

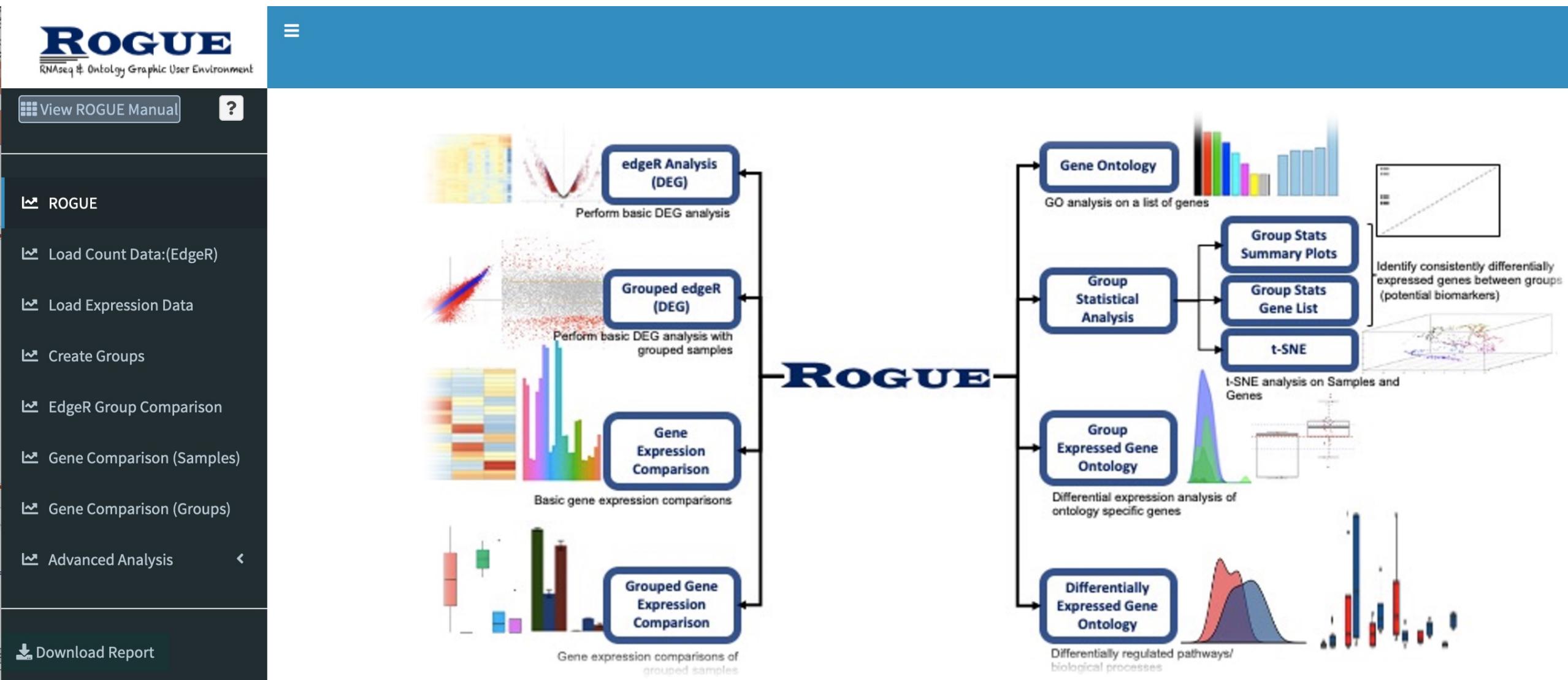


Basic Tutorial

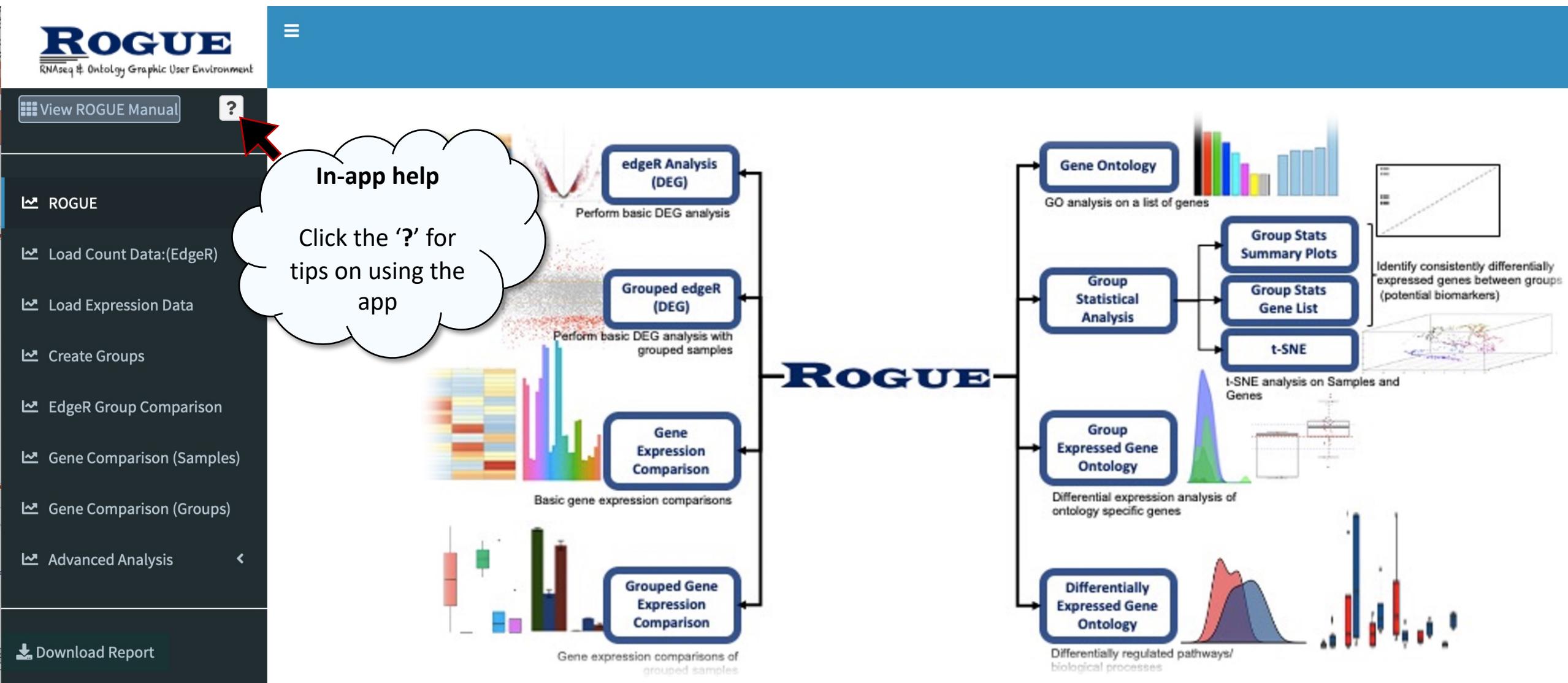
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Getting Started



Getting Started



Getting Started

The screenshot shows the ROGUE RNAseq & Ontology Graphic User Environment interface. On the left, a sidebar lists various analysis options: ROGUE, Load Count Data:(EdgeR), Load Expression Data, Create Groups, EdgeR Group Comparison, Gene Comparison (Samples), Gene Comparison (Groups), Advanced Analysis, and Download Report. A red arrow points from a question mark icon in the top right to a white cloud-shaped callout box.

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

The main area displays several analysis tools:

- edgeR Analysis (DEG)**: Perform basic DEG analysis.
- Grouped edgeR (DEG)**: Perform basic DEG analysis with grouped samples.
- Gene Expression Comparison**: Basic gene expression comparisons.
- Grouped Gene Expression Comparison**: Gene expression comparisons of grouped samples.
- Gene Ontology**: GO analysis on a list of genes.
- Group Statistical Analysis**: Identify consistently differentially expressed genes between groups (potential biomarkers).
- Group Stats Summary Plots**: t-SNE
- Group Stats Gene List**: I-SNE analysis on Samples and Genes
- Group Expressed Gene Ontology**: Differential expression analysis of ontology specific genes.
- Differentially Expressed Gene Ontology**: Differentially regulated pathways/biological processes.

A red circle with the number 1 is positioned over the 'Group Statistical Analysis' section. A callout box with a red border contains the following text:

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.

At the bottom of the callout box are three small dots, followed by 'Skip', '← Back', and 'Next →' buttons.

Getting Started – Session ID

The screenshot shows the ROGUE interface with a sidebar on the left containing various analysis options like 'edgeR Analysis (DEG)', 'Gene Ontology', and 'Advanced Analysis'. A red arrow points to the bottom-left corner of the sidebar where the session ID is displayed: '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 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

The screenshot shows the ROGUE web application interface. On the left is a dark sidebar with various analysis options. At the top right is a blue header bar. Below the header is the main content area.

Main Content Area:

- Session Info (Restore/Load)**: A section showing the current session ID: 159.14.228.161_2023.01.19_1.
- Enter Session ID**: A form field labeled "Enter Session ID" with a placeholder "Enter Session ID".
- Restore Session**: A button labeled "Restore Session" located next to the session ID input field.
- Choose 'Session' File (Rdata)**: A section with a "Browse..." button and a "No file selected" label.
- Upload Session**: A button labeled "Upload Session" located next to the file selection field.
- Download Session**: A button labeled "Download Session" with a download icon.

Left Sidebar:

- 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

Annotations:

- (1)** Go to the **'Restore/Load Session'** page
- (2)** Enter a previous session ID in the '**Enter Session ID**' field and click the '**Restore Session**' button.
Note: sessions are only stored on the server temporarily and will be removed often
- (3)** 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

The screenshot shows the ROGUE web application interface. On the left is a dark sidebar with various analysis options. The main area is titled 'Session Info (Restore/Load)' and displays the current session ID: 159.14.228.161_2023.01.19_1. It includes fields for 'Enter Session ID' and 'Choose 'Session' File (Rdata)'. A 'Download Session' button is on the left, and an 'Upload Session' button is on the right. Red arrows and callout boxes provide instructions:

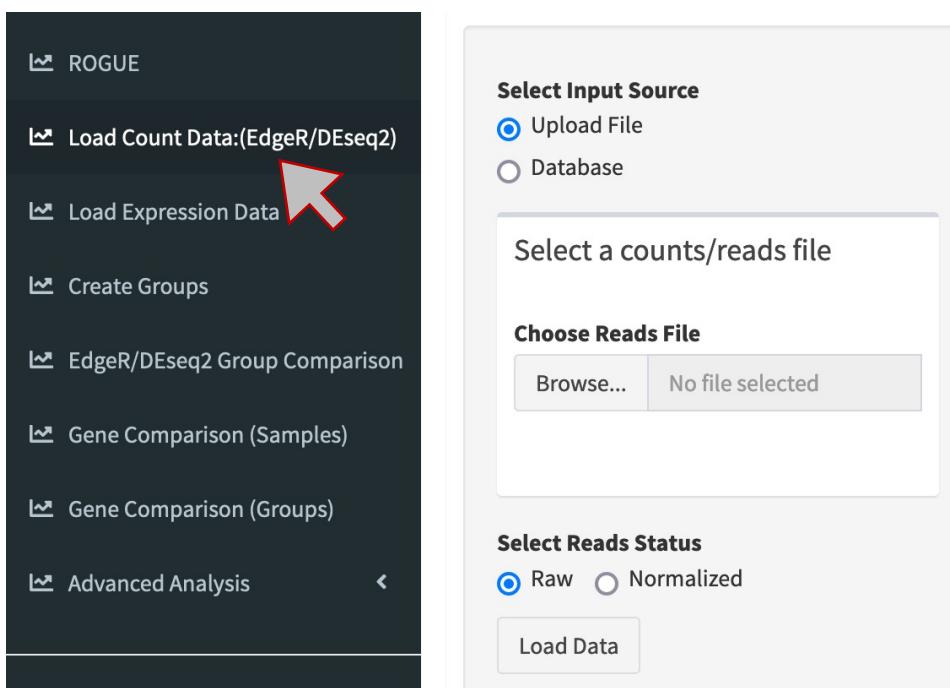
- (1)** Go to the **'Restore/Load Session'** page
- (2)** A session can be downloaded to your local computer by clicking the **'Download session'** button. The session will be a 'rdata' file
- (3)** If a session file (rdata) has been downloaded to your local computer, it can be uploaded using the upload 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.

Session ID: 159.14.228.161_2023.01.19_1

Data Input

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

Counts/Reads Data



ROGUE

Load Count Data:(EdgeR/DEseq2)

Load Expression Data 

Create Groups

EdgeR/DEseq2 Group Comparison

Gene Comparison (Samples)

Gene Comparison (Groups)

Advanced Analysis 

Select Input Source

Upload File

Database

Select a counts/reads file

Choose Reads File

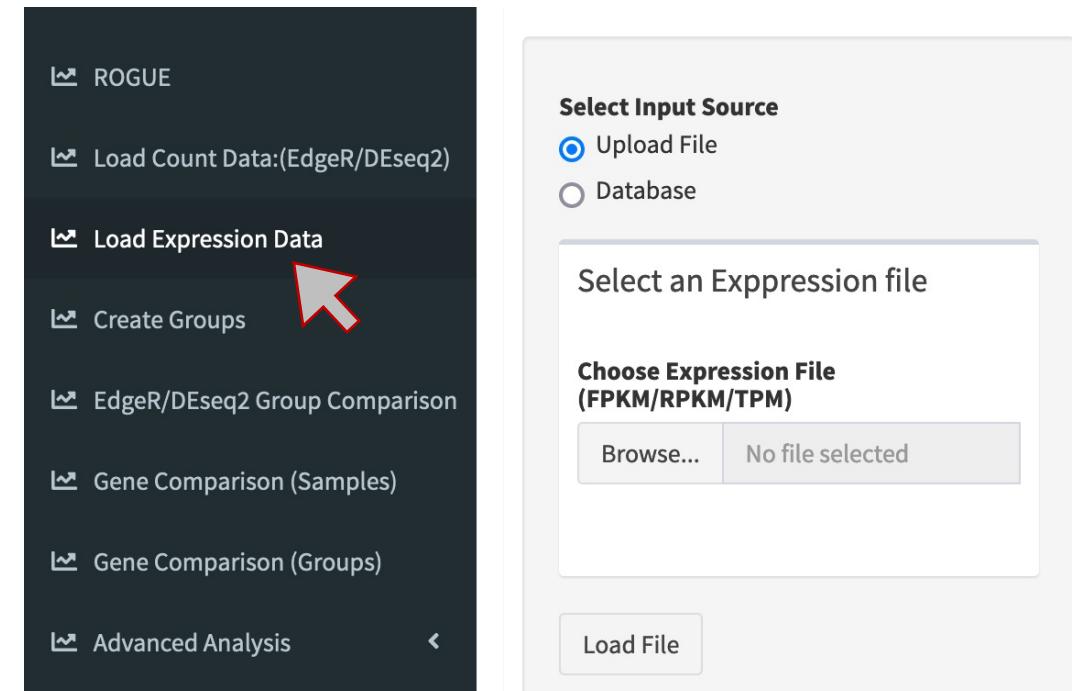
Browse... No file selected

Select Reads Status

Raw Normalized

Load Data

Expression Data



ROGUE

Load Count Data:(EdgeR/DEseq2)

Load Expression Data 

Create Groups

EdgeR/DEseq2 Group Comparison

Gene Comparison (Samples)

Gene Comparison (Groups)

Advanced Analysis 

Select Input Source

Upload File

Database

Select an Expression file

Choose Expression File (FPKM/RPKM/TPM)

Browse... No file selected

Load File

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

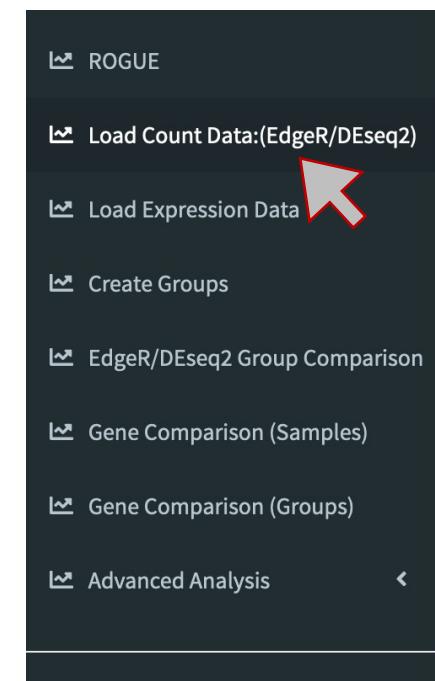
symbol	Sample_1	Sample_2	Sample_3
Wwp1	687	579	761
Pak2	5194	4724	5962
Tsr1	1204	1421	2334

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 use 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

Browse... No file selected

Select Reads Status

Raw Normalized

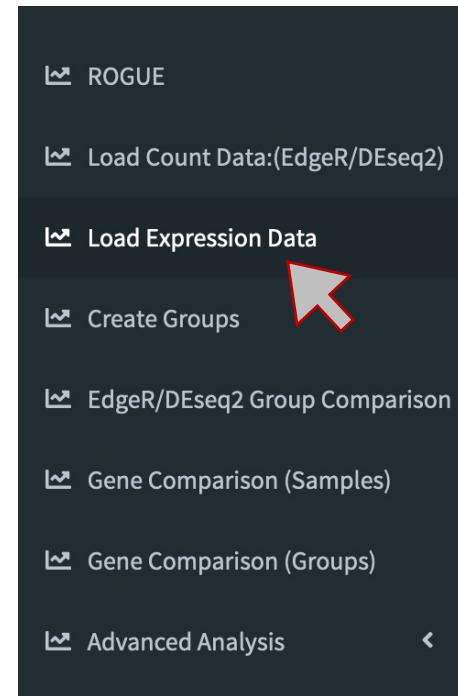
Load Data

Data Formats

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

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).



Select Input Source

Upload File
 Database

Select an Expression file

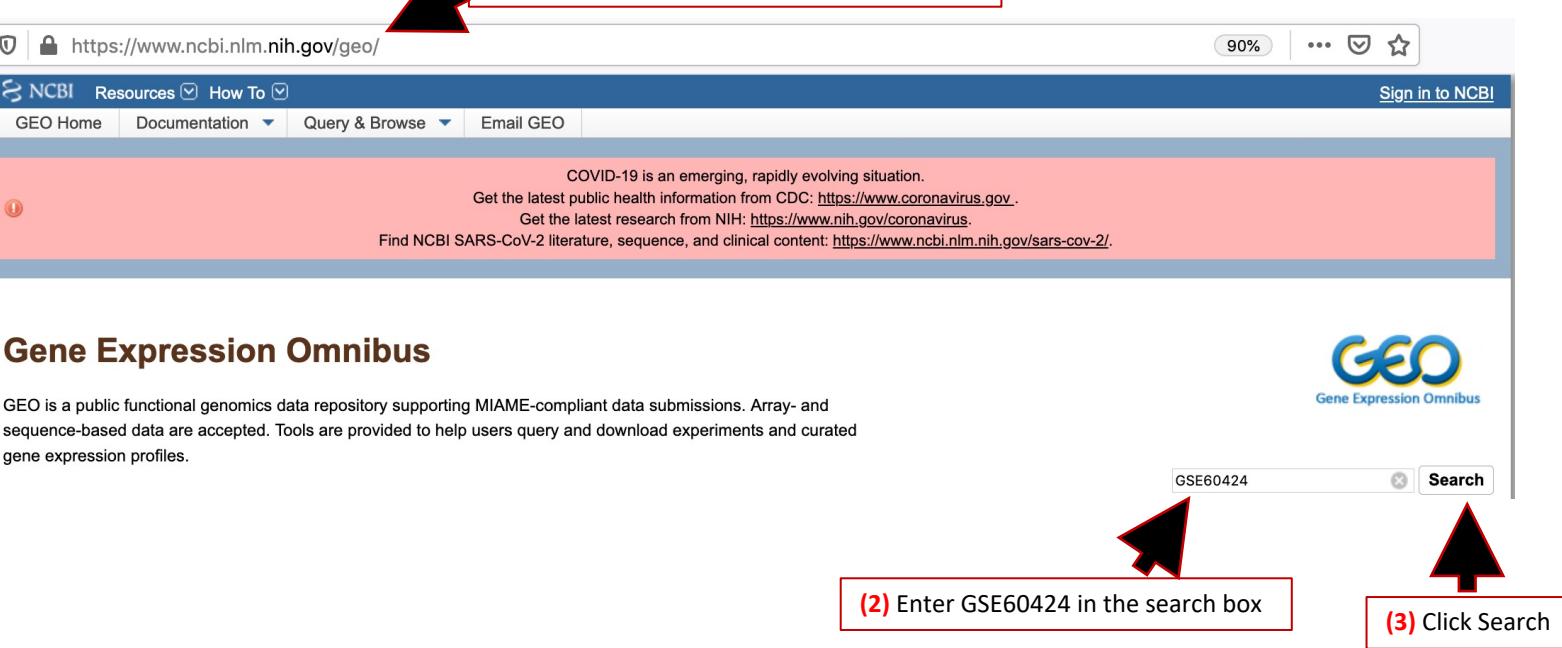
Choose Expression File (FPKM/RPKM/TPM)

Browse... No file selected

Load File

Tutorial-Getting Data from GEO

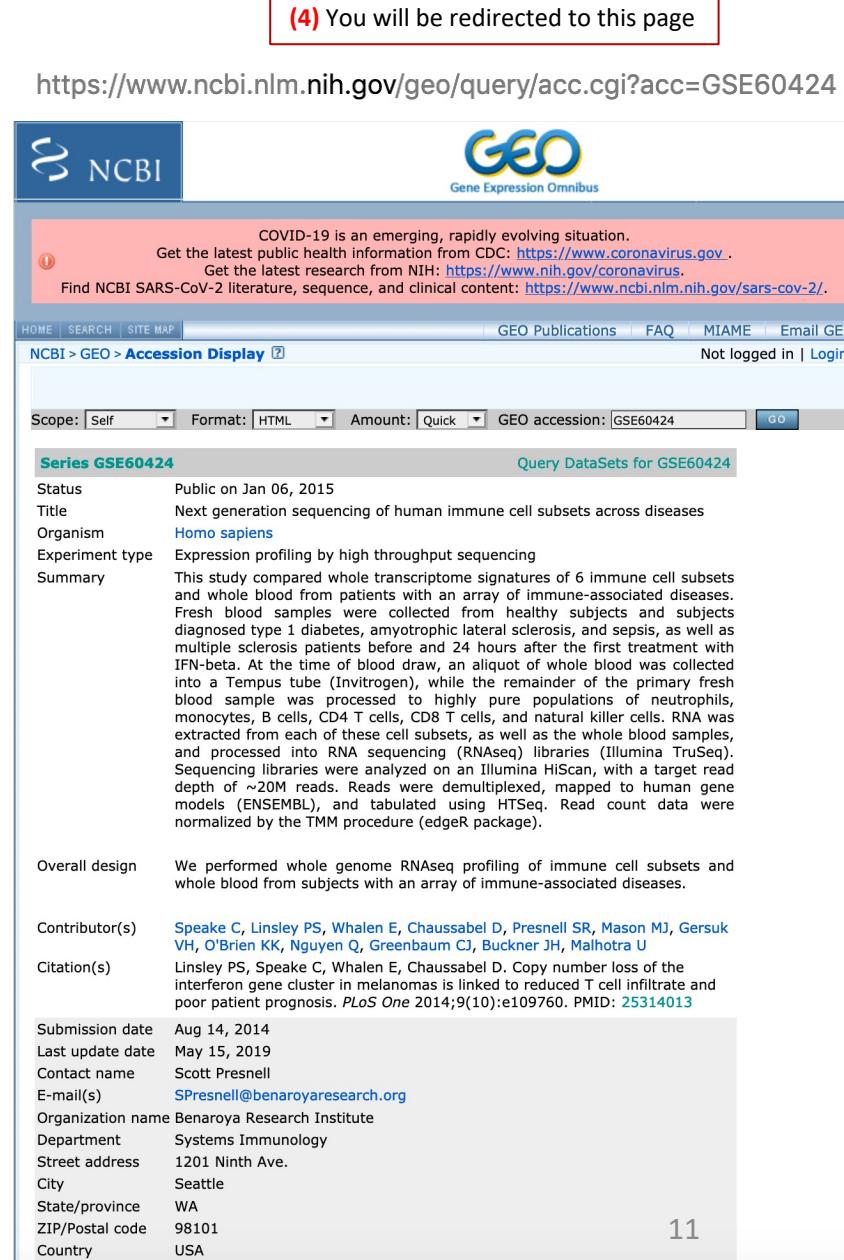
(1) Go to <https://www.ncbi.nlm.nih.gov/geo/>



(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>

NCBI Gene Expression Omnibus

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/>.

GEO Home Documentation Query & Browse Email GEO Sign in to NCBI

GEO Gene Expression Omnibus

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

Series GSE60424

Status	Public on Jan 06, 2015
Title	Next generation sequencing of human immune cell subsets across diseases
Organism	<i>Homo sapiens</i>
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. <i>PLoS One</i> 2014;9(10):e109760. PMID: 25314013
Submission date	Aug 14, 2014
Last update date	May 15, 2019
Contact name	Scott Presnell
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ZIP/Postal code	98101
Country	USA

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.

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Submission date Aug 14, 2014

Last update date May 15, 2019

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ZIP/Postal code 98101

Country USA

Platforms (1) [GPL15456](#) Illumina HiScanSQ (*Homo sapiens*)

Samples (134) [GSM1479433](#) lib221
[More...](#) [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 ?

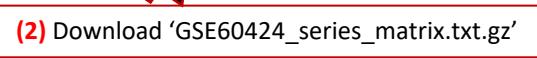
Supplementary file

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)'



(2) Download 'GSE60424_series_matrix.txt.gz'



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



Tutorial-Getting Data from GEO

<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

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

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
GSM1479449 GSM1479450 GSM1479451 GSM1479452 GSM1479453 GSM1479454 GSM1479455 GSM1479456 GSM1479457 GSM1479458 GSM1479459 GSM1479460 GSM1479461 GSM1479462 GSM1479463 GSM1479464 GSM1479465 GSM1479466 GSM1479467
GSM1479468 GSM1479469 GSM1479470 GSM1479471 GSM1479472 GSM1479473 GSM1479474 GSM1479475 GSM1479476 GSM1479477 GSM1479478 GSM1479479 GSM1479480 GSM1479481 GSM1479482 GSM1479483 GSM1479484 GSM1479485 GSM1479486
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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" "lib304" "lib305" "lib306" "lib307" "lib308" "lib309" "lib310" "lib311"
"lib312" "lib313" "lib314" "lib315" "lib316" "lib317" "lib318" "lib319" "lib320" "lib321" "lib322" "lib323" "lib324"
"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"
```

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.

Tutorial-Getting Data from GEO

Go to 'ROGUE_Companion' to prepare files.

reslnmaris01.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

The screenshot shows a web browser window for the GEO ROGUE Companion tool at the URL <https://reslnmaris01.research.chop.edu/ROGUE.Companion/>. The page has a header "GEO ROGUE Companion". On the left, there's a section for selecting data type ("Single Matrix" selected) and uploading a GSE Series Matrix File, with a note to upload files from GSE60424. A file selection dialog is open on the right, showing a list of files in the "Downloads" folder. The file "GSE60424_series_matrix.txt" is highlighted with a blue selection bar. Three red boxes with numbered labels provide instructions: (1) "Upload 'Series Matrix File'" points to the upload input field; (2) "Select GSE60424_series_matrix.txt" points to the highlighted file in the dialog; and (3) "Click 'Open'" points to the "Open" button at the bottom of the dialog.

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

(1) Upload 'Series Matrix File'

Note: Upload the files downloaded from GSE60424.

Select Series Matrix field to Match data columns/files

Downloads

Name

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

(2) Select GSE60424_series_matrix.txt

Cancel Open

(3) Click 'Open'

Tutorial-Getting Data from GEO

The screenshot shows the 'GEO ROGUE Companion' application interface. On the left, there's a sidebar with file upload options: 'Upload GSE Series Matrix File' (with 'GSE60424_series_matrix.txt' selected) and 'Upload Data matrix' (empty). A red box labeled '(1) Upload 'Counts Matrix File'' surrounds the 'Upload Data matrix' section, with a black arrow pointing to its 'Browse...' button. Below this is a note: 'Note: Upload the files downloaded from GSE60424.' On the right, under 'Select Series Matrix field to Match data columns/files', a dropdown menu shows 'Sample_title'. A file selection dialog is open over the application, showing files in 'Downloads': 'lib221', 'lib222', 'lib223', 'lib224', '...', and 'GSE60424_GEOSubmit_FC1to11_normalized_counts.txt' (which is highlighted with a blue selection bar). A red box labeled '(2) Select GSE60424_GEOSubmit_FC1to11_normalized_counts.txt' surrounds this file. At the bottom of the dialog are 'Cancel' and 'Open' buttons, with a red box labeled '(3) Click 'Open'' surrounding the 'Open' button.

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

Name

lib221
lib222
lib223
lib224
...
GSE60424_GEOSubmit_FC1to11_normalized_counts.txt

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

Data Matrix Sample Names/

The Sample names or Color appear here

Select Common Columns

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.

(1) Upload 'Counts Matrix File'

(2) Select GSE60424_GEOSubmit_FC1to11_normalized_counts.txt

(3) Click 'Open'

Tutorial-Getting Data from GEO

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_.txt
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
- Genentechovid_oh1
- 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_.txt
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

Create Groups

Group Name

Type Group Name here

(1) Enter a group name.



Select Features

age

32

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



Add Group Feature

Group Features

Selected group features will appear here

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



Create_Group

Groups

Groups appear here

(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 created, 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

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

Create Groups

Group Name

(1) Type 'Healthy_CD4' as the Group Name

Select Features

celtype

- age
- cellcount
- celtype
- collectiondate
- diseasestatus
- donorid
- gender
- index
- Create_Group

Select Detail

Whole Blood

Create Groups

Group Name

Select Features

celtype

Add Group Feature

Group Features

Selected group features will appear here

Create_Group

Select Detail

Whole Blood

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

Create Groups

Group Name

Select Features

celtype

Select Detail

CD4

Add Group Feature



(4) Click 'Add Group Feature'

Group Features

Selected group features will appear here

Create Groups

Group Name

Select Features

celtype

Select Detail

CD4

Add Group Feature

Group Features

celtype: CD4

Tutorial-Getting Data from GEO

Create Groups

Group Name
Healthy_CD4

Select Features
celtype

- age
- cellcount
- celtype
- collectiondate
- diseasestatus
- donorid
- gender
- index_Group

Select Detail
CD4

(6) Choose 'diseasestatus'

Create Groups

Group Name
Healthy_CD4

Select Features
diseasestatus

Add Group Feature

Group Features

celtype: CD4

Create_Group

(7) Choose 'Healthy Control'

Create Groups

Group Name
Healthy_CD4

Select Features
diseasestatus

Select Detail
Healthy Control

Add Group Feature

Group Features

celtype: CD4
diseasestatus: Healthy Control

(8) Click 'Add Group Feature'

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

Tutorial-Getting Data from GEO

Create Groups

Group Name
Healthy_CD4

Select Features
diseasestatus

Select Detail
Healthy Control

Add Group Feature

Group Features

celltype: CD4
diseasestatus: Healthy Control

Create_Group

Groups
Groups appear here

(10) Click 'Create_Group'

Create Groups

Group Name
Healthy_CD4

Select Features
diseasestatus

Select Detail
Healthy Control

Add Group Feature

Group Features

Selected group features will appear here

Create_Group

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

Add Group Feature

Group Features

Selected group features will appear here

Create_Group

Groups

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

(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

[Download Data Table](#)

[Download Groups](#)

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

[Download Data Table](#)

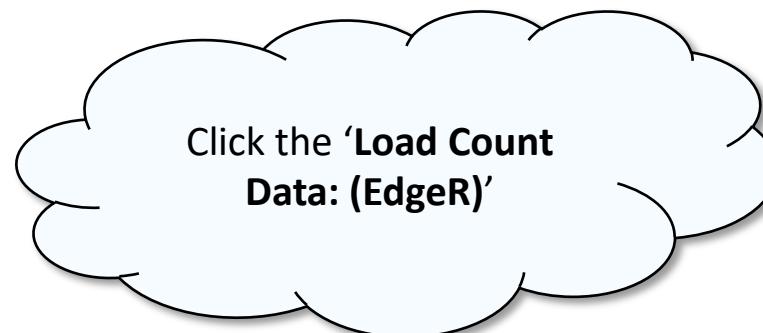
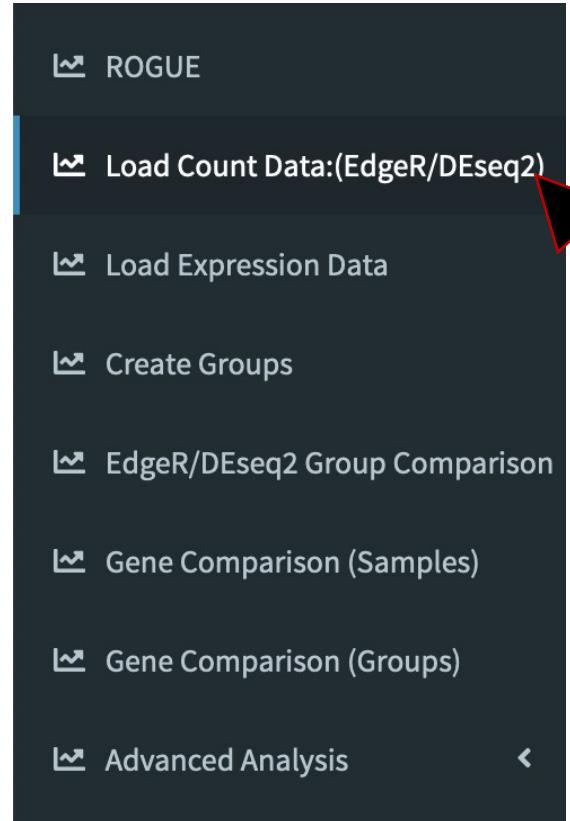
[Download Groups](#)

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

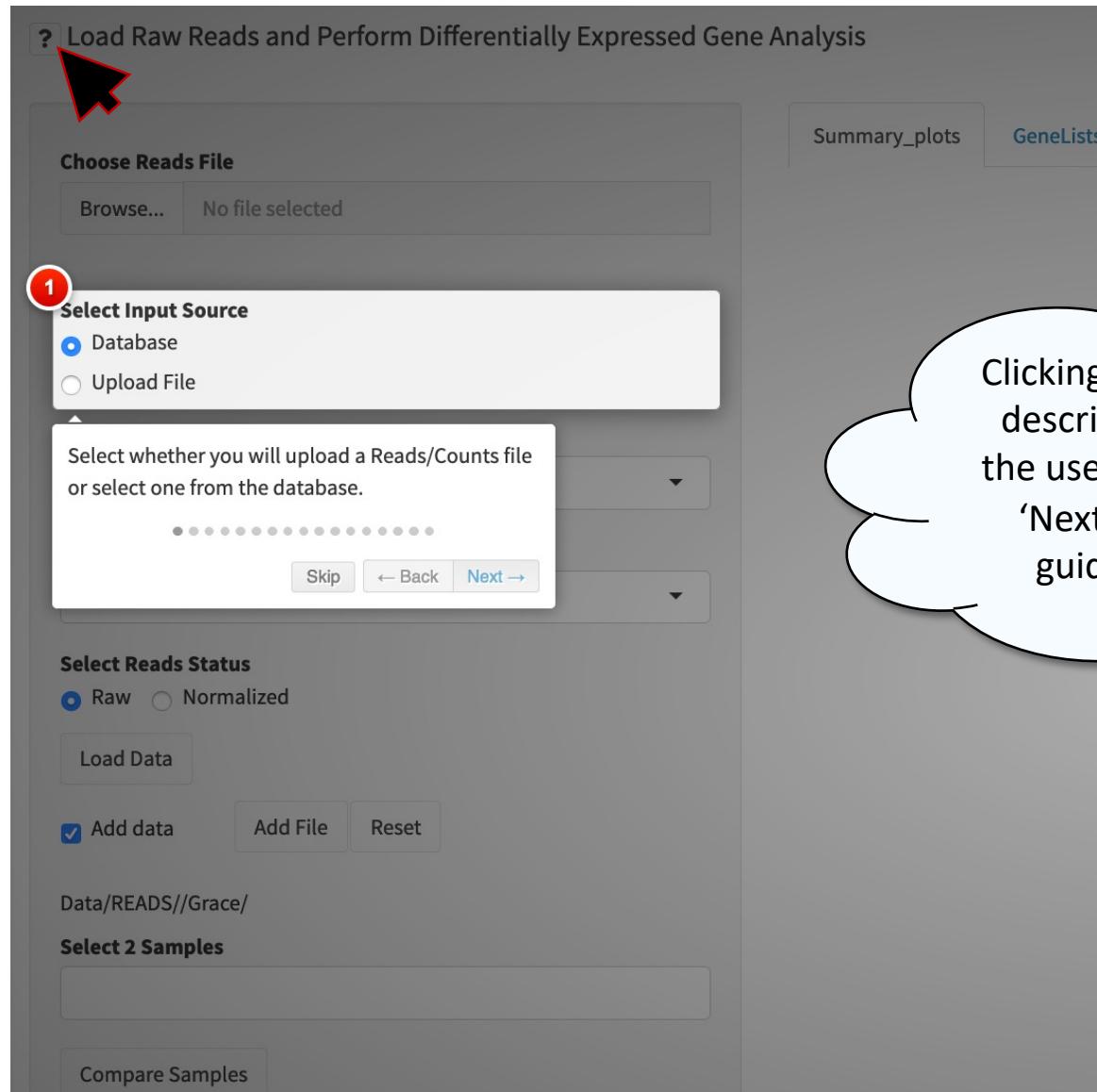
Note: Download the Data Table as 'Data_Matrix.txt' and the groups file as 'Groups_File.txt'

ROGUE – Differential Expression Analysis

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



ROGUE – Differential Expression Analysis



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

No file selected

Select Input Source

Database
 Upload File

Select Data Library

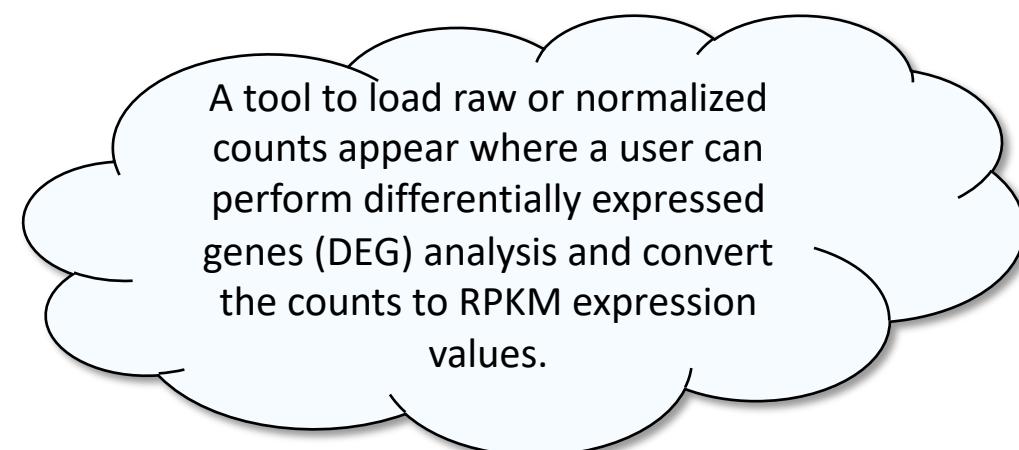
Select Dataset

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

No file selected



Select Input Source

Database
 Upload File



Select Data Library

GSE60424

Select Dataset

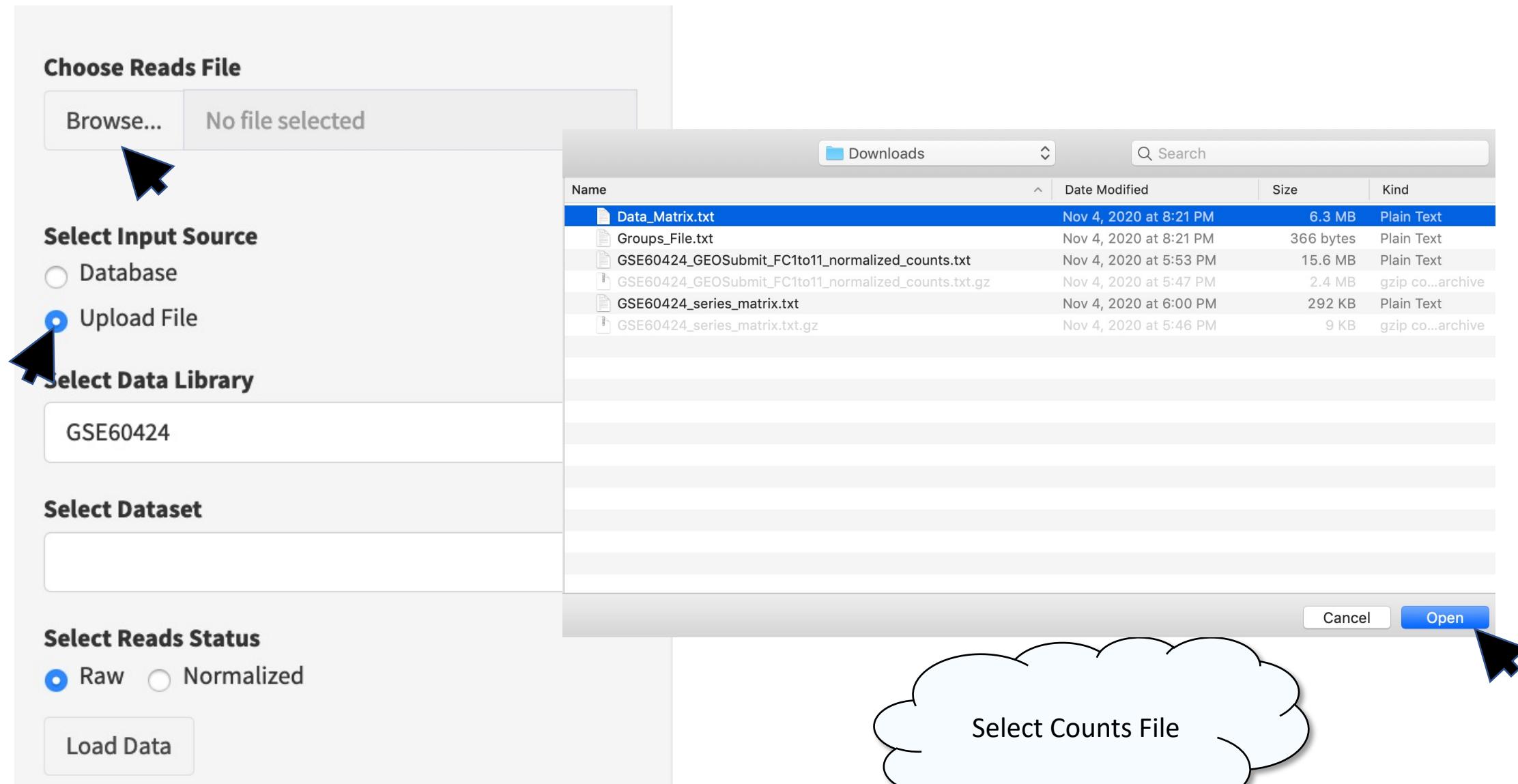
Select Reads Status

Raw Normalized

Load Data

Check the upload radio button
and click 'Browse' to
select a Counts file

ROGUE – Differential Expression Analysis



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

Data_Matrix.txt
Upload complete

Select Input Source

Database
 Upload File

Select Data Library

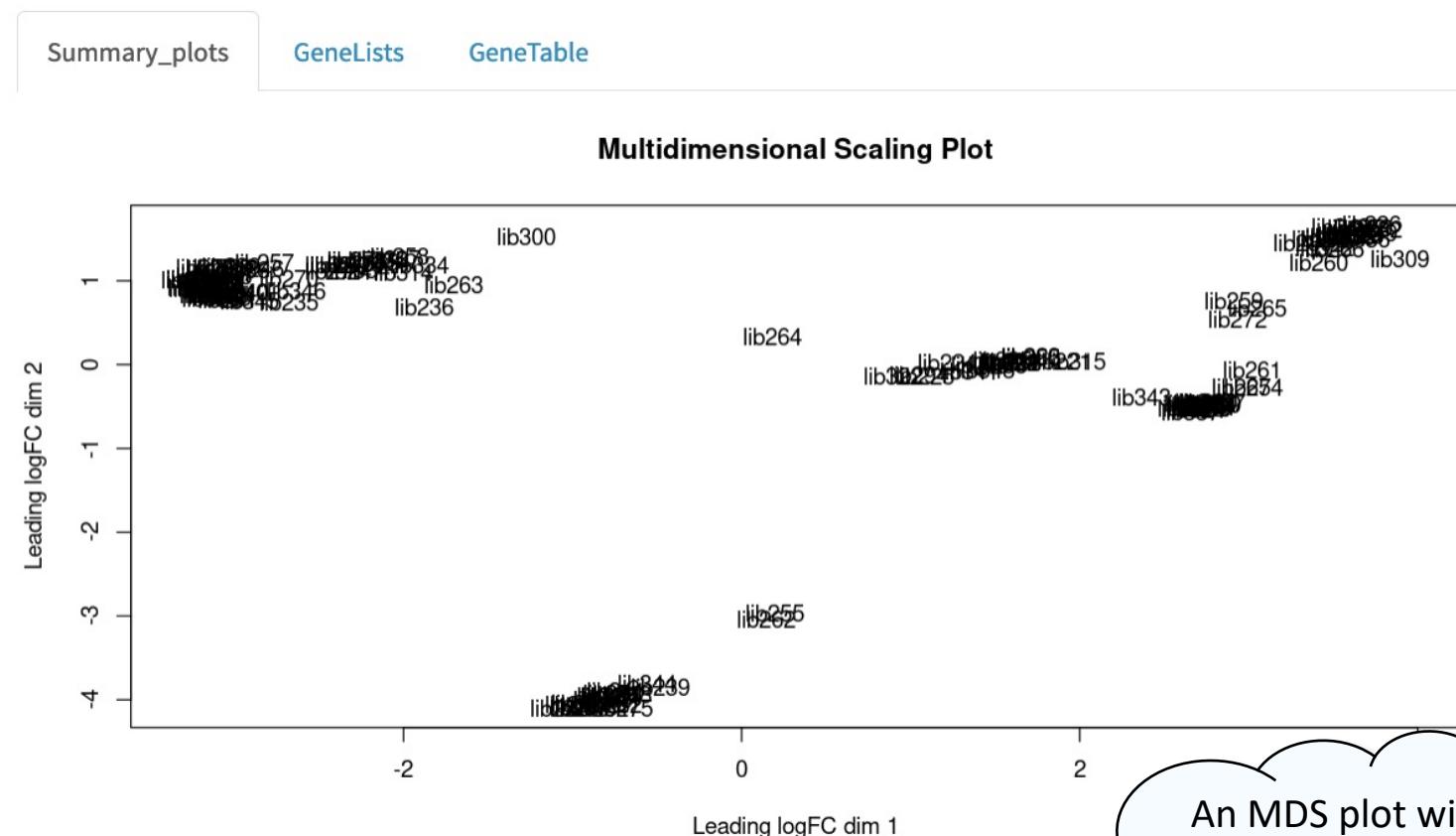
GSE60424

Select Dataset

▼

Select Reads Status

Raw Normalized



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

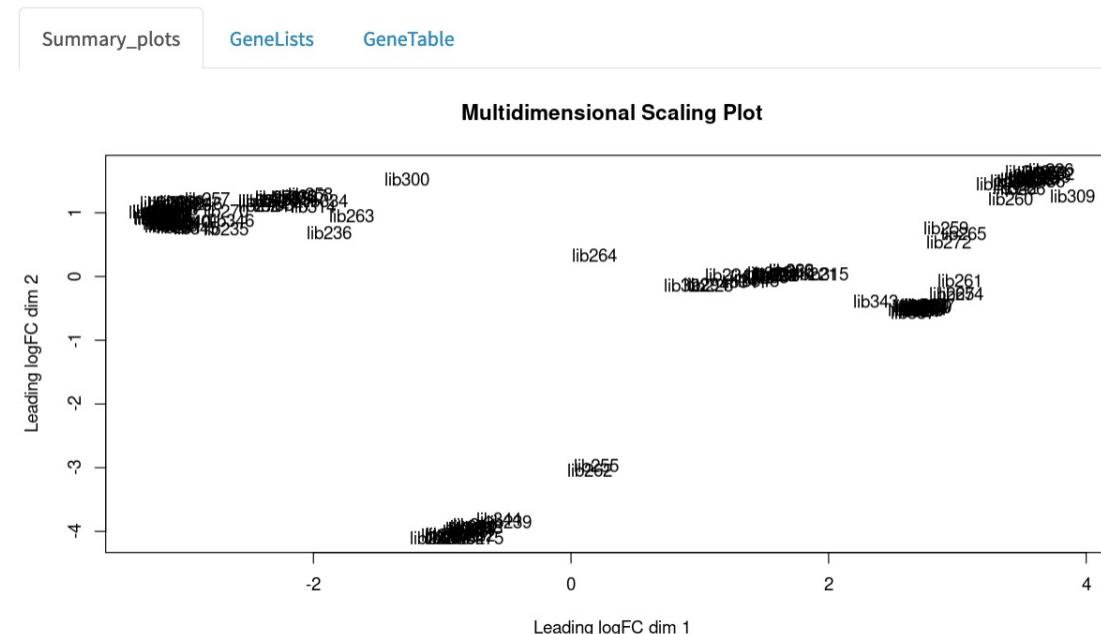
Load Data

Add data

Data/READS//GSE60424/

Select 2 Samples
lib221 lib222 lib223 lib224 lib225 lib226 lib227 lib228

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

Data_Matrix.txt

Upload complete

Select Input Source

Database

Upload File

Select Data Library

GSE60424 ▼

Select Dataset

▼

Select Reads Status

Raw Normalized

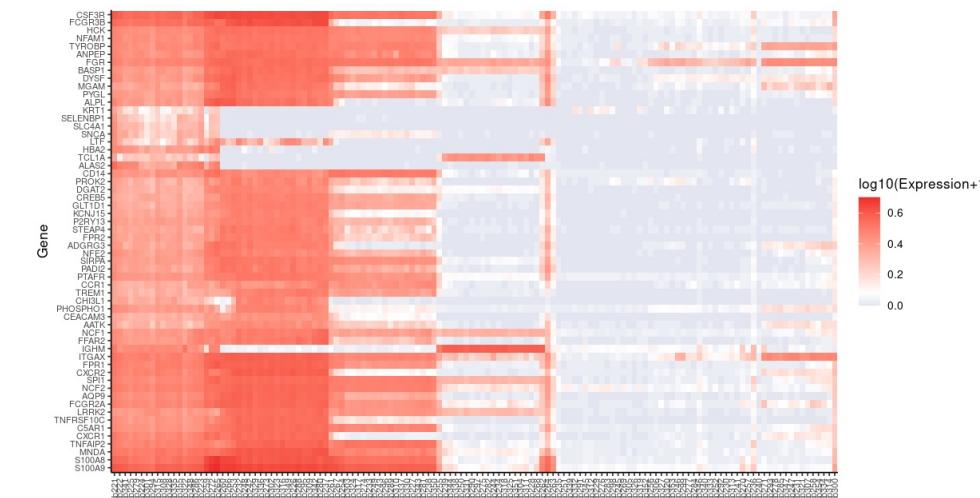
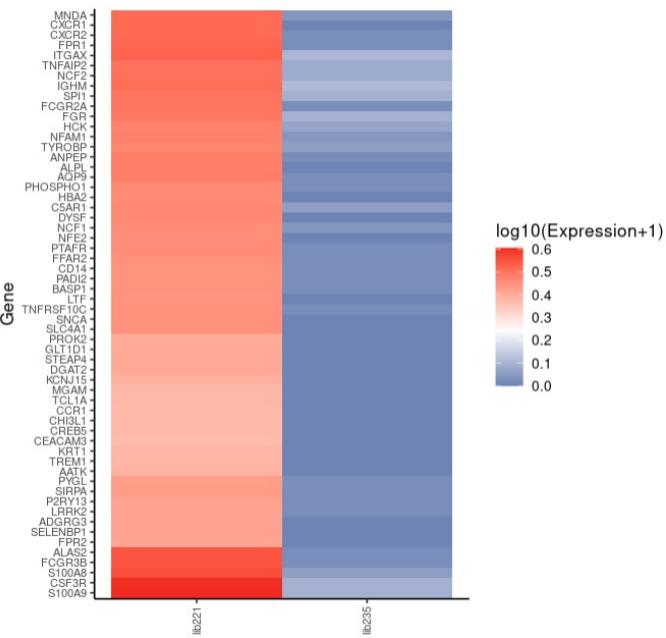
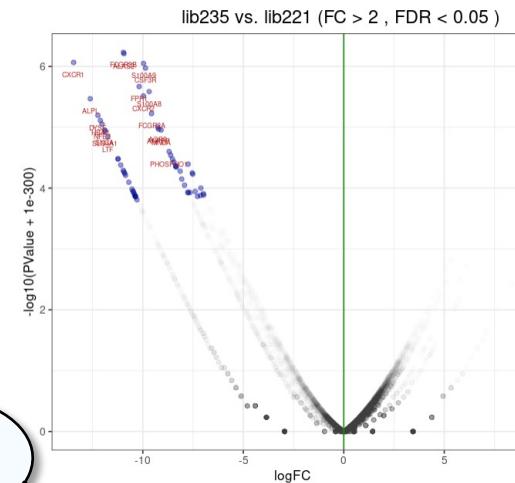
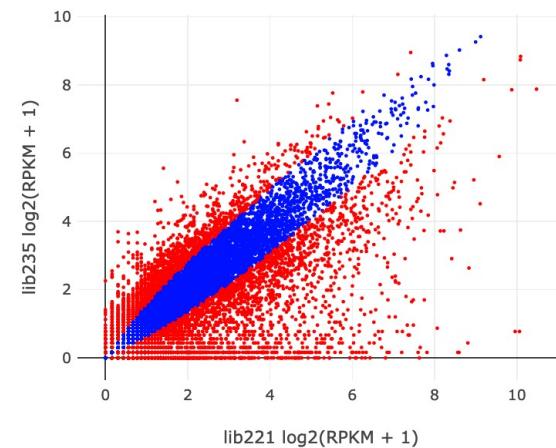
Add data

Data/READS//GSE60424/

Select 2 Samples

lib221 lib235

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

Summary_plots GeneLists

[Download FC Gene List](#) [Download RPKM Table](#)

[Download Upregulated FC Gene List](#) [Download Upregulated RPKM Data](#)

[Download Downregulated Gene List](#) [Download Downregulated RPKM Data](#)

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

The screenshot shows the ROGUE software interface. On the left, a dark sidebar lists various analysis options: View ROGUE Manual, ROGUE, Load Count Data:(EdgeR), Load Expression Data, Create Groups (highlighted with a red arrow), EdgeR Group Comparison, Gene Comparison (Samples), Gene Comparison (Groups), Advanced Analysis, and Download Report. The main panel has a blue header with a menu icon. Below the header, a large button labeled 'Create Groups' is highlighted with a red arrow. The interface includes fields for 'Group Name' (with a placeholder 'Enter Group Name') and 'Select Group Members'. It also features a 'Create Group' button, a 'Choose 'Groups' File' section with 'Browse...' and 'No file selected' buttons, and 'Upload Groups' and 'Confirm Groups' buttons. A 'Groups' section is currently empty. At the bottom is a 'Download Groups' button. Two callout bubbles provide instructions: one pointing to the 'Create Groups' button says 'Click the 'Create Groups' tab.', and another pointing to the '?' button in the top right corner says 'Click '?' button for step by step tips on using this tool'.

Click the 'Create Groups' tab.

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

ROGUE – Create Groups

The screenshot shows the ROGUE software interface with a dark theme. On the left, a vertical sidebar contains links: View ROGUE Manual, ROGUE, Load Count Data:(EdgeR), Load Expression Data, Create Groups (selected), EdgeR Group Comparison, Gene Comparison (Samples), Gene Comparison (Groups), Advanced Analysis, and Download Report. Below these are two buttons: Download Groups and Download Report.

The main page title is 'Create Groups'. It features a 'Group Name' input field with placeholder 'Enter Group Name' and a 'Create Group' button. Below this is a 'Choose 'Groups' File' section with 'Browse...' and 'No file selected' buttons, and an 'Upload Groups' button. A 'Select Group Members' dropdown menu is open, listing sample names: lib221 (highlighted with a red arrow), lib222, lib223, lib224, lib225, lib226, lib227, and lib228. At the bottom right of the dropdown is a 'Confirm Groups' button with the value '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

Create Group



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

ROGUE – Create Groups

? Create Groups

Group Name **Select Group Members**

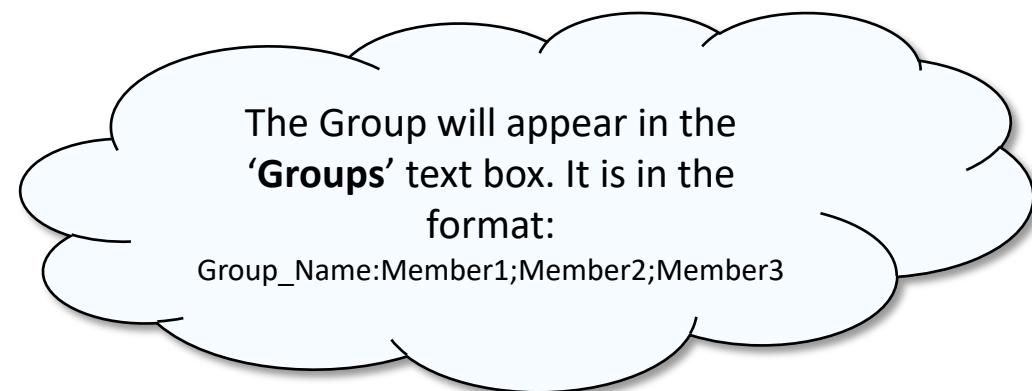
Create Group

Choose 'Groups' File

Upload Groups **Confirm Groups**

Groups
Group1:lib221;lib222;lib223;lib224

 **Download Groups**



ROGUE – Create Groups

? Create Groups

Group Name **Select Group Members**

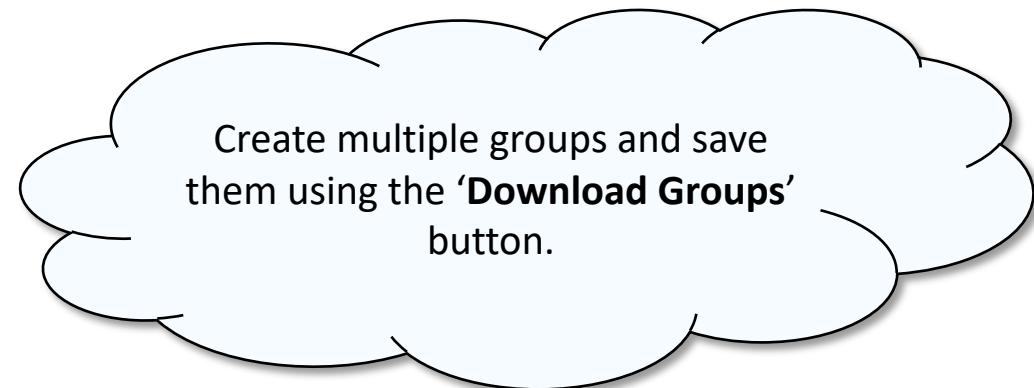
Create Group

Choose 'Groups' File

Upload Groups **Confirm Groups**

Groups
Group1:lib221;lib222;lib223;lib224

Download Groups 



ROGUE – Create Groups

Create Groups

Group Name
Enter Group Name

Select Group Members

Create Group

Choose 'Groups' File
Browse... No file selected

Upload Groups **Confirm Groups**

Groups
Group1:lib221;lib222;lib223;lib224

Download 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

Enter groups manually by typing in the format:
Group_Name:Member1;Member2;Member3
Or upload saved groups by clicking '**Browse**' button

ROGUE – Create Groups

Create Groups

Group Name
Enter Group Name

Select Group Members

Create Group

Choose 'Groups' File
Browse... No file selected

Upload Groups **Confirm Groups**

Groups
Group1:lib221;lib222;lib223;lib224

Download 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

Select the Groups file

Cancel

Open



ROGUE – Create Groups

? Create Groups

Group Name **Select Group Members**

Enter Group Name

Create Group

Choose 'Groups' File

Browse... Groups_File
Upload complete

Upload Groups Confirm Groups

Groups

Group1:lib221;lib222;lib223;lib224

Download Groups

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

ROGUE – Create Groups

? Create Groups

Group Name

Enter Group Name

Select Group Members

Create Group

Choose 'Groups' File

Browse... Groups_File

Upload complete

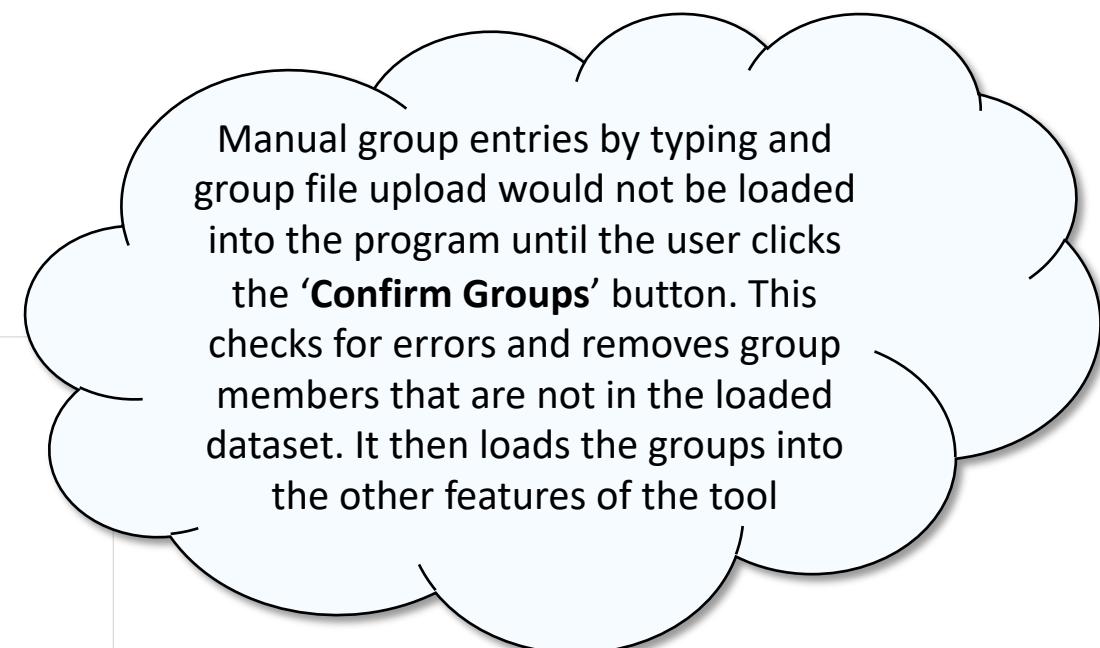
Upload Groups

Confirm Groups

Groups

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

Download Groups



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

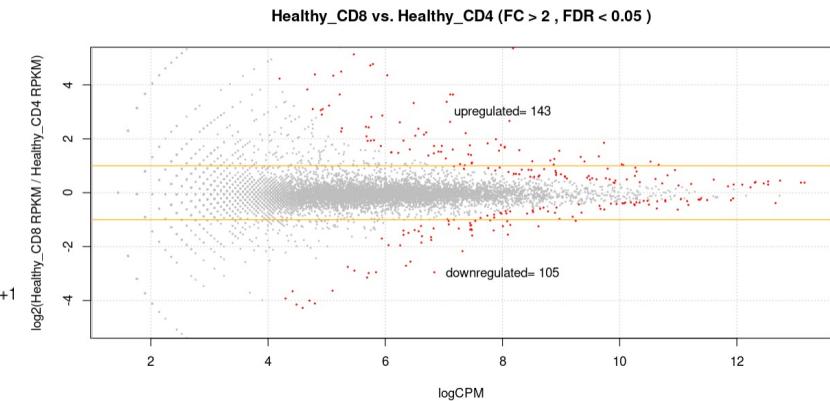
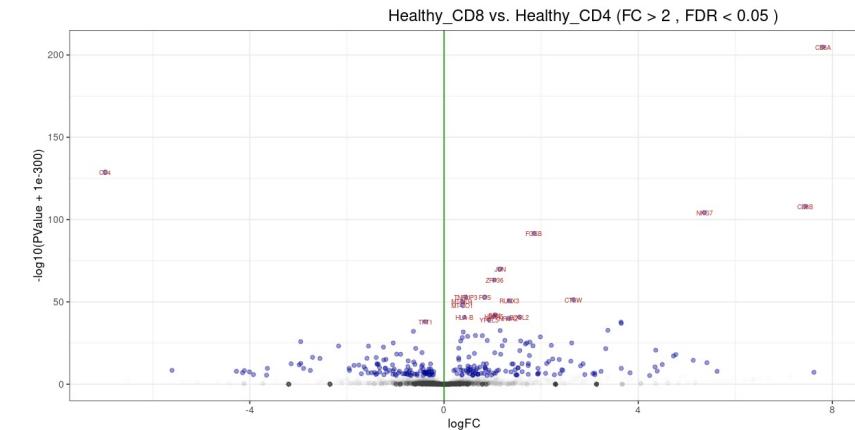
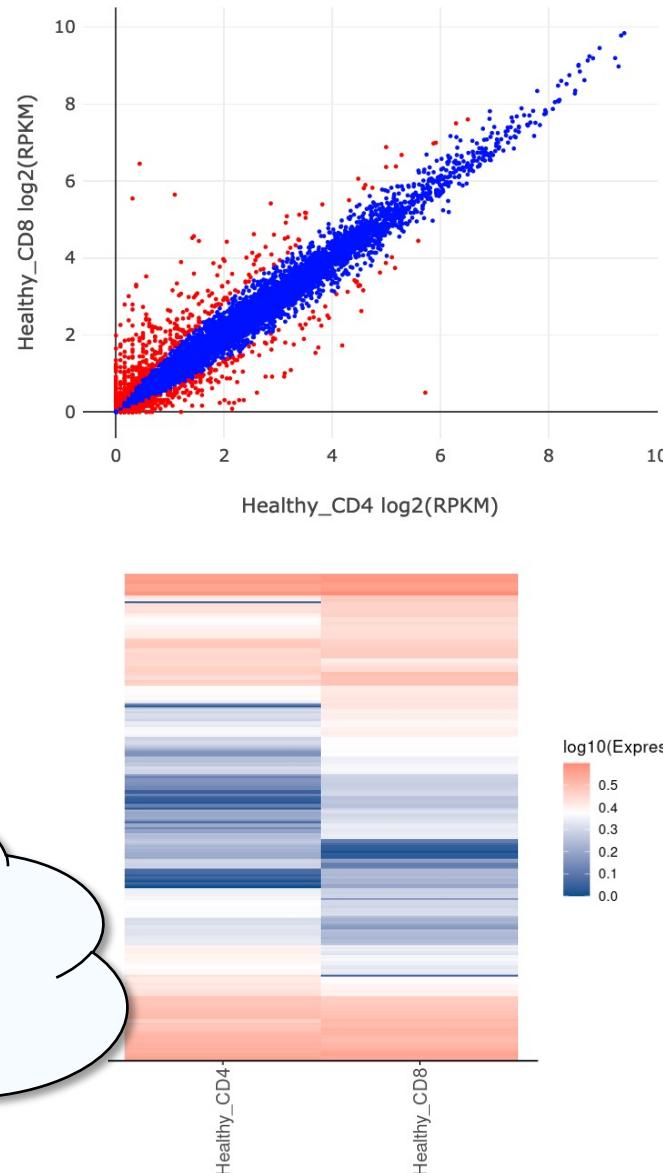
Select Group 2

Healthy_CD8

Compare Groups

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

The screenshot shows the ROGUE software interface with the 'Gene Comparison (Samples)' tab selected. The left sidebar contains navigation links: View ROGUE Manual, View ROGUE Help, ROGUE, Load Count Data:(EdgeR), Load Expression Data, Create Groups, EdgeR Group Comparison, Gene Comparison (Samples) (highlighted with a red arrow), Gene Comparison (Groups) (highlighted with a red arrow), Advanced Analysis, and Download Report.

The main panel has the following sections:

- Select Genes (max:12):** A dropdown menu containing "IL24", "PPIL2", "FADS2", "stat5a", and "STAT5A". A red arrow points to the "STAT5A" entry.
- Paste Gene List (max:12):** A text input field containing "IL21R", "MYC", and "IL15". A red arrow points to the "IL15" entry.
- Select Samples:** A dropdown menu containing "lib229", "lib230", and "lib231". A red arrow points to the "lib231" entry.

Below these sections are various configuration controls:

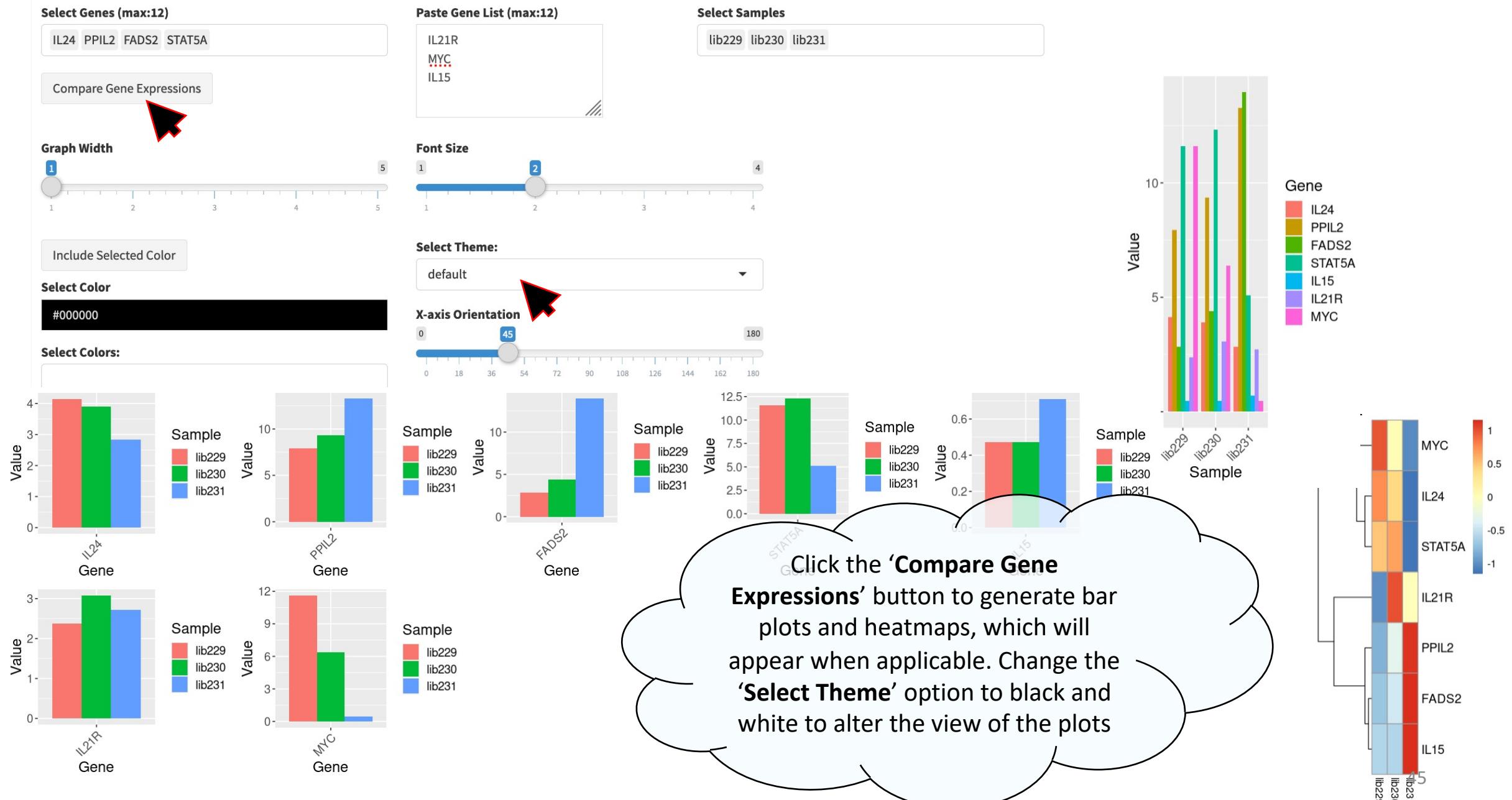
- Graph Width:** A slider set to value 2.
- Font Size:** A slider set to value 2.
- Select Theme:** A dropdown menu set to "default".
- X-axis Orientation:** A slider set to value 45.

A large callout bubble on the right side provides instructions:

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.

Page number 44 is located in the bottom right corner.

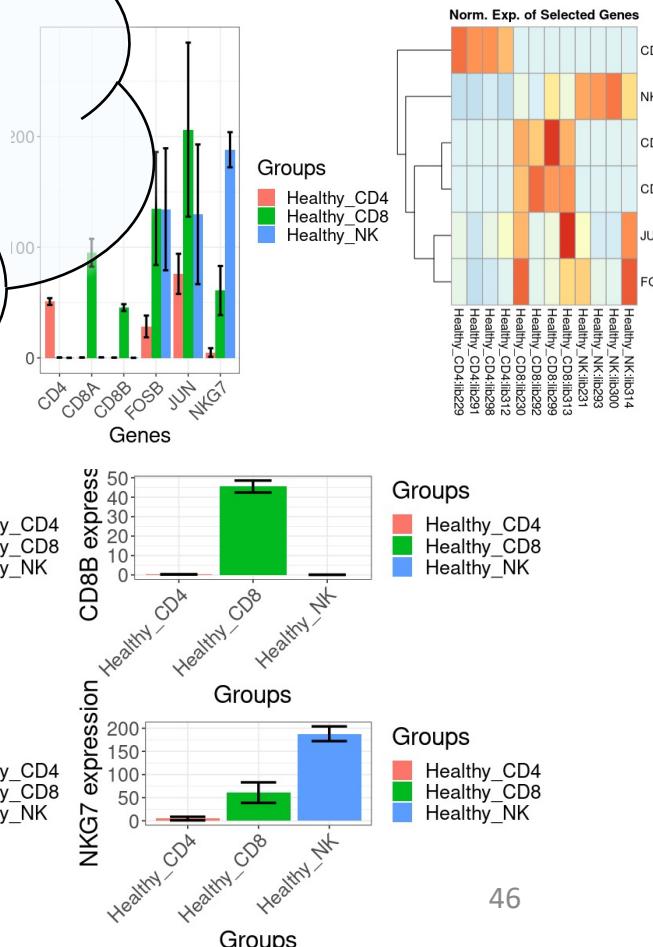
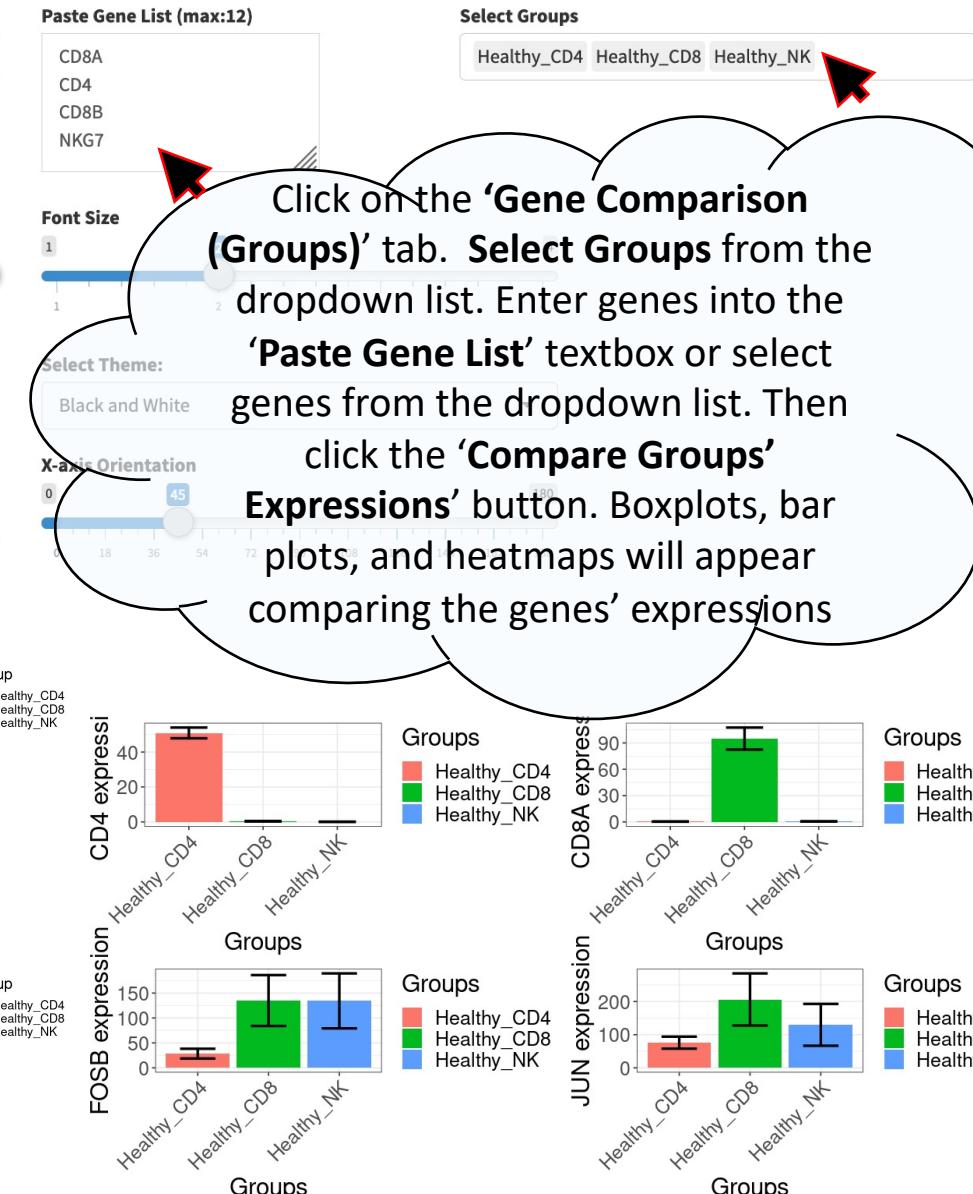
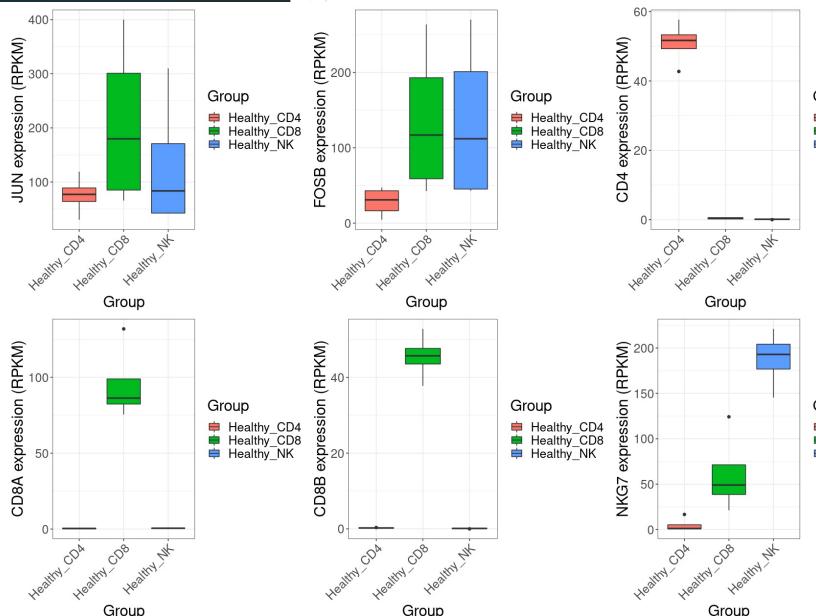
ROGUE – Single Sample Gene Expression Analysis



- [View ROGUE Manual](#)
- [?](#)

- [ROGUE](#)
- [Load Count Data:\(EdgeR\)](#)
- [Load Expression Data](#)
- [Create Groups](#)
- [EdgeR Group Comparison](#)
- [Gene Comparison \(Samples\)](#)
- [Gene Comparison \(Groups\)](#)
- [Advanced Analysis](#)

[Download Report](#)



ROGUE – Gene Set Enrichment Analysis

The screenshot shows the ROGUE software interface. On the left is a dark sidebar with a list of menu items: View ROGUE Manual, ROGUE, Load Count Data:(EdgeR), Load Expression Data, Create Groups, EdgeR Group Comparison, Gene Comparison (Samples), Gene Comparison (Groups), Advanced Analysis, Gene Set Enrichment Analysis (selected), Gene Ontology, Group Statistical Comparison, Group Expr. Ontologies, Differentially Expressed Ontolog, and Download Report. A red arrow points to the 'Advanced Analysis' item. In the main panel, there is a large blue header bar with the ROGUE logo. Below it is a form titled 'Perform Gene Set Enrichment Analysis'. It includes sections for 'Analyze Groups or Samples?' (radio buttons for Groups and Sample, with Sample selected), 'Select Control' (dropdown menu set to lib221), 'Select Subjects' (dropdown menu set to lib355), 'Upregulated Enriched GeneSets' (button set to 10), 'Downregulated Enriched GeneSets' (button set to 10), 'Select GSEA Collection' (dropdown menu with options hallmark gene sets and GO gene sets, with hallmark gene sets selected), 'Find Enriched Gene Signatures' (button), 'Select Gene Set' (dropdown menu), and 'Select Gene Limit' (a slider from 0 to 1 with a value of 0). A red arrow also points to the question mark icon in the top right corner of the main panel.

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 Signatures’ button.

Perform Gene Set Enrichment Analysis

Analyze Groups or Samples?

Groups Sample

Select Control

Healthy_CD4

Select Subjects

Healthy_CD8

Upregulated Enriched GeneSets

10

Downregulated Enriched GeneSets

10

Select GSEA Collection

hallmark gene sets GO gene sets

Find Enriched Gene Signatures

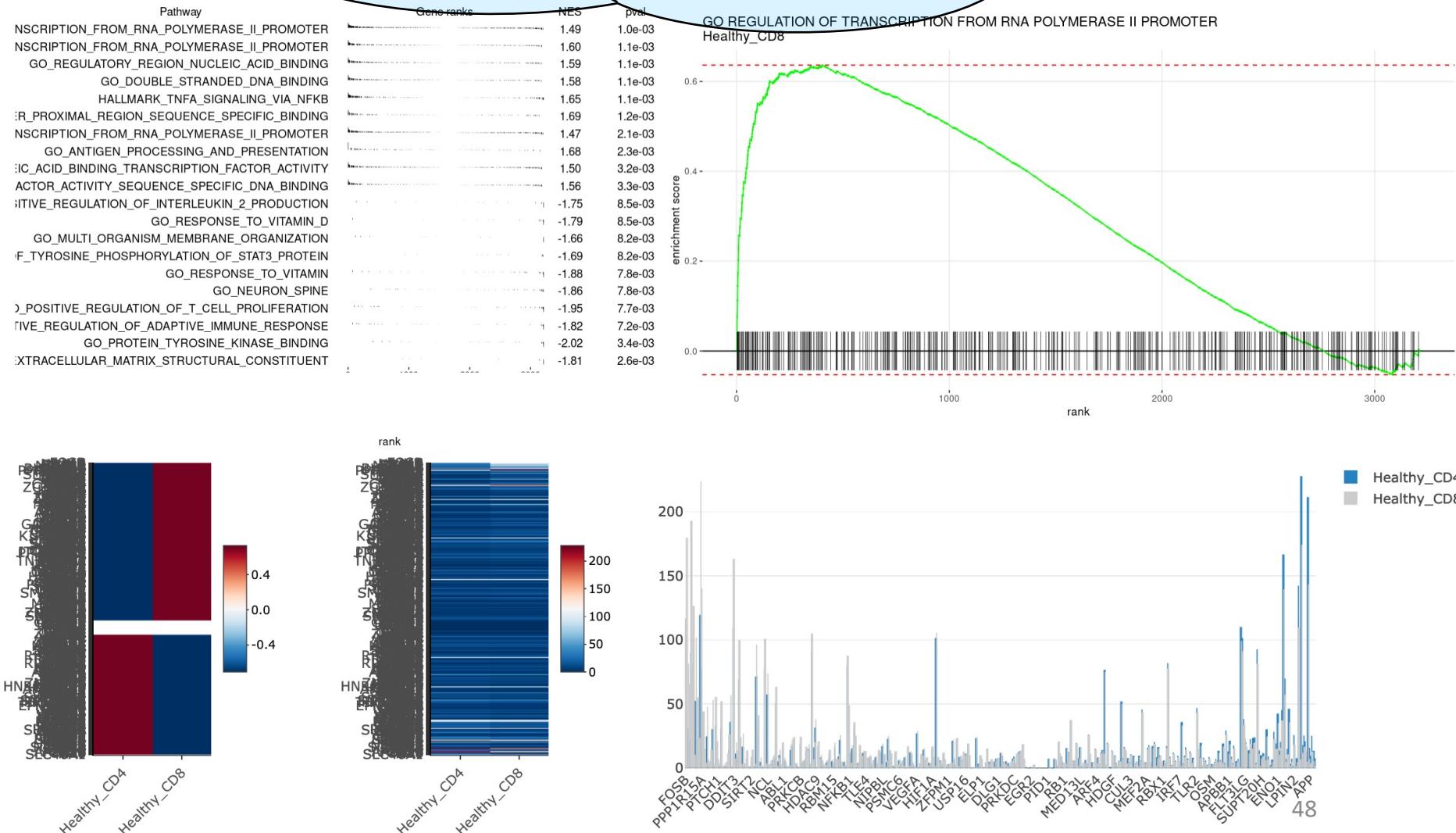
Select Gene Set

GO_REGULATION_OF_TRANSCRIPTION_FROM_RNA_P

Select Gene Limit

2

1,784



ROGUE – Gene Ontology Analysis

The screenshot shows the ROGUE RNAseq+ Ontology Graphic User Environment. The left sidebar contains a navigation menu with items like ROGUE, Load Count Data:(EdgeR), Load Expression Data, Create Groups, EdgeR Group Comparison, Gene Comparison (Samples), Gene Comparison (Groups), Advanced Analysis, Gene Set Enrichment Analysis, Gene Ontology (selected), Group Statistical Comparison, Group Expr. Ontologies, Differentially Expressed Ontologs, and Download Report. The main panel displays the 'Gene Ontology Analysis' page. It includes a 'Select Organism' section with checkboxes for Human (checked) and Mouse (checked). A 'Enter Gene List' text input field contains 'GZMB' and 'ITGAX', with a red arrow pointing to it. Below this is a 'Select Ontology Type' section with radio buttons for molecular_function (unchecked), biological_process (checked), and cellular_component (unchecked). There are also 'P-value' (0.05) and 'Correction' (none) inputs. Under 'Select X-axis Labels', there are radio buttons for GO ID (checked) and GO Name (unchecked). A 'Get Ontologies' button is highlighted with a red arrow. At the bottom, a 'Select Ontology Confidence Code' section lists several options with checkboxes, all of which are checked: 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, and 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 P-val adj

0.05 none▼

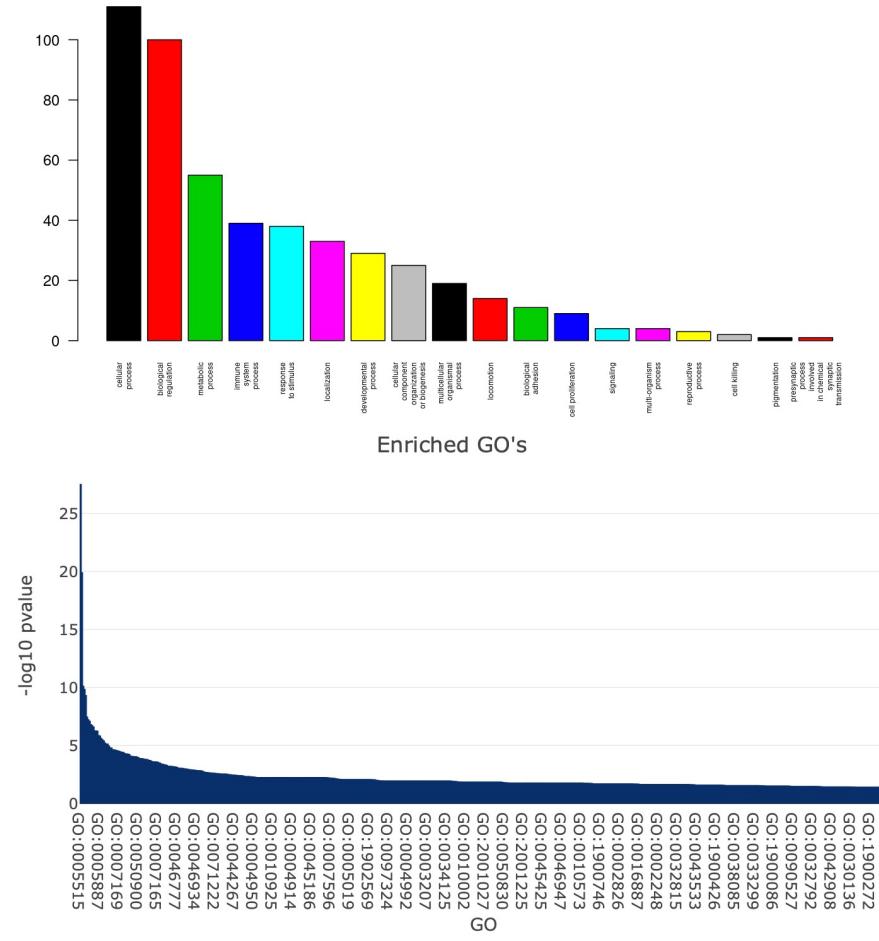
Select X-axis Labels

GO ID GO Name

Get Ontologies

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

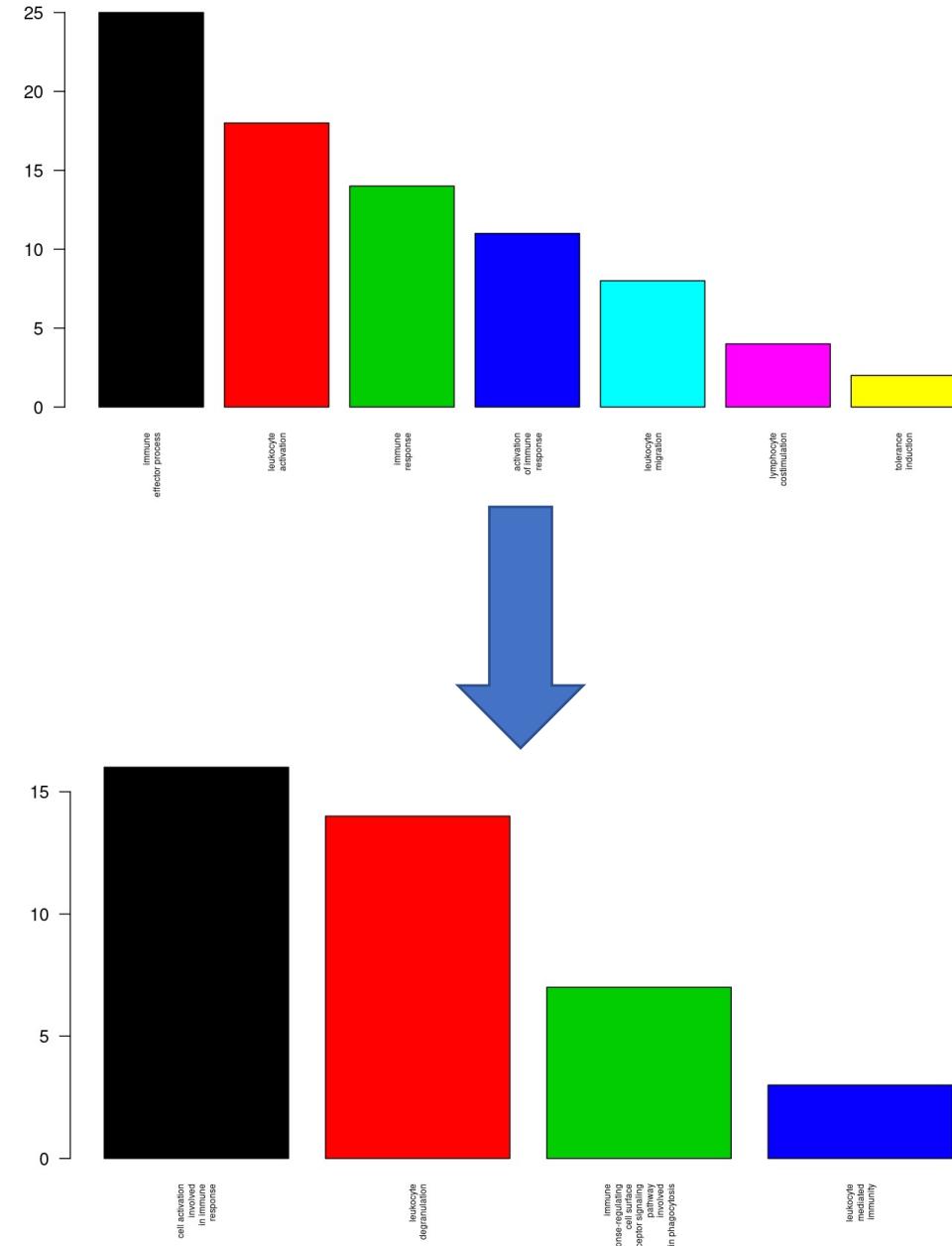
Select GO

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



Go Back

Select Ontologies

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

Select GO

immune system process:Represented Genes

MGAM
TYROBP



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

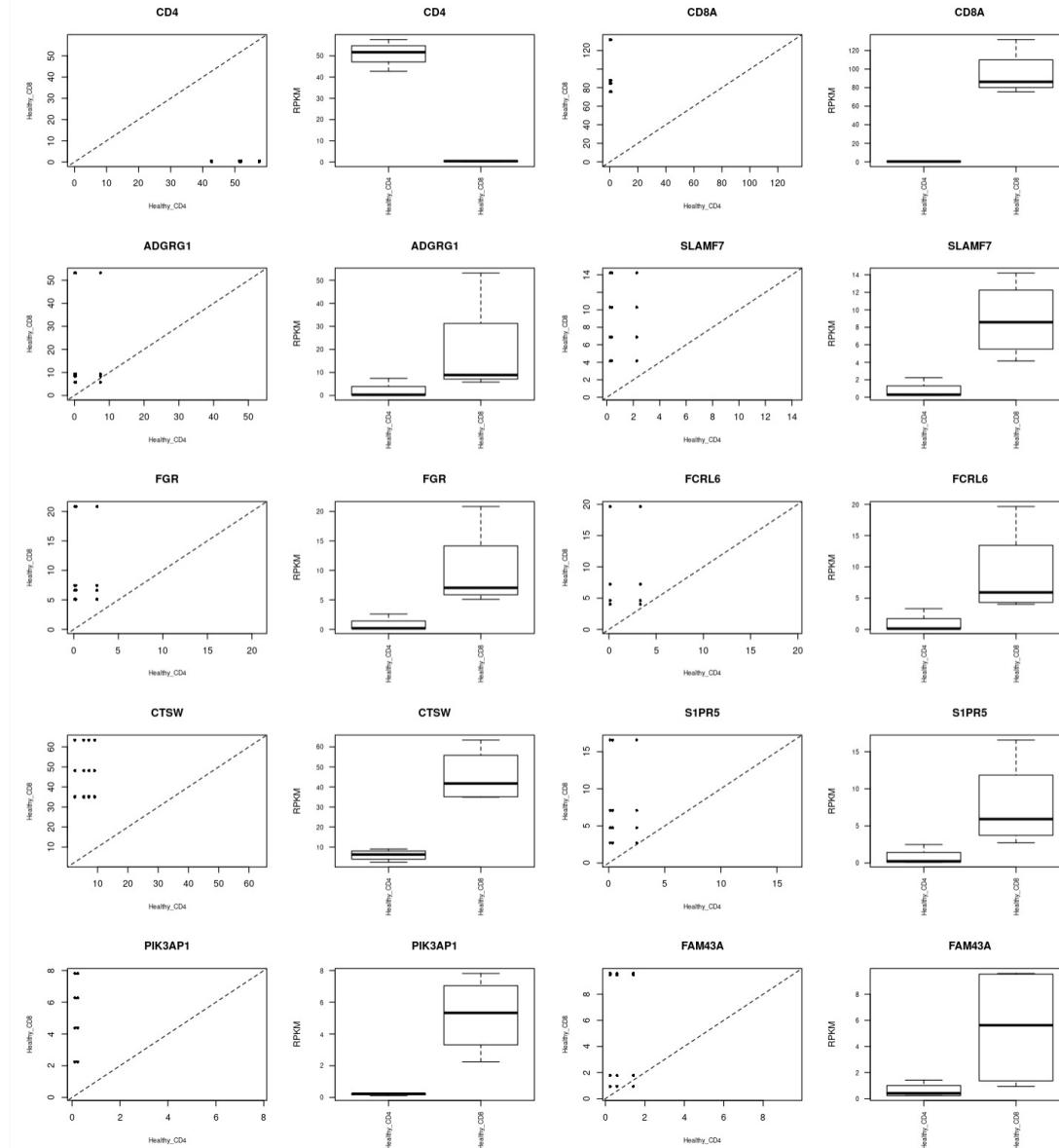
Gene Ontology bar plots appear. Choose ontology subcategories and click 'Select GO' button to explore even deeper subcategories within the selected ontology.

ROGUE – Statistical Group Comparison (Biomarker Discovery)

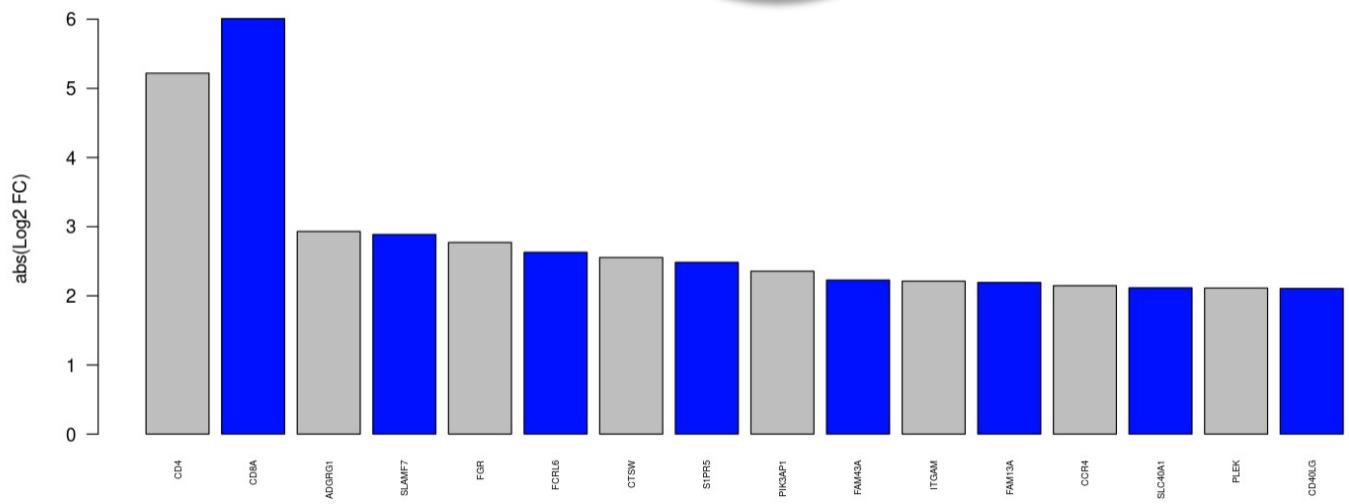
The screenshot shows the ROGUE software interface. On the left, a sidebar lists various analysis options. A red arrow points to the 'Group Statistical Comparison' option. The main panel displays the 'Statistical Biomarker Prediction Tool' for 'Group_Stats_GeneList'. It includes dropdown menus for 'Select Group1' (Healthy_CD4) and 'Select Group2' (Healthy_CD8), a checkbox for 'Fold Change' (checked), a checkbox for 'Mean/SD' (checked), and a 'Find Genes' button. Below these are sliders for 'Select Pvalue' (set to 0.05) and 'Min RPKM' (set to 5). The 'Display Top Genes' field is set to 10, and the 'Show log2FC in gene list?' checkbox is checked. At the top right, tabs for 'Group_Stats_Summary_plots', 'Group_Stats_GeneList', and 't_SNE' are visible.

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 be changed. All data and plots can be downloaded to a pdf file.



[Download All Data](#)

ROGUE – Statistical Group Comparison (Biomarker Discovery)

Select Group1

Healthy_CD4

Select Group2

Healthy_CD8

Select Tests

Fold Change

Mean/SD

Wilcox

Ttest

Find Genes

Select Pvalue

0 1

Min RPKM

5

Min log2FC

2

Display Top Genes

10

Show log2FC in gene list?

[Group_Stats_Summary_plots](#)

[Group_Stats_GeneList](#)

[t_SNE](#)

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

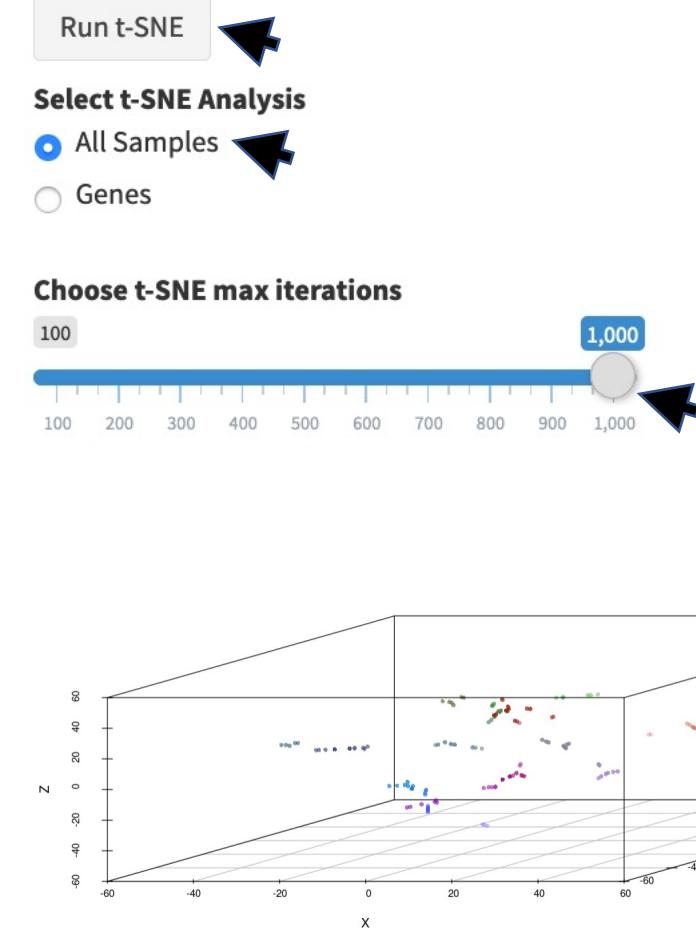
Click on
'Group_Stats_GeneList' tab.
A list of genes and the log2 fold change will appear in a textbox ranked from highest to lowest fitting the parameters set in the left panel.

ROGUE – tSNE

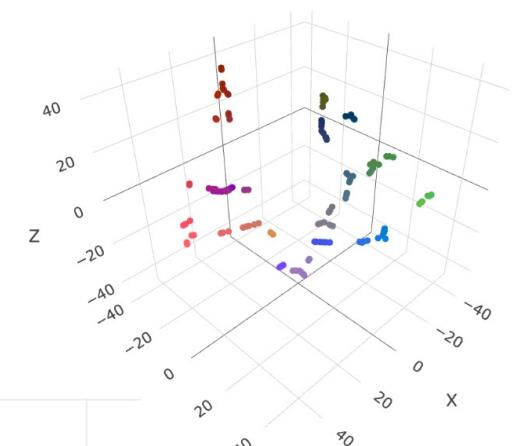
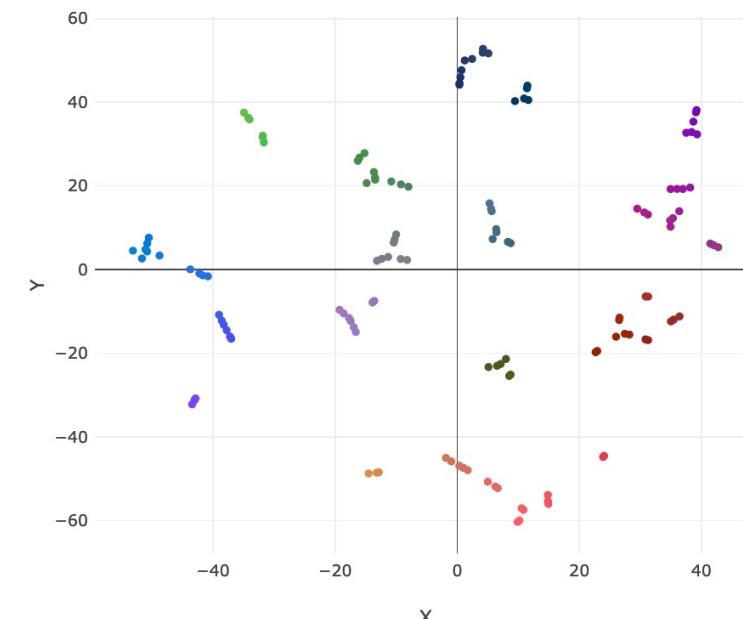
The screenshot shows the ROGUE interface with the t-SNE tool tab selected. The left sidebar contains various analysis options like ROGUE, Load Count Data, Create Groups, etc. The main panel has sections for Select Group1 (Healthy_CD4), Select Group2 (Healthy_CD8), Select Tests (Fold Change, Mean/SD checked; Wilcox, Ttest unchecked), Find Genes, Select Pvalue (0.05), Min RPKM (5), Min log2FC (2), Display Top Genes (10), and Show log2FC in gene list? (checked). The t-SNE tool section includes tabs for Group_Stats_Summary_plots, Group_Stats_GeneList, and t_SNE (highlighted with a red arrow). It features a Run t-SNE button, Select t-SNE Analysis (All Samples selected, Genes unselected), Choose t-SNE max iterations (set to 1,000), Choose point size (set to 3), Set Seed (4000), and a checkbox for Generate distance p-value lists. Below these are optional fields for Enter Gene List and Highlight Gene/Group.

Click on 't-SNE' tab

ROGUE – tSNE



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.

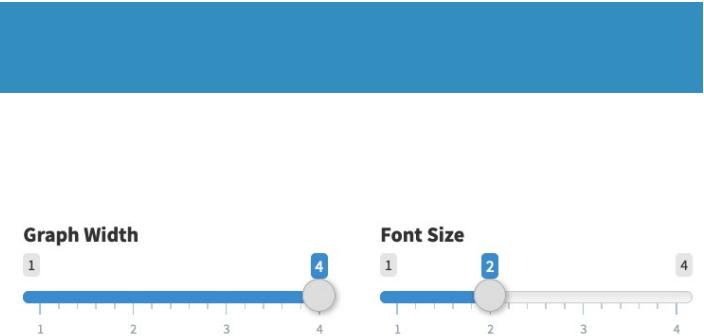


ROGUE – Gene Ontology Expression Comparison

The screenshot shows the ROGUE software interface with the 'Expressed Ontologies Tool' tab selected. On the left, a sidebar lists various analysis options. The main panel contains the following configuration fields:

- Analyze Groups or Samples?**: Radio buttons for 'Groups' (selected) and 'Samples'.
- Select Control**: A dropdown menu showing 'Healthy_CD4'.
- Select Subjects**: A dropdown menu showing 'Healthy_CD8' and 'Healthy_NK'.
- Select GO Class**: A dropdown menu showing 'immune system process'.
- Select GO**: An empty input field.
- Select GO Class**: A dropdown menu showing 'molecular_function'.
- Combine GOs with - Optional:(Push GO Class Button)**: An empty input field.
- Get GO Gene's Fold Change**: A button at the bottom.

Red arrows point to the 'Groups' radio button, the 'Healthy_CD4' control, the 'Healthy_CD8' and 'Healthy_NK' subjects, and the 'immune system process' GO class.



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

innate immune response

innate immunity memory response

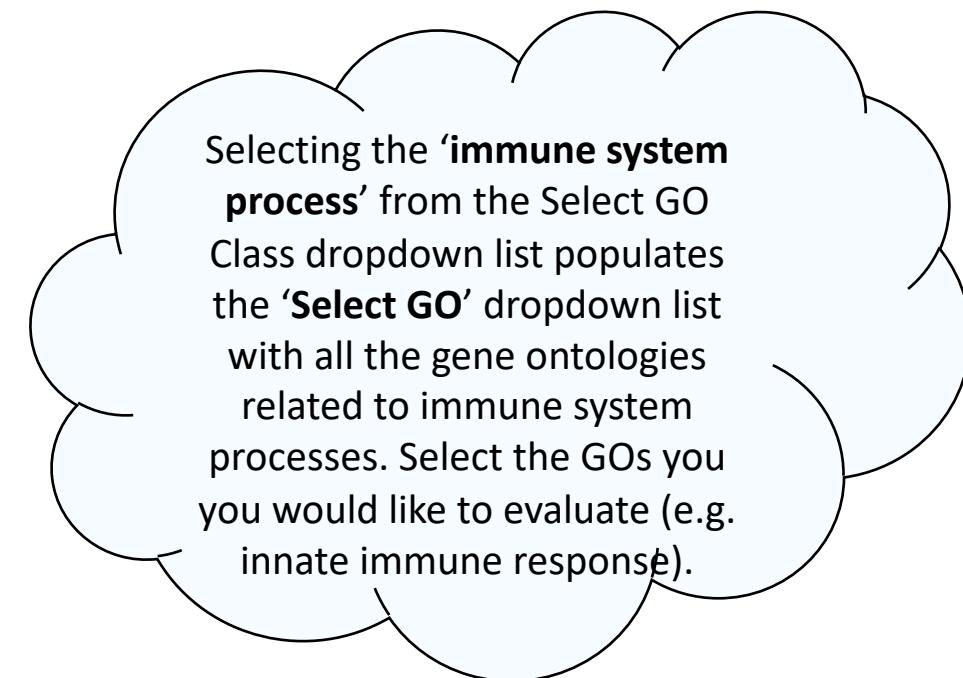
innate immune response in mucosa

innate immune response-activating signal transduction

innate immune response activating cell surface receptor signaling pathway

antifungal innate immune response

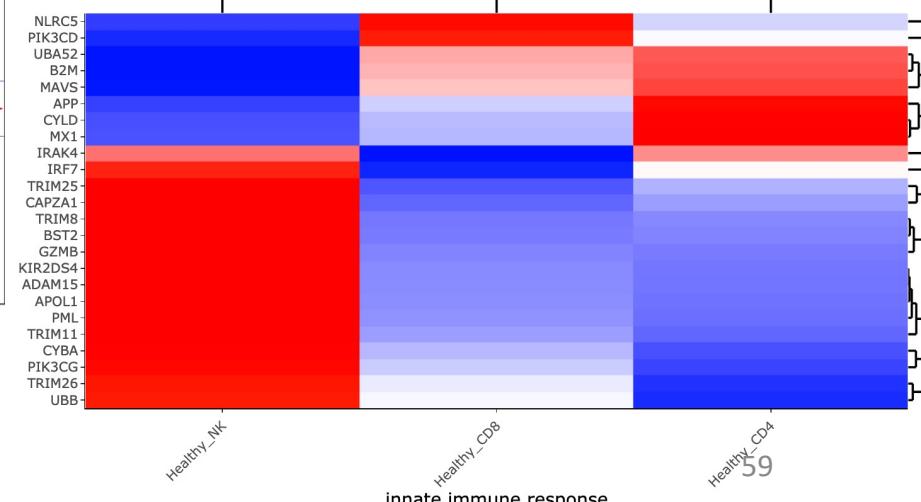
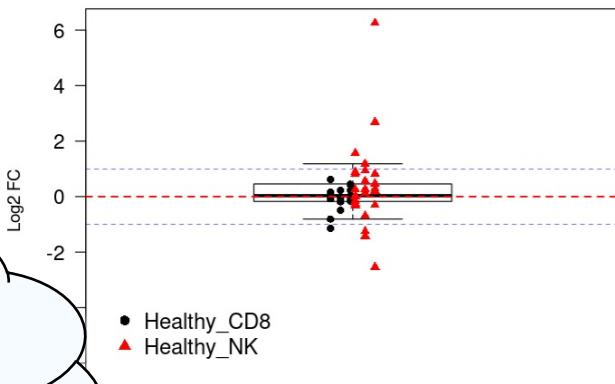
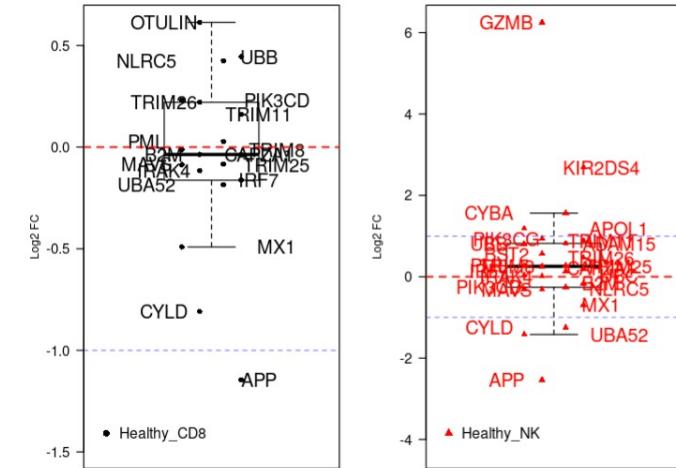
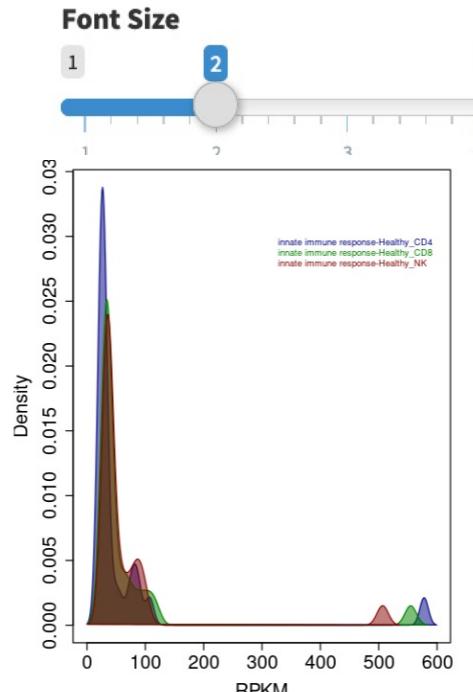
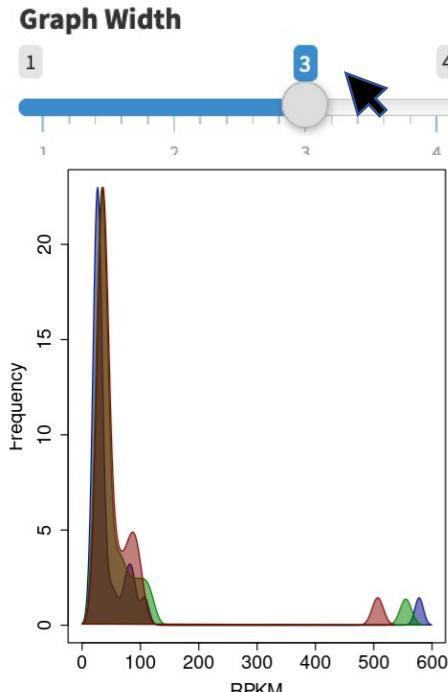
activation of innate immune response



ROGUE – Gene Ontology Expression Comparison

? Expressed Ontologies Tool

Analyze Groups or Samples?

 Groups Samples

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

ROGUE
RNAseq + Ontology Graphic User Environment

[View ROGUE Manual](#) [?](#)

- [ROGUE](#)
- [Load Count Data:\(EdgeR\)](#)
- [Load Expression Data](#)
- [Create Groups](#)
- [EdgeR Group Comparison](#)
- [Gene Comparison \(Samples\)](#)
- [Gene Comparison \(Groups\)](#)
- [Advanced Analysis](#)
 - [Gene Set Enrichment Analysis](#)
 - [Gene Ontology](#)
 - [Group Statistical Comparison](#)
 - [Group Expr. Ontologies](#)
- [Differentially Expressed Ontolog](#)
- [Download Report](#)

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 Keywords

Find Differentially Expressed GOs

Label absFC Threshold

0

Min RPKM base

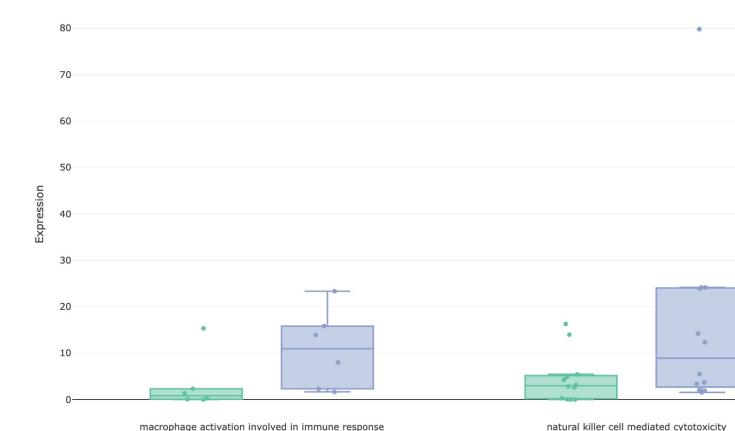
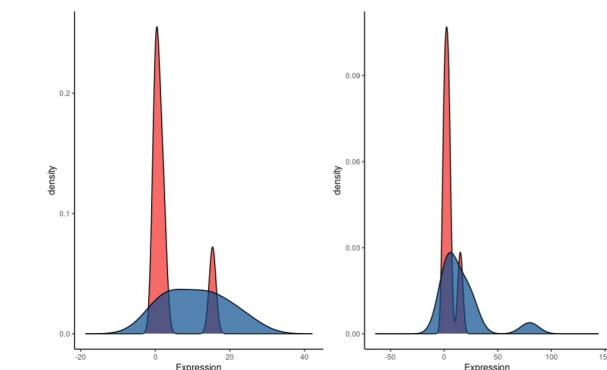
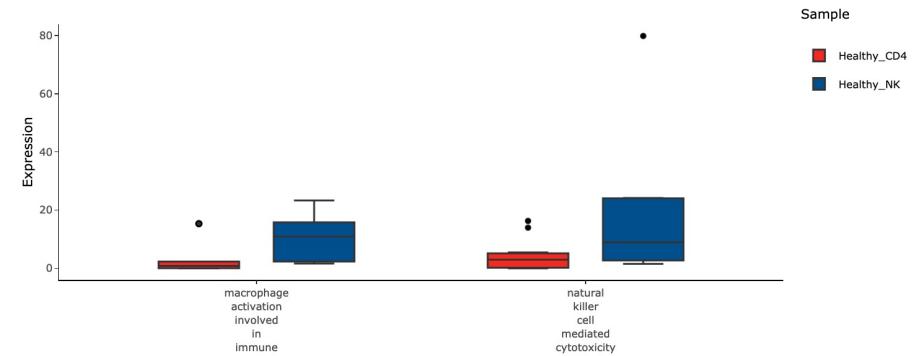
3

Min log2 Fold Change

0.7

Max p-value

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

Sample

- Healthy_CD4
- Healthy_NK

ROGUE
RNAseq & Ontology Graphic User Environment

[View ROGUE Manual](#) [?](#)

[ROGUE](#)

[Load Count Data:\(EdgeR\)](#)

[Load Expression Data](#)

[Create Groups](#)

[EdgeR Group Comparison](#)

[Gene Comparison \(Samples\)](#)

[Gene Comparison \(Groups\)](#)

[Advanced Analysis](#)

[Gene Set Enrichment Analysis](#)

[Gene Ontology](#)

[Group Statistical Comparison](#)

[Group Expr. Ontologies](#)

[Differentially Expressed Ontolog](#) [?](#)

Differentially Expressed Ontologies Tool

Analyze Groups or Samples?
 Groups Samples

Select Control
Healthy_CD4 [?](#)

Select Subjects
Healthy_NK [?](#)

Select GO Class
biological regulation [?](#)

Choose Keywords

Find Differentially Expressed GOs [?](#)

Label absFC Threshold
0

Perform analysis
searching for
differences in
biological regulation

