PROMO – Profiler of Multi-Omics Data

Integrative Multi-omic Analysis Tutorial

Introduction

PROMO (Profiler of Multi-Omics data) is an interactive tool, designed to analyze large versatile datasets together with their clinical labels. This tutorial demonstrates PROMO's more advanced features for integrative analysis of multi-omic datasets (multiple datasets describing the same set of patients but generated using different omic technologies).

Use this tutorial if you wish to:

- Assemble a multi-omic collection by importing several different omic matrices using PROMO.
- Edit your multi-omic dataset collection by adding/removing datasets or by editing a specific dataset within the dataset collection.
- Intersect the collection datasets in order to keep only samples appearing in all collection datasets.
- Merge the collection datasets to yield a single feature-concatenated matrix.
- **Inter-omic feature correlations** - Identify correlations of features in two different omics.
- **Multi-omic sample clustering** - Cluster the patients using any subset of the datasets in your collection.

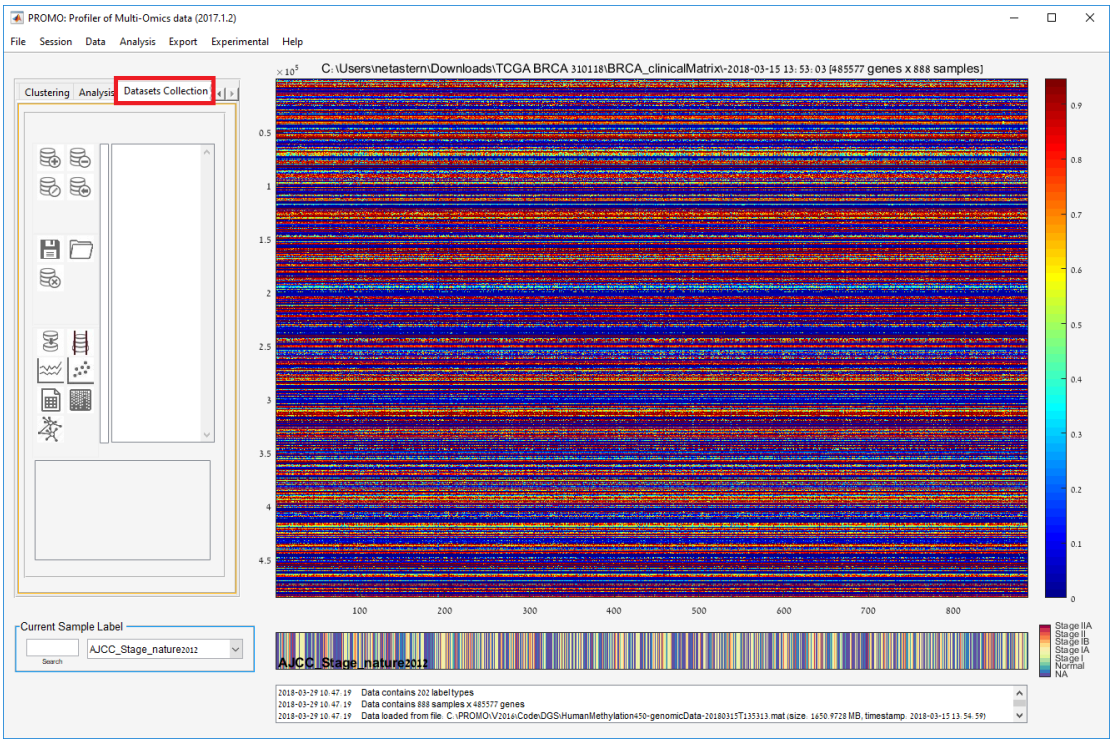
Multi-Omic Dataset Collection Management

Multi-omic dataset collection is a set of datasets for the same group of paitents, each possibly generated by a different omic technology. Datasets in a collection can be used in PROMO for various integrative multi-omic analyses.

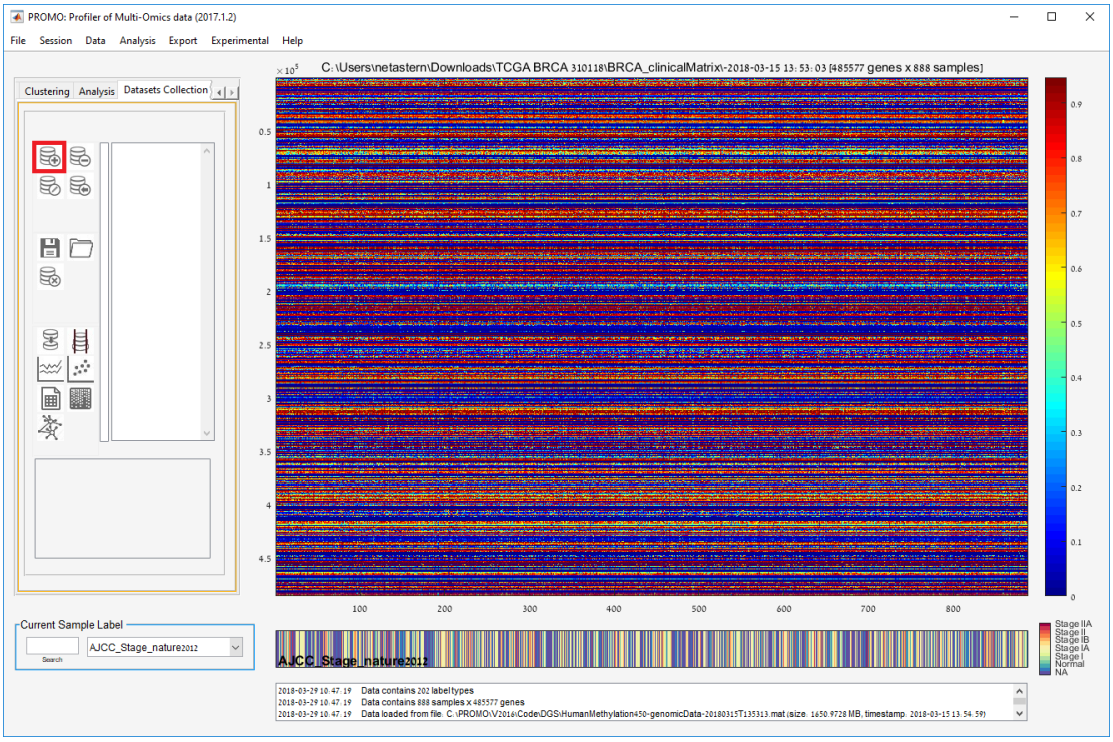
Follow these instructions to assemble a dataset collection:

1. Import the first dataset as described in section 'Step 1 - Importing data' in PROMO's basic tutorial: http://acgt.cs.tau.ac.il/promo/tutorial/PROMO_Example_Tutorial.pdf
   The dataset you imported is now your active dataset.

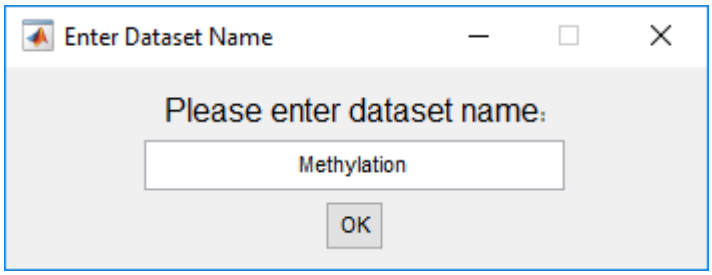
2. On the left panel of the main form, switch to the tab 'Datasets Collection':



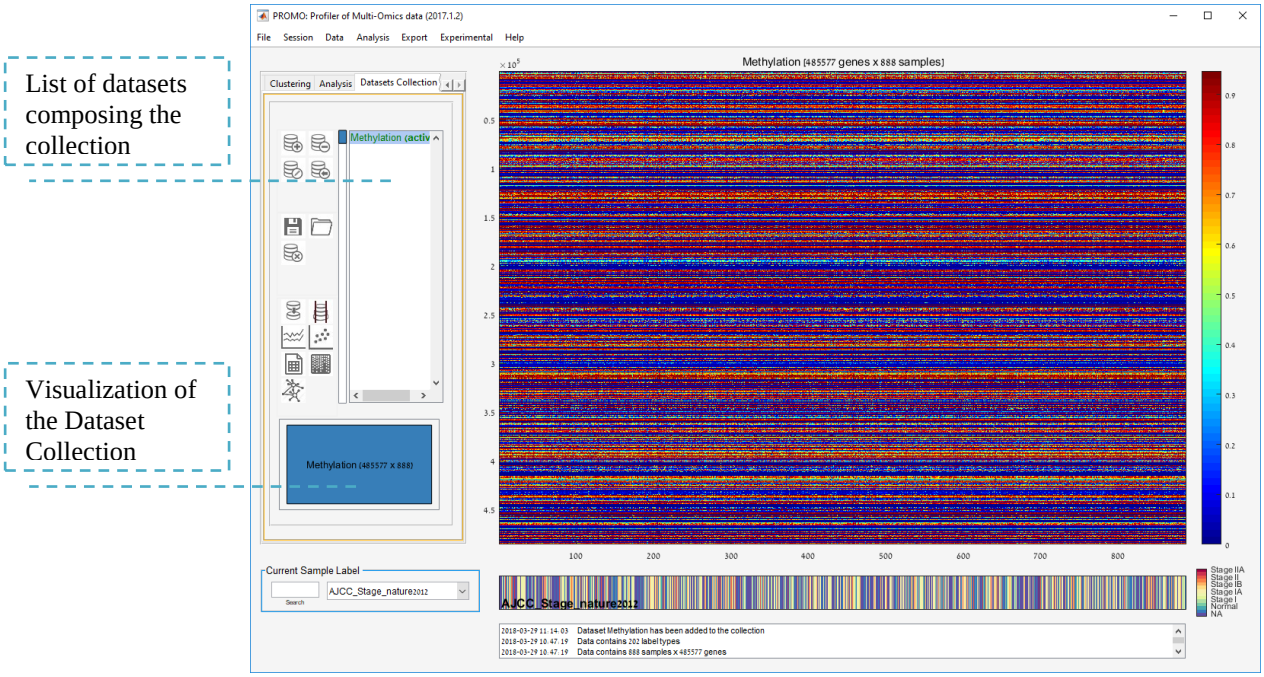
3. Add the active dataset to your dataset collection by pressing the 'Add Dataset' button: 



4. Enter a name for the dataset:

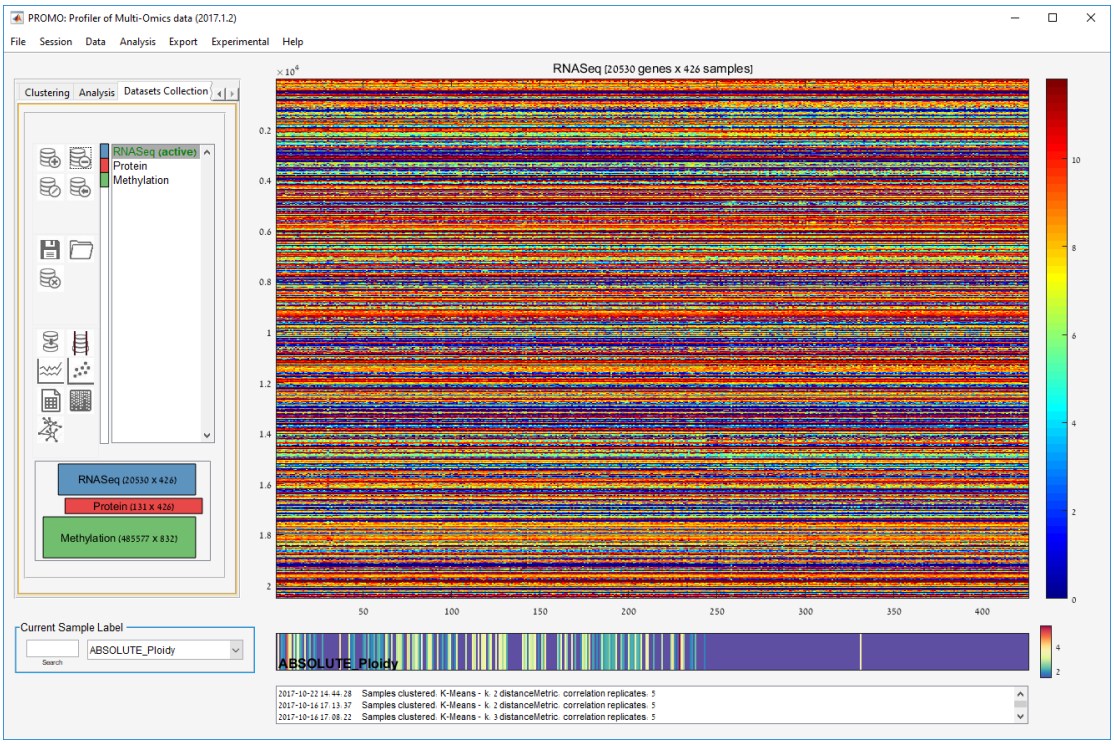


5. Press 'OK'. The active dataset will now be added to the dataset collection. You can see the names of the datasets composing the dataset collection on the list box under the 'Dataset Collection' tab on the left side of the screen.



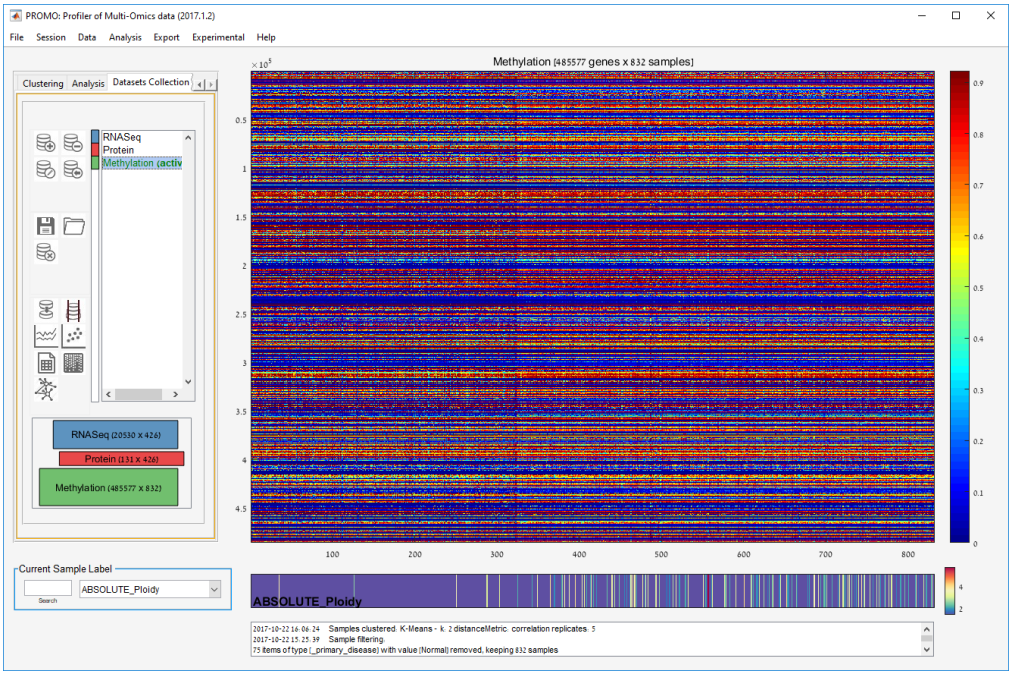
6. Repeat steps 1-5 for every dataset you wish to add to your collection.

The following screenshot shows a dataset collection containing three different omics for TCGA lung cancer cohort: RNA-Seq, Protein, and Methylation. Notice that the name of the currently active dataset (RNA-Seq) is colored in green.

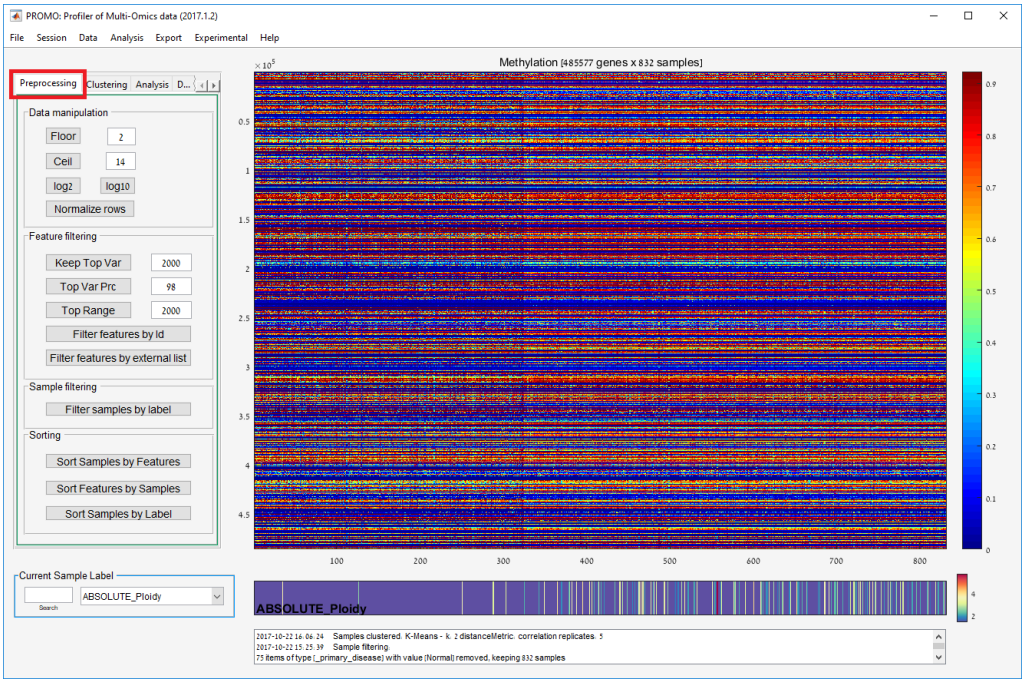


We will now edit and update one of the datasets within our dataset collection.

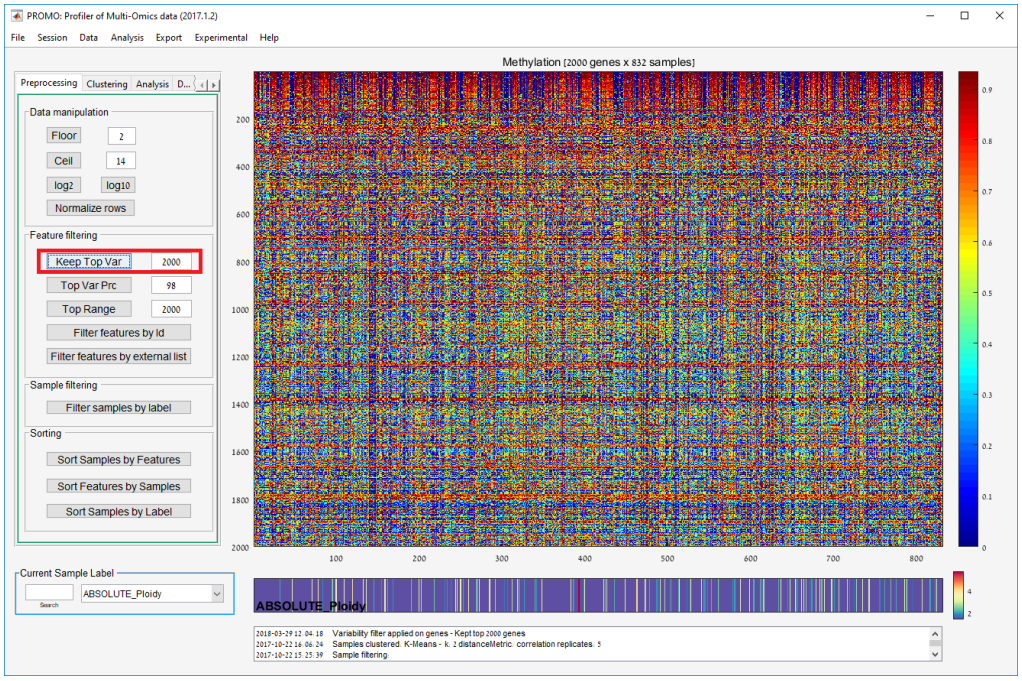
7. On the left panel, double-click the dataset that you wish to edit in order to make it the active dataset. In this example, we will edit the Methylation dataset. Notice that the dataset name on the left is now colored in green. Methylation is now the **active** dataset and we can make changes to this dataset using the various single-omic methods provided by PROMO.



8. On the left panel of the main form, switch to tab 'Preprocessing':

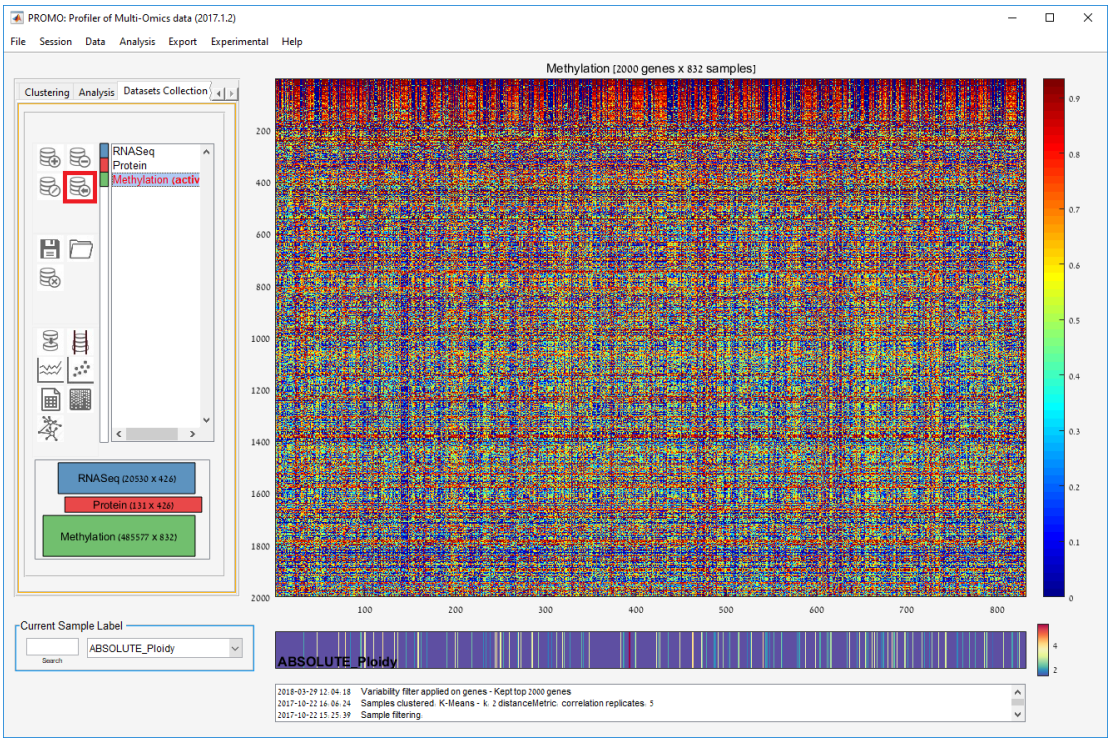


9. Apply row filtering for keeping the features with the highest variance: On the adjacent text box to the "Keep Top Var" Button, Enter the number of features to keep. In our example, we kept the top 2000 most variable features:



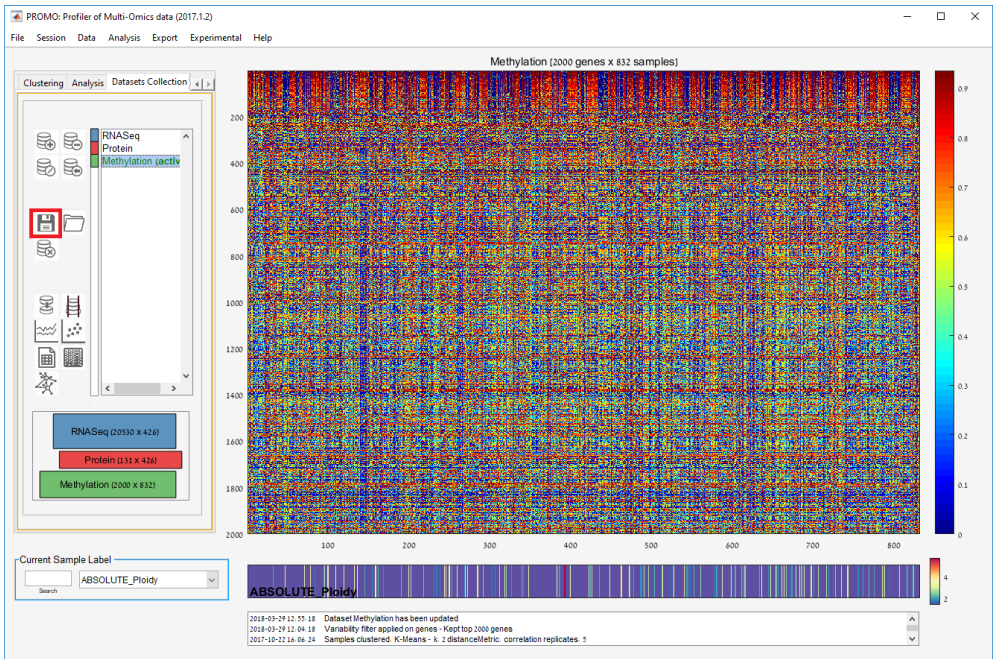
10. Switch back to the datasets collection tab. Notice that the Methylation dataset name is now colored in red, indicating the active dataset contains pending changes that were not saved to the collection. You can perform similar data filtering steps on the other datasets in the collection.

11. Update your dataset collection using the update button: 

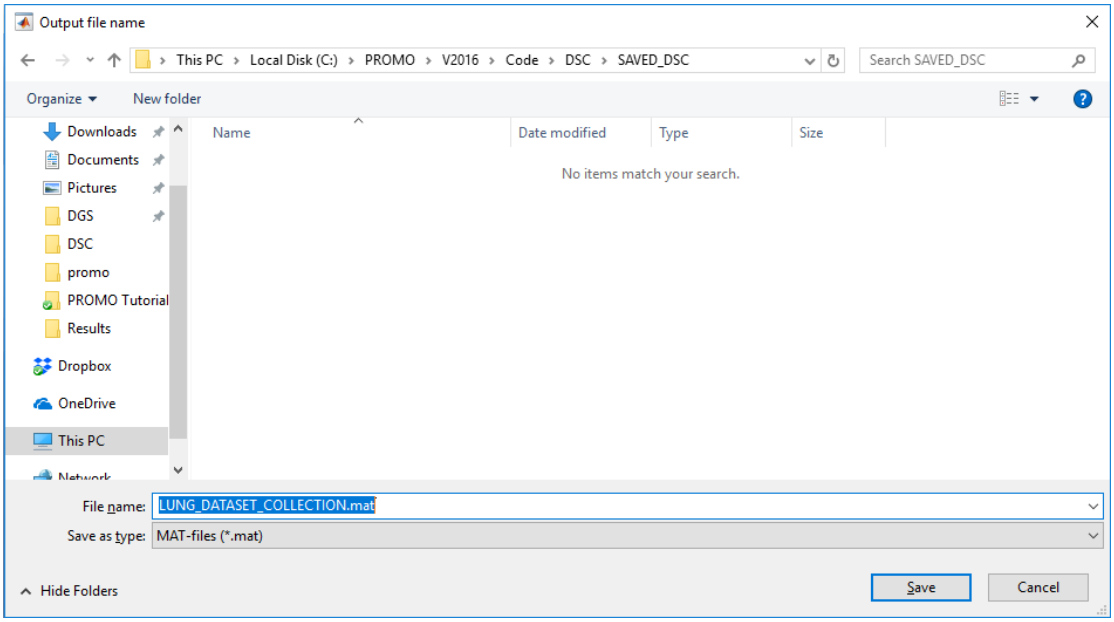


Notice the active dataset name (Methylation) is now colored in green. The changes made to the methylation dataset are now saved and our dataset collection has been updated.

12. Save the dataset collection to your local disk using the save button: 



13. Choose a name for your dataset collection file and press "save":



Congratulations! You are now the official owner of a dataset collection. Other buttons you might find useful while working on your dataset collection are:

	Remove the selected dataset from the dataset collection.
	Remove the whole dataset collection

	Rename the selected dataset.
	Load dataset collection from file.
	Intersect common samples (Leave only the samples that appear in all selected datasets).
	Merge all selected datasets by intersecting the samples and concatenating the data of all of the selected datasets. The result is a unified feature-concatenated matrix.

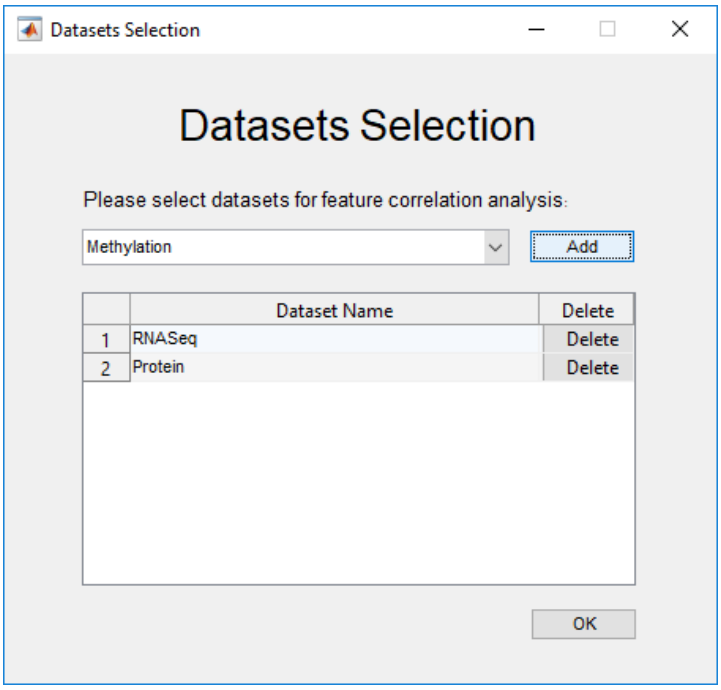
More advanced features will be discussed further in the tutorial.

Inter-Omic Feature Correlation Analysis

PROMO enables the identification of features that are correlated on two different datasets contained in a dataset collection.

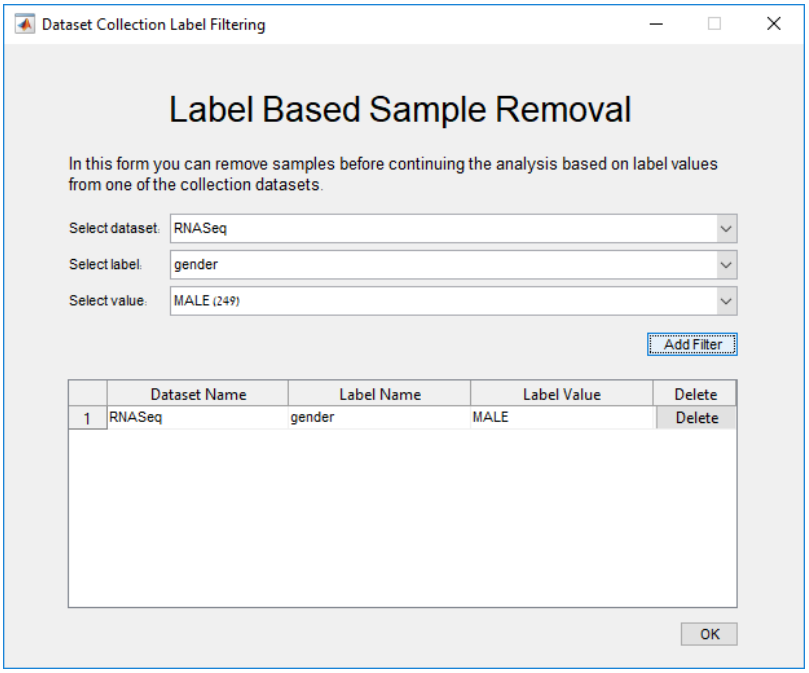
Using the dataset collection described on the previous section (TCGA's lung cancer datasets), we will demonstrate how to identify the most correlated features between the RNA-Seq and Protein datasets.

1. On the 'Dataset Collection' tab, press the inter-omic correlation button on the left panel: 
2. In the 'Dataset Selection' window, select the two datasets you wish to use (this type of analysis requires exactly two datasets) and add them to the list using the add button.
   Here, we selected and added the RNASeq and Protein datasets.



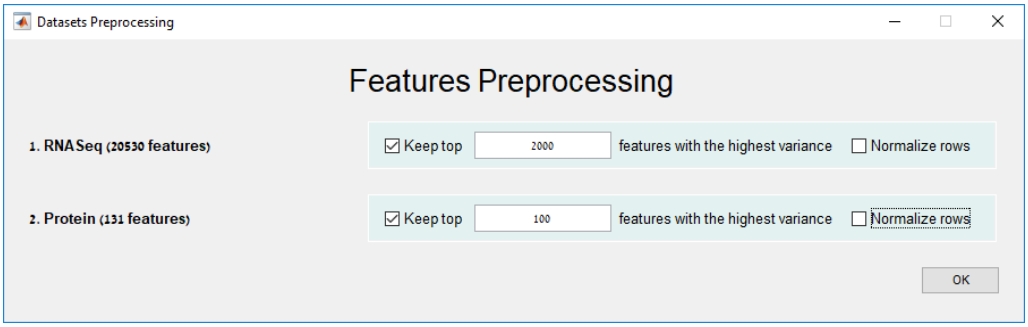
3. Label based sample filtering - At this point, you can select which samples you wish to remove from the data before continuing the analysis, based on their clinical label values.
   This filtering will not affect the original dataset collection. Therefore, you can repeat this analysis using different filters on the original dataset collection.

   In this example, we chose to remove all the male samples from the data used in the following analysis.



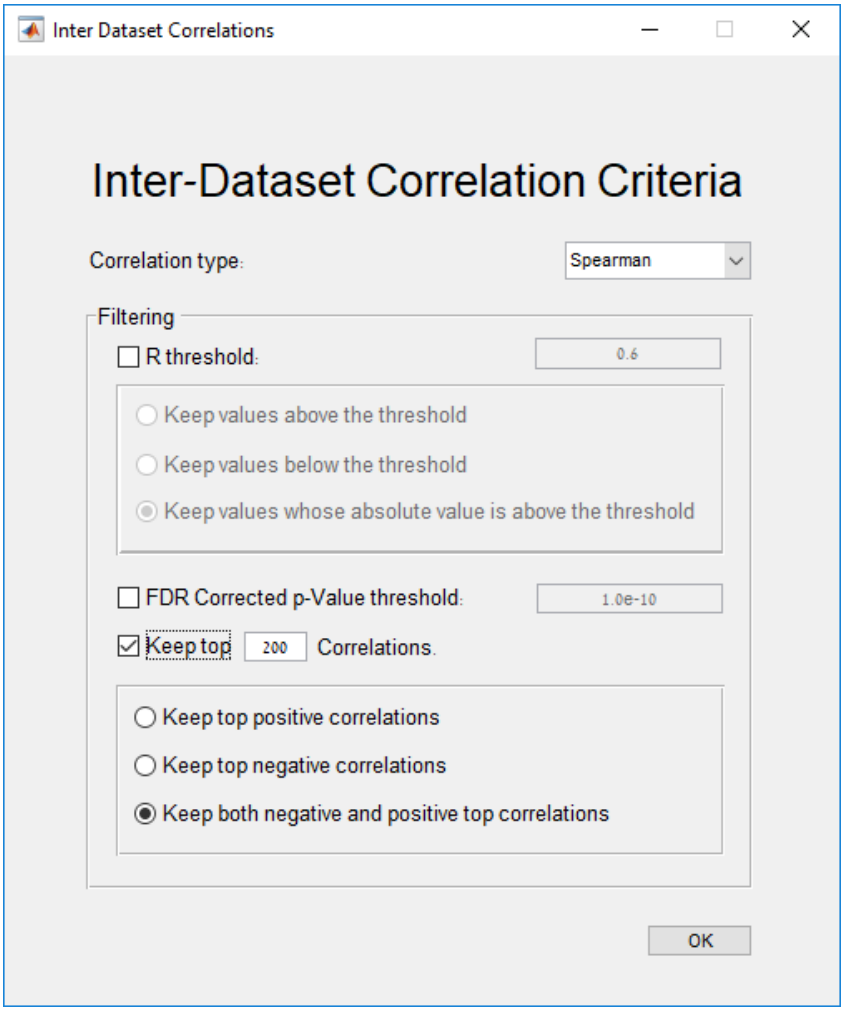
4. Feature Preprocessing – at this point you can define the number of the most variable features in each dataset to be included in the analysis, and indicate whether to normalize the features before starting the analysis. This preprocessing will not affect the original dataset collection, only the data used in the current analysis.

   In this example, we chose to keep the top 2000 features from the RNA-Seq dataset and the top 100 features from the Protein dataset.



5. We will now select the criteria for the analysis.
   a. Select the type of correlation you want to use. Available options are Spearman or Pearson. In this example, we will use Spearman correlation.
   b. Define filters to be applied on the results. You can choose to filter by R values, FDR corrected p-values, or keep a predefined number of top results. You can also combine several types of

filtering techniques to make sure all the correlations you will get meet all the conditions specified.
In our example we chose to keep the top 200 correlations, both negative and positive.



6. The results of the analysis appear on the 'Dataset Correlation Results' window, which contains the list of top inter-omic correlations as well as various visualizations of the top correlations:

Dataset Correlation Results

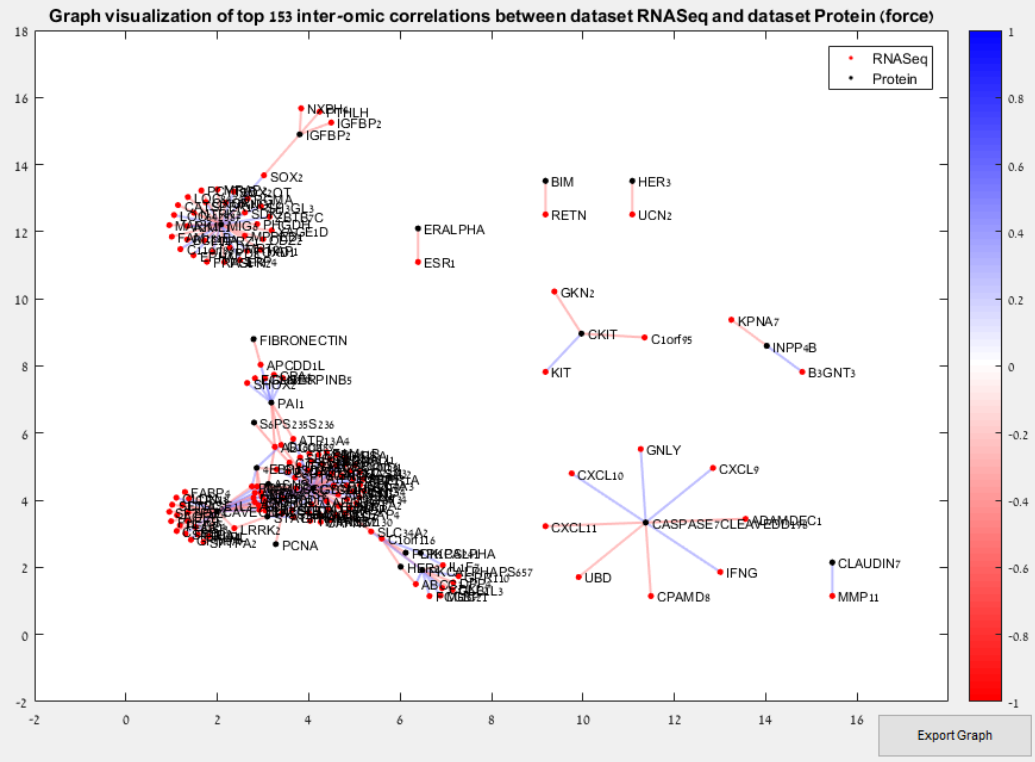
Inter Correlation Summary | Results Table | Correlation Results - Graph Visualization (force) | Correlation Results - Graph Visualization (layered)

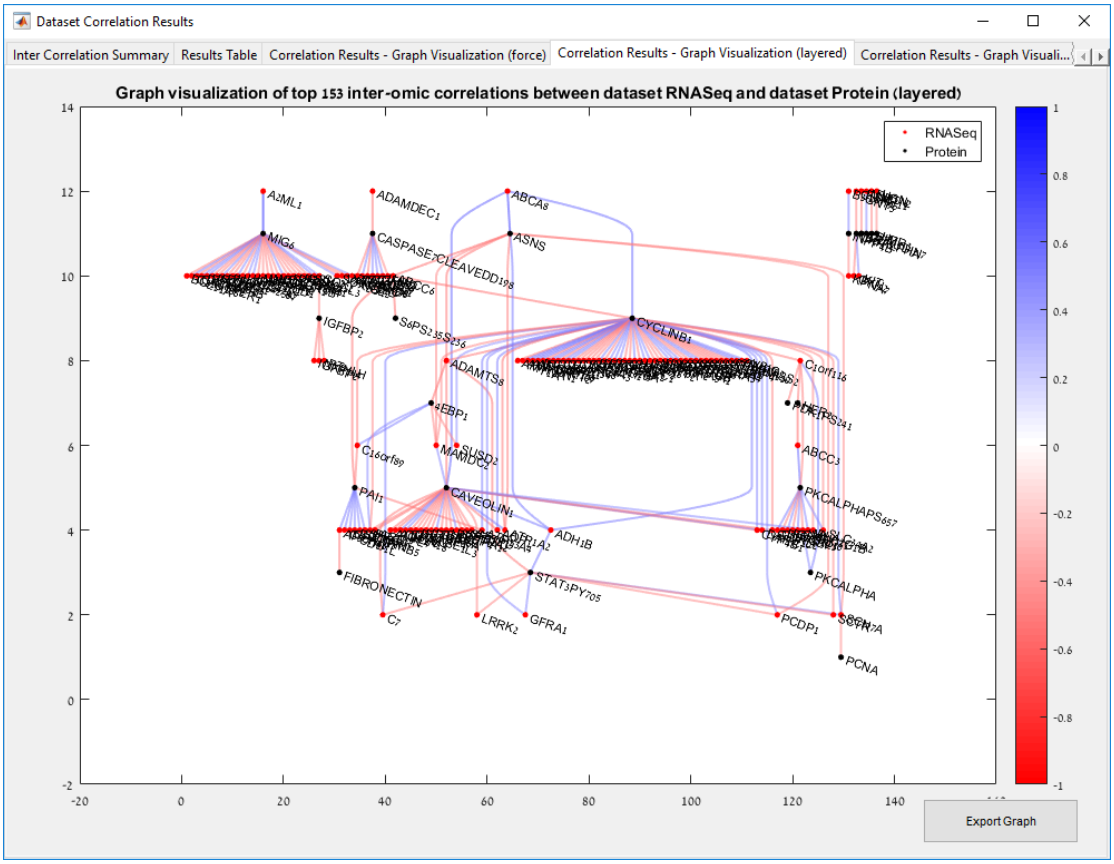
Top Inter-Dataset Correlations

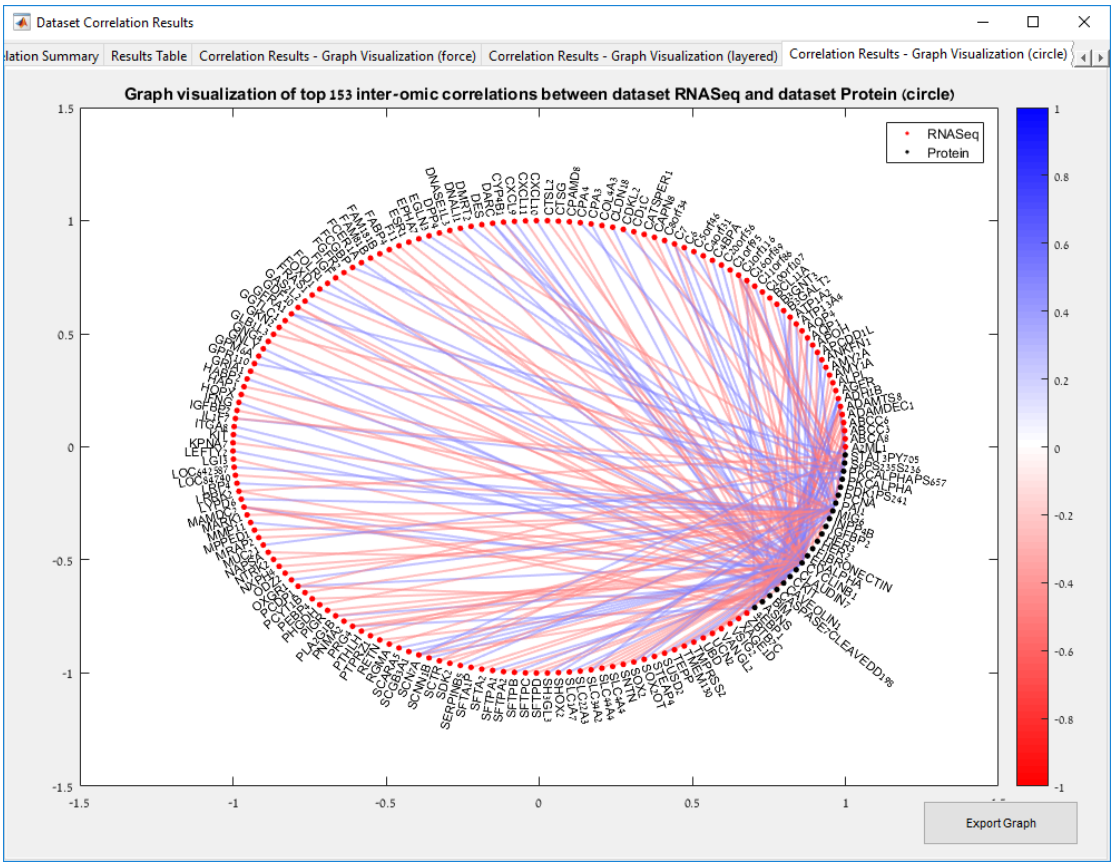
	PS1	GS1	MEAN1	PS2	GS2	MEAN2	RHO	P-VAL	Show Plot
1	IGFBP2	IGFBP2	11.42	IGFBP2	IGFBP2	0.28	0.9016	2.7071e-60	Show Plots
2	KIT	KIT	9.20	CKIT	CKIT	0.75	0.7936	1.3220e-34	Show Plots
3	ADH1B	ADH1B	8.22	CAVEOLIN1	CAVEOLIN1	1.28	0.6409	4.9274e-17	Show Plots
4	IFNG	IFNG	3.17	CASPASE7C...	CASPASE7CLE	0.31	0.6174	2.7904e-15	Show Plots
5	SCN7A	SCN7A	6.35	CYCLINB1	CYCLINB1	-0.49	-0.6055	1.7528e-14	Show Plots
6	SFTPC	SFTPC	10.66	CAVEOLIN1	CAVEOLIN1	1.28	0.6034	2.0485e-14	Show Plots
7	C16orf89	C16orf89	9.77	CYCLINB1	CYCLINB1	-0.49	-0.5910	1.3329e-13	Show Plots
8	ADAMTS8	ADAMTS8	5.23	CYCLINB1	CYCLINB1	-0.49	-0.5903	1.3329e-13	Show Plots
9	SOX2	SOX2	7.85	MIG6	MIG6	0.05	-0.5843	2.5783e-13	Show Plots
10	PCDP1	PCDP1	6.29	CYCLINB1	CYCLINB1	-0.49	-0.5839	2.5783e-13	Show Plots
11	SUSD2	SUSD2	9.98	CYCLINB1	CYCLINB1	-0.49	-0.5838	2.5783e-13	Show Plots
12	ABCA8	ABCA8	5.69	CYCLINB1	CYCLINB1	-0.49	-0.5835	2.5783e-13	Show Plots
13	SFTA1P	SFTA1P	6.38	CYCLINB1	CYCLINB1	-0.49	-0.5747	9.2503e-13	Show Plots
14	ATP1A2	ATP1A2	4.12	CYCLINB1	CYCLINB1	-0.49	-0.5742	9.3783e-13	Show Plots
15	OXGR1	OXGR1	2.19	MIG6	MIG6	0.05	-0.5708	1.4542e-12	Show Plots
16	ADH1B	ADH1B	8.22	CYCLINB1	CYCLINB1	-0.49	-0.5701	1.5151e-12	Show Plots
17	C7	C7	9.56	CAVEOLIN1	CAVEOLIN1	1.28	0.5683	1.8636e-12	Show Plots
18	GRIA1	GRIA1	3.29	CAVEOLIN1	CAVEOLIN1	1.28	0.5655	2.6534e-12	Show Plots
19	C1orf116	C1orf116	11.01	PKCALPHAP...	PKCALPHAPS6	0.02	0.5650	2.6964e-12	Show Plots
20	IL1F7	IL1F7	3.10	PKCALPHAP...	PKCALPHAPS6	0.02	0.5630	3.2113e-12	Show Plots
21	ABCC6	ABCC6	7.30	CYCLINB1	CYCLINB1	-0.49	-0.5627	3.2113e-12	Show Plots
22	C7	C7	9.56	CYCLINB1	CYCLINB1	-0.49	-0.5626	3.2113e-12	Show Plots
23	MARK1	MARK1	7.34	MIG6	MIG6	0.05	-0.5623	3.2113e-12	Show Plots
24	GNLY	GNLY	7.06	CASPASE7C...	CASPASE7CLE	0.31	0.5622	3.2113e-12	Show Plots
25	APCDD1L	APCDD1L	4.46	PAI1	PAI1	0.55	0.5556	7.6006e-12	Show Plots

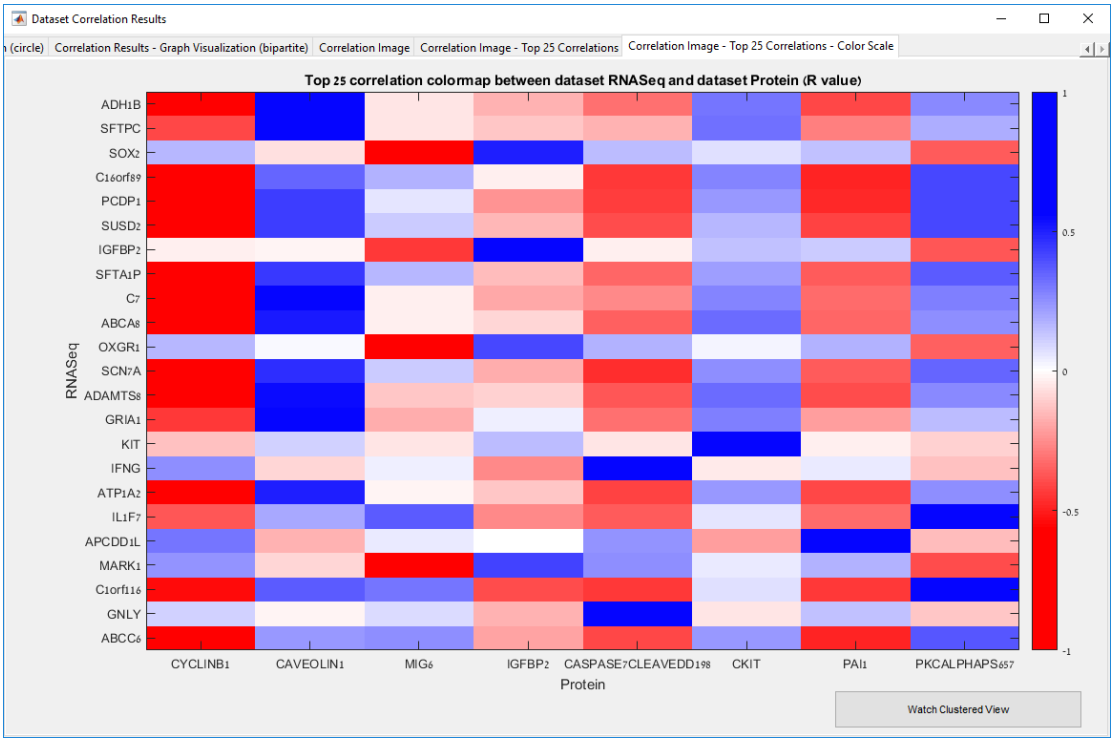
Dataset Correlation Results

Inter Correlation Summary | Results Table | Correlation Results - Graph Visualization (force) | Correlation Results - Graph Visualization (layered) | Correlation Results - ...




Dataset Correlation Results
Inter Correlation Summary
Results Table
Correlation Results - Graph Visualization (force)
Correlation Results - Graph Visualization (layered)
Correlation Results - Graph Visuali...
Graph visualization of top 153 inter-omic correlations between dataset RNASeq and dataset Protein (layered)
RNASeq
Protein
AJML1
MIG6
ADAMDEC1
CASPASE7CLEAVED198
ABCA8
ASNS
IGFBP2
S6PS235S236
CYCLINB1
ADAMTS8
C1orf116
4EBP1
C16orf59
SUSD2
MANDC2
ABCC3
PAI1
CAVEOLIN1
ADH1B
PKCALPHAPS657
FIBRONECTIN
STAT3PY705
PKCALPHA
C7
LRRK2
GFRA1
PCDP1
PCNA
Export Graph


Dataset Correlation Results
lation Summary
Results Table
Correlation Results - Graph Visualization (force)
Correlation Results - Graph Visualization (layered)
Correlation Results - Graph Visualization (circle)
Graph visualization of top 153 inter-omic correlations between dataset RNASeq and dataset Protein (circle)
RNASeq
Protein
Export Graph


Dataset Correlation Results
(circle)
Correlation Results - Graph Visualization (bipartite)
Correlation Image
Correlation Image - Top 25 Correlations
Correlation Image - Top 25 Correlations - Color Scale
Top 25 correlation colormap between dataset RNASeq and dataset Protein (R value)
RNASeq
ADH1B
SFTPC
SOX2
C16orf89
PCDP1
SUSD2
IGFBP2
SFTA1P
C7
ABCA8
OXGR1
SCN7A
ADAMTS8
GRIA1
KIT
IFNG
ATP1A2
IL1F7
APCDD1L
MARK1
C1orf116
GNLY
ABCC6
CYCLINB1
CAVEOLIN1
MIG6
IGFBP2
CASPASE7CLEAVEDD198
CKIT
PAI1
PKCALPHAPS657
Protein
1
0.5
0
-0.5
-1
Watch Clustered View

Multi-Omic Clustering

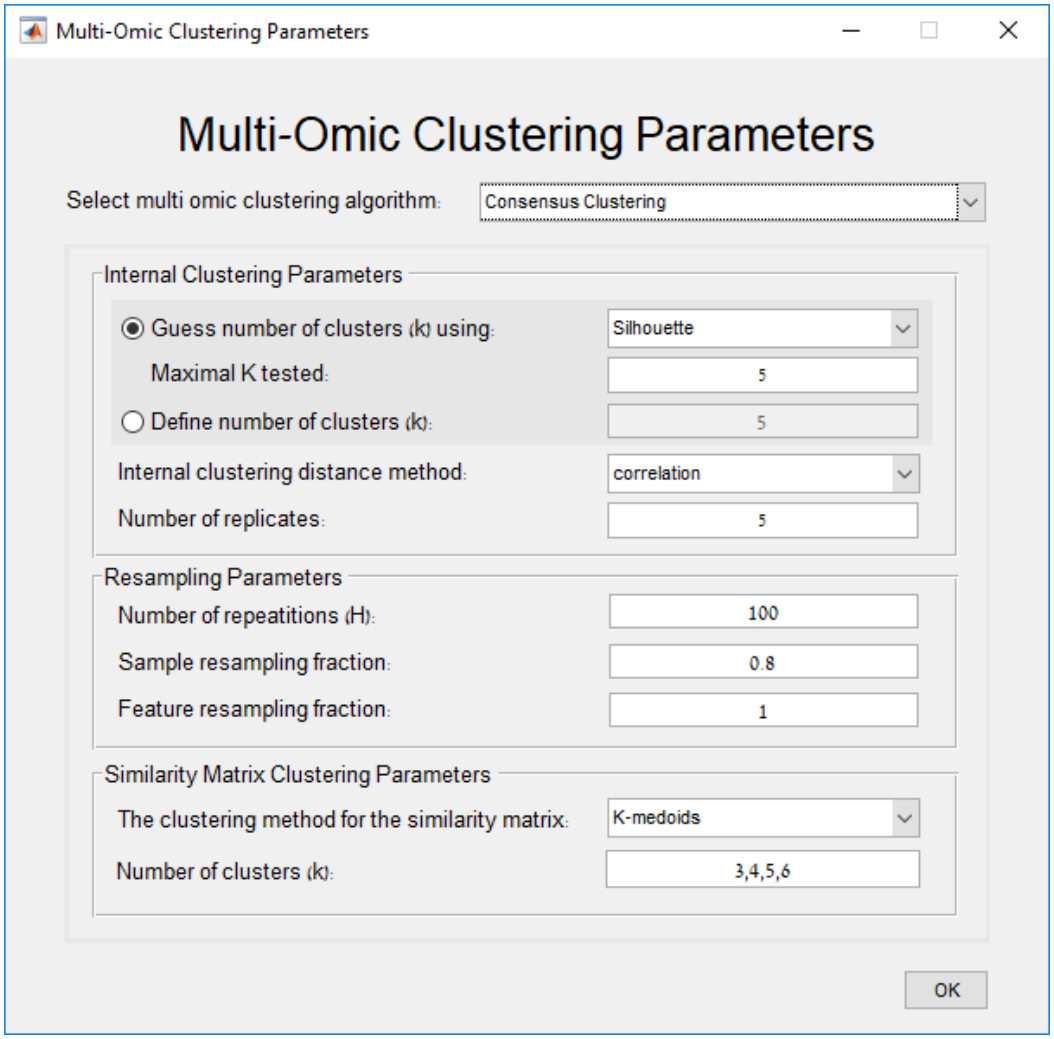
PROMO offers several methods for multi-omic clustering. Such methods cluster the samples based on several different datasets describing the same set of samples.

Here we show how to apply the multi-omic consensus clustering implemented in PROMO.

Consensus Clustering can be initiated by clicking the Consensus Clustering button on the 'Dataset Collection' tab: 

1. The next three steps allow selection of the datasets to be included in the analysis, sample filtering and feature preprocessing similarly to the process described in sections 2-4. Notice that this time you can select up to 10 datasets.

2. The next screen enables choosing the parameters for the Consensus Clustering:



3. Once the clustering completes, a figure displaying the Consensus Clustering results is displayed. The figure includes the original data, iterations matrix, consensus distance matrix and the resulting consensus clustering solution.

   Further, a unified feature-concataenated matrix containing all datasets included in the anlsysis will be displayed. In addition, new label representing the assignment of the multi-omic clustering will be added to labels list box.

   The following example uses Methylation, Protein and RNA-Seq datasets and K=5 as the final number of clusters:

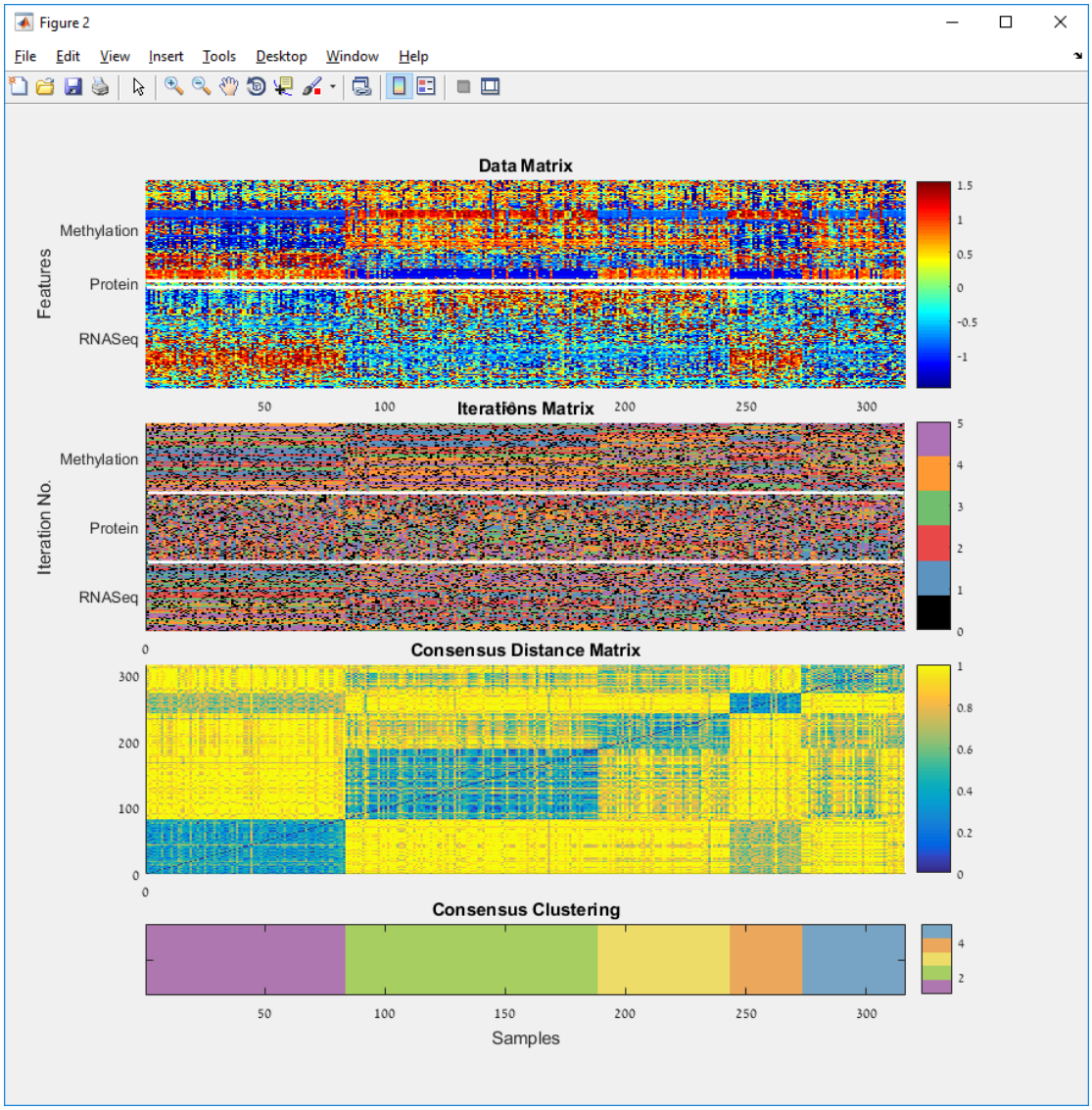


Figure 2
File Edit View Insert Tools Desktop Window Help
Data Matrix
Features
Methylation
Protein
RNASeq
Iterations Matrix
Iteration No.
Consensus Distance Matrix
Consensus Clustering
Samples