Supplementary material for: Rodriguez-Mena, A., Tarragó-Claramunt, X., Castellani, G., Méndez-Viera, J., & Monleón-Getino, A. (2025). Gango + BioFunctional: A Computational tool for efficient functional gene analysis. *The European chemistry and biotechnology journal, 4*, 69-80. https://doi.org/10.62063/ecb-63. Corresponce: Antonio Monleón-Getino, Department of Genetics, Microbiology and Statistics, Universitat de Barcelona, Barcelona, Spain. E-mail: amonleong@ub.edu (A.M.G.); Ph.: ++34-678329864.

Appendix section

Tutorial: GANGO + BioFunctional

This tutorial provides a step-by-step guide on using GANGO and BioFunctional in R with a real example.

The RNA-Seq data used were retrieved from the study "Dysregulated transcriptional responses to SARS-CoV-2 in the periphery" (McClain et al, 2020), which analyzed samples from subjects affected by SARS-CoV-2 infection, seasonal coronavirus, influenza, bacterial pneumonia, as well as healthy controls.

The RNA Sequencing dataset and associated sample metadata were obtained from the public repository GEO (https://www.ncbi.nlm.nih.gov/geo/) under accession number GSE161731.

To identify genes differentially expressed between Bacterial and Healthy cohorts, RNA-seq count data were processed using the **DESeq2** package (Love et al., 2014).

To focus the analysis on protein-coding genes, gene biotypes were retrieved using the **biomaRt** package. Only genes annotated as protein_coding in the Ensembl database (GRCh38, hsapiens_gene_ensembl) were retained.

Normalization and differential expression analysis were performed using the DESeq() function. Genes with an adjusted p-value (padj) < 0.1 were considered statistically significant. These genes were further stratified by their log2 fold-change direction: genes upregulated in the Bacterial group (log2FC > 0) and those upregulated in the Healthy group (log2FC < 0) were saved separately.

The final output was a table containing significant genes with their associated Ensembl IDs, affiliation group (Bacterial or Healthy), and organism taxonomy (*Homo sapiens*).

This file was used as input for GANGO.

The GANGO + BioFuncional demo showcases a user-friendly, R-based application designed to streamline the functional analysis of gene expression data. It's important to note that the demo is derived from data presented in a study on transcriptional responses, and focuses on the functional analysis between a group of healthy individuals and a group with bacterial infection. The source of this data is McClain et al. (2020; https://pubmed.ncbi.nlm.nih.gov/32743603/).

For this demo, all necessary files for the analysis are provided in the correct format and can be download in https://github.com/amonleong/Biofunctional.

The demo illustrates how the application processes gene lists or taxonomic classifications to elucidate Gene Ontology (GO) term and KEGG pathway enrichment. It highlights the two main modules:

- **GANGO:** Efficiently maps input data (genes/taxa/groups) to GO terms and KEGG pathways.
- **BioFuncional:** Interprets the output from GANGO, incorporating hierarchical information, generating interactive networks, and producing bar plot visualizations.

1. First Steps

• **Download example files for BioFunctional:** Download sample files from the repository https://github.com/amonleong/Biofunctional.

2. Run the BioFunctional Application

Execute the BioFunctional application in https://alexub.shinyapps.io/BioFunctional/. This will launch the application and display its main menu.



This screen contains the menu:

BIOFunctional	
🛧 НОМЕ	
GANGO Data Explorer	
E KEGG	<
⊠ Gene Ontologies	<
Ê HELP	

GANGO



5. GANGO: Gene/Taxon/Group to Gene Ontologies and KEGG

- Initial Screen: GANGO's interface allows you to upload your data.
- Input: GANGO takes as input a file containing your taxon, gene and group identifiers.
- Using the example: Load the example dataset provided with BioFunctional to familiarize yourself with the input format: "GANGO_BACTERIA_HEALTHY_2.csv"
- Submit: Run GANGO to map your identifiers to Gene Ontology (GO) terms and KEGG pathways.

IMPORTANT: The process can take 2-3 hours, resulting in file "GANGO_significant_results_2025-05-09.csv". (You can skip this step and check the result file directly if preferred.) Move on to the next step.

- Output: GANGO will generate a file containing the mapping of your input identifiers to GO terms and KEGG pathways: "GANGO_significant_results_2025-05-09.csv"
- **Download results:** Download the results file. This file serves as the input for BioFunctional.

BIOFunctional		=
📌 НОМЕ		Query Parameters
GANGO Data Explorer		Enter Taxon
Е KEGG	<	Homo sapiens, Mus musculus, Saccharomyces cerevisiae
Z Gene Ontologies	<	Enter Gene
B HELP		BRCA1, Tp53, CDC28
		Enter Group
		Mammals, Mammals, Fungi
		Choose CSV File
		Browse No file selected
		Submit

GANGO screen with defect example

Upload the required data in the correct format (eg. GANGO_BACTERIA_HEALTHY_2.csv) and click Submit

Screen with the results of the user example (Bacteria vs Health) after 2h processing:

Enter	Group										
Mat	nmals, Mammals	s, Fungi									
Choo	e CSV File										
Bro	wse No file	selected									
Sut	mit										
Que	y Results										
Show	10 v entrie									Search:	
	ONTOLOGY	EA_VALUE	pvalue	GROUP_1	GROUP_2	GROUP	pvalue_corrected	FOR	UP_DOWN	ONT_NAME	ONT_DESCRIPTION
1	G0:000082	0.003510858256931307	2.158112297062982e-34	count_Mammals	count_Fungi	count_Mammais_vs_count_Fungi	4.940998104125697e-31	4.940998104125697e-31	UP	G1/S transition of mitotic cell cycle	biological_process
2	GO:0000086	0.0009296605484878411	3.163945439579864e-17	count_Mammals	count_Fungi	count_Mammals_vs_count_Fungi	2.897541233567239e-14	2.897541233567239e-14	UP	G2/M transition of mitotic cell cycle	biological_process
3	G0:000111	0.01610119414476545	0.0003534021535587202	count_Mammals	count_Fungi	count_Mammais_vs_count_Fungi	0.03302507063562	0.03302507063562	UP	incipient cellular bud site	cellular_component
4	G0:0000307	0.004351898228421243	5.309386448741623e-25	count_Mammals	count_Fungi	count_Mammals_vs_count_Fungi	8.10389351676263e-22	8.10389351626263e-22	UP	cyclin-dependent protein kinase holoenzyme complex	cellular_component
5	G0:0000749	0.0186929075229834	0.0000249534043135164	count_Mammals	count_Fungi	count_Mammals_vs_count_Fungi	0.003940056494882469	0.003940056494882469	UP	response to pheromone triggering conjugation with cellular fusion	biological_process
6	G0:0004693	-0.002591713378217946	1.041518641792662e-8	count_Mammals	count_Fungi	count_Mammals_vs_count_Fungi	0.00000433555805524418	0.00000433555805524418	DOWN	cyclin-dependent protein serine/threonine kinase activity	molecular_function
7	60:0005543	0.01610119414476545	0.0003534021535587202	count_Mammals	count_Fungl	count_Mammals_vs_count_Fungi	0.03302507063562	0.03302507063562	UP	phospholipid binding	molecular_function
8	G0:0005634	-0.02320099769391685	0.0000160433762893852	count_Mammals	count_Fungi	count_Mammals_vs_count_Fungi	0.003194026957786731	0.003194026957786731	DOWN	nucleus	cellular_component
9	60:0005635	-0.00470929739575942	1.982240186793022e-9	count_Mammals	count_Fungi	count_Mammais_vs_count_Fungi	9.584229236345302e-7	9.584229236345302e-7	DOWN	nuclear envelope	cellular_component



The computational time can be high in this process (ex: for 9000 genes use 2.50 hours). The results also present a volcano plot (A volcano plot is a graph that combines the magnitude of change (X-axis) and statistical significance (Y-axis) to quickly visualize which elements (like genes) show large and statistically important differences in a data analysis.)

Download FILE RESULTS: "GANGO_significant_results_2025-05-09.csv"

A	т т	: ×	$\sqrt{-f_X}$	ONTOLO	OGY,"EA_	VALUE","p	value","GRO	UP_1","GRO	JP_2","GRO	UP","pvalue_	corrected","	FDR","UP_DO	WN","ONT_NA	ME","ONT_D	ESCRIPTION"							
	A	в	c	D		E	F	G	н	1	J J	к	L	м	N	0	Р	Q	R	s	т	U
1	ONTOLOGY,	A VALUE	,"pvalue","	GROUP 1",	"GROUP	2","GROU	P","pvalue	corrected","F	DR", "UP DO	WN","ONT I	NAME","ON	DESCRIPTIO	N"									
2	GO:0000049,-0	.02069354	83551545,6	4689987339	99715e-0	7,"count h	ealthy","con	unt Bacterial	","count he	althy vs cou	int Bacterial	",0.000231975	21412555,0.00	023197521412	555,"DOWN"	"tRNA bind	ing ", "molecu	lar function				
3	GO:0000387,-0	.03190282	12353485,5	.074277526	64983e-0	6,"count h	ealthy","co	unt Bacterial	","count he	althy vs cou	int Bacterial	",0.001389521	70597951,0.00	138952170597	951,"DOWN"	,"spliceosor	nal snRNP ass	embly ","bio	logical proc	ess"		
4	GO:0000398,-0	.02566201	81853156,2	496794332	5704e-15	,"count he	althy","cou	nt Bacterial"	,"count hea	althy vs cour	nt Bacterial"	,2.6860156744	8877e-12,2.68	601567448877	e-12,"DOWN	","mRNA sp	licing, via splie	eosome ","	biological pr	ocess"		
5	GO:0000462,-0	.03190282	12353485,5	.0742775266	64983e-0)6,"count h	ealthy","co	unt Bacterial	","count he	althy vs cou	int Bacterial	",0.001389521	70597951,0.00	138952170597	951,"DOWN"	,"maturatio	n of SSU-rRNA	from tricisti	ronic rRNA tr	anscript (SSU-ri	RNA, 5.8S rRI	NA, LSU-rF
6	GO:0000463,-0	.02844114	78414554,0	.0001988552	28388332	26,"count_h	nealthy","co	unt_Bacterial	","count_he	ealthy_vs_cou	int_Bacteria	",0.02620849	55426923,0.026	208496542692	3,"DOWN","	maturation o	of LSU-rRNA fr	om tricistro	nic rRNA tran	script (SSU-rRN	A, 5.8S rRNA	, LSU-rRN
7	GO:0000723,-0	.01798653	67595952,0	.0003169159	94219186	56,"count_h	nealthy","co	unt_Bacteria	","count_he	ealthy_vs_cou	Int_Bacteria	",0.03849250	31076749,0.038	492508107674	9,"DOWN","	telomere ma	aintenance ","	biological_p	rocess"			
8	GO:0000724,-0	.01569993	29401571,0	.0002312384	46650683	31,"count_h	nealthy","co	unt_Bacteria	","count_he	ealthy_vs_cou	int_Bacteria	",0.02951425	8479609,0.029	514258847960	9,"DOWN","	double-strar	nd break repai	r via homolo	ogous recomb	ination ","biol	ogical_proce	ess"
9	GO:0000978,-0	.00746332	945012145,	2.312536544	423462e-	-05,"count_	healthy","co	ount_Bacteria	al","count_h	nealthy_vs_co	unt_Bacteria	al",0.00424745	279179484,0.0	042474527917	9484,"DOWN	","RNA poly	merase II cis-	regulatory re	egion sequen	ce-specific DN	A binding ","	molecula
10	GO:000981,-0	.00810398	769474756,	8.565899122	290866e-	-07,"count_	healthy","co	ount_Bacteria	al","count_h	nealthy_vs_co	unt_Bacteria	al",0.00029320	6833386653,0.	000293206833	386653,"DOV	VN","DNA-b	inding transcr	iption factor	activity, RNA	polymerase II	-specific ","r	nolecular
11	GO:0001650,-0	.01330023	37006064,1	9506324309	91512e-0)5,"count_h	ealthy","co	unt_Bacterial	","count_he	ealthy_vs_cou	int_Bacterial	",0.004247452	79179484,0.00	424745279179	484,"DOWN"	,"fibrillar ce	nter ","cellula	ir_compone	nt"			
12	GO:0002102,0	01060116	7984623,0.0	0001874713	4360270	6,"count_h	ealthy","cou	nt_Bacterial	',"count_he	althy_vs_cou	nt_Bacterial	,0.025668235	5090942,0.0256	68235509094	2,"UP","podo	some ","cell	ular_compon	ent"				
13	GO:0002181,-0	.03810093	20693606,1	1569514840	06886e-1	L9,"count_h	ealthy","co	unt_Bacterial	","count_he	ealthy_vs_cou	int_Bacteria	",1.584076930	50556e-16,1.5	840769365055	6e-16,"DOW	N","cytoplas	mic translatio	n ","biologic	al_process"			
14	GO:0002523,0	023114444	1882249,0.0	0003785611	7758251	5,"count_h	ealthy","cou	nt_Bacterial	',"count_he	althy_vs_cou	nt_Bacterial	,0.041922866	8791931,0.0419	22866879193	L,"UP","leuko	ocyte migrati	ion involved i	n inflammat	ory response	","biological_p	rocess"	
15	GO:0003677,-0	.00756672	112782535,	6.04018722	122731e-	-06,"count_	healthy","co	ount_Bacteria	al","count_h	nealthy_vs_co	unt_Bacteria	al",0.00162448	678105187,0.0	016244867810	5187,"DOWN	I","DNA bind	ling ","molecu	lar_function	1"			
16	GO:0003684,-0	.01749675	78040983,0	.0001152234	45961685	57,"count_h	nealthy","co	unt_Bacterial	l","count_he	ealthy_vs_cou	int_Bacteria	",0.01684835	614461,0.0168	48354614461,	"DOWN","da	maged DNA	binding ","mo	lecular_fun	ction"			
17	GO:0003697,-0	.01432607	51411014,0	.000245320:	18727888	33,"count_h	nealthy","co	unt_Bacteria	l","count_he	ealthy_vs_cou	int_Bacteria	",0.030358944	672339,0.0303	58944672339,	"DOWN","sir	gle-strande	d DNA binding	;,"molecul	ar_function"			
18	GO:0003723,-0	.01275350	94249421,2	442288570	54503e-3	35,"count_h	ealthy","co	unt_Bacterial	","count_he	ealthy_vs_cou	int_Bacteria	",1.226110272	03262e-31,1.2	261102720326	2e-31,"DOWI	N","RNA bin	ding ","molec	ular_functio	n"			
19	GO:0003735,-0	.05036584	97515445,9	1339975338	87608e-3	36,"count_h	ealthy","co	unt_Bacterial	","count_he	ealthy_vs_cou	int_Bacteria	",6.878356842	88538e-32,6.8	783568428853	8e-32,"DOWI	N","structura	al constituent	of ribosome	","molecula	r_function"		
20	GO:0003779,0	003304484	06071434,7	.105361755	685201e-(05,"count_H	nealthy","co	unt_Bacteria	l","count_h	ealthy_vs_cou	unt_Bacteria	",0.01091978	09596824,0.010	919780959682	4,"UP","actir	n binding ","	molecular_fu	nction"				
21	GO:0003899,-0	.03056236	93815985,3	.0760358623	31981e-0)5,"count_h	ealthy","co	unt_Bacterial	","count_he	ealthy_vs_cou	int_Bacteria	",0.005205413	04746052,0.00	520541304746	052,"DOWN"	,"DNA-direc	ted 5'-3' RNA	polymerase	activity ","m	olecular_functi	on"	
22	GO:0004519,-0	.02360422	71437218,0	.0002363678	85821460	05,"count_h	nealthy","co	unt_Bacteria	l","count_he	ealthy_vs_cou	int_Bacteria	",0.02966613	9380847,0.029	666135938084	7,"DOWN","	endonucleas	se activity ","n	nolecular_fu	inction"			
23	GO:0004674,0	003769290	38023568,6	.366518588	805597e-0	07,"count_l	nealthy","co	unt_Bacteria	l","count_h	ealthy_vs_cou	unt_Bacteria	1",0.00023197	521412555,0.00	023197521412	2555,"UP","pr	otein serine	/threonine ki	nase activity	","molecula	r_function"		
24	GO:0004676,0	003945549	09882275,2	.249776855	616967e-0	05,"count_H	nealthy","co	unt_Bacteria	l","count_h	ealthy_vs_cou	unt_Bacteria	",0.00424745	279179484,0.00	424745279179	484,"UP","3-	phosphoino	sitide-depend	dent protein	kinase activi	ty ","molecular	_function"	
25	GO:0004677,0	003945549	09882275,2	.249776855	516967e-(05,"count_H	nealthy","co	unt_Bacteria	l","count_h	ealthy_vs_cou	unt_Bacteria	",0.00424745	279179484,0.00	424745279179	484,"UP","DI	NA-depende	ent protein kir	nase activity	","molecular	function"		
26	GO:0004679,0	00376135	68682045,3	.335372019	28766e-0	05,"count_l	nealthy","co	unt_Bacteria	l","count_h	ealthy_vs_cou	unt_Bacteria	",0.00558155	77583239,0.00	558155977583	239,"UP","AI	MP-activated	d protein kina	se activity ",	"molecular_f	unction"		
27	GO:0004694,0	003945549	09882275,2	.249776855	516967e-0	05,"count_l	nealthy","co	unt_Bacteria	l","count_h	ealthy_vs_cou	unt_Bacteria	1",0.00424745	279179484,0.00	424745279179	484,"UP","et	ukaryotic tra	nslation initia	tion factor 2	alpha kinase	activity ","mole	ecular_funct	ion"
28	GO:0004711,0	003945549	09882275,2	.249776855	516967e-0	05,"count_l	nealthy","co	unt_Bacteria	l","count_h	ealthy_vs_cou	unt_Bacteria	1",0.00424745	279179484,0.00	424745279179	484,"UP","ril	bosomal pro	tein S6 kinase	activity ","n	nolecular_fu	nction"		
29	GO:0005634,-0	.00773071	390233566,	1.961896359	995156e-	-29,"count_	healthy","co	ount_Bacteria	al","count_h	nealthy_vs_co	unt_Bacteria	al",5.90962421	.544608e-26,5.	909624215446	08e-26,"DOV	/N","nucleu	s ","cellular_o	omponent"				
30	GO:0005654,-0	.00772828	357250891,	6.499016323	30308e-2	24,"count_h	ealthy","co	unt_Bacterial	","count_he	ealthy_vs_cou	int_Bacteria	",1.398309783	44524e-20,1.3	983097834452	4e-20,"DOWI	N","nucleop	lasm ","cellula	ar_compone	nt"			
			-																			

"GANGO_significant_results_2025-05-09.csv" file

BIOFunctional	
♠ НОМЕ	
GANGO Data Explorer	
E KEGG	<
Z Gene Ontologies	<
≫ Filter	
≫ Functional Analysis	
≫ Network Analysis	
≫ BarPlot	
B HELP	

BIOFUNCTIONAL

The program is also capable of analyzing KEGG, but in this example we will focus on Gene Ontologies.

3. BioFunctional: Gene Ontology Analysis

- Gene Ontologies (Input): BioFunctional uses the output file from GANGO (or a manually created file) containing gene ontologies: "GANGO_significant_results_2025-05-09.csv"
- **Column Selection:** BioFunctional automatically selects essential columns from the input file to guarantee the proper operation of the different algorithms. You can select additional columns of your interest for filtering and visualization in subsequent steps.
- **Download Preprocessed Files (OUTPUT):** Download the preprocessed data. This is an intermediate step to prepare the data for functional análisis: "filtered_data_2025-05-13.csv"

BIOFunctional	=								
🏫 НОМЕ	Filter	Data							
GANGO Data Explorer	Uploa	CSV File							
LE KEGG K	Brow	se GANGO_	significant_results_2025-05-07.csv						
₩Gene Ontologies <						Upload	complete		
>> Filter	Select	additional filter	columns:						
		lue_corrected							
	D FDF	t							
≫ BarPlot									
B HELP	± 0	ownload Processe	ed File						
	Filter	ed Data							
	Show	10 v entries							Search:
		ONTOLOGY	¢ EA_VALUE ¢	GROUP_1	GROUP_2	GROUP	UP_DOWN	ONT_NAME	ONT_DESCRIPTION
	1	GO:000082	0.00351085825693131	count_Mammals	count_Fungi	count_Mammals_vs_count_Fungi	UP	G1/S transition of mitotic cell cycle	biological_process
	2	GO:000086	0.000929660548487841	count_Mammals	count_Fungi	count_Mammals_vs_count_Fungi	UP	G2/M transition of mitotic cell cycle	biological_process
	3	GO:0000131	0.0161011941447655	count_Mammals	count_Fungi	count_Mammals_vs_count_Fungi	UP	incipient cellular bud site	cellular_component
	4	GO:0000307	0.00435189822842124	count_Mammals	count_Fungi	count_Mammals_vs_count_Fungi	UP	cyclin-dependent protein kinase holoenzyme complex	cellular_component
	5	GO:0000749	0.0186929075229834	count_Mammals	count_Fungi	count_Mammals_vs_count_Fungi	UP	response to pheromone triggering conjugation with cellular fusion	biological_process
	6	GO:0004693	-0.00259171337821795	count_Mammals	count_Fungi	count_Mammals_vs_count_Fungi	DOWN	cyclin-dependent protein serine/threonine kinase activity	molecular_function
	7	GO:0005543	0.0161011941447655	count_Mammals	count_Fungi	count_Mammals_vs_count_Fungi	UP	phospholipid binding	molecular_function

BioFunctional pre-selects essential columns from the input file. You can select additional columns for filtering and visualization in subsequent steps.

DOWNLOAD PREPROCESS FILES: "filtered_data_2025-05-13.csv"

A	1 *		× v	f_X	ONTOLOGY	,"EA_VA	LUE", "GR	OUP_1","G	ROUP_2",	'GROUP",'	UP_DOW	N","ONT_	NAME","ONT	_DESCRIPTIO	N"						
	A	В		с	D		E	F	G		- I	1.1	1	к	L	м	N	0	P	Q	R
1	ONTOLOGY,	EA VAL	UE", "GR	OUP 1"	"GROUP 2",	GROUP	","UP DO	WN","ONT	NAME","	ONT DES	RIPTION'										
2	GO:0000049.	-0.02069	93548355	1545."0	ount healthy	"."count	Bacteria	I","count	healthy v	count B	acterial"."	DOWN".	tRNA binding	z","molecular	r function"						
3	GO:0000387.	-0.03190	02821235	3485."co	ount healthy	"."count	Bacteria	I"."count	healthy v	count B	acterial"."	DOWN"	"spliceosomal	snRNP assen	nbly ","biolog	ical process					
4	GO:0000398.	-0.02566	52018185	3156."co	ount healthy	"."count	Bacteria	il"."count	healthy v	count B	acterial".'	DOWN".	mRNA splicin	ng, via spliceo	some ","biolo	gical proce	ss"				
5	GO:0000462.	-0.03190	02821235	3485."0	ount healthy	"."count	Bacteria	I"."count	healthy v	count B	acterial".	DOWN".	maturation o	f SSU-rRNA fr	om tricistron	c rRNA trans	cript (SSU-rRM	VA. 5.8S rRN/	A. LSU-rRNA)	","biological	process"
6	GO:0000463.	-0.02844	11147841	4554."0	ount healthy	"."count	Bacteria	I"."count	healthy y	count B	acterial"."	DOWN".	maturation o	f I SU-rRNA fr	om tricistroni	c rRNA trans	cript (SSU-rRN	A. 5.85 rRN4	ALISU-rRNA)	", "biological	process"
7	GO:0000723.	-0.01798	36536759	5952."co	ount healthy	"."count	Bacteria	il"."count	healthy v	count B	acterial".'	DOWN".	'telomere ma	intenance "."	biological pr	ocess"			i i		
8	GO:0000724.	-0.01569	99932940	1571."co	ount healthy	"."count	Bacteria	il"."count	healthy v	count B	, acterial".'	DOWN".	double-stran	d break repai	r via homolos	ous recomb	ination "."bio	logical proce	ess"		
9	GO:0000978.	-0.00746	53329450	12145."	ount health	v"."cour	t Bacter	ial","count	healthy	s count	Bacterial"	"DOWN"	"RNA polyme	erase II cis-re	ulatory regio	n sequence	specific DNA	binding "."m	olecular fur	nction"	
10	GO:000981.	-0.00810	13987694	74756."	ount health	v"."cour	t Bacter	ial","count	healthy	s count	Bacterial"	"DOWN"	"DNA-bindin	g transcriptio	n factor activ	ty, RNA poly	merase II-spe	cific "."mole	cular functi	on"	
11	60:0001650	-0.01330	0233700	6064."co	ount healthy	"."count	Bacteria	d"."count	healthy y	count B	acterial".	DOWN"	fibrillar cente	er"."cellular	component"	.,,		,	_		
12	GO:0002102.	0.01060	11607984	623."co	unt healthy	"count	Bacteria	"."count h	ealthy vs	count Ba	cterial"."	UP"."pod	osome "."cellu	ular compon	ent"						
13	GO:0002181.	-0.03810	0932069	3606."0	ount healthy	"."count	Bacteria	I"."count	healthy y	count B	acterial".	DOWN".	cytoplasmic t	translation "."	biological pr	ocess"					
14	60:0002523	0.02311	44481882	249."co	unt healthy!	"count	Bacterial	"."count h	ealthy vs	count Ba	cterial"."	UP","leuk	ocyte migratio	on involved i	n inflammato	ry response	"biological	process"			
15	60:0003677	-0.00756	56721127	82535."	ount health	v"."cour	t Bacter	al"."count	healthy	s count	Bacterial"	"DOWN"	"DNA bindin	g","molecula	r function"	,,	/				
16	GO:0003684	-0.01749	96757804	0983 "0	unt healthy	" "count	Bacteria	I" "count	healthy y	count B	acterial"	DOWN"	damaged DN	A hinding " "	nolecular fu	oction"					
17	GO:0003697.	-0.01432	26075141	1014."co	ount healthy	"."count	Bacteria	I"."count	healthy y	count B	acterial".	DOWN".	'single-strand	led DNA bind	ing ", "molecu	lar function					
18	60:0003723	-0.0127	53509424	9421."0	ount healthy	"."count	Bacteria	d"."count	healthy v	count B	acterial"."	DOWN"	RNA binding	"."molecular	function"	-					
19	60:0003735	-0.05036	55849751	5445 "0	ount healthy	" "count	Bacteria	d"."count	healthy v	count B	acterial"	DOWN"	structural cor	stituent of ri	hosome " "m	olecular fur	ction"				
20	GO:0003779.	0.00330	44840607	1434."0	ount healthy	","coun	Bacteria	al", "count	healthy y	s count E	acterial".	"UP", "act	in binding "."r	molecular fu	nction"						
21	GO:0003899.	-0.03056	52369381	5985."0	ount healthy	"."count	Bacteria	I"."count	healthy y	count B	acterial".	DOWN".	DNA-directer	d 5'-3' RNA po	lymerase act	vity "."mole	cular function	n"			
22	60:0004519	-0.02360	14227143	7218."0	ount healthy	"."count	Bacteria	d"."count	healthy v	count B	acterial"."	DOWN"	endonucleas	e activity "."n	nolecular fur	ction"	_				
23	GO:0004674	0.00376	92903802	3568 "0	ount health	/" "coun	Bacteri	al" "count	healthy y	s count P	lacterial"	"UP" "ord	tein serine/th	preonine kina	se activity "	molecular f	unction"				
24	60:0004676	0.00394	55490988	2275 "0	ount health	/" "coun	Barteri	al" "count	healthy y	s count P	lactorial"	"IIP" "3-n	hosphoinositi	ide-denende	nt protein kin	ase activity	"molecular	function"			
25	60:0004677	0.00394	55490988	2275 "0	ount health	/" "coun	Barteri	al" "count	healthy y	s count P	lactorial"	"IIP" "DN	A-dependent	protein kina	e activity " "	nolecular fi	nction"	anotion			
26	60:0004679	0.00376	13516868	2045."0	ount health	/"."coun	t Bacteriu	al","count	healthy v	s count P	lacterial".	"UP"."AN	P-activated n	rotein kinase	activity "."m	olecular fun	rtion"				
27	GO:0004694	0.00394	55490988	2275 "0	ount health	/" "coun	t Bacteria	al" "count	healthy v	s count P	lacterial"	"UP" "eul	arvotic transl	ation initiatio	on factor 2aln	ha kinase art	ivity " "moler	ular functio	in"		
28	60:0004711	0.00394	55490988	2275 "0	ount health	/" "coun	t Barteri:	al" "count	healthy v	s count P	lactorial"	"UP" "rib	osomal protei	in S6 kinase a	rtivity " "mole	a kinase aci	ion"	.unar_runctio			
20	60:0005634	-0.00773	30713902	33566 "	ount health	v" "cour	t Barter	ial" "count	healthy i	s count	Ractorial"	"DOWN"	"nucleus " "c	ellular como	onent"	.conor_ronec					
20	60:0005654	-0.00773	28283572	50891 "	ount health	w" "cour	t Bacter	ial" "count	healthy	is count	Bactorial"	"DOWN"	"nucleonlass	n " "cellular i	component"						
21	GO:0005656	-0.02793	22569051	5067 "0	ount healthu	" "count	- Pactoria	d" "count	healthy u	count P	actorial"	DOWN"	PNA polymer	rase III compl	ex " "cellular	component					
32	60:0005681	-0.02148	33005603	5787 "0	ount healthy	" "count	Bactoria	d" "count	healthy v	count B	acterial"	DOWN"	spliceosomal	complex " "c	ex , central	onent"					
33	60:0005682	-0.02783	22568951	5067 "0	ount healthy	" "count	Bacteria	d" "count	healthy w	count B	actorial"	DOWN"	"US snRNP " "	cellular.com	onent"	onen					
34	GO:0005684	-0.0314	75587512	0923 "co	ount healthy	" "count	Bacteria	d" "count	healthy v	count B	acterial" '	DOWN"	"U2-type splic	eosomal com	nlex " "cellul	ar compone	nt"				
35	60:0005685	-0.02844	11147841	4554 "0	ount healthy	" "count	Bacteria	d" "count	healthy y	count B	acterial"	DOWN"	"U1 snRNP " "	cellular com	onent"	ar_compone					
36	GO:0005686.	-0.03056	52369381	5985."cc	ount healthy	"."count	Bacteria	d"."count	healthy y	count B	acterial".	DOWN".	"U2 snRNP "."	cellular com	onent"						
37	60:0005689	-0.02955	57683854	7302."0	ount healthy	", "count	Bacteria	d"."count	healthy v	count B	acterial".	DOWN".	U12-type spli	iceosomal co	molex "."cellu	ilar compon	ent"				
38	GO:0005730	-0.0130	57071825	9853."m	ount healthy	","count	Bacteria	I","count	healthy v	count B	acterial"	DOWN"	"nucleolus " "	cellular com	ponent"	acompon					
39	GO:0005737.	-0.00552	20133957	4334."0	ount healthy	"."count	Bacteria	d","count	healthy y	count B	acterial".	DOWN".	cytoplasm "."	cellular com	ponent"						
40	GO:0005739.	-0.0130	72945728	2131."co	ount healthy	"."count	Bacteria	I"."count	healthy v	count B	acterial".	DOWN".	mitochondrig	on "."cellular	component"						

4. Functional Analysis

- Input: Use the preprocessed file obtained in the previous step: "filtered_data_2025-05-13.csv"
- **Process:** BioFunctional analyzes the Gene Ontology terms and determines their hierarchies for each group in your data.
- Output: The tool adds columns to the table, indicating the GO term hierarchies:" go.csv"
- **Download Processed File:** Download the processed file, which now includes GO term hierarchy information.

BIOFunctional	=
Н ОМЕ	Functional Analysis
GANGO Data Explorer	Upload File
L≣ KEGG <	Browse filtered_data_2025-05-13.csv
≫ Filter ≫ Functional Analysis ≫ Network Analysis ≫ HeatMap	Lownload Processed File
⊠ Gene Ontologies <	
B HELP	€ Cear Data

GET THE HIERARCHIES OF THE GENE ONTOLOGIES AND INDICATE THEM IN THE TABLE FOR EACH GROUP:

П НОМЕ	Fund	ctional Analysis									
GANGO Data Explorer	Uplos	ad File									
LEE. KEGG K	Bro	wsefiltered_data_2025-05-13.csv									
Z Gene Ontologies <						Upload complete					
30 Eilter	*	Download Processed File									
> Functional Analysis											
>> Network Analysis	Show	10 v entries									Search:
≫ BarPlot		GROUP	ONTOLOGY	UP DOWN	ONT DESCRIPTION	ONT NAME	EA VALUE	GROUP 1	GROUP 2	first ancestor	first ancestor name
BHELP	1	count healthy vs count Bacterial	60.000049	DOWN	molecular function	19NA binding	0.0206935483551545	count healthy	count Racterial	60:0005488	binding
	-	count healthy as count Barterial	60-000387	DOWN	biological percess	enlineersmal exPMP accombin	0.0319028212353485	count healthu	court Bacterial	60-0009987	callular opposes
	-	count healthy us count Basterial	00.0000388	DOWN	biological process	aprocessment and a collectronic statements	0.0356630181853156	count healthu	count Bastedal	60.00000000	callular process
	-	count_memory_vii_count_bacterial	00.000.000	DOWN	biological process	menter apriling, ma aprilanzarine	0.02100201010101010	count_health	Count_Databala	00.00000007	- Bular process
	-	count_nearby_vs_count_bacterial	00000492	DOWN	biological_process	maturation of SSU-rena from tricitonic rena transcript (SSU-rena, 5.65 rena, LSU-rena)	-0.0319020212353465	count_nealthy	count_bacteriat	60:0009987	cellular process
	3	count_neariny_vs_count_bacterial	000000465	DOWN	biological_process	maturation of LSO-rene from tricistronic rene transcript (SSO-rene, S.65 rene, LSO-rene)	-010504411410414334	count_nearthy	count_bactenat	00:0003381	cenurar process
	6	count_healthy_vs_count_Bacterial	G0:0000723	DOWN	biological_process	telomere maintenance	-0.0179865367595952	count_healthy	count_Bacterial	GO:0009987	cellular process
	7	count_healthy_vs_count_Bacterial	G0:000724	DOWN	biological_process	double-strand break repair via homologous recombination	-0.0156999329401571	count_healthy	count_Bacterial	GO:0009987	cellular process
	8	count_healthy_vs_count_Bacterial	GO:0000978	DOWN	molecular_function	RNA polymerase II cis-regulatory region sequence-specific DNA binding	-0.00746332945012145	count_healthy	count_Bacterial	G0:0005488	binding
	9	count_healthy_vs_count_Bacterial	GO:000981	DOWN	molecular_function	DNA-binding transcription factor activity, RNA polymerase II-specific	-0.00810398769474756	count_healthy	count_Bacterial	GO:0140110	transcription regulator activity
	10	count_healthy_vs_count_Bacterial	GO:0001650	DOWN	cellular_component	fibrillar center	-0.0133002337006064	count_healthy	count_Bacterial	GO:0110165	cellular anatomical structure
	Show	ing 1 to 10 of 141 entries							Previous	1 2 3	4 5 15 Next
		Clear Data									

DOWNLOAD PROCESSED FILE: go.csv

A1	~		< √ fx	GROUP,"ONT	OLOGY","UP	_DOWN","O	NT_DESCRIPT	ION","ONT_1	NAME","E	A_VALUE","	GROUP_1","GI	GROUP_2"	"first_anc	estor","first_	ancestor_nar	ne"						
	А	В	с	D	E	F	G	н	1.1	- L	ј к	ĸ	L	м	N	0	Р	Q	R	s	т	
1 GRC	UP,"ON	OLOGY'	,"UP_DOWN"	,"ONT_DESCRIPT	ION","ONT_N	NAME","EA_	VALUE","GRO	UP_1","GROU	JP_2","firs	st_ancestor'	","first_ancest	stor_name	e"									
2 cou	nt_health	y_vs_co	unt_Bacteria	l,"GO:0000049","	DOWN","mol	lecular_func	tion","tRNA b	inding ",-0.0	206935483	551545,"cou	unt_healthy",'	,"count_B	acterial","	GO:0005488",	"binding"							
3 cou	nt_health	y_vs_co	unt_Bacteria	I,"GO:0000387","I	DOWN","biol	logical_proce	ss","spliceos	omal snRNP	assembly	",-0.031902	8212353485,"c	count_he	althy","cou	int_Bacterial	","GO:000998	7","cellular	process"					
4 cou	nt_health	y_vs_co	unt_Bacteria	l,"GO:0000398","	DOWN","biol	logical_proci	ss","mRNA s	olicing, via sp	liceosom	e ",-0.02566	20181853156,	,"count_h	ealthy","co	ount_Bacteri	al","GO:0009	987","cellula	ar process"					
5 cou	nt_health	y_vs_co	unt_Bacteria	l,"GO:0000462","	DOWN","biol	logical_proce	ess","maturat	ion of SSU-rR	NA from t	tricistronic r	RNA transcrip	pt (SSU-rR	NA, 5.8S rF	RNA, LSU-rRN	A) ",-0.03190	2821235348	5,"count_heal	thy","count_B	acterial","GO	:0009987","c	ellular process	
6 cou	nt_health	y_vs_co	unt_Bacteria	l,"GO:0000463","	DOWN","biol	logical_proce	ss","maturat	ion of LSU-rR	NA from t	ricistronic r	RNA transcrip	pt (SSU-rR	NA, 5.8S rF	INA, LSU-rRN	A) ",-0.02844	11478414554	4,"count_heal	thy","count_B	acterial","GO	:0009987","o	Ilular process	•
7 cou	nt_health	y_vs_co	unt_Bacteria	l,"GO:0000723","	DOWN","biol	logical_proce	ss","telomer	e maintenan	ce ",-0.01	79865367595	5952,"count_h	healthy",	count_Bac	terial","GO:0	009987","cel	lular proces	s"					
8 cou	nt_health	y_vs_co	unt_Bacteria	l,"GO:0000724","	DOWN","biol	logical_proce	ess","double-	strand break	repair via	homologou	is recombinat	tion ",-0.0	156999329	401571,"cour	nt_healthy","	count_Bacte	erial","GO:000	9987","cellula	r process"			
9 cou	nt_health	y_vs_co	unt_Bacteria	l,"GO:0000978","	DOWN","mol	lecular_func	tion","RNA po	lymerase II o	is-regulat	tory region s	sequence-spe	ecific DNA	binding ",	-0.007463329	45012145,"co	ount_health	y","count_Bac	terial","GO:00	05488","bind	ing"		
10 cou	nt_health	y_vs_co	unt_Bacteria	l,"GO:0000981","	DOWN","mol	lecular_func	tion","DNA-bi	nding transc	ription fac	ctor activity,	, RNA polyme	erase II-sp	ecific ",-0.	008103987694	474756,"coun	t_healthy",'	"count_Bacter	ial","GO:01401	10","transcri	otion regulat	or activity"	
11 cou	nt_health	y_vs_co	unt_Bacteria	l,"GO:0001650","	DOWN","cell	ular_compoi	nent","fibrilla	r center ",-0.	013300233	37006064,"ci	ount_healthy'	","count	Bacterial",	"GO:0110165	","cellular ar	natomical str	ructure"					
12 cou	nt_health	y_vs_co	unt_Bacteria	l,"GO:0002102","	UP","cellular	_component	","podosome	",0.01060116	507984623	,"count_hea	althy","count_	_Bacteria	l","GO:011	0165","cellul	ar anatomica	l structure"						
13 cou	nt_health	y_vs_co	unt_Bacteria	l,"GO:0002181","	DOWN","biol	logical_proce	ess","cytoplas	mic translati	on ",-0.03	8100932069	3606,"count_h	healthy",	'count_Bac	terial","GO:	009987","cel	lular proces	s"					
14 cou	nt_health	y_vs_co	unt_Bacteria	l,"GO:0002523","	UP","biologic	al_process",	"leukocyte m	igration invo	lved in in	flammatory	response ",0.	0.02311444	81882249,	"count_healt	hy","count_E	Bacterial","G	iO:0002376","i	mmune syster	m process"			
15 cou	nt_health	y_vs_co	unt_Bacteria	l,"GO:0003677","	DOWN","mol	lecular_func	tion","DNA bi	nding ",-0.00	756672112	2782535,"co	unt_healthy",	',"count_E	lacterial","	GO:0005488"	,"binding"							
16 cou	nt_health	y_vs_co	unt_Bacteria	l,"GO:0003684","	DOWN","mol	lecular_func	tion","damage	ed DNA bind	ing ",-0.01	7496757804	0983,"count_h	healthy",	"count_Ba	cterial","GO:	0005488","bii	nding"						
17 cou	nt_health	y_vs_co	unt_Bacteria	l,"GO:0003697","	DOWN","mol	lecular_func	tion","single-	stranded DN	A binding	",-0.014326	0751411014,"c	count_he	althy","cou	int_Bacterial	","GO:000548	8","binding						
18 cou	nt_health	y_vs_co	unt_Bacteria	l,"GO:0003723","	DOWN","mol	lecular_func	tion","RNA bi	nding ",-0.01	275350942	249421,"cou	nt_healthy","	"count_Ba	cterial","G	O:0005488",'	'binding"							
19 cou	nt_health	y_vs_co	unt_Bacteria	l,"GO:0003735","	DOWN","mol	lecular_func	tion","structu	ral constitue	nt of ribos	some ",-0.05	5036584975154	i445,"cou	nt_healthy	","count_Bac	terial","GO:0	005198","str	ructural molec	ule activity"				
20 cou	nt_health	y_vs_co	unt_Bacteria	l,"GO:0003779","	UP","molecul	lar_function	',"actin bindir	ng ",0.003304	484060714	434,"count_	healthy","cou	unt_Bacte	rial","GO:0	005488","bir	iding"							
21 cou	nt_health	y_vs_co	unt_Bacteria	l,"GO:0003899","	DOWN","mol	lecular_func	tion","DNA-di	rected 5'-3' F	RNA polyn	nerase activ	ity ",-0.030562	523693815	985,"count	_healthy","o	ount_Bacteri	al","GO:000	9987","cellula	process"				
22 cou	nt_health	y_vs_co	unt_Bacteria	l,"GO:0004519","	DOWN","mol	lecular_func	tion","endonu	uclease activ	ity ",-0.02	3604227143	7218,"count_h	healthy",'	'count_Bac	terial","GO:0	003824","cat	alytic activit	:y"					
23 cou	nt_health	y_vs_co	unt_Bacteria	l,"GO:0004674","	UP","molecul	lar_function	',"protein ser	ine/threonir	ie kinase a	activity ",0.0	037692903802	23568,"cc	unt_health	ny","count_B	acterial","GC	:0003824","	catalytic activi	ty"				
24 cou	nt_health	y_vs_co	unt_Bacteria	l,"GO:0004676","	UP","molecul	lar_function	',"3-phosphoi	nositide-dep	pendent p	rotein kinas	se activity ",0.	.00394554	909882275	,"count_heal	thy","count_	Bacterial","	GO:0003824",'	catalytic activ	ity"			
25 cou	nt_health	y_vs_co	unt_Bacteria	l,"GO:0004677","	UP","molecul	lar_function	',"DNA-deper	ndent protei	n kinase a	ctivity ",0.00	0394554909883	32275,"coi	unt_health	y","count_Ba	cterial","GO:	:0003824","c	atalytic activit	Y"				
26 cou	nt_health	y_vs_co	unt_Bacteria	l,"GO:0004679","	UP","molecul	lar_function	',"AMP-activa	ted protein l	kinase act	ivity ",0.003	761351686820	045,"cour	t_healthy"	,"count_Bact	erial","GO:0	003824","cat	alytic activity					
27 cou	nt_health	y_vs_co	unt_Bacteria	l,"GO:0004694","	UP","molecul	lar_function	',"eukaryotic	translation in	nitiation fa	actor 2alpha	i kinase activit	ity ",0.003	945549098	82275,"count	_healthy","c	ount_Bacter	rial","GO:0003	824","catalytic	activity"			
28 cou	nt_health	y_vs_co	unt_Bacteria	l,"GO:0004711","	UP","molecul	lar_function	',"ribosomal p	protein S6 kir	nase activi	ity ",0.00394	1554909882275	5,"count_	healthy","	count_Bacter	rial","GO:000	3824","catal	ytic activity"					
29 cou	nt_health	y_vs_co	unt_Bacteria	l,"GO:0005634","	DOWN","cell	ular_compoi	nent","nucleu	s ",-0.007730	71390233	566,"count_	healthy","cou	unt_Bacte	rial","GO:0	0110165","cel	lular anatom	ical structur	e"					
30 cou	nt_health	y_vs_co	unt_Bacteria	l,"GO:0005654","	DOWN","cell	ular_compoi	nent","nucleo	plasm ",-0.0	077282835	7250891,"co	ount_healthy"	","count_	Bacterial",	"GO:0110165	","cellular an	atomical str	ucture"					
31 cou	nt_health	y_vs_co	unt_Bacteria	l,"GO:0005666","	DOWN","cell	ular_compoi	nent","RNA pi	olymerase III	complex	",-0.0278325	5689515067,"c	count_he	althy","cou	int_Bacterial	","GO:011016	5","cellular	anatomical st	ucture"				
32 cou	nt_health	y_vs_co	unt_Bacteria	l,"GO:0005681","	DOWN","cell	ular_compoi	nent","spliced	somal comp	lex ",-0.02	21483005603	5787,"count_	_healthy"	"count_Ba	cterial","GO:	0032991","pr	otein-conta	ining comple>					
33 cou	nt_health	y_vs_co	unt_Bacteria	l,"GO:0005682","	DOWN","cell	ular_compoi	nent","U5 snR	NP ",-0.0278	325689515	067,"count	healthy","co	ount_Bact	erial","GO:	0110165","ce	Ilular anaton	nical structu	re"					
34 cou	nt_health	y_vs_co	unt_Bacteria	l,"GO:0005684","I	DOWN","cell	ular_compoi	nent","U2-typ	e spliceoson	nal comple	ex ",-0.0314	755875120923,	3,"count_	nealthy","o	ount_Bacter	ial","GO:0110	165","cellul	ar anatomical	structure"				
35 cou	nt_health	y_vs_co	unt_Bacteria	l,"GO:0005685","	DOWN","cell	ular_compoi	nent","U1 snR	NP ",-0.0284	411478414	1554,"count_	healthy","co	ount_Bact	erial","GO:	0110165","ce	Ilular anaton	nical structu	re"					
36 cou	nt_health	y_vs_co	unt_Bacteria	l,"GO:0005686","I	DOWN","cell	ular_compoi	nent","U2 snR	NP ",-0.0305	623693815	985,"count	_healthy","coi	ount_Bact	erial","GO:	0110165","ce	Ilular anaton	nical structu	re"					
37 cou	nt_health	y_vs_co	unt_Bacteria	l,"GO:0005689","	DOWN","cell	ular_compoi	nent","U12-ty	pe spliceoso	mal comp	lex ",-0.029	557683854730	02,"count	healthy",	'count_Bacte	rial","GO:011	.0165","cellu	ular anatomica	structure				
38 cou	nt_health	y_vs_co	unt_Bacteria	l,"GO:0005730","	DOWN","cell	ular_compoi	nent","nucleo	lus ",-0.0130	570718259	9853,"count	_healthy","co	ount_Bact	erial","GO:	0110165","ce	Ilular anator	nical structu	re"					
39 cou	nt_health	y_vs_co	unt_Bacteria	l,"GO:0005737","I	DOWN","cell	ular_compoi	nent","cytopla	asm ",-0.0055	20133957	4334,"count	healthy","co.	ount_Bac	erial","GO	:0110165","c	ellular anator	mical structu	ire"					
40 cou	nt_health	y_vs_co	unt_Bacteria	l,"GO:0005739","	DOWN","cell	ular_compoi	nent","mitoch	iondrion ",-0	.01307294	57282131,"c	ount_healthy	y","count	Bacterial"	,"GO:011016	5","cellular a	natomical st	ructure"					

5. Network Analysis

- Input: Use the ontology table FILE from the previous step: "go.csv"
- **Selection:** BioFunctional allows you to select GO terms based on:
 - GO category (Biological Process, Cellular Component, Molecular Function).
 - Hierarchy filters.
- Visualization: BioFunctional creates a network graph that represents the relationships between chosen GO terms. This allows users to examine how ontology relationships vary across different experimental groups. The tool provides extensive options for filtering and visualizing the network, such as selecting by ontology and reordering groups to highlight specific comparisons.

BIOFunctional	=	
🟫 НОМЕ	Network Analysis Filters	Network
GANGO Data Explorer	Upload File	GO Network
E KEGG K	Browse go.csv	Salarshu first and
X Gene Ontologies <	Upload complete	Groups
≫ Filter	Ontology Description	
>> Functional Analysis	fraunt -	
>> BarPlot	All groups	
BHELP	k Download Network	A 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4
	Clear Data	
	Larrand	
	Arrows	
	Up-regulated	count healthy
	Down-regulated	
	reutral	
		count_Bacterial Count_Bacterial
		2 <u>2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 </u>
		<u></u>
		· · · · · · · · · · · · · · · · · · ·

GO Network



There are different options and drop-down menus to SELECT BY GO BIOLOGICAL, FUNCTION, ETC

Upload File Browse go.csv Upload complete Ontology Description molecular_function biological_process cellular_component & Download Network Clear Data Legend ArrowsUp-regulatedDown-regulatedNeutral	Network Ana	alysis Filters
Browse go.csv Upload complete Ontology Description molecular_function biological_process cellular_component & Download Network Clear Data Legend Arrows Up-regulated Down-regulated Neutral	Upload File	
Upload complete Ontology Description molecular_function biological_process cellular_component Clear Data Legend Arrows Up-regulated Down-regulated Neutral	Browse	go.csv
Ontology Description molecular_function biological_process cellular_component > Download Network Clear Data Legend Arrows Up-regulated Down-regulated Neutral		Upload complete
molecular_function molecular_function biological_process cellular_component Download Network Clear Data Legend Arrows Up-regulated Down-regulated Neutral	Ontology Desc	ription
molecular_function biological_process cellular_component Download Network Clear Data Legend Arrows Up-regulated Down-regulated Neutral	molecular_fu	nction 🔺
biological_process cellular_component Download Network Clear Data Legend Arrows Up-regulated Down-regulated Neutral	molecular_fu	nction
cellular_component Download Network Clear Data Legend Arrows Up-regulated Down-regulated Neutral	biological_pr	ocess
Download Network Clear Data Legend Arrows Up-regulated Down-regulated Neutral	cellular_com	ponent
Clear Data Legend Arrows Up-regulated Down-regulatedNeutral	🛓 Download	Network
Legend Arrows Up-regulated Down-regulated Neutral	👕 Clear Data	
Arrows — Up-regulated — Down-regulated — Neutral	Legend	
Up-regulated Down-regulated Neutral	Arrows	
Down-regulated Neutral	Up-regulate	d
Neutral	Down-regul	ated
	Neutral	



ALSO FOR DIFFERENT HIERARCHY FILTERS

6. Bar Plot

• Input: Use the GO term file from the functional análisis: "go.csv"

• Visualization: BioFunctional creates a bar plot showing the enrichment of specific GO terms.





DOWNLOAD FILE: "DOWN_data_zscore_2025-05-13.csv"

7.Interpreting Gene Ontology (GO) Data obtained in Biofunctional: Challenges and Manual Interpretation

Gene Ontology (GO) provides a structured vocabulary to describe gene functions across different organisms. It categorizes these functions into three domains:

- **Biological Process:** Describes biological objectives to which the gene contributes. (e.g., "cell cycle")
- Cellular Component: Describes where the gene product acts. (e.g., "nucleus")
- Molecular Function: Describes the gene product's biochemical activity. (e.g., "DNA binding")

Why is GO Interpretation Difficult?

Interpreting GO data, especially after a GO enrichment analysis (identifying over-represented GO terms in a set of genes), can be challenging for several reasons:

- **Hierarchical Complexity:** GO terms are organized in a complex, hierarchical structure (a Directed Acyclic Graph, or DAG). A gene can be associated with multiple terms at different levels, making it difficult to pinpoint the most relevant functions.
- **Redundancy:** Due to the hierarchical structure, some GO terms may be redundant, with broader terms encompassing more specific ones. This can make it hard to extract precise biological insights.
- **Context Dependence:** The biological significance of a GO term can vary depending on the specific experimental context, organism, or tissue type.
- Annotation Bias: Some genes are better studied and annotated than others, leading to bias in GO term representation. Well-studied genes may show up more frequently in GO enrichment results, potentially overshadowing the roles of less-studied but equally important genes.
- Large Datasets: High-throughput experiments often generate long lists of significantly enriched GO terms, making it difficult to manually sift through and identify the most meaningful ones.

7.1. How to Perform Manual Interpretation

Despite these challenges, manual interpretation by experts is crucial for drawing accurate and biologically relevant conclusions. Here's how a biologist or clinician might approach it:

1. Prioritize Relevant GO Terms:

- Focus on the most significantly enriched GO terms (based on statistical measures like p-values or Z-values in Biofunctional using the barplots).
- Consider the specific biological question or experimental context to narrow down the list.

2. Navigate the GO Hierarchy:

- Use GO browsers (like AmiGO) to explore the relationships between GO terms.
- Identify parent and child terms to understand the broader and more specific functions associated with the gene set.
- Look for the "sweet spot" in the hierarchy: terms that are specific enough to be informative but not so granular that they become overwhelming.

3. Literature Review (Bibliography):

- Consult scientific literature (PubMed, etc.) to understand the biological functions associated with the identified GO terms.
- Investigate the genes annotated with those terms and their known roles in the relevant biological processes or disease conditions.
- Look for supporting evidence that connects the GO terms to the experimental findings.
- 4. Cross-referencing and Validation (Checking Results):
 - Compare the GO analysis results with other relevant data, such as:
 - Other functional enrichment analyses (e.g., KEGG pathway analysis).
 - Gene expression data.
 - Protein-protein interaction networks.
 - Phenotype data.
 - Validate the findings by checking if the identified GO terms align with the expected biological functions based on prior knowledge.
 - Look for consistency and convergence of evidence from multiple sources.

By combining computational analysis with expert knowledge and thorough literature review, biologists and clinicians can effectively interpret GO data and gain valuable insights into gene function and their roles in biological systems.

7.2-GO Term Interpretation with CURIE Technology

The GO terms of interest can also be interpreted using a novel technology developed by the BIOST3 group at the University of Barcelona, called CURIE. This technology is currently in an experimental phase and pending patent or registration. While highly efficient and requiring very few cases to be effectively utilized, it remains experimental and may be subject to potential errors or inconsistencies

The GO of interest in the plot. In this example we are interested in the GO enriched significatively in the bacteria group for biological process (Negatives, or regulation down) can be downloaded for study using option "Download DOWN DATA (Z-SCORE)" button. Obtain file: "DOWN_data_zscore_2025-05-13.csv"



This gene ontologies can be extracted manually (expert criteria) or using an special R script (SEE "go selection.R"):

In the regulation down plot (related to bacterial infection and biological process), the displayed ontologies serve as an illustrative example of the many that were detected. It's crucial to note that users must determine the most relevant ontologies for their specific research (RED ARROWS). For instance, this could involve selecting all detected ontologies, or focusing solely on those with the most positive or negative Z-score values. The preceding graph highlights the most positively enriched ontologies, and as a further example, CURIE has interpreted one of these ontologies. It's important to understand that the actual interpretation work is far more extensive than what's shown in this brief example.

Ontologies in the regulation down plot (related to bacterial infection and biological process):

ONTOLOGY	ONT_DESCRIPTION	ONT_NAME
12 GO:0002181	biological_process	cytoplasmic translation
55 GO:0006357	biological_process	regulation of transcription by RNA polymerase II
89 GO:0032543	biological_process	mitochondrial translation
106 GO:0042274	biological_process	ribosomal small subunit biogenesis

GO Term Codes: These GO terms can be converted into a comma-separated list of description codes using an R script. This is crucial for interpreting the results and use an artificial intelligence:

"cytoplasmic translation ,regulation of transcription by RNA polymerase II ,mitochondrial translation ,ribosomal small subunit biogénesis"

EXAMPLE INTERPRETATION USING AN EXTERNAL ARTIFICIAL INTELLIGENCE (CURIE AI) FOR GO TERMS: "cytoplasmic translation and bacterial infection"



CURIE AI 09:42

The study of cytoplasmic translation is crucial in understanding bacterial infection mechanisms. It involves the process by which ribosomes translate messenger RNA (mRNA) into proteins, which are essential for bacterial growth, survival, and pathogenicity. During bacterial infection, pathogens employ various strategies to manipulate the host's cellular machinery, including hijacking the translation machinery for their own benefit. This can involve the production of effector proteins that modulate host cell functions or the use of virulence factors that directly damage the host. Recent studies have highlighted the role of specific bacterial proteins in interacting with host ribosomes to enhance translation efficiency. For example, the jumbophage protein interacts with both the ribosome and the phage nucleus, facilitating efficient propagation of the phage within the host cell. Additionally, the GTPase BipA plays a dual role in ribosome assembly and surface structure biogenesis in Salmonella enterica serovar Typhimurium, demonstrating the importance of translation regulation in bacterial Understanding the molecular mechanisms of cytoplasmic translation during bacterial virulence. infection provides opportunities for developing targeted therapies. Interrupting protein synthesis pathways or enhancing the immune response against bacterial pathogens could lead to novel treatment strategies. Future research should focus on elucidating the precise mechanisms by which bacteria manipulate the host's translation machinery. This knowledge will contribute to the development of new antibiotics and therapeutic interventions against bacterial infections.

References:

- A ribosome-interacting jumbophage protein associates with the phage nucleus to facilitate efficient propagation. (PMID: 39992933)

- The dual functions of the GTPase BipA in ribosome assembly and surface structure biogenesis in Salmonella enterica serovar Typhimurium. (PMID: 40203049)

- Molecular basis for the phosphorylation of bacterial tyrosine kinase Wzc. (PMID: 40210632)

- Analysis of cytosolic mtDNA release during Staphylococcus aureus infection. (PMID: 40058964)

- Deficiency in peptidoglycan recycling promotes β -lactam sensitivity in <i>Caulobacter crescentus</i>. (PMID: 40066998)

Was this response helpful? Referenced Articles

A ribosome-interacting jumbophage protein associates with the phage nucleus to facilitate efficient propagation.

propagation.

PMID: 39992933DOI: 10.1371/journal.ppat.1012936

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The dual functions of the GTPase BipA in ribosome assembly and surface structure biogenesis in Salmonella enterica serovar Typhimurium.

PMID: 40203049DOI: 10.1371/journal.ppat.1013047 View Full Text Show Abstract *Molecular basis for the phosphorylation of bacterial tyrosine kinase Wzc.*

PMID: 40210632DOI: 10.1038/s41467-025-58693-7 View Full Text Show Abstract

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McClain, M. T., Constantine, F. J., Henao, R., Liu, Y., Tsalik, E. L., Burke, T. W., Steinbrink, J. M., Petzold, E., Nicholson, B. P., Rolfe, R., Kraft, B. D., Kelly, M. S., Saban, D. R., Yu, C., Shen, X., Ko, E. M., Sempowski, G. D., Denny, T. N., Ginsburg, G. S., & Woods, C. W. (2021). Dysregulated transcriptional responses to SARS-CoV-2 in the periphery. *Nat Commun* **12**, 1079. https://doi.org/10.1038/s41467-021-21289-y

Love, M. I., Huber, W., & Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology*, *15*, 550. https://doi.org/10.1186/s13059-014-0550-8