
gseapy Documentation

Release 1.1.3

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TABLE OF CONTENTS

1	GSEAPY: Gene Set Enrichment Analysis in Python.	1
2	Citation	3
3	Installation	5
4	GSEAPy is a Python/Rust implementation of GSEA and wrapper for Enrichr.	7
5	Why GSEAPY	9
6	Indices and tables	79
	Python Module Index	81
	Index	83

GSEAPY: GENE SET ENRICHMENT ANALYSIS IN PYTHON.

Release notes : <https://github.com/zqfang/GSEAPy/releases>

CITATION

Zhuoqing Fang, Xinyuan Liu, Gary Peltz, GSEAPy: a comprehensive package for performing gene set enrichment analysis in Python, *Bioinformatics*, 2022;, btac757, <https://doi.org/10.1093/bioinformatics/btac757>

INSTALLATION

Install gseapy package from bioconda or pypi.

```
# if you have conda (MacOS_x86-64 and Linux only)  
$ conda install -c bioconda gseapy  
  
# or use pip to install the latest release  
$ pip install gseapy
```


GSEAPY IS A PYTHON/RUST IMPLEMENTATION OF GSEA AND WRAPPER FOR ENRICHR.

GSEAPy has multiple subcommands: `gsea`, `prerank`, `ssgsea`, `gsva`, `replot` `enrichr`, `biomart`.

1. The `gsea` module produces **GSEA** results. The input requires a txt file(FPKM, Expected Counts, TPM, et.al), a `cls` file, and `gene_sets` file in `gmt` format.
2. The `prerank` module produces **Prerank tool** results. The input expects a pre-ranked gene list dataset with correlation values, which in `.rnk` format, and `gene_sets` file in `gmt` format. `prerank` module is an API to *GSEA* pre-rank tools.
3. The `ssgsea` module performs **single sample GSEA(ssGSEA)** analysis. The input expects a gene list with expression values(same with `.rnk` file, and `gene_sets` file in `gmt` format. ssGSEA enrichment score for the gene set as described by [D. Barbie et al 2009](#).
4. The `gsva` module performs **GSVA** analysis, which described by [Hänzelmann et al](#).
5. The `replot` module reproduces GSEA desktop version results. The only input for GSEAPY is the location to GSEA Desktop output results.
6. The `enrichr` module enables you to perform gene set enrichment analysis using **Enrichr** API. Enrichr is open source and freely available online at: <http://amp.pharm.mssm.edu/Enrichr> . It runs very fast and generates results in `txt` format.
7. The `biomart` module helps you convert gene ids using BioMart API.

GSEAPy could be used for **RNA-seq, ChIP-seq, Microarray** data. It's used for convenient GO enrichments and produce **publishable quality figures** in python.

The full GSEA is far too extensive to describe here; see [GSEA](#) documentation for more information. All files' formats for GSEAPy are identical to GSEA desktop version.

WHY GSEAPY

I would like to use Pandas to explore my data, but I did not find a convenient tool to do gene set enrichment analysis in python. So, here are my reasons:

- **Ability to run inside python interactive console without having to switch to R!!!**
- User friendly for both wet and dry lab users.
- Produce or reproduce publishable figures.
- Perform batch jobs easy.
- Easy to use in bash shell or your data analysis workflow, e.g. snakemake.

5.1 Welcome to GSEAPY's documentation!

5.1.1 GSEAPY: Gene Set Enrichment Analysis in Python.

5.1.2 GSEAPy is a Python/Rust implementation of GSEA and wrapper for Enrichr.

It's used for convenient GO enrichments and produce **publication-quality figures** from python.

GSEAPy could be used for **RNA-seq, ChIP-seq, Microarray** data.

Gene Set Enrichment Analysis (GSEA) is a computational method that determines whether an a priori defined set of genes shows statistically significant, concordant differences between two biological states (e.g. phenotypes).

The full GSEA is far too extensive to describe here; see [GSEA](#) documentation for more information.

Enrichr is open source and freely available online at: <http://amp.pharm.mssm.edu/Enrichr> .

5.1.3 Citation

Zhuoqing Fang, Xinyuan Liu, Gary Peltz, GSEAPy: a comprehensive package for performing gene set enrichment analysis in Python, *Bioinformatics*, 2022;, btac757, <https://doi.org/10.1093/bioinformatics/btac757>

5.1.4 Installation

Install gseapy package from bioconda or pypi.

```
# if you have conda (MacOS_x86-64 and Linux only)
$ conda install -c bioconda gseapy

# or use pip to install the latest release
$ pip install gseapy
```

5.1.5 GSEA Java version output:

This is an example of GSEA desktop application output

5.1.6 GSEAPy Prerank module output

Using the same data from GSEA, GSEAPy reproduces the example above.

Using Prerank or replot module will reproduce the same figure for GSEA Java desktop outputs

5.1.7 GSEAPy enrichr module

A graphical introduction of Enrichr

The only thing you need to prepare is a gene list file in txt format(one gene id per row), or a python list object.

Note: Enrichr uses a list of Entrez gene symbols as input. You should convert all gene names to uppercase.

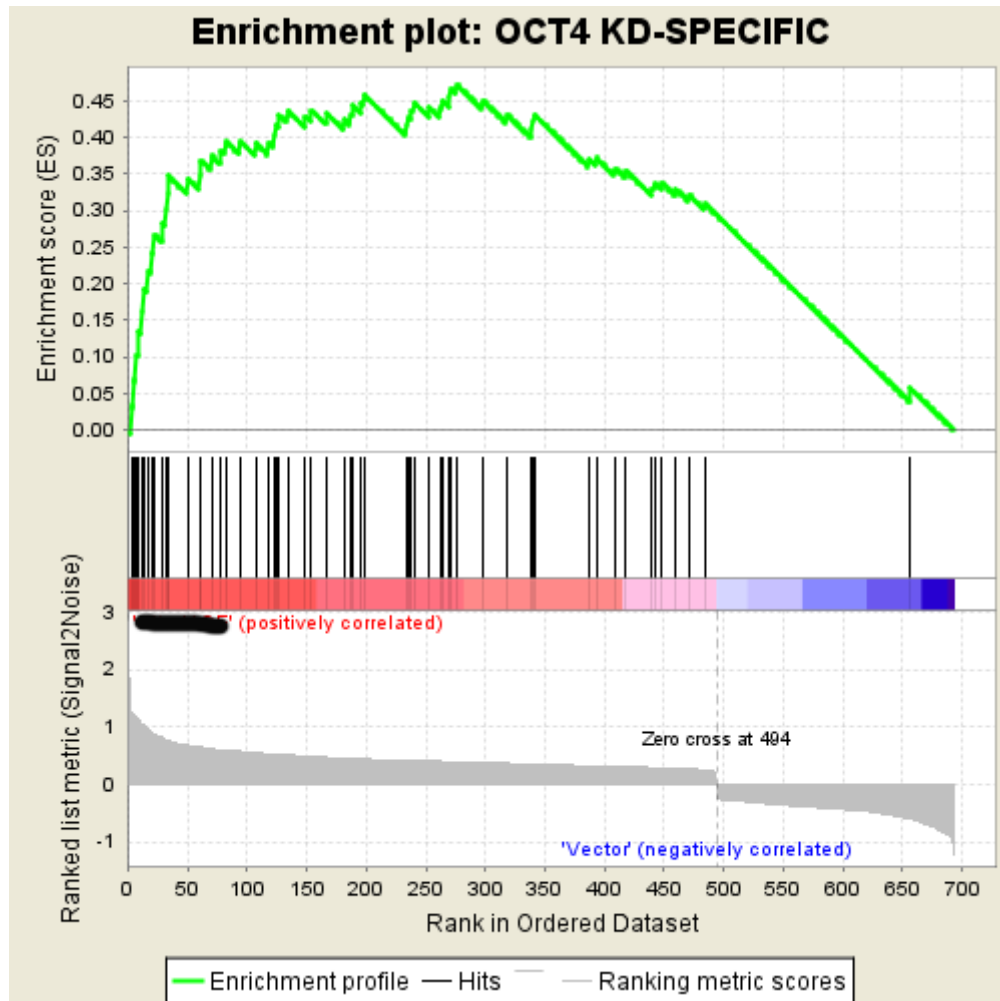
For example, both a list object and txt file are supported for enrichr API

```
# if you prefer to run gseapy.enrchr() inside python console, you could assign a list_
↳object to
# gseapy like this.
gene_list = ['SCARA3', 'LOC100044683', 'CMBL', 'CLIC6', 'IL13RA1', 'TACSTD2', 'DKKL1',
            'CSF1', 'CITED1', 'SYNPO2L']
```

```
# an alternative way is that you could provide a gene list txt file which looks like_
↳this:
with open('data/gene_list.txt') as genes:
    print(genes.read())
```

```
CTLA2B
SCARA3
```

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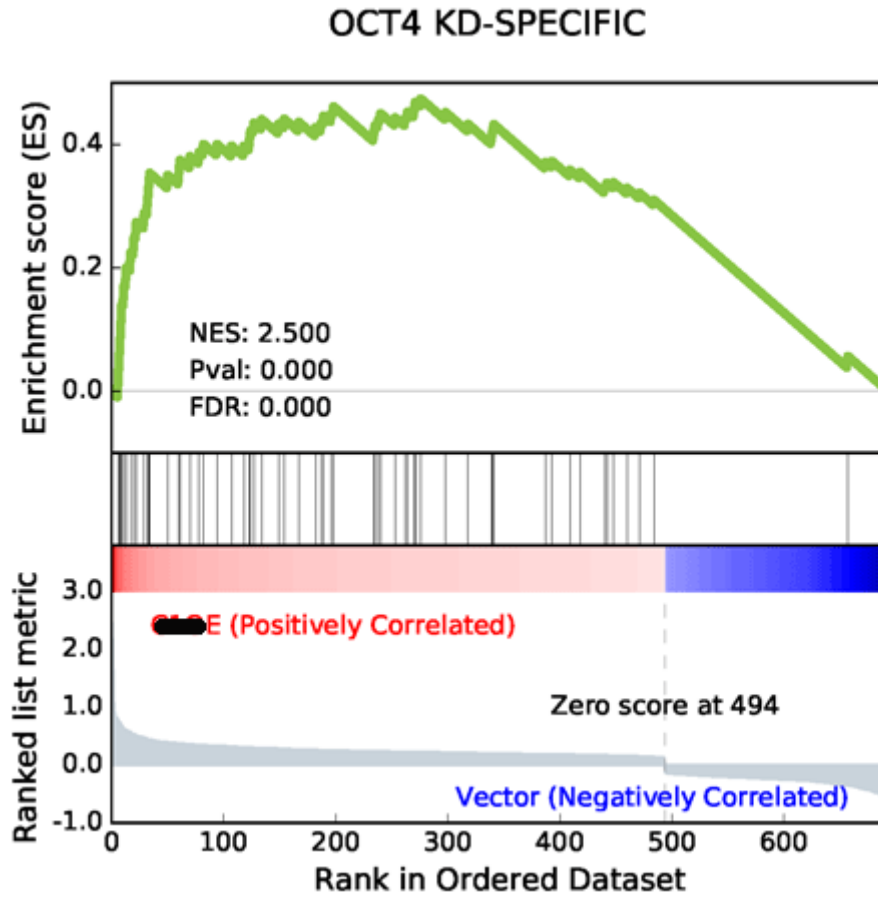
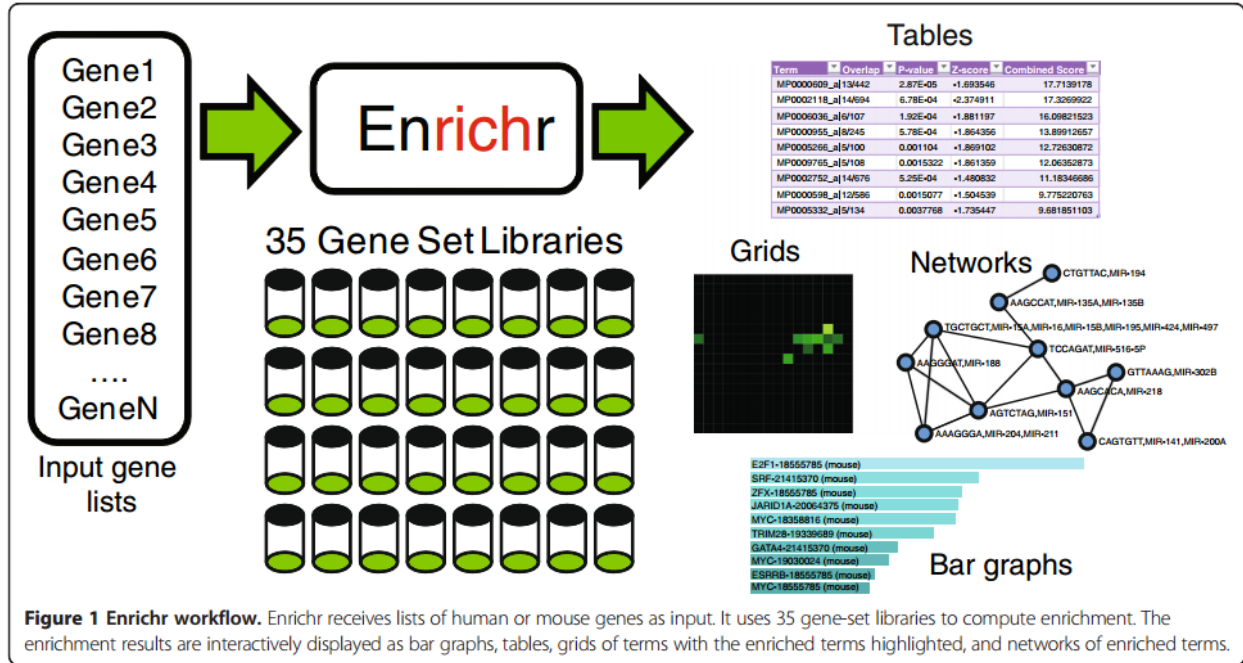


Fig. 1: Generated by GSEAPY
GSEAPy figures are supported by all matplotlib figure formats.
You can modify GSEA plots easily in .pdf files. Please Enjoy.



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```

LOC100044683
CMBL
CLIC6
IL13RA1
TACSTD2
DKKL1
CSF1
CITED1
SYNPO2L
TINAGL1
PTX3

```

5.1.8 Installation

Install gseapy package from bioconda or pypi.

```

# if you have conda
$ conda install -c conda-forge -c bioconda gseapy

# or use pip to install the latest release
$ pip install gseapy

```

For API information to use this library, see the *Developmental Guide*.

5.2 GSEAPY Example

Examples to use GSEAPY inside python console

```
[1]: # %matplotlib inline
# %config InlineBackend.figure_format='retina' # mac
%load_ext autoreload
%autoreload 2
import pandas as pd
import gseapy as gp
import matplotlib.pyplot as plt
```

Check gseapy version

```
[2]: gp.__version__
[2]: '1.1.0'
```

5.2.1 Biomart API

Don't use this if you don't know Biomart

Warning: This API has limited support now

Convert gene identifiers

```
[3]: from gseapy import Biomart
bm = Biomart()
```

```
[4]: ## view validated marts
# marts = bm.get_marts()
## view validated dataset
# datasets = bm.get_datasets(mart='ENSEMBL_MART_ENSEMBL')
## view validated attributes
# attrs = bm.get_attributes(dataset='hsapiens_gene_ensembl')
## view validated filters
# filters = bm.get_filters(dataset='hsapiens_gene_ensembl')
## query results
queries = {'ensembl_gene_id': ['ENSG00000125285', 'ENSG00000182968']} # need to be a dict_
↪object
results = bm.query(dataset='hsapiens_gene_ensembl',
                  attributes=['ensembl_gene_id', 'external_gene_name', 'entrezgene_id',
↪'go_id'],
                  filters=queries)
results.tail()
```

```
[4]:   ensembl_gene_id  external_gene_name  entrezgene_id   go_id
36  ENSG00000182968                SOX1           6656  GO:0021884
37  ENSG00000182968                SOX1           6656  GO:0030900
38  ENSG00000182968                SOX1           6656  GO:0048713
39  ENSG00000182968                SOX1           6656  GO:1904936
40  ENSG00000182968                SOX1           6656  GO:1990830
```

```
[5]: results.dtypes
[5]: ensembl_gene_id      object
external_gene_name      object
entrezgene_id           Int32
go_id                    object
dtype: object
```

Mouse gene symbols maps to Human, or Vice Versa

This is useful when you have troubles to convert gene symbols between human and mouse

```
[6]: from gseapy import Biomart
bm = Biomart()
# note the dataset and attribute names are different
m2h = bm.query(dataset='mmusculus_gene_ensembl',
               attributes=['ensembl_gene_id', 'external_gene_name',
                           'hsapiens_homolog_ensembl_gene',
                           'hsapiens_homolog_associated_gene_name'])

h2m = bm.query(dataset='hsapiens_gene_ensembl',
               attributes=['ensembl_gene_id', 'external_gene_name',
                           'mmusculus_homolog_ensembl_gene',
                           'mmusculus_homolog_associated_gene_name'])
```

```
[7]: # h2m.sample(10)
```

Gene Symbols Conversion for the GMT file

This is useful when running GSEA for non-human species

e.g. Convert Human gene symbols to Mouse.

```
[8]: # get a dict symbol mappings
h2m_dict = {}
for i, row in h2m.loc[:, ["external_gene_name", "mmusculus_homolog_associated_gene_name"]].iterrows():
    if row.isna().any(): continue
    h2m_dict[row['external_gene_name']] = row["mmusculus_homolog_associated_gene_name"]
# read gmt file into dict
kegg = gp.read_gmt(path="tests/extdata/enrichr.KEGG_2016.gmt")
print(kegg['MAPK signaling pathway Homo sapiens hsa04010'][:10])

['EGF', 'IL1R1', 'IL1R2', 'HSPA1L', 'CACNA2D2', 'CACNA2D1', 'CACNA2D4', 'CACNA2D3',
 ↪ 'MAPK8IP3', 'MAPK8IP1']
```

```
[9]: kegg_mouse = {}
for term, genes in kegg.items():
    new_genes = []
    for gene in genes:
        if gene in h2m_dict:
```

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```

        new_genes.append(h2m_dict[gene])
    kegg_mouse[term] = new_genes
print(kegg_mouse['MAPK signaling pathway Homo sapiens hsa04010'][:10])
['Egf', 'I11r1', 'I11r2', 'Hspa11', 'Cacna2d2', 'Cacna2d1', 'Cacna2d4', 'Cacna2d3',
↪ 'Mapk8ip3', 'Mapk8ip1']

```

5.2.2 Msigdb API

Down load gmt file from: <https://data.broadinstitute.org/gsea-msigdb/msigdb/release/>

```
[10]: from gseapy import Msigdb
```

```
[11]: msig = Msigdb()
      # mouse hallmark gene sets
      gmt = msig.get_gmt(category='mh.all', dbver="2023.1.Mm")

```

two helper method

```

# list msigdb version you wanna query
msig.list_dbver()
# list categories given dbver.
msig.list_category(dbver="2023.1.Hs") # mouse

```

```
[12]: print(gmt['HALLMARK_WNT_BETA_CATENIN_SIGNALING'])
['Ctnnb1', 'Jag1', 'Myc', 'Notch1', 'Ptch1', 'Trp53', 'Axin1', 'Ncstn', 'Rbpj', 'Psen2',
↪ 'Wnt1', 'Axin2', 'Hey2', 'Fzd1', 'Frat1', 'Csnk1e', 'Dvl2', 'Hey1', 'Gnai1', 'Lef1',
↪ 'Notch4', 'Ppard', 'Adam17', 'Tcf7', 'Numb', 'Cnd2', 'Ncor2', 'Kat2a', 'Nkd1', 'Hdac2',
↪ 'Dkk1', 'Wnt5b', 'Wnt6', 'Dl11', 'Skp2', 'Hdac5', 'Fzd8', 'Dkk4', 'Cul1', 'Jag2',
↪ 'Hdac11', 'Maml1']

```

5.2.3 Enrichr API

See all supported enrichr library names

Select database from { 'Human', 'Mouse', 'Yeast', 'Fly', 'Fish', 'Worm' }

```
[13]: # default: Human
      names = gp.get_library_name()
      names[:10]
```

```
[13]: ['ARCHS4_Cell-lines',
      'ARCHS4_IDG_Coexp',
      'ARCHS4_Kinases_Coexp',
      'ARCHS4_TFs_Coexp',
      'ARCHS4_Tissues',
      'Achilles_fitness_decrease',
      'Achilles_fitness_increase',
      'Aging_Perturbations_from_GEO_down',
      'Aging_Perturbations_from_GEO_up',
      'Allen_Brain_Atlas_10x_scrNA_2021']

```

```
[14]: # yeast
yeast = gp.get_library_name(organism='Yeast')
yeast[:10]

[14]: ['Cellular_Component_AutoRIF',
'Cellular_Component_AutoRIF_Predicted_zscore',
'GO_Biological_Process_2018',
'GO_Biological_Process_AutoRIF',
'GO_Biological_Process_AutoRIF_Predicted_zscore',
'GO_Cellular_Component_2018',
'GO_Cellular_Component_AutoRIF',
'GO_Cellular_Component_AutoRIF_Predicted_zscore',
'GO_Molecular_Function_2018',
'GO_Molecular_Function_AutoRIF']
```

Parse Enrichr library into dict

```
[15]: ## download library or read a .gmt file
go_mf = gp.get_library(name='GO_Molecular_Function_2018', organism='Yeast')
print(go_mf['ATP binding (GO:0005524)'])

['MLH1', 'ECM10', 'RLI1', 'SSB1', 'SSB2', 'YTA12', 'MSH2', 'CDC6', 'HMI1', 'YNL247W',
↪ 'MSH6', 'SSQ1', 'MCM7', 'SRS2', 'HSP104', 'SSA1', 'MCX1', 'SSC1', 'ARP2', 'ARP3', 'SSE1
↪ ', 'SMC2', 'SSZ1', 'TDA10', 'ORC5', 'VPS4', 'RBK1', 'SSA4', 'NEW1', 'ORC1', 'SSA2',
↪ 'KAR2', 'SSA3', 'DYN1', 'PGK1', 'VPS33', 'LHS1', 'CDC123', 'PMS1']
```

Over-representation analysis by Enrichr web services

The only requirement of input is a list of gene symbols.

For online web services, gene symbols are not case sensitive.

- `gene_list` accepts
 - `pd.Series`
 - `pd.DataFrame`
 - list object
 - txt file (one gene symbol per row)
- `gene_sets` accepts:
 - Multi-libraries names supported, separate each name by comma or input a list.

For example:

```
# gene_list
gene_list="./data/gene_list.txt",
gene_list=glist
# gene_sets
gene_sets='KEGG_2016'
gene_sets='KEGG_2016,KEGG_2013'
gene_sets=['KEGG_2016', 'KEGG_2013']
```

```
[16]: # read in an example gene list
gene_list = pd.read_csv("./tests/data/gene_list.txt",header=None, sep="\t")
gene_list.head()
```

```
[16]:
      0
0      IGKV4-1
1      CD55
2      IGKC
3      PPFIBP1
4      ABHD4
```

```
[17]: # convert dataframe or series to list
glist = gene_list.squeeze().str.strip().to_list()
print(glist[:10])
```

```
['IGKV4-1', 'CD55', 'IGKC', 'PPFIBP1', 'ABHD4', 'PCSK6', 'PGD', 'ARHGDI1', 'ITGB2',
 → 'CARD6']
```

Over-representation analysis via Enrichr web services

This is an Example of the Enrichr analysis

NOTE: 1. Enrichr Web Services need gene symbols as input 2. Gene symbols will convert to upcases automatically.
3. (Optional) Input an user defined background gene list

Enrichr Web Services (without a background input)

```
[18]: # if you are only intrested in dataframe that enrichr returned, please set outdir=None
enr = gp.enrichr(gene_list=gene_list, # or "./tests/data/gene_list.txt",
                gene_sets=['MSigDB_Hallmark_2020','KEGG_2021_Human'],
                organism='human', # don't forget to set organism to the one you desired!_
 → e.g. Yeast
                outdir=None, # don't write to disk
                )
```

```
[19]: # obj.results stores all results
enr.results.head(5)
```

```
[19]:
```

	Gene_set	Term	Overlap	P-value	\
0	MSigDB_Hallmark_2020	IL-6/JAK/STAT3 Signaling	19/87	1.197225e-09	
1	MSigDB_Hallmark_2020	TNF-alpha Signaling via NF-kB	27/200	3.220898e-08	
2	MSigDB_Hallmark_2020	Complement	27/200	3.220898e-08	
3	MSigDB_Hallmark_2020	Inflammatory Response	24/200	1.635890e-06	
4	MSigDB_Hallmark_2020	heme Metabolism	23/200	5.533816e-06	

	Adjusted P-value	Old P-value	Old Adjusted P-value	Odds Ratio	\
0	5.986123e-08	0	0	6.844694	
1	5.368163e-07	0	0	3.841568	
2	5.368163e-07	0	0	3.841568	
3	2.044862e-05	0	0	3.343018	
4	5.533816e-05	0	0	3.181358	

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	Combined Score	Genes
0	140.612324	IL4R;TGFB1;IL1R1;IFNGR1;IL10RB;ITGB3;IFNGR2;IL...
1	66.270963	BTG2;BCL2A1;PLEK;IRS2;LITAF;IFIH1;PANX1;DRAM1;...
2	66.270963	FCN1;LRP1;PLEK;LIPA;CA2;CASP3;LAMP2;S100A12;FY...
3	44.540108	LYN;IFITM1;BTG2;IL4R;CD82;IL1R1;IFNGR2;ITGB3;F...
4	38.509172	SLC22A4;MPP1;BNIP3L;BTG2;ARHGEF12;NEK7;GDE1;FO...

Enrichr Web Service (with background input)

NOTE: Missing Overlap column in final output

```
[20]: # background only reconigized a gene list input.
enr_bg = gp.enrichr(gene_list=gene_list,
                   gene_sets=['MSigDB_Hallmark_2020', 'KEGG_2021_Human'],
                   # organism='human', # organism argment is ignored because user input a
↳background
                   background="tests/data/background.txt",
                   outdir=None, # don't write to disk
                   )
```

```
[21]: enr_bg.results.head() #
```

```
[21]:
```

	Gene_set	Term	P-value	\
0	MSigDB_Hallmark_2020	IL-6/JAK/STAT3 Signaling	3.559435e-11	
1	MSigDB_Hallmark_2020	TNF-alpha Signaling via NF-kB	3.401526e-10	
2	MSigDB_Hallmark_2020	Complement	3.813953e-10	
3	MSigDB_Hallmark_2020	Inflammatory Response	3.380686e-08	
4	MSigDB_Hallmark_2020	heme Metabolism	8.943634e-08	

	Adjusted P-value	Old P-value	Old adjusted P-value	Odds Ratio	\
0	1.779718e-09	0	0	8.533251	
1	6.356588e-09	0	0	4.824842	
2	6.356588e-09	0	0	4.796735	
3	4.225857e-07	0	0	4.197067	
4	8.943634e-07	0	0	4.111306	

	Combined Score	Genes
0	205.300064	IL4R;TGFB1;IL1R1;IFNGR1;IL10RB;ITGB3;IFNGR2;IL...
1	105.189414	BTG2;BCL2A1;PLEK;IRS2;LITAF;IFIH1;PANX1;DRAM1;...
2	104.027683	FCN1;LRP1;PLEK;LIPA;CA2;CASP3;LAMP2;S100A12;FY...
3	72.200480	LYN;IFITM1;BTG2;IL4R;CD82;IL1R1;IFNGR2;ITGB3;F...
4	66.725423	SLC22A4;MPP1;BNIP3L;BTG2;ARHGEF12;NEK7;GDE1;FO...

Over-representation analysis (hypergeometric test) by offline

This API **DO NOT** use Enrichr web services.

NOTE: 1. The input gene symbols are **case sensitive**. 2. You need to **match the type of the gene identifiers** which used in your `gene_list` input and GMT file. 3. Input a `.gmt` file or `gene_set` dict object for the argument `gene_sets`

For example:

```
gene_sets="./data/genes.gmt",
gene_sets={'A':['gene1', 'gene2',...],
          'B':['gene2', 'gene4',...],
          ...}
```

```
[22]: # NOTE: `enrich` instead of `enrichr`
enr2 = gp.enrich(gene_list="./tests/data/gene_list.txt", # or gene_list=glist
                gene_sets=["./tests/data/genes.gmt", "unknown", kegg ], # kegg is a
↳dict object
                background=None, # or "hsapiens_gene_ensembl", or int, or text file, or
↳a list of genes
                outdir=None,
                verbose=True)
```

```
2023-10-25 10:46:28,796 [INFO] User defined gene sets is given: ./tests/data/genes.gmt
2023-10-25 10:46:28,813 [INFO] Input dict object named with gs_ind_2
2023-10-25 10:46:29,289 [WARNING] Input library not found: unknown. Skip
2023-10-25 10:46:29,291 [INFO] Run: genes.gmt
2023-10-25 10:46:29,293 [INFO] Background is not set! Use all 682 genes in genes.gmt.
2023-10-25 10:46:29,302 [INFO] Run: gs_ind_2
2023-10-25 10:46:29,327 [INFO] Done.
```

```
[23]: enr2.results.head()
```

```
[23]:
```

	Gene_set	Term	Overlap	P-value	Adjusted P-value	Odds Ratio	\
0	genes.gmt	BvA_UpIN_A	8/139	0.457390	0.568432	1.161982	
1	genes.gmt	BvA_UpIN_B	12/130	0.026744	0.187208	2.160059	
2	genes.gmt	CvA_UpIN_A	1/12	0.481190	0.568432	2.266479	
3	genes.gmt	DvA_UpIN_A	16/284	0.426669	0.568432	1.127395	
4	genes.gmt	DvA_UpIN_D	13/236	0.487227	0.568432	1.084567	

```

Combined Score                                     Genes
0      0.908925   PCSK6;MAP3K5;MBOAT2;MSRB2;IQGAP2;HAL;PADI2;IL1R1
1      7.822534   FAM65B;MBNL3;GPX8;DYSF;KCTD12;HEBP1;SUOX;ARHG...
2      1.657913                                     MBOAT2
3      0.960255   PCSK6;FXVD6;IFNGR2;MAP3K5;MBOAT2;VNN1;IQGAP2;H...
4      0.779830   GNB4;FAM198B;FAM65B;TXNDC5;GLIPR2;MBNL3;GPX8;D...
```


About Background genes

By default, all genes in the `gene_sets` input will be used as background.

However, a better background genes would be the following:

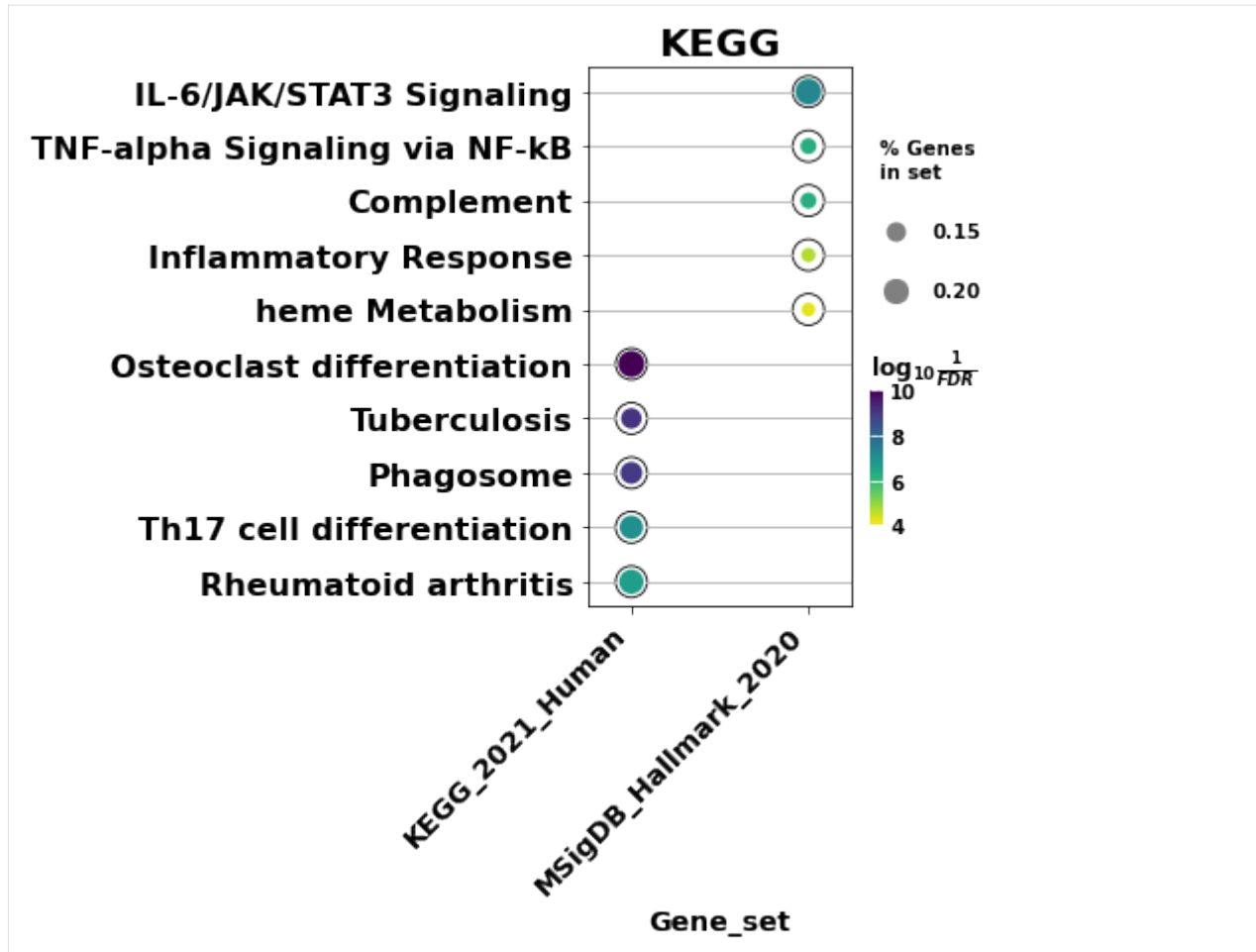
1. (Recommended) Input a list of background genes: ['gene1', 'gene2',...]
 - The background gene list is defined by your experiment. e.g. the expressed genes in your RNA-seq.
 - The gene identifier in `gmt/dict` should be the same type to the background genes.
2. Specify a number: e.g. 20000. (the number of total expressed genes).
 - This works, but not recommend. It assumes that all your genes could be found in background.
 - If genes exist in `gmt` but not included in background provided, they will affect the significance of the statistical test.
3. Set a Biomart dataset name: e.g. "hsapiens_gene_ensembl"
 - The background will use all annotated genes from the BioMart datasets you've chosen.
 - The program will try to retrieve the background information automatically.

Plotting

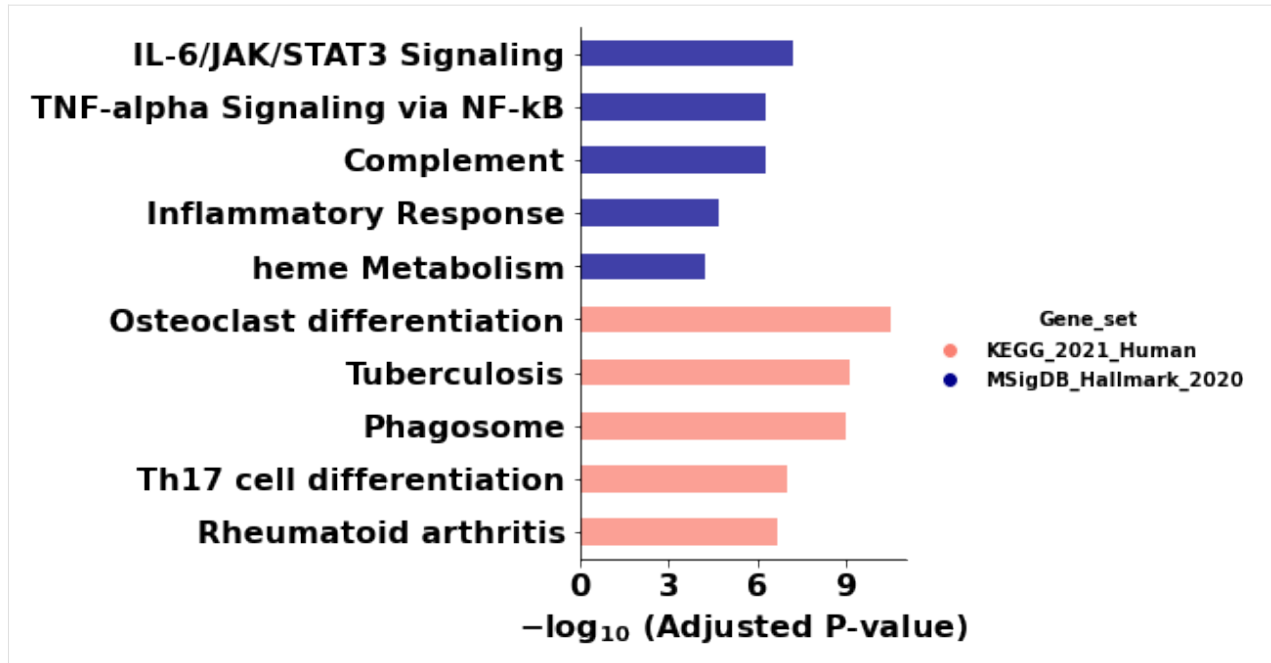
Show top 5 terms of each `gene_set` ranked by "Adjusted P-value"

```
[24]: # simple plotting function
from gseapy import barplot, dotplot
```

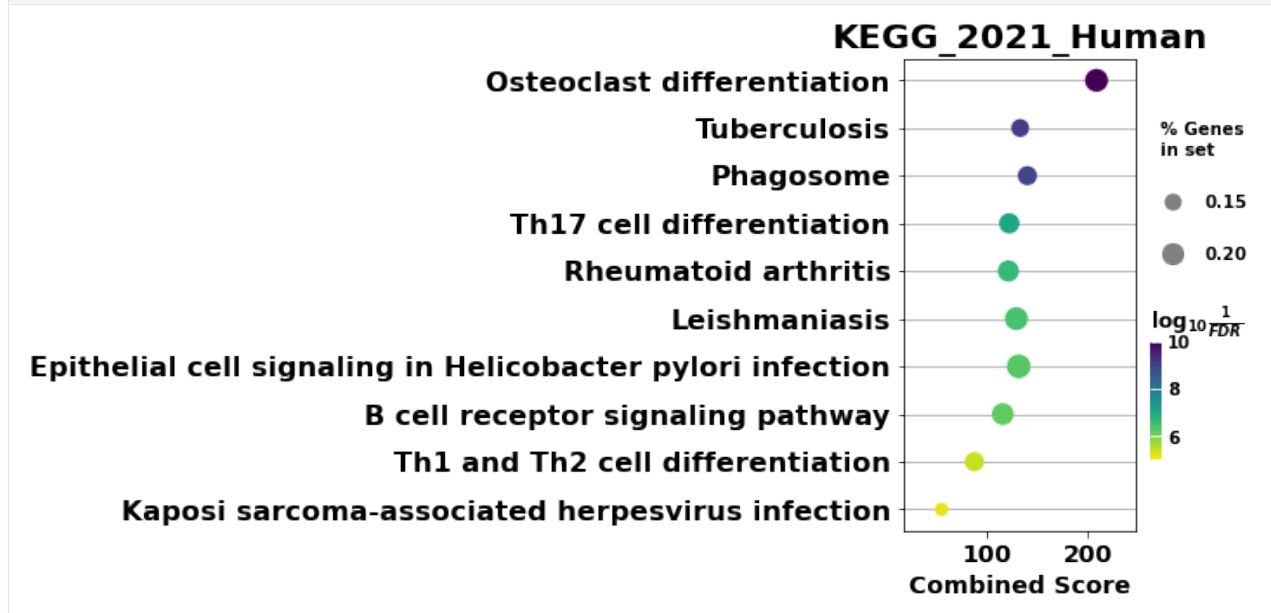
```
[25]: # categorical scatterplot
ax = dotplot(enr.results,
             column="Adjusted P-value",
             x='Gene_set', # set x axis, so you could do a multi-sample/library_
             ↪comparsion
             size=10,
             top_term=5,
             figsize=(3,5),
             title = "KEGG",
             xticklabels_rot=45, # rotate xtick labels
             show_ring=True, # set to False to remove outer ring
             marker='o',
             )
```



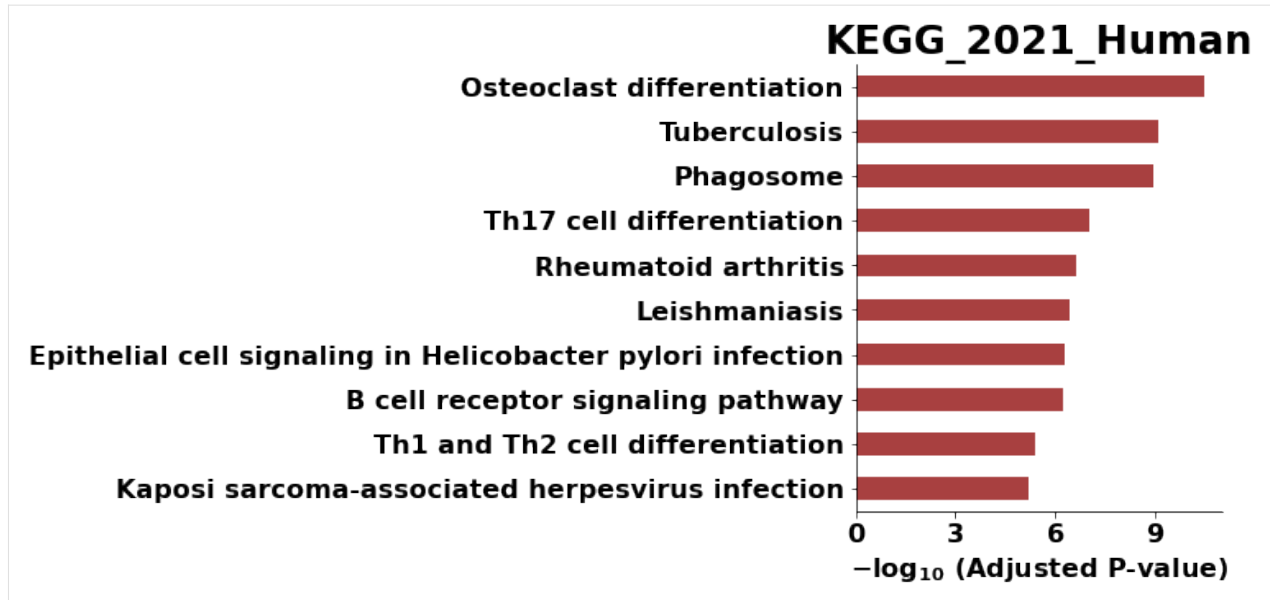
```
[26]: # categorical scatterplot
ax = barplot(enr.results,
             column="Adjusted P-value",
             group='Gene_set', # set group, so you could do a multi-sample/library_
             ↪comparsion
             size=10,
             top_term=5,
             figsize=(3,5),
             #color=['darkred', 'darkblue'] # set colors for group
             color = {'KEGG_2021_Human': 'salmon', 'MSigDB_Hallmark_2020': 'darkblue'})
```



```
[27]: # to save your figure, make sure that ``ofname`` is not None
ax = dotplot(enr.res2d, title='KEGG_2021_Human', cmap='viridis_r', size=10, figsize=(3,5))
```



```
[28]: # to save your figure, make sure that ``ofname`` is not None
ax = barplot(enr.res2d, title='KEGG_2021_Human', figsize=(4, 5), color='darkred')
```



Command line usage

the option `-v` will print out the progress of your job

```
[29]: # !gseapy enrichr -i ./data/gene_list.txt \
#           -g GO_Biological_Process_2017 \
#           -v -o test/enrichr_BP
```

5.2.4 Prerank example

Assign prerank() with

- `pd.DataFrame`: Only contains two columns, or one column with `gene_name` indexed
- `pd.Series`
- a txt file:
 - GSEAPy will skip any data after “#”.
 - Do not include header in your gene list !

NOTE: UPCASES for gene symbols by Default

1. Gene symbols are all “UPCASES” in the Enrichr Libraries. You should convert your input gene identifier to “UPCASES” first.
2. If input `gmt`, `dict` object, please refer to 1.2 Mouse gene symbols maps to Human, or Vice Versa (in this page) to convert gene identifier

Supported gene_sets input

For example:

```
gene_sets="KEGG_2016",
gene_sets="KEGG_2016,KEGG2013",
gene_sets="./data/genes.gmt",
gene_sets=["KEGG_2016","./data/genes.gmt"],
gene_sets={'A':['gene1', 'gene2',...],
           'B':['gene2', 'gene4',...],
           ...}
```

```
[30]: rnk = pd.read_csv("./tests/data/temp.rnk", header=None, index_col=0, sep="\t")
      rnk.head()
```

```
[30]:      1
      0
      ATXN1  16.456753
      UBQLN4 13.989493
      CALM1  13.745533
      DLG4   12.796588
      MRE11A 12.787631
```

```
[31]: rnk.shape
```

```
[31]: (22922, 1)
```

```
[32]: # # run prerank
      # # enrichr libraries are supported by prerank module. Just provide the name
      # # use 4 process to acceralate the permutation speed
      pre_res = gp.prerank(rnk="./tests/data/temp.rnk", # or rnk = rnk,
                          gene_sets='KEGG_2016',
                          threads=4,
                          min_size=5,
                          max_size=1000,
                          permutation_num=1000, # reduce number to speed up testing
                          outdir=None, # don't write to disk
                          seed=6,
                          verbose=True, # see what's going on behind the scenes
                          )
```

```
2023-10-25 10:46:30,863 [WARNING] Duplicated values found in preranked stats: 4.97% of
↳ genes
The order of those genes will be arbitrary, which may produce unexpected results.
2023-10-25 10:46:30,864 [INFO] Parsing data files for GSEA...
2023-10-25 10:46:30,865 [INFO] Enrichr library gene sets already downloaded in: /home/
↳ fangzq/.cache/gseapy, use local file
2023-10-25 10:46:30,902 [INFO] 0001 gene_sets have been filtered out when max_size=1000
↳ and min_size=5
2023-10-25 10:46:30,903 [INFO] 0292 gene_sets used for further statistical testing...
2023-10-25 10:46:30,903 [INFO] Start to run GSEA...Might take a while...
2023-10-25 10:46:43,563 [INFO] Congratulations. GSEApY runs successfully...
```

[]:

How to generate your GSEA plot inside python console

Visualize it using gseaplot

Make sure that ofname is not None, if you want to save your figure to the disk

```
[33]: pre_res.res2d.head(5)
```

```
[33]:
```

	Name	Term	ES	\
0	prerank	Adherens junction Homo sapiens hsa04520	0.784625	
1	prerank	Glioma Homo sapiens hsa05214	0.784678	
2	prerank	Estrogen signaling pathway Homo sapiens hsa04915	0.766347	
3	prerank	Thyroid hormone signaling pathway Homo sapiens...	0.7577	
4	prerank	Long-term potentiation Homo sapiens hsa04720	0.778249	

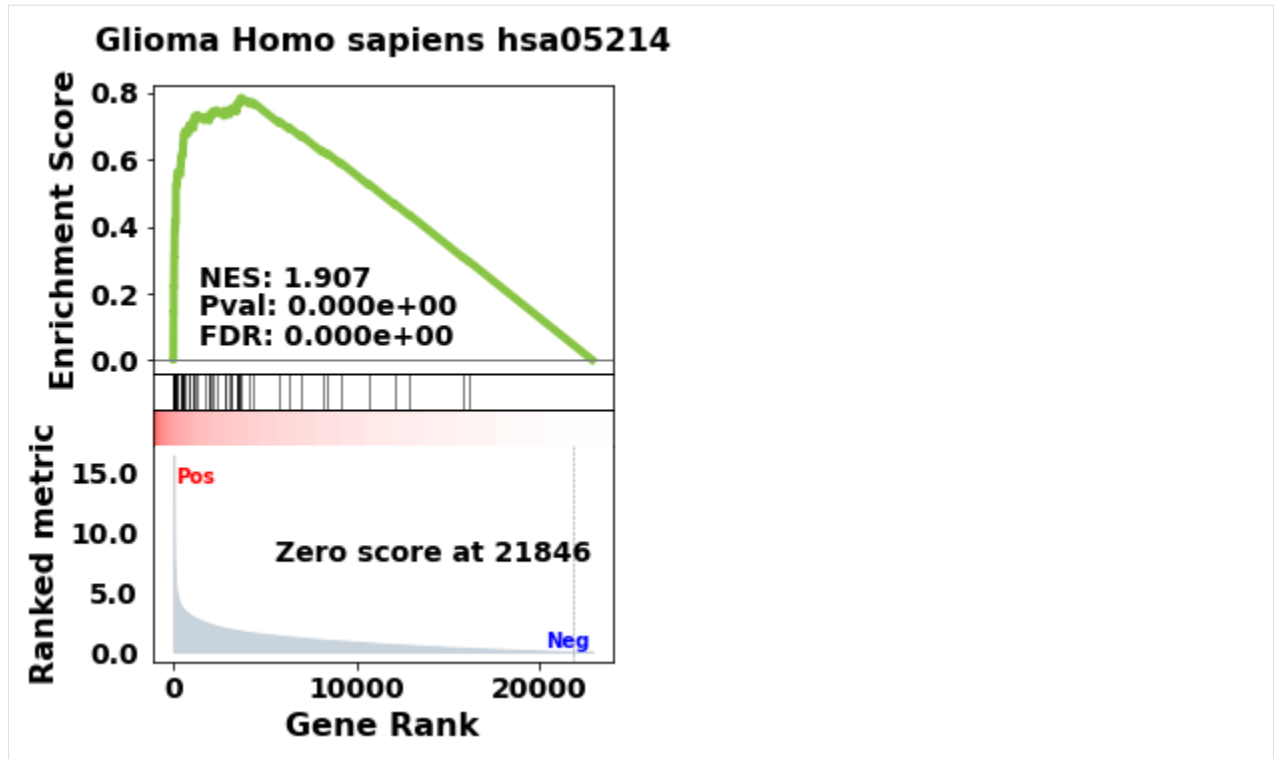
	NES	NOM	p-val	FDR	q-val	FWER	p-val	Tag %	Gene %	\
0	1.912548		0.0		0.0		0.0	47/74	10.37%	
1	1.906706		0.0		0.0		0.0	52/65	16.29%	
2	1.897957		0.0		0.0		0.0	74/99	16.57%	
3	1.891815		0.0		0.0		0.0	84/118	16.29%	
4	1.888739		0.0		0.0		0.0	42/66	9.01%	


```

                                Lead_genes
0  CTNNB1;EGFR;RAC1;TGFB1;SMAD4;MET;EP300;CDC42;...
1  CALM1;GRB2;EGFR;PRKCA;KRAS;HRAS;TP53;MAPK1;PRK...
2  CALM1;PRKACA;GRB2;SP1;EGFR;KRAS;HRAS;HSP90AB1;...
3  CTNNB1;PRKACA;PRKCA;KRAS;NOTCH1;EP300;CREBBP;H...
4  CALM1;PRKACA;PRKCA;KRAS;EP300;CREBBP;HRAS;PRKA...

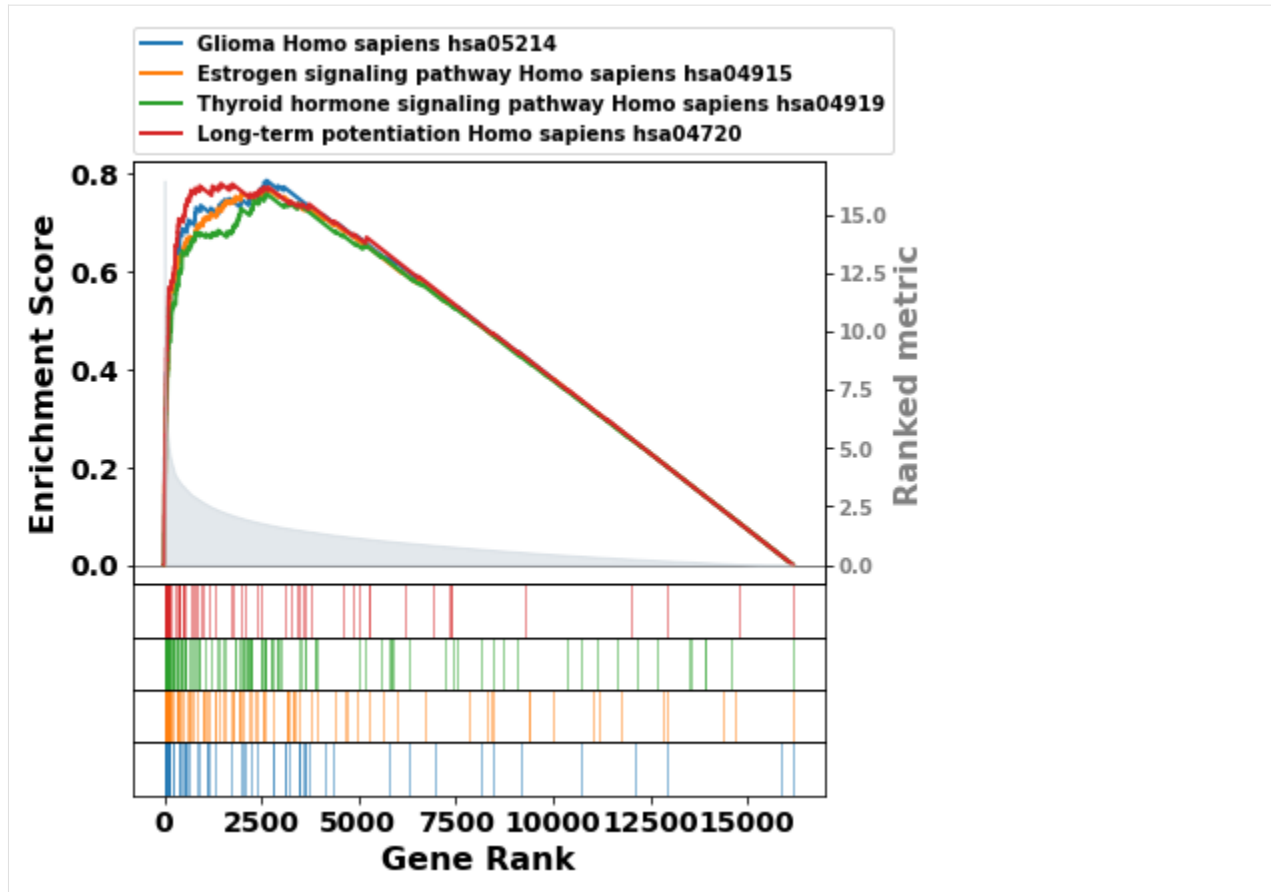
```

```
[34]: ## easy way
terms = pre_res.res2d.Term
axs = pre_res.plot(terms=terms[1]) # v1.0.5
# to make more control on the plot, use
# from gseapy import gseaplot
# gseaplot(rank_metric=pre_res.ranking, term=terms[0], ofname='your.plot.pdf', **pre_res.
↪results[terms[0]])
```



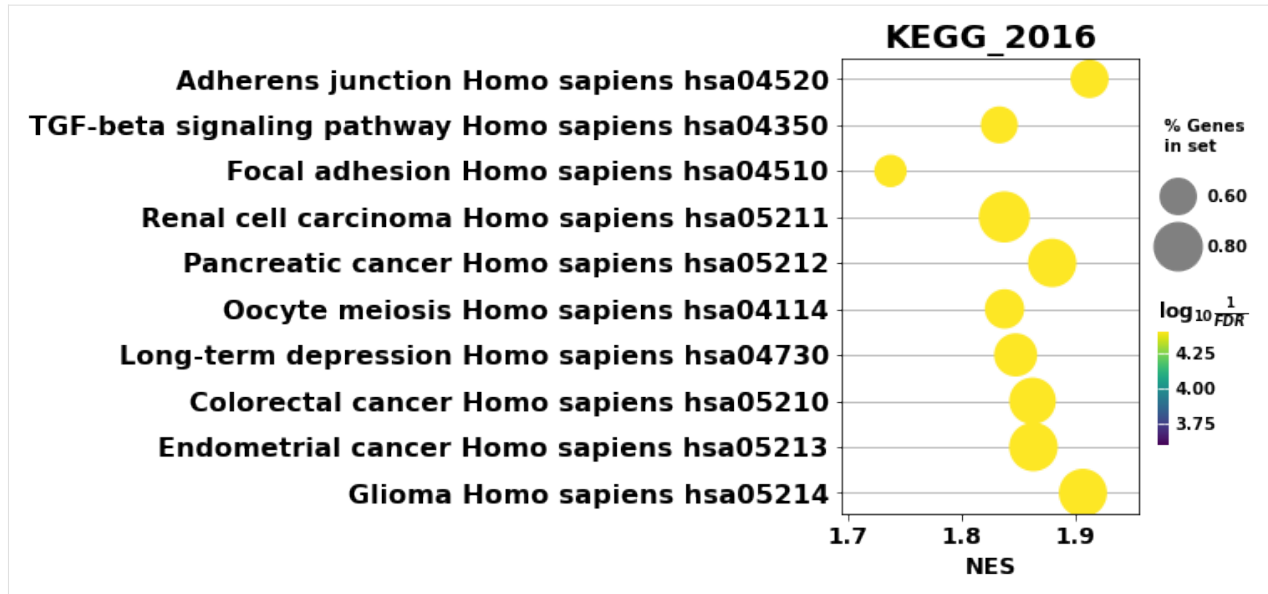
or multi pathway in one

```
[35]: ax = pre_res.plot(terms=terms[1:5],
                      #legend_kws={'loc': (1.2, 0)}, # set the legend loc
                      show_ranking=True, # whether to show the second yaxis
                      figsize=(3,4)
                      )
# or use this to have more control on the plot
# from gseapy import gseaplot2
# terms = pre_res.res2d.Term[1:5]
# hits = [pre_res.results[t]['hits'] for t in terms]
# runes = [pre_res.results[t]['RES'] for t in terms]
# fig = gseaplot2(terms=terms, res=runes, hits=hits,
#                 rank_metric=gs_res.ranking,
#                 legend_kws={'loc': (1.2, 0)}, # set the legend loc
#                 figsize=(4,5)) # rank_metric=pre_res.ranking
```



dotplot for GSEA results

```
[36]: from gseapy import dotplot
# to save your figure, make sure that ``ofname`` is not None
ax = dotplot(pre_res.res2d,
             column="FDR q-val",
             title='KEGG_2016',
             cmap=plt.cm.viridis,
             size=6, # adjust dot size
             figsize=(4,5), cutoff=0.25, show_ring=False)
```

Network Visualization

- use `enrichment_map` to build network
- save the nodes and edges. They could be used for cytoscape visualization.

```
[37]: from gseapy import enrichment_map
# return two dataframe
nodes, edges = enrichment_map(pre_res.res2d)
```

```
[38]: import networkx as nx
```

```
[39]: # build graph
G = nx.from_pandas_edgelist(edges,
                           source='src_idx',
                           target='targ_idx',
                           edge_attr=['jaccard_coef', 'overlap_coef', 'overlap_genes'])
```

```
[40]: fig, ax = plt.subplots(figsize=(8, 8))

# init node coordinates
pos=nx.layout.spiral_layout(G)
#node_size = nx.get_node_attributes()
# draw node
nx.draw_networkx_nodes(G,
                       pos=pos,
                       cmap=plt.cm.RdYlBu,
                       node_color=list(nodes.NES),
                       node_size=list(nodes.Hits_ratio * 1000))

# draw node label
nx.draw_networkx_labels(G,
                        pos=pos,
                        labels=nodes.Term.to_dict())

# draw edge
```

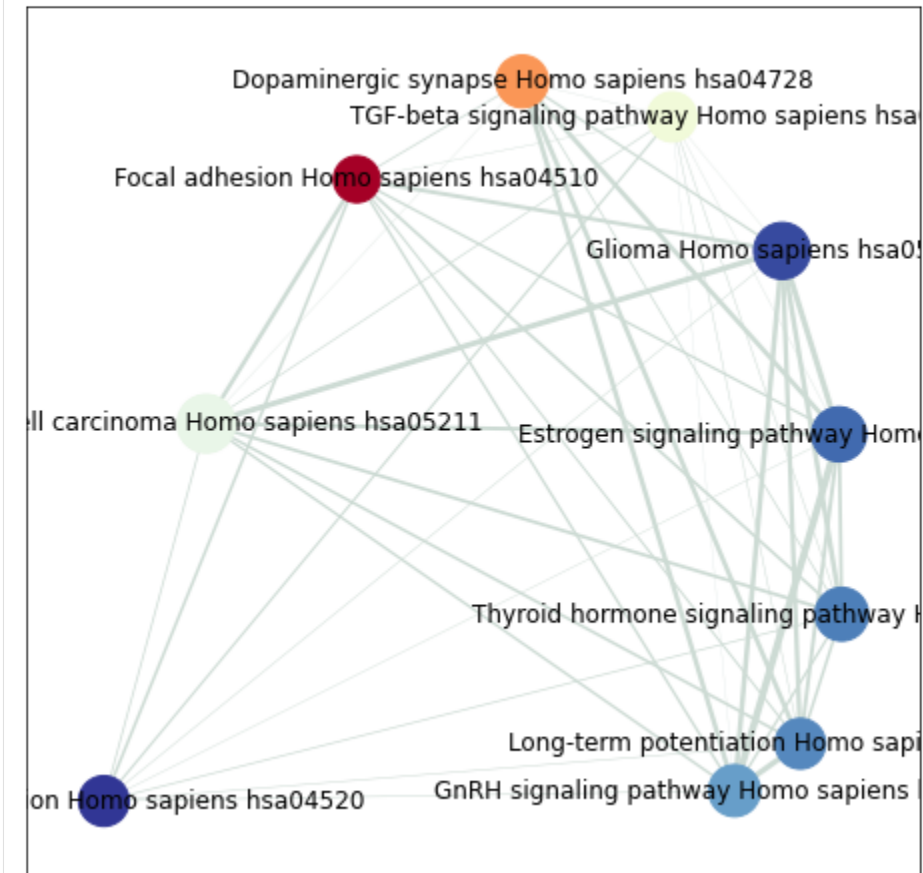
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```

edge_weight = nx.get_edge_attributes(G, 'jaccard_coef').values()
nx.draw_networkx_edges(G,
                       pos=pos,
                       width=list(map(lambda x: x*10, edge_weight)),
                       edge_color='#CDDBD4')
plt.show()

```



Command line usage

You may also want to use prerank in command line

```
[41]: # !gseapy prerank -r temp.rnk -g temp.gmt -o prerank_report_temp
```

5.2.5 GSEA Example

Inputs

Assign gsea()

- data with:
 - pandas DataFrame
 - .gct format file, or a text file
- cls with:
 - a list
 - a .cls format file
- gene_sets with:

```
gene_sets="KEGG_2016",
gene_sets="KEGG_2016,KEGG2013",
gene_sets="./data/genes.gmt",
gene_sets=["KEGG_2016","./data/genes.gmt"],
gene_sets={'A':['gene1', 'gene2',...],
           'B':['gene2', 'gene4',...],
           ...}
```

NOTE: UPCASES for gene symbols by Default

1. Gene symbols are all “UPCASES” in the Enrichr Libraries. You should convert your input gene identifier to “UPCASES” first.
2. If input gmt, dict object, please refer to 1.2 Mouse gene symbols maps to Human, or Vice Versa (in this page) to convert gene identifier

```
[42]: import gseapy as gp
      phenoA, phenoB, class_vector = gp.parser.gsea_cls_parser("./tests/extdata/Leukemia.cls")
```

```
[43]: #class_vector used to indicate group attributes for each sample
      print(class_vector)

['ALL', 'ALL', 'ALL', 'ALL', 'ALL', 'ALL', 'ALL', 'ALL', 'ALL', 'ALL', 'ALL', 'ALL', 'ALL', 'ALL', 'ALL',
↪', 'ALL', 'ALL', 'ALL', 'ALL', 'ALL', 'ALL', 'ALL', 'ALL', 'ALL', 'ALL', 'ALL', 'ALL', 'ALL', 'ALL', 'AML',
↪'AML', 'AML', 'AML', 'AML', 'AML', 'AML', 'AML', 'AML', 'AML', 'AML', 'AML', 'AML', 'AML', 'AML', 'AML',
↪'AML', 'AML', 'AML', 'AML', 'AML', 'AML', 'AML', 'AML', 'AML', 'AML', 'AML', 'AML']
```

```
[44]: gene_exp = pd.read_csv("./tests/extdata/Leukemia_hgu95av2.trim.txt", sep="\t")
      gene_exp.head()
```

```
[44]:
```

	Gene	NAME	ALL_1	ALL_2	ALL_3	ALL_4	ALL_5	ALL_6	ALL_7	\
0	MAPK3	1000_at	1633.6	2455.0	866.0	1000.0	3159.0	1998.0	1580.0	
1	TIE1	1001_at	284.4	159.0	173.0	216.0	1187.0	647.0	352.0	
2	CYP2C19	1002_f_at	285.8	114.0	429.0	-43.0	18.0	366.0	119.0	
3	CXCR5	1003_s_at	-126.6	-388.0	143.0	-915.0	-439.0	-371.0	-448.0	
4	CXCR5	1004_at	-83.3	33.0	195.0	85.0	54.0	-6.0	55.0	

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```

    ALL_8  ...  AML_15  AML_16  AML_17  AML_18  AML_19  AML_20  AML_21  \
0  1955.0  ...  1826.0  2849.0  2980.0  1442.0  3672.0  294.0  2188.0
1  1224.0  ...  1556.0  893.0  1278.0  301.0  797.0  248.0  167.0
2  -88.0   ...  -177.0  64.0  -359.0  68.0  2.0  -464.0  -127.0
3  -862.0  ...  237.0  -834.0  -1940.0  -684.0  -1236.0  -1561.0  -895.0
4  101.0   ...  86.0  -5.0  487.0  102.0  33.0  -153.0  -50.0

    AML_22  AML_23  AML_24
0  1245.0  1934.0  13154.0
1  941.0  1398.0  -502.0
2  -279.0  301.0  509.0
3  -1016.0  -2238.0  -1362.0
4  257.0  439.0  386.0

```

[5 rows x 50 columns]

```
[45]: print("positively correlated: ", phenoA)
```

positively correlated: ALL

```
[46]: print("negtively correlated: ", phenoB)
```

negtively correlated: AML

```
[47]: # run gsea
# enrichr libraries are supported by gsea module. Just provide the name
gs_res = gp.gsea(data=gene_exp, # or data='./P53_resampling_data.txt'
                gene_sets='./tests/extdata/h.all.v7.0.symbols.gmt', # or enrichr_
                ↪library names
                cls= './tests/extdata/Leukemia.cls', # cls=class_vector
                # set permutation_type to phenotype if samples >=15
                permutation_type='phenotype',
                permutation_num=1000, # reduce number to speed up test
                outdir=None, # do not write output to disk
                method='signal_to_noise',
                threads=4, seed= 7)
```

```
2023-10-25 10:46:47,125 [WARNING] Found duplicated gene names, values averaged by gene_
↪names!
```

You can set pheno_pos, and pheno_neg manually

```
[48]: # example
from gseapy import GSEA
gs = GSEA(data=gene_exp,
          gene_sets='KEGG_2016',
          classes = class_vector, # cls=class_vector
          # set permutation_type to phenotype if samples >=15
          permutation_type='phenotype',
          permutation_num=1000, # reduce number to speed up test
          outdir=None,
          method='signal_to_noise',
```

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```

        threads=4, seed= 8)
gs.pheno_pos = "AML"
gs.pheno_neg = "ALL"
gs.run()

```

```

2023-10-25 10:46:50,381 [WARNING] Found duplicated gene names, values averaged by gene_
↳names!

```

Show the gsea plots

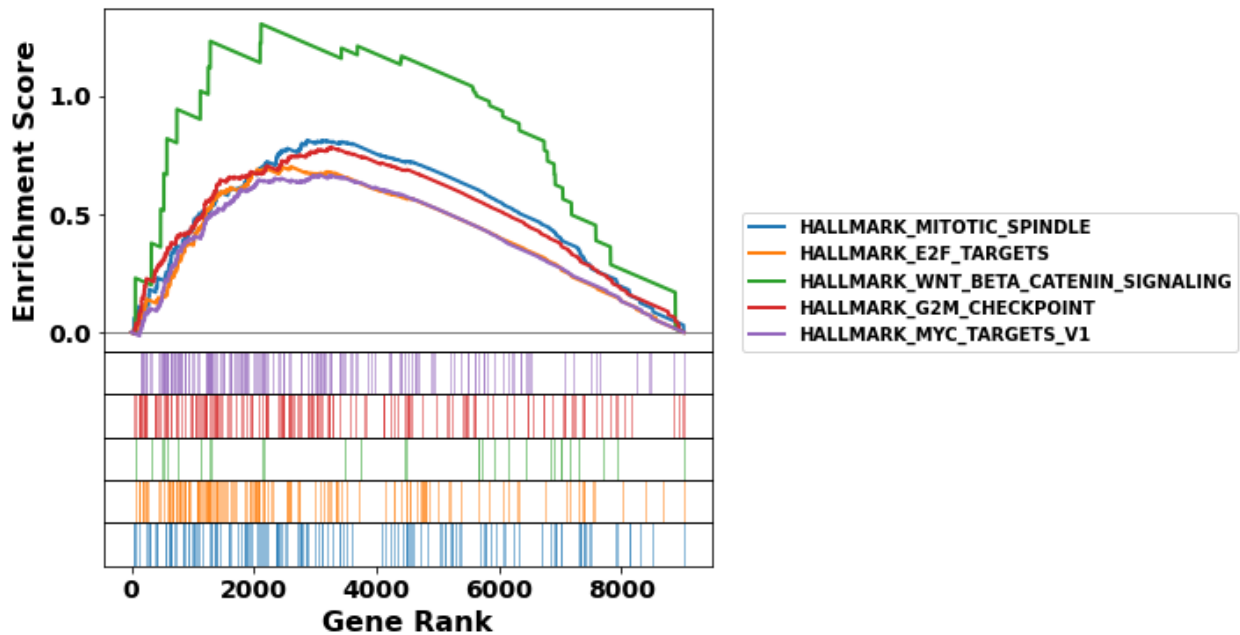
The `gsea` module will generate heatmap for genes in each gene sets in the background.

But if you need to do it yourself, use the code below

```

[49]: terms = gs_res.res2d.Term
      axs = gs_res.plot(terms[:5], show_ranking=False, legend_kws={'loc': (1.05, 0)}, )

```



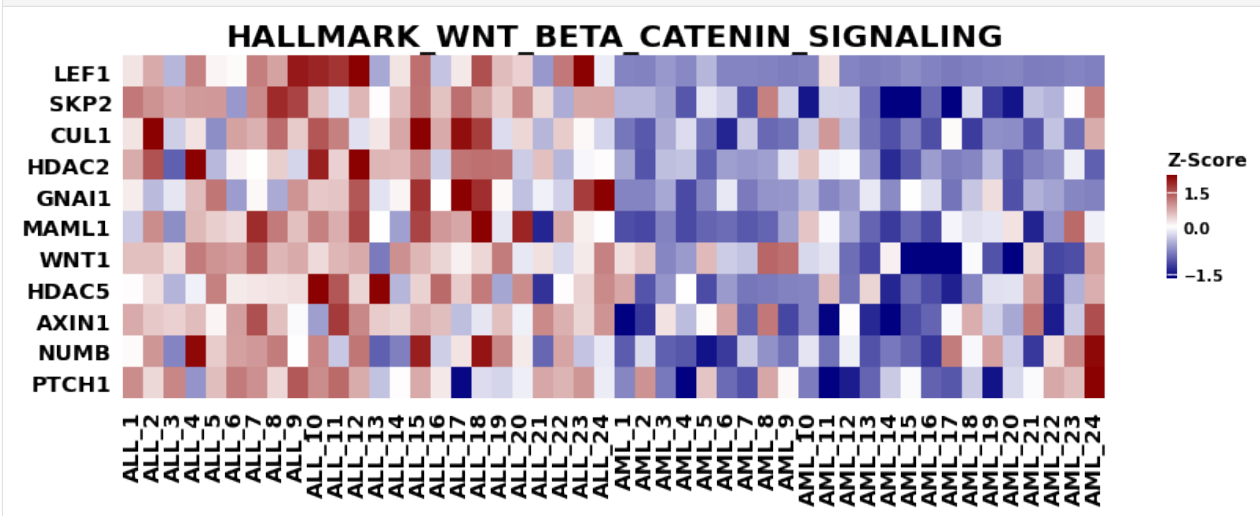
```

[50]: # or use
      # from gseapy import gseaplot2

      # # multi in one
      # terms = gs_res.res2d.Term[:5]
      # hits = [gs_res.results[t]['hits'] for t in terms]
      # runes = [gs_res.results[t]['RES'] for t in terms]
      # fig = gseaplot2(terms=terms, res=runes, hits=hits,
      #                 rank_metric=gs_res.ranking,
      #                 legend_kws={'loc': (1.2, 0)}, # set the legend loc
      #                 figsize=(4,5)) # rank_metric=pre_res.ranking

```

```
[51]: from gseapy import heatmap
# plotting heatmap
i = 2
genes = gs_res.res2d.Lead_genes[i].split(";")
# Make sure that `ofname` is not None, if you want to save your figure to disk
ax = heatmap(df = gs_res.heatmat.loc[genes], z_score=0, title=terms[i], figsize=(14,4))
```



```
[52]: gs_res.heatmat.loc[genes]
```

```
[52]:
```

Gene	ALL_1	ALL_2	ALL_3	ALL_4	ALL_5	ALL_6	ALL_7	ALL_8	\
LEF1	8544.10	12552.0	2869.0	15265.0	7446.0	6991.0	15520.0	13114.0	
SKP2	23.80	-45.0	-95.0	-71.0	-65.0	-547.0	-24.0	230.0	
CUL1	1712.75	3309.0	1273.5	1726.5	947.5	2160.0	2065.0	2524.5	
HDAC2	4542.90	6030.0	1195.0	9368.0	2281.0	3407.0	3175.0	3962.0	
GNAI1	588.50	163.0	364.0	882.0	1317.0	17.0	518.0	89.0	
MAML1	871.40	1871.0	578.0	1589.0	1448.0	1364.0	2494.0	1989.0	
WNT1	-872.50	-875.0	-1012.0	-535.0	-654.0	-694.0	-421.0	-827.0	
HDAC5	2137.20	2374.0	1651.0	2012.0	3132.0	2279.0	2314.0	2349.0	
AXIN1	-433.50	-722.0	-808.0	-623.0	-1167.0	-326.0	448.0	-661.0	
NUMB	1033.60	1474.0	600.0	2106.0	1239.0	1430.0	1468.0	1594.0	
PTCH1	352.60	86.0	372.5	-326.5	181.5	413.5	337.0	94.5	

Gene	ALL_9	ALL_10	...	AML_15	AML_16	AML_17	AML_18	AML_19	AML_20	\
LEF1	22604.0	21795.0	...	682.0	152.0	-348.0	30.0	210.0	350.0	
SKP2	159.0	-162.0	...	-865.0	-642.0	-1005.0	-413.0	-733.0	-812.0	
CUL1	1882.5	2684.5	...	851.5	614.5	1560.0	523.0	952.0	935.0	
HDAC2	2616.0	6848.0	...	1072.0	1918.0	1545.0	1653.0	2328.0	1061.0	
GNAI1	1136.0	816.0	...	470.0	313.0	-163.0	210.0	684.0	-331.0	
MAML1	1538.0	1946.0	...	390.0	233.0	1075.0	962.0	997.0	1316.0	
WNT1	-770.0	-1001.0	...	-2506.0	-2791.0	-2249.0	-1201.0	-1819.0	-2599.0	
HDAC5	2376.0	5455.0	...	1215.0	1024.0	760.0	1368.0	1923.0	1927.0	
AXIN1	-1315.0	-1991.0	...	-2590.0	-2417.0	-1321.0	-466.0	-1628.0	-1910.0	
NUMB	1014.0	1549.0	...	491.0	342.0	1594.0	990.0	1436.0	841.0	
PTCH1	534.0	354.5	...	-62.5	-454.0	-487.0	-185.0	-663.5	-150.0	

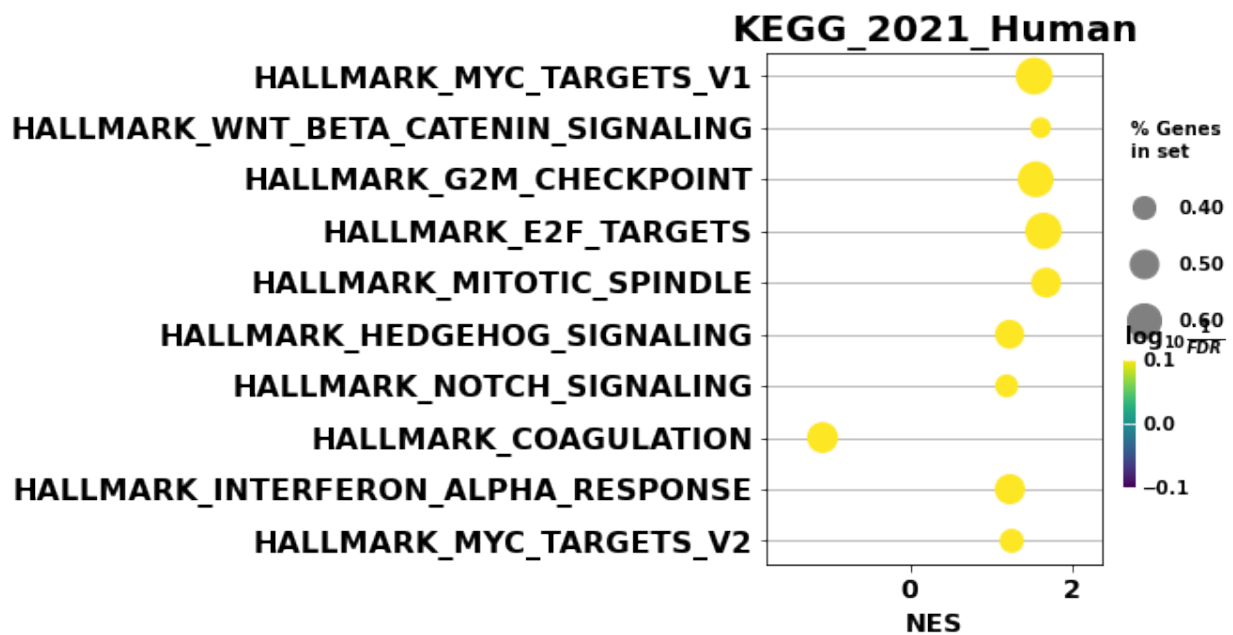
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Gene	AML_21	AML_22	AML_23	AML_24
LEF1	-242.0	-47.0	176.0	14.0
SKP2	-464.0	-490.0	-333.0	7.0
CUL1	646.0	1214.5	770.0	2088.5
HDAC2	1571.0	1749.0	2942.0	1174.0
GNAI1	115.0	55.0	-80.0	-94.0
MAML1	48.0	609.0	2090.0	1056.0
WNT1	-995.0	-1861.0	-1835.0	-714.0
HDAC5	2872.0	848.0	1629.0	2763.0
AXIN1	93.0	-2951.0	-1666.0	471.0
NUMB	352.0	1158.0	1541.0	2109.0
PTCH1	-58.0	252.0	178.5	1061.0

[11 rows x 48 columns]

```
[53]: from gseapy import dotplot
# to save your figure, make sure that `ofname` is not None
ax = dotplot(gs_res.res2d,
             column="FDR q-val",
             title='KEGG_2021_Human',
             cmap=plt.cm.viridis,
             size=5,
             figsize=(4,5), cutoff=1)
```



Command line usage

You may also want to use gsea in command line

```
[54]: # !gseapy gsea -d ./data/P53_resampling_data.txt \
#         -g KEGG_2016 -c ./data/P53.cls \
#         -o test/gsea_reprot_2 \
#         -v --no-plot \
#         -t phenotype
```

5.2.6 Single Sample GSEA example

What's ssGSEA? Which one should I use? Prerank or ssGSEA

see FAQ [here](#)

Assign - data with - a txt file, gct file, - pd.DataFrame - pd.Seires(gene name as index)

- gene_sets with:

```
gene_sets="KEGG_2016",
gene_sets="KEGG_2016,KEGG2013",
gene_sets="./data/genes.gmt",
gene_sets=["KEGG_2016","./data/genes.gmt"],
gene_sets={'A':['gene1', 'gene2',...],
           'B':['gene2', 'gene4',...],
           ...}
```

1. Gene symbols are all “UPCASES” in the Enrichr Libraries. You should convert your input gene identifier to “UPCASES” first.
2. If input gmt, dict object, please refer to 1.2 Mouse gene symbols maps to Human, or Vice Versa (in this page) to convert gene identifier

```
[55]: import gseapy as gp
# txt, gct file input
ss = gp.ssgsea(data='./tests/extdata/Leukemia_hgu95av2.trim.txt',
              gene_sets='./tests/extdata/h.all.v7.0.symbols.gmt',
              outdir=None,
              sample_norm_method='rank', # choose 'custom' will only use the raw value.
# of `data`
              no_plot=True)
```

```
2023-10-25 10:46:59,844 [WARNING] Found duplicated gene names, values averaged by gene.
names!
```

```
[56]: ss.res2d.head()
```

```
[56]:
```

	Name	Term	ES	NES
0	ALL_2	HALLMARK_MYC_TARGETS_V1	3393.823575	0.707975
1	ALL_12	HALLMARK_MYC_TARGETS_V1	3385.626111	0.706265
2	AML_11	HALLMARK_MYC_TARGETS_V1	3359.186716	0.700749
3	ALL_14	HALLMARK_MYC_TARGETS_V1	3348.938881	0.698611
4	ALL_17	HALLMARK_MYC_TARGETS_V1	3335.065348	0.695717


```
[57]: # or assign a dataframe, or Series to ssgsea()
ssdf = pd.read_csv("./tests/data/temp.rnk", header=None, index_col=0, sep="\t")
ssdf.head()
```

```
[57]:      1
0
ATXN1  16.456753
UBQLN4 13.989493
CALM1  13.745533
DLG4   12.796588
MRE11A 12.787631
```

```
[58]: # dataframe with one column is also supported by ssGSEA or Prerank
# But you have to set gene_names as index
ssdf2 = ssdf.squeeze()
```

```
[59]: # Series, DataFrame Example
# supports dataframe and series
temp = gp.ssgsea(data=ssdf2, gene_sets="./tests/data/temp.gmt")
```

Access Enrichment Score (ES) and NES

Results are saved to obj.res2d

```
[60]: # NES and ES
ss.res2d.sort_values('Name').head()
```

```
[60]:      Name      Term      ES      NES
601  ALL_1  HALLMARK_PANCREAS_BETA_CELLS -1280.654659 -0.267153
934  ALL_1      HALLMARK_APOPTOSIS    970.818772  0.202519
1774 ALL_1  HALLMARK_HEDGEHOG_SIGNALING   431.446694  0.090003
279  ALL_1  HALLMARK_INTERFERON_ALPHA_RESPONSE 1721.458034  0.359108
1778 ALL_1  HALLMARK_BILE_ACID_METABOLISM  -429.127871 -0.089519
```

```
[61]: nes = ss.res2d.pivot(index='Term', columns='Name', values='NES')
nes.head()
```

```
[61]: Name      ALL_1      ALL_10      ALL_11      ALL_12  \
Term
HALLMARK_ADIPOGENESIS      0.287384  0.274548  0.290059  0.285388
HALLMARK_ALLOGRAFT_REJECTION  0.06177  0.028062  0.096589  0.080713
HALLMARK_ANDROGEN_RESPONSE  0.133453  0.113911  0.193074  0.201531
HALLMARK_ANGIOGENESIS      -0.113481 -0.182411 -0.195637 -0.094817
HALLMARK_APICAL_JUNCTION    0.051372  0.063763  0.054601  0.014385

Name      ALL_13      ALL_14      ALL_15      ALL_16  \
Term
HALLMARK_ADIPOGENESIS      0.322757  0.305239  0.275686  0.266209
HALLMARK_ALLOGRAFT_REJECTION  0.082701  0.102735  0.12525  0.147262
HALLMARK_ANDROGEN_RESPONSE  0.151001  0.12967  0.173563  0.144836
HALLMARK_ANGIOGENESIS      -0.163717 -0.139243 -0.119084 -0.154526
HALLMARK_APICAL_JUNCTION    0.049019  0.05269  0.064787  0.052192
```

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Name	ALL_17	ALL_18	...	AML_22	AML_23	\
Term			...			
HALLMARK_ADIPOGENESIS	0.315803	0.282617	...	0.277755	0.261477	
HALLMARK_ALLOGRAFT_REJECTION	0.124621	0.091077	...	0.185738	0.157852	
HALLMARK_ANDROGEN_RESPONSE	0.180214	0.180801	...	0.180443	0.188891	
HALLMARK_ANGIOGENESIS	-0.06829	-0.121156	...	0.054883	-0.023782	
HALLMARK_APICAL_JUNCTION	0.05607	0.064936	...	0.10927	0.090065	

Name	AML_24	AML_3	AML_4	AML_5	\
Term					
HALLMARK_ADIPOGENESIS	0.200083	0.312948	0.342963	0.253282	
HALLMARK_ALLOGRAFT_REJECTION	0.055585	0.218827	0.172395	0.199077	
HALLMARK_ANDROGEN_RESPONSE	0.197979	0.174892	0.14285	0.184843	
HALLMARK_ANGIOGENESIS	0.119022	-0.067741	0.04843	0.012808	
HALLMARK_APICAL_JUNCTION	0.155801	0.091556	0.110045	0.101659	

Name	AML_6	AML_7	AML_8	AML_9
Term				
HALLMARK_ADIPOGENESIS	0.298924	0.410395	0.387433	0.343606
HALLMARK_ALLOGRAFT_REJECTION	0.158945	0.13835	0.110787	0.121643
HALLMARK_ANDROGEN_RESPONSE	0.157449	0.162843	0.180475	0.181878
HALLMARK_ANGIOGENESIS	0.032505	-0.024058	-0.039492	-0.043769
HALLMARK_APICAL_JUNCTION	0.128808	0.095511	0.080076	0.098644

[5 rows x 48 columns]

Warning !!!

if you set `permutation_num > 0`, `ssgsea` will become `prerank` with `ssGSEA` statistics. **DO NOT** use this, unless you known what you are doing !

```
ss_permut = gp.ssgsea(data="./tests/extdata/Leukemia_hgu95av2.trim.txt",
                    gene_sets="./tests/extdata/h.all.v7.0.symbols.gmt",
                    outdir=None,
                    sample_norm_method='rank', # choose 'custom' for your custom metric
                    permutation_num=20, # set permutation_num > 0, it will act like prerank.
                    ↪ tool
                    no_plot=True, # skip plotting, because you don't need these figures
                    processes=4, seed=9)
ss_permut.res2d.head(5)
```

Command line usage of ssGSEA

```
[62]: # !gseapy ssgsea -d ./data/testSet_rand1200.gct \
#           -g data/temp.gmt \
#           -o test/ssgsea_report2 \
#           -p 4 --no-plot
```

5.3 GSVA example

```
[63]: import gseapy as gp
# txt, gct file input
es = gp.gsva(data='./tests/extdata/Leukemia_hgu95av2.trim.txt',
            gene_sets='./tests/extdata/h.all.v7.0.symbols.gmt',
            outdir=None)
```

```
2023-10-25 10:47:01,160 [WARNING] Found duplicated gene names, values averaged by gene_
↳names!
```

```
[64]: es.res2d.pivot(index='Term', columns='Name', values='ES').head()
```

```
[64]: Name          ALL_1    ALL_10    ALL_11    ALL_12  \
Term
HALLMARK_ADIPOGENESIS      -0.21331  -0.08096  0.003289 -0.017909
HALLMARK_ALLOGRAFT_REJECTION -0.210468 -0.373787 -0.086016 -0.169623
HALLMARK_ANDROGEN_RESPONSE  -0.13633 -0.308572  0.008126  0.04849
HALLMARK_ANGIOGENESIS       0.035895 -0.287645 -0.214951 -0.291145
HALLMARK_APICAL_JUNCTION    -0.088652 -0.128757 -0.050282 -0.248682

Name          ALL_13    ALL_14    ALL_15    ALL_16  \
Term
HALLMARK_ADIPOGENESIS       0.207841  0.023294 -0.085392 -0.221273
HALLMARK_ALLOGRAFT_REJECTION -0.158775 -0.016488 -0.050703  0.10443
HALLMARK_ANDROGEN_RESPONSE  -0.061181 -0.203036  0.070416 -0.12524
HALLMARK_ANGIOGENESIS       -0.311917 -0.236717 -0.345662 -0.250202
HALLMARK_APICAL_JUNCTION    -0.145164  0.001997 -0.082962 -0.091691

Name          ALL_17    ALL_18    ...    AML_22    AML_23  \
Term
HALLMARK_ADIPOGENESIS       0.16147  -0.01825  ...    0.03344 -0.190436
HALLMARK_ALLOGRAFT_REJECTION -0.075816 -0.193654  ...    0.023653  0.032892
HALLMARK_ANDROGEN_RESPONSE   0.080075  0.022248  ...    0.031898  0.064394
HALLMARK_ANGIOGENESIS       -0.233296 -0.318353  ...    0.244374 -0.076852
HALLMARK_APICAL_JUNCTION    -0.168941 -0.139766  ...    0.005859 -0.067385

Name          AML_24    AML_3     AML_4     AML_5  \
Term
HALLMARK_ADIPOGENESIS      -0.0985  0.105208  0.196799 -0.296305
HALLMARK_ALLOGRAFT_REJECTION -0.113577  0.30703  0.134581  0.188905
HALLMARK_ANDROGEN_RESPONSE   0.070232  0.199349 -0.079399 -0.016658
HALLMARK_ANGIOGENESIS       -0.010928 -0.210787  0.387912  0.269447
HALLMARK_APICAL_JUNCTION     0.062719 -0.022434  0.076593  0.138664

Name          AML_6     AML_7     AML_8     AML_9
Term
HALLMARK_ADIPOGENESIS      -0.084042  0.450832  0.226921  0.209835
HALLMARK_ALLOGRAFT_REJECTION  0.132169  0.024078 -0.092054 -0.195987
HALLMARK_ANDROGEN_RESPONSE  -0.127327  0.018847  0.121426  0.163149
HALLMARK_ANGIOGENESIS       0.34823  0.157249  0.075479 -0.064515
HALLMARK_APICAL_JUNCTION     0.240647  0.039307  0.016764  0.057512
```

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```
[5 rows x 48 columns]
```

```
[65]: # !gseapy ssgsea -d ./tests/data/expr.gsva.csv \  
#           -g ./tests/data/geneset.gsva.gmt \  
#           -o test/gsva_report
```

5.3.1 Replot Example

Locate your directory

Notes: replot module need to find edb folder to work properly. keep the file tree like this:

```
data  
|--- edb  
|   |--- C10E.cls  
|   |--- gene_sets.gmt  
|   |--- gsea_data.gsea_data.rnk  
|   |--- results.edb
```

```
[66]: # run command inside python console  
rep = gp.replot(indir="./tests/data", outdir="test/replot_test")
```

Command line usage of replot

```
[67]: # !gseapy replot -i data -o test/replot_test
```

5.4 scRNA-seq Example

Examples to use GSEAPy for scRNA-seq data

```
[1]: %load_ext autoreload  
%autoreload 2  
import os  
import numpy as np  
import pandas as pd  
import matplotlib.pyplot as plt
```

```
[2]: import gseapy as gp  
import scanpy as sc
```

```
[3]: gp.__version__
```

```
[3]: '1.0.5'
```

5.4.1 Read Demo Data

Convert demo data from `seurat` to `scanpy`

```
## R code
library(Seurat)
library(SeuratDisk)
ifnb = SeuratData::LoadData("ifnb")
SaveH5Seurat(ifnb, "ifnb.h5seurat", overwrite = T)
Convert("ifnb.h5seurat", "ifnb.h5ad", overwrite = T)
```

```
[4]: adata = sc.read_h5ad("data/ifnb.h5ad") # data from SeuratData::ifnb
```

```
[5]: adata.obs.head()
```

```
[5]:
```

	orig.ident	nCount_RNA	nFeature_RNA	stim	\
AAACATACATTCC.1	IMMUNE_CTRL	3017.0	877	CTRL	
AAACATACCAGAAA.1	IMMUNE_CTRL	2481.0	713	CTRL	
AAACATACCTCGCT.1	IMMUNE_CTRL	3420.0	850	CTRL	
AAACATACCTGGTA.1	IMMUNE_CTRL	3156.0	1109	CTRL	
AAACATACGATGAA.1	IMMUNE_CTRL	1868.0	634	CTRL	


```

seurat_annotatons
AAACATACATTCC.1      CD14 Mono
AAACATACCAGAAA.1    CD14 Mono
AAACATACCTCGCT.1    CD14 Mono
AAACATACCTGGTA.1    pDC
AAACATACGATGAA.1    CD4 Memory T
```

```
[6]: adata.layers['counts'] = adata.X # Save raw counts
```

```
[7]: # preprocessing
sc.pp.normalize_total(adata, target_sum=1e4)
sc.pp.log1p(adata)
adata.layers['lognorm'] = adata.X
```

```
[8]: adata.obs.groupby('seurat_annotatons')['stim'].value_counts()
```

```
[8]: seurat_annotatons  stim
```

B	STIM	571
	CTRL	407
B Activated	STIM	203
	CTRL	185
CD14 Mono	CTRL	2215
	STIM	2147
CD16 Mono	STIM	537
	CTRL	507
CD4 Memory T	STIM	903
	CTRL	859
CD4 Naive T	STIM	1526
	CTRL	978
CD8 T	STIM	462
	CTRL	352

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DC	CTRL	258
	STIM	214
Eryth	STIM	32
	CTRL	23
Mk	STIM	121
	CTRL	115
NK	STIM	321
	CTRL	298
T activated	STIM	333
	CTRL	300
pDC	STIM	81
	CTRL	51

Name: stim, dtype: int64

```
[9]: # set STIM as class 0, CTRL as class 1, to make categorical
adata.obs['stim'] = pd.Categorical(adata.obs['stim'], categories=["STIM", "CTRL"],
↳ordered=True)
indices = adata.obs.sort_values(['seurat_annotatons', 'stim']).index
adata = adata[indices,:]
```

```
[10]: # # # subset and write GCT and CLS file
# outdir = "ifnb/"
# for cell in adata.obs.seurat_annotatons.unique():
#     bdata = adata[adata.obs.seurat_annotatons == cell ]
#     groups = bdata.obs['stim'].to_list()
#     cls_dict = bdata.obs['stim'].to_dict()
#     gs = bdata.to_df().T
#     gs.index.name = "NAME"

#     gs_std = gs.groupby(by=cls_dict, axis=1).std()
#     gs = gs[gs_std.sum(axis=1) > 0]
#     gs = gs + 1e-08 # we don't like zeros!!!

#     gs.insert(0, column="Description", value=cell,)
#     outname = os.path.join( outdir, cell + ".gct")
#     outcls = os.path.join(outdir, cell + ".cls")
#     s_len = gs.shape[1] - 1
#     with open(outname,"w") as correct:
#         line1="#1.2\n"+f"{gs.shape[0]}\t{s_len}\n"
#         correct.write(line1)
#         gs.to_csv(correct, sep="\t")

#     with open(outcls, "w") as cl:
#         line = f"{len(groups)} 2 1\n# STIM CTRL\n"
#         cl.write(line)
#         cl.write(" ".join(groups) + "\n")
#     print(outname)
```

```
[11]: # subset data
bdata = adata[adata.obs.seurat_annotatons == "CD14 Mono"].copy()
bdata
```

```
[11]: AnnData object with n_obs × n_vars = 4362 × 14053
      obs: 'orig.ident', 'nCount_RNA', 'nFeature_RNA', 'stim', 'seurat_annotatons'
      var: 'features'
      uns: 'log1p'
      layers: 'counts', 'lognorm'
```

5.4.2 GSEA

```
[12]: import time
      t1 = time.time()
      # NOTE: To speed up, use gp.prerank instead with your own ranked list.
      res = gp.gsea(data=bdata.to_df().T, # row -> genes, column-> samples
                  gene_sets="GO_Biological_Process_2021",
                  cls=bdata.obs.stim,
                  permutation_num=1000,
                  permutation_type='phenotype',
                  outdir=None,
                  method='s2n', # signal_to_noise
                  threads= 16)
      t2=time.time()
      print(t2-t1)

      60.238986015319824
```

```
[13]: res.res2d.head(10)
```

```
[13]:
```

	Name	Term	ES	\
0	gsea	cytokine-mediated signaling pathway (GO:0019221)	0.685491	
1	gsea	innate immune response (GO:0045087)	0.784391	
2	gsea	regulation of immune response (GO:0050776)	0.759354	
3	gsea	defense response to virus (GO:0051607)	0.903464	
4	gsea	response to cytokine (GO:0034097)	0.718931	
5	gsea	defense response to symbiont (GO:0140546)	0.904717	
6	gsea	cellular response to interferon-gamma (GO:0071...	0.792726	
7	gsea	regulation of interferon-beta production (GO:0...	0.856704	
8	gsea	RNA splicing, via transesterification reaction...	-0.626583	
9	gsea	gene expression (GO:0010467)	-0.70455	

	NES	NOM	p-val	FDR	q-val	FWER	p-val	Tag %	Gene %	\
0	3.759972		0.0		0.0	0.0	0.0	140/490	9.03%	
1	3.66143		0.0		0.0	0.0	0.0	56/188	6.30%	
2	3.549856		0.0		0.0	0.0	0.0	49/140	8.77%	
3	3.438759		0.0		0.0	0.0	0.0	42/108	2.85%	
4	3.37735		0.0		0.0	0.0	0.0	37/120	7.26%	
5	3.362051		0.0		0.0	0.0	0.0	49/100	4.90%	
6	3.327923		0.0		0.0	0.0	0.0	49/99	7.18%	
7	3.259412		0.0		0.0	0.0	0.0	14/44	4.94%	
8	-3.225436		0.0		0.0	0.0	0.0	128/234	19.45%	
9	-3.219153		0.0		0.0	0.0	0.0	134/322	10.13%	


```

                                Lead_genes
0  ISG15; IFIT3; IFIT1; RSAD2; ISG20; CXCL10; IFITM3; CX...
```

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```

1 ISG15;IFIT1;CXCL10;IFITM3;APOBEC3A;MX1;IFI6;OA...
2 RSAD2;IRF7;PLSCR1;HERC5;IL4I1;SLAMF7;IFITM1;HL...
3 ISG15;IFIT3;IFIT1;RSAD2;ISG20;CXCL10;IFITM3;AP...
4 ISG15;IFITM3;MX1;IFITM2;PLSCR1;MX2;BST2;EIF2AK...
5 ISG15;IFIT3;IFIT1;RSAD2;ISG20;IFITM3;APOBEC3A;...
6 CCL8;OAS1;MT2A;OASL;IRF7;GBP1;GBP4;CCL2;OAS3;O...
7 ISG15;OAS1;IRF7;DDX58;IFIH1;OAS3;OAS2;DHX58;HS...
8 YBX1;PABPC1;HNRNPA1;DDX5;SRSF9;HNRNPM;RBMX;SF3...
9 RPL6;RPL7;RPL15;RPL10;RPS3A;RPS6;RPL8;RPL21;RP...

```

```
[14]: res.ranking.shape # raking metric
```

```
[14]: (13216,)
```

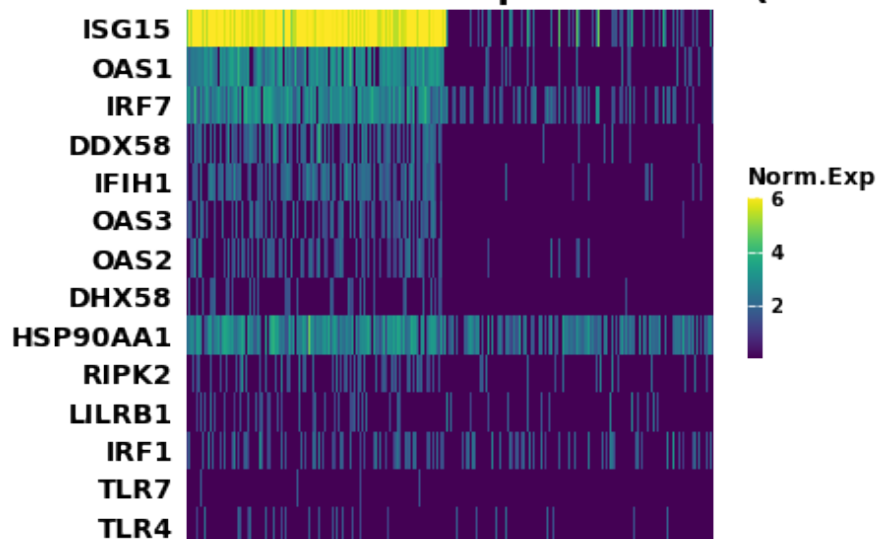
```
[15]: ## Heatmap of gene expression
```

```

i = 7
genes = res.res2d.Lead_genes.iloc[i].split(";")
ax = gp.heatmap(df = res.heatmap.loc[genes],
                z_score=None,
                title=res.res2d.Term.iloc[i],
                figsize=(6,5),
                cmap=plt.cm.viridis,
                xticklabels=False)

```

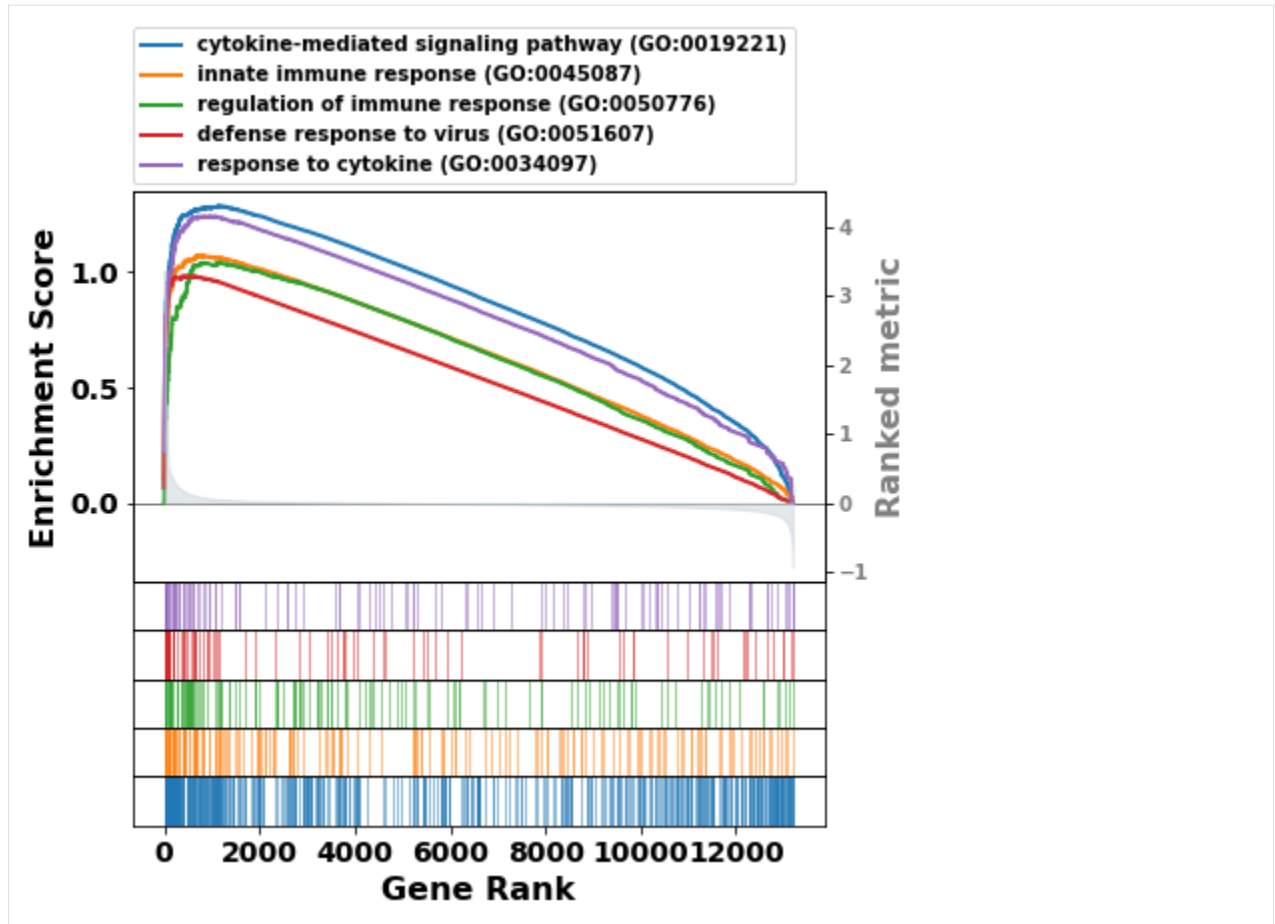
regulation of interferon-beta production (GO:0032648)



```

[16]: term = res.res2d.Term
# gp.gseaplot(res.ranking, term=term[i], **res.results[term[i]])
axs = res.plot(terms=term[:5])

```

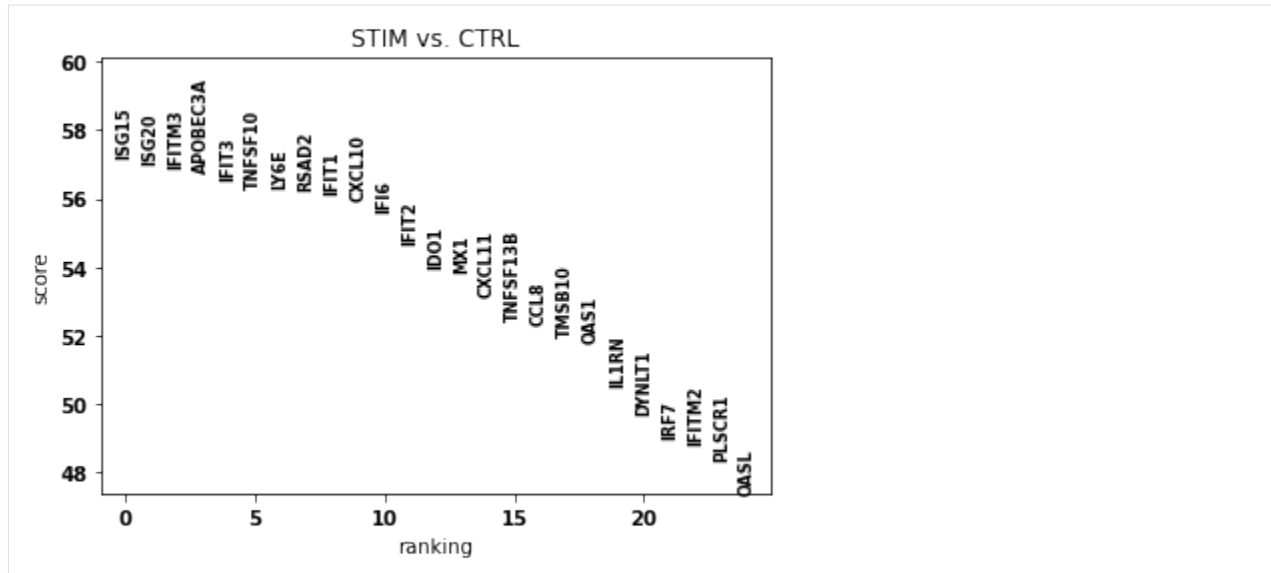
5.4.3 DEG Analysis

```
[17]: # find degs
sc.tl.rank_genes_groups(bdata,
                        groupby='stim',
                        use_raw=False,
                        layer='lognorm',
                        method='wilcoxon',
                        groups=["STIM"],
                        reference='CTRL')
```

```
[18]: bdata.X.max() # already log1p
```

```
[18]: 8.065909516515664
```

```
[19]: sc.pl.rank_genes_groups(bdata, n_genes=25, sharey=False)
```



```
[20]: # get deg result
result = bdata.ans['rank_genes_groups']
groups = result['names'].dtype.names
degs = pd.DataFrame(
    {group + '_' + key: result[key][group]
     for group in groups for key in ['names', 'scores', 'pvals', 'pvals_adj', 'logfoldchanges'
     ↪ ]})
```

```
[21]: degs.head()
```

```
[21]:  STIM_names  STIM_scores  STIM_pvals  STIM_pvals_adj  STIM_logfoldchanges
0      ISG15      57.165920         0.0           0.0           8.660480
1      ISG20      57.010372         0.0           0.0           6.850681
2      IFITM3      56.890392         0.0           0.0           6.320490
3  APOBEC3A      56.770397         0.0           0.0           6.616682
4      IFIT3      56.569122         0.0           0.0           8.313443
```

```
[22]: degs.shape
```

```
[22]: (14053, 5)
```

5.4.4 Over-representation analysis (Enrichr API)

```
[23]: # subset up or down regulated genes
degs_sig = degs[degs.STIM_pvals_adj < 0.05]
degs_up = degs_sig[degs_sig.STIM_logfoldchanges > 0]
degs_dw = degs_sig[degs_sig.STIM_logfoldchanges < 0]
```

```
[24]: degs_up.shape
```

```
[24]: (687, 5)
```

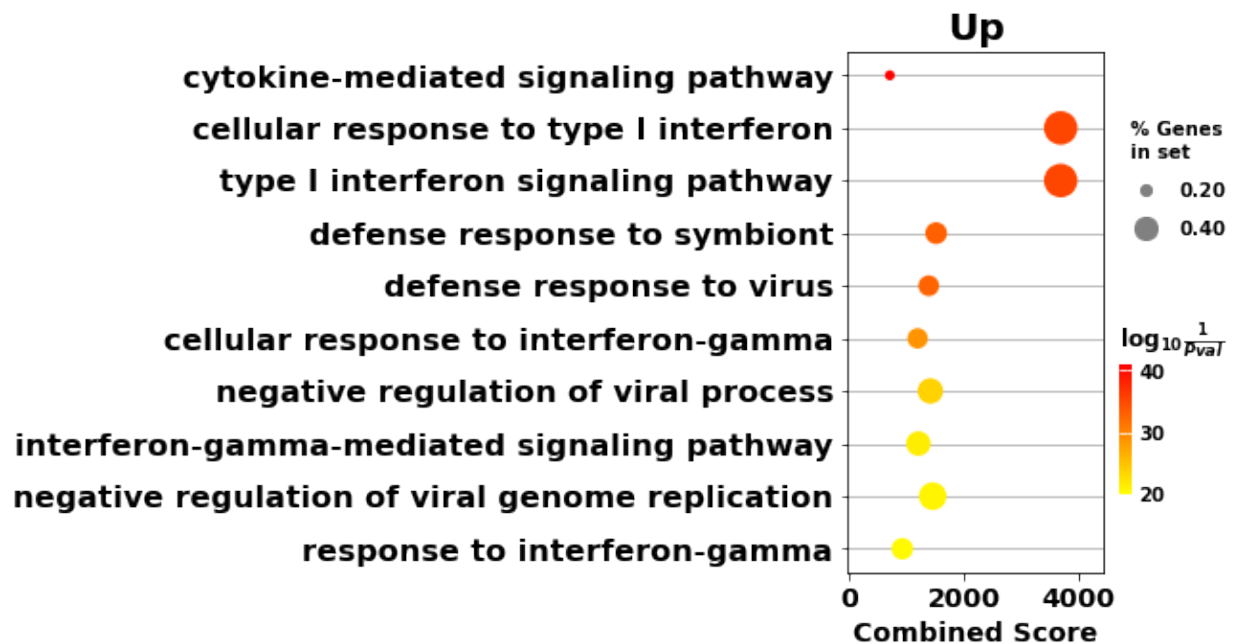
```
[25]: degs_dw.shape
```

```
[25]: (1030, 5)
```

```
[26]: # Enrichr API
enr_up = gp.enrichr(degs_up.STIM_names,
                    gene_sets='GO_Biological_Process_2021',
                    outdir=None)
```

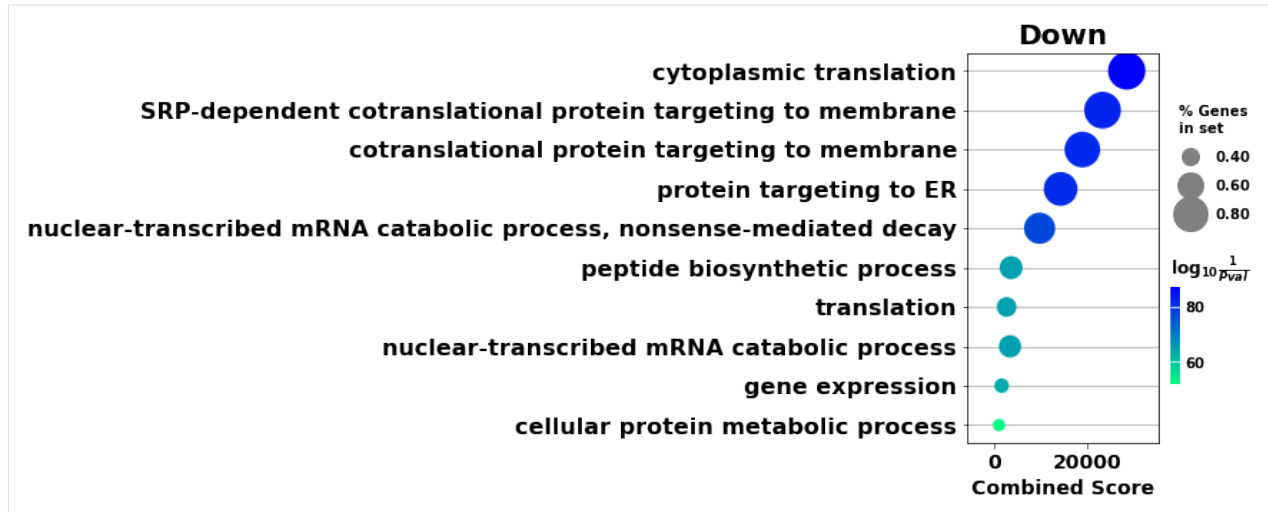
```
[27]: # trim (go:...)
enr_up.res2d.Term = enr_up.res2d.Term.str.split(" \((GO)").str[0]
```

```
[28]: # dotplot
gp.dotplot(enr_up.res2d, figsize=(3,5), title="Up", cmap = plt.cm.autumn_r)
plt.show()
```



```
[29]: enr_dw = gp.enrichr(degs_dw.STIM_names,
                          gene_sets='GO_Biological_Process_2021',
                          outdir=None)
```

```
[30]: enr_dw.res2d.Term = enr_dw.res2d.Term.str.split(" \((GO)").str[0]
gp.dotplot(enr_dw.res2d,
           figsize=(3,5),
           title="Down",
           cmap = plt.cm.winter_r,
           size=5)
plt.show()
```



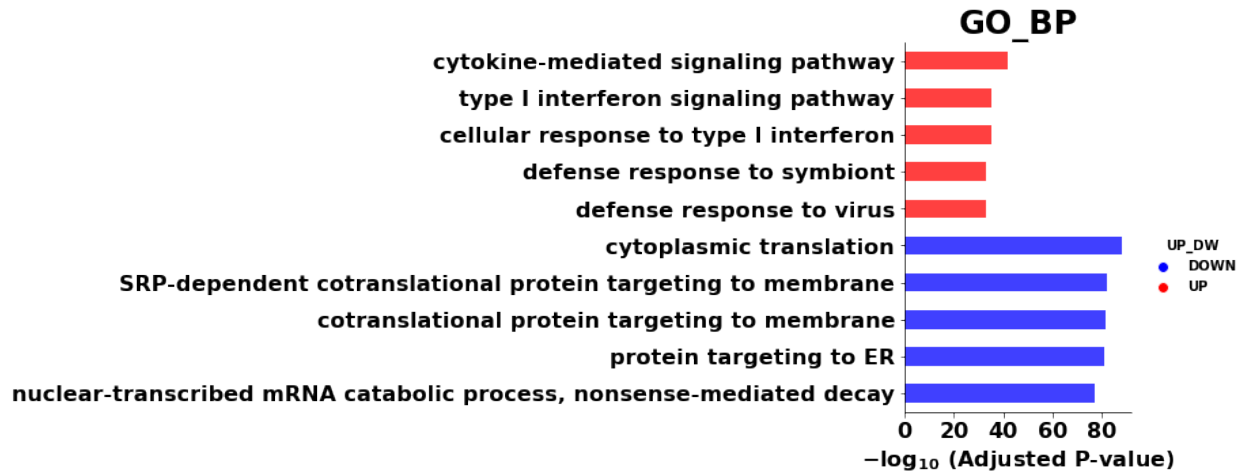
```
[31]: # concat results
enr_up.res2d['UP_DW'] = "UP"
enr_dw.res2d['UP_DW'] = "DOWN"
enr_res = pd.concat([enr_up.res2d.head(), enr_dw.res2d.head()])
```

```
[32]: from gseapy.scipalette import SciPalette
sci = SciPalette()
NbDr = sci.create_colormap()
# NbDr
```

```
[33]: # display multi-datasets
ax = gp.dotplot(enr_res, figsize=(3,5),
               x='UP_DW',
               x_order = ["UP", "DOWN"],
               title="GO_BP",
               cmap = NbDr.reversed(),
               size=3,
               show_ring=True)
ax.set_xlabel("")
plt.show()
```



```
[34]: ax = gp.barplot(enr_res, figsize=(3,5),
                    group = 'UP_DW',
                    title = "GO_BP",
                    color = ['b', 'r'])
```



5.4.5 Network Visualization

```
[35]: import networkx as nx
```

```
[36]: res.res2d.head()
```

```
[36]:
```

	Name	Term	ES	NES	\
0	gsea	cytokine-mediated signaling pathway (GO:0019221)	0.685491	3.759972	
1	gsea	innate immune response (GO:0045087)	0.784391	3.66143	
2	gsea	regulation of immune response (GO:0050776)	0.759354	3.549856	
3	gsea	defense response to virus (GO:0051607)	0.903464	3.438759	
4	gsea	response to cytokine (GO:0034097)	0.718931	3.37735	

	NOM p-val	FDR	q-val	FWER p-val	Tag %	Gene %	\
0	0.0	0.0	0.0	0.0	140/490	9.03%	
1	0.0	0.0	0.0	0.0	56/188	6.30%	
2	0.0	0.0	0.0	0.0	49/140	8.77%	
3	0.0	0.0	0.0	0.0	42/108	2.85%	
4	0.0	0.0	0.0	0.0	37/120	7.26%	

	Lead_genes
0	ISG15; IFIT3; IFIT1; RSAD2; ISG20; CXCL10; IFITM3; CX...
1	ISG15; IFIT1; CXCL10; IFITM3; APOBEC3A; MX1; IFI6; OA...
2	RSAD2; IRF7; PLSCR1; HERC5; IL4I1; SLAMF7; IFITM1; HL...
3	ISG15; IFIT3; IFIT1; RSAD2; ISG20; CXCL10; IFITM3; AP...
4	ISG15; IFITM3; MX1; IFITM2; PLSCR1; MX2; BST2; EIF2AK...

```
[37]: # res.res2d.to_csv("data/test.out.txt", sep="\t", index=False)
```

```
[38]: nodes, edges = gp.enrichment_map(res.res2d)
```

[39]: nodes.head()

```
[39]:
```

	Name	Term	ES	\
node_idx				
0	gsea	gene expression (GO:0010467)	-0.70455	
1	gsea	RNA splicing, via transesterification reaction...	-0.626583	
2	gsea	regulation of interferon-beta production (GO:0...	0.856704	
3	gsea	cellular response to interferon-gamma (GO:0071...	0.792726	
4	gsea	defense response to symbiont (GO:0140546)	0.904717	

	NES	NOM	p-val	FDR	q-val	FWER	p-val	Tag %	Gene %	\
node_idx										
0	-3.219153		0.0	0.000009		0.0		134/322	10.13%	
1	-3.225436		0.0	0.000009		0.0		128/234	19.45%	
2	3.259412		0.0	0.000009		0.0		14/44	4.94%	
3	3.327923		0.0	0.000009		0.0		49/99	7.18%	
4	3.362051		0.0	0.000009		0.0		49/100	4.90%	

	Lead_genes	p_inv	\
node_idx			
0	RPL6;RPL7;RPL15;RPL10;RPS3A;RPS6;RPL8;RPL21;RP...	5.061359	
1	YBX1;PABPC1;HNRNPA1;DDX5;SRSF9;HNRNPM;RBMX;SF3...	5.061359	
2	ISG15;OAS1;IRF7;DDX58;IFIH1;OAS3;OAS2;DHX58;HS...	5.061359	
3	CCL8;OAS1;MT2A;OASL;IRF7;GBP1;GBP4;CCL2;OAS3;O...	5.061359	
4	ISG15;IFIT3;IFIT1;RSAD2;ISG20;IFITM3;APOBEC3A;...	5.061359	

	Hits_ratio
node_idx	
0	0.416149
1	0.547009
2	0.318182
3	0.494949
4	0.490000

[40]: edges.head()

```
[40]:
```

	src_idx	targ_idx	src_name	\
0	0	1	gene expression (GO:0010467)	
1	0	8	gene expression (GO:0010467)	
2	1	8	RNA splicing, via transesterification reaction...	
3	2	3	regulation of interferon-beta production (GO:0...	
4	2	4	regulation of interferon-beta production (GO:0...	

	targ_name	jaccard_coef	\
0	RNA splicing, via transesterification reaction...	0.110169	
1	cellular macromolecule biosynthetic process (G...	0.645390	
2	cellular macromolecule biosynthetic process (G...	0.022624	
3	cellular response to interferon-gamma (GO:0071...	0.105263	
4	defense response to symbiont (GO:0140546)	0.188679	

	overlap_coef	overlap_genes
0	0.203125	POLR2F,RBM8A,HNRNPK,POLR2E,SYNCRIP,SRSF6,SRRM1...
1	0.928571	RPL26,RPL36,RPS2,RPL41,POLR2J,RPL24,RPL29,RPS2...

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```

2      0.051020          POLR2J,POLR2G,POLR2F,POLR2E,POLR2L
3      0.428571          IRF1,OAS2,OAS1,TLR4,OAS3,IRF7
4      0.714286  IRF1,TLR7,DDX58,OAS2,ISG15,LILRB1,OAS1,IFIH1,0...
```

```

[41]: # build graph
G = nx.from_pandas_edgelist(edges,
                           source='src_idx',
                           target='targ_idx',
                           edge_attr=['jaccard_coef', 'overlap_coef', 'overlap_genes'])

# Add missing node if there is any
for node in nodes.index:
    if node not in G.nodes():
        G.add_node(node)
```

```

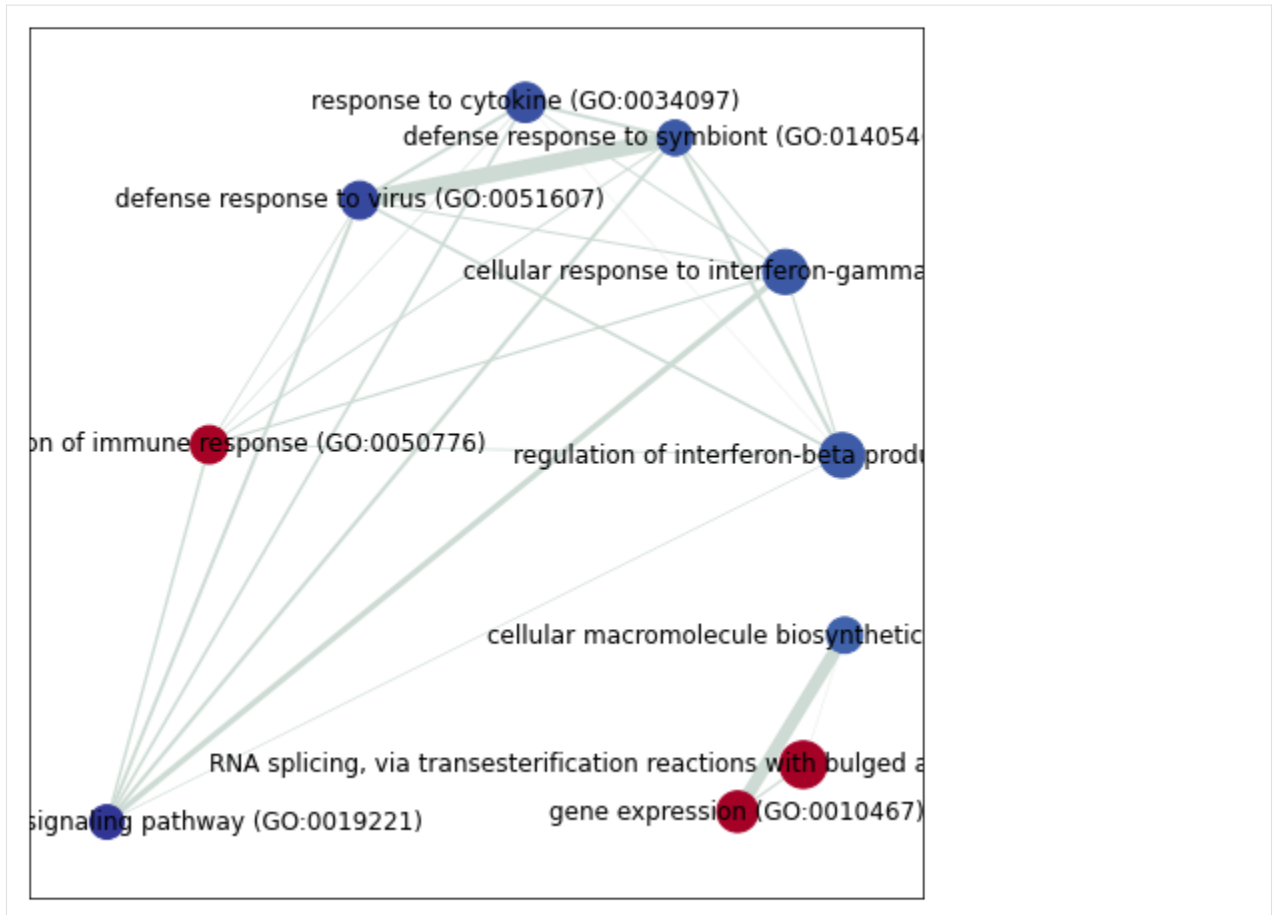
[42]: fig, ax = plt.subplots(figsize=(8, 8))

# init node coordinates
pos=nx.layout.spiral_layout(G)
#node_size = nx.get_node_attributes()
# draw node
nx.draw_networkx_nodes(G,
                       pos=pos,
                       cmap=plt.cm.RdYlBu,
                       node_color=list(nodes.NES),
                       node_size=list(nodes.Hits_ratio *1000))

# draw node label
nx.draw_networkx_labels(G,
                        pos=pos,
                        labels=nodes.Term.to_dict())

# draw edge
edge_weight = nx.get_edge_attributes(G, 'jaccard_coef').values()
nx.draw_networkx_edges(G,
                       pos=pos,
                       width=list(map(lambda x: x*10, edge_weight)),
                       edge_color='#CDDBD4')

plt.show()
```



[]:

5.5 A Protocol to Prepare files for GSEAPy

As a biological researcher, I like protocols.

Here is a short tutorial for you to walk you through gseapy.

For file format explanation, please see [here](#)

In order to run gseapy successfully, install gseapy use pip.

```
pip install gseapy
```

```
# if you have conda
```

```
conda install -c bioconda gseapy
```


5.5.1 Use gsea command, or gsea()

Follow the steps blow.

One thing you should know is that the gseapy input files are the same as GSEA desktop required. You can use these files below to run GSEA desktop, too.

Prepare an tabular text file of gene expression like this:

RNA-seq,ChIP-seq, Microarry data are all supported.

Here is to see what the structure of expression table looks like

```
import pandas as pd
df = pd.read_table('./test/gsea_data.txt')
df.head()

#or assign dataframe to the parameter 'data'
```

An cls file is also expected.

This file is used to specify column attributes in step 1, just like GSEA asked.

An example of cls file looks like below.

```
with open('gsea/edb/C10E.cls') as cls:
    print(cls.read())

# or assign a list object to parameter 'cls' like this
# cls=['C10E', 'C10E', 'C10E', 'Vector', 'Vector', 'Vector']
```

```
6 2 1
# C10E Vector
C10E C10E C10E Vector Vector Vector
```

The first line specify the total samples and phenotype numbers. Leave number 1 always be 1.

The second line specify the phenotype class(name).

The third line specify column attributes in step 1.

So you could prepare the cls file in python like this

```
groups = ['C10E', 'C10E', 'C10E', 'Vector', 'Vector', 'Vector']
with open('gsea/edb/C10E.cls', "w") as cl:
    line = f"{len(groups)} 2 1\n# C10E Vector\n"
    cl.write(line)
    cl.write(" ".join(groups) + "\n")
```

Gene_sets file in gmt format.

All you need to do is to download gene set database file from GSEA or Enrichr website.

Or you could use enrichr library. In this case, just provide library name to parameter 'gene_sets'

If you would like to use you own gene_sets.gmts files, build such a file use excel:

An example of gmt file looks like below:

```
with open('gsea/edb/gene_sets.gmt') as gmt:
    print(gmt.read())
```

```
ES-SPECIFIC Arid3a_used ACTA1 CALML4 CORO1A DHX58 DPYS EGR1 ESRRB GLI2
↳ GPX2 HCK INHBB
HDAC-UNIQUE Arid3a_used 1700017B05RIK 8430427H17RIK ABCA3 ANKRD44 ARL4A BNC2
↳ CLDN3
XEN-SPECIFIC Arid3a_used 1110036003RIK A130022J15RIK B2M B3GALNT1
↳ CBX4 CITED1 CLU CTSH CYP26A1
GATA-SPECIFIC Arid3a_used 1200009I06RIK 5430407P10RIK BAIAP2L1
↳ BMP8B CITED1 CLDN3 COBLL1 CORO1A CRYAB CTDSPL DKKL1
TS-SPECIFIC Arid3a_used 5430407P10RIK AFAP1L1 AHNAK ANXA2 ANXA3 ANXA5 B2M
↳ BIK BMP8B CAMK1D CBX4 CLDN3 CSRP1 DKKL1 DSC2
```

5.5.2 Use enrichr command, or enrichr()

The only thing you need to prepare is a gene list file.

Note: Enrichr uses a list of Entrez gene symbols as input.

For `enrichr`, you could assign a list object

```
# assign a list object to enrichr
l = ['SCARA3', 'LOC100044683', 'CMBL', 'CLIC6', 'IL13RA1', 'TACSTD2', 'DKKL1', 'CSF1',
     'SYNPO2L', 'TINAGL1', 'PTX3', 'BGN', 'HERC1', 'EFNA1', 'CIB2', 'PMP22', 'TMEM173']

gseapy.enrichr(gene_list=l, gene_sets='KEGG_2016', outfile='test')
```

or a gene list file in txt format(one gene id per row)

```
gseapy.enrichr(gene_list='gene_list.txt', gene_sets='KEGG_2016', outfile='test')
```

Let's see what the txt file looks like.

```
with open('data/gene_list.txt') as genes:
    print(genes.read())
```

```
CTLA2B
SCARA3
LOC100044683
CMBL
CLIC6
IL13RA1
TACSTD2
```

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```
DKKL1
CSF1
CITED1
SYNPO2L
TINAGL1
PTX3
```

Select the library you want to do enrichment analysis. To get a list of all available libraries, run

```
#s get_library_name(), it will print out all library names.
import gseapy
names = gseapy.get_library_name()
print(names)
```

```
['Genome_Browser_PWMs',
'TRANSFAC_and_JASPAR_PWMs',
'ChEA_2013',
'Drug_Perturbations_from_GEO_2014',
'ENCODE_TF_ChIP-seq_2014',
'BioCarta_2013',
'Reactome_2013',
'WikiPathways_2013',
'Disease_Signatures_from_GEO_up_2014',
'KEGG_2013',
'TF-LOF_Expression_from_GEO',
'TargetScan_microRNA',
'PPI_Hub_Proteins',
'GO_Molecular_Function_2015',
'GeneSigDB',
'Chromosome_Location',
'Human_Gene_Atlas',
'Mouse_Gene_Atlas',
'GO_Cellular_Component_2015',
'GO_Biological_Process_2015',
'Human_Phenotype_Ontology',
'Epigenomics_Roadmap_HM_ChIP-seq',
'KEA_2013',
'NURSA_Human_Endogenous_Complexome',
'CORUM',
'SILAC_Phosphoproteomics',
'MGI_Mammalian_Phenotype_Level_3',
'MGI_Mammalian_Phenotype_Level_4',
'Old_CMAP_up',
'Old_CMAP_down',
'OMIM_Disease',
'OMIM_Expanded',
'VirusMINT',
'MSigDB_Computational',
'MSigDB_Oncogenic_Signatures',
'Disease_Signatures_from_GEO_down_2014',
'Virus_Perturbations_from_GEO_up',
'Virus_Perturbations_from_GEO_down',
```

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```
'Cancer_Cell_Line_Encyclopedia',
'NCI-60_Cancer_Cell_Lines',
'Tissue_Protein_Expression_from_ProteomicsDB',
'Tissue_Protein_Expression_from_Human_Proteome_Map',
'HMDB_Metabolites',
'Pfam_InterPro_Domains',
'GO_Biological_Process_2013',
'GO_Cellular_Component_2013',
'GO_Molecular_Function_2013',
'Allen_Brain_Atlas_up',
'ENCODE_TF_ChIP-seq_2015',
'ENCODE_Histone_Modifications_2015',
'Phosphatase_Substrates_from_DEPOD',
'Allen_Brain_Atlas_down',
'ENCODE_Histone_Modifications_2013',
'Achilles_fitness_increase',
'Achilles_fitness_decrease',
'MGI_Mammalian_Phenotype_2013',
'BioCarta_2015',
'HumanCyc_2015',
'KEGG_2015',
'NCI-Nature_2015',
'Panther_2015',
'WikiPathways_2015',
'Reactome_2015',
'ESCAPE',
'HomoloGene',
'Disease_Perturbations_from_GEO_down',
'Disease_Perturbations_from_GEO_up',
'Drug_Perturbations_from_GEO_down',
'Genes_Associated_with_NIH_Grants',
'Drug_Perturbations_from_GEO_up',
'KEA_2015',
'Single_Gene_Perturbations_from_GEO_up',
'Single_Gene_Perturbations_from_GEO_down',
'ChEA_2015',
'dbGaP',
'LINCS_L1000_Chem_Pert_up',
'LINCS_L1000_Chem_Pert_down',
'GTEx_Tissue_Sample_Gene_Expression_Profiles_down',
'GTEx_Tissue_Sample_Gene_Expression_Profiles_up',
'Ligand_Perturbations_from_GEO_down',
'Aging_Perturbations_from_GEO_down',
'Aging_Perturbations_from_GEO_up',
'Ligand_Perturbations_from_GEO_up',
'MCF7_Perturbations_from_GEO_down',
'MCF7_Perturbations_from_GEO_up',
'Microbe_Perturbations_from_GEO_down',
'Microbe_Perturbations_from_GEO_up',
'LINCS_L1000_Ligand_Perturbations_down',
'LINCS_L1000_Ligand_Perturbations_up',
'LINCS_L1000_Kinase_Perturbations_down',
```

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```
'LINCS_L1000_Kinase_Perturbations_up',
'Reactome_2016',
'KEGG_2016',
'WikiPathways_2016',
'ENCODE_and_ChEA_Consensus_TFs_from_ChIP-X',
'Kinase_Perturbations_from_GEO_down',
'Kinase_Perturbations_from_GEO_up',
'BioCarta_2016',
'Humancyc_2016',
'NCI-Nature_2016',
'Panther_2016']
```

For more details, please track the official links: <http://amp.pharm.mssm.edu/Enrichr/>

5.5.3 Use replot Command, or replot()

You may also want to use `replot()` to reproduce GSEA desktop plots.

The only input of `replot()` is the directory of GSEA desktop output.

The input directory(e.g. `gsea`), must contained **edb** folder, gseapy need 4 data files inside edb folder.The `gsea` document tree looks like this:

```
gsea
├── edb
│   ├── test.cls
│   ├── gene_sets.gmt
│   ├── gsea_data.rnk
│   └── results.edb
```

After this, you can start to run `gseapy`.

```
import gseapy
gseapy.replot(indir='gsea', outdir='gseapy_out')
```

If you prefer to run in command line, it's more simple.

```
gseapy replot -i gsea -o gseapy_out
```

For advanced usage of library, see the *Developmental Guide*.

5.6 Developmental Guide

5.6.1 Module APIs

`gseapy.gsea()`

Run Gene Set Enrichment Analysis.

Parameters

- **data** – Gene expression data table, Pandas DataFrame, gct file.
- **gene_sets** – Enrichr Library name or .gmt gene sets file or dict of gene sets. Same input with GSEA.
- **cls** – A list or a .cls file format required for GSEA.
- **outdir** (*str*) – Results output directory. If None, nothing will write to disk.
- **permutation_num** (*int*) – Number of permutations. Default: 1000. Minimal possible nominal p-value is about 1/nperm.
- **permutation_type** (*str*) – Type of permutation reshuffling, choose from {"phenotype": 'sample.labels', "gene_set": gene.labels}.
- **min_size** (*int*) – Minimum allowed number of genes from gene set also the data set. Default: 15.
- **max_size** (*int*) – Maximum allowed number of genes from gene set also the data set. Default: 500.
- **weight** (*float*) – Refer to `algorithm.enrichment_score()`. Default:1.
- **method** – The method used to calculate a correlation or ranking. Default: 'signal_to_noise'. Others methods are:
 1. 'signal_to_noise'

You must have at least three samples for each phenotype to use this metric. The larger the signal-to-noise ratio, the larger the differences of the means (scaled by the standard deviations); that is, the more distinct the gene expression is in each phenotype and the more the gene acts as a "class marker."
 2. 't_test'

Uses the difference of means scaled by the standard deviation and number of samples. Note: You must have at least three samples for each phenotype to use this metric. The larger the tTest ratio, the more distinct the gene expression is in each phenotype and the more the gene acts as a "class marker."
 3. 'ratio_of_classes' (also referred to as fold change).

Uses the ratio of class means to calculate fold change for natural scale data.
 4. 'diff_of_classes'

Uses the difference of class means to calculate fold change for nature scale data
 5. 'log2_ratio_of_classes'

Uses the log2 ratio of class means to calculate fold change for natural scale data. This is the recommended statistic for calculating fold change for log scale data.
- **ascending** (*bool*) – Sorting order of rankings. Default: False.
- **threads** (*int*) – Number of threads you are going to use. Default: 4.
- **figsize** (*list*) – Matplotlib figsize, accept a tuple or list, e.g. [width,height]. Default: [6.5,6].
- **format** (*str*) – Matplotlib figure format. Default: 'pdf'.
- **graph_num** (*int*) – Plot graphs for top sets of each phenotype.
- **no_plot** (*bool*) – If equals to True, no figure will be drawn. Default: False.
- **seed** – Random seed. expect an integer. Default:None.

- **verbose** (*bool*) – Bool, increase output verbosity, print out progress of your job, Default: False.

Returns

Return a GSEA obj. All results store to a dictionary, obj.results, where contains:

```
| {
|   term: gene set name,
|   es: enrichment score,
|   nes: normalized enrichment score,
|   pval: Nominal p-value (from the null distribution of the gene set,
|   fdr: FDR qvalue (adjusted False Discory Rate),
|   fwerp: Family wise error rate p-values,
|   tag %: Percent of gene set before running enrichment peak (ES),
|   gene %: Percent of gene list before running enrichment peak (ES),
|   lead_genes: leading edge genes (gene hits before running enrichment.
|   ↪peak),
|   matched genes: genes matched to the data,
| }
```

gseapy.prerank()

Run Gene Set Enrichment Analysis with pre-ranked correlation defined by user.

Parameters

- **rnk** – pre-ranked correlation table or pandas DataFrame. Same input with GSEA .rnk file.
- **gene_sets** – Enrichr Library name or .gmt gene sets file or dict of gene sets. Same input with GSEA.
- **outdir** – results output directory. If None, nothing will write to disk.
- **permutation_num** (*int*) – Number of permutations. Default: 1000. Minimal possible nominal p-value is about 1/nperm.
- **min_size** (*int*) – Minimum allowed number of genes from gene set also the data set. Default: 15.
- **max_size** (*int*) – Maximum allowed number of genes from gene set also the data set. Defaults: 500.
- **weight** (*str*) – Refer to `algorithm.enrichment_score()`. Default: 1.
- **ascending** (*bool*) – Sorting order of rankings. Default: False.
- **threads** (*int*) – Number of threads you are going to use. Default: 4.
- **figsize** (*list*) – Matplotlib figsize, accept a tuple or list, e.g. [width,height]. Default: [6.5,6].
- **format** (*str*) – Matplotlib figure format. Default: 'pdf'.
- **graph_num** (*int*) – Plot graphs for top sets of each phenotype.
- **no_plot** (*bool*) – If equals to True, no figure will be drawn. Default: False.
- **seed** – Random seed. expect an integer. Default: None.
- **verbose** (*bool*) – Bool, increase output verbosity, print out progress of your job, Default: False.

Returns

Return a Prerank obj. All results store to a dictionary, obj.results, where contains:

```
| {
|   term: gene set name,
|   es: enrichment score,
|   nes: normalized enrichment score,
|   pval: Nominal p-value (from the null distribution of the gene set,
|   fdr: FDR qvalue (adjusted False Discory Rate),
|   fwerp: Family wise error rate p-values,
|   tag %: Percent of gene set before running enrichment peak (ES),
|   gene %: Percent of gene list before running enrichment peak (ES),
|   lead_genes: leading edge genes (gene hits before running enrichment_
↪peak),
|   matched genes: genes matched to the data,
| }
```

gseapy.ssgsea()

Run Gene Set Enrichment Analysis with single sample GSEA tool

Parameters

- **data** – Expression table, pd.Series, pd.DataFrame, GCT file, or .rnk file format.
- **gene_sets** – Enrichr Library name or .gmt gene sets file or dict of gene sets. Same input with GSEA.
- **outdir** – Results output directory. If None, nothing will write to disk.
- **sample_norm_method** (*str*) – Sample normalization method. Choose from {'rank', 'log', 'log_rank', None}. Default: rank. this argument will be used for ordering genes.
 1. 'rank': Rank your expression data, and transform by $10000 * \text{rank_dat} / \text{gene_numbers}$
 2. 'log' : Do not rank, but transform data by $\log(\text{data} + \exp(1))$, while $\text{data} = \text{data}[\text{data} < 1] = 1$.
 3. 'log_rank': Rank your expression data, and transform by $\log(10000 * \text{rank_dat} / \text{gene_numbers} + \exp(1))$
 4. None or 'custom': Do nothing, and use your own rank value to calculate enrichment score.

see here: <https://github.com/GSEA-MSigDB/ssGSEAProjection-gpmodule/blob/master/src/ssGSEAProjection.Library.R>, line 86

Parameters

- **correl_norm_type** (*str*) – correlation normalization type. Choose from {'rank', 'sym-rank', 'zscore', None}. Default: rank. After ordering genes by sample_norm_method, further data transformed could be applied to get enrichment score.

when weight == 0, sample_norm_method and correl_norm_type do not matter; when weight > 0, the combination of sample_norm_method and correl_norm_type dictate how the gene expression values in input data are transformed to obtain the score – use this setting with care (the transformations can skew scores towards +ve or -ve values)

sample_norm_method will first transformed and rank original data. the data is named correl_vector for each sample. then correl_vector is transformed again by

1. correl_norm_type is None or 'rank' : do nothing, genes are weighted by actual correl_vector.

2. `correl_norm_type == 'symrank'`: symmetric ranking.
 3. `correl_norm_type == 'zscore'`: standardizes the `correl_vector` before using them to calculate scores.
- **`min_size`** (*int*) – Minimum allowed number of genes from gene set also the data set. Default: 15.
 - **`max_size`** (*int*) – Maximum allowed number of genes from gene set also the data set. Default: 2000.
 - **`permutation_num`** (*int*) – For ssGSEA, default is 0. However, if you try to use `ssgsea` method to get `pval` and `fdr`, set to an interger.
 - **`weight`** (*str*) – Refer to `algorithm.enrichment_score()`. Default:0.25.
 - **`ascending`** (*bool*) – Sorting order of rankings. Default: False.
 - **`threads`** (*int*) – Number of threads you are going to use. Default: 4.
 - **`figsize`** (*list*) – Matplotlib figsize, accept a tuple or list, e.g. [width,height]. Default: [7,6].
 - **`format`** (*str*) – Matplotlib figure format. Default: 'pdf'.
 - **`graph_num`** (*int*) – Plot graphs for top sets of each phenotype.
 - **`no_plot`** (*bool*) – If equals to True, no figure will be drawn. Default: False.
 - **`seed`** – Random seed. expect an integer. Default:None.
 - **`verbose`** (*bool*) – Bool, increase output verbosity, print out progress of your job, Default: False.

Returns

Return a ssGSEA obj. All results store to a dictionary, access enrichment score by `obj.resultsOnSamples`, and normalized enrichment score by `obj.res2d`. if `permutation_num > 0`, additional results contain:

```
{
  term: gene set name,
  es: enrichment score,
  nes: normalized enrichment score,
  pval: Nominal p-value (from the null distribution of the gene set,
  ↪(if permutation_num > 0),
  fdr: FDR qvalue (adjusted FDR) (if permutation_num > 0),
  fwerp: Family wise error rate p-values (if permutation_num > 0),
  tag %: Percent of gene set before running enrichment peak (ES),
  gene %: Percent of gene list before running enrichment peak (ES),
  lead_genes: leading edge genes (gene hits before running enrichment,
  ↪peak),
  matched genes: genes matched to the data,
}
```

`gseapy.enrichr()`

Enrichr API.

Parameters

- **`gene_list`** – str, list, tuple, series, dataframe. Also support input txt file with one gene id per row. The input *identifier* should be the same type to *gene_sets*.

- **gene_sets** – str, list, tuple of Enrichr Library name(s). or custom defined gene_sets (dict, or gmt file).

Examples:

Input Enrichr Libraries (<https://maayanlab.cloud/Enrichr/#stats>):

str: 'KEGG_2016' list: ['KEGG_2016','KEGG_2013'] Use comma to separate each other, e.g. "KEGG_2016,huMAP,GO_Biological_Process_2018"

Input custom files:

dict: gene_sets={'A':['gene1', 'gene2',...],
'B':['gene2', 'gene4',...], ... }

gmt: "genes.gmt"

see also the online docs: https://gseapy.readthedocs.io/en/latest/gseapy_example.html#2.-Enrichr-Example

- **organism** – Enrichr supported organism. Select from (human, mouse, yeast, fly, fish, worm). This argument only affects the Enrichr library names you've chosen. No any affects to gmt or dict input of *gene_sets*.

see here for more details: <https://maayanlab.cloud/modEnrichr/>.

- **outdir** – Output file directory
- **background** – int, list, str. Background genes. This argument works only if *gene_sets* has a type Dict or gmt file. If your input are just Enrichr library names, this argument will be ignored.

However, this argument is not straightforward when *gene_sets* is given a custom input (a gmt file or dict).

By default, all genes listed in the *gene_sets* input will be used as background.

There are 3 ways to tune this argument:

- (1) (Recommended) Input a list of background genes: ['gene1', 'gene2',...] The background gene list is defined by your experment. e.g. the expressed genes in your RNA-seq. The gene identifier in gmt/dict should be the same type to the background genes.
- (2) Specify a number: e.g. 20000. (the number of total expressed genes). This works, but not recommend. It assumes that all your genes could be found in background. If genes exist in gmt but not included in background provided, they will affect the significance of the statistical test.
- (3) Set a Biomart dataset name: e.g. "hsapiens_gene_ensembl" The background will be all annotated genes from the *BioMart datasets* you've chosen. The program will try to retrieve the background information automatically.

Enrichr module use the code below to get the background genes:

```
>>> from gseapy.parser import Biomart
>>> bm = Biomart()
>>> df = bm.query(dataset=background, # e.g. 'hsapiens_gene_
↳ ensembl'
                    attributes=['ensembl_gene_id', 'external_gene_name',
↳ 'entrezgene_id'],
                    filename=f'~/ .cache/gseapy/{background}.background.
↳ genes.txt')
>>> df.dropna(subset=["entrezgene_id"], inplace=True)
```

So only genes with entrezid above will be the background genes if not input specify by user.

- **cutoff** – Show enriched terms which Adjusted P-value < cutoff. Only affects the output figure, not the final output file. Default: 0.05
- **format** – Output figure format supported by matplotlib, ('pdf', 'png', 'eps'...). Default: 'pdf'.
- **figsize** – Matplotlib figsize, accept a tuple or list, e.g. (width,height). Default: (6.5,6).
- **no_plot** (*bool*) – If equals to True, no figure will be drawn. Default: False.
- **verbose** (*bool*) – Increase output verbosity, print out progress of your job, Default: False.

Returns

An Enrichr object, which `obj.res2d` stores your last query, `obj.results` stores your all queries.

`gseapy.enrich()`

Perform over-representation analysis (hypergeometric test).

Parameters

- **gene_list** – str, list, tuple, series, dataframe. Also support input txt file with one gene id per row. The input *identifier* should be the same type to *gene_sets*.
- **gene_sets** – str, list, tuple of Enrichr Library name(s). or custom defined *gene_sets* (dict, or gmt file).

Examples:

```
dict: gene_sets={'A':['gene1', 'gene2',...],
                'B':['gene2', 'gene4',...], ... }
```

```
gmt: "genes.gmt"
```

- **outdir** – Output file directory
- **background** – None | int | list | str. Background genes. This argument works only if *gene_sets* has a type Dict or gmt file.

However, this argument is not straightforward when *gene_sets* is given a custom input (a gmt file or dict).

By default, all genes listed in the *gene_sets* input will be used as background.

There are 3 ways to tune this argument:

- (1) (Recommended) Input a list of background genes: ['gene1', 'gene2',...] The background gene list is defined by your experiment. e.g. the expressed genes in your RNA-seq. The gene identifier in gmt/dict should be the same type to the background genes.
- (2) Specify a number: e.g. 20000. (the number of total expressed genes). This works, but not recommend. It assumes that all your genes could be found in background. If genes exist in gmt but not included in background provided, they will affect the significance of the statistical test.
- (3) Set a Biomart dataset name: e.g. "hsapiens_gene_ensembl" The background will be all annotated genes from the *BioMart datasets* you've chosen. The program will try to retrieve the background information automatically.

Enrichr module use the code below to get the background genes:

```
>>> from gseapy.parser import Biomart
>>> bm = Biomart()
>>> df = bm.query(dataset=background, # e.g. 'hsapiens_gene_
↳ensembl'
           attributes=['ensembl_gene_id', 'external_gene_name',
↳ 'entrezgene_id'],
           filename=f'~/ .cache/gseapy/{background}.background.
↳genes.txt')
>>> df.dropna(subset=["entrezgene_id"], inplace=True)
```

So only genes with entrezid above will be the background genes if not input specify by user.

- **cuttoff** – Show enriched terms which Adjusted P-value < cutoff. Only affects the output figure, not the final output file. Default: 0.05
- **format** – Output figure format supported by matplotlib, ('pdf', 'png', 'eps'...). Default: 'pdf'.
- **figsize** – Matplotlib figsize, accept a tuple or list, e.g. (width,height). Default: (6.5,6).
- **no_plot** (*bool*) – If equals to True, no figure will be drawn. Default: False.
- **verbose** (*bool*) – Increase output verbosity, print out progress of your job, Default: False.

Returns

An Enrichr object, which obj.res2d stores your last query, obj.results stores your all queries.

gseapy.replot()

The main function to reproduce GSEA desktop outputs.

Parameters

- **indir** – GSEA desktop results directory. In the sub folder, you must contain edb file folder.
- **outdir** – Output directory.
- **weight** (*float*) – weighted score type. choose from {0,1,1.5,2}. Default: 1.
- **figsize** (*list*) – Matplotlib output figure figsize. Default: [6.5,6].
- **format** (*str*) – Matplotlib output figure format. Default: 'pdf'.
- **min_size** (*int*) – Min size of input genes presented in Gene Sets. Default: 3.
- **max_size** (*int*) – Max size of input genes presented in Gene Sets. Default: 5000. You are not encouraged to use min_size, or max_size argument in *replot()* function. Because gmt file has already been filtered.
- **verbose** – Bool, increase output verbosity, print out progress of your job, Default: False.

Returns

Generate new figures with selected figure format. Default: 'pdf'.

5.6.2 GSEA Statistics

```
class gseapy.gsea.GSEA(data: DataFrame | str, gene_sets: List[str] | str | Dict[str, str], classes: List[str] | str | Dict[str, str], outdir: str | None = None, min_size: int = 15, max_size: int = 500, permutation_num: int = 1000, weight: float = 1.0, permutation_type: str = 'phenotype', method: str = 'signal_to_noise', ascending: bool = False, threads: int = 1, figsize: Tuple[float, float] = (6.5, 6), format: str = 'pdf', graph_num: int = 20, no_plot: bool = False, seed: int = 123, verbose: bool = False)
```

GSEA main tool

```
calc_metric(df: DataFrame, method: str, pos: str, neg: str, classes: Dict[str, str], ascending: bool) → Tuple[List[int], Series]
```

The main function to rank an expression table. works for 2d array.

Parameters

- **df** – gene_expression DataFrame.
- **method** – The method used to calculate a correlation or ranking. Default: 'log2_ratio_of_classes'. Others methods are:
 1. 'signal_to_noise' (s2n) or 'abs_signal_to_noise' (abs_s2n)

You must have at least three samples for each phenotype. The more distinct the gene expression is in each phenotype, the more the gene acts as a “class marker”.
 2. 't_test'

Uses the difference of means scaled by the standard deviation and number of samples. Note: You must have at least three samples for each phenotype to use this metric. The larger the t-test ratio, the more distinct the gene expression is in each phenotype and the more the gene acts as a “class marker.”
 3. 'ratio_of_classes' (also referred to as fold change).

Uses the ratio of class means to calculate fold change for natural scale data.
 4. 'diff_of_classes'

Uses the difference of class means to calculate fold change for natural scale data
 5. 'log2_ratio_of_classes'

Uses the log2 ratio of class means to calculate fold change for natural scale data. This is the recommended statistic for calculating fold change for log scale data.
- **pos** (*str*) – one of labels of phenotype's names.
- **neg** (*str*) – one of labels of phenotype's names.
- **classes** (*dict*) – column id to group mapping.
- **ascending** (*bool*) – bool or list of bool. Sort ascending vs. descending.

Returns

returns argsort values of a tuple where 0: argsort positions (indices) 1: pd.Series of correlation value. Gene_name is index, and value is rankings.

visit here for more docs: <http://software.broadinstitute.org/gsea/doc/GSEAUUserGuideFrame.html>

```
load_classes(classes: str | List[str] | Dict[str, Any])
```

Parse group (classes)

load_data() → Tuple[DataFrame, Dict]

pre-processed the data frame.new filtering methods will be implement here.

run()

GSEA main procedure

```
class gseapy.gsea.Prerank(rnk: DataFrame | Series | str, gene_sets: List[str] | str | Dict[str, str], outdir: str | None = None, pheno_pos='Pos', pheno_neg='Neg', min_size: int = 15, max_size: int = 500, permutation_num: int = 1000, weight: float = 1.0, ascending: bool = False, threads: int = 1, figsize: Tuple[float, float] = (6.5, 6), format: str = 'pdf', graph_num: int = 20, no_plot: bool = False, seed: int = 123, verbose: bool = False)
```

GSEA prerank tool

load_ranking()

parse rnk input

run()

GSEA prerank workflow

```
class gseapy.gsea.Replot(indir: str, outdir: str = 'GSEApY_Replot', weight: float = 1.0, min_size: int = 3, max_size: int = 1000, figsize: Tuple[float, float] = (6.5, 6), format: str = 'pdf', verbose: bool = False)
```

To reproduce GSEA desktop output results.

gsea_edb_parser(results_path)

Parse results.edb file stored under **edb** file folder.

Parameters

results_path – the path of results.edb file.

Returns

a dict contains { enrichment_term: [es, nes, pval, fdr, fwer, hit_ind]}

run()

main replot function

```
class gseapy.base.GMT(mapping: Dict[str, str] | None = None, description: str | None = None)
```

apply(func)

apply function in place

write(ofname: str)

write gmt file to disk

```
class gseapy.base.GSEAbase(outdir: str | None = None, gene_sets: List[str] | str | Dict[str, str] = 'KEGG_2016', module: str = 'base', threads: int = 1, enrichr_url: str = 'http://maayanlab.cloud', verbose: bool = False)
```

base class of GSEA.

```
enrichment_score(gene_list: Iterable[str], correl_vector: Iterable[float], gene_set: Dict[str, List[str]], weight: float = 1.0, nperm: int = 1000, seed: int = 123, single: bool = False, scale: bool = False)
```

This is the most important function of GSEApY. It has the same algorithm with GSEA and ssGSEA.

Parameters

- **gene_list** – The ordered gene list gene_name_list, rank_metric.index.values

- **gene_set** – gene_sets in gmt file, please use gmt_parser to get gene_set.
- **weight** – It's the same with gsea's weighted_score method. Weighting by the correlation is a very reasonable choice that allows significant gene sets with less than perfect coherence. options: 0(classic),1,1.5,2. default:1. if one is interested in penalizing sets for lack of coherence or to discover sets with any type of nonrandom distribution of tags, a value $p < 1$ might be appropriate. On the other hand, if one uses sets with large number of genes and only a small subset of those is expected to be coherent, then one could consider using $p > 1$. Our recommendation is to use $p = 1$ and use other settings only if you are very experienced with the method and its behavior.
- **correl_vector** – A vector with the correlations (e.g. signal to noise scores) corresponding to the genes in the gene list. Or rankings, rank_metric.values
- **nperm** – Only use this parameter when computing esnull for statistical testing. Set the esnull value equal to the permutation number.
- **seed** – Random state for initializing gene list shuffling. Default: seed=None

Returns

ES: Enrichment score (real number between -1 and +1)

ESNULL: Enrichment score calculated from random permutations.

Hits_Indices: Index of a gene in gene_list, if gene is included in gene_set.

RES: Numerical vector containing the running enrichment score for all locations in the gene list .

get_libraries() → List[str]

return active enrichr library name. Official API

load_gmt(gene_list: Iterable[str], gmt: List[str] | str | Dict[str, str]) → Dict[str, List[str]]

load gene set dict

load_gmt_only(gmt: List[str] | str | Dict[str, str]) → Dict[str, List[str]]

parse gene_sets. gmt: List, Dict, Strings

However, this function will merge different gene sets into one big dict to save computation time for later.

make_unique(rank_metric: DataFrame, col_idx: int) → DataFrame

make gene id column unique by adding a digit, similar to R's make.unique

parse_gmt(gmt: str) → Dict[str, List[str]]

gmt parser when input is a string

plot(terms: str | List[str], colors: str | List[str] | None = None, legend_kws: Dict[str, Any] | None = None, figsize: Tuple[float, float] = (4, 5), show_ranking: bool = True, ofname: str | None = None)

terms: str, list. terms/pathways to show colors: str, list. list of colors for each term/pathway legend_kws: kwargs to pass to ax.legend. e.g. loc, bbox_to_anchor. ofname: savefig

prepare_outdir()

create temp directory.

property results

compatible to old style

to_df(gsea_summary: List[Dict], gmt: Dict[str, List[str]], rank_metric: Series | DataFrame, indices: List | None = None)

Convert GSEASummary to DataFrame

rank_metric: if a Series, then it must be sorted in descending order already
if a DataFrame, indices must not None.

indices: Only works for DataFrame input. Stores the indices of sorted array

5.6.3 Over-representation Statistics

`gseapy.stats.calc_pvalues(query, gene_sets, background=20000, **kwargs)`

calculate pvalues for all categories in the graph

Parameters

- **query** (*set*) – set of identifiers for which the p value is calculated
- **gene_sets** (*dict*) – gmt file dict after background was set
- **background** (*set*) – total number of genes in your annotated database.

Returns

pvalues x: overlapped gene number n: length of gene_set which belongs to each terms hits:
overlapped gene names.

For 2*2 contingency table:

in query | not in query | row total

=> in gene_set | a | b | a+b => not in gene_set | c | d | c+d

column total | a+b+c+d = anno database

Then, in R

x=a the number of white balls drawn without replacement

from an urn which contains both black and white balls.

m=a+b the number of white balls in the urn n=c+d the number of black balls in the urn k=a+c the number
of balls drawn from the urn

In Scipy: for args in `scipy.hypergeom.sf(k, M, n, N, loc=0)`:

M: the total number of objects, n: the total number of Type I objects. k: the random variate represents
the number of Type I objects in N drawn

without replacement from the total population.

Therefore, these two functions are the same when using parameters from 2*2 table: R: `> phyper(x-1, m, n, k,
lower.tail=FALSE)` Scipy: `>>> hypergeom.sf(x-1, m+n, m, k)`

For Odds ratio in Enrichr (see <https://maayanlab.cloud/Enrichr/help#background&q=4>)

$oddsRatio = (1.0 * x * d) / Math.max(1.0 * b * c, 1)$

where:

x are the overlapping genes, b (m-x) are the genes in the annotated set - overlapping genes, c (k-x)
are the genes in the input set - overlapping genes, d (bg-m-k+x) are the 20,000 genes (or total genes
in the background) - genes in the annotated set - genes in the input set + overlapping genes

`gseapy.stats.fdr correction(pvals, alpha=0.05)`

benjamini hochberg fdr correction. inspired by statsmodels

`gseapy.stats.multiple_testing_correction(ps, alpha=0.05, method='benjamini-hochberg', **kwargs)`
 correct pvalues for multiple testing and add corrected q value

Parameters

- **ps** – list of pvalues
- **alpha** – significance level default : 0.05
- **method** – multiple testing correction method [bonferroni|benjamini-hochberg]

Returns (q, rej)

two lists of q-values and rejected nodes

5.6.4 Enrichr API

`class gseapy.enrichr.Enrichr(gene_list: Iterable[str], gene_sets: List[str] | str | Dict[str, str], organism: str = 'human', outdir: str | None = 'Enrichr', background: List[str] | int | str = 'hsapiens_gene_ensembl', cutoff: float = 0.05, format: str = 'pdf', figsize: Tuple[float, float] = (6.5, 6), top_term: int = 10, no_plot: bool = False, verbose: bool = False)`

Enrichr API

`check_genes(gene_list: List[str], usr_list_id: str)`

Compare the genes sent and received to get successfully recognized genes

`enrich(gmt: Dict[str, List[str]])`

use local mode

p = p-value computed using the Fisher exact test (Hypergeometric test) z = z-score (Odds Ratio) combine score = $-\log(p) \cdot z$

see here: <http://amp.pharm.mssm.edu/Enrichr/help#background&q=4>

columns contain:

Term Overlap P-value Odds Ratio Combinde Score Adjusted_P-value Genes

`filter_gmt(gmt, background)`

the gmt values should be filtered only for genes that exist in background this substantially affect the significance of the test, the hypergeometric distribution.

Parameters

- **gmt** – a dict of gene sets.
- **background** – list, set, or tuple. A list of custom background genes.

`get_background()` → Set[str]

get background gene

`get_libraries()` → List[str]

return active enrichr library name. Official API

`get_results(gene_list: List[str])` → Tuple[AnyStr, DataFrame]

Enrichr API

`parse_background(gmt: Dict[str, List[str]] | None = None)`

set background genes

parse_genelists() → str

parse gene list

parse_genesets(*gene_sets=None*)

parse gene_sets input file type

prepare_outdir()

create temp directory.

run()

run enrichr for one sample gene list but multi-libraries

send_genes(*payload, url*) → Dict

send gene list to enrichr server

set_organism()

Select Enrichr organism from below:

Human & Mouse, H. sapiens & M. musculus Fly, D. melanogaster Yeast, S. cerevisiae Worm, C. elegans
Fish, D. rerio

5.6.5 BioMart API

class gseapy.biomart.**Biomart**(*host: str = 'www.ensembl.org', verbose: bool = False*)

query from BioMart

add_filter(*name: str, value: Iterable[str]*)

key: filter names value: Iterable[str]

get_attributes(*dataset: str = 'hsapiens_gene_ensembl'*)

Get available attributes from dataset you've selected

get_datasets(*mart: str = 'ENSEMBL_MART_ENSEMBL'*)

Get available datasets from mart you've selected

get_filters(*dataset: str = 'hsapiens_gene_ensembl'*)

Get available filters from dataset you've selected

get_marts()

Get available marts and their names.

query(*dataset: str = 'hsapiens_gene_ensembl', attributes: List[str] | None = [], filters: Dict[str, Iterable[str]] | None = {}, filename: str | None = None*)

mapping ids using BioMart.

Parameters

- **dataset** – str, default: 'hsapiens_gene_ensembl'
- **attributes** – str, list, tuple
- **filters** – dict, {'filter name': list(filter value)}
- **host** – www.ensembl.org, asia.ensembl.org, useast.ensembl.org

Returns

a dataframe contains all attributes you selected.

Example:

```
>>> queries = {'ensembl_gene_id': ['ENSG00000125285', 'ENSG00000182968']} #_
↳ need to be a python dict
>>> results = bm.query(dataset='hsapiens_gene_ensembl',
                       attributes=['ensembl_gene_id', 'external_gene_name',
↳ 'entrezgene_id', 'go_id'],
                       filters=queries)
```

query_simple(dataset: str = 'hsapiens_gene_ensembl', attributes: List[str] = [], filters: Dict[str, Iterable[str]] = {}, filename: str | None = None)

This function is a simple version of BioMart REST API. same parameter to query().

However, you could get cross page of mapping. such as Mouse 2 human gene names

Note: it will take a couple of minutes to get the results. A xml template for querying biomart. (see <https://gist.github.com/keithshep/7776579>)

Example::

```
>>> from gseapy import Biomart
>>> bm = Biomart()
>>> results = bm.query_simple(dataset='mmusculus_gene_ensembl',
                              attributes=['ensembl_gene_id',
                              'external_gene_name',
                              'hsapiens_homolog_associated_
↳ gene_name',
                              'hsapiens_homolog_ensembl_gene'])
```

5.6.6 Parser

gseapy.parser.download_library(name: str, organism: str = 'human') → Dict[str, List[str]]

download enrichr libraries.

Parameters

- **name** (str) – the enrichr library name. see `gseapy.get_library_name()`.
- **organism** (str) – Select one from { 'Human', 'Mouse', 'Yeast', 'Fly', 'Fish', 'Worm' }

Return dict

gene_sets of the enrichr library from selected organism

gseapy.parser.get_library(name: str, organism: str = 'Human', min_size: int = 0, max_size: int = 2000, gene_list: List[str] | None = None) → Dict[str, List[str]]

Parse gene_sets.gmt(gene set database) file or download from enrichr server.

Parameters

- **name** (str) – the gene_sets.gmt file or an enrichr library name. checkout full enrichr library name here: <https://maayanlab.cloud/Enrichr/#libraries>
- **organism** (str) – choose one from { 'Human', 'Mouse', 'Yeast', 'Fly', 'Fish', 'Worm' }. This argument has not effect if input is a .gmt file.
- **min_size** – Minimum allowed number of genes for each gene set. Default: 0.
- **max_size** – Maximum allowed number of genes for each gene set. Default: 2000.

- **gene_list** – if input a gene list, min and max overlapped genes between gene set and gene_list are kept.

Return dict

Return a filtered gene set database dictionary.

Note: **DO NOT** filter gene sets, when use `replot()`. Because GSEA Desktop have already done this for you.

`gseapy.parser.get_library_name(organism: str = 'Human') → List[str]`

return enrichr active enrichr library name. see also: <https://maayanlab.cloud/modEnrichr/>

Parameters

organism (*str*) – Select one from { 'Human', 'Mouse', 'Yeast', 'Fly', 'Fish', 'Worm' }

Returns

a list of enrichr libraries from selected database

`gseapy.parser.gsea_cls_parser(cls: str) → Tuple[str]`

Extract class(phenotype) name from .cls file.

Parameters

cls – the a class list instance or .cls file which is identical to GSEA input .

Returns

phenotype name and a list of class vector.

`gseapy.parser.gsea_edb_parser(results_path: str) → Dict[str, List[str]]`

Parse results.edb file stored under **edb** file folder.

Parameters

results_path – the path of results.edb file.

Returns

a dict contains { enrichment_term: [es, nes, pval, fdr, fwer, hit_ind]}

`gseapy.parser.read_gmt(path: str) → Dict[str, List[str]]`

Read GMT file

Parameters

path (*str*) – the path to a gmt file.

Returns

a dict object

5.6.7 Visualization

`class gseapy.plot.MidpointNormalize(vmin=None, vmax=None, vcenter=None, clip=False)`

`gseapy.plot.barplot(df: DataFrame, column: str = 'Adjusted P-value', group: str | None = None, title: str = "", cutoff: float = 0.05, top_term: int = 10, figsize: Tuple[float, float] = (4, 6), color: str | List[str] | Dict[str, str] = 'salmon', ofname: str | None = None, **kwargs)`

Visualize GSEApY Results. When multiple datasets exist in the input dataframe, the *group* argument is your friend.

Parameters

- **df** – GSEApY DataFrame results.
- **column** – column name in *df* to map the x-axis data. Default: Adjusted P-value
- **group** – group by the variable in *df* that will produce bars with different colors.

- **title** – figure title.
- **cutoff** – terms with *column* value < cut-off are shown. Work only for (“Adjusted P-value”, “P-value”, “NOM p-val”, “FDR q-val”)
- **top_term** – number of top enriched terms grouped by *hue* are shown.
- **figsize** – tuple, matplotlib figsize.
- **color** – color or list or dict of matplotlib.colors. Must be reconigzed by matplotlib. if dict input, dict keys must be found in the *group*
- **ofname** – output file name. If None, don’t save figure

Returns

matplotlib.Axes. return None if given ofname. Only terms with *column* <= *cut-off* are plotted.

```
gseapy.plot.dotplot(df: DataFrame, column: str = 'Adjusted P-value', x: str | None = None, y: str = 'Term',
                    x_order: List[str] | bool = False, y_order: List[str] | bool = False, title: str = "", cutoff:
                    float = 0.05, top_term: int = 10, size: float = 5, figsize: Tuple[float, float] = (4, 6), cmap:
                    str = 'viridis_r', ofname: str | None = None, xticklabels_rot: float | None = None,
                    yticklabels_rot: float | None = None, marker: str = 'o', show_ring: bool = False,
                    **kwargs)
```

Visualize GSEApY Results with categorical scatterplot When multiple datasets exist in the input dataframe, the *x* argument is your friend.

Parameters

- **df** – GSEApY DataFrame results.
- **column** – column name in *df* that map the dot colors. Default: Adjusted P-value.
- **x** – Categorical variable in *df* that map the x-axis data. Default: None.
- **y** – Categorical variable in *df* that map the y-axis data. Default: Term.
- **x_order** – bool, array-like list. Default: False. If True, peformed hierarchical_clustering on X-axis. or input a array-like list of *x* categorical levels.
- **y_order** – bool, array-like list. Default: False. If True, peformed hierarchical_clustering on Y-axis. or input a array-like list of *y* categorical levels.
- **title** – Figure title.
- **cutoff** – Terms with *column* value < cut-off are shown. Work only for (“Adjusted P-value”, “P-value”, “NOM p-val”, “FDR q-val”)
- **top_term** – Number of enriched terms to show (based on values in the *column* (colormap)).
- **size** – float, scale the dot size to get proper visualization.
- **figsize** – tuple, matplotlib figure size.
- **cmap** – Matplotlib colormap for mapping the *column* semantic.
- **ofname** – Output file name. If None, don’t save figure
- **marker** – The matplotlib.markers. See https://matplotlib.org/stable/api/markers_api.html
- **bool** (*show_ring*) – Whether to draw outer ring.

Returns

matplotlib.Axes. return None if given ofname. Only terms with *column* <= *cut-off* are plotted.

```
gseapy.plot.enrichment_map(df: DataFrame, column: str = 'Adjusted P-value', cutoff: float = 0.05, top_term:
    int = 10, **kwargs) → Tuple[DataFrame, DataFrame]
```

Visualize GSEapy Results. Node size corresponds to the percentage of gene overlap in a certain term of interest. Colour of the node corresponds to the significance of the enriched terms. Edge size corresponds to the number of genes that overlap between the two connected nodes. Gray edges correspond to both nodes when it is the only colour edge. When there are two different edge colours, red corresponds to positive nodes and blue corresponds to negative nodes.

Parameters

- **df** – GSEapy DataFrame results.
- **column** – column name in *df* to map the node colors. Default: Adjusted P-value or FDR q-val. choose from (“Adjusted P-value”, “P-value”, “FDR q-val”, “NOM p-val”).
- **group** – group by the variable in *df* that will produce bars with different colors.
- **title** – figure title.
- **cutoff** – nodes with *column* value < cut-off are shown. Work only for (“Adjusted P-value”, “P-value”, “NOM p-val”, “FDR q-val”)
- **top_term** – number of top enriched terms are selected as nodes.

Returns

tuple of dataframe (nodes, edges)

```
gseapy.plot.gseaplot(term: str, hits: Sequence[int], nes: float, pval: float, fdr: float, RES: Sequence[float],
    rank_metric: Sequence[float] | None = None, pheno_pos: str = "", pheno_neg: str = "",
    color: str = '#88C544', figsize: Tuple[float, float] = (6, 5.5), cmap: str = 'seismic',
    ofname: str | None = None, **kwargs) → List[Axes] | None
```

This is the main function for generating the gsea plot.

Parameters

- **term** – gene_set name
- **hits** – hits indices of rank_metric.index presented in gene set S.
- **nes** – Normalized enrichment scores.
- **pval** – nominal p-value.
- **fdr** – false discovery rate.
- **RES** – running enrichment scores.
- **rank_metric** – pd.Series for rankings, rank_metric.values.
- **pheno_pos** – phenotype label, positive correlated.
- **pheno_neg** – phenotype label, negative correlated.
- **color** – color for RES and hits.
- **figsize** – matplotlib figsize.
- **ofname** – output file name. If None, don’t save figure

return matplotlib.Figure.

```
gseapy.plot.gseaplot2(terms: List[str], hits: List[Sequence[int]], RESs: List[Sequence[float]], rank_metric:
    Sequence[float] | None = None, colors: str | List[str] | None = None, figsize:
    Tuple[float, float] = (6, 4), legend_kws: Dict[str, Any] | None = None, ofname: str |
    None = None, **kwargs) → List[Axes] | None
```

Trace plot for combining multiple terms/pathways into one plot :param terms: list of terms to show in trace plot :param hits: list of hits indices correspond to each term. :param RESs: list of running enrichment scores correspond to each term. :param rank_metric: Optional, rankings. :param figsize: matplotlib figsize. :param legend_kws: Optional, control the location of legends :param ofname: output file name. If None, don't save figure

return matplotlib.Figure.

```
gseapy.plot.heatmap(df: DataFrame, z_score: int | None = None, title: str = "", figsize: Tuple[float, float] = (5, 5), cmap: str | None = None, xticklabels: bool = True, yticklabels: bool = True, ofname: str | None = None, **kwargs)
```

Visualize the dataframe.

Parameters

- **df** – DataFrame from expression table.
- **z_score** – 0, 1, or None. z_score axis{0, 1}. If None, not scale.
- **title** – figure title.
- **figsize** – heatmap figsize.
- **cmap** – matplotlib colormap. e.g. “RdBu_r”.
- **xticklabels** – bool, whether to show xticklabels.
- **yticklabels** – bool, whether to show yticklabels.
- **ofname** – output file name. If None, don't save figure

```
gseapy.plot.ringplot(df: DataFrame, column: str = 'Adjusted P-value', x: str | None = None, title: str = "", cutoff: float = 0.05, top_term: int = 10, size: float = 5, figsize: Tuple[float, float] = (4, 6), cmap: str = 'viridis_r', ofname: str | None = None, xticklabels_rot: float | None = None, yticklabels_rot: float | None = None, marker='o', show_ring: bool = True, **kwargs)
```

ringplot is deprecated, use dotplot instead

Parameters

- **df** – GSEApY DataFrame results.
- **x** – Group by the variable in *df* that will produce categorical scatterplot.
- **column** – column name in *df* to map the dot colors. Default: Adjusted P-value
- **title** – figure title
- **cutoff** – terms with *column* value < cut-off are shown. Work only for (“Adjusted P-value”, “P-value”, “NOM p-val”, “FDR q-val”)
- **top_term** – number of enriched terms to show.
- **size** – float, scale the dot size to get proper visualization.
- **figsize** – tuple, matplotlib figure size.
- **cmap** – matplotlib colormap for mapping the *column* semantic.
- **ofname** – output file name. If None, don't save figure
- **marker** – the matplotlib.markers. See https://matplotlib.org/stable/api/markers_api.html
- **bool** (*show_ring*) – whether to show outer ring.

Returns

matplotlib.Axes. return None if given ofname. Only terms with *column* <= *cut-off* are plotted.

`gseapy.plot.zscore(data2d: DataFrame, axis: int | None = 0)`

Standardize the mean and variance of the data axis Parameters.

Parameters

- **data2d** – DataFrame to normalize.
- **axis** – int, Which axis to normalize across. If 0, normalize across rows, if 1, normalize across columns. If None, don't change data

Returns

Normalized DataFrame. Normalized data with a mean of 0 and variance of 1 across the specified axis.

5.6.8 Scientific Journal and Sci- themed Color Palettes

5.6.9 Utils

5.7 Frequently Asked Questions

5.7.1 Q: What kind of gene identifiers are supported in GSEAPy?

A:

- If you select Enrichr library as your input `gene_sets` (gmt format), then gene symbols in upper cases are needed.
- If you use your own GMT file, you need to use the same type of your gene identifiers in GMT and input gene list.

5.7.2 Q: Why gene symbols in Enrichr library are all UPPER cases for mouse, fly, fish, worm ?

A: GSEAPy can't change the Enrichr databases. So convert your gene symbols into UPPER cases first, then run the analysis you want.

5.7.3 Q: Why P-value or FDR is 0, not a very small number?

A: GSEA methodology use random permutation procedure (e.g. 1000 permutation) to obtain a null distribution. Then, an observed ES is compared to the 1000 shuffled ES to calculate a P-value. When observed ES is not within the null ESs, you'll get 0s. if you don't want 0, you could

- set the smallest pvalue to $1 / (\text{number of permutations})$
- increase the permutation number (but more running time needed)

5.7.4 Q: What are gene %, and tag % mean in the output?

5.7.5 Q: What Enrichr database are supported?

A: Support modEnrich (<https://amp.pharm.mssm.edu/modEnrichr/>) . Now, Human, Mouse, Fly, Yeast, Worm, Fish are all supported.

5.7.6 Q: Use custom defined GMT file input in Jupyter ?

A: argument gene_sets accept dict input. This is useful when define your own gene_sets. An example dict looks like this:

```
gene_sets = {
    "term_1": ["gene_A", "gene_B", ...],
    "term_2": ["gene_B", "gene_C", ...],
    ...
    "term_100": ["gene_A", "gene_T", ...]
}
```

APIs support dict object input: gsea, prerank, ssgsea, enrichr

5.7.7 Q: How to use Yeast database in gseapy.enrichr()?

Because some library names are the same in different Enrichr database, you have to set an additional augment organism when no use **Human**

```
gss = gseapy.get_library_name(organism='Yeast')
enr = gseapy.enrichr(gene_list=...,
                    gene_sets=gss,
                    organism='Yeast', # don't forget to set organism="Yeast"
                    )
```

5.7.8 Q: How to use Yeast database in gseapy.prerank()?

There is no augment organism in prerank, gsea, ssgsea, but you could input these Enrichr libraries as follow:

```
# get libraries you'd like to use
gss = gseapy.get_library_name(organism='Yeast')
# get a custom gmt_dict
gmt_dict = gseapy.parser.gsea_gmt_parser('GO_Biological_Process_2018', organism='Yeast')
# run
prn_res = gseapy.prerank( ..., gene_sets=gmt_dict, ...)
```

5.7.9 Q: How to save plots using gseaplot, barplot, dotplot, ``heatmap`` in Jupyter ?

A: e.g. `gseaplot(..., ofname='your.plot.pdf')`. That's it

5.7.10 Q: What cutoff mean in functions, like `enrichr()`, `dotplot`, `barplot` ?

A: This argument control the terms (e.g $FDR < 0.05$) that will be shown on figures, not the result table output.

5.7.11 Q: ssGSEA missing p value and FDR?

A: The original ssGSEA algorithm will not give you pval or FDR, so, please ignore the gseaplot generated by `ssgsea`. It's useless and misleading, therefore, `fd`, and `pval` are not shown on the plot. If you're seeking for ssGSEA with p-value output, please see here: <https://github.com/broadinstitute/ssGSEA2.0> Actually, ssGSEA2.0 use the same method with GSEAPy to calculate P-value, but FDR is not.

5.7.12 Q: What the difference between ssGSEA and Prerank

A: In short, - prerank is used for comparing **two group of samples** (e.g. control and treatment), where the gene ranking are defined by your custom rank method (like t-statistic, signal-to-noise, et.al). - ssGSEA is used for comparing individual samples to the rest of all, trying to find the gene signatures which samples shared the same (use ssGSEA when you have a lot of samples).

The statistic between prerank (GSEA) and ssGSEA are different. Assume that we have calculated each *running enrichment score* of your ranked input genes, then

- es for GSEA: $\max(\text{running enrichment scores})$ or $\min(\text{running enrichment scores})$
- es for ssGSEA: $\text{sum}(\text{running enrichment scores})$

INDICES AND TABLES

- genindex
- modindex
- search

PYTHON MODULE INDEX

g

`gseapy`, 57
`gseapy.base`, 66
`gseapy.biomart`, 70
`gseapy.enrichr`, 69
`gseapy.gsea`, 65
`gseapy.parser`, 71
`gseapy.plot`, 72
`gseapy.scipalette`, 76
`gseapy.stats`, 68

A

add_filter() (*gseapy.biomart.Biomart method*), 70
 apply() (*gseapy.base.GMT method*), 66

B

barplot() (*in module gseapy.plot*), 72
 Biomart (*class in gseapy.biomart*), 70

C

calc_metric() (*gseapy.gsea.GSEA method*), 65
 calc_pvalues() (*in module gseapy.stats*), 68
 check_genes() (*gseapy.enrichr.Enrichr method*), 69

D

dotplot() (*in module gseapy.plot*), 73
 download_library() (*in module gseapy.parser*), 71

E

enrich() (*gseapy.enrichr.Enrichr method*), 69
 enrich() (*in module gseapy*), 63
 enrichment_map() (*in module gseapy.plot*), 73
 enrichment_score() (*gseapy.base.GSEAbase method*), 66
 Enrichr (*class in gseapy.enrichr*), 69
 enrichr() (*in module gseapy*), 61

F

fdr correction() (*in module gseapy.stats*), 68
 filter_gmt() (*gseapy.enrichr.Enrichr method*), 69

G

get_attributes() (*gseapy.biomart.Biomart method*), 70
 get_background() (*gseapy.enrichr.Enrichr method*), 69
 get_datasets() (*gseapy.biomart.Biomart method*), 70
 get_filters() (*gseapy.biomart.Biomart method*), 70
 get_libraries() (*gseapy.base.GSEAbase method*), 67
 get_libraries() (*gseapy.enrichr.Enrichr method*), 69
 get_library() (*in module gseapy.parser*), 71
 get_library_name() (*in module gseapy.parser*), 72
 get_marts() (*gseapy.biomart.Biomart method*), 70

get_results() (*gseapy.enrichr.Enrichr method*), 69
 GMT (*class in gseapy.base*), 66
 GSEA (*class in gseapy.gsea*), 65
 gsea() (*in module gseapy*), 57
 gsea_cls_parser() (*in module gseapy.parser*), 72
 gsea_edb_parser() (*gseapy.gsea.Replot method*), 66
 gsea_edb_parser() (*in module gseapy.parser*), 72
 GSEAbase (*class in gseapy.base*), 66
 gseaplot() (*in module gseapy.plot*), 74
 gseaplot2() (*in module gseapy.plot*), 74
 gseapy
 module, 57
 gseapy.base
 module, 66
 gseapy.biomart
 module, 70
 gseapy.enrichr
 module, 69
 gseapy.gsea
 module, 65
 gseapy.parser
 module, 71
 gseapy.plot
 module, 72
 gseapy.scipalette
 module, 76
 gseapy.stats
 module, 68

H

heatmap() (*in module gseapy.plot*), 75

L

load_classes() (*gseapy.gsea.GSEA method*), 65
 load_data() (*gseapy.gsea.GSEA method*), 65
 load_gmt() (*gseapy.base.GSEAbase method*), 67
 load_gmt_only() (*gseapy.base.GSEAbase method*), 67
 load_ranking() (*gseapy.gsea.Prerank method*), 66

M

make_unique() (*gseapy.base.GSEAbase method*), 67
 MidpointNormalize (*class in gseapy.plot*), 72

module

- gseapy, 57
- gseapy.base, 66
- gseapy.biomart, 70
- gseapy.enrichr, 69
- gseapy.gsea, 65
- gseapy.parser, 71
- gseapy.plot, 72
- gseapy.scipalette, 76
- gseapy.stats, 68

multiple_testing_correction() (in module *gseapy.stats*), 68

P

parse_background() (*gseapy.enrichr.Enrichr* method), 69

parse_genelists() (*gseapy.enrichr.Enrichr* method), 69

parse_genesets() (*gseapy.enrichr.Enrichr* method), 70

parse_gmt() (*gseapy.base.GSEAbase* method), 67

plot() (*gseapy.base.GSEAbase* method), 67

prepare_outdir() (*gseapy.base.GSEAbase* method), 67

prepare_outdir() (*gseapy.enrichr.Enrichr* method), 70

Prerank (class in *gseapy.gsea*), 66

prerank() (in module *gseapy*), 59

Q

query() (*gseapy.biomart.Biomart* method), 70

query_simple() (*gseapy.biomart.Biomart* method), 71

R

read_gmt() (in module *gseapy.parser*), 72

Replot (class in *gseapy.gsea*), 66

replot() (in module *gseapy*), 64

results (*gseapy.base.GSEAbase* property), 67

ringplot() (in module *gseapy.plot*), 75

run() (*gseapy.enrichr.Enrichr* method), 70

run() (*gseapy.gsea.GSEA* method), 66

run() (*gseapy.gsea.Prerank* method), 66

run() (*gseapy.gsea.Replot* method), 66

S

send_genes() (*gseapy.enrichr.Enrichr* method), 70

set_organism() (*gseapy.enrichr.Enrichr* method), 70

ssgsea() (in module *gseapy*), 60

T

to_df() (*gseapy.base.GSEAbase* method), 67

W

write() (*gseapy.base.GMT* method), 66

Z

zscore() (in module *gseapy.plot*), 75