

A01 - Manual Cluster Annotation



- 1 Clone the Workshop V and add the initials of your name to it.

As we have done in the previous workshops.

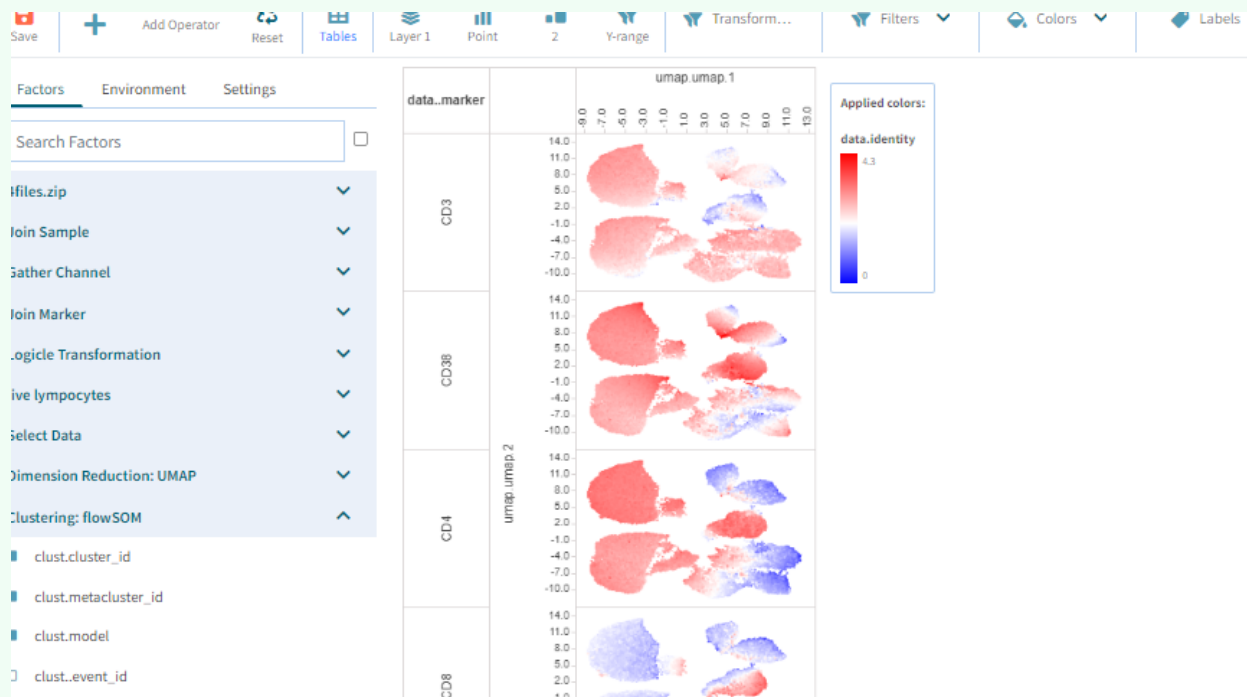
This will open the new cloned workshop.

- 2 Download to you local drive, the file called "cluster_annot_manual.csv", You will find in the "files" folder

- 3 Go to the download folder and edit the file with excel



Tip! Before filling out the population names of each cluster, find out what markers are expressed for which cluster by using the umap colored by marker expression in the workflow.



4

Fill in population names and save the file, below is an example, you can fill in what you think are the correct population names.

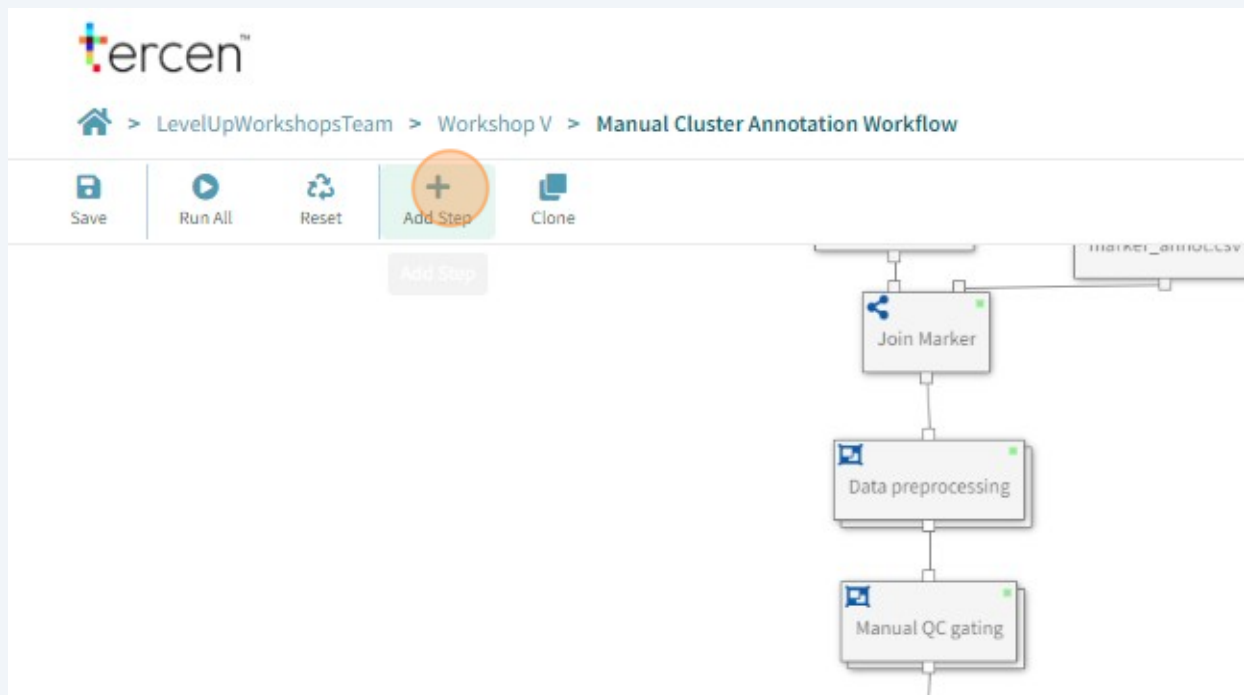
	A	B	
1	metacluster_id	population_name	
2	c1	CD4+	
3	c2	Unknown	
4	c3	CD8+	
5			

5

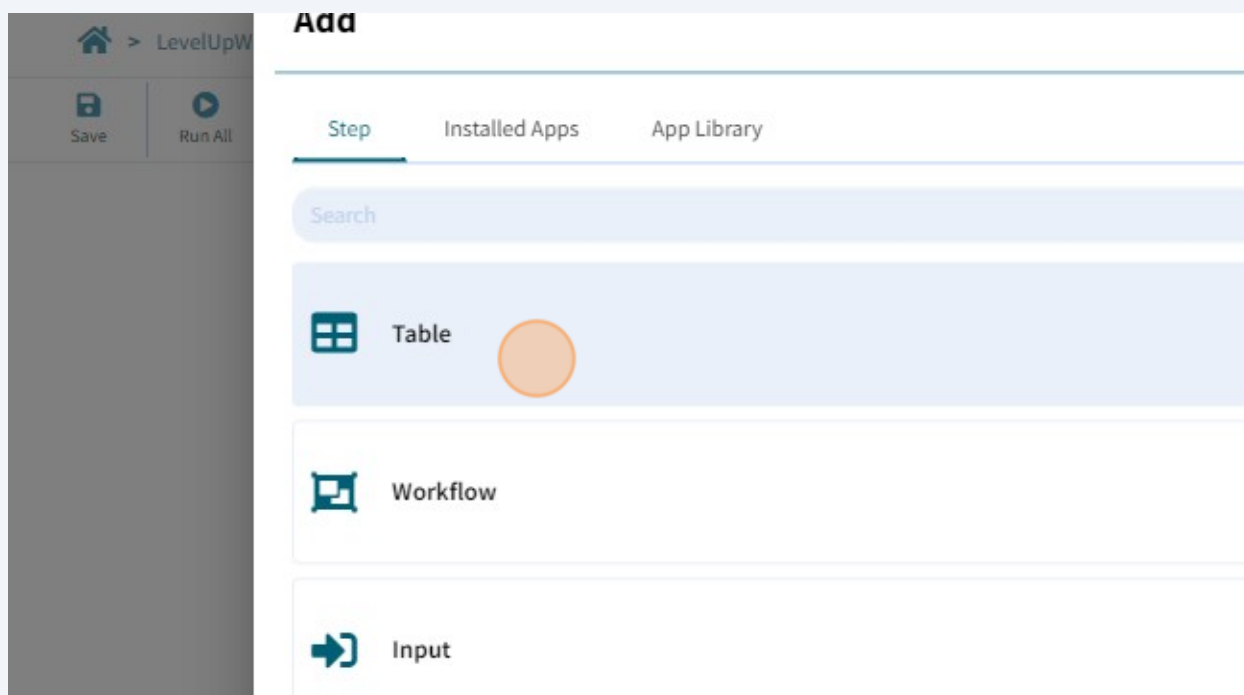
Upload the file (as a delimited file) to your personal workshop V project.

6 Open the workflow called "Manual Cluster Annotation Workflow"

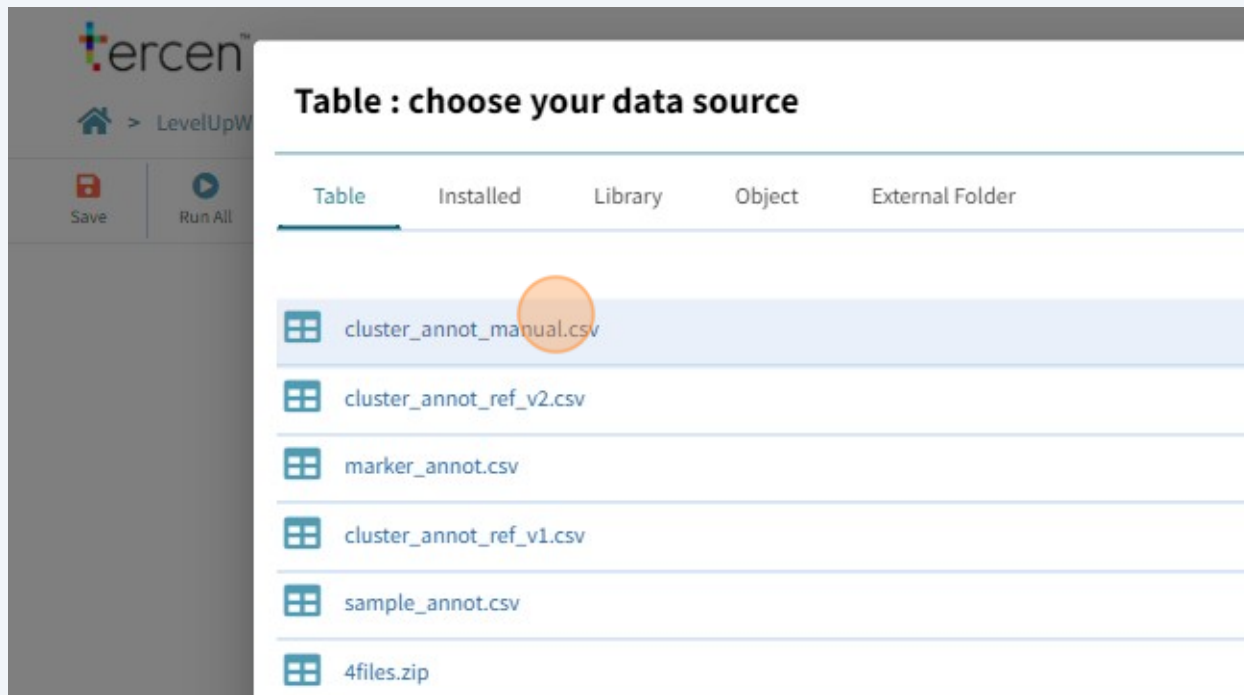
We will add a Table step in order to use the new uploaded file.



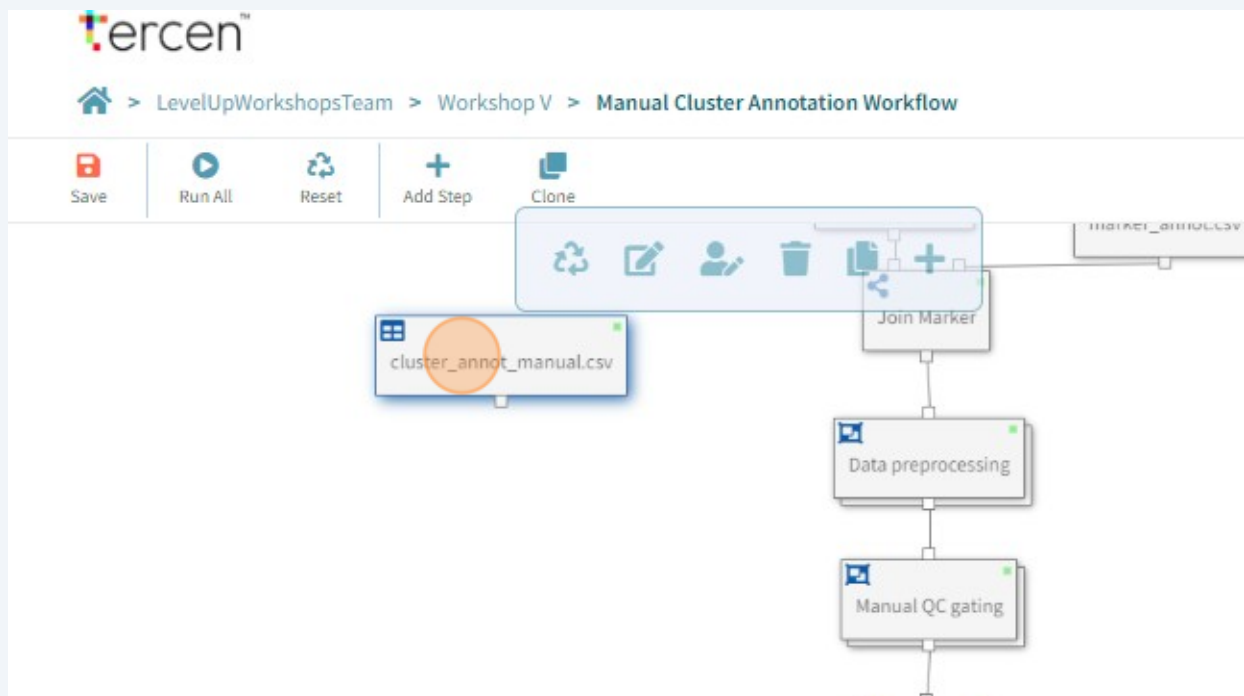
7 Click here.



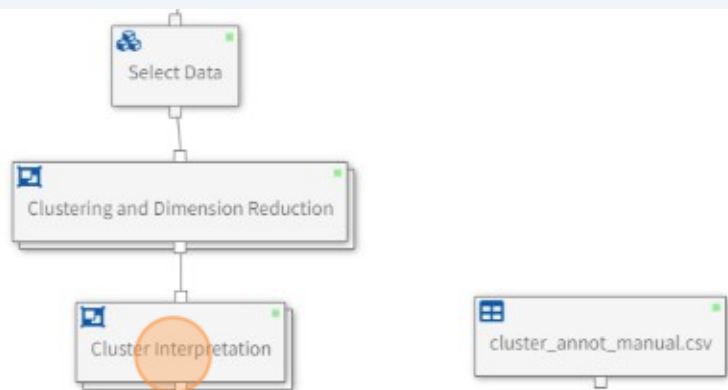
- 8 Click "cluster_annot_manual.csv"



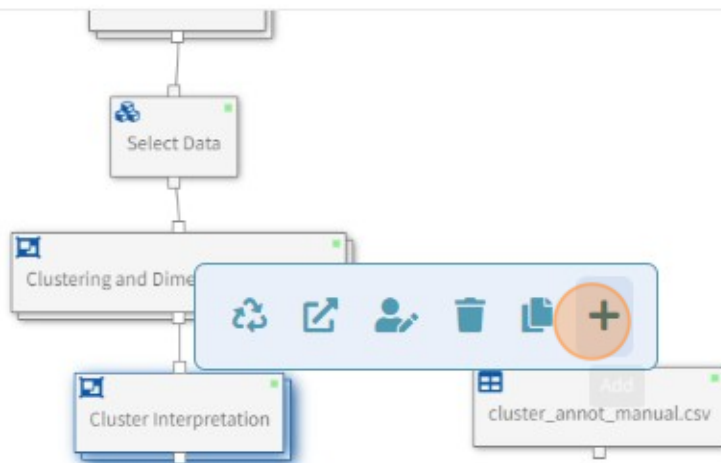
- 9 You should see the file in your workflow, move it to a more convenient location like the bottom right of the workflow.




10 Click here.



11 Click here.



12 Click "Join two data sets"



Data step data
Perform computation on user defined projection

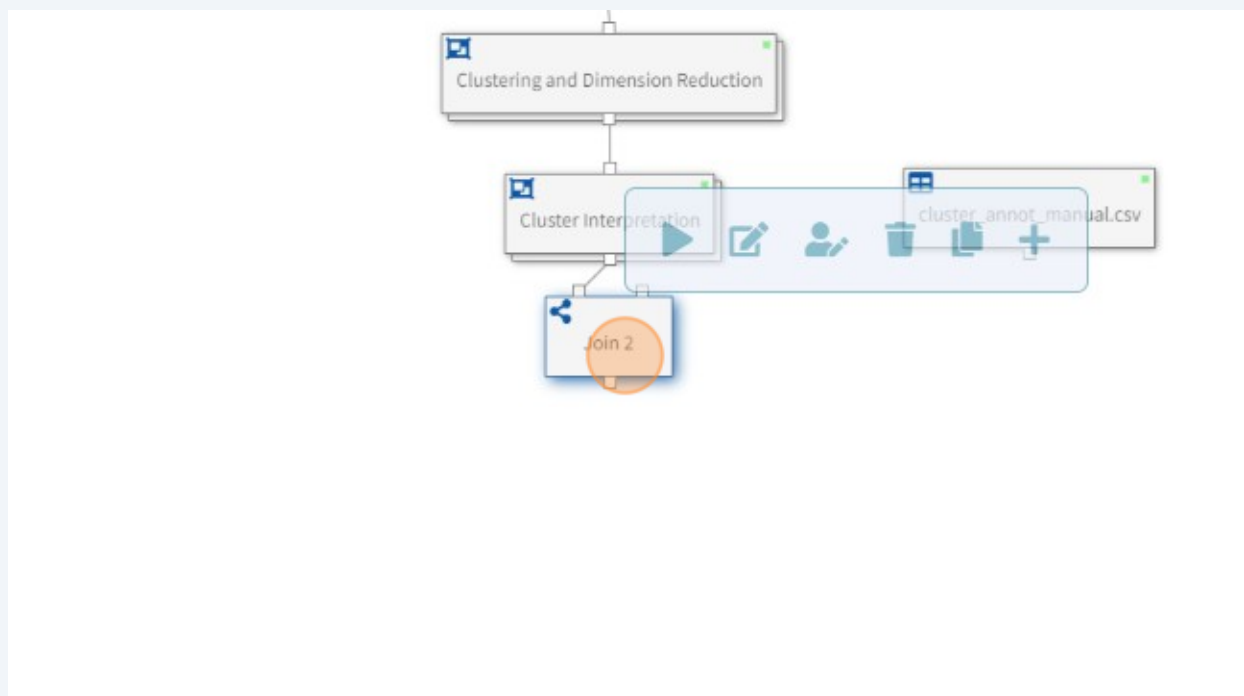
Multi data step data
Perform computation on user defined projection

Join leftTable
Join two data sets

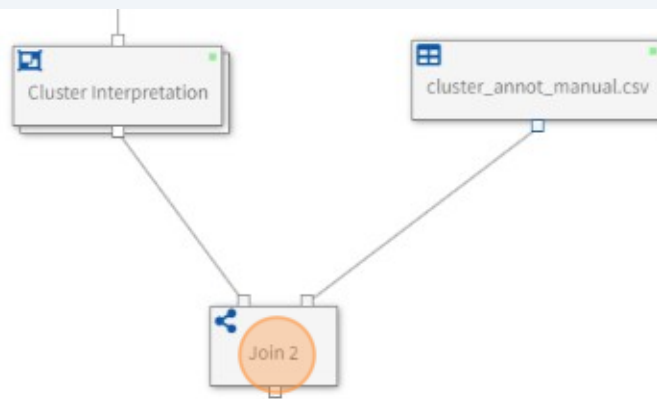
Join rightTable
Join two data sets

Gather table
Convert data from a wide format to a long format

13 Click here.



14 Connect the new table to the join step



15 Select the metacluster_id and the right

Right join factors

0

Right

4

metacluster_id	+
population_name	+
rowId	+
tableId	+

- 16 Click this text field on the left and type "meta"

ercen

Home > LevelUpWorkshopsTeam > Workshop V > Manual Cluster Annotation Workflow > Join 2

Namespace js0

Left

29

filename +

sample.filename +

sample.sample_id +

Left join factors

0

- 17 select "clust.metacluster_id"

Namespace js0

Left

meta

2

clust.metacluster_id +

enrich..metacluster_id +

Left join factors

0

- 18 Click this text field and enter the text "manual" and run the step.

Search Terce

ual Cluster Annotation Workflow > Join 2

Namespace js0

Left join factors

1

clust.metacluster_id

Right join factors

1

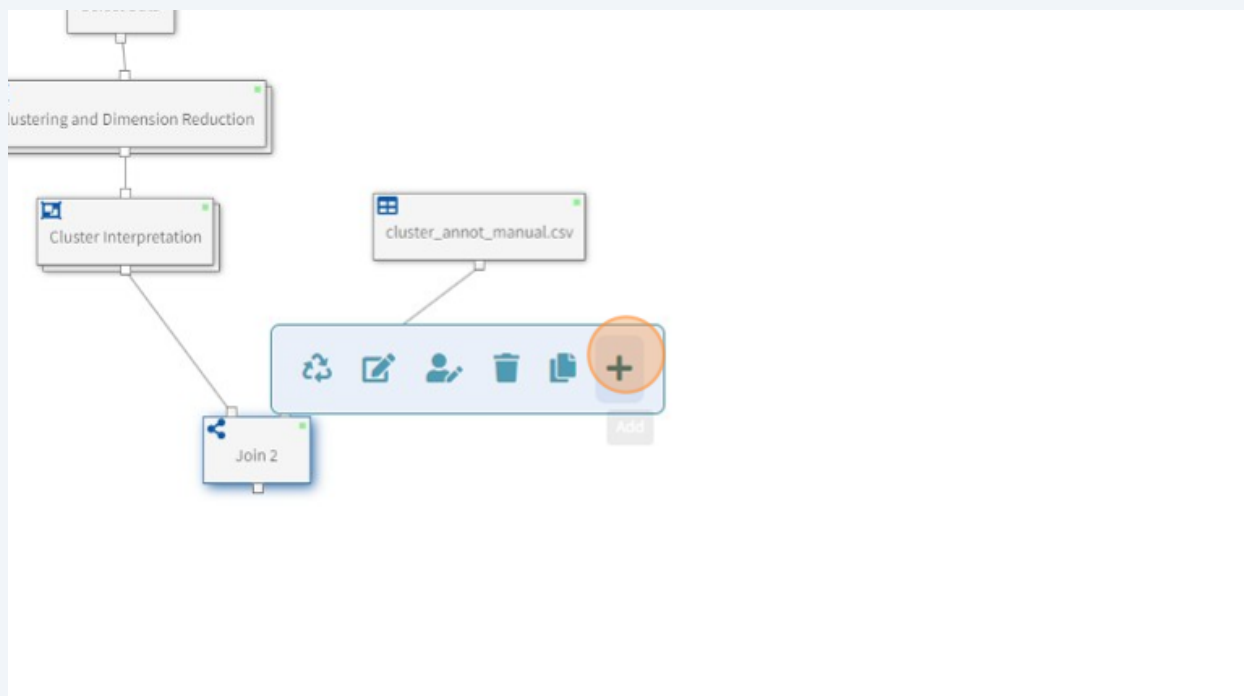
metacluster_id

population

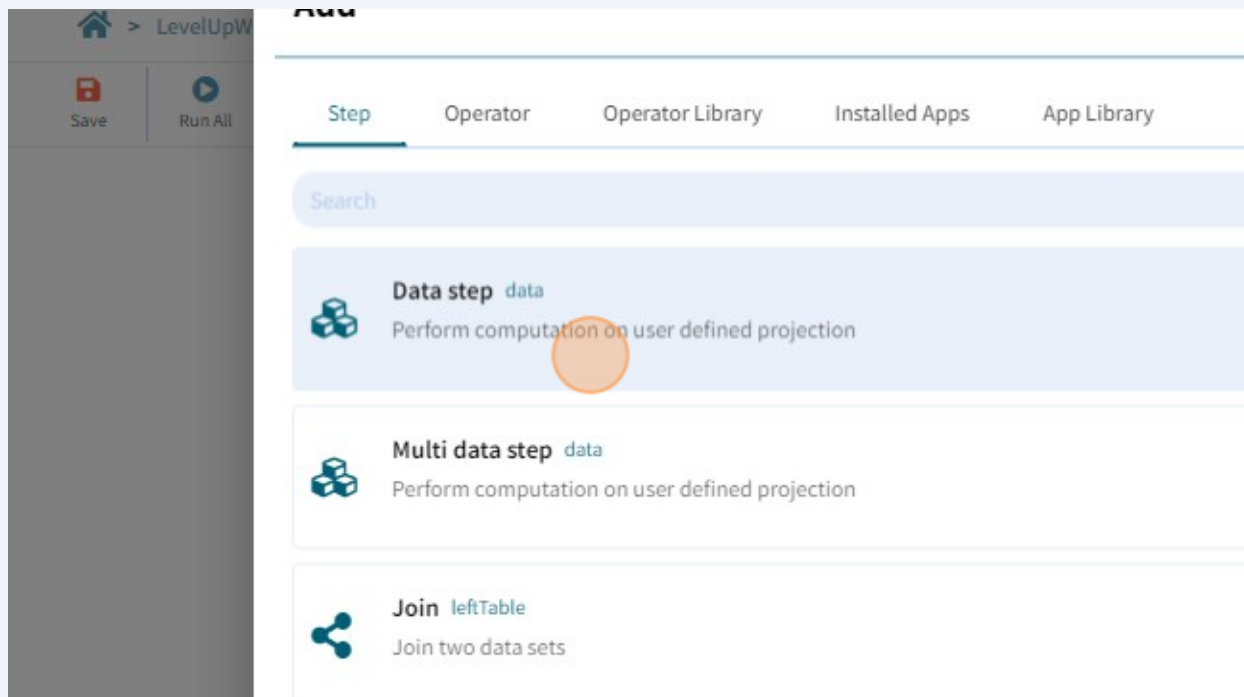
rowId

tableId

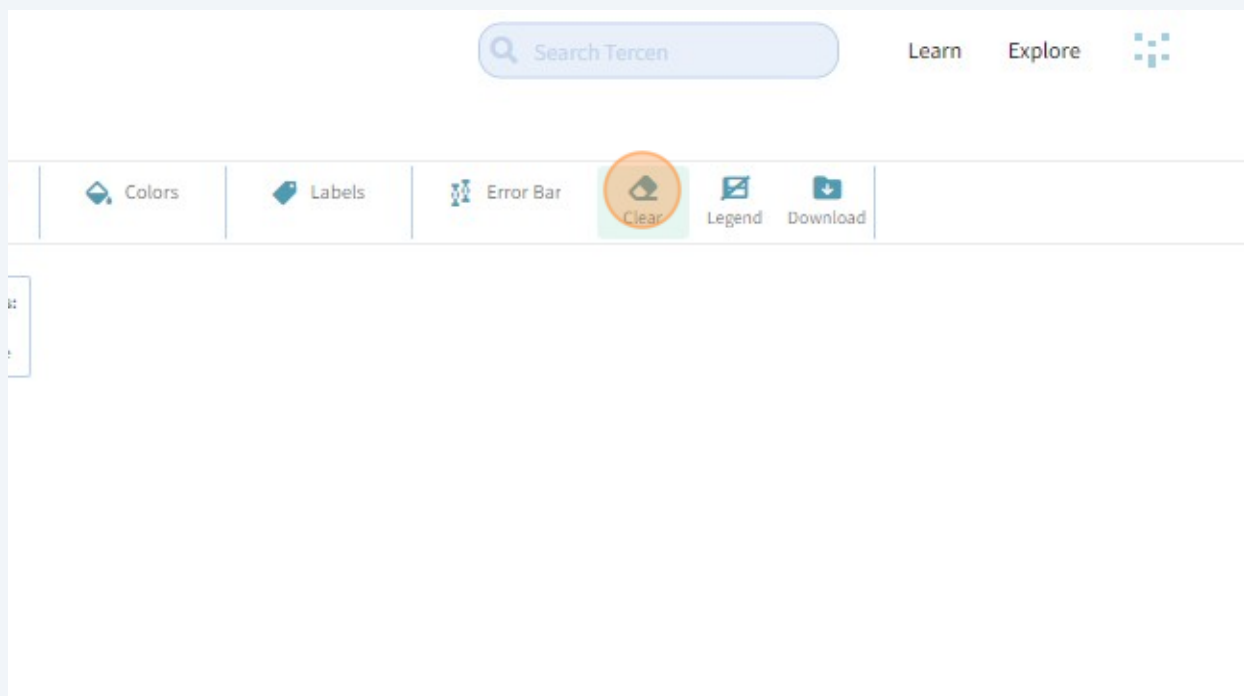
- 19 Click here.



20 Click here.



21 Click here to Clear the cross-tab view



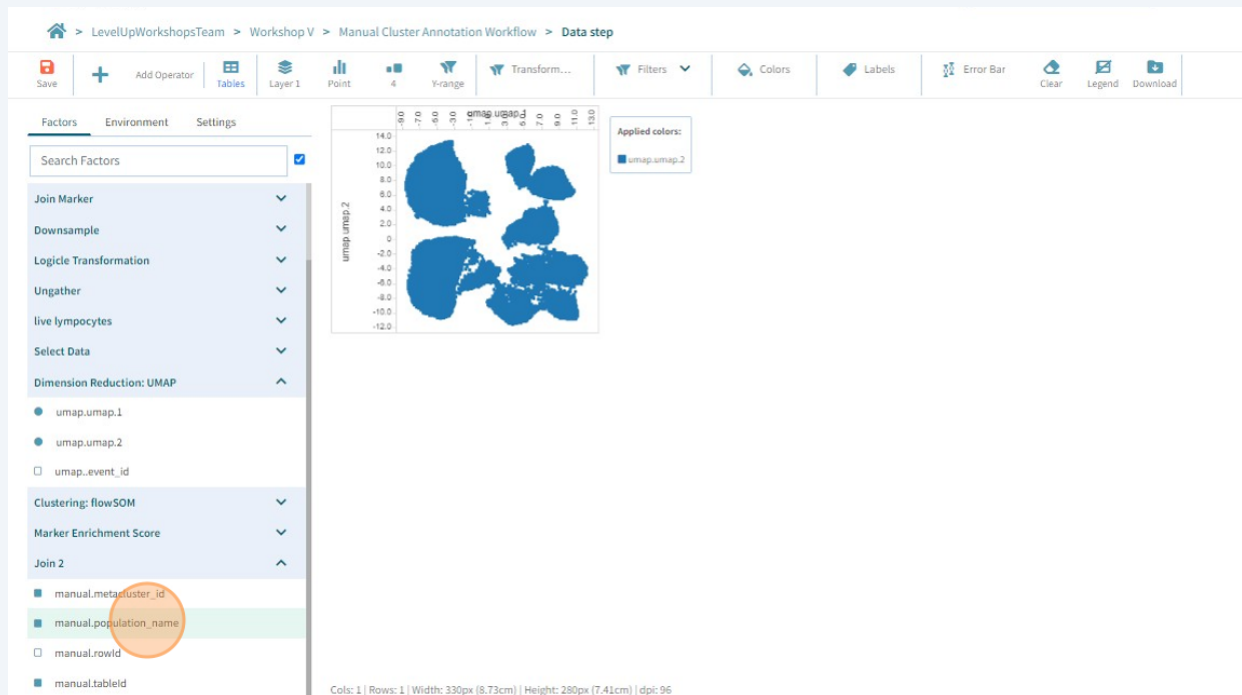
22 Click this checkbox, to show all the factors

The screenshot shows the terecen software interface. The breadcrumb navigation at the top reads: LevelUpWorkshopsTeam > Workshop V > Manual Cluster Annotation Workflow > Data step. The main toolbar includes icons for Save, Add Operator, Tables, Layer 1, Point, a count of 4, Transform..., and Filters. Below the toolbar, there are three tabs: Factors, Environment, and Settings. The 'Factors' tab is active, displaying a search bar labeled 'Search Factors' and a list of factors: 4files.zip, Join Sample, Gather Channel, Join Marker, Logicle Transformation, and live lvm0ocvtes. Each factor has a downward arrow to its right. A small orange circle highlights a checkbox icon next to the search bar, with a tooltip labeled 'Show All' appearing over it. To the right of the factors list is a large empty rectangular area.

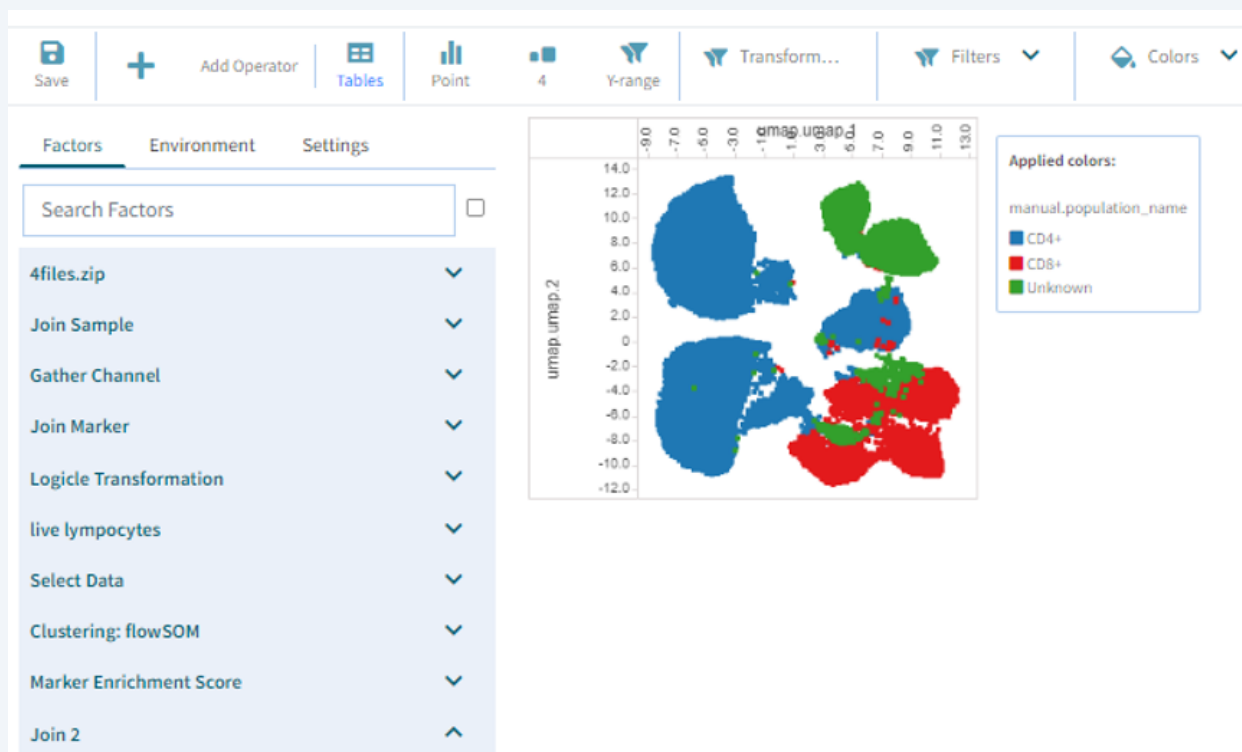
23 Create a umap1 vs. umap2 plot

The screenshot shows the terecen software interface with the 'Factors' tab active. The list of factors includes: Downsample, Logicle Transformation, Ungather, live lymphocytes, Select Data, Dimension Reduction: UMAP, umap.umap.1, umap.umap.2, umap..event_id, Clustering: flowSOM, Marker Enrichment Score, Join 2, and manual.metacluster_id. The 'umap.umap.1' factor is highlighted with a green background and a blue dot, and an orange circle is drawn around it. The 'umap.umap.2' factor also has a blue dot. The 'umap..event_id' factor has an unchecked checkbox. The 'manual.metacluster_id' factor has a blue square icon. At the bottom right of the interface, the following text is displayed: Cols: 1 | Rows: 1 | Width: 330px (8.73cm) | Height: 280px (7.41cm) | dpi: 96.

24 Color by "manual.population_name"



25 Congrats you have manually annotated clusters





Discuss!

Why do you think there are many lobes/sub clusters within each population group CD4+

Look at the enrichment scores of each cluster, do you notice anything about c1?