

R for toxicology (RNA-Seq)

How to use Metascape?



Objective

- Learning how to use Metascape for RNA-Seq analysis

What is Metascape?

- Very useful tool for enrichment analysis
- Metascape is an online-based tool (webpage)
- URL: <https://metascape.org/>

What is Metascape?



Step 1

☐ Multiple Gene Lists

Drag & drop your file (.xls,.xlsx,.csv,.txt)

Select File...

Or paste a gene list

Accept Gene ID/Symbol/RefSeq/
Ensembl/UniProt/UCSC

Upload File Format

Single List:
.xls/xlsx .csv .txt

Multiple List:
.xls/xlsx .csv .txt

Test Upload
single list
3 gene lists

Test Identifiers
Gene Symbol try it!
RefSeq
Entrez Gene ID

Step 2

Step 3

Express Analysis Custom Analysis Batch Analysis?

Please cite Zhou et al. [Nature Commun. 2019 10\(1\):1523](#) within any publication that makes use of analyses inspired by Metascape.

Public questions: [forum](#). Private questions: [metascape.team at gmail.com](#)

News & Updates

- 📍 Current version **v3.5.20240101**
- 🗨️ [Code Release History](#)
- 🗨️ 2021-12-18 Release MSBio.
- 🗨️ 2021-02-01 Include STRING, EggNog, WikiPathways.
- 🗨️ 2018-11-11 Include DisGeNET, TRRUST, HPO, PaGenBase, L1000.
- 🗨️ 2017-09-15 Include CORUM, rearchitect GPEC beta.
- 🗨️ 2016-11-2 Support model organisms and PPI analysis!
- 🗨️ 2015-12-9 First Metascape Publication [[link](#)]
- 🗨️ 2015-10-8 Launch of metascape.org at UCSD.

Message Board

- 🗨️ 2024-02-19 Alpha release of a new MSBio, support Singularity technology.
- 🗨️ 2023-12-12 New blog: behind the scene of the enrichment clustering algorithm.
- 🗨️ 2023-12-04 Database updated to release 2024-01-01 (StringDB 12.0). MSBio updated accordingly.
- 🗨️ 2023-06-22 ChatGPT-based gene annotation added (see [blog](#)).

Step 1: add DEGs

Step 1

Or paste a gene list

ENSDARG00000077360
ENSDARG00000093145
ENSDARG00000020594
ENSDARG00000094237
ENSDARG00000102885

Submit Cancel

Upload File Format

Single List:
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Multiple List:
.xls/xlsx .csv .txt

Test Upload

single list
3 gene lists

Test Identifiers

Gene Symbol [try it!](#)
RefSeq
Entrez Gene ID

Step 2

Step 3

Express Analysis Custom Analysis Batch Analysis?

Ensembl ID of DEGs

Step 2: click submit

Step 1

Upload File Format

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Multiple List:

.xls/xlsx .csv .txt

Test Upload

single list

3 gene lists

Test Identifiers

Gene Symbol [try it!](#)

RefSeq

Entrez Gene ID

Or paste a gene list

ENSDARG00000077360

ENSDARG00000093145

ENSDARG00000020594

ENSDARG00000094237

ENSDARG00000102885

Submit Cancel

Step 2

Step 3

Express Analysis Custom Analysis Batch Analysis?

Click here

Step 3: select species

Step 1

Upload File Format

Single List:

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Multiple List:

.xls/xlsx .csv .txt

Test Upload

single list

3 gene lists

Test Identifiers

Gene Symbol [try it!](#)

RefSeq

Entrez Gene ID

Or paste a gene list

ENSDARG00000077360

ENSDARG00000093145

ENSDARG00000020594

ENSDARG00000094237

ENSDARG00000102885

Submit Cancel

Your id type: Ensembl Gene .

Step 2

Optional if you only consider human species in your study.

Input as species:

Any Species

Analysis as species:

Any Species

D. rerio (319)

Step 3

Express Analysis Custom Analysis Batch Analysis?

Select D. rerio in 'Input as species'

Step 3: select species

Step 1

Upload File Format

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Multiple List:

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Test Upload

single list

3 gene lists

Test Identifiers

Gene Symbol [try it!](#)

RefSeq

Entrez Gene ID

Or paste a gene list

ENSDARG00000077360

ENSDARG00000093145

ENSDARG00000020594

ENSDARG00000094237

ENSDARG00000102885

Submit Cancel

Your id type: Ensembl Gene .

Step 2

Optional if you only consider human species in your study.

Input as species: D. rerio (319)

Analysis as species: D. rerio (319)

Step 3

Express Analysis

D. rerio (319)

R. norvegicus (219)

Select D. rerio in 'Analysis as species'

Step 3: select species

Step 1

Upload File Format

Single List:

[.xls/xlsx](#) [.csv](#) [.txt](#)

Multiple List:

[.xls/xlsx](#) [.csv](#) [.txt](#)

Test Upload

[single list](#)

[3 gene lists](#)

Test Identifiers

[Gene Symbol](#) [try it!](#)

[RefSeq](#)

[Entrez Gene ID](#)

Or paste a gene list

ENSDARG00000077360
ENSDARG00000093145
ENSDARG00000020594
ENSDARG00000094237
ENSDARG00000102885

Submit

Cancel

Your id type: Ensembl Gene .

Step 2

Optional if you only consider human species in your study.

Input as species:

D. rerio (319)

Analysis as species:

D. rerio (319)

Step 3

Express Analysis

Custom Analysis

Batch Analysis?

Click 'Express Analysis'

Step 4: Wait

Step 1

Upload File Format

Single List:

[.xls/xlsx](#) [↓](#) [.csv](#) [↓](#) [.txt](#) [↓](#)

Multiple List:

[.xls/xlsx](#) [↓](#) [.csv](#) [↓](#) [.txt](#) [↓](#)

Test Upload

[single list](#)

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ENSDARG00000094237

ENSDARG00000102885

Submit

Cancel

Your id type: Ensembl Gene .

Step 2

Optional if you only consider human species in your study.

Input as species:

D. rerio (319) ▼

Analysis as species:

D. rerio (319) ▼

Step 3

Express Analysis

Custom Analysis

Batch Analysis?

Please click the button below to see reports

Analysis Report Page

100

When analysis is completed, click
'Analysis Report Page'

Step 5: download

Gene List Report Excel Sheets

Gene List Report PPT file

All in One Zip File

Warning: this report page can be accessed within the next 72 hours; all data (session id: t1wpuhtxo) will be deleted afterwards.

Due to database update, bug fix, and continuous features improvement, Metascape does not provide a mechanism to reproduce the exact same analysis results for the same input data. It is important to save the .zip file as your complete record.

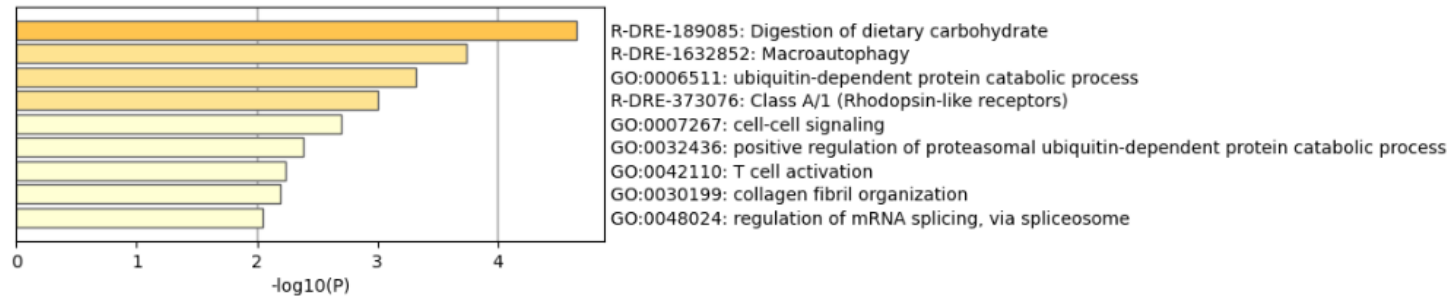
Metascape Gene List Analysis Report

metascape.org¹

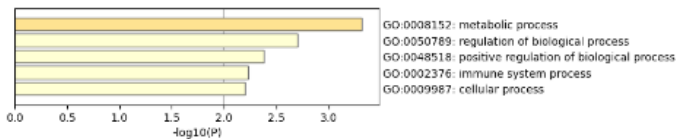
Bar Graph Summary

Click 'All in One Zip File' to download all the information

Figure 1. Bar graph of enriched terms across input gene lists, colored by p-values.



The top-level Gene Ontology biological processes can be viewed here.



Step 6: check files

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S
1	MyList	Gene ID	Type	Tax ID	Homolog	Homolog	Gene Syn	Descripti	Biological	Kinase	CIR-DRE-1	R-DRE-1	GO:00065	R-DRE-3	GO:00072	GO:00324	GO:00421	GO:00301	GO:00486
2	ENSDARG559768	ensembl_gene	D. rerio	559768	D. rerio	fth30	ferritin, hez	GO:0006880	intracellu	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	ENSDARG00000093145					None	None	None	None	nan	nan	nan	nan	nan	nan	nan	nan	nan	nan
4	ENSDARG563018	ensembl_gene	D. rerio	563018	D. rerio	txlnba	taxilin beta a			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5	ENSDARG795068	ensembl_gene	D. rerio	795068	D. rerio	taar12h	trace amin	GO:0007186	G protein	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0
6	ENSDARG553276	ensembl_gene	D. rerio	553276	D. rerio	rsu1	Ras suppre	GO:0007165	signal tra	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
7	ENSDARG100033772	ensembl_gene	D. rerio	100033772	D. rerio	dre-mir-20c	microRNA	GO:0035195	miRNA-n	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
8	ENSDARG00000103689					None	None	None	None	nan	nan	nan	nan	nan	nan	nan	nan	nan	nan
9	ENSDARG560867	ensembl_gene	D. rerio	560867	D. rerio	lin9	lin-9 DREA	GO:0006351	DNA-ten	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
10	ENSDARG445285	ensembl_gene	D. rerio	445285	D. rerio	psmc5	proteasome	GO:0043161	proteaso	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
11	ENSDARG334337	ensembl_gene	D. rerio	334337	D. rerio	ubqln4	ubiquilin 4	GO:0006511	ubiquitin-	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
12	ENSDARG00000101429					None	None	None	None	nan	nan	nan	nan	nan	nan	nan	nan	nan	nan
13	ENSDARG00000100587					None	None	None	None	nan	nan	nan	nan	nan	nan	nan	nan	nan	nan
14	ENSDARG00000105093					None	None	None	None	nan	nan	nan	nan	nan	nan	nan	nan	nan	nan
15	ENSDARG00000097733					None	None	None	None	nan	nan	nan	nan	nan	nan	nan	nan	nan	nan
16	ENSDARG566921	ensembl_gene	D. rerio	566921	D. rerio	mcama	melanoma	GO:0008150	biological	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
17	ENSDARG797196	ensembl_gene	D. rerio	797196	D. rerio	dnajc12	DnaJ (Hsp40) homolog, subfamily			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
18	ENSDARG562092	ensembl_gene	D. rerio	562092	D. rerio	vgl14l	vestigial lik	GO:0070121	Kupffer's	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
19	ENSDARG00000084034					None	None	None	None	nan	nan	nan	nan	nan	nan	nan	nan	nan	nan
20	ENSDARG00000085610					None	None	None	None	nan	nan	nan	nan	nan	nan	nan	nan	nan	nan
21	ENSDARG368924	ensembl_gene	D. rerio	368924	D. rerio	fkbp5	FKBP proly	GO:0000413	protein p	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
22	ENSDARG00000093863					None	None	None	None	nan	nan	nan	nan	nan	nan	nan	nan	nan	nan
23	ENSDARG81587	ensembl_gene	D. rerio	81587	D. rerio	cldn3c	claudin 3c	GO:0060116	vestibula	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
24	ENSDARG100009626	ensembl_gene	D. rerio	100009626	D. rerio	sv2ba	synaptic ve	GO:0007268	chemical	0.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0
25	ENSDARG100534918	ensembl_gene	D. rerio	100534918	D. rerio	pell1a	pellino E3	GO:0008592	regulatio	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
26	ENSDARG00000096121					None	None	None	None	nan	nan	nan	nan	nan	nan	nan	nan	nan	nan
27	ENSDARG497173	ensembl_gene	D. rerio	497173	D. rerio	gja1.2.2	gap junctio	GO:0007267	cell-cell	0.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0
28	ENSDARG00000115752					None	None	None	None	nan	nan	nan	nan	nan	nan	nan	nan	nan	nan
29	ENSDARG00000099829					None	None	None	None	nan	nan	nan	nan	nan	nan	nan	nan	nan	nan
30	ENSDARG100033646	ensembl_gene	D. rerio	100033646	D. rerio	mir130c-1	microRNA 130c-1			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
31	ENSDARG00000097124					None	None	None	None	nan	nan	nan	nan	nan	nan	nan	nan	nan	nan
32	ENSDARG00000099009					None	None	None	None	nan	nan	nan	nan	nan	nan	nan	nan	nan	nan
33	ENSDARG568810	ensembl_gene	D. rerio	568810	D. rerio	tanc2a	tetratricop	GO:0061001	regulatio	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
34	ENSDARG00000091938					None	None	None	None	nan	nan	nan	nan	nan	nan	nan	nan	nan	nan
35	ENSDARG00000097045					None	None	None	None	nan	nan	nan	nan	nan	nan	nan	nan	nan	nan
36	ENSDARG641324	ensembl_gene	D. rerio	641324	D. rerio	ube2v1	ubiquitin-c	GO:0070534	protein K	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
37	ENSDARG563776	ensembl_gene	D. rerio	563776	D. rerio	deaf1	DEAF1 trar	GO:0006357	regulatio	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
38	ENSDARG00000117419					None	None	None	None	nan	nan	nan	nan	nan	nan	nan	nan	nan	nan

Check if 'metascape_result.xlsx' file is included
We need this file for further analysis of RNA-Seq