frontiers | Frontiers in Medicine

TYPE Technology and Code
PUBLISHED 11 August 2022
DOI 10.3389/fmed.2022.965908

OPEN ACCESS

EDITED BY
Thomas H. Hampton,
Dartmouth College, United States

REVIEWED BY
Britton Goodale,
Dartmouth College, United States
Zhongyou Li,
University of Colorado Anschutz
Medical Campus, United States

*CORRESPONDENCE
Antonio Mora
antoniocmora@gzhmu.edu.cn

†These authors have contributed equally to this work

SPECIALTY SECTION
This article was submitted to Gene and Cell Therapy, a section of the journal Frontiers in Medicine

RECEIVED 10 June 2022
ACCEPTED 15 July 2022
PUBLISHED 11 August 2022

CITATION
Huang X, Lu X, Xie C, Jauhari S, Xie Z, Mei S and Mora A (2022) GSA Central—A web platform to perform, learn, and discuss gene set analysis. *Front. Med.* 9:965908. doi: 10.3389/fmed.2022.965908

GSA Central—A web platform to perform, learn, and discuss gene set analysis

Xiaowei Huang[1†], Xuanyi Lu[1†], Chengshu Xie[1†], Shaurya Jauhari[1], Zihong Xie[1], Songqing Mei[2] and Antonio Mora[1*]

[1]Joint School of Life Sciences, Guangzhou Medical University and Guangzhou Institutes of Biomedicine and Health (Chinese Academy of Sciences), Guangzhou, China, [2]School of Biomedical Engineering, Guangzhou Medical University, Guangzhou, China

Gene Set Analysis (GSA) is one of the most commonly used strategies to analyze *omics* data. Hundreds of GSA-related papers have been published, giving birth to a GSA field in Bioinformatics studies. However, as the field grows, it is becoming more difficult to obtain a clear view of all available methods, resources, and their quality. In this paper, we introduce a web platform called "GSA Central" which, as its name indicates, acts as a focal point to centralize GSA information and tools useful to beginners, average users, and experts in the GSA field. "GSA Central" contains five different resources: A Galaxy instance containing GSA tools ("Galaxy-GSA"), a portal to educational material ("GSA Classroom"), a comprehensive database of articles ("GSARefDB"), a set of benchmarking tools ("GSA BenchmarKING"), and a blog ("GSA Blog"). We expect that "GSA Central" will become a useful resource for users looking for introductory learning, state-of-the-art updates, method/tool selection guidelines and insights, tool usage, tool integration under a Galaxy environment, tool design, and tool validation/benchmarking. Moreover, we expect this kind of platform to become an example of a "thematic platform" containing all the resources that people in the field might need, an approach that could be extended to other bioinformatics topics or scientific fields.

KEYWORDS

web platform, gene set analysis, pathway analysis, database, galaxy, benchmarking, education

Introduction

"Gene Set Analysis" (GSA) is an annotation-based approach for *omics* data analysis. It has been defined as the statistical comparison of a query gene set to a database of annotated gene sets to transform gene-level experimental results into gene-set-level experimental results. In other words, a statistical method to interpret a query gene set in terms of biological pathways or functionally related gene sets from a reference database

(1). If the query set is made of genes differentially expressed between two experimental conditions, a GSA result can be understood as the gene sets, ontology terms, or pathways significantly enriched between those experimental conditions (1).

GSA has become one of the standard analyses of current *omics* data analysis workflows. Therefore, numerous independent tools have been created to perform GSA on different types of datasets or from different programming environments, such as GSEA (2), DAVID (3), Enrichr (4), clusterProfiler (5), GOseq (6), and ClueGO (7). Multiple reviews have been written and multiple courses have been offered, and, consequently, a GSA sub-field with its own jargon, methods, tools, and opposing schools has appeared. In this context, our goal is to create a web platform that can serve as a focal point for GSA practitioners, both novice and experts. In this platform, biomedical researchers can use simplified versions of the existing tools, find information about all existing methods and reviews, discuss new developments, follow online lessons, and more.

Here we introduce "GSA Central," a web platform to perform, learn, and discuss GSA, which is divided into five sections (**Figure 1**): (i) "Galaxy-GSA" (a collection of a variety of GSA tools inside a Galaxy environment), (ii) "GSA Classroom" (a database of online GSA courses and videos), (iii) "GSARefDB" (a comprehensive database of all published GSA papers), (iv) "GSA BenchmarKING" (a repository of tools to benchmark GSA methods and software), and (v) "GSA Blog" (a place to discuss novel GSA-related topics).

Materials and methods

GSA Central

GSA Central's website was built as a GitHub website[1] using HTML, CSS, and javascript. The code is open source and can be reviewed or cloned from https://github.com/gsa-central/gsa-central.github.io.

Galaxy-GSA

Galaxy is one of the most popular multi-purpose bioinformatics platforms. It was originally built as a platform for researchers without programming experience and started as a public server with an emphasis on simplicity.[2] The original Galaxy server offered data search and manipulation options, multiple bioinformatics tools, and the possibility of easily combining different analyses thanks to an element called the "History" which allowed the storage of every intermediate result (8). Soon after, it was clear that Galaxy offered more than simplicity, and quickly became one of the best platforms to guarantee the transparency and reproducibility of full computational analyses for biologists and bioinformaticians (9). More recently, Galaxy has evolved into an open environment that facilitates the development of independent and specialized Galaxy instances and servers, as well as the wrapping of multiple bioinformatics packages or libraries inside the Galaxy framework to take advantage of its simplicity, transparency, and reproducibility. Due to that, a vast ecosystem of Galaxy servers[3] and individual tools[4] has been built, which nowadays includes Galaxy tools for the most common tasks found in Bioinformatics.

Several GSA tools have been added to the Galaxy public server, including "g:Profiler" (10), "DAVID" (3), "goseq" (6), "EGSEA" (11), "fgsea" (12), "GOEnrichment" (13), and "KOBAS" (14). Using the galaxy toolshed, it is possible to find other popular GSA tools authored by the community, such as "clusterProfiler" (5) and "TopGO" (15). However, other kinds of experimental designs may require more sophisticated GSA tools. For example, an experiment with thousands of patients and only one sample per patient would be better suited for using "single-sample" GSA methods; an experiment generating multi-omics datasets would better use "integrative" GSA methods; *omics* data different to mRNA, such as ncRNA, ChIP-seq, or methylation data, would need an additional mapping procedure before the specific GSA method is applied; and, following the same reasoning, we can think of multiple scenarios that justify using specialized types of GSA methods. Therefore, we created a set of galaxy tools that we called "Galaxy-GSA" to fulfill the need for such methods inside the Galaxy environment. Galaxy-GSA wraps multiple R packages into Galaxy and gives them the chance to communicate with each other and with all other Galaxy tools. We have made such tools individually available in the galaxy toolshed and collectively available as a virtual machine, a Docker image, and a web platform. Also, we have distributed a few Galaxy workflows for specific scenarios and practical tasks. All of this will allow the user to leverage the results of both general and specialized GSA methods inside Galaxy's environment.

All Galaxy-GSA tools are initially written in R and integrated into Galaxy using XML and tools such as Planemo.[5] Full tutorials are included on our website with detailed explanations on how to use Galaxy-GSA,[6] how we built our Galaxy instance,[7] and how we created the new Galaxy tools.[8]

[1] https://gsa-central.github.io/

[2] https://usegalaxy.org

[3] https://galaxyproject.org/use/

[4] https://galaxyproject.org/toolshed/

[5] https://planemo.readthedocs.io/en/latest/

[6] https://github.com/gsa-central/galaxy-gsa/blob/main/tutorials/using-galaxy-gsa.md

[7] https://github.com/mora-lab/installing/tree/main/galaxy

[8] https://github.com/mora-lab/installing/tree/main/planemo

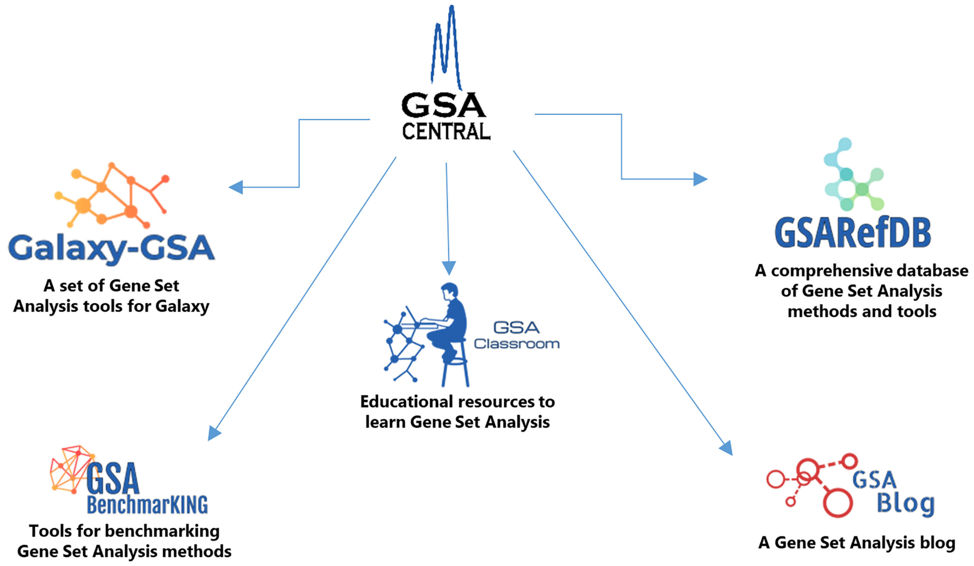

GSA CENTRAL
Galaxy-GSA
A set of Gene Set Analysis tools for Galaxy
GSA Classroom
Educational resources to learn Gene Set Analysis
GSARefDB
A comprehensive database of Gene Set Analysis methods and tools
GSA BenchmarKING
Tools for benchmarking Gene Set Analysis methods
GSA Blog
A Gene Set Analysis blog

FIGURE 1
GSA Central architecture.

GSA Classroom

For beginners, educational material for learning GSA methods and tools might be the most important resource.

There are multiple educational slides and videos scattered all over the Internet. We have collected information and links to such resources in a single place, including courses (slides), courses (animations), and online videos. Regarding online videos, we have added a search engine to facilitate GSA video search according to several descriptors, including: title, author, keywords, language (English or Chinese), video platform, and year, besides the links to such resources.

GSA Classroom can be accessed at: https://gsa-central.github.io/education.html. The animated GSA lessons were created using Vyond[9] and Adobe After Effects.[10] The video search engine was built using R 4.0.0 and its "shiny" package. Its code is open source and can be accessed at https://github.com/gsa-central/gsaclassroom. The shiny app reads the information from an excel table located at its "data" folder[11]; therefore, the app can be easily updated by updating such table.

GSARefDB

Some GSA reviews have previously tried to reference all existing GSA methods and tools; however, previous to our work, the most comprehensive report included merely 68 GSA tools (16). In 2019, we introduced the "Gene Set Analysis Reference Database" (GSARefDB), the most comprehensive compilation of available methods, tools, and reviews for GSA. GSARefDB is not only a list of papers but also includes valuable meta-data, such as references (author, title, year), classification (types of GSA methods), popularity (citation counts), method details, and other descriptors. Our first version included 445 papers [version used in Mora (17)], while our second version contained 503 papers [version used in Xie et al.(1)]. Our most recent version contains 641 papers, which makes GSARefDB the largest database of GSA methods and tools. In addition, its associated meta-data (such as method classification and popularity) makes it one of a kind.

GSARefDB was initially built as an excel spreadsheet. Citation information was extracted from Google scholar, while all other information was manually extracted from the papers. The spreadsheet versions of the database can be downloaded at https://github.com/gsa-central/gsarefdb/tree/master/archive/. We also created a "GSARefDB" app, which can be found at https://gsa-central.github.io/gsarefdb.html. The app was built using R version 3.6.3 and "shiny" version 1.4.0.2. Its code is open source and can be accessed at https://github.com/gsa-central/gsarefdb.

GSA BenchmarKING

Computational method or tool selection is fundamental to the process of generating high-quality scientific results, and the two most common selection strategies are (i) following

[9] https://www.vyond.com/

[10] https://www.adobe.com/products/aftereffects.html

[11] https://github.com/gsa-central/gsaclassroom/blob/master/data/videos.xlsx

the method's popularity or (ii) following objective performance tests. GSARefDB is the best existing resource to find out the popularity of GSA tools. However, popular methods are not necessarily the best and, therefore, we should always consider the recommendations of performance studies, such as benchmark and simulation studies (1). That is not an easy task because the existing benchmarks are very few and only evaluate a few methods at the time [see Table 1 of Xie et al. (1)], while performing new benchmark studies is a labor-intensive task. Because of that, we have introduced some benchmarking guidelines, together with "GSA BenchmarKING," which is a repository of tools to perform an easy benchmark of GSA tools.

The "GSA-BenchmarKING" repository[12] stores tools to measure GSA method performance which are expected to have the following attributes: (i) Be open software; (ii) Have a clear reason for selecting the group of GSA methods under comparison; for example, because all of them belong to the same type of methods; (iii) Include both a gold standard dataset and options to upload user-selected gold standard datasets (an example of this is showed in the Results section, GSA BenchmarKING sub-section); (iv) Include either a list of "target pathways" linked to the gold standard dataset or "disease relevance scores" per pathway for the diseases related to the gold standard [this is explained and discussed in Xie et al. (1)]; (v) Give the user the option of selecting different benchmarking metrics (such as precision, sensitivity, prioritization, and specificity); (vi) Options for selecting ensemble results; (vii) Flexibility for easily adding new GSA methods to the code in the future.

The current tools in GSA BenchmarKING include jupyter notebooks and shiny apps for benchmarking both "single-sample GSA" tools and "genomic-range GSA" tools. The "ss-shiny" app was built using R 3.6.2, while the "gr-shiny" app was built using R 4.0.0. Their code is open source and can be accessed at https://github.com/mora-lab/ss-shiny and https://github.com/mora-lab/gr-shiny, respectively. The performance metrics (precision, sensitivity, and specificity) are defined as below:

$$\text{Precision} = \text{TP}/\text{TP} + \text{FP}$$

$$\text{Sensitivity} = \text{TP}/\text{TP} + \text{FN}$$

$$\text{Specificity} = \text{TN}/\text{TN} + \text{FP}$$

where TP = True Positives, FP = False Positives, TN = True Negatives, and FN = False Negatives.

Our benchmark studies always include an ensemble of all methods under consideration, which is built by combining their individual *p*-values. The combination of *p*-values is performed through the "metap" R package, which allows the user to choose between Fisher's method (sum of log), Stouffer's method (sum of z), or a simple average of *p*-values.

TABLE 1 A list of current servers and individual tools for Galaxy-GSA.

	Tool name	Tool type	Location
1	Galaxy-GSA	WEB	https://gsa-central.github.io/galaxy.html
2	Galaxy-GSA	Docker	https://gsa-central.github.io/galaxy.html
3	Galaxy-GSA	Virtual machine	https://gsa-central.github.io/galaxy.html
4	Gene set uploader	Data uploader	Toolshed
5	ReactomePA	ORA	Toolshed
6	SPIA	PT	Toolshed
7	PLAGE	SS	Toolshed
8	ZSCORE	SS	Toolshed
9	SSGSEA	SS	Toolshed
10	GSVA	SS	Toolshed
11	ChIPEnrich	GR	Toolshed
12	PolyEnrich	GR	Toolshed
13	BroadEnrich	GR	Toolshed
14	methylGSA	GR	Toolshed
15	Mogsa	INTEG	Toolshed
16	WW	FCS	Toolshed
17	KS	FCS	Toolshed
18	Agg-F	FCS	Toolshed
19	GSNCA	FCS	Toolshed

WEB, Web platform; Docker, Docker image; VM, Virtual Machine; ORA, Over-representation analysis; FCS, Functional-class scoring; PT, Pathway topology-based; SS, Single-sample; GR, Genomic Region; INTEG, Integra-tive; Toolshed, Galaxy toolshed (https://galaxyproject.org/toolshed/or https://toolshed.g2.bx.psu.edu/).

GSA Blog

We have created the GSA Blog as a space where both beginners and experts can find discussions on topics of interest in the field. The GSA Blog was also built as a GitHub website,[13] using HTML, CSS, and javascript. The code is open source and can be reviewed or cloned from https://github.com/gsa-blog/gsa-blog.github.io.

Results

Galaxy-GSA

Galaxy-GSA is a collection of Gene Set Analysis tools for different types of Bioinformatics projects inside a Galaxy environment. It is built as a toolbox that contains original tools, wrappers for existing R packages, and workflows with various goals. Users are offered several options for functional interpretation of *omics* data to choose one depending on the goals of their study. For example, having mRNA data, transcription factor data, or multiple *omics* datasets; having several replicates of a given cell line or just one sample for each of many patients; focusing on pathway

[12] https://gsa-central.github.io/benchmarKING.html

[13] https://gsa-blog.github.io

structure or ontology term annotation; and so on. Galaxy-GSA includes (i) popular general-purpose GSA tools, (ii) specialized tools built for specific experimental designs, (iii) specialized tools for datasets different from mRNA, (iv) GSA-related auxiliary tools, and (v) Workflows for specific types of Bioinformatics projects.

The current tools include:

(1) Gene set uploader: Tool to select a collection of gene sets or pathways from either MSigDB or KEGG and use them as reference gene sets for other gene set analysis tools.
(2) ReactomePA: Over-representation analysis tool that finds over-representation of Reactome pathways in a list of Entrez gene IDs through the hypergeometric model (18).
(3) SPIA: Pathway-Topology tool that considers the topology of the pathways by combining an over-representation score with a perturbation factor (which, in turn, combines the fold change of a gene with the fold change of the genes upstream) (19).
(4) GSVA: Tool containing four different single-sample (or sample-specific) methods: PLAGE, ZSCORE, SSGSEA, and GSVA. Such methods compute single-sample-specific enrichment statistics and do not generate a single pathway ranking but a sample-pathway matrix instead (20).
(5) ChIPEnrich: Tool containing four different GSA methods for genomic regions (i.e., peaks coming from ChIP-seq and similar technologies). ChIPEnrich and PolyEnrich can be applied to narrow peaks such as transcription factor binding sites, while BroadEnrich has been designed to work with broad peaks such as histone marks (21).
(6) methylGSA: Tool that performs GSA for DNA methylation data using logistic regression (22).
(7) mogsa: Tool that performs GSA on multiple *omics* data by integrative clustering (23).
(8) GSAR: Tool that includes a series of methods that compare hypotheses different to the equality of means of a gene set between two conditions. For example, WW is a test of differential distribution; the aggregated *F*-test is a test of differential variance; and GSNCA is a test of differential co-expression that measures the change in the net correlation structure (24).

Each Galaxy-GSA tool gives visibility to all the software options, parameters, and help from the original packages. The user can leverage Galaxy's infrastructure to upload data, format data, choose parameters and options through a simple interface, and send results to the history to allow comparisons between different runs or link results to other bioinformatics tools. The current Galaxy-GSA server version contains one original tool for downloading gene sets and seven wrappers for R packages. It has been organized into different GSA categories, and those categories also include other GSA-related Galaxy tools not developed for us, such as g:Profiler, DAVID, and others.

For experienced Galaxy users, we have built three solutions:

(i) Individual Galaxy-GSA tools can be downloaded from the "Galaxy toolshed",[14]
(ii) A Galaxy-GSA Docker image can be downloaded from "Docker hub",[15]
(iii) A virtual machine image can be downloaded from Zenodo.[16]

For new Galaxy users, we have provided instructions on how to install Galaxy, and we have built a Galaxy-GSA platform on our website.[17] The website is ideal for beginners and quick testing, as it does not require any installation whatsoever; however, it does not allow saving your histories or installing new tools.

Besides that, Galaxy-GSA has also been included in the Galaxy Platform Directory (see text footnote 3) as one of the 125 Galaxy platforms currently available. You can find Galaxy-GSA under "Public Servers," "Containers," and "VMs." A list of Galaxy-GSA implementations and individual tools is shown in **Table 1**. **Figure 2** shows screenshots of Galaxy-GSA.

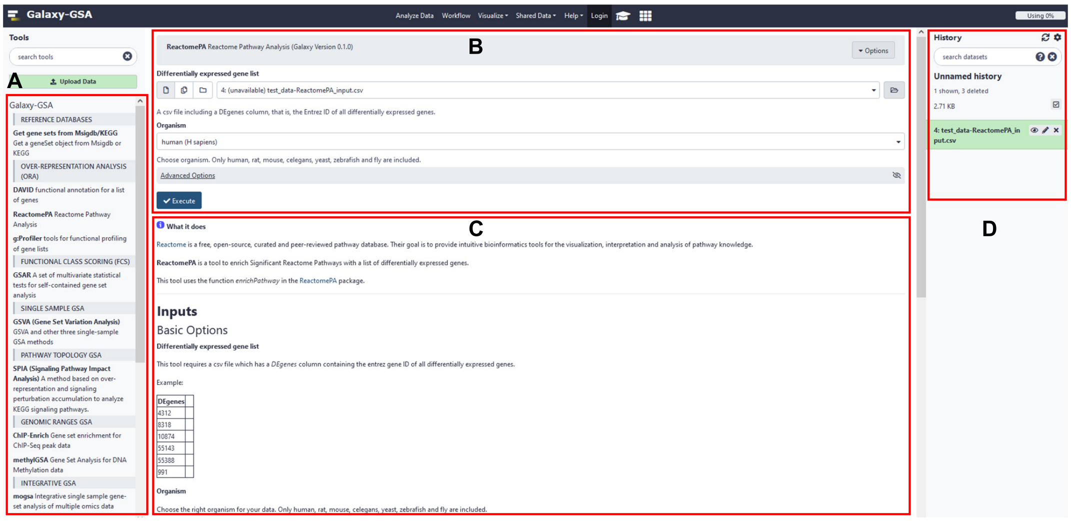
GSA Classroom

GSA Classroom is a gateway to slides, videos, and animations to learn GSA. In its first version, it is made of links to some online courses (both slides and animations) and a database to find online videos.

Our GSA animated lessons are seven lessons covering annotation databases, over-representation analysis (ORA), and functional class scoring (FCS), which include both theoretical knowledge and software usage.

Our shiny app allows us to search by title, author, keywords, language, video platform, and year. It does feature a "Search" button that retrieves all the rows in the table whose information partially matches the query. Each column has an option that allows ordering in ascending or descending order (two small arrows next to the column name). The database includes both academic software and proprietary software, as well as English and Chinese videos. The first version of the video database contains 77 resources, 38 in English and 39 in Chinese, with 60 videos corresponding to academic works and 17 corresponding to commercial products. **Figure 3** shows screenshots of GSA Classroom.

[14] https://toolshed.g2.bx.psu.edu/
[15] https://github.com/gsa-central/galaxy-gsa/tree/main/docker
[16] https://zenodo.org/record/5091267#.Yl5wXNNByUk
[17] https://gsa-central.github.io/galaxy.html



Galaxy-GSA

REFERENCE DATABASES

Get gene sets from Msigdb/KEGG Get a geneSet object from Msigdb or KEGG

OVER-REPRESENTATION ANALYSIS (ORA)

DAVID functional annotation for a list of genes

ReactomePA Reactome Pathway Analysis

g:Profiler tools for functional profiling of gene lists

FUNCTIONAL CLASS SCORING (FCS)

GSAR A set of multivariate statistical tests for self-contained gene set analysis

SINGLE SAMPLE GSA

GSVA (Gene Set Variation Analysis) GSVA and other three single-sample GSA methods

PATHWAY TOPOLOGY GSA

SPIA (Signaling Pathway Impact Analysis) A method based on over-representation and signaling perturbation accumulation to analyze KEGG signaling pathways.

GENOMIC RANGES GSA

ChIP-Enrich Gene set enrichment for ChIP-Seq peak data

methylGSA Gene Set Analysis for DNA Methylation data

INTEGRATIVE GSA

mogsa Integrative single sample gene-set analysis of multiple omics data

ReactomePA Reactome Pathway Analysis (Galaxy Version 0.1.0)

Differentially expressed gene list

4: (unavailable) test_data-ReactomePA_input.csv

A csv file including a DEgenes column, that is, the Entrez ID of all differentially expressed genes.

Organism

human (H sapiens)

Choose organism. Only human, rat, mouse, celegans, yeast, zebrafish and fly are included.

Advanced Options

✓ Execute

What it does

Reactome is a free, open-source, curated and peer-reviewed pathway database. Their goal is to provide intuitive bioinformatics tools for the visualization, interpretation and analysis of pathway knowledge.

ReactomePA is a tool to enrich Significant Reactome Pathways with a list of differentially expressed genes.

This tool uses the function *enrichPathway* in the ReactomePA package.

Inputs

Basic Options

Differentially expressed gene list

This tool requires a csv file which has a *DEgenes* column containing the entrez gene ID of all differentially expressed genes.

Example:

DEgenes	
4312	
8318	
10874	
55143	
55388	
991	

Organism

Choose the right organism for your data. Only human, rat, mouse, celegans, yeast, zebrafish and fly are included.

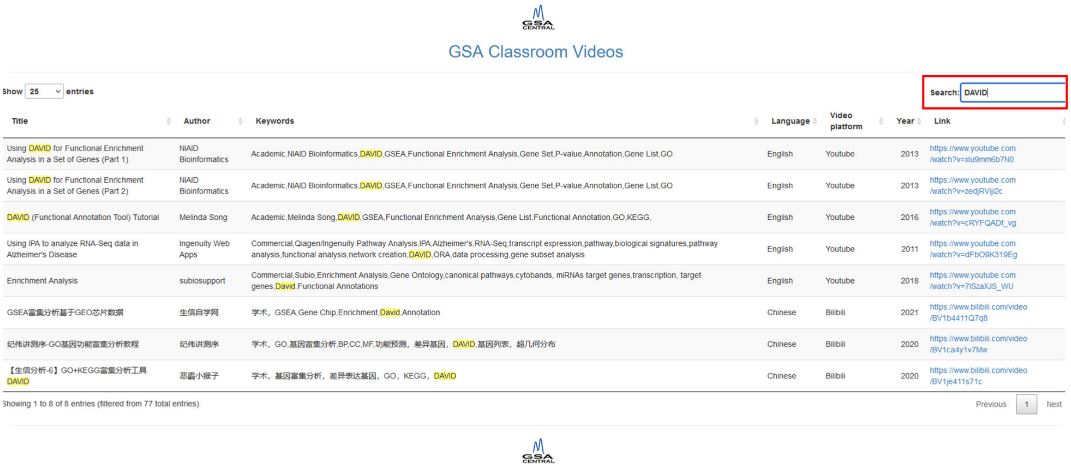
Unnamed history

1 shown, 3 deleted

2.71 KB

4: test_data-ReactomePA_input.csv

FIGURE 2
Galaxy-GSA screenshot. **(A)** All installed Galaxy tools (including all Galaxy-GSA tools). **(B)** Input boxes for the currently open tool (here, ReactomePA). **(C)** Help for the currently open tool. **(D)** History (including intermediate and final results).



GSA Classroom Videos

Show 25 entries

Search: DAVID

Title	Author	Keywords	Language	Video platform	Year	Link
Using DAVID for Functional Enrichment Analysis in a Set of Genes (Part 1)	NIAID Bioinformatics	Academic,NIAID Bioinformatics,DAVID,GSEA,Functional Enrichment Analysis,Gene Set,P-value,Annotation,Gene List,GO	English	Youtube	2013	https://www.youtube.com/watch?v=xlu9mm6b7N0
Using DAVID for Functional Enrichment Analysis in a Set of Genes (Part 2)	NIAID Bioinformatics	Academic,NIAID Bioinformatics,DAVID,GSEA,Functional Enrichment Analysis,Gene Set,P-value,Annotation,Gene List,GO	English	Youtube	2013	https://www.youtube.com/watch?v=zedjRViji2c
DAVID (Functional Annotation Tool) Tutorial	Melinda Song	Academic,Melinda Song,DAVID,GSEA,Functional Enrichment Analysis,Gene List,Functional Annotation,GO,KEGG,	English	Youtube	2016	https://www.youtube.com/watch?v=cRYFQADf_vg
Using IPA to analyze RNA-Seq data in Alzheimer's Disease	Ingenuity Web Apps	Commercial,Qiagen/Ingenuity Pathway Analysis,IPA,Alzheimer's,RNA-Seq,transcript expression,pathway,biological signatures,pathway analysis,functional analysis,network creation,DAVID,ORA,data processing,gene subset analysis	English	Youtube	2011	https://www.youtube.com/watch?v=dFbO9K319Eg
Enrichment Analysis	subiosupport	Commercial,Subio,Enrichment Analysis,Gene Ontology,canonical pathways,cytobands, miRNAs target genes,transcription, target genes,David,Functional Annotations	English	Youtube	2018	https://www.youtube.com/watch?v=7ISzaXJS_WU
GSEA富集分析基于GEO芯片数据	生信自学网	学术，GSEA,Gene Chip,Enrichment,David,Annotation	Chinese	Bilibili	2021	https://www.bilibili.com/video/BV1b4411Q7q8
纪伟讲测序-GO基因功能富集分析教程	纪伟讲测序	学术，GO,基因富集分析,BP,CC,MF,功能预测，差异基因，DAVID,基因列表，超几何分布	Chinese	Bilibili	2020	https://www.bilibili.com/video/BV1ca4y1v7Mw
【生信分析-6】GO+KEGG富集分析工具 DAVID	恶霸小猴子	学术，基因富集分析，差异表达基因，GO，KEGG，DAVID	Chinese	Bilibili	2020	https://www.bilibili.com/video/BV1je411s71c

Showing 1 to 8 of 8 entries (filtered from 77 total entries)

FIGURE 3
GSA Classroom screenshot. In this example, we searched for the keyword “DAVID” and found all online videos where it appears either in the title or in the keyword columns.

GSARefDB

GSARefDB is the most comprehensive database of GSA methods and tools currently available. It is available as an excel file or as a shiny app, and contains information regarding the publication, website or software tool (if they exist), and an index of each paper’s popularity.

GSARefDB is made of seven main tables or menu options:

(i) “General methods and tools” contains information about general GSA methods, platforms, and software tools, usually applied to mRNA data.

(ii) “Reviews and benchmarks” includes information on reviews of the field and tool comparisons.

(iii) “Genomic GSA” contains information on methods and tools for GSA applied to genomic range data, such as ChIP-seq data, SNP data, and DNA methylation data.

(iv) “ncRNA GSA” includes methods and tools for GSA applied to ncRNA data, such as miRNA, lncRNA, and others.

(v) “MS-based GSA” includes methods and tools for GSA applied to proteomic, metabolomic, glucomic, and lipidomic data.

(vi) “Metagenomics GSA” includes methods and tools for GSA applied to metagenomic and metatranscriptomic data.

(vii) "Integromics GSA" includes methods and tools for GSA of experiments involving multiple *omics* datasets.

The app also includes a few other menu options, such as a "FAQ" tab and an "Analysis" tab (which offers summary plots of the entire database).

Each of the tables has columns with relevant information, including method or tool name, reference (title, author, year, DOI), popularity (citation count), type of GSA, programming language, and website information. In the shiny app, each of the seven tables has a "Search" and a "Download" button to explore that table only. The search retrieves all the rows whose information partially matches the query. Each column in each table has an option for ordering that column in ascending or descending order (two small arrows next to the column name). For example, to see the popularity ranking of GSA tools, we can order the "Citations" column in descending order. A statistical summary, including the number of collected papers and the amount of collective citations by such papers, can be found in **Table 2**. **Figure 4** shows screenshots of GSARefDB.

GSA BenchmarKING

"GSA BenchmarKING" is a collection of tools to benchmark groups of GSA methods. Benchmarking tools include jupyter notebooks (with full workflows for benchmarking GSA methods) and shiny apps that allow benchmarking with the click of a few buttons. In the first version, we include two Jupyter notebooks (for "single-sample GSA" and "genomic-region GSA," respectively) and two shiny apps (also for single-sample GSA and genomic-region GSA). Here we will explain the single-sample tools (Jupyter notebook and shiny app).

The Jupyter notebook compares five single-sample GSA methods [PLAGE (25), ZSCORE (26), SSGSEA (27), GSVA (20), and GRAPE (28)] plus an ensemble of all five. The workflow is designed to predict the pathways for four respiratory diseases that we know beforehand (non-small cell lung cancer, chronic obstructive pulmonary disease, asthma, and tuberculosis). In the beginning, datasets are downloaded and formatted to be read by the different methods. Then, each of the datasets is used as input of each of the methods to produce one predicted pathway ranking per dataset per method; in addition, a combination of their *p*-values is used as a sixth method to produce additional rankings. After that, each ranking is compared to the "target pathways" (the reported pathways for the four diseases) and precision, sensitivity, and specificity, are computed as specified before. Finally, box plots are built to compare the performance of each of the methods and each performance metric. With a minimum knowledge of R programming, Jupyter notebooks (or Rstudio notebooks) allow any part of the workflow to be modified; for example, using different datasets/gold standards or adding new GSA methods to the comparison.

The shiny app ("ss-shiny") compares the same methods but gives more flexibility to a non-experienced user: The app allows the user to upload disease-related RNA expression datasets, either from a sample file for illustration purposes, the gold standard of disease-related RNA expression datasets introduced by Tarca et al. (29), or the user's own dataset. The app also allows the user to determine its own disease/target pathways, either from a sample file or its own pathway file. The user can choose any number of methods among the five methods provided, as well as any method for *p*-value combination. Finally, the user can also choose the performance metrics (precision, sensitivity, specificity) to be evaluated. The app offers three tabs to, respectively, preview the input datasets, preview the results, and download the results, together with a menu containing an example of a benchmark study and a help menu.

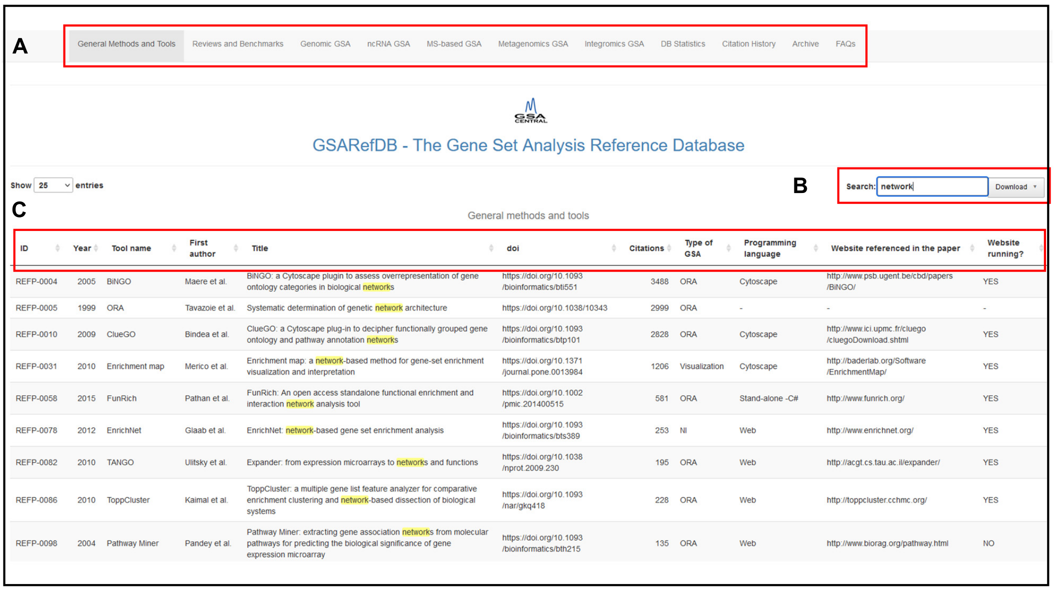
Benchmarks and performance evaluation are not an easy task, and there is abundant discussion regarding which should be the parameters to compare and the procedures to follow (1), but "GSA BenchmarKING" makes such procedures transparent and allows the user to change every aspect they disagree with, which nowadays is not easy due to the closed ways in which benchmarks are practiced. We expect "GSA BenchmarKING" can become a place, or at least an inspiration, for more advanced users to develop their own open benchmarks or to validate their own GSA methods. **Figure 5** shows some screenshots of GSA BenchmarKING.

GSA Blog

We introduced "GSA Blog" as a space to share current and useful information related to the GSA field. Most specifically, at the GSA Blog, we will share posts including discussions on both old and new GSA methods and tools, as well as useful code.

TABLE 2 Statistics of GSARefDB v.2.0.

	General methods and tools	Reviews and benchmarks	Genomic GSA	ncRNA GSA	MS-based GSA	Meta-omics GSA	Integromics GSA	Total
Number of papers	386	85	65	33	29	23	20	641
Number of citations	131,332	21,727	9,657	4,329	8,656	17,985	1,065	194,751



GSARefDB - The Gene Set Analysis Reference Database

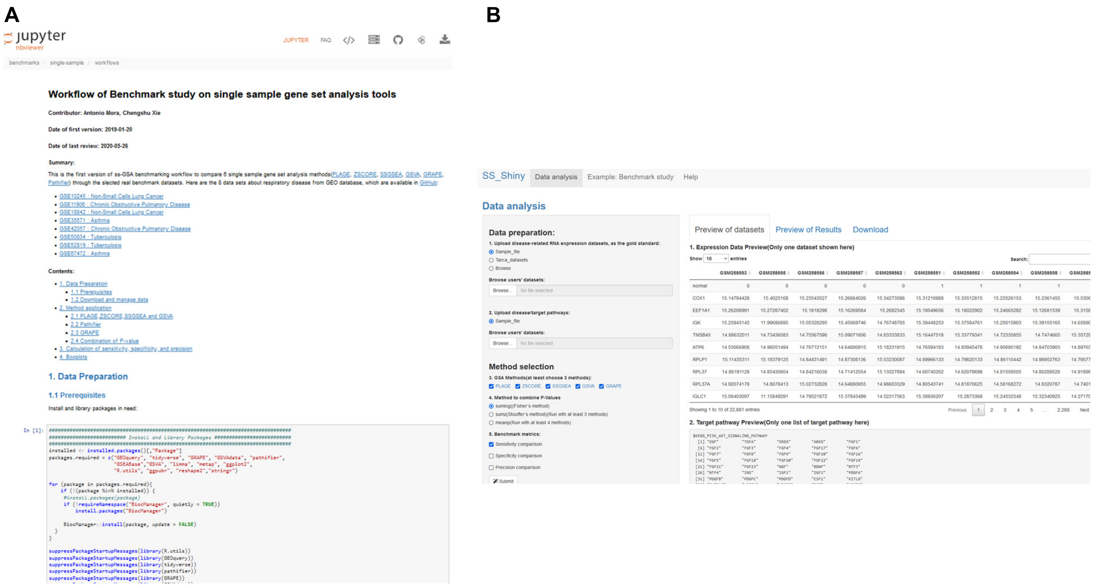
Show 25 entries

Search: network

General methods and tools

ID	Year	Tool name	First author	Title	doi	Citations	Type of GSA	Programming language	Website referenced in the paper	Website running?
REFP-0004	2005	BiNGO	Maere et al.	BiNGO: a Cytoscape plugin to assess overrepresentation of gene ontology categories in biological networks	https://doi.org/10.1093/bioinformatics/bti551	3488	ORA	Cytoscape	http://www.psb.ugent.be/cbd/papers/BiNGO/	YES
REFP-0005	1999	ORA	Tavazoie et al.	Systematic determination of genetic network architecture	https://doi.org/10.1038/10343	2999	ORA	-	-	-
REFP-0010	2009	ClueGO	Bindea et al.	ClueGO: a Cytoscape plug-in to decipher functionally grouped gene ontology and pathway annotation networks	https://doi.org/10.1093/bioinformatics/btp101	2828	ORA	Cytoscape	http://www.ici.upmc.fr/cluego/cluegoDownload.shtml	YES
REFP-0031	2010	Enrichment map	Merico et al.	Enrichment map: a network-based method for gene-set enrichment visualization and interpretation	https://doi.org/10.1371/journal.pone.0013984	1206	Visualization	Cytoscape	http://baderlab.org/Software/EnrichmentMap/	YES
REFP-0058	2015	FunRich	Pathan et al.	FunRich: An open access standalone functional enrichment and interaction network analysis tool	https://doi.org/10.1002/pmic.201400515	581	ORA	Stand-alone -C#	http://www.funrich.org/	YES
REFP-0078	2012	EnrichNet	Glaab et al.	EnrichNet: network-based gene set enrichment analysis	https://doi.org/10.1093/bioinformatics/bts389	253	NI	Web	http://www.enrichnet.org/	YES
REFP-0082	2010	TANGO	Ulitsky et al.	Expander: from expression microarrays to networks and functions	https://doi.org/10.1038/nprot.2009.230	195	ORA	Web	http://acgt.cs.tau.ac.il/expander/	YES
REFP-0086	2010	ToppCluster	Kaimal et al.	ToppCluster: a multiple gene list feature analyzer for comparative enrichment clustering and network-based dissection of biological systems	https://doi.org/10.1093/nar/gkq418	228	ORA	Web	http://toppcluster.cchmc.org/	YES
REFP-0098	2004	Pathway Miner	Pandey et al.	Pathway Miner: extracting gene association networks from molecular pathways for predicting the biological significance of gene expression microarray	https://doi.org/10.1093/bioinformatics/bth215	135	ORA	Web	http://www.biorag.org/pathway.html	NO

FIGURE 4
GSARefDB screenshot. **(A)** Main Menu: The user can choose between the database of general methods and tools, the database of reviews and benchmarks, the specialized databases (genomic GSA, nc-RNA GSA, MS-based GSA, meta-omics GSA, and integromics GSA), general GSARefDB statistics, the archive, and FAQs. **(B)** The search box: Here, we search for papers with the keyword "network". **(C)** The columns with all the information we can obtain from each paper: ID, publication year, tool name, first author, paper title, DOI, number of citations, type of GSA, programming language, and website info.



Workflow of Benchmark study on single sample gene set analysis tools

Contributor: Antonio Mora, Chengshu Xie

Date of first version: 2019-01-20

Date of last review: 2020-05-26

Summary:

This is the first version of ss-GSA benchmarking workflow to compare 6 single sample gene set analysis methods(PLAGE, ZSCORE, SSGSEA, GSVA, GRAPE, Pathifier) through the slected real benchmark datasets. Here are the 8 data sets about respiratory disease from GEO database, which are available in GitHub:

- GSE10245 : Non-Small Cells Lung Cancer
- GSE11906 : Chronic Obstructive Pulmanory Disease
- GSE18842 : Non-Small Cells Lung Cancer
- GSE35571 : Asthma
- GSE42057 : Chronic Obstructive Pulmanory Disease
- GSE50834 : Tuberculosis
- GSE52819 : Tuberculosis
- GSE67472 : Asthma

Contents:

1. Data Preparation

1.1 Prerequisites

Install and library packages in need:

```
In [1]: ####################################################################################
        ########################### Install and Library Packages ###########################
        ####################################################################################
        installed <- installed.packages()[,"Package"]
        packages.required = c("GEOquery", "tidyverse", "GRAPE", "GSVAdata", "pathifier",
                              "GSEABase","GSVA", "limma", "metap", "ggplot2",
                              "R.utils", "ggpubr", "reshape2","stringr")

        for (package in packages.required){
            if (!(package %in% installed)) {
             #install.packages(package)
             if (!requireNamespace("BiocManager", quietly = TRUE))
                 install.packages("BiocManager")

             BiocManager::install(package, update = FALSE)
          }
        }

        suppressPackageStartupMessages(library(R.utils))
        suppressPackageStartupMessages(library(GEOquery))
        suppressPackageStartupMessages(library(tidyverse))
        suppressPackageStartupMessages(library(pathifier))
        suppressPackageStartupMessages(library(GRAPE))
```

Data analysis

Data preparation:

1. Upload disease-related RNA expression datasets, as the gold standard:
● Sample_file
○ Tarca_datasets
○ Browse

Browse users' datasets: No file selected

2. Upload disease/target pathways:
● Sample_file

Browse users' datasets: No file selected

Method selection

3. GSA Methods(at least choose 3 methods):
☑ PLAGE ☑ ZSCORE ☑ SSGSEA ☑ GSVA ☑ GRAPE

4. Method to combine P-Values
● sumlog((Fisher's method)
○ sumz(Stouffer's method)(Run with at least 3 methods)
○ meanp(Run with at least 4 methods)

5. Benchmark metrics:
☑ Sensitivity comparison
□ Specificity comparison
□ Precision comparison

1. Expression Data Preview(Only one dataset shown here)

Show 10 entries Search:

	GSM258553	GSM258555	GSM258556	GSM258557	GSM258562	GSM258551	GSM258552	GSM258554	GSM258558	GSM2585
normal	0	0	0	0	0	1	1	1	1	
COX1	15.14794428	15.4025168	15.23543527	15.26664026	15.34273586	15.31218988	15.33512815	15.23526153	15.2361455	15.530
EEF1A1	15.26208991	15.27287402	15.1818298	15.16269584	15.2682345	15.18049656	15.18022902	15.24665282	15.12681539	15.315
IGK	15.25943145	11.99686895	15.05328295	15.45069746	14.76748705	15.39448253	15.37584761	15.25015903	15.39105165	14.6589
TMSB4X	14.88632011	14.73436083	14.75567598	15.09071606	14.83333833	15.16447518	15.33779341	14.72335855	14.7474665	15.3572
ATP6	14.53066908	14.96051494	14.76712151	14.64806915	15.18231915	14.76594183	14.93945478	14.95695192	14.84703903	14.8976
RPLP1	15.11435311	15.18379125	14.84431491	14.87308136	15.03230087	14.89966133	14.79820133	14.86110442	14.98952763	14.7957
RPL37	14.86191128	14.85430604	14.84216038	14.71412054	15.13527894	14.60740202	14.62079698	14.81559505	14.80289528	14.9189
RPL37A	14.92074178	14.8078413	15.02732828	14.64880955	14.98603329	14.80543741	14.81870625	14.58168272	14.8320787	14.740
IGLC1	15.08403097	11.15849291	14.79521872	15.37843499	14.52317563	15.38936207	15.2873368	15.24532348	15.32340925	14.2717

Showing 1 to 10 of 22,881 entries Previous 1 2 3 4 5 … 2,289 Next

2. Target pathway Preview(Only one list of target pathway here)

```
$KEGG_PI3K_AKT_SIGNALING_PATHWAY
  [1] "EGF"      "TGFA"     "EREG"     "AREG"     "FGF1"
  [6] "FGF2"     "FGF3"     "FGF4"     "FGF17"    "FGF6"
 [11] "FGF7"     "FGF8"     "FGF9"     "FGF10"    "FGF16"
 [16] "FGF5"     "FGF18"    "FGF20"    "FGF22"    "FGF19"
 [21] "FGF21"    "FGF23"    "NGF"      "BDNF"     "NTF3"
 [26] "NTF4"     "INS"      "IGF1"     "IGF2"     "PDGFA"
 [31] "PDGFB"    "PDGFC"    "PDGFD"    "CSF1"     "KITLG"
```

FIGURE 5
GSA BenchmarKING screenshot. **(A)** A jupyter notebook with an R workflow for the benchmark of six different single-sample GSA tools using datasets related to respiratory disease. **(B)** A shiny app to benchmark five different single-sample GSA tools using different gold standards, target pathways, and benchmark metrics.

Our initial posts included discussions on gene set annotation databases, how to understand and handle *p*-values, time-course GSA tools, and single-cell GSA tools.

In the future, we expect "GSA Blog" to become a space open to the GSA community.

Discussion

We have built an education and research resource useful for GSA beginners, average users, and tool creators alike. Beginners have access to "GSA Classroom," where they can watch lessons, conferences and other types of videos for different types of GSA or even for specific academic or commercial software. Average users can use "Galaxy-GSA," a Galaxy instance with multiple GSA tools, including tools to download reference databases, generic popular GSA tools, and tools tailored to analyze single-sample designs, genomic regions, DNA methylation, integrative *omics* datasets, and others. Expert users, that is, creators of GSA methods and tools, may find that "GSA BenchmarKING" is a useful resource to test their methods compared to already tested methods. Both GSA average users and tool creators will find useful to visit "GSARefDB," a database that allows the user to explore all available papers, finding all available reviews or benchmarks, finding all tools written for a specific type of analysis or programming software, discovering the popularity of methods and tools, and other information that will help the user make better decisions regarding method and tool selection. Finally, all three types of users might find it interesting to access "GSA Blog," a space to post comments on current GSA software and current discussions of interest.

We have designed this platform with the purpose of covering all different types of resources that users of a given scientific field might be looking for, such as (i) introductory learning, (ii) state-of-the-art updates, (iii) method/tool selection guidelines and insights, (iv) easy-to-use tools, (v) tool integration strategies (Galaxy environment), (vi) tool design examples, and (vii) method/tool validation/benchmarking tools. Therefore, we introduce "GSA Central" as an example of a "thematic platform" containing all the resources that researchers might need in a given field.

"GSA Central" tools are relatively easy to update and maintain. Therefore, we plan to keep adding more methods and workflows in the near future.

In a previous paper, we suggested that "GSARefDB" and "GSA BenchmarKING" can be seen as the instruments of a methodology to permanently follow up the popularity and the performance status of all the tools in any bioinformatics sub-field (1). Going one step beyond, we believe that a platform like "GSA Central" is more than a website and can be considered as the main instrument of a novel approach to systematically and comprehensively contribute to the development of any scientific discipline.

Software availability

"GSA Central" can be found at: https://gsa-central.github.io/.

"Galaxy-GSA" can be found at https://gsa-central.github.io/galaxy.html (general page), http://www.moralab.science:8080/ (web platform), https://github.com/gsa-central/galaxy-gsa/tree/main/docker (Docker image), and https://zenodo.org/record/5091267#.Yl5wXNNByUk (Virtual machine).

"GSA Classroom" can be found at https://gsa-central.github.io/education.html.

"GSARefDB" can be found at https://gsa-central.github.io/gsarefdb.html.

"GSA BenchmarKING" can be found at https://gsa-central.github.io/benchmarKING.html.

"GSA Blog" can be found at https://gsa-blog.github.io/index.html.

"Galaxy-GSA" has also been included as part of the "Galaxy community hub" (https://galaxyproject.org/use/galaxy-gsa/).

"GSA Central" contains open software under a Creative Commons license.

Data availability statement

The original contributions presented in this study are publicly available. This data can be found here: https://gsa-central.github.io/.

Author contributions

XH and AM built the GSA Central's website. Galaxy-GSA was designed by AM and programmed by XH (including the individual tools, website, Docker image, and virtual machine). SM provided Galaxy and server technical support. GSA classroom was conceived by AM and built by XL and ZX based on code written by CX. GSA animated lessons were written by AM and programmed by XL. GSARefDB was conceived and built by AM, and its shiny app was written by CX. GSA BenchmarKING was conceived by AM and built by CX and SJ. GSA Blog was created by AM and its website was assembled by XH. AM conceived and supervised the project and wrote the manuscript. All authors contributed to the article and approved the submitted version.

Funding

AM was funded by Guangzhou Medical University, high-level talent fund. SJ was funded by China Postdoctoral Science Foundation grant: 2019M652847.

Acknowledgments

We thank Mette Dahl Pausgaard for her help with logo and animation design, as well as all researchers and students at Guangzhou Medical University for their support and suggestions.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

1. Xie C, Jauhari S, Mora A. Popularity and performance of bioinformatics software: the case of gene set analysis. *BMC Bioinformat.* (2021) 22:191. doi: 10.1186/s12859-021-04124-5

2. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc Natl Acad Sci USA.* (2005) 102:15545–50. doi: 10.1073/pnas.0506580102

3. Huang DW, Sherman BT, Tan Q, Kir J, Liu D, Bryant D, et al. DAVID bioinformatics resources: expanded annotation database and novel algorithms to better extract biology from large gene lists. *Nucleic Acids Res.* (2007) 35:W169–75. doi: 10.1093/nar/gkm415

4. Kuleshov MV, Jones MR, Rouillard AD, Fernandez NF, Duan Q, Wang Z, et al. Enrichr: a comprehensive gene set enrichment analysis web server 2016 update. *Nucleic Acids Res.* (2016) 44:W90–7. doi: 10.1093/nar/gkw377

5. Yu G, Wang LG, Han Y, He QY. clusterProfiler: an R package for comparing biological themes among gene clusters. *OMICS.* (2012) 16:284–7. doi: 10.1089/omi.2011.0118

6. Young MD, Wakefield MJ, Smyth GK, Oshlack A. Gene ontology analysis for RNA-seq: accounting for selection bias. *Genome Biol.* (2010) 11:R14. doi: 10.1186/gb-2010-11-2-r14

7. Bindea G, Mlecnik B, Hackl H, Charoentong P, Tosolini M, Kirilovsky A, et al. ClueGO: a Cytoscape plug-in to decipher functionally grouped gene ontology and pathway annotation networks. *Bioinformatics.* (2009) 25:1091–3. doi: 10.1093/bioinformatics/btp101

8. Giardine B, Riemer C, Hardison RC, Burhans R, Elnitski L, Shah P, et al. Galaxy: a platform for interactive large-scale genome analysis. *Genome Res.* (2005) 15:1451–5. doi: 10.1101/gr.4086505

9. Goecks J, Nekrutenko A, Taylor J, Galaxy Team. Galaxy: a comprehensive approach for supporting accessible, reproducible, and transparent computational research in the life sciences. *Genome Biol.* (2010) 11:R86. doi: 10.1186/gb-2010-11-8-r86

10. Raudvere U, Kolberg L, Kuzmin I, Arak T, Adler P, Peterson H, et al. g:Profiler: a web server for functional enrichment analysis and conversions of gene lists (2019 update). *Nucleic Acids Res.* (2019) 47:W191–8. doi: 10.1093/nar/gkz369

11. Alhamdoosh M, Law CW, Tian L, Sheridan JM, Ng M, Ritchie ME. Easy and efficient ensemble gene set testing with EGSEA. *F1000Res.* (2017) 6:2010. doi: 10.12688/f1000research.12544.1

12. Korotkevich G, Sukhov V, Sergushichev A. Fast gene set enrichment analysis. *bioRxiv.* (2019). [Preprint].

13. Faria D. *GOEnrichment*. San Francisco, CA: GitHub (2017).

14. Bu D, Luo H, Huo P, Wang Z, Zhang S, He Z, et al. KOBAS-i: intelligent prioritization and exploratory visualization of biological functions for gene enrichment analysis. *Nucleic Acids Res.* (2021) 49:W317–25. doi: 10.1093/nar/gkab447

15. Alexa A, Rahnenfuhrer J. *topGO: Enrichment Analysis for Gene Ontology*. Vienna: R Core Team (2021).

16. Huang W, Sherman BT, Lempicki RA. Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. *Nucleic Acids Res.* (2009) 37:1–13. doi: 10.1093/nar/gkn923

17. Mora A. Gene set analysis methods for the functional interpretation of non-mRNA data-Genomic range and ncRNA data. *Brief Bioinform.* (2020) 21:1495–508. doi: 10.1093/bib/bbz090

18. Yu G, He QY. ReactomePA: an R/Bioconductor package for reactome pathway analysis and visualization. *Mol Biosyst.* (2016) 12:477–9. doi: 10.1039/C5MB00663E

19. Tarca AL, Draghici S, Khatri P, Hassan SS, Mittal P, Kim JS, et al. A novel signaling pathway impact analysis. *Bioinformatics.* (2009) 25:75–82. doi: 10.1093/bioinformatics/btn577

20. Hanzelmann S, Castelo R, Guinney J. GSVA: gene set variation analysis for microarray and RNA-seq data. *BMC Bioinformat.* (2013) 14:7. doi: 10.1186/1471-2105-14-7

21. Welch RP, Lee C, Imbriano PM, Patil S, Weymouth TE, Smith RA, et al. ChIP-Enrich: gene set enrichment testing for ChIP-seq data. *Nucleic Acids Res.* (2014) 42:e105. doi: 10.1093/nar/gku463

22. Ren X, Kuan PF. methylGSA: a Bioconductor package and Shiny app for DNA methylation data length bias adjustment in gene set testing. *Bioinformatics.* (2019) 35:1958–9. doi: 10.1093/bioinformatics/bty892

23. Meng C, Basunia A, Peters B, Gholami AM, Kuster B, Culhane AC. MOGSA: integrative Single Sample Gene-set Analysis of Multiple Omics Data. *Mol Cell Proteom.* (2019) 18:S153–68. doi: 10.1074/mcp.TIR118.001251

24. Rahmatallah Y, Zybailov B, Emmert-Streib F, Glazko G. GSAR: bioconductor package for Gene Set analysis in R. *BMC Bioinformat.* (2017) 18:61. doi: 10.1186/s12859-017-1482-6

25. Tomfohr J, Lu J, Kepler TB. Pathway level analysis of gene expression using singular value decomposition. *BMC Bioinformat.* (2005) 6:225. doi: 10.1186/1471-2105-6-225

26. Lee E, Chuang HY, Kim JW, Ideker T, Lee D. Inferring pathway activity toward precise disease classification. *PLoS Comput Biol.* (2008) 4:e1000217. doi: 10.1371/journal.pcbi.1000217

27. Barbie DA, Tamayo P, Boehm JS, Kim SY, Moody SE, Dunn IF, et al. Systematic RNA interference reveals that oncogenic KRAS-driven cancers require TBK1. *Nature.* (2009) 462:108–12. doi: 10.1038/nature08460

28. Klein MI, Stern DF, Zhao H. GRAPE: a pathway template method to characterize tissue-specific functionality from gene expression profiles. *BMC Bioinformat.* (2017) 18:317. doi: 10.1186/s12859-017-1711-z

29. Tarca AL, Bhatti G, Romero R. A comparison of gene set analysis methods in terms of sensitivity, prioritization and specificity. *PLoS One.* (2013) 8:e79217. doi: 10.1371/journal.pone.0079217