



Research & Innovation – Nevada Bioinformatics Center

Tutorial: Differential expression analysis using GEO2R

Database URL: <https://www.ncbi.nlm.nih.gov/geo>

Example dataset: GSE48350 (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE48350>)

The URL for analysis will be: <https://www.ncbi.nlm.nih.gov/geo/geo2r/?acc=GSE48350>

The interface:

GEO accession Set Alzheimer's Disease Dataset The title of the dataset

▼ Samples Define groups Create groups for analysis Selected 0 out of 253 samples

Sample ID Columns Set

Group	Accession	Title	Source name	Individual	Brain region	Gender	Characteristics	Bra
-	GSM300166	PostcentralGyrus_female_91yrs_indiv10	brain, postcentral gyrus, female, 91 years	10, C	postcentral gyrus	female	age (yrs): 91	
-	GSM300167	SuperiorFrontalGyrus_female_91yrs_indiv10	brain, superior frontal gyrus, female, 91 years	10, C	superior frontal gyrus	female	age (yrs): 91	
-	GSM300168	Hippocampus_female_96yrs_indiv105	brain, hippocampus, female, 96 years	105, C	hippocampus	female	age (yrs): 96	
-	GSM300169	Hippocampus_male_82yrs_indiv106	brain, hippocampus, male, 82 years	106, C	hippocampus	male	age (yrs): 82	
-	GSM300170	Hippocampus_male_84yrs_indiv108	brain, hippocampus, male, 84 years	108, C	hippocampus	male	age (yrs): 84	
-	GSM300171	Hippocampus_female_87yrs_indiv109	brain, hippocampus, female, 87 years	111, C	hippocampus	female	age (yrs): 87	
-	GSM300172	Hippocampus_female_82yrs_indiv111	brain, hippocampus, female, 82 years	111, C	hippocampus	female	age (yrs): 82	
-	GSM300173	EntorhinalCortex_male_45yrs_indiv12	brain, entorhinal cortex, male, 45 years	12, AA	entorhinal cortex	male	age (yrs): 45	
-	GSM300174	Hippocampus_male_45yrs_indiv12	brain, hippocampus, male, 45 years	12, AA	hippocampus	male	age (yrs): 45	
-	GSM300175	PostcentralGyrus_male_45yrs_indiv12	brain, postcentral gyrus, male, 45 years	12, AA	postcentral gyrus	male	age (yrs): 45	
-	GSM300176	SuperiorFrontalGyrus_male_45yrs_indiv12	brain, superior frontal gyrus, male, 45 years	12, AA	superior frontal gyrus	male	age (yrs): 45	
-	GSM300177	EntorhinalCortex_male_95yrs_indiv14	brain, entorhinal cortex, male, 95 years	14, C	entorhinal cortex	male	age (yrs): 95	
-	GSM300178	Hippocampus_male_95yrs_indiv14	brain, hippocampus, male, 95 years	14, C	hippocampus	male	age (yrs): 95	
-	GSM300179	PostcentralGyrus_male_95yrs_indiv14	brain, postcentral gyrus, male, 95 years	14, C	postcentral gyrus	male	age (yrs): 95	
-	GSM300180	SuperiorFrontalGyrus_male_95yrs_indiv14	brain, superior frontal gyrus, male, 95 years	14, C	superior frontal gyrus	male	age (yrs): 95	

Step 1: Create groups for the analysis

Let's create two groups: "condition" and "control"

▼ Samples Define groups Selected 0 out of 253 samples

Enter a group name: List

Cancel selection

☒ condition ☐ control

Group	Accession	Title	Source name	Individual	Brain region	Gender	Characteristics	Bra
-	GSM300166	PostcentralGyrus_female_91yrs_indiv10	brain, postcentral gyrus, female, 91 years	10, C	postcentral gyrus	female	age (yrs): 91	
-	GSM300167	SuperiorFrontalGyrus_female_91yrs_indiv10	brain, superior frontal gyrus, female, 91 years	10, C	superior frontal gyrus	female	age (yrs): 91	
-	GSM300168	Hippocampus_female_96yrs_indiv105	brain, hippocampus, female, 96 years	105, C	hippocampus	female	age (yrs): 96	

Step 2: Select samples for each group

In this tutorial, let's only consider samples from the Entorhinal Cortex region.

Selected 0 out of 253 samples

Click to sort

Group	Accession	Title	Source name	Individual	Brain region	Gender	Characteristics
-	GSM300173	EntorhinalC	brain, entorhinal cortex, male, 45 years	12, AA	entorhinal cortex	male	age (yrs): 45
-	GSM300177	EntorhinalC	brain, entorhinal cortex, male, 95 years	14, C	entorhinal cortex	male	age (yrs): 95
-	GSM300181	EntorhinalCortex_male_80yrs_indiv15	brain, entorhinal cortex, male, 80 years	15, C	entorhinal cortex	male	age (yrs): 80
-	GSM300186	EntorhinalCortex_female_45yrs_indiv17	brain, entorhinal cortex, female, 45 years	17, C	entorhinal cortex	female	age (yrs): 45

Find the sample with the suffix “AD” and add them to the “condition” group. To select multiple samples next to each other, select the first sample, hold the shift button, and select the last sample. After the samples are selected, click on the group “condition” to add all selected samples to this group.

Selected 15 out of 253 samples

Group	Accession	Title	Source name	Individual	Brain region	Gender	Characteristics
-	GSM300338	Entorhinal	brain, entorhinal cortex, female, 82 years	98, C	entorhinal cortex	female	age (yrs): 82
condition	GSM1176196	entorhinal	entorhinal cortex_female_AD		entorhinal cortex	female	age (yrs): 76
condition	GSM1176197	entorhinal	entorhinal cortex_female_AD		entorhinal cortex	female	age (yrs): 86
condition	GSM1176198	entorhinal	entorhinal cortex_female_AD		entorhinal cortex	female	age (yrs): 82
condition	GSM1176199	entorhinal cortex_female_85_AD_24	entorhinal cortex_female_AD		entorhinal cortex	female	age (yrs): 85
condition	GSM1176200	entorhinal cortex_female_90_AD_36	entorhinal cortex_female_AD		entorhinal cortex	female	age (yrs): 90
condition	GSM1176201	entorhinal cortex_female_90_AD_6	entorhinal cortex_female_AD		entorhinal cortex	female	age (yrs): 90
condition	GSM1176202	entorhinal cortex_female_90_AD_75	entorhinal cortex_female_AD		entorhinal cortex	female	age (yrs): 90
condition	GSM1176203	entorhinal cortex_female_91_AD_11	entorhinal cortex_female_AD		entorhinal cortex	female	age (yrs): 91
condition	GSM1176204	entorhinal cortex_male_76_AD_69	entorhinal cortex_male_AD		entorhinal cortex	male	age (yrs): 76

Next, select the remaining samples of this brain region for the “control” group.

Selected 54 out of 253 samples

Group	Accession	Title	Source name	Individual	Brain region	Gender	Characteristics
control	GSM300173	Entorhinal	brain, entorhinal cortex, male, 45 years	12, AA	entorhinal cortex	male	age (yrs): 45
control	GSM300177	Entorhinal	brain, entorhinal cortex, male, 95 years	14, C	entorhinal cortex	male	age (yrs): 95
control	GSM300181	EntorhinalCortex_male_80yrs_indiv15	brain, entorhinal cortex, male, 80 years	15, C	entorhinal cortex	male	age (yrs): 80
control	GSM300186	EntorhinalCortex_female_45yrs_indiv17	brain, entorhinal cortex, female, 45 years	17, C	entorhinal cortex	female	age (yrs): 45
control	GSM300189	EntorhinalCortex_female_74yrs_indiv18	brain, entorhinal cortex, female, 74 years	18, C	entorhinal cortex	female	age (yrs): 74
control	GSM300192	EntorhinalCortex_female_99yrs_indiv2	brain, entorhinal cortex, female, 99 years	2, C	entorhinal cortex	female	age (yrs): 99
control	GSM300196	EntorhinalCortex_female_74yrs_indiv21	brain, entorhinal cortex, female, 74 years	21, C	entorhinal cortex	female	age (yrs): 74
control	GSM300204	EntorhinalCortex_male_83yrs_indiv28	brain, entorhinal cortex, male, 83 years	28, C	entorhinal cortex	male	age (yrs): 83

Step 3: Select options:

GEO2R

Options

Profile graph

R script

Apply adjustment to the P-values. [More...](#)
☒ Benjamini & Hochberg (False discovery rate)
☐ Benjamini & Yekutieli
☐ Bonferroni
☐ Hochberg
☐ Holm
☐ Hommel
☐ None

Apply log transformation to the data. [More...](#)
☒ Auto-detect
☐ Yes
☐ No

Apply limma precision weights (vooma). [More...](#)
☐ Yes
☒ No

Force normalization. [More...](#)
☐ Yes
☒ No

Category of Platform annotation to display on results.
☐ Submitter supplied
☒ NCBI generated

Plot displays. [More...](#)
Significance level cut-off
(enter number between 0 and 1)
Volcano and MA plot contrasts (select up to 5)
0 selected ([clear](#))
☐ condition vs control

Step 4: Start the analysis:

GEO2R

Options

Profile graph

R script

Quick start

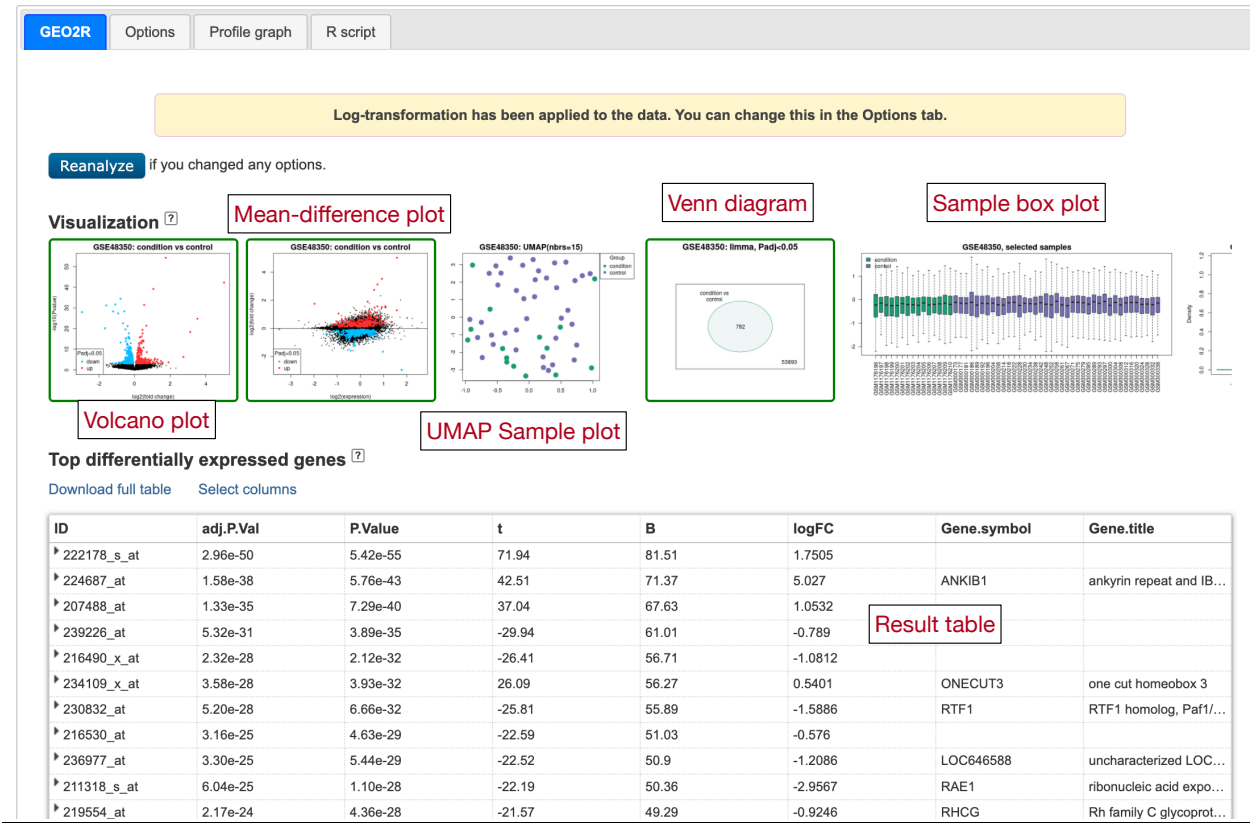
- Specify a GEO Series accession and a Platform if prompted.
- Click 'Define groups' and enter names for the groups of Samples you plan to compare, e.g., test and control.
- Assign Samples to each group. Highlight Sample rows then click the group name to assign those Samples to the group. Use the Sample metadata (title, source and characteristics) columns to help determine which Samples belong to which group.
- Click 'Analyze' to perform the calculation with default settings.
- You may change settings in the Options tab.

How to use

Analyze

Click the analyze button. This process can take from 3-10 minutes depending on how many samples are selected.

Step 4: Download the results



Visualization ?

Mean-difference plot

Venn diagram

Sample box plot

Volcano plot

UMAP Sample plot

Top differentially expressed genes ?

Result table