IDEA: a web server for Interactive Differential Expression Analysis with R Packages

Qi Zhao^{1,†}, Yubin Xie^{1,†}, Peng Nie^{1,†}, Rucheng Diao¹, Lichen Sun¹, Zhixiang Zuo^{1,*},
Jian Ren^{1,*}

¹State Key Laboratory of Oncology in South China, Cancer Center, Collaborative Innovation Center for Cancer Medicine, School of Life Sciences, Sun Yat-sen University, China

*To whom correspondence should be addressed.

[†]The authors wish it to be known that, in their opinion, those authors contributed equally to this work.

Abstract

Differential expression (DE) analysis is a fundamental task in the downstream analysis of the next-generation sequencing (NGS) data. Up to now, a number of R packages have been developed for detecting differentially expressed genes. Although R language has an interaction-oriented programming design, for many biology researchers, a lack of basic programming skills has greatly hindered the application of these R packages. To address this issue, we developed the Interactive Differential Expression Analyzer (IDEA), a Shiny-based web application integrating the differential expression analysis related R packages into a graphical user interface (GUI), allowing users to run the analysis without writing any new code. A wide variety of charts and tables are generated to facilitate the interpretation of the results. In addition, IDEA also provides a combined analysis framework which helps to reconcile any discrepancy from different computational methods. As a public data analysis server, IDEA is implemented in HTML, CSS and JavaScript, and is freely available at <http://idea.renlab.org>.

Keywords: differential expression analysis, interactively analysis, integrated analysis.

Introduction

High-throughput sequencing technology is rapidly becoming the standard method for measuring gene expression at the transcriptional level. One of the major goals of such work is to identify differentially expressed (DE) genes under two or more conditions. A number of computational tools, such as DESeq2 [1], edgeR [2], NOISeq [3], PoissonSeq [4], SAMseq [5] and Cuffdiff [6] have been developed for the analysis of differential gene expression from RNA-seq data. Most of these tools are implemented in R language, which is commonly used for the analysis of high-dimensional expression data. However, a fairly high level of programming skill is required when applying these R tools to screen out differentially expressed genes, which greatly hinders the application of these tools since many biology researchers have little programming experience. In recent year, a number of software, such as easyRNASeq[7], RNASeqGUI[8] and RobiNA[9] et al, was developed to address this issue. Although the above tools have provided convenient GUI-based computer platforms for back-end R packages to some extent, none of them implements an interactive interface that can not only facilitate the understanding of analysis process for a web-bench scientist, but also inconvenient to adjust the analytical parameters, even for advanced users. Moreover, since different packages will generate inconsistent results, it is difficult for the users to decide which DE algorithm to use. Thus, an interactive platform that can combine these tools together is necessary for obtaining more solid analysis results.

In this regards, we present the Interactive Differential Expression Analyzer (IDEA), a Shiny-based web application dedicated to the identification of differential expression genes in an interactive way. IDEA was built as a user-friendly and highly interactive utility using the Shiny package in R. Besides, five relevant R packages are integrated into IDEA. IDEA is capable of visualizing the results with plenty of charts and tables, as well as providing great ease of interaction during the analysis

55 **Features and options**

56 *Framework*

57 IDEA provides a well-designed data analysis framework to help identify reliable differential
 58 expressed features (Figure 1, 2A). Briefly, three kinds of experimental comparison between
 59 groups can be performed using IDEA, namely “standard comparison”, “multifactor design” and
 60 “comparison without replicates”. To have a quick start, IDEA requires users to specify the
 61 experimental design and can automatically determine available packages for further analysis.
 62 For input dataset, a read count matrix and a design matrix which presents the relationship
 63 between samples and conditions were mandatory for an independent analysis. Users were
 64 encouraged to load feature length file to obtain length-normalized expression values, such as
 65 (Reads Per Kilobase Million) RPKM and (Fragments Per Kilobase Million) FPKM. After that,
 66 data exploration panel was introduced to help assessing the data quality and normalizing the
 67 reads count dataset for further analysis. Differential expression analysis can be sequentially
 68 selected for running separately or not. As different algorithms may generate inconsistent
 69 differential expression feature list, we further developed a combine analysis module in IDEA to
 70 integrate result from each selected analysis packages. Additionally, a build-in example data
 71 that enabled users to run demo analysis and download demo report was established.

72

73 *Data exploration*

74 Data quality assessment and quality control are essential steps for expression analysis.
 75 Beyond the reads base quality and mapping quality evaluation in raw data preprocessing
 76 stage, IDEA implements a quality control panel based on reads count distribution in terms of
 77 genes and samples. In this panel, we provide several normalized methods including TPM,
 78 upper quartile (Default), RPKM and “none” to help exploring potential calculating bias from
 79 different normalization approaches. We also provide three visualization strategies: *density*
 80 *area plot*, *bar plot* and *box plot* to help assessing the data quality (Figure 2B). These plots
 81 illustrate the feature expression distribution in each sample, allowing users to identify samples
 82 with extremely low or high expressed gene number.

83 Expression-based principal component analysis (PCA) and correlation analysis were provided
 84 for studying the sample relationship. *Scatter plot* with sample point and *heat map* presenting
 85 spearman correlation coefficients were generated respectively. Specifically, if the samples
 86 come from the same condition, we expect to observe a closer sample distance in the scatter
 87 plot, and to find the tested samples under the resembling branch within clustered heat map.
 88 Generally, biological or technical replicates exhibit higher correlation than samples from
 89 different sources, thus an extra correlation scatter plot containing detail relationships between
 90 couple of samples was included. It gives a further investigation of correlation analysis result
 91 from clustered heat map.
 92 It's also crucial for biologist to verify the known or some housekeeping genes in the experiment.
 93 By setting negative and positive control in a "wet" experiment, features with known expression
 94 patterns can be applied for assessing the quality of sequencing library, such as low expression
 95 of a gene in knockout library against wild type. IDEA, therefore, provides a *bar plot* interface to
 96 help users query the average abundance of a specified gene in each condition, while some
 97 internal control features can be checked with the help of this function.

98

99 *Multiple DE methods integration*

100 To allow users with little programming experience to easily perform DE analysis using the
 101 most commonly used R packages, the current implementation of IDEA has integrated DESeq2,
 102 edgeR, NOISeq, PossionSeq and SAMseq into a convenient web-based GUI. To avoid
 103 tedious operations, parameters of each method were optimized to fit most cases, and only few
 104 ones were required be adjusted in special scenario. When the analysis is finished, important
 105 information involved in each method was also presented, including model parameters
 106 estimation, normalized factor and statistic descriptions. Plots, like *MA plot*, *volcano plot* (Figure
 107 2C), clustered *heat map* and *FDR/P-value distribution bar plot*, are presented to summarize
 108 the identified DE genes.
 109 Different packages may generate discrepant results on the same dataset. To reconcile any
 110 such discrepancy, we developed a combination analysis module to integrate the results from

the different packages in an unbiased manner. Using a set of rank aggregation methods [10], such as Robust Rank Aggregation and Stuart P-value Integration, the integration score of a given DE feature is calculated as the mean of the integrated rank numbers generated by each of the packages. To visualize the combined results, a *bar plot* showing the DE features identified by each packages (Figure 2D) and a Venn diagram demonstrating the distribution and reliability of the verified features (Figure 2E) were developed. Finally, the combined result was also presented in an interactive table to help exploring the most confidential DE features (Figure 2F).

119

120 *Interactive analysis and fancy analysis report*

Interactive features involved in IDEA consist of dynamic data operation, parameter adjustment and real-time plot rendering. First, dynamic data table enables interactively filtering, sorting and querying genes from input and out data frames by sorts of attributes (Figure 3A). Second, parameter adjustment enables users to refine analysis with modified parameters in real time without rerun the irrelevant step. For example, in DEseq analysis section, modifying DE gene filtering criterion only require to trigger transformation of the result table, and the DEseq will not be executed again. Similarly, in combine analysis, modifying the parameters of one specific tool do not affect others. This may greatly reduce the reanalysis time for exploring analysis, and especially suitable for screening gene lists that are stable under different parameters. For interactive plot rendering, we added some switchable panels in plot frame for refining plot setting in real time. Samples, colors, comparisons and plot data can be dynamically adjusted as well (Figure 3B, 3C). Those options can just trigger re-execution of the plot function while leave the computing function unchanged. Plots can be interactive repainted while the corresponding parameters altered.

Using R markdown and knitr package, IDEA summarized analysis results into a well-structured fancy report with all parameters, figures and citations listed. To meet the publication requirement, high quality figures with 300 dpi and result table can be downloaded from the final report, separately.

139

140 **Implementation**

141 IDEA was initially implemented in R programming language [<https://www.r-project.org/>] with
 142 shiny [<http://shiny.rstudio.com/>] for building interactive web applications. It hosted in a Unix-like
 143 system which have both R and shiny server pre-installed. The front-end code was elaborately
 144 reconstructed with JavaScript, CSS and html to optimize the interactive experience of users.
 145 We also implemented an R package version of IDEA that allows users to run analysis locally
 146 under R environment. The package can be freely accessed and installed from
 147 <https://github.com/likelet/IDEA>. Detailed usage information was presented at the 'help' section
 148 of the web page.

149

150 **Future developments**

151 As IDEA was developed under shiny web framework, its web utilities were greatly limited by
 152 shiny server performance. Therefore, to lift the limitation of server resources, we encourage
 153 users to install the local version when performing differential expression analysis. Besides, for
 154 interactive analysis, plots were dynamically rendered when data or parameters have changed,
 155 while a true sense of interactive plot have not yet been implemented due to a possible heavy
 156 browser loading. We will overcome this with reduced data points and customized data
 157 structure in the near future. Popular data visualization JavaScript library like D3
 158 (<https://d3js.org>), plotly (<https://plot.ly>) and Highchart (<https://www.highcharts.com>) will be
 159 integrated as well. For the extension of more functions, extra analysis features, such as gene
 160 annotation, function enrichment and co-expression analysis, will be included into IDEA in the
 161 near future.

162 Nowadays, various third-party tools were developed to perform differential expression analysis
 163 [11], while some of them were not implemented in R. In the current version, IDEA only
 164 selected five most popular and efficient R-based tools in the analysis pipeline. For future
 165 development, packages in low computing efficiency will be integrated in R package version as
 166 an optional analysis module. However, non R-based tools like gfold[12], cufflinks[13] and

Stringtie[14] etc, will not directly supported in our platform. Instead, to cope alternately with this issue, we are going to provide a combine analysis framework based on results generated by those tools.

170

171 **Conclusion**

IDEA is a user-friendly web application for differential expression analysis, which combines the five commonly used DE analysis packages into a highly interactive web-based framework using the Shiny package. The abundant visualization functions in IDEA enable the users to gain both deeper insight into their original data and the ability to interpret the results in a more intuitive way. Furthermore, in contrast to the static image generated by R language, IDEA can produce diagrams in real time, which makes the analytical process more convenient and efficient. Taken together, we introduce IDEA as an effective application that can facilitate and simplify the process of differential expression analysis in high-dimension expression data.

180

181 **Funding**

This work was supported by grants from the National Natural Science Foundation of China [31471252, 31771462, 81772614 and U1611261]; National Key Research and Development Program [2017YFA0106700]; Guangdong Natural Science Foundation [2014TQ01R387 and 2017A030313134]; Science and Technology Program of Guangzhou, China [201604020003 and 201604046001]; and China Postdoctoral Science Foundation [2017M610573, 2017M622864 and 2018T110907].

188

189 **References**

- 190 1. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for
191 RNA-seq data with DESeq2, Genome Biol 2014;15:550.
- 192 2. Zhou X, Lindsay H, Robinson MD. Robustly detecting differential expression in RNA
193 sequencing data using observation weights, Nucleic Acids Res 2014;42:e91.
- 194 3. Tarazona S, Garcia-Alcalde F, Dopazo J et al. Differential expression in RNA-seq: a

- 195 matter of depth, *Genome Res* 2011;21:2213-2223.
- 196 4. Li J, Witten DM, Johnstone IM et al. Normalization, testing, and false discovery rate
197 estimation for RNA-sequencing data, *Biostatistics* 2012;13:523-538.
- 198 5. Li J, Tibshirani R. Finding consistent patterns: a nonparametric approach for identifying
199 differential expression in RNA-Seq data, *Stat Methods Med Res* 2013;22:519-536.
- 200 6. Trapnell C, Hendrickson DG, Sauvageau M et al. Differential analysis of gene regulation
201 at transcript resolution with RNA-seq, *Nat Biotechnol* 2013;31:46-53.
- 202 7. Delhomme N, Padiou I, Furlong EE et al. easyRNASeq: a bioconductor package for
203 processing RNA-Seq data, *Bioinformatics* 2012;28:2532-2533.
- 204 8. Russo F, Angelini C. RNASeqGUI: a GUI for analysing RNA-Seq data, *Bioinformatics*
205 2014;30:2514-2516.
- 206 9. Lohse M, Bolger AM, Nagel A et al. RobiNA: a user-friendly, integrated software solution
207 for RNA-Seq-based transcriptomics, *Nucleic Acids Res* 2012;40:W622-627.
- 208 10. Kolde R, Laur S, Adler P et al. Robust rank aggregation for gene list integration and
209 meta-analysis, *Bioinformatics* 2012;28:573-580.
- 210 11. Conesa A, Madrigal P, Tarazona S et al. A survey of best practices for RNA-seq data
211 analysis, *Genome Biol* 2016;17:13.
- 212 12. Feng J, Meyer CA, Wang Q et al. GFOLD: a generalized fold change for ranking
213 differentially expressed genes from RNA-seq data, *Bioinformatics* 2012;28:2782-2788.
- 214 13. Trapnell C, Roberts A, Goff L et al. Differential gene and transcript expression analysis of
215 RNA-seq experiments with TopHat and Cufflinks, *Nat Protoc* 2012;7:562-578.
- 216 14. Pertea M, Kim D, Pertea GM et al. Transcript-level expression analysis of RNA-seq
217 experiments with HISAT, StringTie and Ballgown, *Nat Protoc* 2016;11:1650-1667.

218

219

220 **Figure legends**

221

222 **Figure 1.** Differential expression analysis framework implemented in IDEA.

223 **Figure 2.** Selected analysis plots generated by IDEA. A, screen shot of IDEA web page. B, a
 224 box plot presents the normalized reads count distribution among samples. C, Differential
 225 expression analysis result was shown in a volcano plot; each point represents a feature for
 226 statistic test, the green points are significantly differential expressed with adjusted P value <
 227 0.05. D, Overlap analysis based on the DE result from five analysis packages. E, overview of
 228 differential expressed feature count by each package shown in a bar plot. F. combine analysis
 229 result data frame, only top 50 features were displayed as default.

230 **Figure 3.** Interactive features provided in IDEA. A. dynamic data table JavaScript library was
 231 applied to help interactively exploring features stored in data frame. B. The floating control
 232 panel that enables modifying parameters in real-time.

233

234 **Figure 1**

235

236

237 **Figure 2**

A

B

C

D

E

F

Feature Weight Table

Feature	DESeq	edgeR	NOIseq	PoissonSeq	SAMseq	Weight	Rank
1	1	1	1	1	1	1	1
2	1	1	1	1	1	1	2
3	1	1	1	1	1	1	3
4	1	1	1	1	1	1	4
5	1	1	1	1	1	1	5
6	1	1	1	1	1	1	6
7	1	1	1	1	1	1	7
8	1	1	1	1	1	1	8
9	1	1	1	1	1	1	9
10	1	1	1	1	1	1	10
11	1	1	1	1	1	1	11
12	1	1	1	1	1	1	12
13	1	1	1	1	1	1	13
14	1	1	1	1	1	1	14
15	1	1	1	1	1	1	15
16	1	1	1	1	1	1	16
17	1	1	1	1	1	1	17
18	1	1	1	1	1	1	18
19	1	1	1	1	1	1	19
20	1	1	1	1	1	1	20
21	1	1	1	1	1	1	21
22	1	1	1	1	1	1	22
23	1	1	1	1	1	1	23
24	1	1	1	1	1	1	24
25	1	1	1	1	1	1	25
26	1	1	1	1	1	1	26
27	1	1	1	1	1	1	27
28	1	1	1	1	1	1	28
29	1	1	1	1	1	1	29
30	1	1	1	1	1	1	30
31	1	1	1	1	1	1	31
32	1	1	1	1	1	1	32
33	1	1	1	1	1	1	33
34	1	1	1	1	1	1	34
35	1	1	1	1	1	1	35
36	1	1	1	1	1	1	36
37	1	1	1	1	1	1	37
38	1	1	1	1	1	1	38
39	1	1	1	1	1	1	39
40	1	1	1	1	1	1	40
41	1	1	1	1	1	1	41
42	1	1	1	1	1	1	42
43	1	1	1	1	1	1	43
44	1	1	1	1	1	1	44
45	1	1	1	1	1	1	45
46	1	1	1	1	1	1	46
47	1	1	1	1	1	1	47
48	1	1	1	1	1	1	48
49	1	1	1	1	1	1	49
50	1	1	1	1	1	1	50
51	1	1	1	1	1	1	51
52	1	1	1	1	1	1	52
53	1	1	1	1	1	1	53
54	1	1	1	1	1	1	54
55	1	1	1	1	1	1	55
56	1	1	1	1	1	1	56
57	1	1	1	1	1	1	57
58	1	1	1	1	1	1	58
59	1	1	1	1	1	1	59
60	1	1	1	1	1	1	60
61	1	1	1	1	1	1	61
62	1	1	1	1	1	1	62
63	1	1	1	1	1	1	63
64	1	1	1	1	1	1	64
65	1	1	1	1	1	1	65
66	1	1	1	1	1	1	66
67	1	1	1	1	1	1	67
68	1	1	1	1	1	1	68
69	1	1	1	1	1	1	69
70	1	1	1	1	1	1	70
71	1	1	1	1	1	1	71
72	1	1	1	1	1	1	72
73	1	1	1	1	1	1	73
74	1	1	1	1	1	1	74
75	1	1	1	1	1	1	75
76	1	1	1	1	1	1	76
77	1	1	1	1	1	1	77
78	1	1	1	1	1	1	78
79	1	1	1	1	1	1	79
80	1	1	1	1	1	1	80
81	1	1	1	1	1	1	81
82	1	1	1	1	1	1	82
83	1	1	1	1	1	1	83
84	1	1	1	1	1	1	84
85	1	1	1	1	1	1	85
86	1	1	1	1	1	1	86
87	1	1	1	1	1	1	87
88	1	1	1	1	1	1	88
89	1	1	1	1	1	1	89
90	1	1	1	1	1	1	90
91	1	1	1	1	1	1	91
92	1	1	1	1	1	1	92
93	1	1	1	1	1	1	93
94	1	1	1	1	1	1	94
95	1	1	1	1	1	1	95
96	1	1	1	1	1	1	96
97	1	1	1	1	1	1	97
98	1	1	1	1	1	1	98
99	1	1	1	1	1	1	99
100	1	1	1	1	1	1	100

238

239

240 **Figure 3**

241

242