IVAG: An Integrative Visualization Application for Various Types of Genomic Data Based on R-Shiny and the Docker Platform

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Next-generation sequencing (NGS) technology has become a trend in the genomics research area. There are many software programs and automated pipelines to analyze NGS data, which can ease the pain for traditional scientists who are not familiar with computer programming. However, downstream analyses, such as finding differentially expressed genes or visualizing linkage disequilibrium maps and genome-wide association study (GWAS) data, still remain a challenge. Here, we introduce a dockerized web application written in R using the Shiny platform to visualize pre-analyzed RNA sequencing and GWAS data. In addition, we have integrated a genome browser based on the JBrowse platform and an automated intermediate parsing process required for custom track construction, so that users can easily build and navigate their personal genome tracks with in-house datasets. This application will help scientists perform series of downstream analyses and obtain a more integrative understanding about various types of genomic data by interactively visualizing them with customizable options.

Keywords: docker, genome browser, genome-wide association study, RNA sequencing, Shiny, visualization

Availability: A docker image of IVAG can be downloaded at <https://hub.docker.com/r/leetaerim/ivag/>. Pre-processed example input data and the manual file are available at <https://github.com/jmoa/IVAG>.

Introduction

Since its advent, high throughput next-generation sequencing (NGS) technology has revolutionized the genomics research area, including transcriptome analysis and genome-wide association studies (GWASs) taking advantage of accelerated sequencing speed with reduced cost [1, 2]. Even though many bioinformatics software programs have been developed to handle and analyze the massive data generated from NGS, most of them are based on a command-line interface and require quite a high level of computational power [3], which creates a high barrier for wet lab biologists to enter into this field. Thanks to web-based analysis platforms, including Galaxy [4] and BIOEXPRESS [5], this barrier has been lowered. However, there still are problems. Downstream analyses, such as finding differentially expressed

genes (DEGs), conducting Gene Ontology (GO) enrichment analysis, calculating linkage disequilibrium (LD), annotating gene information into GWAS results, and finally visualizing the resulting data, still require significant computer programming skills.

In this study, we present a dockerized application, IVAG. It provides a user-friendly web interface in which all downstream analyses mentioned above can be carried out without any programming knowledge. Detailed parameters for each analysis step can be adjusted with simple click-and-drag operation. IVAG interactively outputs publication-quality plots in response to the given parameters, and all of these plots can be downloaded. Also, a variety of data types, ranging from RNA sequencing (RNA-seq) and GWAS results to sequence read alignments, gene annotation, variant call information, and peak information, can be uploaded into the embedded genome browser and then

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visualized together to help users gain greater integrative insights into their data. Furthermore, IVAG is lightweight, allowing it to be deployed on a desktop computer, as well as a server application.

Methods

IVAG is mostly written in the R programming language [6] and dockerized [7] with all required dependencies to avoid compatibility issues. It uses the Shiny package [8] to build a user-friendly web interface and several other packages to analyze and visualize RNA-seq and GWAS data (Supplementary Table 1). VCFtools [9] and PLINK (v1.90b4.6) [10] were used for the LD analysis. The JBrowse platform [11] was integrated into IVAG, and all intermediate steps required for custom track construction were automated using a custom BASH script. Three publicly available plugins (Supplementary Table 2), with slight modification, were incorporated into the genome browser to build GWAS, GC content, and browser extensible data (BED) tracks. Gene transfer format (GTF)-to-general feature format 3 (GFF3) format conversion was carried out with Cufflinks (2.2.1) [12]. Binary sequence alignment map (BAM) and variant call format (VCF) files were sorted with SAMtools [13]. Example data were prepared using publicly available RNA-seq and GWAS data (Supplementary Table 3).

Results

Workflow

Fig. 1 shows a graphical overview of the pre-analysis steps and the IVAG workflow. The blue line (Fig. 1A) denotes a schematic representation of the external pipelines required for RNA-seq and GWAS data. These parts are prerequisites for downstream analyses prior to IVAG analysis. The red box in the right panel (Fig. 1B) illustrates the IVAG workflow.

The orange items are input files for IVAG, and their detailed formats are described in Supplementary Figs. 1–29.

RNA-seq

IVAG DEG analysis requires raw count RNA sequence data that can be generated using open source software, such as Htseq [14]. DEG analysis outputs a DEG results table generated with user-specified parameters based on the R Bioconductor package edgeR [15]. The output table consists of multiple columns, such as log2 fold-change, log2 count per million, associated p-value, and associated false discovery rate (Fig. 2A), and it can be visualized as a heatmap, a volcano plot, and a principal component analysis plot (Fig. 2B). The heatmap is generated using raw count data, which are converted to counts per million and normalized to a have row-based percentage value. The volcano plot is generated using log2 fold-change and the associated false discovery rate. Principal component analysis is generated using the raw count of each sample normalized to the log2 count per million. The heatmap and volcano plot can be interactively updated based on user-specified filtering criteria, such as false discovery rate or absolute fold-change. GO enrichment analysis uses the DEG analysis results table, GO annotation file, and GTF file. The DEG analysis results table can be generated using IVAG DEG analysis, or a pre-analyzed DEG analysis result can also be used. The GO annotation file consists of two columns: gene ID and GO category. A GTF file is needed to generate the gene length of each gene in the DEG analysis table. However, it can be omitted if a user wishes not to take gene length bias into account. IVAG GO enrichment analysis outputs over-represented and under-represented GO terms among DEGs (Fig. 2C) using the R Bioconductor package goseq [16]. It also shows a histogram of DEGs in each GO category based on its ontology: biological process, cellular component, and molecular function.

Fig. 1. Graphical overview of IVAG workflow. (A) External pre-calculation and automated pipelines for RNA sequencing and genome-wide association study (GWAS) analysis. (B) Schematic representation of the App pipeline. DEG, differentially expressed gene; GO, Gene Ontology; GTF, gene transfer format; SNP, single nucleotide polymorphism; LD, linkage disequilibrium; PCA, principal component analysis; QQ, quantile-quantile. ^aThese data can be uploaded directly to the genome browser. The orange items are input files for IVAG, while the yellow ones are output files.

Fig. 2. Functions and results of IVAG. (A) Single-factor differential expression analysis. (B) Heatmap, volcano, and principal component analysis plot drawn with specified parameters. (C) Result of gene ontology enrichment analysis. Histogram shows how many differentially expressed genes are allocated to specific Gene Ontology categories. (D) Manhattan and quantile-quantile plots drawn with customizable options. (E) Linkage disequilibrium (LD) analysis generating LD matrix. (F) Pairwise LD heatmap. A group of single nucleotide polymorphisms of interest can be the subset. (G) Genome browser track with integrated view of differentially expressed gene and genome-wide association study results.

GWAS

Gene ID annotation requires a tab-separated GWAS result file comprising marker ID, chromosome ID, base position, and p-value columns in order, and a GTF file that contains strand and position information of genes. It returns a new GWAS result file in which gene, upstream, and downstream columns are added. Both GWAS result files, before and after

this annotation, can be visualized in Manhattan and quantile-quantile plot with customizable options (Fig. 2D). One can see all information for a specific dot of one's interest if he clicks on the interactive plots. The LD analysis part is read in a VCF file with several detailed options to generate an LD matrix and a marker information file, which can be visualized in the LD heatmap (Fig. 2E and 2F).

Genome browser

Constructing a custom genome browser with a reference genome sequence is the first step. After selecting one of the genome browsers configured in IVAG, various types of genomic data, including GTF, GFF3, BAM, BED, BigWig, and VCF, can be uploaded and visualized all together (Fig. 2G). Also, this genome browser receives RNA-seq and GWAS results generated from IVAG as inputs.

Discussion

IVAG is an easy-to-use, web-based application with three modules, including RNA-seq, GWAS, and a genome browser. This application enables scientists with little computational proficiency to analyze and visualize their data easily. Some web applications provide similar functions for RNA-seq and GWAS, but they have some limitations. For example, DEApp [17] provides differential expression analysis using three different methods—edgeR, limma-voom, and DESeq2—while a heatmap or a principal component analysis plot is not provided. START [18] can output several plots, but it does not offer a GO enrichment analysis function. LocusTrack [19] can visualize GWAS data and annotate multiple tracks on them, but it is limited to only one species, human. Zbrowse [20] can be used over every species. However, because it focuses on plotting multiple GWAS results in one panel to enable users to detect genotype-environment interactions, the number of markers that can be plotted for one trait is limited to 5,000. IVAG is not limited to a specific organism or the number of markers [14]. Most importantly, IVAG combines a genome browser with analysis and visualization modules so that users can analyze, visualize, and finally navigate their entire data interactively in one application. We offer only two analysis and visualization modules now, but several more modules are in development and will be included in the near future.

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Authors' contribution

Conceptualization: SK
 Formal analysis: TRL, JMA, GK
 Funding acquisition: SK
 Writing – original draft: TRL, JMA, GK, SK
 Writing – review & editing: TRL, SK

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Supplementary materials

Supplementary data including three tables and 29 figures can be found with this article online at <http://www.genominfo.org/src/sm/gni-15-178-s001.pdf>.

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SUPPLEMENTARY INFORMATION

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Supplementary Table 1. R packages used in IVAG

Name	Reference
shiny	Chang W, Cheng J, Allaire JJ, Xie Y, McPherson J (2017). shiny: Web Application Framework for R. R package version 1.0.5. https://CRAN.R-project.org/package=shiny
shinydashboard	Chang W, Ribeiro BB (2017). shinydashboard: Create Dashboards with 'Shiny'. R package version 0.6.1. https://CRAN.Rproject.org/package=shinydashboard
shinycssloaders	Sali A (2017). shinycssloaders: add CSS loading animations to 'shiny' outputs. R package version 0.2.0. https://CRAN.R-project.org/package=shinycssloaders
shinyjs	Attali D (2017). shinyjs: easily improve the user experience of your shiny apps in seconds. R package version 0.9.1. https://CRAN.R-project.org/package=shinyjs
RColorBrewer	Neuwirth E (2014). RColorBrewer: ColorBrewer Palettes. R package version 1.1-2. https://CRAN.R-project.org/package=RColorBrewer
gplots	Warnes GR, Bolker B, Bonebakker L, Gentleman R, Liaw WH, Lumley T, Maechler M, Magnusson A, Moeller S, Schwartz M, Venables B (2016). gplots: various R programming tools for plotting data. R package version 3.0.1. https://CRAN.R-project.org/package=gplots
ggplot2	Wickham H. ggplot2: elegant graphics for data analysis. Springer-Verlag, New York, 2009
easyGgplot2	Kassambara A (2014). easyGgplot2: perform and customize easily a plot with ggplot2. R package version 1.0.0.9000. http://www.sthda.com
rmarkdown	Allaire JJ, Cheng J, Xie Y, McPherson J, Chang W, Allen J, Wickham H, Atkins A, Hyndman R, Arslan R (2017). rmarkdown: dynamic documents for R. R package version 1.6. https://CRAN.R-project.org/package=rmarkdown
rtracklayer	Lawrence M, Gentleman R, Carey V. "rtracklayer: an {R} package for interfacing with genome browsers". <i>Bioinformatics</i> 2009;25:1841-1842.
DT	Xie Y (2016). DT: a wrapper of the JavaScript library 'DataTables'. R package version 0.2. https://CRAN.R-project.org/package=DT
data.table	Dowle M, Srinivasan A (2017). data.table: extension of `data.frame`. R package version 1.10.4. https://CRAN.R-project.org/package=data.table
gridExtra	Auguie B (2017). gridExtra: miscellaneous functions for "Grid" graphics. R package version 2.3. https://CRAN.R-project.org/package=gridExtra
GenomicFeatures	Lawrence M, Huber W, Pagés H, Aboyoun P, Carlson M, Gentleman R, et al. Software for computing and annotating genomic ranges. <i>PLoS Comput Biol</i> 2013;9:e1003118
edgeR	Robinson MD, McCarthy DJ, Smyth GK. edgeR: a bioconductor package for differential expression analysis of digital gene expression data. <i>Bioinformatics</i> 2010;26:139-140
goseq	Young MD, Wakefield MJ, Smyth GK, Oshlack A. Gene ontology analysis for RNA-seq: accounting for selection bias. <i>Genome Biol</i> 2010;11:R14
pheatmap	Kolde R (2015). pheatmap: Pretty Heatmaps. R package version 1.0.8. https://CRAN.R-project.org/package=pheatmap
plotly	Sievert C, Parmer C, Hocking T, Chamberlain S, Ram K, Corvellec M, Despouy P (2017). plotly: create interactive web graphics via 'plotly.js'. R package version 4.7.1. https://CRAN.R-project.org/package=plotly
manhattanly	Bhatnagar S (2016). manhattanly: interactive Q-Q and Manhattan plots using 'plotly.js'. R package version 0.2.0. https://CRAN.R-project.org/package=manhattanly
LDheatmap	Shin JH, Blay S, McNeney B, Graham J (2006). LDheatmap: an R function for graphical display of pairwise linkage disequilibria between single nucleotide polymorphisms. <i>J Stat Softw</i> 2006;16:Code Snippet 3
GWASTools	Gogarten SM, Bhangale T, Conomos MP, Laurie CA, McHugh CP, Painter I, et al. GWASTools: an R/Bioconductor package for quality control and analysis of

genetics	genome-wide association studies. <i>Bioinformatics</i> 2012;28:3329-3331 Warnes G, with contributions from Gregor Gorjanc, Leisch F, Man M (2013). Genetics: population genetics. R package version 1.3.8.1. https://CRAN.R-project.org/package=genetics
org.At.tair.db	Carlson M (2015). org.At.tair.db: Genome wide annotation for Arabidopsis. R package version 3.2.3.

Supplementary Table 2. Plugins attached in JBrowse

Name	Reference
gccontent	https://github.com/elsiklab/gccontent
narrowpeak	https://github.com/cmdcolin/narrowpeak
gwasviewer	https://github.com/elsiklab/gwasviewer
bedGraph to BigWig	https://github.com/ENCODE-DCC/kentUtils

Supplementary Table 3. Example data source

Name	Reference
RNA-seq single factor	https://www.ebi.ac.uk/arrayexpress/experiments/E-MTAB-4298/
RNA-seq multiple factor	https://www.ebi.ac.uk/arrayexpress/experiments/E-MTAB-4243/
GWAS and LD analysis	Tutorial data of R package GAPIT http://zzlab.net/GAPIT/GAPIT_Tutorial_Data.zip numeric to vcf format conversion was carried out using TASSEL

Index

1. RNA-Seq

01. DEG analysis for single factor	03
02. DEG Visualization	04
03. Gene Ontology Enrichment Analysis	06
04. DEG analysis for multiple factor	09
05. DEG visualization & Gene Ontology Enrichment for pre-analyzed data	11
06. Input files	12

2. GWAS

01. Upload Data	14
02. Manhattan and QQ Plot	15
03. LD heatmap Visualization	16
04. Input files	17

3. Genome Browser

01. Docker installation	20
02. Launching Docker Image	20
03. Upload Data - Build	21
04. Upload Data - Upload	22
05. Upload Data – Build_folder	23
06. Remove Data - Track	23
07. Remove Data - Dataset	24
08. RNA-Seq & GWAS	24
09. RNA-Seq	24
10. GWAS	26

<1.1 DEG analysis for single factor>

The screenshot shows the 'DEG analysis for single factor' interface. It consists of a sidebar on the left with navigation options like 'Introduction to DEG', 'DEG analysis Multiple factor', 'Upload Data', 'Charts', 'GO Enrichment Analysis', and 'Pre-analysis'. The main area has three panels: 'Upload Count table' (A), 'Count Table Meta information' (B), and 'Normalization' (C). Panel A has a file upload button. Panel B has input fields for 'Control start column number' and 'Control end column number'. Panel C has input fields for 'CPM cutoff' and 'Number of sample to contain above CPM cutoff'. Below these panels is a table showing the results of the analysis, with columns for gene ID, logFC, logCPM, LR, PValue, and FDR. At the bottom, there are buttons for 'Start analysis' (D), 'Download', 'Visualization', and 'GO analysis'.

<Figure 1. DEG single factor analysis page >

Ⓐ: Upload count table generated using htseq-count or similar software after mapping RNA-Seq data to reference Genome.

Ⓑ: Specify the range of control columns.

Ⓒ: In order to analyze differentially expressed genes, each genes need at least 6~10 counts. However, in raw count table, there are lots of genes with 0 counts which need to be filtered out. We use count per million to filter out genes with low or no counts. CPM cutoff criteria filters gene out using the smallest library size. For example, if the smallest library size was 4,000,000, CPM cutoff 2 would filter out genes with less than 8 counts. Number of samples to contain above CPM cutoff criteria can be specified as to how many samples must satisfy above filtering criteria. For example, raw count table with samples having 3 replicates each, if we specify Number of sample to contain above CPM as 3, at least 3 samples must meet the filtering criteria in order to proceed.

Ⓓ: Clicking the Start analysis button will run DEG analysis and produce the results as a table. Users can click Download button to get the results as text file format. Visualization button will lead the users to a page where they can generate Heatmap, Volcano plot, PCA analysis plot with the DEG analysis result. Clicking the GO analysis button will lead to a page where Gene Ontology Enrichment analysis can be done.

<1.2 DEG Visualization>

<Figure 2. Heatmap visualization>

Ⓐ: Specify False Discovery Rate to filter out genes to be used to draw heatmap. Percentage can be set to draw heatmap with top n% of filtered genes. User can specify samples to be drawn on heatmap.

Ⓑ: Generated heatmap

Ⓒ: Shows DEG analysis table of genes used to create heatmap. Click Download button to download table in text file format.

Ⓓ: Specify False Discovery Rate to filter out genes to be used to draw heatmap. Percentage can be set to draw heatmap with top n% of filtered genes. User can specify samples to be drawn on heatmap. Gene list can be uploaded to draw heatmap with genes of interest.

Ⓔ: Generated heatmap.

Ⓕ: Shows DEG analysis table of genes used to create heatmap. Click Download button to download table in text file format.

<Figure 3. Volcano plot visualization>

Ⓐ: User can specify False Discovery Rate or Absolute Fold Change to generate Volcano plot.

Ⓑ: Generates volcano plot by plotting log2 fold change versus $-\log_{10}(\text{False Discovery Rate})$. User can download generated plot in PDF format by clicking the Download button.

Ⓒ: DEG analysis result table for genes used to plot volcano plot is shown. This table can be downloaded in text format by clicking the Download button.

<Figure 4. PCA plot visualization>

Ⓐ: Generates Principal analysis plot. This can be downloaded in PDF format by clicking the Download button.

<1.3 Gene Ontology Enrichment Analysis>

GO enrichment analysis Result for Over represented Genes							Search:	
show 10 ▼ entries	category	over_represented_pvalue	under_represented_pvalue	numDEInCat	numInCat	term	ontology	
822	GO:0005576	7.749356296295e-20	1	156	1265	extracellular region	CC	
2060	GO:0010167	2.7820180439307e-19	1	44	191	response to nitrate	BP	
2439	GO:0018706	3.874799993541e-15	1	45	201	nitrate transport	BP	
289	GO:0003700	3.37407945845e-14	1	121	1031	transcription factor activity, sequence-specific DNA binding	MF	
1789	GO:0003736	4.6226830992023e-11	1	21	66	cytosolin-activated signaling pathway	BP	
1049	GO:0006355	7.3450058522947e-10	1	98	926	regulation of transcription, DNA-templated	BP	
47	GO:0006196	1.3689388395235e-9	0.9999999923296	12	24	phosphorelay response regulator activity	MF	
1223	GO:0006426	1.4512166506056e-9	0.99999999733971	26	113	iron ion transport	BP	
2022	GO:0012006	1.4399461744761e-9	0.99999999724662	26	113	cellular response to iron-ion starvation	BP	
782	GO:0006216	4.11914516839277e-9	0.99999999593631	33	197	transporter activity	MF	
Showing 1 to 10 of 33 entries							Previous	1 2 3 4 Next
Download								

GO enrichment analysis Result for Under represented Genes							Search:	
show 10 ▼ entries	category	over_represented_pvalue	under_represented_pvalue	numDEInCat	numInCat	term	ontology	
1698	GO:0009570	0.999961947391618	0.000064754769575025	12	535	chloroplast stroma	CC	
1513	GO:0009941	0.99997721046774	0.000078948909756931	10	469	chloroplast envelope	CC	
1679	GO:0009634	0.99998380062613	0.0000208738682365	1	176	chloroplast thylakoid	CC	
1110	GO:0006811	0.99998188218623	0.000106047925245407	2	236	ubiquitin-dependent protein catabolic process	BP	
885	GO:0005870	0.999980882832499	0.0000778174478933789	3	275	nucleus	CC	
1090	GO:0006467	0.99993492471202	0.0000629611316106231	1	247	protein folding	BP	
1062	GO:0006364	0.999997139566762	0.0000407406824774373	2	211	rRNA processing	BP	
1680	GO:0009838	0.9999905612961	0.0000176072734862478	2	253	chloroplast thylakoid membrane	CC	
128	GO:000674	0.999990046774103	0.00000166727393659326	177	587	molecular_function	MF	
900	GO:0005623	0.99999020309	0.00000164011962073651	41	1412	cytosol	CC	
Showing 1 to 10 of 23 entries							Previous	1 2 3 Next
Download

F

G

H

<Figure 5. Gene Ontology Enrichment analysis page>

Ⓐ: Specify filtering criteria(False Discovery Rate) for differentially expressed genes which will go through Gene Ontology Enrichment analysis.

Ⓔ: Specify False Discovery Rate to be used in Gene Ontology Enrichment analysis.

Ⓒ: Upload Gene Ontology annotation file and GTF file.

Ⓓ: Shows over represented Gene Ontology Enrichment analysis result among differentially expressed genes. 5th column(numInCat) shows how many genes are allocated to the specific category and 4th column(numDEInCat) shows among those genes, how many of them are differentially expressed. Users can click Download button to retrieve the results in text file format.

Ⓔ: Shows under represented Gene Ontology Enrichment analysis result among differentially expressed genes. 5th column(numInCat) shows how many genes are allocated to the specific category and 4th column(numDEInCat) shows among those genes, how many of them are differentially expressed. Users can click Download button to retrieve the results in text file format.

Ⓕ ~ Ⓖ: Shows the histogram of differentially expressed genes with specific Gene Ontology term and ontology such as biological process, cellular component and molecular function. These graphs can be downloaded in PDF format by clicking the Download button.

<1.4 DEG analysis for multiple factor>

Upload

Choose Raw count data

Browse... Ara_root_raw_count.txt Upload complete

Choose Meta data

Browse... meta_data.txt Upload complete

Show 10 entries

group	lib.size	norm.factors
root_C1	6840004	1
root_C2	4897925	1
root_C3	4948455	1
root_BA1	7655196	1
root_BA2	1220960	1
root_BA3	6314525	1

Showing 1 to 6 of 6 entries

Submit

Design Matrix

Show 10 entries

	In	met	water
root_C1	1	0	0
root_C2	1	0	0
root_C3	0	1	0
root_BA1	0	1	0
root_BA2	0	0	1
root_BA3	0	0	1

Showing 1 to 6 of 6 entries

Previous 1 Next

Normalization

CPM cutoff
2

Number of sample to contain above CPM cutoff
3

Show: 10 entries

group	lib.size	norm.factors
rest_C1	8224304	1.00758779112994
rest_C2	4682794	0.59377650090159
rest_C3	4682794	1.04397761842499
rest_B41	7827824	0.96302192677723
rest_B42	12178938	0.96220678861377
rest_B43	6296930	1.01178803489319

Showing 1 to 6 of 6 entries

Choose two levels to compare

In: rest, water

Level 1
water

Level 2
In

Start DEG analysis

DEG output

	logFC	logCPM	PValue	FDR
AT1001010	-1.01017629428887	5.5289577449092	0.00748434113463	0.407154118932168
AT1001020	-0.338473622978611	3.97446237178613	0.29787788628466	1
AT1001040	-0.148743065821946	5.58178274430061	0.826838724107497	1
AT1001080	-0.182433667781127	6.88239431139089	0.44884584973742	1
AT1001090	-0.058942388538328	5.54480278727781	0.870738976478718	1
AT1001070	0.448363113052771	4.1845433267316	0.223251707544371	1
AT1001080	-0.062189098328596	3.28031392470948	0.906674475151342	1
AT1001090	-0.094708854731126	7.82232321462109	0.69032100717949	1
AT1001100	0.087897889622893	0.25248789472227	0.825746211112343	1
AT1001110	-0.187171317138867	3.13940450405036	0.74581545788876	1

Showing 1 to 10 of 11 DEG entries

Download | Visualize | GO analysis

<Figure 6. Multi factor DEG analysis multi factor page>

Ⓐ: Upload count table generated using htseq-count or similar software after mapping RNA-Seq data to reference Genome with meta information file.

Ⓑ: Generates design matrix when all the files are uploaded.

Ⓒ: In order to analyze differentially expressed genes, each genes need at least 6~10 counts. However, in raw count table, there are lots of genes with 0 counts which need to be filtered out. We use count per million to filter out genes with low or no counts. CPM cutoff criteria filters gene out using the smallest library size. For example, if the smallest library size was 4,000,000, CPM cutoff 2 would filter out genes with less than 8 counts. Number of samples to contain above CPM cutoff criteria can be specified as to how many samples must satisfy above filtering criteria. For example, raw count table with samples having 3 replicates each, if we specify Number of sample to contain above CPM as 3, at least 3 samples must meet the filtering criteria in order to proceed.

Ⓓ: Among multiple samples, choose two samples to proceed DEG analysis.

Ⓔ: Clicking the Start analysis button will run DEG analysis and produce the results as a table. Users can click Download button to get the results as text file format. Visualization button will lead the users to a page where they can generate Heatmap, Volcano plot, PCA analysis plot with the DEG analysis result. Clicking the GO analysis button will lead to a page where Gene Ontology Enrichment analysis can be done.

<1.5 DEG visualization and Gene Ontology Enrichment analysis for pre-analyzed data>

<Figure 7. Pre-analyzed data upload page>

Ⓐ: Upload count table generated using htseq-count or similar software after mapping RNA-Seq data to reference Genome.

Ⓑ: Upload DEG analysis result table.

Ⓒ: Visualization button will lead the users to a page where they can generate Heatmap, Volcano plot, PCA analysis plot with the DEG analysis result. Clicking the GO analysis button will lead to a page where Gene Ontology Enrichment analysis can be done.

<1.6 Input files>

1	ln1	ln2	meth1	meth2	water1	water2		
2	AT1G01010	440	366	419	625	334	482	
3	AT1G01020	1095		1030	856	1447		759 1183
4	AT1G01030	0	0	1	0	0	2	
5	AT1G01040	2455		1706	2672		4204	1834 3098
6	AT1G01046	11	10	20	52	17	30	
7	AT1G01050	11180		11030	10013	16152	8180	11682
8	AT1G01060	652	473	800	1410	642	996	
9	AT1G01070	109	97	91	119	58	113	
10	AT1G01073	0	0	0	0	0	0	
11	AT1G01080	2209		1951	2116	3061	1655	2390
12	AT1G01090	8647		8519	7271	10956	5500	8672
13	AT1G01100	15938		13942	10564	16053	8958	12852
14	AT1G01110	0	0	0	0	0	0	
15	AT1G01115	0	0	0	0	0	0	
16	AT1G01120	8	1	15	17	6	19	
17	AT1G01130	68	65	51	62	43	60	
18	AT1G01140	478	463	624	1003	529	829	
19	AT1G01150	8	1	21	19	12	6	
20	AT1G01160	3483		2737	3300	4585	2468	3396
21	AT1G01170	5226		4862	5953	7873	4204	5819
22	AT1G01180	5	3	15	34	20	19	
23	AT1G01183	0	0	0	0	0	0	
24	AT1G01190	0	3	4	3	0	1	
25	AT1G01200	26	24	11	5	2	8	
26	AT1G01210	604	628	539	898	441	645	
27	AT1G01220	3277		2761	4580	7004	3652	5094
28	AT1G01225	779	686	810	1294	754	1085	
29	AT1G01230	2199		2120	1979	2881	1673	2318
30	AT1G01240	310	304	1098	1665	922	1442	
31	AT1G01250	2	0	0	1	0	0	
32	AT1G01260	1340		1136	1258	1943	1035	1632
33	AT1G01270	0	0	0	0	0	0	
34	AT1G01280	0	0	0	0	0	0	

1	ln
2	ln
3	met
4	met
5	water
6	water

B

A

1	"logFC"	"logCPM"	"LR"	"PValue"	"FDR"
2	"AT1G01010"	1.19688737736099	5.52688808335964	39.3089106372121	3.61786523882999e-10 4.80222720383886e-08
3	"AT1G01020"	0.0822804977656206	3.97165716089378	0.111434656713485	0.738516674183035 0.992950029705138
4	"AT1G01030"	-0.162468882715308	0.0823715495057947	0.0610995702734676	0.804766390210944 0.994580412175897
5	"AT1G01040"	0.106377858075599	5.57910426454308	0.37042230935759	0.542774163320339 0.974600223816041
6	"AT1G01050"	0.0153574773648363	6.67932177116587	0.00814645735631103	0.92808241163436 0.995983980196374
7	"AT1G01060"	0.0456180046924779	5.54156373113906	0.019399476207294	0.889227311214195 0.995931204225289
8	"AT1G01070"	-0.544012070552485	4.18302675004016	5.48539400852262	0.0191759975507977 0.211398693538396
9	"AT1G01080"	0.174667523566418	3.26203485385487	0.299539868880617	0.584171025511136 0.978544418926473
10	"AT1G01090"	0.0770064556487513	7.82001219000772	0.206235076914759	0.649734453606224 0.987057549001829
11	"AT1G01100"	-0.194374421491345	8.24999782635235	0.450922121063243	0.501897378775529 0.967135588725176
12	"AT1G01110"	-0.0356166922910766	3.18109685561797	0.011510637873462	0.914560789141767 0.995983980196374
13	"AT1G01120"	0.641291331519332	6.3387621639768	11.0961455503092	0.000865073303584205 0.0196921958851816
14	"AT1G01130"	0.467864553000374	1.76456938585317	0.977622941476459	0.322786369058292 0.904971916676201
15	"AT1G01140"	-0.542815616073306	2.63788851959664	2.13563384343845	0.143910966721881 0.68944588214333
16	"AT1G01160"	-0.0296144956218807	5.15484537653471	0.0203745510116882	0.88649595793983 0.995931204225289
17	"AT1G01170"	0.0959514365512992	3.64734295039377	0.15817628576344	0.69084114621376 0.987813981559841
18	"AT1G01180"	0.24596413317094	3.7881988269758	0.650354637870404	0.419985915804817 0.950470048855293
19	"AT1G01190"	-0.375672462290263	3.86317903951983	0.635708343635596	0.425269154293829 0.951393075845317
20	"AT1G01200"	0.246507464818891	3.01178043499523	0.779877708445121	0.377178543497332 0.933754390534284

C

1	AT1G01010	GO:0006888	1	#!genome-build TAIR10	
2	AT1G01010	GO:0007275	2	#!genome-version TAIR10	
3	AT1G01010	GO:0043090	3	#!genome-date 2010-09	
4	AT1G01020	GO:0006665	4	#!genome-build-accession GCA_000001735.1	
5	AT1G01020	GO:0006665	5	#!genomebuild-last-updated 2010-09	
6	AT1G01020	GO:0016125	6	1 tair gene 3631 5899 . +	. gene_id "AT1G01010"; gene_version "1"; gene_name "NAC001"; gene_source "ta
7	AT1G01020	GO:0016125	7	1 tair transcript 3631 5899 . +	. gene_id "AT1G01010"; gene_version "1"; transcript_id "AT1G01010.1"; tr
8	AT1G01030	GO:0006355	8	1 tair exon 3631 3913 . +	. gene_id "AT1G01010"; gene_version "1"; transcript_id "AT1G01010.1"; transc
9	AT1G01030	GO:0006355	9	1 tair CDS 3760 3913 . + 0	gene_id "AT1G01010"; gene_version "1"; transcript_id "AT1G01010.1"; transcript
10	AT1G01030	GO:0006355	10	1 tair start_codon 3760 3762 .	+ 0 gene_id "AT1G01010"; gene_version "1"; transcript_id "AT1G01010.1"; tr
11	AT1G01030	GO:0006355	11	1 tair exon 3996 4276 . +	. gene_id "AT1G01010"; gene_version "1"; transcript_id "AT1G01010.1"; transc
12	AT1G01030	GO:0006355	12	1 tair CDS 3996 4276 . + 2	gene_id "AT1G01010"; gene_version "1"; transcript_id "AT1G01010.1"; transcript
13	AT1G01030	GO:0009908	13	1 tair exon 4486 4605 . +	. gene_id "AT1G01010"; gene_version "1"; transcript_id "AT1G01010.1"; transc
14	AT1G01030	GO:0048366	14	1 tair CDS 4486 4605 . + 0	gene_id "AT1G01010"; gene_version "1"; transcript_id "AT1G01010.1"; transcript
15	AT1G01040	GO:0000226	15	1 tair exon 4706 5095 . +	. gene_id "AT1G01010"; gene_version "1"; transcript_id "AT1G01010.1"; transc
16	AT1G01040	GO:0000226	16	1 tair CDS 4706 5095 . + 0	gene_id "AT1G01010"; gene_version "1"; transcript_id "AT1G01010.1"; transcript
17	AT1G01040	GO:0000226	17	1 tair exon 5174 5326 . +	. gene_id "AT1G01010"; gene_version "1"; transcript_id "AT1G01010.1"; transc
18	AT1G01040	GO:0000278	18	1 tair CDS 5174 5326 . + 0	gene_id "AT1G01010"; gene_version "1"; transcript_id "AT1G01010.1"; transcript
19	AT1G01040	GO:0000911	19	1 tair exon 5439 5899 . +	. gene_id "AT1G01010"; gene_version "1"; transcript_id "AT1G01010.1"; transc
20	AT1G01040	GO:0006306	20	1 tair CDS 5439 5627 . + 0	gene_id "AT1G01010"; gene_version "1"; transcript_id "AT1G01010.1"; transcript
21	AT1G01040	GO:0006306	21	1 tair stop_codon 5628 5630 .	+ 0 gene_id "AT1G01010"; gene_version "1"; transcript_id "AT1G01010.1"; tr
22	AT1G01040	GO:0006306	22	1 tair five_prime_utr 3631 3759 .	. gene_id "AT1G01010"; gene_version "1"; transcript_id "AT1G01010.1"
23	AT1G01040	GO:0006306	23	1 tair three_prime_utr 5631 5899 .	+ . gene_id "AT1G01010"; gene_version "1"; transcript_id "AT1G01010.1"
24	AT1G01040	GO:0006342	24	1 tair gene 5928 8737 . -	. gene_id "AT1G01020"; gene_version "1"; gene_name "ARV1"; gene_source "ta
25	AT1G01040	GO:0006342	25	1 tair transcript 5928 8737 . -	. gene_id "AT1G01020"; gene_version "1"; transcript_id "AT1G01020.1"; tr
26	AT1G01040	GO:0006342	26	1 tair exon 8571 8737 . -	. gene_id "AT1G01020"; gene_version "1"; transcript_id "AT1G01020.1"; transc
27	AT1G01040	GO:0006396	27	1 tair CDS 8571 8666 . - 0	gene_id "AT1G01020"; gene_version "1"; transcript_id "AT1G01020.1"; transcript
28	AT1G01040	GO:0006396	28	1 tair start_codon 8664 8666 .	- 0 gene_id "AT1G01020"; gene_version "1"; transcript_id "AT1G01020.1"; tr
29	AT1G01040	GO:0006396	29	1 tair exon 8417 8464 . -	. gene_id "AT1G01020"; gene_version "1"; transcript_id "AT1G01020.1"; transc
30	AT1G01040	GO:0006396	30	1 tair CDS 8417 8464 . - 0	gene_id "AT1G01020"; gene_version "1"; transcript_id "AT1G01020.1"; transcript
31	AT1G01040	GO:0006396	31	1 tair exon 8236 8325 . -	. gene_id "AT1G01020"; gene_version "1"; transcript_id "AT1G01020.1"; transc
32	AT1G01040	GO:0007267	32	1 tair CDS 8236 8325 . - 0	gene_id "AT1G01020"; gene_version "1"; transcript_id "AT1G01020.1"; transcript
33	AT1G01040	GO:0009616	33	1 tair exon 7942 7987 . -	. gene_id "AT1G01020"; gene_version "1"; transcript_id "AT1G01020.1"; transc
34	AT1G01040	GO:0009616	34	1 tair CDS 7942 7987 . - 0	gene_id "AT1G01020"; gene_version "1"; transcript_id "AT1G01020.1"; transcript
35	AT1G01040	GO:0009616			
36	AT1G01040	GO:0009616			
37	AT1G01040	GO:0009616			
38	AT1G01040	GO:0009630			
39	AT1G01040	GO:0009880			
40	AT1G01040	GO:0009908			

<Figure 8. >

Ⓐ: Raw count table / Used in DEG analysis

Ⓑ: Meta information specifying which column belongs to which group in raw count table / Used in DEG analysis multiple factor.

Ⓒ: DEG analysis result / pre analyzed DEG result table can be uploaded. Column header must be the same as above figure but, 'logCPM' and 'LR' can be omitted.

Ⓓ: Gene Ontology annotation file / Text file containing Gene ID and corresponding Gene Ontology category. Gene ID must be the same as the ones used in raw count table.

Ⓔ: GTF file / Used in Gene Ontology Enrichment analysis to calculate gene length.

2. GWAS & LD heatmap

<2.1 Upload Data >

IVAG: an Integrative Visualization Application for various types of Genomic data based on R and Shiny platform

Introduction to IVAG
RNA-Seq
GWAS & LD
Upload Data
Manhattan Plot
QQ Plot
LD Heatmap
Re-analyze
Genome Browser

Upload Data

Choose GWAS result data

Browse... No file selected

Visualize Manhattan Plot

Choose the type of input file:

☒ unzipped.tsv
☐ gzipped.tsv

Add into Genome Browser

Choose LD matrix data

Browse... No file selected

Visualize LD-heatmap

Choose the type of input file:

☒ unzipped.tsv
☐ gzipped.tsv

Add into Genome Browser

☐ GeneID Annotation with GTF file

☐ Make LD matrix with your VCF file

Annotate Gene ID

Choose GTF file

Browse... No file selected

Start Annotation

Download

Visualize Manhattan Plot

Up & Downstream flanking size(bp):

3000

Choose the type of Gene nomenclature:

☒ Gene ID(example: ENSG00000139618.10)
☐ Gene Name(example: BRCA2)

Make LD matrix

Choose your VCF file to make LD(r2) matrix

ALL.chr6.phase3_shapeit2_mvncall_integrated_v5a.20130502.genotypes.vcf

Choose the type of input file:

☒ unzipped.vcf
☐ gzipped.vcf

VCF subsetting options

CHR: 1 From(bp): 1 To(bp): 10000

SNP-pruning options

Window size in SNPs: 50 Number of SNPs to shift the window at each step: 5 R2 threshold to keep: 0.5

Start LD Analysis

Show analysis log

Download LD-matrix(Please wait!!)

Download marker information

Visualize LD-heatmap

<Fig. 9. GWAS & LD Upload and Analysis page>

Ⓐ: GWAS result and LD matrix files (TSV format) can be uploaded. Users can move into plotting page by clicking Visualize buttons. Also, uploaded files can be added into pre-built genome browser.

Ⓐ: Users can annotate gene information into all markers in the GWAS result file.

Ⓒ: Choose a VCF file you want to use in the LD analysis.

Ⓓ: Subset the region of your interest by assigning CHR, Start, and END parameters.

Ⓔ: All markers will be pruned using PLINK analysis option "--indep-pairwise" to reduce the number of markers to be used in LD calculation. Detailed information for this analysis can be found at <http://zzz.bwh.harvard.edu/plink/summary.shtml#prune>.

<2.2 Manhattan and QQ Plot Visualization >

<Fig. 10. Manhattan and QQ plot visualization page>

- Ⓐ: Users can select what type of plot to draw; Interactive or Non-Interactive.
- Ⓑ: Adjust specific parameters to customize your plot. You can subset the plot by chromosome and highlight the SNPs of your interest.
- Ⓒ: Detailed information will be shown when the cursor is hovered over a specific point of interest.
- Ⓓ: If you click a specific dot, all information for that dot will be recoded here.
- Ⓔ: User can move directly into the genome browser to search the SNP selected at the previous step. To use this function, genome browser construction should be done in advance.
- Ⓕ: Information of all markers that passed the cutoff will be shown here.
- Ⓖ: QQ plot can also be drawn. Light blue shade indicates the confidence interval for the null hypothesis that assumes there is no association between SNP and a trait.

<2.3 LD heatmap Visualization>

<Fig. 12. LD heatmap visualization page>

- Ⓐ: All markers in the LD matrix (uploaded or generated) will be plotted on the upper panel.
- Ⓑ: Genomic coordinate map can be added when genomic coordinate information is given.
- Ⓒ: The LD heatmap can be subset by uploading a list of markers of your interest

⑩: r2 value for each SNP-SNP pair can be added on the plot.

<2.4 Input Files >

GWAS

SNP	Chromosome	Position	P.value
PZA01946.7	7	123601837	1.61E-12
PZA00947.1	9	96885738	1.66E-12
PZA00527.10	2	216833071	9.81E-11
PZA00702.5	3	185760526	1.09E-08
PZA00498.5	8	48775713	9.61E-08
PZA00069.4	5	214424975	4.17E-07
PZA01209.1	8	52678845	8.63E-07
PZA00498.3	8	48775756	1.02E-06
PZA00663.5	4	141267246	1.06E-06
PZA02808.12	2	44606596	0.000143238
tb1.11	1	264848126	0.000483664

<Fig. 13. GWAS_Result.tsv -1 >

1. IVAG intakes GWAS result summary statistics file that comprise Marker ID, Chromosome, position, and p-value columns in order.

SNP	Chromosome	Position	P.value	GENE	UPSTREAM	DOWNSTREAM
PZA01946.7	7	123601837	1.61E-12	NA	NA	NA
PZA00947.1	9	96885738	1.66E-12	Zm00001d046581[-]	NA	NA
PZA00527.10	2	216833071	9.81E-11	NA	NA	NA
PZA00702.5	3	185760526	1.09E-08	NA	NA	NA
PZA00498.5	8	48775713	9.61E-08	NA	NA	NA
PZA00069.4	5	214424975	4.17E-07	NA	NA	NA
PZA01209.1	8	52678845	8.63E-07	NA	NA	NA
PZA00498.3	8	48775756	1.02E-06	NA	NA	NA
PZA00663.5	4	141267246	1.06E-06	NA	NA	NA
PZA02808.12	2	44606596	0.000143238	NA	NA	NA
tb1.11	1	264848126	0.000483664	NA	NA	NA

<Fig. 13. GWAS_Result.tsv - 2 >

2. Another version of the input file contains additional three columns which could be annotated from IVAG Gene ID annotation function.

LD heatmap - 1

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
1		PZA02388.1	PZA02174.2	PZA03316.4	PZA03316.1	PZA03316.3	PZA00368.17	PZA00368.1	PZA00058.1	PZA00058.5	PZA00632.1	PZA00416.4	PZA00416.2	PZA01601.1	PZA03178.3	PZA03178.1
2	PZA02388.1	NA	0.003131425	0.001322904	1.89E-05	0.010741308	0.00236276	6.80E-05	0.004041285	0.00030022	0.012297859	0.011686865	0.007863762	1.12E-06	0.004849854	0.001331938
3	PZA02174.2	NA	NA	0.0004783	0.021465195	0.001231729	0.00992238	0.024481744	0.010871922	0.00101117	0.002377562	0.016651214	0.029450244	0.055942213	0.005134748	0.002694006
4	PZA03316.4	NA	NA	NA	0.06055998	0.003111189	7.71E-05	4.94E-05	0.000890745	0.007548125	0.002149237	0.005553662	0.000882867	0.006944157	0.000186331	0.001078003
5	PZA03316.1	NA	NA	NA	NA	0.031768088	0.00528988	0.004006694	0.030093192	0.00026265	0.000316417	0.008766957	0.020765621	0.009565184	0.000657431	0.015585438
6	PZA03316.3	NA	NA	NA	NA	NA	0.025351076	0.000510142	0.014710731	0.007110129	0.00223847	0.000121821	0.000939886	0.002376762	0.000639039	0.001826396
7	PZA00368.17	NA	NA	NA	NA	NA	NA	0.23464068	0.015698233	0.006223222	0.000859469	0.006282812	1.15E-05	0.002566236	5.83E-06	1.23E-05
8	PZA00368.1	NA	NA	NA	NA	NA	NA	NA	0.022382311	0.001951984	0.000375188	0.001289014	2.77E-05	8.82E-08	0.000300585	0.005470765
9	PZA00058.1	NA	NA	NA	NA	NA	NA	NA	NA	0.079778481	0.0092099	0.010593625	0.017851142	0.000487344	8.59E-05	0.025033994
10	PZA00058.5	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.007402666	0.029095655	0.010838279	0.003178828	0.003044483	8.38E-05
11	PZA00632.1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.000169487	0.01006222	0.012087334	1.87E-05	0.001650081
12	PZA00416.4	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.510533148	0.037531322	0.015276814	0.004631683
13	PZA00416.2	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.02832356	0.012447245	0.000960156
14	PZA01601.1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.021422767	0.016685048
15	PZA03178.3	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.150240822
16	PZA03178.1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

<Fig. 14. LD_Matrix_Rsquare.tsv >

1. LD matrix file is tab-separated and has r2 values for all SNP-SNP pairs in an upper triangular matrix form.
2. The first row (header) is SNPIDs sorted by their position.
3. The first column, colored in blue, should not be included in the input LD matrix file, but I just showed it to help you better understand this format. SNP IDs in column header and row header are ordered equally and you can interpret this LD matrix like r2 value between PZA00368.17 and PZA03316.3 is 0.025351076 as described in green.
4. Note that your input file **SHOULD** look like the red boxed one.

LD heatmap - 2

```

chr8_SNPid - 메모장
파일(F) 편집(E) 서식(O) 보기(V) 도움말(H)
PZB01094.3
PZA02927.1
PZA03114.2
PZA03381.2
PZA03381.1
PZB02114.1
PZB02114.2
PZB02114.3
PZB00145.1
PZA00498.5
PZA00498.3
PZA01209.1
PZA02203.1
PZA03126.1
PZA00417.3

```

<Fig. 15. SNPid.txt >

1. This text file has the list of SNP ids of your interest. You can extract a subset of LD heatmap that contains only SNPs specified with this file.

LD heatmap - 3

	A	B	C
1	SNP	Chromosome	Position
2	PZA02388.1	8	169137
3	PZA02174.2	8	4101256
4	PZA03316.4	8	4766593
5	PZA03316.1	8	4766694
6	PZA03316.3	8	4766801
7	PZA00368.17	8	5632196
8	PZA00368.1	8	5632308
9	PZA00058.1	8	5966657
10	PZA00058.5	8	5966698
11	PZA00632.1	8	6017018
12	PZA00416.4	8	8098163
13	PZA00416.2	8	8098271
14	PZA01601.1	8	8404207
15	PZA03178.3	8	11602192

<Fig. 16. SNP_Pos_Info.tsv >

1. genomic coordinate information file requires three columns; SNP, Chromosome, and position.

3. JBrowse

<3.1 Docker installation >

Install the docker before running IVAG. Docker is available on multiple platforms, on cloud and on-premises. Choose the best installation path for you and install it from the URL link below.

(<https://docs.docker.com/engine/installation/>)

<3.2 Launching Docker Image >

<Figure 17. Example folder mounted on a docker >

1. Create DATA directory for mounting docker.
2. Create subdirectory raw, json, plink on Data directory.
 - raw : Upload file for JBrowse
 - json : Information about data uploaded on JBrowse
 - plink : Save tmp, log files generated from IVAG LD analysis
3. Create subdirectory for each species under raw directory and put each data.
(fasta, bam, gtf, gff3, bed, bw, vcf)
4. Specify DATA directory when launching docker image

DATA directory	C:\Users\USER\Desktop\DATA
Docker image launch commend	docker run -ti -v C:\Users\USER\Desktop\DATA:/jbrowse/my_data -p 8080:80 -p 8383:3838 leetaerim/ivag:v1 /bin/bash -c "Rscript load.R"

<Table 1. Example of launching docker image >

<3.3 Upload Data – Build >

The screenshot shows the 'Build' tab of the 'My Genome browser' section in the IVAG application. The left sidebar has 'Upload Data' highlighted. The main content area includes a 'Name the dataset' text input (A), a 'Choose directory' dropdown (B) showing 'Arabidopsis', and a 'fasta' file selection dropdown (C) showing 'Arabidopsis_thaliana.TAIR10.dna.toplevel.fa'. At the bottom are 'Update list' (D) and 'Build browser' (E) buttons. A 'Make browser' link is in the top right. A red box (F) encloses the top header area.

<Figure 18. Build Genome Browser page>

Ⓐ: Specify genome browser name

Ⓑ: Shows subdirectory of raw directory mounted on docker. Among the list, select subdirectory to construct genome browser.

Ⓒ: Shows files of subdirectory chosen from Ⓑ. Choose fasta file.

Ⓓ: Click Update list button to refresh list on Ⓑ.

Ⓔ: Click Build browser button to construct genome browser.

Ⓕ: Leads to JBrowse page

<3.4 Upload Data – Upload >

<Figure 19. Upload data to browser page>

Ⓐ: Specify file type to upload.

➔ vcf : Takes long time to index data for ID search. So asks whether user will index or not.

➔ bam : If bam file size is large, asks whether to divide file into pieces to upload.

Ⓑ: Select genome browser to upload.

Ⓒ: Specify track name.

Ⓓ: Specify data to upload.

Ⓔ: Click Update list button to refresh list on Ⓑ, Ⓓ.

Ⓕ: Upload to genome browser.

<3.5 Upload Data – Build_folder >

IVAG: an Integrative Visualization Application for various types of Genomic data based on R and Shiny platform

My Genome browser

Build Upload **Build_folder** Make browser

Make new dataset,
upload all files in folder.

Choose directory Arabidopsis A

Name the dataset B

Update list C Build browser D

<Figure 20. Build and Upload folder to browser page>

Ⓐ: Build genome browser using fasta file in specified directory and upload all the file in the directory automatically.

Ⓑ: Specify genome browser name.

Ⓒ: Click Update list button to refresh list on Ⓐ.

Ⓓ: Build genome browser.

<3.6 Remove Data - Track >

IVAG: an Integrative Visualization Application for various types of Genomic data based on R and Shiny platform

My Genome browser

Track Dataset Remove

Remove track

Dataset : Arabidopsis track name A

Remove track B

<Figure 21. Remove Data - Track page>

Ⓐ: Specify genome browser name and type track name to be deleted.

Ⓑ: Remove track.

<3.7 Remove Data - Dataset >

<Figure 22. Remove Data - Dataset page>

Ⓐ: Select genome browser to be removed.

Ⓑ: Remove genome browser.

<3.8 RNA-Seq & GWAS >

IVAG RNA-Seq and GWAS analysis result can be uploaded to IVAG genome browser. Results can be uploaded via file but can be omitted by clicking "Add into Genome Browser" button on analysis page.

<3.9 RNA-Seq & GWAS – RNA-Seq >

<Figure 23. JBrowse RNAseq peak track >

<Figure 24. JBrowse RNAseq peak with logFC track >

IVAG RNA-Seq analysis result can be uploaded in 3 track types.

① peak : DEG list is uploaded on JBrowse in bed file format (Figure 23)

② peak with logFC : Uploaded to JBrowse in BigWig format. (Figure 24)

③ peak with logCPM : Uploaded to JBrowse in BigWig format.

IVAG: an Integrative Visualization Application for various types of Genomic data based on R and Shiny platform

My Genome browser

Rna_seq

Upload Rna_seq data

Browse... No file selected

Type of track :

- peak
- peak with logFC
- peak with logCPM

Unit of analysis :

- gene
- transcript

Option :

- with cutoff

Upload Data to :

Arabidopsis

track name

DEG_RESULT

Choose directory

Arabidopsis

gtf or gff

Arabidopsis_thaliana.TAIR10.30.gff3

Upload data

Refresh input file & list

<Figure 25. RNAseq&GWAS - RNAseq >

Ⓐ: Upload DEG analysis result table.

Ⓑ: Specify track type.

Ⓒ: Specify analysis level(gene, transcript).

Ⓓ: Can specify FDR cutoff for DEG.

Ⓔ: Select genome browser to be uploaded.

Ⓕ: Set track name.

Ⓖ: Genome browser needs gene positions in order to process DEG list. Therefore GTF file needs to be specified.

Ⓖ: Create RNA-Seq track.

<3.10 RNA-Seq & GWAS - GWAS >

<Figure 26. JBrowse GWAS track >

JBrowse GWAS track. X axis shows Genome coordinate and t axis shows $-\log(p\text{-value})$. Each dot represents SNP. Double click the dot to see the ID and coordinate.

<Figure 27. JBrowse GWAS with LD track >

Uploading GWAS data with LD data will produce default GWAS track (Figure 27). Dot represents SNP. Double clicking the dot will show R^2 value between other dots (Figure 27). Double clicked dot will be shown as pink diamond.

A screenshot of the GWAS upload interface. The interface has a blue header bar with the text 'GWAS'. Below the header, there are several input fields and buttons. A red box labeled 'A' highlights the 'Upload GWAS data' section, which includes a 'Browse...' button and a 'No file selected' message. A red box labeled 'B' highlights the 'with LD data' checkbox. A red box labeled 'C' highlights the 'Upload Data to:' dropdown menu, which is currently set to 'Arabidopsis'. A red box labeled 'D' highlights the 'track name' input field, which is currently set to 'GWAS_RESULT'. A red box labeled 'E' highlights the 'max Height (minimum p value)' input field, which is currently set to '1e-20'. A red box labeled 'F' highlights the 'Upload data' button. There is also a 'Refresh input file & list' button.

<Figure 28. RNAseq&GWAS - GWAS >

- Ⓐ: Upload IVAG GWAS analysis file.
- Ⓑ: LD analysis file can be uploaded as well.
- Ⓒ: Select genome browser to be uploaded.
- Ⓓ: Set track name.
- Ⓔ: JBrowse GWAS track's y axis represents $-\log(p\text{-value})$. Specify the maximum value for y axis.
- Ⓕ: Upload GWAS track.

LD

Upload LD data

Browse... No file selected Ⓐ

Upload Data to :

Arabidopsis GWAS track GFF Ⓑ

Upload data Ⓒ Refresh input file & list

<Figure 29. RNAseq&GWAS – LD >

- Ⓐ: Upload IVAG LD analysis result file.
- Ⓑ: Select GWAS track to be uploaded.
- Ⓒ: Click Upload data to add LD information to the specified GWAS track.