

# GSEA

GSEA is a bioinformatics tool that determines whether a set of genes (e.g. a gene ontology (GO) group or a pathway) shows statistically significant, concordant differences between two experimental groups (1,2). Briefly, the goal of GSEA is to determine whether the genes belonging to a gene set are randomly distributed throughout the ranked (by expression) list of all the genes that should be taken into consideration (e.g. gene model), or are primarily found at the top or at the bottom of the list.

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## Prerequisites

To run GSEA, your project has to contain at least one categorical factor with exactly two levels (e.g. Treated and Control). If you are running GSEA on RNA-seq data, note that some common normalisation transformations, such as fragments/reads per kilobase of transcript per million mapped reads (FPKM /RPKM) or transcripts per million (TPM) are not considered suitable for GSEA (for more information, please see [GSEA documentation](#)). Instead, you should use an approach such as DESeq2 normalisation, trimmed means of M (TMM), or geometric mean.

## Running GSEA

To launch GSEA, select the data node with normalised data and then go to **Biological interpretation > GSEA** (Figure 1).

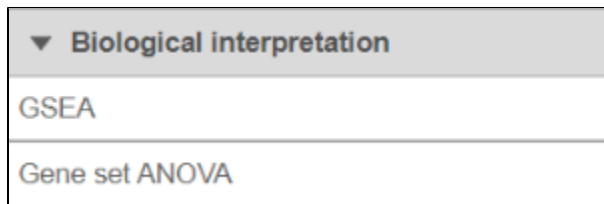


Figure 13. Gene Set Enrichment Analysis task in the toolbox

Use the first dialog (Figure 2) to specify gene sets. You can run GSEA on pathways (currently based on Kyoto Encyclopedia of Genes and Genomes ([KEGG](#)) pathways) or on other gene set databases. When using the KEGG option, the *KEGG database* (i.e. the species) is automatically set, based on the upstream nodes. The *Gene set size* option allows you to restrict your analysis on gene sets of certain size (i.e. number of genes).

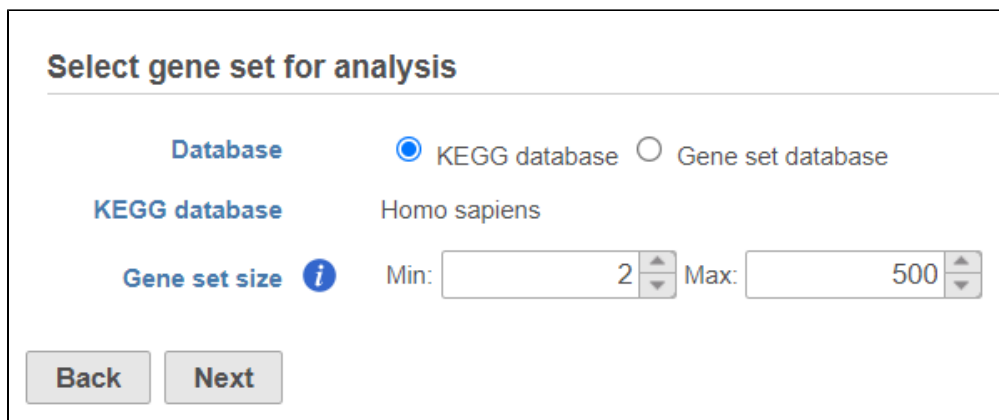
A screenshot of a dialog box titled 'Select gene set for analysis'. The dialog has a light blue header. Below the header, there are two radio buttons: 'KEGG database' (selected) and 'Gene set database'. Under 'KEGG database', there is a text field showing 'Homo sapiens'. Below that, there is a section for 'Gene set size' with an information icon. It includes 'Min:' and 'Max:' labels with corresponding input fields. The 'Min' field contains the number '2' and the 'Max' field contains the number '500'. At the bottom of the dialog, there are two buttons: 'Back' and 'Next'.

Figure 14. Select gene set for analysis dialog is used to specify gene sets

If you select *Gene set database*, two additional options will appear. *Genome build* will be detected automatically, based on the upstream nodes. The gene sets that are available for that build are listed in the drop down list (Figure 3). Custom databases will be labeled by their name as specified in the [Library file management](#), while GO database will be labeled by the release date (as seen in Figure 3).

Figure 15. Specifying gene set database for GSEA analysis

Once your choices are made, push **Next** to proceed.

In the second part of the set up (Figure 4) pick the experimental factor for GSEA (three are available in this example: *Condition*, *Stim*, *Numeric*). The dialog will list only the factors with two categories; if your project contains additional factors, which have a single category or more than two categories, a warning message will be displayed at the top.

Figure 16. Select factor for analysis dialog. Only factors with two categories are enabled. The warning message indicates that some of the factors in the current project have either a single category, or more than two categories

If the warning message is displayed, click on the **details** link to learn more about unavailable factors (an example is shown in Figure 5).

Figure 17. Excluded attributes details page listing experimental factors which are not available for the GSEA analysis

Select the experimental factor that you want to run GSEA on and push **Next**.

The third dialog is *Define comparisons* (Figure 6). The box on the left side displays the categories of the selected factor (shown as *Factor*). Use the arrow buttons (>) to move one of the factors to the *Denominator* box (that factor should be interpreted as the reference category) and the other factor to the *Numerator* box. Confirm your selection by pushing the **Add comparison** button and the comparison will be added to the *Comparisons* table (Figure 6).

Low value filter is turned on by default and will remove all the genes with the lowest average coverage of 1.0 or below; if a filter feature task was performed before this task, the default low-value filter is set to *None* (for details please see the [GSA](#) chapter) .

Define comparisons i

Factor Treatment

Control

Treated

Treated

vs

Control

☒ Combine i

☐ Pairwise i

Add comparison

Reset comparison

Comparisons

Comparison	Delete
Treated vs. Control	✖

Figure 18. Use the dialog to specify the reference category (Denominator) and the test category (Numerator). In the example, the Dependent group is the Numerator and is compared relative to the Control group (Denominator)

Push **Finish** to launch GSEA with the default settings.

Alternatively, click on the **Configure** icon to access the advanced options (Figure 7). Number of data permutations (needed to calculate the normalised enrichment scores) can be controlled using the *Permutations* option. Permutation is to randomly permute the group assignment across a given gene. For each permutation, a random order is computed, that order is used to compute the score for each gene. Finally, if you start your project by importing a count matrix (i.e. as opposed to generating the count matrix using Partek Flow), you need to specify whether the expression values were log transformed before the import (use the *Data has been log transformed with base* drop down).

Advanced options

▼ GSEA

Permutations

100

▼ Report options

Data has been log transformed with base

None

Apply

Save as new

Cancel

Figure 19. Advanced GSEA options. Default settings are shown

## GSEA Results

When the task completes, double click on the **GSEA** task node (Figure 8) to view the report.

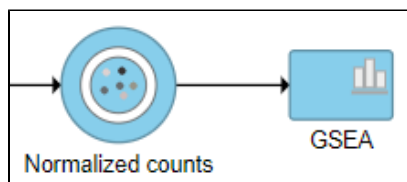


Figure 20. GSEA task node. Double click to view the report

The report consists of two parts: the GSEA result table on the right and the filter panel on the left (Figure 9).

							Treated vs Control		
View	Gene set ID	Gene set description	Gene set size	Enrichment score	Normalized enrichment score	P-value	FDR		
1	path:hsa04940	Type I diabetes mellitus	12	0.764	1.862	0.043	0.085		
2	path:hsa04672	Intestinal immune network for IgA production	12	0.712	1.781	0	0.115		
3	path:hsa03440	Homologous recombination	38	0.497	1.706	0	0.172		
4	path:hsa00430	Taurine and hypotaurine metabolism	7	0.762	1.643	0	0.242		
5	path:hsa05320	Autoimmune thyroid disease	9	0.840	1.593	0.047	0.362		
6	path:hsa05323	Rheumatoid arthritis	44	0.568	1.574	0.075	0.343		
7	path:hsa02010	ABC transporters	25	0.584	1.551	0.020	0.342		
8	path:hsa05332	Graft-versus-host disease	9	0.863	1.551	0.049	0.299		
9	path:hsa05330	Allograft rejection	8	0.855	1.544	0.047	0.282		
10	path:hsa03030	DNA replication	34	0.622	1.538	0.044	0.268		
11	path:hsa05340	Primary immunodeficiency	15	0.709	1.537	0	0.245		
12	path:hsa04061	Viral protein interaction with cytokine and cytokine receptor	27	0.446	1.525	0.077	0.250		
13	path:hsa04612	Antigen processing and presentation	41	0.567	1.463	0.156	0.357		
14	path:hsa04610	Complement and coagulation cascades	31	0.451	1.446	0.157	0.375		
15	path:hsa03430	Mismatch repair	22	0.583	1.443	0.093	0.358		
16	path:hsa04064	NF-kappa B signaling pathway	70	0.392	1.428	0.085	0.376		
17	path:hsa03460	Fanconi anemia pathway	50	0.520	1.425	0.023	0.360		
18	path:hsa03450	Non-homologous end-joining	12	0.451	1.400	0.104	0.398		
19	path:hsa05150	Staphylococcus aureus infection	22	0.489	1.395	0.176	0.390		
20	path:hsa00603	Glycosphingolipid biosynthesis - globo and isoglobo series	10	0.565	1.357	0.042	0.479		
21	path:hsa00470	D-Amino acid metabolism	4	0.783	1.335	0.061	0.510		
22	path:hsa04060	Cytokine-cytokine receptor interaction	95	0.377	1.333	0.182	0.493		
23	path:hsa04657	IL-17 signaling pathway	63	0.333	1.318	0.128	0.516		
24	path:hsa04145	Phagosome	91	0.391	1.263	0.222	0.650		
25	path:hsa04970	Salivary secretion	46	0.329	1.243	0.157	0.692		

Figure 21. GSEA report table with the filter panel on the left

The comparison (i.e. Denominator vs. Numerator) is given at the top of the GSEA table. To download the table to your local computer as a text file, use the **Download** link in the bottom right. Each row of the table corresponds to one gene set and the gene sets are ranked by the P-value, ascending (lowest values at the top). The icon ( ) in the column headers are used for sorting. The columns of the table are as follows.

- **View.** The icons in the *View* column open the enrichment plot ( ) or the extra details report ( ) (explanations below).
- **Gene set ID.** The Gene set IDs are based on the gene set file that was selected during set up. Each ID is a link to the [geneontology.org](http://geneontology.org) page of the selected set.
- **Gene set size.** Number of genes in the set (as specified in the gene set file).
- **Enrichment score.** The enrichment score is the primary result of GSEA; it reflects the degree to which the current gene set is overrepresented at the top or the bottom of the ranked list of all the genes in the gene model (for details, see the *References*). The higher the enrichment score the more overrepresented (enriched) the gene set is.
- **Normalised score.** Normalisation of the enrichment score takes into account the size of the gene set. We recommend to use normalised values for filtering.
- **P-value.** P-value estimates the statistical significance of the enrichment score.
- **FDR.** False discovery rate (FDR) is used to control for multiple testing. We recommend to use FDR values for filtering.

The filter panel is used to narrow the list of gene sets. The *Results* shows the number of gene sets currently in the table. Filtering can be performed on: *Gene set ID* (search for the numeric ID), *Gene set description* (search for a key word), *Gene set size* (number of genes in the set), *Enrichment score*, *Normalised enrichment score*, *P-value*, *FDR*. Click on the black triangle ▼ to open the controls for each filter (Figure 10). To remove all the filters, click on the **Clear filter** link. If you commonly use a filter, you can save the filter settings by clicking the **Save filter** button. The saved filter will be shown under *Saved filters*.

The cogwheel icon ( ) is a link to Settings > *Filter management* page.

Results: 12211

Filter

☐ Gene set ID
 

+

(All gene set IDs)

☐ Gene set description
 

+

(All gene set descriptions)

☐ Gene set size
 

Greater than
 

2

2
 
 496

☐ Enrichment score
 

Greater than or  $\epsilon$ 

0

-10000
 
 10000

☐ Normalized enrichment score
 

Greater than or  $\epsilon$ 

0

-10000
 
 10000

☐ P-value
 

Less than or equ
 

1

0
 
 1


☐ FDR
 

Less than or equ
 

1

0
 
 1

Figure 22. GSEA table: filter fine tuning options. The Results show the number of gene sets currently filtered in

Click on the **View enrichment report** icon () to open a new *Data viewer* session with the per-gene set report. The current gene set is in the title, at the top of the canvas (*Enrichment profile*). To quickly switch to another gene set, use the *Axis > Content* drop-down list. The individual plots are as follows (Figure 11; from top to bottom).

- *Enrichment score*. The algorithm walks down the ranked list of all the genes in the model, increasing the running sum (y axis) each time when a gene in the current gene set is encountered. Conversely, the running-sum is decreased each time a gene not in the current gene set is encountered. The magnitude of the increment depends on the correlation of the gene with the experimental factor. The enrichment score is then the maximum deviation from zero encountered in the random walk (the summit of the curve).
- *Gene set hits*. Each column shows the location of a gene from the current gene set, within the ranked list of all the genes in the model.
- *Rank metric*. The plot shows the value of the ranking metric (y axis) as you move down the ranked list of all the genes in the model (x axis). The ranking metric measures a gene's correlation with a phenotype. A positive value of the metric indicates correlation with the first category (*Numerator*) and a negative value indicates correlation with the second category (*Denominator*).

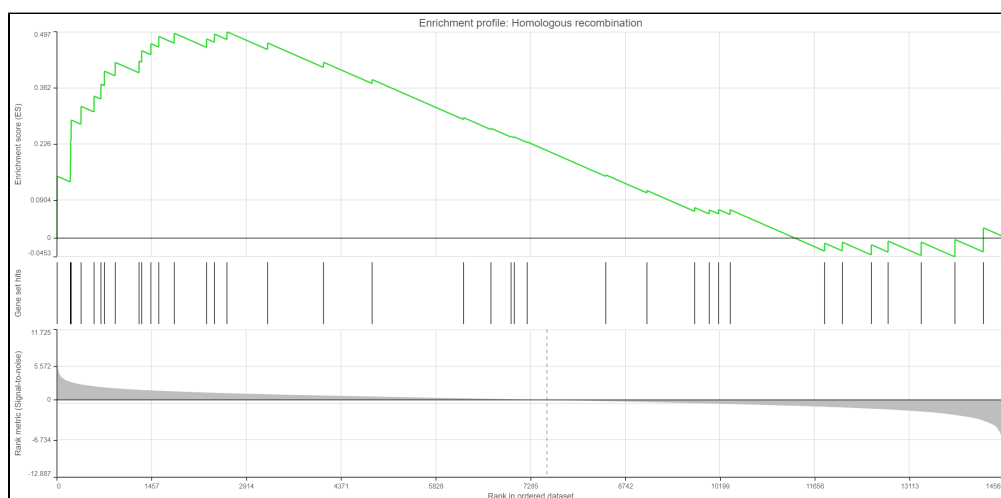



Figure 23. Enrichment plots. Three plots are generated for each gene set: enrichment score, gene set hits, and rank metric

Click on the **View extra details** plot (  ) to open a gene set-specific report page (Figure 12).

Leading edge genes: it is a subset of genes that contribute most to the ES. For a positive ES, the leading edge subset is the set of members that appear in the ranked list prior to the peak score. For a negative ES, it is the set of genes that appear subsequent to the peak score.

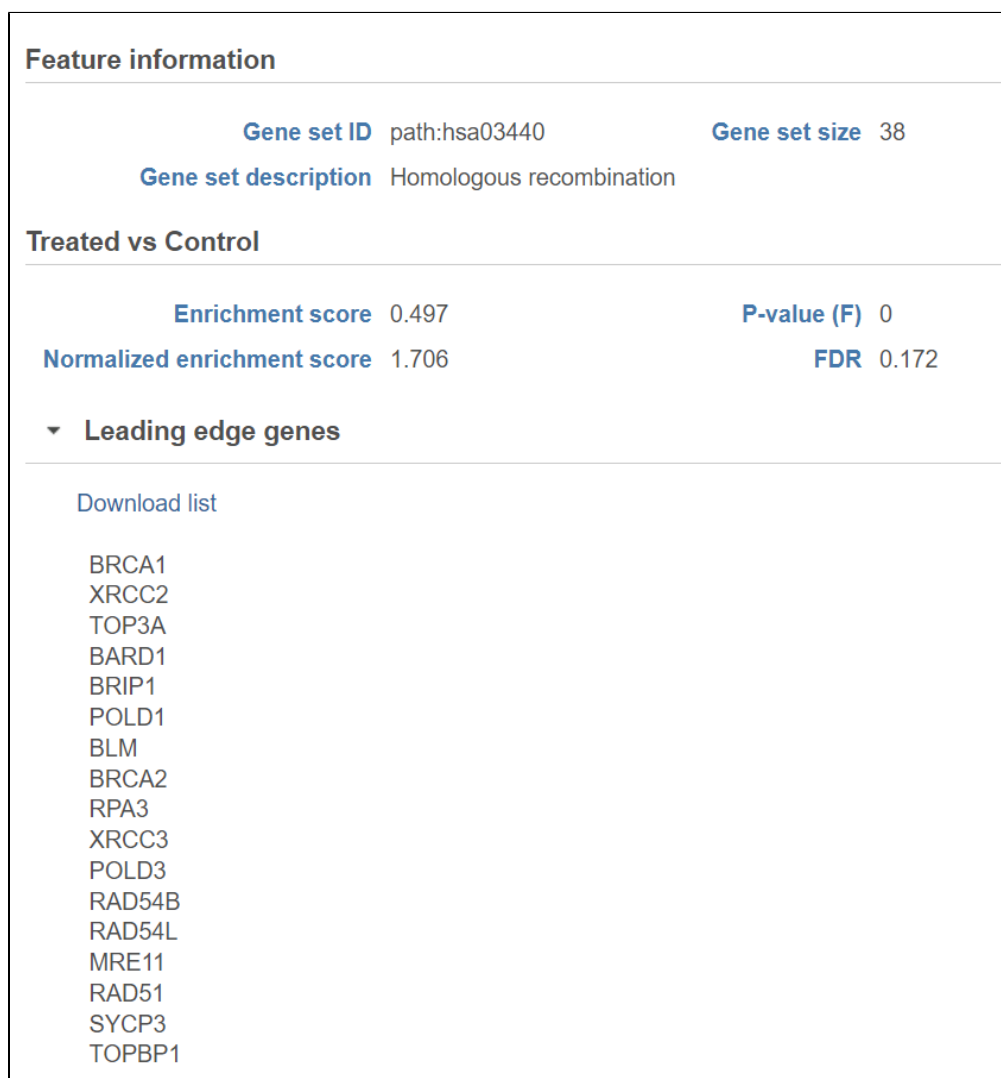


Figure 24. Extra details report with key metrics for each gene set. The figure shows an example set (rRNA binding)

## References

1. Subramanian A, Tamayo P, Mootha VK, et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc Natl Acad Sci U S A*. 2005;102(43):15545-15550. doi:10.1073/pnas.0506580102
2. Mootha VK, Lindgren CM, Eriksson KF, et al. PGC-1alpha-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. *Nat Genet*. 2003;34(3):267-273. doi:10.1038/ng1180