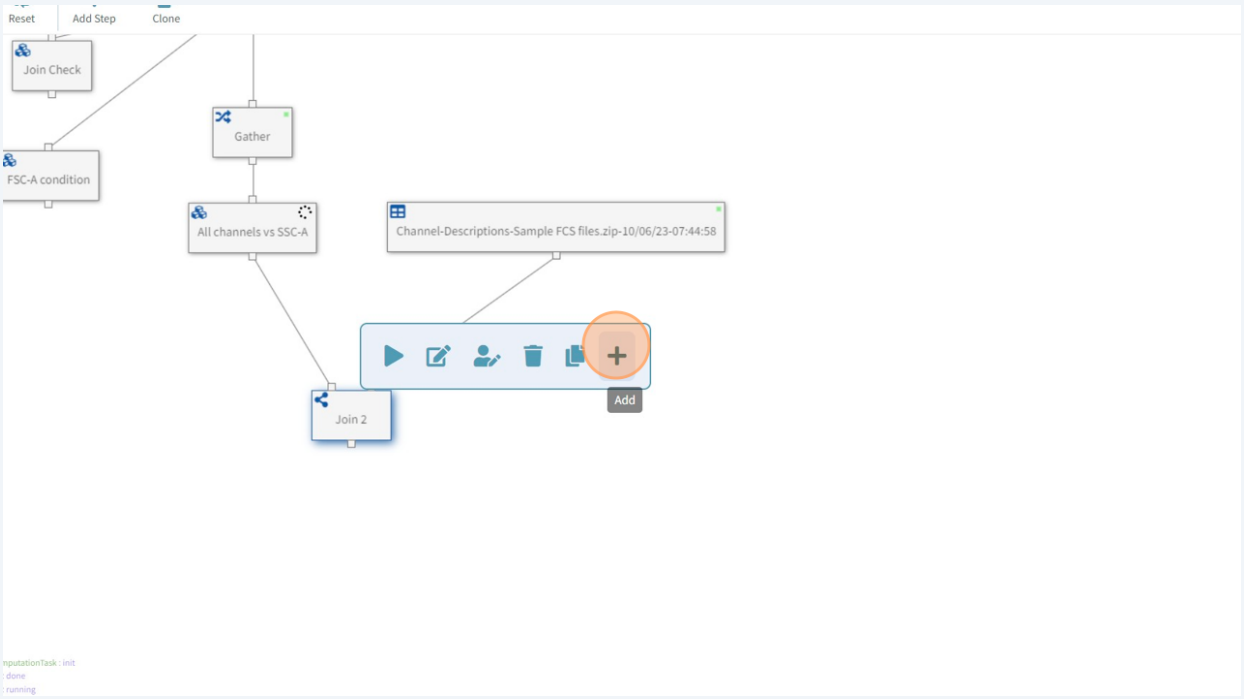
18 And **channel_name (character)** on the right-hand side. These two represent the keys (name of colors used) on which the join will be performed.



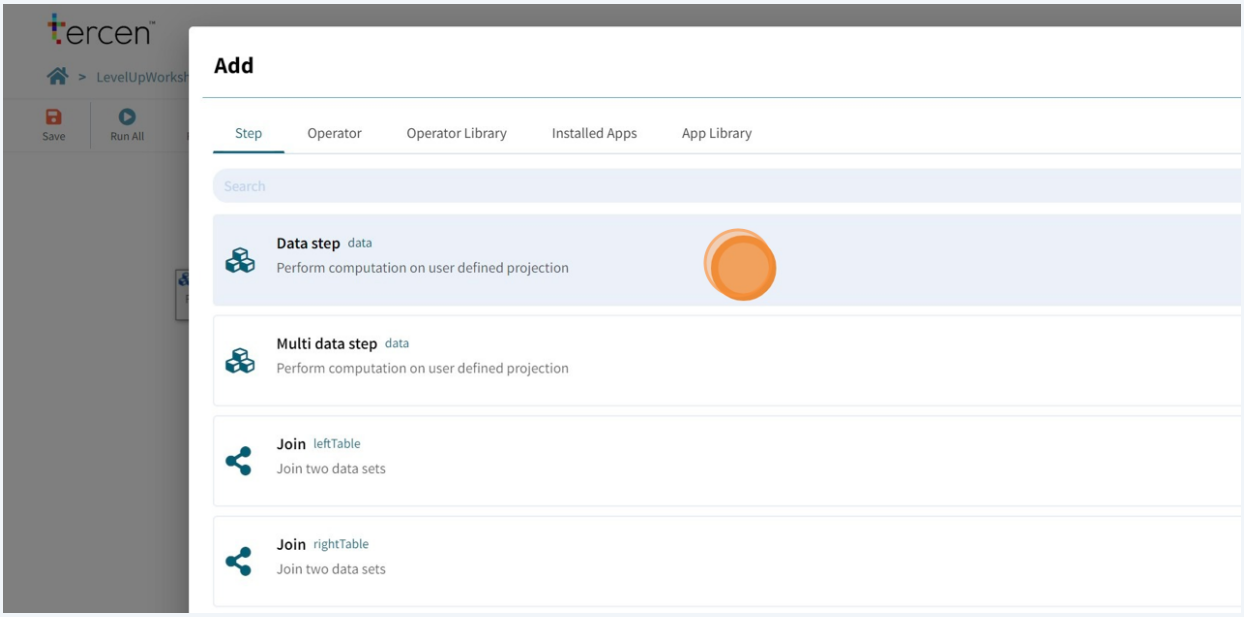
19 Click "Save & Run Step"



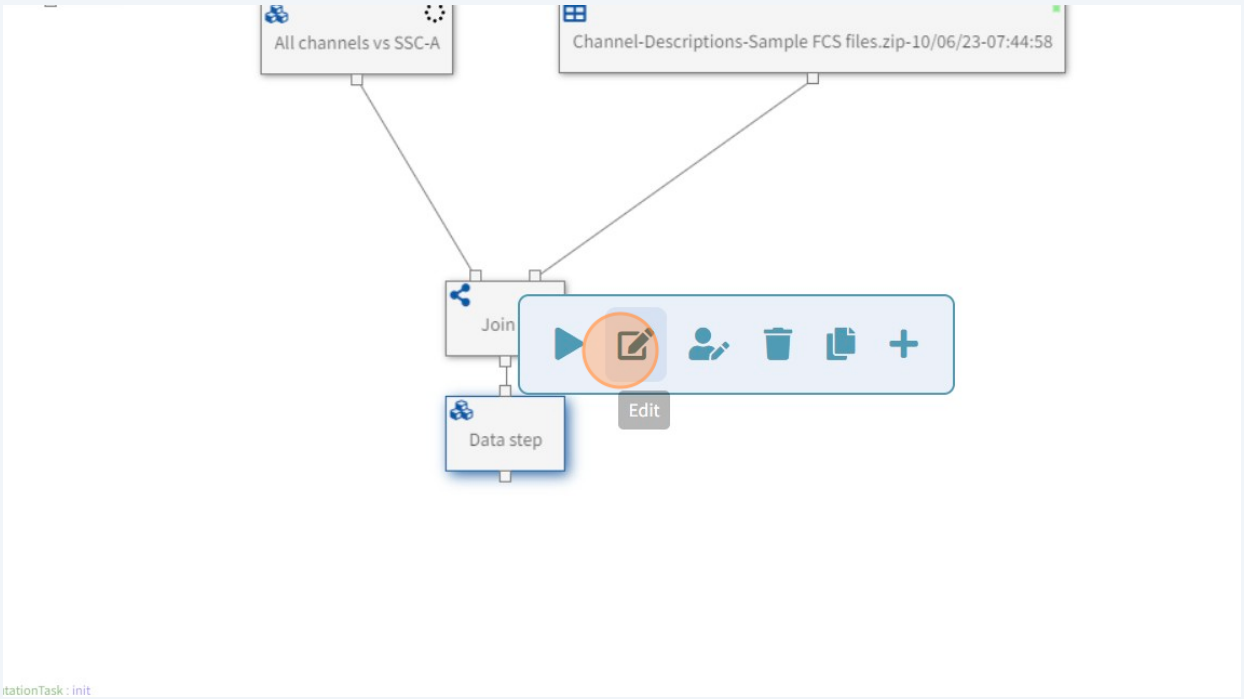
20 Click "Add" on the local toolabr of the **Join 2** step.



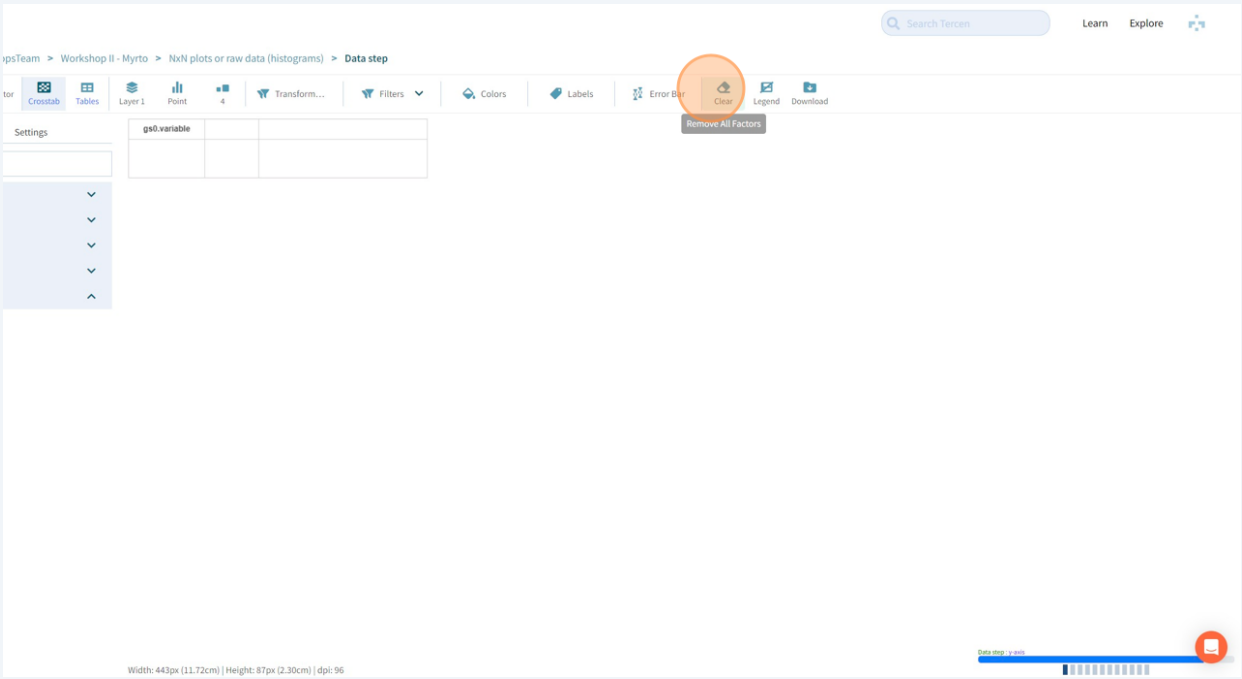
21 Select "Data step"



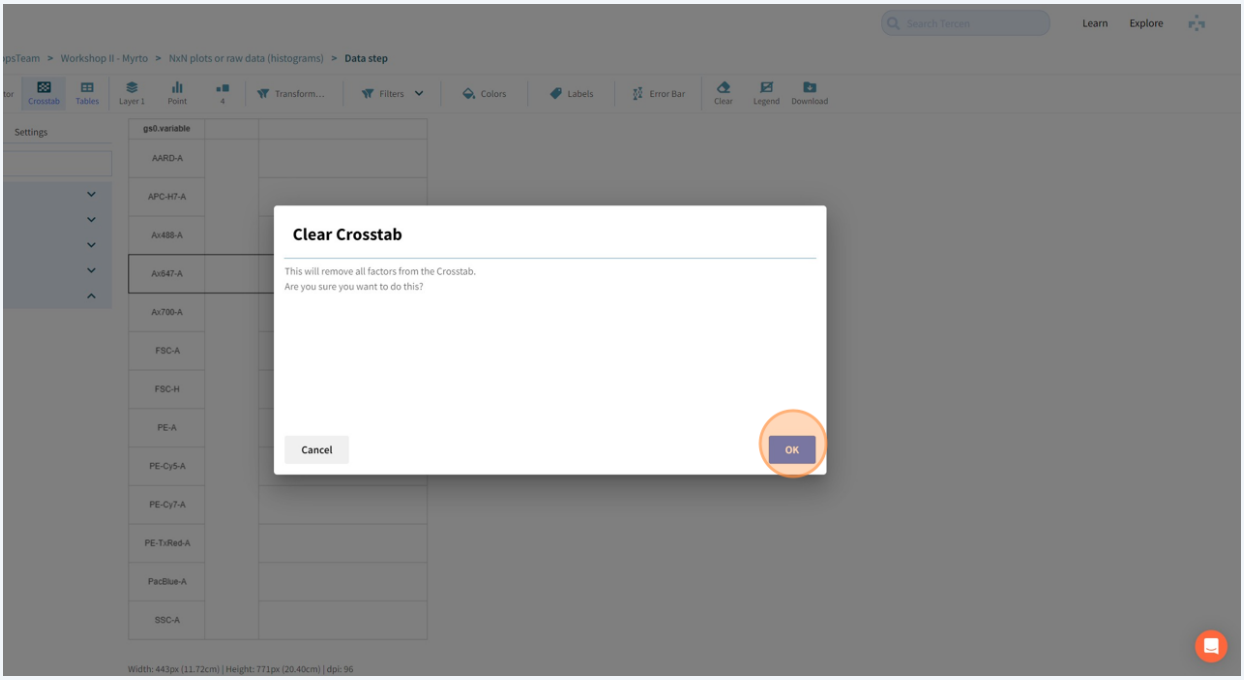
22 Click "Edit"



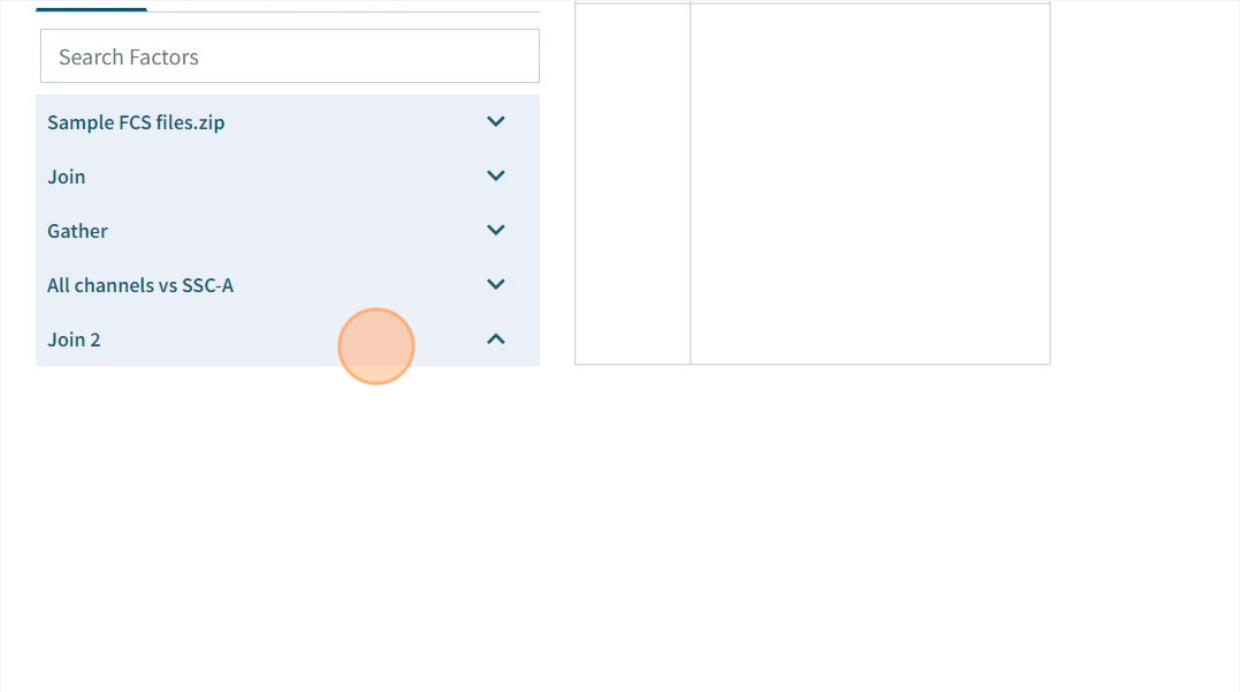
23 Clear any pre-defined data projections by clicking the "Clear" button on the toolbar.



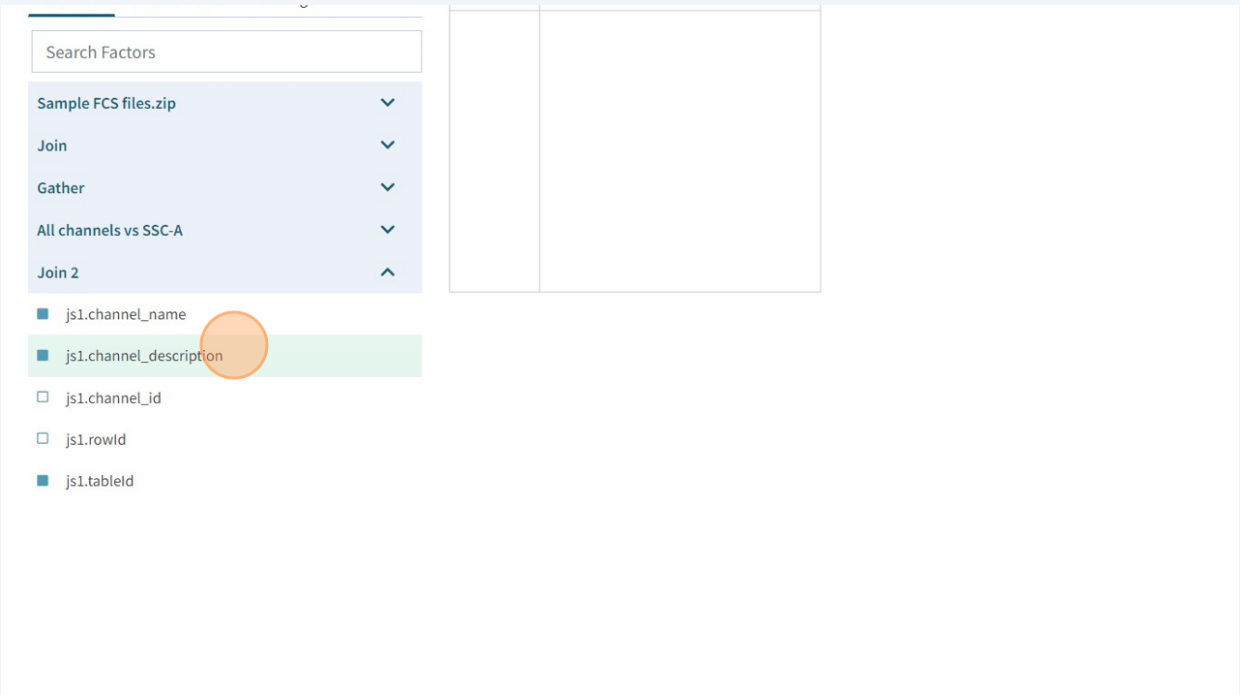
24 Click "OK"



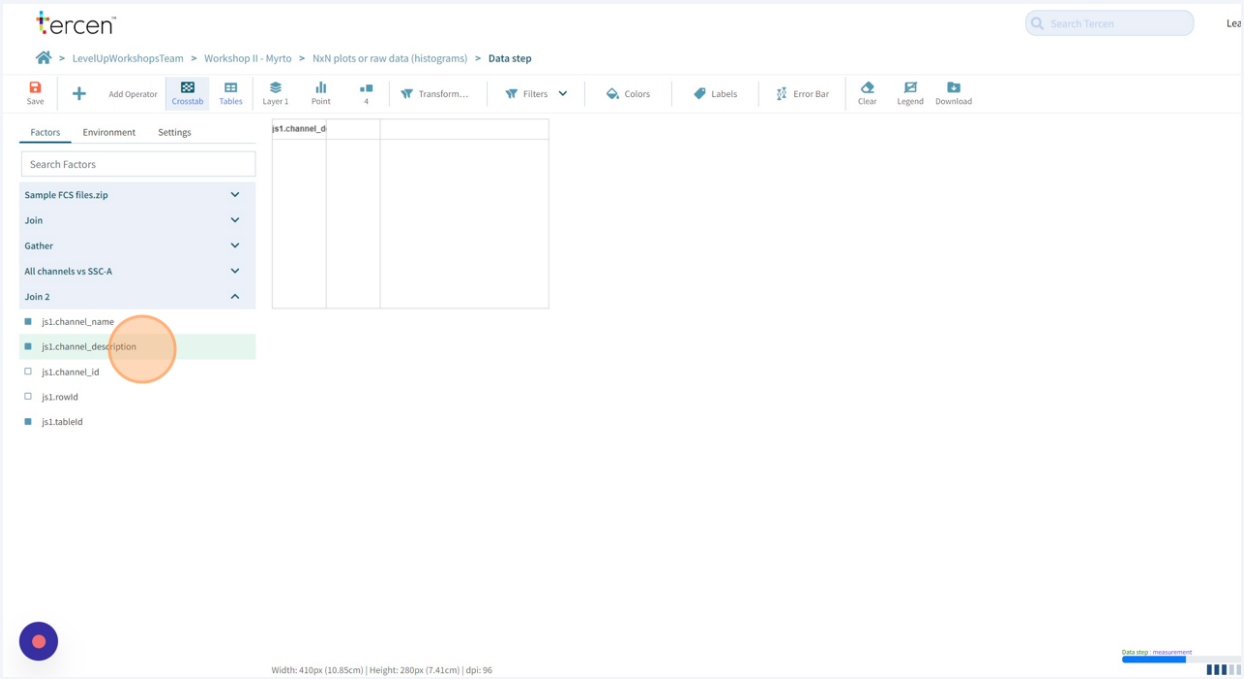
25 Expand the "Join 2" factors by clicking on it on the Factors list.



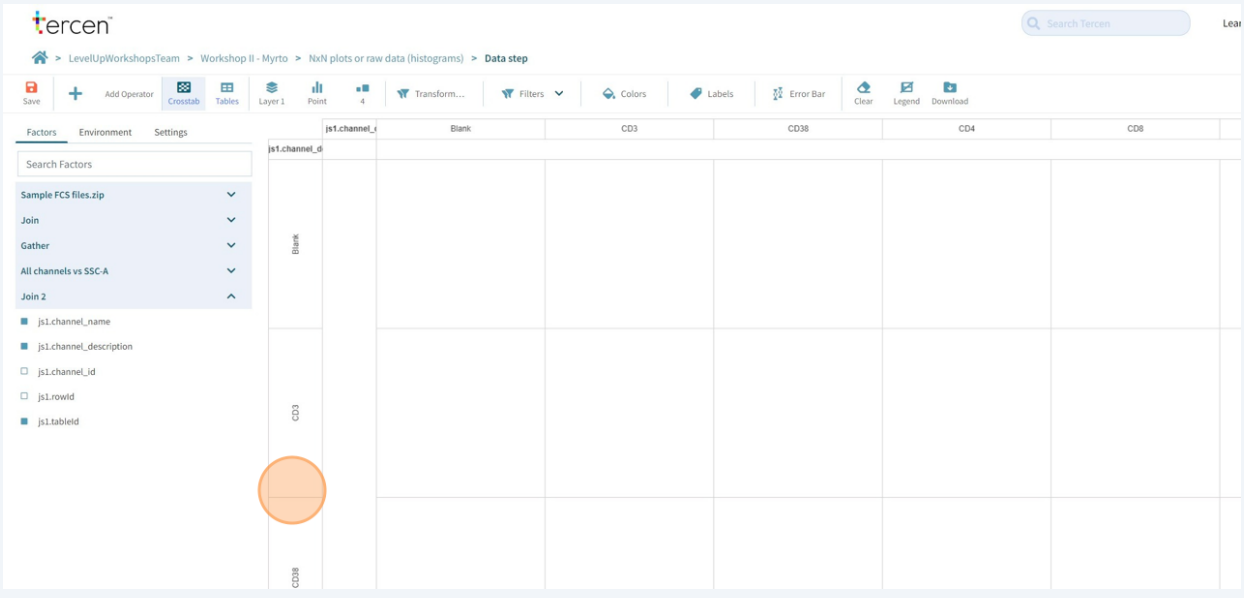
26 Drag and drop the **js1.channel_description** factor to Row.



27 Again, drag and drop the **js1.channel_description** factor to Column this time.

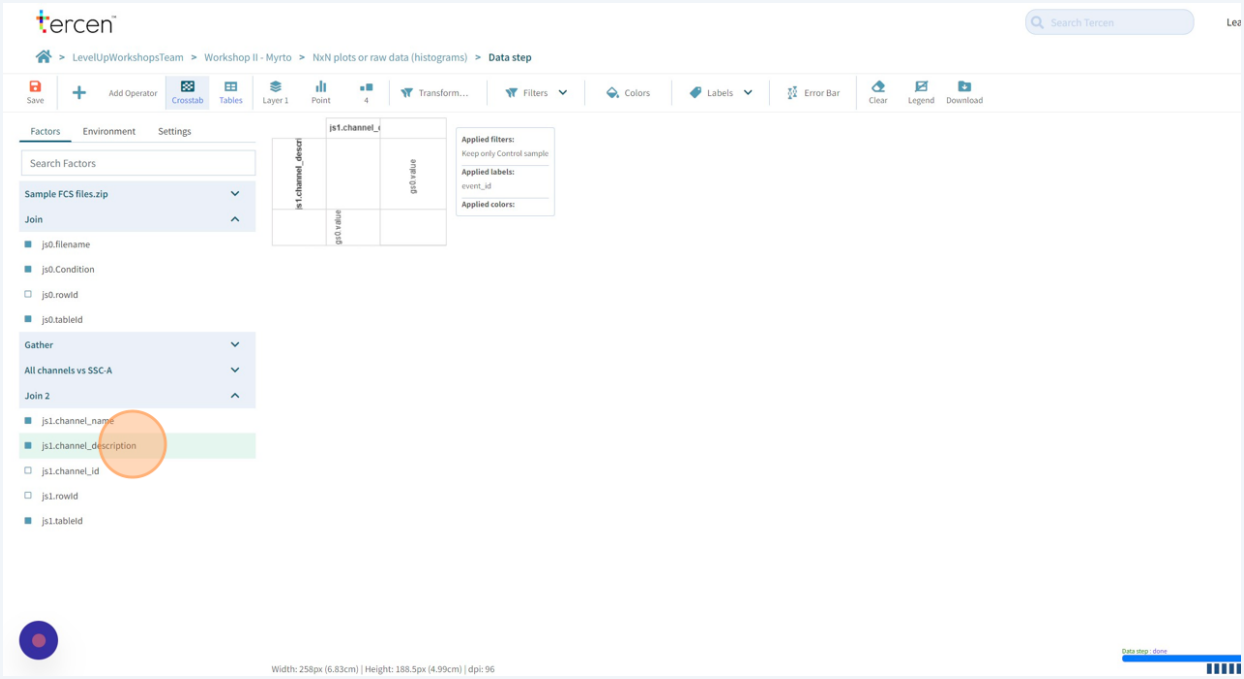


28 Adjust the height of rows & columns, as desired.



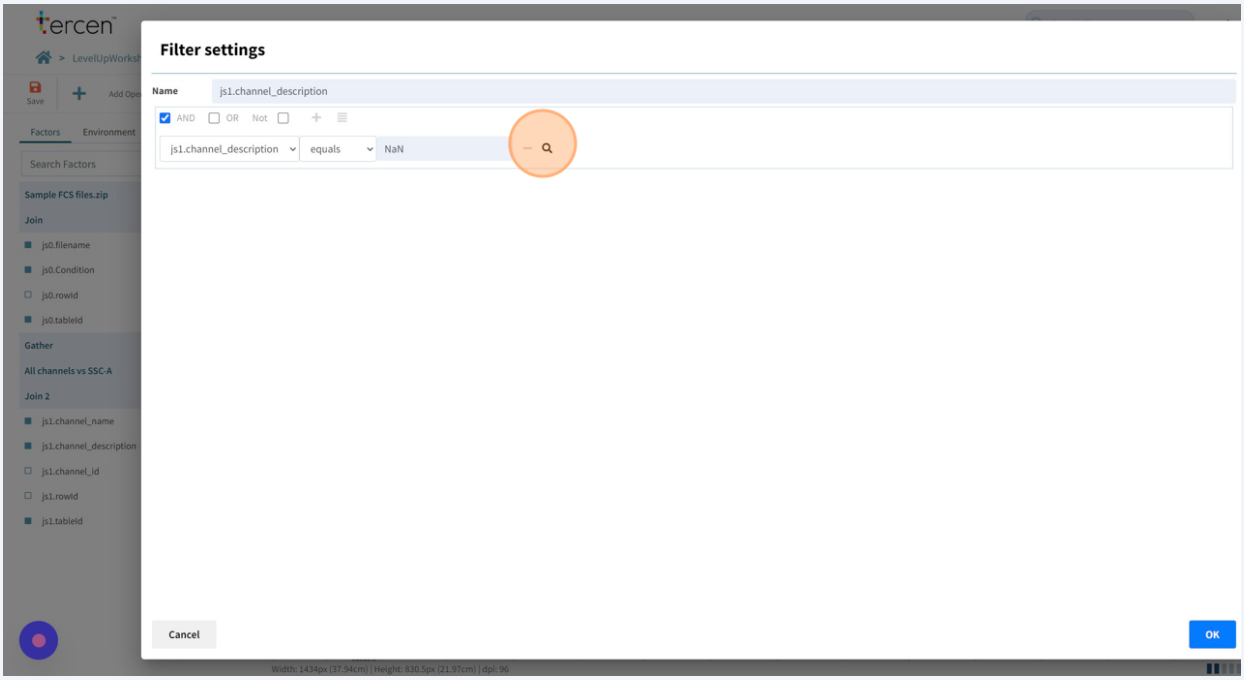
29 We will now set up a filter based on the names of the markers to filter down the markers for which the NxN plot will be generated.

Drag and drop the **js1.channel_description** factor to the Filters area.

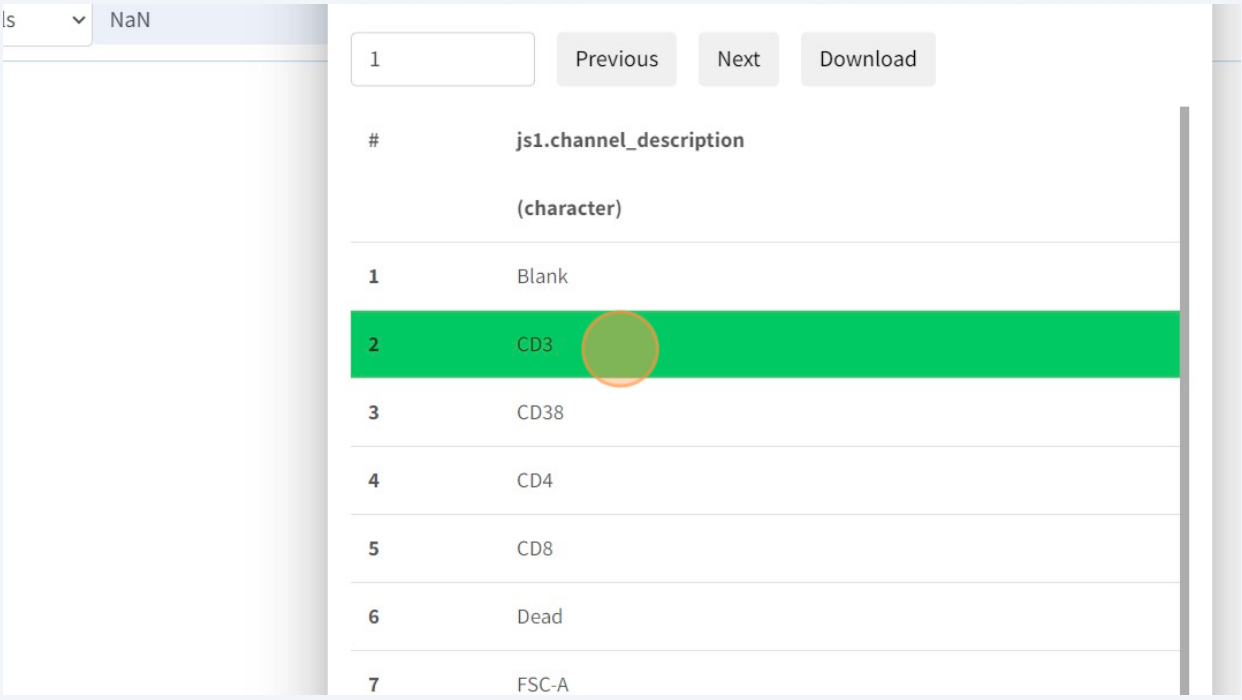


30 On the popup window, we will build a boolean filter to select a subset of the available markers. For our example, we will choose CD3, CD4 and CD8.

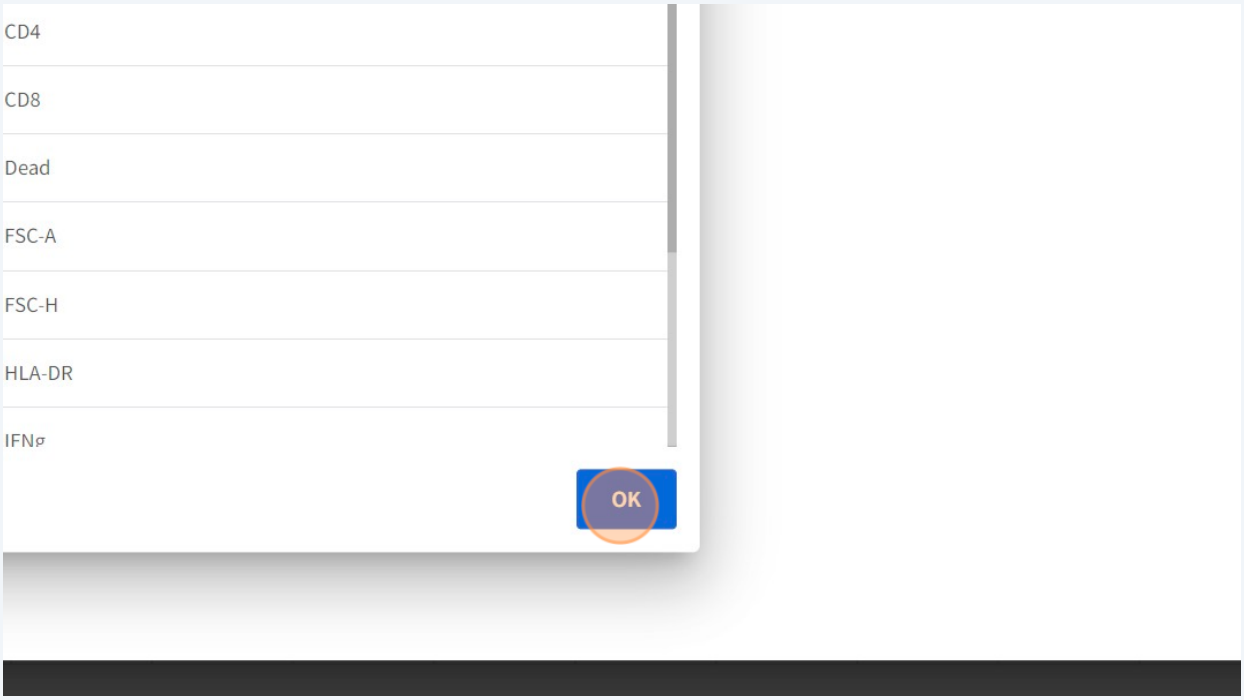
We start building the filter by clicking on the magnifying glass to select the desired marker from the list.



31 Click "CD3"

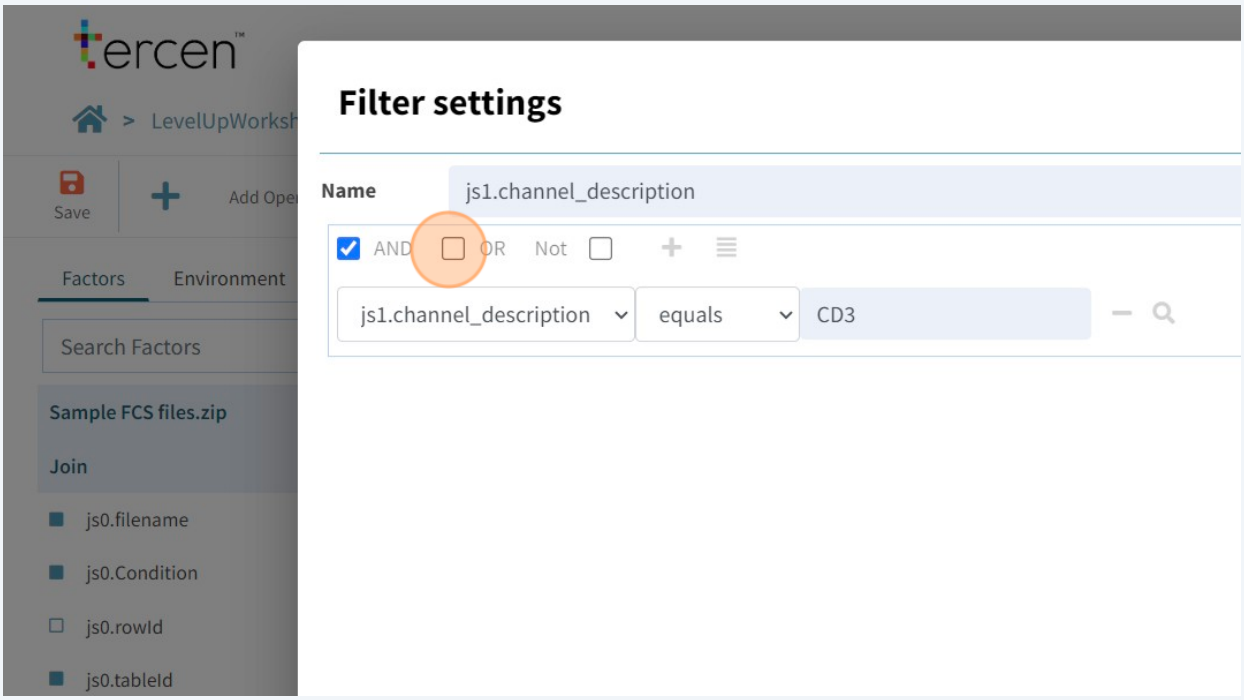


32 Click "OK"

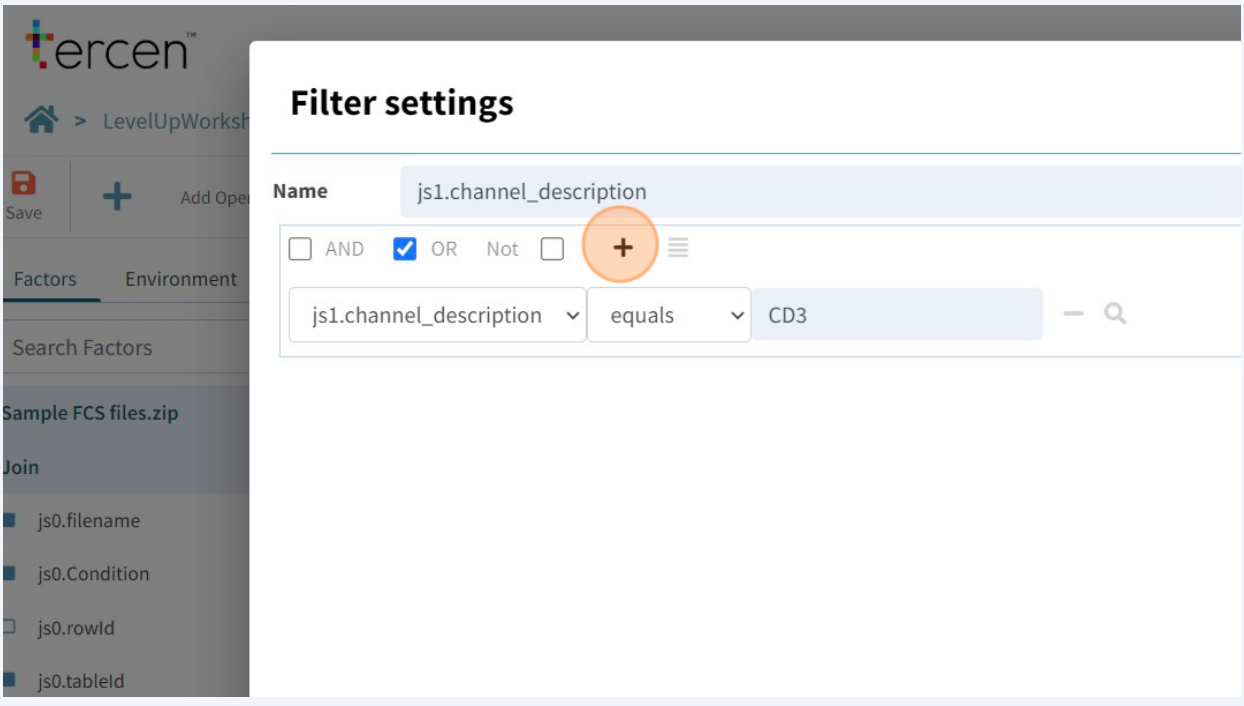


33 Now, on to expanding the filter to add the other two markers.

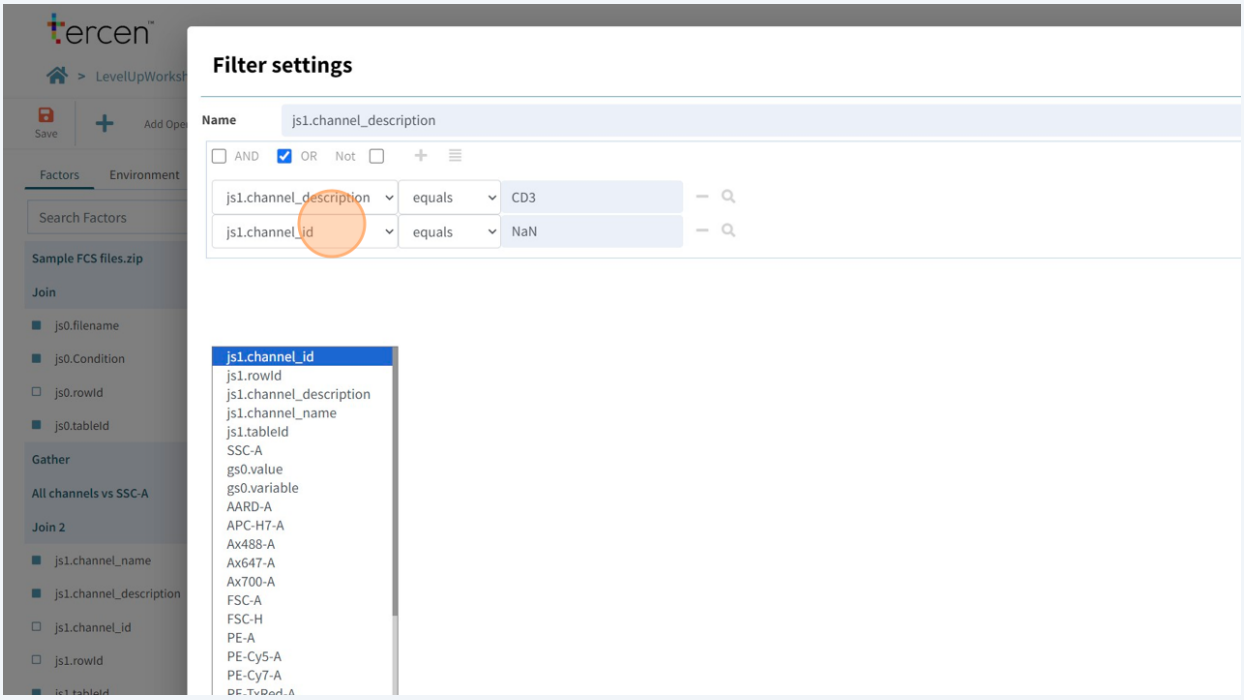
Select 'OR'.



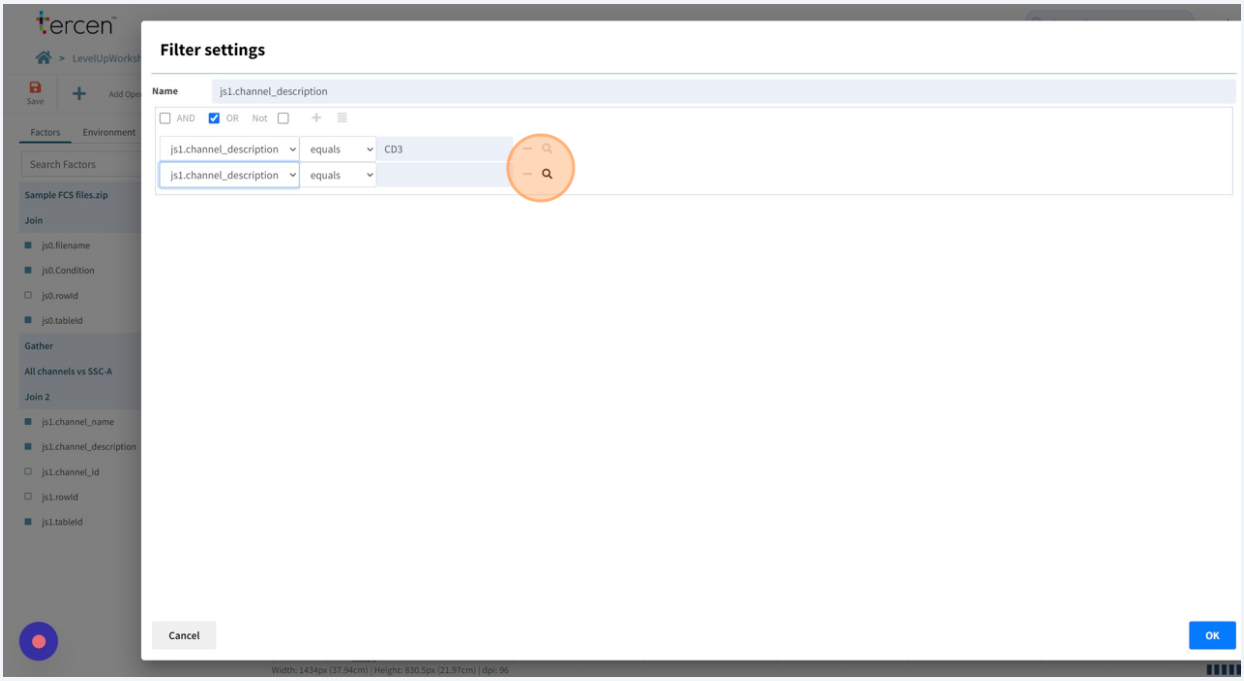
34 Click the '**+**' button.



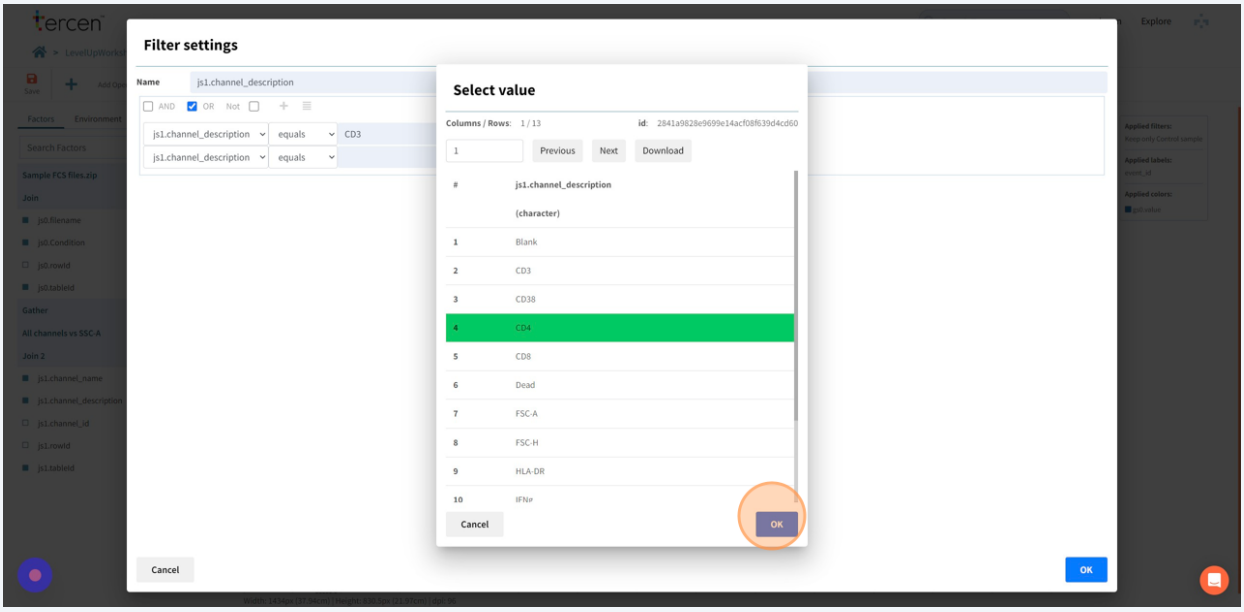
35 Change the default factor to **js1.channel_description** from the dropdown menu.



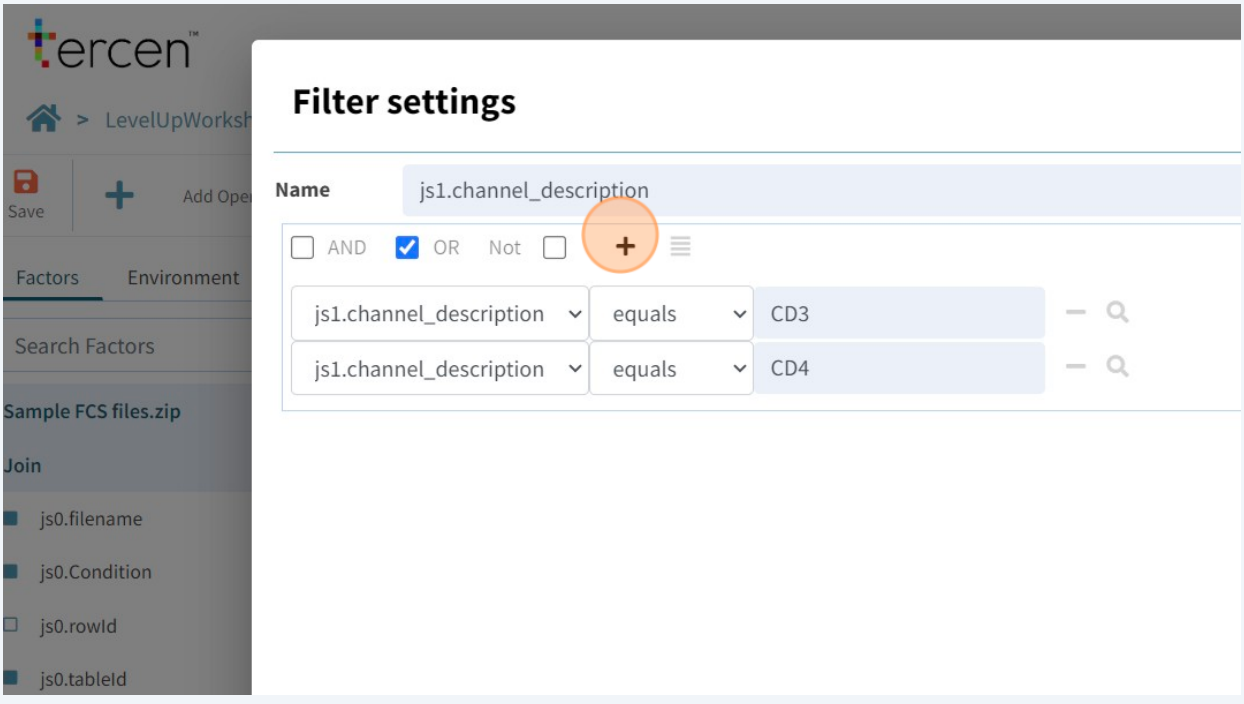
36 Click on the magnifying glass again.



37 Select "CD4" and then click 'OK'.

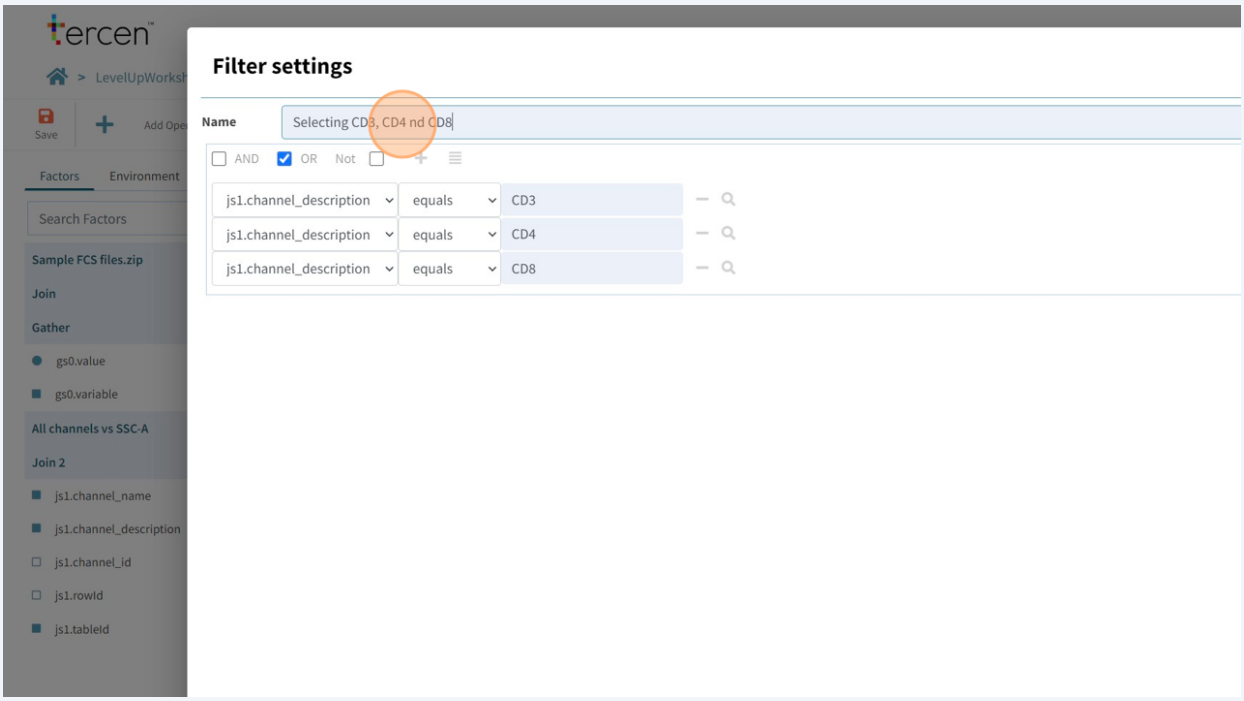


38 Repeat these steps to add the "CD8" marker to the filter.

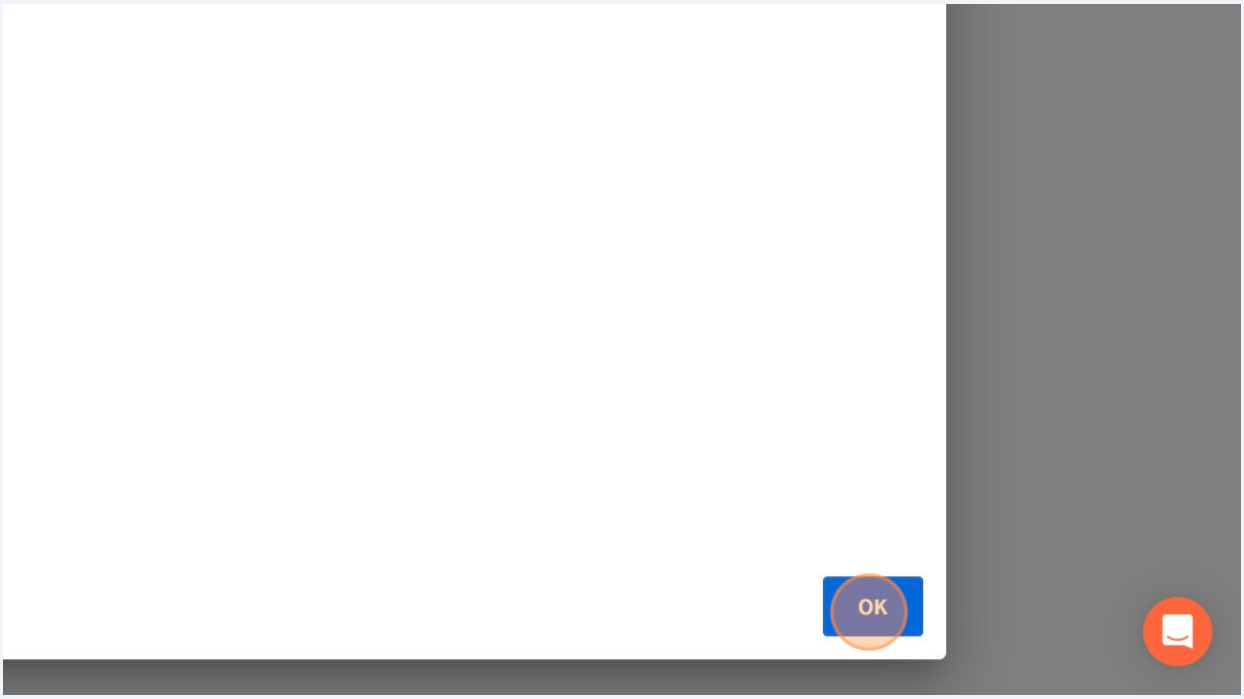


39 Once you have added all desired markers to the filter, we will edit its name.

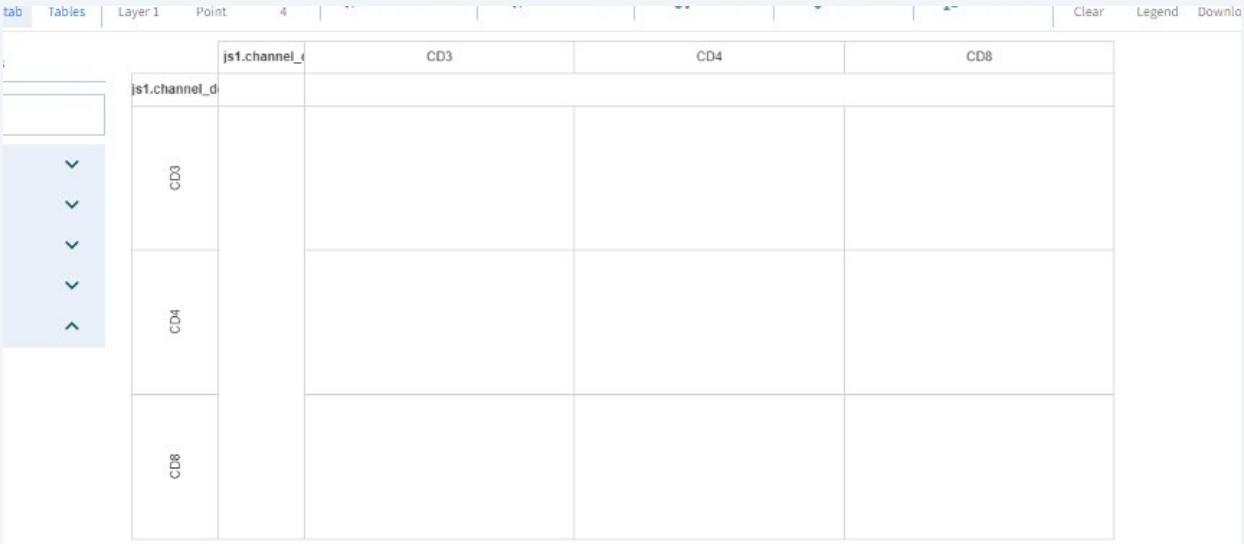
Type "Selecting CD3, CD4 and CD8" in the relevant text box.



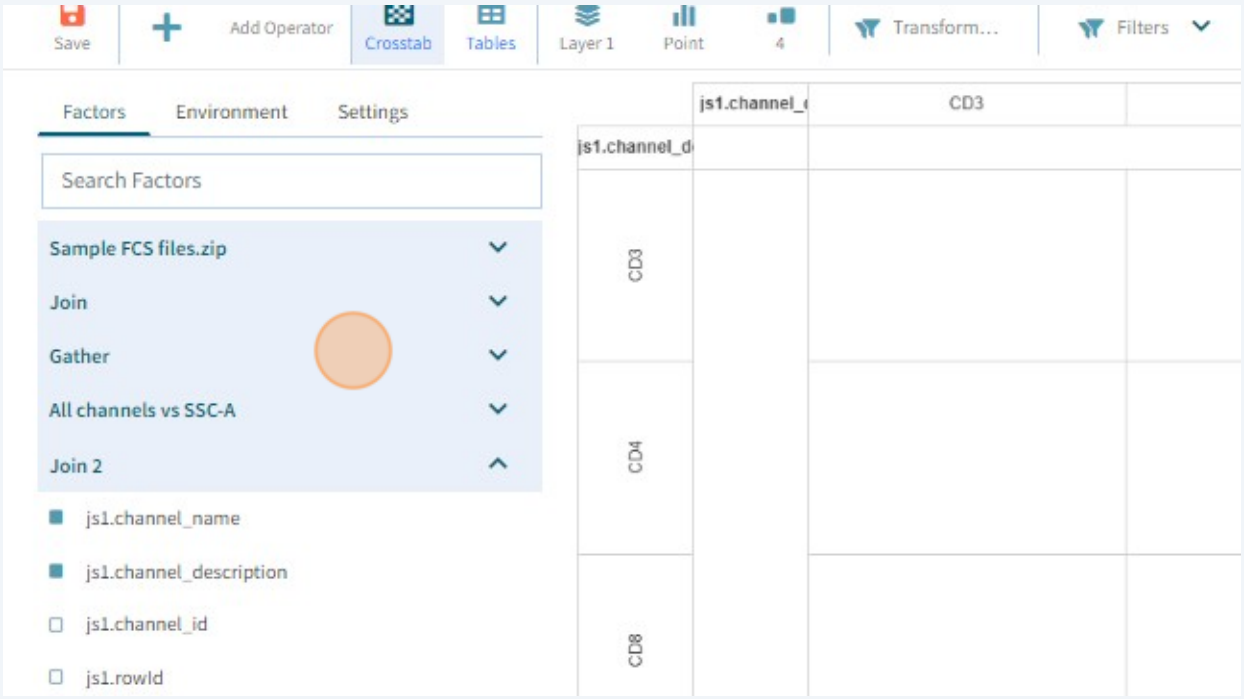
40 Click "OK"



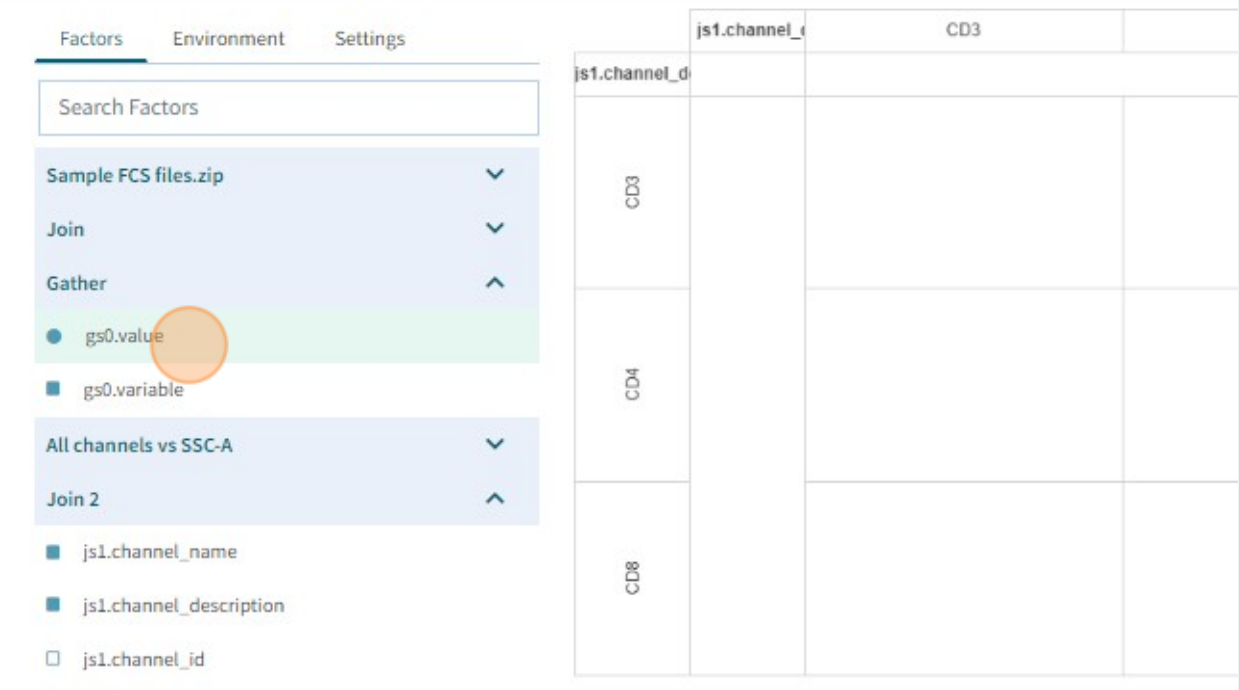
41 Notice how the row & columns now contain only the three selected markers.



42 Click "Gather"



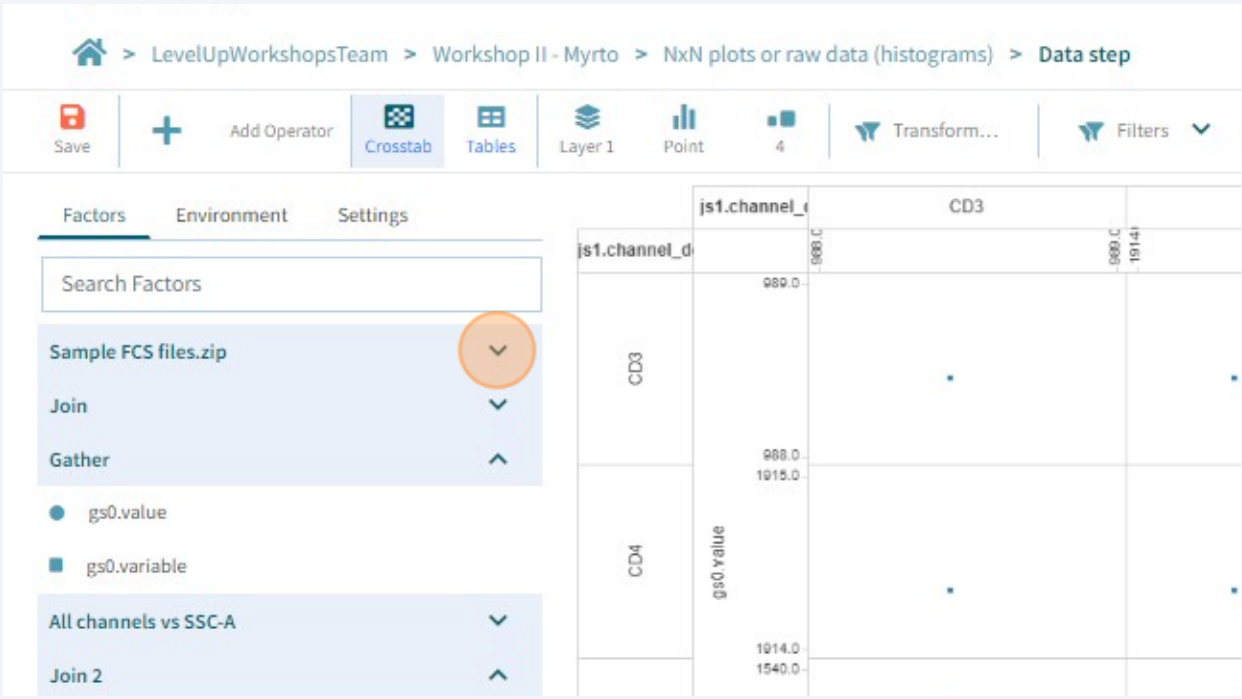
43 Drag and drop the **gs0.value** factor to the Y-axis to project the marker measurements.



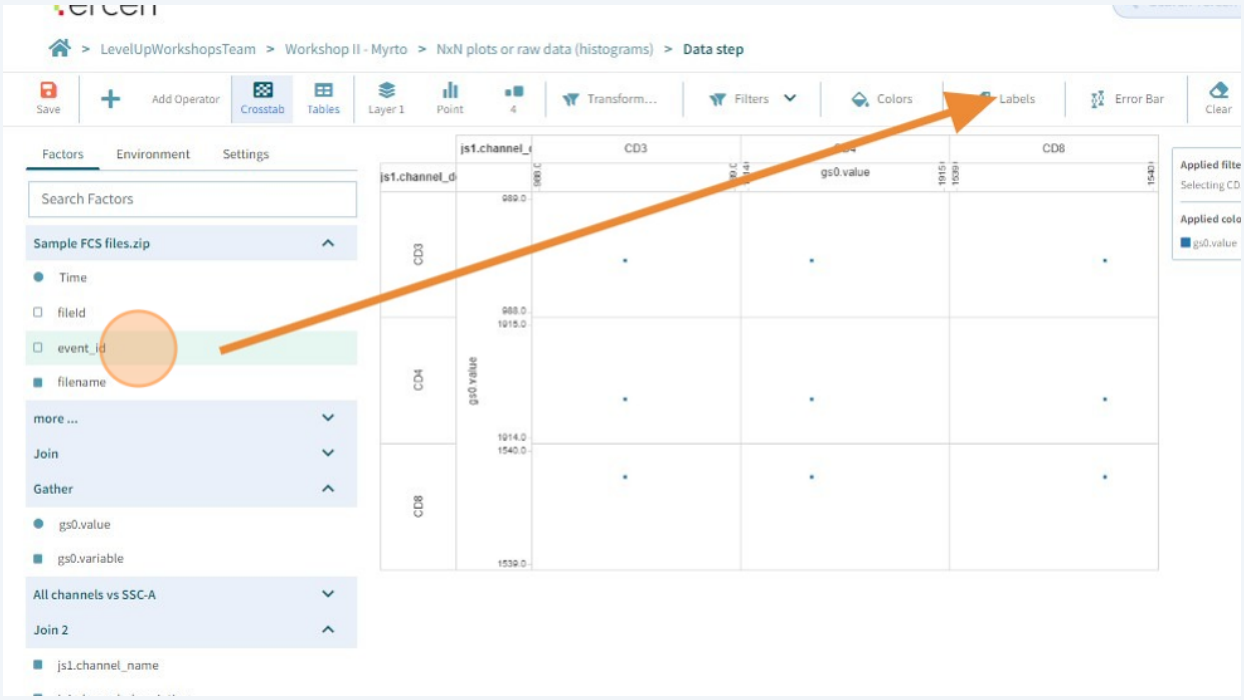
Note that for pairwise plots, like the NxN plots we are building, Tercen also adds the gs0.value to the X-axis.

You will also notice only one data point appears in each data cell. We require one last action to complete the pairwise plot.

44 Click 'Sample FCS files.zip' to expand the factors listed under it.



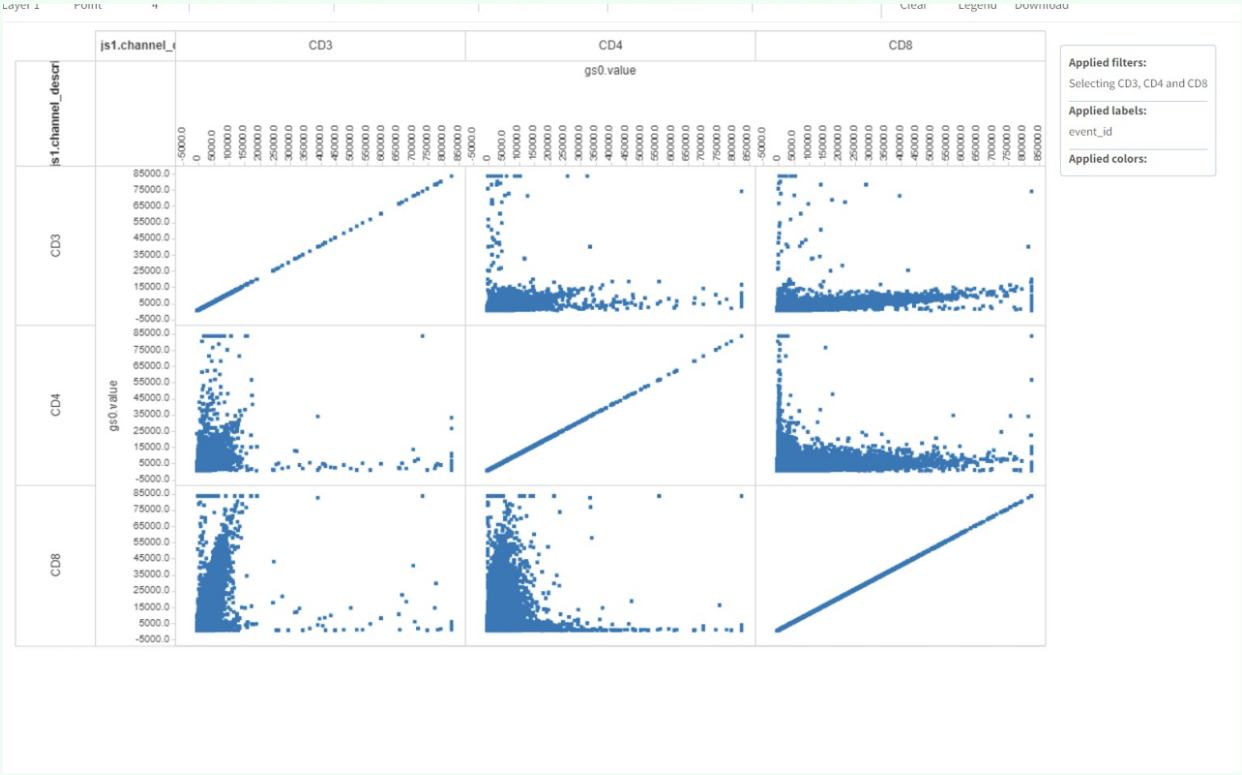
45 Drag the **event_id** factor and drop it on 'Labels'.



You have just created a pairwise plot in Tercen. Every dot is an event from the uploaded .FCS file(s).

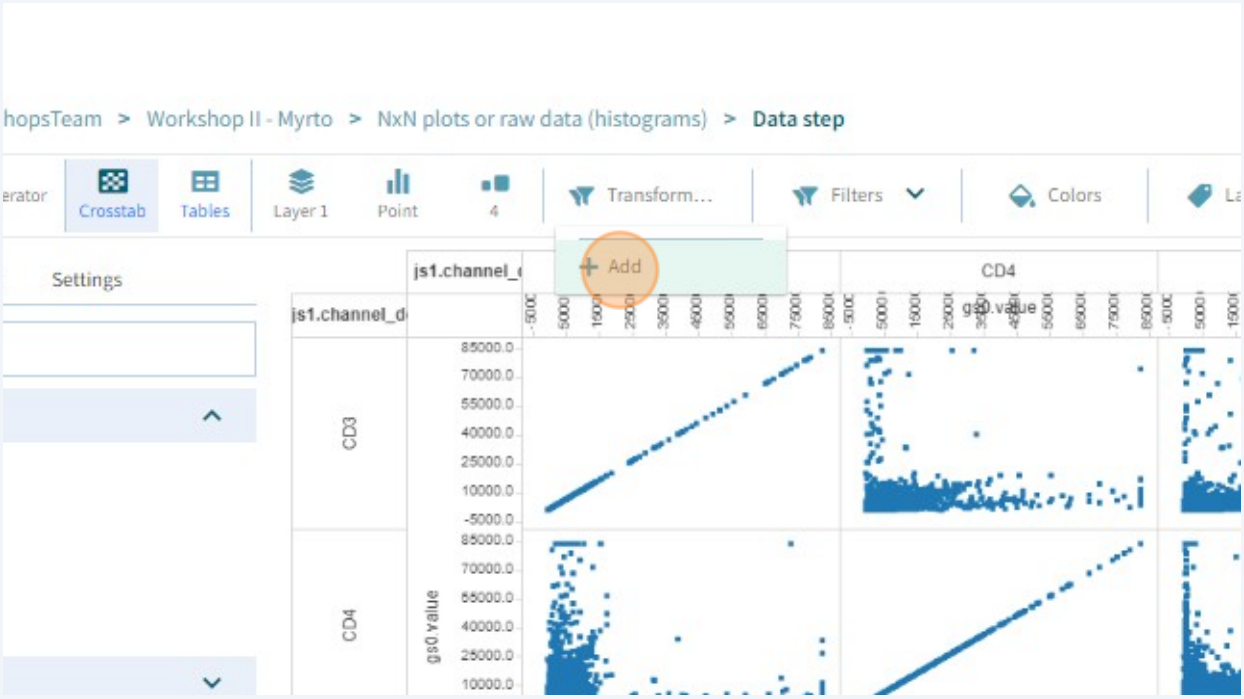
It is far more useful though to create a summary of the data rather than looking at individual data points, where in many cases one will be falling on top of the other when they have similar values.

We will therefore display histograms, which provide a count summary on an interval scale and help illustrate the major features of the data distribution.

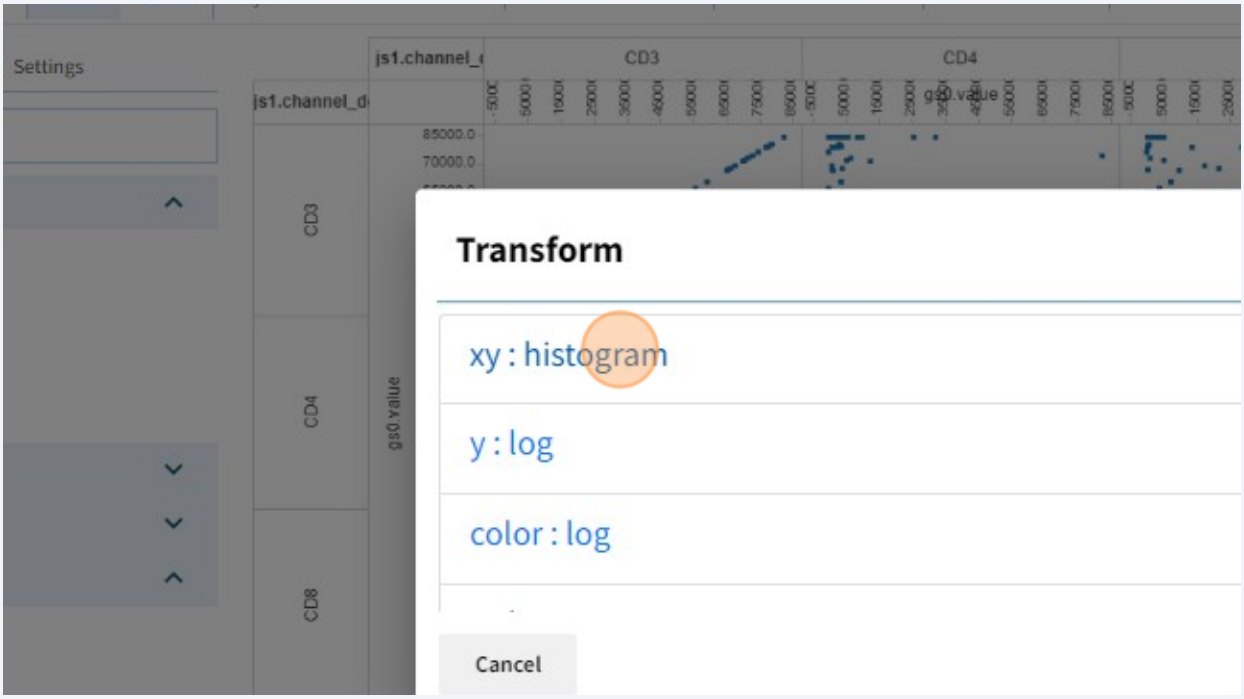


46 To transform the values visualized on the fly Tercen includes a "Transformation" tab on the toolbar.

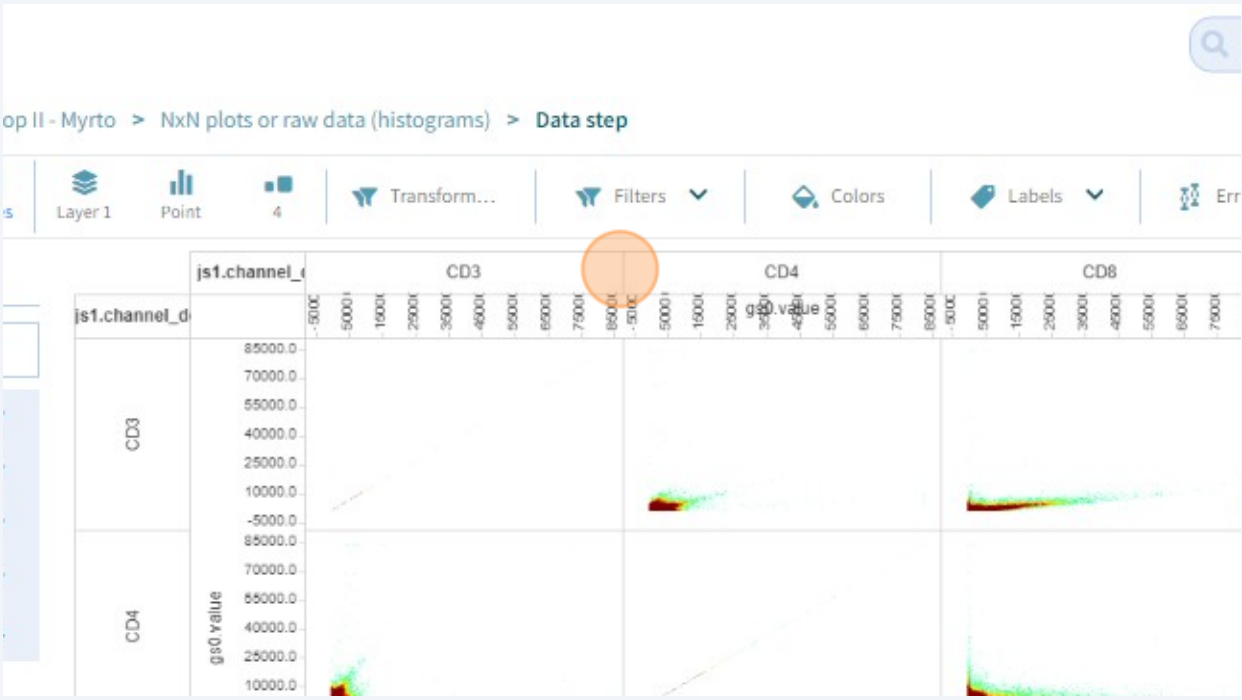
Mouse over "Transform" and click "Add".



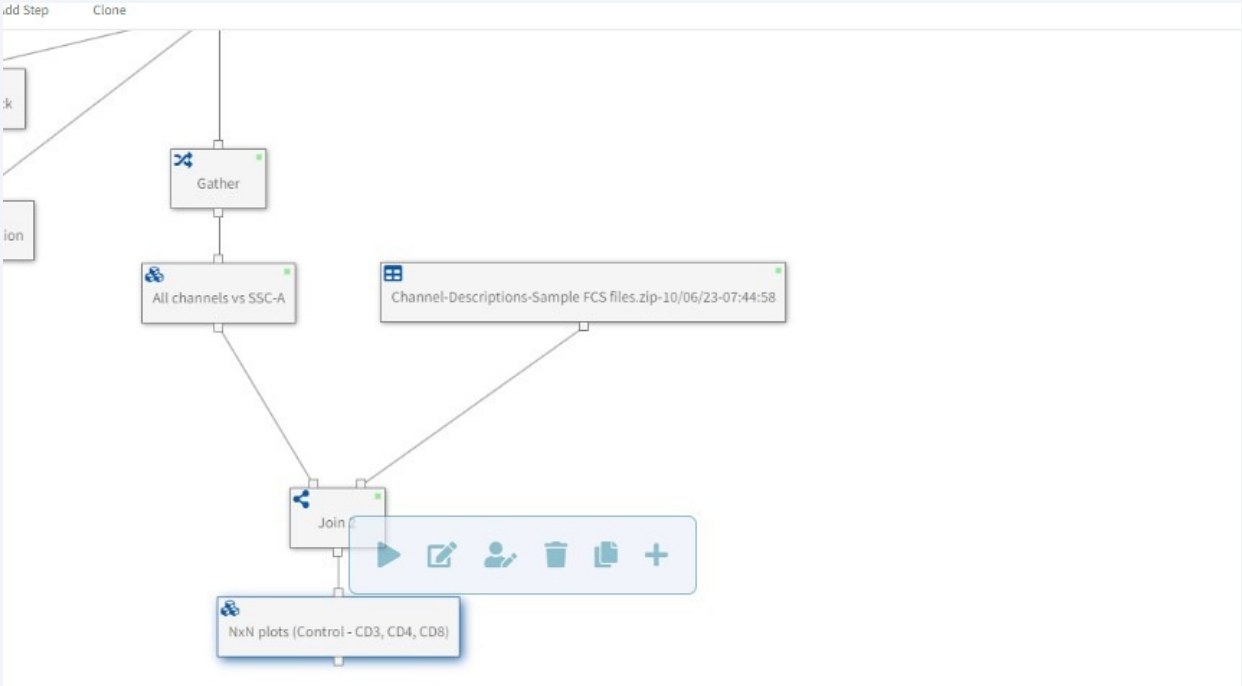
47 Select "xy : histogram"



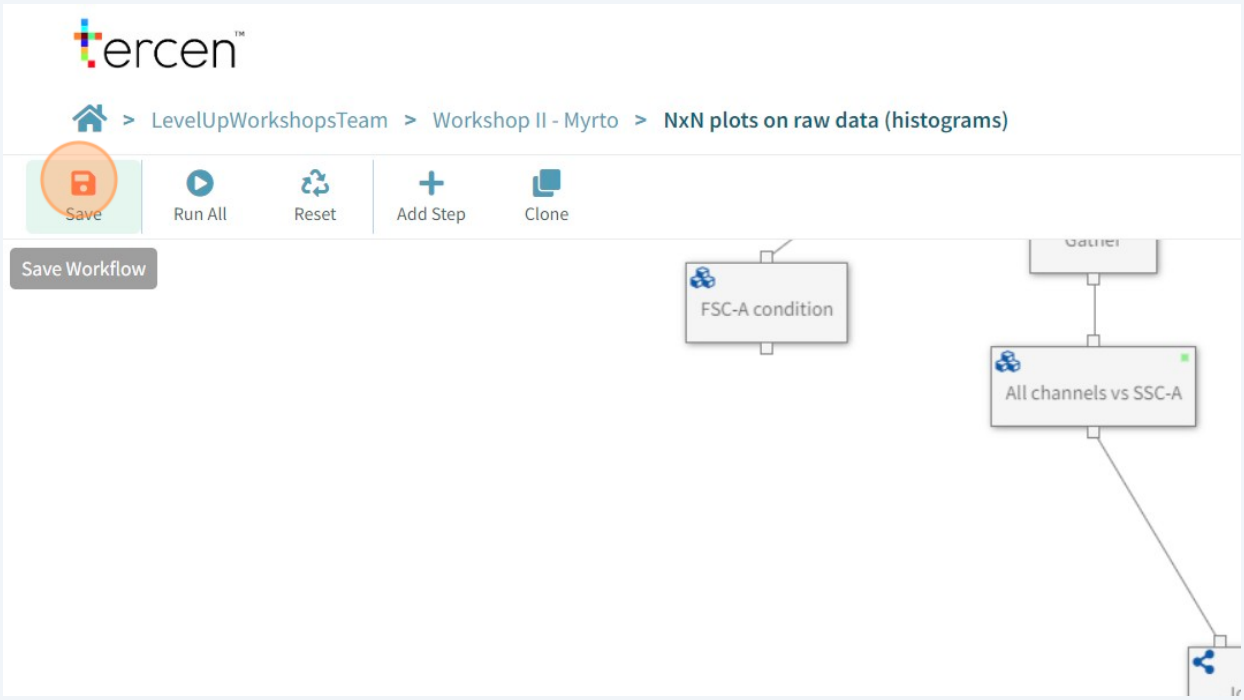
48 Adjust the height of the X-axis as desired.



49 Go up to the workflow and rename the Data Step to "NxN plots (Control - CD3, CD4, CD8)"



50 Do not forget to save your progress by clicking on the Save button on the leftmost side of the toolbar.



51 You have now created a pairwise plot of three channels against three channels. The raw FCS data is shown.

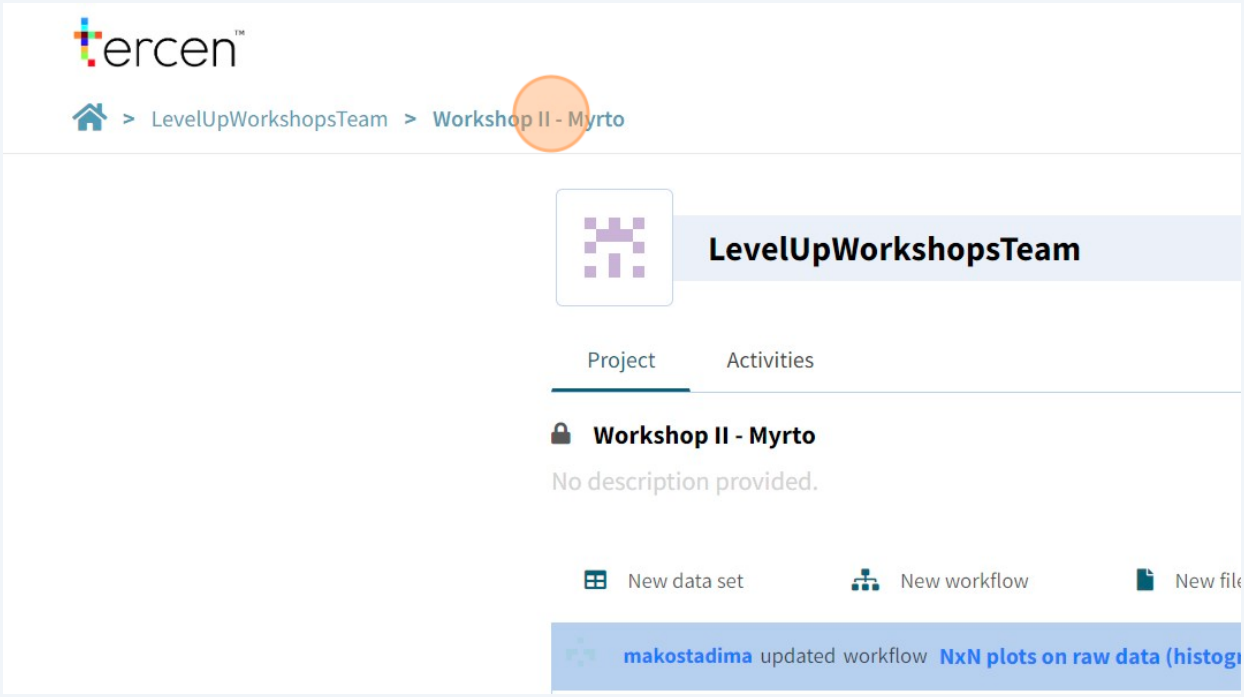
The raw data has negative values, and the data points are squashed on the axis.

To make this more useful, we will need to further transform the data. Stay tuned!

0206 - Transform Data

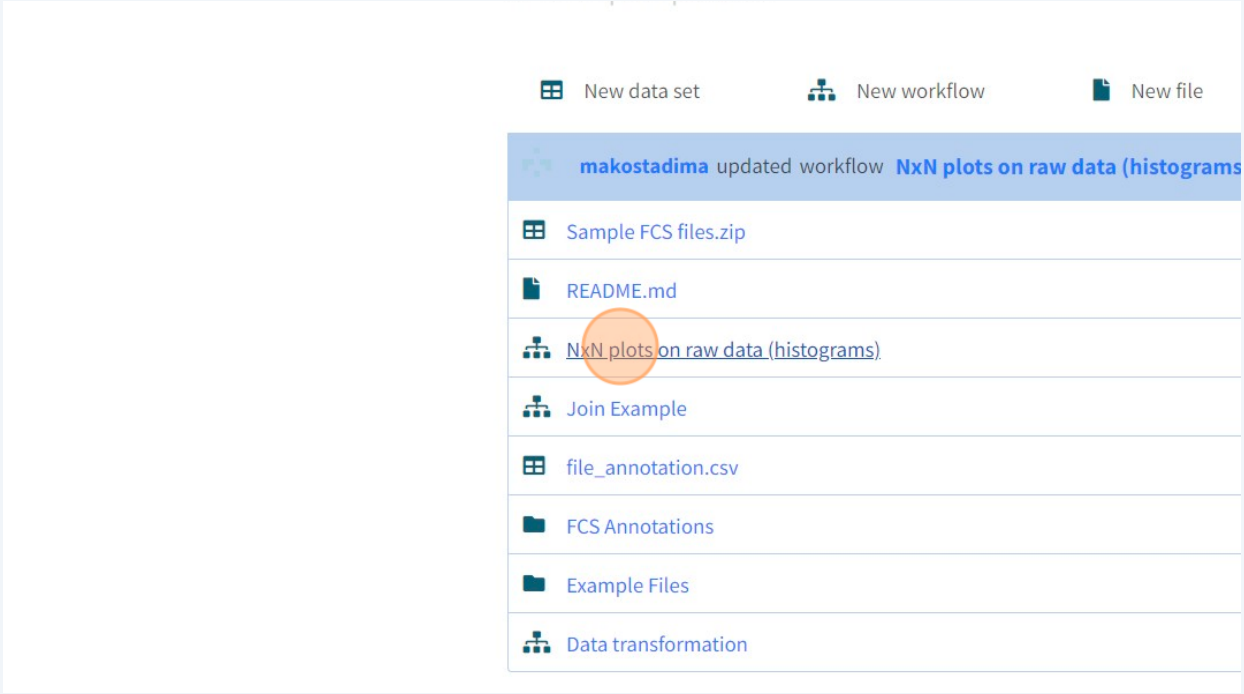
In this lesson, we will perform data transformation using the logicle operator in Tercen.

1 Click on your copy of the Workshop II Tercen project.



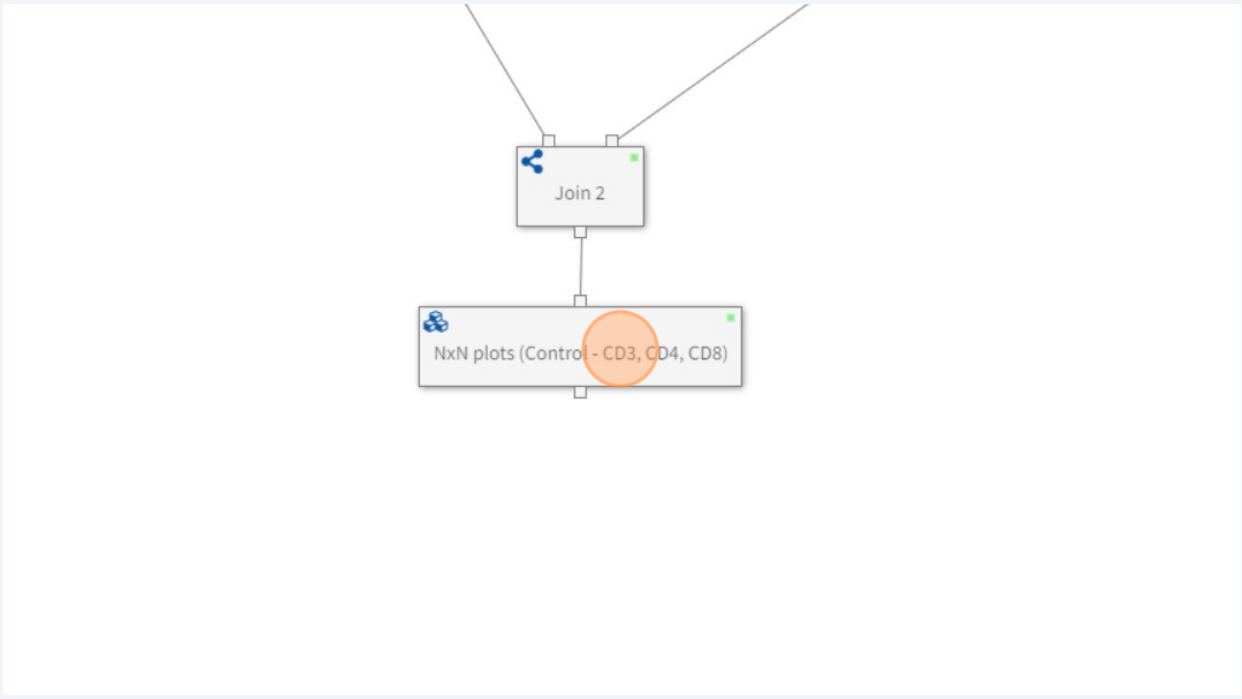
For this lesson we will continue working on the workflow from the previous guide, called **NxN plots on raw data (histograms).**

2 Click "NxN plots on raw data (histograms)".

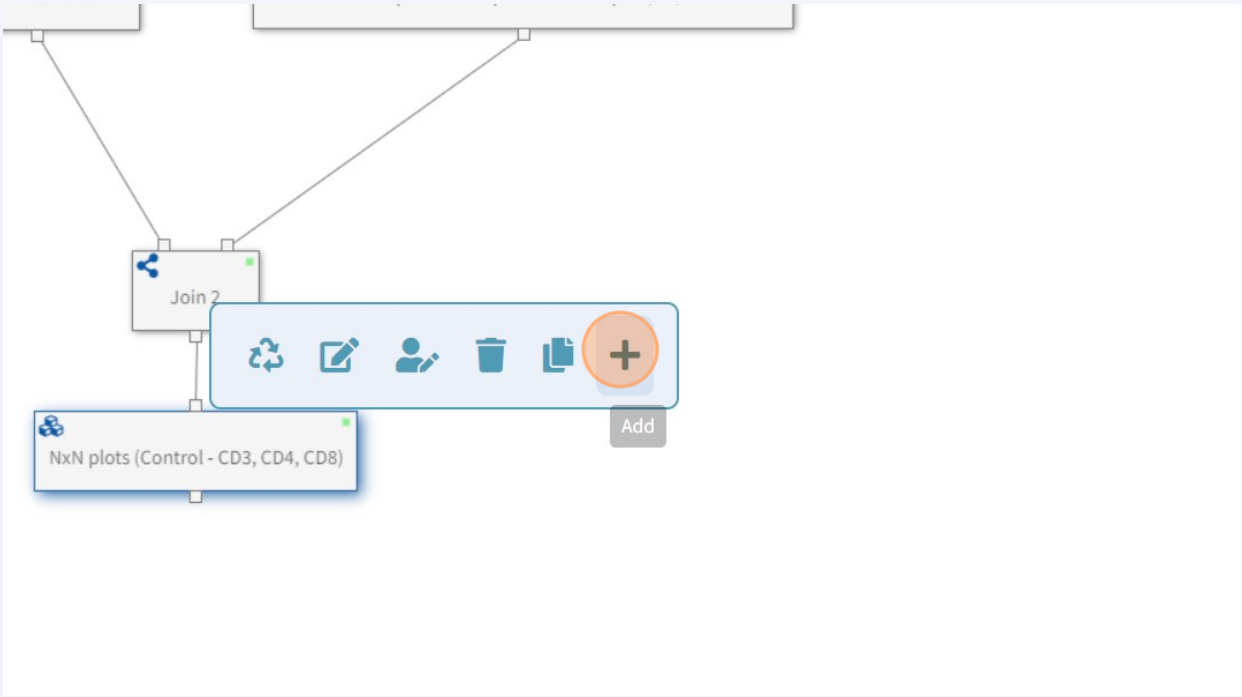


3 Navigate down to the last data step created, called **NxN plots (Control - CD3, CD4, CD8)**.

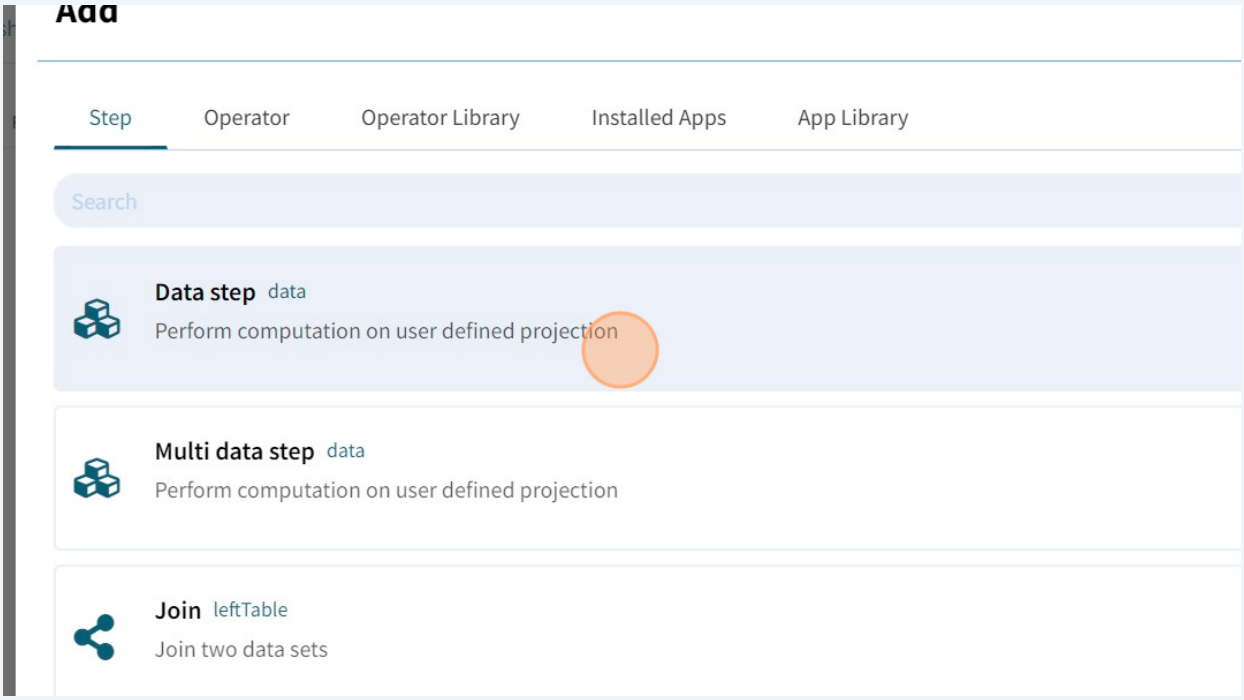
Click on this step so that the local toolbar appears over it.



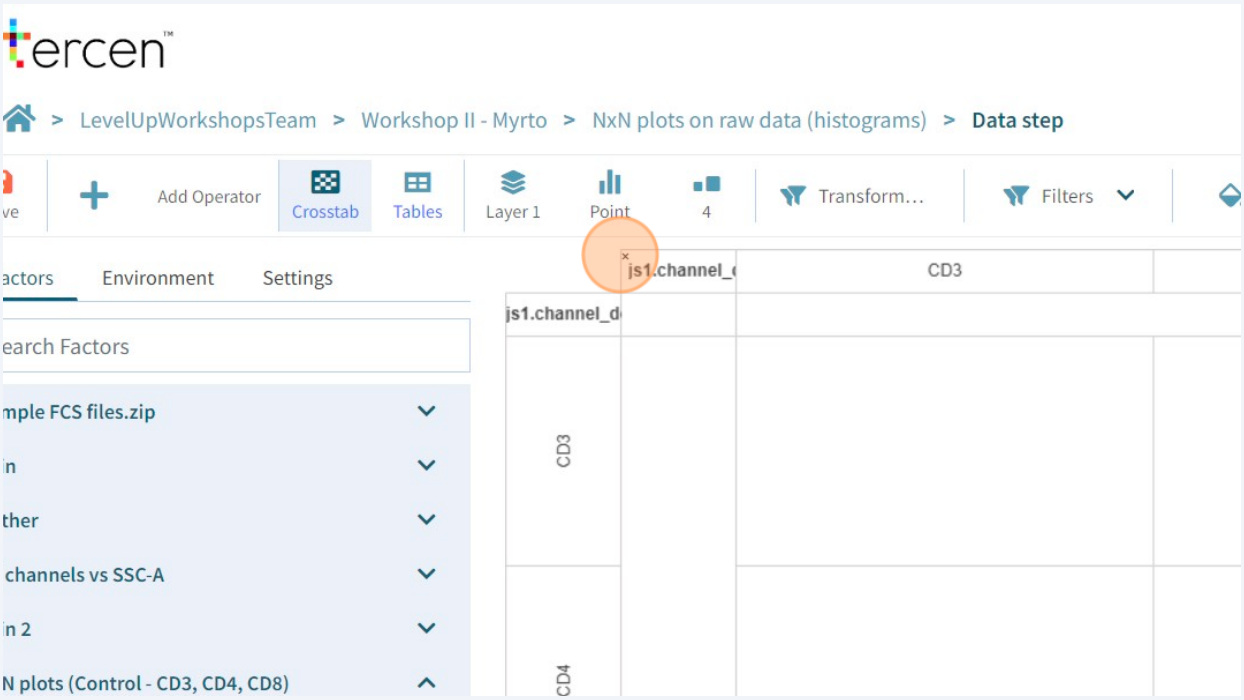
4 Select **Add**.



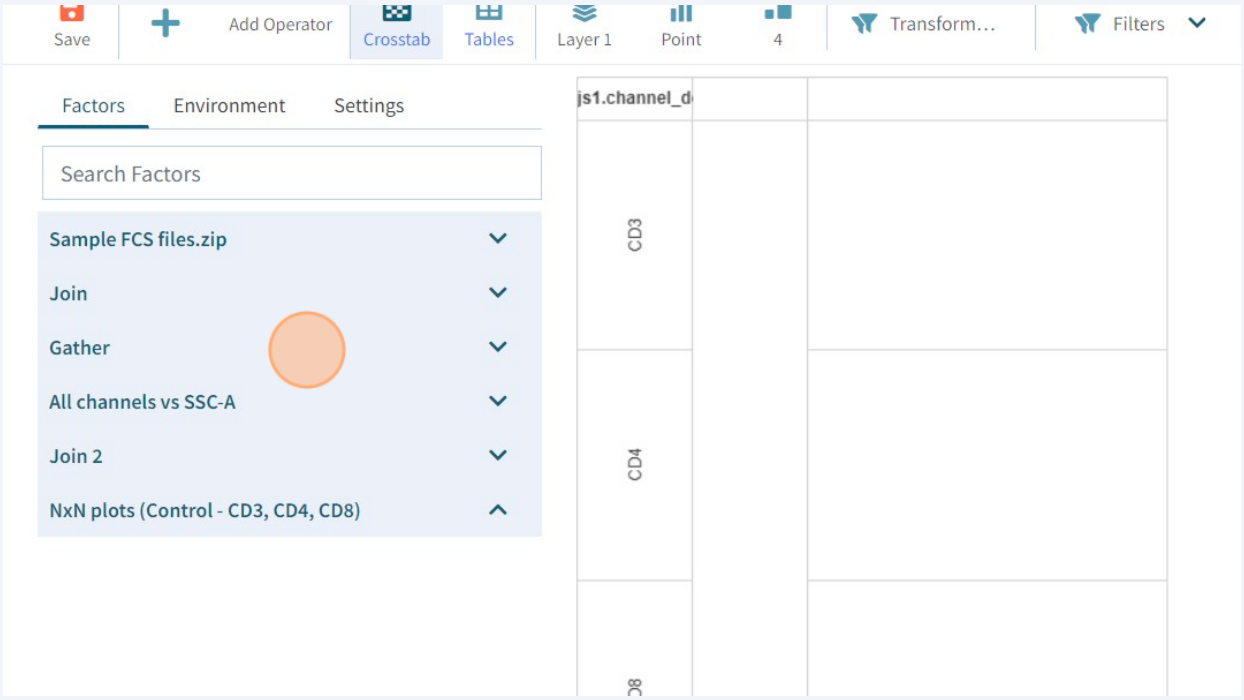
5 Select "Data step".



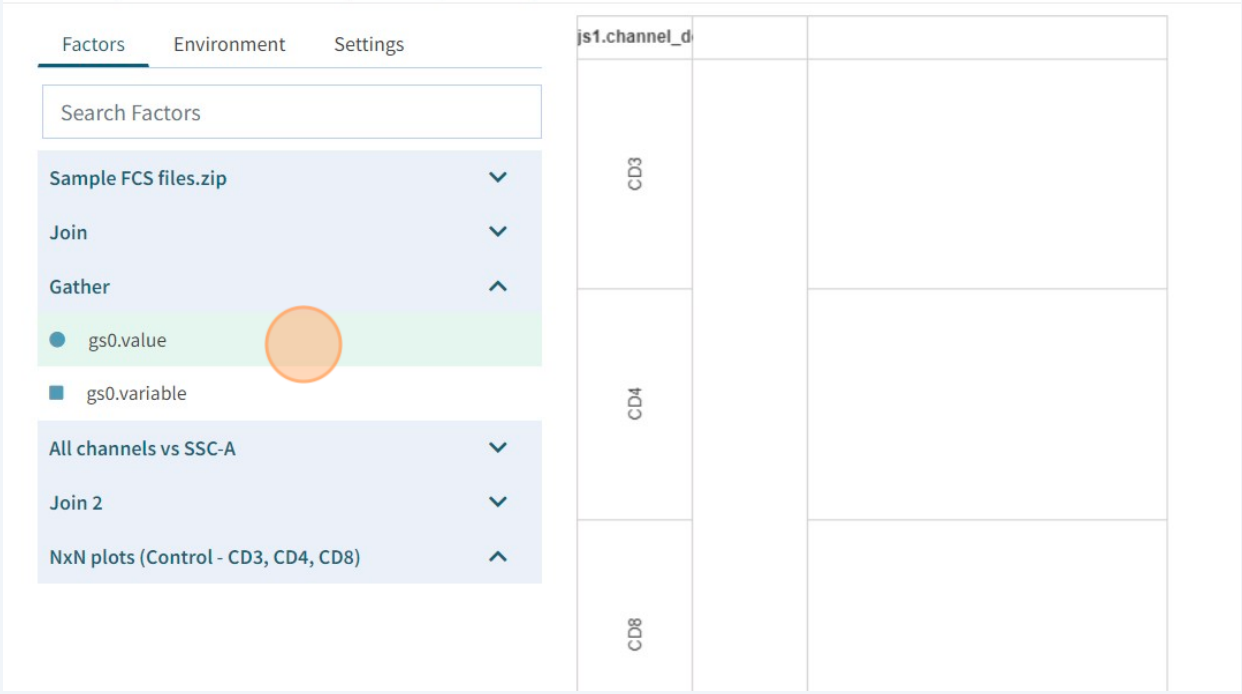
6 Clear the preselected factor on the Columns by clicking on the 'x' on the top left corner when you mouse over, as shown below.



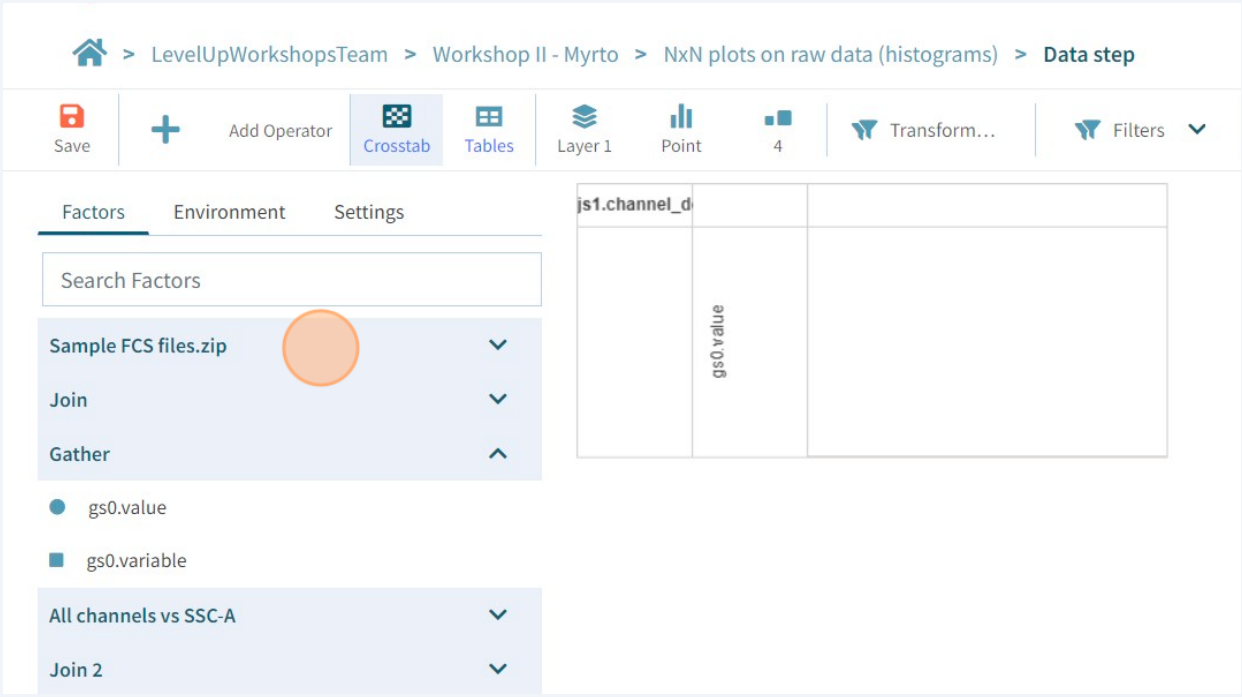
7 Click to expand the factors under the "Gather" step.



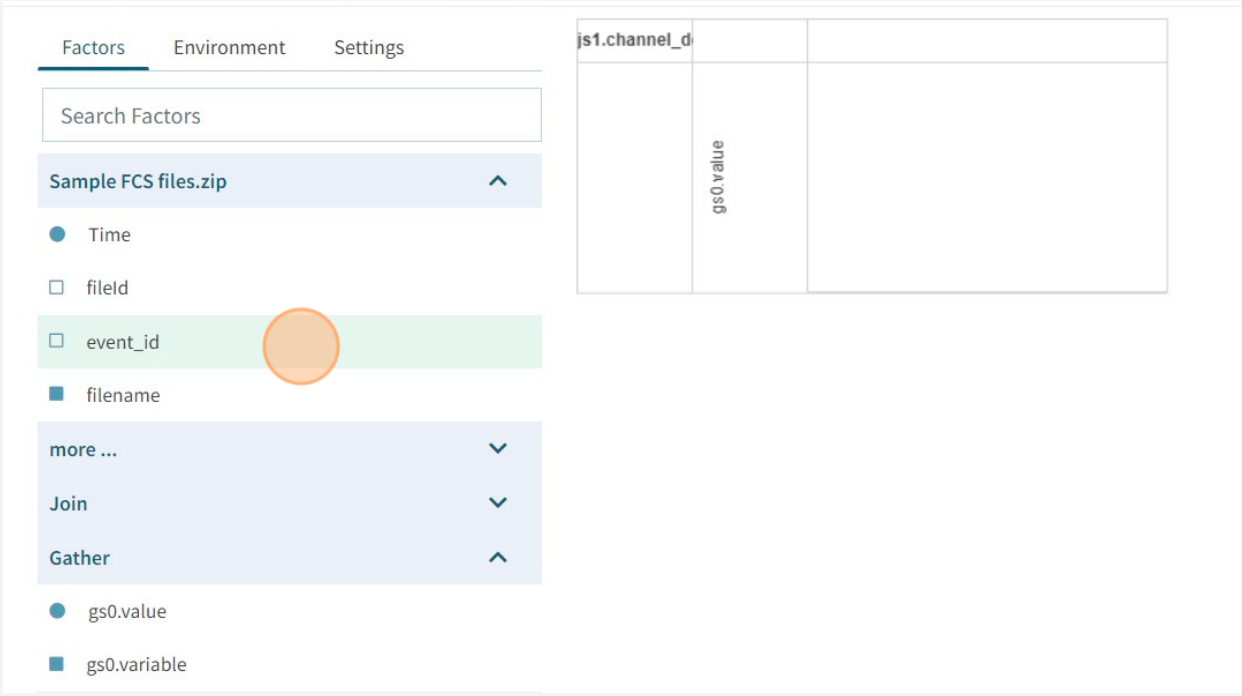
8 Drag and drop the **gs0.value** factor to Y-axis.



9 Expand the "Sample FCS files.zip" factors by clicking on it on the Factors list.

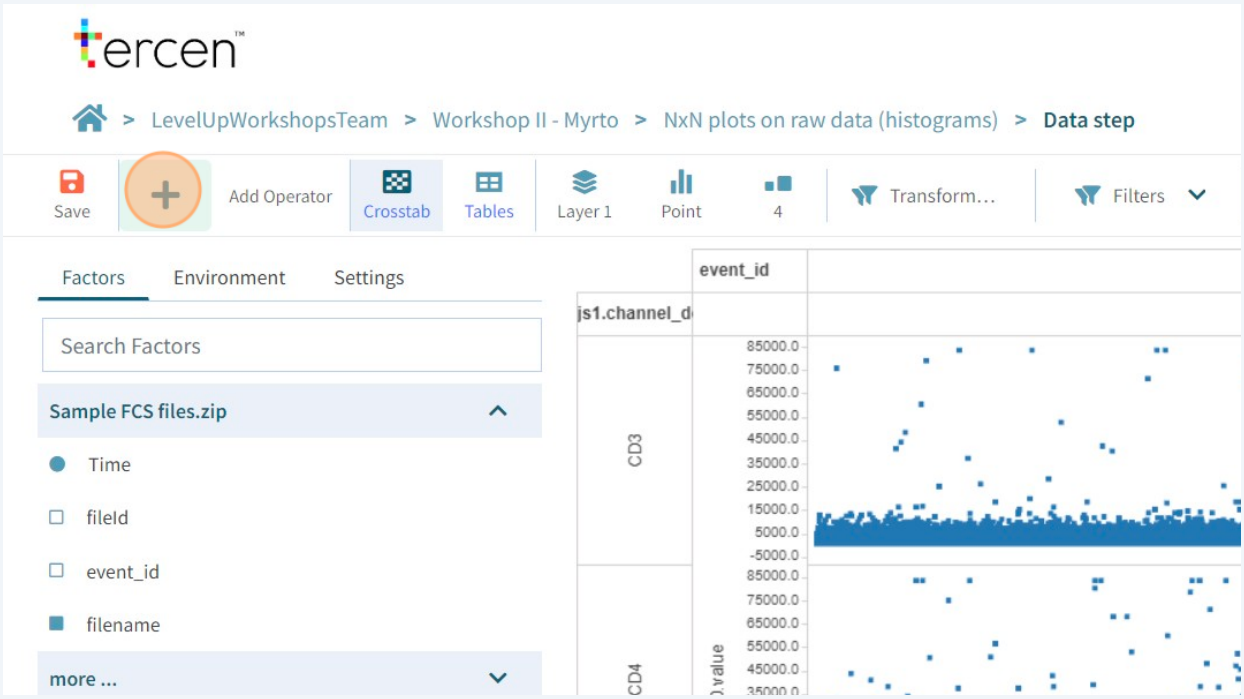


10 Drag and drop the **event_id** factor to Column.

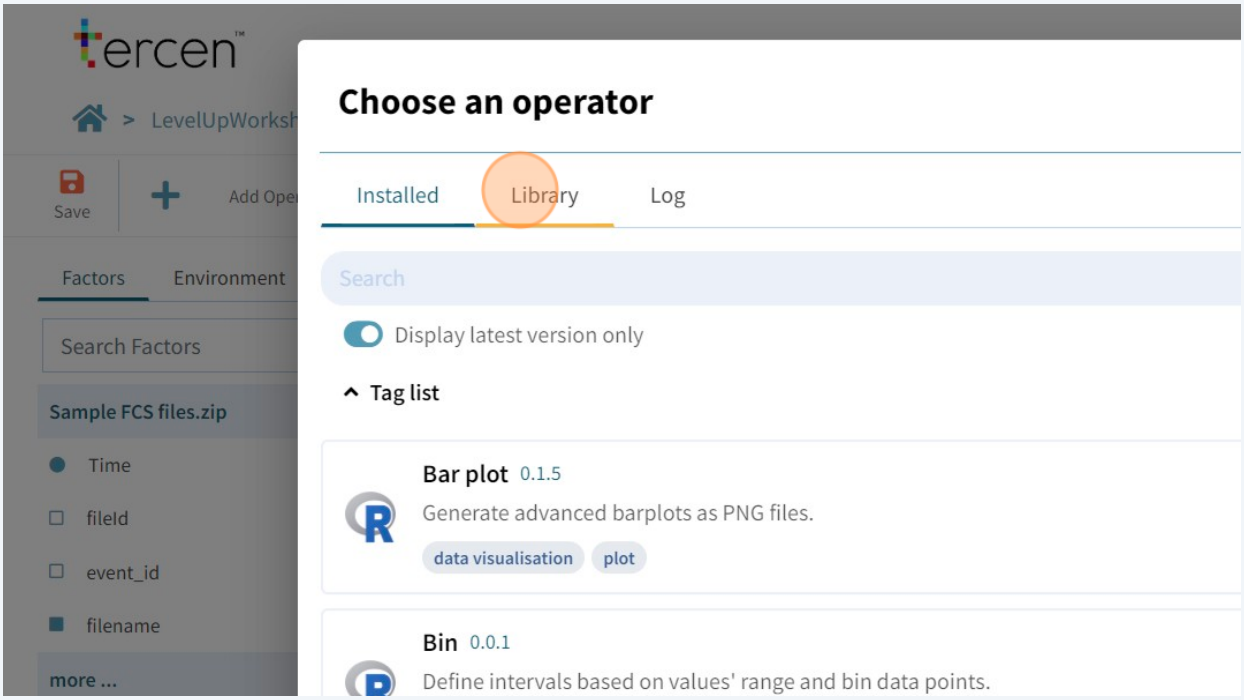


11 You will then add the operator that performs the logicle transformation.

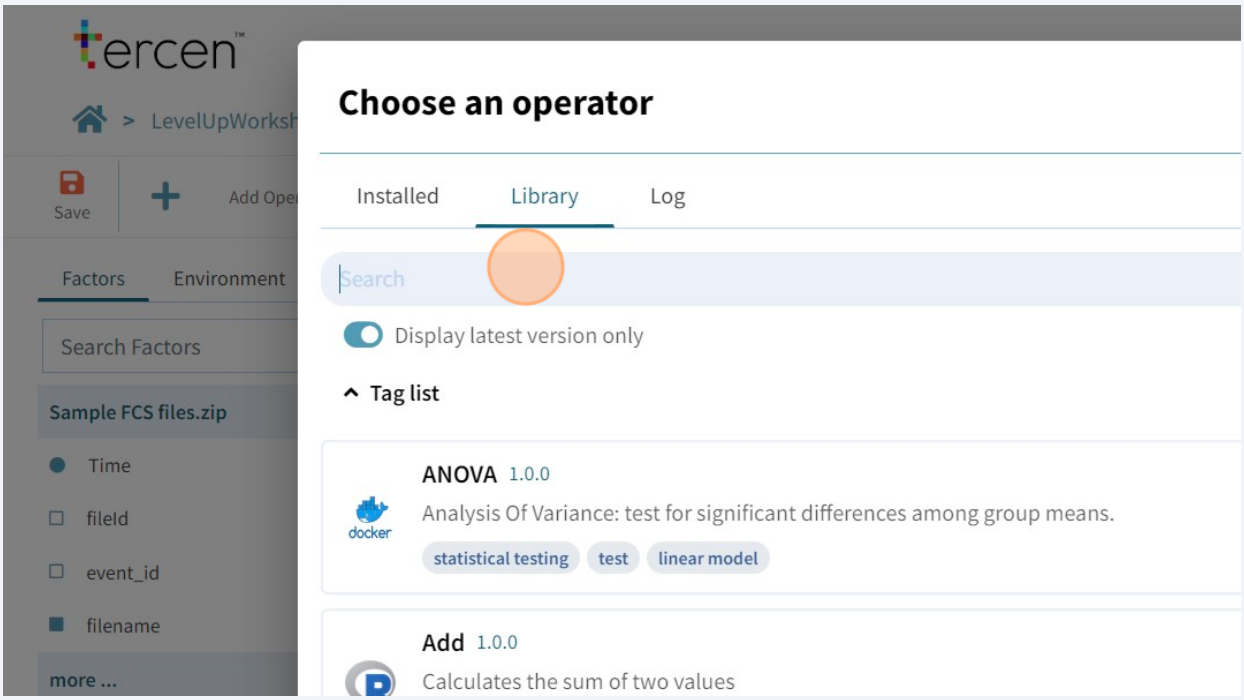
Click the '+' sign next to the Add Operator tab of the toolbar.



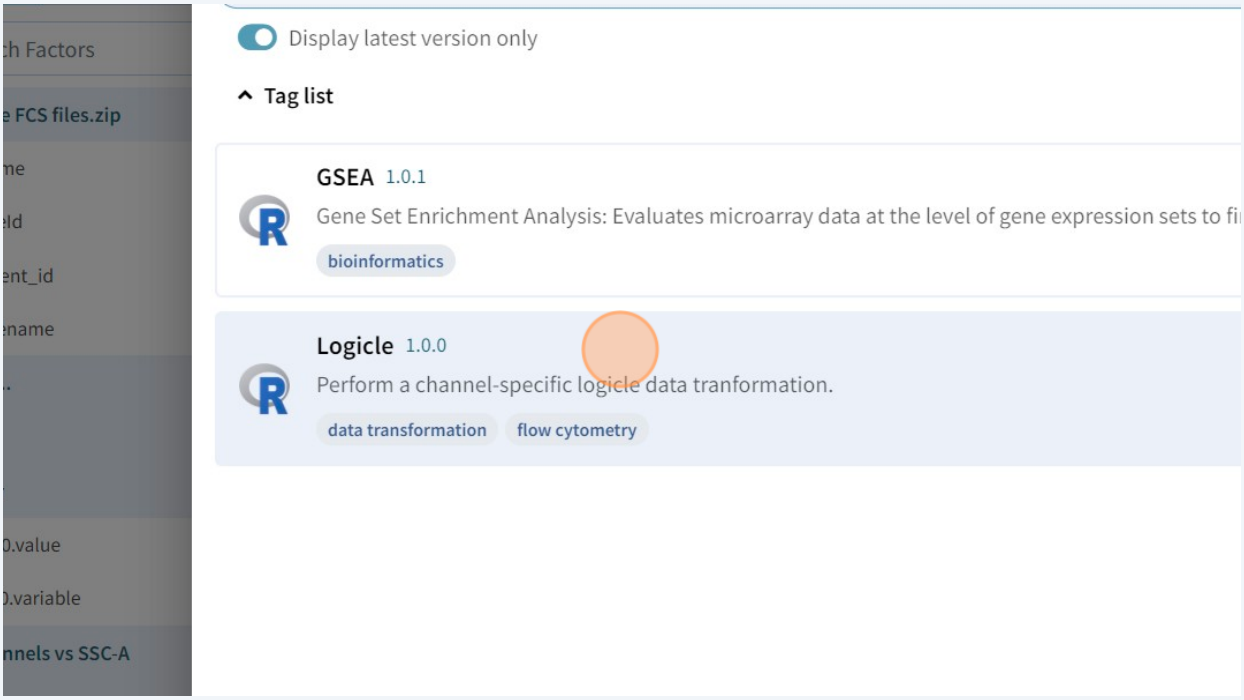
12 Click "Library".



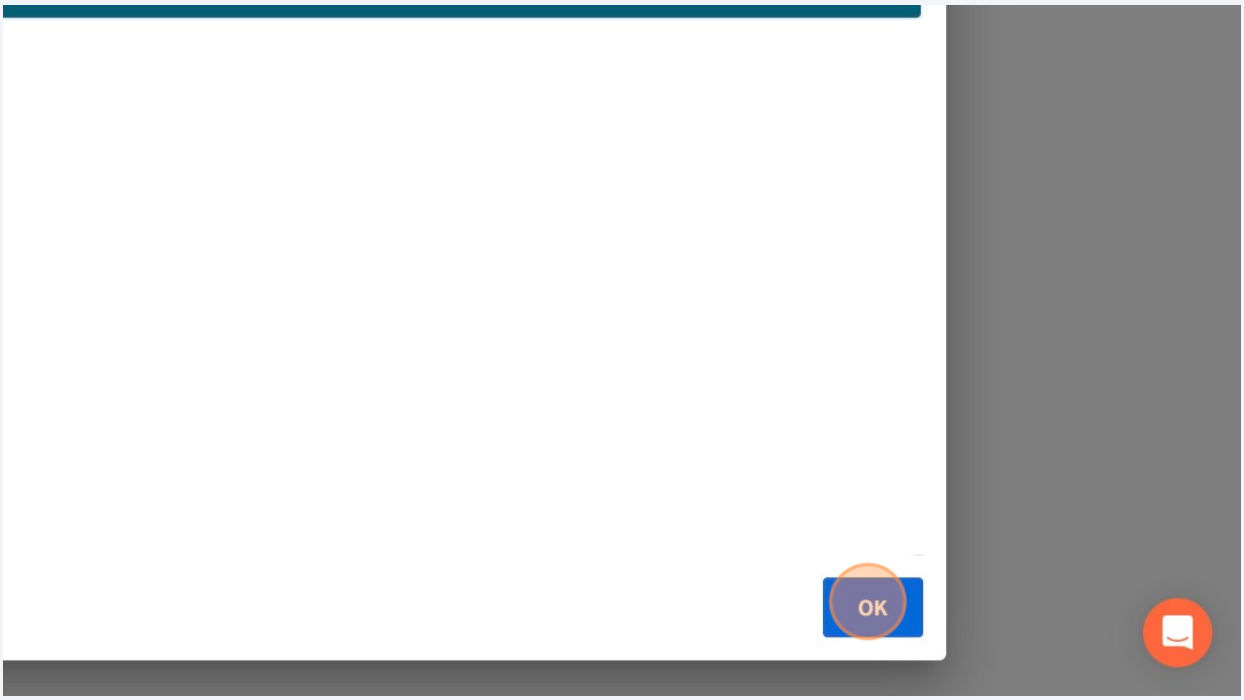
13 In the Search field type 'logic'.



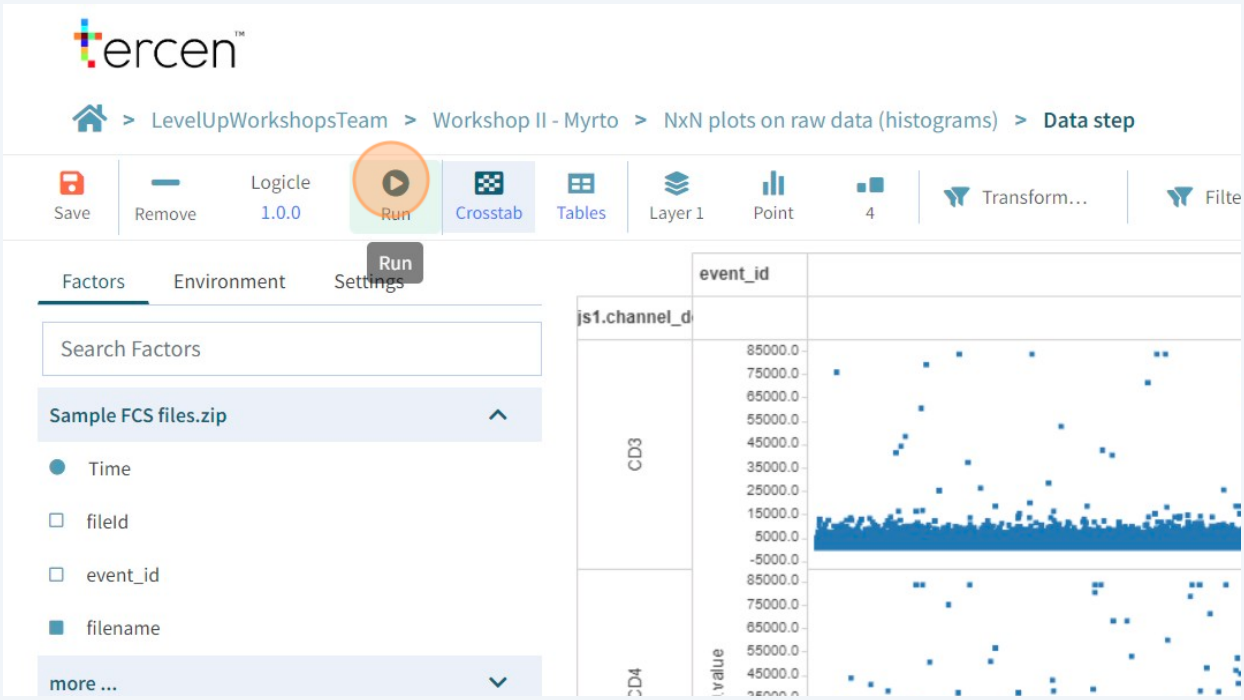
14 Select the **Logicle** operator.



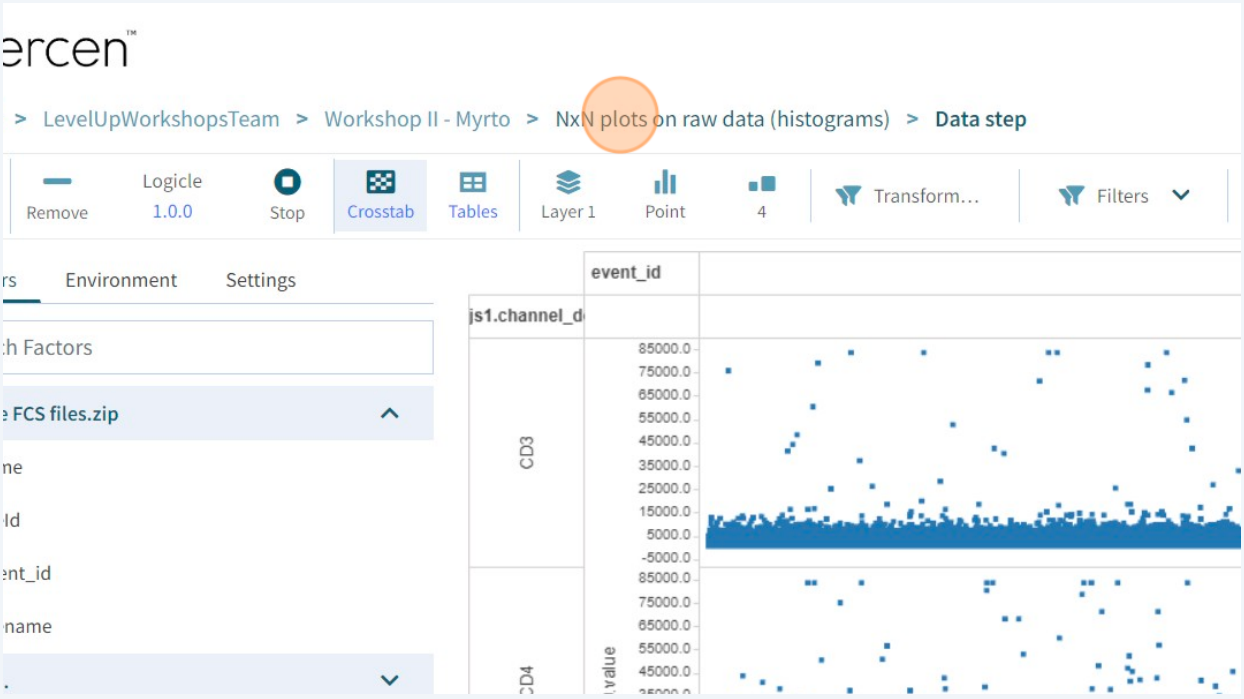
15 Click "OK"



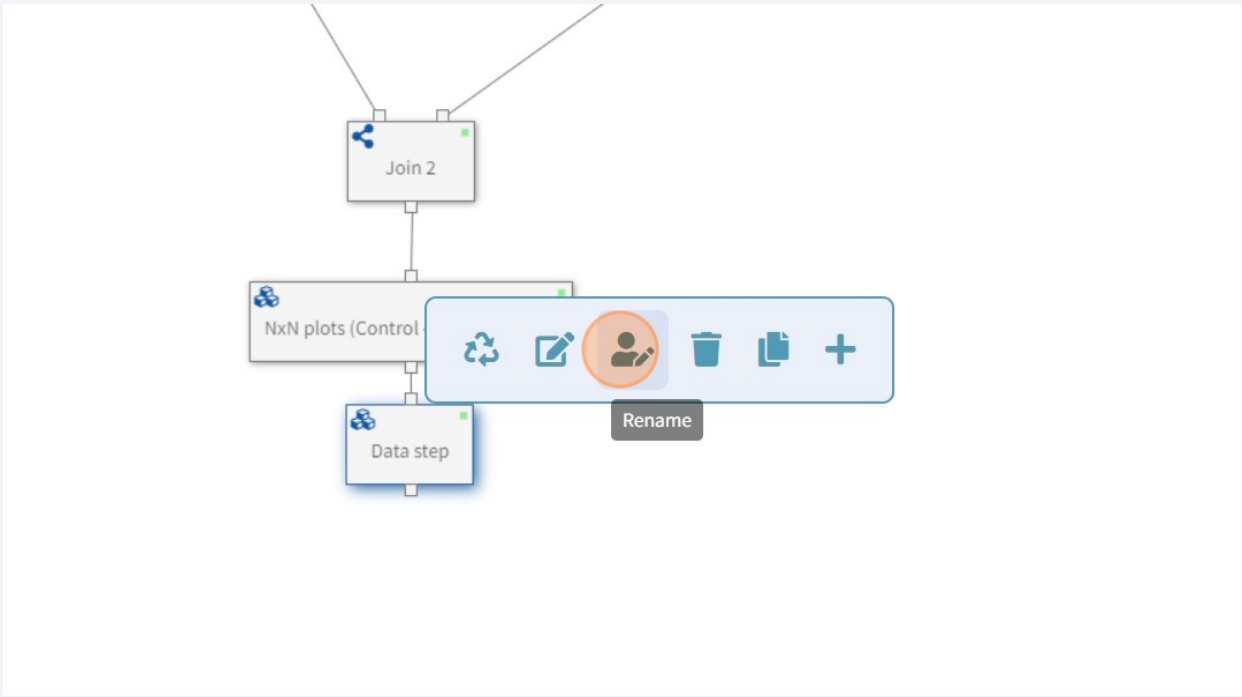
16 Click 'Run'.



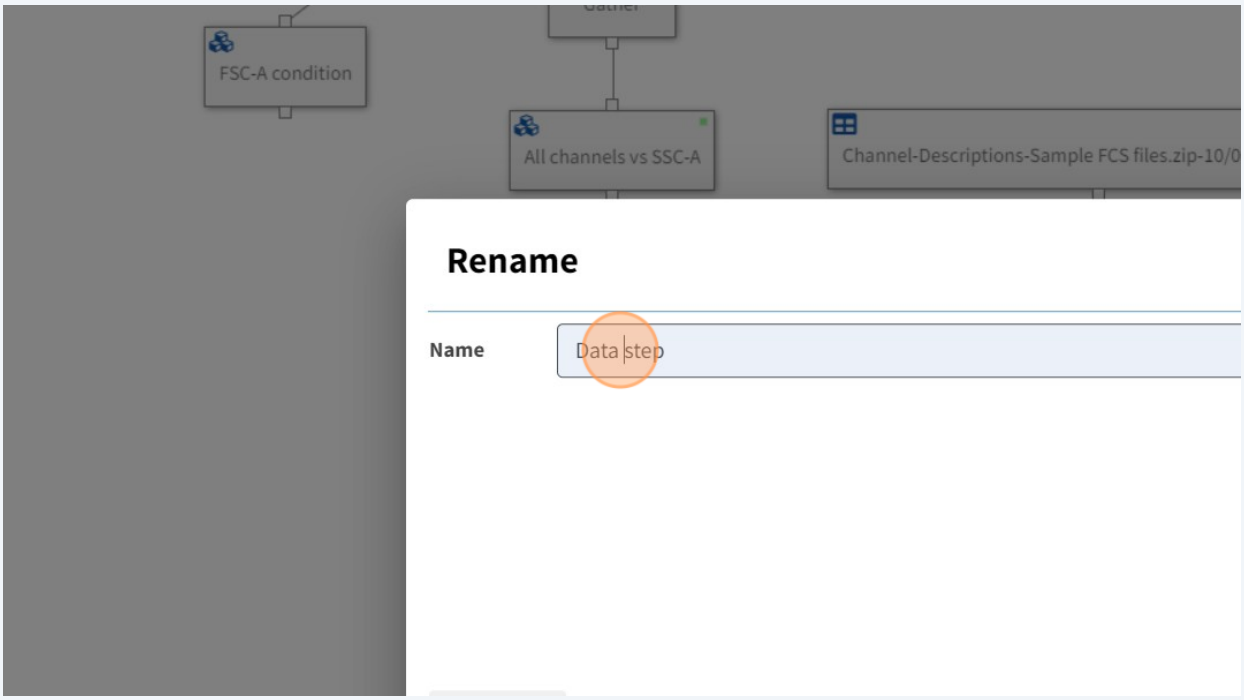
17 Click "NxN plots on raw data (histograms)" to return to the workflow.



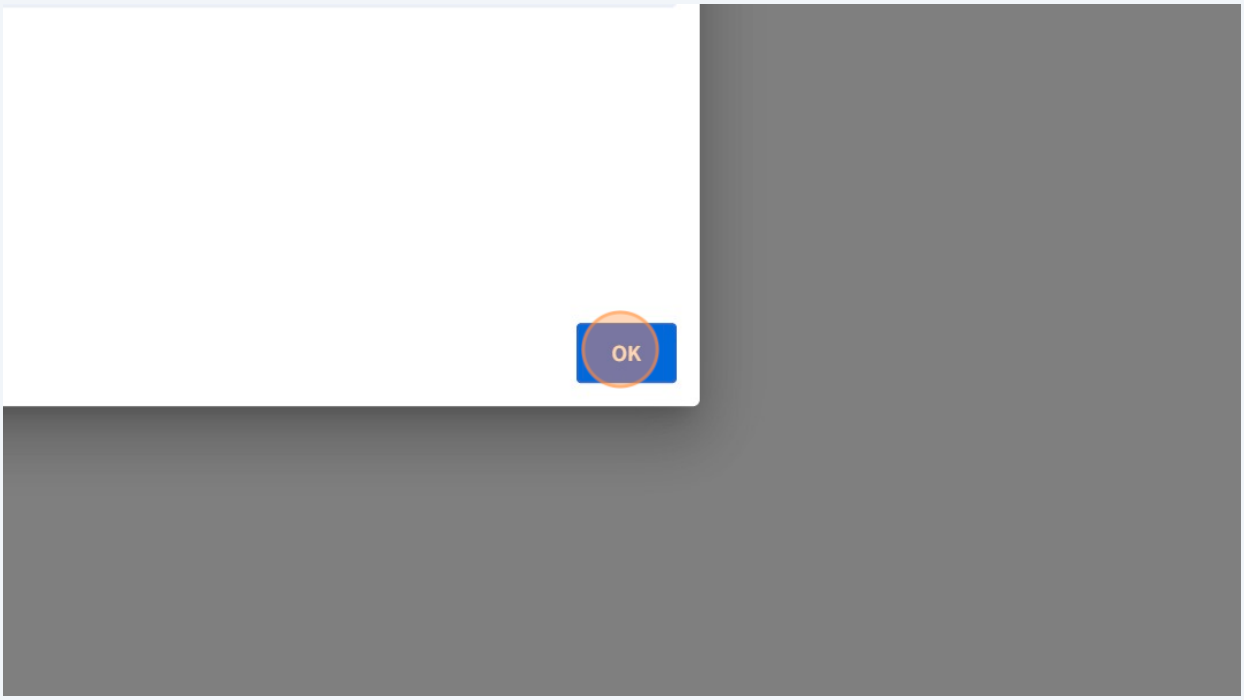
18 Click 'Rename'



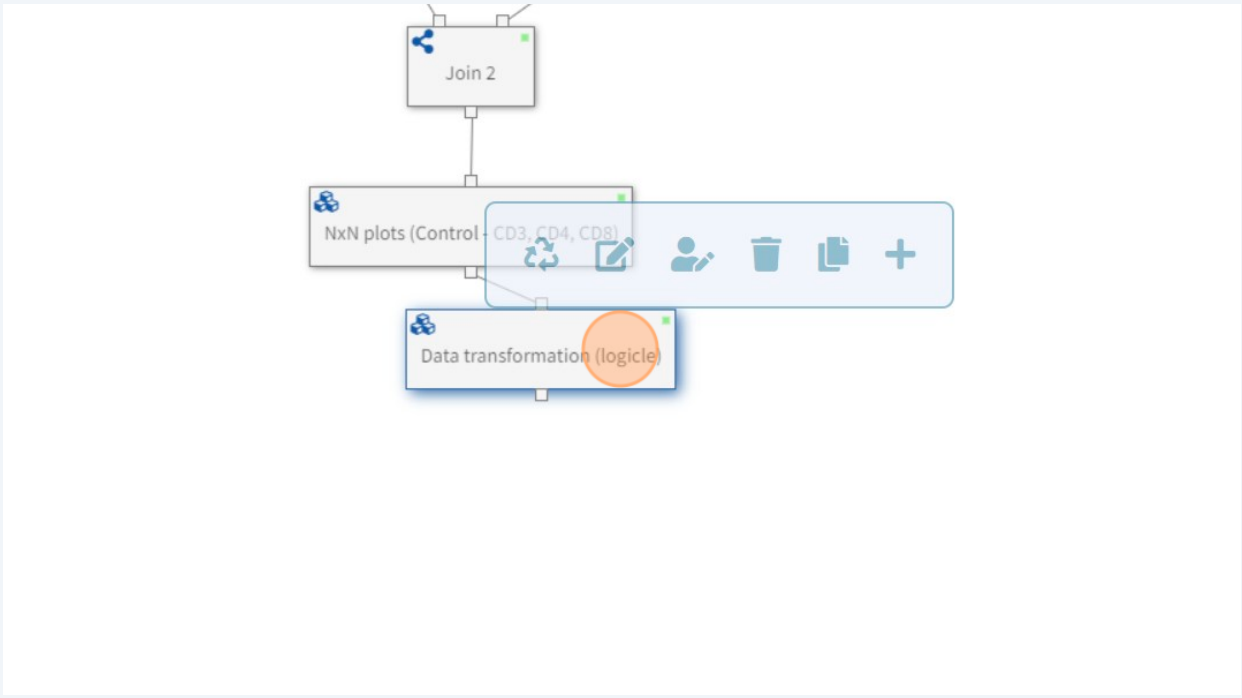
19 Type "Data transformation (logicle)" in the name text box.



20 Click "OK"

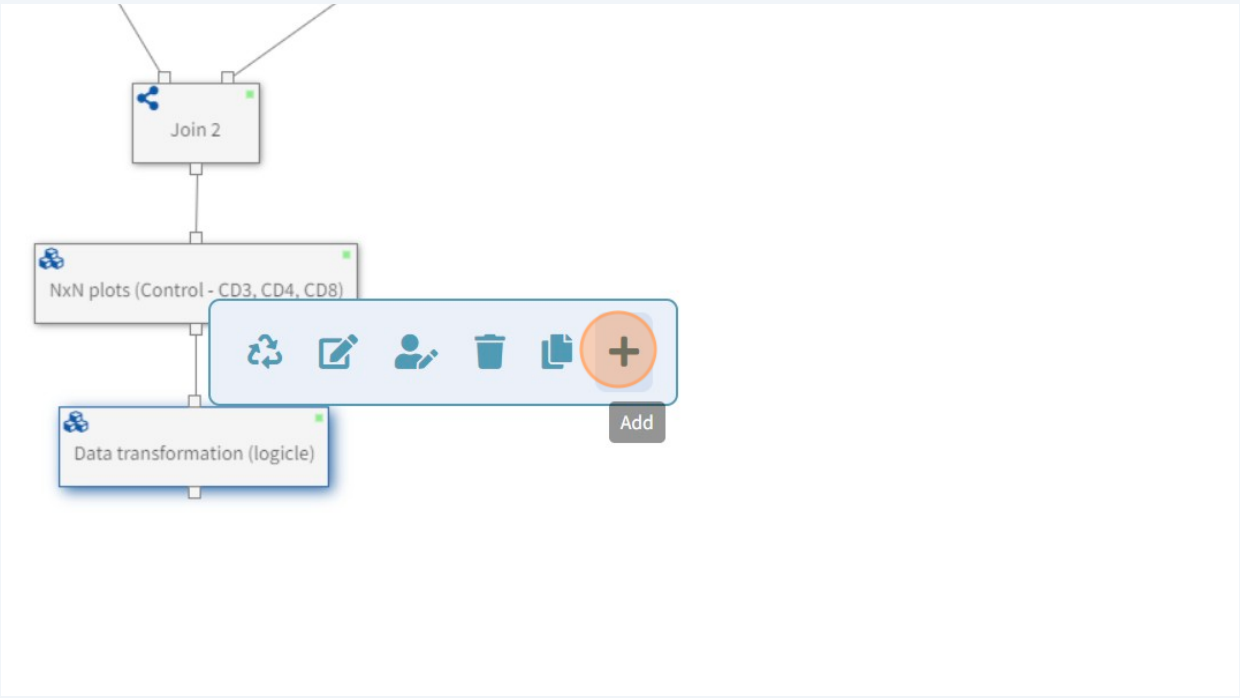


21 (OPTIONAL) If you need to order like me, drag and drop the data step to ensure it aligns perfectly with the previous one.

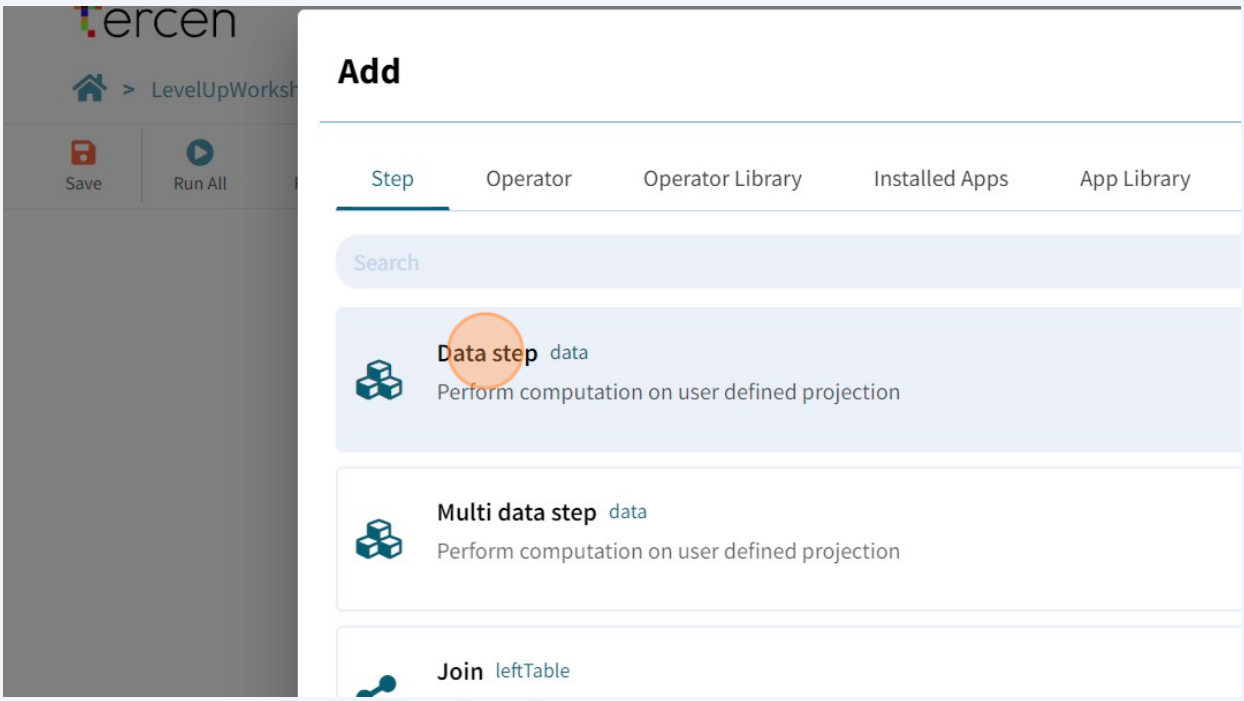


22 Now let us investigate how the logicle transformation has modified our Channels vs Channels (NxN) plots.

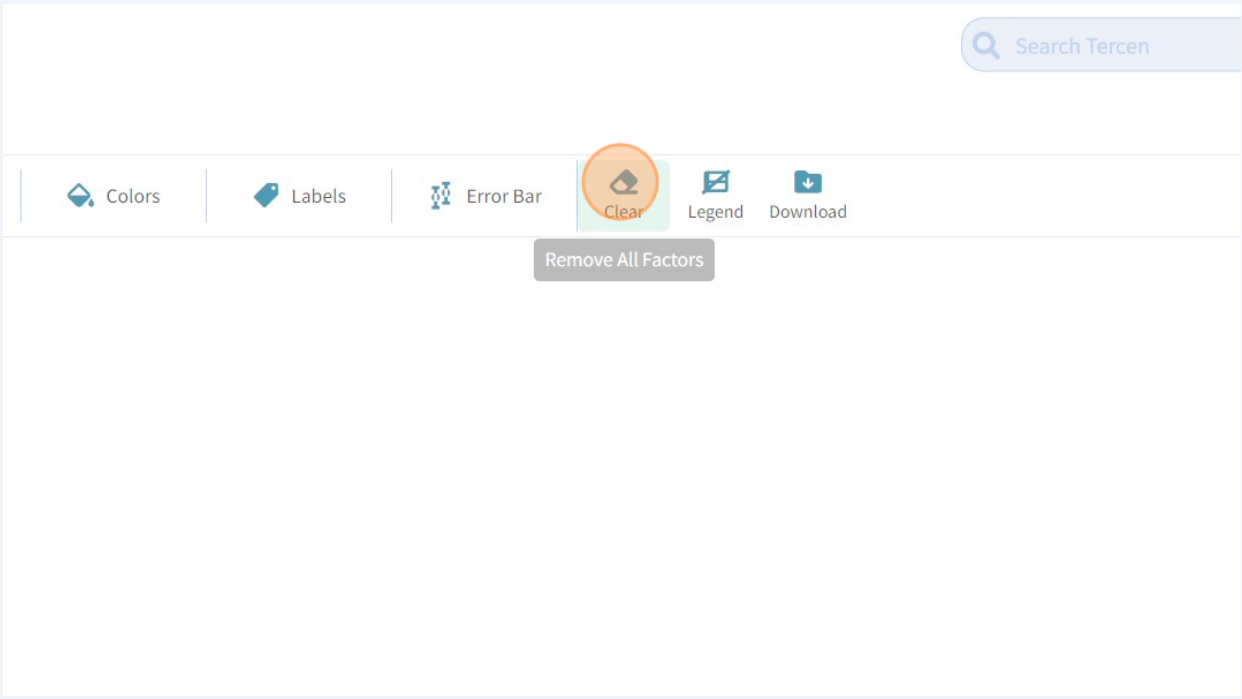
Click "Add".



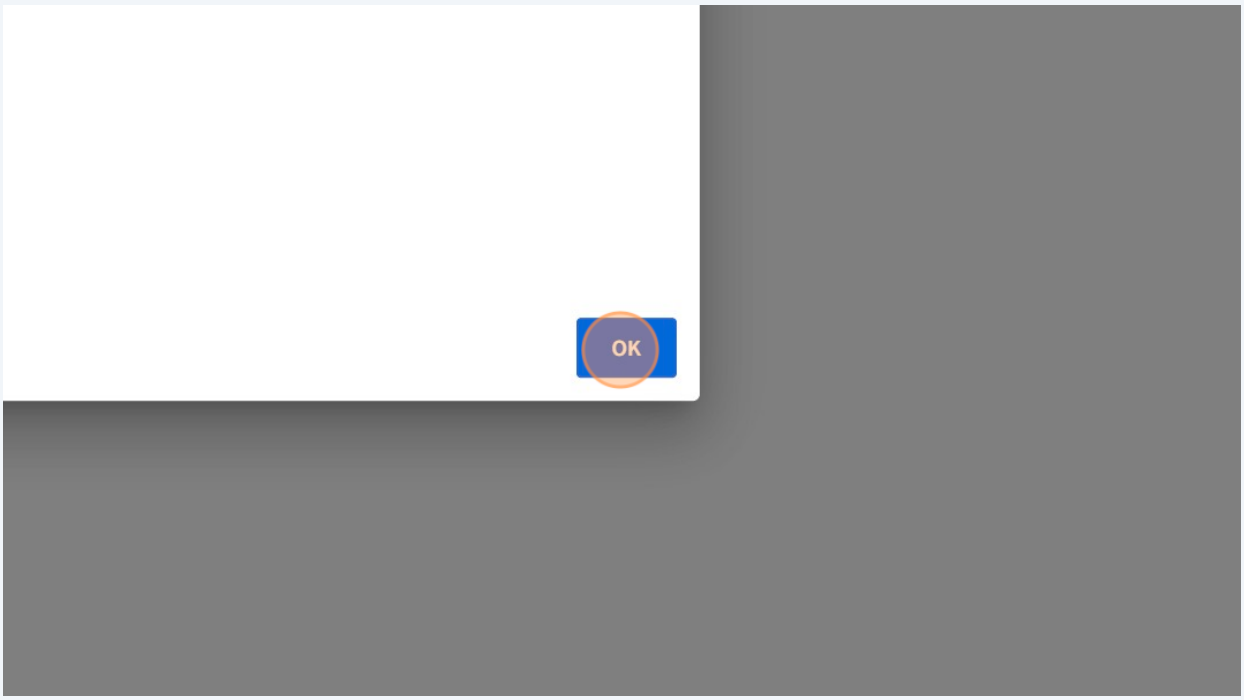
23 Select "Data step (data)"



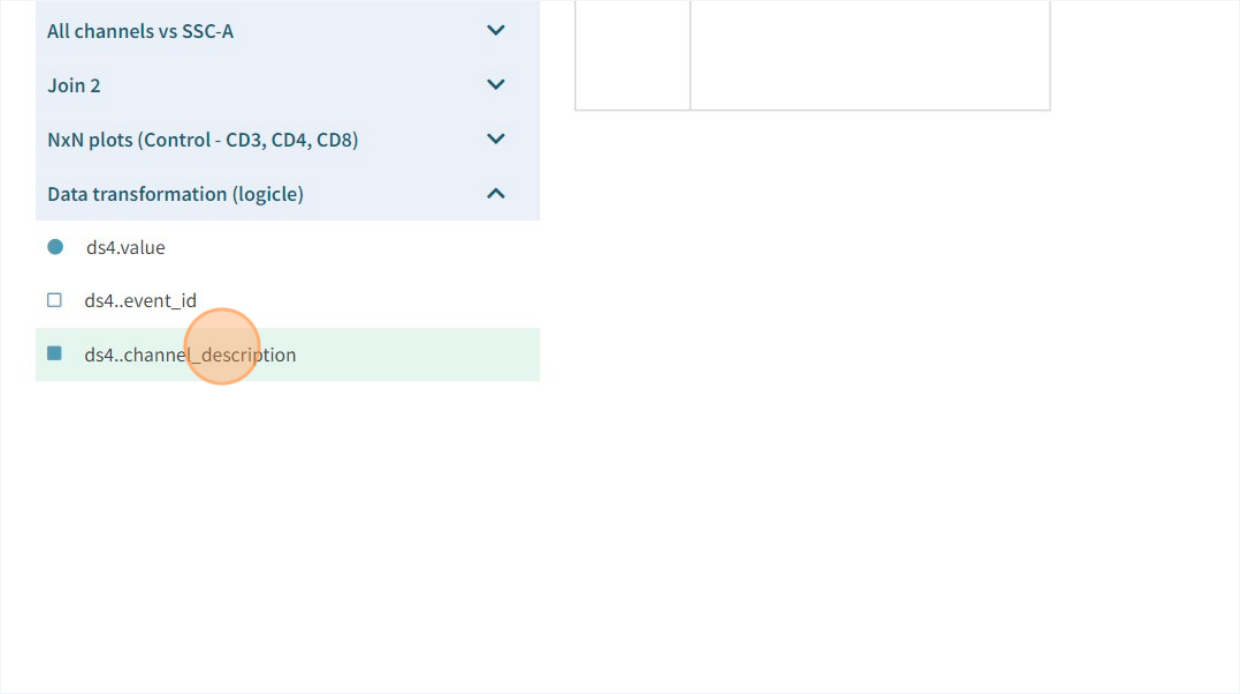
24 Clear any pre-defined data projections by clicking the "Clear" button on the toolbar.



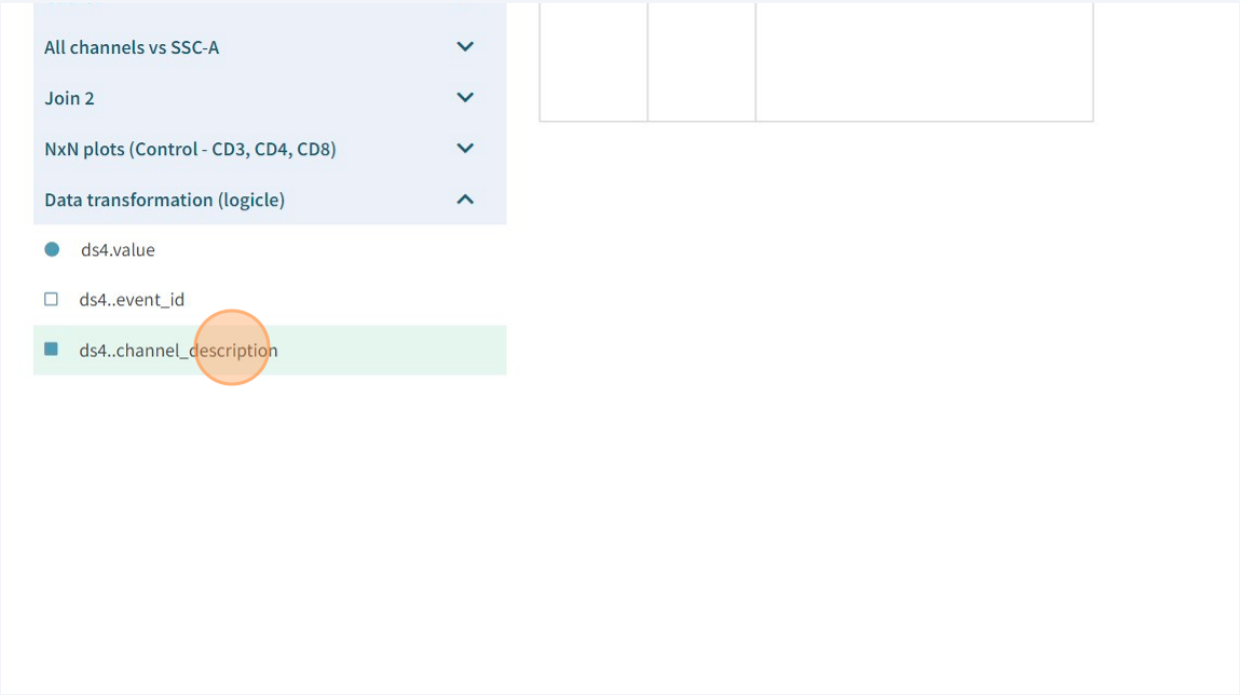
25 Click "OK"



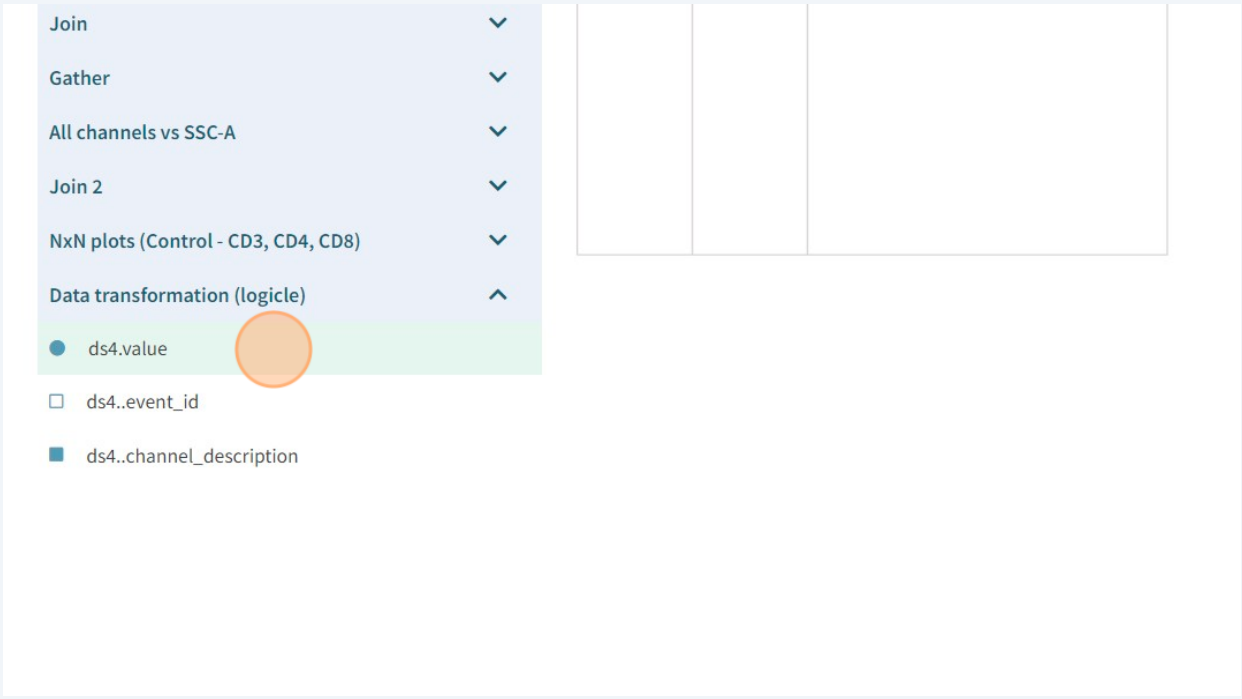
26 Drag and drop the **ds4..channel_description** factor to Row.



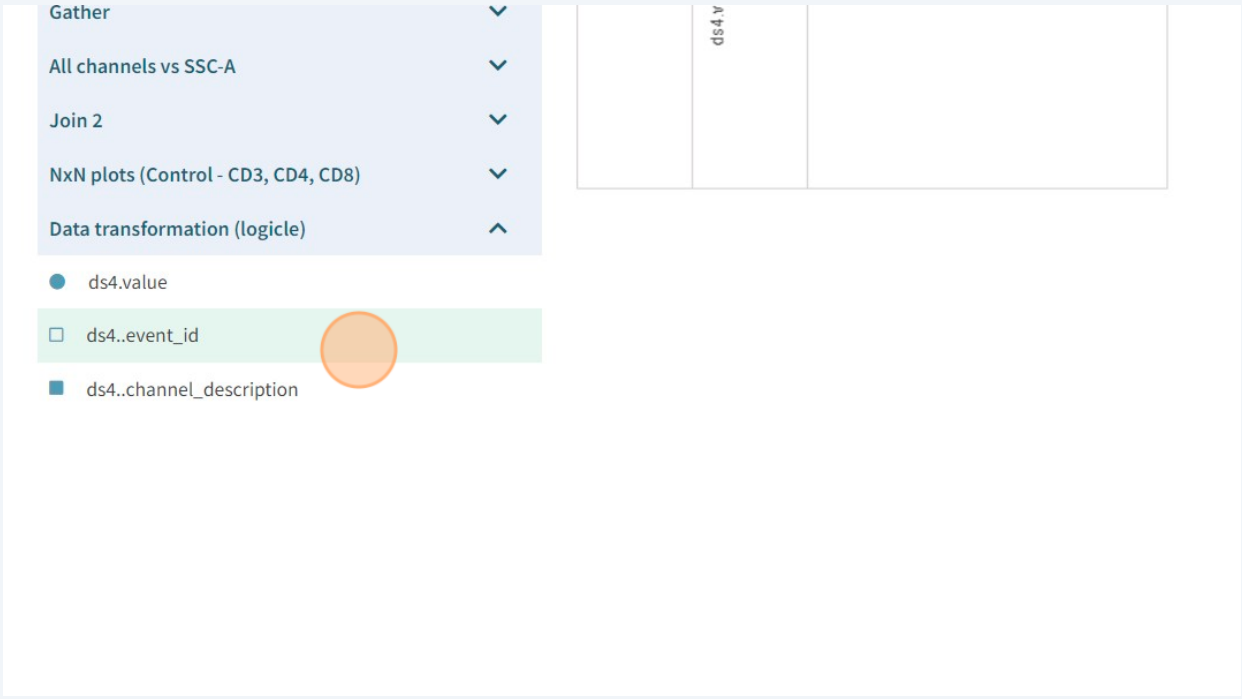
27 Again, drag and drop the ds4..channel_description factor to Column this time.



28 Drag and drop the **ds4.value** factor to Y-axis.



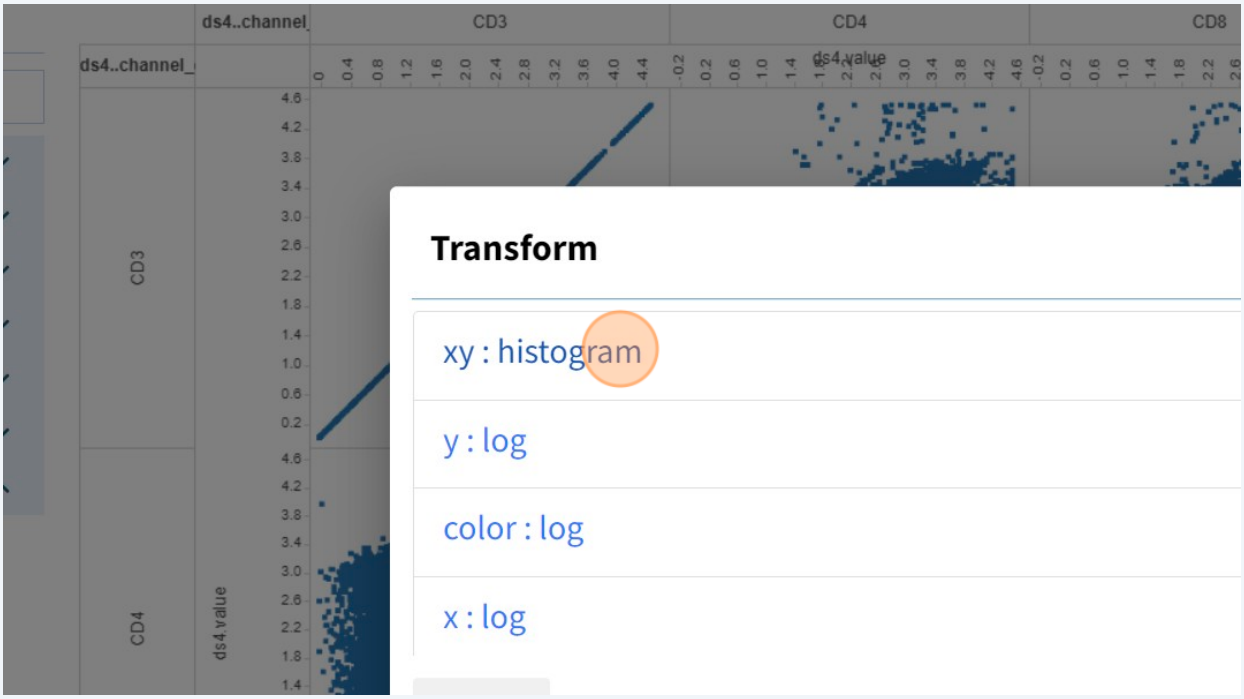
29 Drag and drop the **ds4..event_id** factor to Labels.



30 Again, we will add the histogram transformation to the data in order to visualize them.

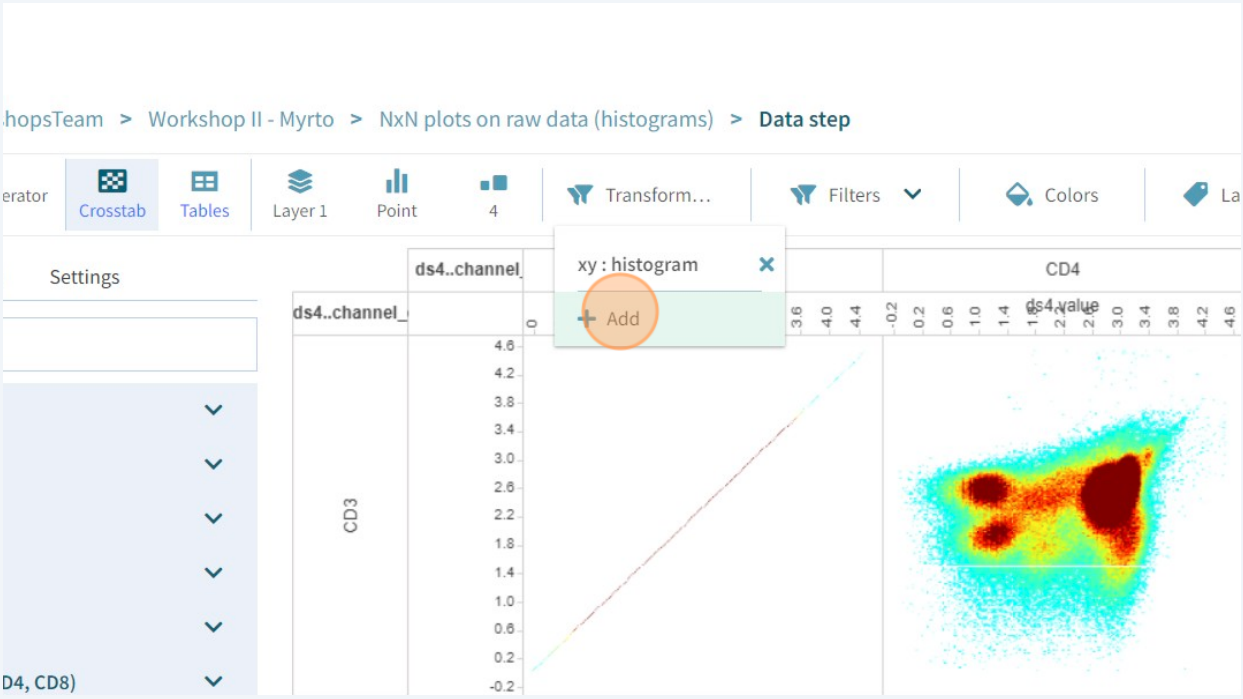
Mouse over "Transform" and click "Add".

31 Select "xy : histogram"

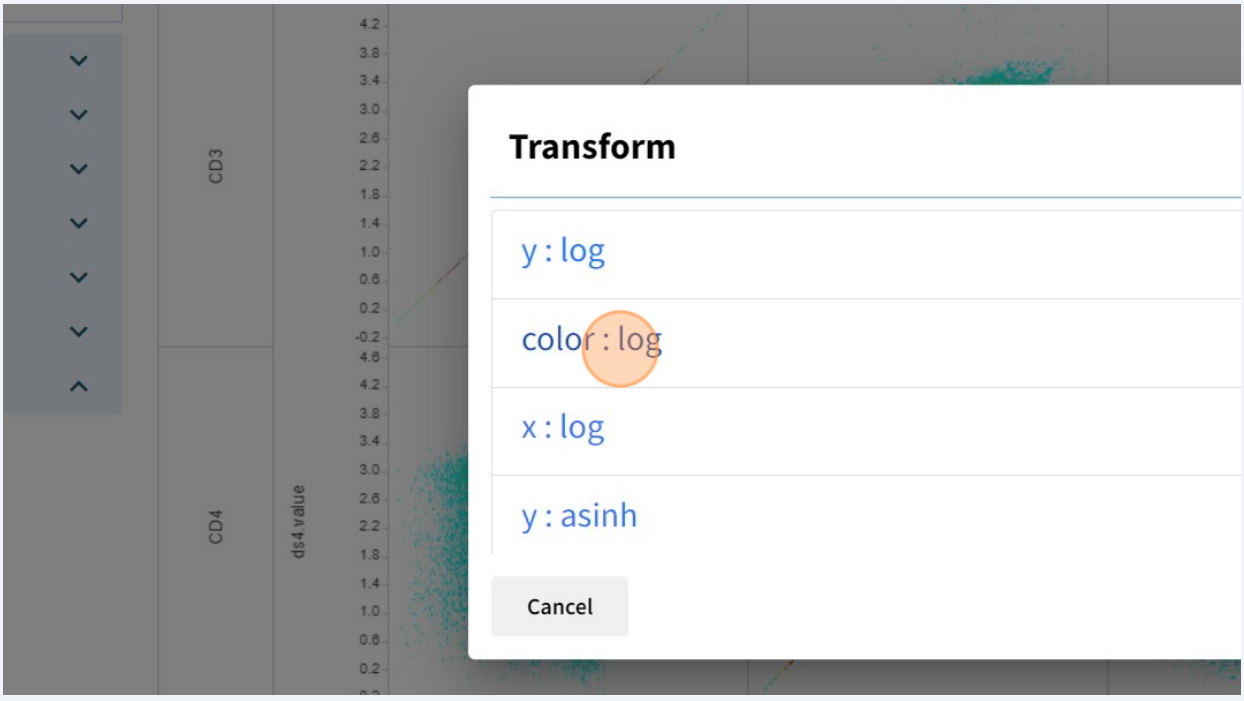


32 In this lesson, we will also see how we can transform the counts per bin (represented by the color).

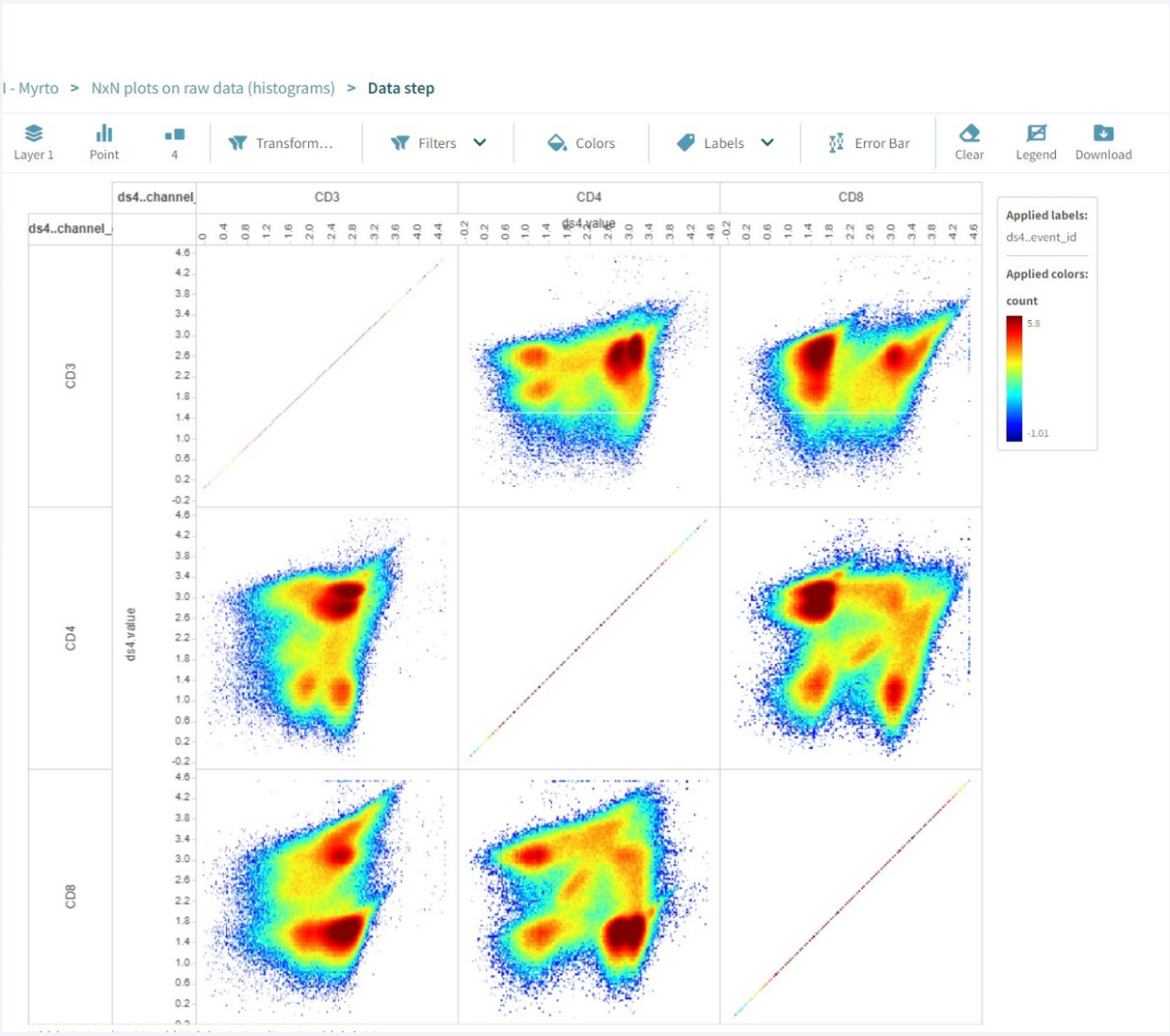
Again, mouse over "Transform" and click "Add".



33 Select "color : log".

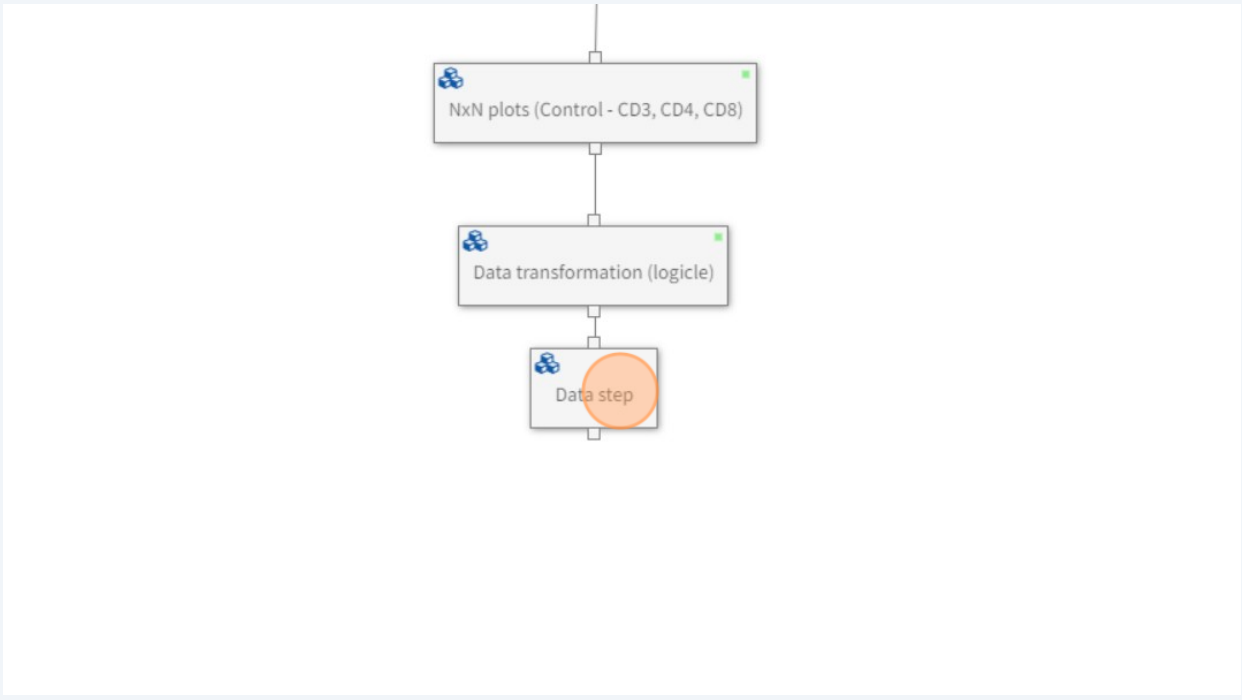


34 Observe how the colors have changed.

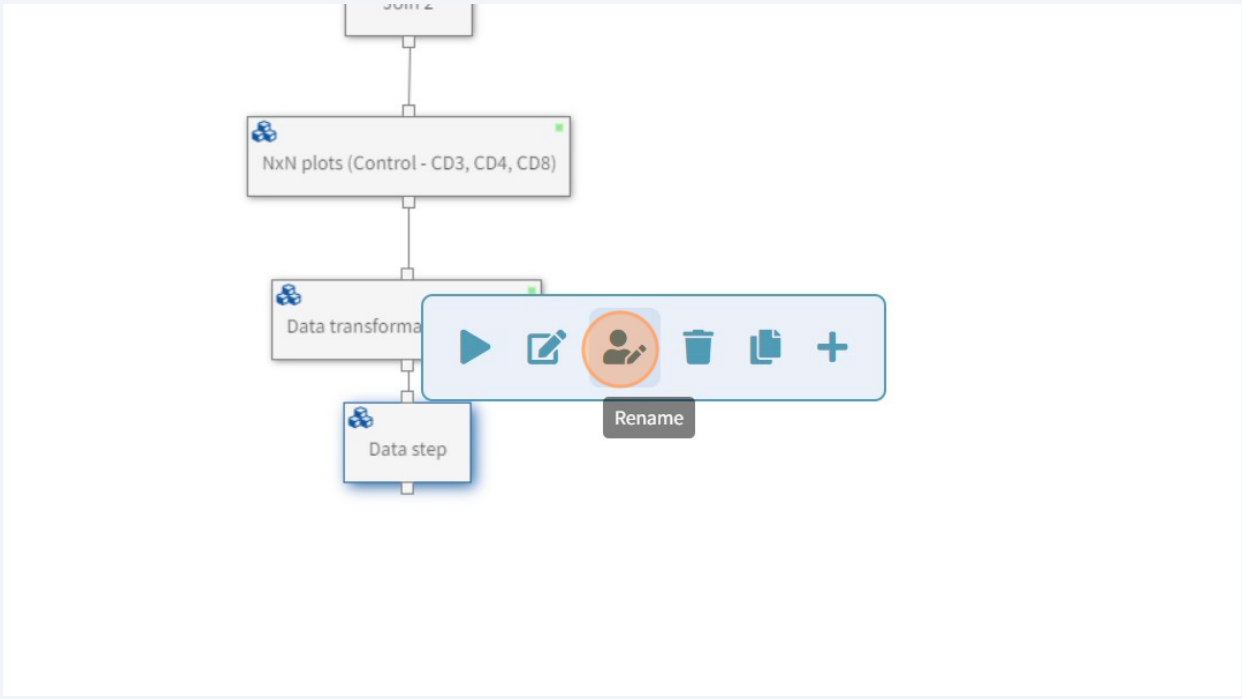


35 Navigate to the workflow by clicking "NxN plots on raw data (histograms)".

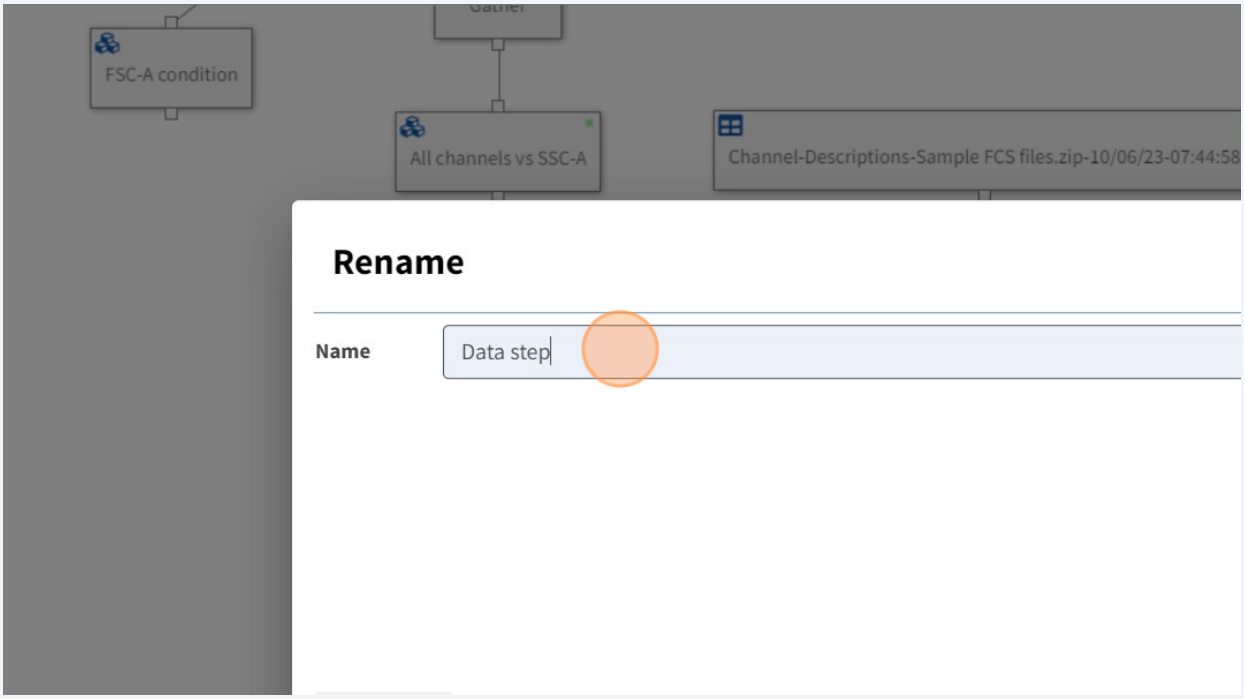
36 Click on the Data step created last.



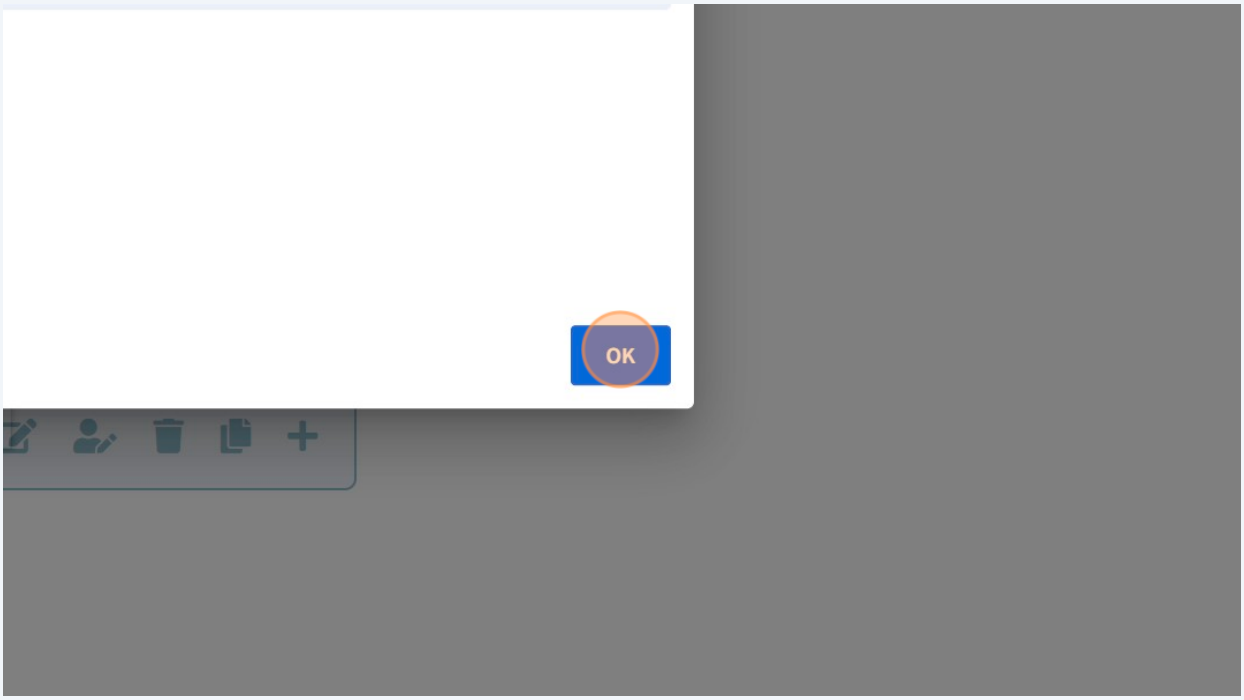
37 Select "Rename".



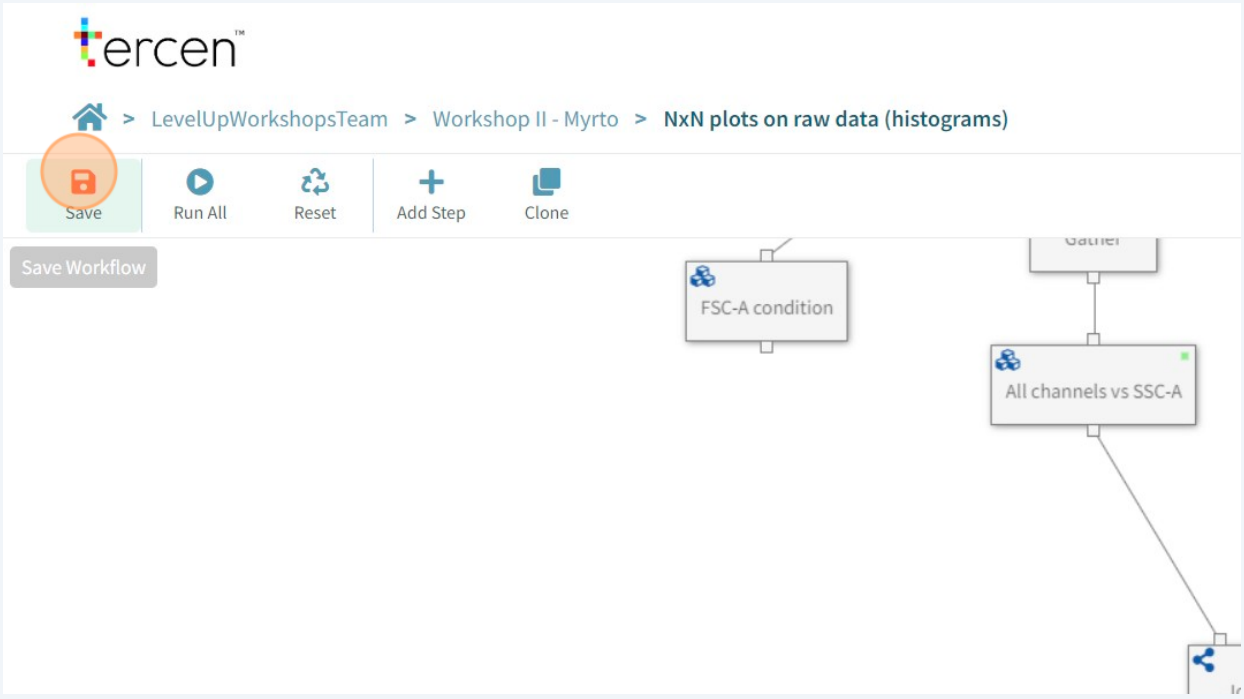
38 Type "NxN plots (logicle transormed CD3, CD4, CD8)" in the name text box.



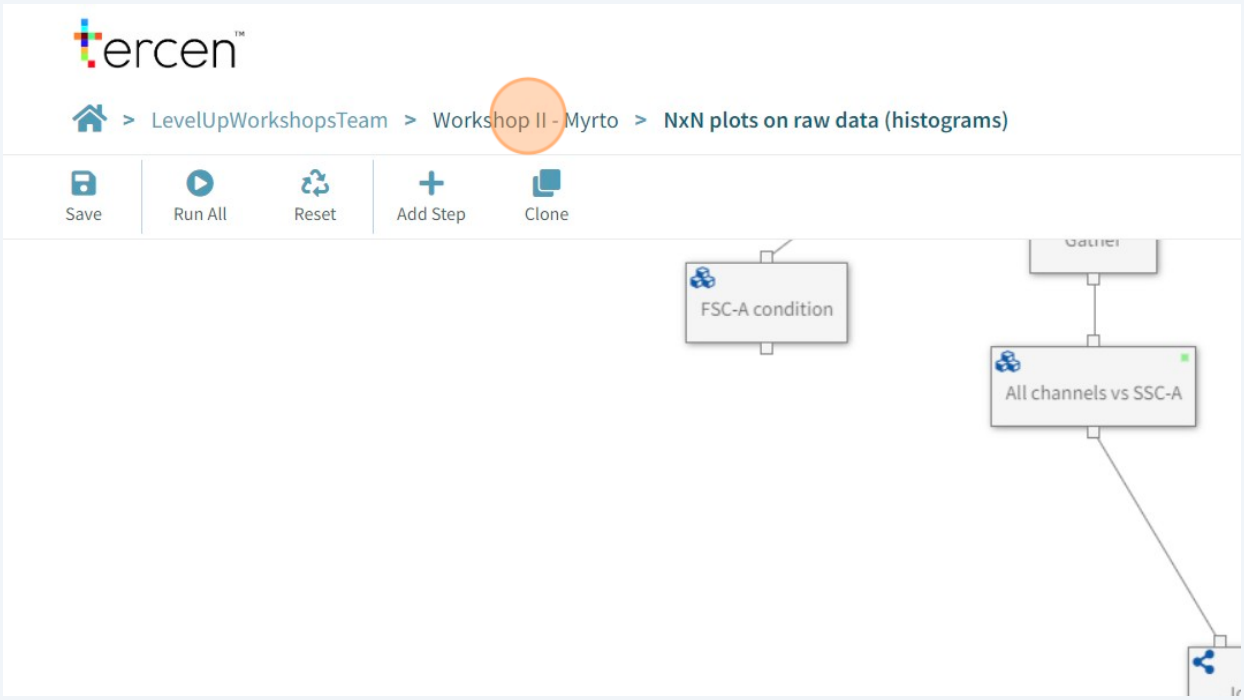
39 Click "OK"



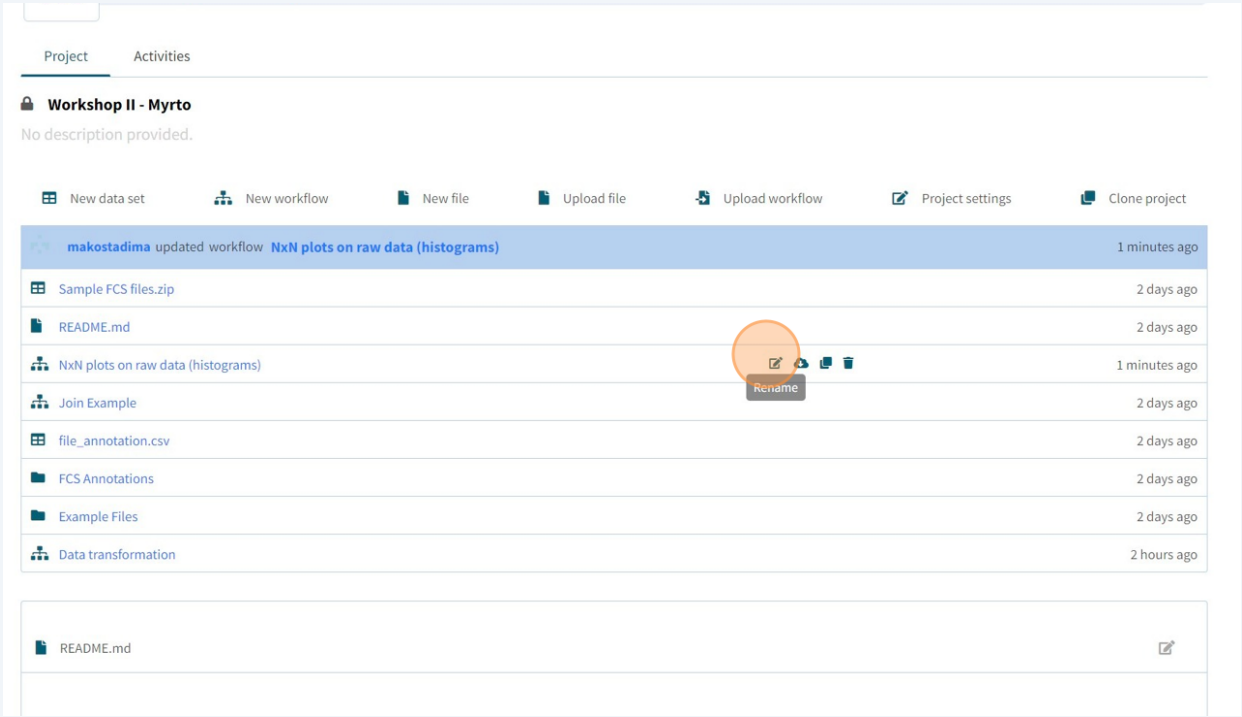
40 Do not forget to save your progress by clicking the Save button on the leftmost side of the Global toolbar.



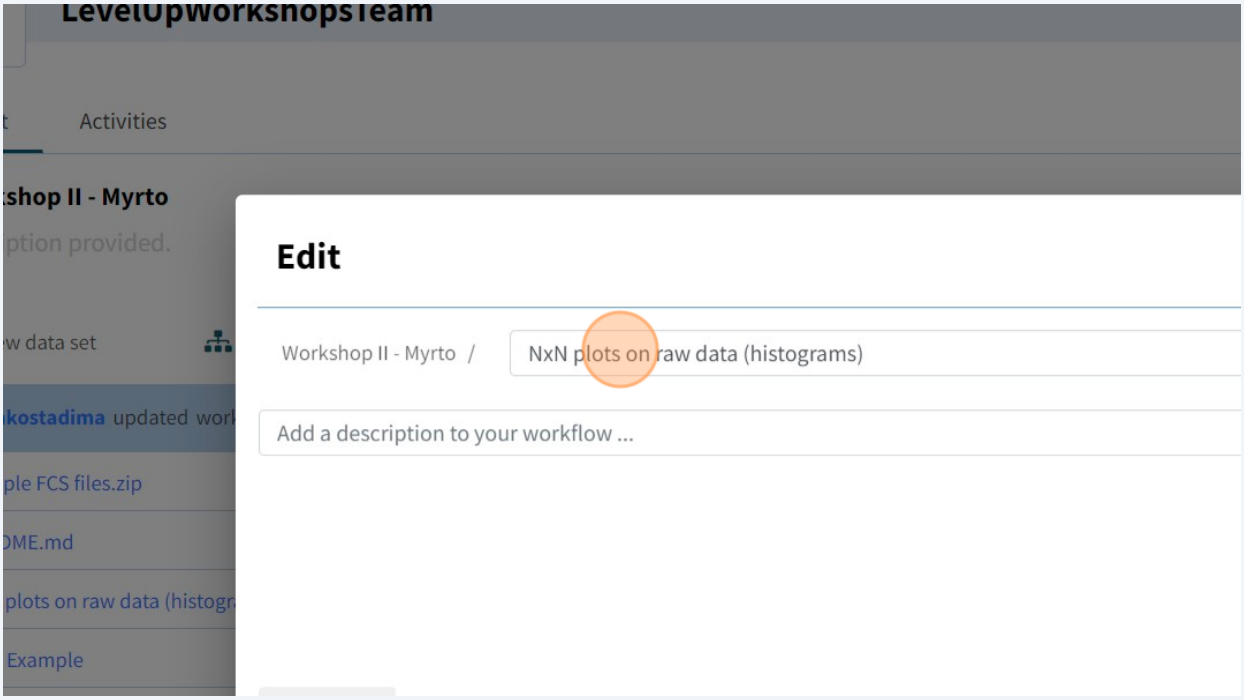
41 Click "Workshop II - Myrto".



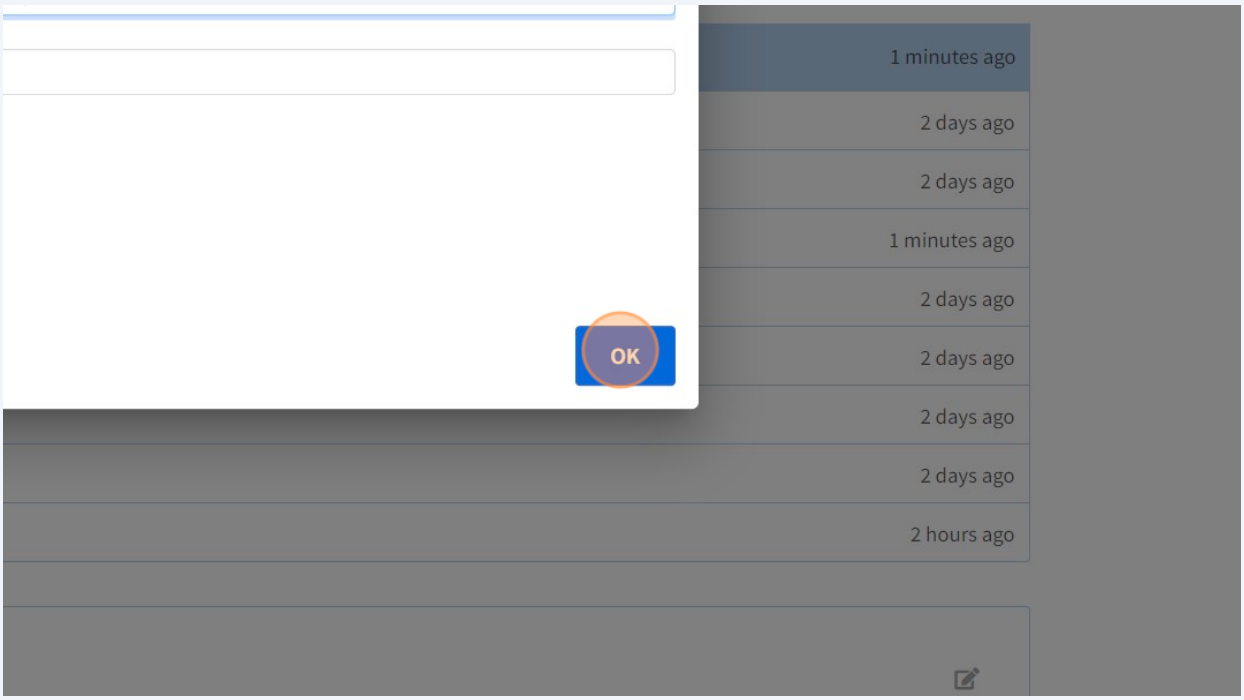
42 Click "Rename"



43 Rename the workflow to "NxN plots (histograms)".



44 Click "OK"



45 This guide has shown you how to transform your flow data.

Well done!

0207 - Clean Data

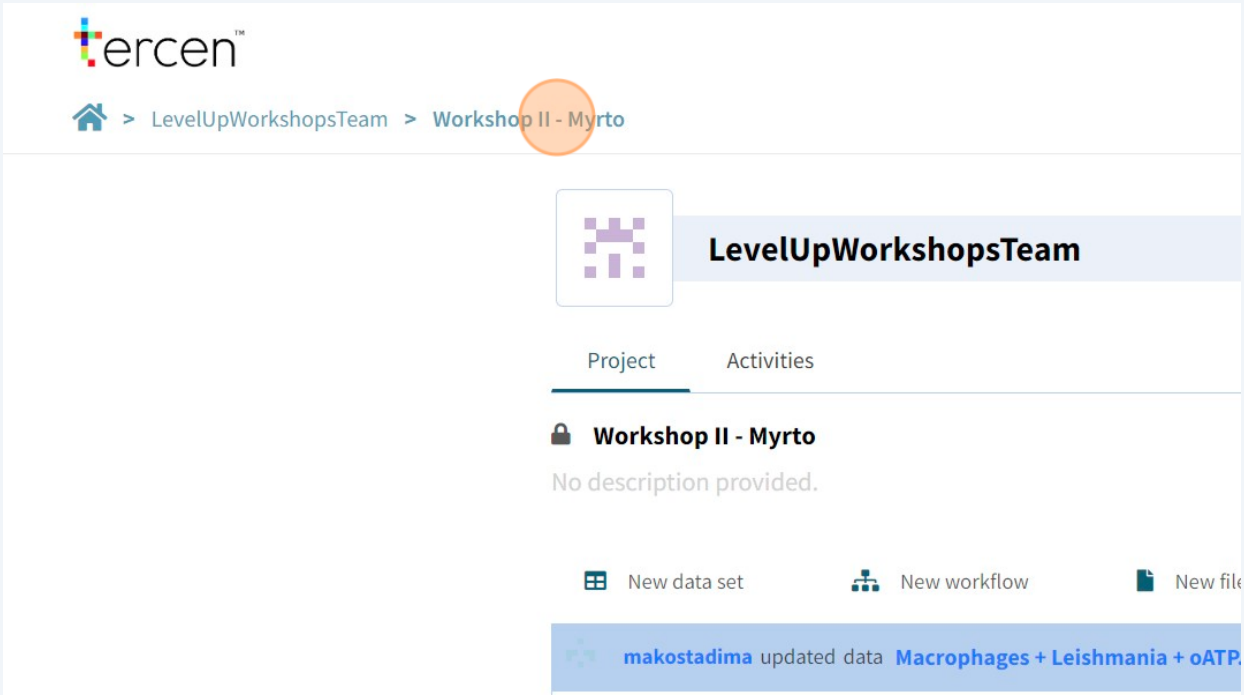
This lesson provides an introduction to automatic data quality control using the flowCut algorithm in Tercen.

Systematic changes throughout the data acquisition can occur because of bubbles, cell clumping, washing, laser malfunction, etc. Identifying and removing such spurious events is crucial for downstream data analysis.

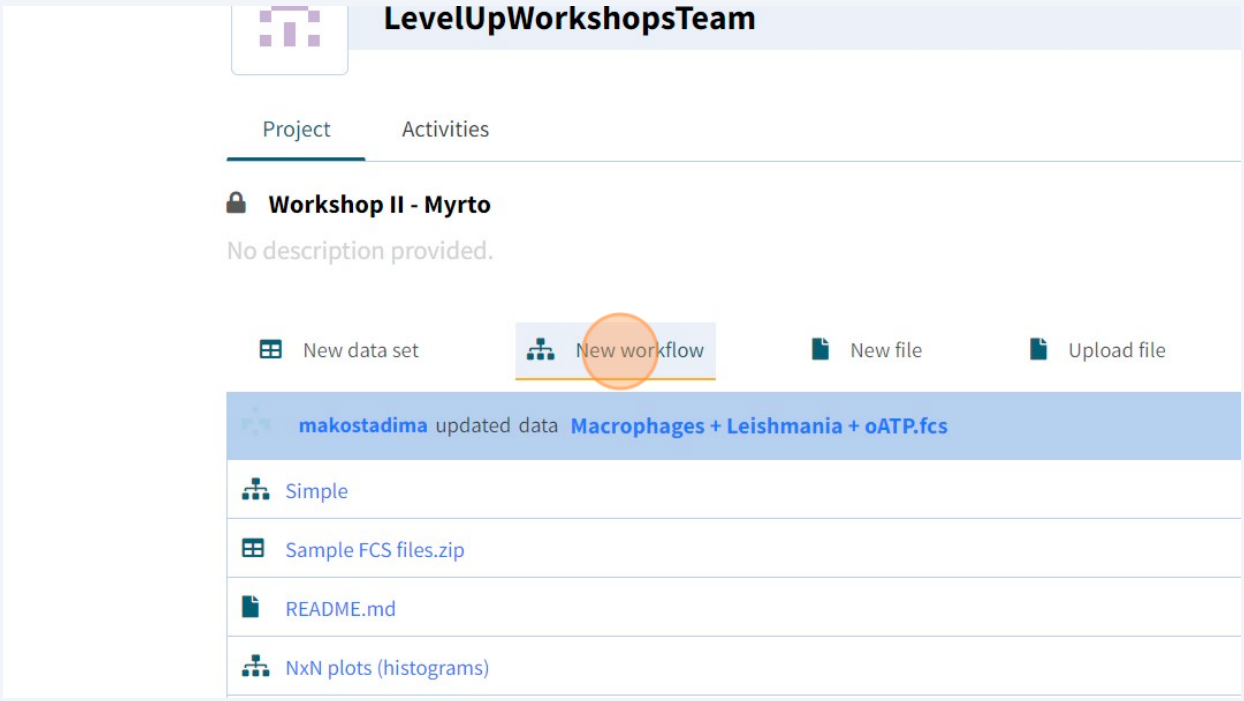
Several algorithms have been published to achieve that automatically, including flowAI, flowClean, flowCut, and PeacoQC.

This introduction will teach you to use flowCut in Tercen.

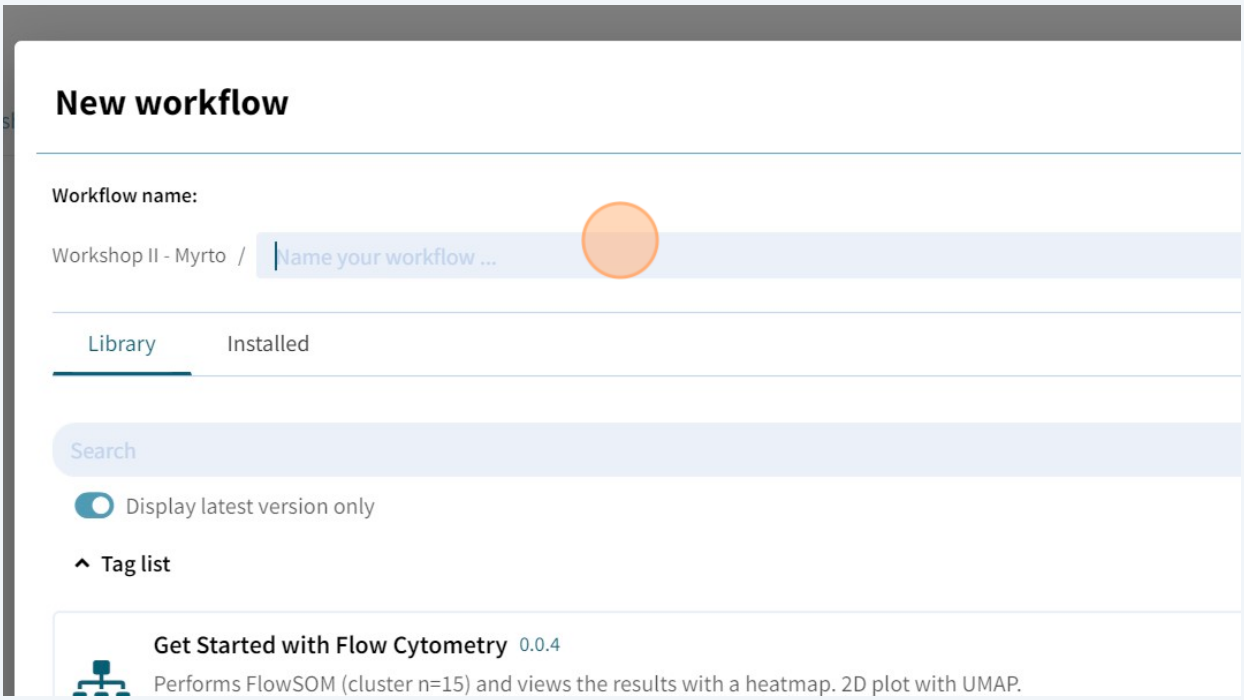
1 Click on your copy of the Workshop II Tercen project.



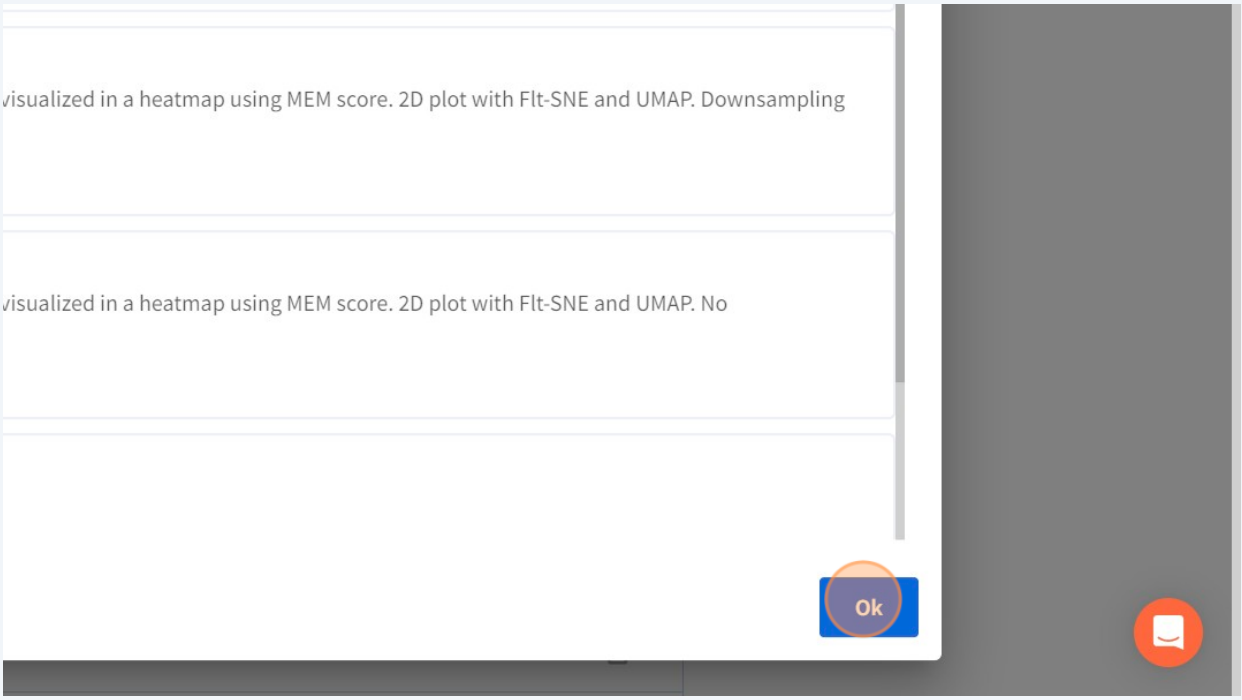
2 Click "New workflow".



3 In the Workflow name text box type "Automatic data clean up".

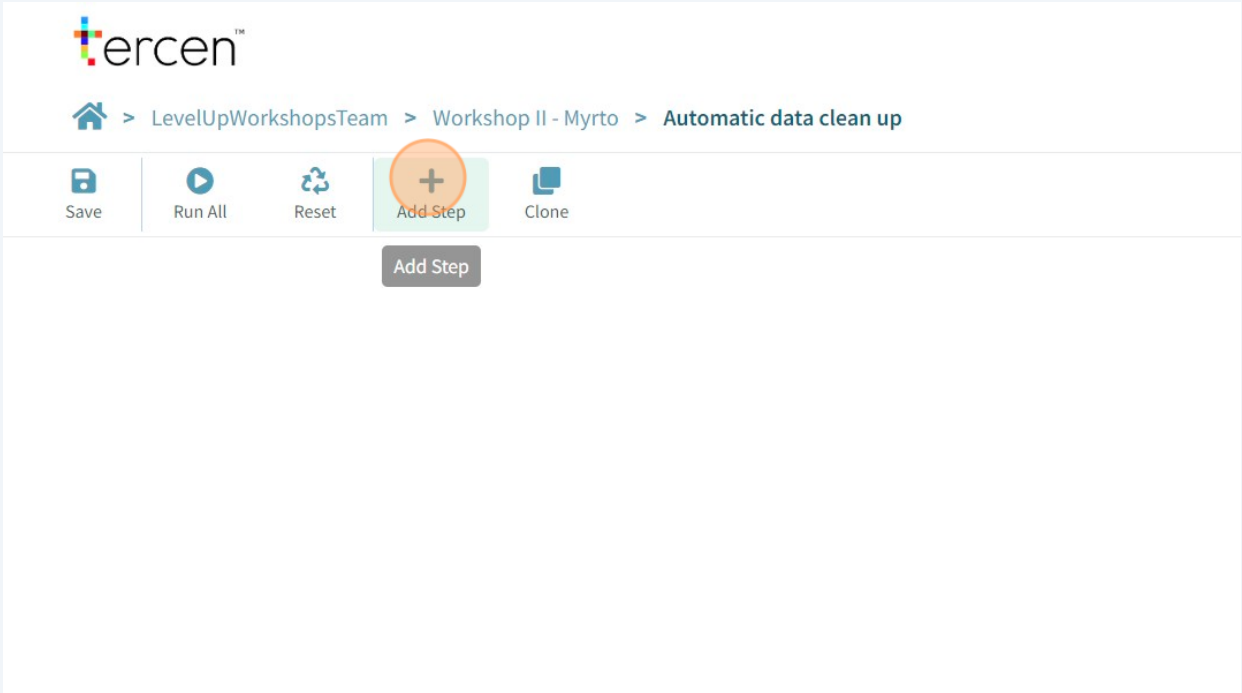


4 Click "Ok".

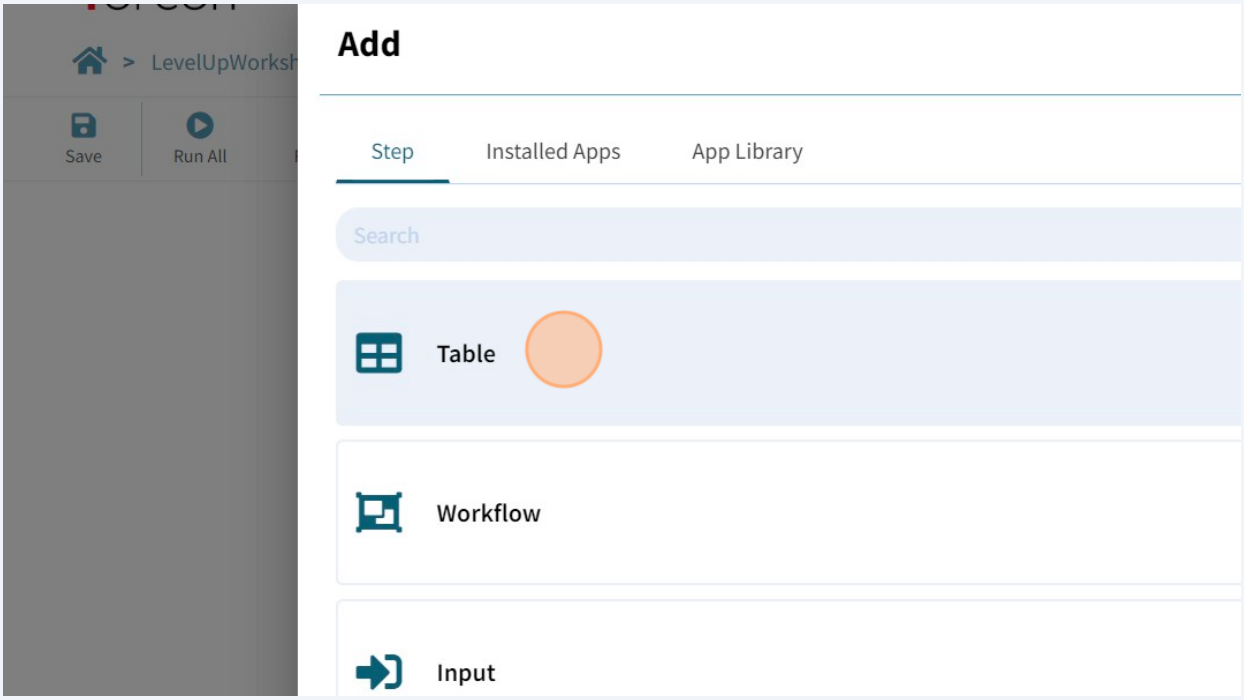


5 You will start by adding a data table to the empty workflow.

Click "Add Step".



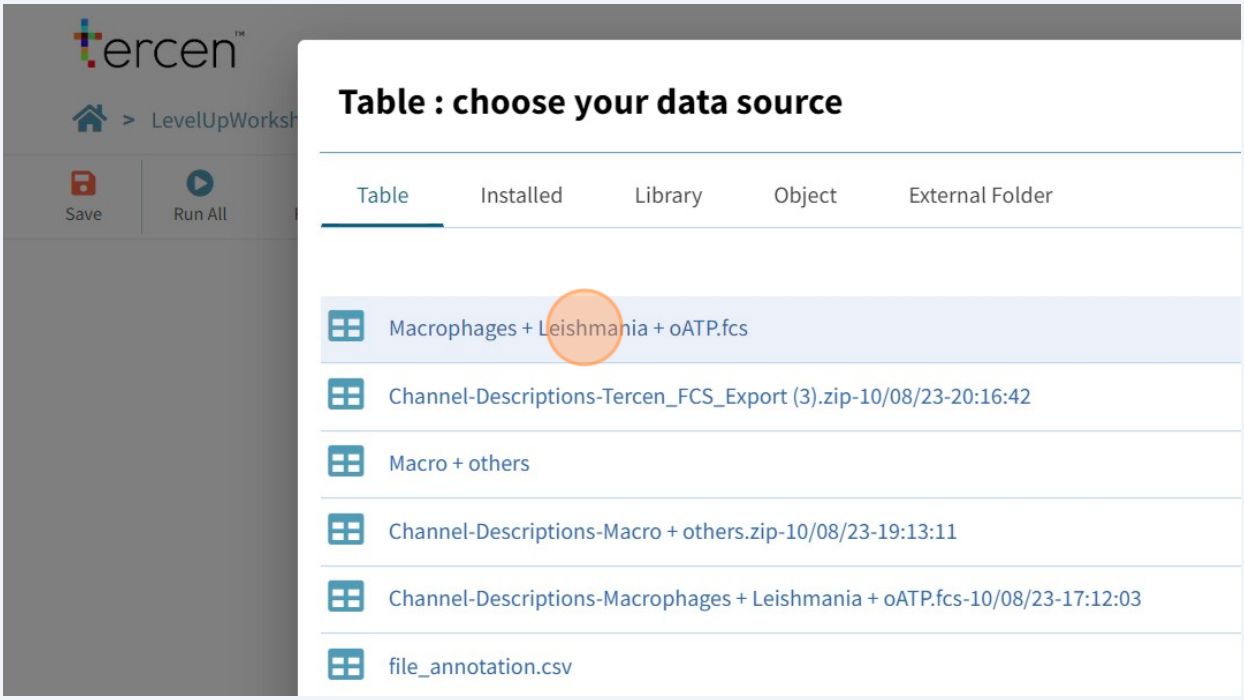
6 Select "Table".



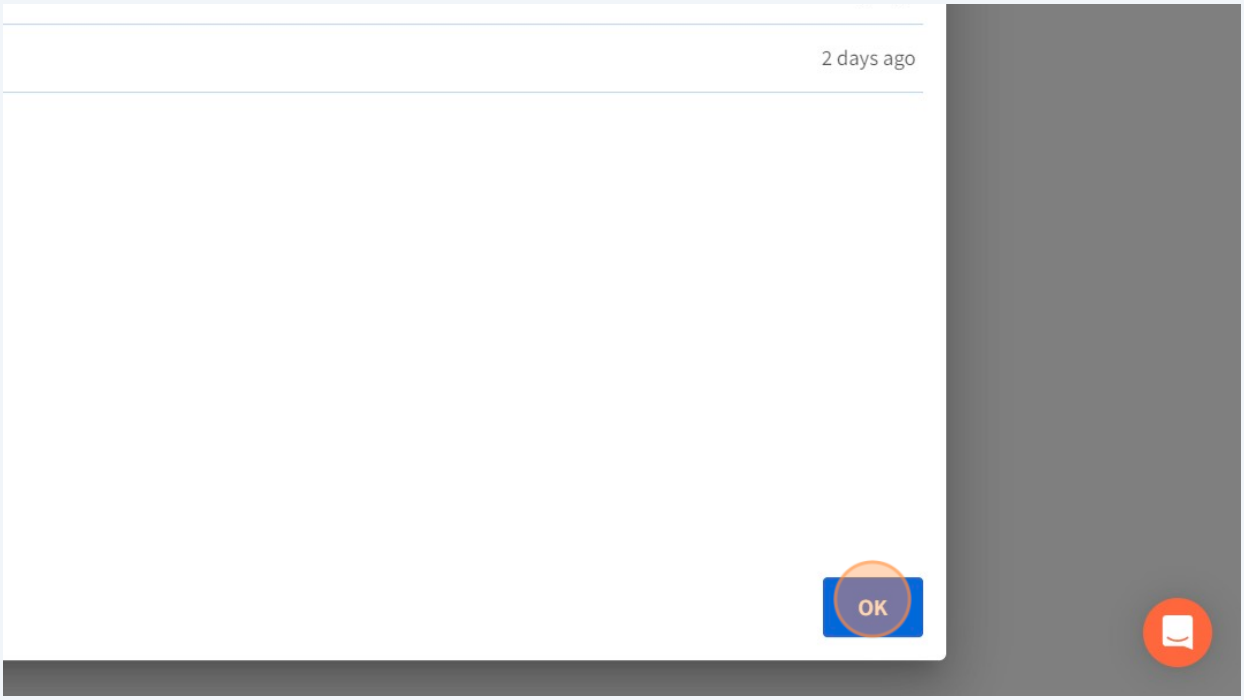
7 For this lesson, we will work with an FCS files that was generated to evaluate the effect of periodate-oxidized ATP (oATP) on parasite control in Leishmania-infected macrophages. The dataset was originally published here and can be accessed on FlowRepository.

We have already read the data into Tercen, which is stored under the "Example Data" folder.

On the popup window, select "Macrophages + Leishmania + oATP.fcs"

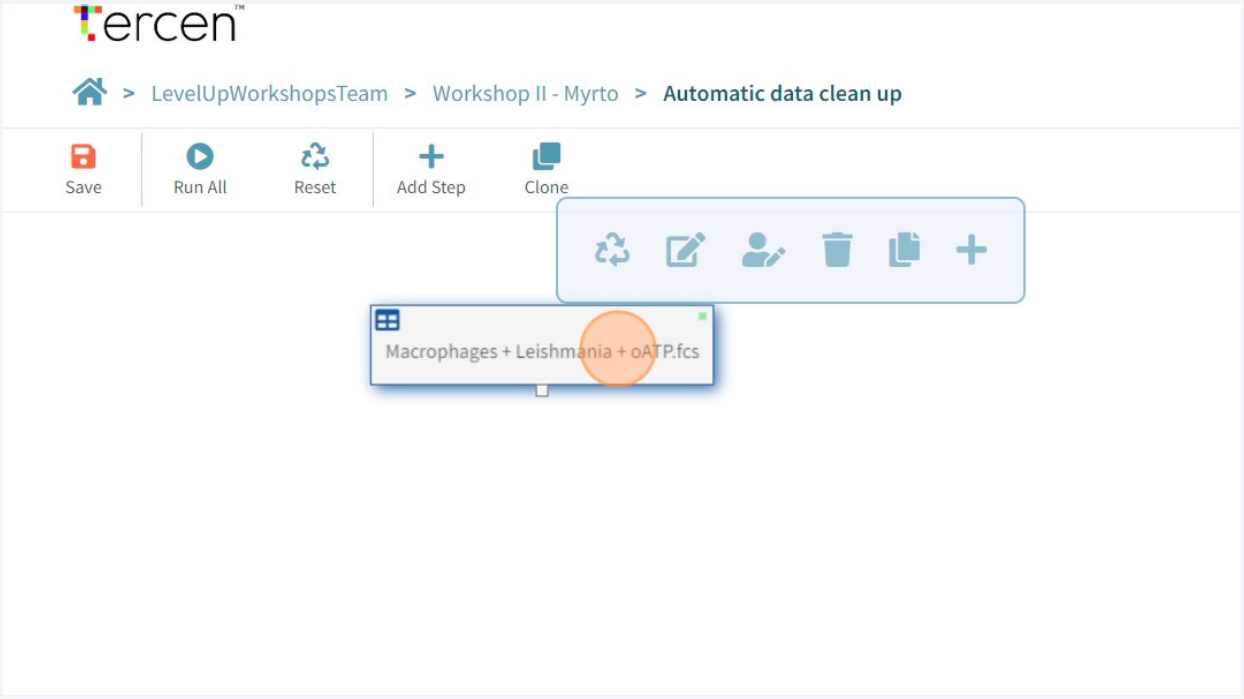


8 Click "OK".

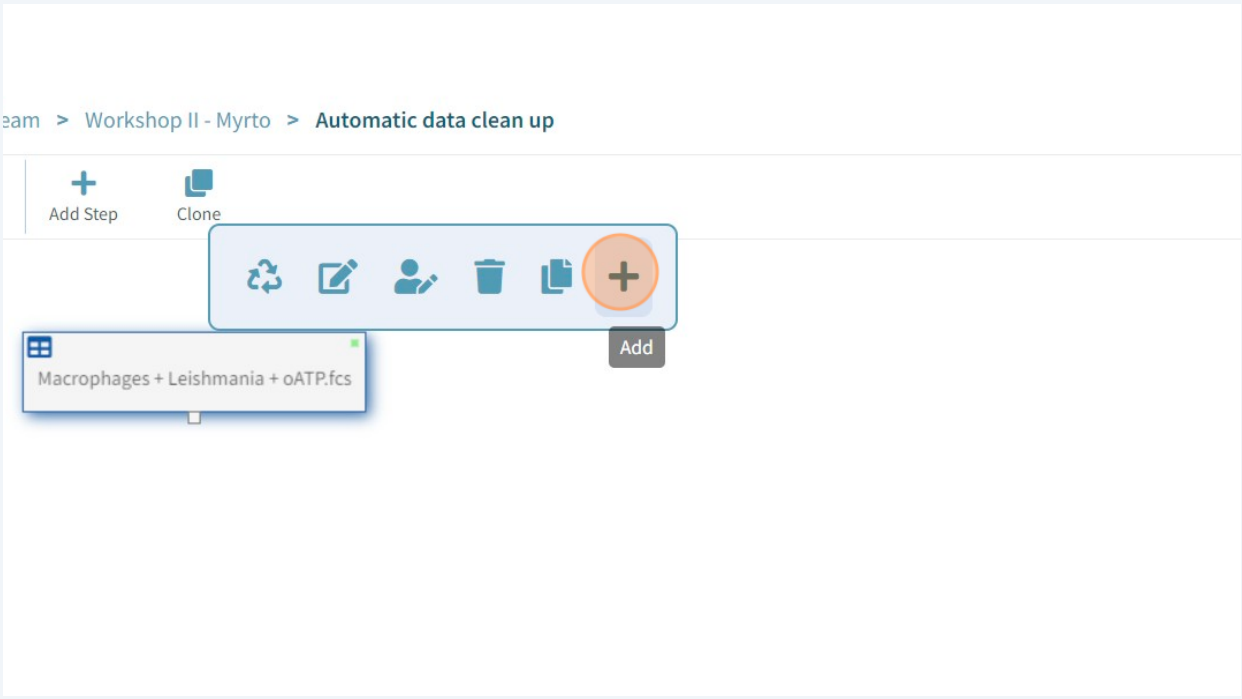


9 You will go on to convert the data into a long format using a **Gather** step.

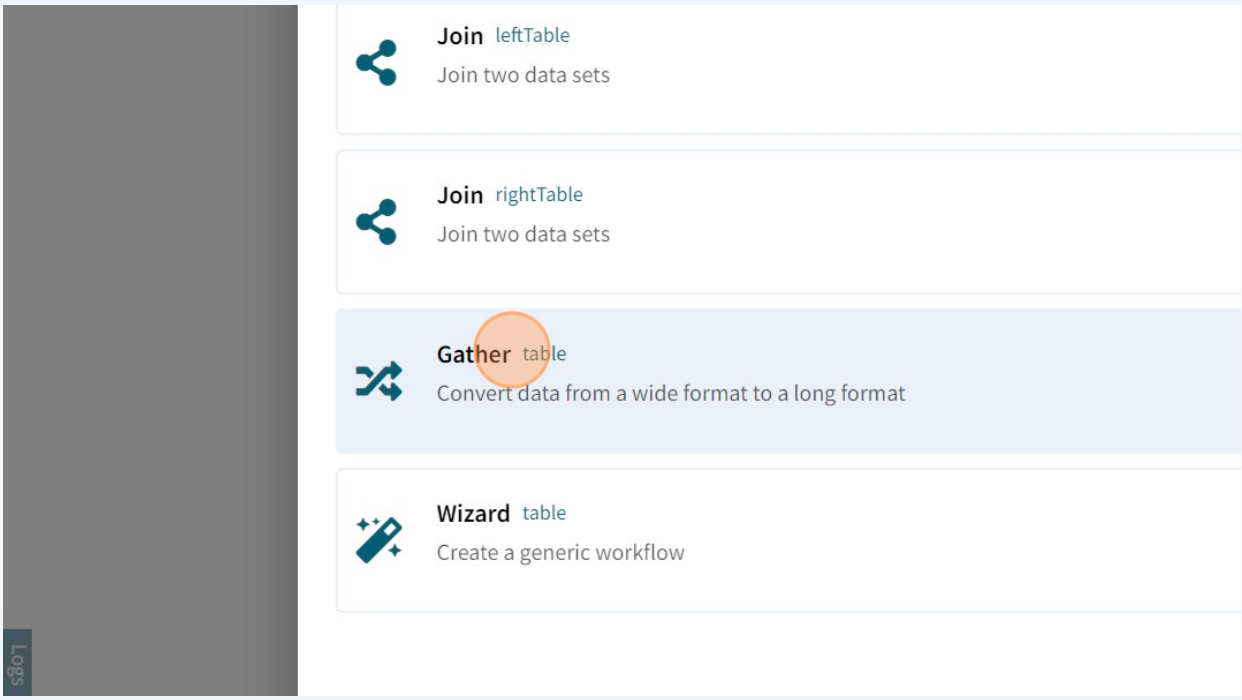
Click on this step so that the local toolbar appears over it.



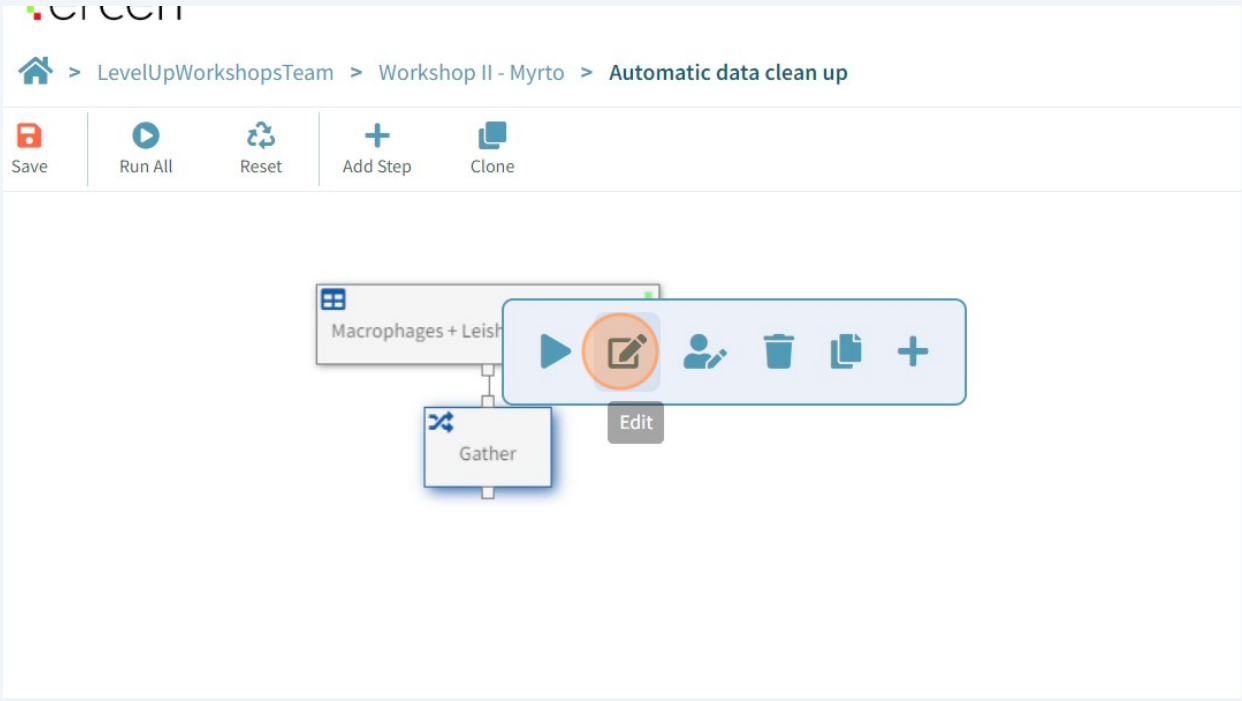
10 Select **Add**.



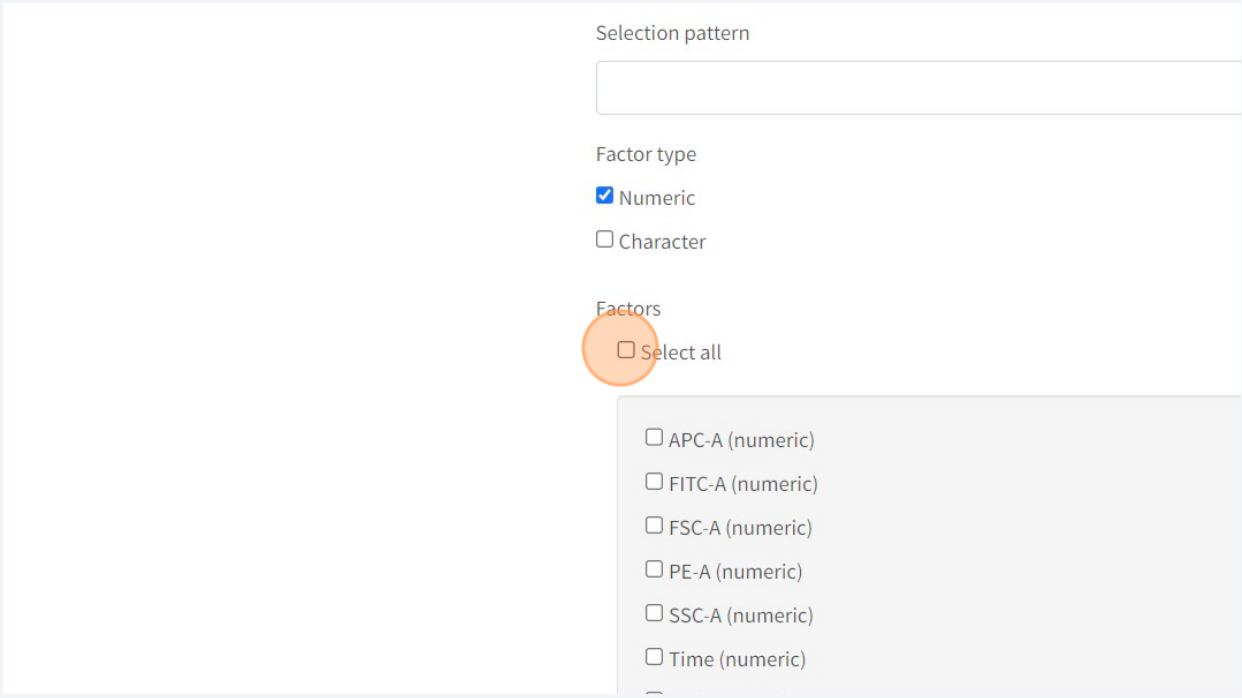
11 Select **Gather.**



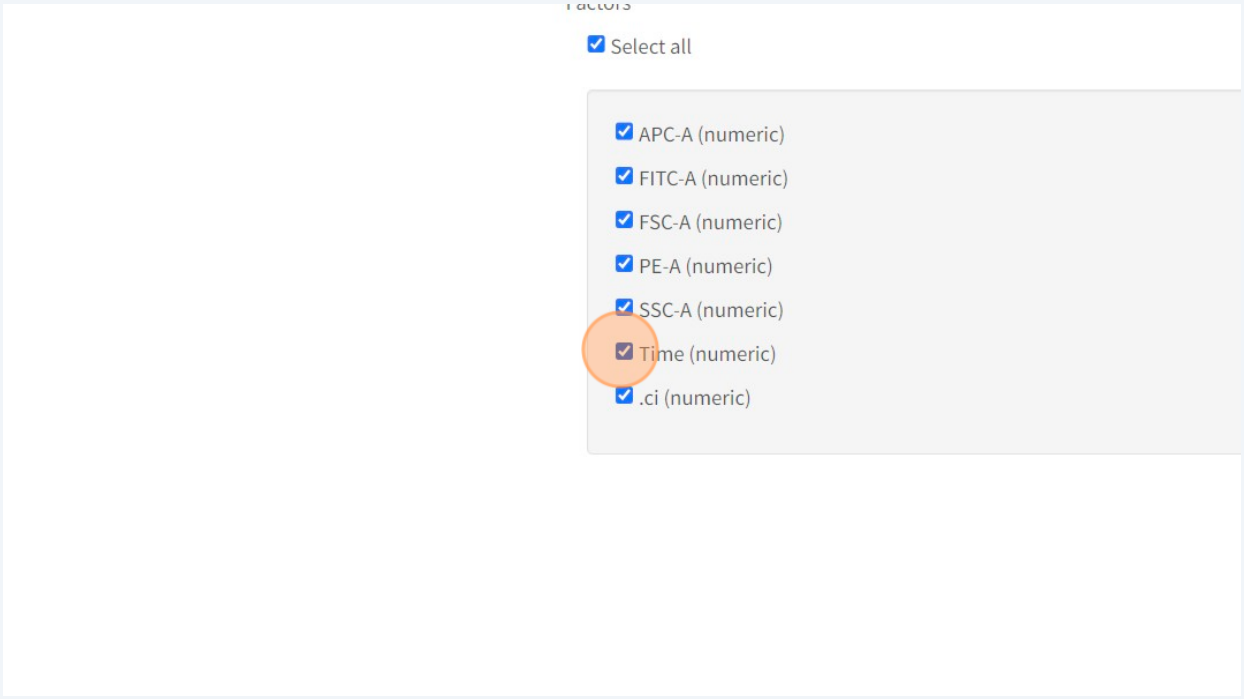
12 Click **Edit.**



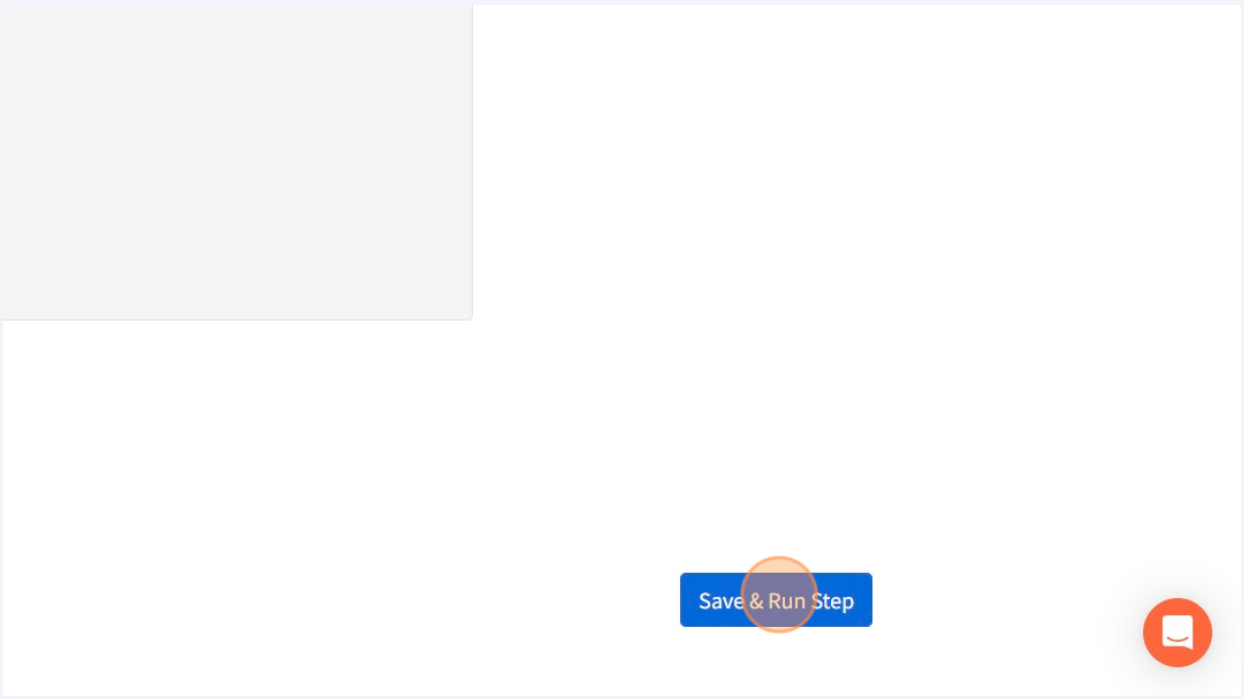
13 Click the "Select all" field.



14 Unselect the "Time (numeric)" & ".ci (numeric)" fields.

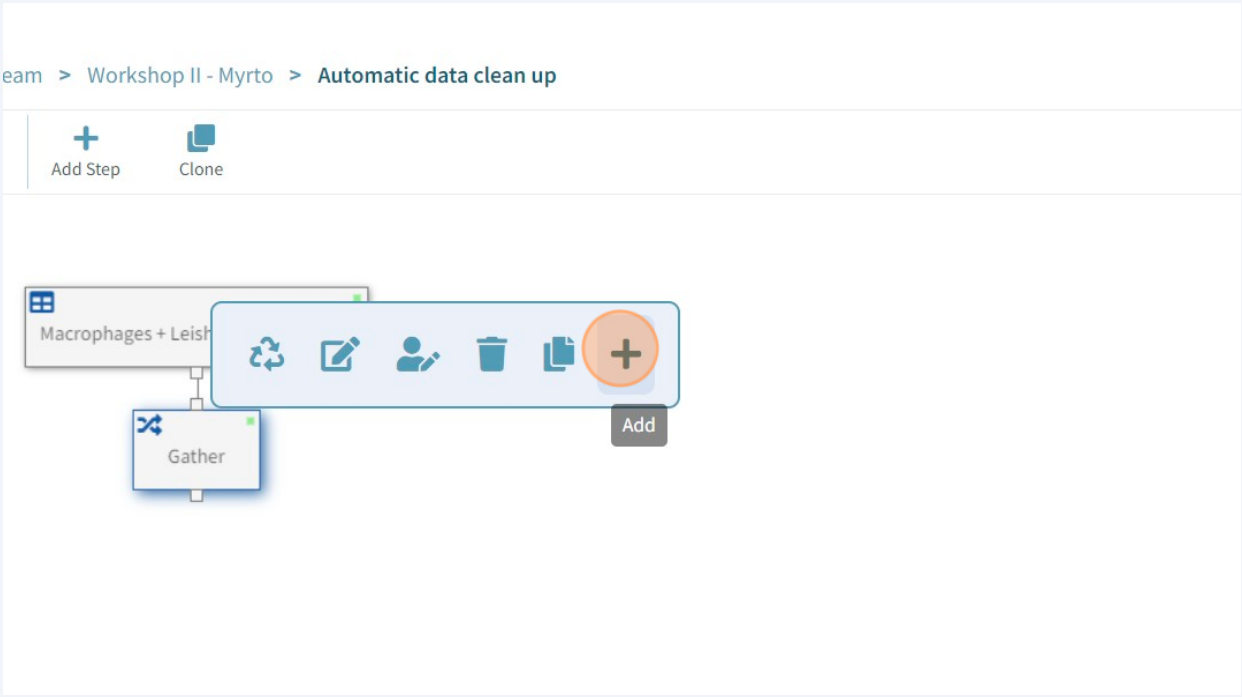


15 Click "Save & Run Step".

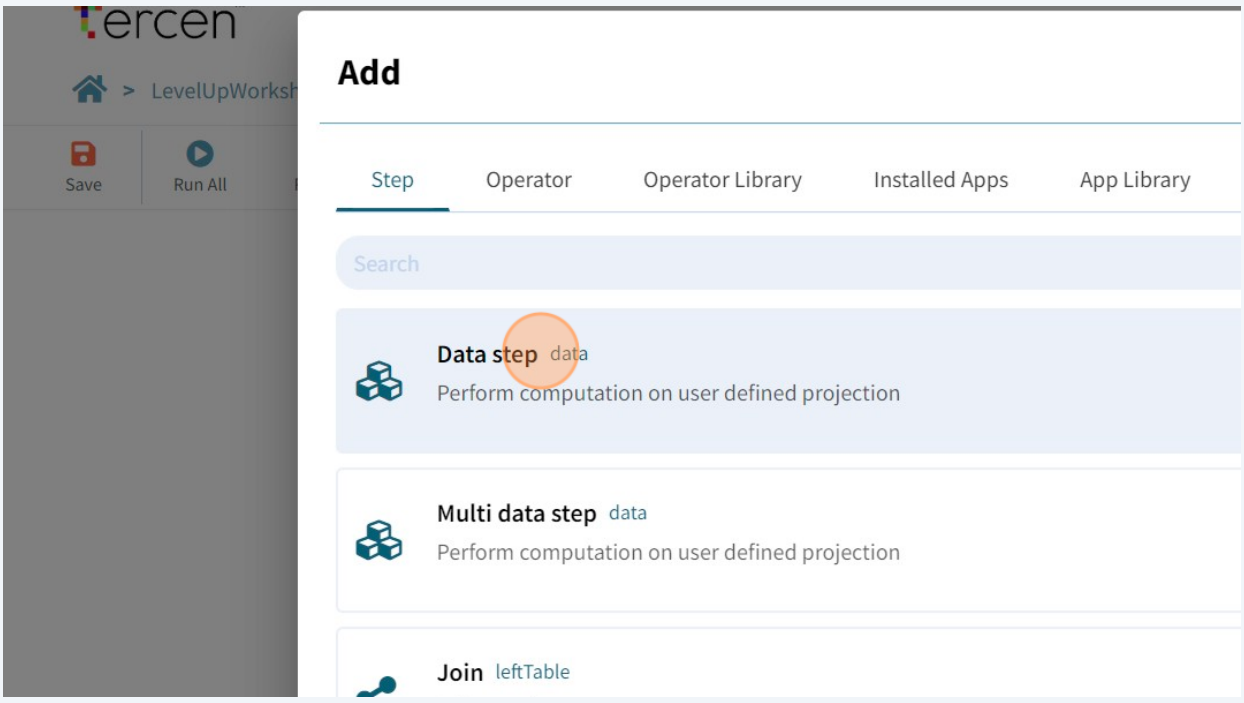


16 You will then proceed to transform the data using the logicle operator.

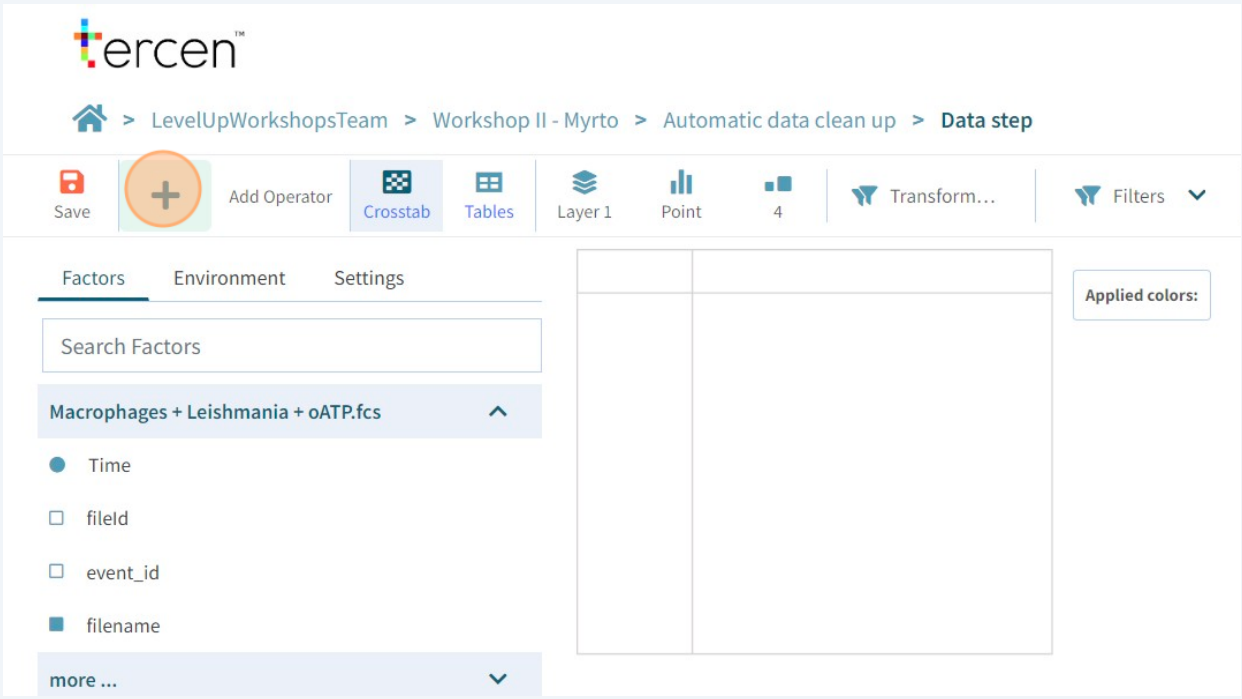
Click **Add**.



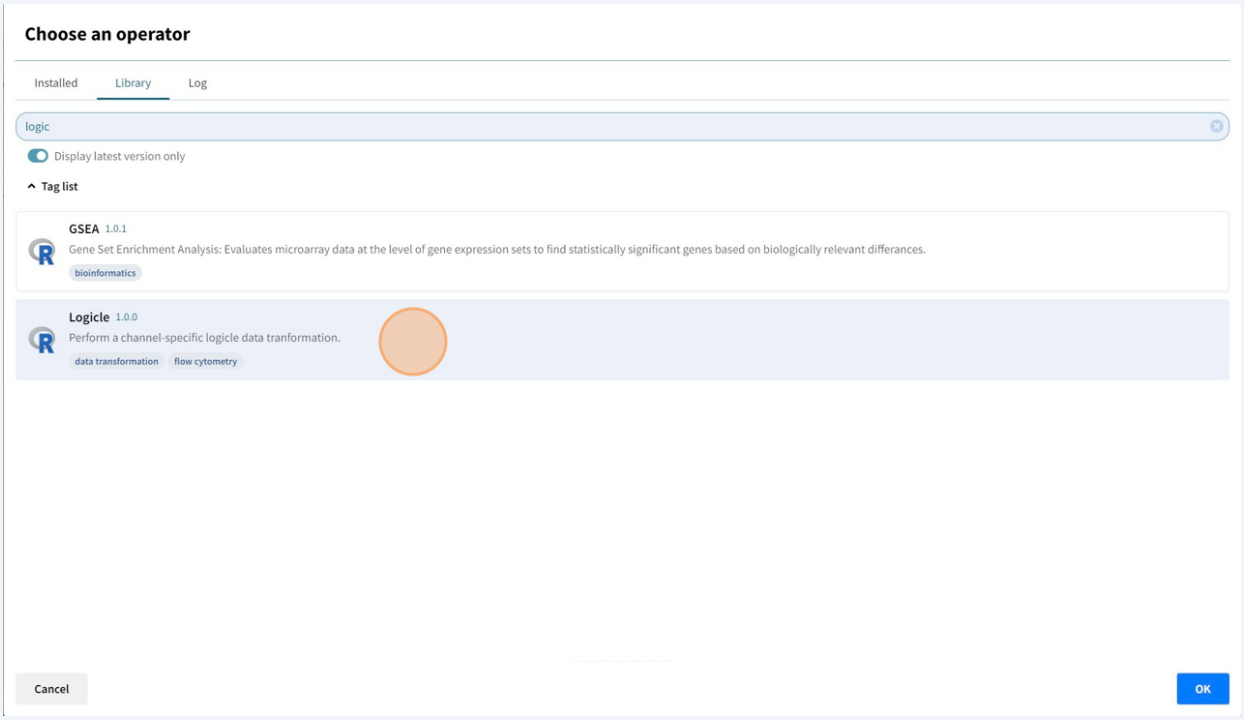
17 Select "Data step".



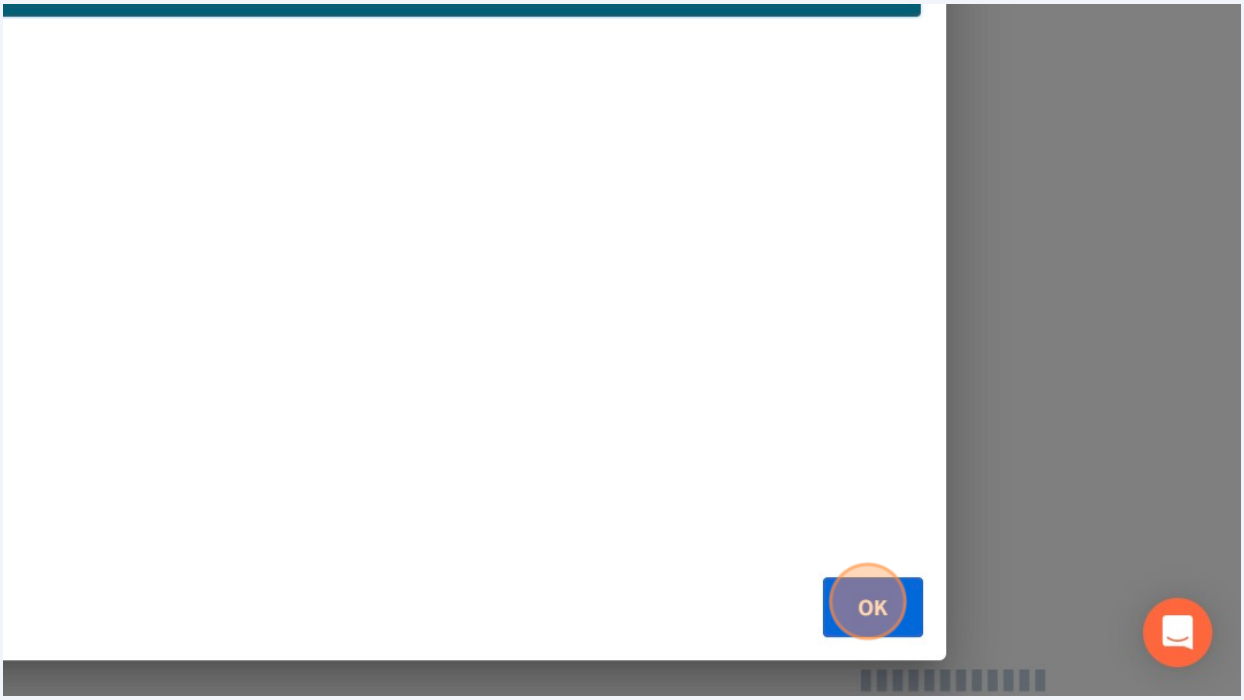
18 Click the '+' button to add the logicle operator.



19 In the Library tab search for and select the Logicle operator.

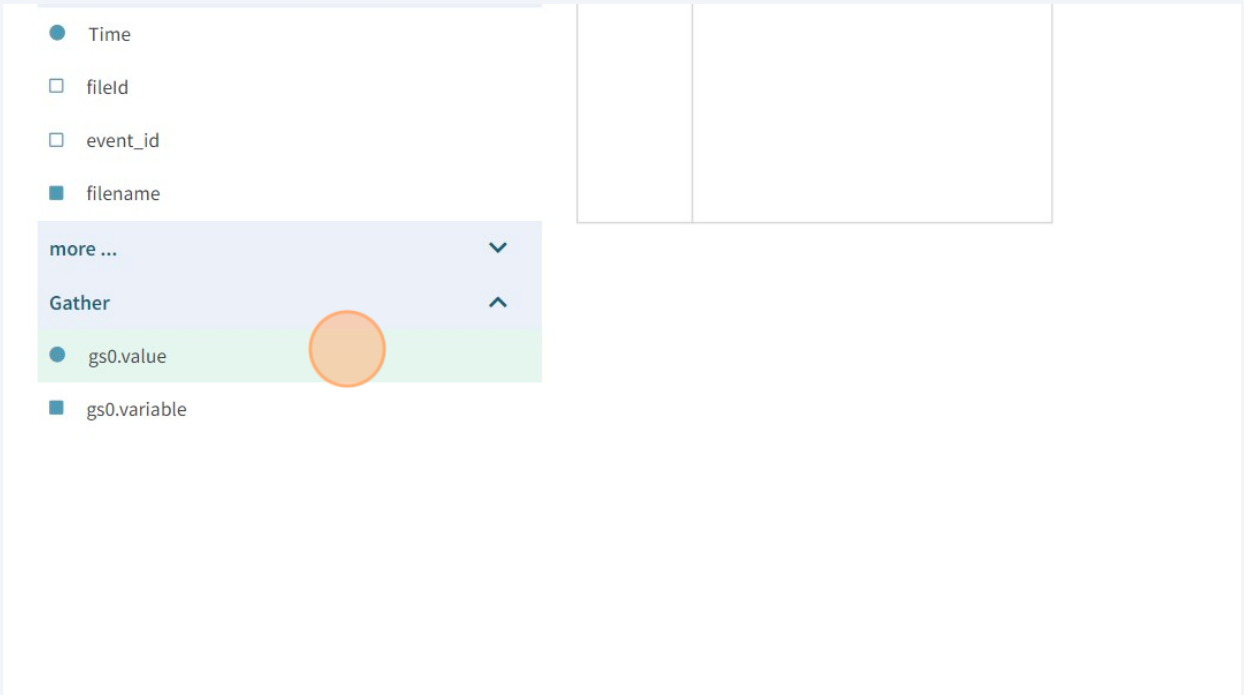


20 Click "OK".

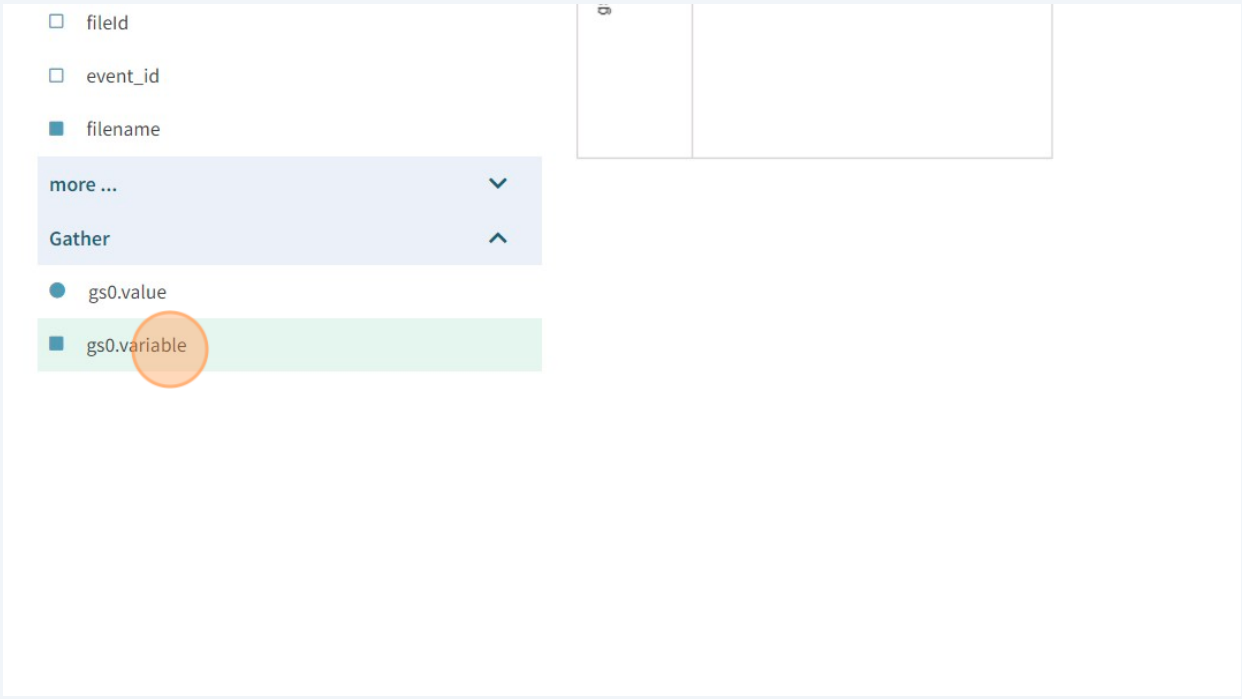


21 Let us start projecting the data in order to run the logicle operator.

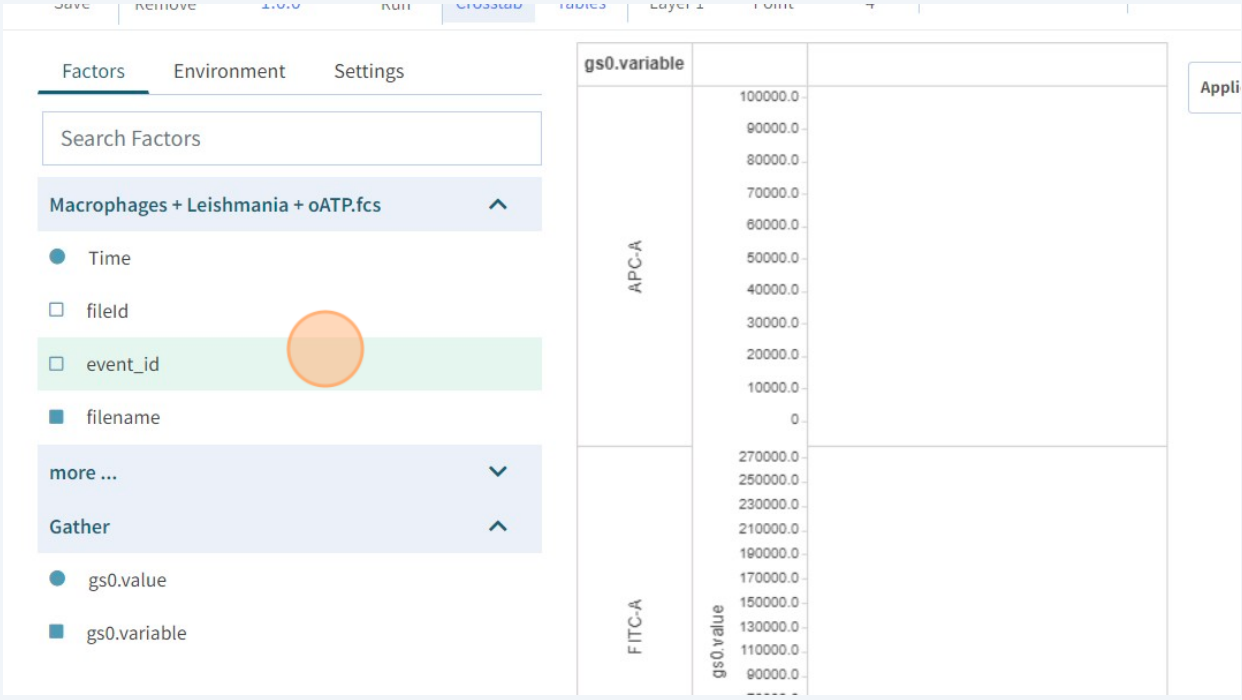
Drag the **gs0.value** factor and drop it in the Y-axis.



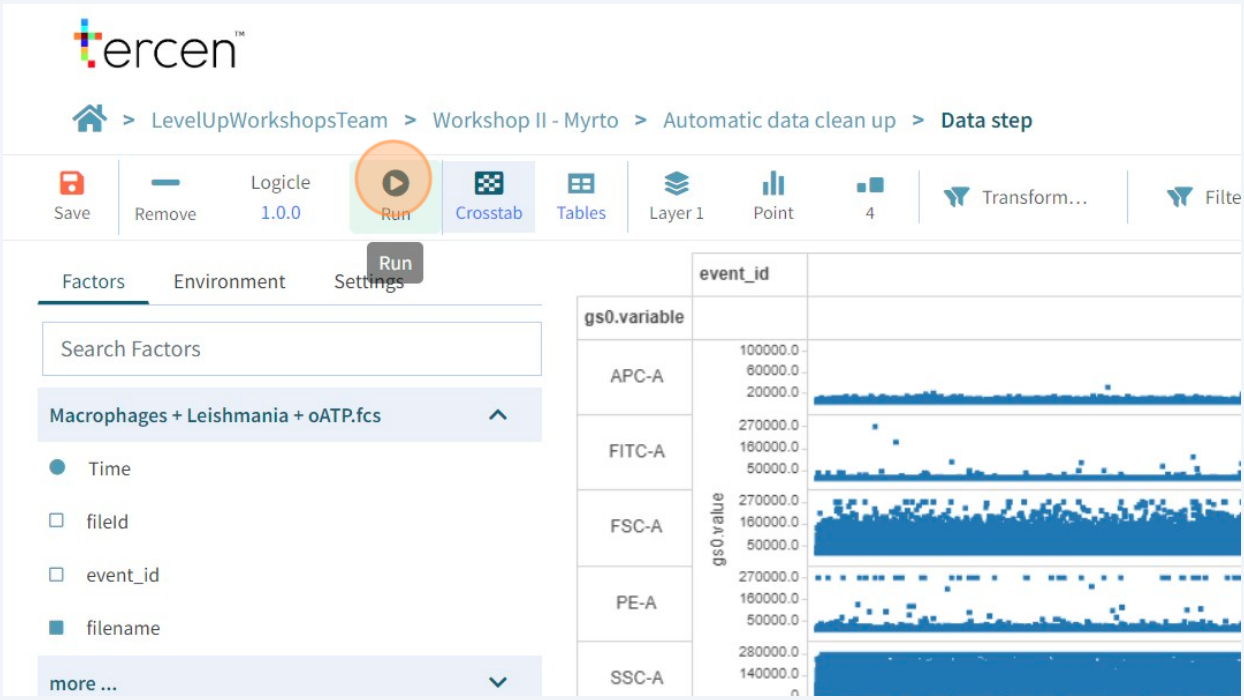
22 Drag the **gs0.variable** factor and drop it in the Row.



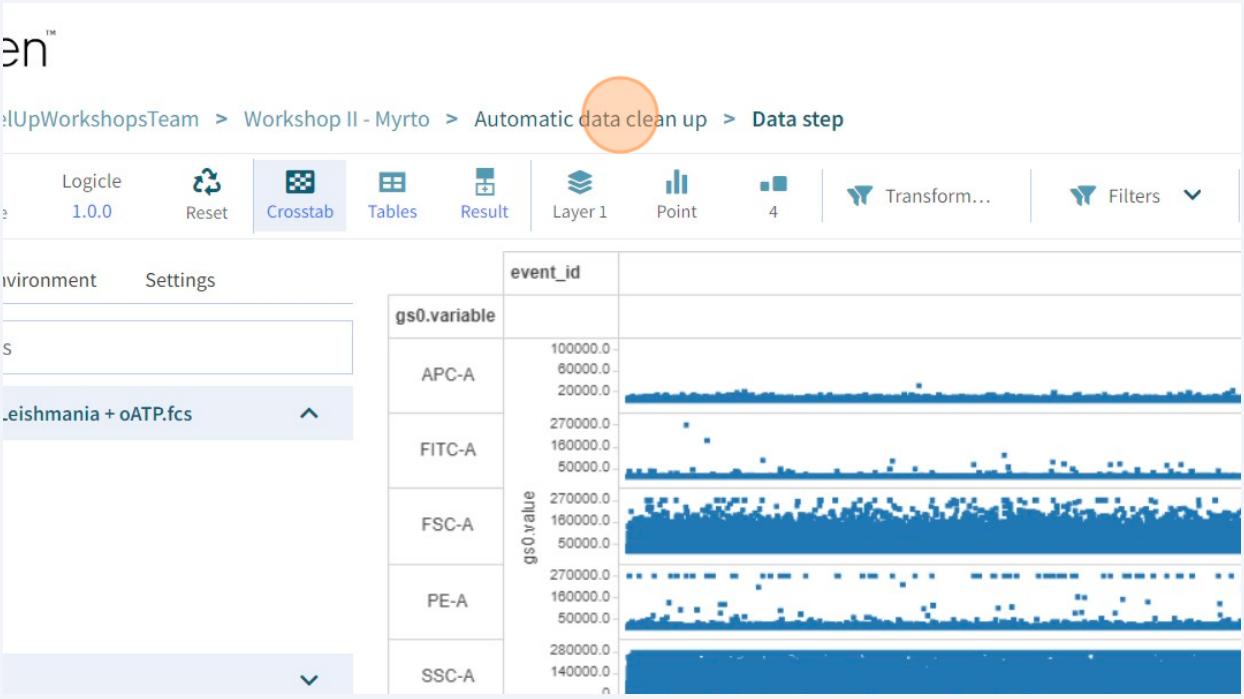
23 Drag the **event_id** factor and drop it in the Column.



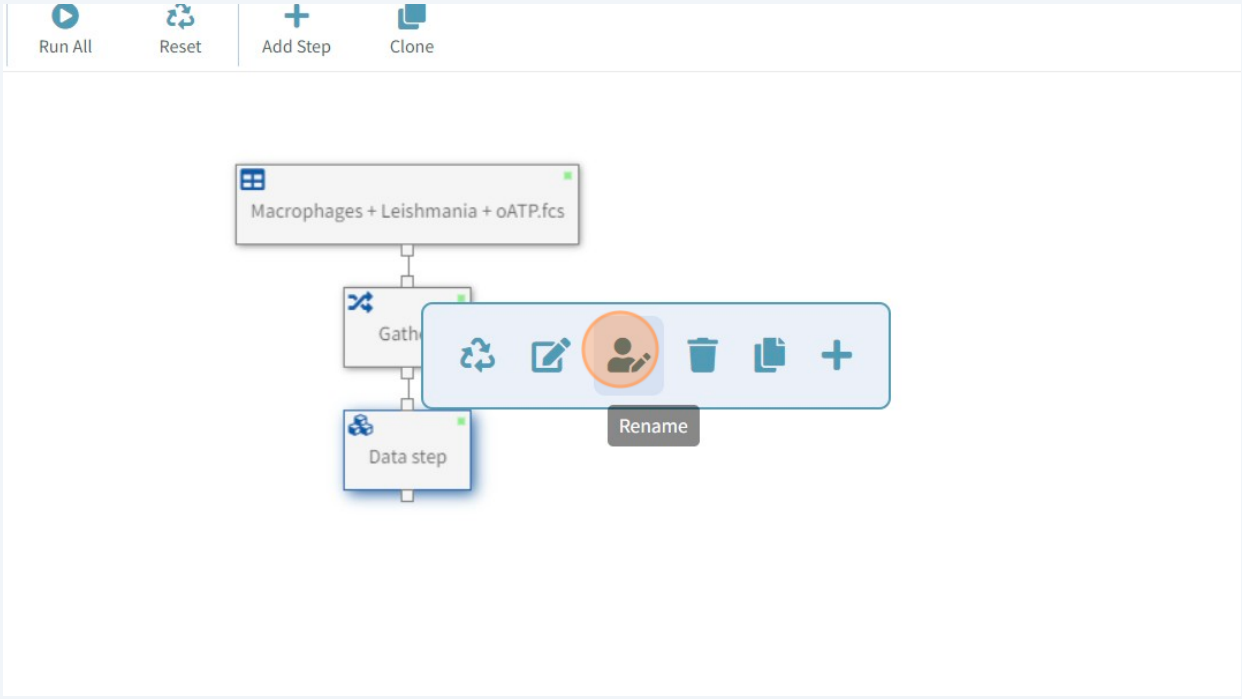
24 Click 'Run'.



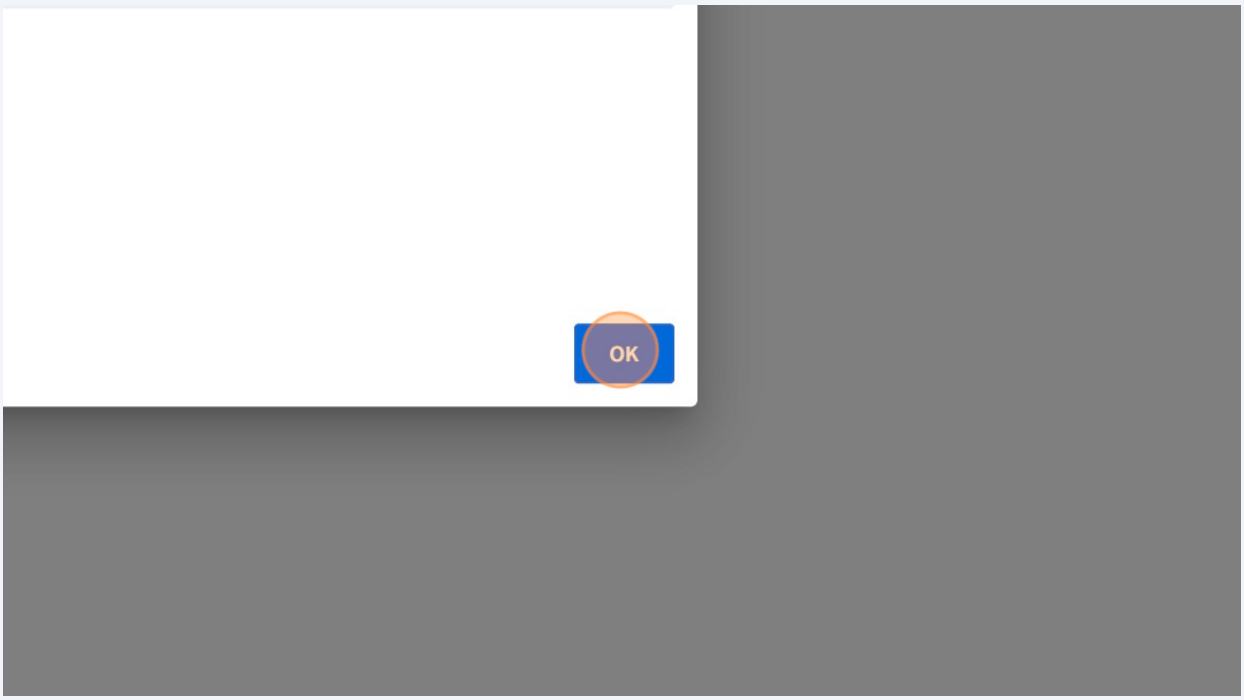
25 Click the "Automatic data clean up" link to return to the workflow.



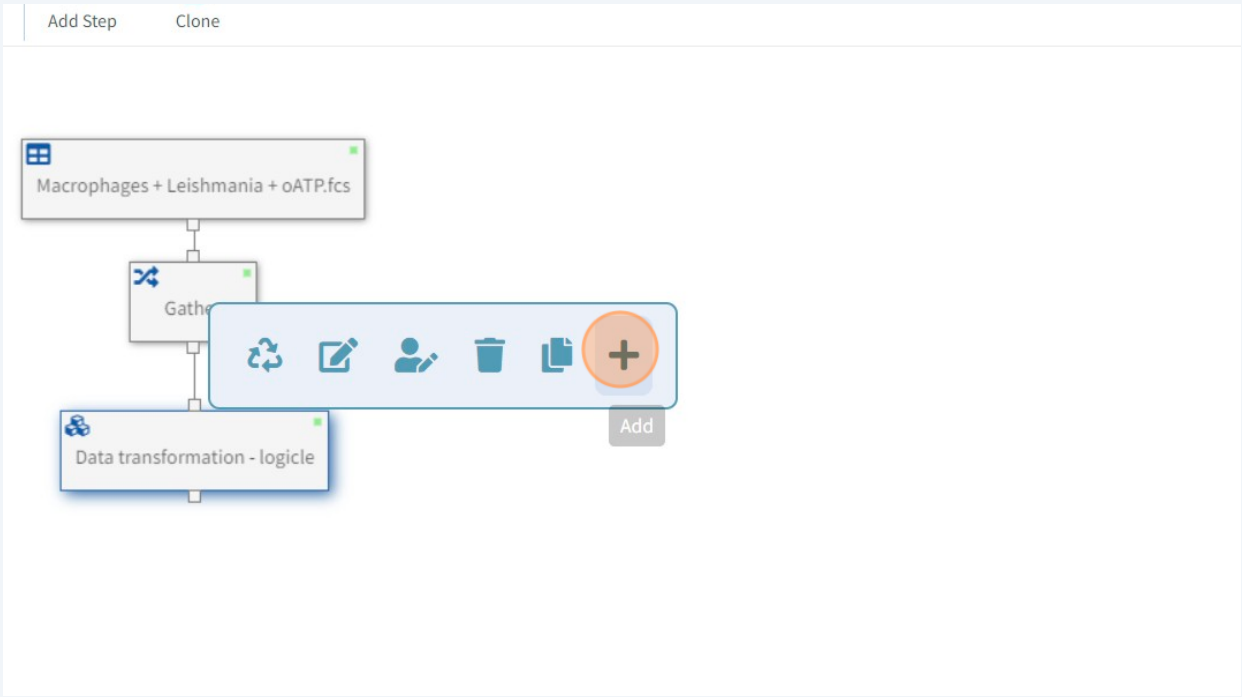
26 Rename the Data step to "Data transformation - logicle".



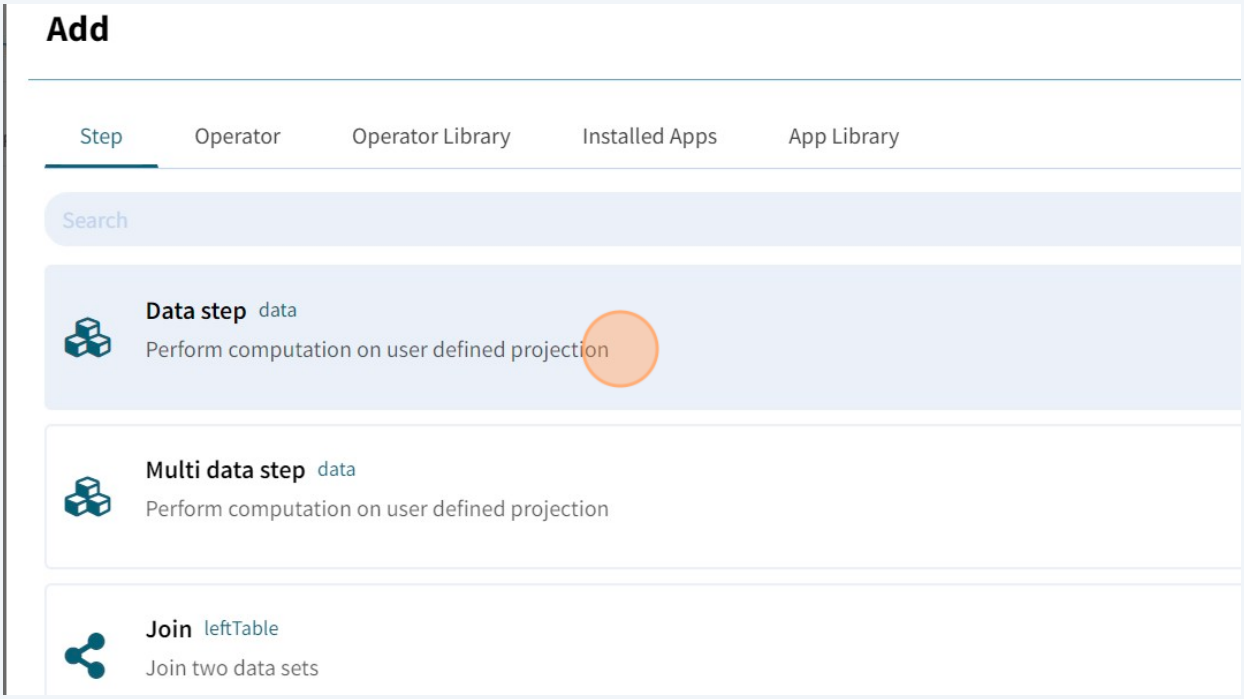
27 Click "OK".



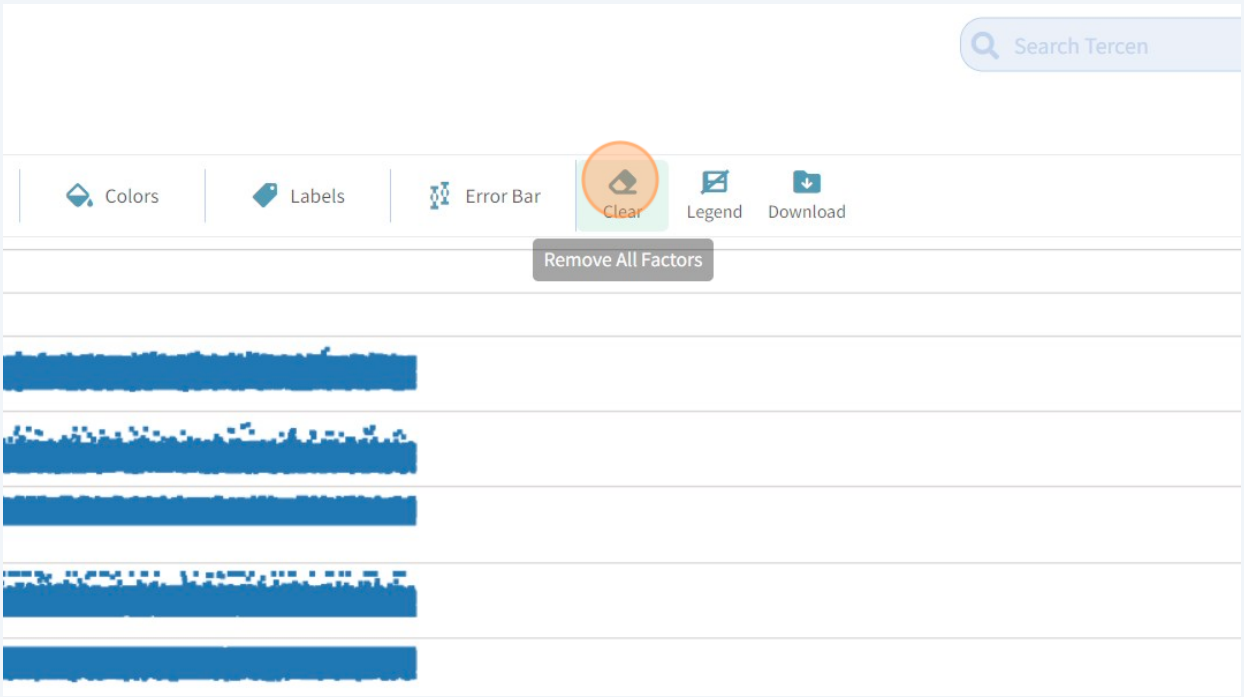
28 Click 'Add'.



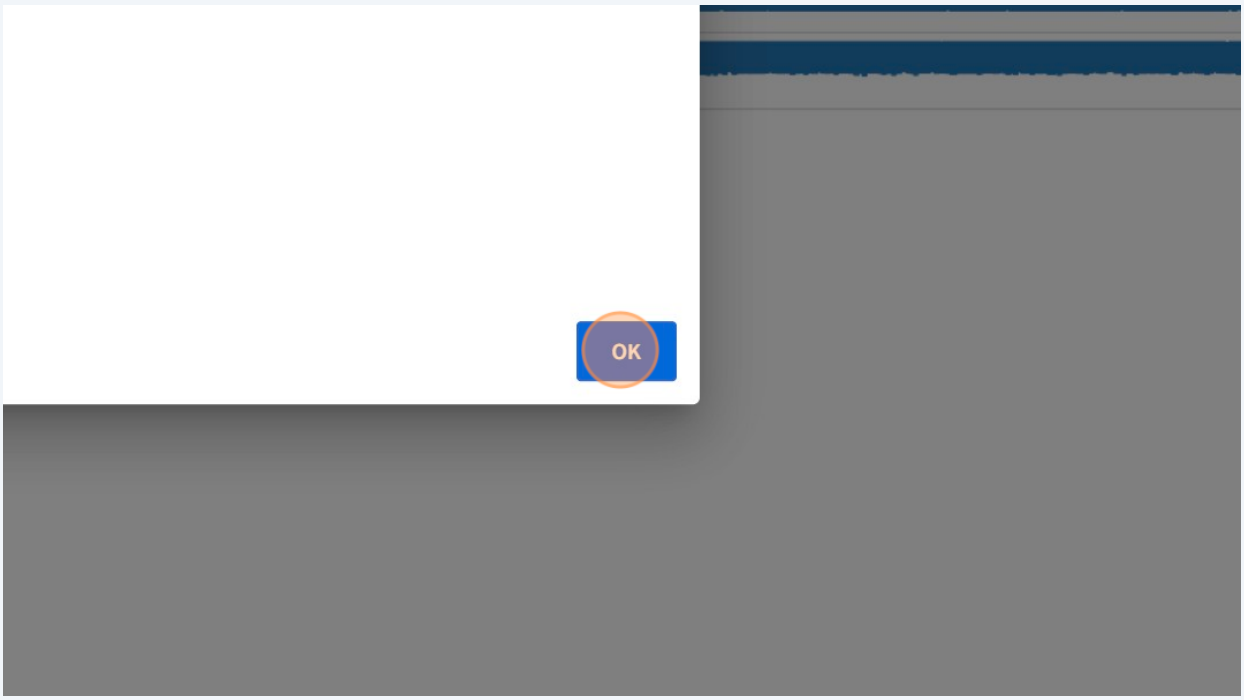
29 Select "Data step".



30 Clear any preselected data projection using the 'Clear' button.

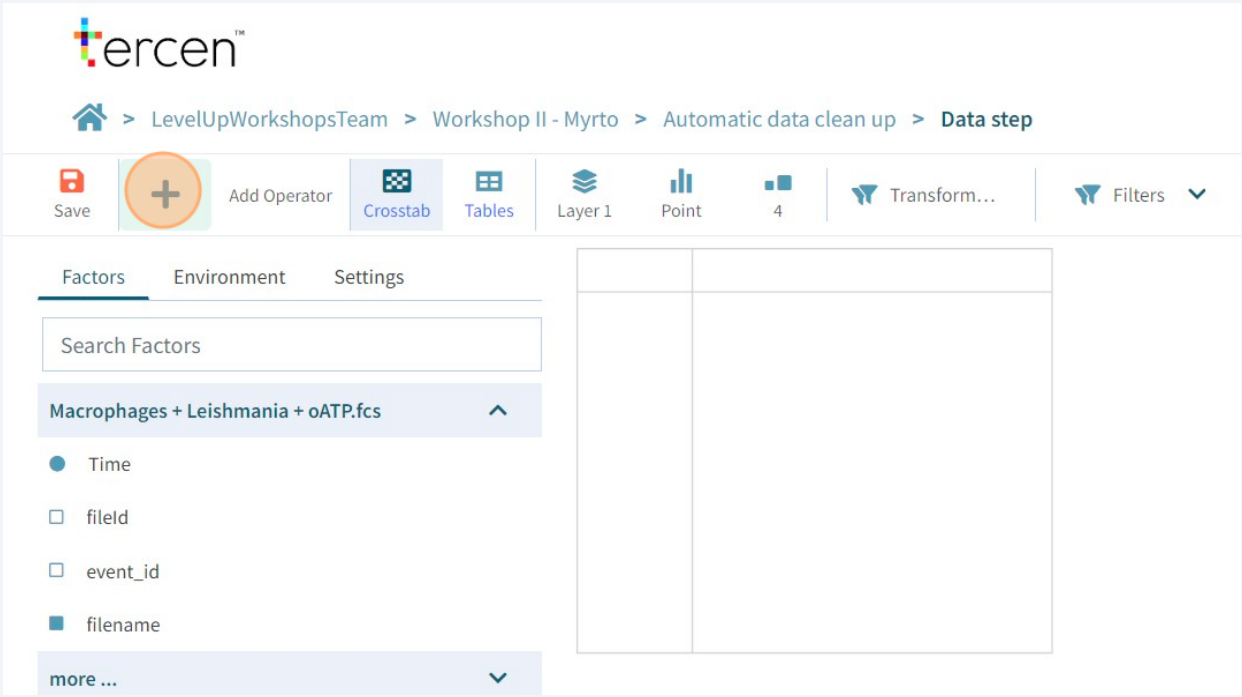


31 Click "OK".

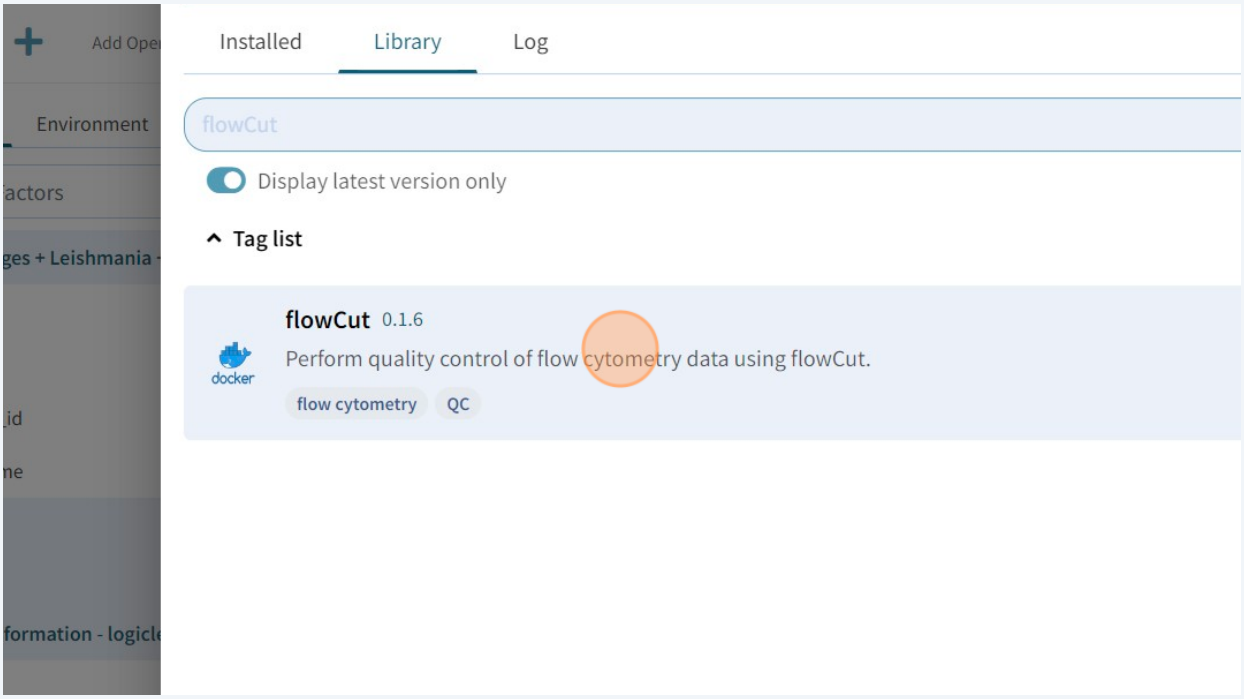


32 Again, you will start by adding the relevant operator to the Data step.

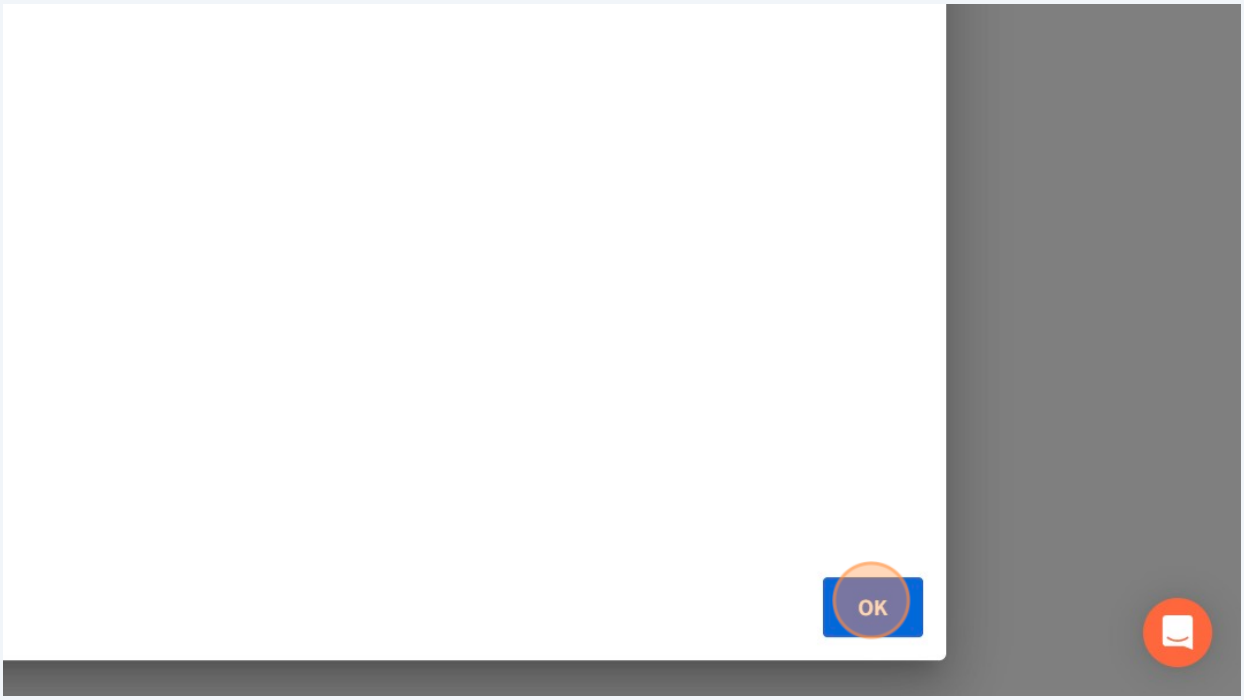
Click the '+' button.



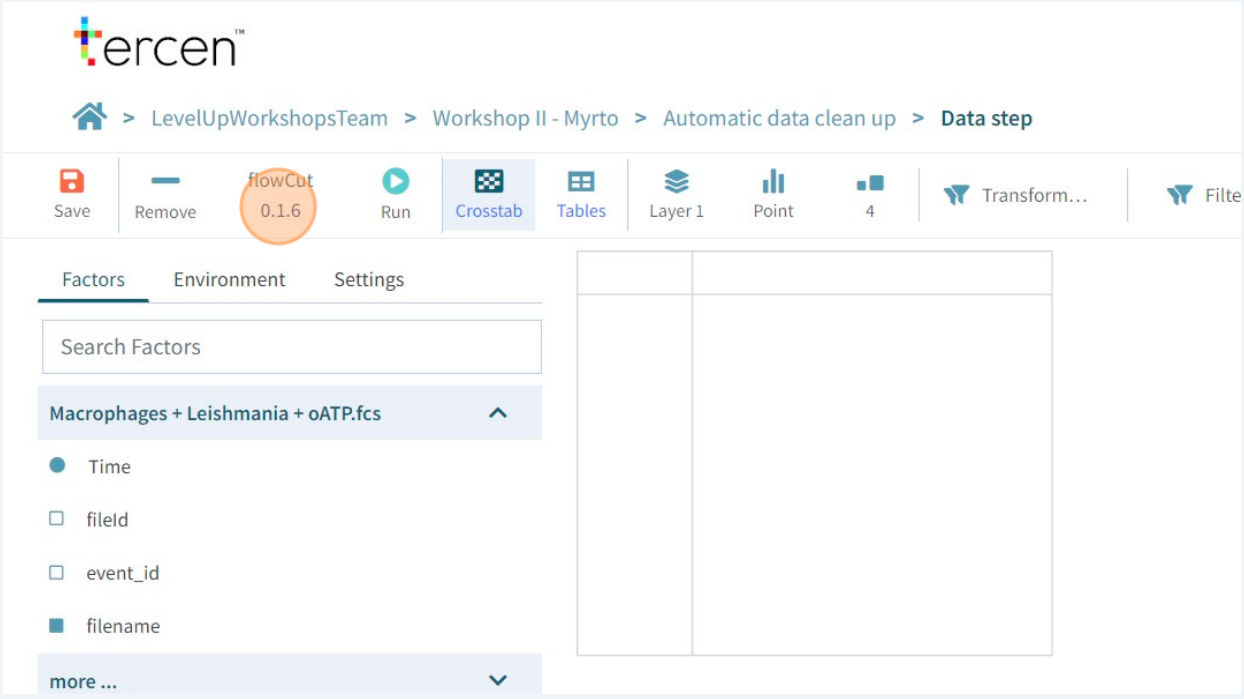
33 In the Library tab search for and select "flowCut".



34 Click "OK".



35 Click on the operator's version, as shown below, to get directed to the operator's Github repo to get information on how to correctly project the data for this operator.

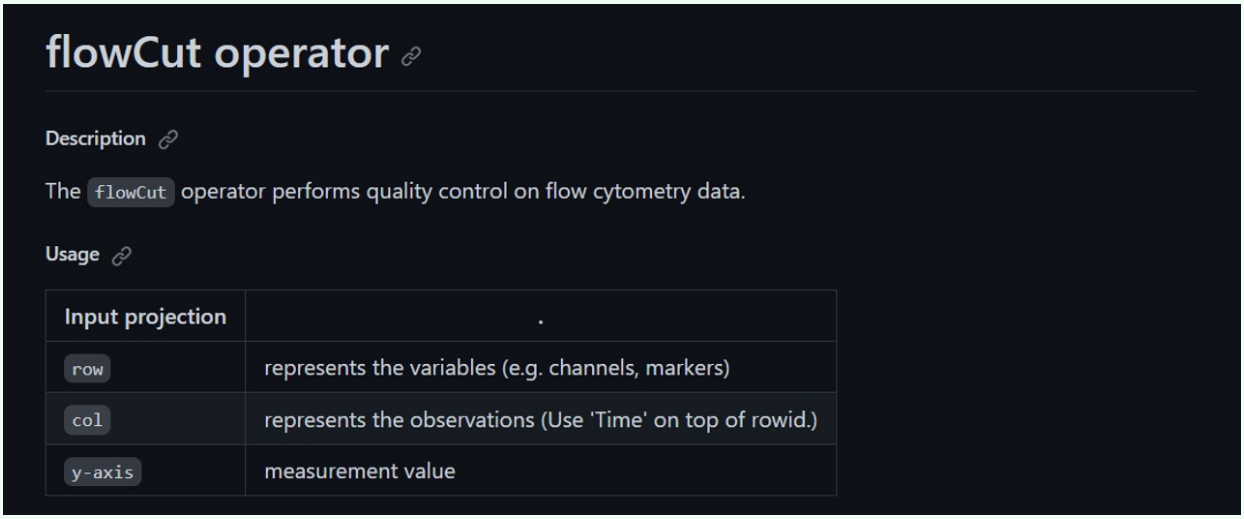


Expected data projection:

Y-axis: the logicle-transformed values

Row: channels

Column: Time + event_id



flowCut operator

Description

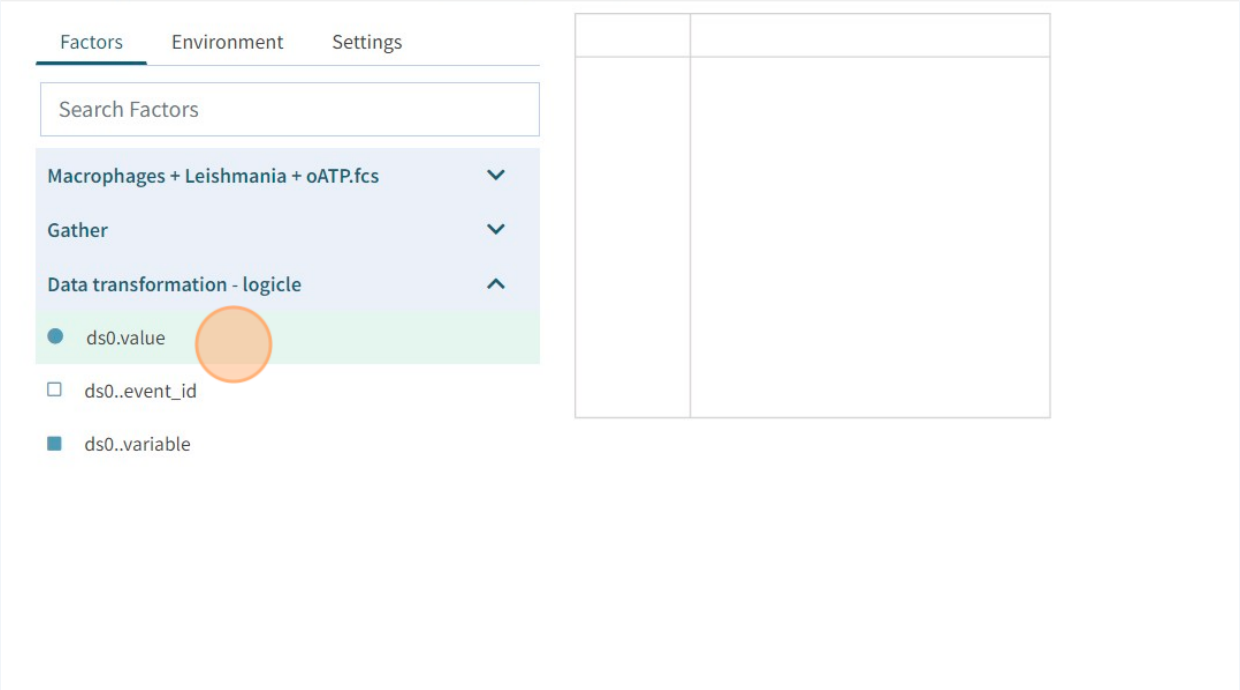
The `flowCut` operator performs quality control on flow cytometry data.

Usage

Input projection	.
`row`	represents the variables (e.g. channels, markers)
`col`	represents the observations (Use 'Time' on top of rowid.)
`y-axis`	measurement value

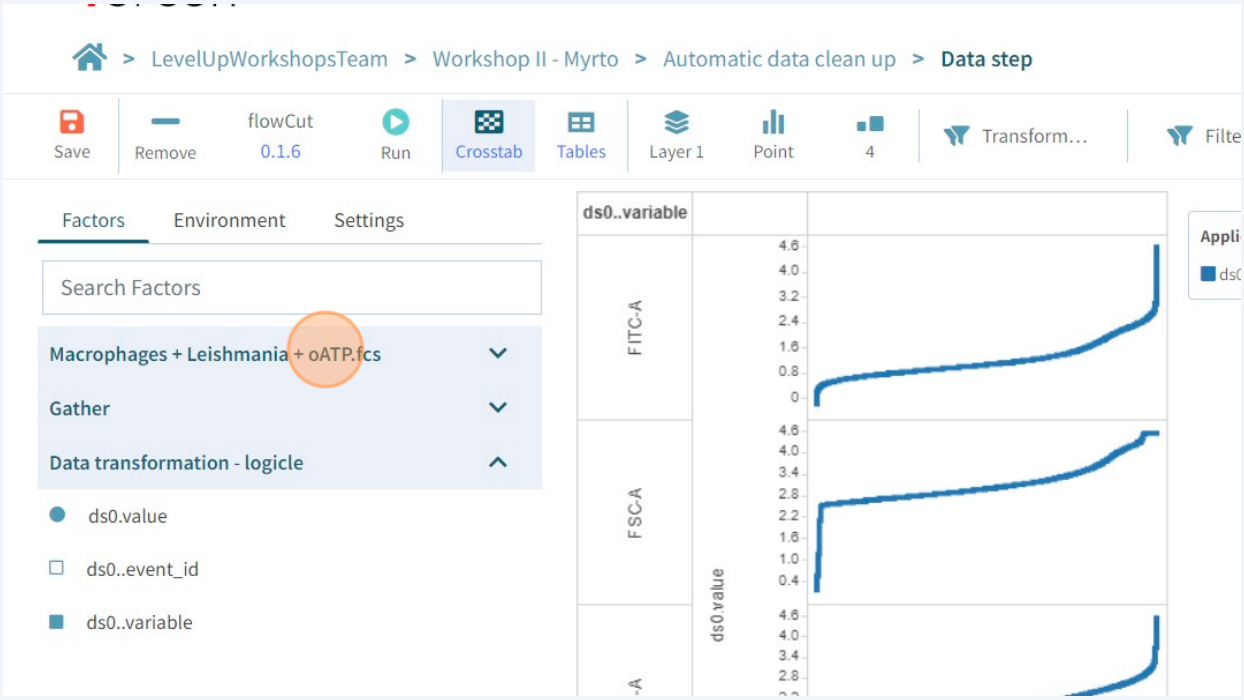
For this operator the order of the column factors is important. Place Time on top of event_id.

36 Drag the **ds0.value** factor and drop it in Y-axis.

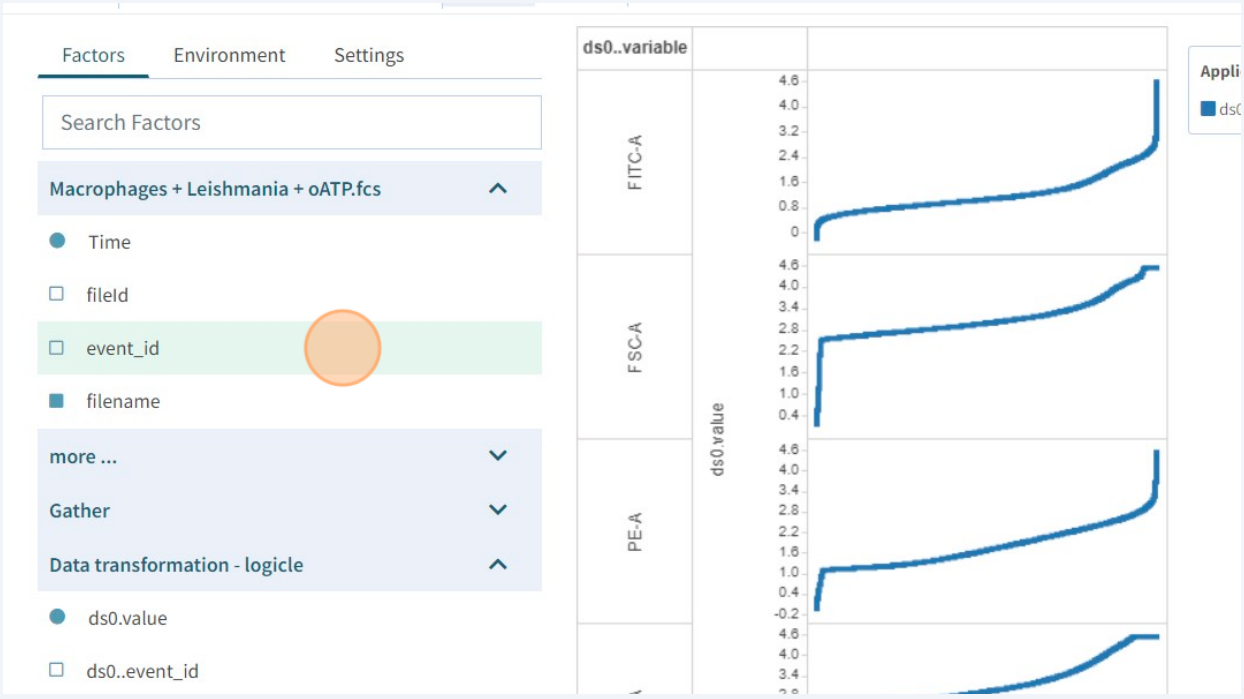


37 Drag the **ds0.variable** factor and drop it in Row.

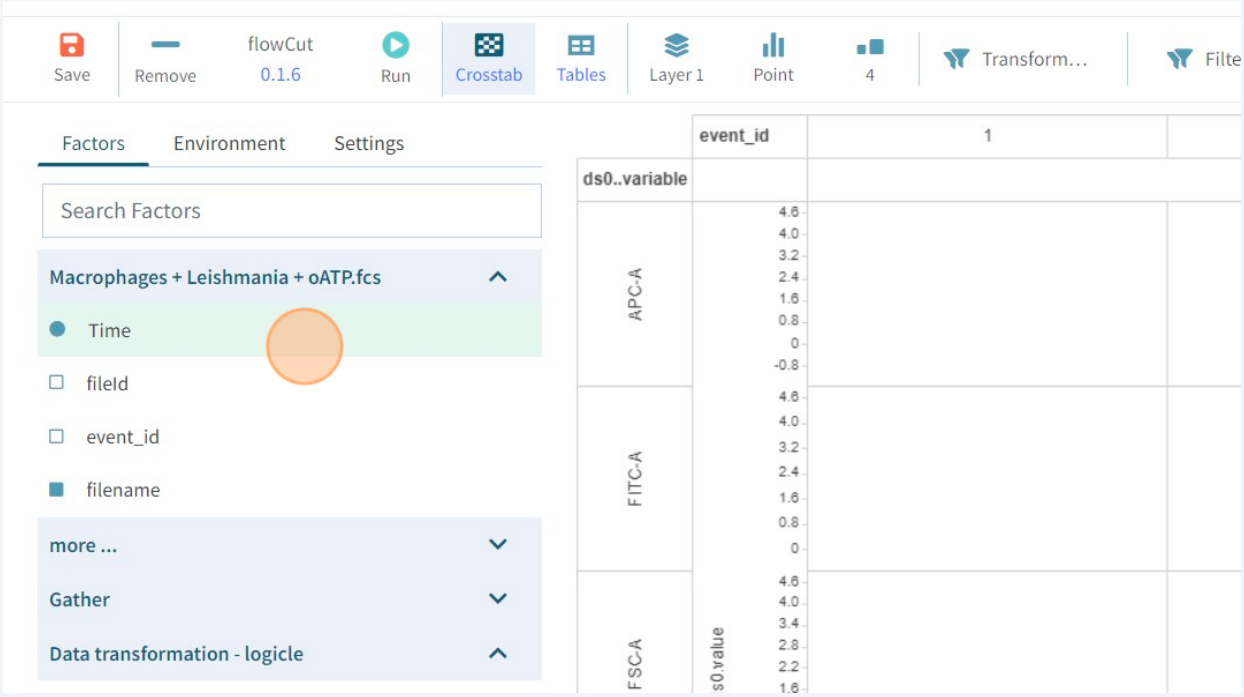
38 Expand the "Macrophages + Leishmania + oATP.fcs" factors list by clicking on it.



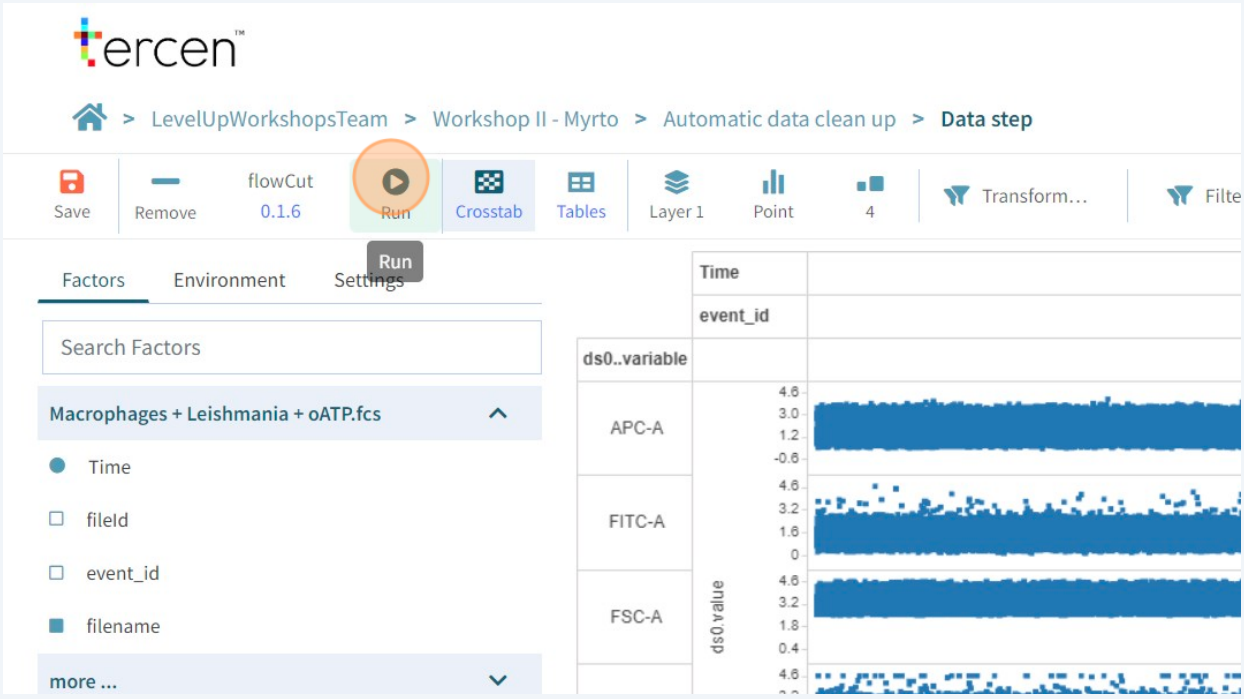
39 Drag the **event_id** factor and drop it in Column.



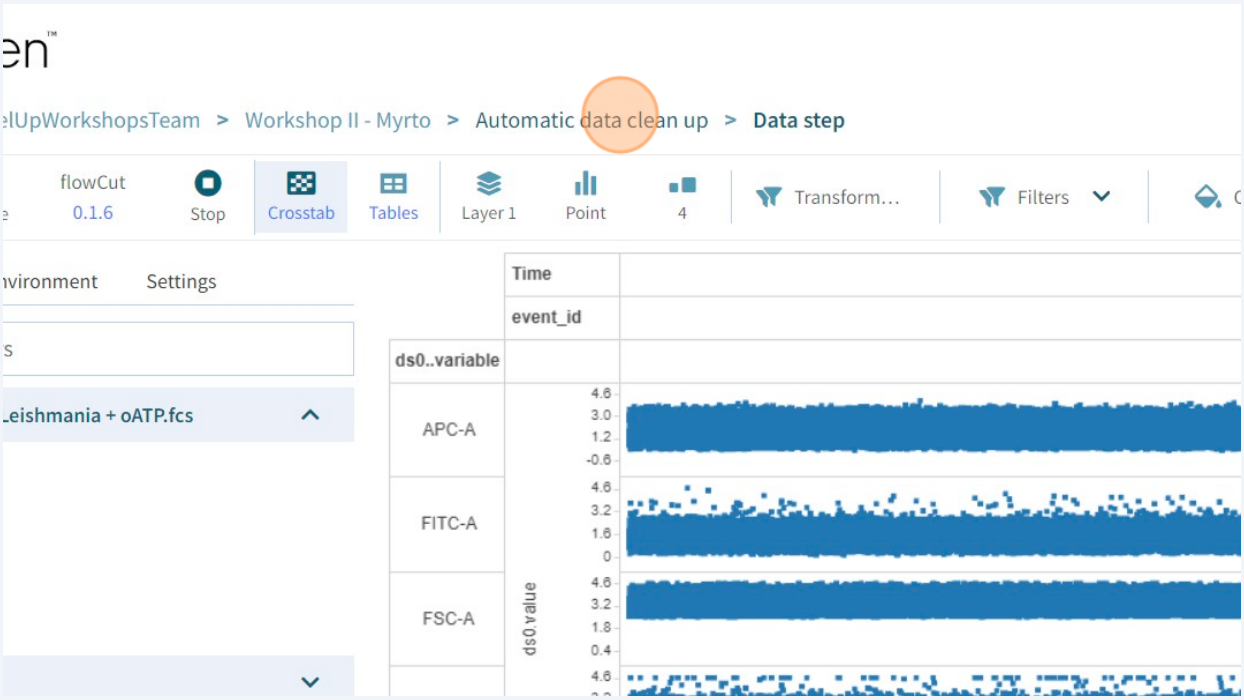
40 Drag the **Time** factor and drop it in Column *ABOVE* the event_id factor.



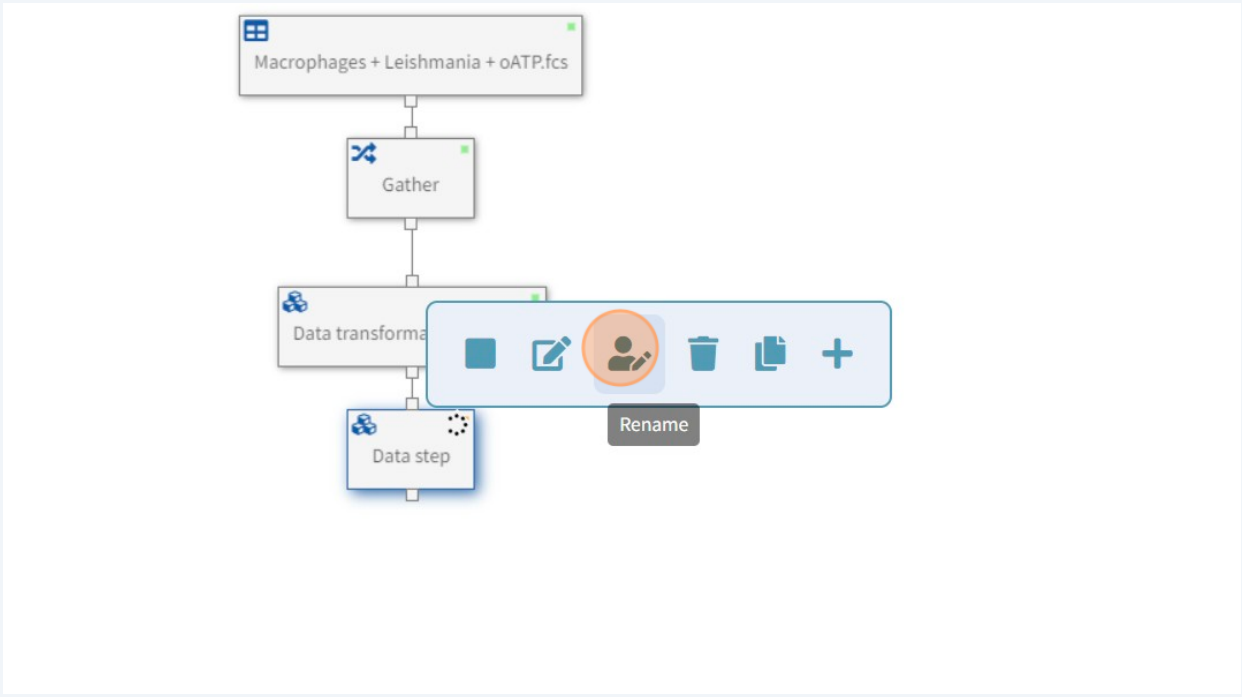
41 Click 'Run' to start the computation.



42 Click the "Automatic data clean up" link to go back to the workflow.



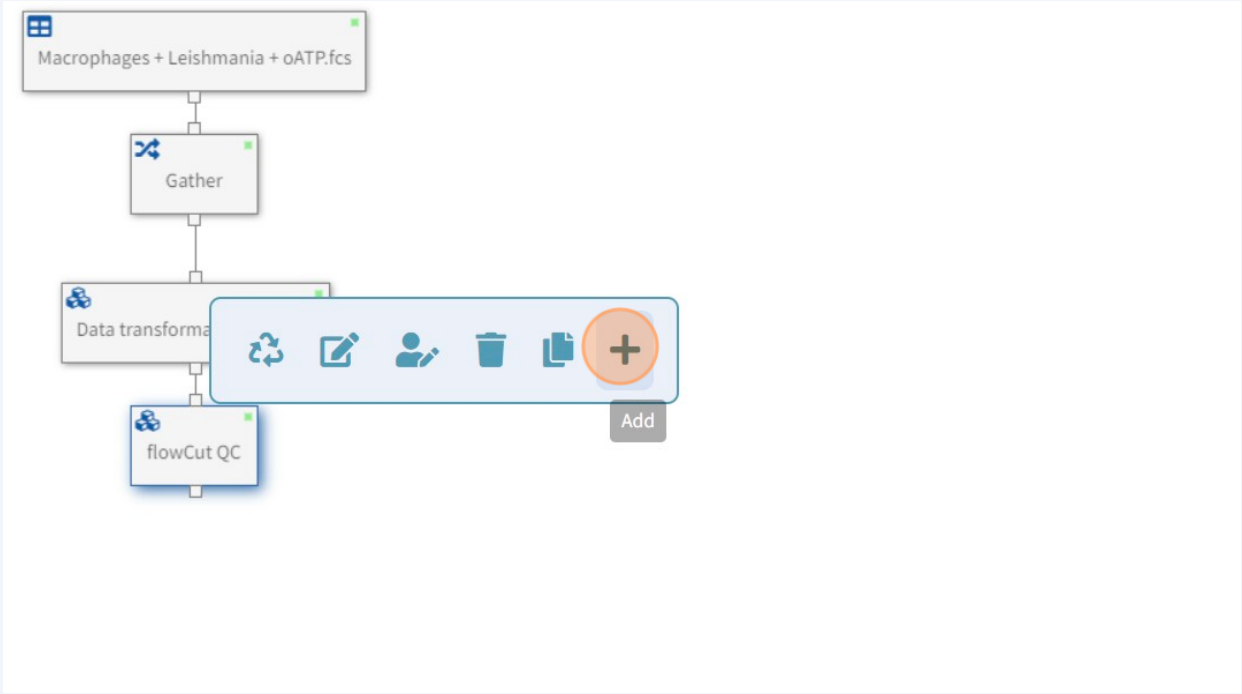
43 Rename the Data step to "flowCut QC".



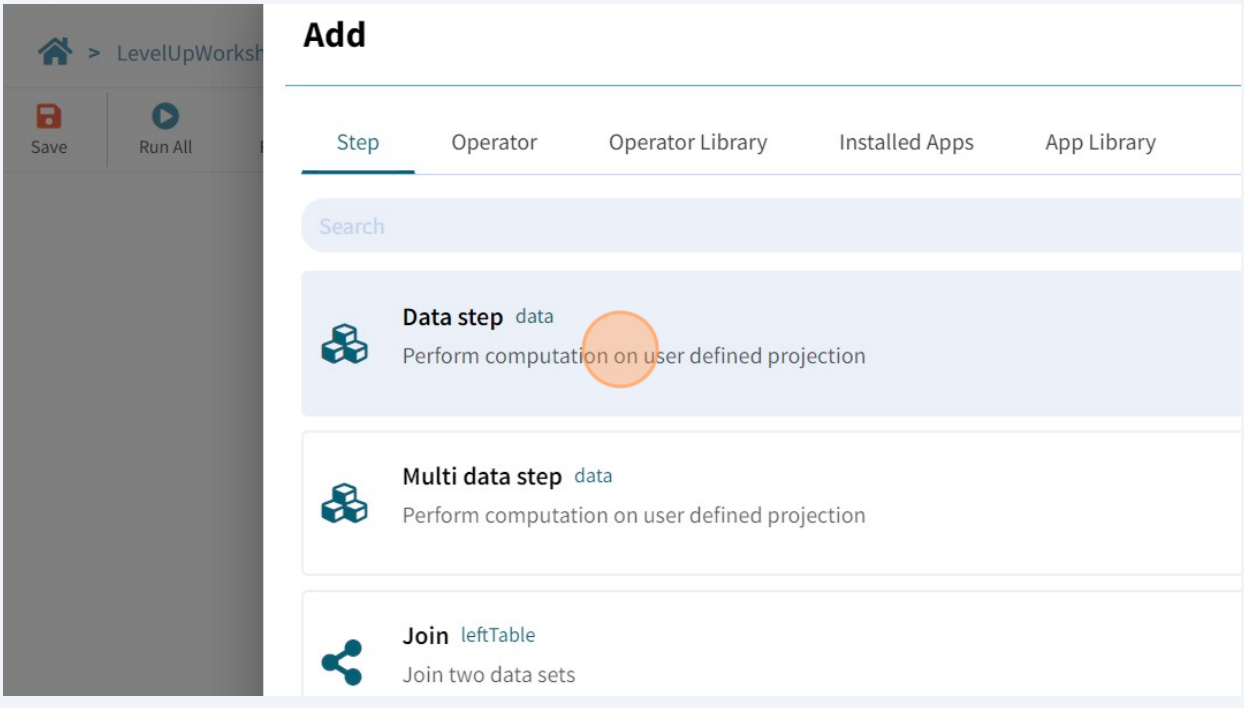
44 Finally, you will build a data step to check the results of flowCut.

For every event, the algorithm returns a quality control flag that can be pass or fail and is stored in the **QC_flag** factor.

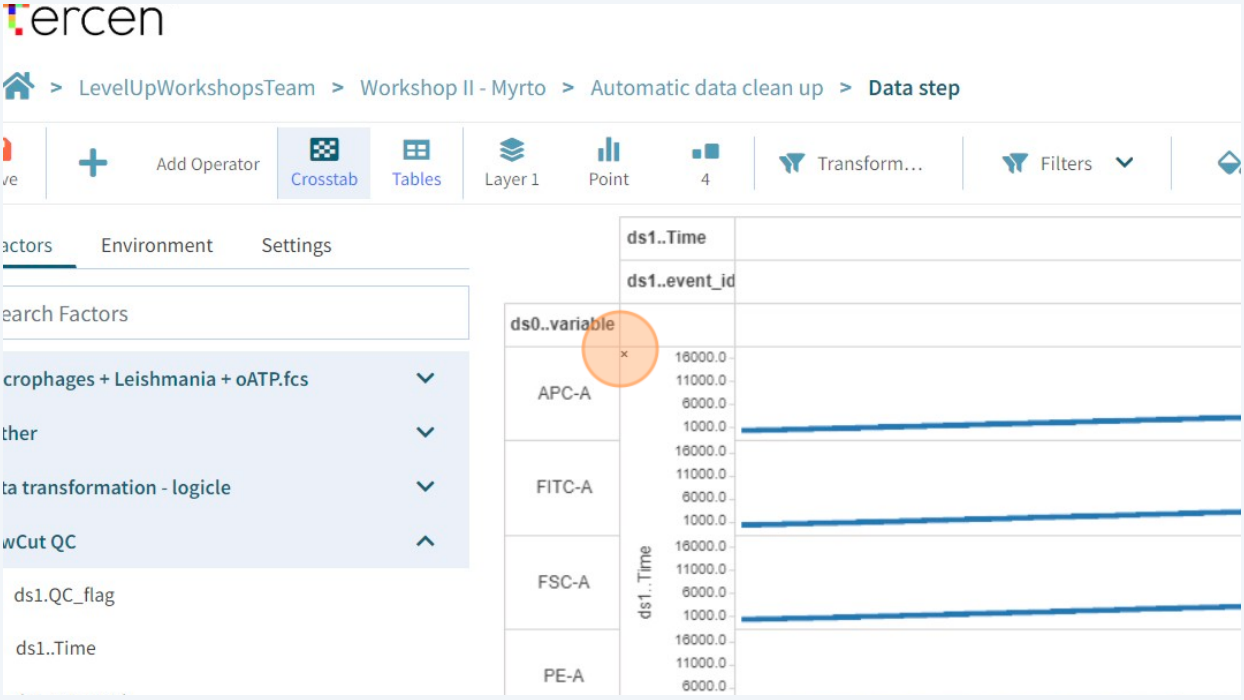
Click 'Add'.



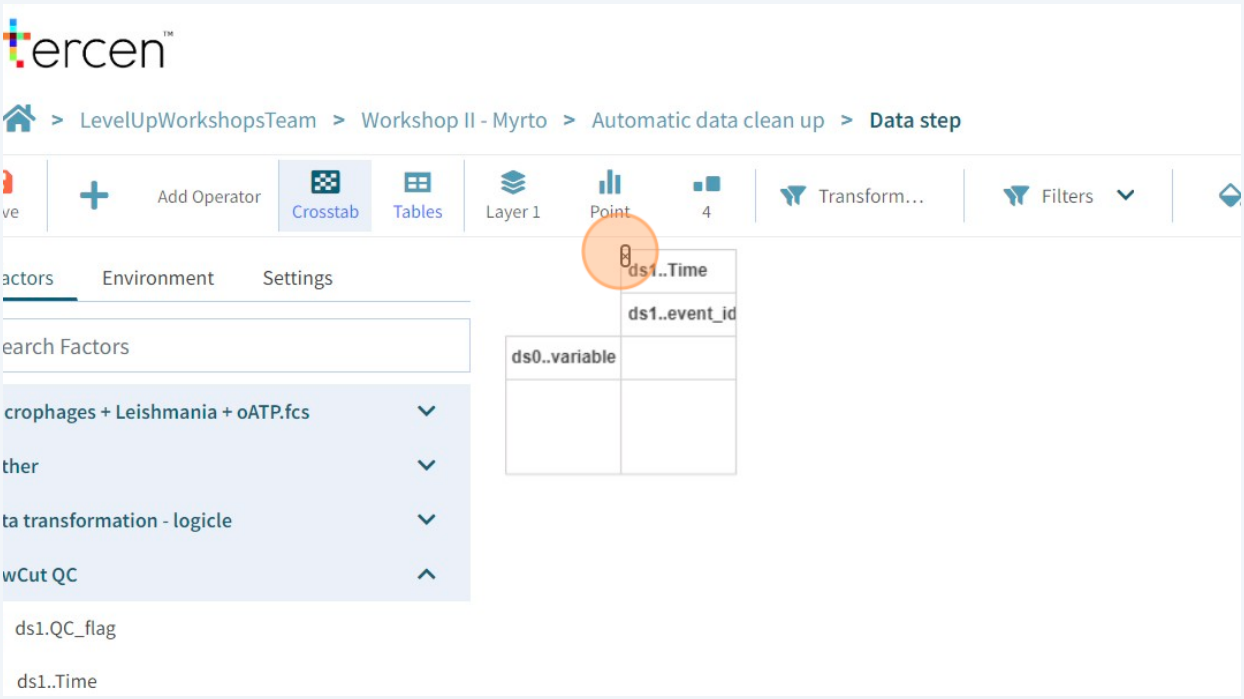
45 Select "Data step".



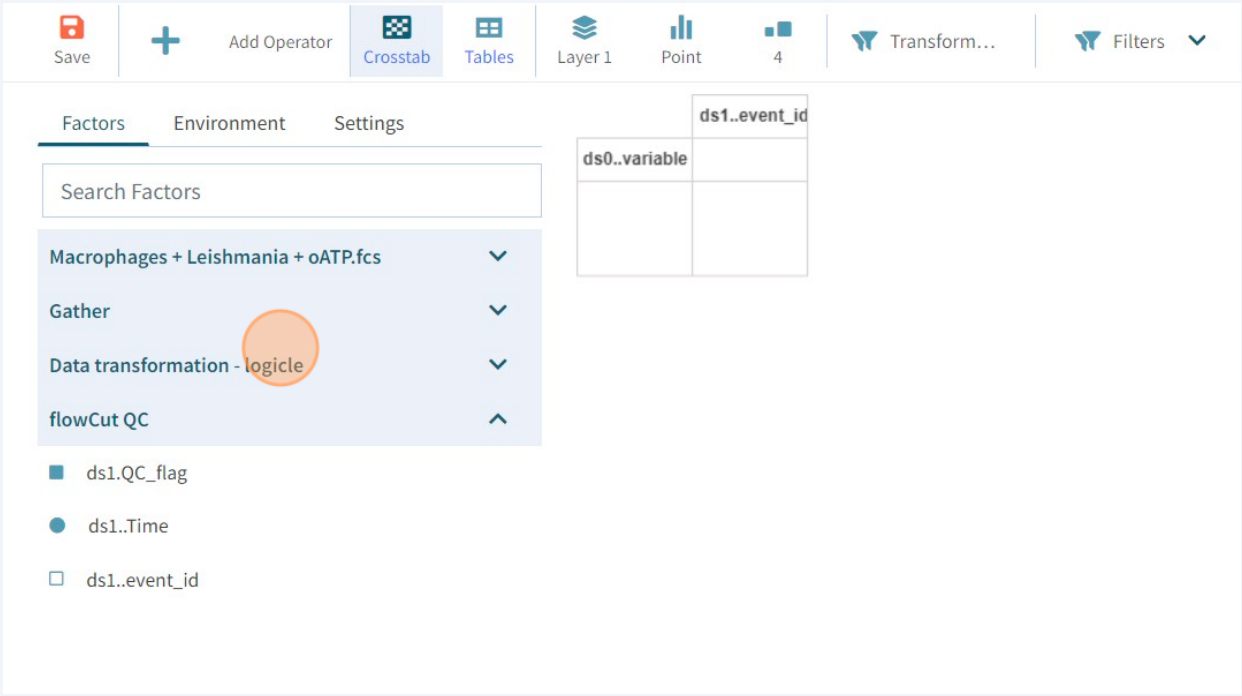
46 Clear the **ds1..Time** factor from the Y-Axis by clicking the "×" button when you mouse over it.



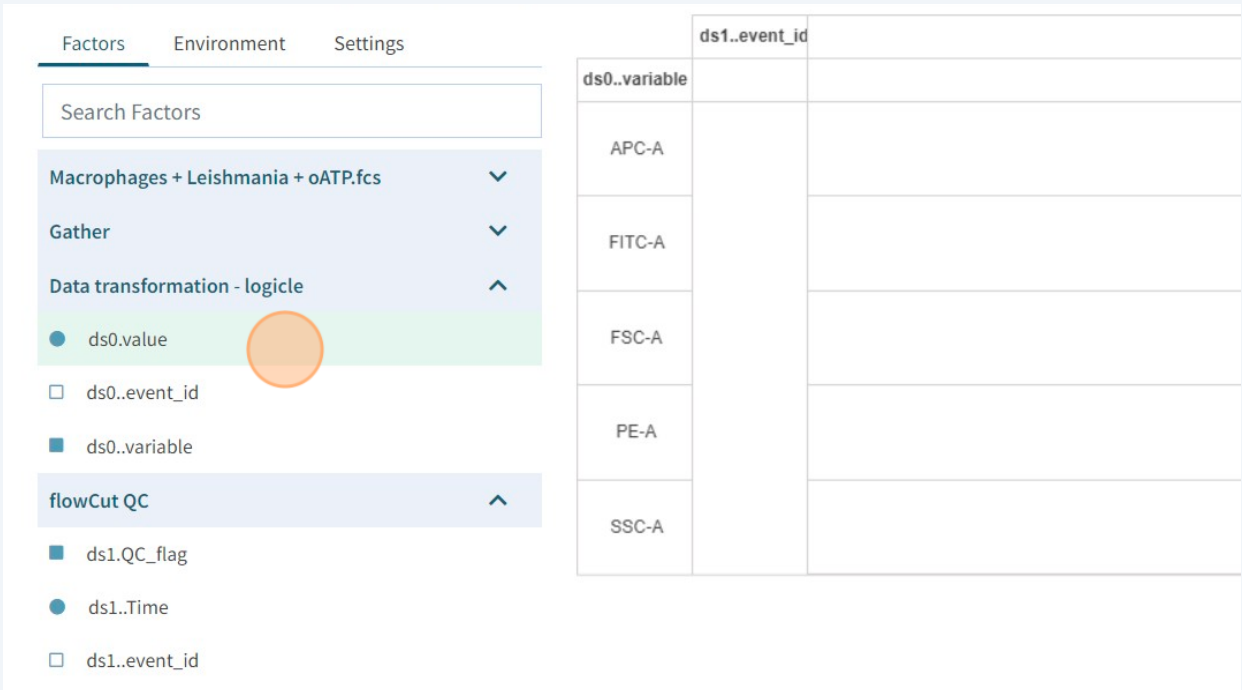
47 Do the same thing for Column.



48 Click the "Data transformation - logicle" data step to expand its factors list.

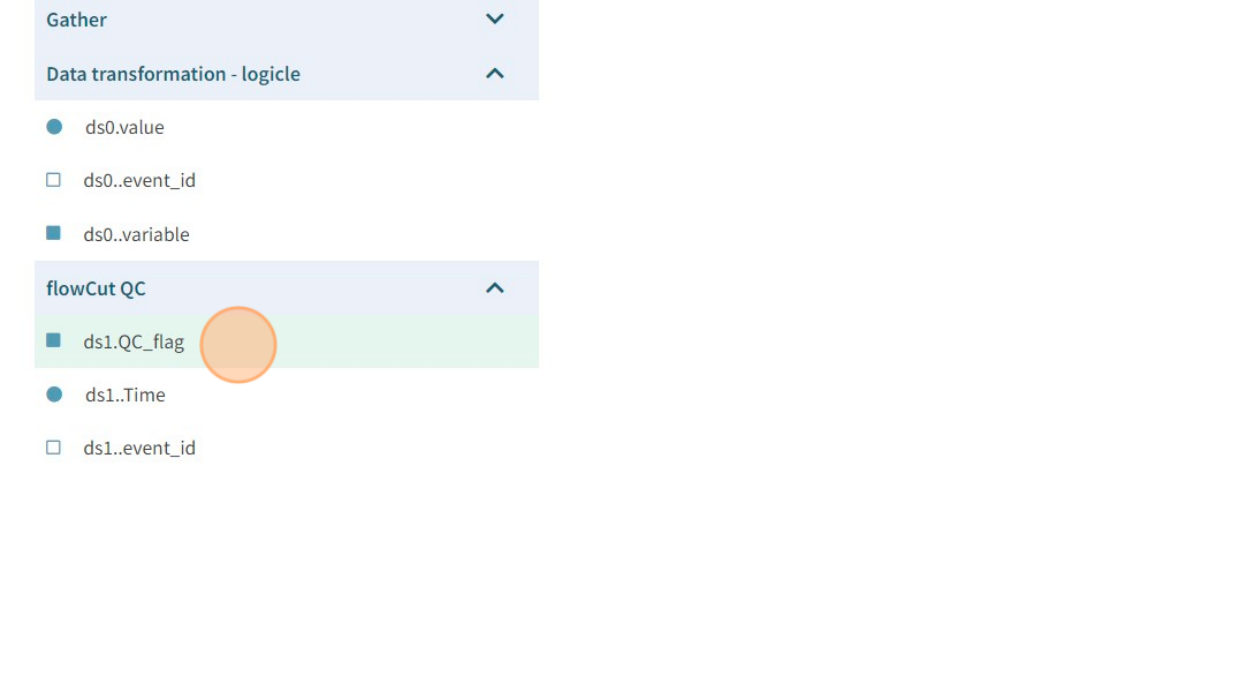


49 Drag the **ds0.value** and drop it in the Y-axis.

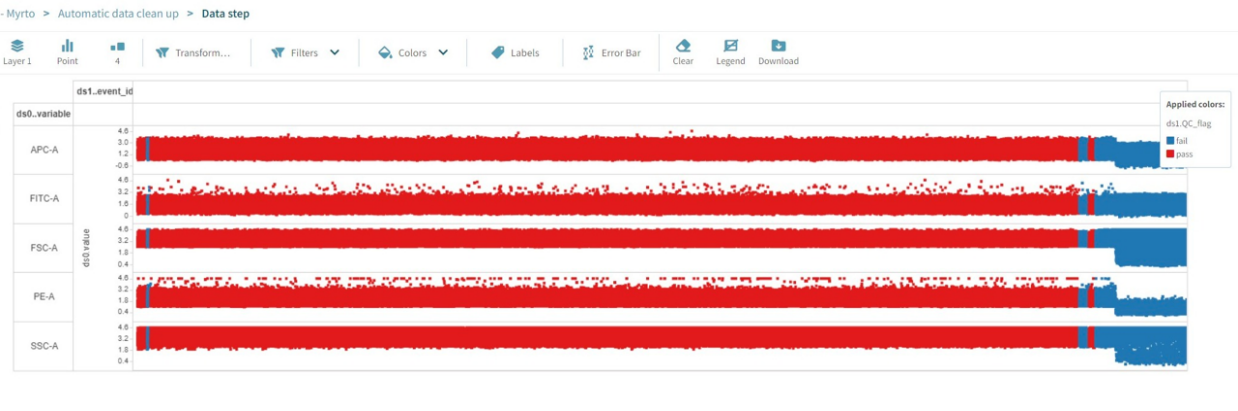


50 Let us know the color of every event based on the QC flag reported by flowCut.

Drag the **ds1.QC_flag** factor and drop it in Colors.

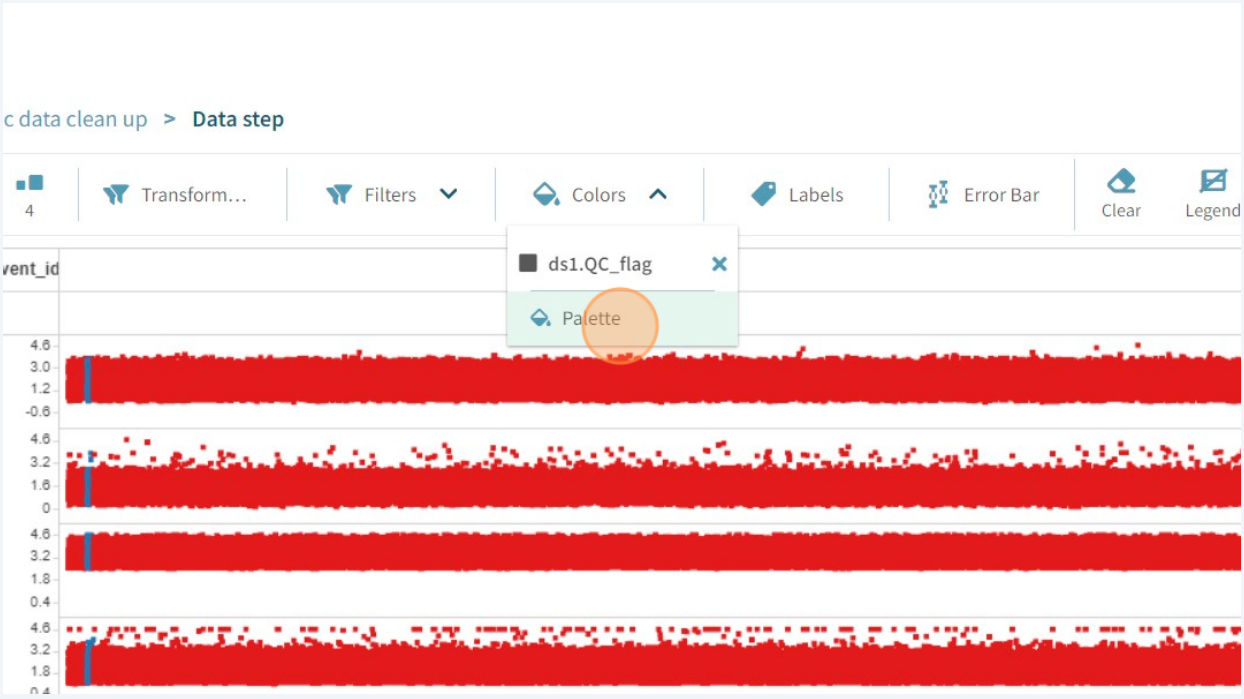


51 Notice how the data points have changed color depending on the flowCut result. Each event has been marked with a pass or fail flag.

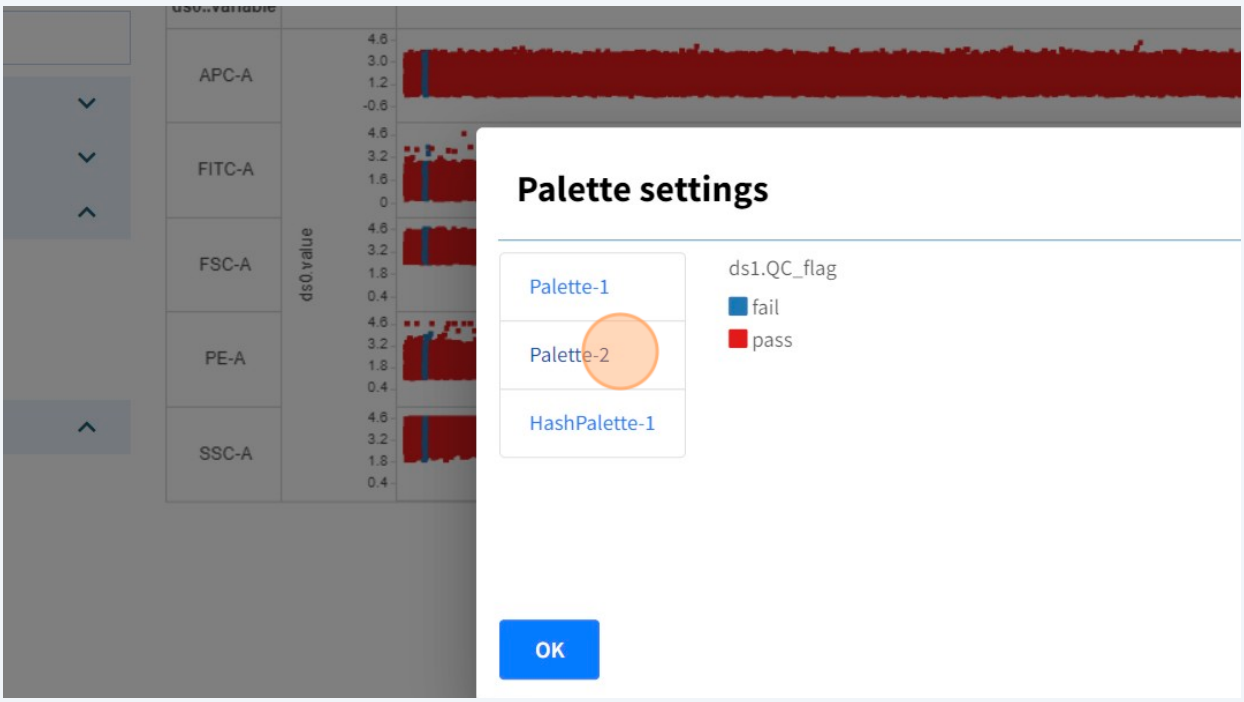


52 Unfortunately, the default choice of colors (red for pass and blue for fail) is not intuitive in this case. Let us change those.

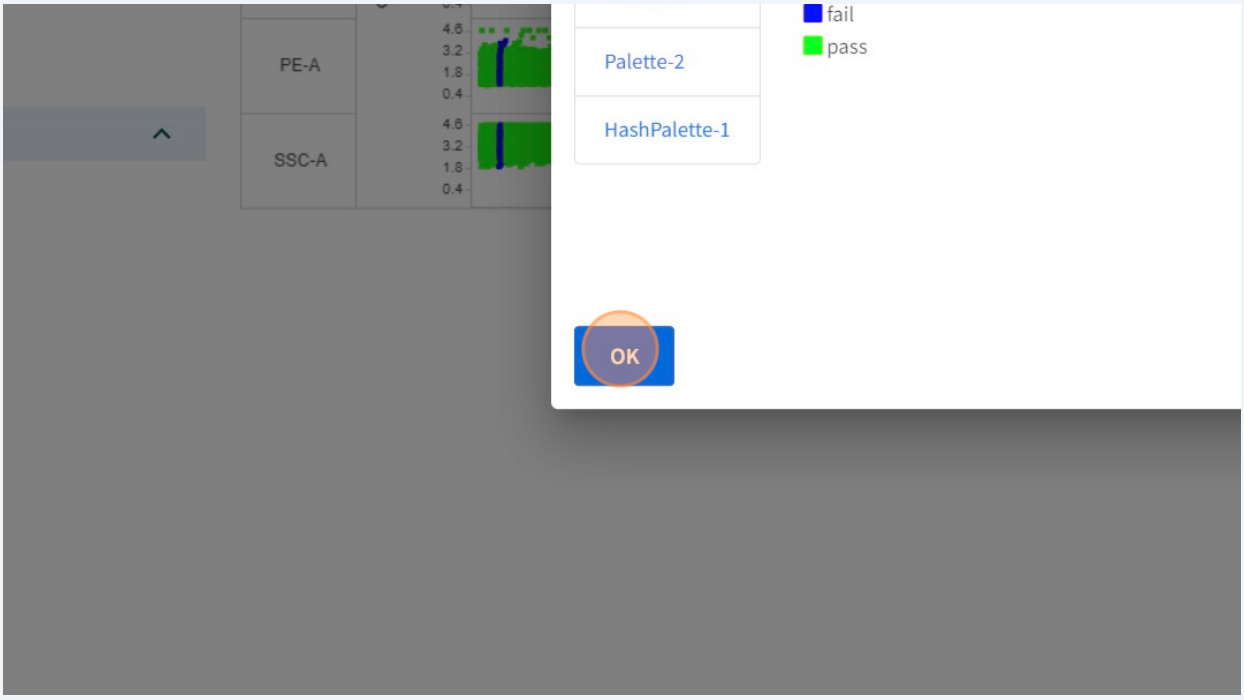
53 Mouse over the Colors button and click 'Palette'.



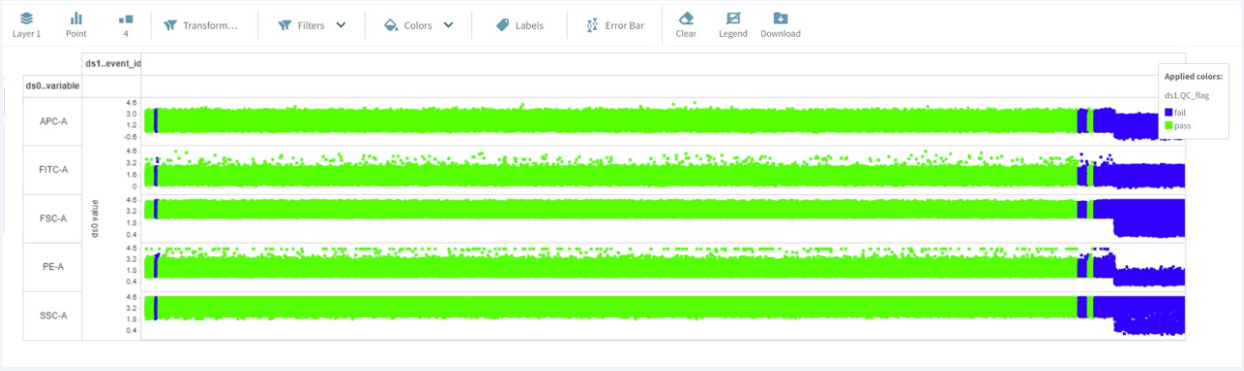
54 Select "Palette-2".



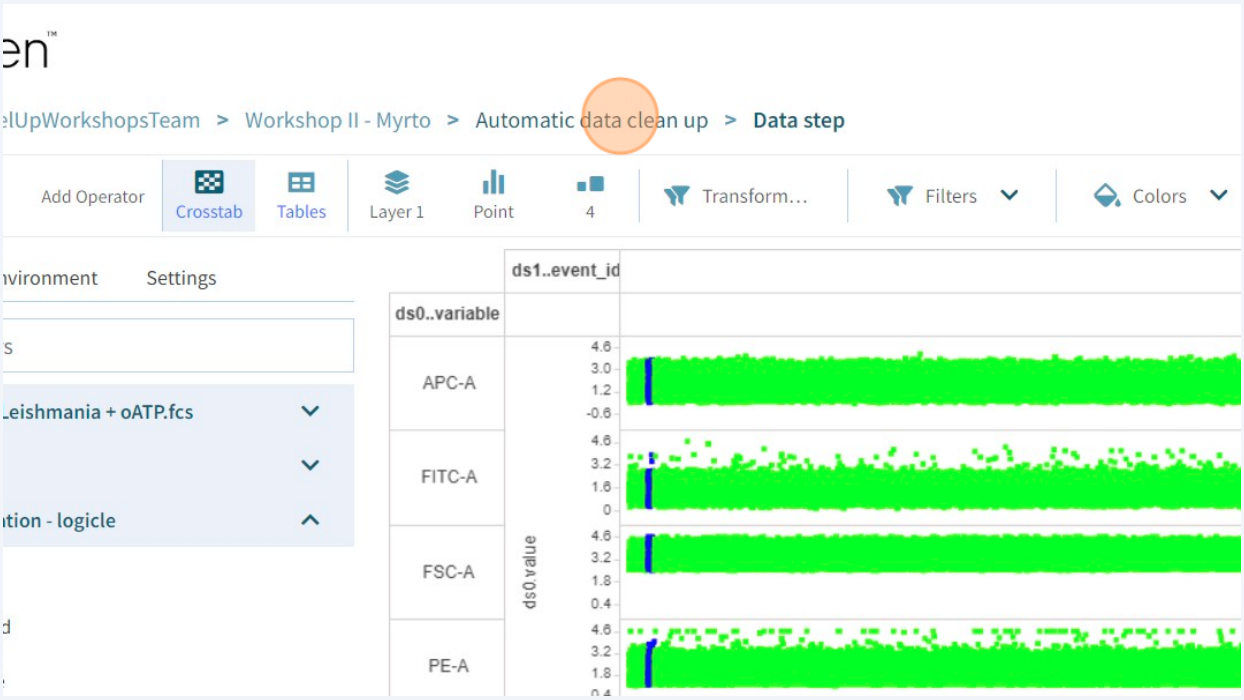
55 Click "OK".



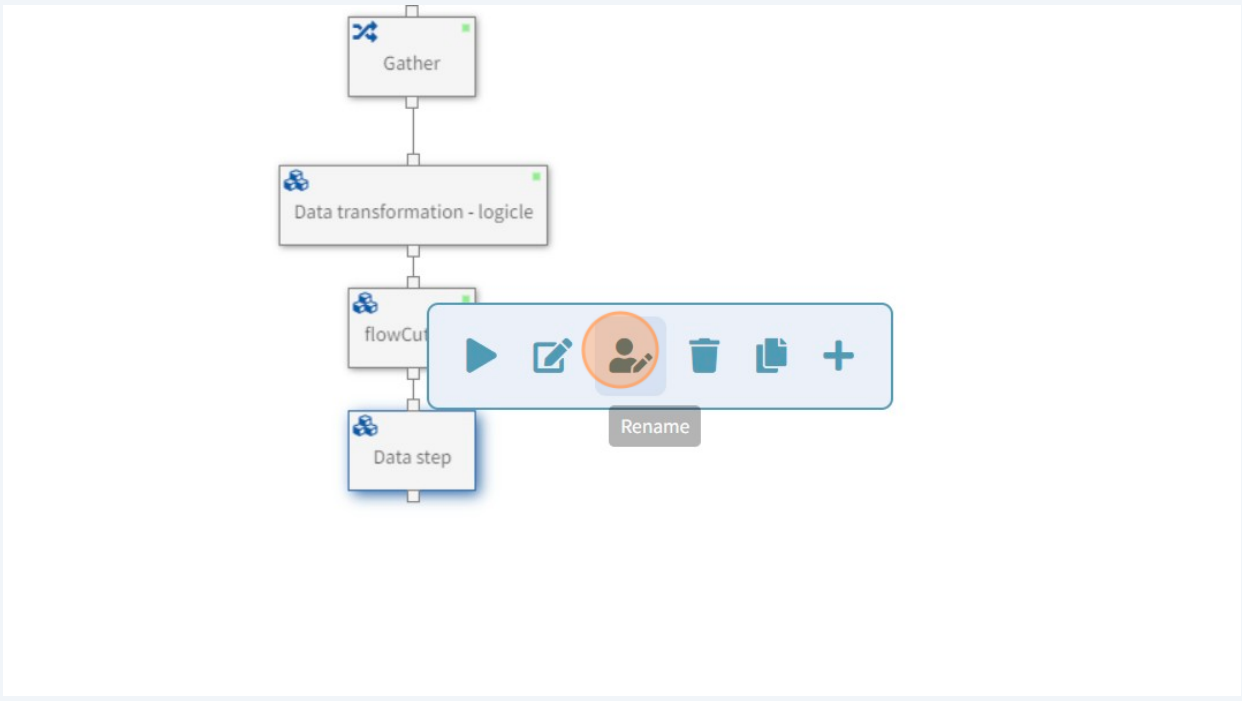
56 Now it is much better!



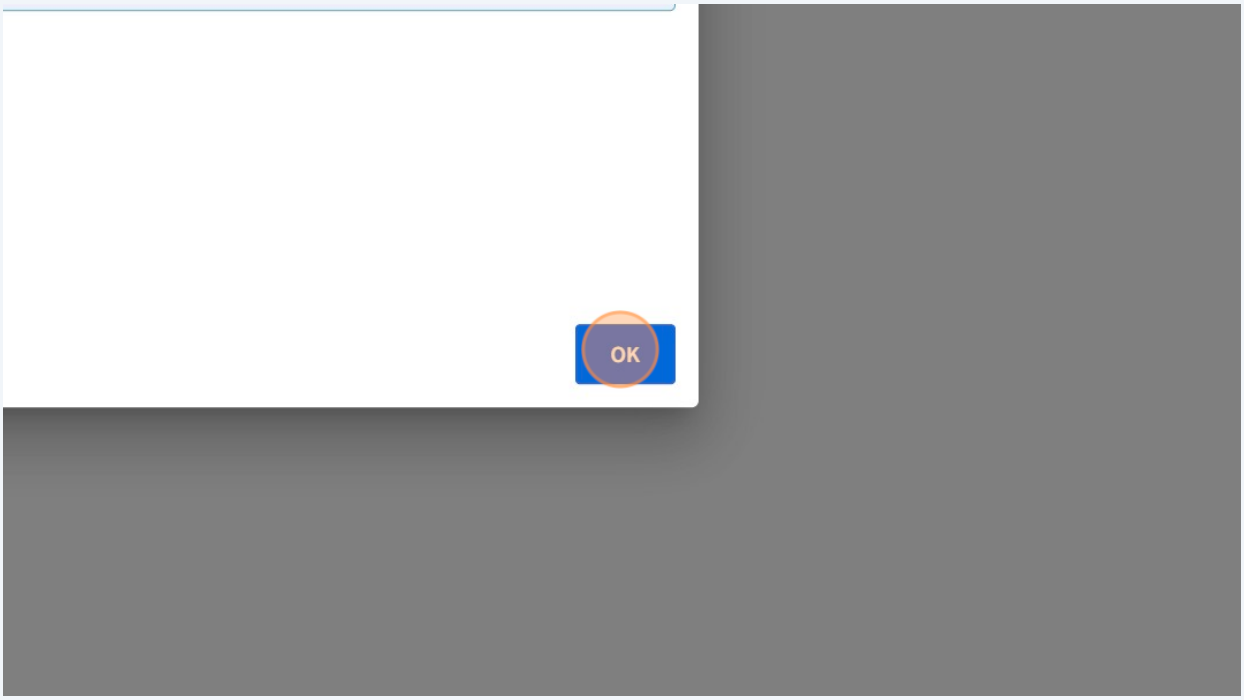
57 Click the "Automatic data clean up" link to return to the workflow.



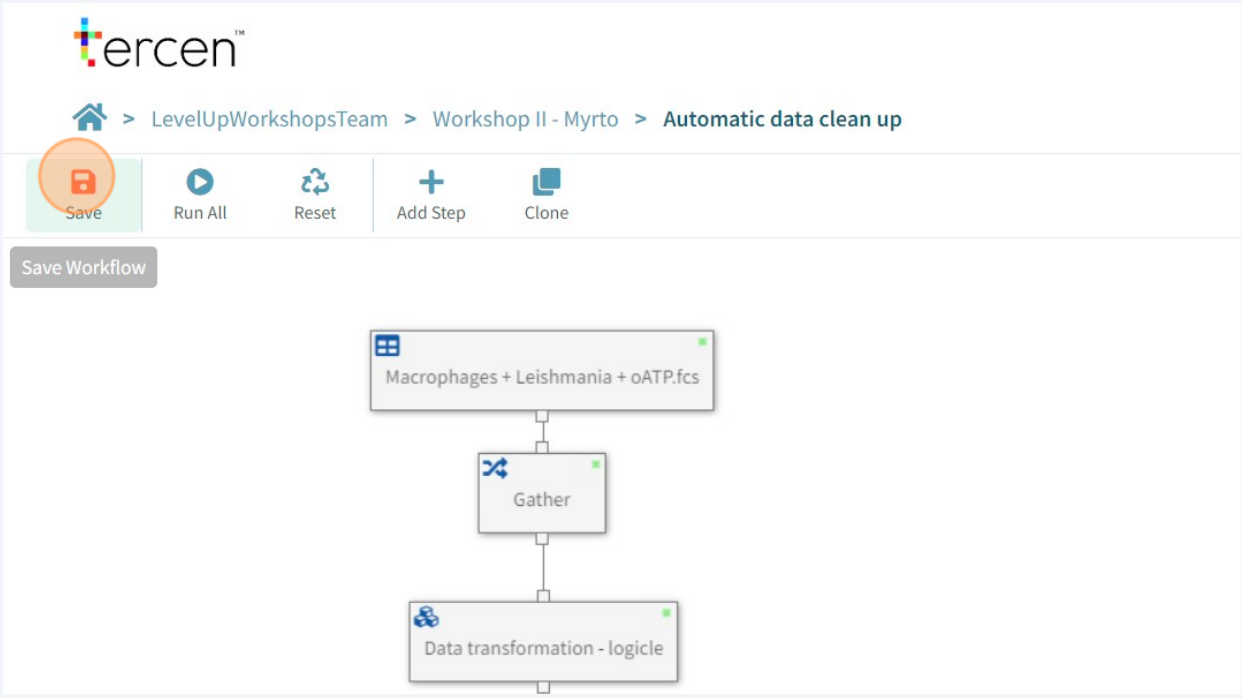
58 Rename the last Data step to "flowCut QC results".



59 Click "OK".



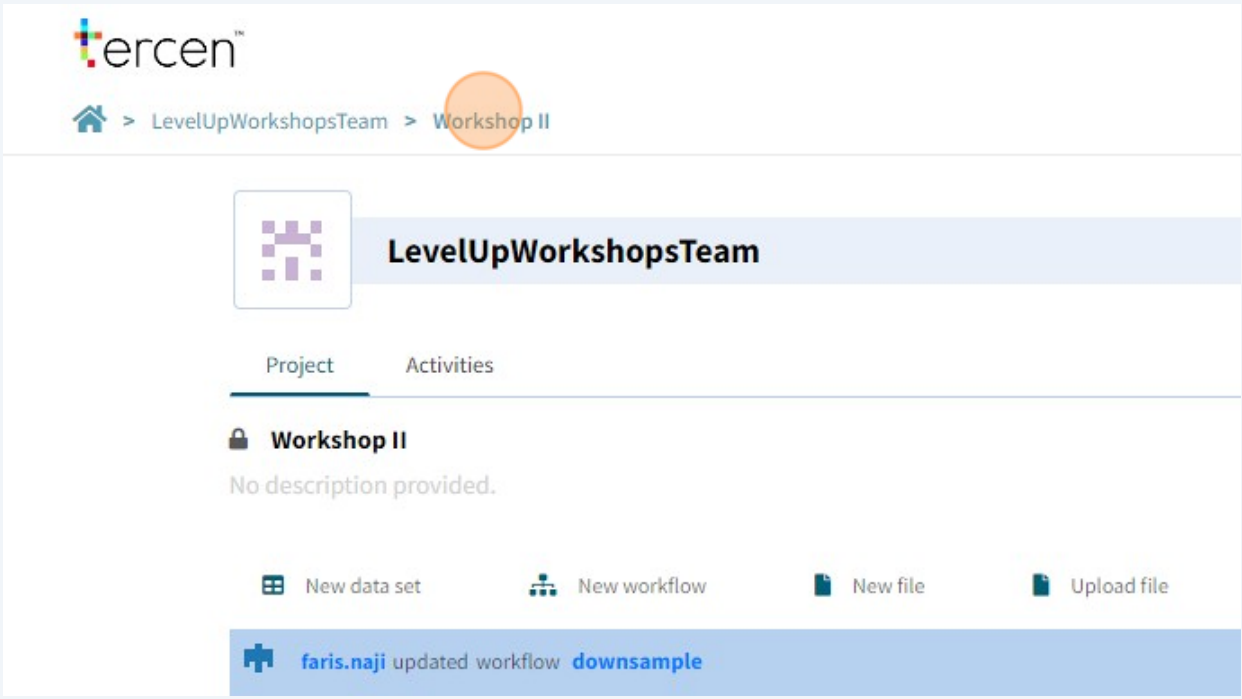
60 Do not forget to save your progress by clicking the Save button on the leftmost side of the Global toolbar.



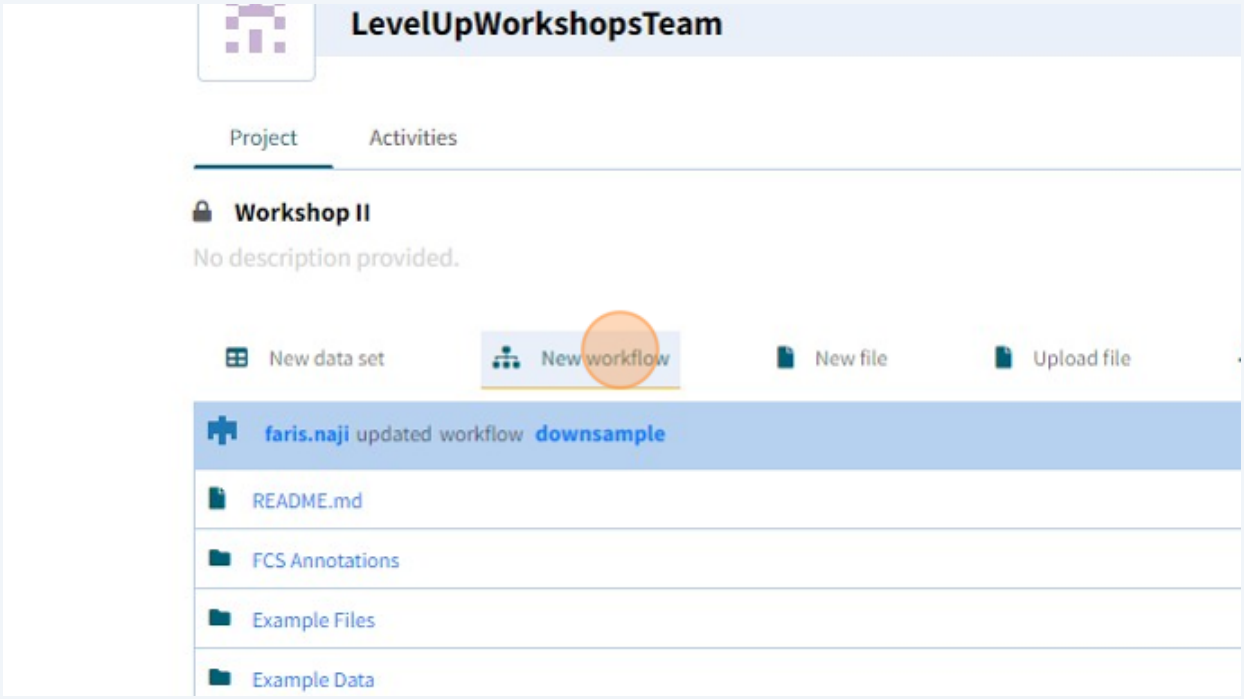
61 This guide has shown you how to automatically clean your FCS files.

0208 - Downsample Data

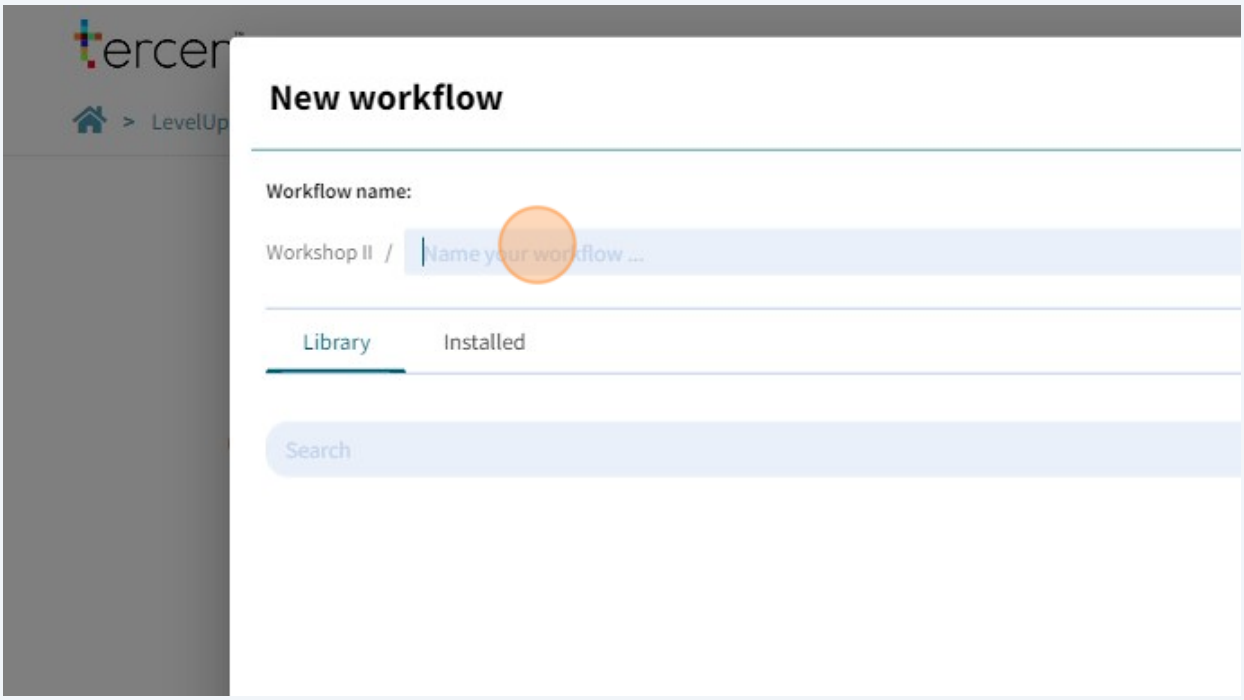
1 Navigate to the top level of "Workshop II"



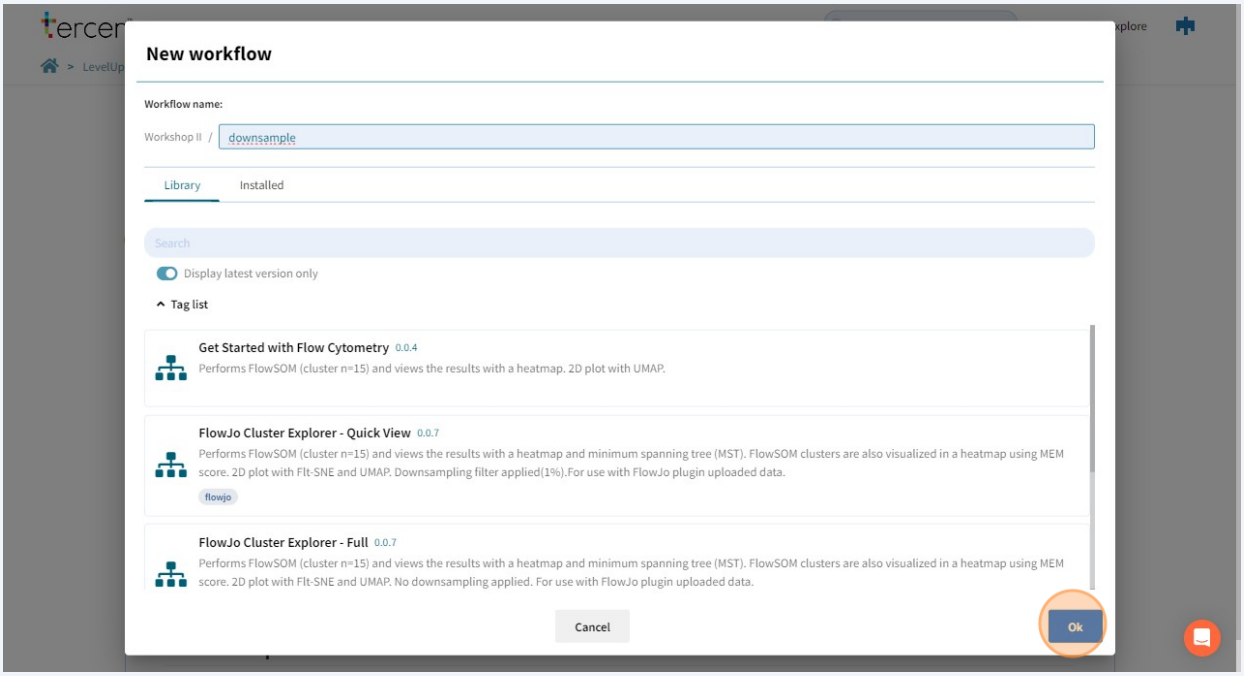
2 Click "New workflow"



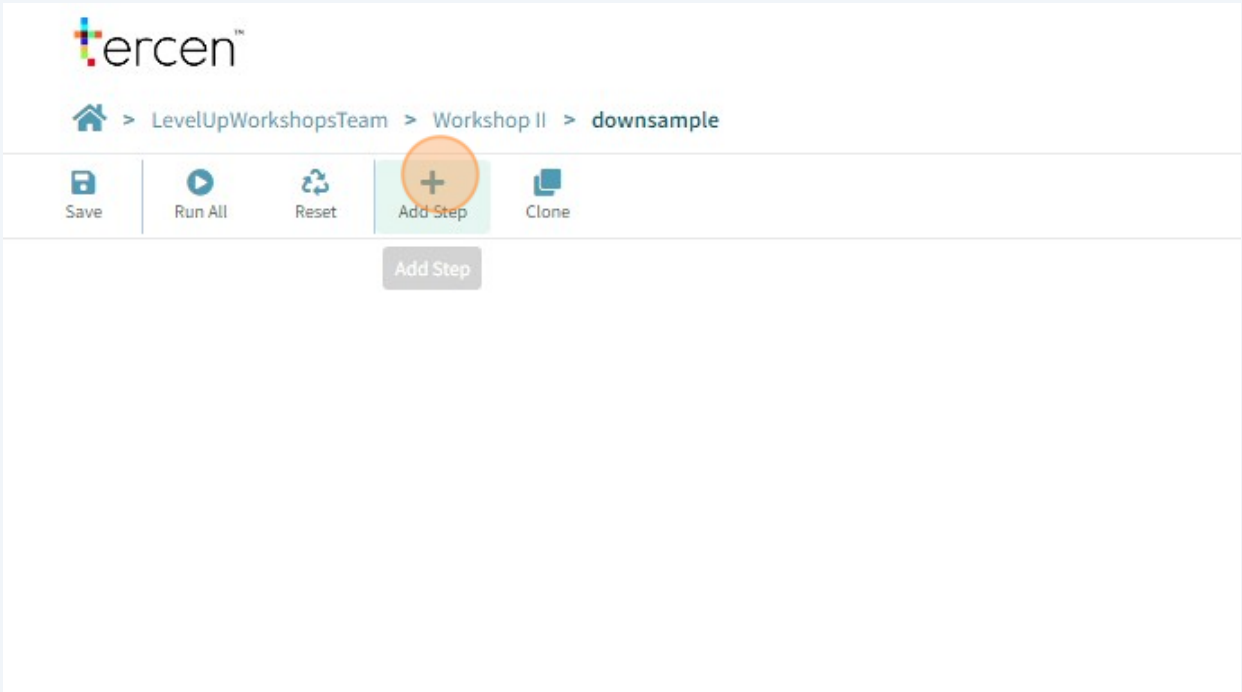
3 Give the name "downsample"



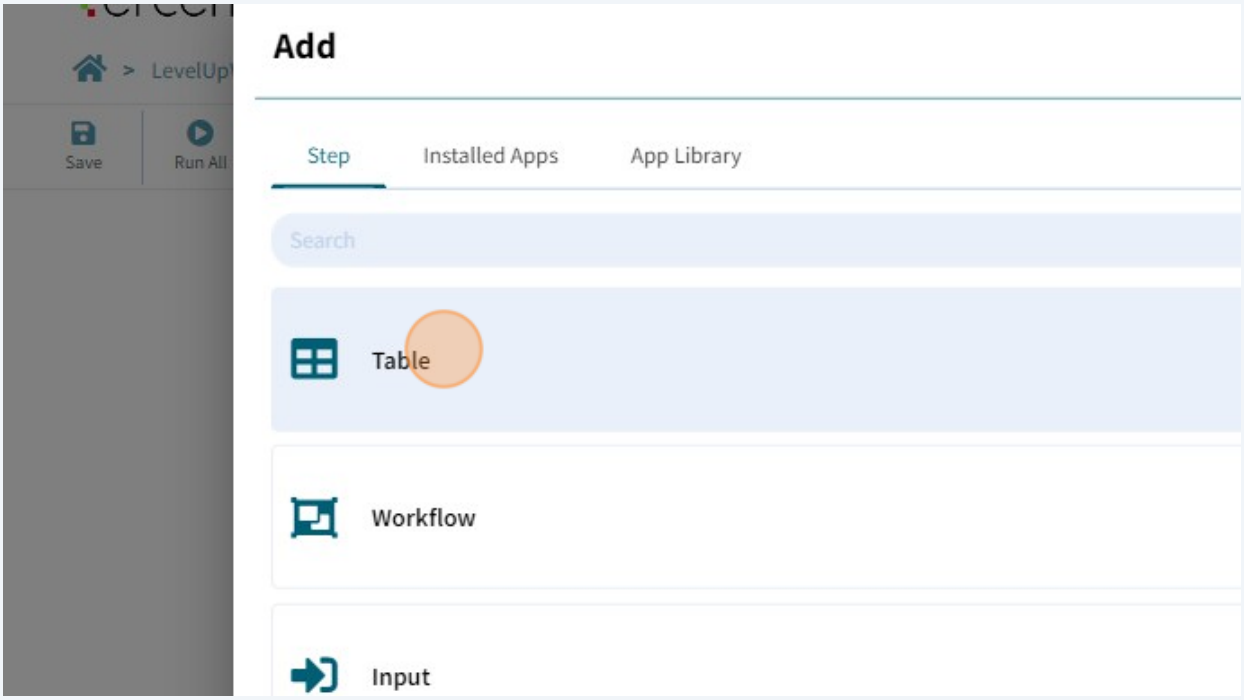
4 Click "Ok"



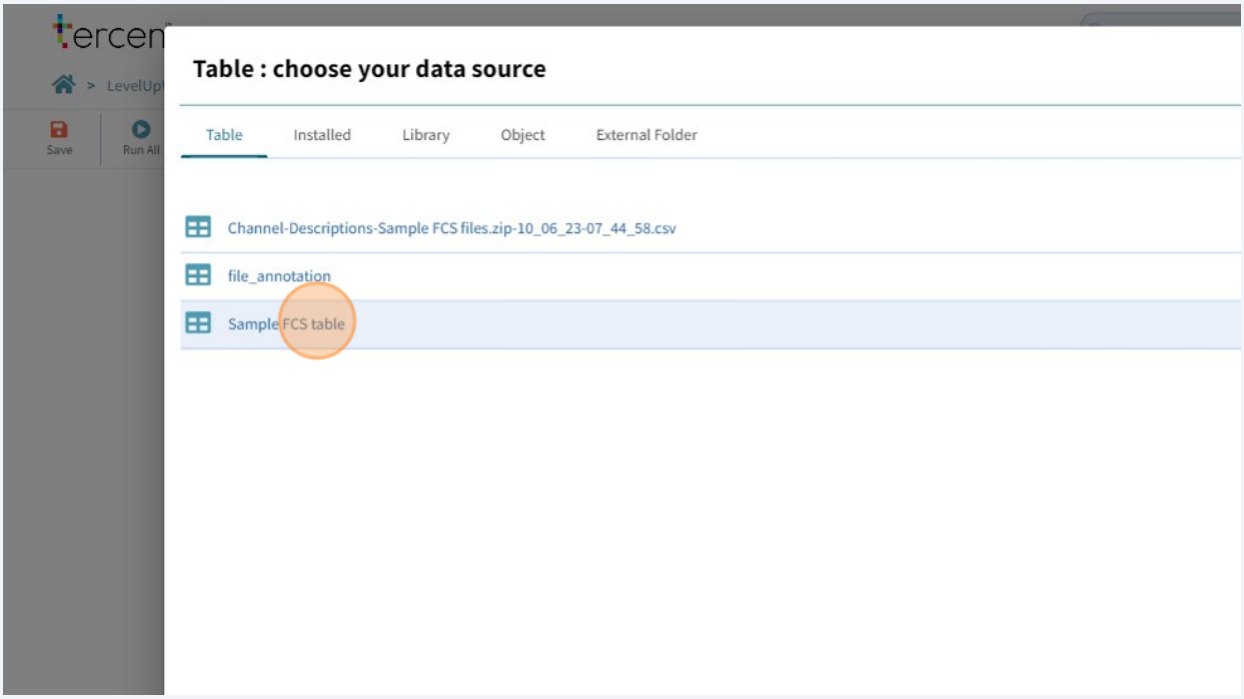
5 We will begin to create a workflow from scratch. Click on the **Add Step** icon



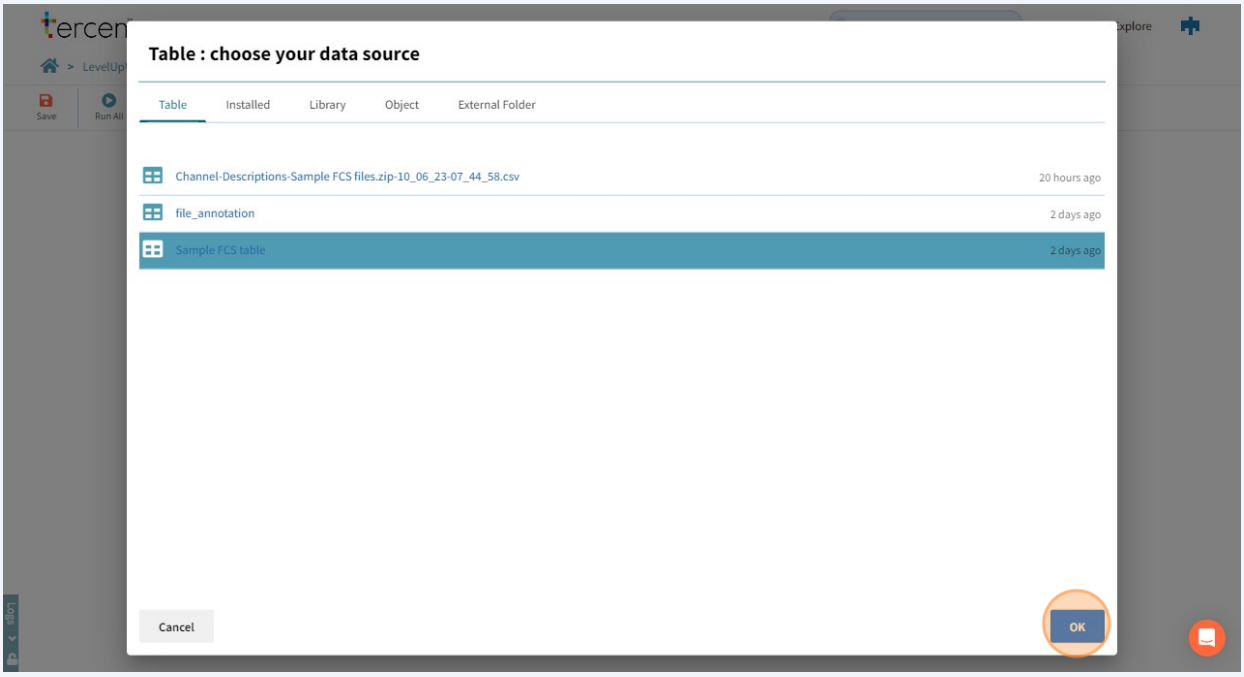
6 Select Table by Click on it.



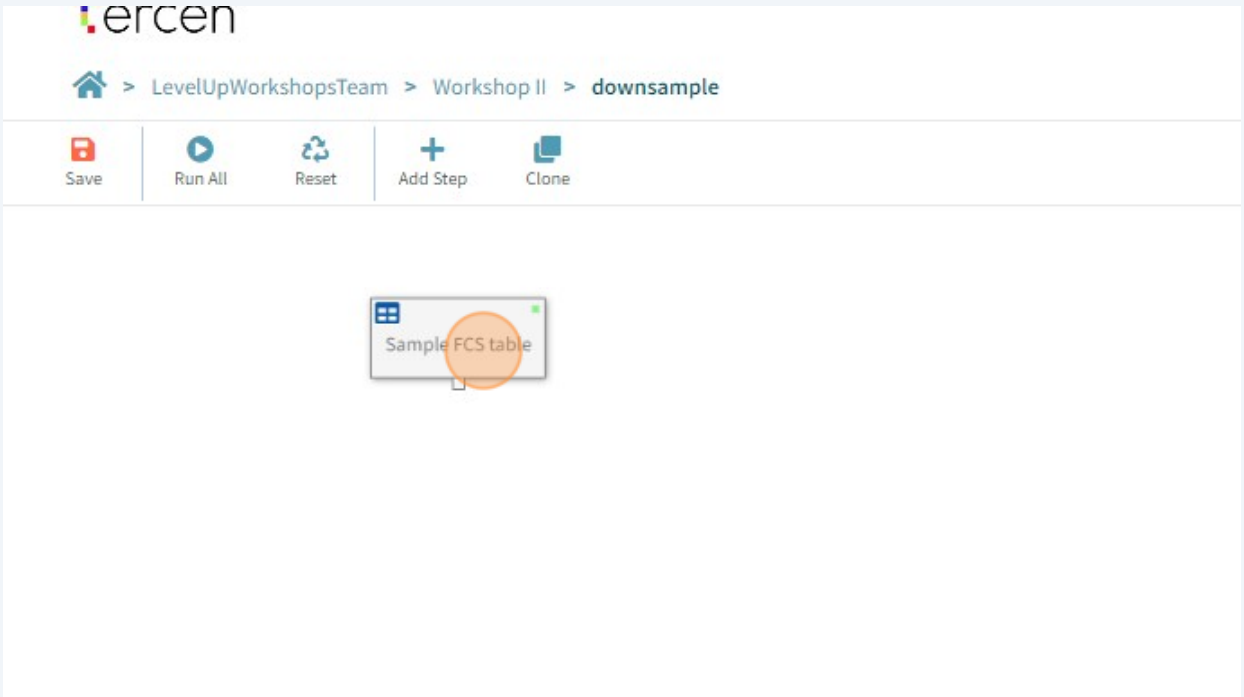
7 Click "Sample FCS table"



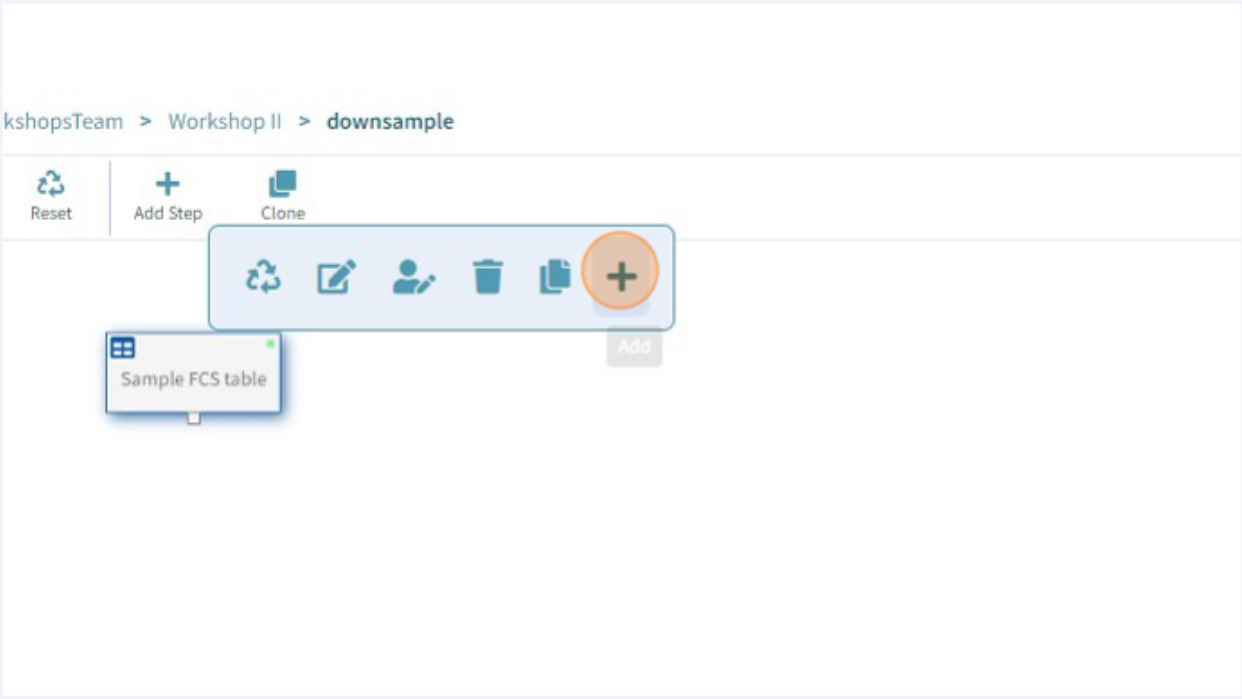
8 Click "OK"



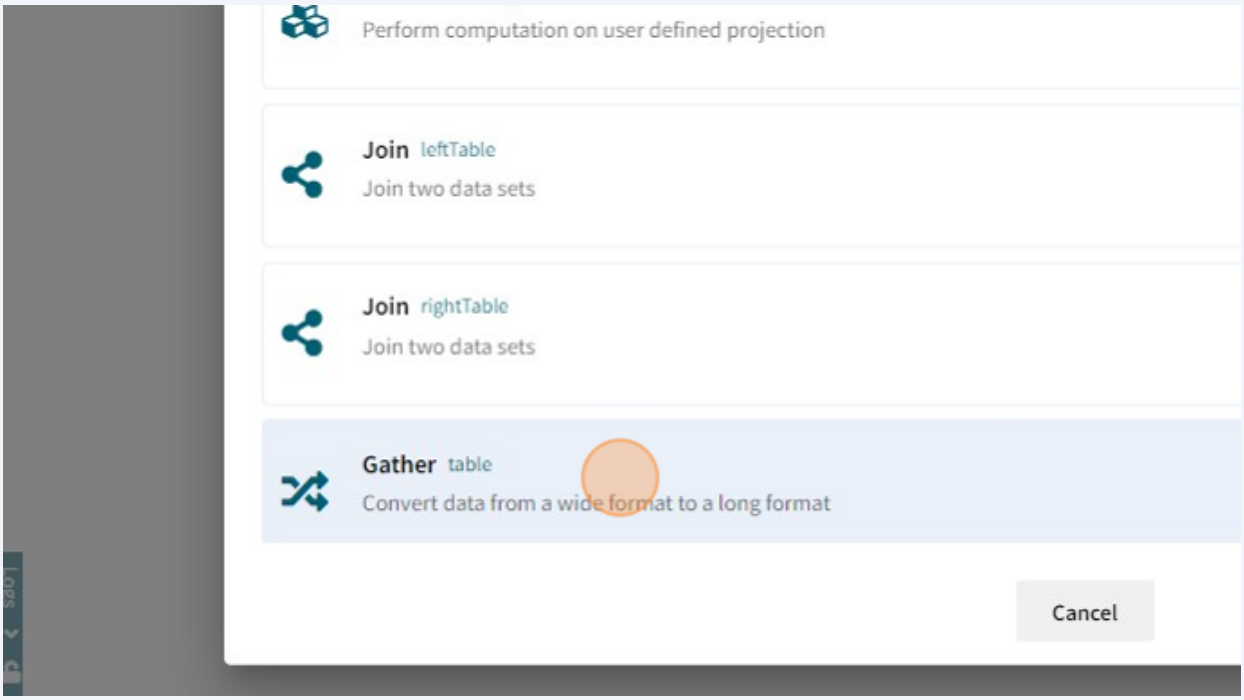
9 Click here.



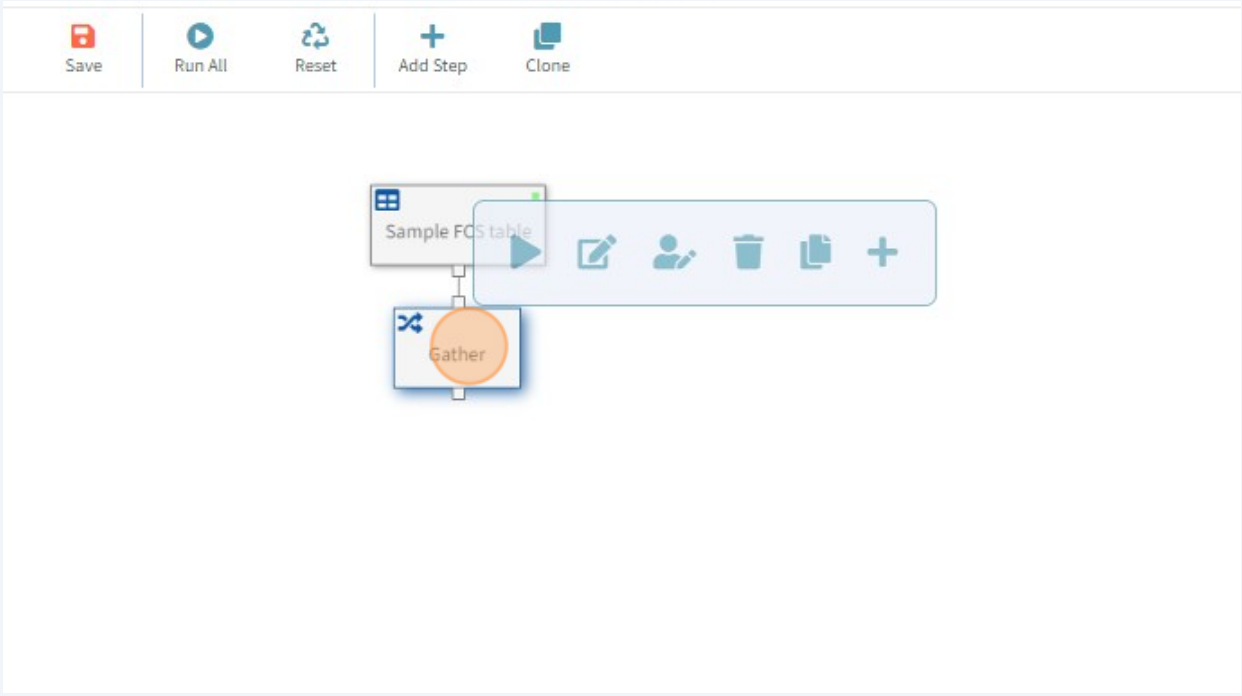
10 Click here.



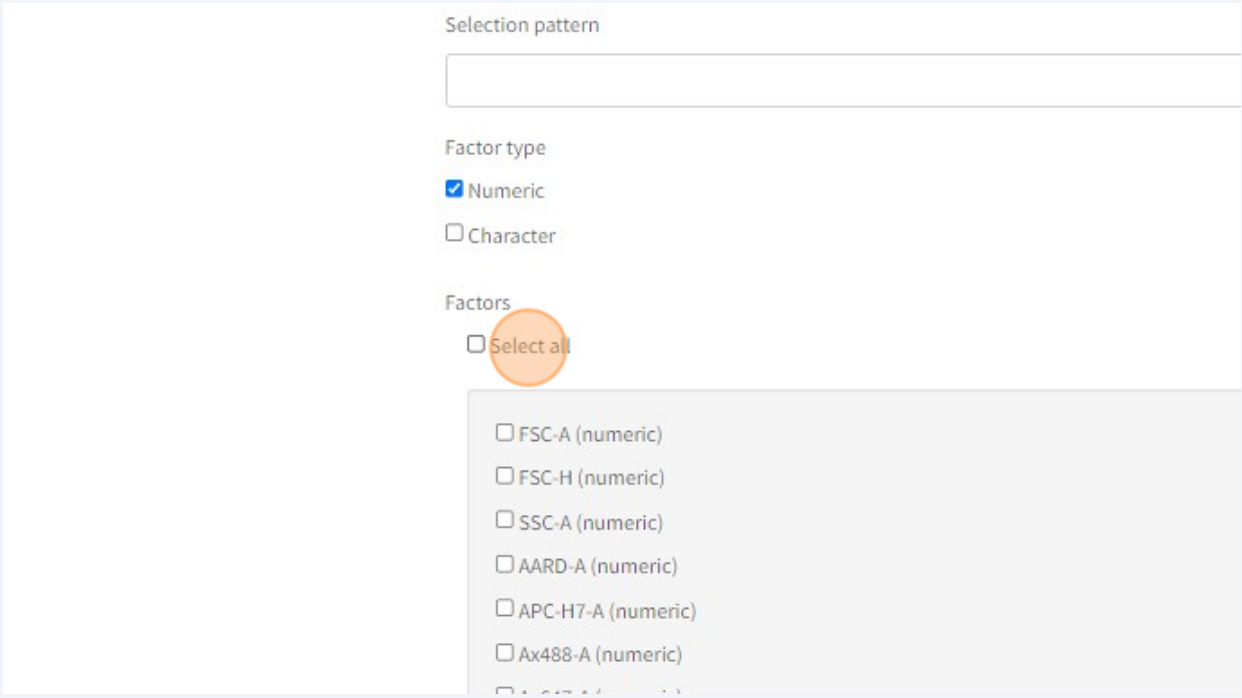
11 Click on the Gather step



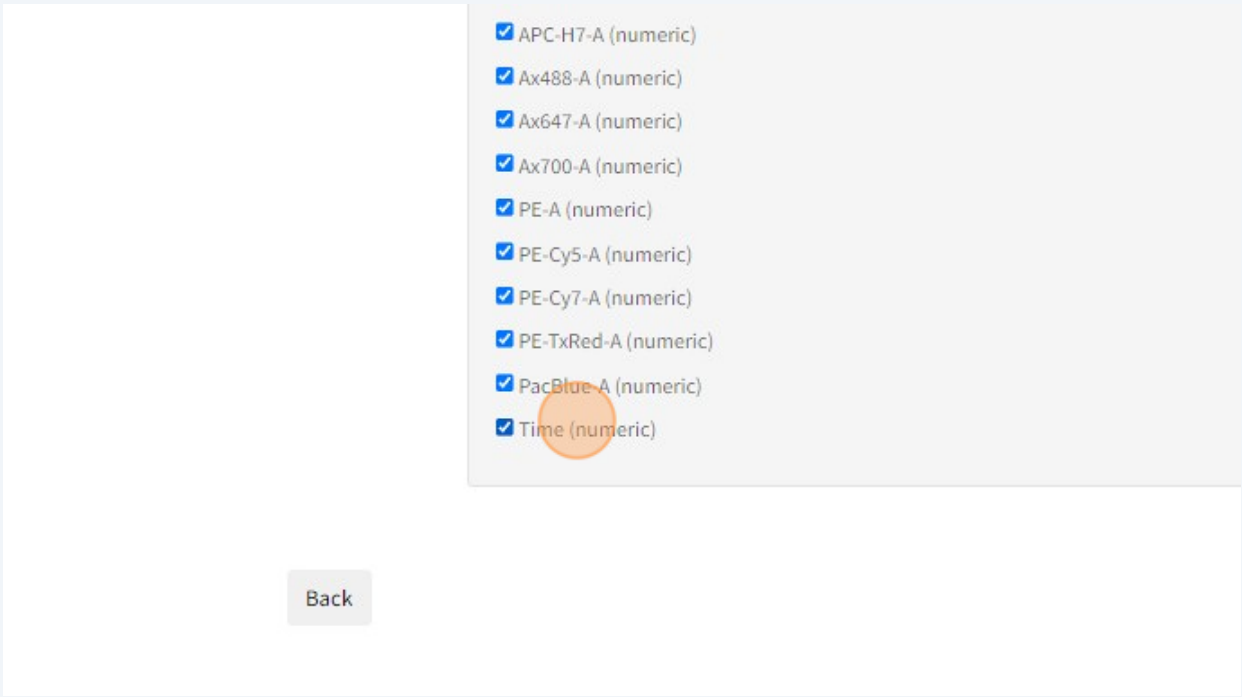
12 Double-click here.



13 Click "Select all"

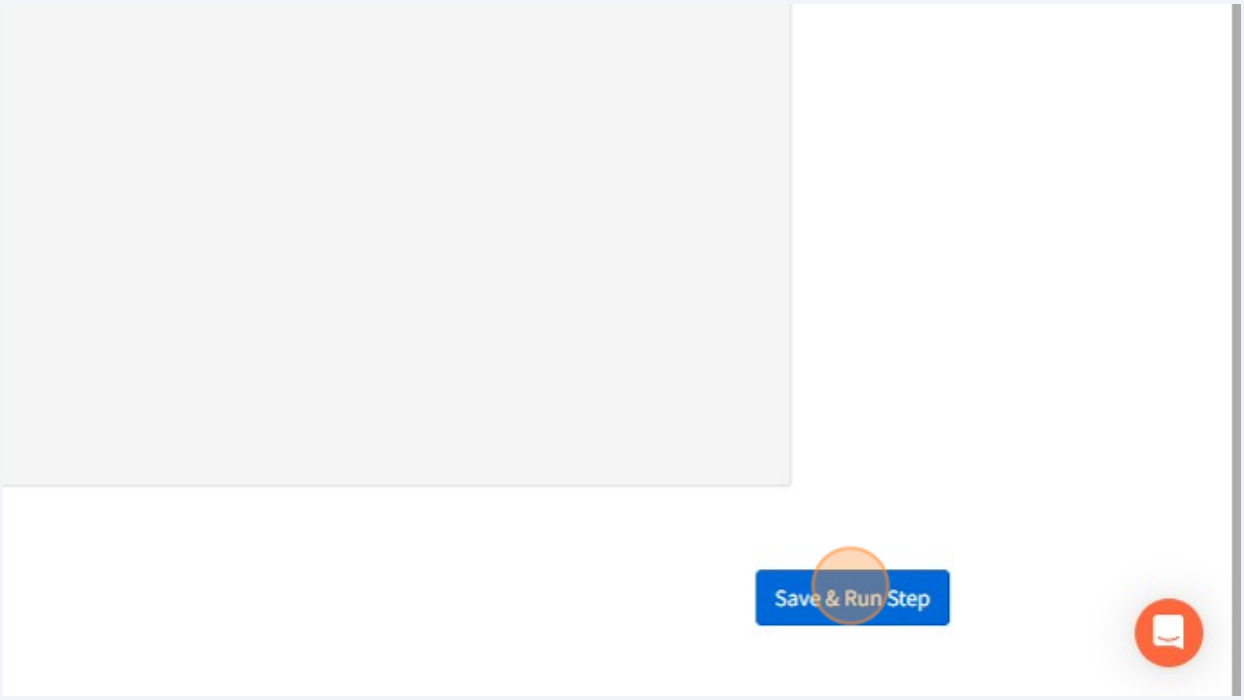


14 Click "Time (numeric)"

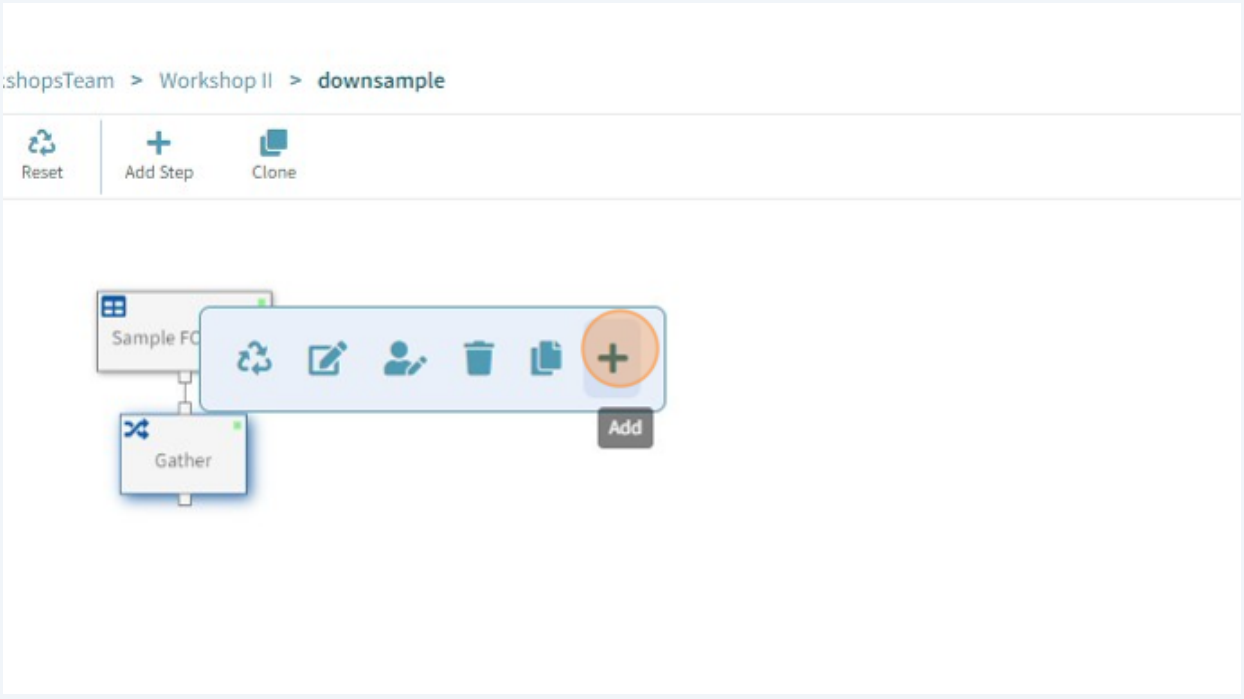


The Time channel needs to be unchecked since we do not need this to be gathered with the other channels. It is represents a different concept. The other channels represents measurements on the biological cells. The time channel represents an annotation of the event.

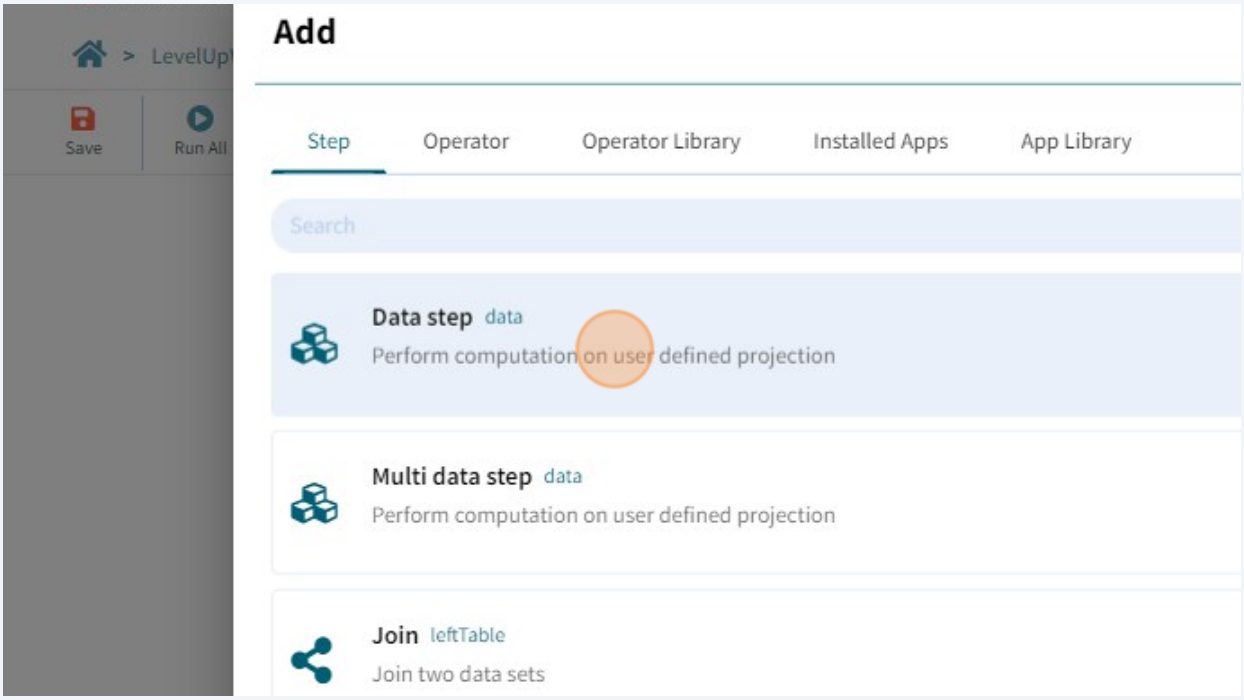
15 Click "Save & Run Step"



16 Click here.

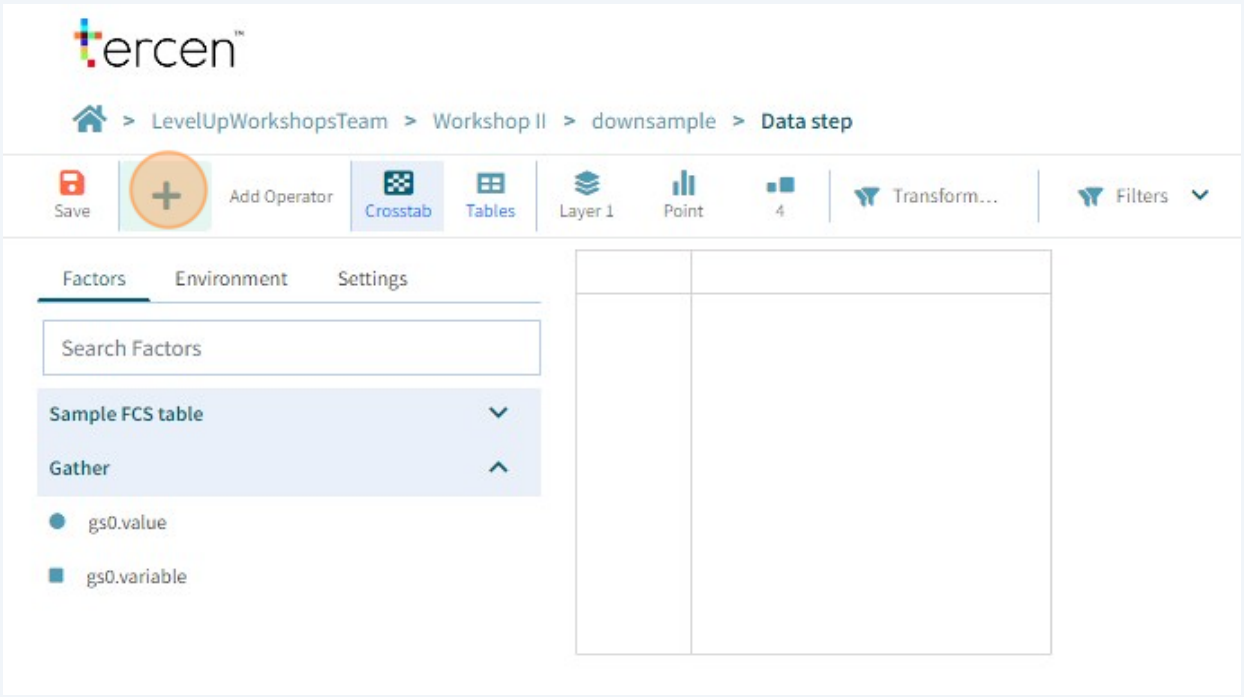


17 Click on "Data step"

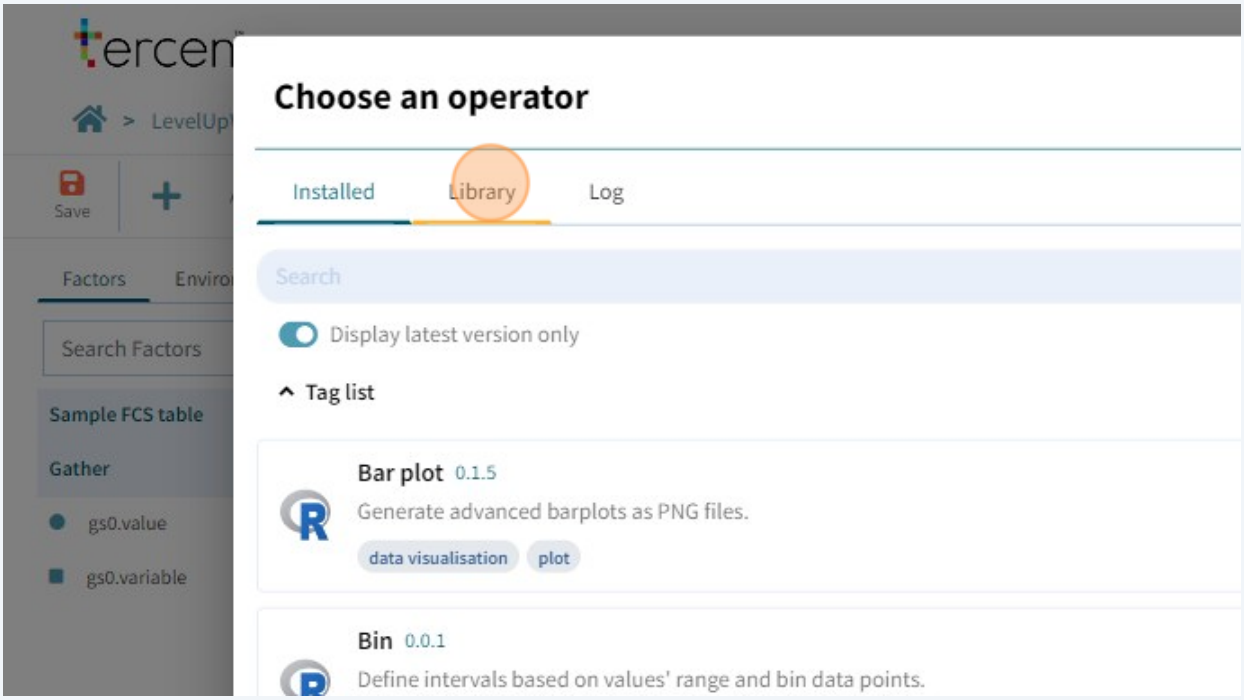


18 Click the large + icon.

This allows us to add an operator.

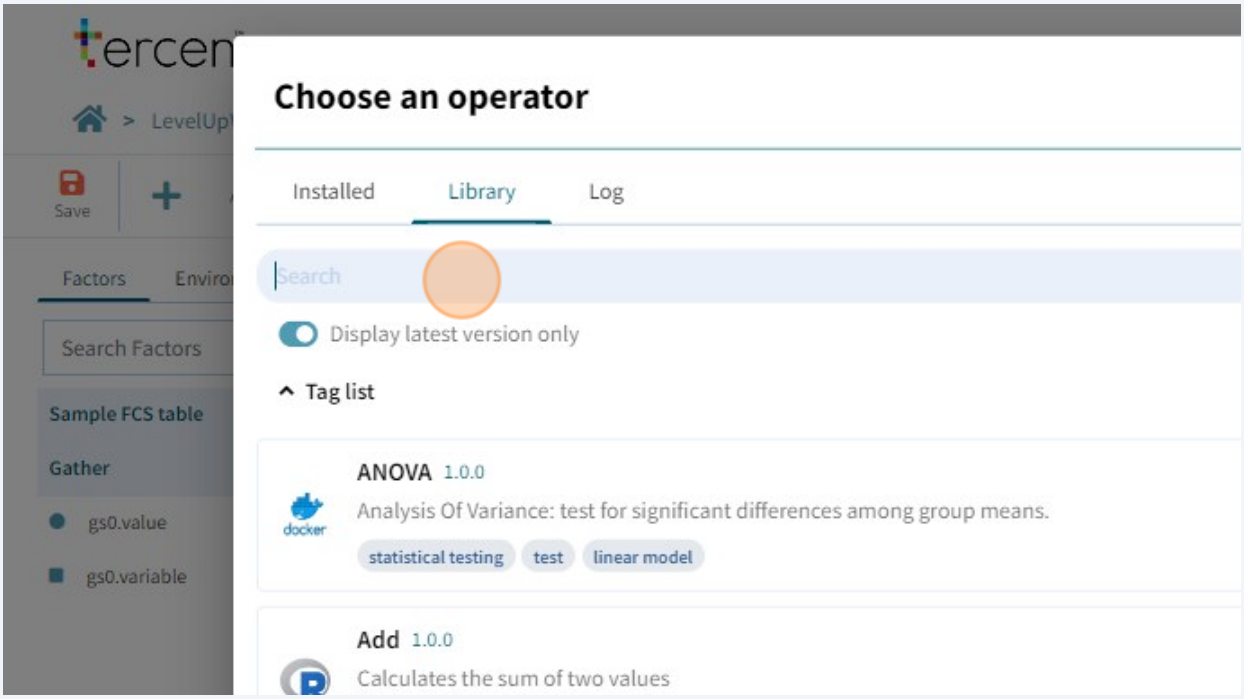


19 Click "Library"

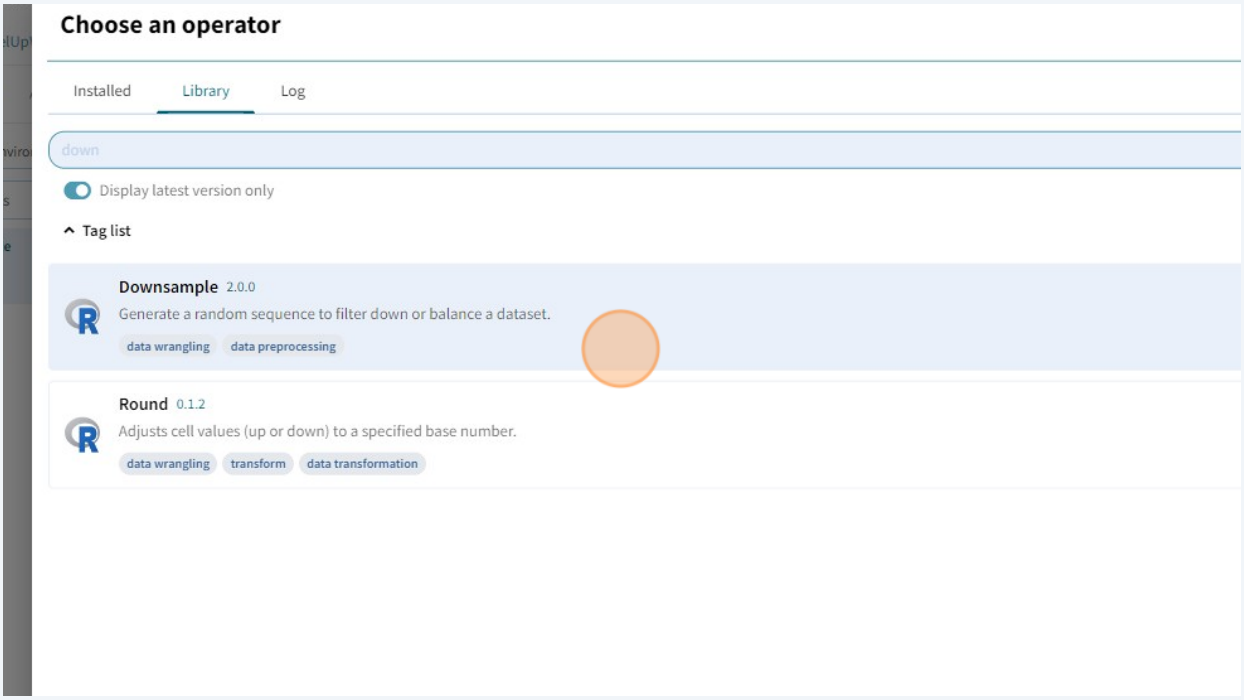


20 Click the "Search" field.

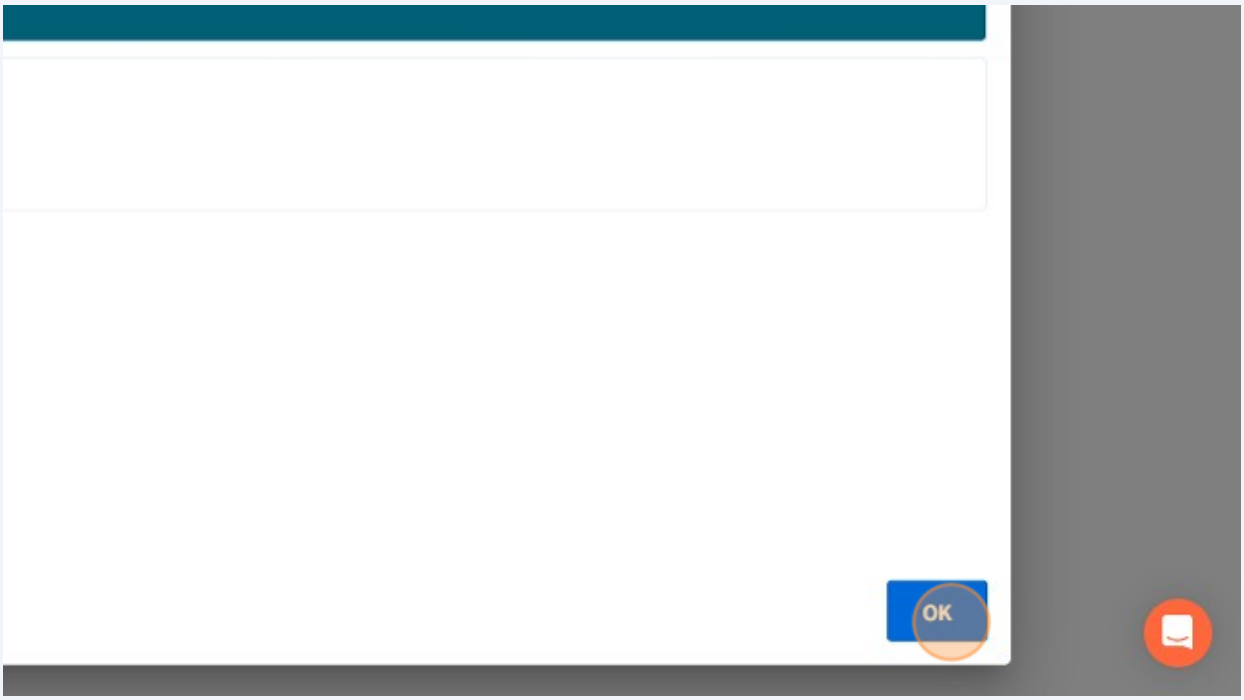
and type "down" this will return a list of operators with the word "down" in it.



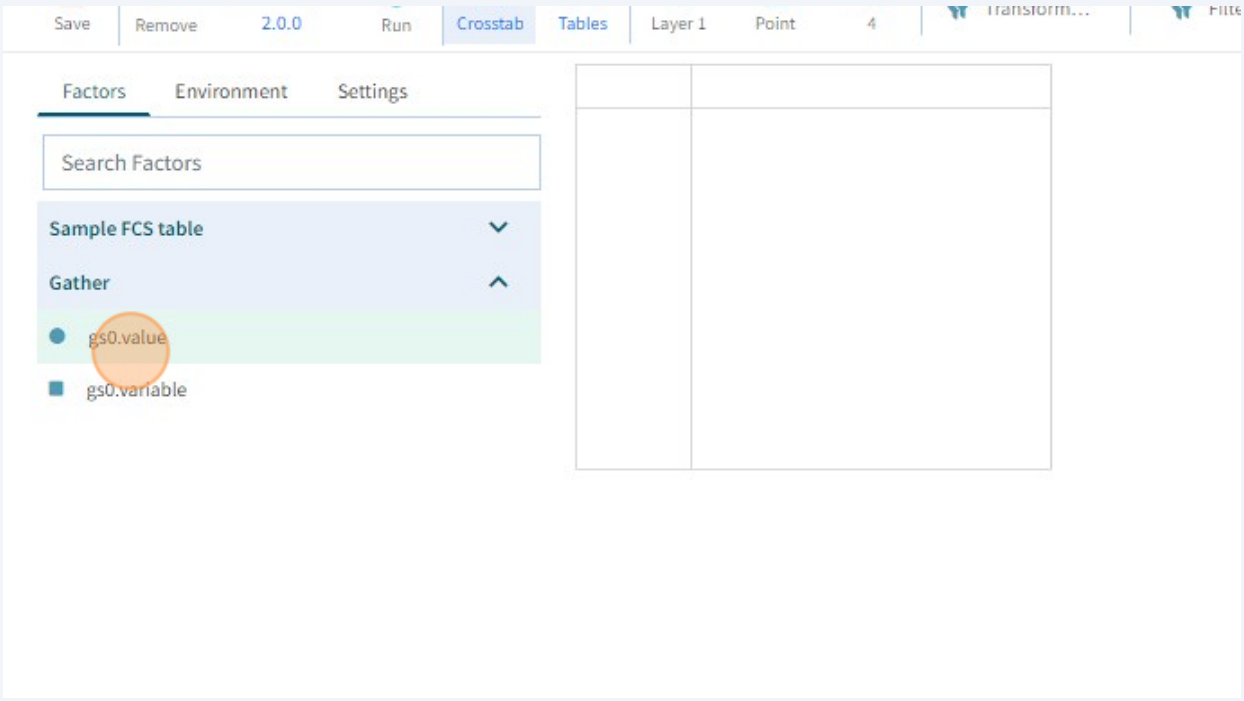
21 Select the "Downsample" operator



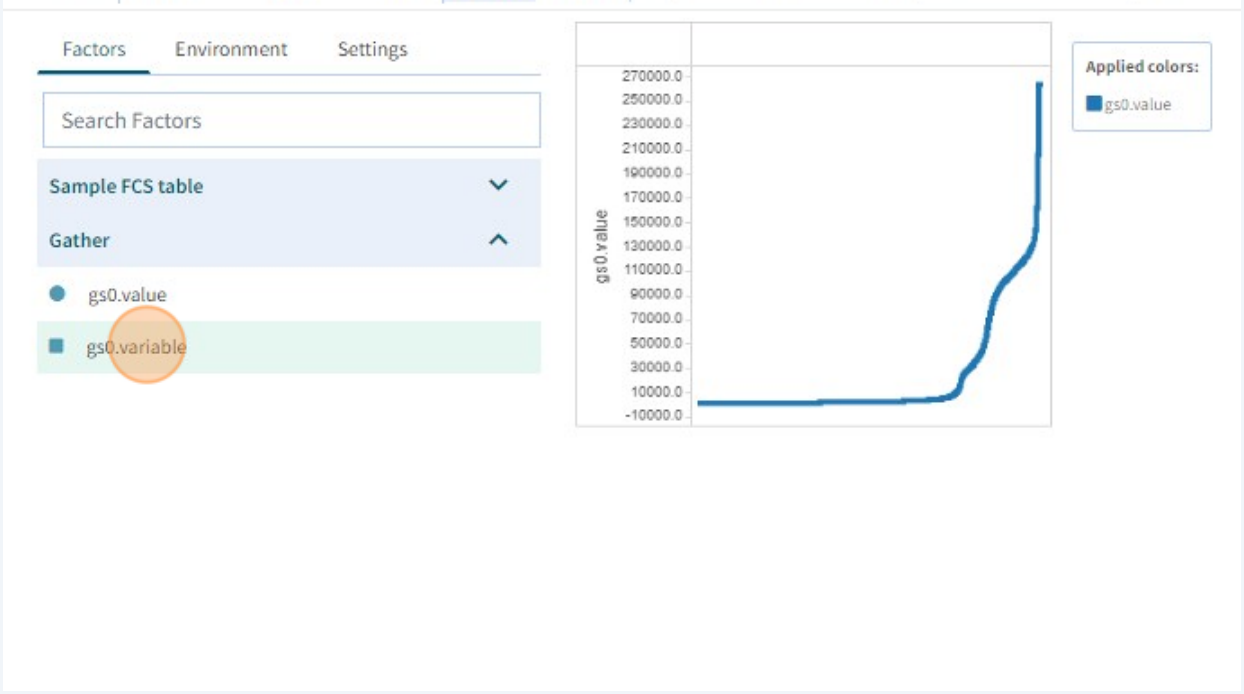
22 Click "OK"



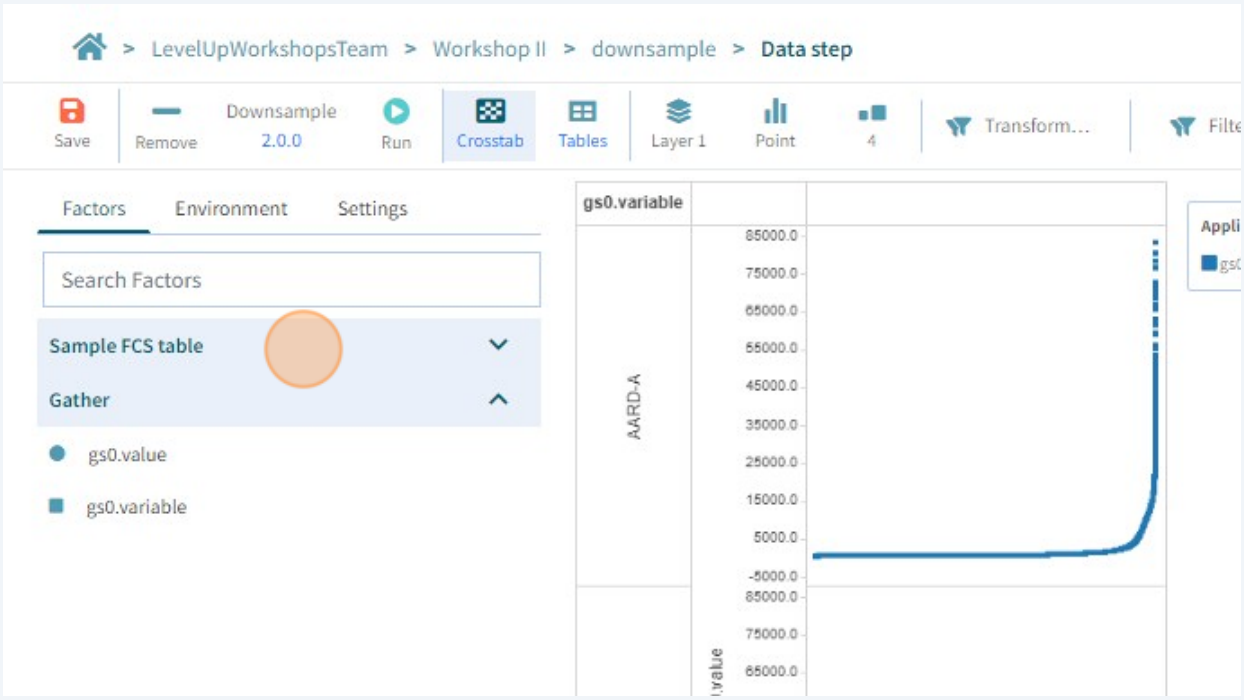
23 Drag **gs0.value** and drop onto the Y-Axis.



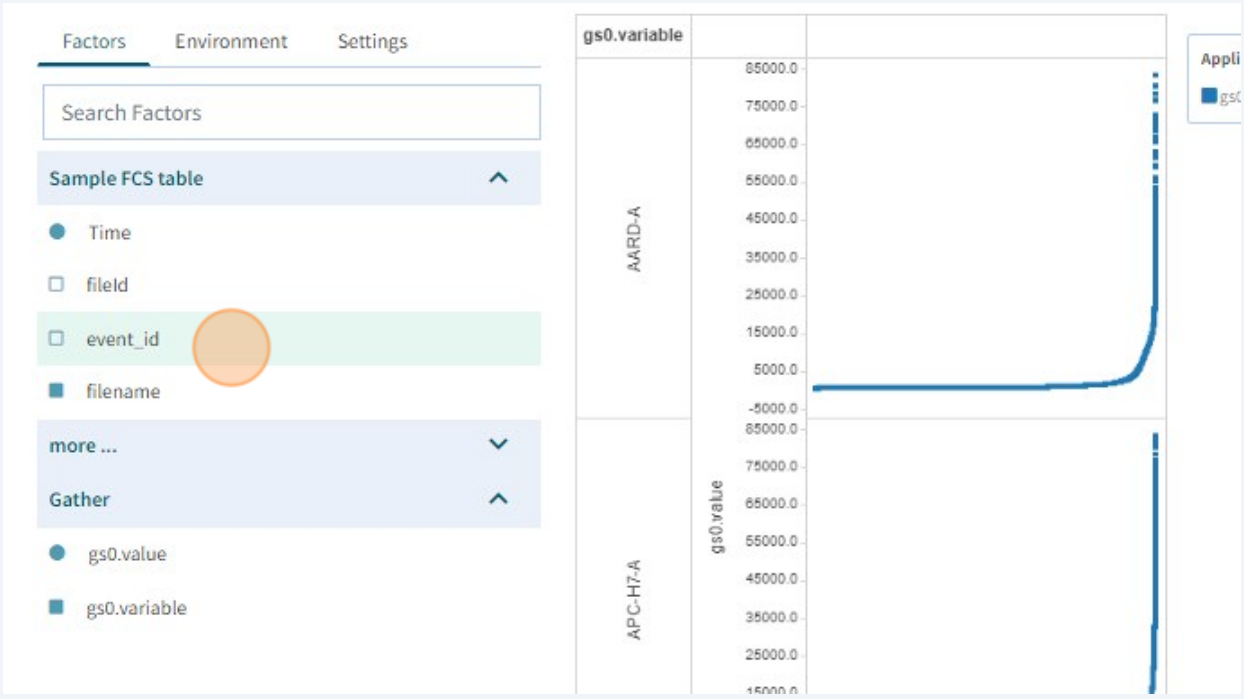
24 Drag **gs0.variable** and drop on the Rows



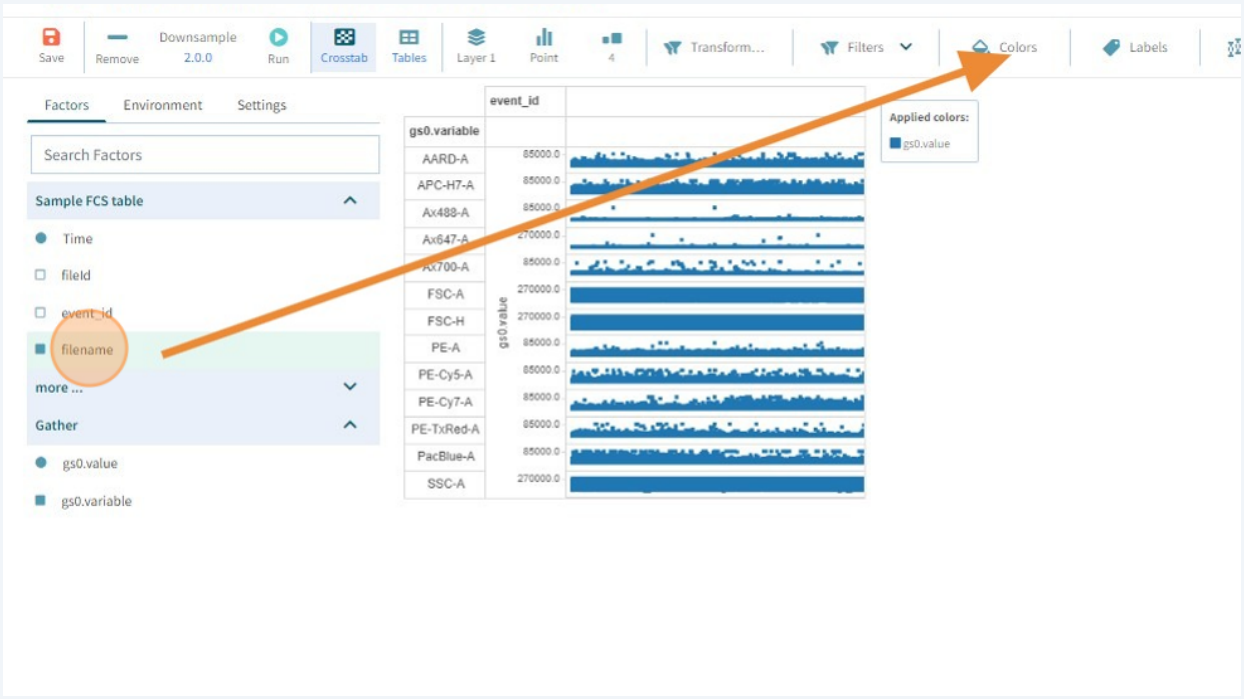
25 Click "Sample FCS table"



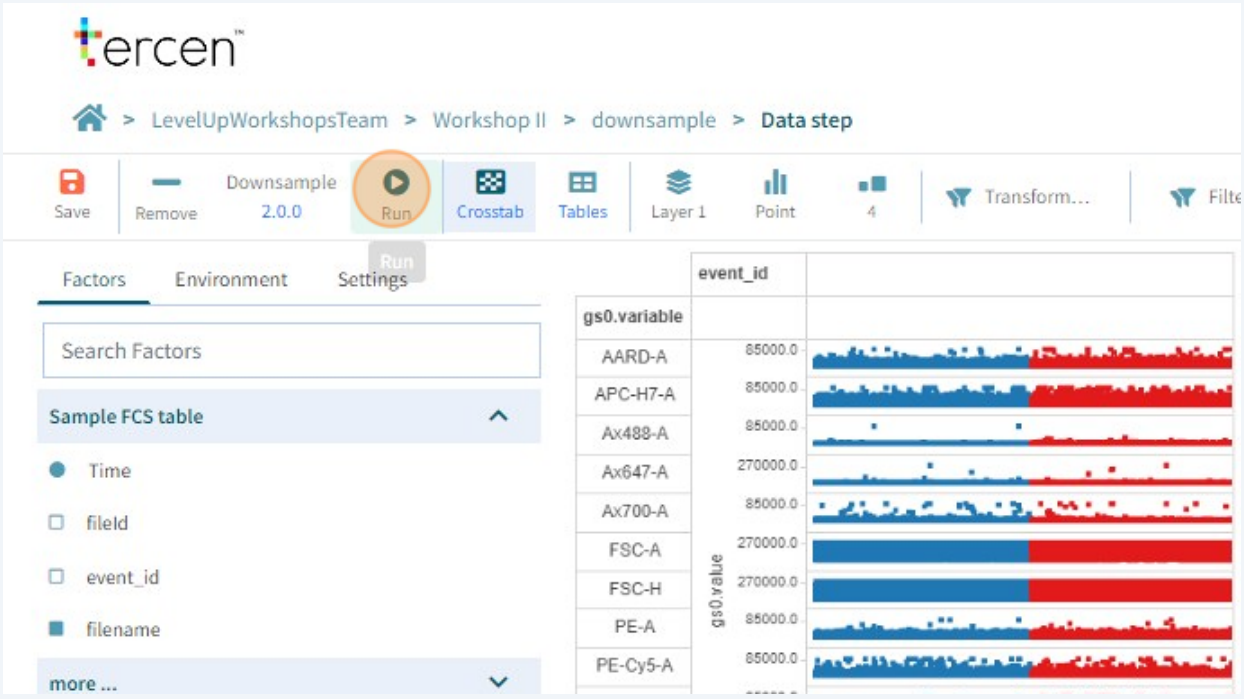
26 Drag **event_id** and drop onto Columns



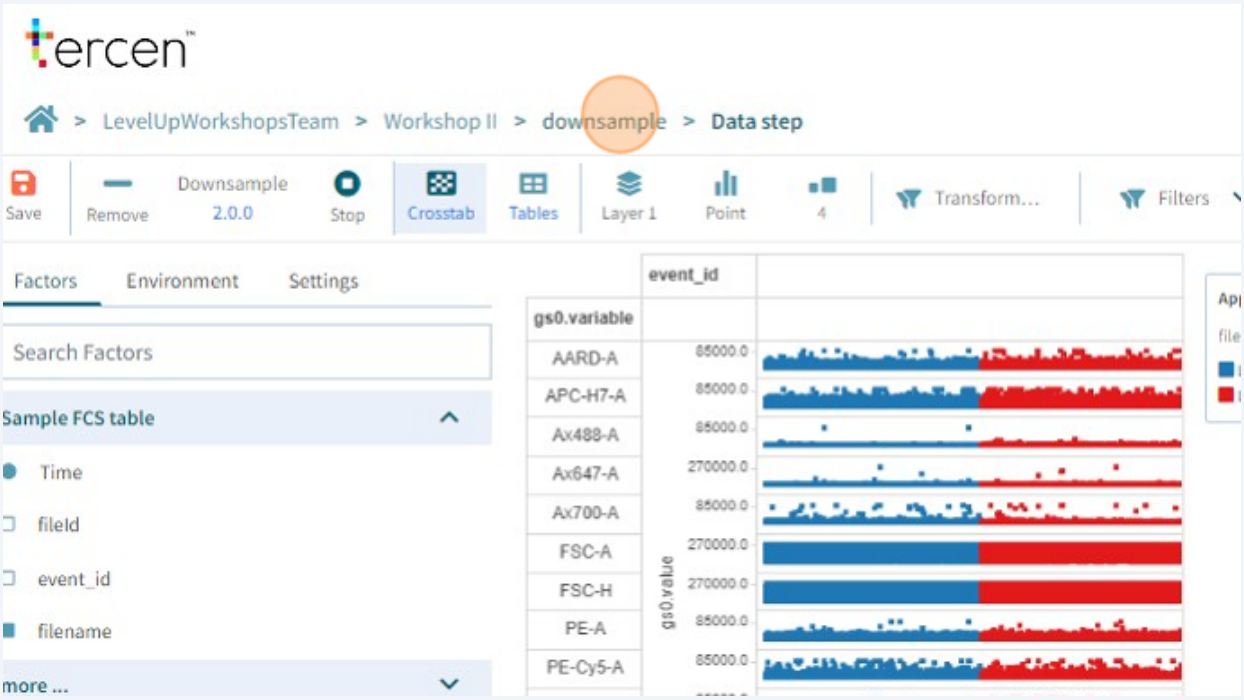
27 Drag "filename" and drop it into Colors



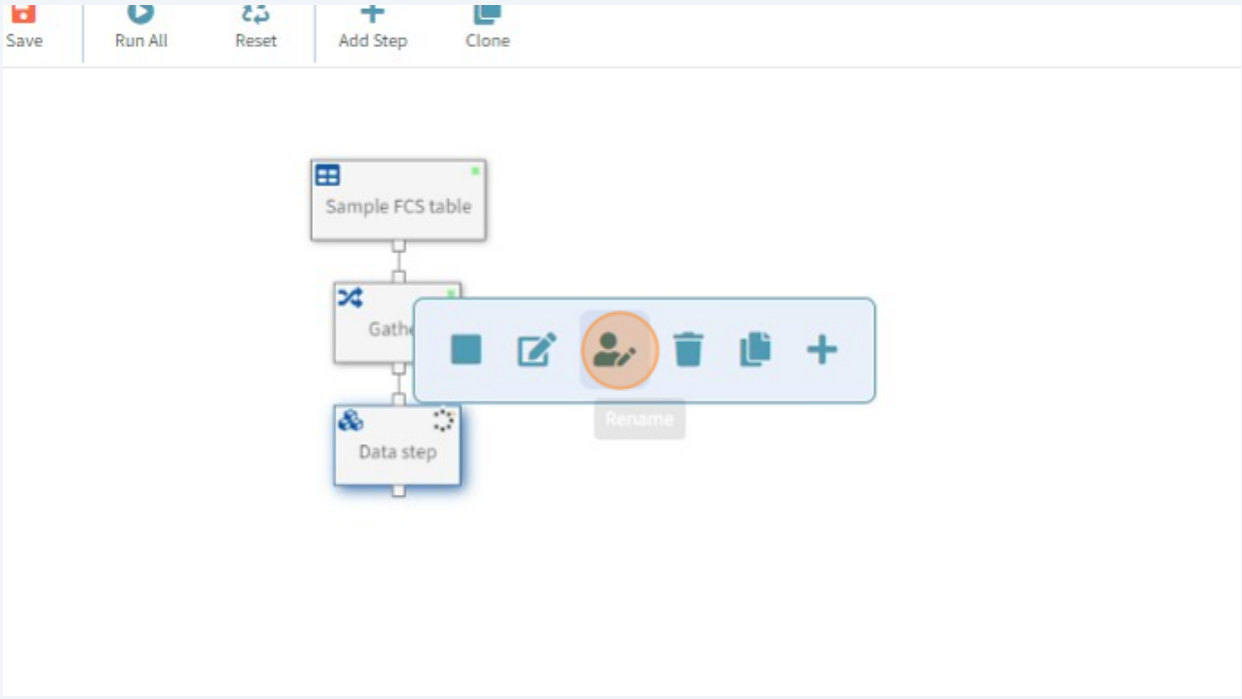
28 Click on the Run icon.



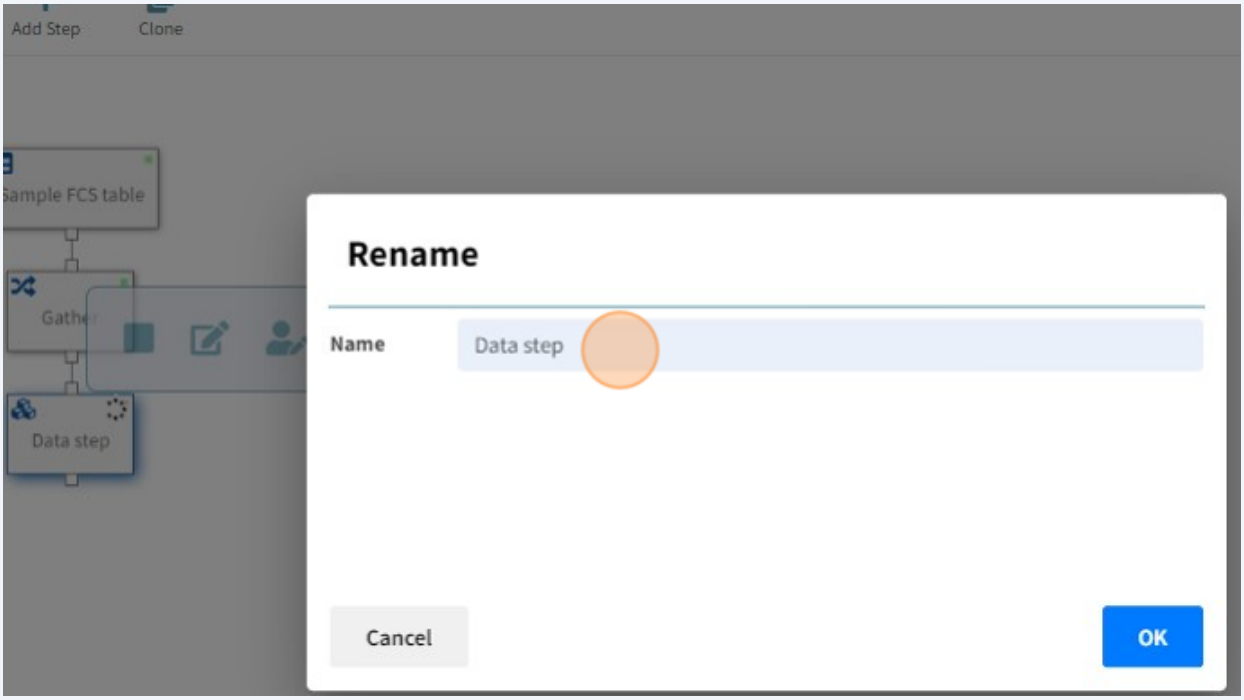
29 Navigate to workflow level by clicking on "downsample" in the breadcrumb.



30 Click here.



31 Click and gave it the name "downsample"



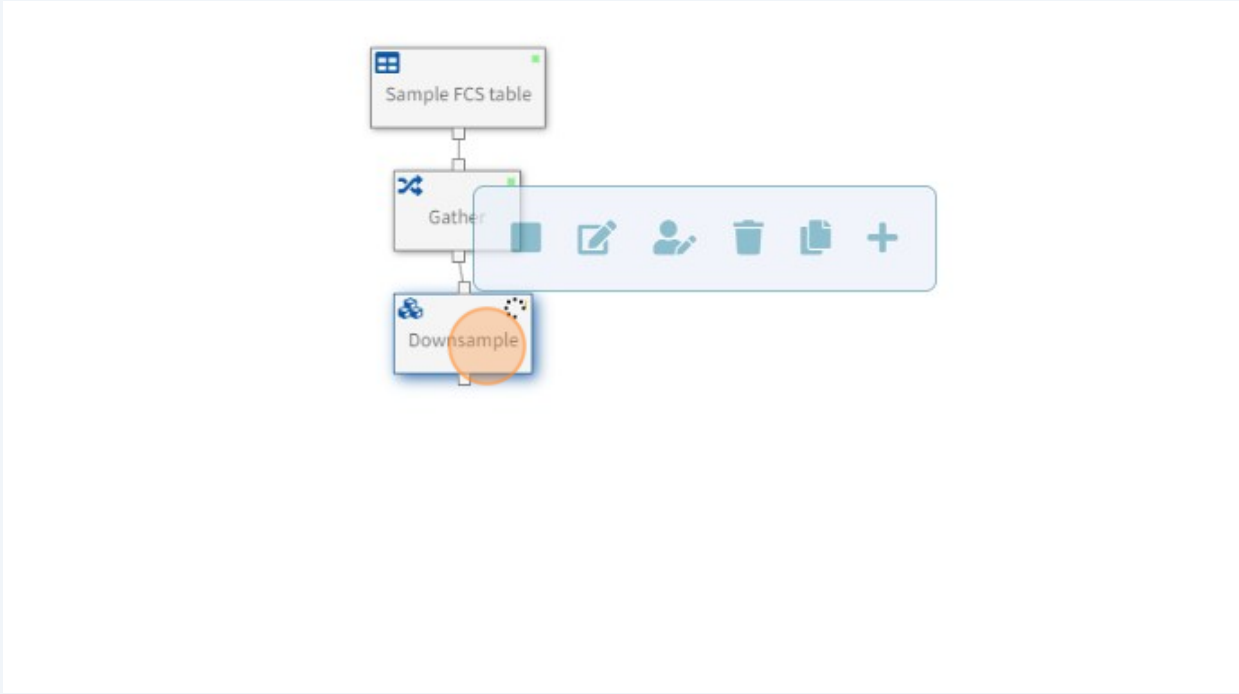
Tip!

You do not have to wait for the operator to finish executing to change the name of the Data Step

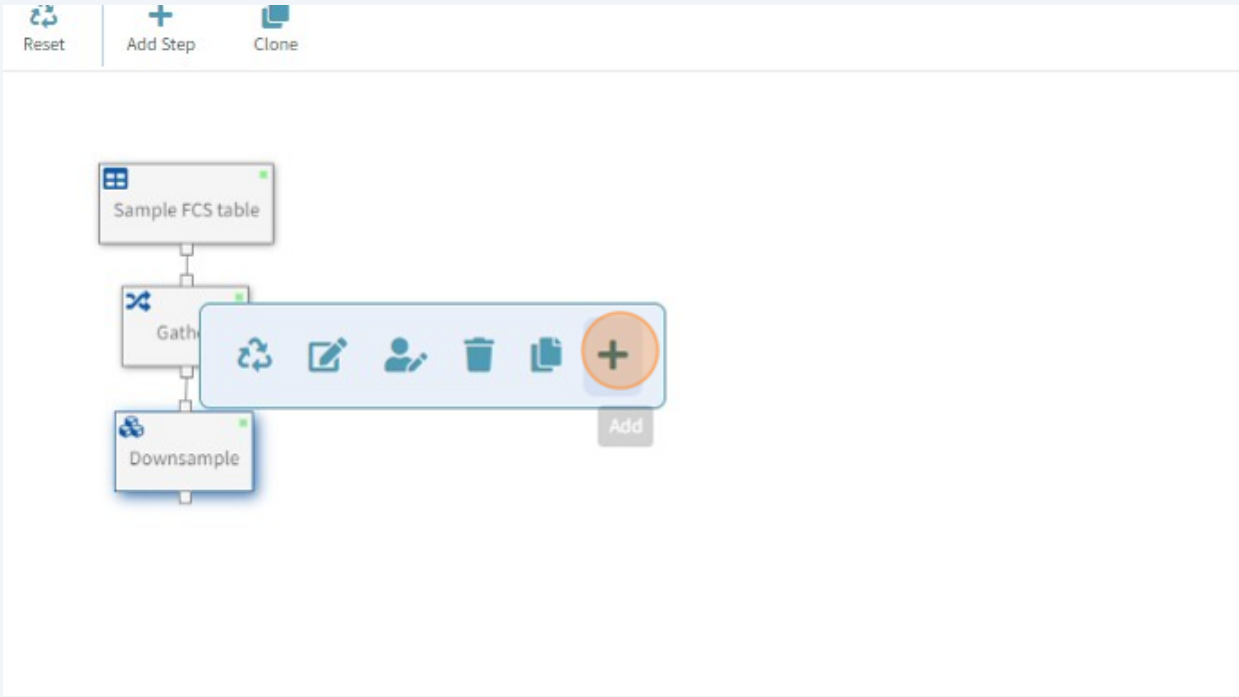
32 Let the downsample operator finish.

We will add another Data step after it.

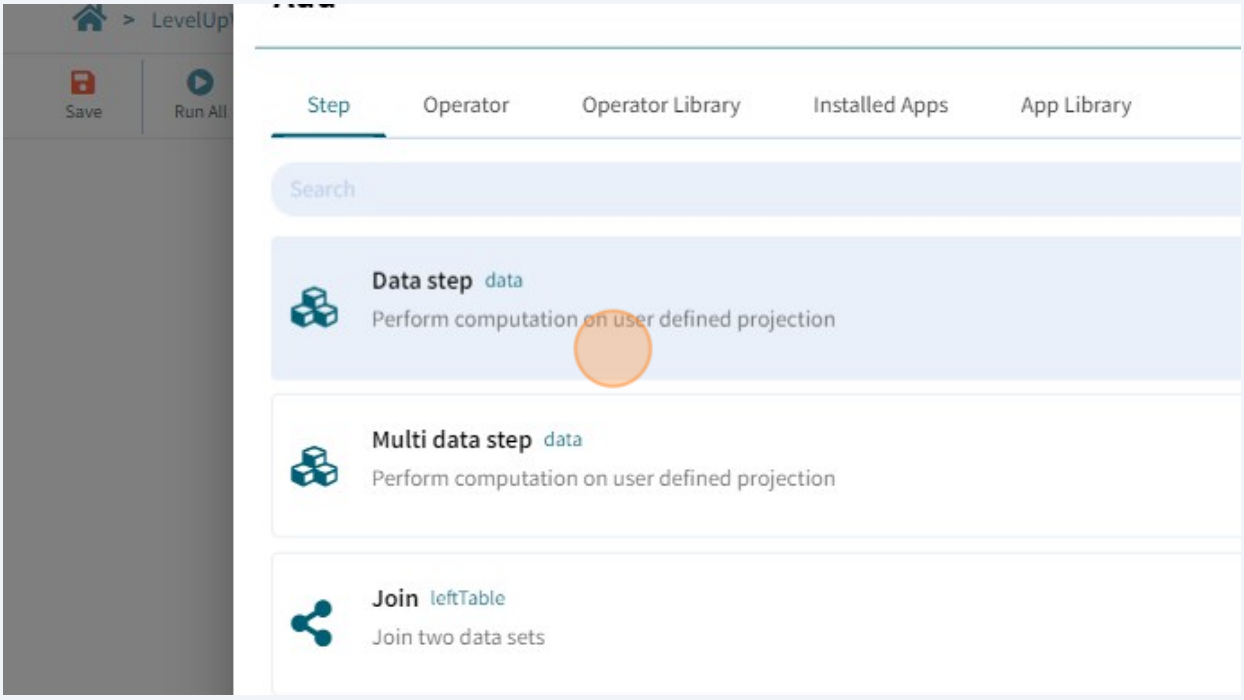
Click on the step the toolbar will appear.



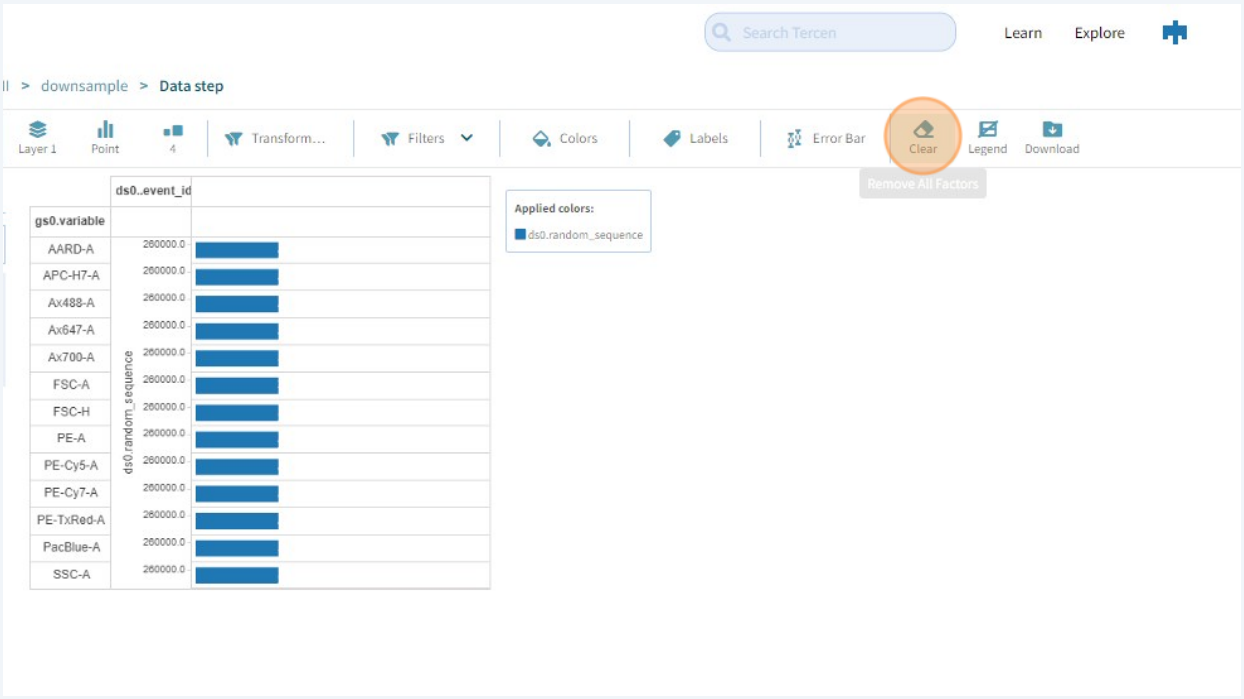
33 Click here.



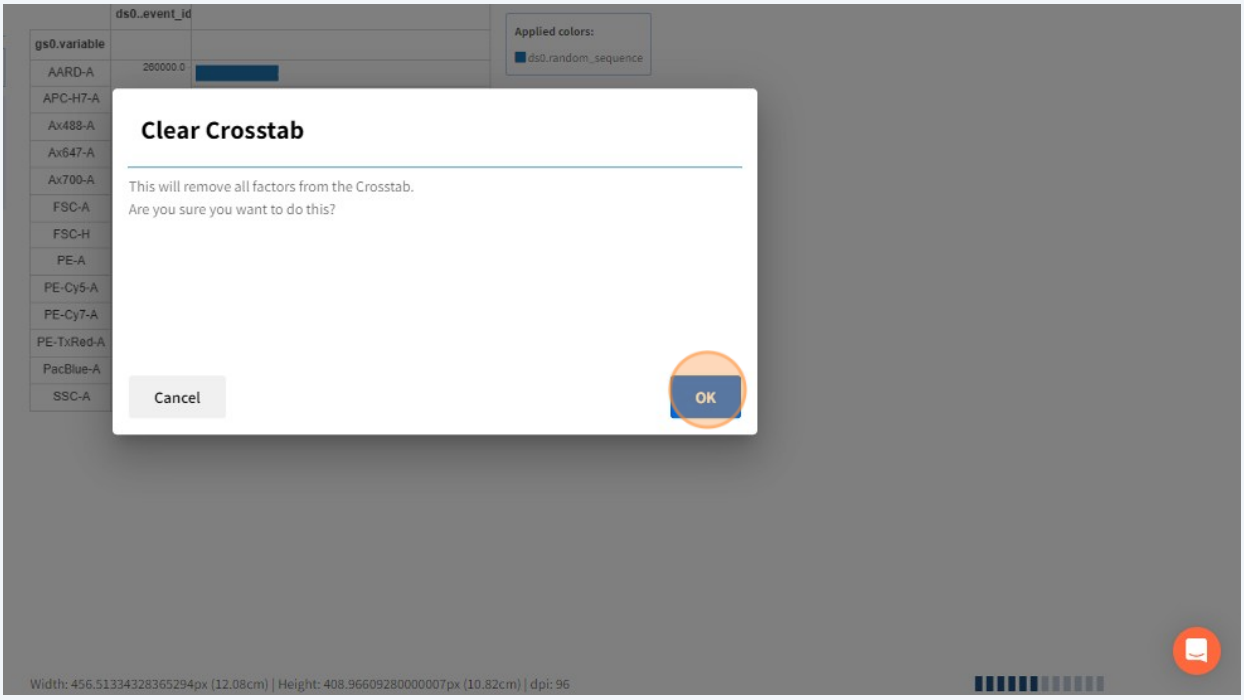
34 Select the Data step.



35 Click here to clear the crosstab window.



36 Click "OK"



Did you notice new entries at the bottom of the Factors list?

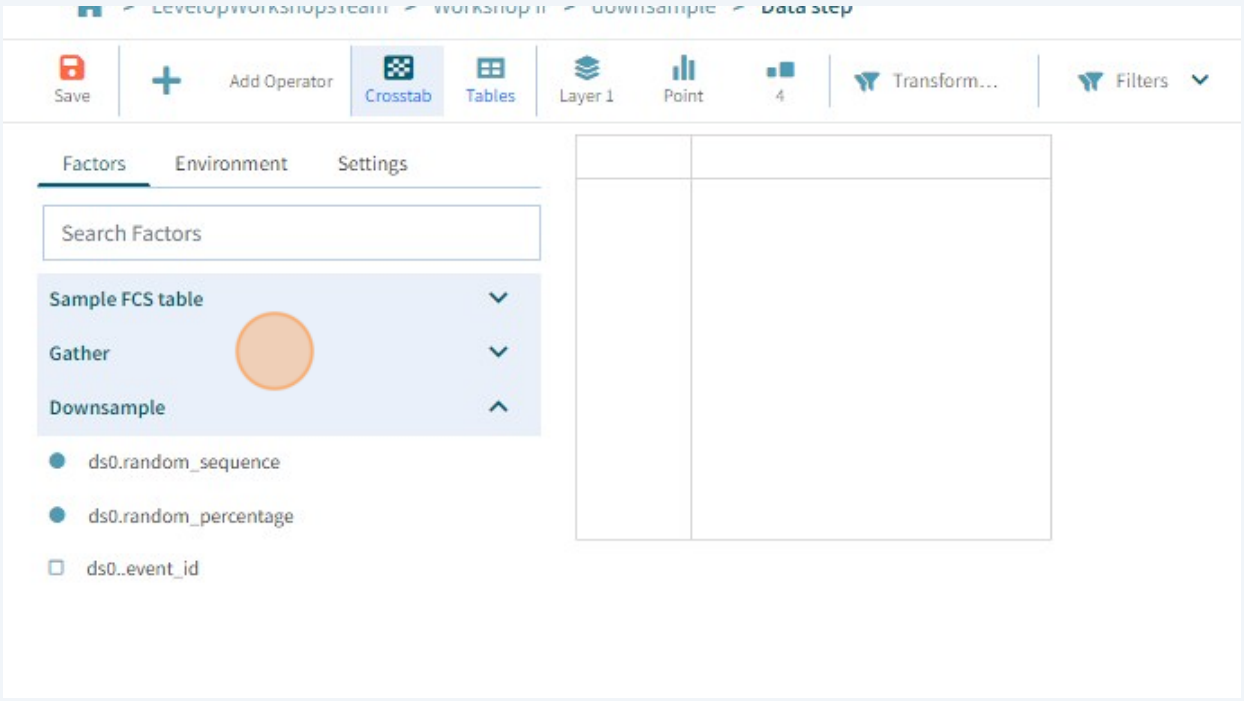
The two important ones are:

ds0.random_sequence

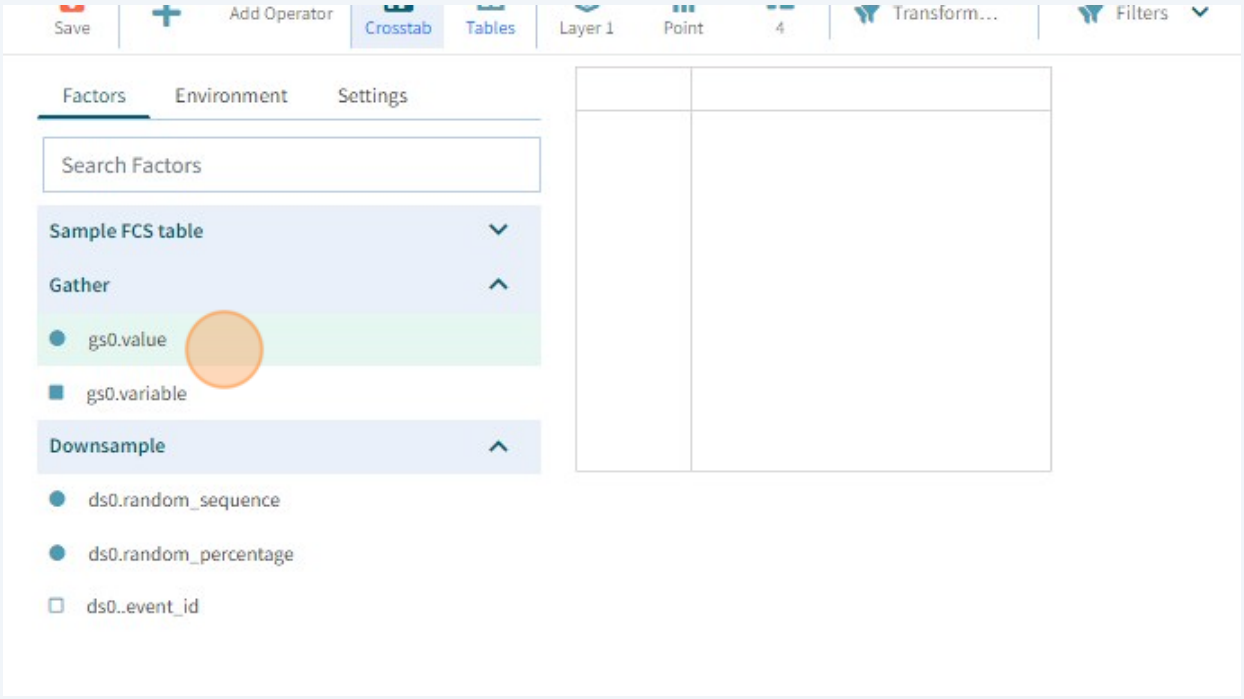
ds0.random_percentage

These were computed by the downsampling operator.

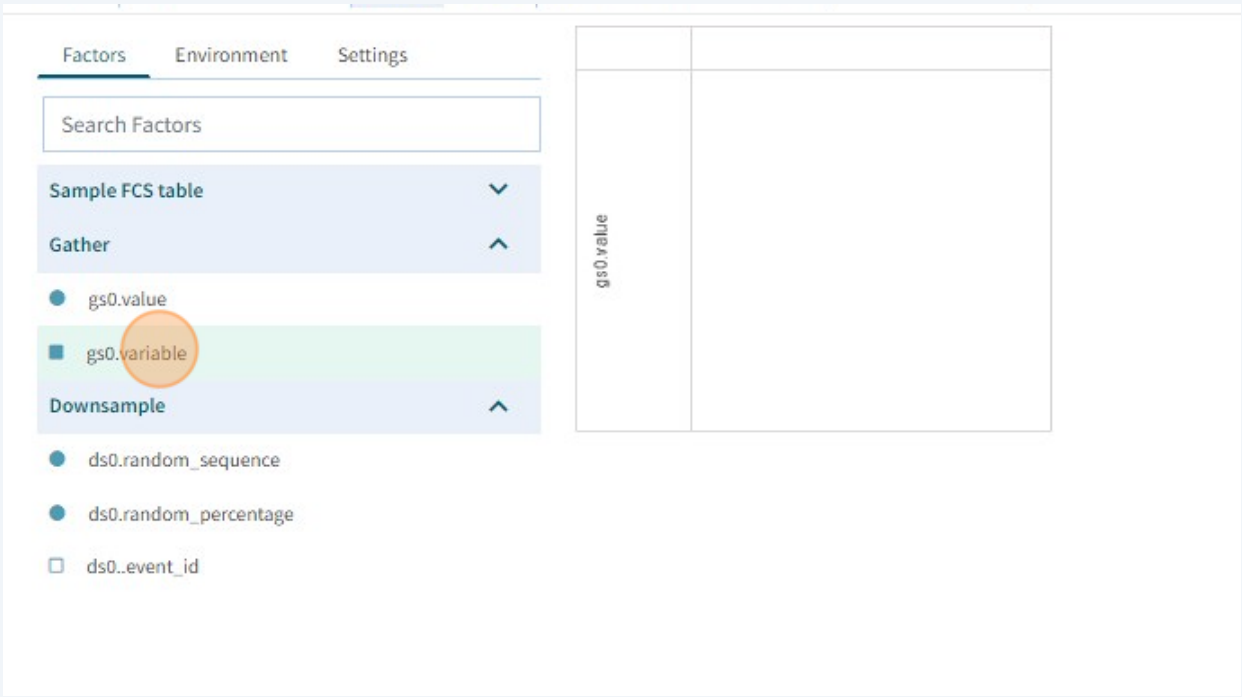
37 Click "Gather" entry in the Factors list to expose the sub-list of factors.



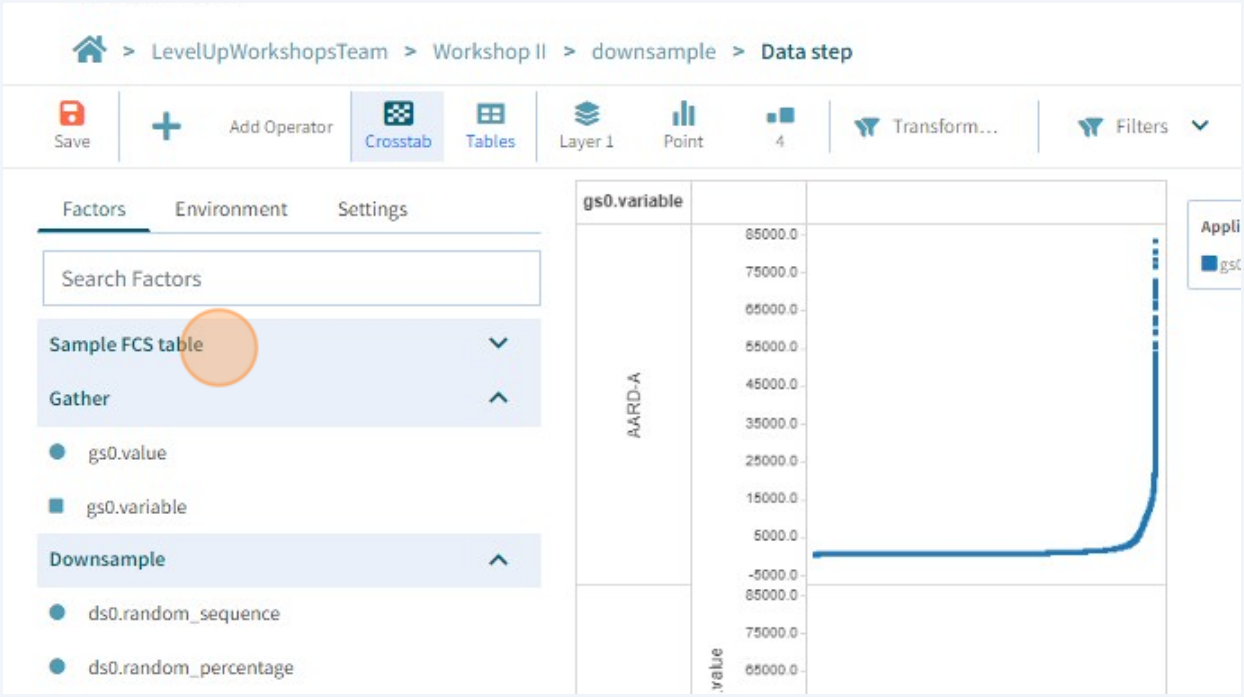
38 Drag **gs0.value** and drop onto the X-axis.



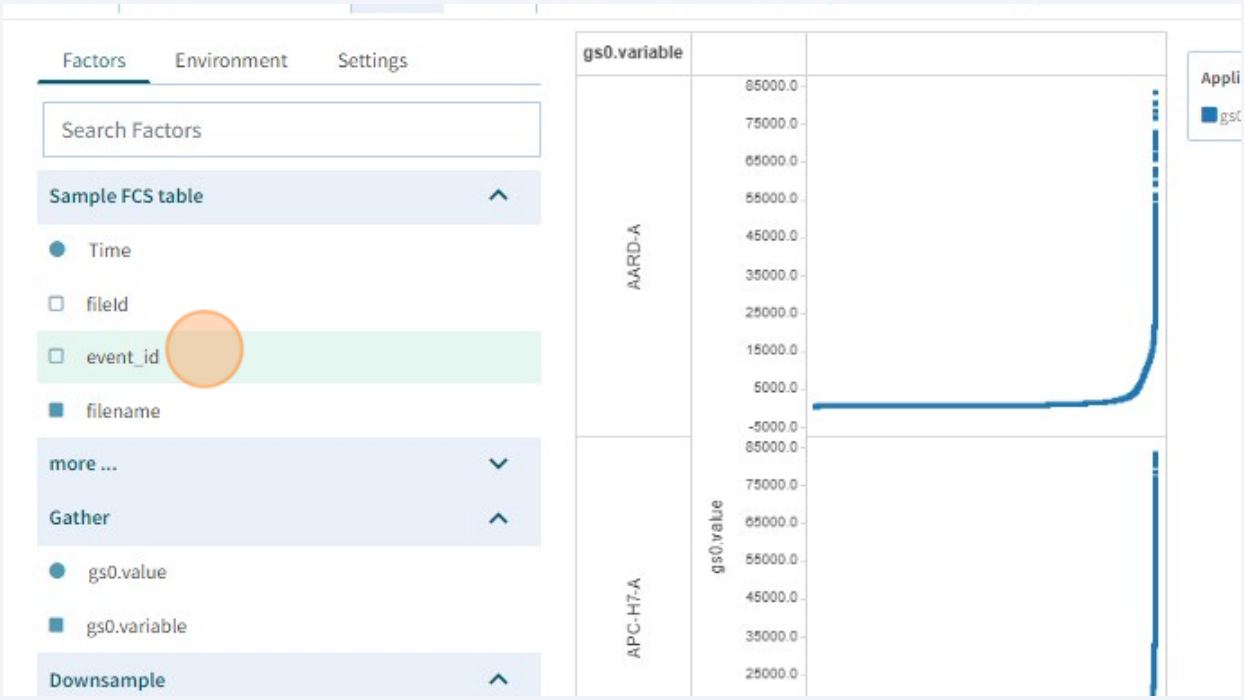
39 Drag the **gs0.variable** and drop onto the Rows.



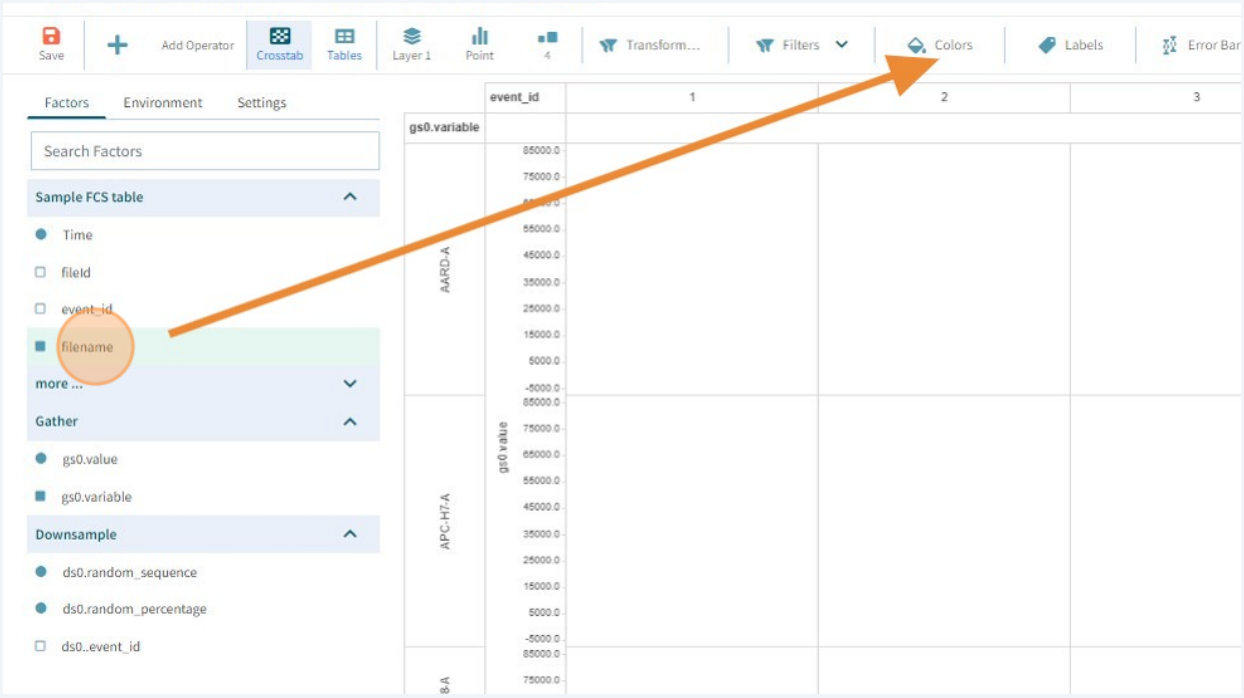
40 Click "Sample FCS table" entry to expose the factor sub-list



41 Drag **event_id** and drop onto the Columns.



42 Drag **filename** and drop into Colors.



Tip!

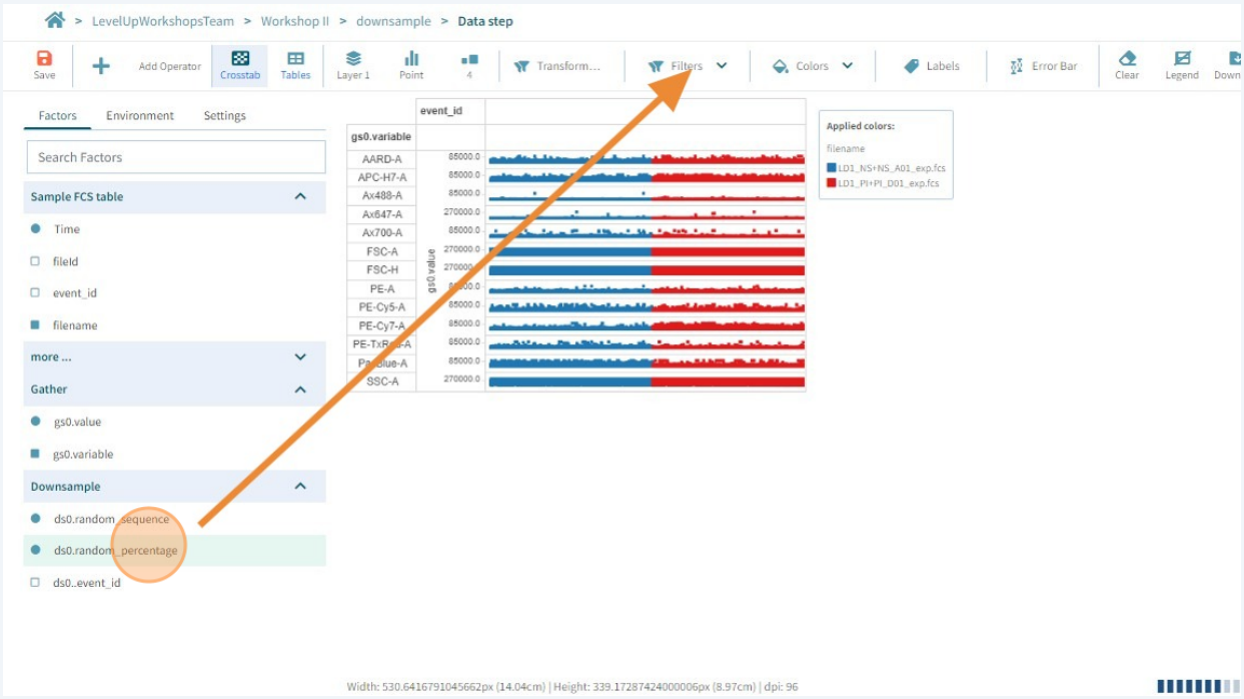
Resize the crosstab window to get an overview of all your data.

Place the mouse on the Row area, and press the CTRL key while using the wheely on your mouse to zoom in. This will squash the window vertically.

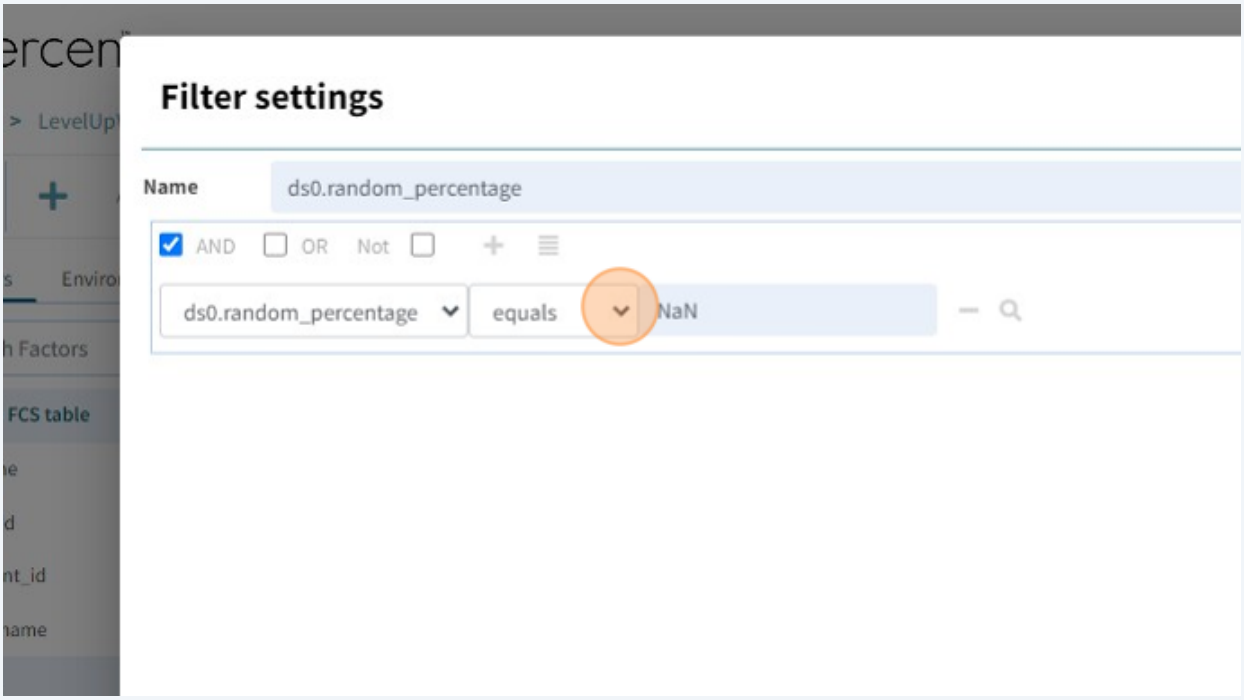
Place the mouse on the Column area, and press the CTRL key while using the wheely on your mouse to zoom in. This will squash the window horizontally.

This gives an overview of all the events and all the channels.

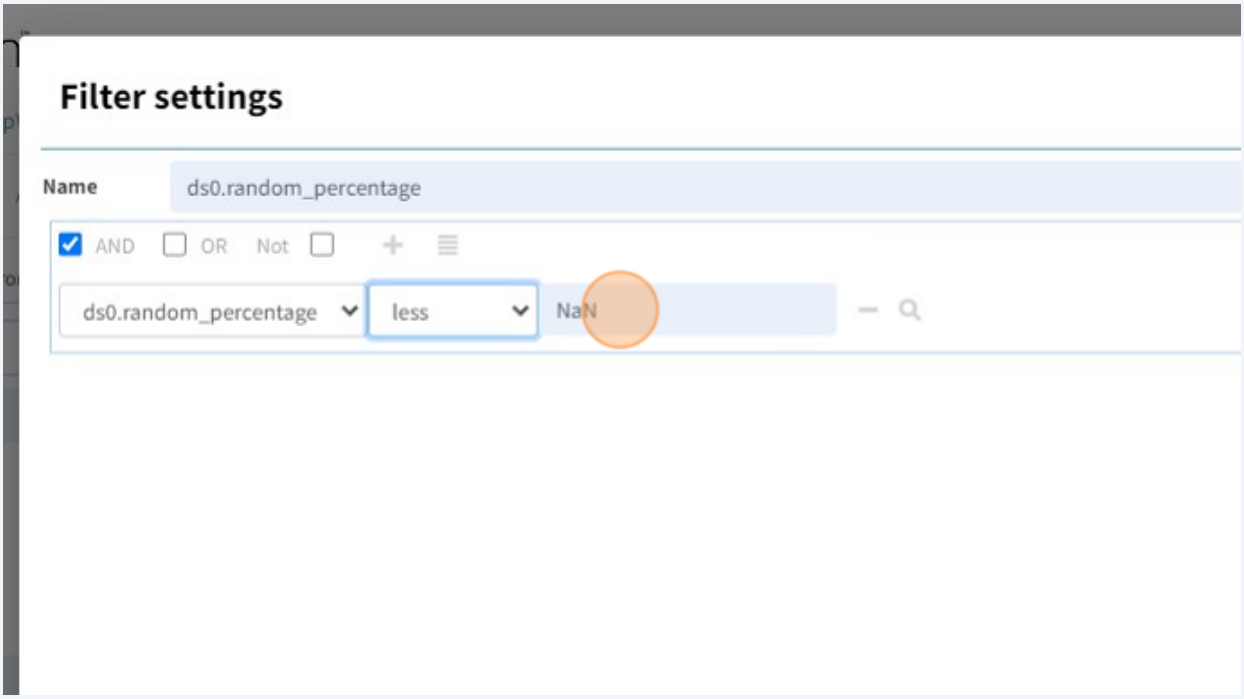
43 Drag **ds0.random_percentage** and drop onto Filters



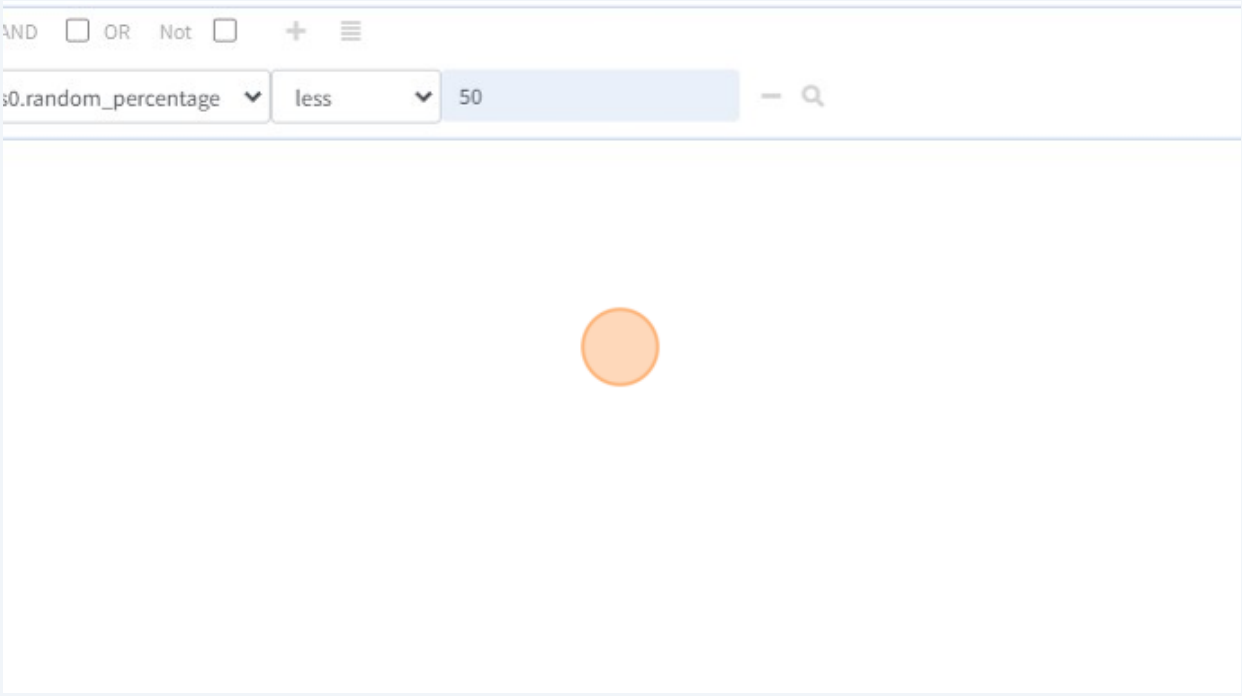
44 Click this dropdown.



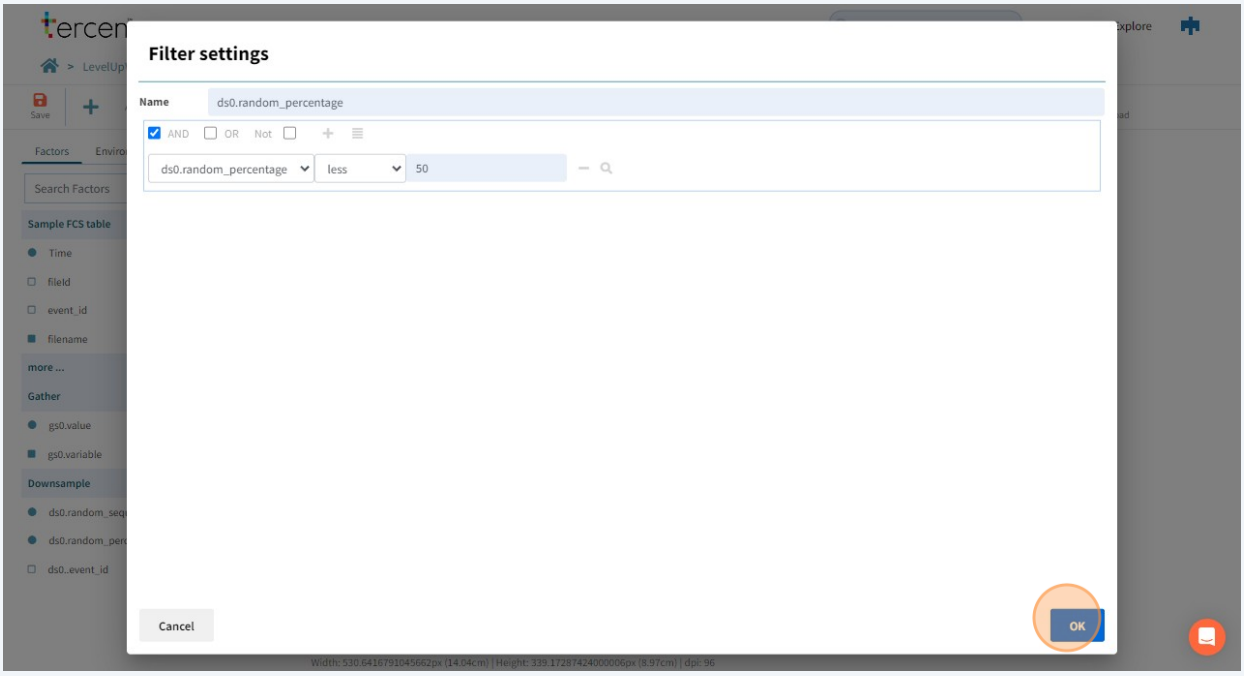
45 Select "less" and type "50"



46 Click here.



47 Click "OK"



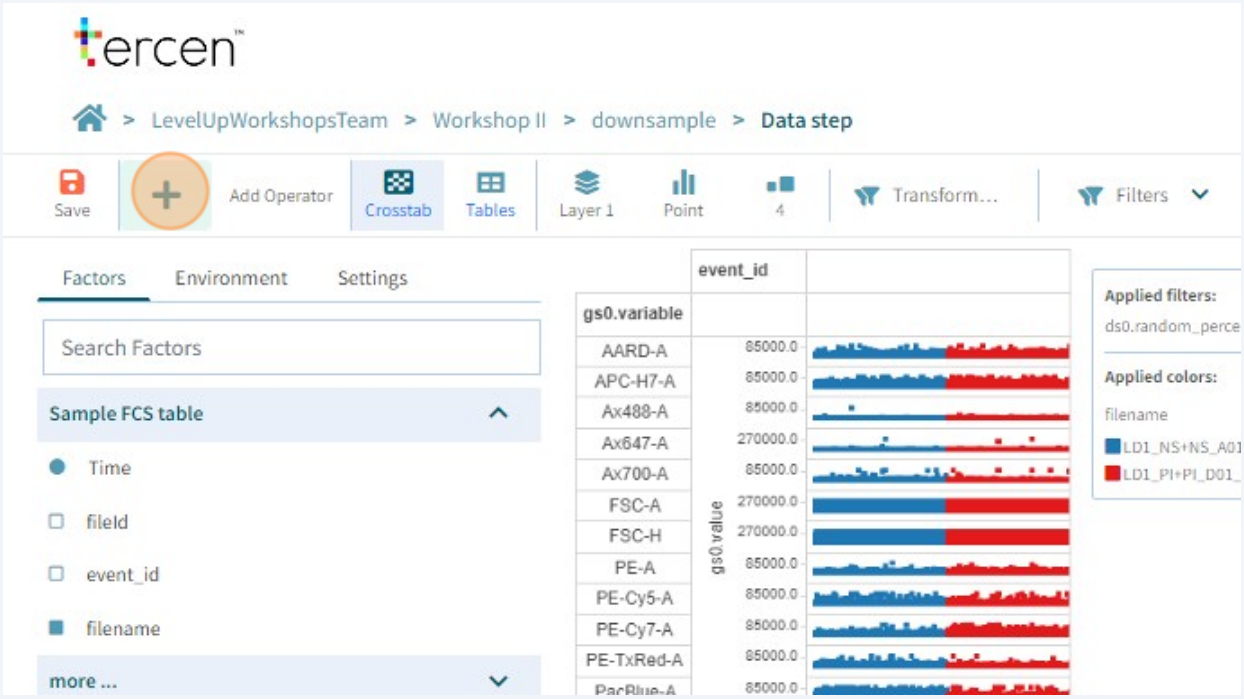
You should now have half the amount of data as before.

The filter setting of 50 reduces the number of events by 50% in each file. There will be an equal number of events as it will use the smallest file as the reference.

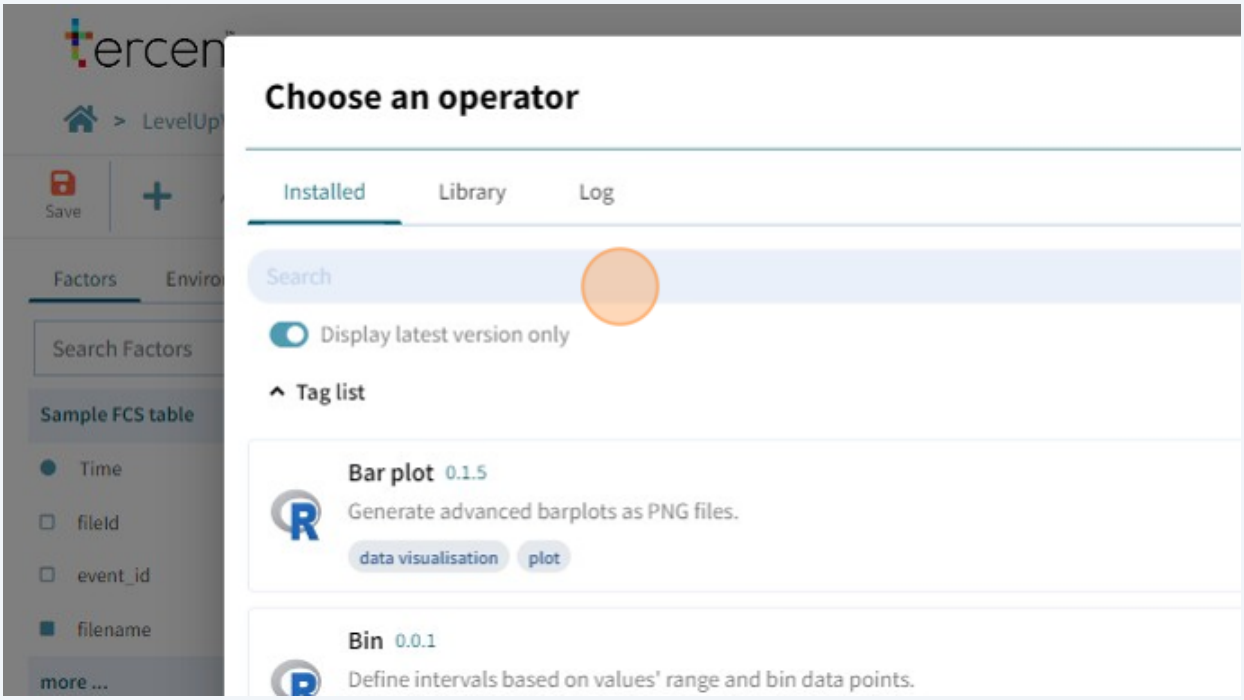
48 We will now export this reduced dataset as two FCS file.

This is done by using the "Export FCS" operator in Tercen.

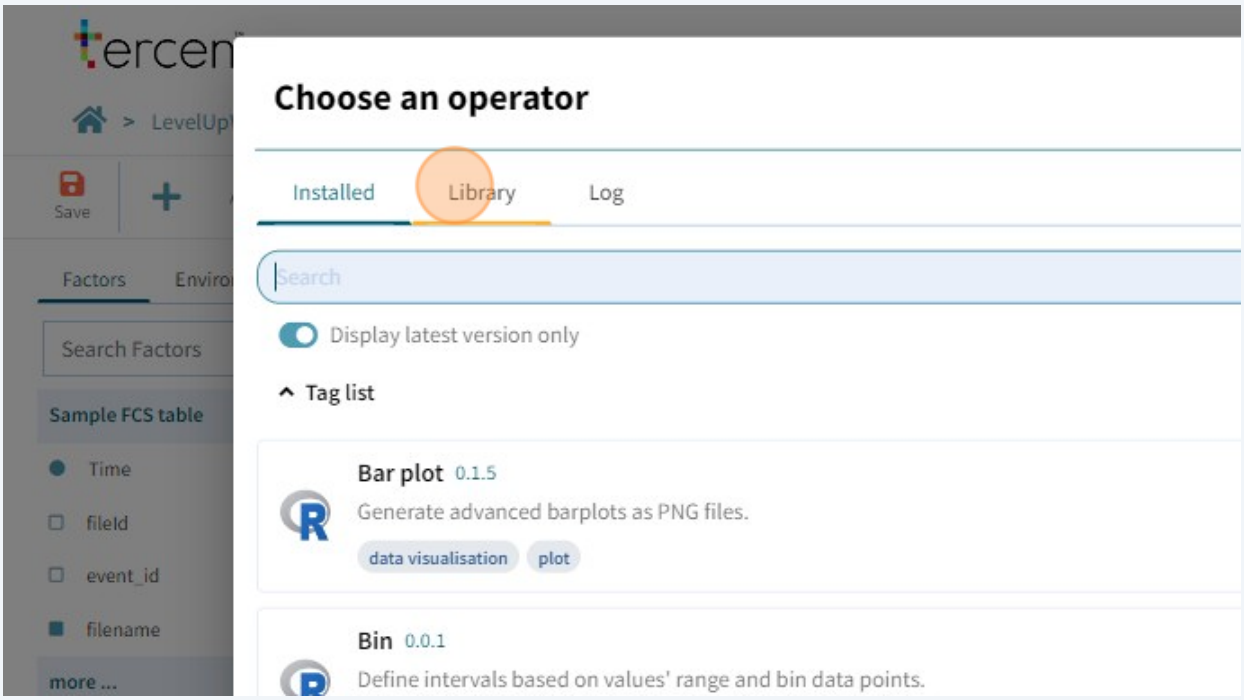
49 Click on the large "+" icon.



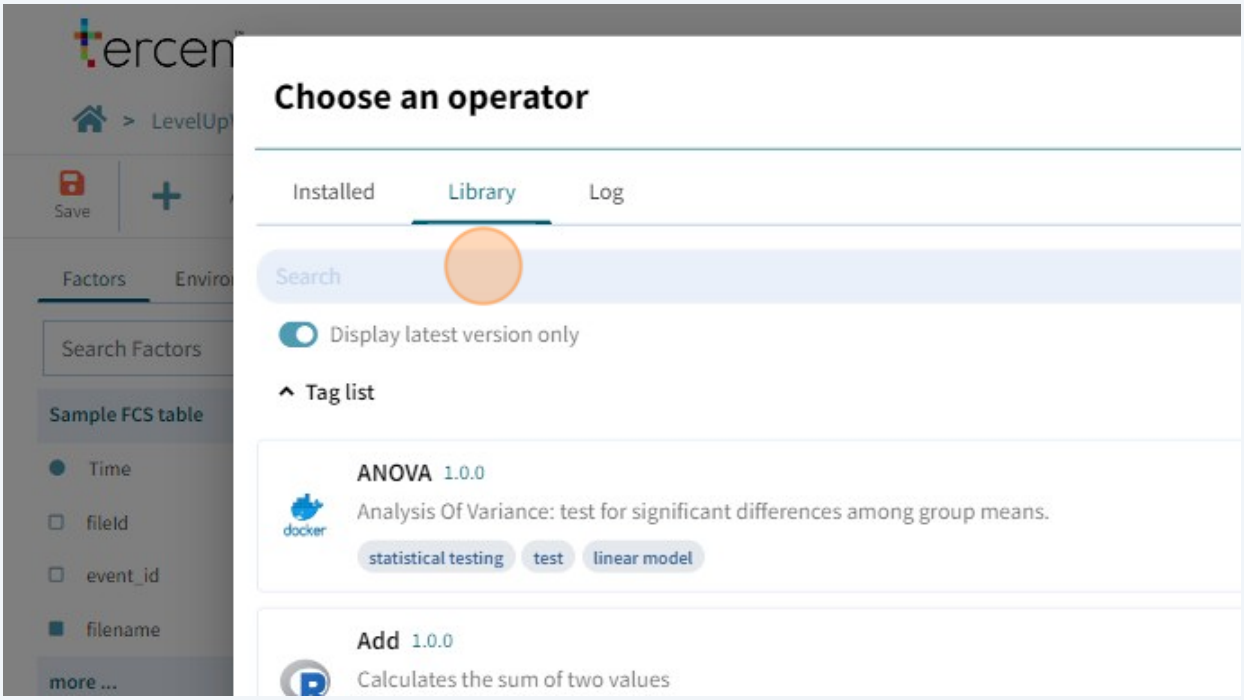
50 Click the "Search" field.



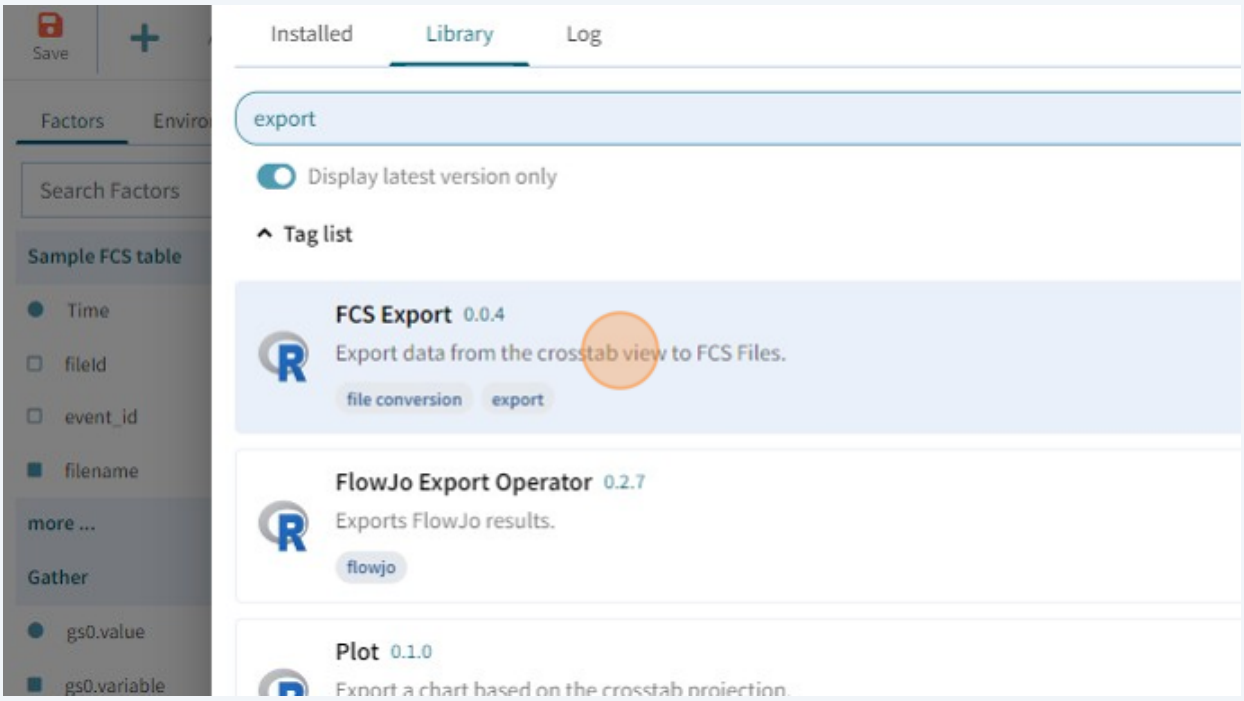
51 Click "Library"



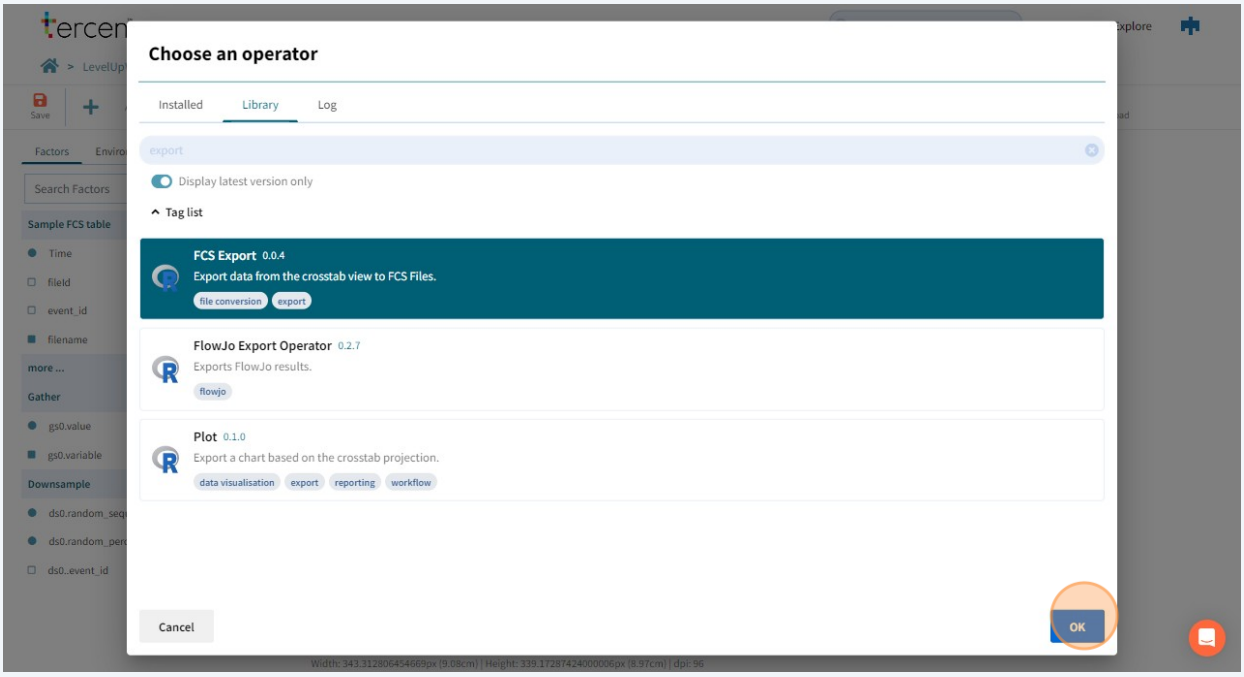
52 Click the "Search" field and type "export"



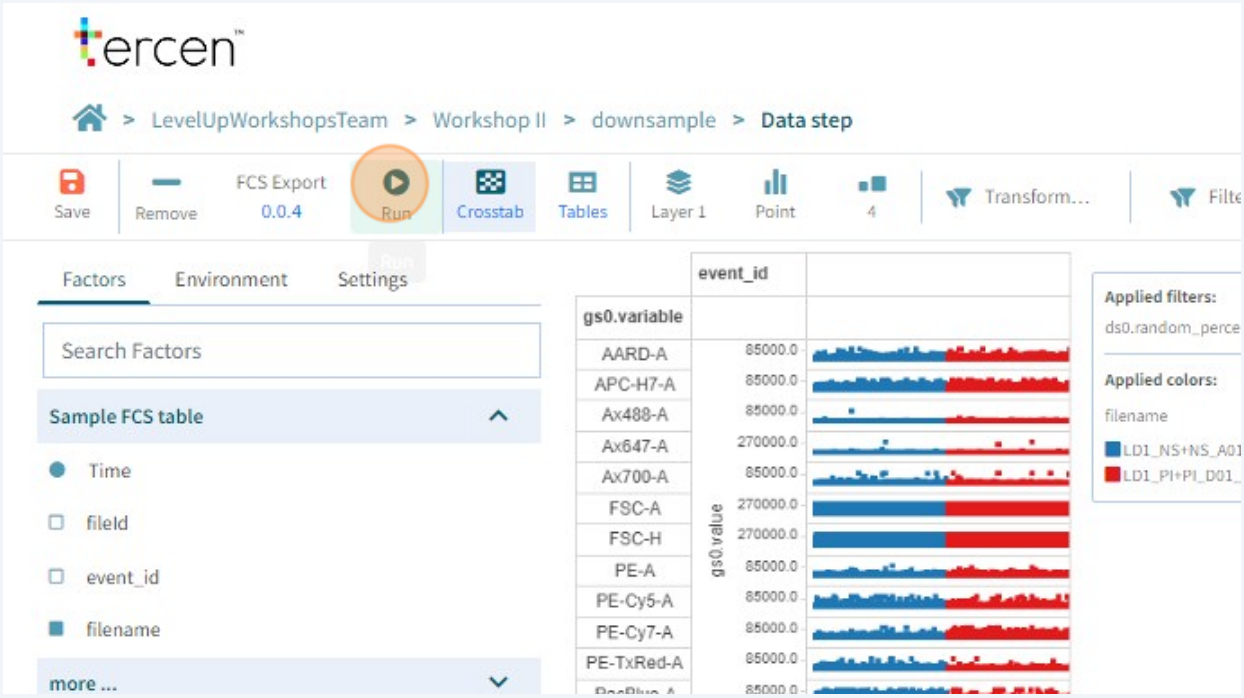
53 Select the "FCS Export" operator



54 Click "OK"

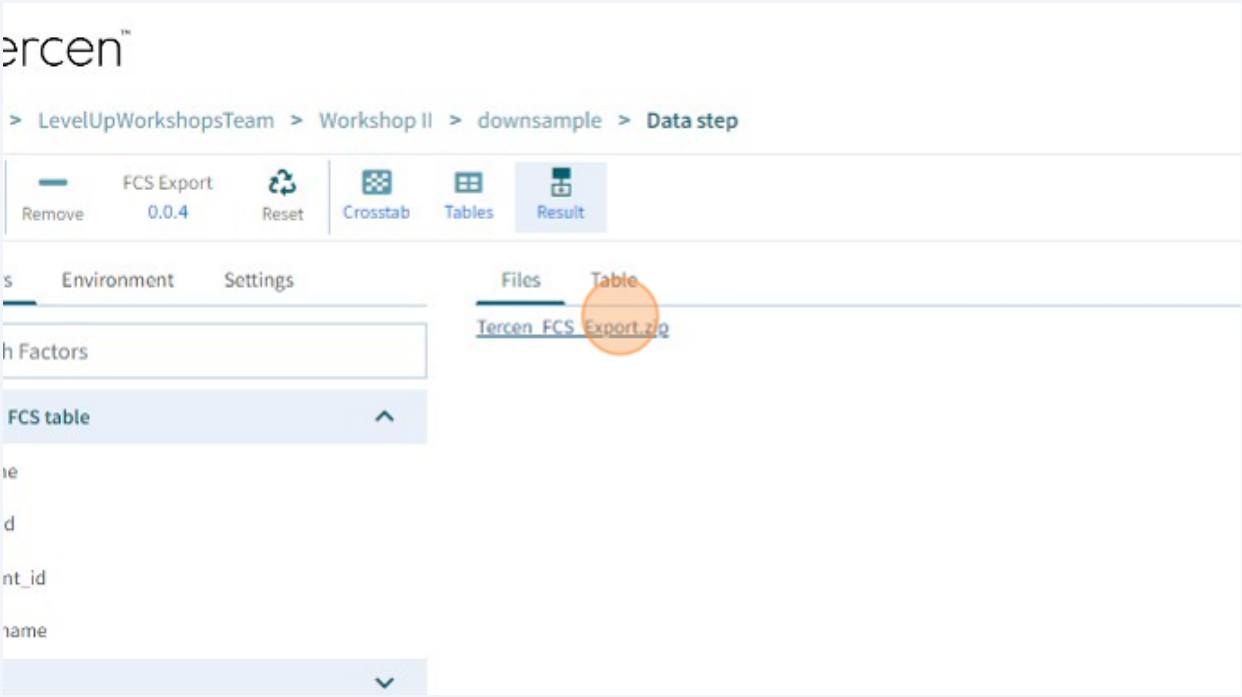


55 Click the "Run" icon to execute the operator and generate the two FCS files.

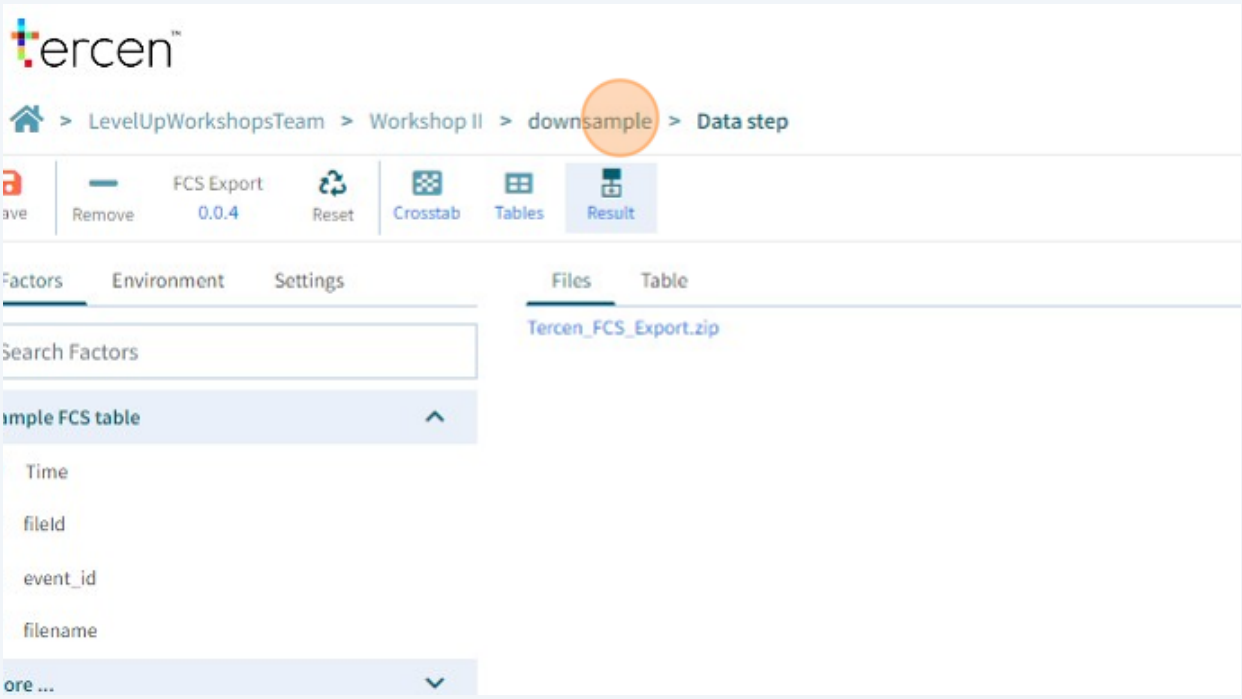


56 A new pane appear with the zip of the two FCS files.

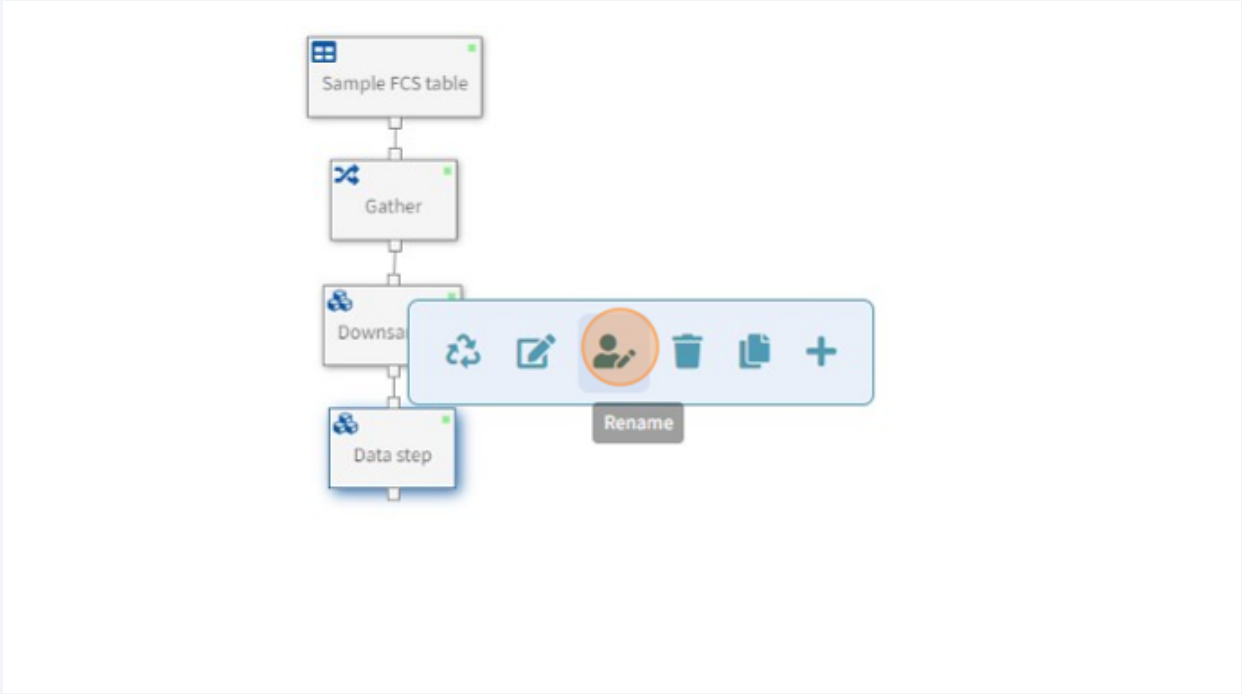
Click "Tercen_FCS_Export.zip" to download it to your drive.



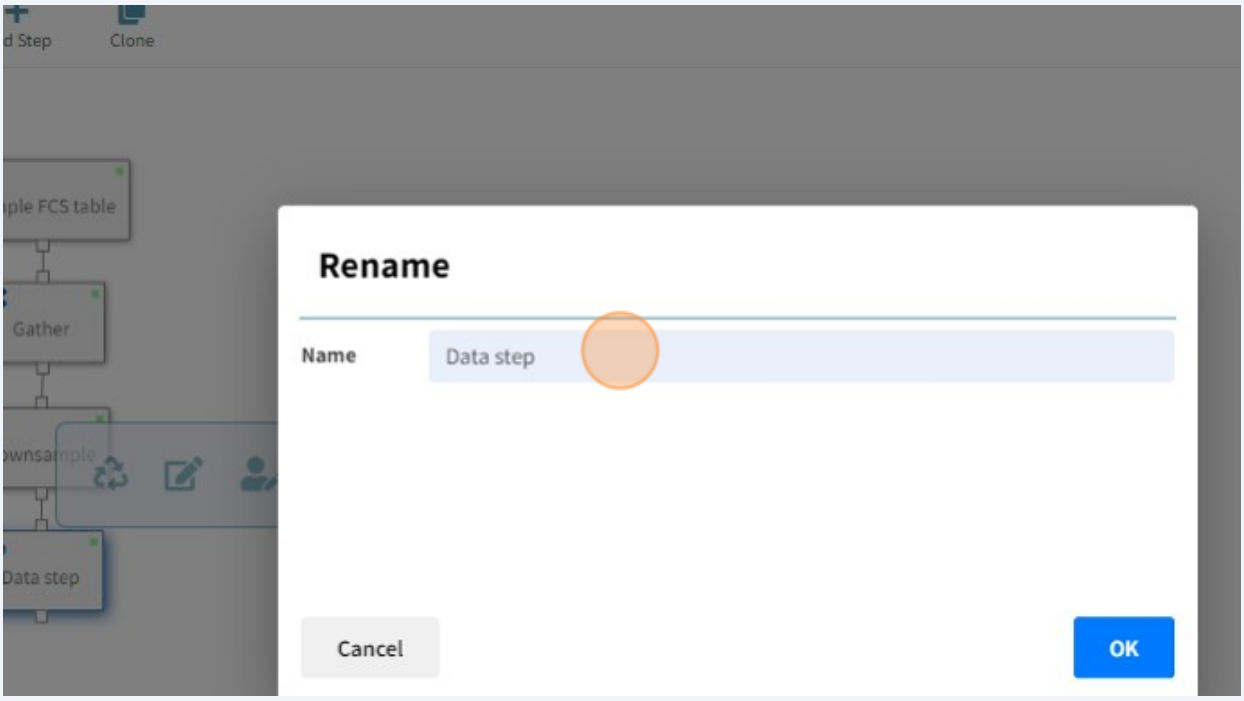
57 Navigate to the workflow level by clicking on "downsample" in the breadcrumb.



58 Click on the Rename icon

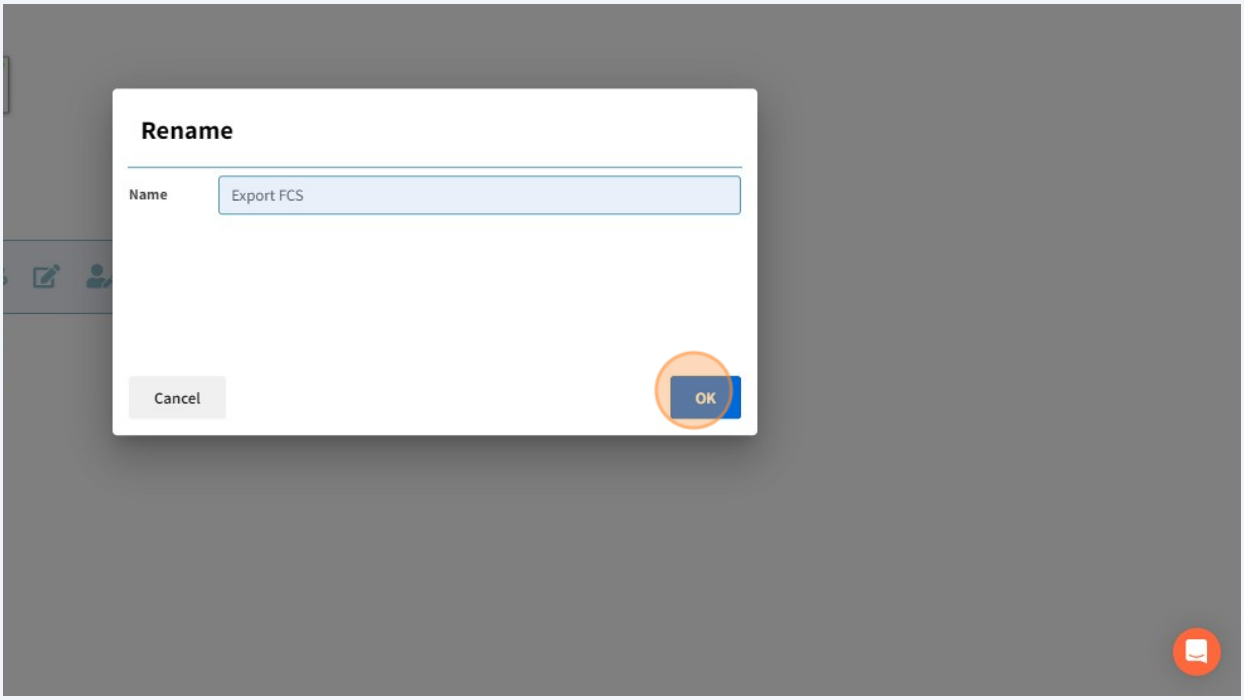


59 Type the text "Export FCS"

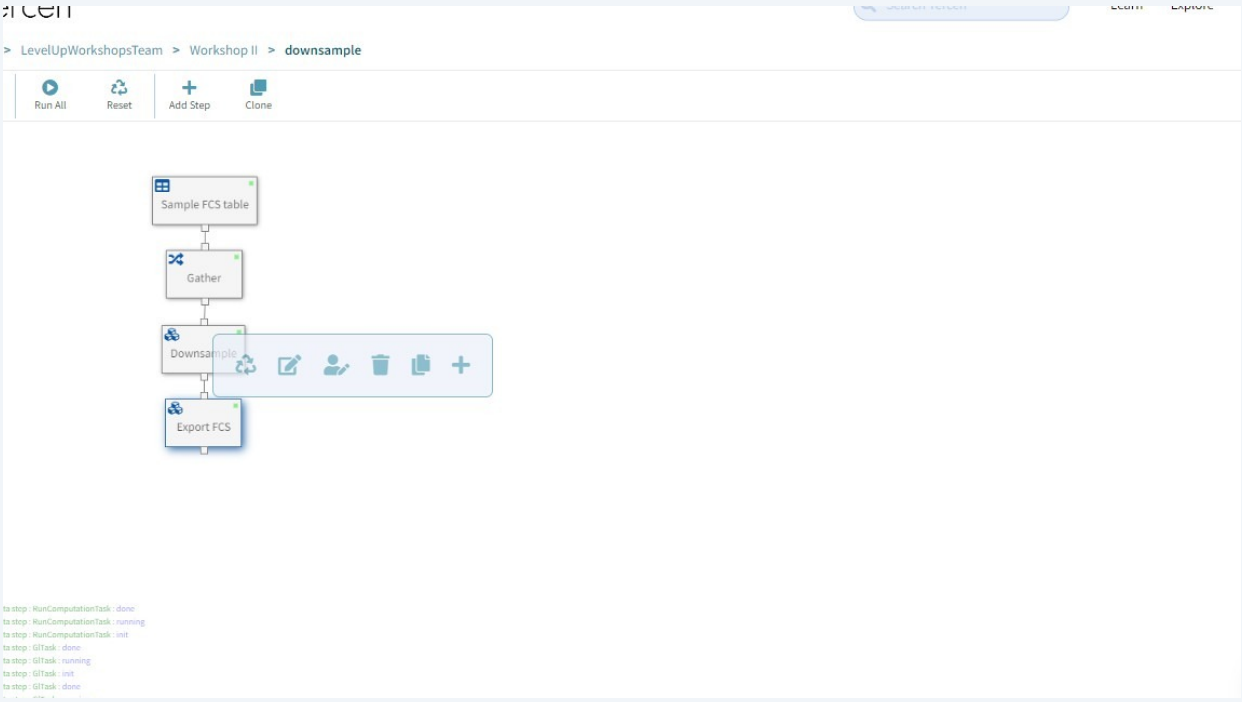


60 Type "xport FCS"

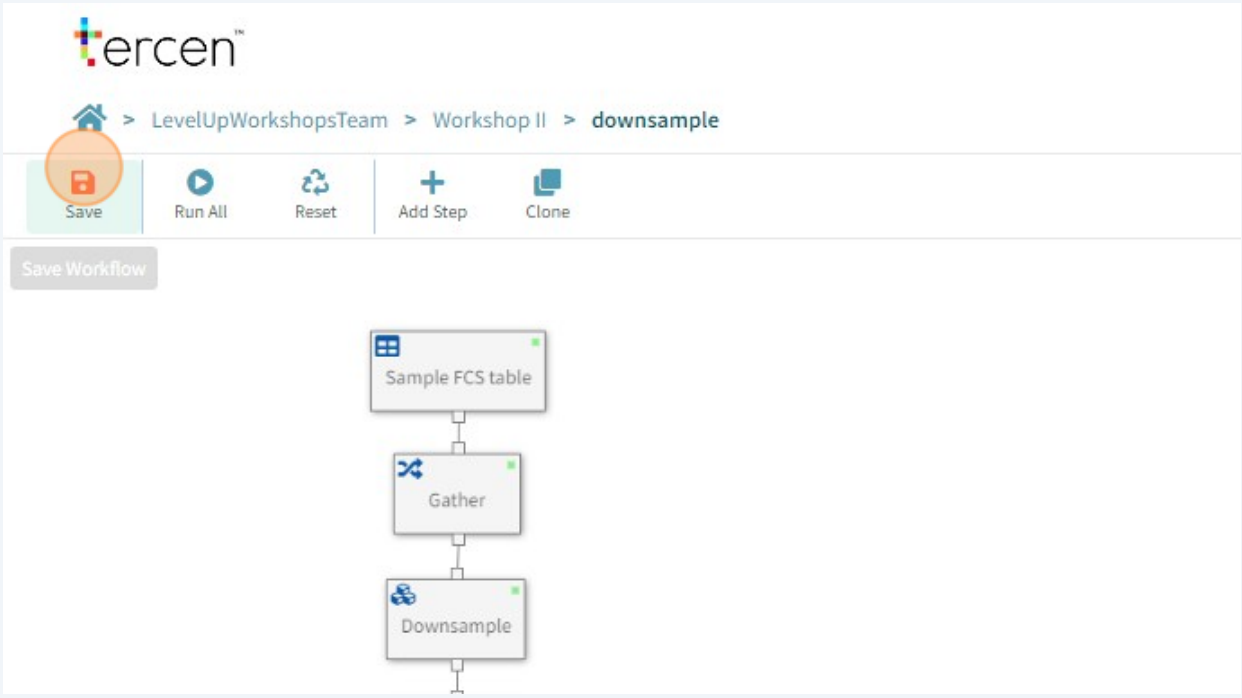
61 Click "OK"



62 Your workflow should look like this.



63 Click on the Save icon to save your workflow.



64 This guide has shown you how to downsample and export two FCS files.

Well done!