FUNKI: Interactive functional footprint-based analysis of omics data

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Abstract

Motivation: Omics data, such as transcriptomics or phosphoproteomics, are broadly used to get a snap-shot of the molecular status of cells. In particular, changes in omics can be used to estimate the activity of pathways, transcription factors and kinases based on known regulated targets, that we call footprints. Then the molecular paths driving these activities can be estimated using causal reasoning on large signaling networks.

Results: We have developed FUNKI, a FUNctional toolKit for footprint analysis. It provides a user-friendly interface for an easy and fast analysis of several omics data, either from bulk or single-cell experiments. FUNKI also features different options to visualise the results and run post-analyses, and is mirrored as a scripted version in R.

Availability: FUNKI is a free and open-source application built on R and Shiny, available in GitHub at https://github.com/saezlab/ShinyFUNKI under GNU v3.0 license and accessible also in https://saezlab.shinyapps.io/funki/

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Supplementary information: We provide data examples within the app, as well as extensive information about the different variables to select, the results, and the different plots in the help page.

1. Introduction

Multiple methods are conceived to infer the activities of specific processes or molecules using the abundance of known targets from omic data (see Supplementary Table for a list of them). We call them footprint-based methods (Dugourd and Saez-Rodriguez, 2019), and we have develop such tools for transcription factor from transcripts of target genes (Garcia-Alonso et al., 2019), kinases from phosphorylated sites (Wirbel et al., 2018), and pathways from downstream responsive genes (Schubert et al., 2018). These activities can then be used to contextualize large signaling networks by identifying paths that can explain the changes in activities via reverse causal reasoning (Liu et al., 2019; Dugourd et al., 2021).

FUNKI (FUNctional analysis toolKit) is an user-friendly interface developed in R (Team, 2020), and designed using Shiny (Chang et al., 2020), to analyze omics data using footprint methods. This application provides an interface for the R implementations (Bioconductor packages) for the aforementioned tools. All methods run on bulk data for human samples, and we have shown that they can also be applied to single-cell transcriptomics (Holland, Tanevski, et al., 2020) and mouse (Holland, Szalai, et al., 2020).
Figure 1. Graphical overview of analysis and visualisation features provided by FUNKI.
FUNKI provides an user interface to upload omics data, and then run DoRothEA, PROGENy, KinAct, CARNIVAL and COSMOS to estimate the activity of pathways, transcription factors, and kinases. The results are visualized in diverse forms.

2. Features

The footprint methods implemented in FUNKI allow users to recover functional insight from several omics data without notions of programming. This application also enhances the analysis with an extended graphic visualisation of the results. Thus, the typical FUNKI pipeline comprises three steps: (i) import user’s omic data, (ii) select the analysis accordingly (DoRothEA, PROGENy, CARNIVAL, COSMOS or KinAct), and (iii) visualise the results in tables and graphical representations (Fig. 1).

2.1 DoRothEA

DoRothEA (Discriminant Regulon Expression Analysis) is a resource that links transcription factors (TFs) with their downstream targets. TFs activities are computed from gene expression where the regulons (the collection of transcriptional targets for each TF) are the underlying gene-sets.
2.2 PROGENy

PROGENy (Pathway RespOnsive GENes) is a footprint method developed to infer pathway activity from gene expression data. The scores are calculated using a linear model with weights based on consensus gene signatures obtained from publicly available perturbation experiments.

2.3 KinAct

KinAct is a resource linking kinases to phosphorylation sites. Kinase activity estimation is performed using the same algorithm as DoRothEA to estimate activity scores. Instead of TF-target interactions, KinAct uses collections of kinase-substrate interactions via OmniPath (Türei et al., 2016) and phosphoproteomic data instead of transcriptomic data.

2.4 CARNIVAL and COSMOS

CARNIVAL (CAusal Reasoning for Network identification using Integer VALue programming) reconstructs signalling networks from downstream TF activities by finding the upstream regulators. COSMOS is an extension of CARNIVAL that provides a multi-omic network to connect different types of omic data together, including transcriptomics, metabolomics, and phosphoproteomics. Both methods identify coherent mechanistic hypotheses (subnetworks) that explain how the measured deregulation may be reached.

3. Implementation

FUNKI is a shiny application developed using R programming language under version 4.0.2 and upgraded to run for 4.1.1 (Team, 2020; Chang et al., 2020). It is directly accessible in the cloud through https://saezlab.shinyapps.io/funki/. The source code is freely available at https://github.com/saezlab/ShinyFUNKI, and it can be run locally in any platform (Windows, macOS and Linux) either downloading the repository or running it directly from GitHub (see https://saezlab.github.io/ShinyFUNKI/ for details).

4. Conclusion

FUNKI provides an intuitive user-friendly interface to run footprint methods from different omics. Together with the analysis implementation, FUNKI also incorporates several graphical representations to explore the results from different perspectives. Users with programming skills can take advantage of an extended script-based version of FUNKI for transcriptomic data (https://github.com/saezlab/transcriptutorial).
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6. Conflict of interests

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**Supplementary table 1**

| Name       | Activity type | Available interface | Reference                                      |
|------------|---------------|---------------------|------------------------------------------------|
| Viper      | TF/Kinase     | No                  | (Alvarez et al., 2016)                         |
| RoKAI      | Kinase        | No                  | (Yılmaz et al., 2021)                         |
| KARP       | Kinase        | No                  | (Wilkes et al., 2017)                         |
| KSEA       | Kinase        | No                  | (Hernandez-Armenta et al., 2017)              |
| KSTAR      | Kinase        | No                  | (Crowl et al., 2021)                         |
| INKA       | Kinase        | No                  | (Beekhof et al., 2019)                        |
| KEA3       | Kinase        | Yes                 | (Kuleshov et al., 2021)                       |
| BART       | TF            | No                  | (Wang et al., 2018)                           |
| TFEA.ChiP  | TF            | Yes                 | (Puente-Santamaria et al., 2019)              |
| oPOSSUM    | TF            | Yes                 | (Kwon et al., 2012)                           |
| CHEA3      | TF            | Yes                 | (Keenan et al., 2019)                         |
| MAGICACT   | TF            | No                  | (Roopra, 2018)                                |
| SPEED      | Pathway       | Yes                 | (Parikh et al., 2010)                         |
| SPEED2     | Pathway       | Yes                 | (Rydenfelt et al., 2020)                      |

List of other methods to infer the activity of proteins from different omics based on the idea of looking at the target molecules (what we call the footprint-based approach). TF = Transcription Factor.

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