




# Knowledge-guided Algorithms in Systems Biology

**KnowEnG BD2K Center**

*Slides by Charles Blatti and Amin Emad*

# Summary

- Our goal in this lab is to use several pipelines of the KnowEnG platform to analyze ‘omic’ data sets and phenotypic spreadsheets

knoweng
BIG DATA TO KNOWLEDGE CENTER OF EXCELLENCE

- We will often try both network-guided and standard modes of operation for the pipelines (if applicable)

- Other network-guided and systems biology analysis tools will also be introduced

# More Specifically

- The structure of this lab is laid out around 3 example datasets
- It is focused on topics, methods, and types of networks from lecture
- It uses browser-based analysis platforms

| Data Sets | Somatic Mutations from Pan-Cancer | Drug Response in Cancer Cell Lines | ER+ Status in Breast Cancer |
|---|---|---|---|
| Topics | Sample Clustering | Gene Prioritization | Gene Expression Signatures, Gene Set Characterization |
| Methods | Network Based Stratification | ProGENI | GeneMANIA, DRaWR, |
| Networks | Integrated | Protein-Protein Interactions | Pathways, Integrated |
| Platforms | KnowEnG | KnowEnG | iLINCS, GeneMANIA, KnowEnG |

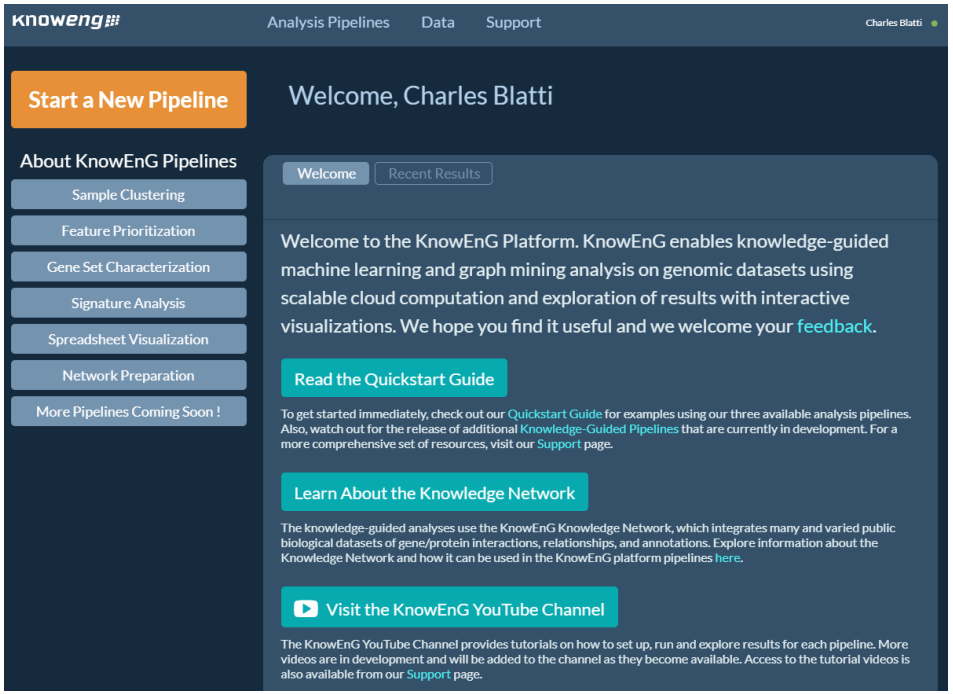
# Some Notes on the KnowEnG Platform

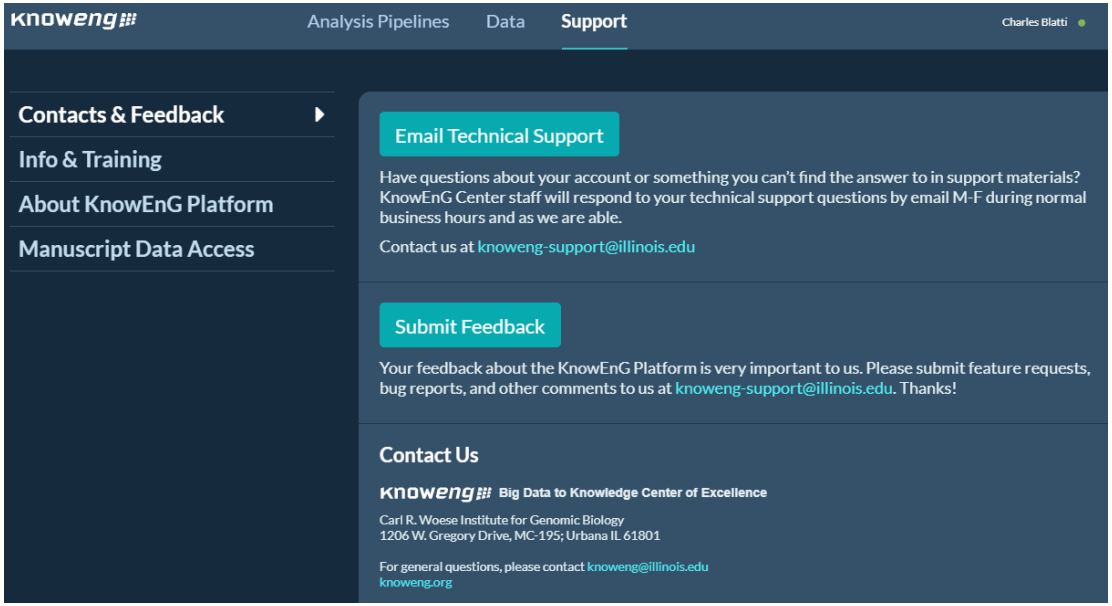
Knowledge-guided analysis of "omics" data using the KnowEnG cloud platform

Charles Blatti III, Amin Emad, Matthew J. Berry, Lisa Gatzke, Milt Epstein, Daniel Lanier, Pramod Rizal, Jing Ge, Xiaoxia Liao, Omar Sobh, Mike Lambert, Corey S. Post, Jinfeng Xiao, [ ... ], Saurabh Sinha [ view all ]

Published: January 23, 2020 • https://doi.org/10.1371/journal.pbio.3000583

- The **home page** has links to many resources
- The "**Support**" tab at the top has even more resources
- Scalable platform using AWS cloud, but requires **some waiting**
- Right before launch, carefully **match** Job summaries to slide stills to avoid errors



# STEP 0A: Start the VM

- Follow instructions for starting VM. (This is the Remote Desktop software.)
- The instructions are different for UIUC and Mayo participants.
- Find the instructions for this on the course website under Lab Set-up: https://publish.illinois.edu/compgenomicscourse/2022-schedule/

# Step 0: Local Files

For viewing and manipulating the files needed for this laboratory exercise, the path on the VM will be denoted as the following:

**[course_directory]**

We will use the files found in:

**[course_directory]**\07_Signatures_and_Characterization

**-and-**

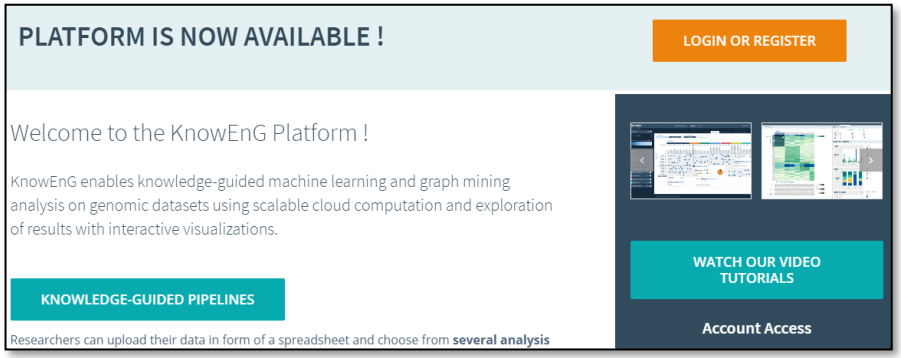
**[course_directory]**\08_Clustering_and_Prioritization

**[course_directory]= Desktop\Labs** **UIUC**

**[course_directory]= Desktop\VM** **Mayo**

# STEP 1: Sign Into KnowEnG Platform

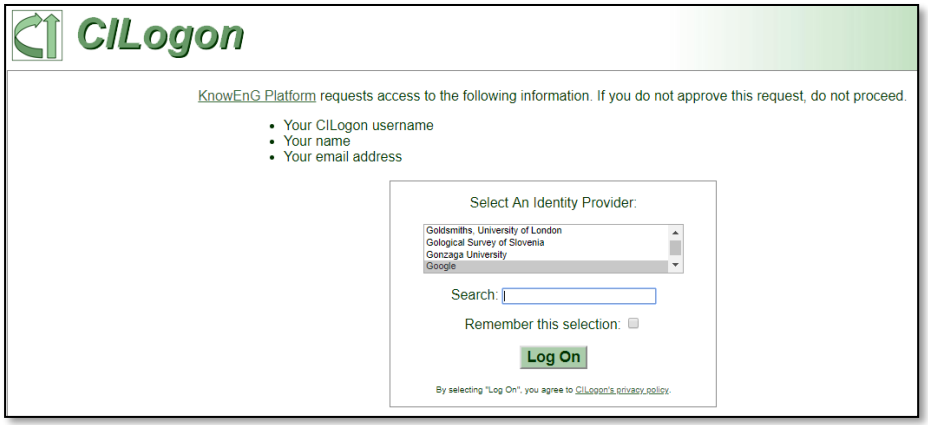
Go to the KnowEnG Platform:
https://knoweng.org/analyze/




Click "**Login or Register**"

Login with **CILogon** - Login service using your other existing accounts

Search for identity provider: **Urbana, Mayo, Google, GitHub**



# Finding Cancer Subtypes with Knowledge Guided Clustering

In this exercise, we will use a subset of somatic mutation data samples from the Cancer Genome Atlas (TCGA) and cluster them into different cancer subtypes.

# STEP 2: Sample Clustering

- We will use KnowEnG's clustering pipeline to perform both network-guided as well as standard clustering of samples

- The network-guided clustering implemented in KnowEnG is inspired by the network-based stratification approach:

Nat Methods. 2013 Nov;10(11):1108-15. doi: 10.1038/nmeth.2651. Epub 2013 Sep 15.

**Network-based stratification of tumor mutations.**

Hofree M[1], Shen JP, Carter H, Gross A, Ideker T.

- We will use some of the samples from the TCGA pancan12 dataset

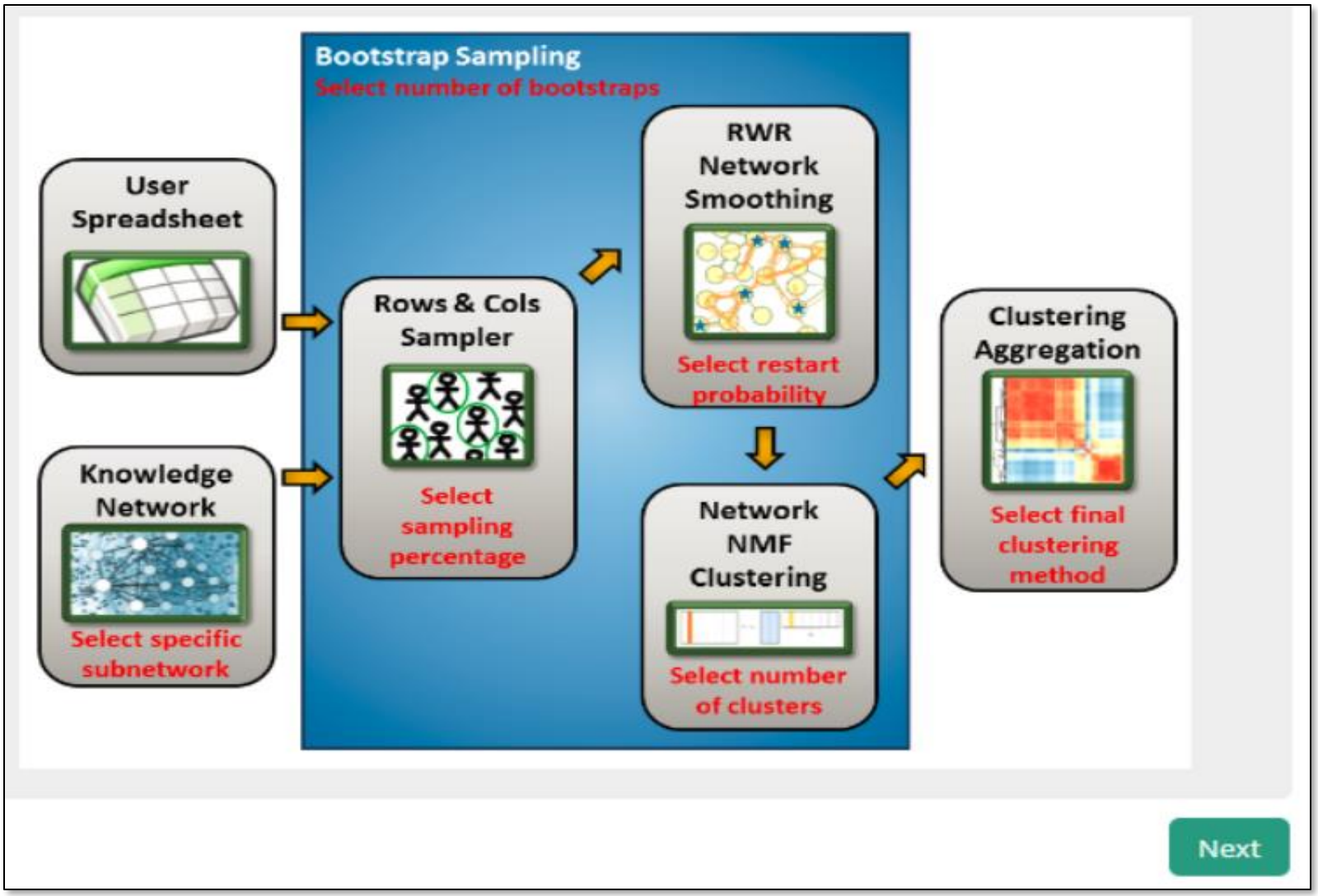
Cell. 2014 Aug 14;158(4):929-944. doi: 10.1016/j.cell.2014.06.049. Epub 2014 Aug 7.

**Multiplatform analysis of 12 cancer types reveals molecular classification within and across tissues of origin.**

Hoadley KA[1], Yau C[2], Wolf DM[3], Cherniack AD[4], Tamborero D[5], Ng S[6], Leiserson MDM[7], Niu B[8], McLellan MD[8], Uzunangelov V[6], Zhang J[9], Kandoth C[8], Akbani R[10], Shen H[11], Omberg L[12], Chu A[13], Margolin AA[12], Van't Veer LJ[3], Lopez-Bigas N[14], Laird PW[11], Raphael BJ[7], Ding L[8], Robertson AG[13], Byers LA[10], Mills GB[10], Weinstein JN[10], Van Waes C[15], Chen Z[16], Collisson EA[17]; Cancer Genome Atlas Research Network, Benz CC[18], Perou CM[19], Stuart JM[20].

# STEP 2: Sample Clustering

- Overview of KnowEnG's Network-based Stratification for Samples:



# STEP 2: Sample Clustering

**Find the files in this slide under [course_directory]/08_Clustering_and_Prioritization**

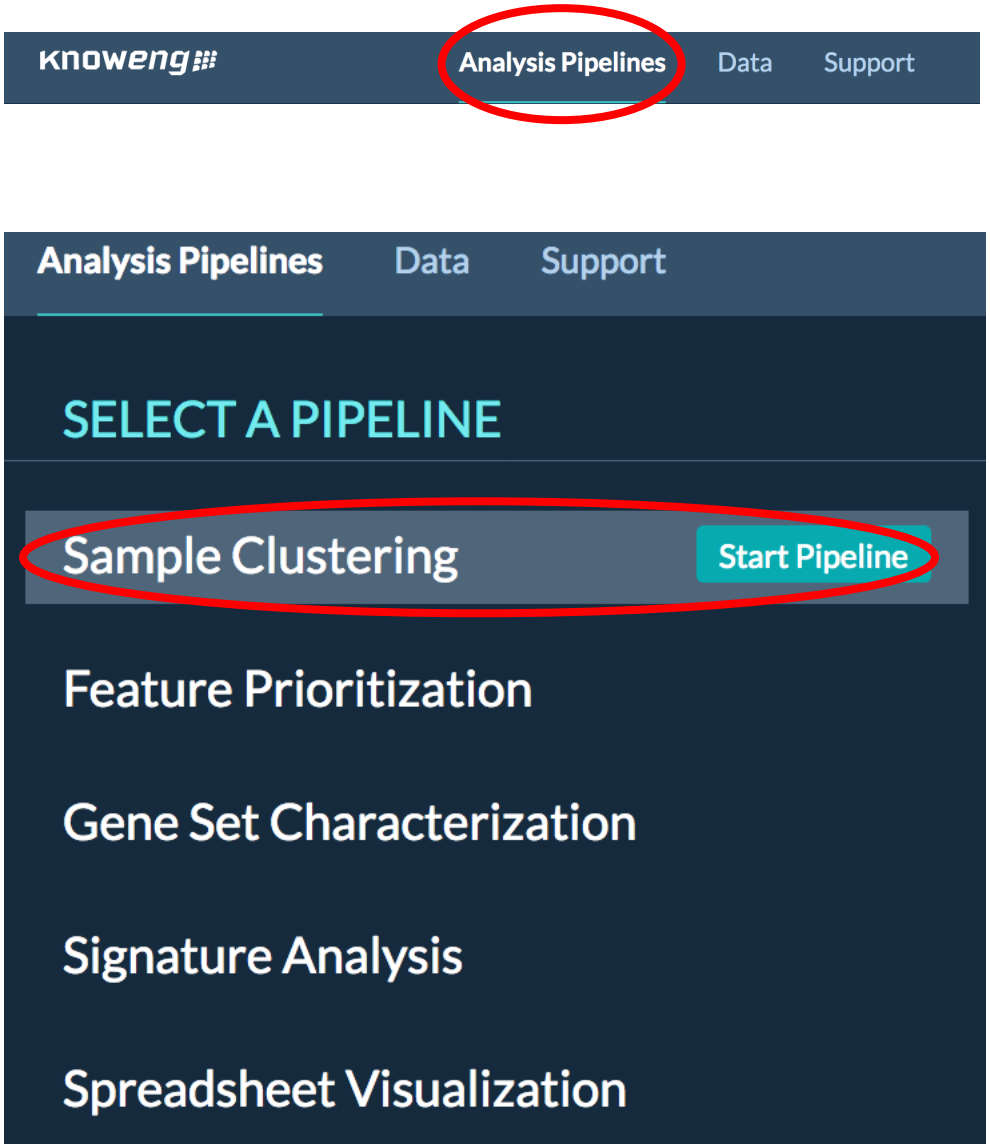
- Dataset characteristics:

| Name | Description |
|---|---|
| Demo2_Mutation_pancan12_30 | A matrix of (gene x samples) containing the somatic mutation status of ~15k protein coding genes in 360 tumor samples from 12 cancer types. |
| Demo2_Clinical_pancan12_30 | A matrix of (samples x clinical phenotypes) including primary disease, PANCAN consensus cluster, survival years, etc. |

# STEP 2A: Sample Clustering (standard)

Select the pipeline:

- Once logged into the KnowEnG Platform
- Select "**Analysis Pipelines**" at the top of the page
- Select "**Sample Clustering**" and Click on "**Start Pipeline**"

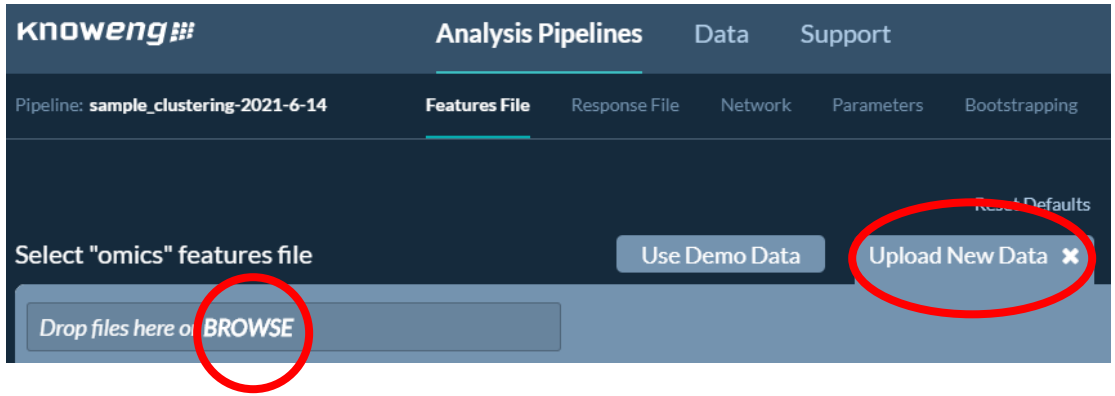


Find the files for this under [course_directory]/08_Clustering_and_Prioritization

# STEP 2A: Sample Clustering (standard)

## Upload the data:

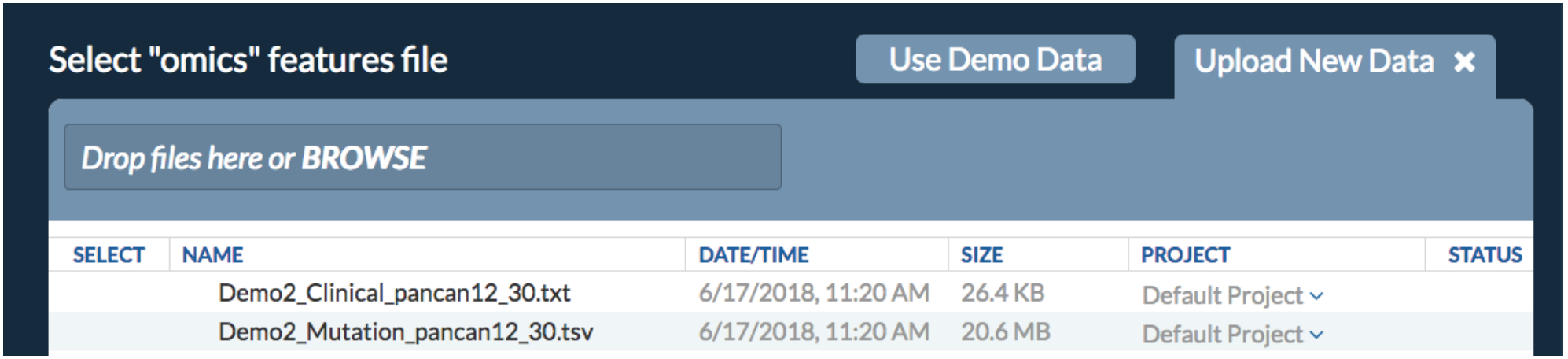
- Click on "**Upload New Data**"




- Click "**BROWSE**" and find the files to upload:

  - Demo2_Clinical_pancan12_30

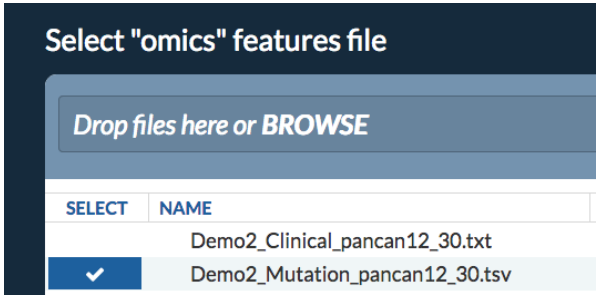
  - Demo2_Mutation_pancan12_30.tsv



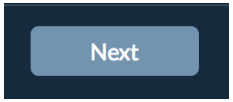
# STEP 2A: Sample Clustering (standard)

Configure the pipeline:

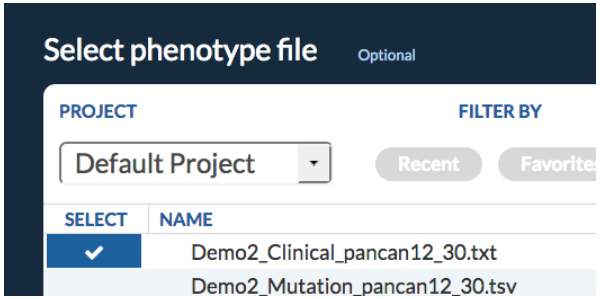
- For the "**omics**" file select:
  - Demo2_Mutation_pancan12_30.tsv



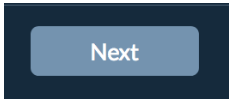

- Click "**Next**" at the bottom right corner




- For the "**phenotype**" file select:
  - Demo2_Clinical_pancan12_30.txt

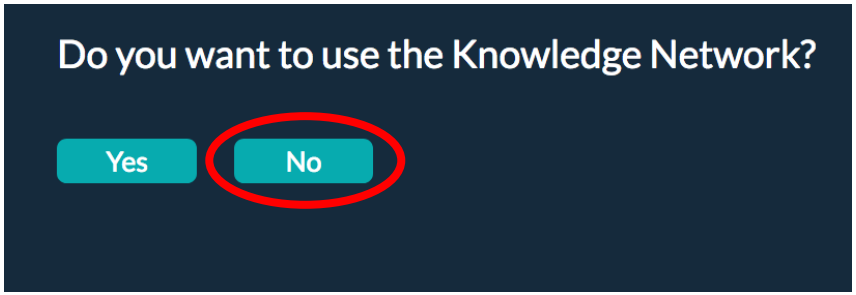


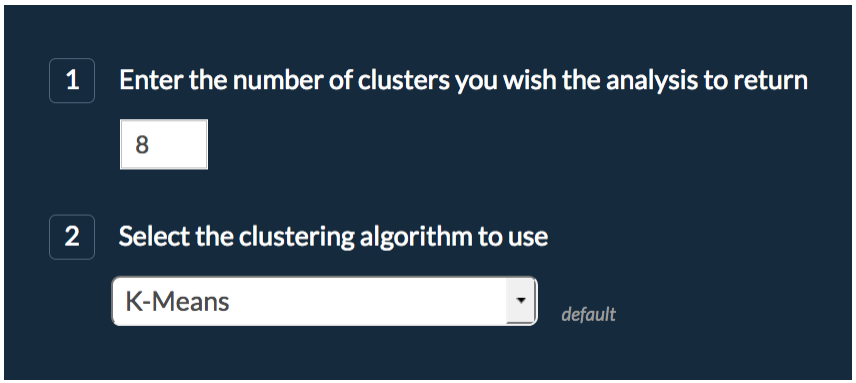

- Click "**Next**" at the bottom right corner

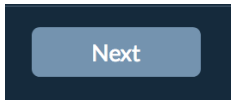


# STEP 2A: Sample Clustering (standard)

- Select "**No**" in response to using the knowledge network:
  - *This allows us to perform standard clustering on the data*
- Click on **"Next"** at the bottom right corner
- Choose **8** as number of clusters
  - *This is what was found as optimal in the TCGA paper*
- We will use the default "**K-Means**" clustering algorithm
- Click on "**Next**" at the bottom right corner

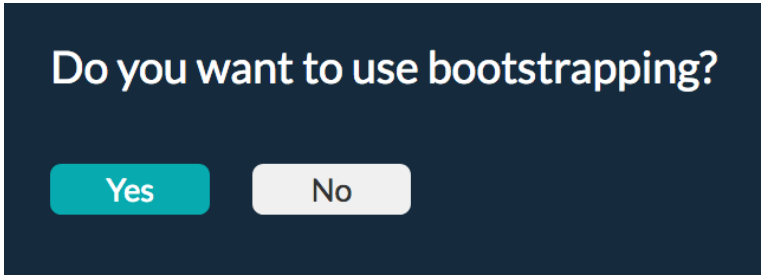


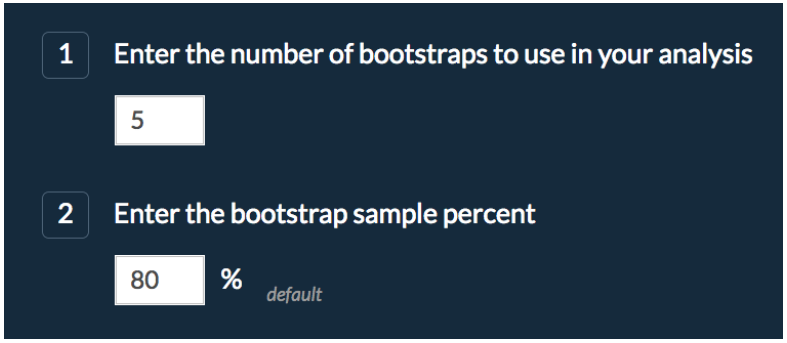



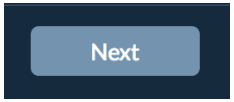



# STEP 2A: Sample Clustering (standard)

- Select "**Yes**" in response to using bootstrap sampling:
  - *This allows us to obtain a more robust final clustering*
- Choose **5** as number of bootstraps.
  - *This is unusually low for the purposes of quicker completion*
- We will use the default **80**% rate to sample the data in each bootstrap
- Click on "**Next**" at the bottom right corner



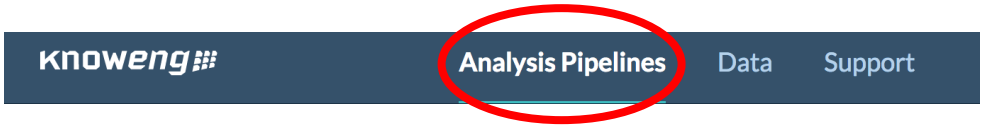
# STEP 2A: Sample Clustering (standard)

- Review the summary of the job and change the default "**Job Name**" to easily recognize later


- **Submit** the job
  - *The job will run for several minutes, so we will return to the results after launching the next job*

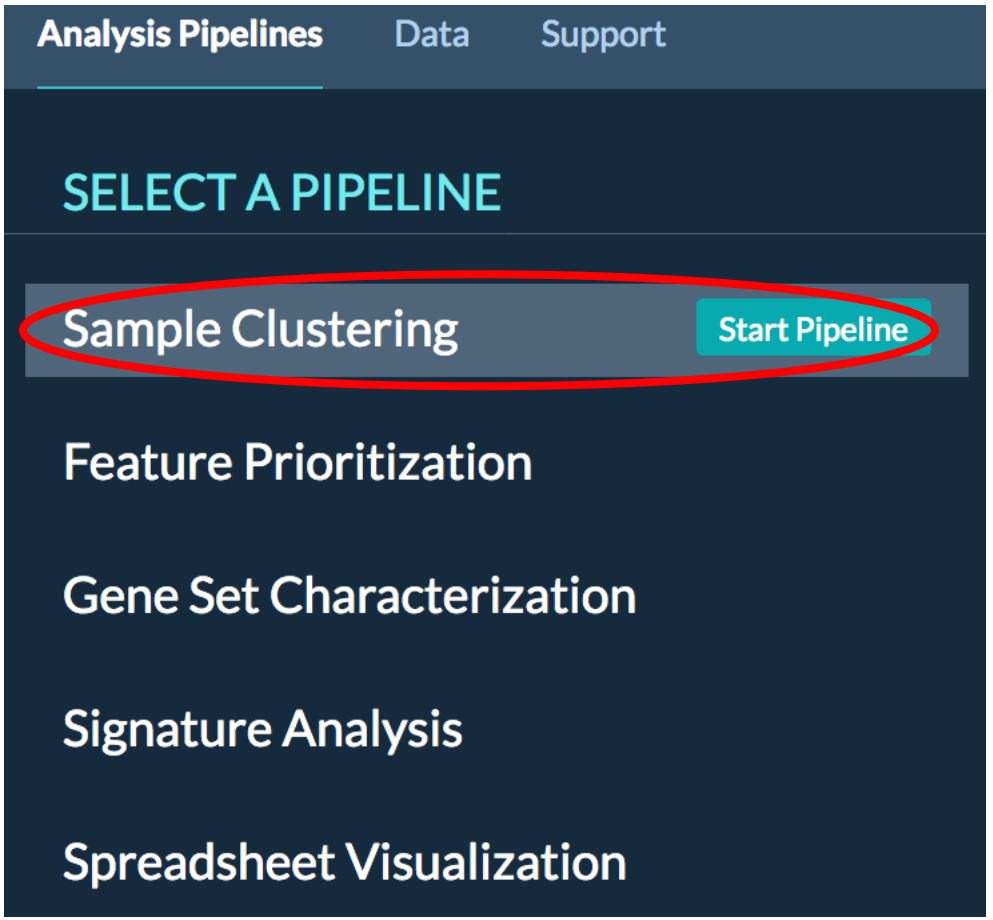
# STEP 2B: Sample Clustering (network-guided)

*Now we are going to repeat the analysis using a knowledge network to provide richer information about the similarity between the sparse mutation samples. Nearly all steps will be the same as before.*




Select the pipeline:

- Select "**Analysis Pipelines**" at the top of the page

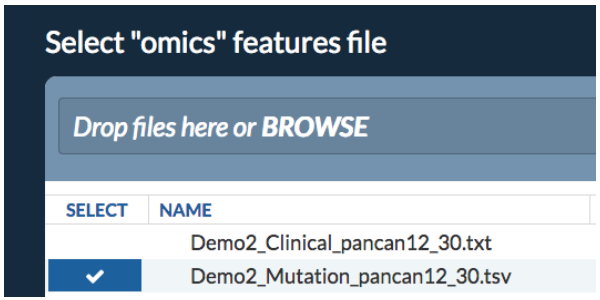



- Select "**Sample Clustering**" and Click on "**Start Pipeline**"

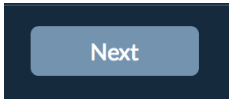
# STEP 2B: Sample Clustering (network-guided)

Configure the pipeline:

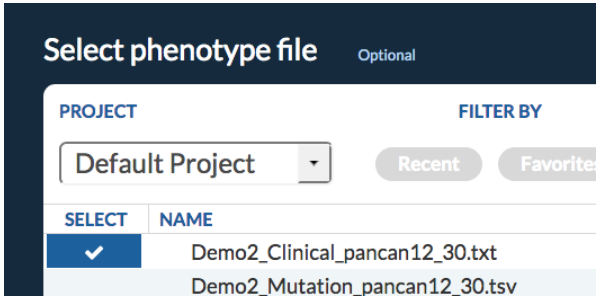
- For the "**omics**" file select:
  - Demo2_Mutation_pancan12_30.tsv



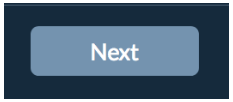

- Click "**Next**" at the bottom right corner




- For the "**phenotype**" file select:
  - Demo2_Clinical_pancan12_30



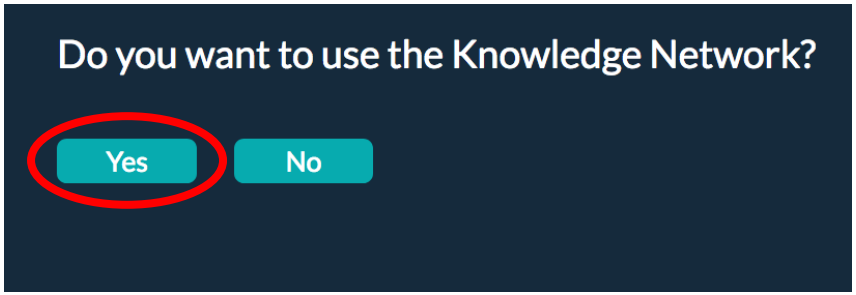

- Click "**Next**" at the bottom right corner

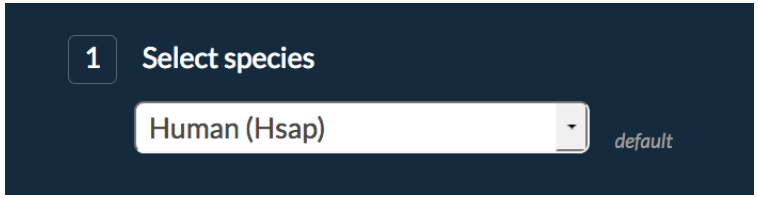


# STEP 2B: Sample Clustering (network-guided)

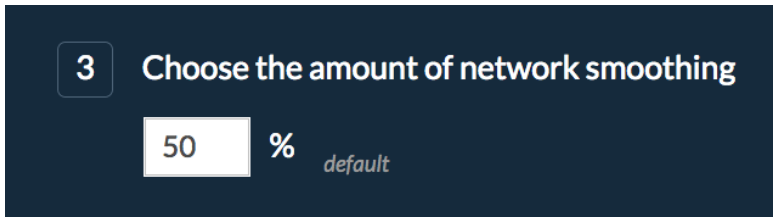
*This is different from the previous run.*

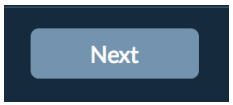
- Select "**Yes**" in response to using the knowledge network:
  - *This allows us to perform network-guided clustering*
- Keep the species as "**Human**"
- Select "**HumanNet Integrated Network**" as the network
  - *This is a network that creates scores pairwise interactions of gene by combining many different types of gene relationships*
- Keep network smoothing at **50**% and **click Next**:
  - *This controls how much importance is put on network connections instead of the somatic mutations*



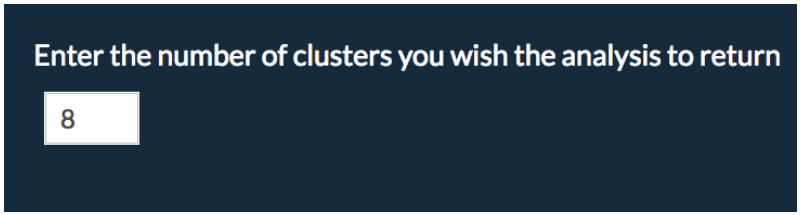




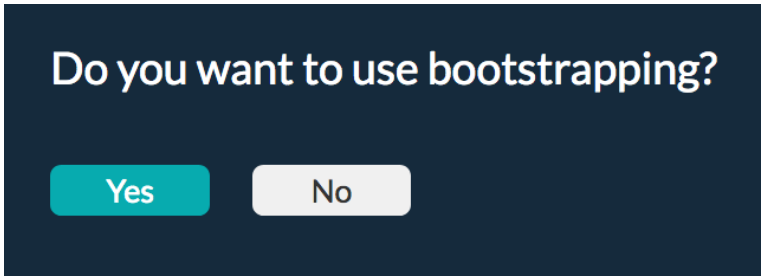



# STEP 2B: Sample Clustering (network-guided)

- Choose **8** as number of clusters and click **Next**

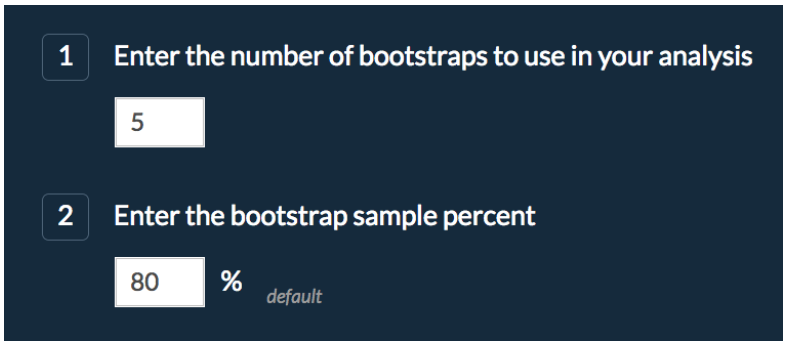



- Select "**Yes**" in response to using bootstrap sampling:




- Choose **5** as number of bootstraps

- We will use the default **80%** rate to sample the data in each bootstrap




- Click **"Next"**

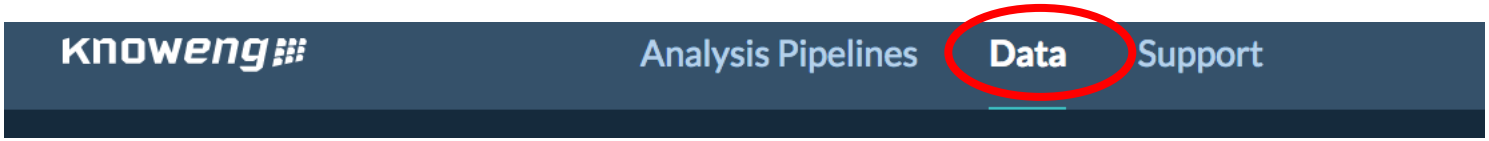
# STEP 2B: Sample Clustering (network-guided)

- Review the summary of the job and change the default "**Job Name**" to easily recognize later

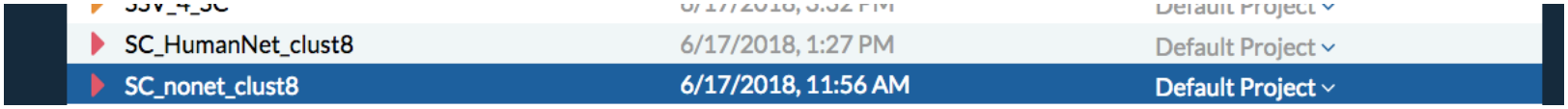

- Press **Submit** Job

# STEP 2C: Standard Clustering Results

- Go to the "**Data**" page:



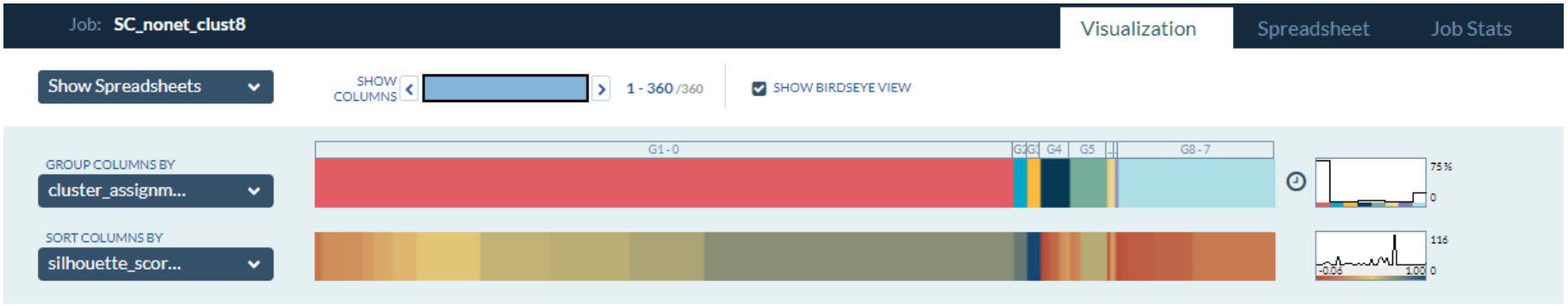

- Select "**SC_nonet_clust8**" (or other name you chose for the first run)



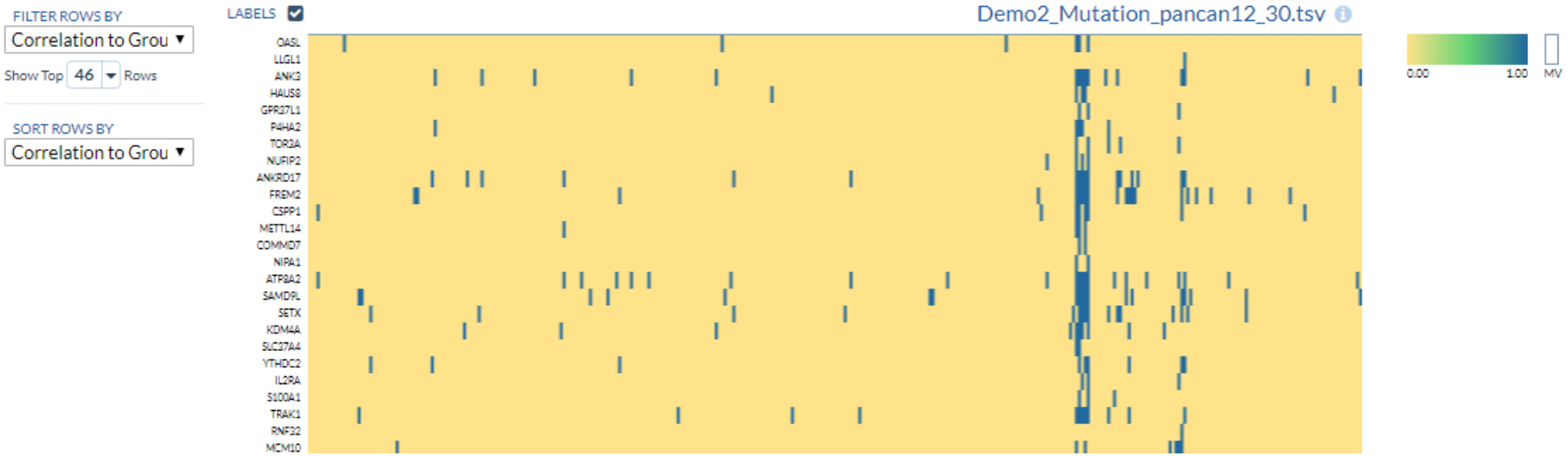

- Select "**View Results**" at the top right corner
  - *The option to view results will not be available if*
    - *The job is still running:*
    - *There was an error:*
  - *If there's an error, try repeating the launch steps*

# STEP 2C: Standard Clustering Results

- Visualization shows the cluster sizes and the match of the samples to the cluster (silhouette_score)



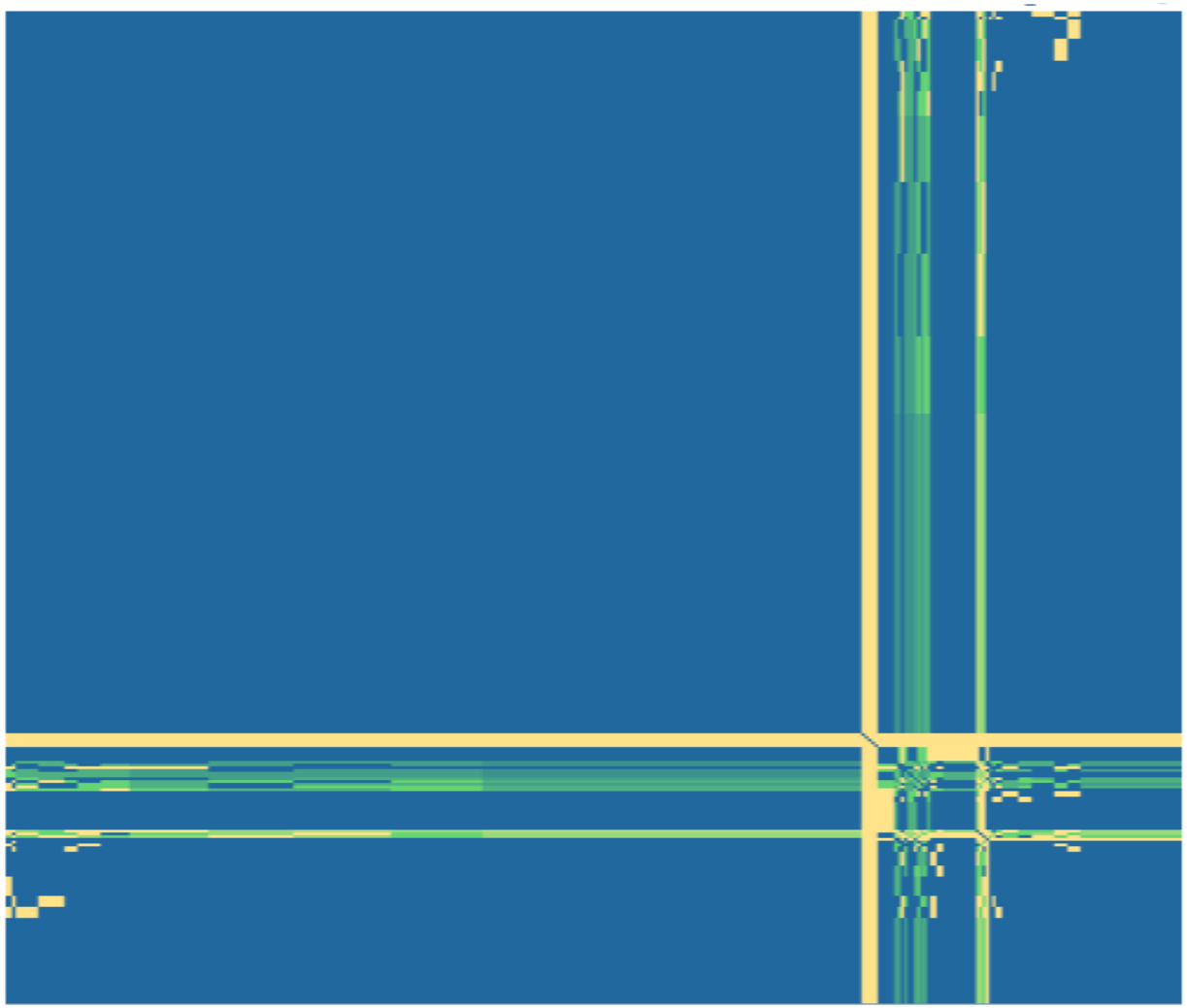

- Heatmap shows genes x samples – significantly correlated mutations



# STEP 2C: Standard Clustering Results

- Heatmap also shows samples x samples co-occurrence

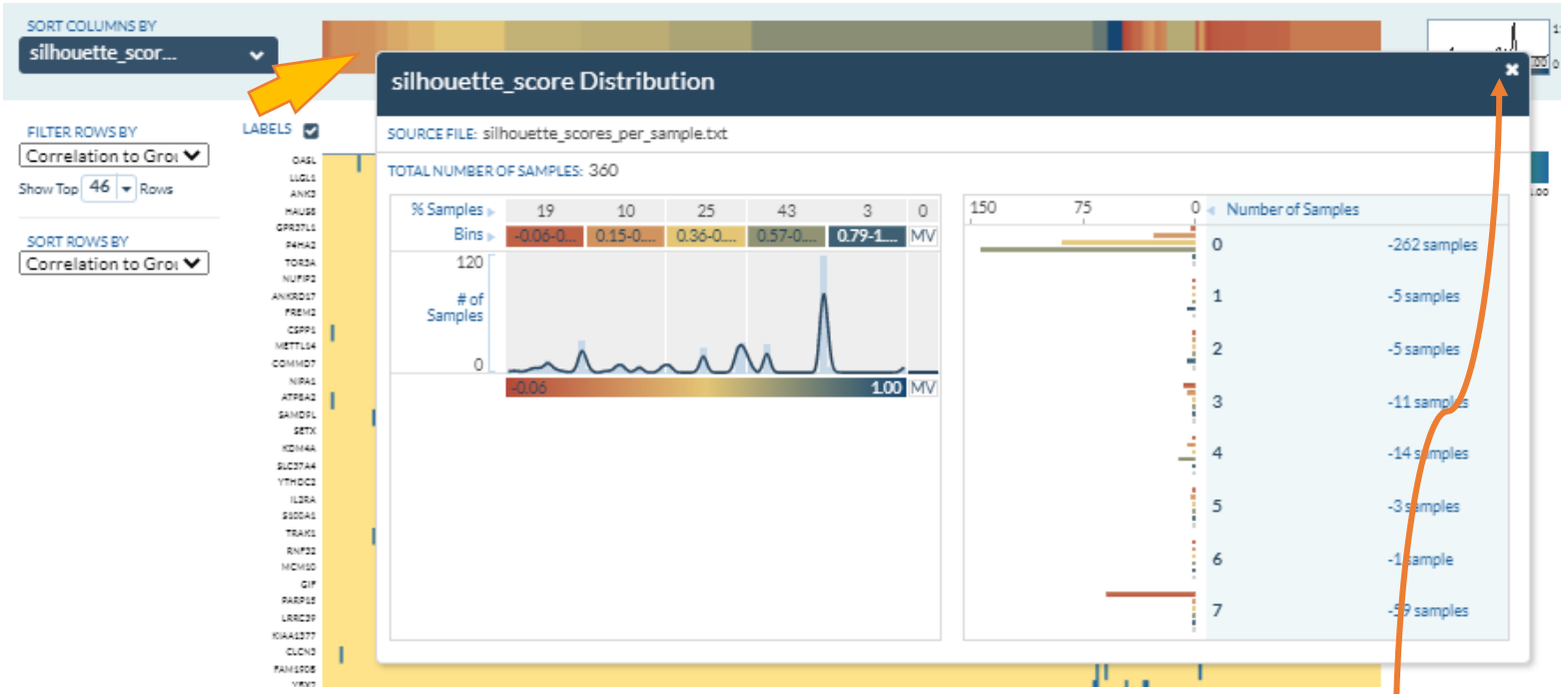
The color of each cell indicates how frequently a pair of patients fell within the same cluster across all samplings

# STEP 2C: Standard Clustering Results

- Click on the **silhouette_score Distribution colorbar**.

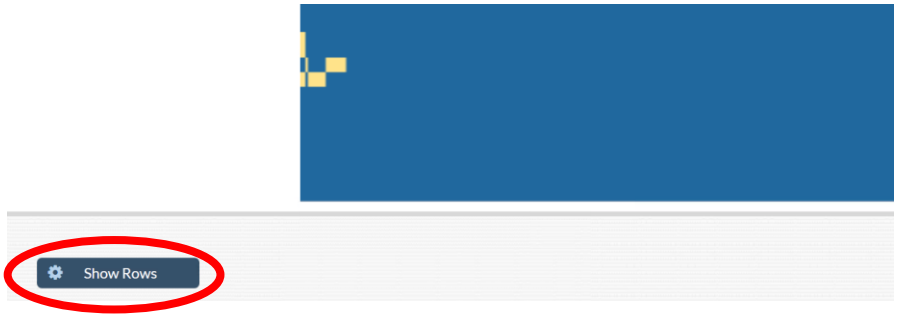
- The **Number of Samples** per cluster show high degree of clustering bias.
  *262 of the 360 samples are in Cluster 0*

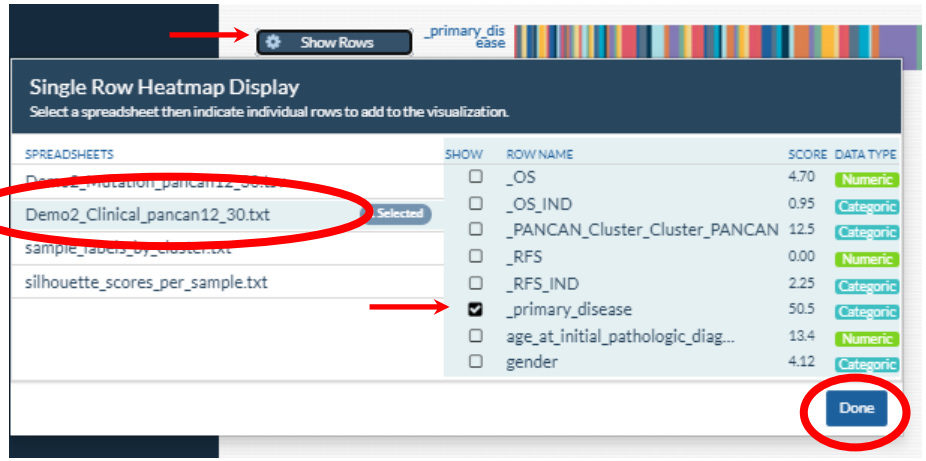


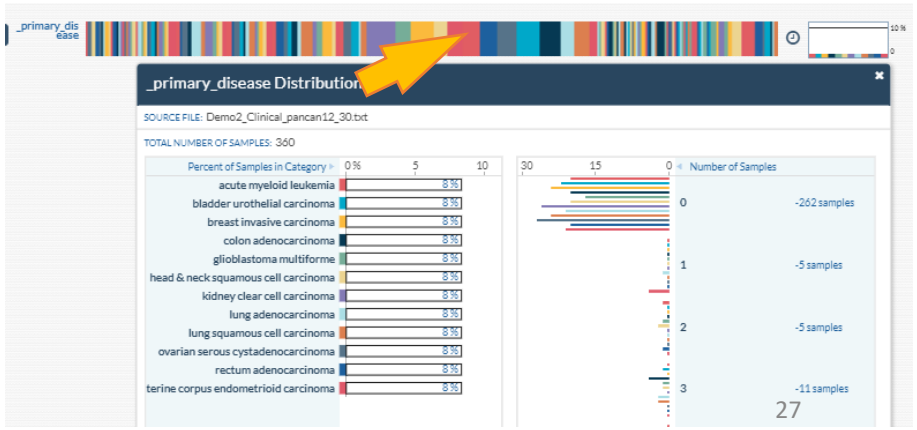

- Close the Distribution panel with the 'X' in the top corner

# STEP 2C: Standard Clustering Results

- You can add a phenotype to compare to the clustering at the very bottom of the page
  - click "**Show Rows**",
  - the name of the clinical file, "**Demo2_Clinical_pancan12_30.txt**",
  - and select an interesting phenotype, like the **"_primary_disease**" type,
  - and click "**Done**"
- This color bar shows the original primary tumor (**_primary_disease**) types. Click on the **colorbar** to show which cancer types are present in which clusters

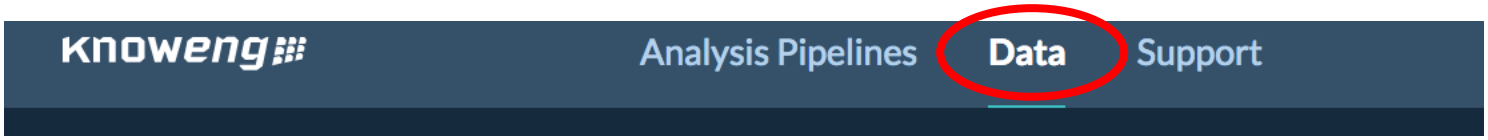


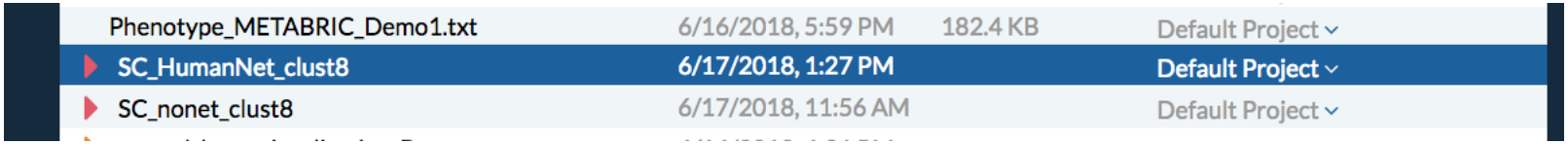



# STEP 2D: Network Clustering Results

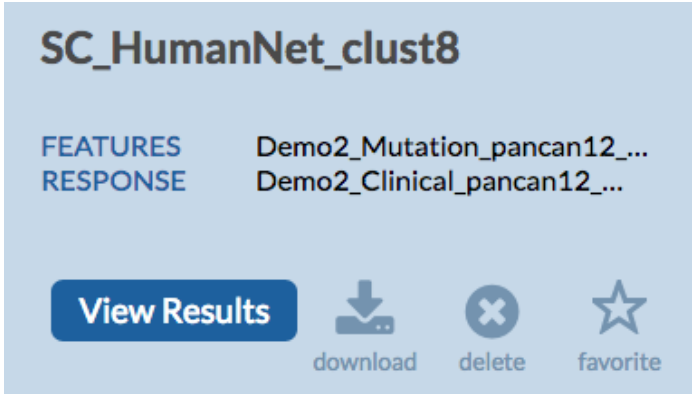
- Go to the "**Data**" page:




- Select "**SC_HumanNet_clust8**" (or any other name you chose)



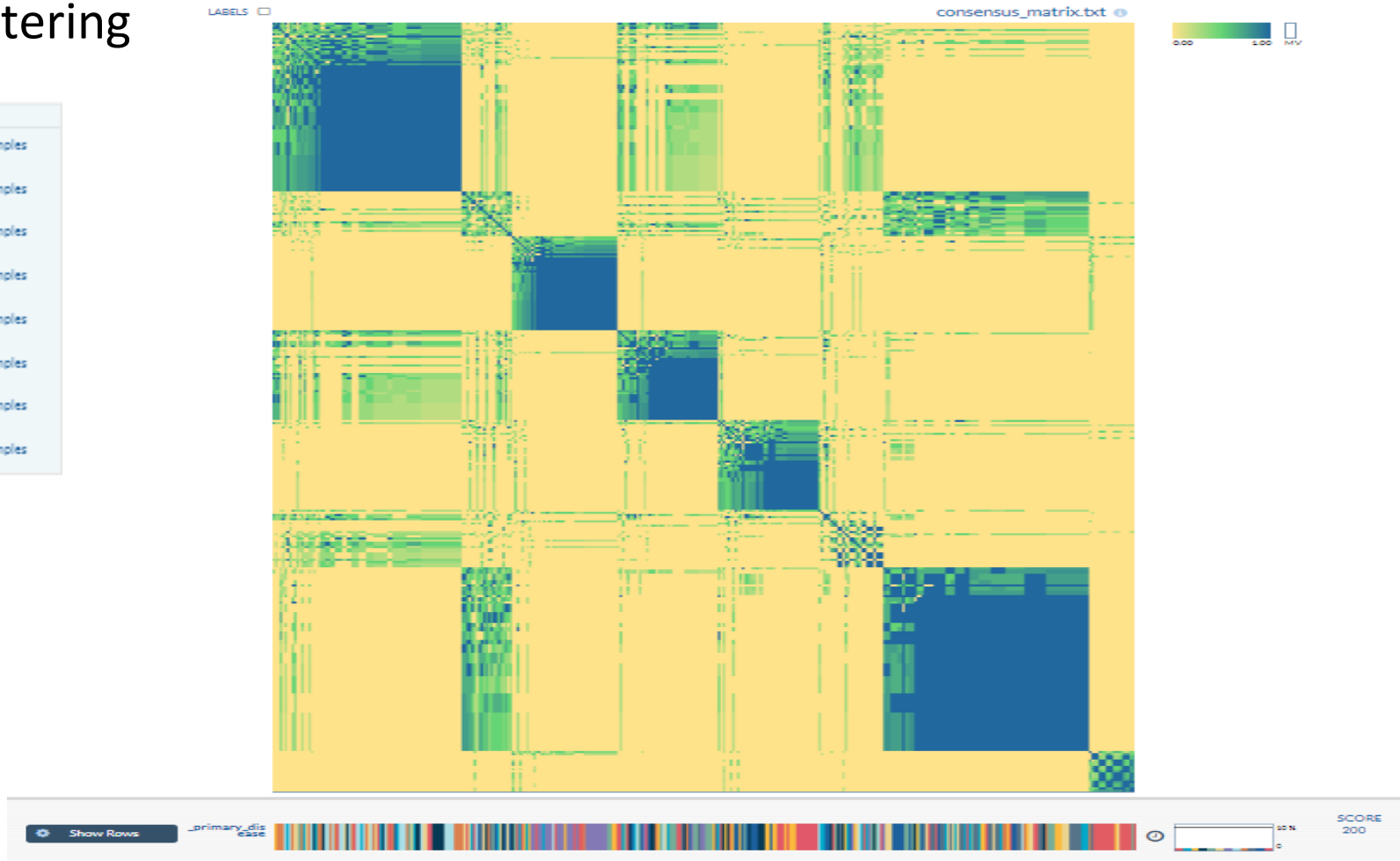

- Select "**View Results**" at the top right corner



# STEP 2D: Network Clustering Results

- As you can see from the sample distribution and the co-occurrence matrix, the network-guided approach provided a more balanced clustering

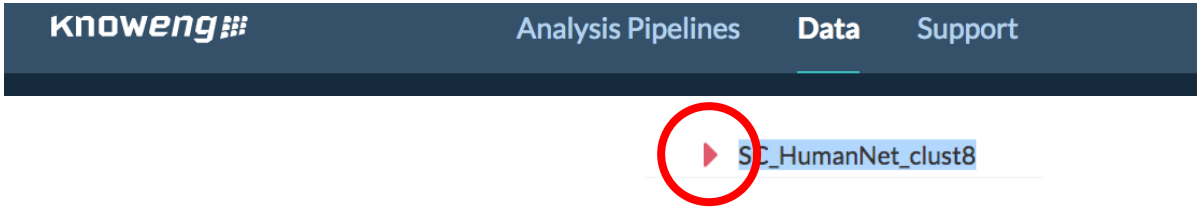
| Number of Samples | |
|---|---|
| 0 | -79 samples |
| 1 | -21 samples |
| 2 | -44 samples |
| 3 | -42 samples |
| 4 | -42 samples |
| 5 | -27 samples |
| 6 | -86 samples |
| 7 | -19 samples |



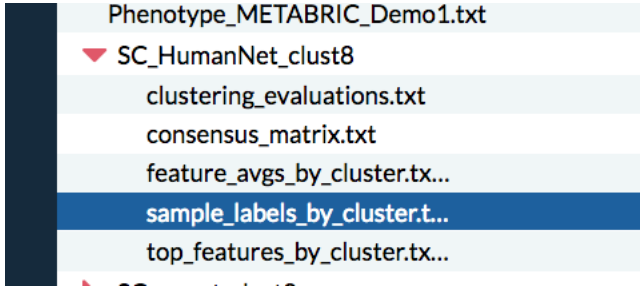
# STEP 2E: Compare in Spreadsheet Visualizer

To Prepare the Files:

- Go to the "**Data**" page




- Click on triangle by "**SC_HumanNet_clust8**" (or any other name you chose)



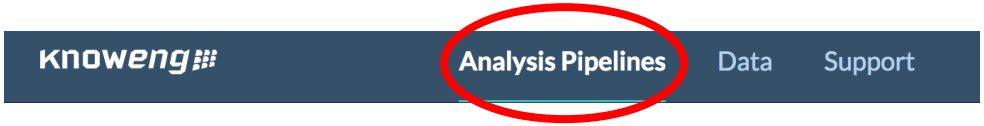

- Select "**sample_labels_by_cluster.txt**" results file of the network run

- Click on the name at the right top corner to edit and add "**_HumanNet**" to the end


- Repeat the same for the file in "**SC_nonet_clust8**" and add "**_nonet**" to the end

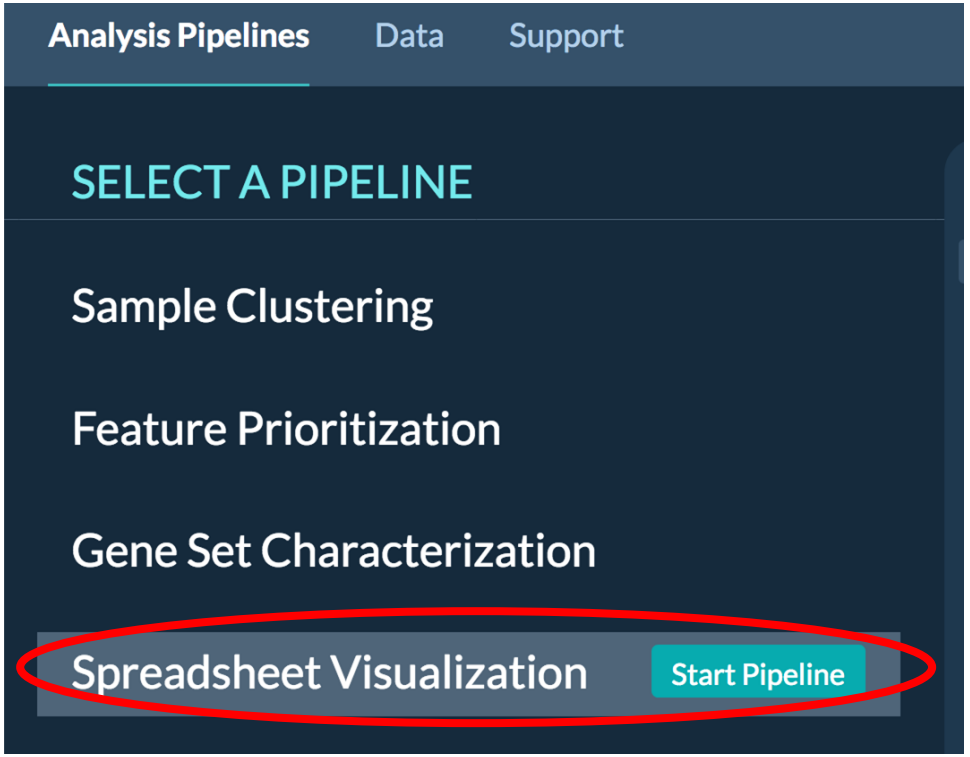
# STEP 2E: Compare in Spreadsheet Visualizer

- Let's compare the two runs in KnowEnG's Spreadsheet Visualization Tool



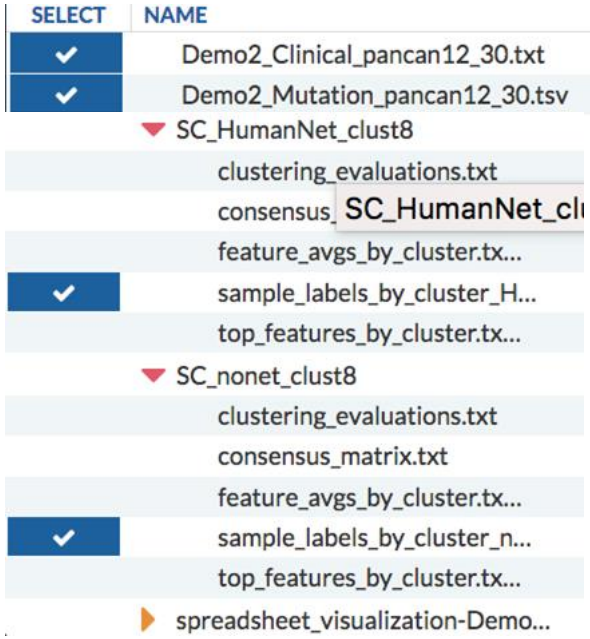

- Select "**Analysis Pipelines**"

- Select "**Spreadsheet Visualization**" and Click on "**Start Pipeline**"

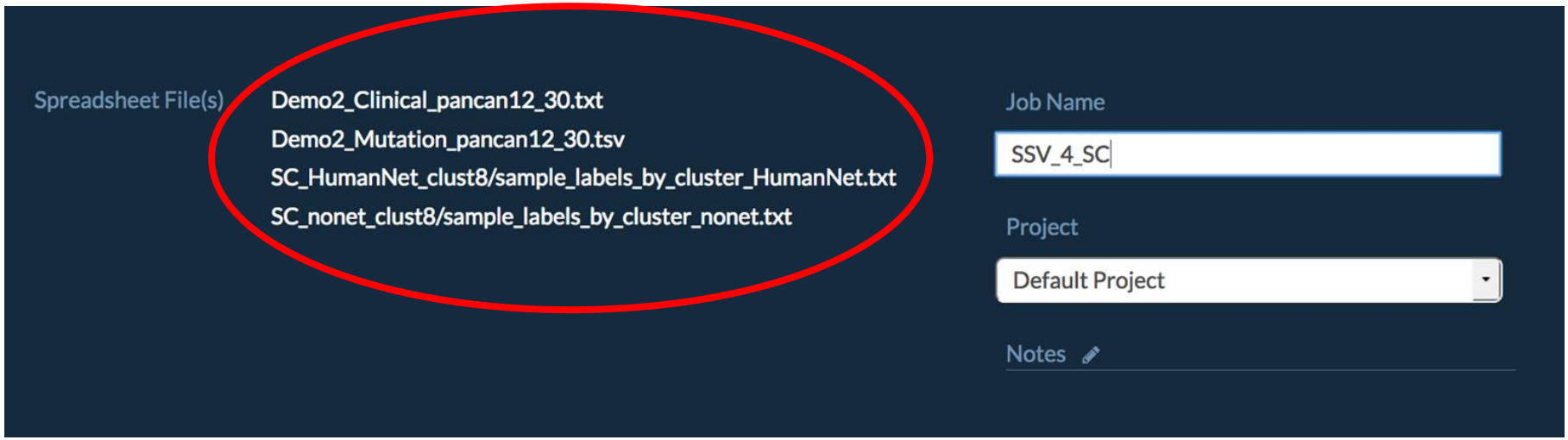


# STEP 2E: Compare in Spreadsheet Visualizer

- Select these four files to evaluate simultaneously and press **Next**:



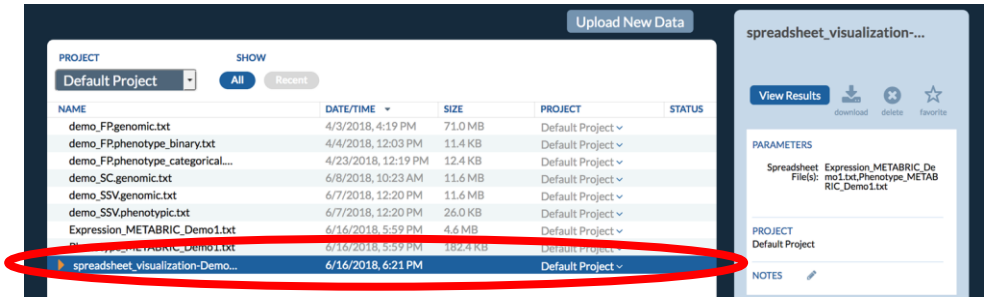

- Check the summary and change the job name if you like. Press **Submit** Job.



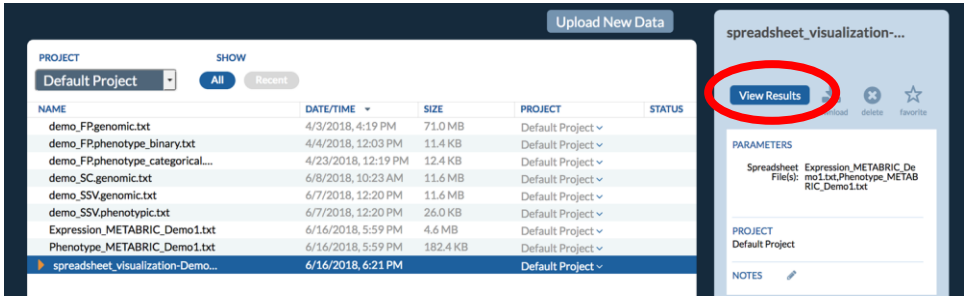
# STEP 2E: Compare in Spreadsheet Visualizer


The results:

- Select "**Go to Data Page**"



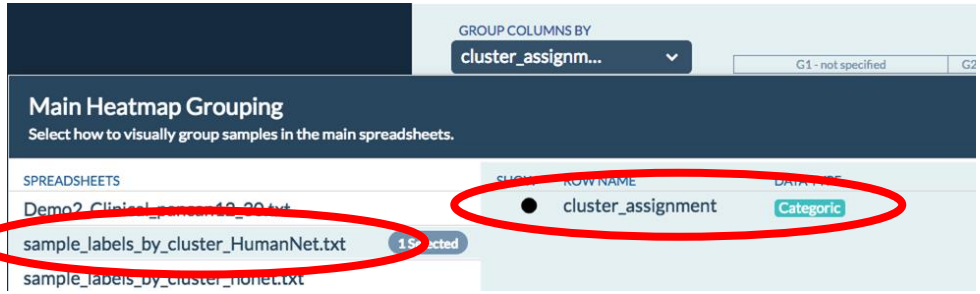

- Select the job you just ran



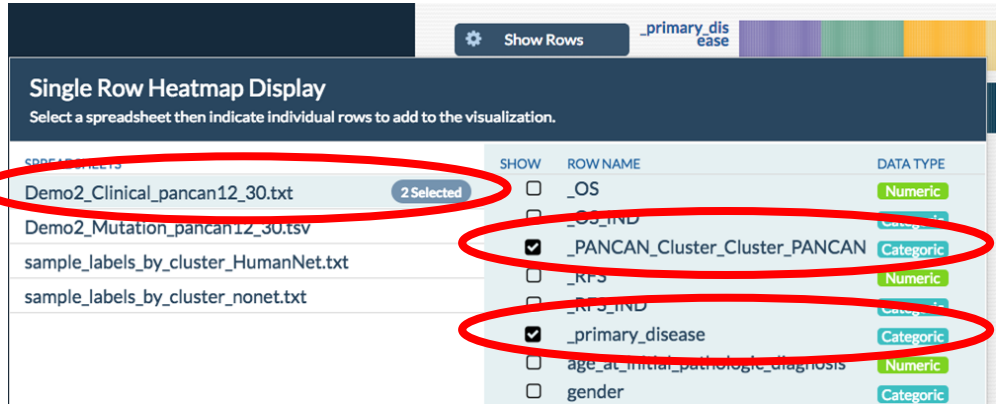

- Then "**View Results**"

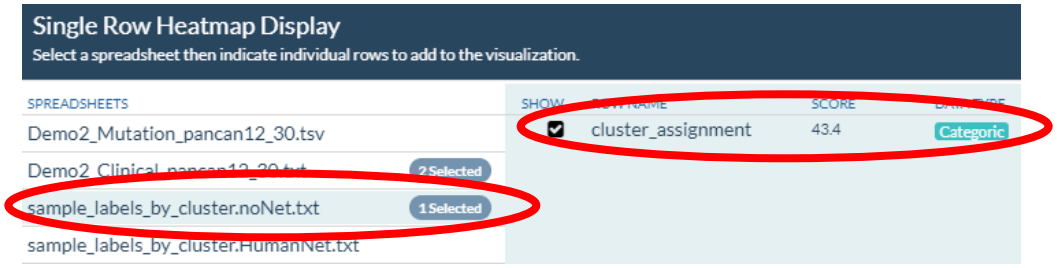
# STEP 2E: Compare in Spreadsheet Visualizer

- In the "**Group Columns By**" drop down click the "**sample_labels_by_cluster_HumanNet.txt**" network-guided clustering results file; then select "**cluster_assignment**"
- Click **"Done"**



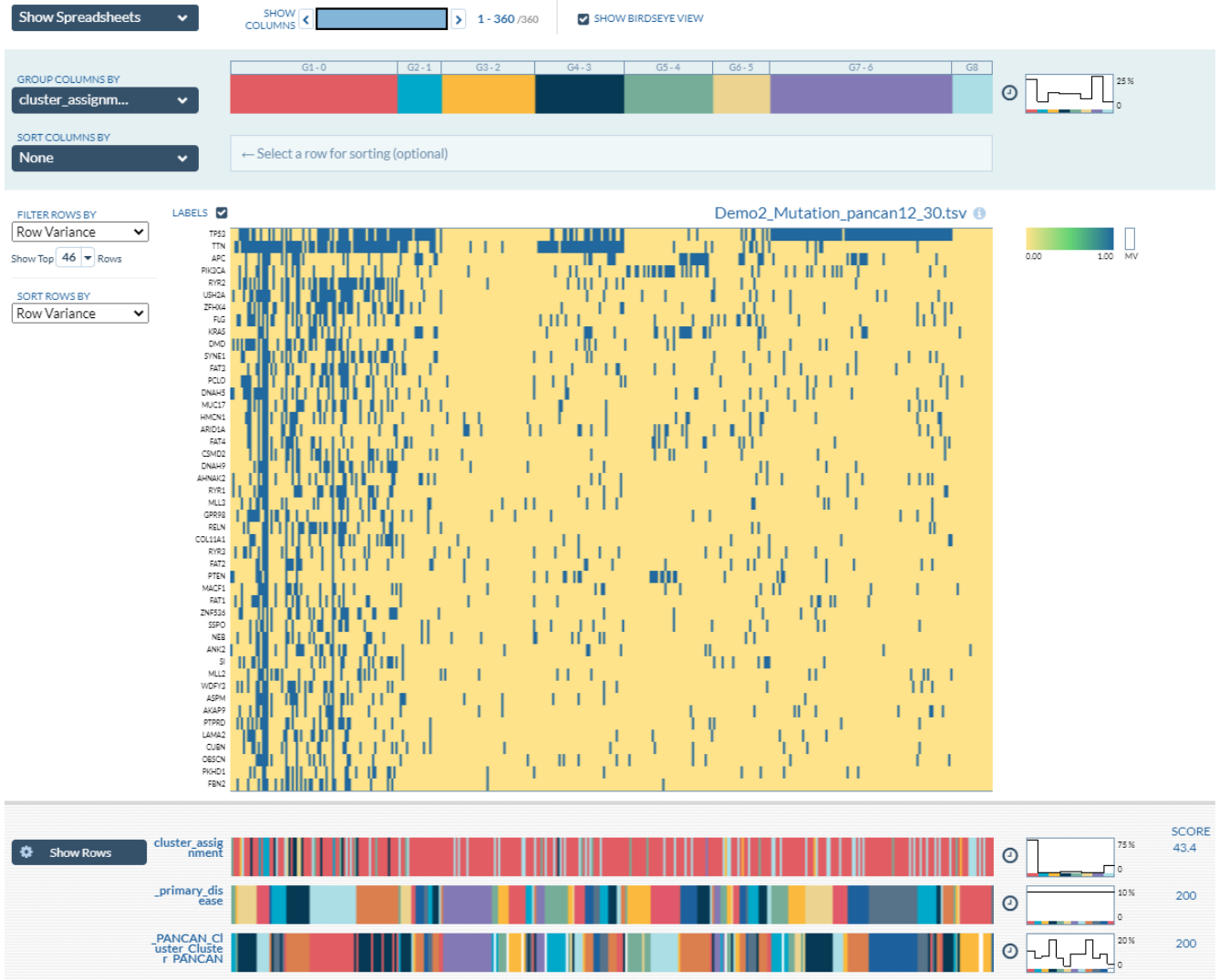

- By clicking on "**Show Rows**" at the very bottom of the page add the colorbars
  - "**_primary_disease**" and "**_PANCAN_Cluster_Cluster_PANCAN**" from "**Demo2_Clinical_pancan12_30.txt**" clinical data
  - and "**cluster_assignment**" from the "**sample_labels_by_cluster_noNet.txt**" standard clustering results file
  - Click **"Done"**



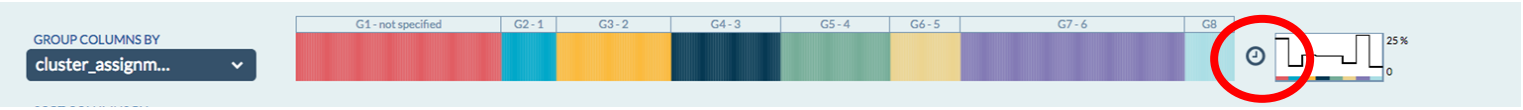
# STEP 2E: Compare in Spreadsheet Visualizer

- You can use this tool to explore top genes, draw Kaplan Meier curves, etc.

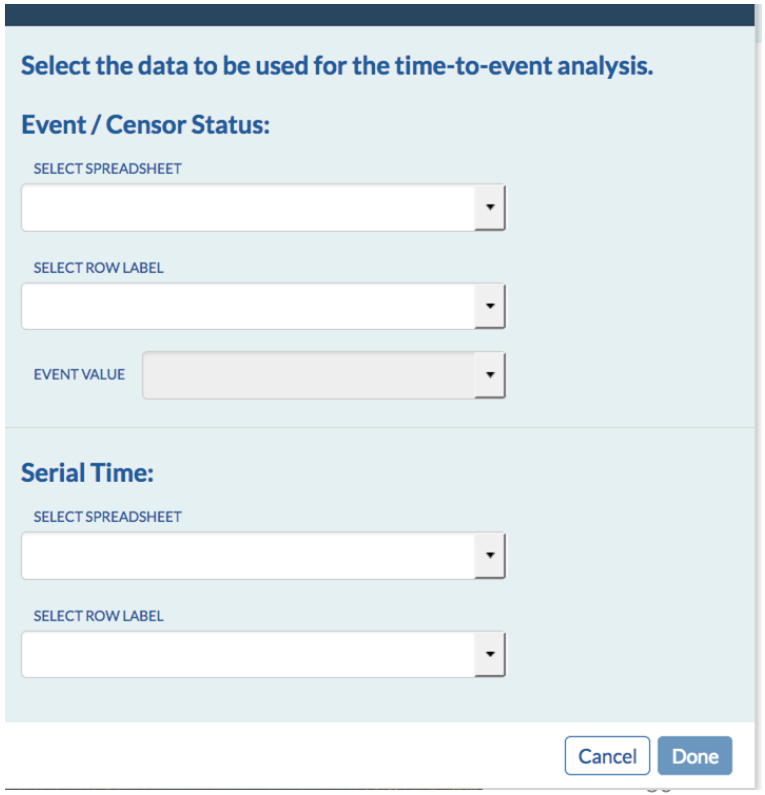


# STEP 2E: Compare in Spreadsheet Visualizer

- Click on the clock sign to perform Kaplan Meier survival analysis using the selected network-guided cancer subtypes

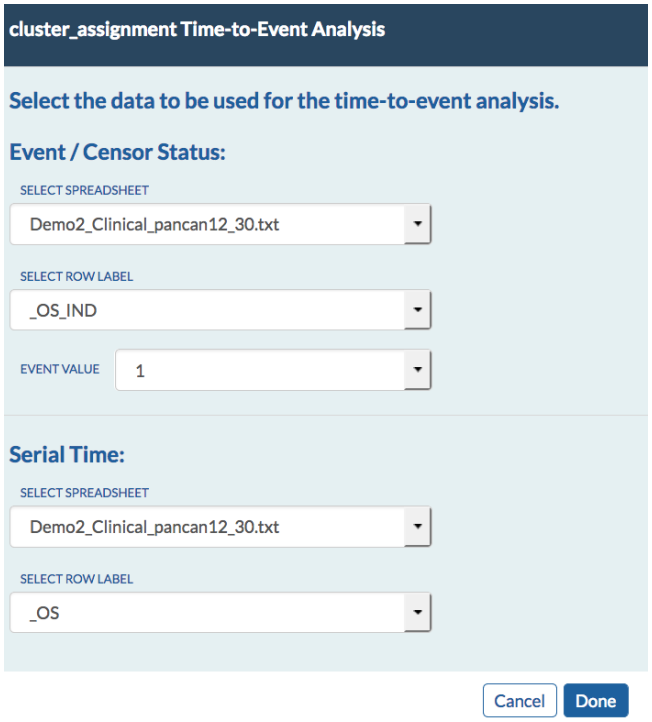


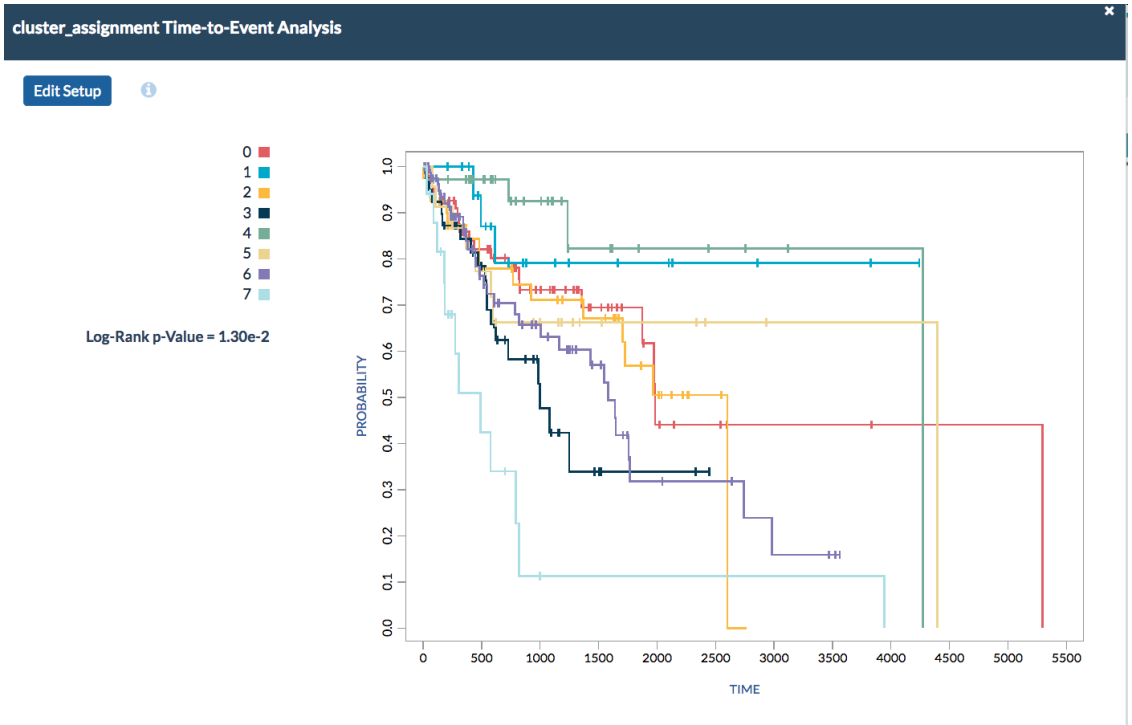

- Use this table to configure Kaplan Meier analysis by selecting the events and time to events



# STEP 2E: Compare in Spreadsheet Visualizer

- Select the parameters below and press **Done** to see Kaplan Meier curves of clusters identified using HumanNet network



# Finding Genes Correlated with Drug Response

In this exercise, we will use cell line gene expression data and cytotoxicity experiments with knowledge-guided methods to find genes that may predict drug response.

# STEP 3: Feature (Gene) Prioritization

- We will use KnowEnG's gene prioritization pipeline to perform network-guided feature (gene) prioritization
- The network-guided gene prioritization implemented in KnowEnG is a method called **ProGENI**:

Genome Biol. 2017 Aug 11;18(1):153. doi: 10.1186/s13059-017-1282-3.

**Knowledge-guided gene prioritization reveals new insights into the mechanisms of chemoresistance.**

Emad A[1], Cairns J[2], Kalari KR[3], Wang L[4], Sinha S[5].

- We will use samples from the Cancer Cell Line Encyclopedia (CCLE)

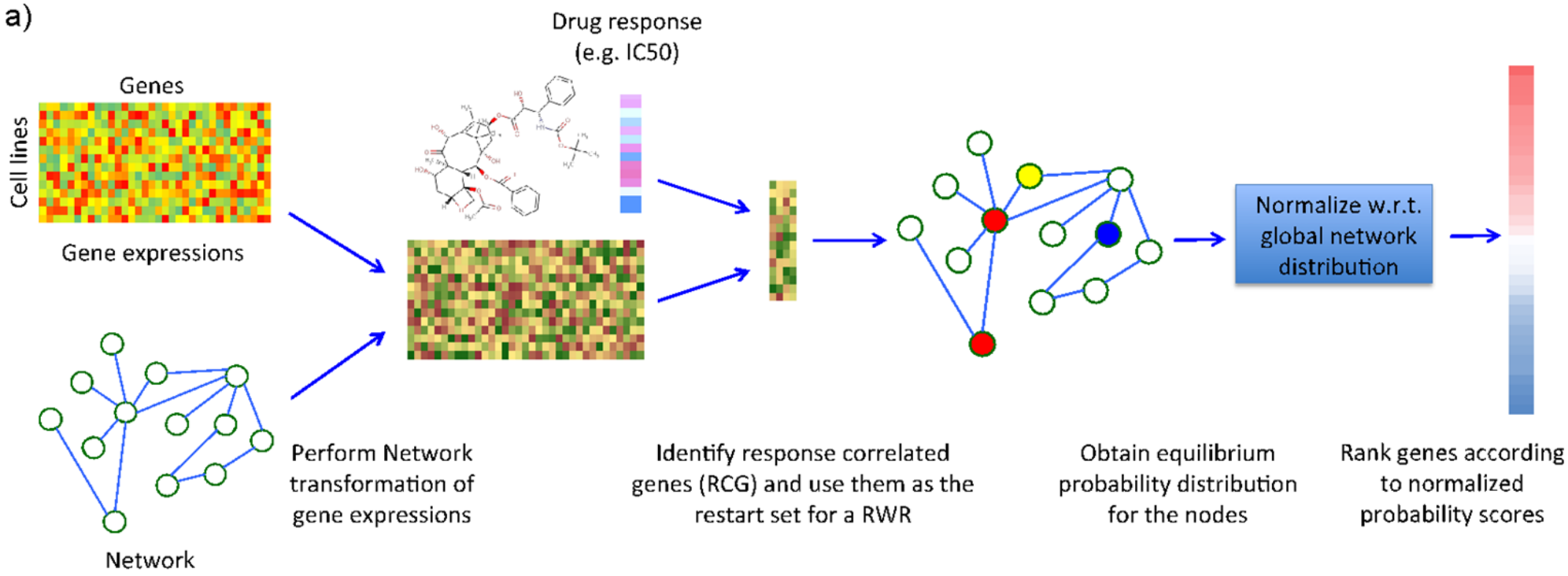
Nature. 2012 Mar 28;483(7391):603-7. doi: 10.1038/nature11003.

**The Cancer Cell Line Encyclopedia enables predictive modelling of anticancer drug sensitivity.**

Barretina J[1], Caponigro G, Stransky N, Venkatesan K, Margolin AA, Kim S, Wilson CJ, Lehár J, Kryukov GV, Sonkin D, Reddy A, Liu M, Murray L, Berger MF, Monahan JE, Morais P, Meltzer J, Korejwa A, Jané-Valbuena J, Mapa FA, Thibault J, Bric-Furlong E, Raman P, Shipway A, Engels IH, Cheng J, Yu GK, Yu J, Aspesi P Jr, de Silva M, Jagtap K, Jones MD, Wang L, Hatton C, Palescandolo E, Gupta S, Mahan S, Sougnez C, Onofrio RC, Liefeld T, MacConaill L, Winckler W, Reich M, Li N, Mesirov JP, Gabriel SB, Getz G, Ardlie K, Chan V, Myer VE, Weber BL, Porter J, Warmuth M, Finan P, Harris JL, Meyerson M, Golub TR, Morrissey MP, Sellers WR, Schlegel R, Garraway LA.

# STEP 3: Gene Prioritization

## Outline of ProGENI:

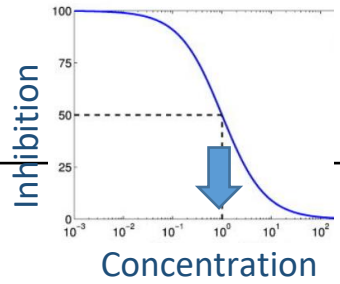


# STEP 3: Gene Prioritization

**Find the files in this slide under [course_directory]/08_Clustering_and_Prioritization**

Dataset characteristics:

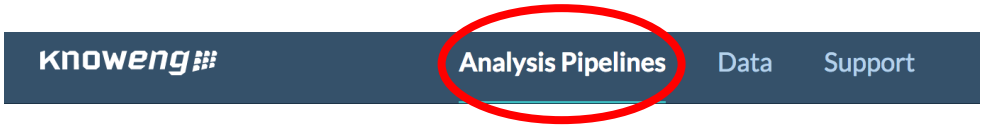
| Name | Description |
|---|---|
| demo_FP.genomic | A matrix of (gene x samples) containing the expression of ~17k genes in ~500 cell lines. The expression profiles are normalized in advance. |
| demo_FP.phenotypic | A matrix of (samples x drugs) containing IC50 values for 24 cytotoxic treatments. |



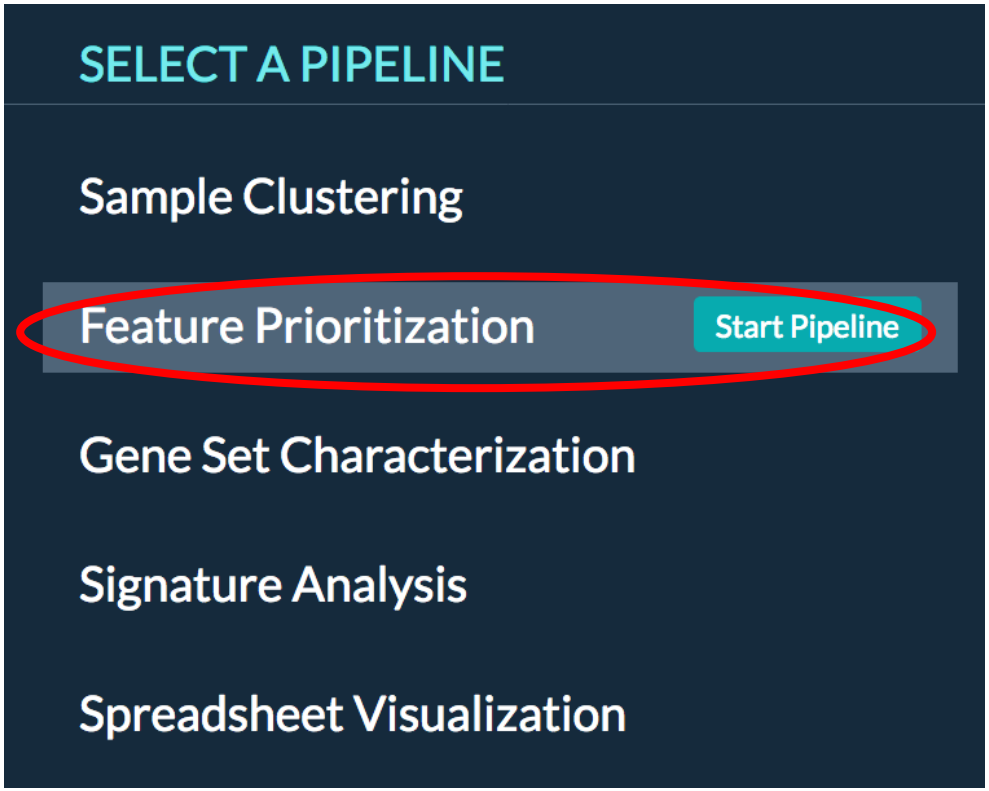
# STEP 3A: Gene Prioritization (network-guided)

Select the pipeline:

- Select "**Analysis Pipelines**" at the top of the page




- Select "**Feature Prioritization**" and Click on "**Start Pipeline**"

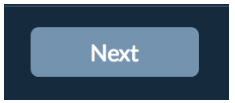


# STEP 3A: Gene Prioritization (network-guided)

Configure the pipeline:

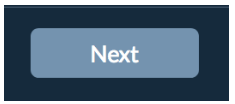
- For the "**omics**" file select "**Use Demo Data**"


- Click "**Next**" at the bottom right corner



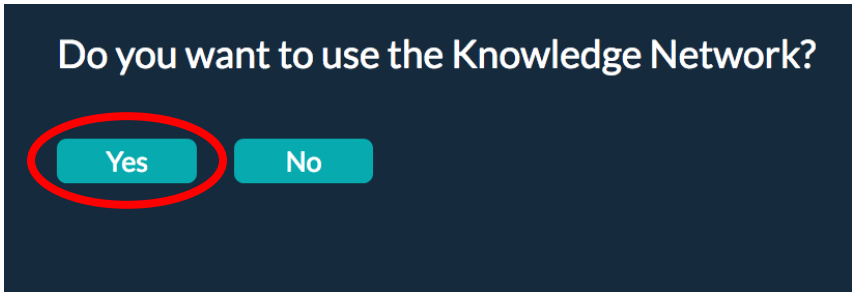

- For the "**response**" file select "**Use Demo Data**"

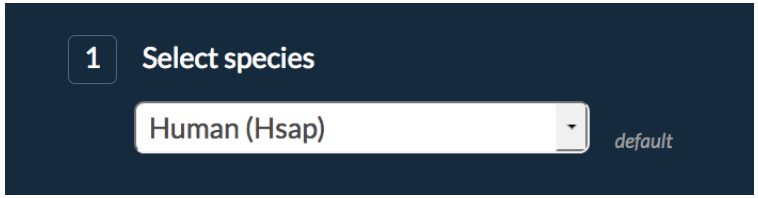

- Click "**Next**" at the bottom right corner

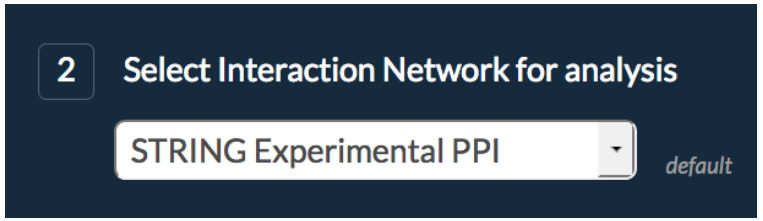


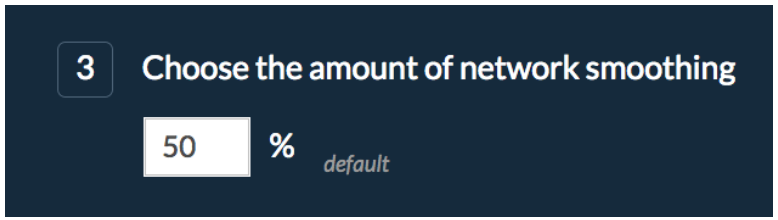
# STEP 3A: Gene Prioritization (network-guided)

- Select "**Yes**" in response to using the knowledge network:
  - *This allows us to perform network-guided prioritization (**ProGENI**)*
- Keep the species as "**Human**"
- Select "**STRING Experimental PPI**" as the network
  - *This network connects genes by the physical protein-protein interactions between their corresponding proteins*
- Keep network smoothing at **50**%:
  - *This controls how much importance is put on network connections instead of the correlation with drug response*



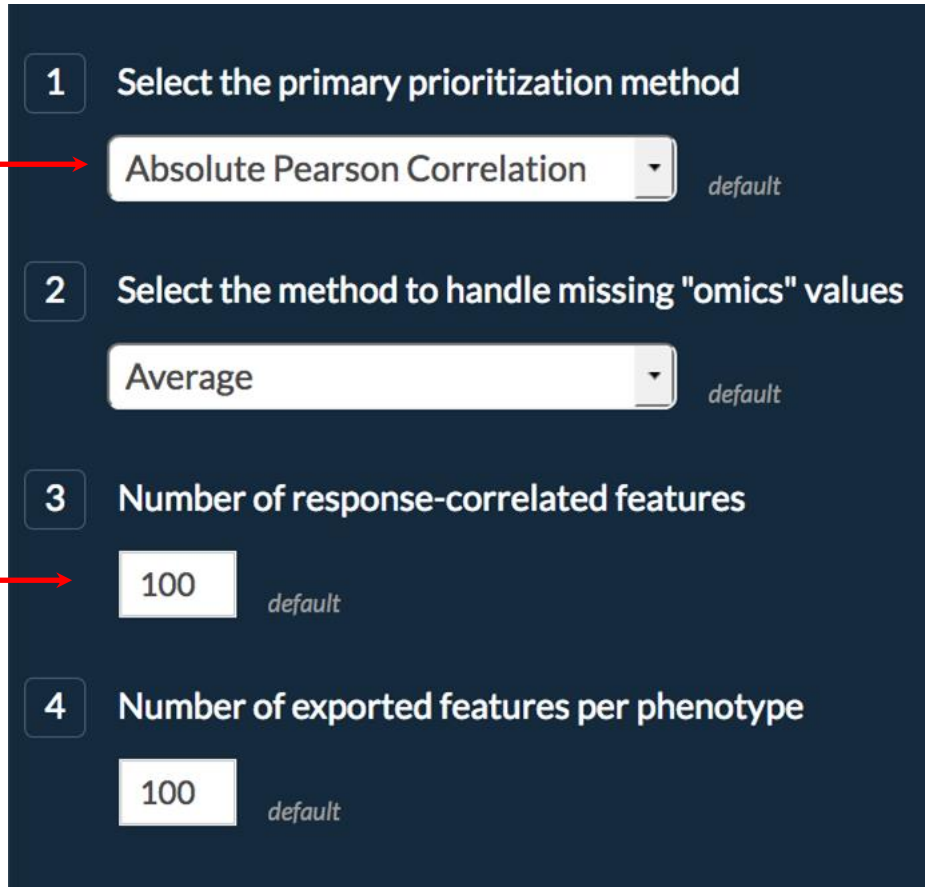



# STEP 3A: Gene Prioritization (network-guided)

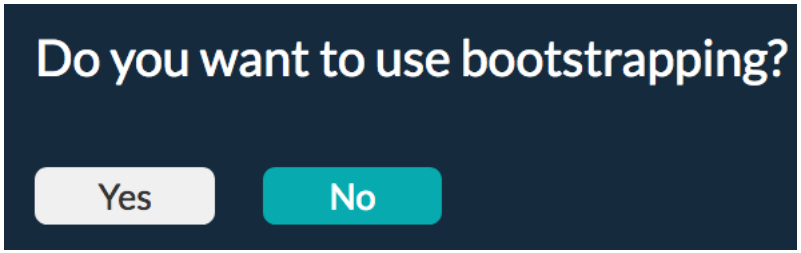
- Keep the default parameters on this page

Used for continuous-valued response



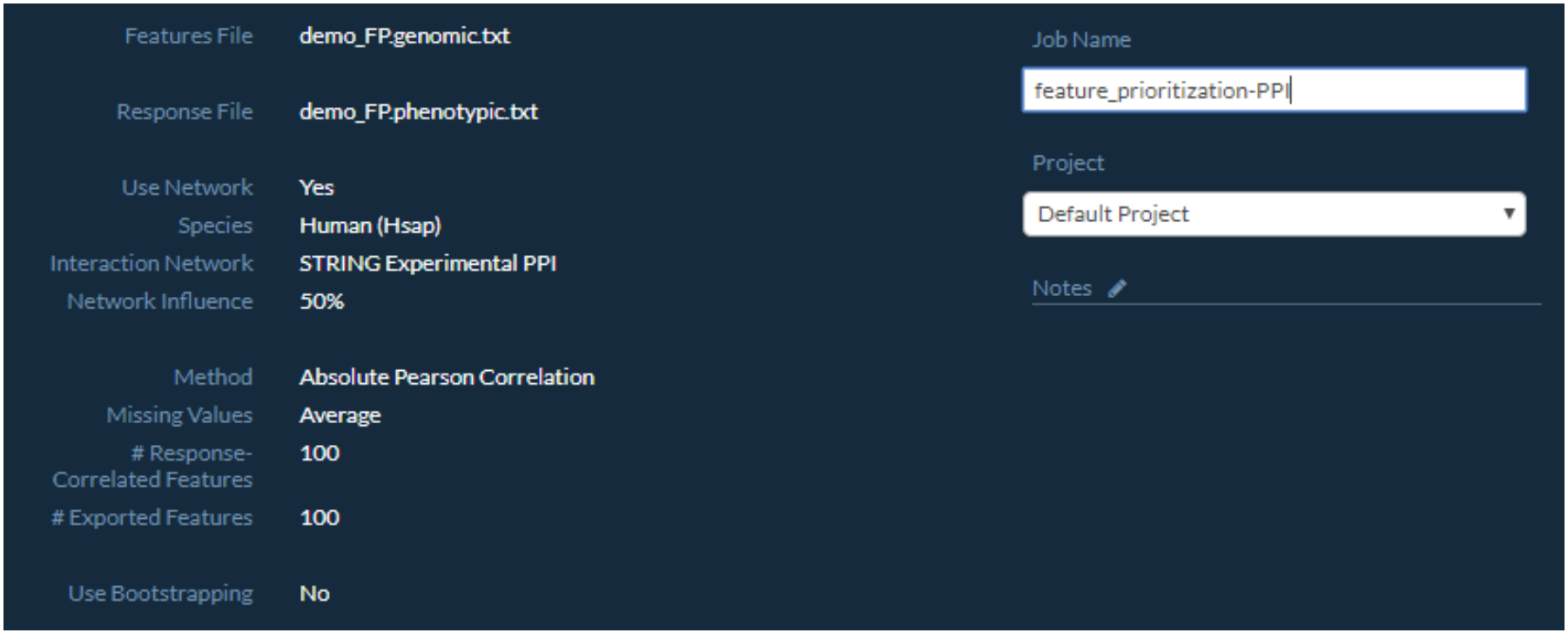

Size of RCG set

- Choose "**No**" for bootstrapping



# STEP 3A: Gene Prioritization (network-guided)

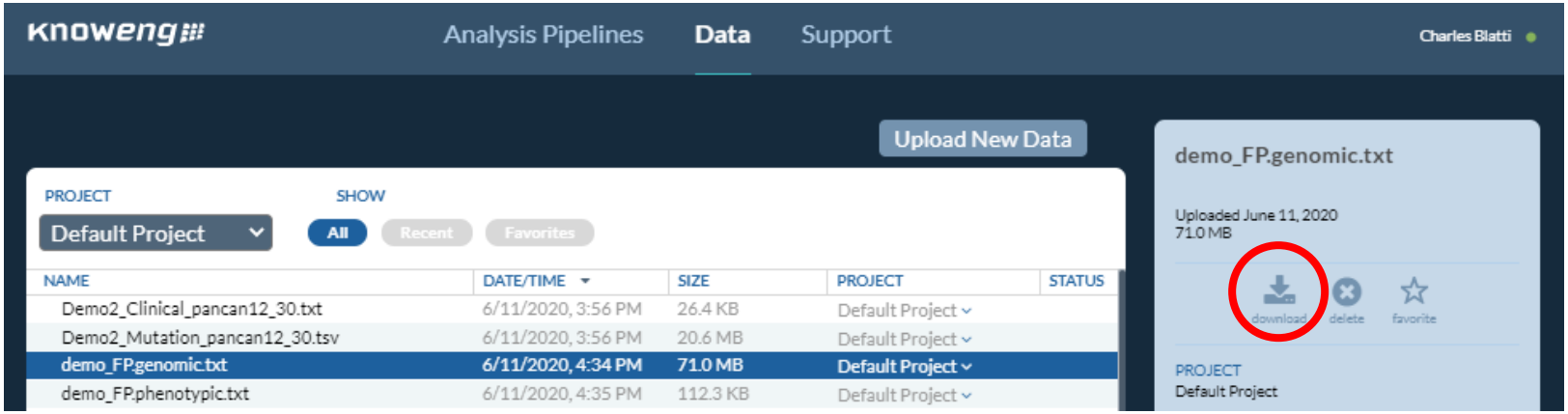
- Review the summary of the job and change its name if you like




- **Submit** the job

# STEP 3A: Gene Prioritization (network-guided)

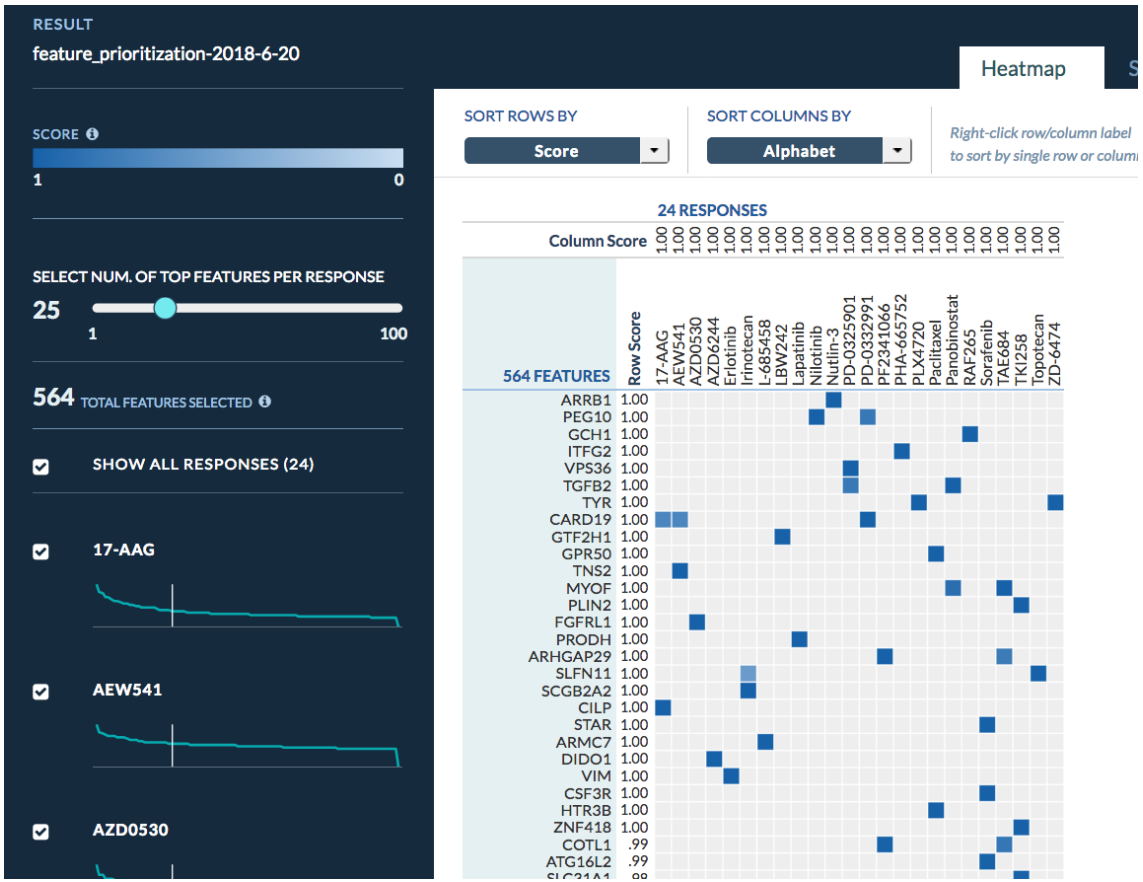
- **Note**: If you ever want to view your data or results outside of the KnowEnG system, just go to the Data page, click on the file or run, and select "download" on the far right panel.




- **Reminder**: The option to view results will not be available if
  - The job is still running:
  - There was an error:
    - If there's an error, repeat the launch steps and check your job summary matches exactly
- *This job takes about five minutes, so you are welcome to skip ahead and start Step 4 on slide 55 and come back later to finish Step 3*

# STEP 3A: Gene Prioritization (network-guided)

- Go to the **Data** page
- Select "**View Results**" when the job is done




Heatmap shows the top gene rows identified for each drug column

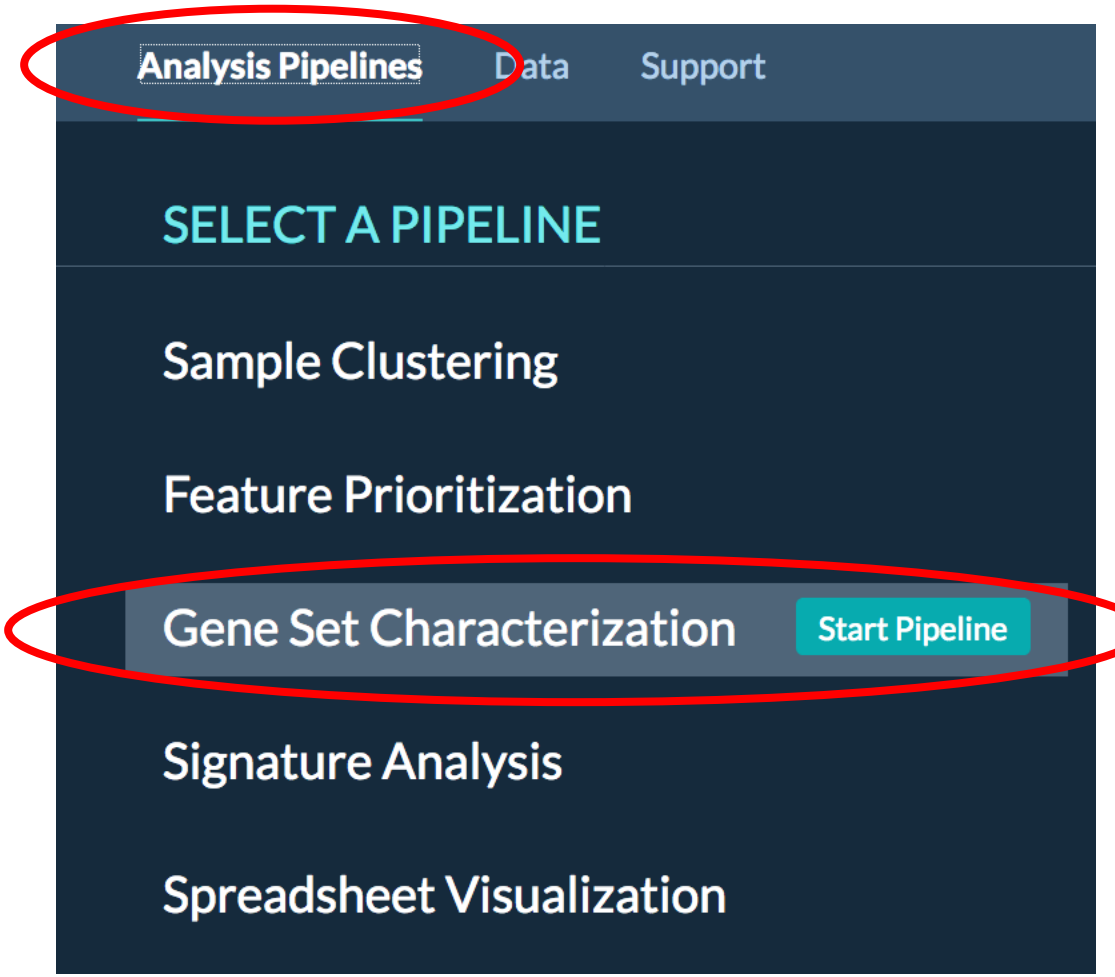
# STEP 3A: Gene Prioritization (network-guided)

- You can “right-click” on a **drug column name** to sort rows and see its top genes


- You can also sort columns by a gene to see drugs for which the gene was among the top list
- *Panobinostat (HDAC inhibitor) prevents chromatin formation which is tied to the transforming growth factor beta signaling pathway (TGFβ2 is top result).*

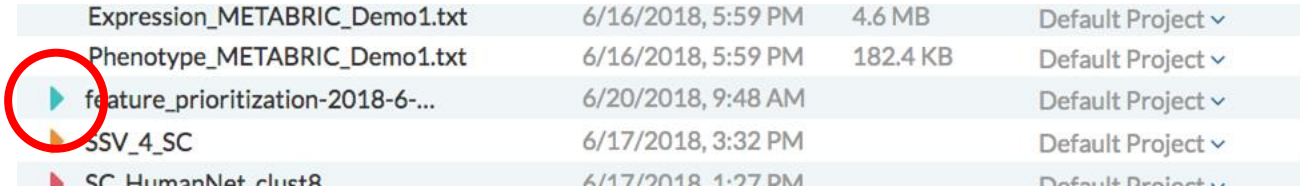
# STEP 3B: Gene Prioritization (network-guided)

- Let's see the enrichment of the top genes in different Gene Ontology (GO) terms
- Go to "**Analysis Pipelines**" page
- Select "**Gene Set Characterization**" pipeline

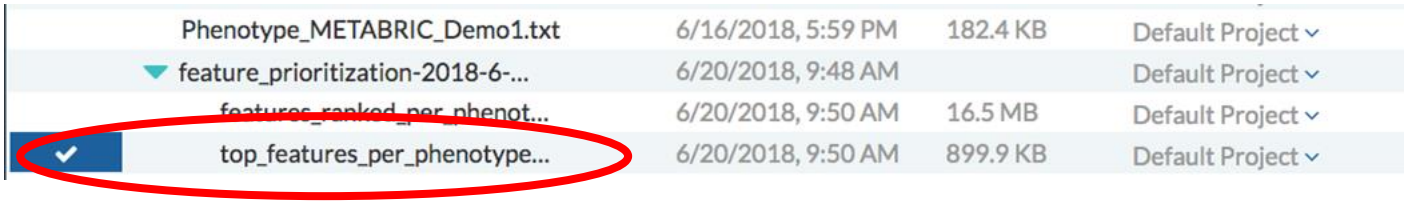


# STEP 3B: Gene Prioritization (network-guided)

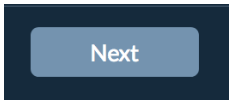
- Select the green triangle by the gene prioritization job you ran




- Select "**top_features_per_phenotype_matrix**" which contains the ProGENI top gene lists for each of the 24 drugs

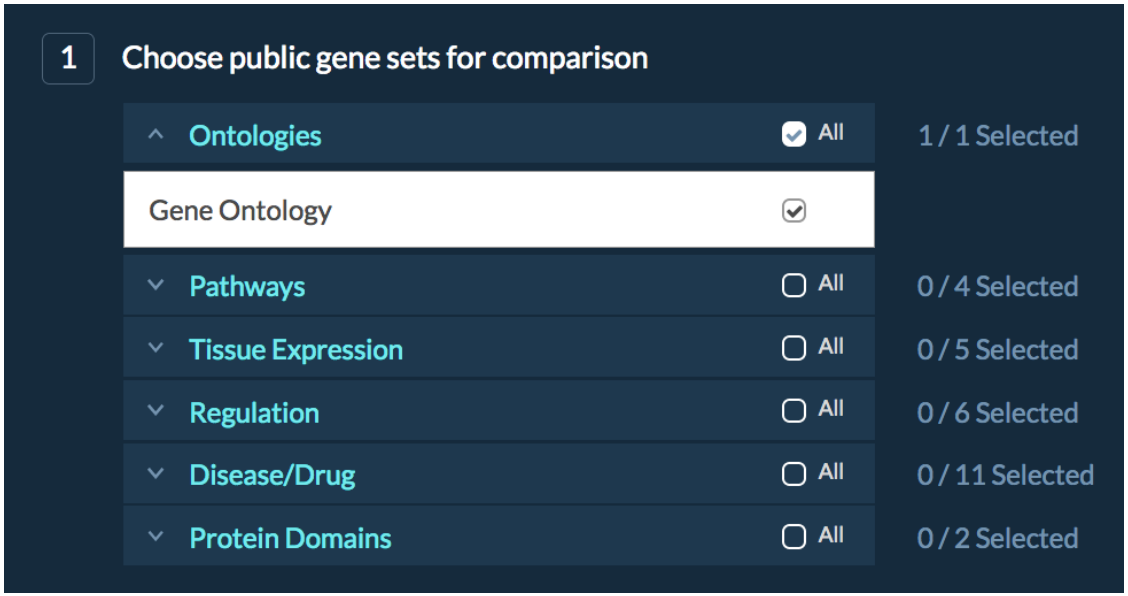



- Press **Next**

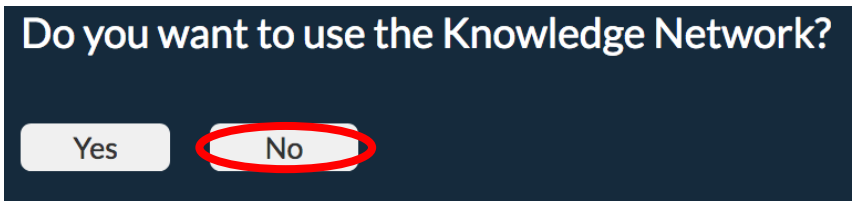


# STEP 3B: Gene Prioritization (network-guided)

- For gene sets, select your gene sets of interest (e.g. GO) and press **Next**



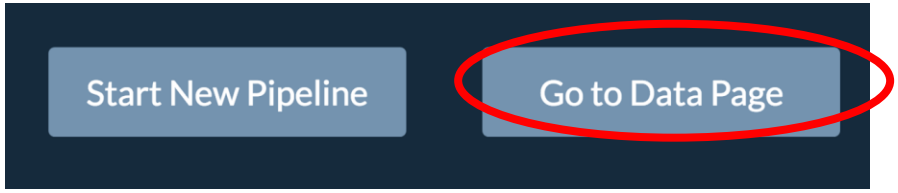

- Say "**No**" to using the knowledge network (we will do that later) and press **Next**. Then press **Submit** Job.
  - *This job should take about one minute.*



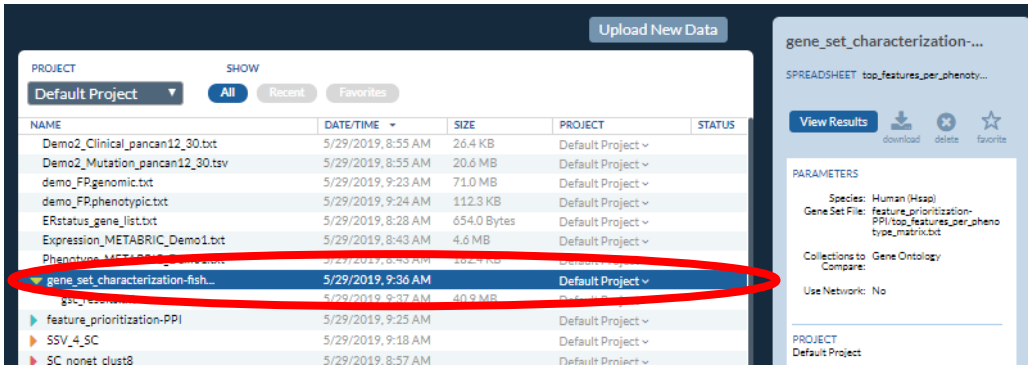
# STEP 3B: Gene Prioritization (network-guided)

The Gene Ontology enrichment results:

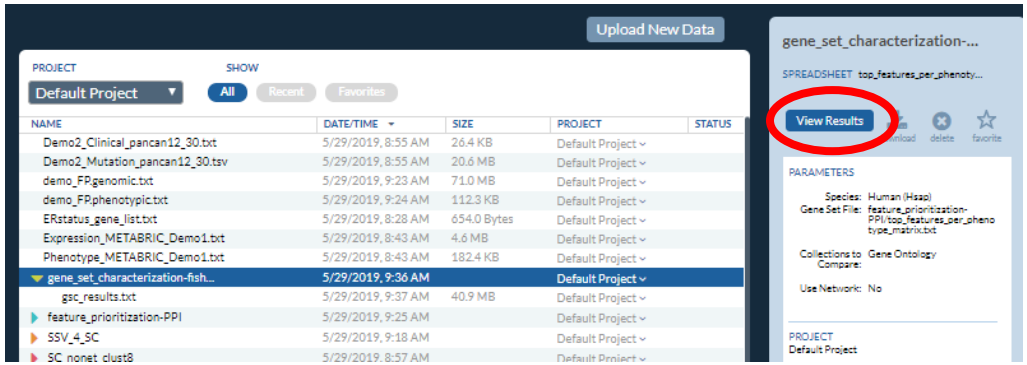
- Select "**Go to Data Page**"




- Select the job you just ran

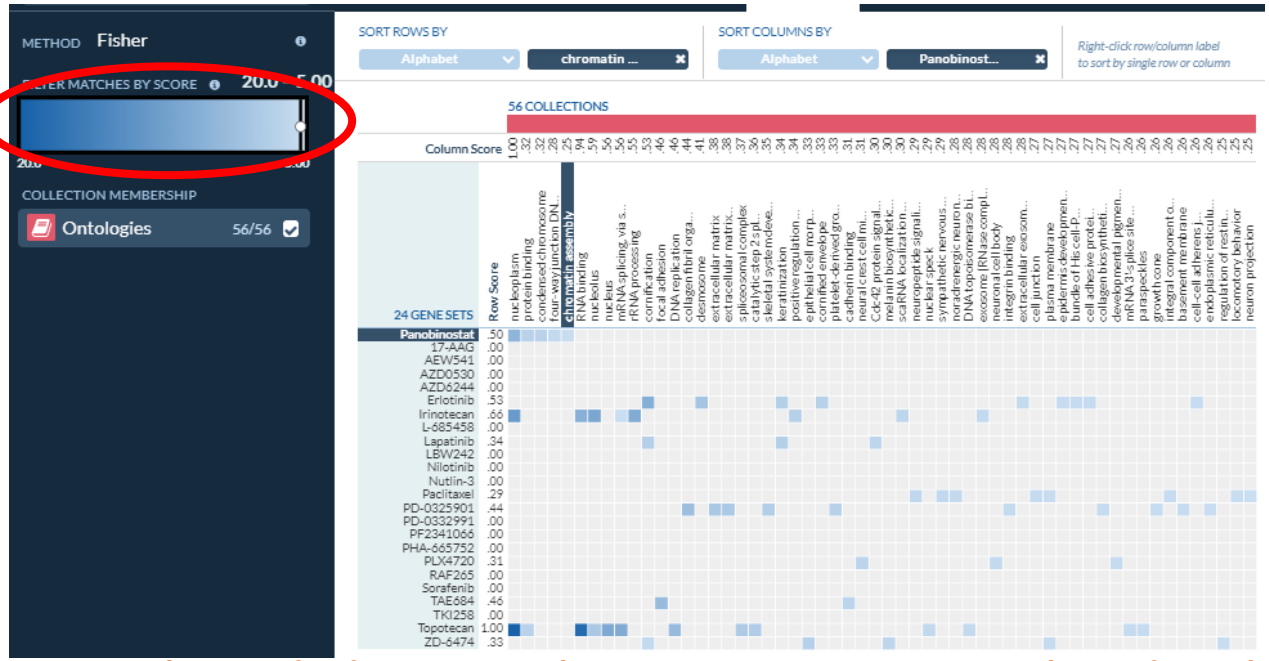



- Then "**View Results**"



# STEP 3B: Gene Prioritization (network-guided)

- This page shows the enriched GO gene sets for each drug to gene list
- You can change the filter (scores represent –log10 (p-value) of enrichment) to see fewer or **more** enriched gene sets




- *The network-guided genes whose expression correlated with the response to Panobinostat are enriched with terms related to chromatin assembly*

# Creating a Novel Gene Expression Signature

In this exercise, we will use the integrative iLINCS data portal to extract gene expression data from TCGA Breast Invasive Carcinoma (BRCA) samples and build a gene signature based on the estrogen receptor status.

# Step 4: Perturbagen and Disease Datasets

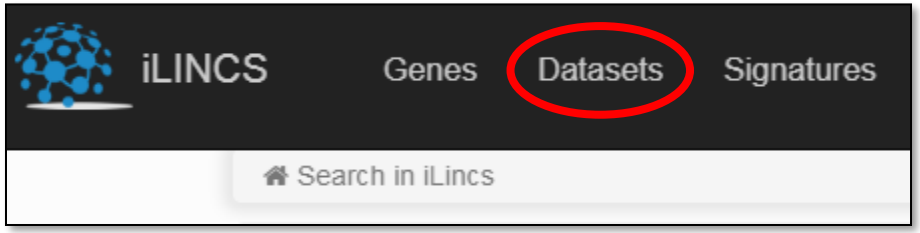
- Open your web browser and go to the iLINCS data portal: http://www.ilincs.org/ilincs/

- This portal, curated by the LINCS Data Coordination and Integration Center, contains transcriptomic and proteomic datasets from the many LINCS affiliated projects, including the LINCS L1000 assay. It also contains several other large public datasets of perturbations to cell lines and samples of disease.

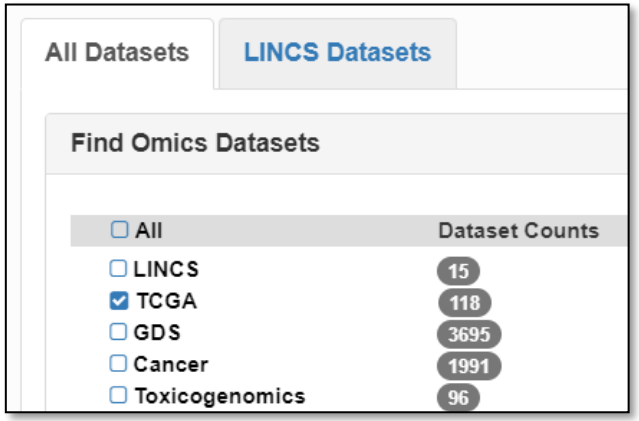
- We will define a custom gene signature from TCGA data and see how it can be used in various network related analyses.

# Step 4A: Select Breast Cancer Dataset

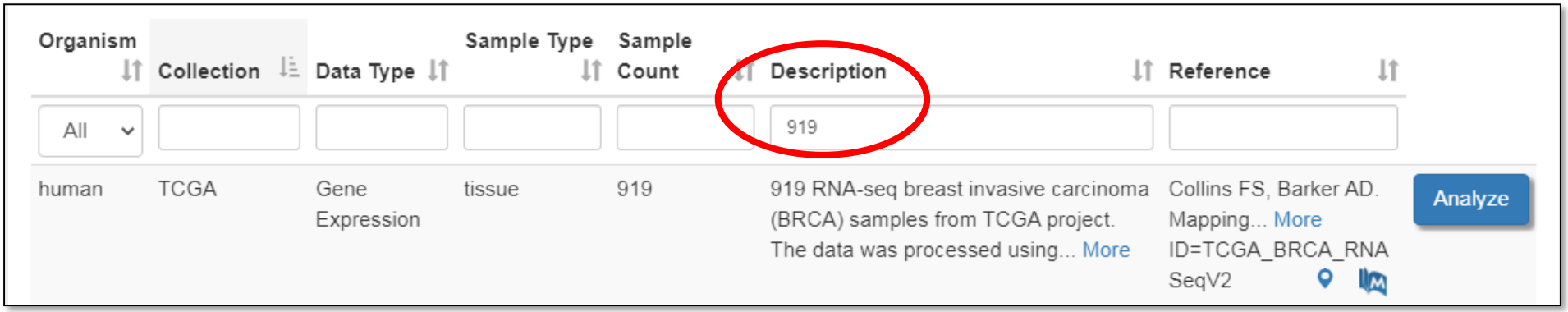
- Click on "**Datasets**" in the header




- In the "**All Datasets**" tab, for Choose Dataset, select only the **TCGA** datasets

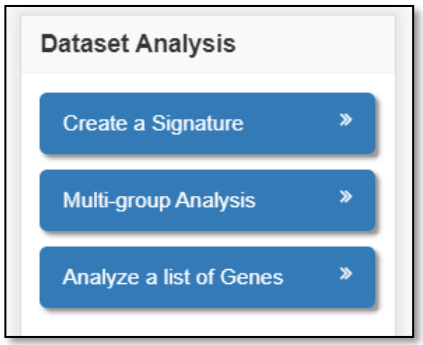


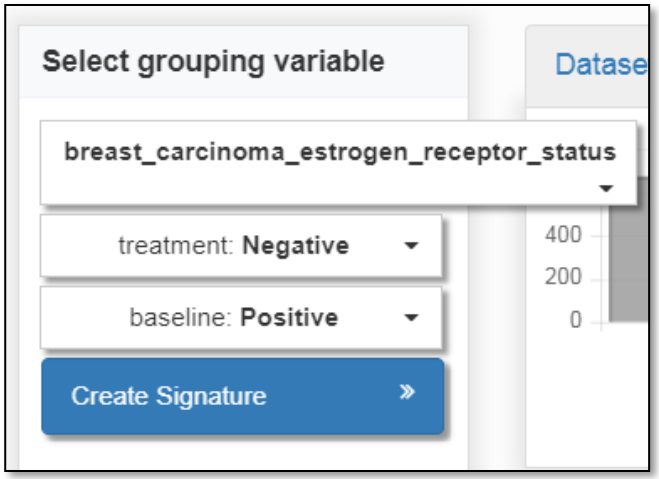

- Scroll down and enter "**919**" on the Description box to find "**919 mRNA-seq breast invasive carcinoma (BRCA) samples from TCGA project**" by Collins, et al. Click "**Analyze**".

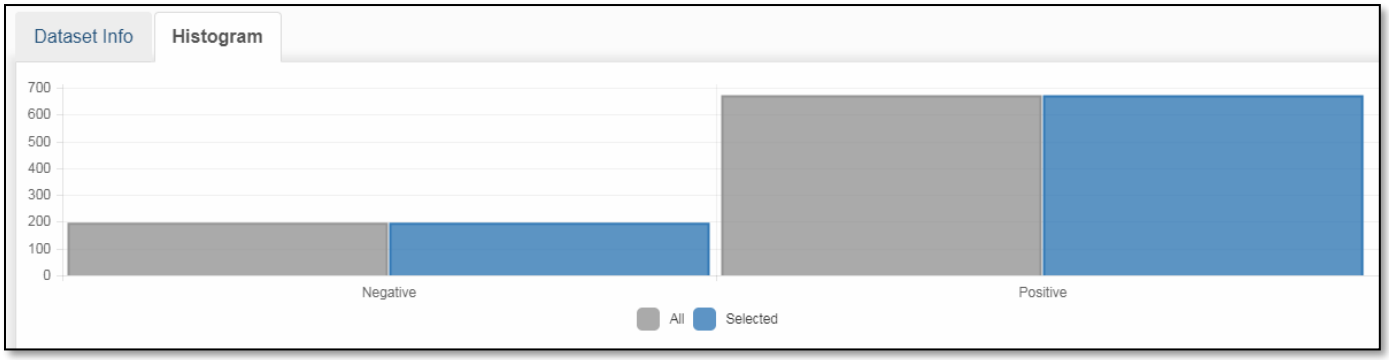


# Step 4B: Creating a Novel Gene Signature

- Click on "**Create a Signature**"
- In "**Select grouping variable**" dropdown select "**breast_carcinoma_estrogen_receptor_status**"
- In "**Select group 1**" dropdown select "**Negative**"
- In "**Select group 2**" dropdown select "**Positive**"
- Finally, click on "**Create Signature**" button

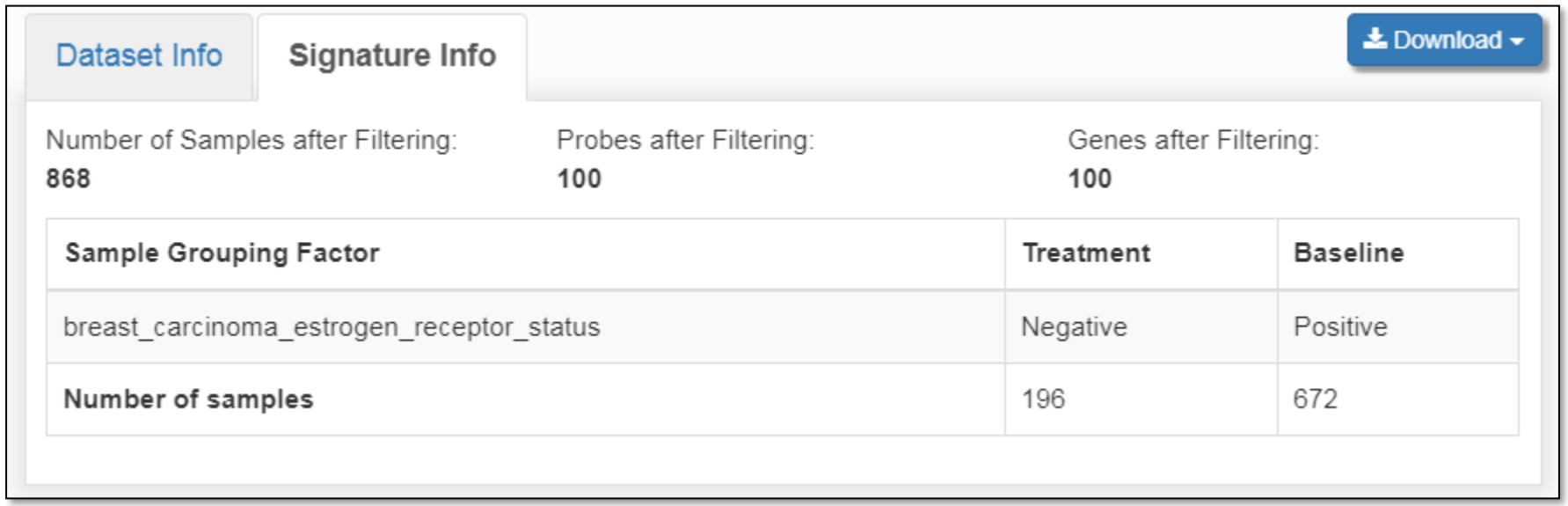


# Step 4B: Our ER Status Gene Signature

- When the signature is calculated, a quick summary of the number of samples from each group is presented in the "**Signature Info**" tab




Next, we will look more closely at the genes involved in our signature.

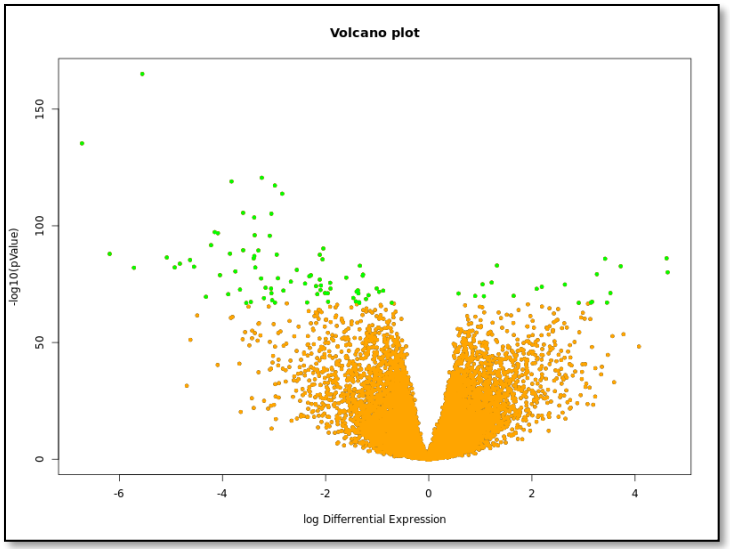
# Step 4C: Examining Gene Expression of our Signature

- To get statistics about how the signature is defined, we will select "**Modify the list of selected genes**" on the left

- We are presented with a volcano plot for the log fold change (x-axis) and differential expression significance (y-axis) of each gene.

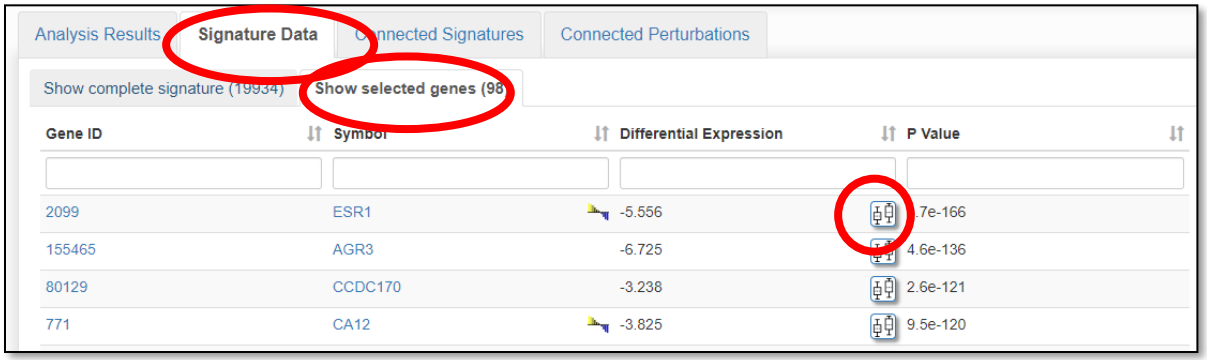
- The green genes are the ones included in the gene expression signature.

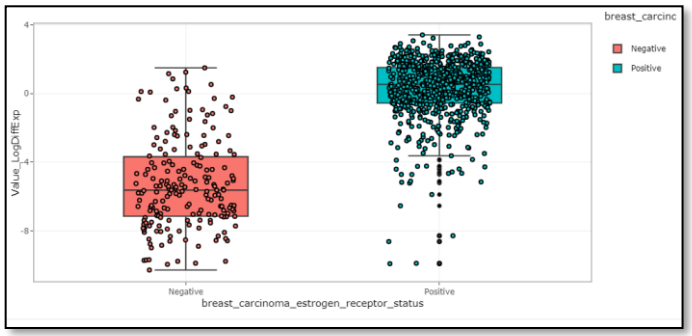
- They have a log Differential Expression of at least +/- 0.6 and significance p-value less than 10^-60.



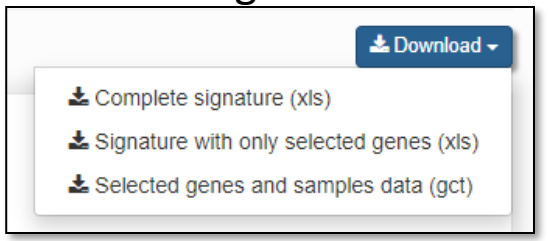
# Step 4C: Examining Gene Expression of our Signature

- Click the "**Signature Data**" tab and "**Show selected genes**" to see the list of selected signature genes




- Note that ESR1, estrogen receptor 1, is the most significantly differentially expressed gene, which is consistent with the immunohistochemical staining assay result that defined the positive and negative groups. Click on the **plot icon** on the right to see ESR1's measured gene expression values.
- Because of the number of samples (868) is high, the differential expression p-values are very significant for these top signature genes
- You can click the "**Download** " button and save "**Signature with only selected genes**" table as an Excel file if you want to save the details of the 100 selected genes. We will use part of this file later.



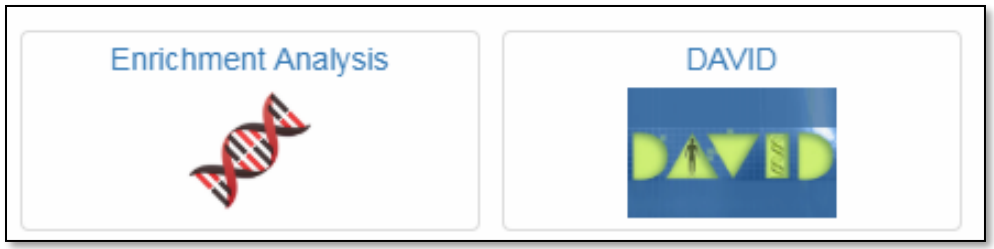
# Discovering Pathways Related to Our Gene Signature

In this section, we will consider some of the characterization resources that are available for gene signatures and gene sets.

# Step 5: Standard Gene Set Enrichment

- Back in iLINCS, the "**Analysis Results**" tab which contains many different methods for analyzing our novel ER status gene signature.

- Two of the tools listed are links to **Enrichr** and **DAVID**.



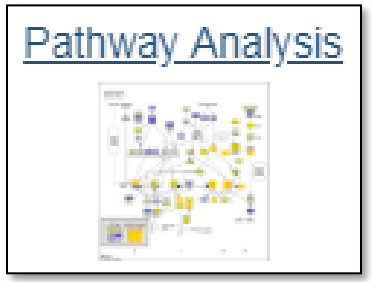

- Both tools use standard statistical enrichment tests to examine the overlap of the 100 genes of our ER status gene signature with Gene Ontology term annotations, pathways, and other gene sets.

- These tools output the results in slightly different ways, so you may want to explore them in your own time.

# Step 5A: Pathway Network Enrichment Test

- Signaling Pathway Enrichment Analysis (**SPIA**) is a method for assessing the impact of a gene set on a pathway. It combines standard enrichment p-values with network perturbation based p-values.

- Click on "**Pathway Analysis**" in the "**Analysis Results**" tab.

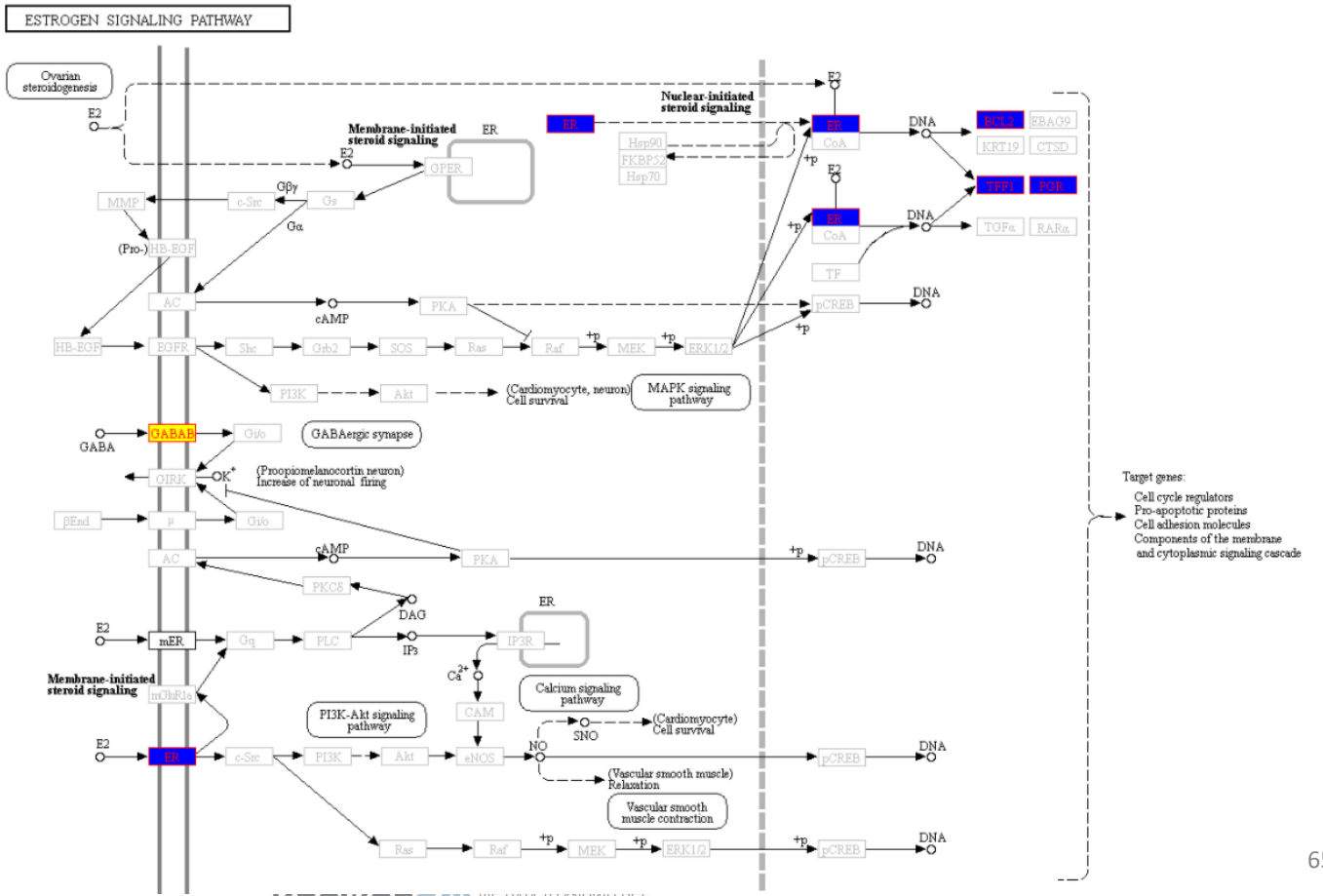



- **Estrogen signaling pathway** is the third result related to our ER status gene signature, although the overall adjusted p-value "SPIA padj" is not significant. Our gene signature is computed to activate the pathway

| KEGG pathway name | KEGG pathway ID | Genes in Pathway | DE Genes in Pathway | ORA pval | Topology Score | Top pval | SPIA pval | SPIA padj | Status | KEGG link |
|---|---|---|---|---|---|---|---|---|---|---|
| Oocyte meiosis | hsa04114 | 114 | 4 | 0.0026 | -2.2173 | 0.783 | 0.0147 | 0.5335 | Inhibited | |
| Morphine addiction | hsa05032 | 54 | 2 | 0.0301 | -3.8089 | 0.076 | 0.0162 | 0.5335 | Inhibited | |
| Estrogen signaling pathway | hsa04915 | 88 | 3 | 0.0099 | 16.4341 | 0.335 | 0.0222 | 0.5335 | Activated | |

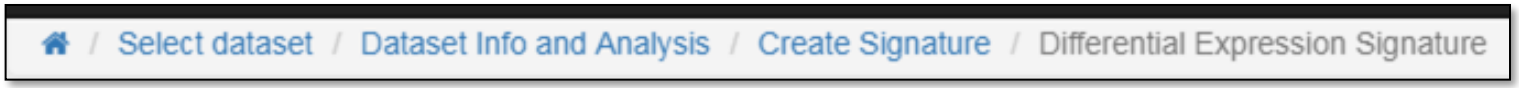
# Step 5A: Pathway Network Enrichment Test

- Click the **KEGG icon** in the last column for “Estrogen signaling pathway”.
- Yellow nodes are up-regulated genes in our signature, blue are down-regulated



# Step 5B: GeneMANIA

- Return to the analysis result by clicking on "**Differential Expression Signature**" in the tool bar at the top



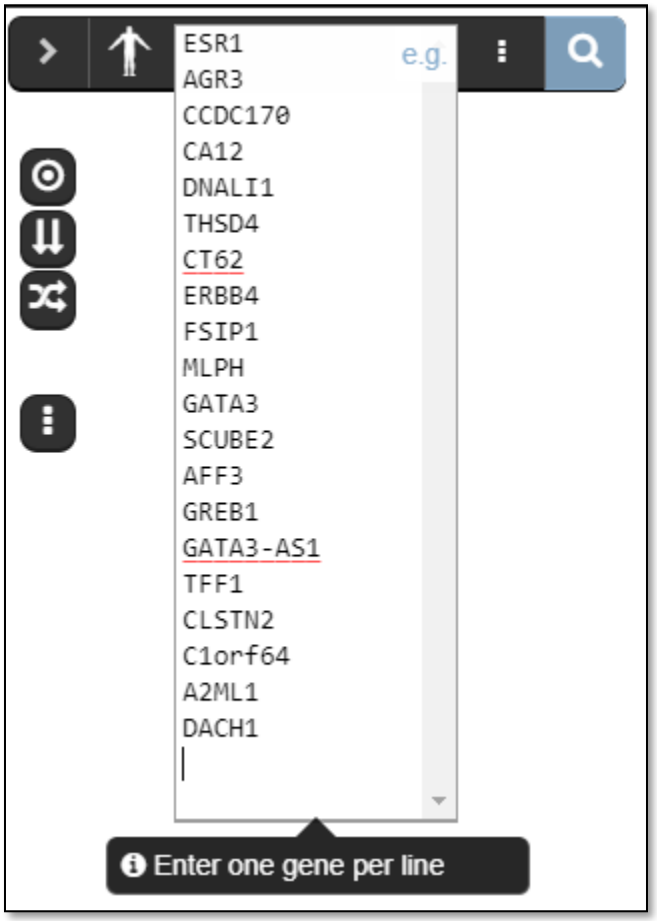

- The last linked tool we will explore today from iLINCS is GeneMANIA.


- GeneMANIA is a network-based guilt-by-association algorithm that finds the network neighbors of an input gene set from a heterogeneous collection of interaction networks

- Go to https://genemania.org/

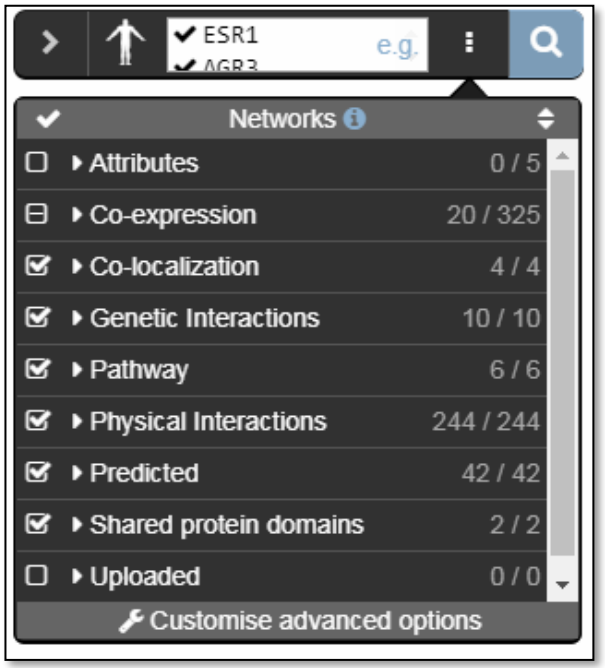
# Step 5B: GeneMANIA

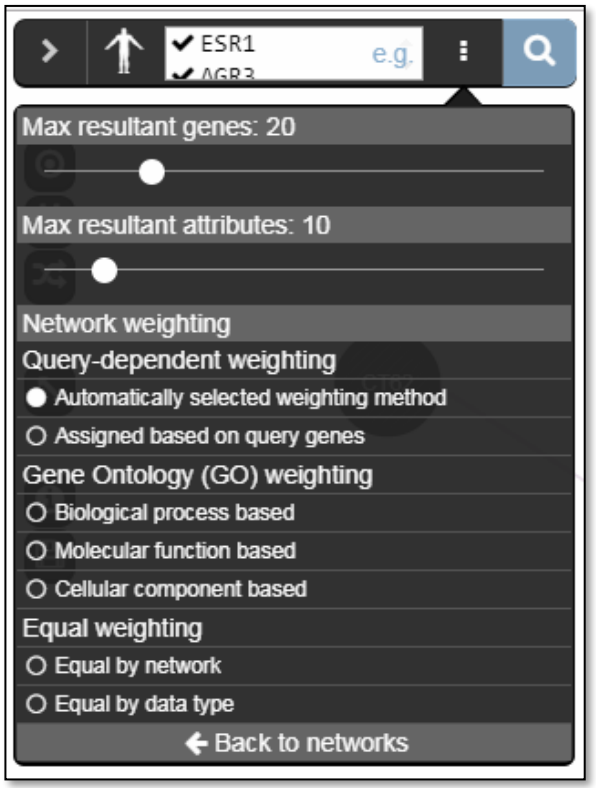
- We are going to enter the top 20 differentially expressed genes from our ER status gene signature. We will use GeneMANIA to return 20 additional network neighbor genes (not necessarily differentially expressed themselves)
- Then we will look at functional enrichment of this combined set of 40 genes.
- Find, open in a editor, and copy the contents of the file: [course_directory]/07_Signatures_and_Characterization/**ERstatus_top20.txt**
- This is the top 20 differentially expressed genes of our ER status signature extracted from the "Name_GeneSymbol" column in the Excel download
- Paste this list into the text box at the top left corner of the main page.



# Step 5B: GeneMANIA

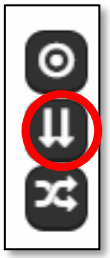
- Click on the **stacked-dots** options button next to where you pasted the list
- This first list shows all the possible networks that GeneMANIA will consider combining for the analysis of our twenty genes
- Select "**Customise advanced options**"
- This menu shows that we are going to find at most 20 neighbors using the automatic network weighting scheme, which is based on our 20 query genes
- Click the **search** magnifying glass.



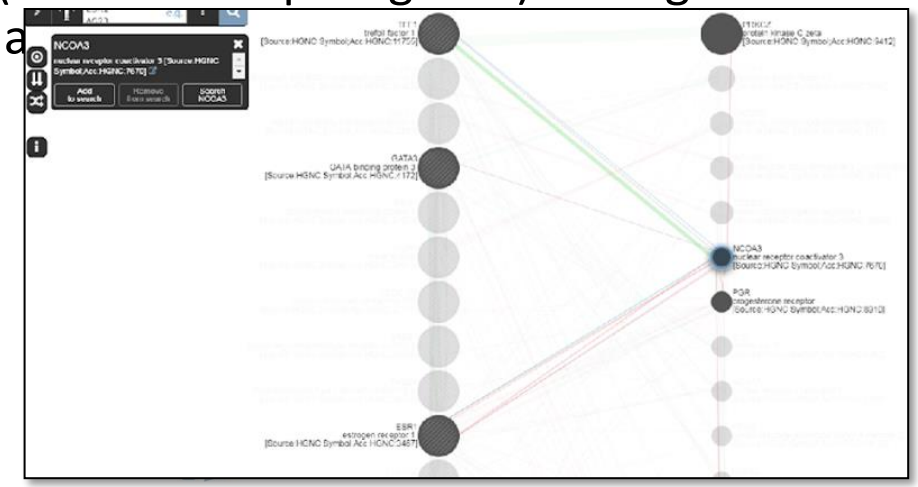



# Step 5B: GeneMANIA

- The resulting network contains our 20 input genes (striped) and our 20 predicted network neighbors (solids). The size of the network neighbors indicates its final guilt-by-association value on the composite affinity network.
- You may choose between three arrangements of the graph. The **stacked** arrangement may be easiest for understanding the nodes. You can hover over any node to highlight its neighbors.

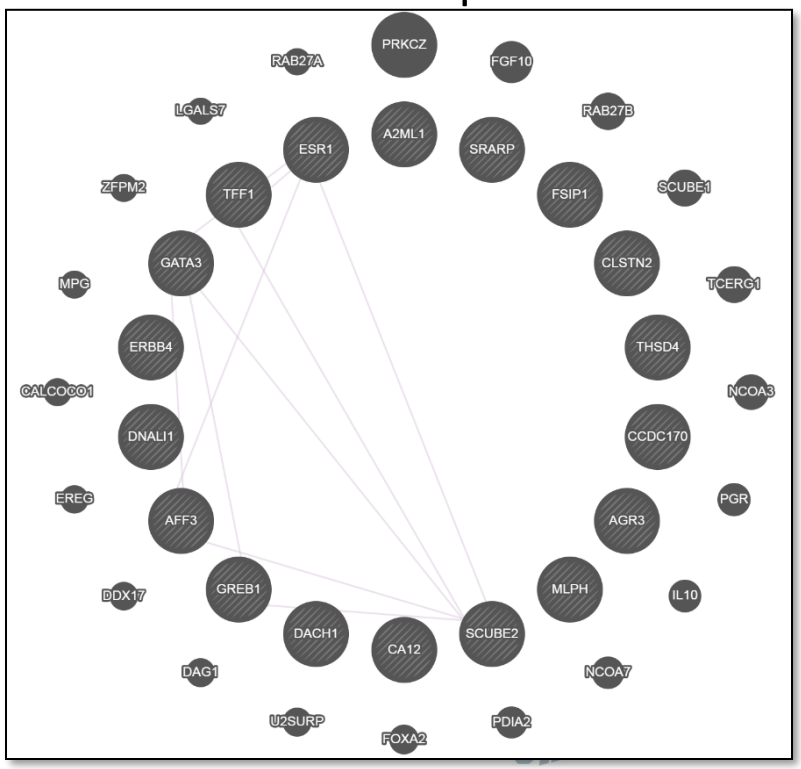


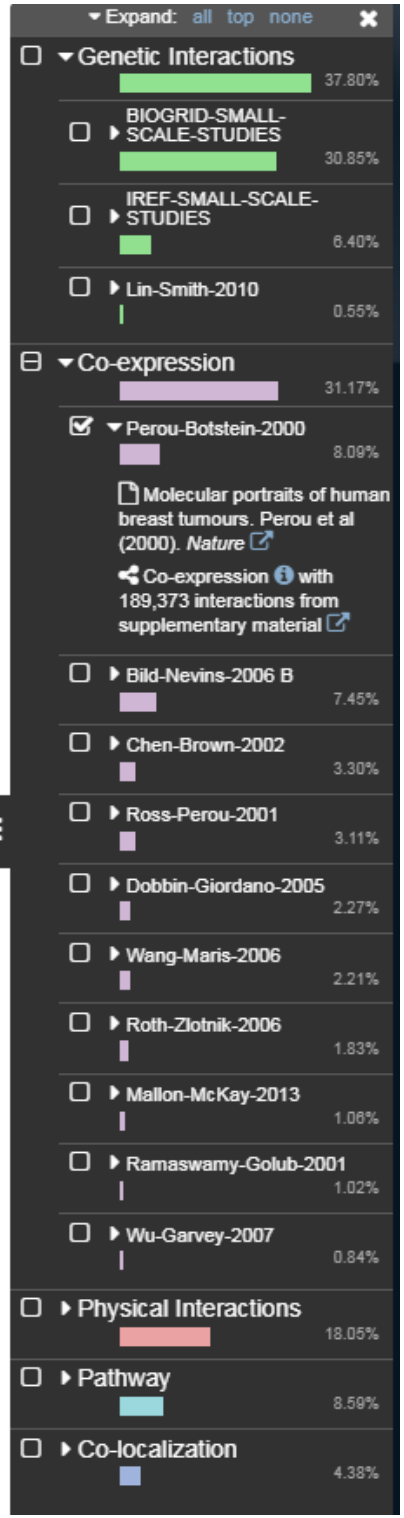
- For example,
  - NCOA7 is also known as Estrogen Nuclear Receptor Coactivator 1 NCOA3 is associated with Estrogen-Receptor Positive Breast Cancer
- Both are connected to ESR1 (and other top 20 genes) through pathways edges and neither a expressed gene signature



# Step 5B: GeneMANIA

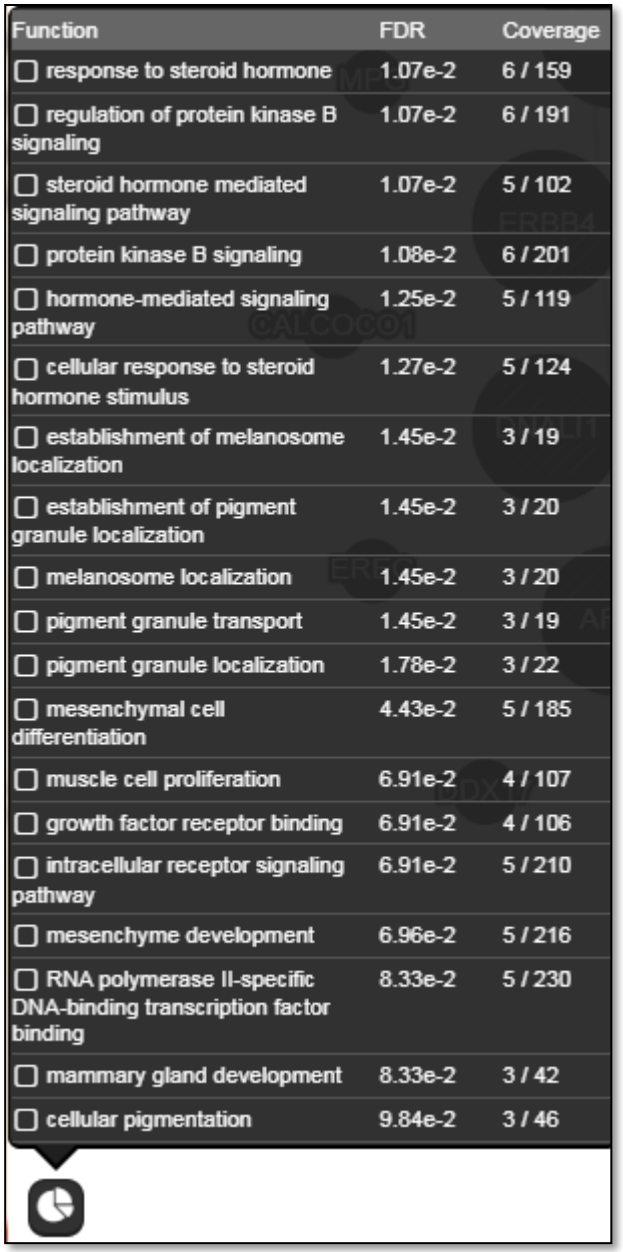
- On the right side (hidden unless you click on the button with 3 horizontal bars) is the selected interaction networks that were relevant to the 20 input genes, sorted by type and by weight. You can toggle the networks to display any set of edges.
- The highest weighted co-expression network is from breast tumors and relates the top 20 genes to each other fairly well, but does not connect them to the predicted 20.



# Step 5B: GeneMANIA

- Finally, we can perform the standard enrichment tests incorporating our predicted neighbors into our gene set.
- Click on the pie chart in the bottom left corner
- We see most of the results relating to hormone and steroid signaling pathways and receptors.



| Function | FDR | Coverage |
|---|---|---|
| response to steroid hormone | 1.07e-2 | 6 / 159 |
| regulation of protein kinase B signaling | 1.07e-2 | 6 / 191 |
| steroid hormone mediated signaling pathway | 1.07e-2 | 5 / 102 |
| protein kinase B signaling | 1.08e-2 | 6 / 201 |
| hormone-mediated signaling pathway | 1.25e-2 | 5 / 119 |
| cellular response to steroid hormone stimulus | 1.27e-2 | 5 / 124 |
| establishment of melanosome localization | 1.45e-2 | 3 / 19 |
| establishment of pigment granule localization | 1.45e-2 | 3 / 20 |
| melanosome localization | 1.45e-2 | 3 / 20 |
| pigment granule transport | 1.45e-2 | 3 / 19 |
| pigment granule localization | 1.78e-2 | 3 / 22 |
| mesenchymal cell differentiation | 4.43e-2 | 5 / 185 |
| muscle cell proliferation | 6.91e-2 | 4 / 107 |
| growth factor receptor binding | 6.91e-2 | 4 / 106 |
| intracellular receptor signaling pathway | 6.91e-2 | 5 / 210 |
| mesenchyme development | 6.96e-2 | 5 / 216 |
| RNA polymerase II-specific DNA-binding transcription factor binding | 8.33e-2 | 5 / 230 |
| mammary gland development | 8.33e-2 | 3 / 42 |
| cellular pigmentation | 9.84e-2 | 3 / 46 |

# Attention!

- For this last section (Slides 74-85), please only continue on if you:

  1. Have **at least 20 min** before we end for the day

  **-AND-**

  2. Feel confident working with KnowEnG on your own

- **If not**, please **stop** here.

# Gene Set Characterization Using Discriminative Random Walks

In this final exercise, we will find terms related to the 100 top differentially expressed genes of our ER status signature using the DRaWR method that incorporates the functional annotation terms directly in the network-based algorithm.

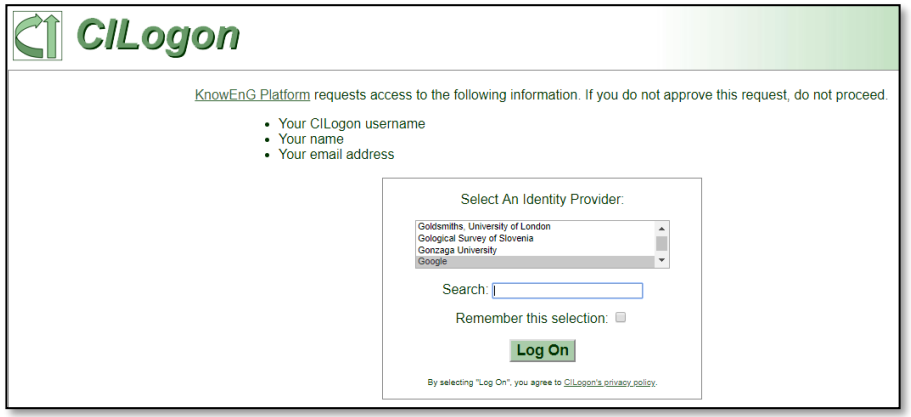
# Step 6: Login Into KnowEnG Platform

Return to KnowEnG Platform:
https://platform.knoweng.org/static/#/home

If necessary,

Login with **CILogon** - Login service through other accounts

Search: **Urbana, Mayo, Google, GitHub**

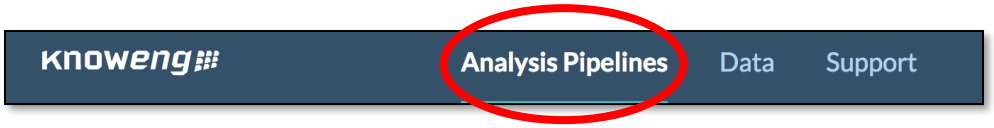


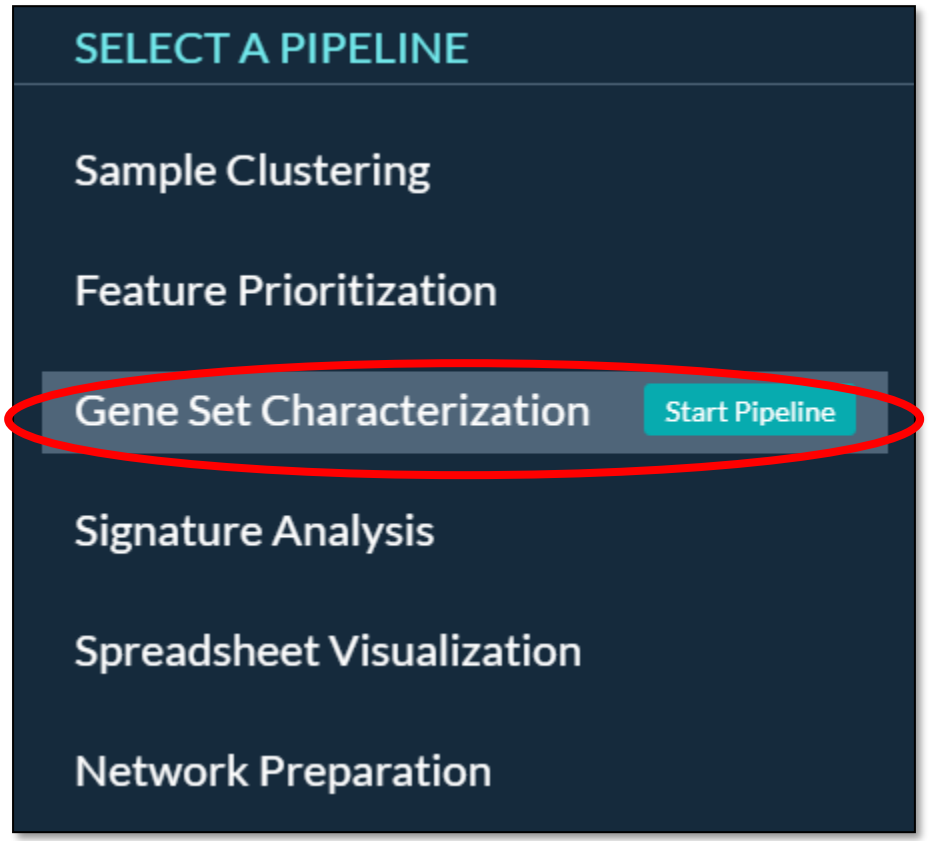
# STEP 6A: Gene Set Characterization

Select the pipeline:

- Select "**Analysis Pipelines**" at the top of the page

- Select "**Gene Set Characterization**" and Click on "**Start Pipeline**"

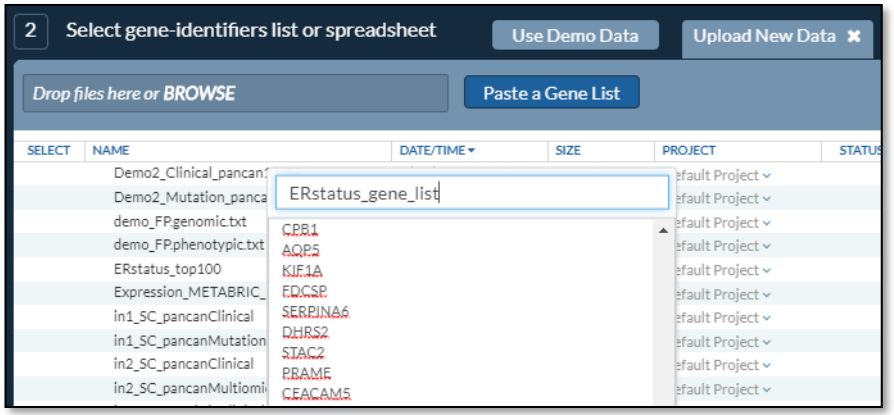


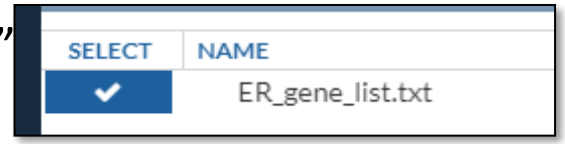
# Step 6A: Upload Data

**Find the file in this slide under [course_directory]/07_Signatures_and_Characterization/**

- Leave the default species "**Human**"
- Find, open in a text editor, and copy the contents of the file [course_directory]/07_Signatures_and_Characterization/**ERstatus_top100.txt**
- This is the top 100 differentially expressed genes of our ER status gene signature extracted from the Name_GeneSymbol column of our earlier Excel download




- Click on the "**Upload New Data**" tab
- Select the "**Paste a Gene List**" button.
- Give your gene list a name, e.g. "**ERstatus_gene_list**"
- Paste the file contents into the gene list text box. Click "**Done**"




- Click "**Select**" next to the name of your pasted list and you should see a checkmark
- Click "**Next**"

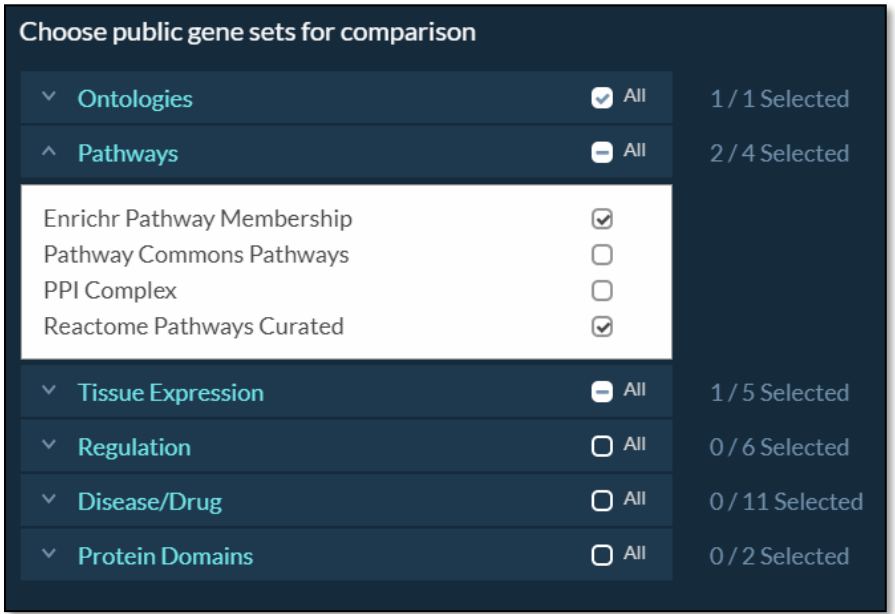
# Step 6A: Configure Algorithm Parameters

- We will choose to use a subset of 4 gene set collections available in the knowledge network

- **Ontologies**: Gene Ontology (default)
- **Pathways**: Enrichr Pathway Membership (must add)
- **Pathways**: Reactome Pathways Curated (must add)
- **Tissue Expression**: GEO Expression Set (must add)

- (unclick **Protein Domains**: PFam Protein Domains)

- Click "**Next**"



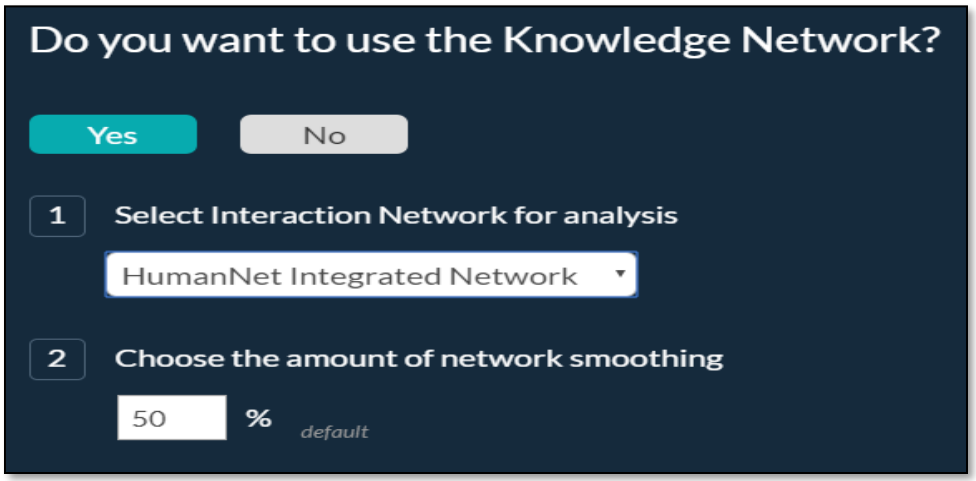
# Step 6A: Configure Network Parameters

- Click "**Yes**" for question about using the Knowledge Network
- The Knowledge Network we will use is an integrated network from the HumanNet project ("**HumanNet Integrated Network**")

- Network size information can be found here

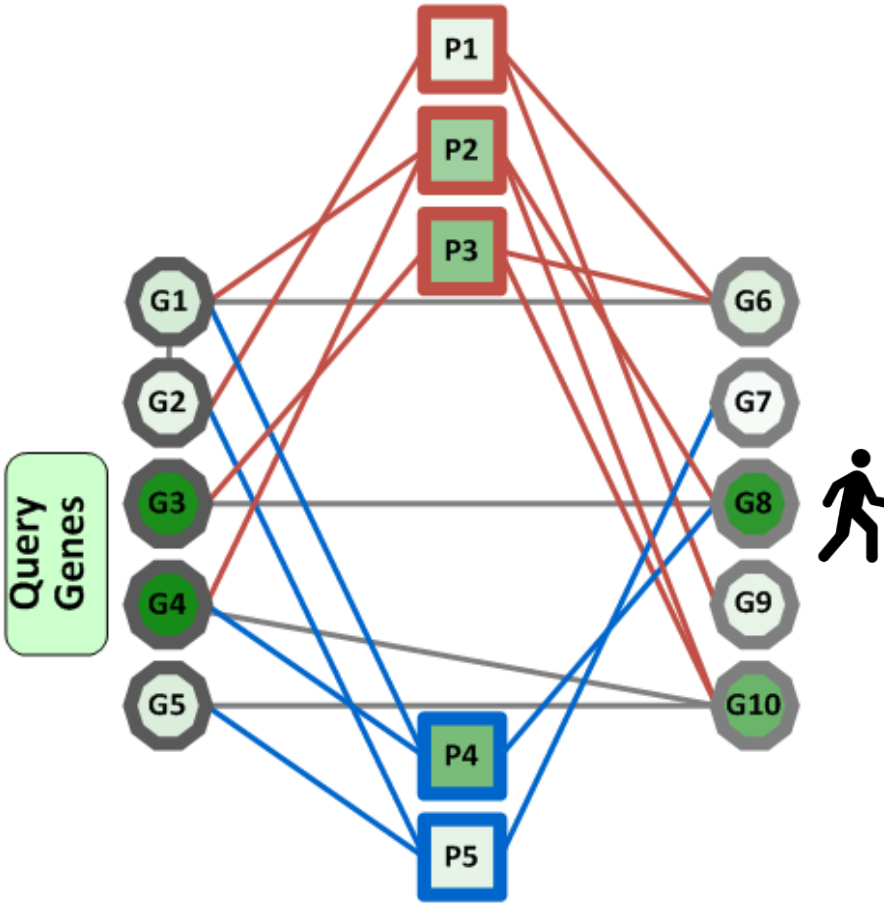
- The amount of network smoothing controls how much importance is put on network connections instead of the original 100 genes. We will use the default of **50**%

- Click "**Next**"



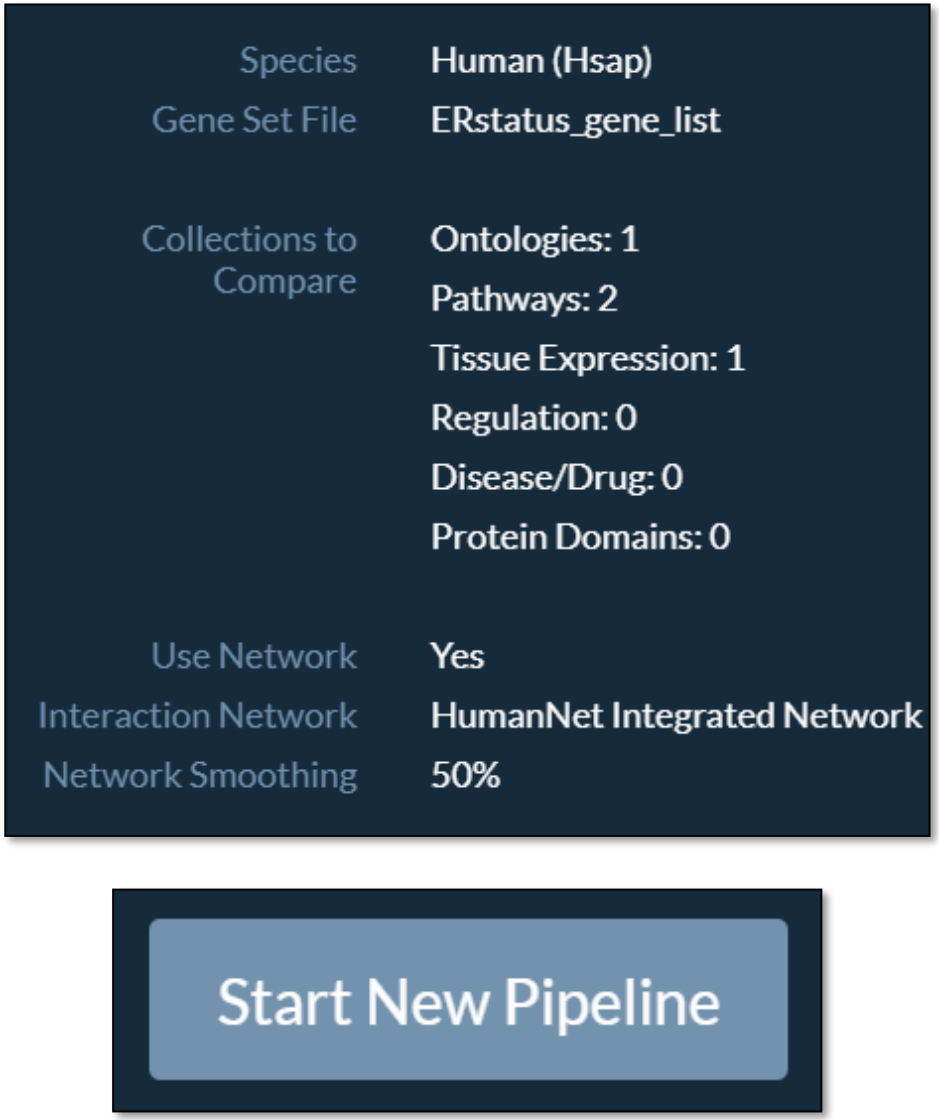
# Step 6A: Reminder about DRaWR Algorithm

- Squares are the Gene Ontology and pathway terms we selected
- Query Genes are our 100 ER status signature genes
- Gray edges are the HumanNet Integrated Network
- We are asking the algorithm to find property squares that a random walker who is forced to restart often at the query genes will visit unusually frequently



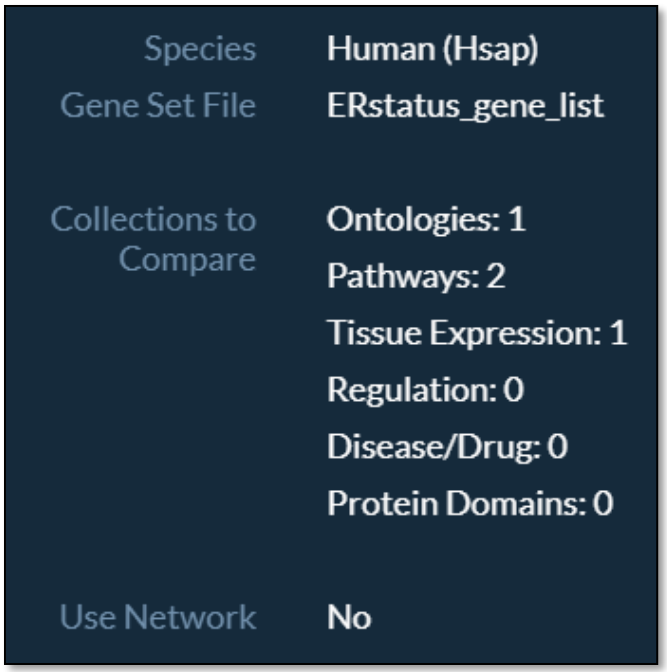
# Step 6A: Launch DRaWR Job

- Change **job name** to "gene_set_characterization-DRaWR-HN"
- Verify all the parameters are correct.
- Click "**Submit Job**"
- While this is running (roughly two minutes), we are going to launch the standard fisher exact enrichment tests with the same data sets.
- Click "**Start New Pipeline**"



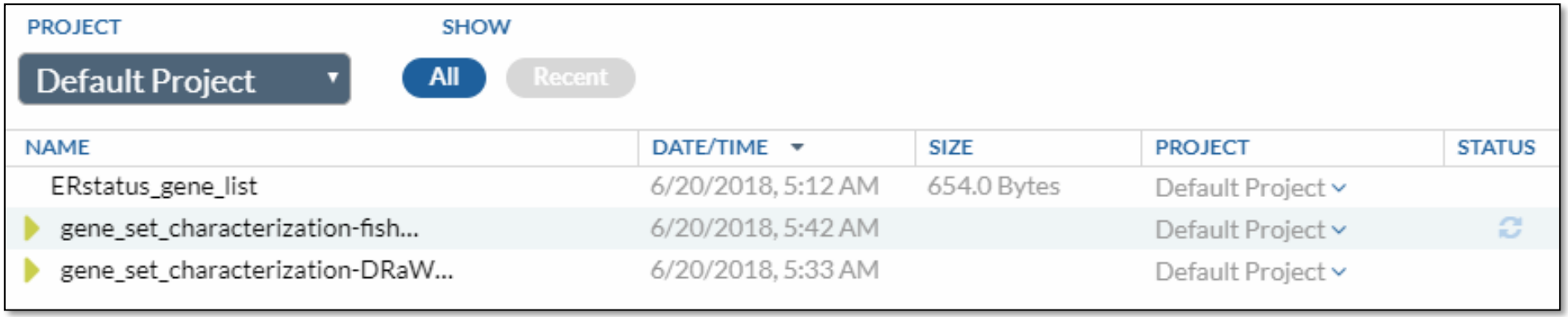
# Step 6B: Launch Standard Enrichment Tests

- Hover over Gene Set Characterization under Analysis Pipelines and click "**Start Pipeline**"
- Click "**Select**" next to the name of your pasted list and you should see a checkmark. Click "**Next**"
- Select same 4 collections:
  - Ontologies: Gene Ontology (default)
  - Pathways: Enrichr Pathway Membership (must add)
  - Pathways: Reactome Pathways Curated (must add)
  - Tissue Expression: GEO Expression Set (must add)
  - (unclick Protein Domains: PFam Protein Domains)
- Click "**Next**"
- Click "**No**" for question about using the Knowledge Network. Click "**Next**"
- Change **job name** to "gene_set_characterization-fisher"
- Verify all the parameters are correct.
- Click "**Submit Job**"



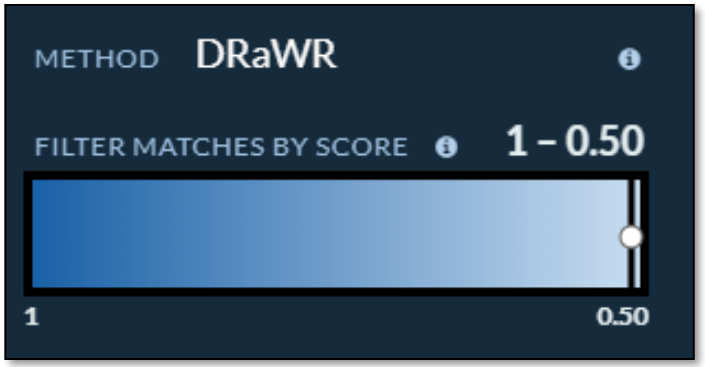
# Step 6C: View DRaWR Results

- Click the “Go to Data Page” button



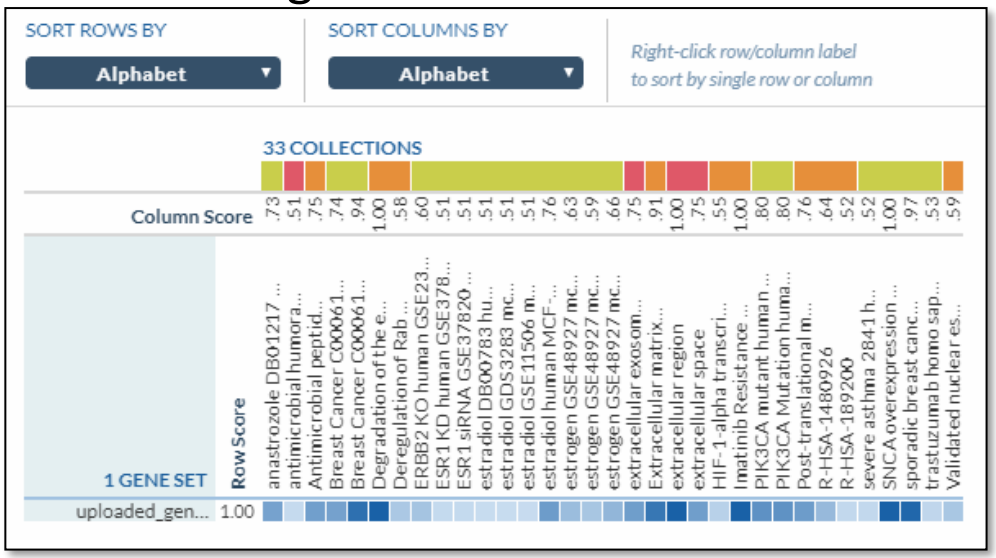

- You can check the status of your jobs here. Gray arrows mean that your job is currently queued or running. A red icon means something went wrong.
- Otherwise, when your job is successfully finished, you should be able to click the green arrow and see the primary result files.
- Click on the DRaWR job “**gene_set_characterization-DRaWR-HN**”
- Then click on the “**View Results**” button

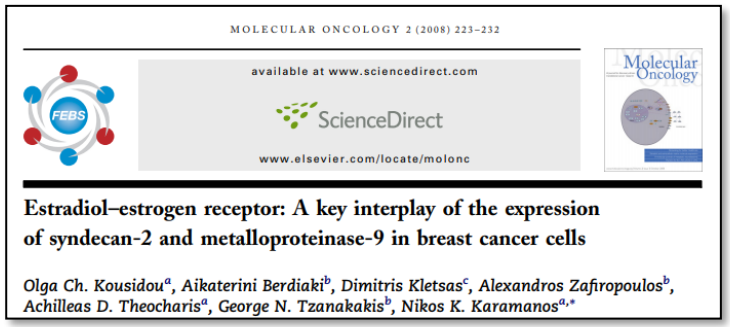
# Step 6C: View DRaWR Results




- Slide the filter slider all the way to the right.

- The DRaWR method picks up many GEO Expression gene sets that relate to ESR1 and estrogen and estradiol.

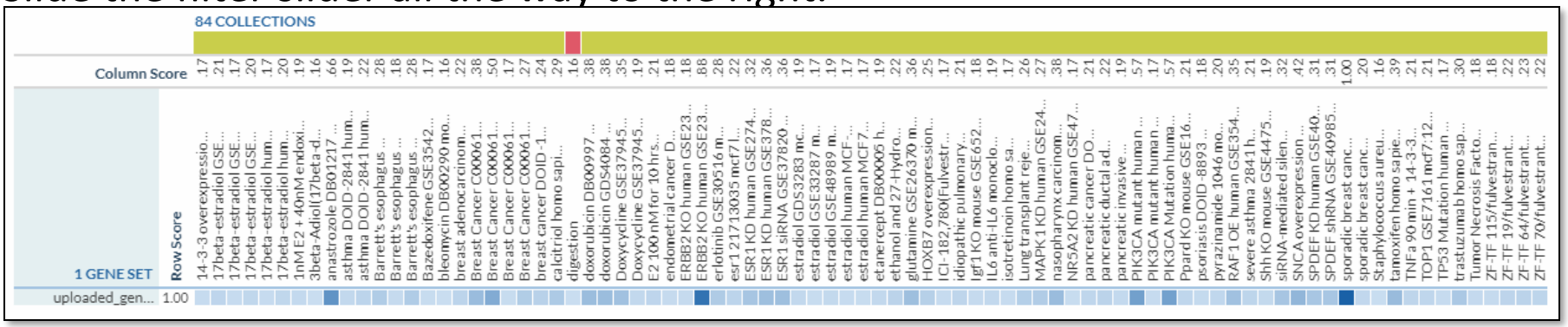



- DRaWR also ranks highly a number of pathway and Gene Ontology terms related to extracellular space, which is known to have many molecules effected by estrogens and related to ER expression

# Step 6C: View Fisher Results

- Click the "**Data**" link at the top of the page
- Click on the DRaWR job "**gene_set_characterization-fisher**"
- Then click on the "**View Results**" button
- Slide the filter slider all the way to the right.




- The Fisher method finds the same GEO Expression gene sets that relate to ESR1 and estrogen and estradiol, as well as some additional estradiol ones that DRaWR missed. It also detects many more less obviously related GEO gene sets.
- The standard enrichment method does not detect any highly significant enrichments with pathways or Gene Ontology terms.
- Since it is missing here the extracellular space terms detected by DRaWR are strongly connected to the signature genes, but mostly through their HumanNet network neighbors and not direct connections.

# Main Lab Take Home Message

- Whether it is
  - Sample Clustering
  - Gene Prioritization
  - Gene Set Characterization
- Omics data can be analyzed
  - in the context of a pathway, interaction, or other affinity network
  - to provide complementary insights to standard approaches

# List of Other KnowEnG Resources

- **Other Pipelines**:
  - **Network Preparation** for uploading your custom network to the platform for analysis
  - **Signature Analysis** for mapping samples to signatures by correlation of omics profiles
- **Tutorials:**
  - Quickstarts: https://knoweng.org/quick-start/
  - YouTube: https://www.youtube.com/channel/UCjyIIolCaZIGtZC20XLBOyg
- **Resources:**
  - Data Preparation Guide: https://github.com/KnowEnG/quickstart-demos/blob/master/pipeline_readmes/README-DataPrep.md
  - Knowledge Network Contents:
    - Summary: https://knoweng.org/kn-data-references/
    - Download: https://github.com/KnowEnG/KN_Fetcher/blob/master/Contents.md
- **Research:**
  - Knowledge-guided analysis of omics Data (KnowEnG cloud platform paper): https://journals.plos.org/plosbiology/article?id=10.1371/journal.pbio.3000583
  - TCGA Analysis Walkthrough: https://github.com/KnowEnG/quickstart-demos/tree/master/publication_data/blatti_et_al_2019
- **Source Code:**
  - Docker Images: https://hub.docker.com/u/knowengdev/
  - GitHub Repos: https://knoweng.github.io/
- **Other Cloud Platforms:**
  - https://cgc.sbgenomics.com/public/apps#q?search=knoweng
- Contact Us with Questions and Feedback: knoweng-support@illinois.edu