

NGSEA website tutorial

1. Analysis: Expression data tab: analysis using an expression matrix

Please submit input data:

Input gene expression table (Format)	Choose File	No file chosen	1
Input cls file (Format)	Choose File	No file chosen	2
Species	Human	▼	3
Gene Set	KEGG pathway	▼	4
Network	HumanNet v2	▼	5
Email (optional)	<input type="text"/>		6

NGSEA process will cost 1~5 minutes, depending on the size of the gene set of your choice.
We'll send you the link of result page to your email after the process is finished.

7

- 1 Upload a gene expression table file.

Input format: Gene Cluster Text file format (*.gct)

Details for the .gct format can be found in the following page:

[http://software.broadinstitute.org/cancer/software/gsea/wiki/index.php/Data_formats#GCT: Gene Cluster Text file format .28.2A.gct.29](http://software.broadinstitute.org/cancer/software/gsea/wiki/index.php/Data_formats#GCT:_Gene_Cluster_Text_file_format_.28.2A.gct.29)

Gene identifiers should be either ENTREZ gene IDs or NCBI official gene symbols.

Values should be raw expression values. (Log-transformed values are not allowed)

- 2 Upload a phenotype data file.

Input format: Categorical class file format (*.cls)

Details for the .cls format can be found in the following page:

[http://software.broadinstitute.org/cancer/software/gsea/wiki/index.php/Data_formats#CLS: Categorical .28e.g_tumor_vs_normal.29_class_file_format .28.2A.cls.29](http://software.broadinstitute.org/cancer/software/gsea/wiki/index.php/Data_formats#CLS:_Categorical_.28e.g_tumor_vs_normal.29_class_file_format_.28.2A.cls.29)

- 3 Select the species of your interest.

- 4 Select the gene set of your interest.

Available genesets: KEGG pathway, Gene Ontology biological pathways, DSigDB FDA-approved drugs (human only), DisGeNET curated annotation of disease genes and DISEASES annotation of disease genes with more than 3-star scores.

- 5 Select the network of your interest.

Available networks: HumanNet v2 (human only), MouseNet v2 (mouse only)

- 6 Insert your email address.

We'll send you an email when the analysis is finished.

- 7 Click 'Run NGSEA' button.

If no problems are found in the uploaded files, the website will provide you a successful upload message along with an analysis code.



The analysis will take for 1~5 minutes, depending on the size of uploaded files and the size of the selected gene set.

2. Analysis: preranked list tab: analysis using pre-calculated values (e.g. log2 fold change)

Please submit input data:

Input preranked table (Format)	Choose File	No file chosen	1
Species	Human		2
Gene Set	KEGG pathway		3
Network	HumanNet v2		4
Email (optional)			5

NGSEA process will cost 1~5 minutes, depending on the size of the gene set of your choice. We'll send you the link of result page to your email after the process is finished.

6 Run NGSEA RESET

- 1 Upload a gene list file.

Input format: Gene Cluster Text file format (*.rnk); [gene ID] [tab] [value]

Gene identifiers should be either ENTREZ gene IDs or NCBI official gene symbols.

Log transformed fold changes between phenotype classes are recommended as the values.

Details for the .rnk format can be found in the following page:

[http://software.broadinstitute.org/cancer/software/gsea/wiki/index.php/Data_formats#RNK: Ranked_list_file_format_.28.2A.rnk.29](http://software.broadinstitute.org/cancer/software/gsea/wiki/index.php/Data_formats#RNK:_Ranked_list_file_format_.28.2A.rnk.29)

- 2 Select the species of your interest.
- 3 Select the gene set of your interest.
- 4 Available genesets: KEGG pathway, Gene Ontology biological pathways, DSigDB FDA-approved drugs (human only), DisGeNET curated annotation of disease genes and DISEASES annotation of

disease genes with more than 3-star scores.

- ⑤ Select the network of your interest.

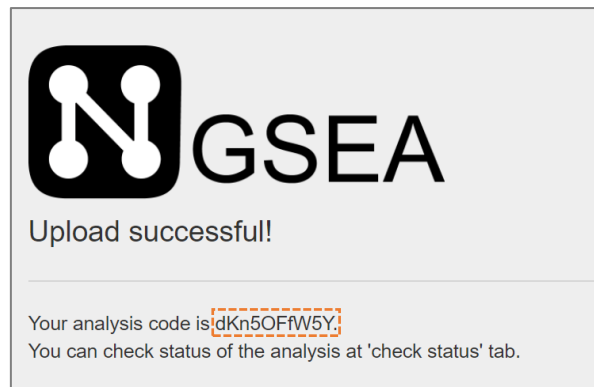
Available networks: HumanNet v2 (human only), MouseNet v2 (mouse only)

- ⑥ Insert your email address.

We'll send you an email when the analysis is finished.

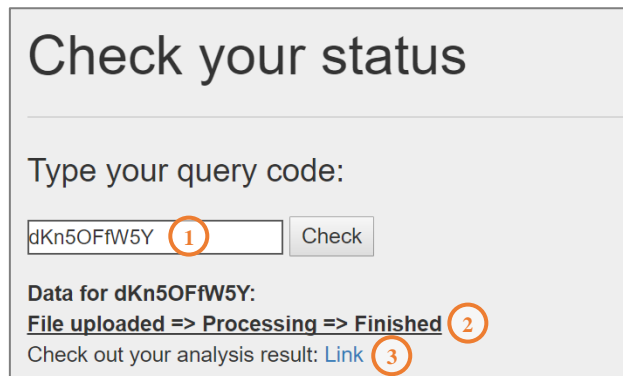
- ⑦ Click 'Run NGSEA' button.

If no problems are found in the uploaded files, the website will provide you a successful upload message along with an analysis code.



The analysis will take for 1~5 minutes, depending on the size of uploaded files and the size of the selected gene set.

3. Check status tab



- ① To check the status of your analysis, insert the code of your analysis and click 'Check' button.
- ② The status can be 'File uploaded', 'Processing' or 'Finished'.
- ③ When the analysis is finished, a link for the result page will appear.

4. Results

Show entries Search:

Gene set	NGSEA result					GSEA result				
	ES	NES	pvalue	FDR	Enrichment plot	ES	NES	pvalue	FDR	Enrichment plot
1 Proteasome	0.673	2.152	0	0	link	-0.724	-2.165	0	0	link
2 Citrate cycle (TCA cycle)	0.676	2.051	0	0	link	-0.717	-2.016	0	0.00028	link
3 Protein export	0.668	1.944	0	0.00063	link	-0.719	-1.941	0	0.00034	link
4 Oxidative phosphorylation	0.551	1.920	0	0.00047	link	-0.593	-1.928	0	0.00036	link
5 Alzheimer's disease	0.500	1.791	0	0.0047	link	-0.543	-1.798	0	0.0026	link
6 Parkinson's disease	0.503	1.766	0	0.006	link	-0.583	-1.911	0	0.00053	link
7 Synaptic vesicle cycle	0.527	1.751	0	0.0064	link	-0.538	-1.650	0	0.014	link
8 RNA transport	0.480	1.707	0	0.011	link	-0.593	-1.970	0	0.00042	link
9 Nucleotide excision repair	0.526	1.682	0	0.014	link	-0.588	-1.740	0	0.0055	link
10 2-Oxocarboxylic acid metabolism	0.598	1.678	0.0041	0.014	link	-0.672	-1.731	0.0037	0.0051	link

Showing 1 to 10 of 273 entries Previous **1** 2 3 4 5 ... 28 Next

- Result columns include gene sets, NGSEA result and GSEA result. Users can sort the result by clicking headers of columns. We suggest users to sort the results by NES values of NGSEA result and compare them with GSEA results.
- By clicking 'link' button in the result, users can check an enrichment plot of each gene set. The enrichment plots are provided only for top/bottom 30 gene sets in each result. Users can observe the ranks and the enrichment pattern of genes in the gene set using the enrichment plots.

