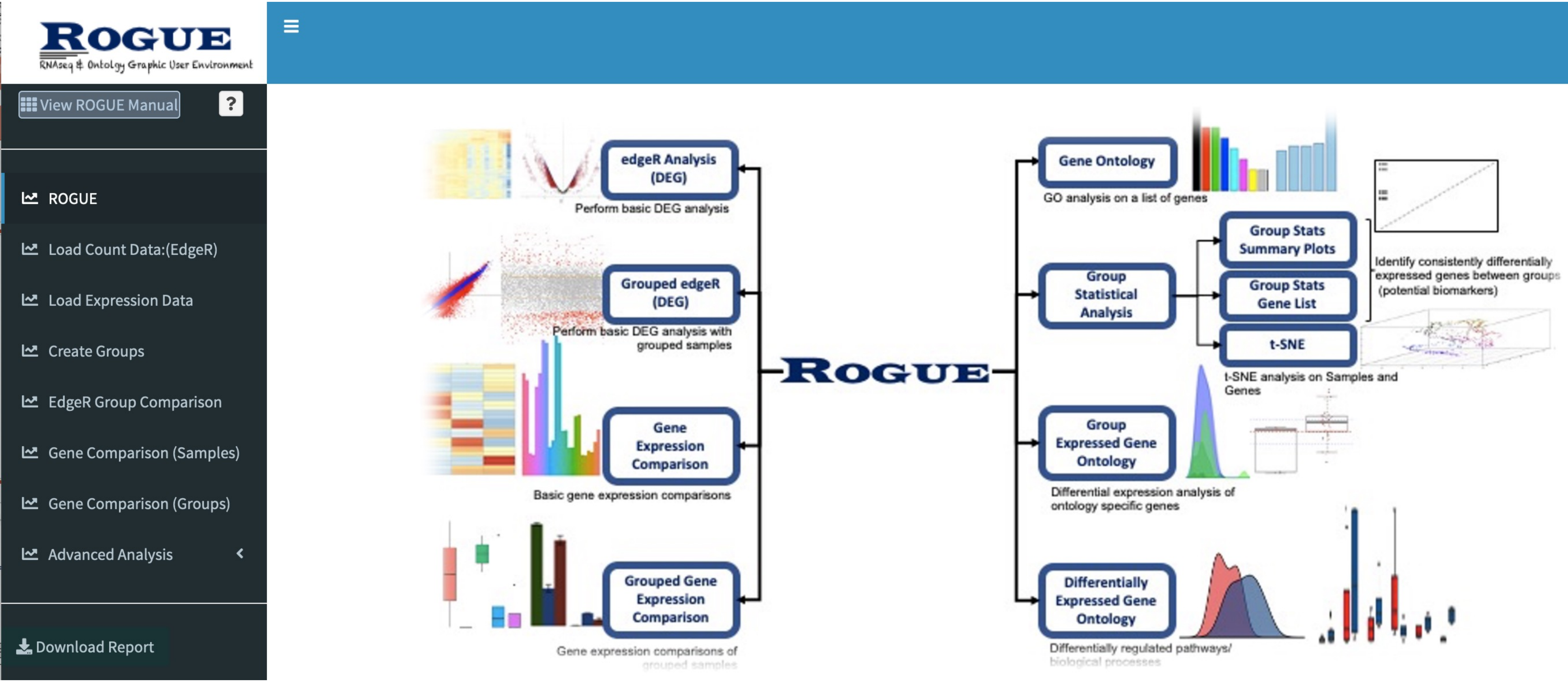
ROGUE

RNAseq & Ontolgy Graphic User Environment

Basic Tutorial

Table of Contents

Getting Started



Perform basic DEG analysis

Perform basic DEG analysis with grouped samples

Basic gene expression comparisons

Gene expression comparisons of grouped samples

GO analysis on a list of genes

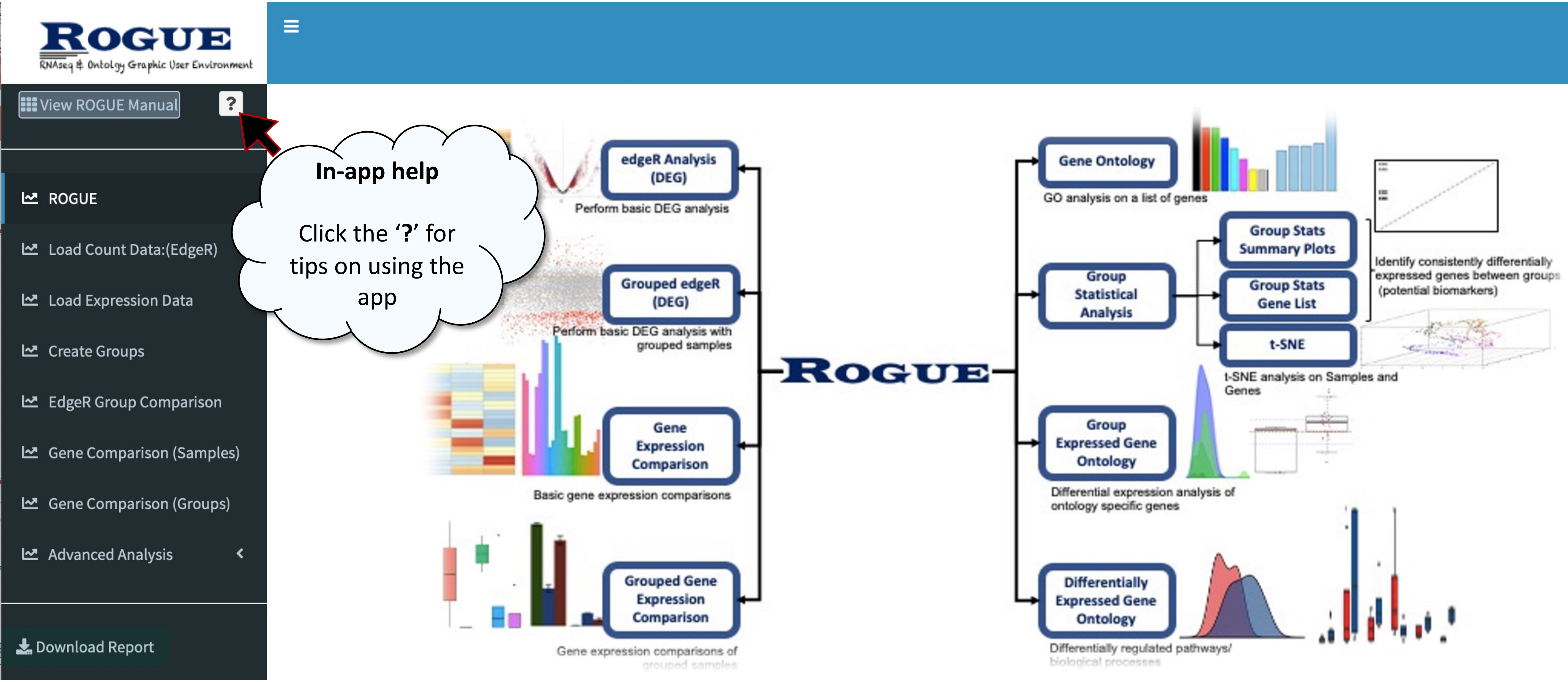
Identify consistently differentially expressed genes between groups (potential biomarkers)

t-SNE analysis on Samples and Genes

Differential expression analysis of ontology specific genes

Differentially regulated pathways/ biological processes

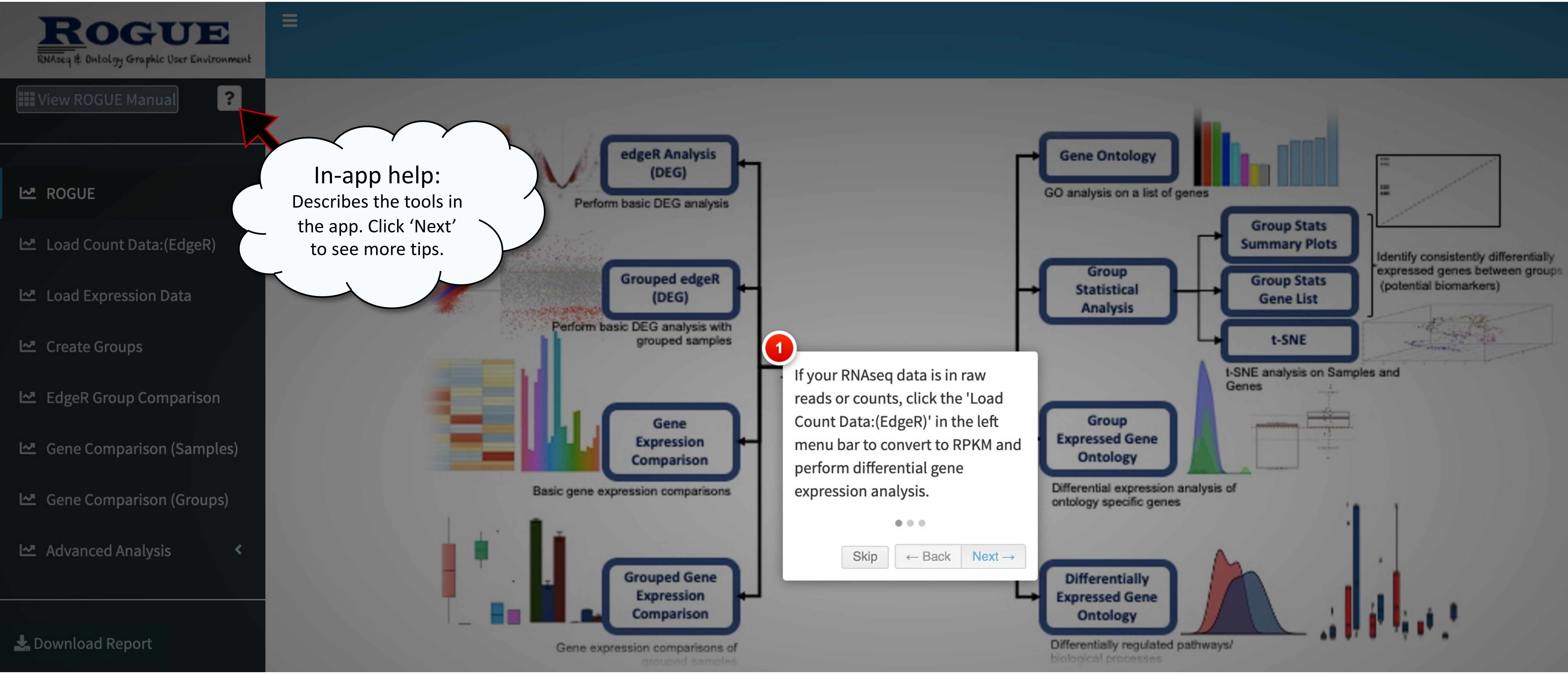
Getting Started



In-app help

Click the '**?**' for tips on using the app

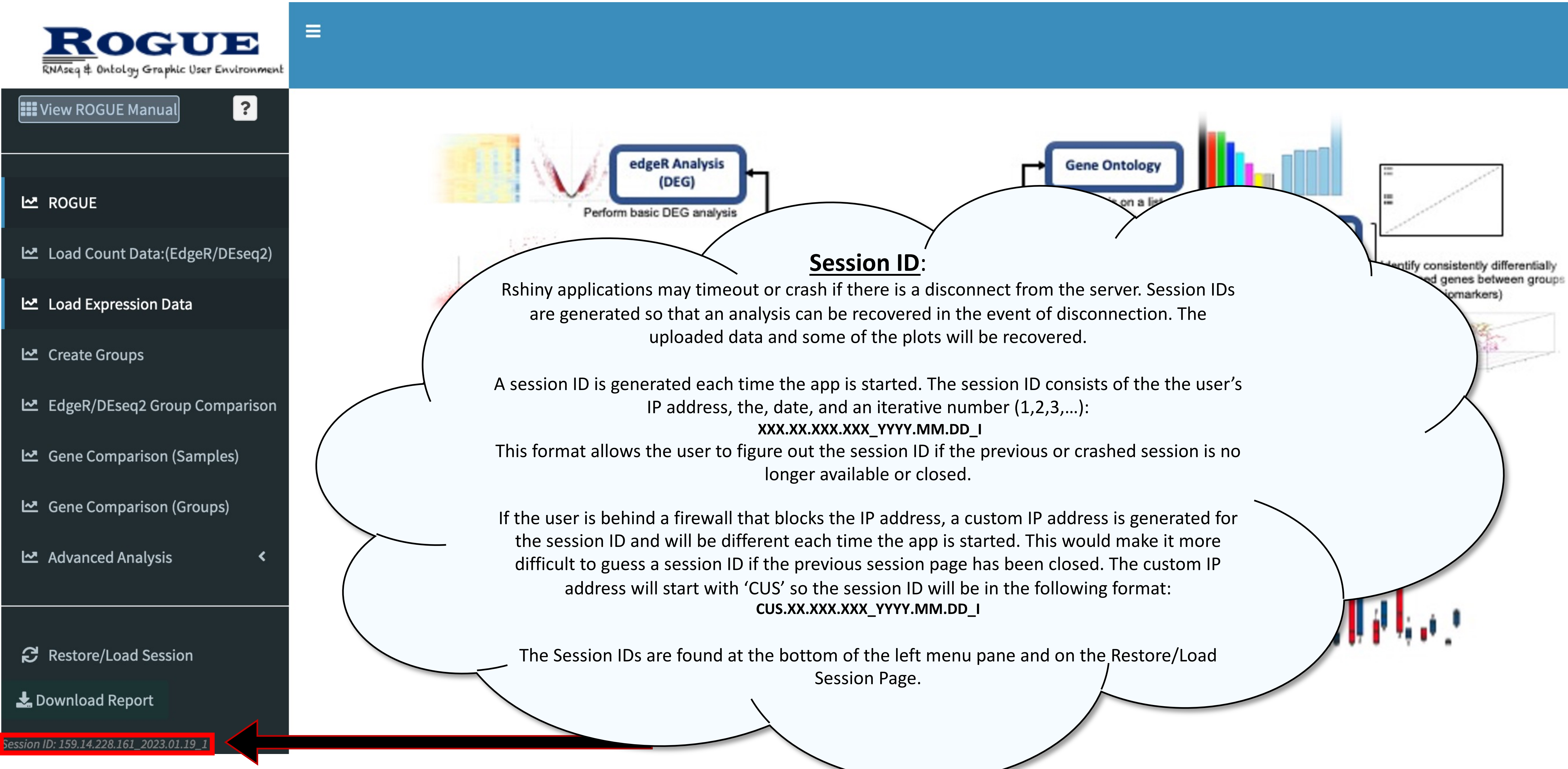
Getting Started



In-app help:
Describes the tools in the app. Click 'Next' to see more tips.

If your RNAseq data is in raw reads or counts, click the 'Load Count Data:(EdgeR)' in the left menu bar to convert to RPKM and perform differential gene expression analysis.

Getting Started – Session ID



Session ID: 159.14.228.161_2023.01.19_1

Session ID:

Rshiny applications may timeout or crash if there is a disconnect from the server. Session IDs are generated so that an analysis can be recovered in the event of disconnection. The uploaded data and some of the plots will be recovered.

A session ID is generated each time the app is started. The session ID consists of the the user's IP address, the, date, and an iterative number (1,2,3,…):

XXX.XX.XXX.XXX_YYYY.MM.DD_I

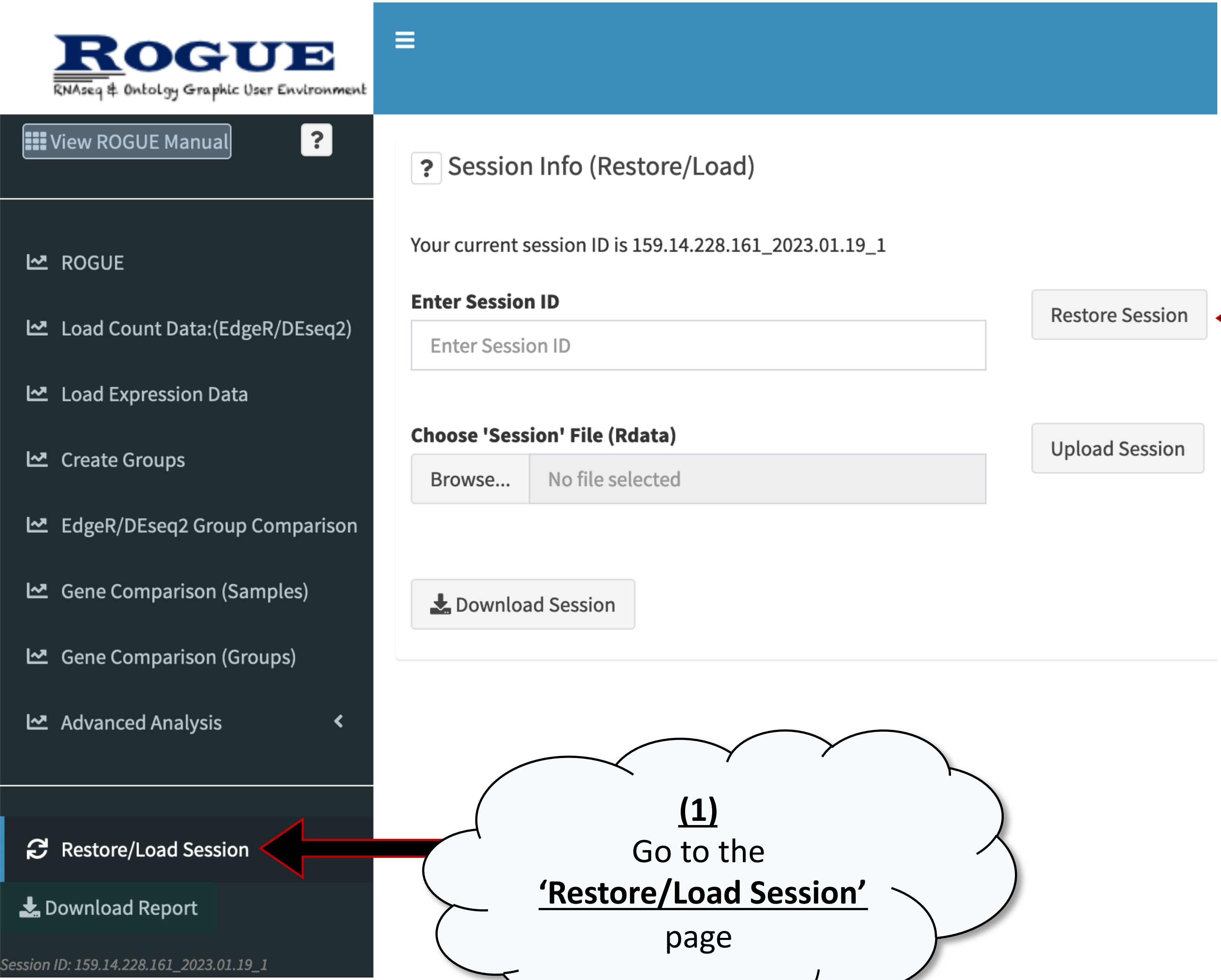
This format allows the user to figure out the session ID if the previous or crashed session is no longer available or closed.

If the user is behind a firewall that blocks the IP address, a custom IP address is generated for the session ID and will be different each time the app is started. This would make it more difficult to guess a session ID if the previous session page has been closed. The custom IP address will start with 'CUS' so the session ID will be in the following format:

CUS.XX.XXX.XXX_YYYY.MM.DD_I

The Session IDs are found at the bottom of the left menu pane and on the Restore/Load Session Page.

Getting Started – Restoring a session with a session ID



ROGUE

RNAseq & Ontolgy Graphic User Environment

View ROGUE Manual ?

- ROGUE
- Load Count Data:(EdgeR/DEseq2)
- Load Expression Data
- Create Groups
- EdgeR/DEseq2 Group Comparison
- Gene Comparison (Samples)
- Gene Comparison (Groups)
- Advanced Analysis
- Restore/Load Session
- Download Report

Session ID: 159.14.228.161_2023.01.19_1

? Session Info (Restore/Load)

Your current session ID is 159.14.228.161_2023.01.19_1

Enter Session ID

Enter Session ID

Restore Session

Choose 'Session' File (Rdata)

Browse... No file selected

Upload Session

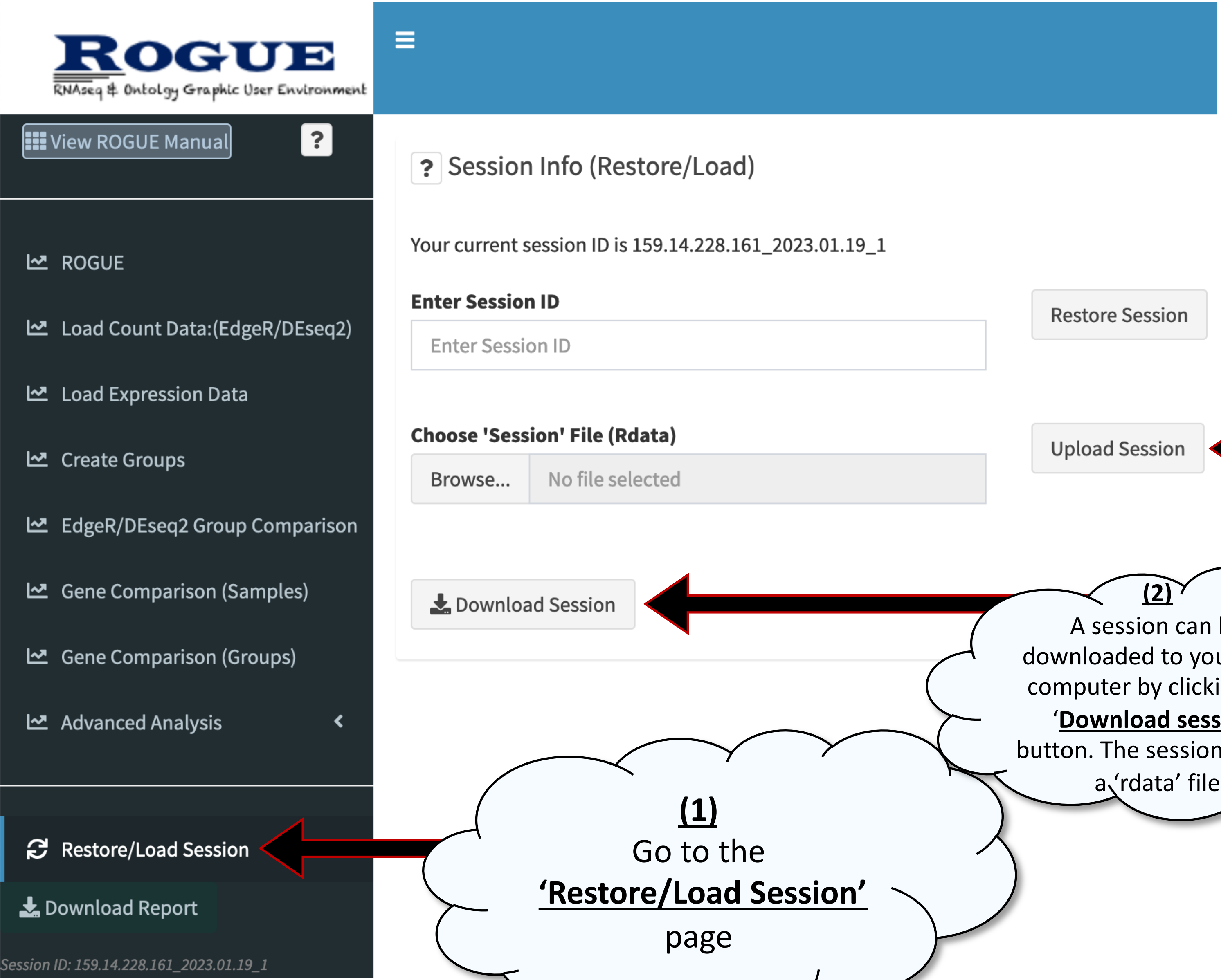
Download Session

<u>(2)</u>
Enter a previous session ID in the **<u>'Enter Session ID'</u>** field and click the '**<u>Restore Session'</u>** button.
Note: sessions are only stored on the server temporarily and will be removed often

<u>(1)</u>
Go to the
<u>'Restore/Load Session'</u>
page

<u>(3)</u>
The 'Sample' and 'Gene' fields in the application will be populated with the restored data.
Some analyses and plots will also be restored.

Getting Started – Downloading and uploading with a session



Your current session ID is 159.14.228.161_2023.01.19_1

(1)
Go to the
'Restore/Load Session'
page

(2)
A session can be downloaded to your local computer by clicking the **'Download session'** button. The session will be a 'rdata' file

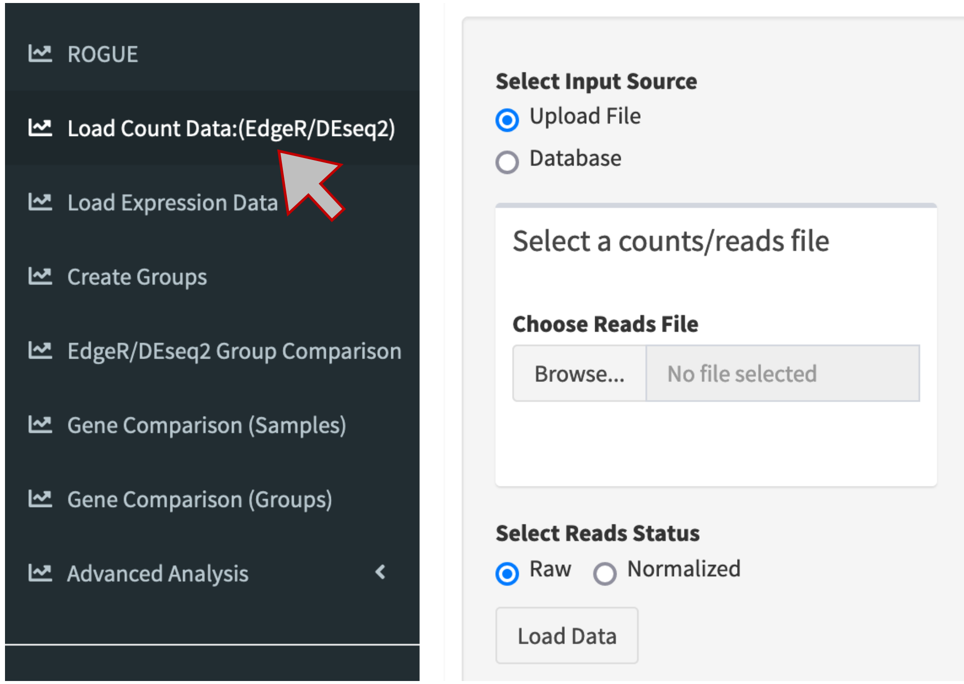
(3)
If a session file (rdata) has been downloaded to your local computer, it can be uploaded using the upload function.

(4)
The 'Sample' and 'Gene' fields in the application will be populated with the restored data. Some analyses and plots will also be restored.

Data Input

ROGUE can use count/reads data (raw or normalized) and expression data (FPKM/RPKM) as input.

Counts/Reads Data



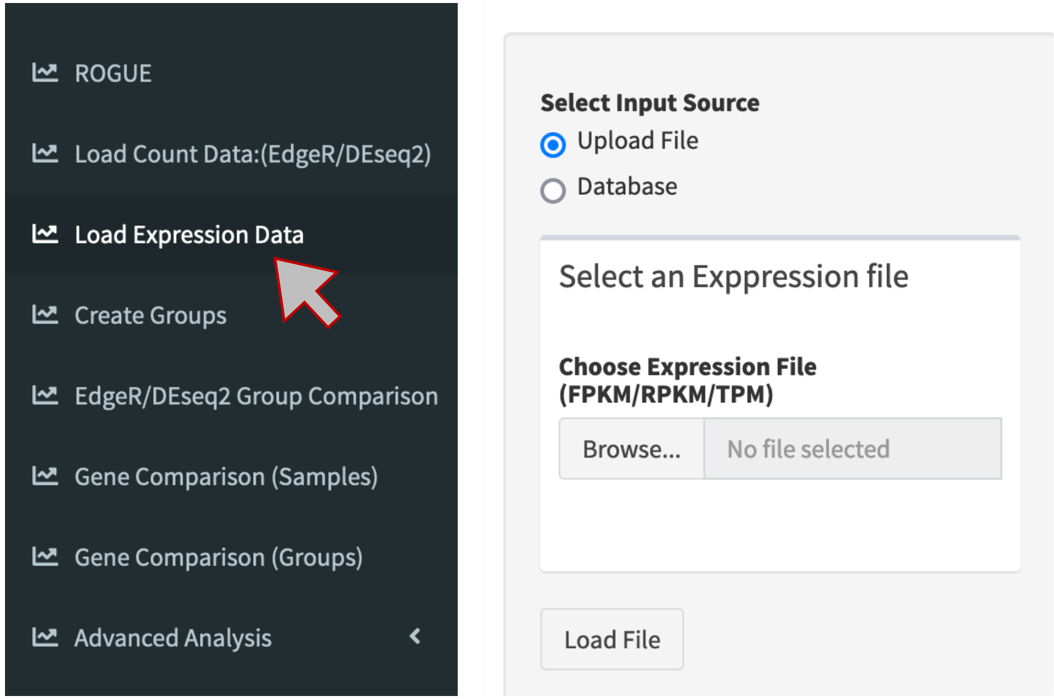
Select Input Source
● Upload File
○ Database

Select a counts/reads file

Choose Reads File
No file selected

Select Reads Status
● Raw ○ Normalized

Expression Data



Select Input Source
● Upload File
○ Database

Select an Exppression file

Choose Expression File (FPKM/RPKM/TPM)
No file selected

Data Formats

Raw Counts

gene_name	symbol	len	Sample_1	Sample_2	Sample_3
NM_177327	Wwp1	5070	687	579	761
NM_177326	Pak2	4099	5194	4724	5962
NM_177325	Tsr1	3385	1204	1421	2334

Note: Tab delimited files: Raw counts can be submitted genenames, symbols, len ('len' column are the gene lengths in bases and needs to be labeled 'len'), followed by sample columns with raw count values

Raw Counts

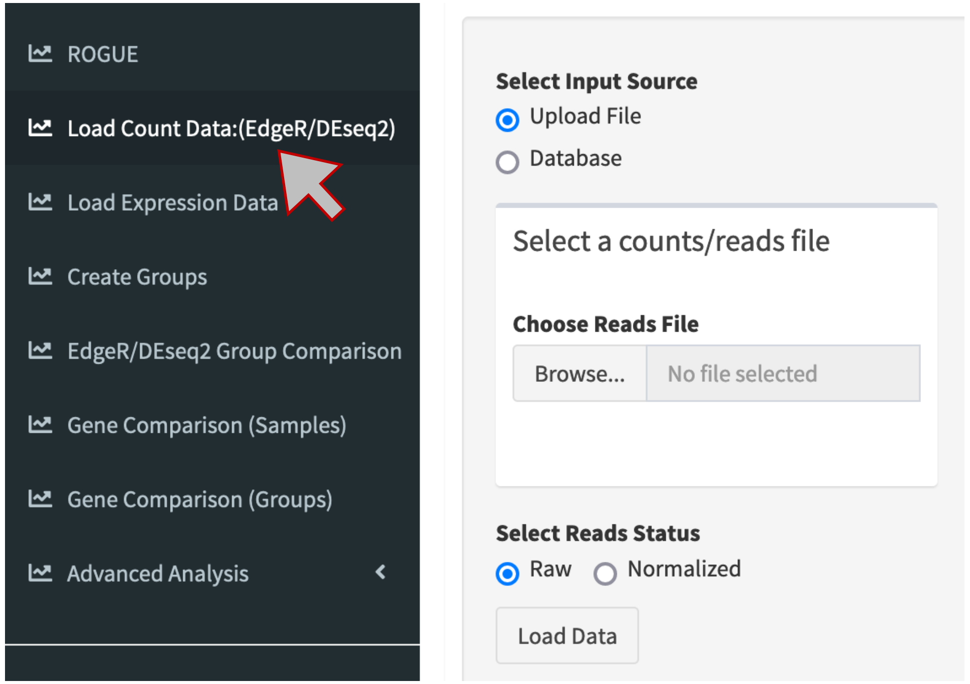
symbol	Sample_1	Sample_2	Sample_3
Wwp1	687	579	761
Pak2	5194	4724	5962
Tsr1	1204	1421	2334

Note: Tab delimited files: Raw counts can be submitted with the genename/symbol column followed by the Sample columns with the raw counts. The tool will used pre-determined gen lengths to normalize counts.

Normalized Counts (by length)

Symbol	Sample_1	Sample_2	Sample_3
ENSG00000000419	687.43	579.63	761.19
ENSG00000000457	5194.64	4724.91	5962.03
ENSG00000000460	1204.01	1421.63	2334.32

Note: Tab delimited files: Normalized counts can be submitted with the genename/symbol column followed by the Sample columns with the normalized counts.



Select Input Source
● Upload File
○ Database

Select a counts/reads file

Choose Reads File
No file selected

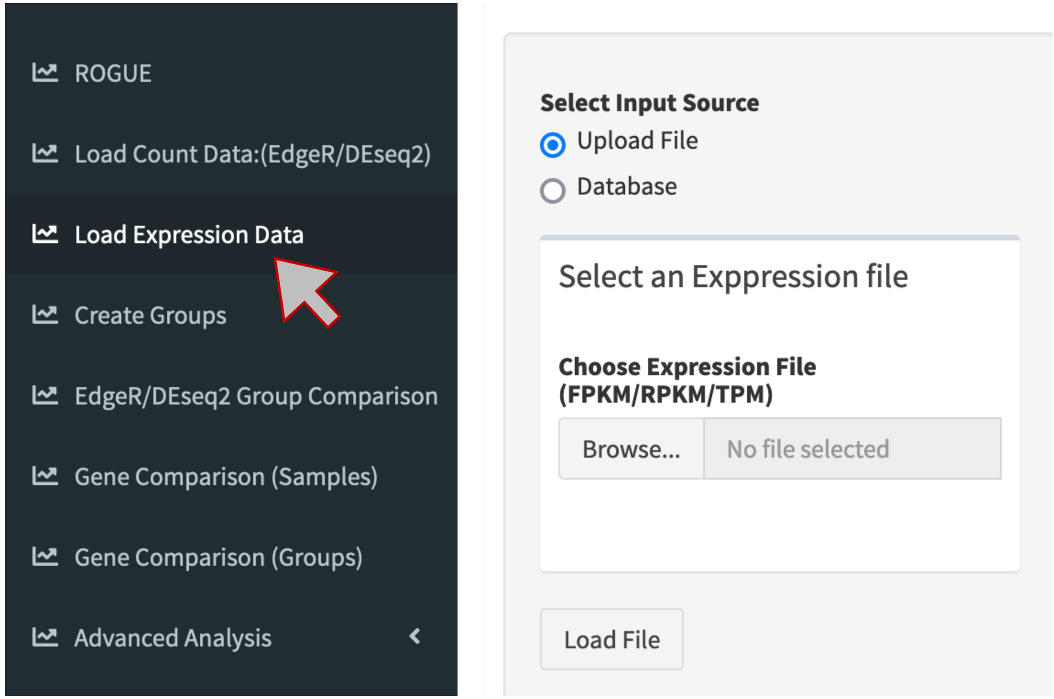
Select Reads Status
● Raw ○ Normalized

Data Formats

Note: Tab delimited files: Expression values can be submitted with the genename/symbol column followed by the Sample columns with the expression values (eg. FPKM/RPKM).

Expression Values

genenames	Sample_1	Sample_2	Sample_3
TSPAN6	17.84	16.37	18.45
TNMD	0	0.18	0
DPM1	23.05	21.24	15.8



Select Input Source

● Upload File

○ Database

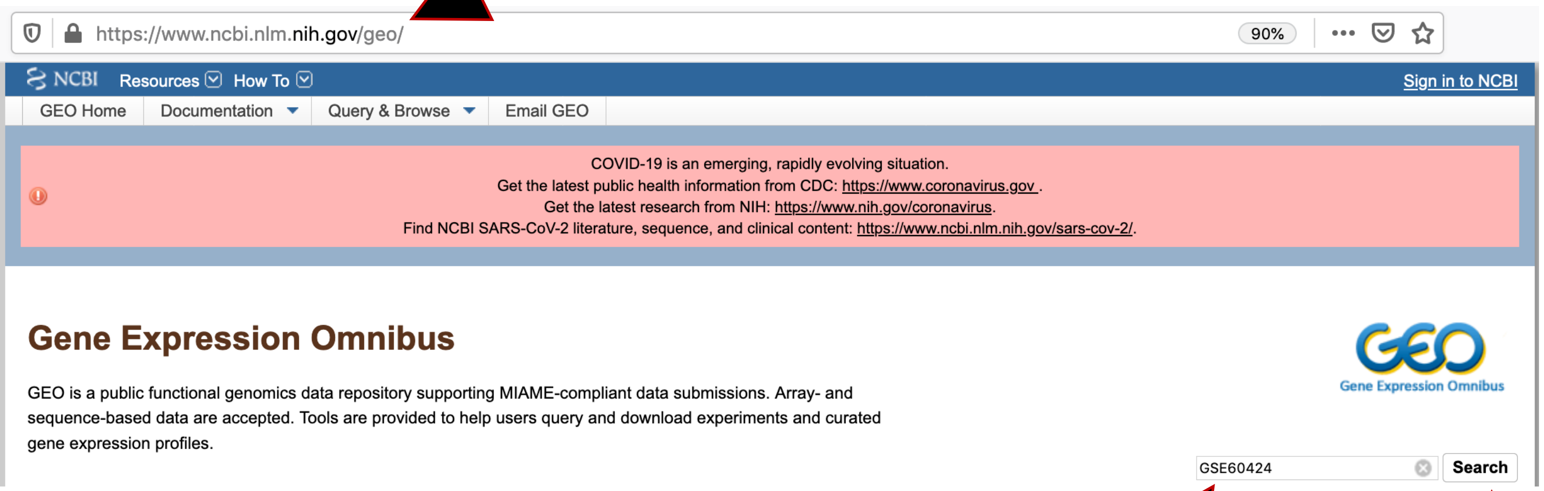
Select an Exppression file

Choose Expression File (FPKM/RPKM/TPM)

No file selected

Tutorial-Getting Data from GEO

(1) Go to https://www.ncbi.nlm.nih.gov/geo/



COVID-19 is an emerging, rapidly evolving situation.
Get the latest public health information from CDC: https://www.coronavirus.gov .
Get the latest research from NIH: https://www.nih.gov/coronavirus.
Find NCBI SARS-CoV-2 literature, sequence, and clinical content: https://www.ncbi.nlm.nih.gov/sars-cov-2/.

Gene Expression Omnibus

GEO is a public functional genomics data repository supporting MIAME-compliant data submissions. Array- and sequence-based data are accepted. Tools are provided to help users query and download experiments and curated gene expression profiles.

GSE60424

(2) Enter GSE60424 in the search box

(3) Click Search

(4) You will be redirected to this page

https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE60424

COVID-19 is an emerging, rapidly evolving situation.
Get the latest public health information from CDC: https://www.coronavirus.gov .
Get the latest research from NIH: https://www.nih.gov/coronavirus.
Find NCBI SARS-CoV-2 literature, sequence, and clinical content: https://www.ncbi.nlm.nih.gov/sars-cov-2/.

Scope: Self
Format: HTML
Amount: Quick
GEO accession: GSE60424

Series GSE60424 Query DataSets for GSE60424

Status	Public on Jan 06, 2015
Title	Next generation sequencing of human immune cell subsets across diseases
Organism	Homo sapiens
Experiment type	Expression profiling by high throughput sequencing
Summary	This study compared whole transcriptome signatures of 6 immune cell subsets and whole blood from patients with an array of immune-associated diseases. Fresh blood samples were collected from healthy subjects and subjects diagnosed type 1 diabetes, amyotrophic lateral sclerosis, and sepsis, as well as multiple sclerosis patients before and 24 hours after the first treatment with IFN-beta. At the time of blood draw, an aliquot of whole blood was collected into a Tempus tube (Invitrogen), while the remainder of the primary fresh blood sample was processed to highly pure populations of neutrophils, monocytes, B cells, CD4 T cells, CD8 T cells, and natural killer cells. RNA was extracted from each of these cell subsets, as well as the whole blood samples, and processed into RNA sequencing (RNAseq) libraries (Illumina TruSeq). Sequencing libraries were analyzed on an Illumina HiScan, with a target read depth of ~20M reads. Reads were demultiplexed, mapped to human gene models (ENSEMBL), and tabulated using HTSeq. Read count data were normalized by the TMM procedure (edgeR package).
Overall design	We performed whole genome RNAseq profiling of immune cell subsets and whole blood from subjects with an array of immune-associated diseases.
Contributor(s)	Speake C, Linsley PS, Whalen E, Chaussabel D, Presnell SR, Mason MJ, Gersuk VH, O'Brien KK, Nguyen Q, Greenbaum CJ, Buckner JH, Malhotra U
Citation(s)	Linsley PS, Speake C, Whalen E, Chaussabel D. Copy number loss of the interferon gene cluster in melanomas is linked to reduced T cell infiltrate and poor patient prognosis. *PLoS One* 2014;9(10):e109760. PMID: 25314013
Submission date	Aug 14, 2014
Last update date	May 15, 2019
Contact name	Scott Presnell
E-mail(s)	SPresnell@benaroyaresearch.org
Organization name	Benaroya Research Institute
Department	Systems Immunology
Street address	1201 Ninth Ave.
City	Seattle
State/province	WA
ZIP/Postal code	98101
Country	USA

Tutorial-Getting Data from GEO

Series GSE60424 Query DataSets for GSE60424

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Submission date	Aug 14, 2014
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Contact name	Scott Presnell
E-mail(s)	SPresnell@benaroyaresearch.org
Organization name	Benaroya Research Institute
Department	Systems Immunology
Street address	1201 Ninth Ave.
City	Seattle
State/province	WA
ZIP/Postal code	98101
Country	USA
Platforms (1)	GPL15456 Illumina HiScanSQ (Homo sapiens)
Samples (134) More...	GSM1479433 lib221 GSM1479434 lib222 GSM1479435 lib223

Relations

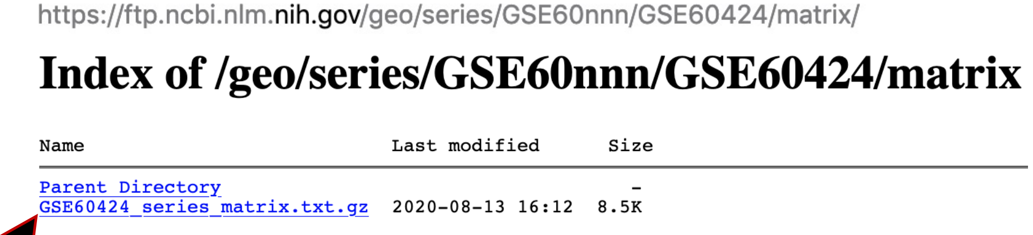
BioProject	PRJNA258216
SRA	SRP045500

Download family	Format
SOFT formatted family file(s)	SOFT
MINiML formatted family file(s)	MINiML
Series Matrix File(s)	TXT

Supplementary file	Size	Download	File type/resource
GSE60424_GEOSubmit_FC1to11_normalized_counts.txt.gz	2.3 Mb	(ftp)(http)	TXT

(1) Click 'Series Matrix File(s)'

(3) Click (ftp) to download the normalized counts file.



https://ftp.ncbi.nlm.nih.gov/geo/series/GSE60nnn/GSE60424/matrix/

Index of /geo/series/GSE60nnn/GSE60424/matrix

Name	Last modified	Size
Parent Directory		-
GSE60424_series_matrix.txt.gz	2020-08-13 16:12	8.5K

(2) Download 'GSE60424_series_matrix.txt.gz'

Tutorial-Getting Data from GEO

Note:
GSE60424_GEOSubmit_FC1to11_normalized_counts.txt is a normalized counts matrix. The file should look like this:

GSE60424_GEOSubmit_FC1to11_normalized_counts.txt

```
genenames       lib221  lib222  lib223  lib224 ...... lib355
ENSG00000000003 1       0       1       1
ENSG00000000005 0       0       0       0
ENSG00000000419 23      22      25      28
ENSG00000000457 11      11      14      17
ENSG00000000460 3       3       3       3
ENSG00000000938 1141    872     1068    629
ENSG00000000971 3       1       1       3
ENSG00000001036 17      21      27      17
ENSG00000001084 19      14      19      19
ENSG00000001167 35      33      33      44

      ...         ...     ...     ...     ...
      ...         ...     ...     ...     ...
      ...         ...     ...     ...     ...
      ...         ...     ...     ...     ...
```

Tutorial-Getting Data from GEO

Note: This is a standard series matrix file that is uploaded with data to GEO. It describes the data using standardized fields defining filenames, sample labels, and sample characteristics, and the related publication.

GSE60424_series_matrix.txt

```
!Series_title	"Next generation sequencing of human immune cell subsets across diseases"
!Series_geo_accession	"GSE60424"
!Series_status	"Public on Jan 06 2015"
!Series_submission_date	"Aug 14 2014"
!Series_last_update_date	"May 15 2019"
!Series_pubmed_id	"25314013"
!Series_summary	"This study compared whole transcriptome signatures of 6 immune cell subsets and whole blood from patients with an array of immune-associated diseases. Fresh blood samples were collected from
healthy subjects and subjects diagnosed type 1 diabetes, amyotrophic lateral sclerosis, and sepsis, as well as multiple sclerosis patients before and 24 hours after the first treatment with IFN-beta. At the
time of blood draw, an aliquot of whole blood was collected into a Tempus tube (Invitrogen), while the remainder of the primary fresh blood sample was processed to highly pure populations of neutrophils,
monocytes, B cells, CD4 T cells, CD8 T cells, and natural killer cells. RNA was extracted from each of these cell subsets, as well as the whole blood samples, and processed into RNA sequencing (RNAseq)
libraries (Illumina TruSeq). Sequencing libraries were analyzed on an Illumina HiScan, with a target read depth of ~20M reads. Reads were demultiplexed, mapped to human gene models (ENSEMBL), and tabulated
using HTSeq. Read count data were normalized by the TMM procedure (edgeR package)."
!Series_overall_design	"We performed whole genome RNAseq profiling of immune cell subsets and whole blood from subjects with an array of immune-associated diseases."
!Series_type	"Expression profiling by high throughput sequencing"
!Series_contributor	"Cate,,Speake"
!Series_contributor	"Peter,S,Linsley"
!Series_contributor	"Elizabeth,,Whalen"
!Series_contributor	"Damien,,Chaussabel"
!Series_contributor	"Scott,R,Presnell"
!Series_contributor	"Michael,J,Mason"
!Series_contributor	"Vivian,H,Gersuk"
!Series_contributor	"Kimm,K,O'Brien"
!Series_contributor	"Quynh-Anh,,Nguyen"
!Series_contributor	"Carla,J,Greenbaum"
!Series_contributor	"Jane,H,Buckner"
!Series_contributor	"Uma,,Malhotra"
!Series_sample_id	"GSM1479433 GSM1479434 GSM1479435 GSM1479436 GSM1479437 GSM1479438 GSM1479439 GSM1479440 GSM1479441 GSM1479442 GSM1479443 GSM1479444 GSM1479445 GSM1479446 GSM1479447 GSM1479448
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GSM1479563 GSM1479564 GSM1479565 GSM1479566 "
!Series_contact_name	"Scott,,Presnell"
!Series_contact_email	"SPresnell@benaroyaresearch.org"
!Series_contact_department	"Systems Immunology"
!Series_contact_institute	"Benaroya Research Institute"
!Series_contact_address	"1201 Ninth Ave."
!Series_contact_city	"Seattle"
!Series_contact_state	"WA"
!Series_contact_zip/postal_code	"98101"
!Series_contact_country	"USA"
!Series_supplementary_file	"ftp://ftp.ncbi.nlm.nih.gov/geo/series/GSE60nnn/GSE60424/suppl/GSE60424_GEOSubmit_FC1to11_normalized_counts.txt.gz"
!Series_platform_id	"GPL15456"
!Series_platform_taxid	"9606"
!Series_sample_taxid	"9606"
!Series_relation	"BioProject: https://www.ncbi.nlm.nih.gov/bioproject/PRJNA258216"
!Series_relation	"SRA: https://www.ncbi.nlm.nih.gov/sra?term=SRP045500"

!
Sample_title	"lib221"	"lib222"	"lib223"	"lib224"	"lib225"	"lib226"	"lib227"	"lib228"	"lib229"	"lib230"	"lib231"	"lib232"
"lib233"	"lib234"	"lib235"	"lib236"	"lib237"	"lib238"	"lib239"	"lib240"	"lib241"	"lib242"	"lib243"	"lib244"	"lib245"
"lib246"	"lib247"	"lib248"	"lib249"	"lib250"	"lib251"	"lib252"	"lib253"	"lib254"	"lib255"	"lib256"	"lib257"	"lib258"
"lib259"	"lib260"	"lib261"	"lib262"	"lib263"	"lib264"	"lib265"	"lib266"	"lib267"	"lib268"	"lib269"	"lib270"	"lib271"
"lib272"	"lib273"	"lib274"	"lib275"	"lib276"	"lib277"	"lib278"	"lib279"	"lib280"	"lib281"	"lib282"	"lib283"	"lib284"
"lib285"	"lib286"	"lib288"	"lib289"	"lib290"	"lib291"	"lib292"	"lib293"	"lib294"	"lib295"	"lib296"	"lib297"	"lib298"
"lib299"	"lib300"	"lib301"	"lib302"	"lib303"	"lib305"	"lib305"	"lib306"	"lib307"	"lib308"	"lib309"	"lib310"	"lib311"
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"lib325"	"lib326"	"lib327"	"lib328"	"lib329"	"lib330"	"lib331"	"lib332"	"lib333"	"lib334"	"lib335"	"lib336"	"lib337"
"lib338"	"lib339"	"lib340"	"lib341"	"lib342"	"lib343"	"lib344"	"lib345"	"lib346"	"lib347"	"lib348"	"lib349"	"lib350"
"lib351"	"lib352"	"lib353"	"lib354"	"lib355"
```
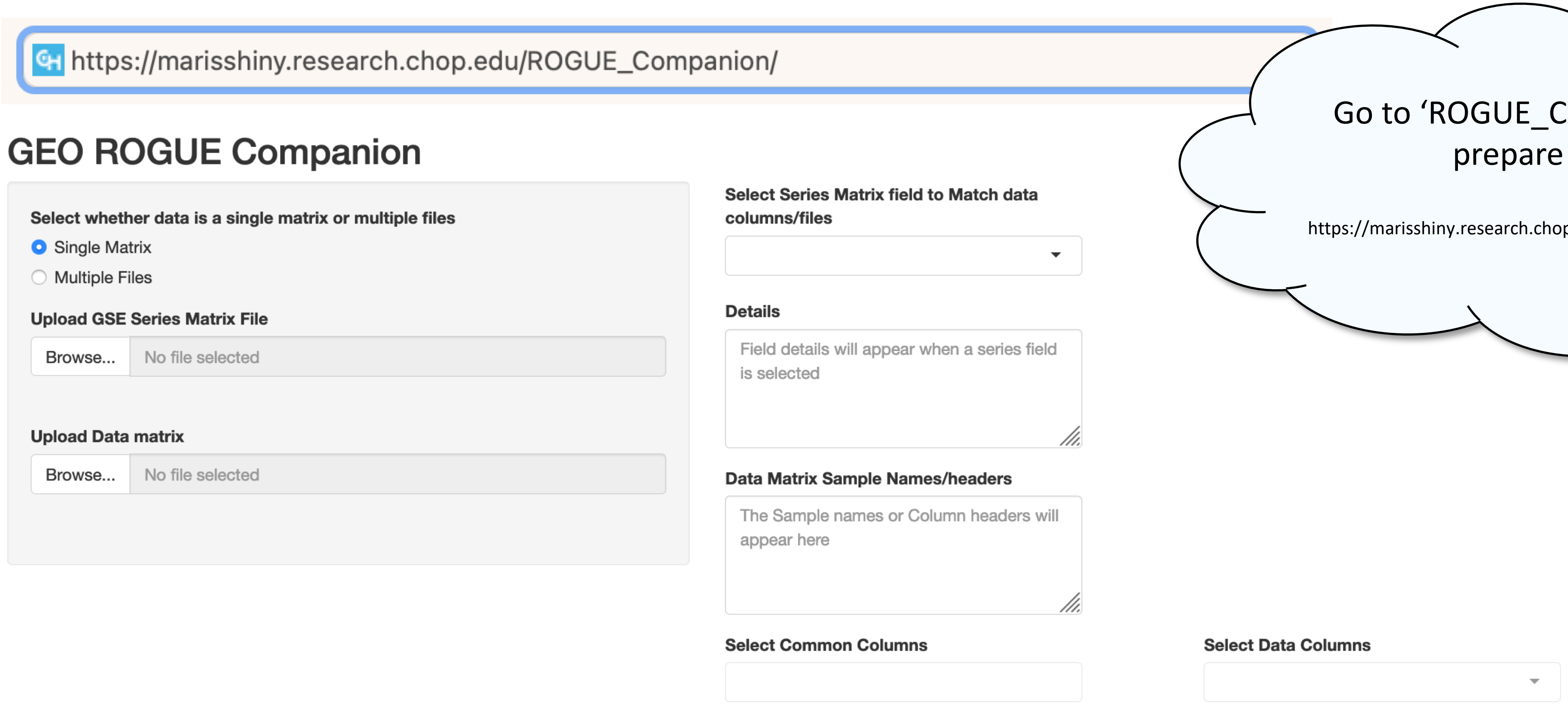

Tutorial-Getting Data from GEO



https://marisshiny.research.chop.edu/ROGUE_Companion/

GEO ROGUE Companion

Select whether data is a single matrix or multiple files

● Single Matrix
○ Multiple Files

Upload GSE Series Matrix File

Browse... No file selected

Upload Data matrix

Browse... No file selected

Select Series Matrix field to Match data columns/files

Details

Field details will appear when a series field is selected

Data Matrix Sample Names/headers

The Sample names or Column headers will appear here

Select Common Columns

Select Data Columns

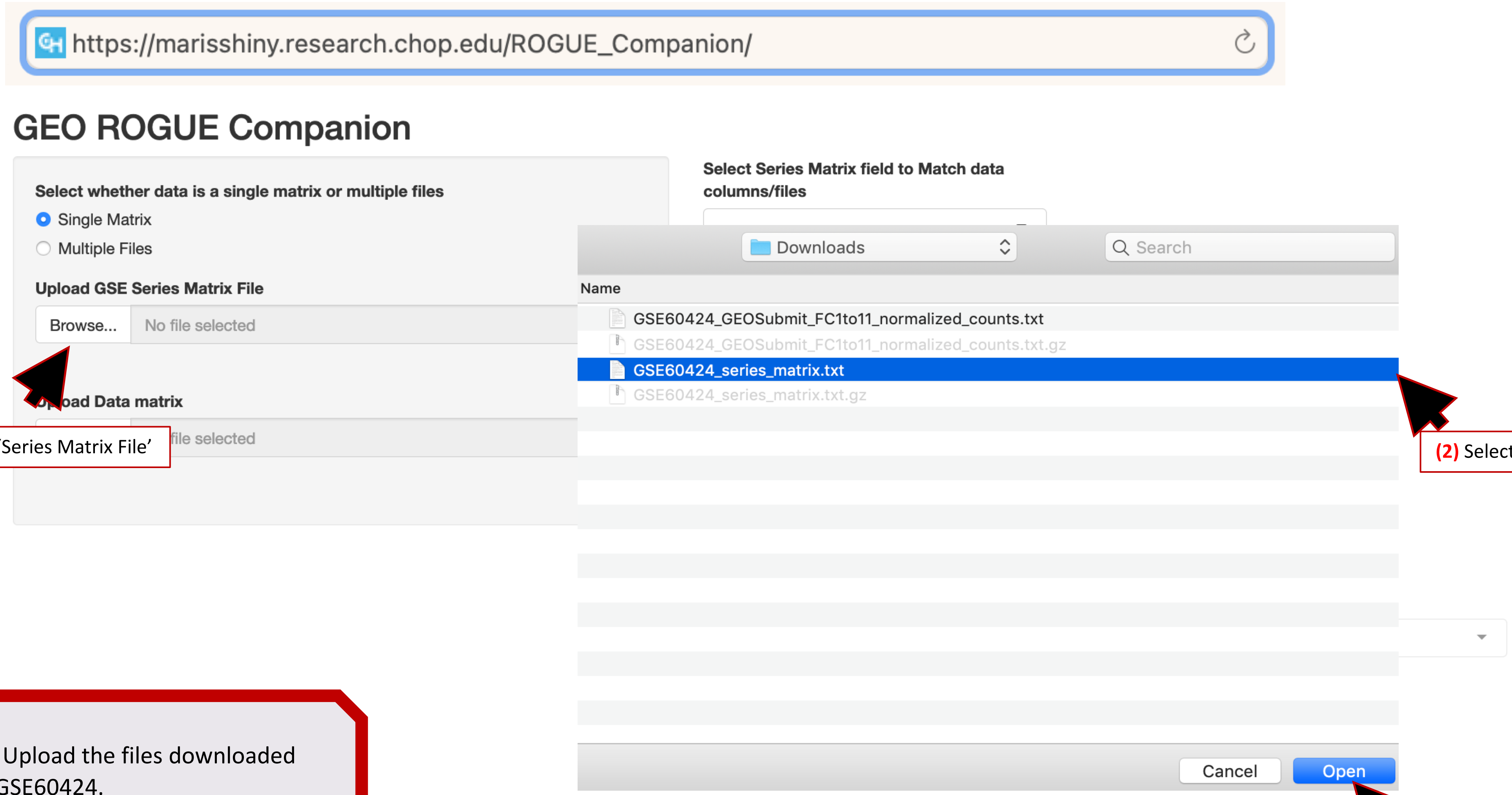
Go to ‘ROGUE_Companion’ to prepare files.

https://marisshiny.research.chop.edu/ROGUE_Companion/

Note: The GEO ROGUE Companion tool was created to help prepare a data matrix and group file from data downloaded from GEO for analysis using ROGUE. Both a data matrix and Series matrix file are required.

Follow this tutorial as an example.

Tutorial-Getting Data from GEO



https://marisshiny.research.chop.edu/ROGUE_Companion/

GEO ROGUE Companion

Select whether data is a single matrix or multiple files

● Single Matrix

○ Multiple Files

Upload GSE Series Matrix File

Browse... No file selected

Upload Data matrix

file selected

Select Series Matrix field to Match data columns/files

GSE60424_GEOSubmit_FC1to11_normalized_counts.txt

GSE60424_GEOSubmit_FC1to11_normalized_counts.txt.gz

GSE60424_series_matrix.txt

GSE60424_series_matrix.txt.gz

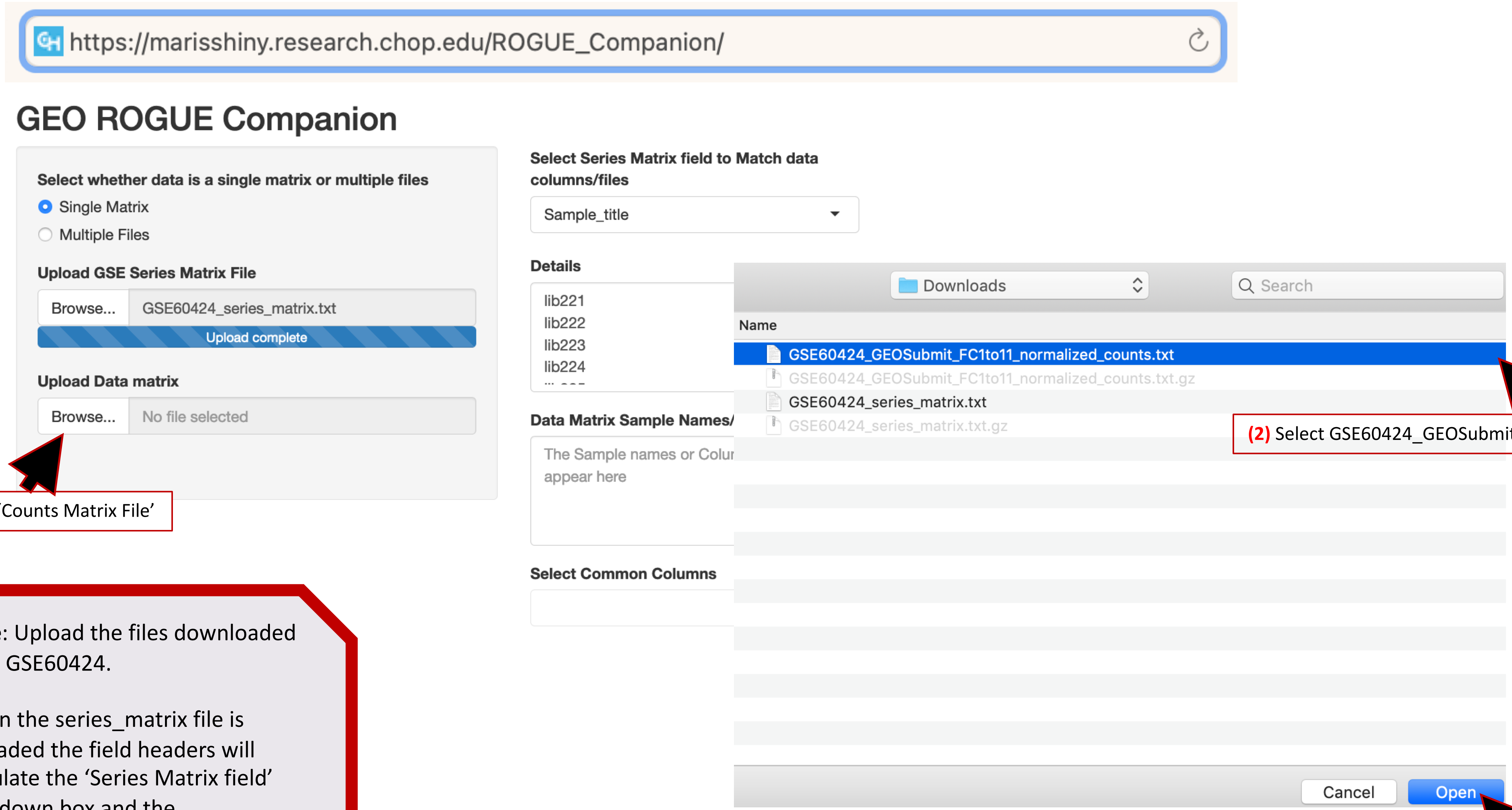
(1) Upload 'Series Matrix File'

(2) Select GSE60424_series_matrix.txt

(3) Click 'Open'

Note: Upload the files downloaded from GSE60424.

Tutorial-Getting Data from GEO



https://marisshiny.research.chop.edu/ROGUE_Companion/

GEO ROGUE Companion

Select whether data is a single matrix or multiple files

● Single Matrix
○ Multiple Files

Upload GSE Series Matrix File

Browse... GSE60424_series_matrix.txt
Upload complete

Upload Data matrix

Browse... No file selected

Select Series Matrix field to Match data columns/files

Sample_title

Details

lib221
lib222
lib223
lib224

Data Matrix Sample Names/

The Sample names or Colu
appear here

Select Common Columns

GSE60424_GEOSubmit_FC1to11_normalized_counts.txt
GSE60424_GEOSubmit_FC1to11_normalized_counts.txt.gz
GSE60424_series_matrix.txt
GSE60424_series_matrix.txt.gz

(1) Upload 'Counts Matrix File'

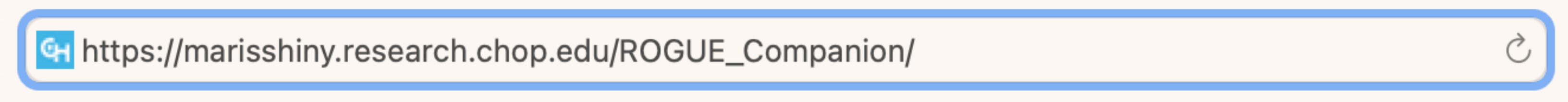
(2) Select GSE60424_GEOSubmit_FC1to11_normalized_counts.txt

(3) Click 'Open'

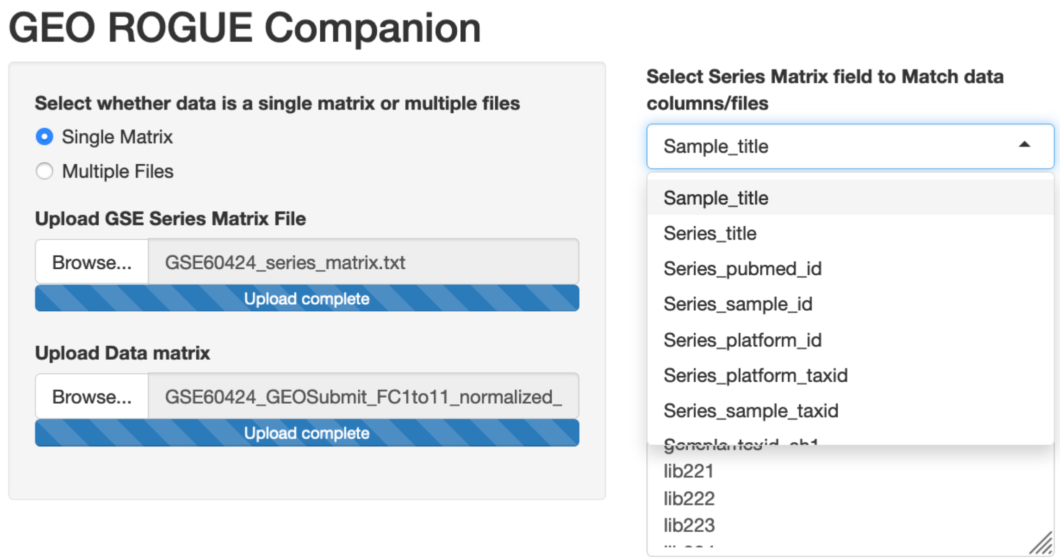
Note: Upload the files downloaded from GSE60424.

When the series_matrix file is uploaded the field headers will populate the 'Series Matrix field' dropdown box and the details/members of the selected field will fill the 'Details' text box.

Tutorial-Getting Data from GEO



https://marisshiny.research.chop.edu/ROGUE_Companion/



GEO ROGUE Companion

Select whether data is a single matrix or multiple files

● Single Matrix

○ Multiple Files

Upload GSE Series Matrix File

Browse... GSE60424_series_matrix.txt

Upload complete

Upload Data matrix

Browse... GSE60424_GEOSubmit_FC1to11_normalized_

Upload complete

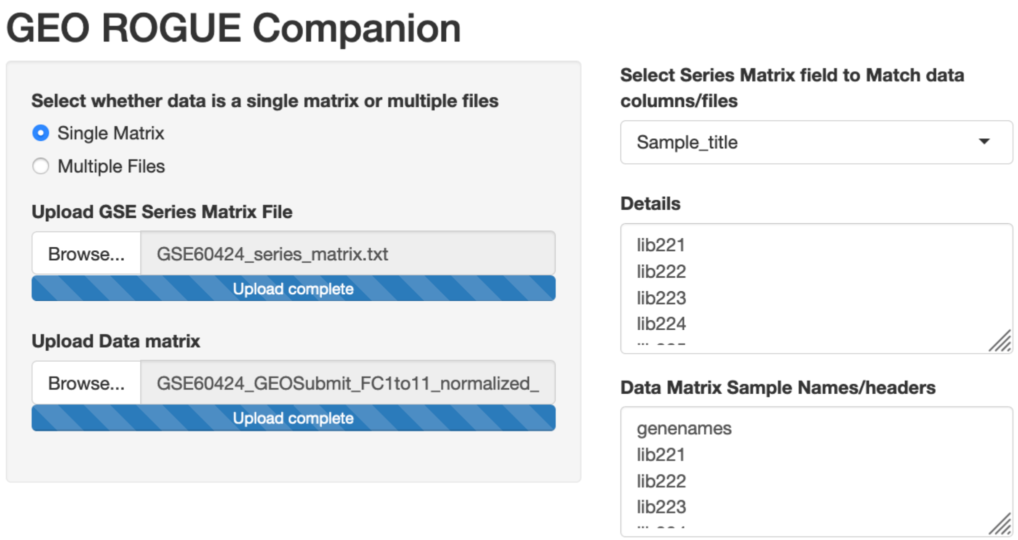
Select Series Matrix field to Match data columns/files

Sample_title

- Sample_title
- Series_title
- Series_pubmed_id
- Series_sample_id
- Series_platform_id
- Series_platform_taxid
- Series_sample_taxid

lib221
lib222
lib223

(1) This Drop down menu is filled with ID and title Fields from the Series Matrix file. When an ID or Title field is selected the 'Details' text box is populated with the members of that field as one line per member.



GEO ROGUE Companion

Select whether data is a single matrix or multiple files

● Single Matrix

○ Multiple Files

Upload GSE Series Matrix File

Browse... GSE60424_series_matrix.txt

Upload complete

Upload Data matrix

Browse... GSE60424_GEOSubmit_FC1to11_normalized_

Upload complete

Select Series Matrix field to Match data columns/files

Sample_title

Details

lib221
lib222
lib223
lib224

Data Matrix Sample Names/headers

genenames
lib221
lib222
lib223

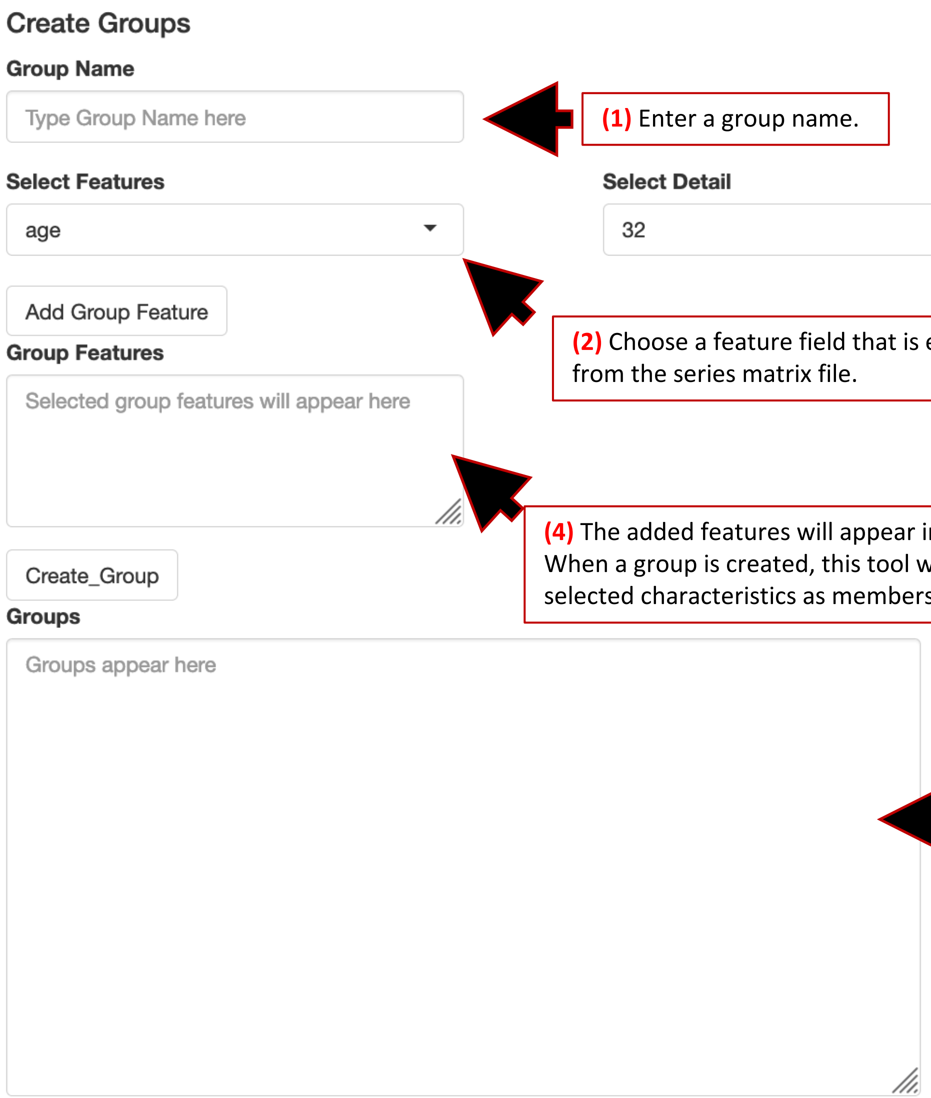
(2) When an ID or Title field is selected the 'Details' text box is populated with the members of that field as one line per member.

It is important that the user chooses the ID or Title field that has details with the same names as the column headers listed in the 'Data Matrix Samples Names/headers' text box.

(3) When a Data matrix is loaded, the column headers fill the 'Data Matrix Samples Names/headers' text box.

Tutorial-Getting Data from GEO

https://marisshiny.research.chop.edu/ROGUE_Companion/



Create Groups

Group Name: Type Group Name here

Select Features: age

Select Detail: 32

Group Features: Selected group features will appear here

Groups: Groups appear here

(1) Enter a group name.

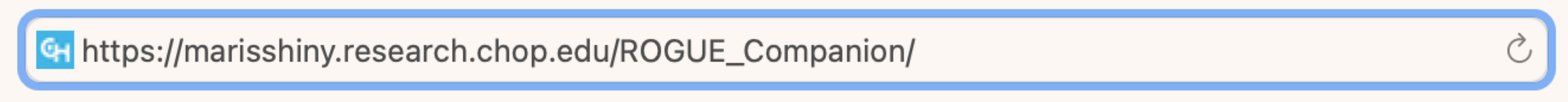
(2) Choose a feature field that is extracted from the "!characteristics" fields from the series matrix file.

(3) Choose the feature details of the members of the group.

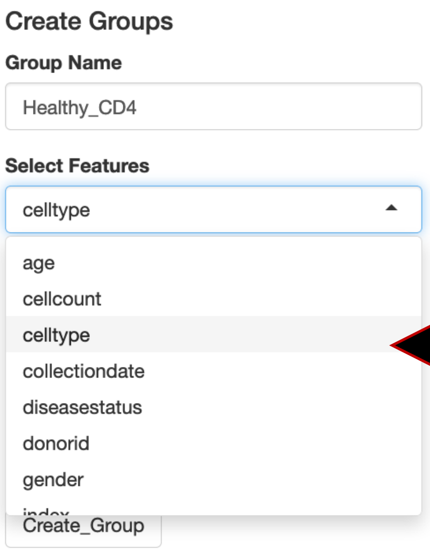
(4) The added features will appear in this box. Multiple features can be added. When a group is created, this tool will include the samples that have **all** the selected characteristics as members of the group.

(5) When 'Create Group' Button is clicked and a group is successfully create, it will appear in the format:
'**Group_Name:Member1;Member2;Member3**'
This is the same group format required by the main ROGUE tool.

Tutorial-Getting Data from GEO


https://marisshiny.research.chop.edu/ROGUE_Companion/

Note: Follow these steps to create groups that will be used in the ROGUE tutorial.


Create Groups

Group Name: Healthy_CD4

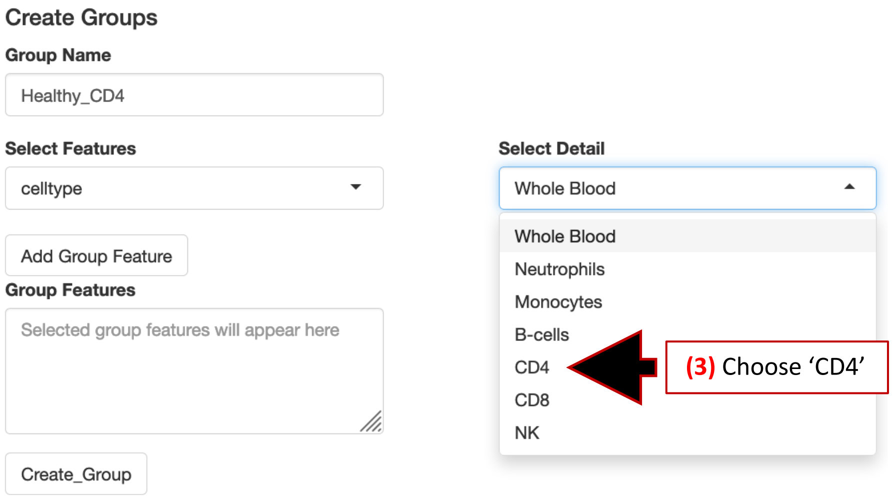
Select Features: celltype

- age
- cellcount
- celltype
- collectiondate
- diseasestatus
- donorid
- gender
- index

Select Detail: Whole Blood

(1) Type ‘Healthy_CD4’ as the Group Name

(2) Choose ‘celltype’


Create Groups

Group Name: Healthy_CD4

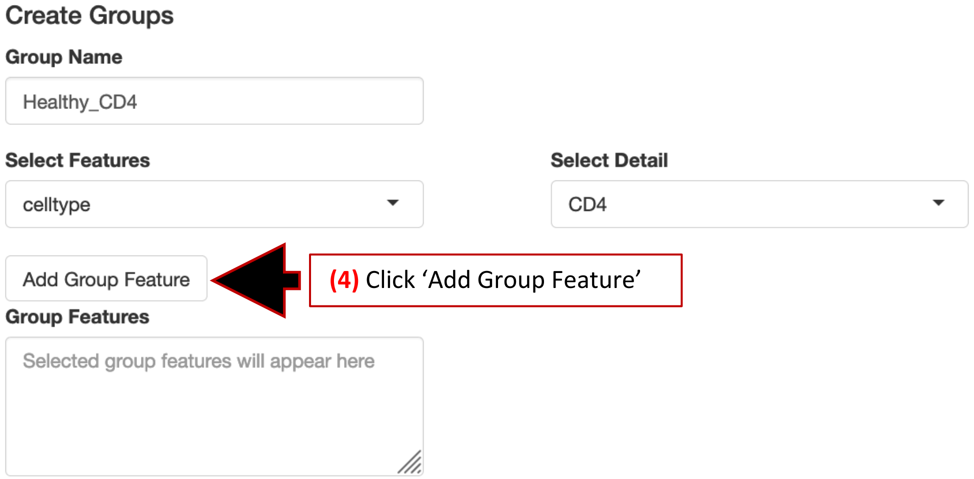
Select Features: celltype

Select Detail: Whole Blood

- Whole Blood
- Neutrophils
- Monocytes
- B-cells
- CD4
- CD8
- NK

Group Features: Selected group features will appear here

(3) Choose ‘CD4’


Create Groups

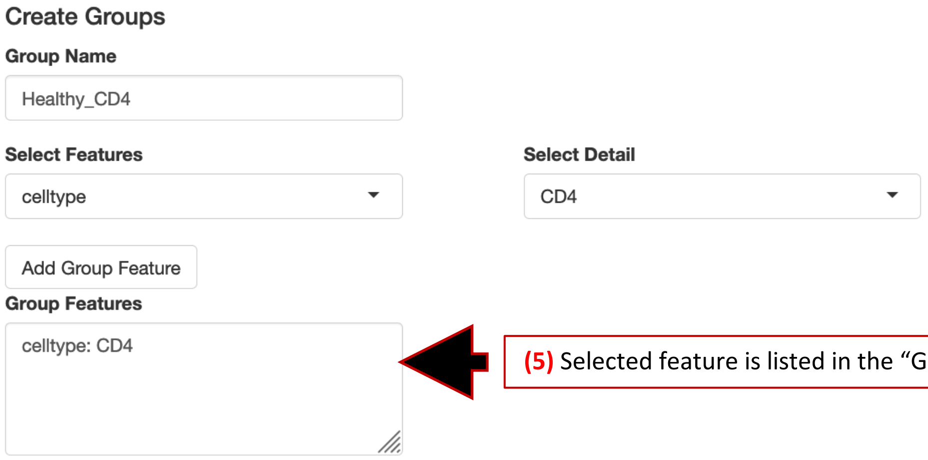
Group Name: Healthy_CD4

Select Features: celltype

Select Detail: CD4

Group Features: Selected group features will appear here

(4) Click ‘Add Group Feature’


Create Groups

Group Name: Healthy_CD4

Select Features: celltype

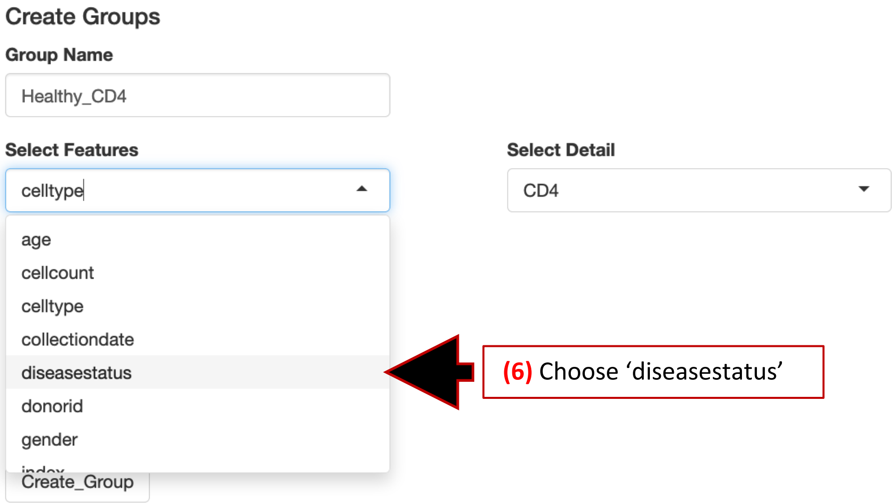
Select Detail: CD4

Group Features: celltype: CD4

(5) Selected feature is listed in the “Group Features” text box.

Tutorial-Getting Data from GEO


https://marisshiny.research.chop.edu/ROGUE_Companion/

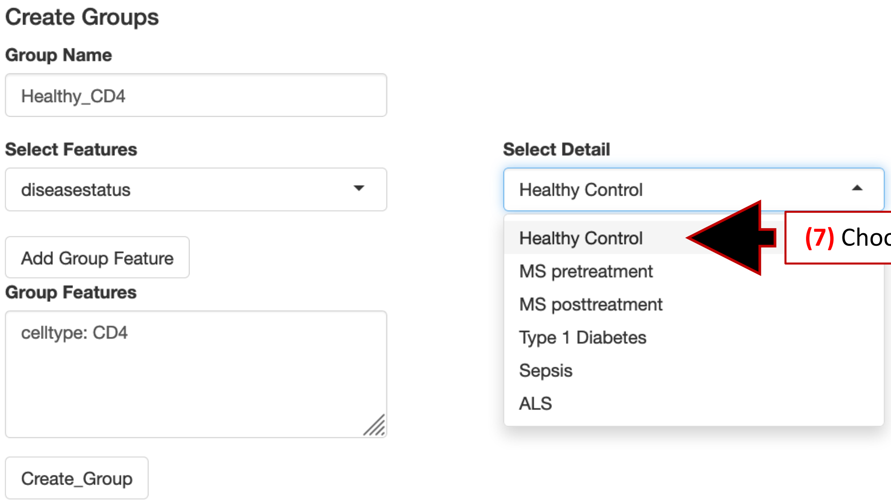

Create Groups

Group Name: Healthy_CD4

Select Features: celltype

Select Detail: CD4

(6) Choose ‘diseasestatus’


Create Groups

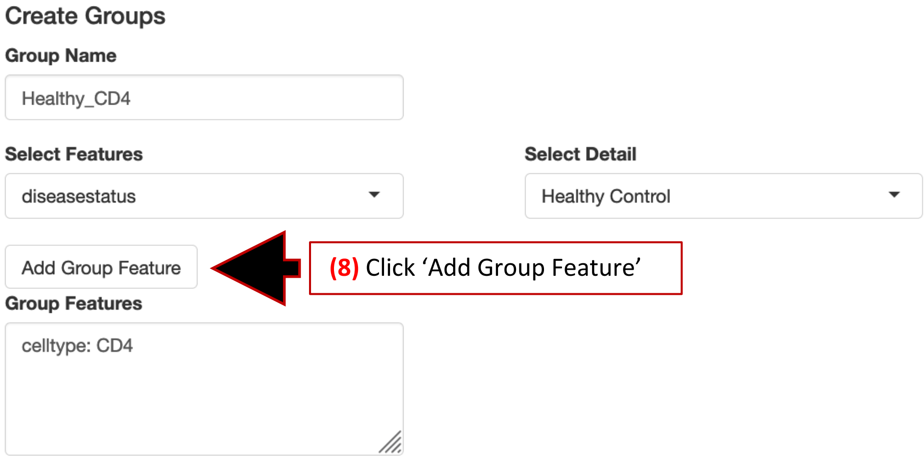
Group Name: Healthy_CD4

Select Features: diseasestatus

Select Detail: Healthy Control

Group Features: celltype: CD4

(7) Choose ‘Healthy Control’


Create Groups

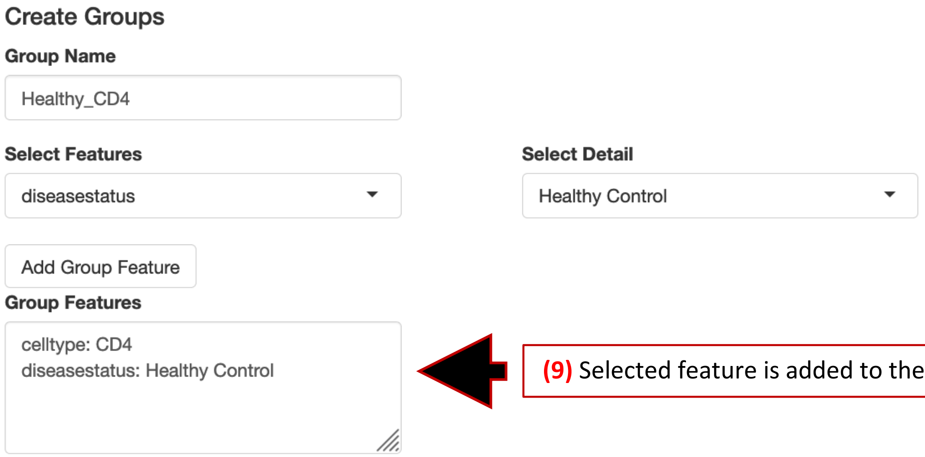
Group Name: Healthy_CD4

Select Features: diseasestatus

Select Detail: Healthy Control

Group Features: celltype: CD4

(8) Click ‘Add Group Feature’


Create Groups

Group Name: Healthy_CD4

Select Features: diseasestatus

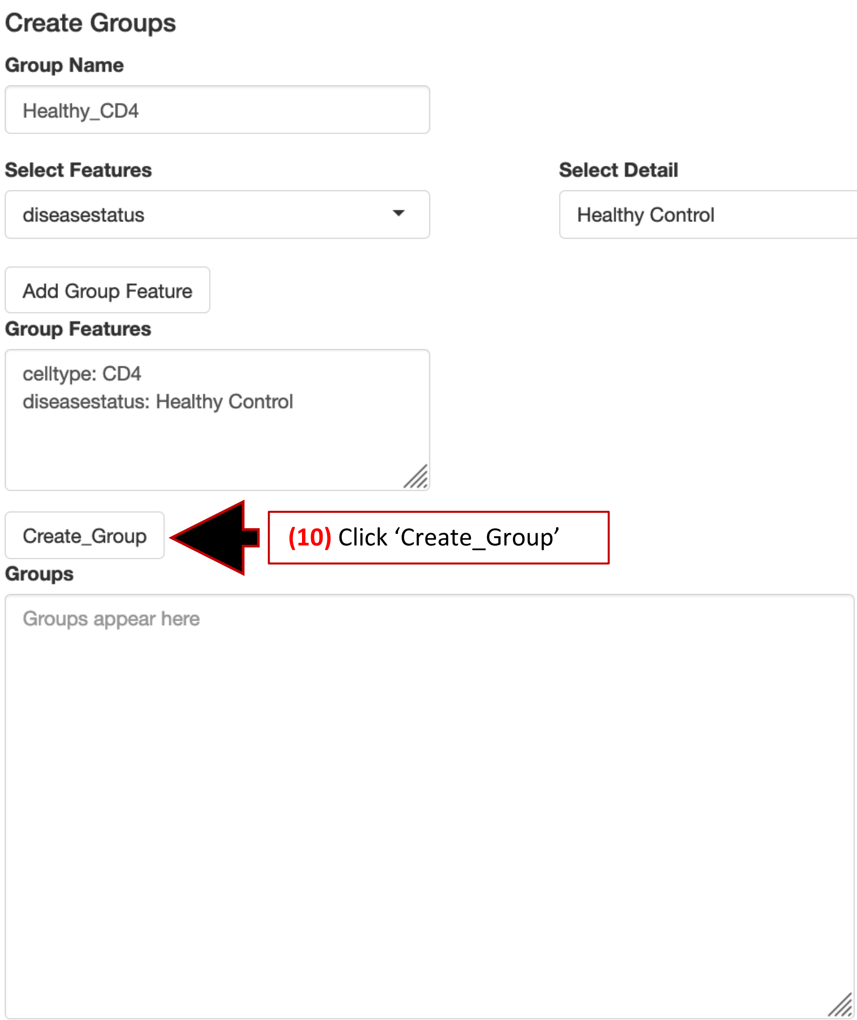
Select Detail: Healthy Control

Group Features:
celltype: CD4
diseasestatus: Healthy Control

(9) Selected feature is added to the “Group Features” text box.

Tutorial-Getting Data from GEO

https://marisshiny.research.chop.edu/ROGUE_Companion/



Create Groups

Group Name

Healthy_CD4

Select Features

diseasestatus

Select Detail

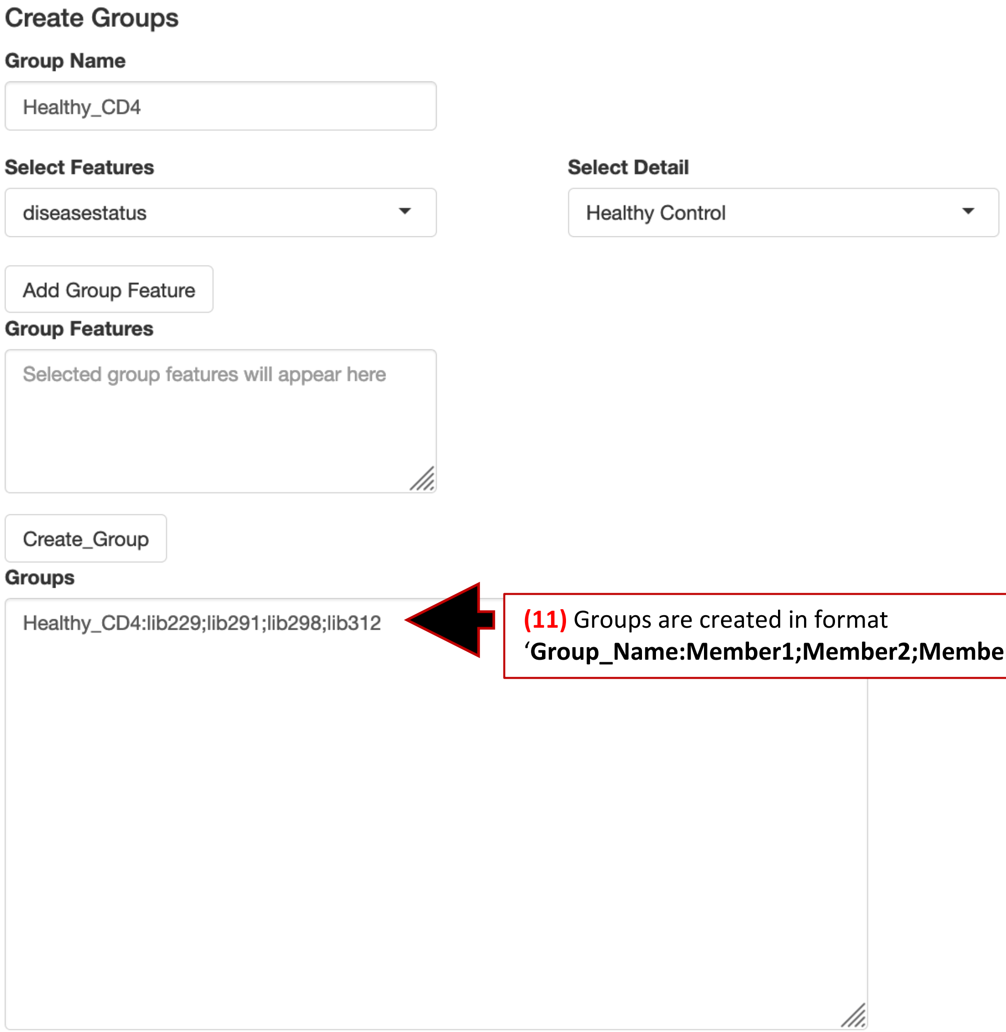
Healthy Control

Group Features

celltype: CD4
diseasestatus: Healthy Control

(10) Click ‘Create_Group’

Groups



Create Groups

Group Name

Healthy_CD4

Select Features

diseasestatus

Select Detail

Healthy Control

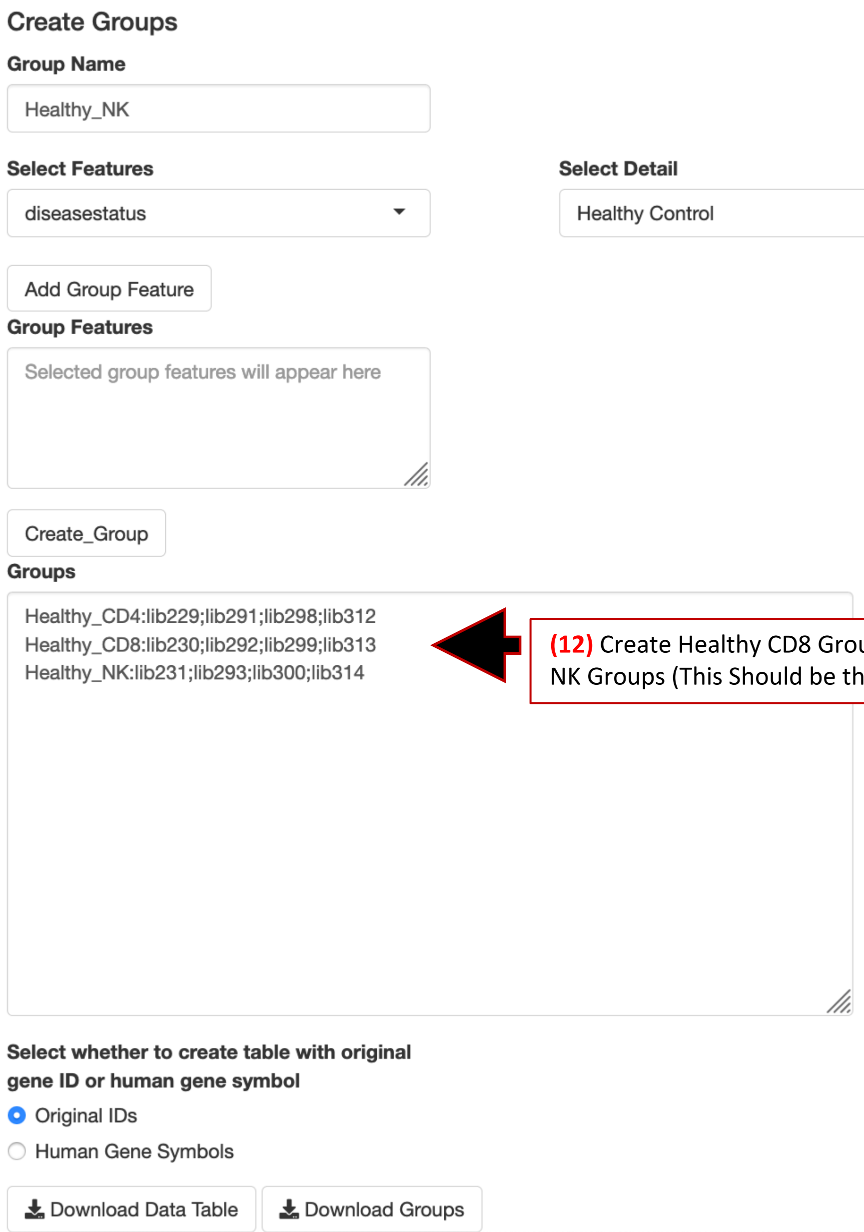
Group Features

Groups

Healthy_CD4:lib229;lib291;lib298;lib312

(11) Groups are created in format ‘**Group_Name:Member1;Member2;Member3**’

Tutorial-Getting Data from GEO



Create Groups

Group Name: Healthy_NK

Select Features: diseasestatus

Select Detail: Healthy Control

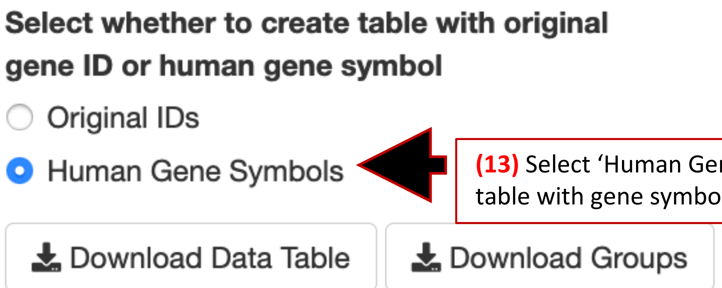
Groups

Healthy_CD4:lib229;lib291;lib298;lib312
Healthy_CD8:lib230;lib292;lib299;lib313
Healthy_NK:lib231;lib293;lib300;lib314

Select whether to create table with original gene ID or human gene symbol

● Original IDs
○ Human Gene Symbols

(12) Create Healthy CD8 Groups and Healthy NK Groups (This Should be the result).



Select whether to create table with original gene ID or human gene symbol

○ Original IDs
● Human Gene Symbols

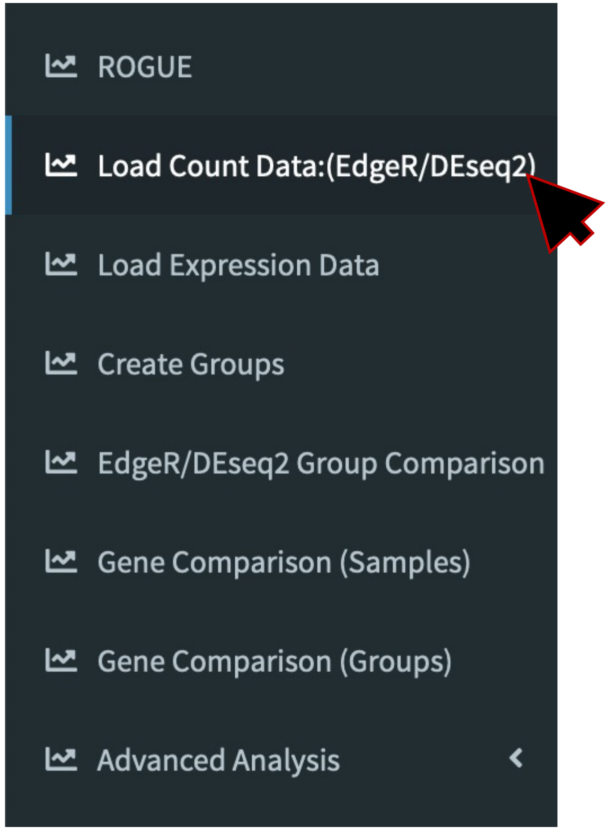
(13) Select 'Human Gene Symbols' if you want to download a table with gene symbols instead of Ensembl IDs

(14) Download Data Tables with Gene Symbols and Group Files

Note: Download the Data Table as 'Data_Matrix.txt' and the groups file as 'Groups_File.txt'

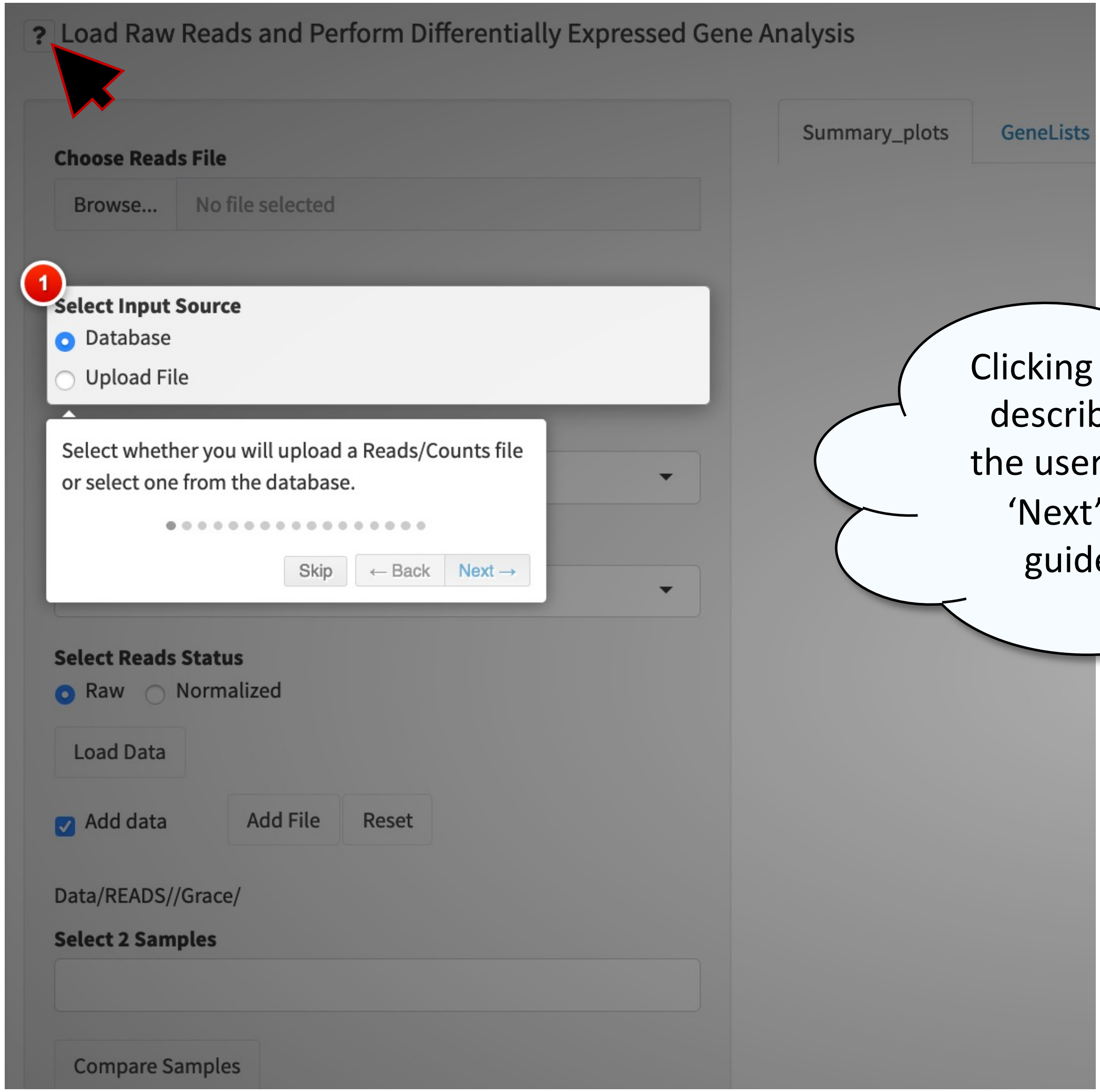
ROGUE – Differential Expression Analysis

https://reslnmaris01.research.chop.edu/ROGUE/



Click the ‘**Load Count Data: (EdgeR)**’

ROGUE – Differential Expression Analysis



? Load Raw Reads and Perform Differentially Expressed Gene Analysis

Summary_plots | GeneLists

Choose Reads File

Browse... No file selected

Select Input Source

● Database

○ Upload File

Select whether you will upload a Reads/Counts file or select one from the database.

Select Reads Status

● Raw ○ Normalized

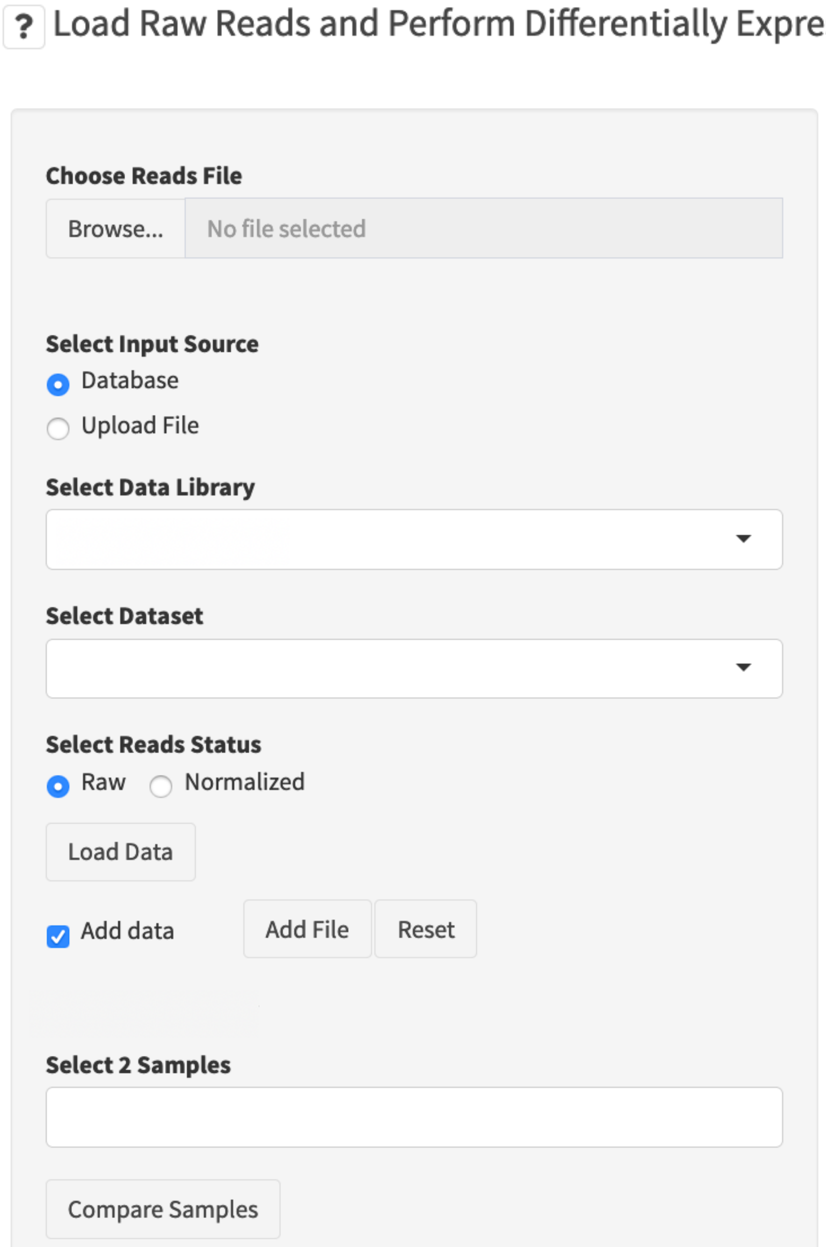
☑ Add data

Data/READS//Grace/

Select 2 Samples

Clicking the in-app help button will describe each options and guide the user through the process. Click ‘Next’ to see more tips and be guided through the options.

ROGUE – Differential Expression Analysis


? Load Raw Reads and Perform Differentially Expressed Gene Analysis

Choose Reads File

Browse... No file selected

Select Input Source

● Database

○ Upload File

Select Data Library

Select Dataset

Select Reads Status

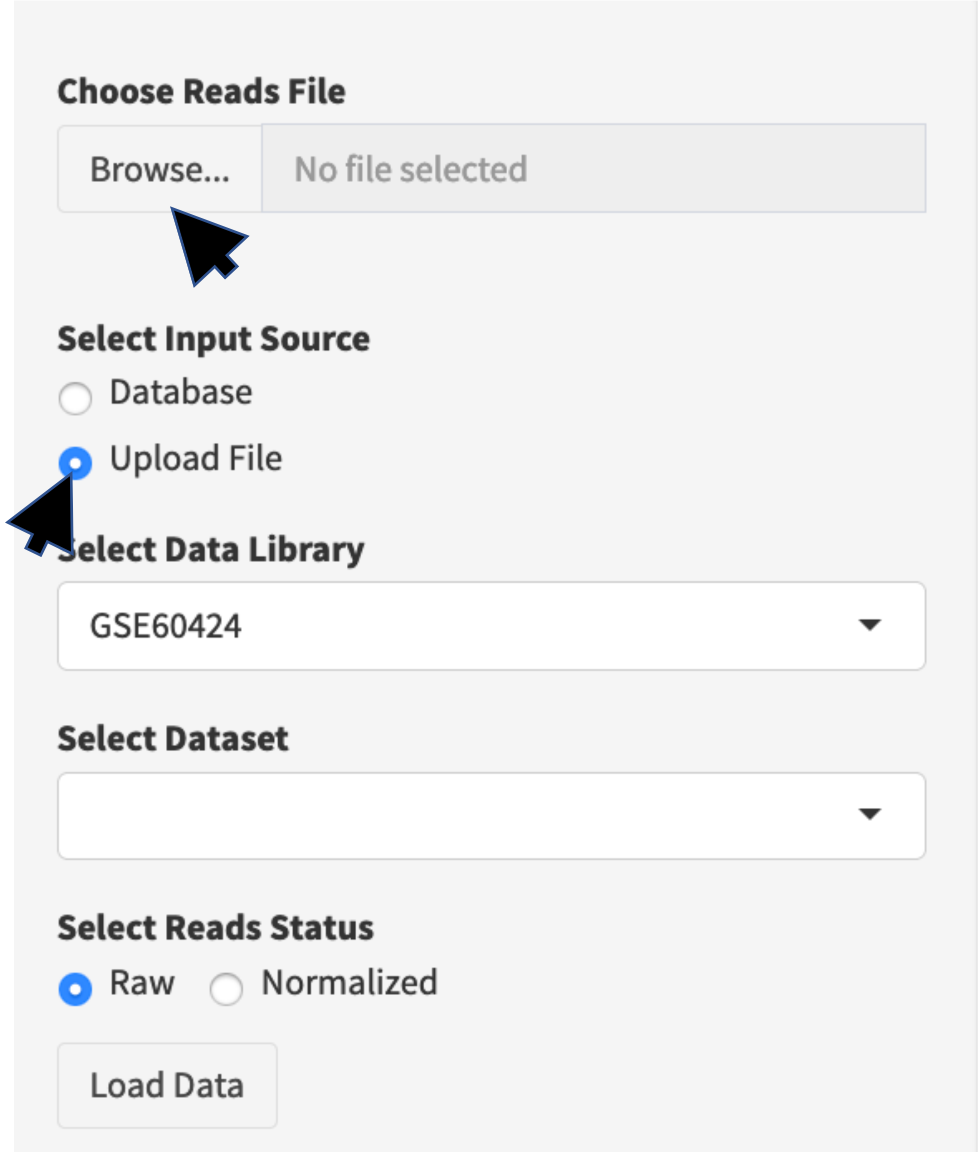
● Raw ○ Normalized

☑ Add data

Select 2 Samples

A tool to load raw or normalized counts appear where a user can perform differentially expressed genes (DEG) analysis and convert the counts to RPKM expression values.

ROGUE – Differential Expression Analysis



Choose Reads File

Browse... No file selected

Select Input Source

○ Database

● Upload File

Select Data Library

GSE60424

Select Dataset

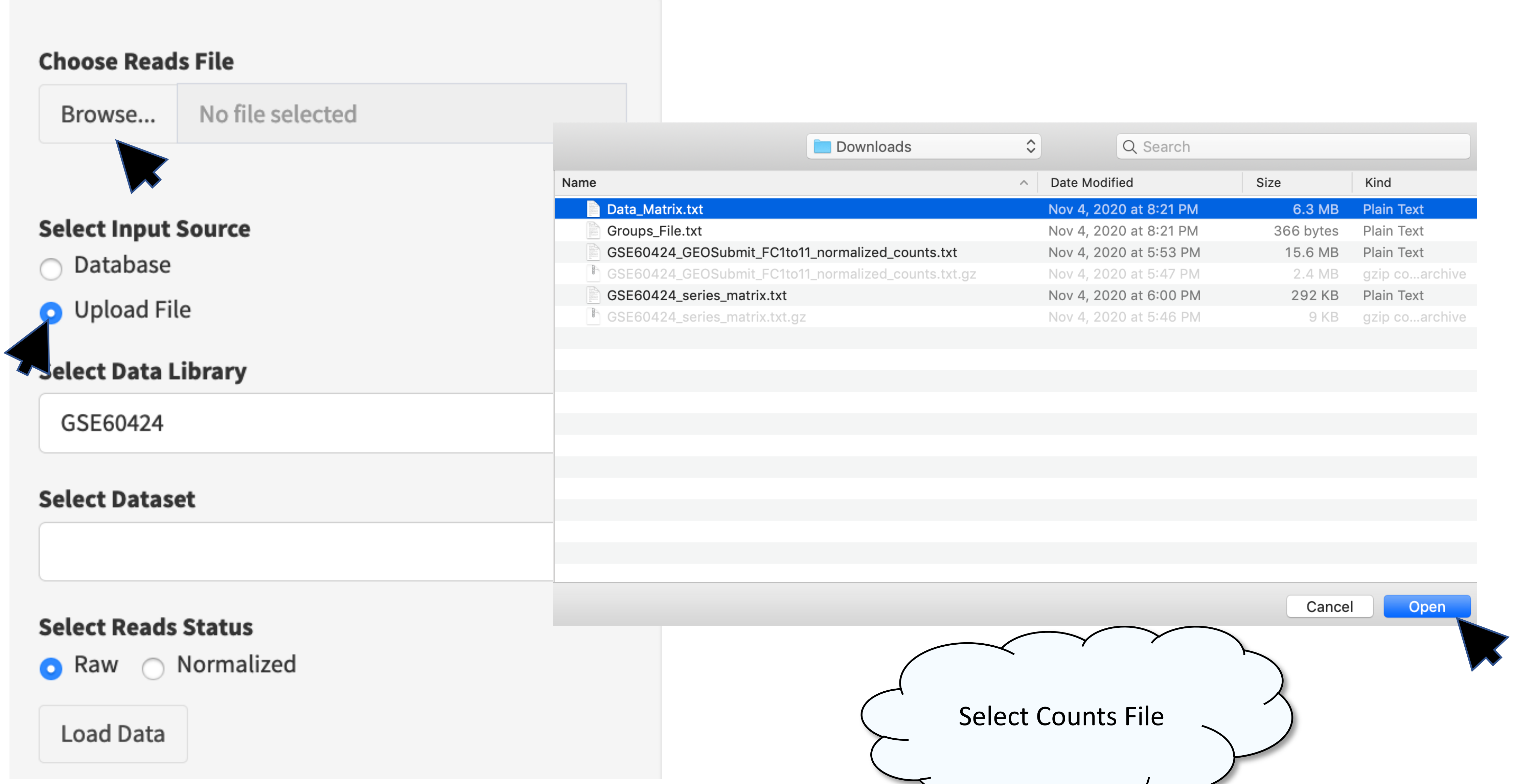
Select Reads Status

● Raw ○ Normalized

Load Data

Check the upload radio button and and click ‘**Browse**’ to select a Counts file

ROGUE – Differential Expression Analysis



Choose Reads File

Browse... No file selected

Select Input Source

○ Database

● Upload File

Select Data Library

GSE60424

Select Dataset

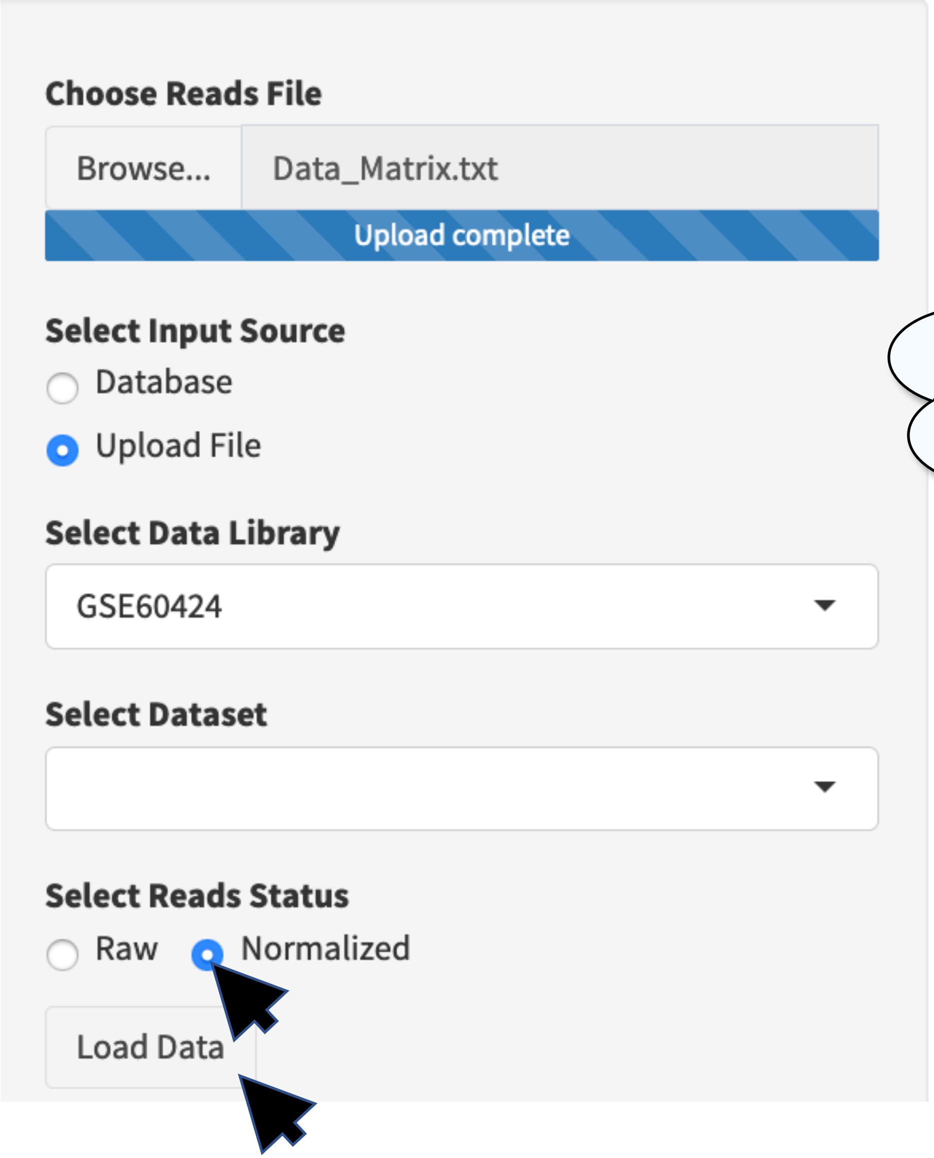
Select Reads Status

● Raw ○ Normalized

Name	Date Modified	Size	Kind
Data_Matrix.txt	Nov 4, 2020 at 8:21 PM	6.3 MB	Plain Text
Groups_File.txt	Nov 4, 2020 at 8:21 PM	366 bytes	Plain Text
GSE60424_GEOSubmit_FC1to11_normalized_counts.txt	Nov 4, 2020 at 5:53 PM	15.6 MB	Plain Text
GSE60424_GEOSubmit_FC1to11_normalized_counts.txt.gz	Nov 4, 2020 at 5:47 PM	2.4 MB	gzip co...archive
GSE60424_series_matrix.txt	Nov 4, 2020 at 6:00 PM	292 KB	Plain Text
GSE60424_series_matrix.txt.gz	Nov 4, 2020 at 5:46 PM	9 KB	gzip co...archive

Select Counts File

ROGUE – Differential Expression Analysis



Choose Reads File

Browse... Data_Matrix.txt

Upload complete

Select Input Source

○ Database

● Upload File

Select Data Library

GSE60424

Select Dataset

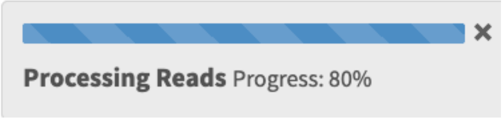
Select Reads Status

○ Raw ● Normalized

Load Data

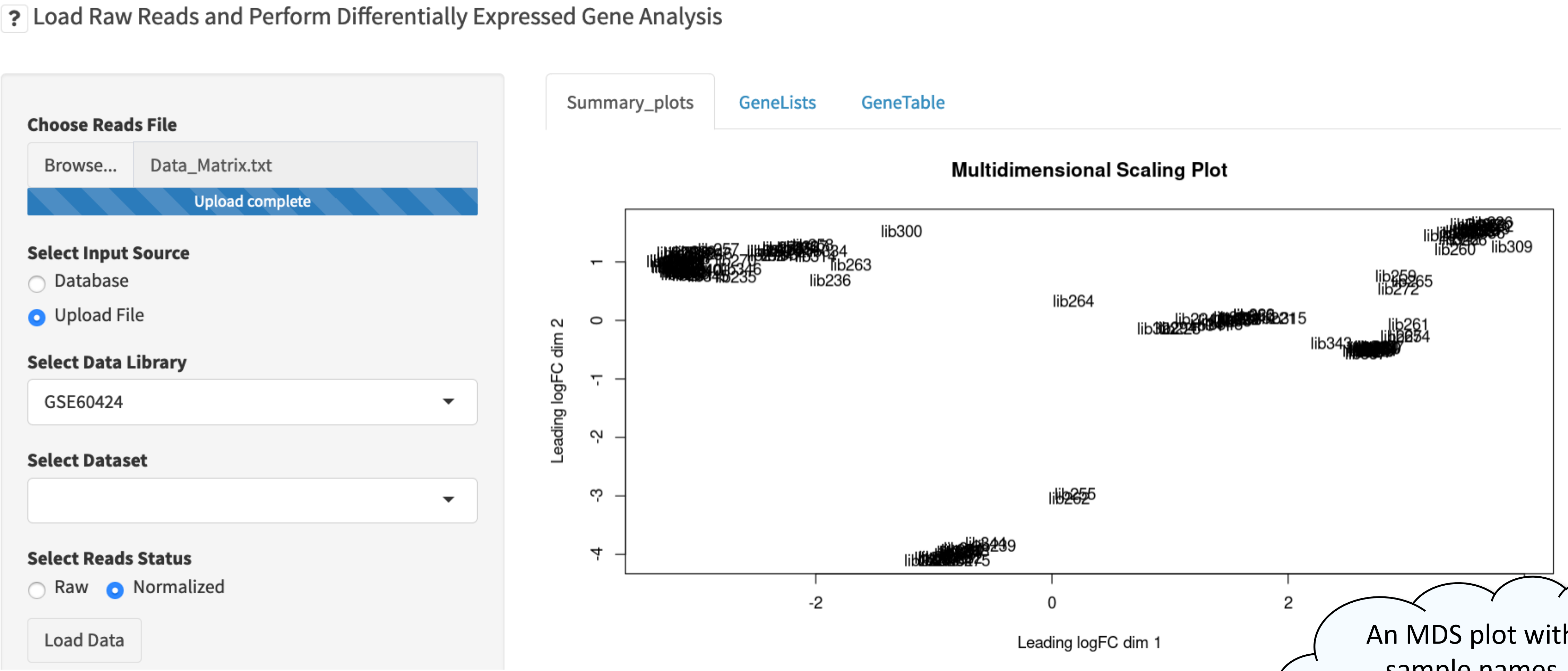
This file has normalized counts (by gene length). Select the ‘**Normalized**’ radio button.

Click the ’**Load Data**’ Button



Processing Reads Progress: 80%

ROGUE – Differential Expression Analysis



? Load Raw Reads and Perform Differentially Expressed Gene Analysis

Choose Reads File

Browse... Data_Matrix.txt

Upload complete

Select Input Source

○ Database

● Upload File

Select Data Library

GSE60424

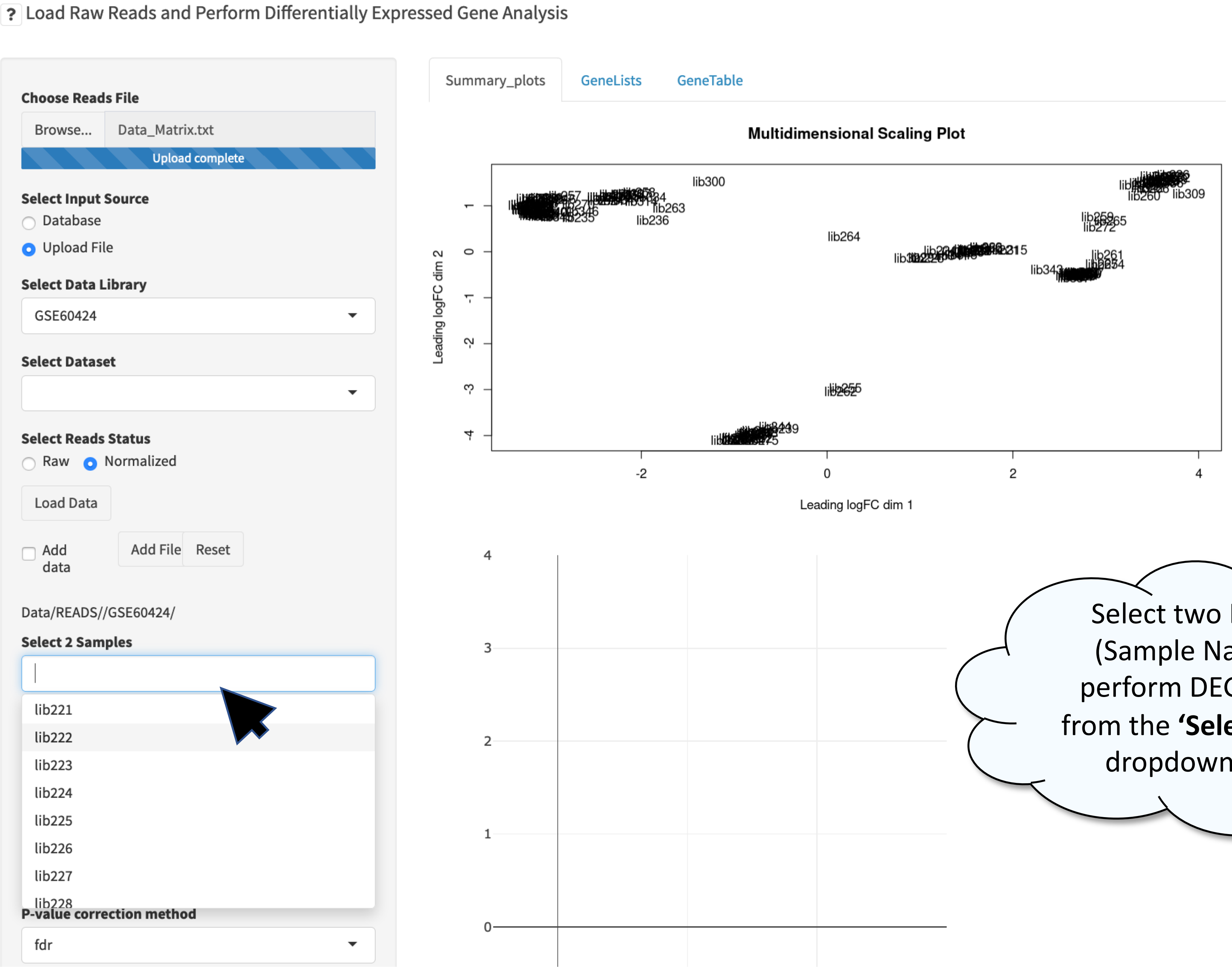
Select Dataset

Select Reads Status

○ Raw ● Normalized

An MDS plot with sample names appears when the data is loaded

ROGUE – Differential Expression Analysis



? Load Raw Reads and Perform Differentially Expressed Gene Analysis

Choose Reads File

Browse... Data_Matrix.txt

Upload complete

Select Input Source

○ Database

● Upload File

Select Data Library

GSE60424

Select Dataset

Select Reads Status

○ Raw ● Normalized

Load Data

☐ Add data

Data/READS//GSE60424/

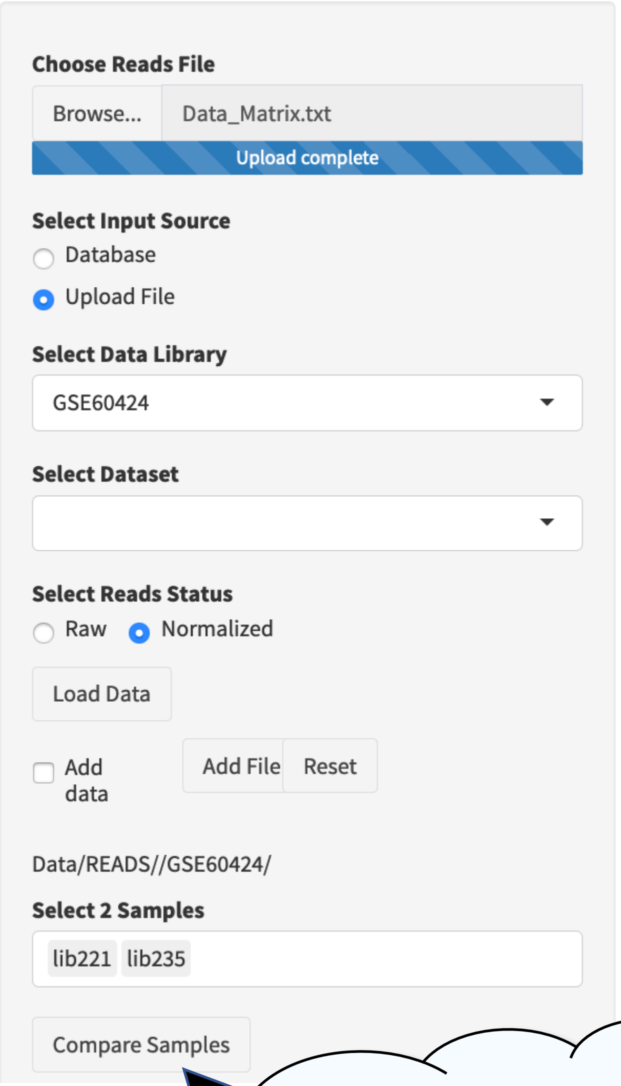
Select 2 Samples

P-value correction method

fdr

Select two Libraries (Sample Names) to perform DEG analysis from the **‘Select Library’** dropdown menu.

ROGUE – Differential Expression Analysis



Choose Reads File

Browse... Data_Matrix.txt

Upload complete

Select Input Source

○ Database

● Upload File

Select Data Library

GSE60424

Select Dataset

Select Reads Status

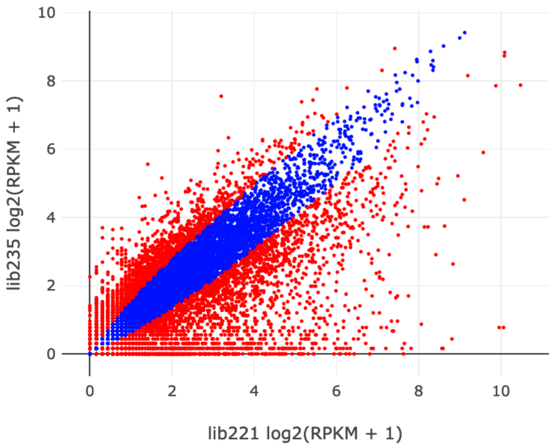
○ Raw ● Normalized

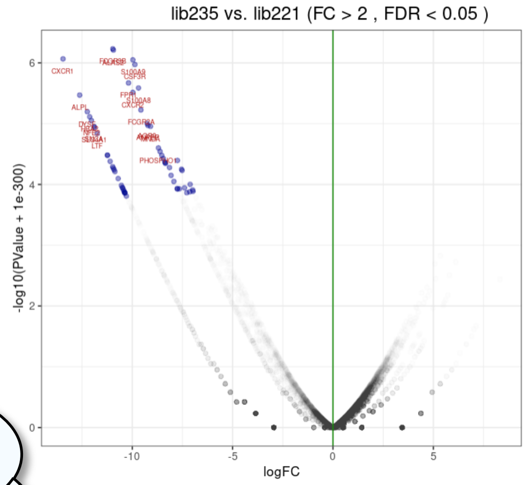
☐ Add data

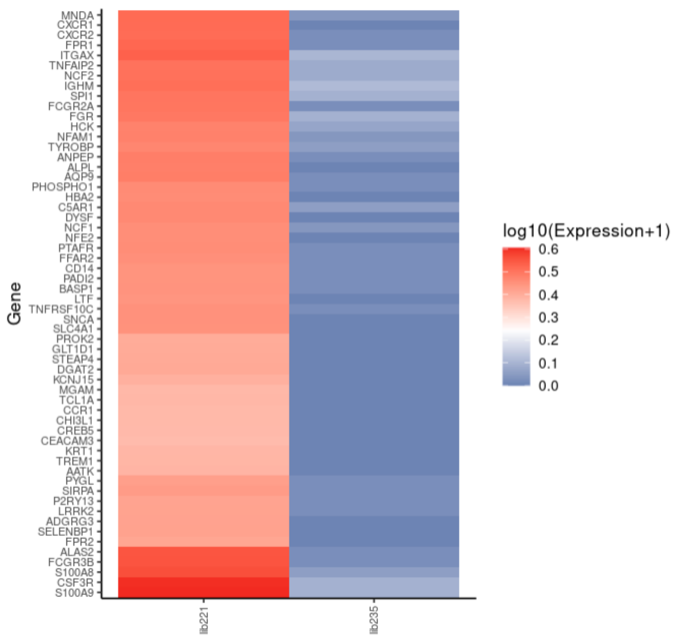
Data/READS//GSE60424/

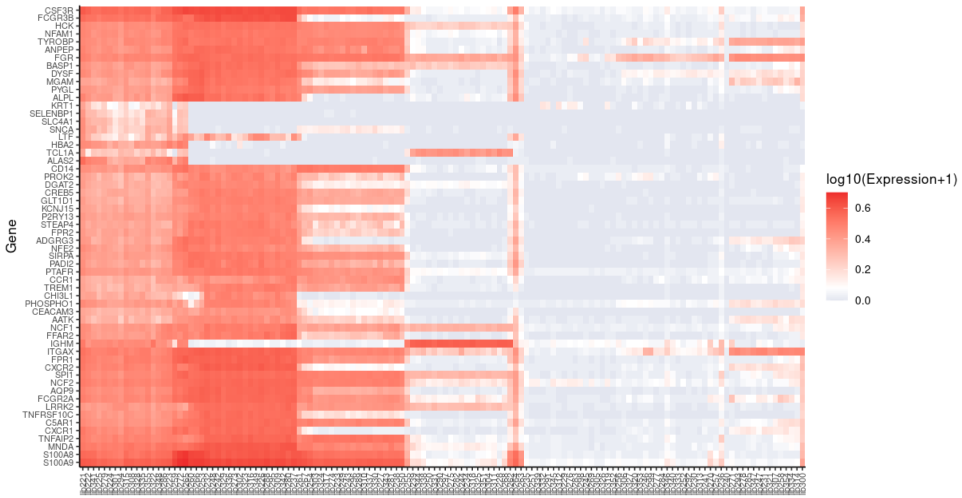
Select 2 Samples

lib221 lib235



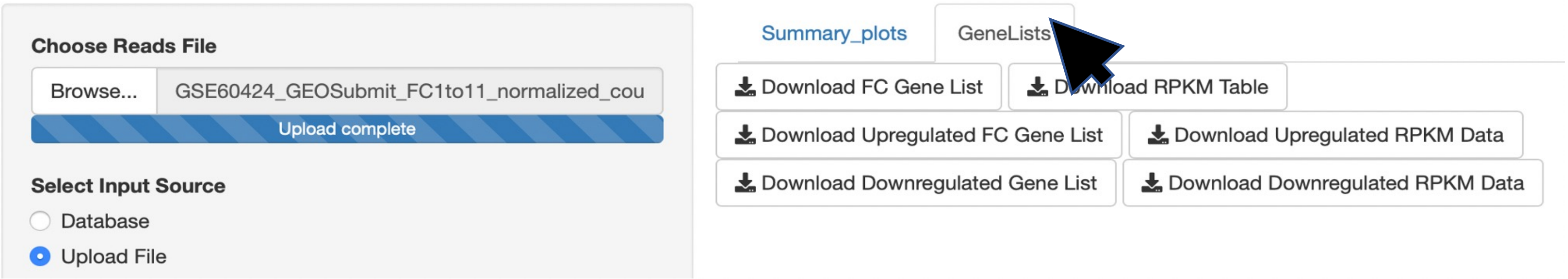

lib235 vs. lib221 (FC > 2 , FDR < 0.05)





Click ‘**Compare Libraries**’ button to perform DEG analysis and display plots.

ROGUE – Differential Expression Analysis



Choose Reads File

Browse... GSE60424_GEOSubmit_FC1to11_normalized_cou

Upload complete

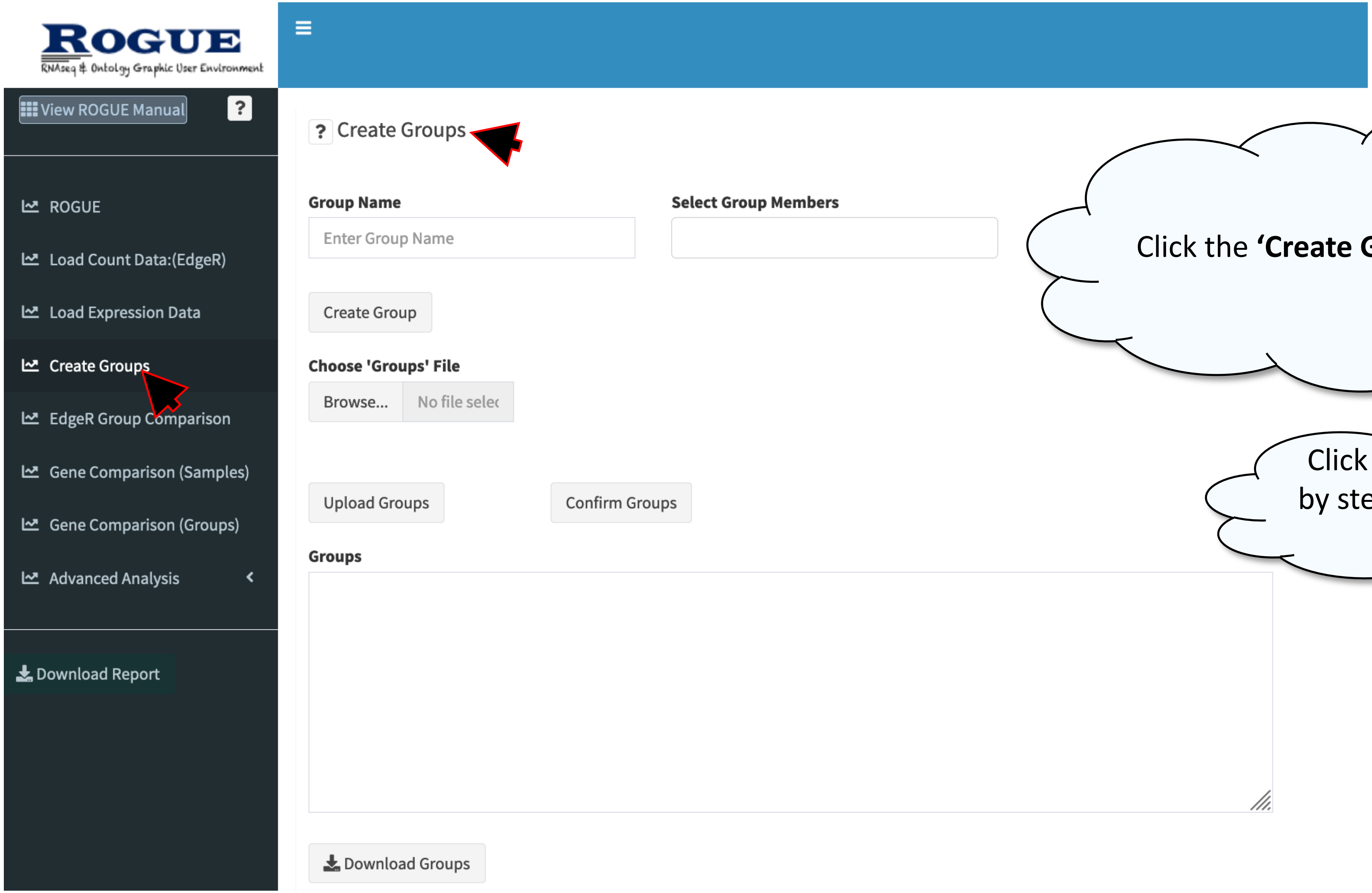
Select Input Source

○ Database

● Upload File

Click **'GeneLists'** tab. User can download the list of genes that are differentially expressed, just the upregulated or downregulated gene lists, or DEG tables with RPKM values

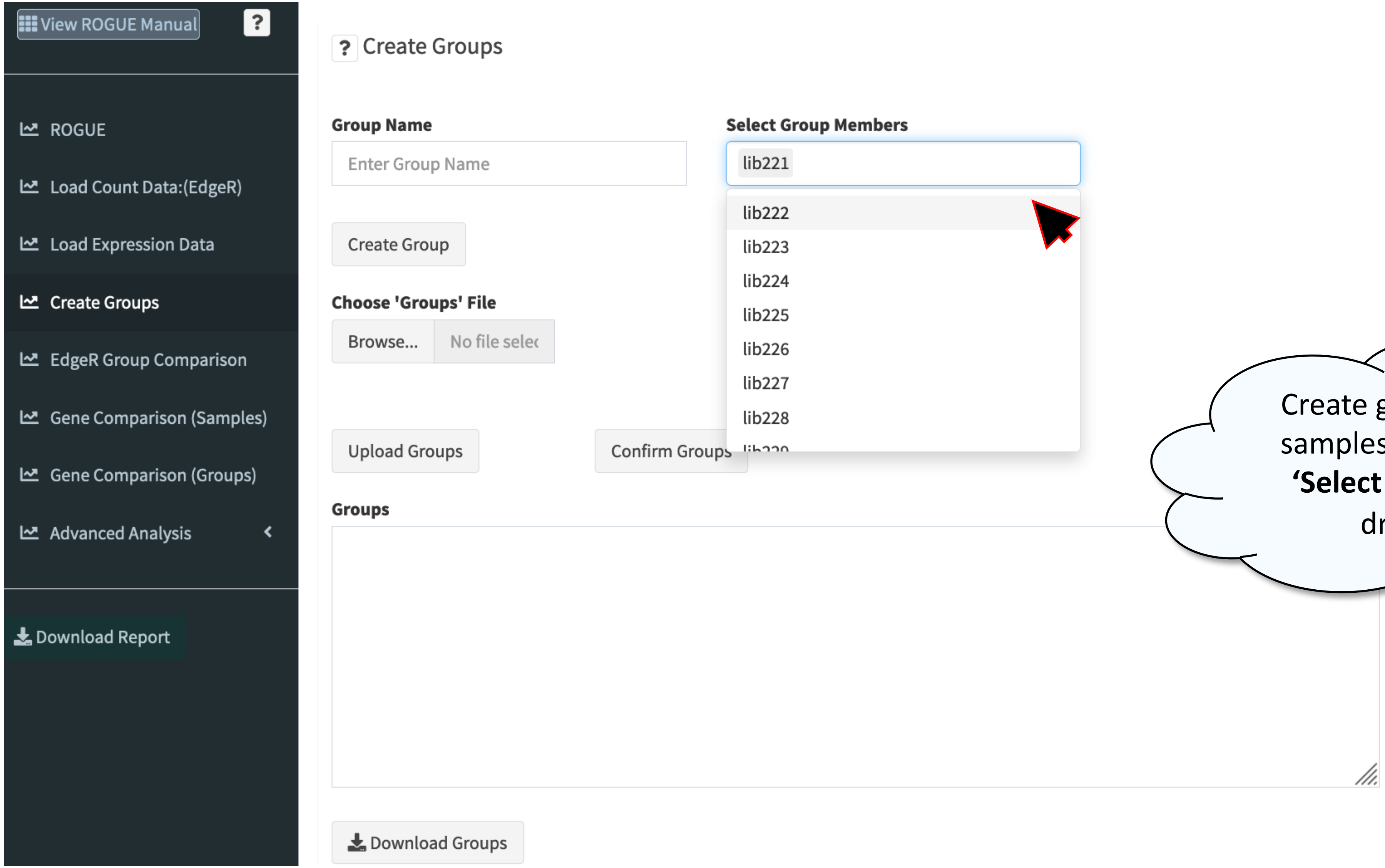
ROGUE – Create Groups



Click the **‘Create Groups’** tab.

Click **‘?’** button for step by step tips on using this tool

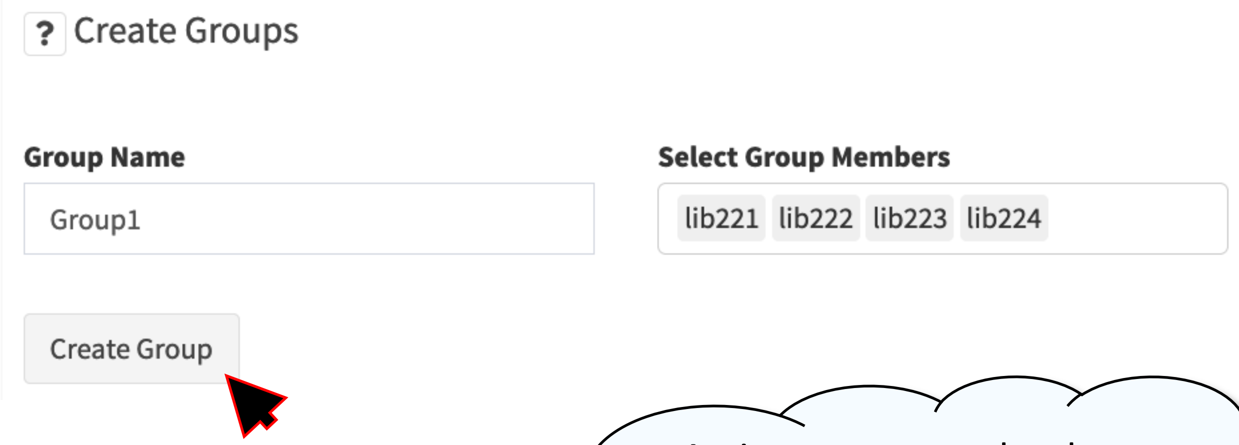
ROGUE – Create Groups



Select Group Members: lib221

Create groups by choosing samples/libraries from the **‘Select Group Members’** dropdown list.

ROGUE – Create Groups



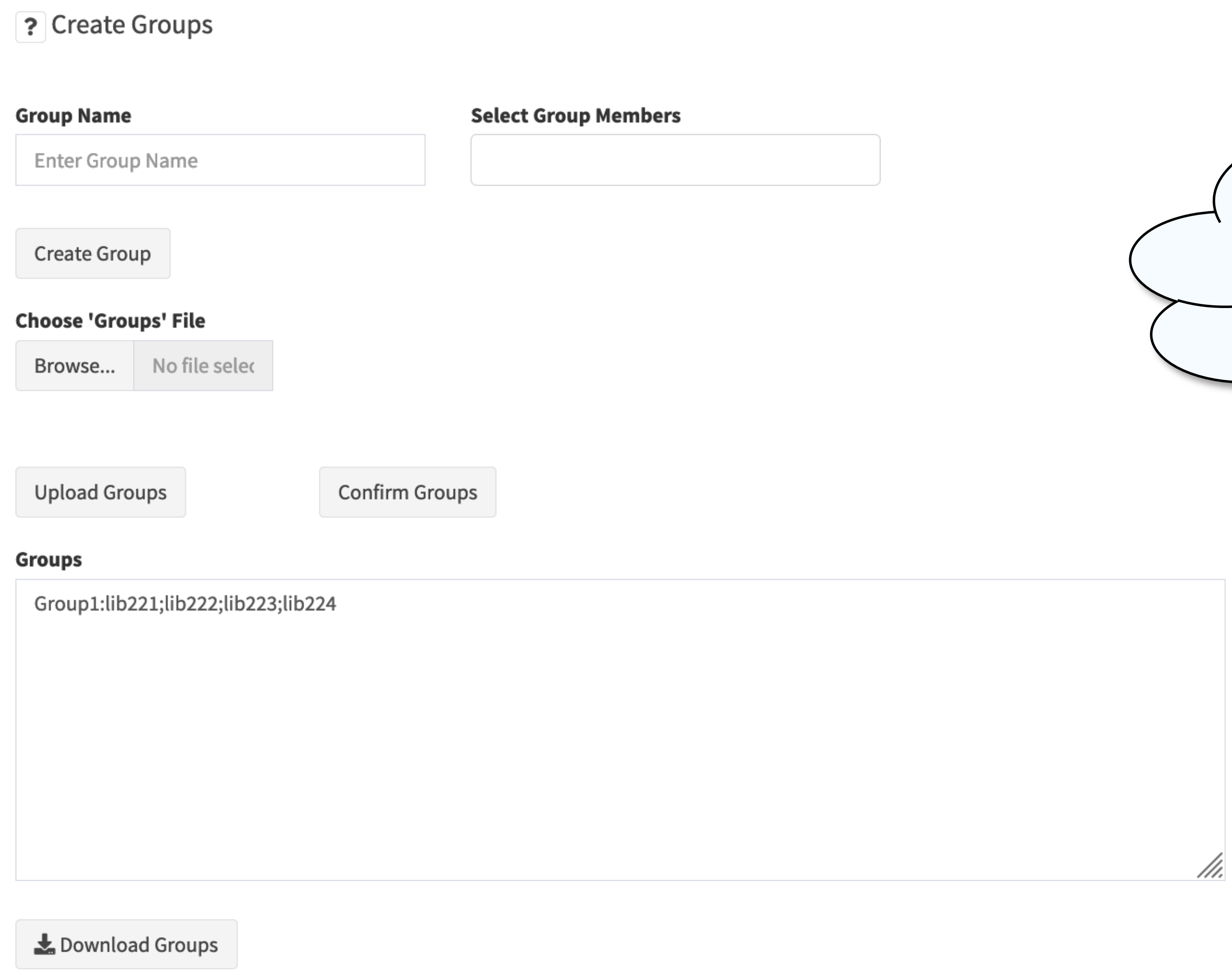
Create Groups

Group Name: Group1

Select Group Members: lib221 lib222 lib223 lib224

Assign a name to the the group in the ‘**Group Name**’ textbox then click the ‘**Create Group**’ button.

ROGUE – Create Groups



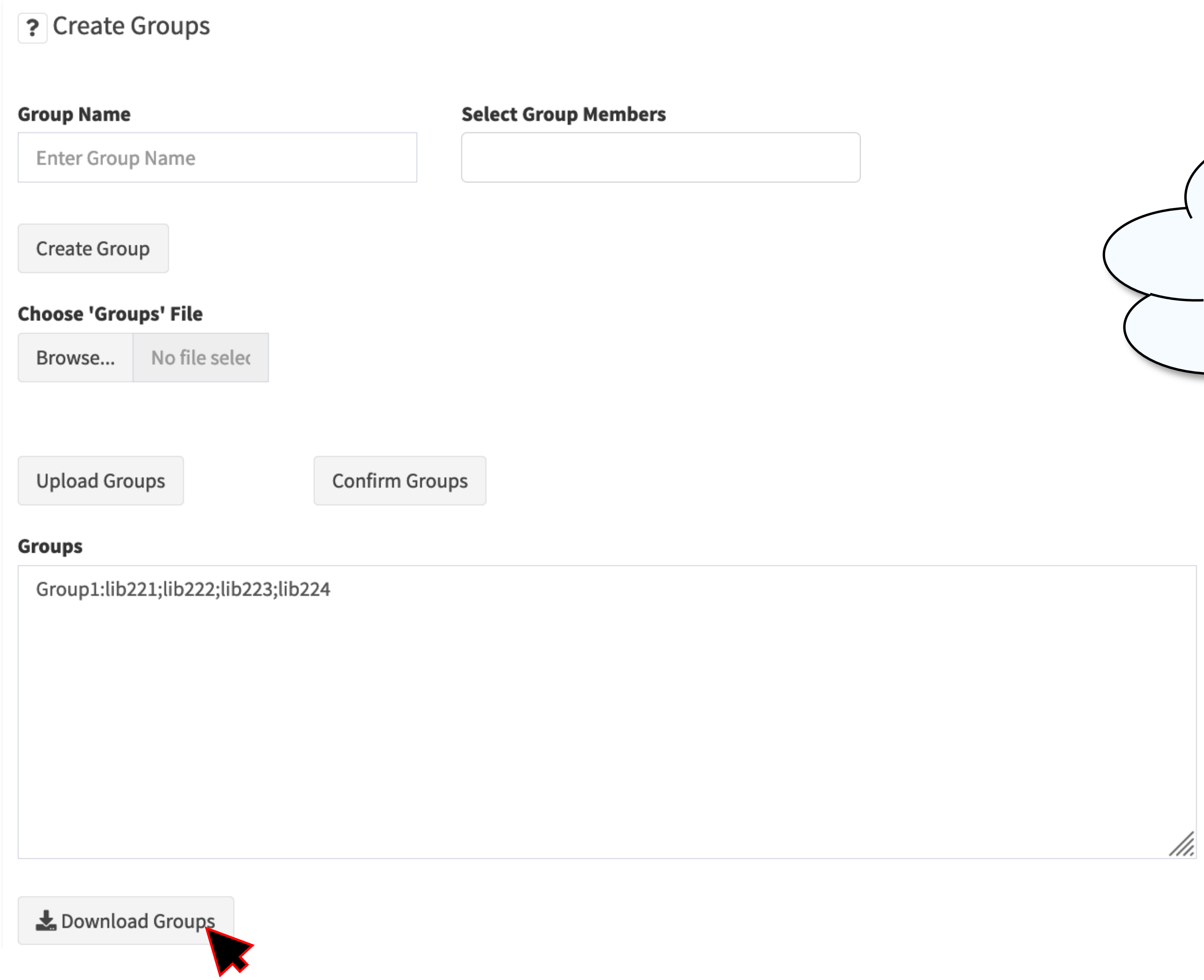
Groups

Group1:lib221;lib222;lib223;lib224

The Group will appear in the ‘**Groups**’ text box. It is in the format:

Group_Name:Member1;Member2;Member3

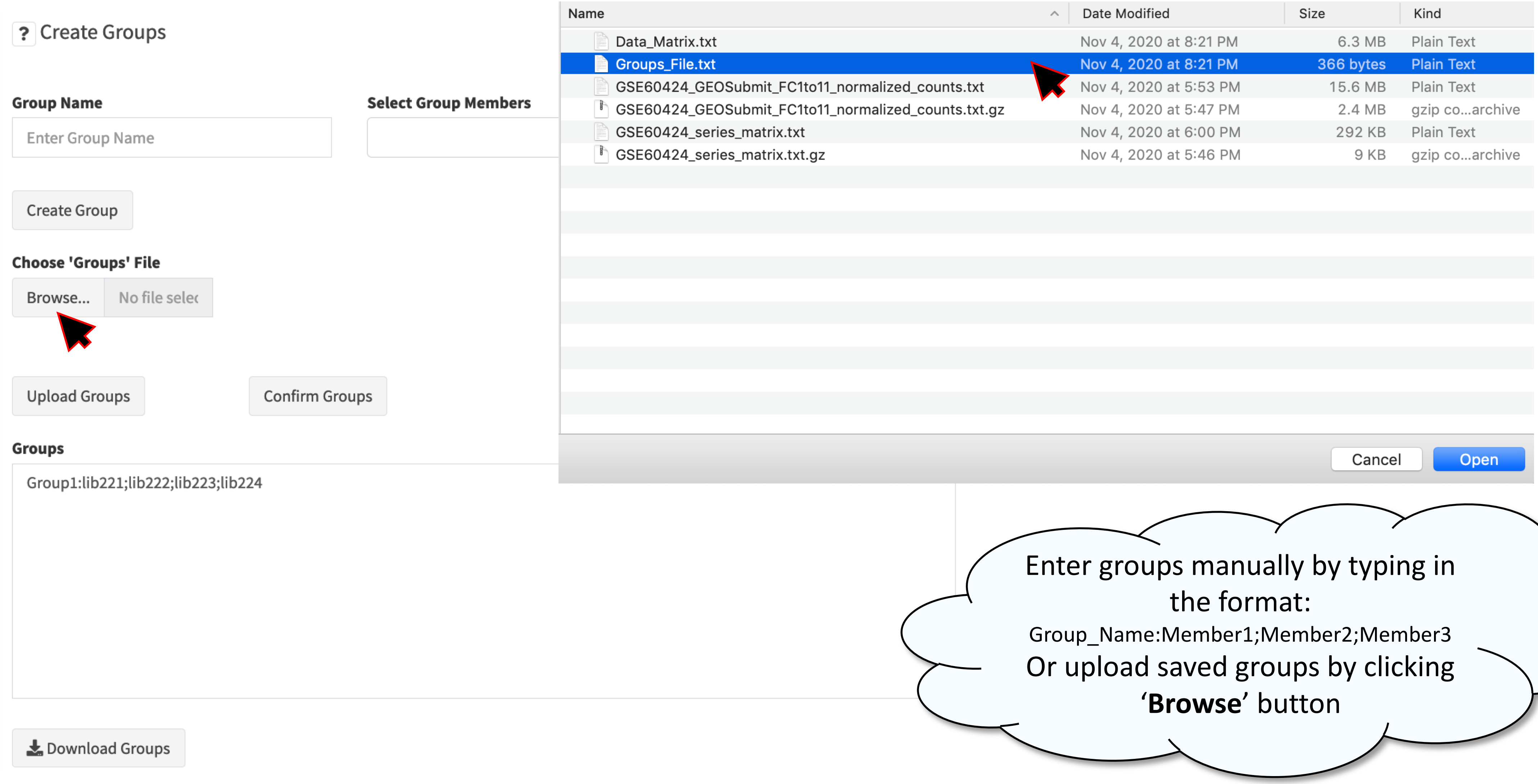
ROGUE – Create Groups



Group1:lib221;lib222;lib223;lib224

Create multiple groups and save them using the ‘**Download Groups**’ button.

ROGUE – Create Groups



Name	Date Modified	Size	Kind
Data_Matrix.txt	Nov 4, 2020 at 8:21 PM	6.3 MB	Plain Text
Groups_File.txt	Nov 4, 2020 at 8:21 PM	366 bytes	Plain Text
GSE60424_GEOSubmit_FC1to11_normalized_counts.txt	Nov 4, 2020 at 5:53 PM	15.6 MB	Plain Text
GSE60424_GEOSubmit_FC1to11_normalized_counts.txt.gz	Nov 4, 2020 at 5:47 PM	2.4 MB	gzip co...archive
GSE60424_series_matrix.txt	Nov 4, 2020 at 6:00 PM	292 KB	Plain Text
GSE60424_series_matrix.txt.gz	Nov 4, 2020 at 5:46 PM	9 KB	gzip co...archive

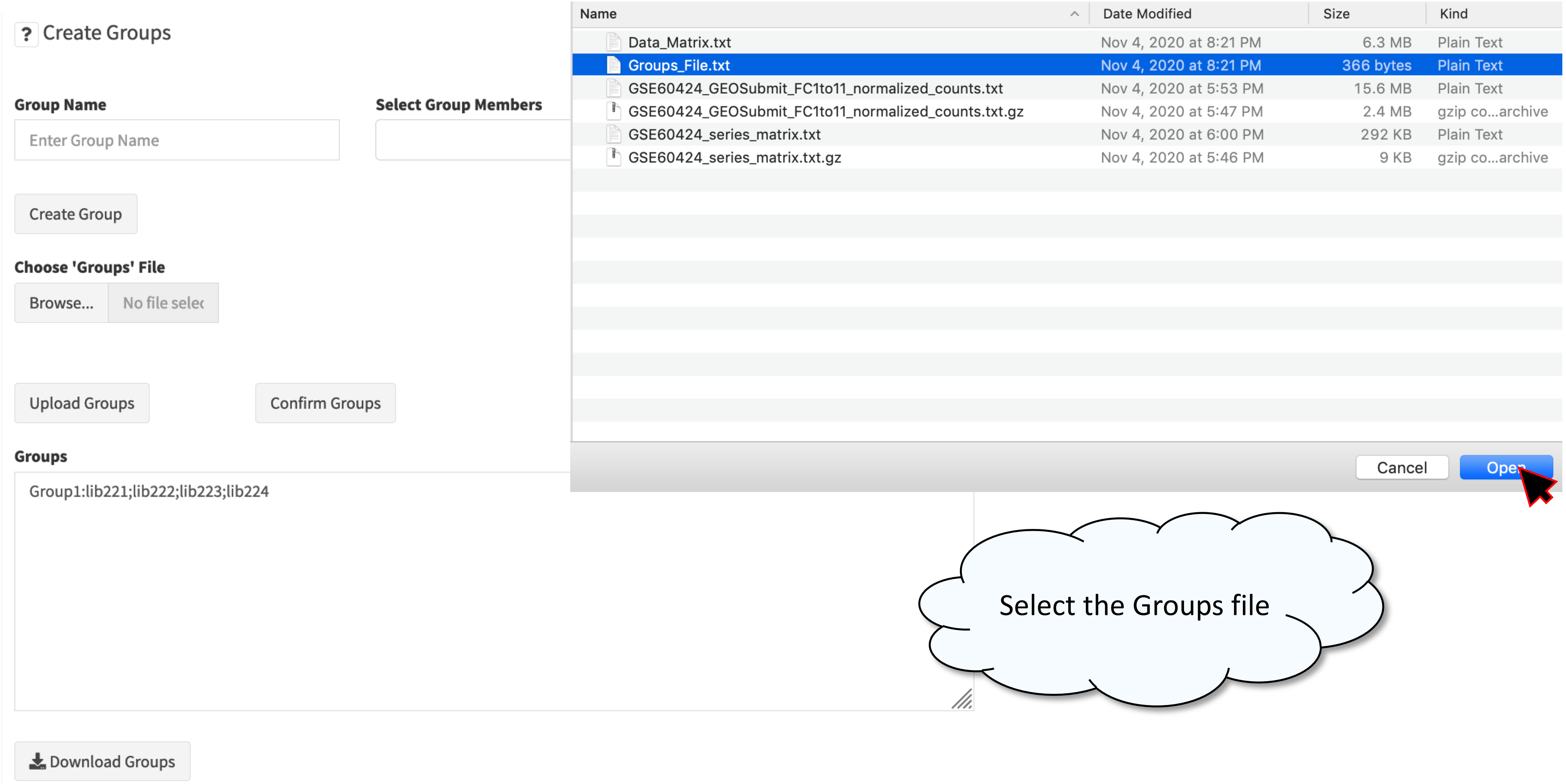
Group1:lib221;lib222;lib223;lib224

Enter groups manually by typing in the format:

Group_Name:Member1;Member2;Member3

Or upload saved groups by clicking '**Browse**' button

ROGUE – Create Groups

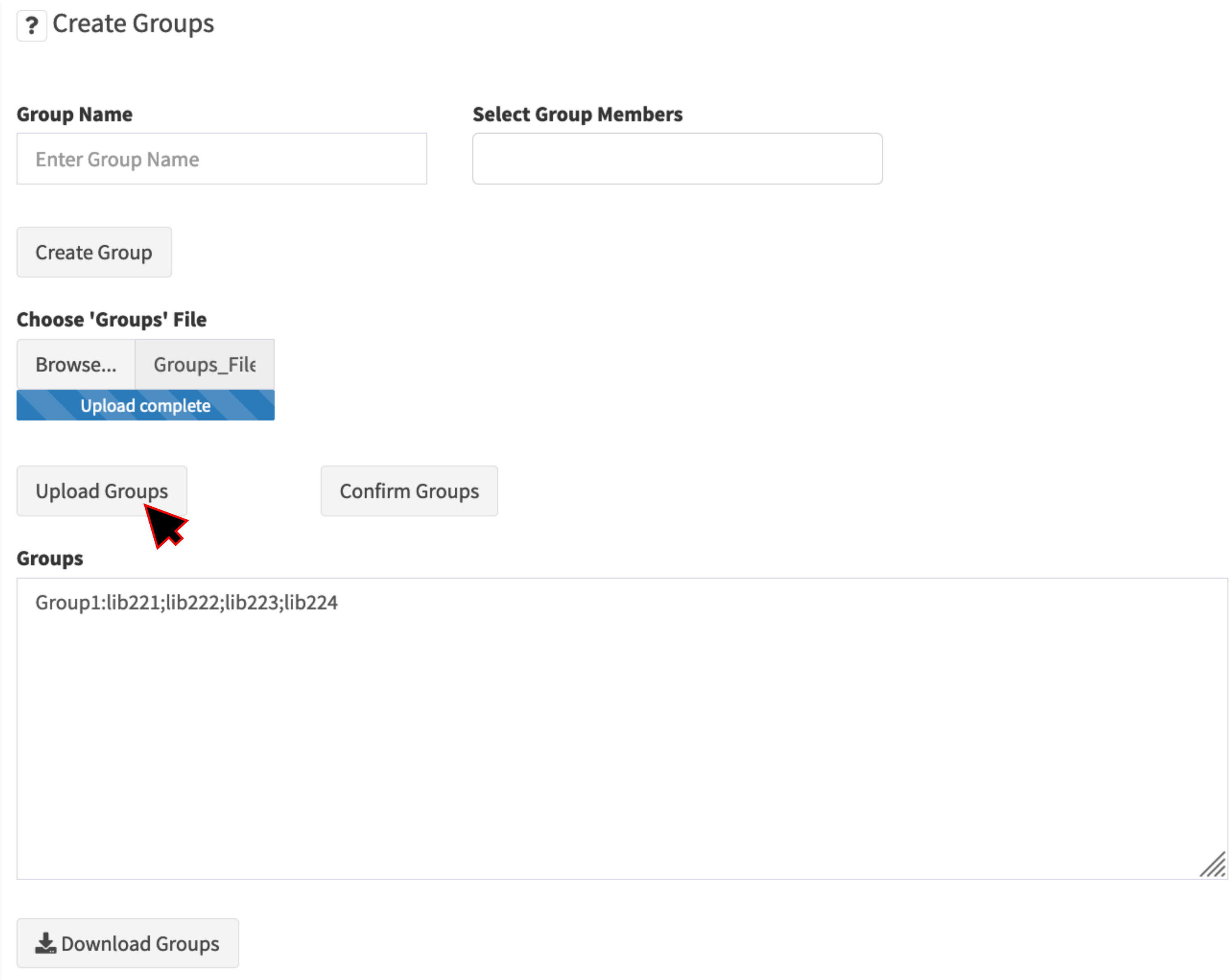


Groups: Group1:lib221;lib222;lib223;lib224

Name	Date Modified	Size	Kind
Data_Matrix.txt	Nov 4, 2020 at 8:21 PM	6.3 MB	Plain Text
Groups_File.txt	Nov 4, 2020 at 8:21 PM	366 bytes	Plain Text
GSE60424_GEOSubmit_FC1to11_normalized_counts.txt	Nov 4, 2020 at 5:53 PM	15.6 MB	Plain Text
GSE60424_GEOSubmit_FC1to11_normalized_counts.txt.gz	Nov 4, 2020 at 5:47 PM	2.4 MB	gzip co...archive
GSE60424_series_matrix.txt	Nov 4, 2020 at 6:00 PM	292 KB	Plain Text
GSE60424_series_matrix.txt.gz	Nov 4, 2020 at 5:46 PM	9 KB	gzip co...archive

Select the Groups file

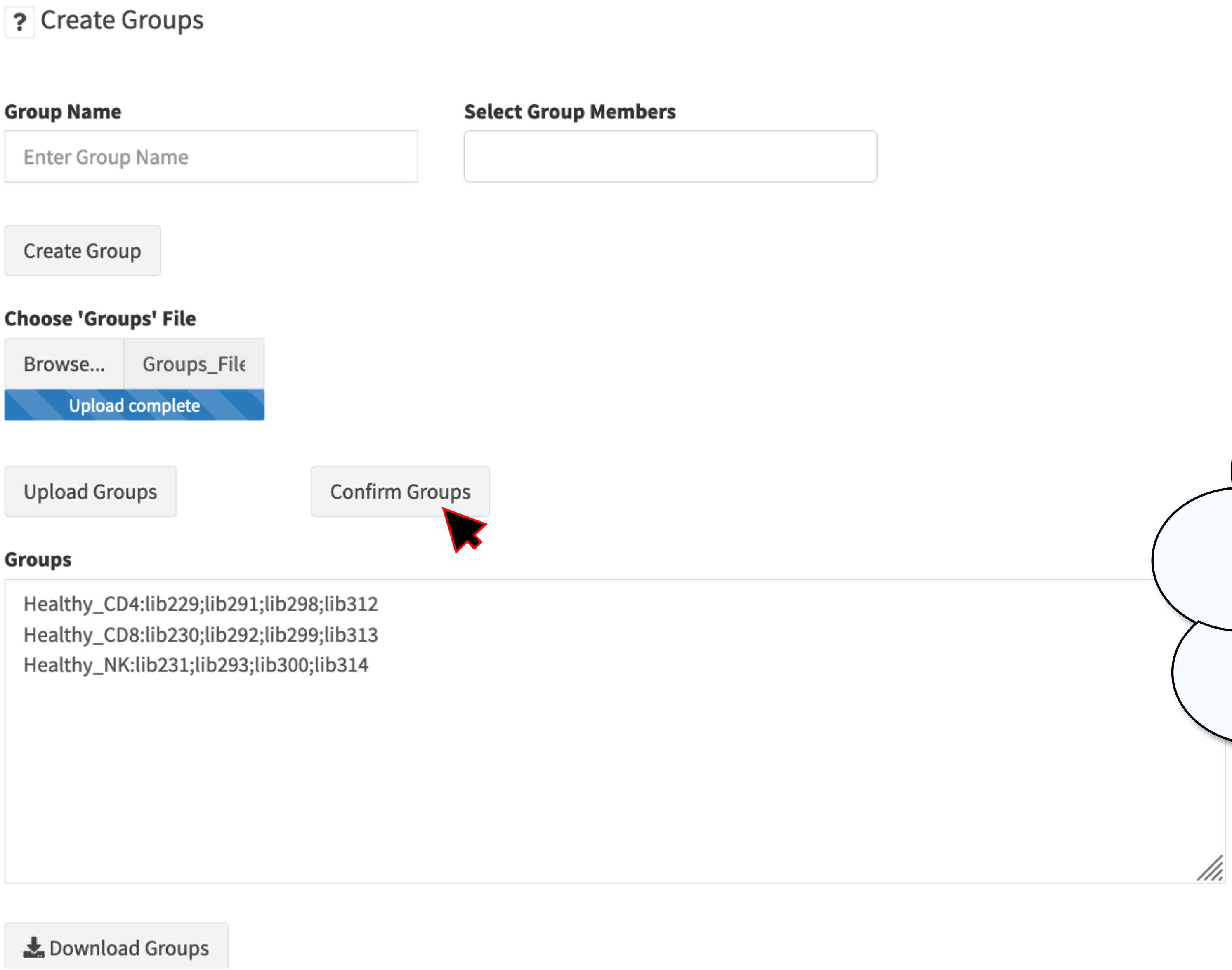
ROGUE – Create Groups



Group1:lib221;lib222;lib223;lib224

When the Browse box says, 'Upload complete' click the ‘**Upload Groups**’ button.

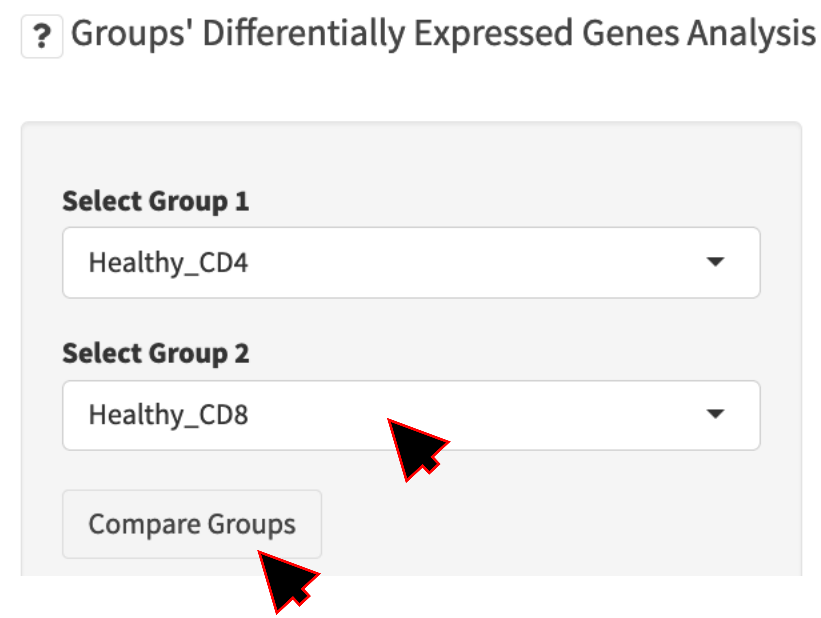
ROGUE – Create Groups



Healthy_CD4:lib229;lib291;lib298;lib312
Healthy_CD8:lib230;lib292;lib299;lib313
Healthy_NK:lib231;lib293;lib300;lib314

Manual group entries by typing and group file upload would not be loaded into the program until the user clicks the ‘**Confirm Groups**’ button. This checks for errors and removes group members that are not in the loaded dataset. It then loads the groups into the other features of the tool

ROGUE – Groups Differential Expression Analysis



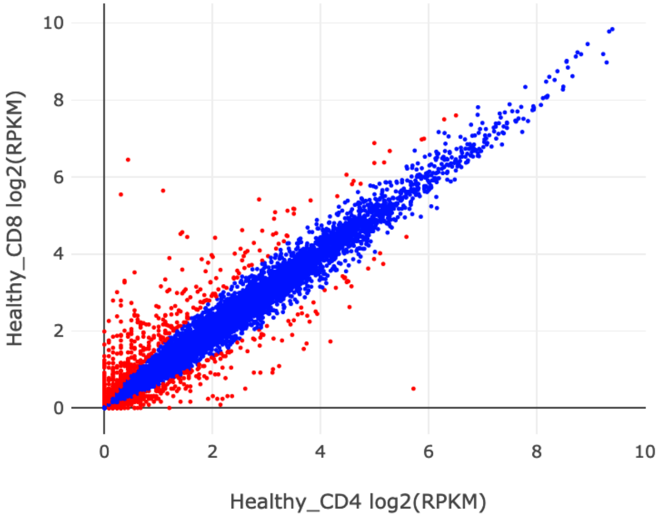
? Groups' Differentially Expressed Genes Analysis

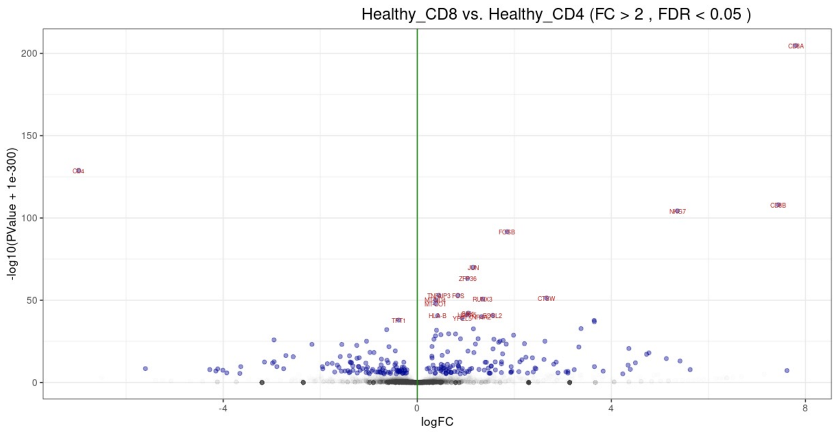
Select Group 1

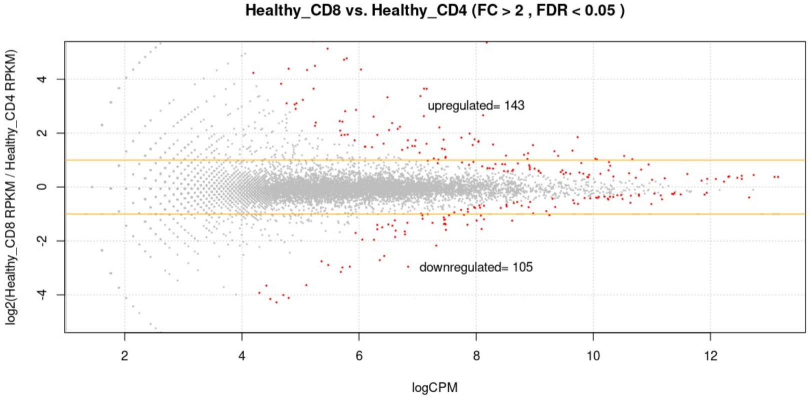
Healthy_CD4

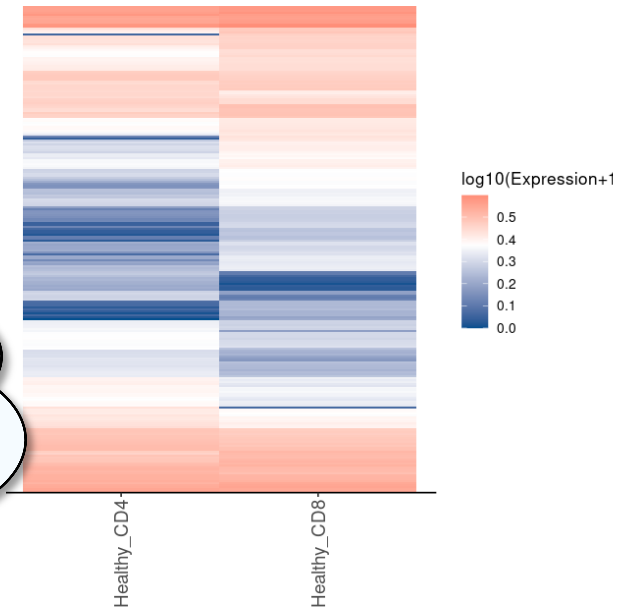
Select Group 2

Healthy_CD8





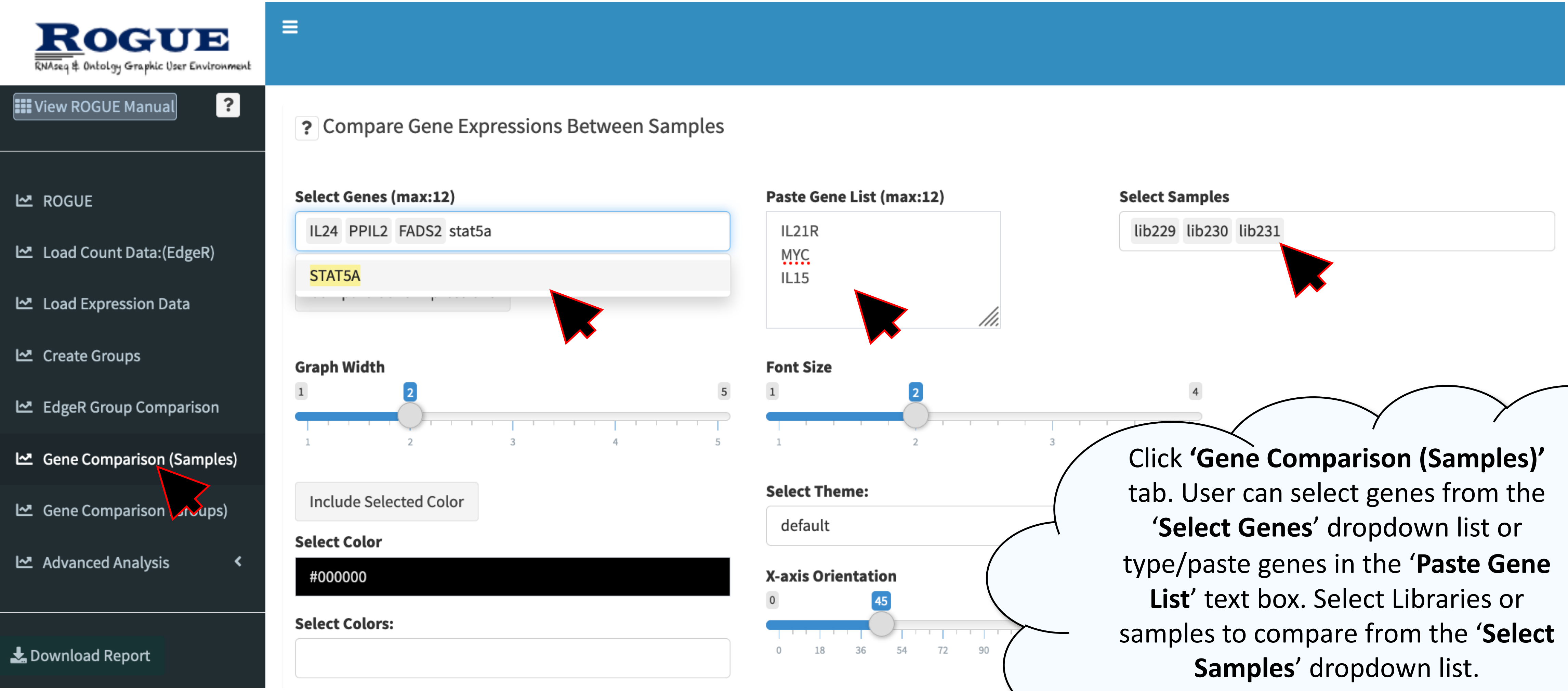




Select 'Healthy_CD8' from the '**Select Group 2'** dropdown box. and click '**Compare Groups'**

Plots will be generated on the summary plots page

ROGUE – Single Sample Gene Expression Analysis



Select Genes (max:12): IL24 PPIL2 FADS2 stat5a (suggestion: STAT5A)

Paste Gene List (max:12):
IL21R
MYC
IL15

Select Samples: lib229 lib230 lib231

Graph Width: 2

Font Size: 2

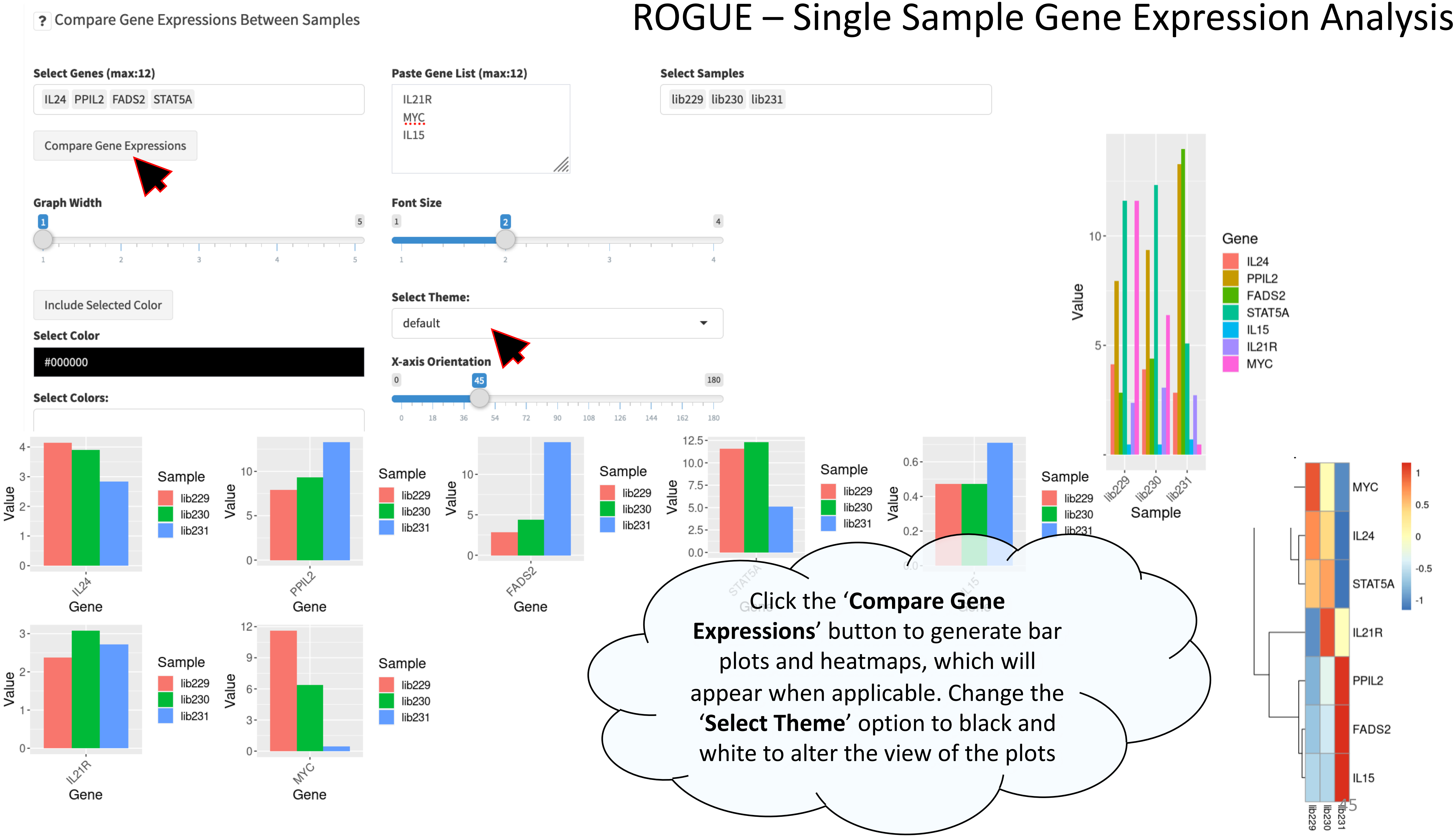
Select Theme: default

X-axis Orientation: 45

Select Color: #000000

Click **'Gene Comparison (Samples)'** tab. User can select genes from the '**Select Genes**' dropdown list or type/paste genes in the '**Paste Gene List**' text box. Select Libraries or samples to compare from the '**Select Samples**' dropdown list.

ROGUE – Single Sample Gene Expression Analysis



? Compare Gene Expressions Between Samples

Select Genes (max:12): IL24 PPIL2 FADS2 STAT5A

Paste Gene List (max:12):
IL21R
MYC
IL15

Select Samples: lib229 lib230 lib231

Compare Gene Expressions

Graph Width: 1

Font Size: 2

Include Selected Color

Select Theme: default

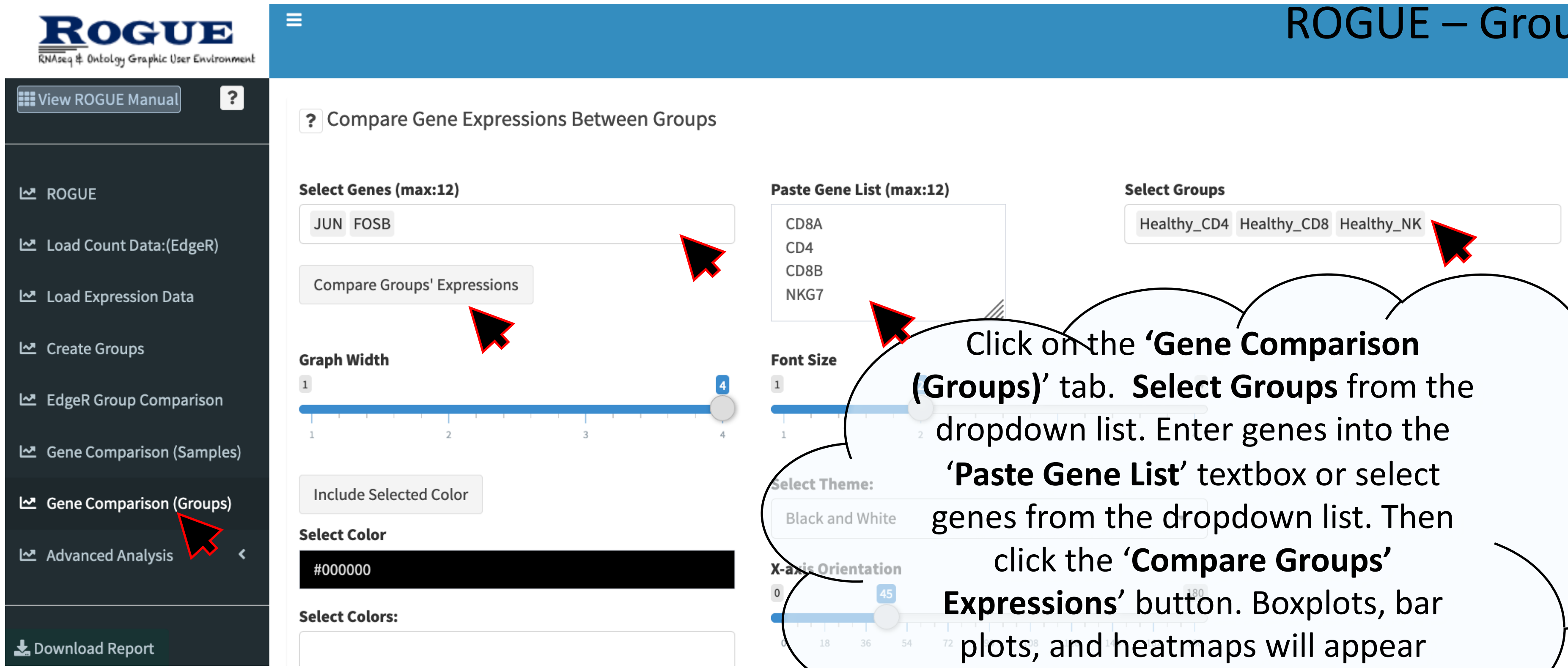
Select Color: #000000

X-axis Orientation: 45

Select Colors:

Click the ‘**Compare Gene Expressions**’ button to generate bar plots and heatmaps, which will appear when applicable. Change the ‘**Select Theme**’ option to black and white to alter the view of the plots

ROGUE – Groups Gene Expression Analysis



? Compare Gene Expressions Between Groups

Select Genes (max:12): JUN FOSB

Paste Gene List (max:12):
CD8A
CD4
CD8B
NKG7

Select Groups: Healthy_CD4 Healthy_CD8 Healthy_NK

Graph Width: 4

Font Size

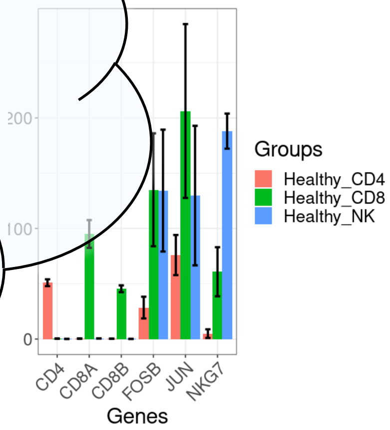
Select Theme: Black and White

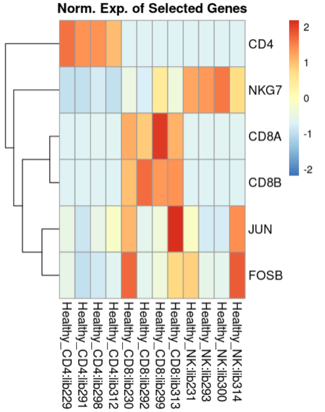
X-axis Orientation: 45

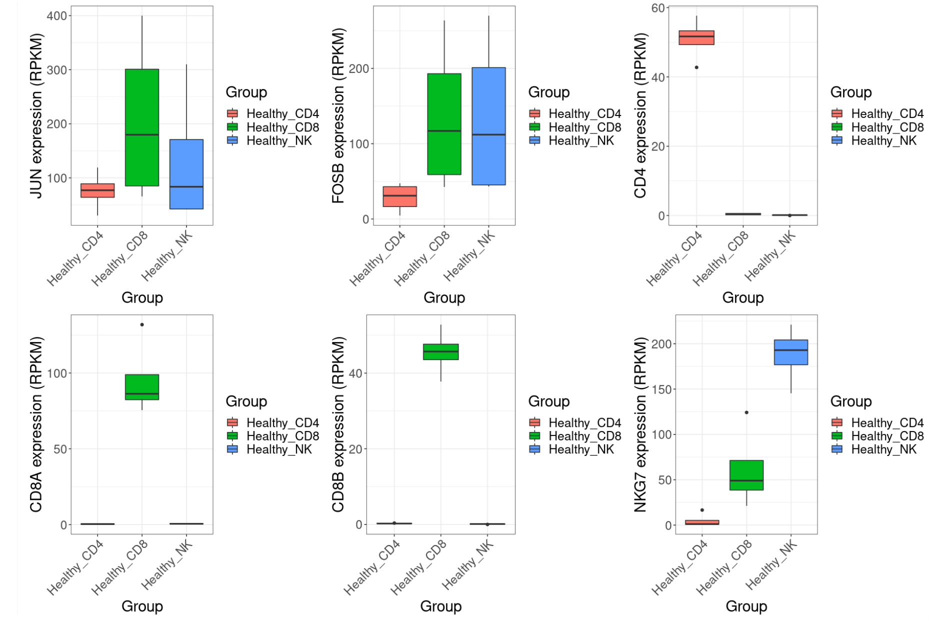
Select Color: #000000

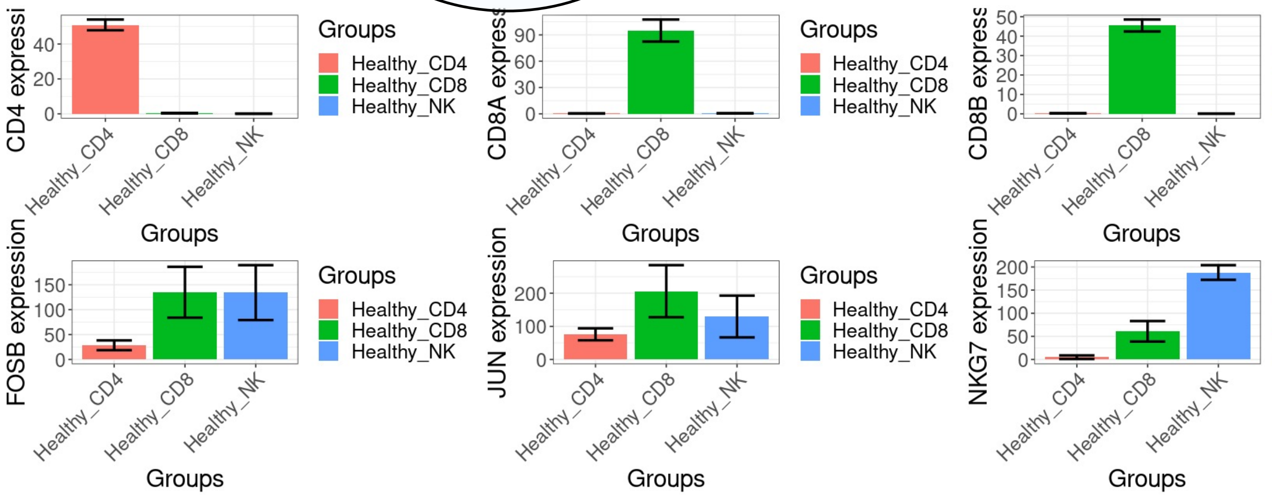
Select Colors:

Click on the **'Gene Comparison (Groups)'** tab. **Select Groups** from the dropdown list. Enter genes into the '**Paste Gene List**' textbox or select genes from the dropdown list. Then click the '**Compare Groups' Expressions**' button. Boxplots, bar plots, and heatmaps will appear comparing the genes' expressions

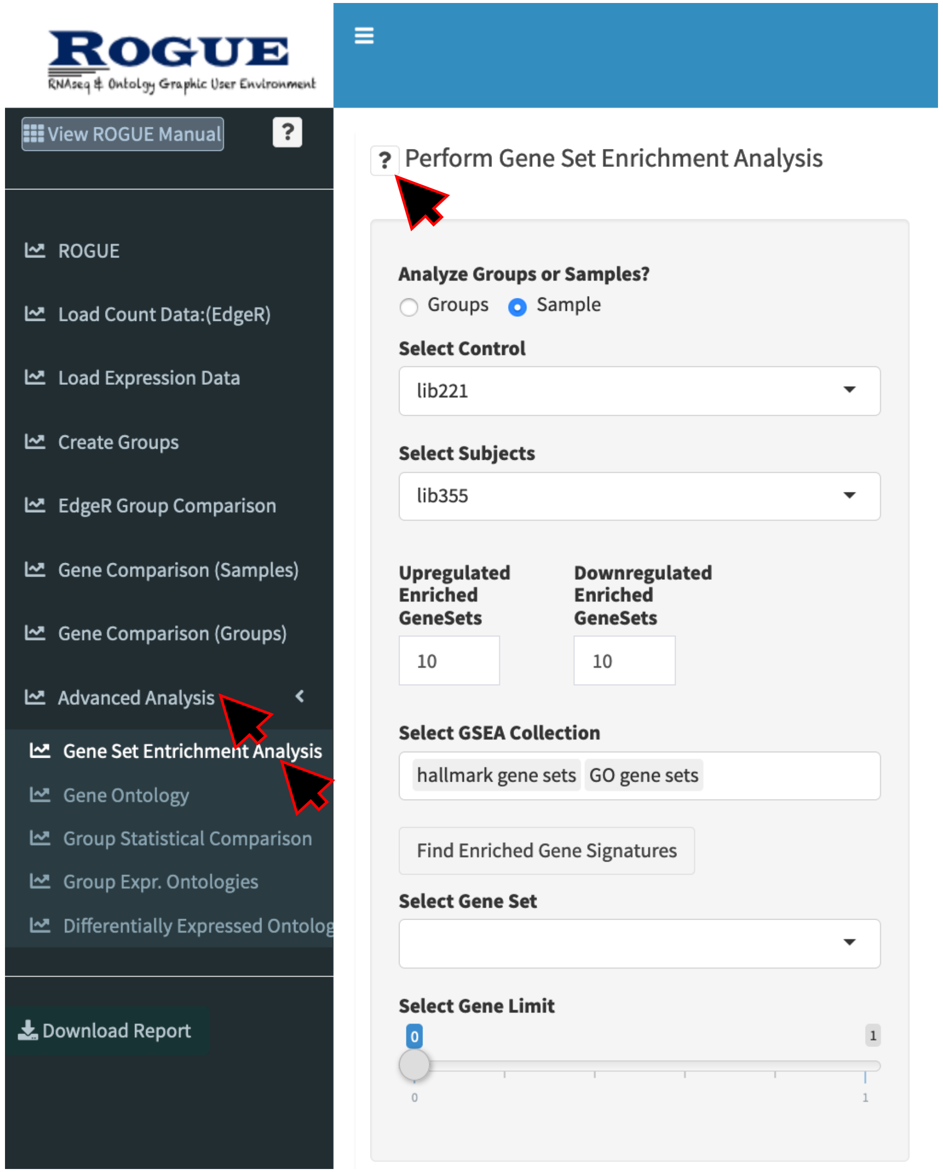








ROGUE – Gene Set Enrichment Analysis



Analyze Groups or Samples?
○ Groups ● Sample

Select Control
lib221

Select Subjects
lib355

Upregulated Enriched GeneSets
10

Downregulated Enriched GeneSets
10

Select GSEA Collection
hallmark gene sets GO gene sets

Select Gene Set

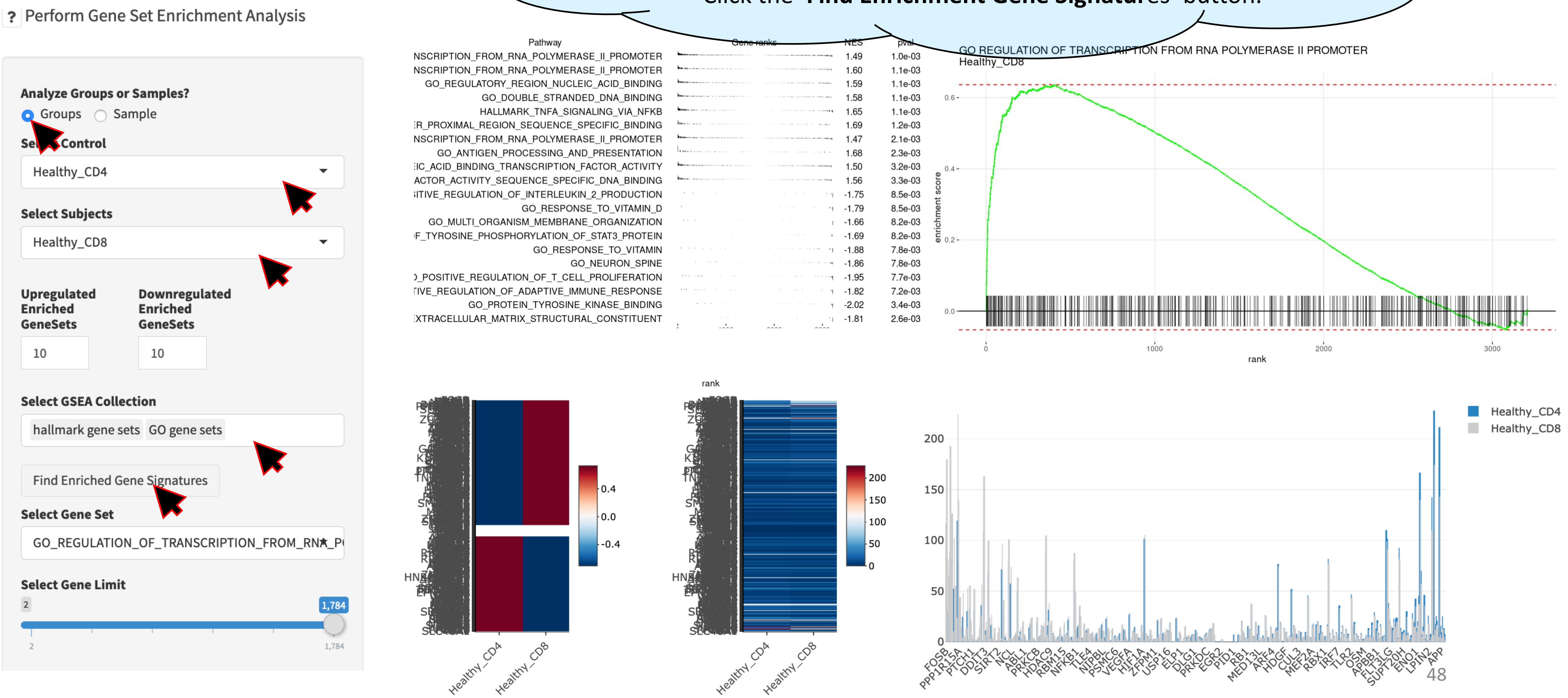
Select Gene Limit
0 1

Click the **'Advanced Analysis'** tab.
Then Click on **'Gene Set Enrichment Analysis'**

Click **'?'** button for step by step tips on using this tool

ROGUE – Gene Set Enrichment Analysis

Select **'Groups'** radio button.
Then select groups for **'Select Control'** and **'Select Subjects'** drop down box. Select GSEA sets from the **'Select GSEA Collection'** drop down box.
Click the '**Find Enrichment Gene Signatur**es' button.



? Perform Gene Set Enrichment Analysis

Analyze Groups or Samples?
● Groups ○ Sample

Se Control
Healthy_CD4

Select Subjects
Healthy_CD8

Upregulated Enriched GeneSets: 10
Downregulated Enriched GeneSets: 10

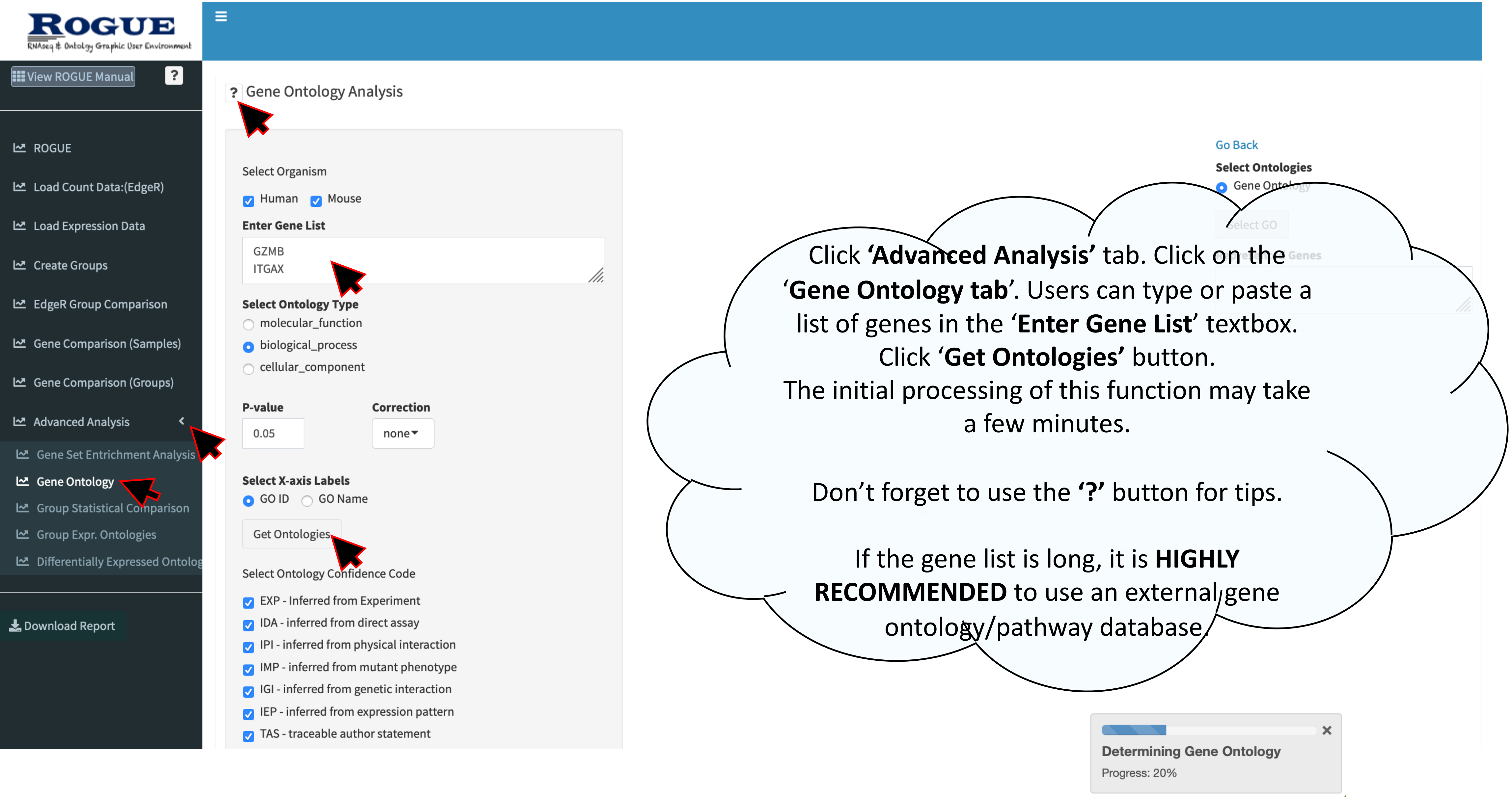
Select GSEA Collection
hallmark gene sets, GO gene sets

Select Gene Set
GO_REGULATION_OF_TRANSCRIPTION_FROM_RNA_P

Select Gene Limit
2 – 1,784

Pathway	Gene ranks	NES	pval
NSCRIPTION_FROM_RNA_POLYMERASE_II_PROMOTER		1.49	1.0e-03
NSCRIPTION_FROM_RNA_POLYMERASE_II_PROMOTER		1.60	1.1e-03
GO_REGULATORY_REGION_NUCLEIC_ACID_BINDING		1.59	1.1e-03
GO_DOUBLE_STRANDED_DNA_BINDING		1.58	1.1e-03
HALLMARK_TNFA_SIGNALING_VIA_NFKB		1.65	1.1e-03
ER_PROXIMAL_REGION_SEQUENCE_SPECIFIC_BINDING		1.69	1.2e-03
NSCRIPTION_FROM_RNA_POLYMERASE_II_PROMOTER		1.47	2.1e-03
GO_ANTIGEN_PROCESSING_AND_PRESENTATION		1.68	2.3e-03
IC_ACID_BINDING_TRANSCRIPTION_FACTOR_ACTIVITY		1.50	3.2e-03
ACTOR_ACTIVITY_SEQUENCE_SPECIFIC_DNA_BINDING		1.56	3.3e-03
SITIVE_REGULATION_OF_INTERLEUKIN_2_PRODUCTION		-1.75	8.5e-03
GO_RESPONSE_TO_VITAMIN_D		-1.79	8.5e-03
GO_MULTI_ORGANISM_MEMBRANE_ORGANIZATION		-1.66	8.2e-03
F_TYROSINE_PHOSPHORYLATION_OF_STAT3_PROTEIN		-1.69	8.2e-03
GO_RESPONSE_TO_VITAMIN		-1.88	7.8e-03
GO_NEURON_SPINE		-1.86	7.8e-03
)_POSITIVE_REGULATION_OF_T_CELL_PROLIFERATION		-1.95	7.7e-03
TIVE_REGULATION_OF_ADAPTIVE_IMMUNE_RESPONSE		-1.82	7.2e-03
GO_PROTEIN_TYROSINE_KINASE_BINDING		-2.02	3.4e-03
EXTRACELLULAR_MATRIX_STRUCTURAL_CONSTITUENT		-1.81	2.6e-03

ROGUE – Gene Ontology Analysis



? Gene Ontology Analysis

Select Organism

☑ Human ☑ Mouse

Enter Gene List

GZMB
ITGAX

Select Ontology Type

○ molecular_function
● biological_process
○ cellular_component

P-value 0.05

Correction none

Select X-axis Labels

● GO ID ○ GO Name

Select Ontology Confidence Code

☑ EXP - Inferred from Experiment
☑ IDA - inferred from direct assay
☑ IPI - inferred from physical interaction
☑ IMP - inferred from mutant phenotype
☑ IGI - inferred from genetic interaction
☑ IEP - inferred from expression pattern
☑ TAS - traceable author statement

Click **'Advanced Analysis'** tab. Click on the **'Gene Ontology tab'**. Users can type or paste a list of genes in the **'Enter Gene List'** textbox. Click **'Get Ontologies'** button. The initial processing of this function may take a few minutes.

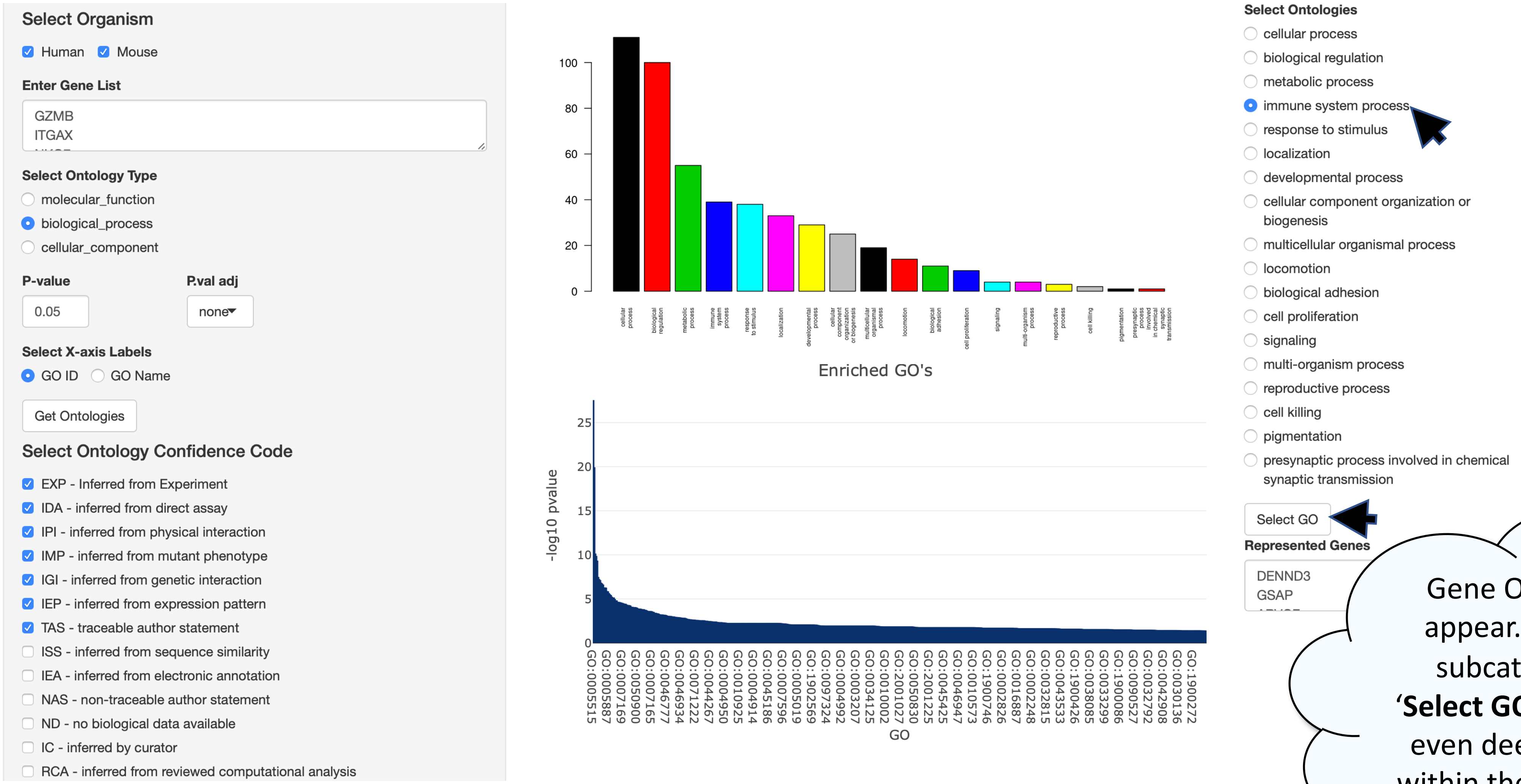
Don't forget to use the **'?'** button for tips.

If the gene list is long, it is **HIGHLY RECOMMENDED** to use an external gene ontology/pathway database.

Determining Gene Ontology

Progress: 20%

ROGUE – Gene Ontology Analysis



Select Organism

☑ Human ☑ Mouse

Enter Gene List

GZMB
ITGAX

Select Ontology Type

- ○ molecular_function
- ● biological_process
- ○ cellular_component

P-value: 0.05

P.val adj: none

Select X-axis Labels

● GO ID ○ GO Name

Select Ontology Confidence Code

- ☑ EXP - Inferred from Experiment
- ☑ IDA - inferred from direct assay
- ☑ IPI - inferred from physical interaction
- ☑ IMP - inferred from mutant phenotype
- ☑ IGI - inferred from genetic interaction
- ☑ IEP - inferred from expression pattern
- ☑ TAS - traceable author statement
- ☐ ISS - inferred from sequence similarity
- ☐ IEA - inferred from electronic annotation
- ☐ NAS - non-traceable author statement
- ☐ ND - no biological data available
- ☐ IC - inferred by curator
- ☐ RCA - inferred from reviewed computational analysis

Select Ontologies

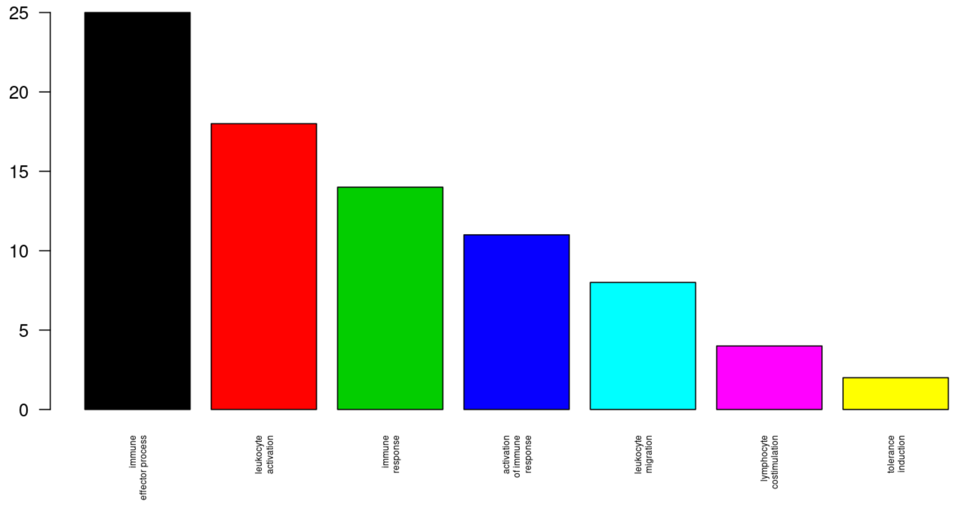
- ○ cellular process
- ○ biological regulation
- ○ metabolic process
- ● immune system process
- ○ response to stimulus
- ○ localization
- ○ developmental process
- ○ cellular component organization or biogenesis
- ○ multicellular organismal process
- ○ locomotion
- ○ biological adhesion
- ○ cell proliferation
- ○ signaling
- ○ multi-organism process
- ○ reproductive process
- ○ cell killing
- ○ pigmentation
- ○ presynaptic process involved in chemical synaptic transmission

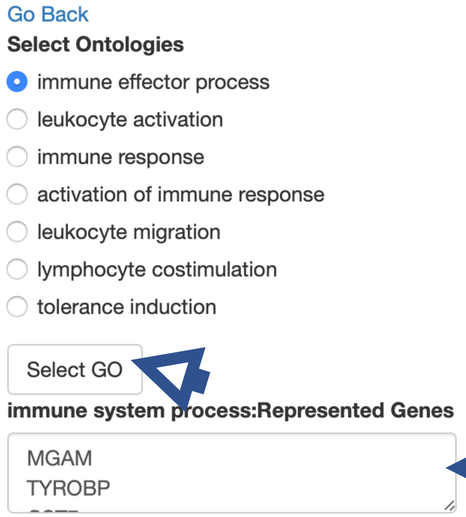
Represented Genes

DENND3
GSAP

Gene Ontology bar plots appear. Choose ontology subcategories and click **'Select GO'** button to explore even deeper subcategories within the selected ontology.

ROGUE – Gene Ontology Analysis





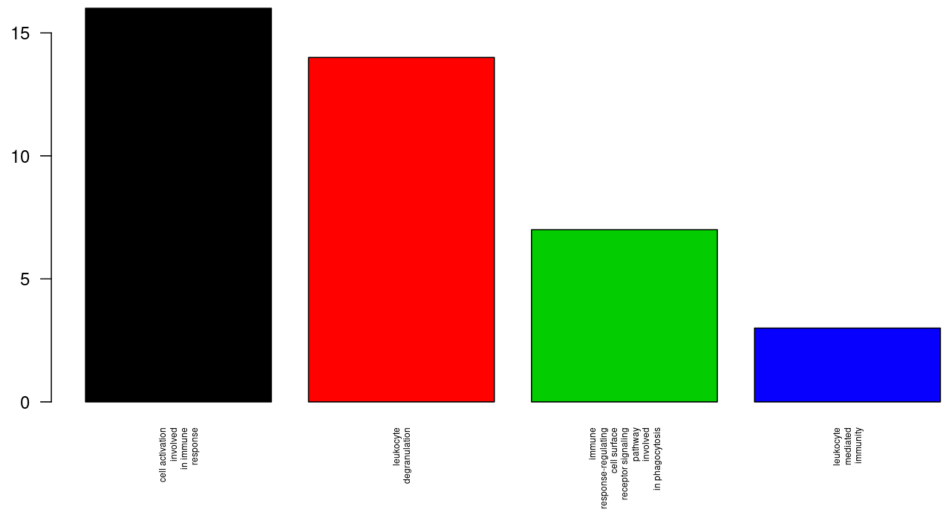
Select Ontologies

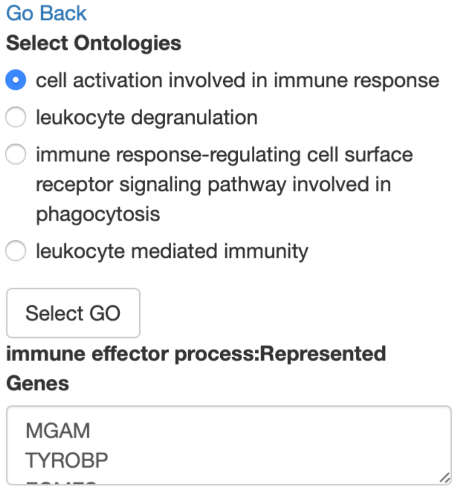
● immune effector process
○ leukocyte activation
○ immune response
○ activation of immune response
○ leukocyte migration
○ lymphocyte costimulation
○ tolerance induction

immune system process:Represented Genes

MGAM
TYROBP

Note: This text box lists the genes represented in the bar plot.





Select Ontologies

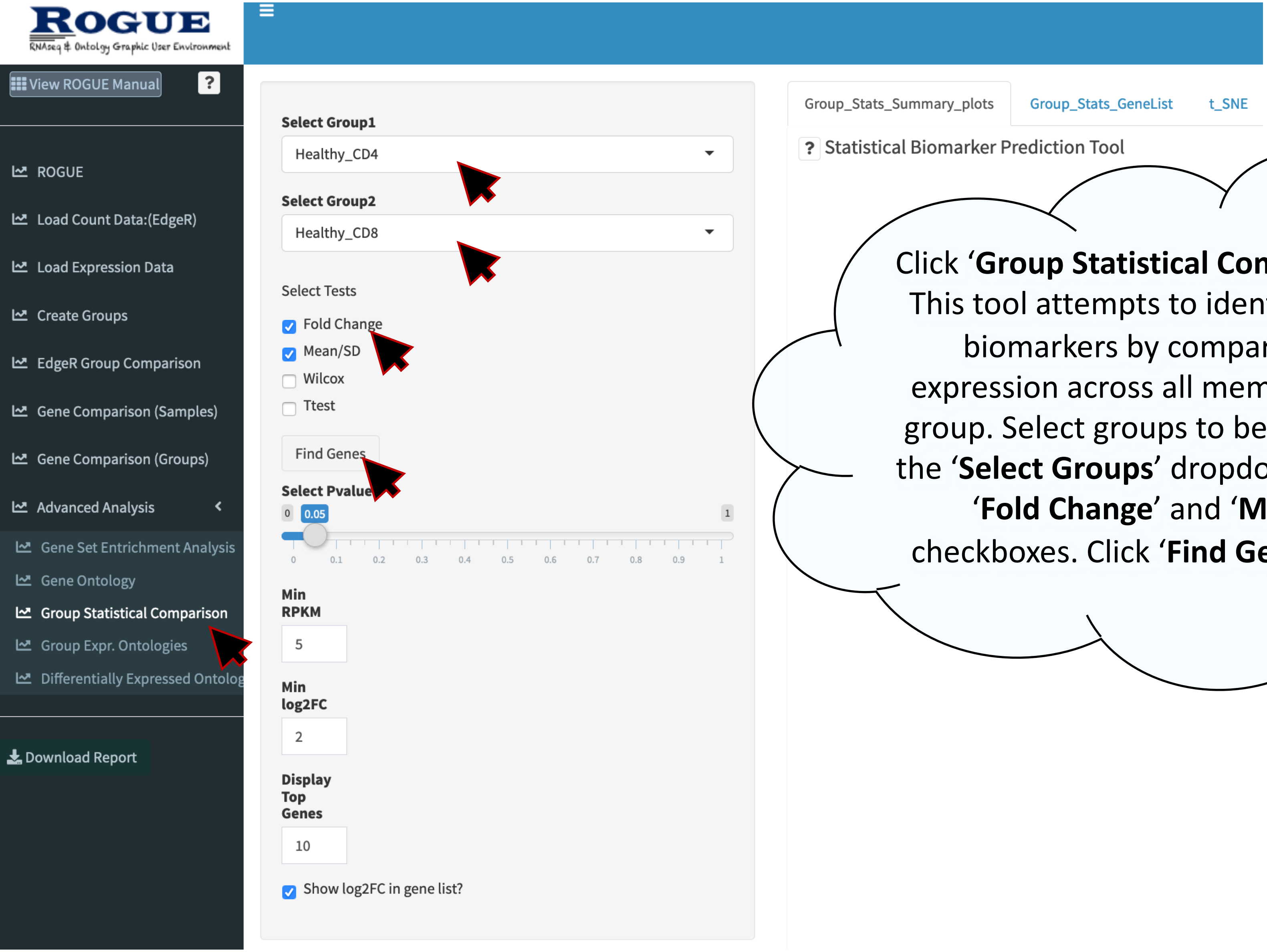
● cell activation involved in immune response
○ leukocyte degranulation
○ immune response-regulating cell surface receptor signaling pathway involved in phagocytosis
○ leukocyte mediated immunity

immune effector process:Represented Genes

MGAM
TYROBP

Gene Ontology bar plots appear. Choose ontology subcategories and click ‘**Select GO**’ button to explore even deeper subcategories within the selected ontology.

ROGUE – Statistical Group Comparison (Biomarker Discovery)



Select Group1: Healthy_CD4

Select Group2: Healthy_CD8

Select Tests
- ☑ Fold Change
- ☑ Mean/SD
- ☐ Wilcox
- ☐ Ttest

Select Pvalue: 0.05

Min RPKM: 5

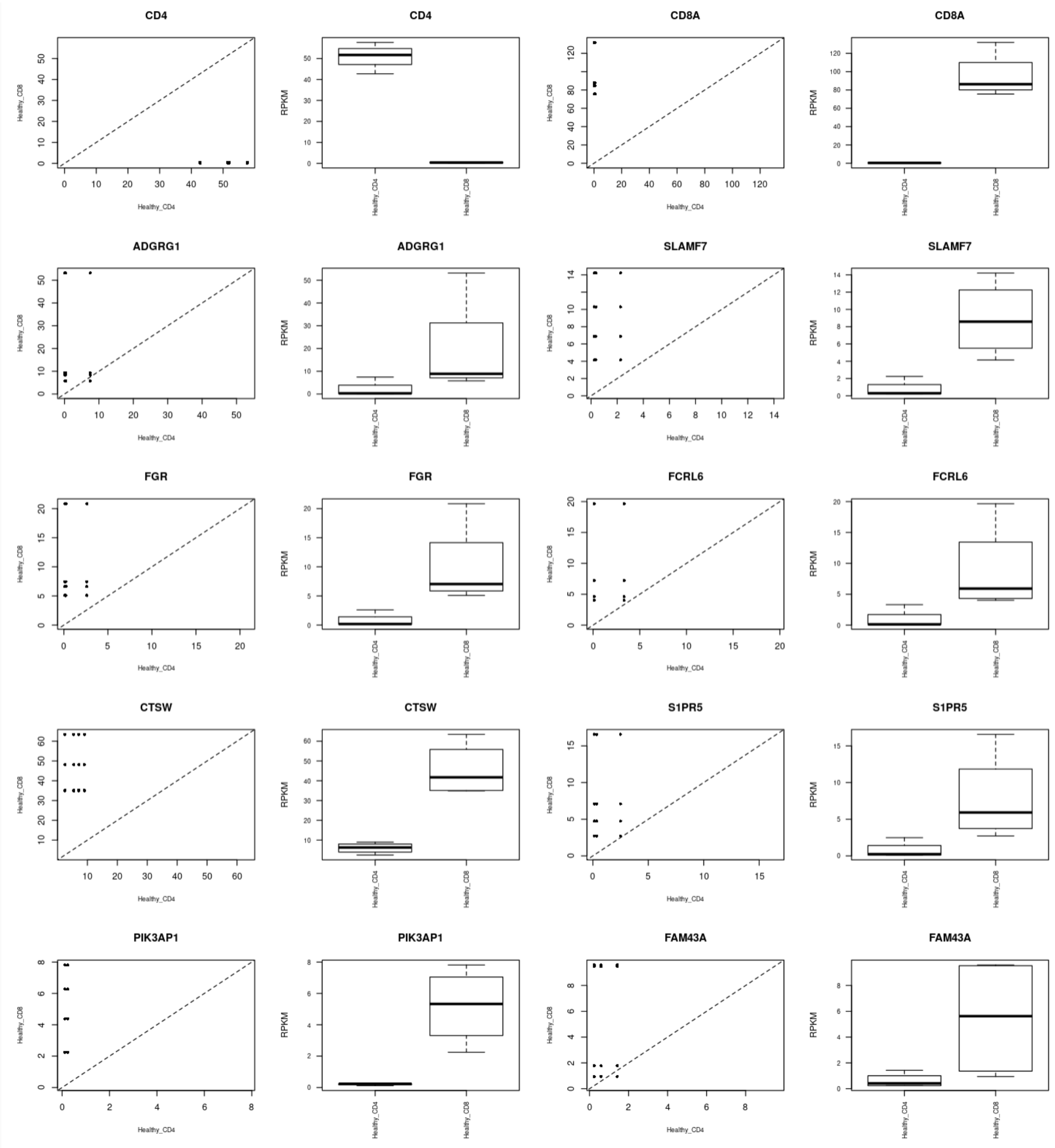
Min log2FC: 2

Display Top Genes: 10

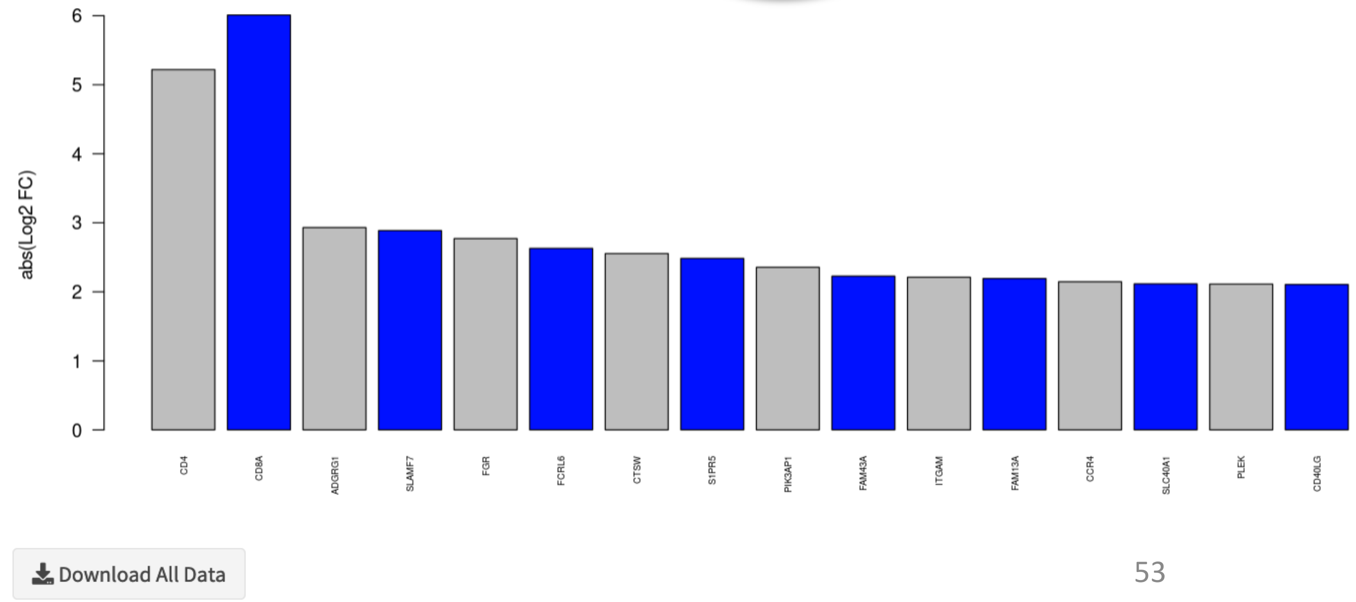
☑ Show log2FC in gene list?

Click ‘**Group Statistical Comparison’** tab. This tool attempts to identify potential biomarkers by comparing gene expression across all members of each group. Select groups to be compared in the ‘**Select Groups**’ dropdown list. check ‘**Fold Change**’ and ‘**Mean/SD**’ checkboxes. Click ‘**Find Genes**’ button.

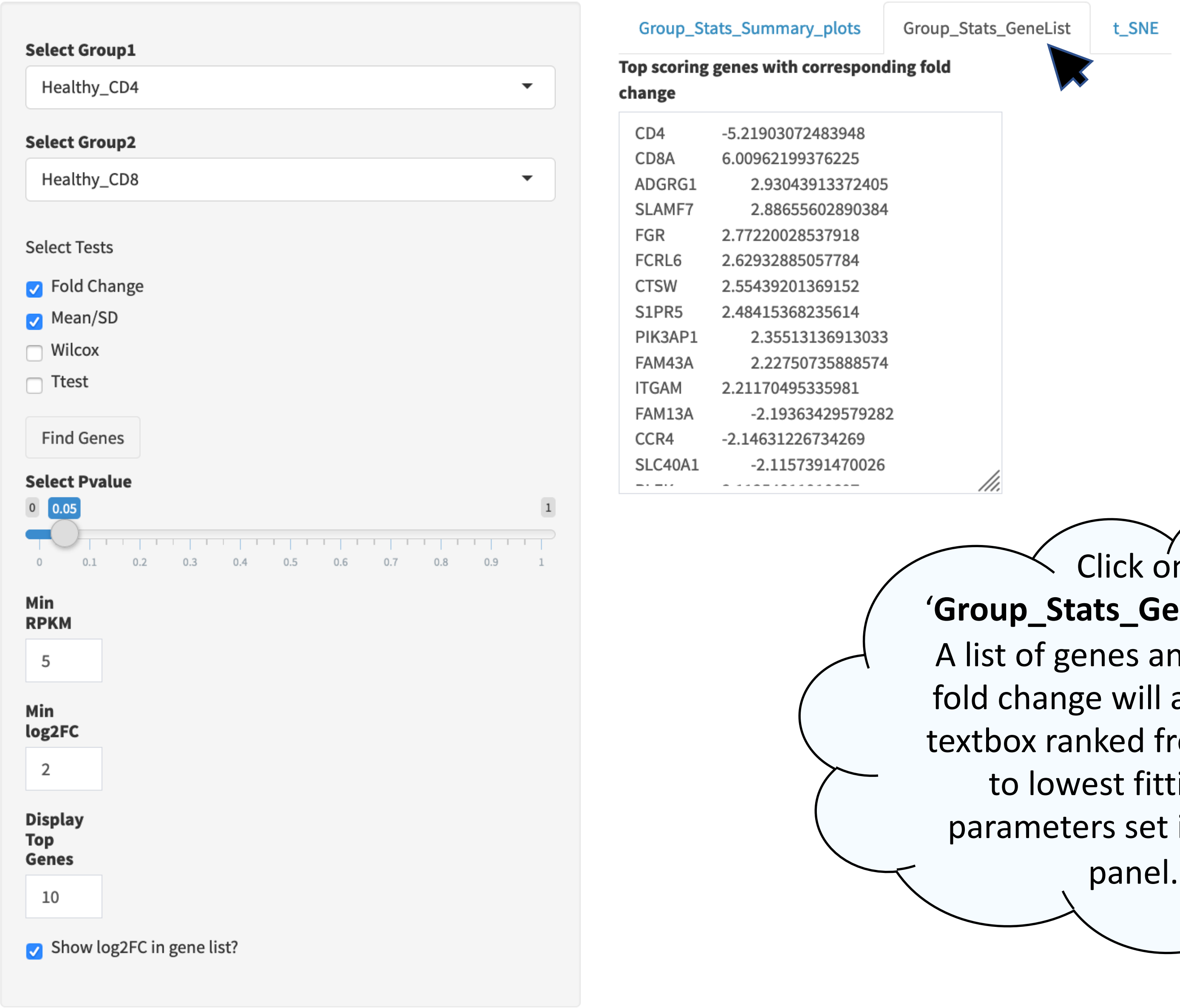
ROGUE – Statistical Group Comparison (Biomarker Discovery)



Plots of expression values and most consistently differentially expressed genes across groups will be displayed. Only the top 10 (default) genes will be displayed in the dot and box plots. This value can you be changed. All data and plots can be downloaded to a pdf file.



ROGUE – Statistical Group Comparison (Biomarker Discovery)



Select Group1
Healthy_CD4

Select Group2
Healthy_CD8

Select Tests
☑ Fold Change
☑ Mean/SD
☐ Wilcox
☐ Ttest

Select Pvalue
0.05

Min RPKM
5

Min log2FC
2

Display Top Genes
10

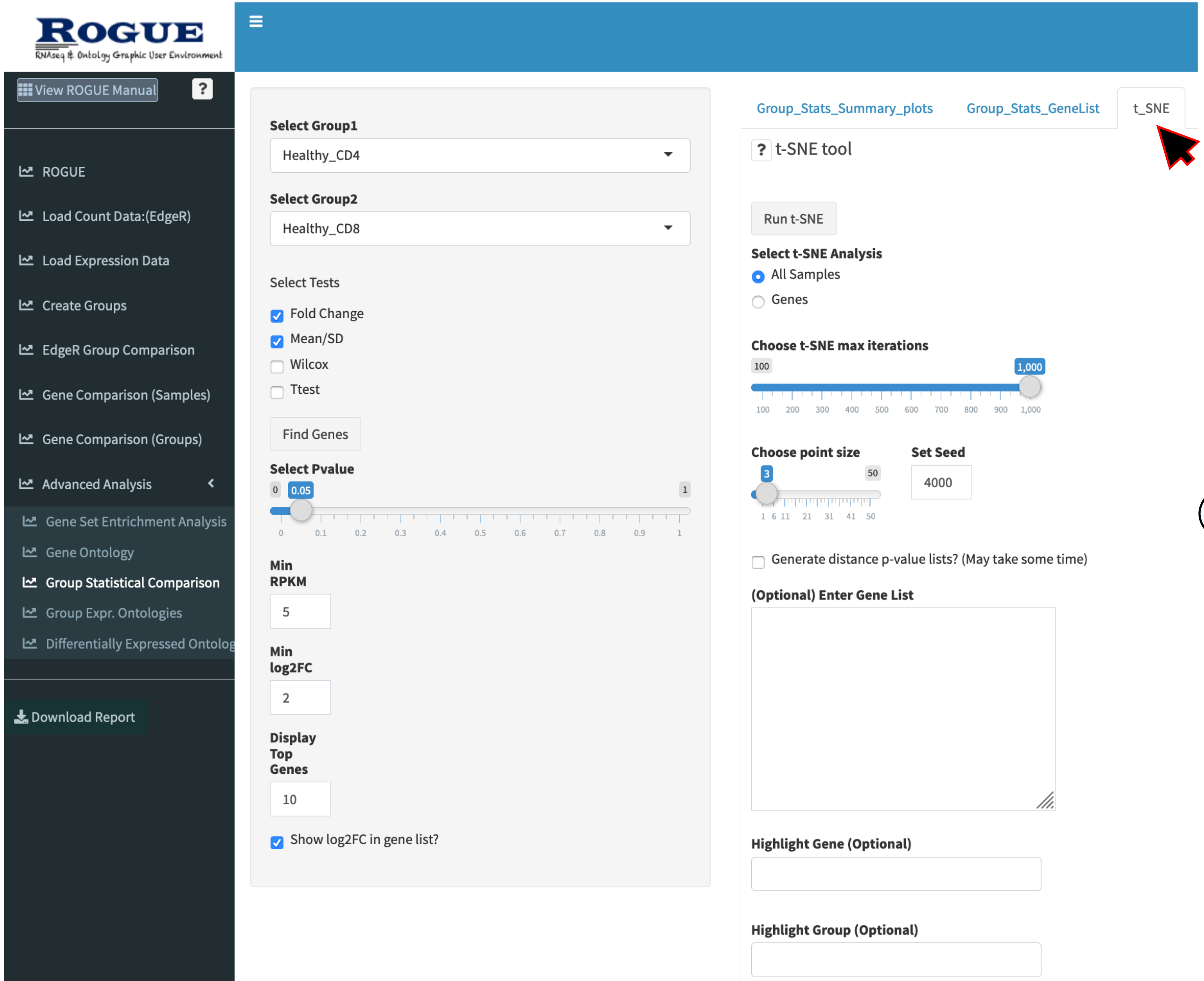
☑ Show log2FC in gene list?

Top scoring genes with corresponding fold change

```
CD4          -5.21903072483948
CD8A         6.00962199376225
ADGRG1            2.93043913372405
SLAMF7            2.88655602890384
FGR          2.77220028537918
FCRL6        2.62932885057784
CTSW         2.55439201369152
S1PR5        2.48415368235614
PIK3AP1           2.35513136913033
FAM43A            2.22750735888574
ITGAM        2.21170495335981
FAM13A            -2.19363429579282
CCR4         -2.14631226734269
SLC40A1           -2.1157391470026
```

Click on ‘**Group_Stats_GeneList**’ tab. A list of genes and the log2 fold change will appear in a textbox ranked from highest to lowest fitting the parameters set in the left panel.

ROGUE – tSNE



Select Group1: Healthy_CD4

Select Group2: Healthy_CD8

Select Tests
- ☑ Fold Change
- ☑ Mean/SD
- ☐ Wilcox
- ☐ Ttest

Select Pvalue: 0.05 (0 to 1)

Min RPKM: 5

Min log2FC: 2

Display Top Genes: 10

☑ Show log2FC in gene list?

Select t-SNE Analysis
- ● All Samples
- ○ Genes

Choose t-SNE max iterations: 1,000 (100 to 1,000)

Choose point size: 3 (1 to 50)

Set Seed: 4000

☐ Generate distance p-value lists? (May take some time)

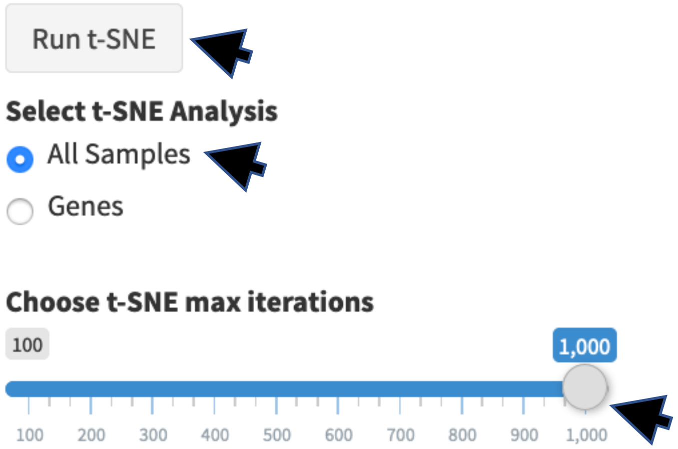
(Optional) Enter Gene List

Highlight Gene (Optional)

Highlight Group (Optional)

Click on ‘**t-SNE**’ tab

ROGUE – tSNE



Run t-SNE

Select t-SNE Analysis

● All Samples

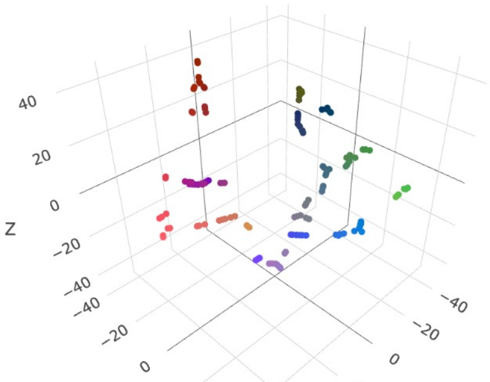
○ Genes

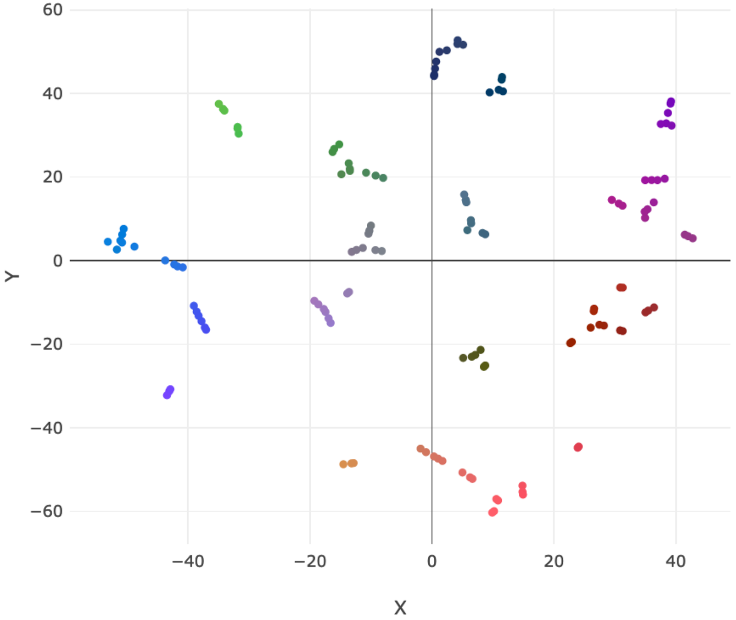
Choose t-SNE max iterations

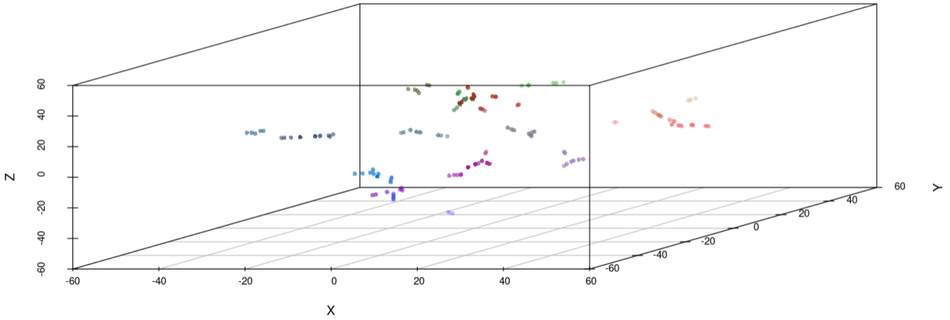
100

1,000

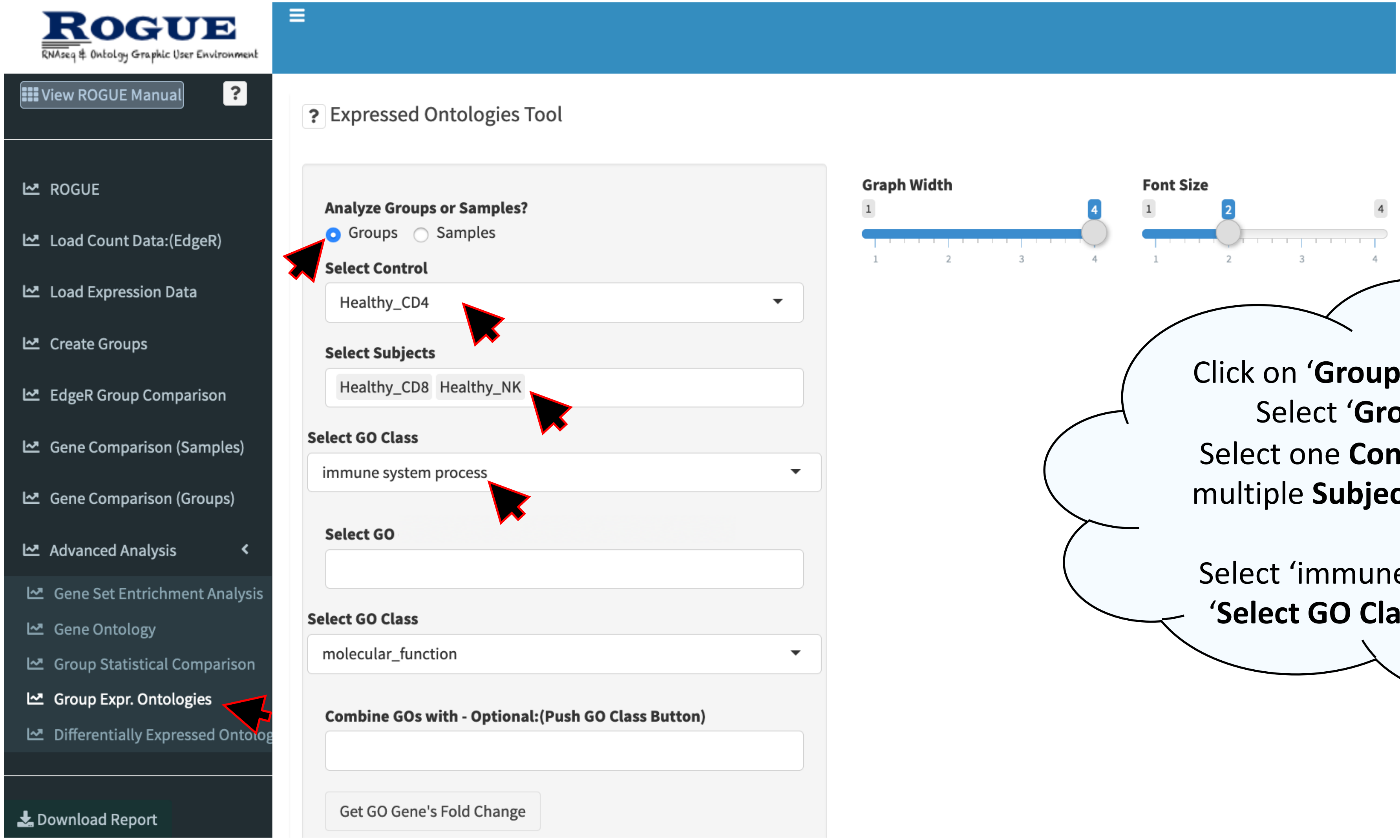
Select ‘**All Samples**’ radio button and change ‘**Choose t-SNE max iterations**’ sliding bar to 1000. 2D and 3D t-SNE plots will be generated.







ROGUE – Gene Ontology Expression Comparison



? Expressed Ontologies Tool

Analyze Groups or Samples?
● Groups ○ Samples

Select Control: Healthy_CD4

Select Subjects: Healthy_CD8 Healthy_NK

Select GO Class: immune system process

Select GO:

Select GO Class: molecular_function

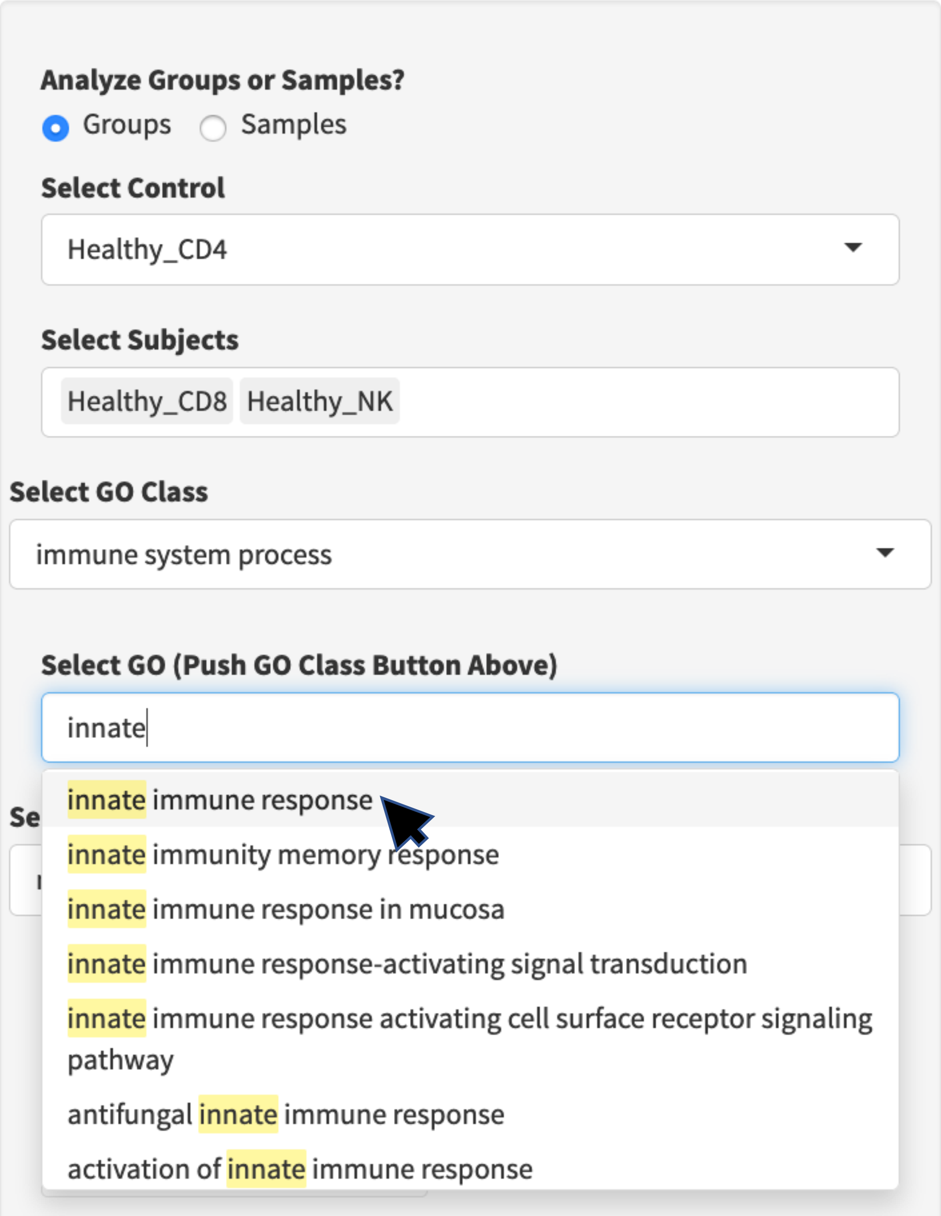
Combine GOs with - Optional:(Push GO Class Button):

Graph Width: 4

Font Size: 2

Click on ‘**Group Expr. Ontologies**’ tab.
Select ‘**Groups**’ radio button
Select one **Control** and either one or multiple **Subjects** from the dropdown lists.
Select ‘immune system process’ from ‘**Select GO Class**’ drop down menu.

ROGUE – Gene Ontology Expression Comparison



Analyze Groups or Samples?
● Groups ○ Samples

Select Control
Healthy_CD4

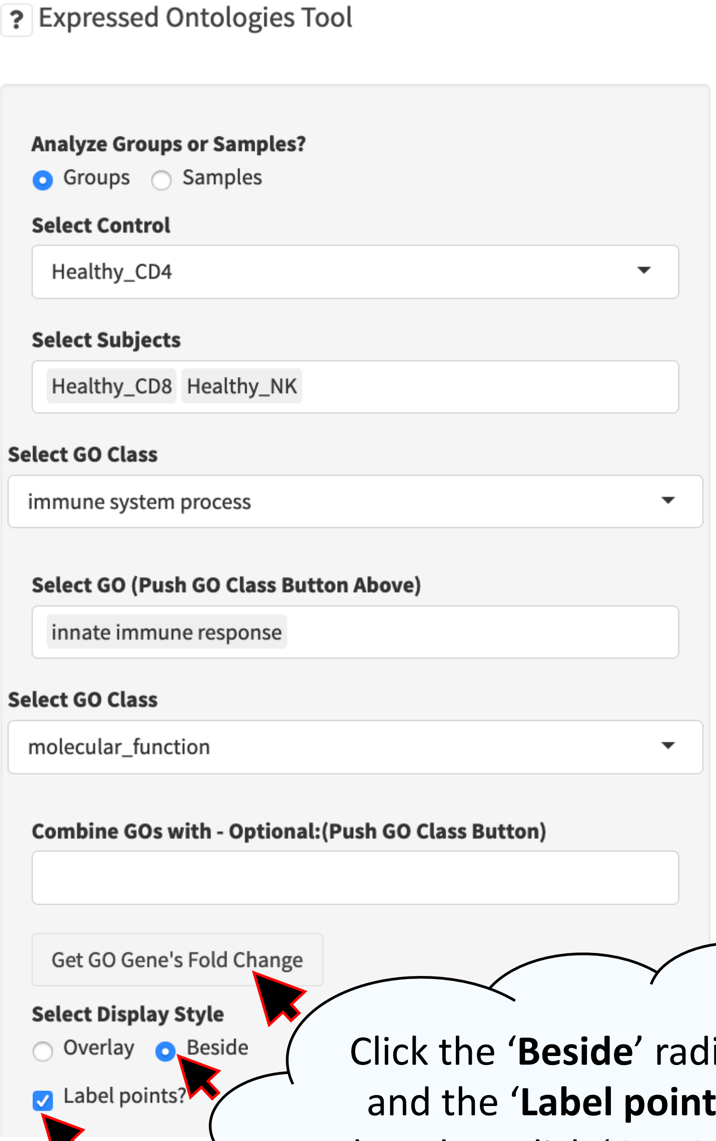
Select Subjects
Healthy_CD8 Healthy_NK

Select GO Class
immune system process

Select GO (Push GO Class Button Above)
innate

Selecting the ‘**immune system process**’ from the Select GO Class dropdown list populates the ‘**Select GO**’ dropdown list with all the gene ontologies related to immune system processes. Select the GOs you you would like to evaluate (e.g. innate immune response).

ROGUE – Gene Ontology Expression Comparison



? Expressed Ontologies Tool

Analyze Groups or Samples?
● Groups ○ Samples

Select Control
Healthy_CD4

Select Subjects
Healthy_CD8 Healthy_NK

Select GO Class
immune system process

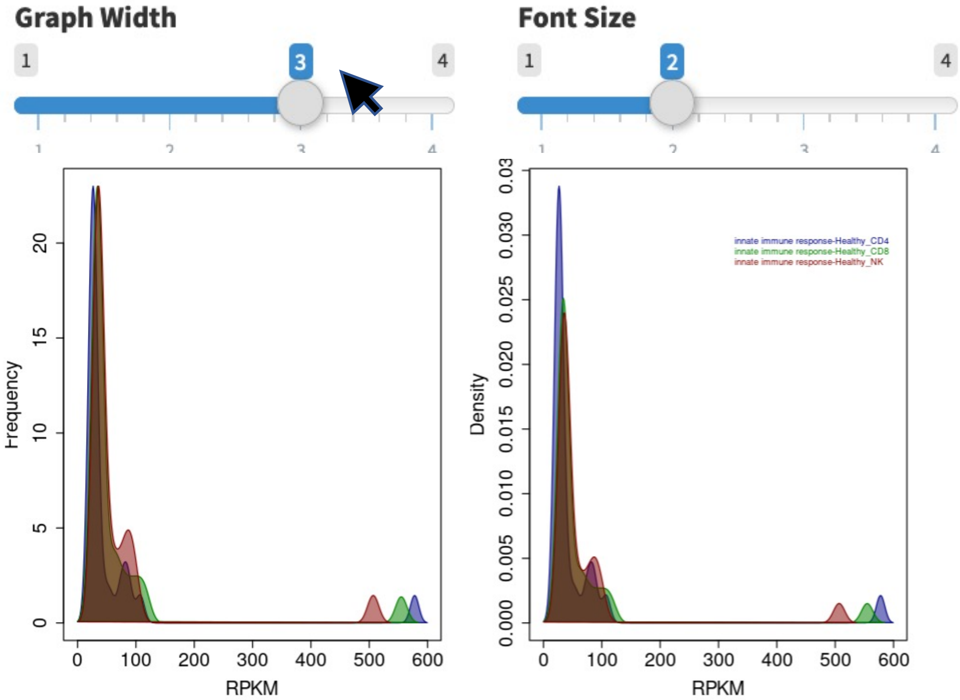
Select GO (Push GO Class Button Above)
innate immune response

Select GO Class
molecular_function

Combine GOs with - Optional:(Push GO Class Button)

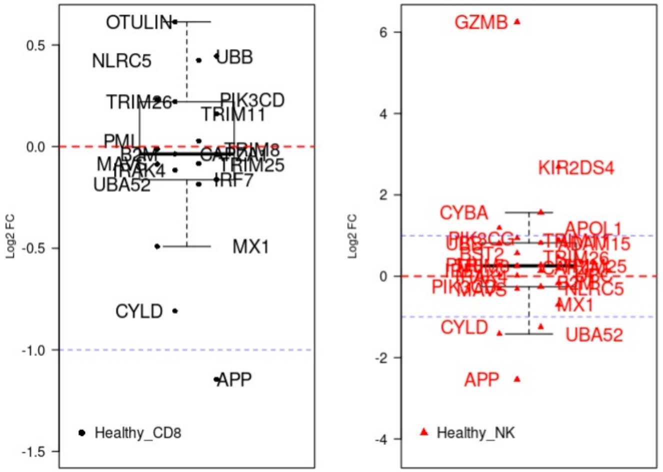
Select Display Style
○ Overlay ● Beside

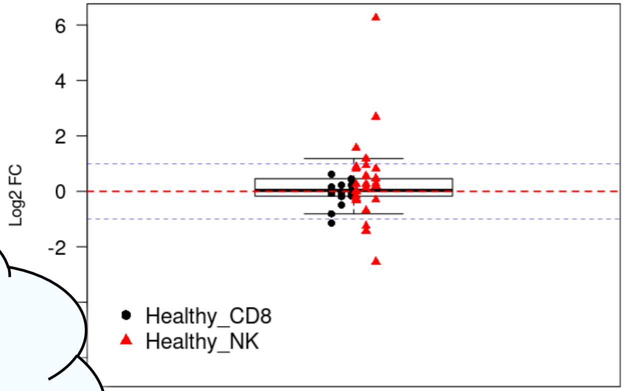
☑ Label points?

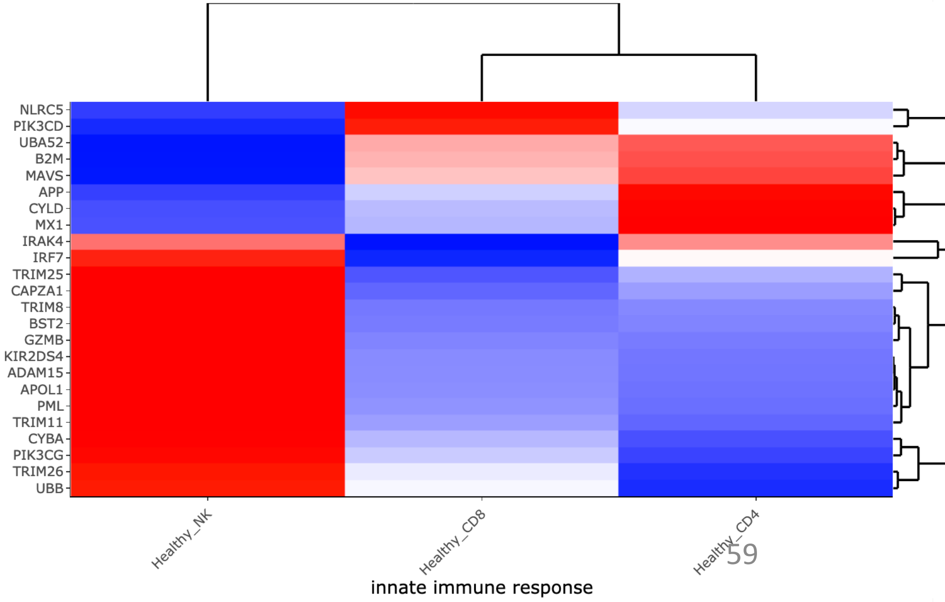


Graph Width
1 — 3 — 4

Font Size
1 — 2 — 4

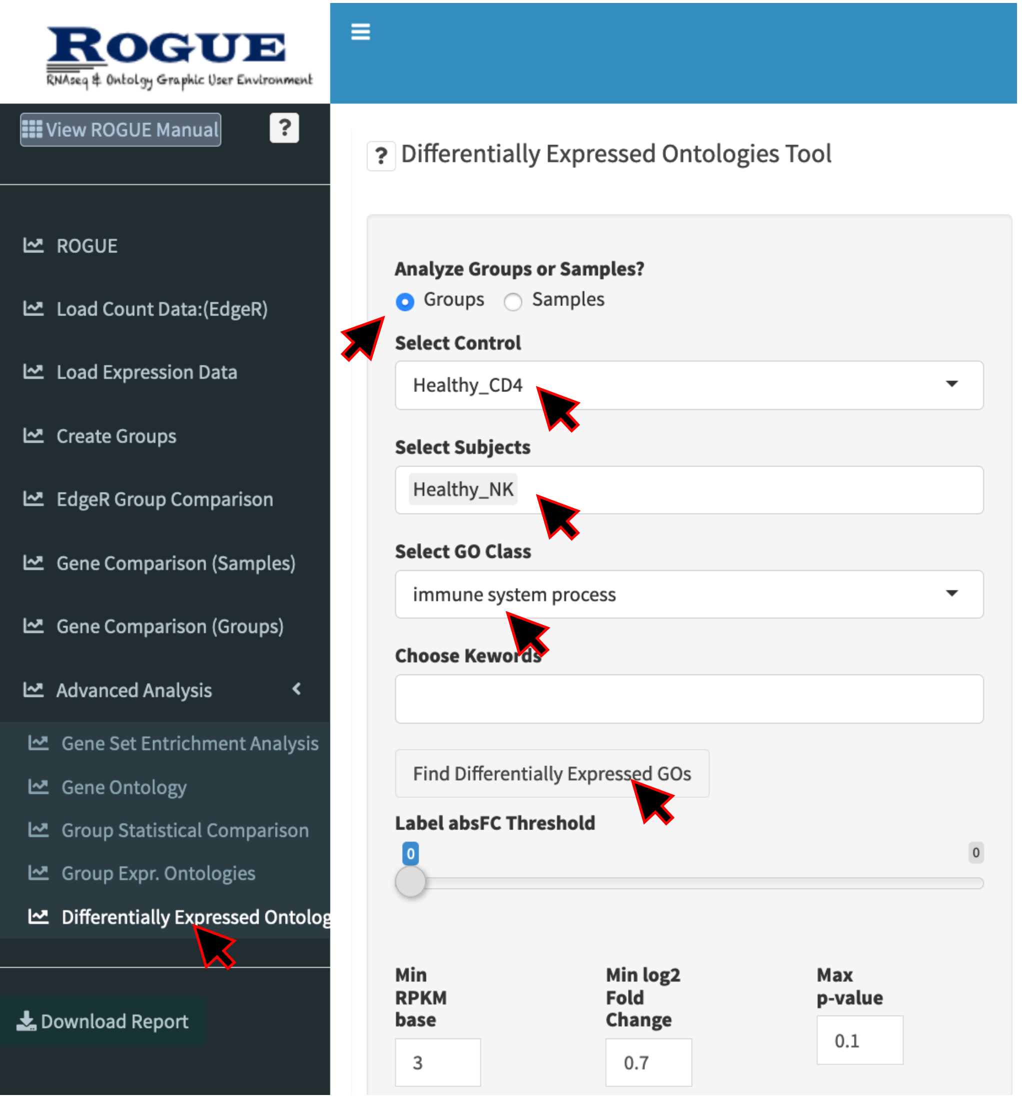






Click the ‘**Beside**’ radio button and the ‘**Label points**’ check box then click ‘**Get GO Gene’s Fold Change**’ button. Adjust the ‘**Graph Width**’ parameter.

ROGUE – Differentially Expressed Gene Ontologies



? Differentially Expressed Ontologies Tool

Analyze Groups or Samples?
● Groups ○ Samples

Select Control
Healthy_CD4

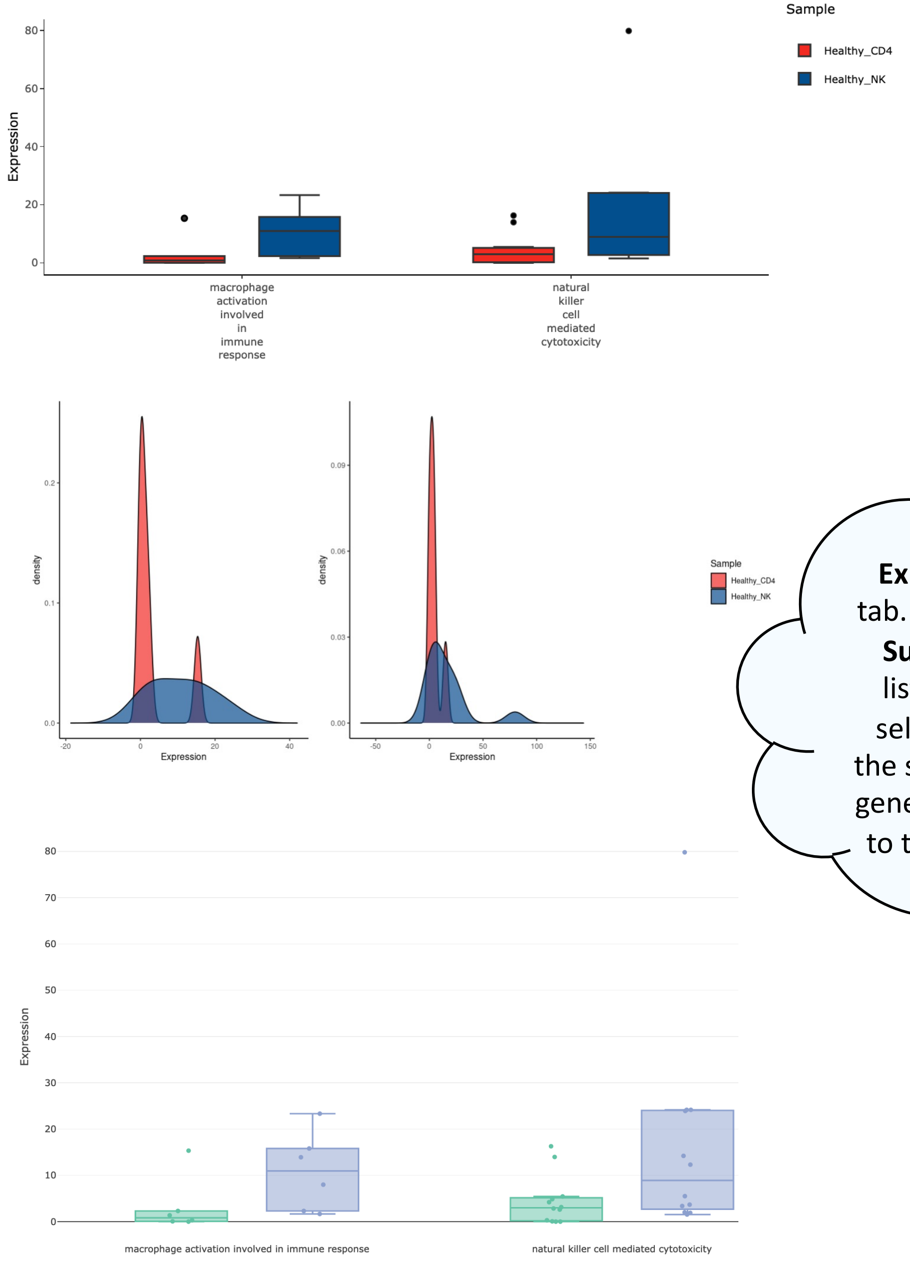
Select Subjects
Healthy_NK

Select GO Class
immune system process

Choose Kewords

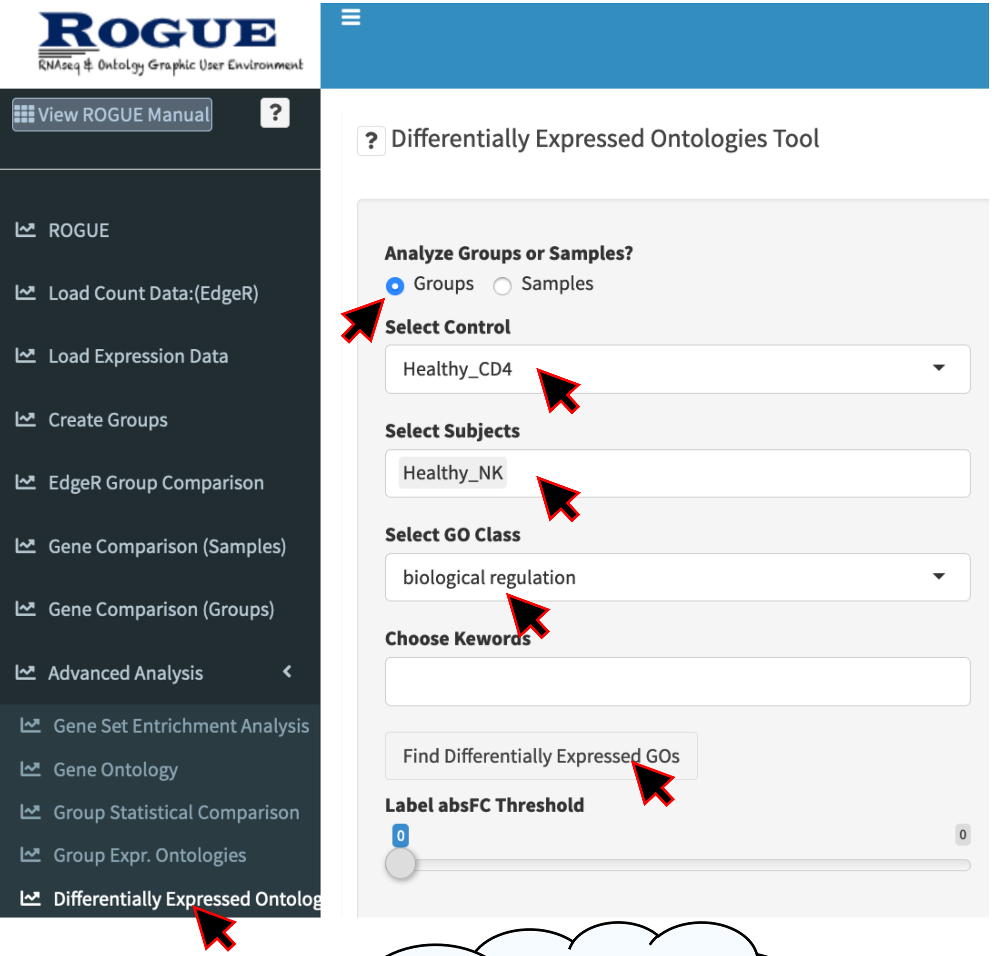
Label absFC Threshold
0 0

Min RPKM base	Min log2 Fold Change	Max p-value
3	0.7	0.1



Click on '**Differentially Expressed Gene Ontologies**' tab. Select one **Control** and one **Subject** from the dropdown lists. Select GO Class and/or select keywords to include in the search. The tool will look for gene ontologies that are related to the GO class or that include the keywords.

ROGUE – Differentially Expressed Gene Ontologies



? Differentially Expressed Ontologies Tool

Analyze Groups or Samples?

● Groups ○ Samples

Select Control: Healthy_CD4

Select Subjects: Healthy_NK

Select GO Class: biological regulation

Choose Kewords

Label absFC Threshold: 0 – 0

Perform analysis searching for differences in biological regulation