DEACT: An Online Tool for Analysing Complementary RNA-Seq Studies
A Case Study of Knockdown and Upregulated FLI1 in Breast Cancer Cells

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Abstract: Understanding the genetic basis of disease may lead to the development of life-saving diagnostics and therapeutics. RNA-sequencing (RNA-seq) gives a snapshot of cellular processes via high-throughput transcriptome sequencing. Meta-analysis of multiple RNA-Seq experiments has the potential to (a) elucidate gene function under different conditions and (b) compare results in replicate experiments. To simplify such meta-analyses, we created the Dataset Exploration And Curation Tool (DEACT), an interactive, user-friendly web application. DEACT allows users to (1) interactively visualize RNA-Seq data, (2) select genes of interest through the user interface, and (3) download subsets for downstream analyses. We tested DEACT using two complementary RNA-seq studies resulting from knockdown and gain-of-function FLI1 in an aggressive breast cancer cell line. We performed fixed gene-set enrichment analysis on four subsets of genes selected through DEACT. Each subset implicated different metabolic pathways, demonstrating the power of DEACT in driving downstream analysis of complementary RNA-Seq studies.

1 INTRODUCTION

Recent advances in next-generation sequencing have enabled researchers to collect genomic data more quickly and cost-efficiently than ever before. RNA-sequencing (RNA-Seq) utilizes next-generation sequencing to identify and quantify transcripts in a cell. Analyzing transcriptome changes between healthy and abnormal cells, or other contrasting phenotypes, is key in understanding diseases and in developing novel molecular therapies and drugs.

Given a reference genome, one of the first stages of analyzing RNA-Seq data involves aligning and quantifying sequenced reads. Next, differential expression is determined by comparing paired groups (e.g., treatment vs control). Several tools have been developed and are widely used for identifying, quantifying, and assessing differential expression of transcripts from the sequenced reads (Trapnell et al., 2012; Zhou et al., 2014; Robinson et al., 2010; Ritchie et al., 2015; Love et al., 2014).

Typically, the following stage in RNA-Seq analysis is pathway enrichment, identification of pathways based on the differentially expressed transcripts in those pathways. Popular tools for pathway enrichment analysis and visualization include iPathwayGuide, GAGE, and Pathview (Draghici et al., 2007; Luo et al., 2009; Luo and Brouwer, 2013).

The transition between these two stages can be problematic for researchers who want to analyze two or more parallel or independent RNA-Seq experiments. Parallel studies may convey, for example, a spectrum of severity of a cell phenotype, while independent studies may contrast two conditions, such as loss-of-function versus gain-of-function of a gene. In such cases, researchers are interested in a subset of transcripts that stand out comparatively across experiments. Subsets of interesting genes could then be fed to downstream pathway enrichment tools.

Visualizing and creating subsets of genes across experiments is difficult for researchers who are not computationally trained. Biclustering is a popular approach for identifying functional genes that behave a similar way in multiple experimental conditions (Pontes et al., 2015). However, successful use of biclustering algorithms depends on having a amount of large data, which is not always the case in compar-

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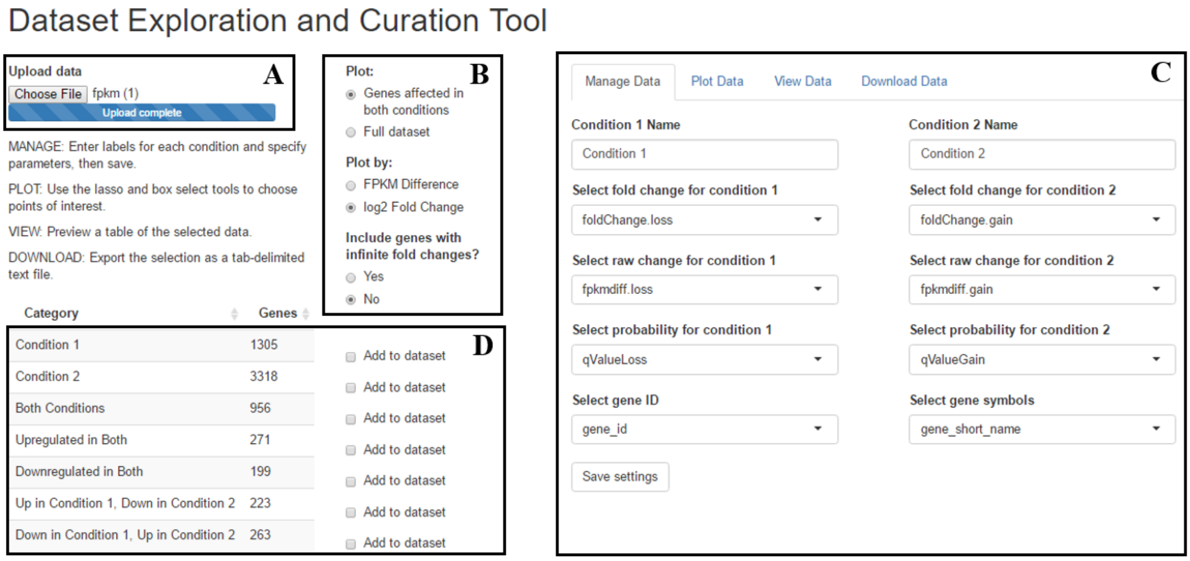

Upload data

fpkm (1)

MANAGE: Enter labels for each condition and specify parameters, then save.

PLOT: Use the lasso and box select tools to choose points of interest.

VIEW: Preview a table of the selected data.

DOWNLOAD: Export the selection as a tab-delimited text file.

Plot:
● Genes affected in both conditions
○ Full dataset

Plot by:
○ FPKM Difference
● log2 Fold Change

Include genes with infinite fold changes?
○ Yes
● No

Category	Genes	
Condition 1	1305	□ Add to dataset
Condition 2	3318	□ Add to dataset
Both Conditions	956	□ Add to dataset
Upregulated in Both	271	□ Add to dataset
Downregulated in Both	199	□ Add to dataset
Up in Condition 1, Down in Condition 2	223	□ Add to dataset
Down in Condition 1, Up in Condition 2	263	□ Add to dataset

Condition 1 Name: Condition 1	Condition 2 Name: Condition 2
Select fold change for condition 1: foldChange.loss	Select fold change for condition 2: foldChange.gain
Select raw change for condition 1: fpkmdiff.loss	Select raw change for condition 2: fpkmdiff.gain
Select probability for condition 1: qValueLoss	Select probability for condition 2: qValueGain
Select gene ID: gene_id	Select gene symbols: gene_short_name

Figure 1: Screenshot of the DEACT web application. DEACTs user interface contains (A) file upload; (B) visualization options; (C) the main panel, in which the user may manage, plot, view, and download the data; and (D) an overview of significant genes in the dataset.

ing only two experimental conditions, or in RNA-Seq analysis in general. Secondly, the lack of an ”optimal” bi-clustering algorithm, as in the case of sequence alignment, is a challenge to experimental biologists who do not have the training to run and compare multiple algorithms.

In order to address this need, we developed a user-friendly web application designed to visualize, compare, select, categorize, and subset differentially expressed transcripts in two complementary experiments. The Dataset Exploration And Curation Tool (DEACT) enables researchers to compare two RNA-Seq studies using a simple interface for managing, viewing, and plotting data. Here we demonstrate its use with a case study of gain-of-function (GOF) and loss-of-function (LOF) of the FLI1 gene, which has been associated with hematological and epithelial cancers (Watson et al., 2010; Scheiber et al., 2014). DEACT allows users to subset data based on gene categories (i.e., whether the gene is significant in both experiments, or has a contrasting expression between two conditions).

Many tools that support multiple studies, such as RNASeqViewer, visualize users data through heat maps, while DEACT allows for more quantitative comparisons. Other applications that utilize scatterplots, like GRACOMICS, do not allow the user to create custom selections or easily export predefined subsets (Rogé and Zhang, 2013; Seo et al., 2015). DEACT offers an interactive data visualization feature which retains quantitative and transcript annotation information. It allows users to quickly and easily compare experimental conditions by visualizing and defining gene subsets that can easily be exported for further analysis.

2 IMPLEMENTATION

2.1 Software

DEACT is an interactive web application implemented using the R shiny package. It uses the R package plotly to create scatterplots (RStudio, Inc, 2014; Plotly Technologies Inc., 2015). DEACT can be used with up to 5 MB of data at https://kduchinski.shinyapps.io/DEACT/. For larger datasets or for deploying customized versions, the source code to use DEACT locally is available at https://github.com/kduchinski/DEACT.

2.2 Workflow

The DEACT workflow consists of three main sections which can be easily navigated using a tab bar in the main panel interface: (a) manage data, (b) view data, and (c) plot data (Figure 1C).

In the MANAGE DATA section, users can upload differential expression data from transcriptome profiling in comma-separated value (csv) or tab-delimited (txt) formats (Figure 1A). Each differential expression observation should have (1) a gene identification number and/or gene symbol, (2) differential ex-

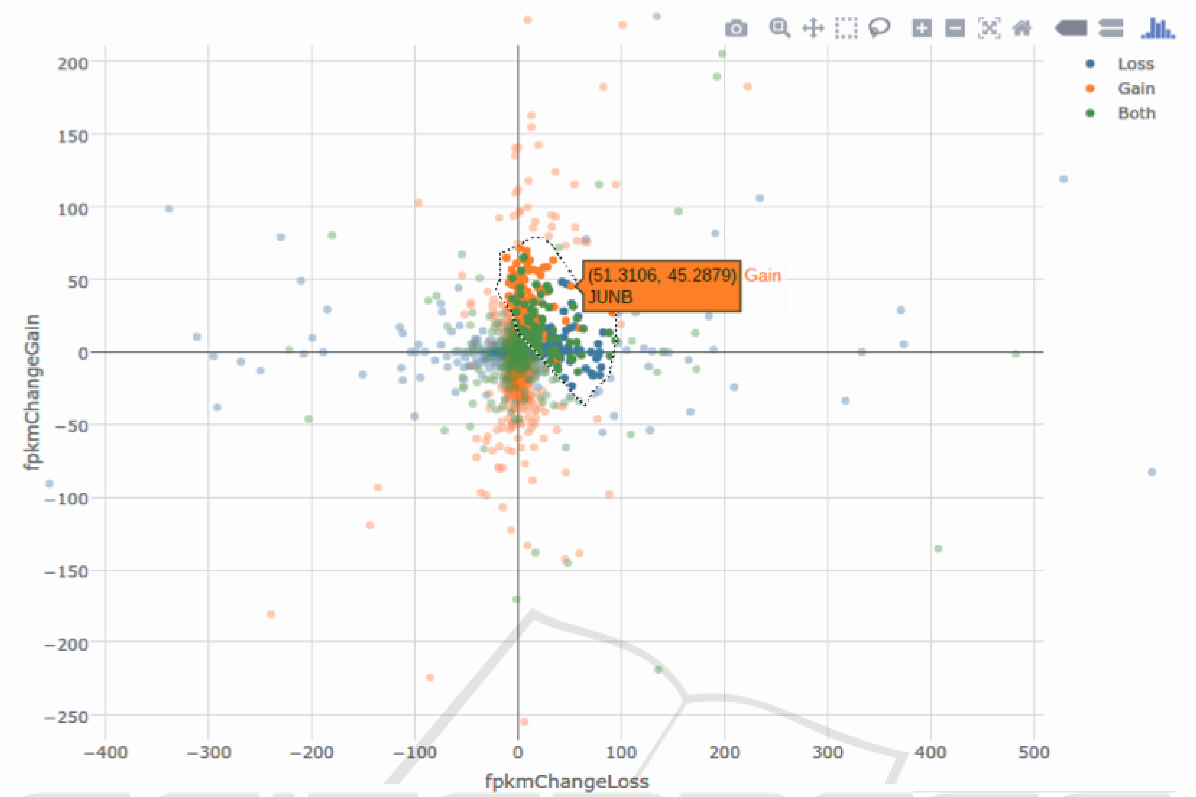

Figure 2: A sample plot generated by DEACT with the Fli1 data. The graph has been zoomed in on the center and an area has been selected through the lasso tool. The hover tag is displayed for one of the points, a gene significantly affected by only the condition named ”Gain” by the user.

pression measured in Fragments Per Kilobase of transcript per Million mapped reads (fpkm) or log fold change, and (3) a measure of significance (i.e. p-value or q-value). Upon uploading data, the fields for these three components must be specified for both experimental conditions using a drop-down selection menu of the data columns (Figure 1C). DEACT automatically subsets transcripts into categories based on their differential expression in the two experimental conditions being studied (Figure 1D). These subsets can be selectively added to the data viewing table. Custom subsets may be added directly from the scatterplot.

The second section, PLOT DATA, plots the entire data set on an interactive scatterplot, which includes zoom, pan, and hover controls (Figure 1). Each significantly differentially expressed gene is plotted by the change in its expression, either in fragments per kilobase per million (fpkm) or by log_2 fold change, which was specified in the MANAGE DATA section. The plot displays the gene symbol/id and differential expression value when the user hovers over a data point (Figure 2). Data can be selected by box (rectangular) or lasso (free shape) selection. All points within the selection will be included in the data viewing table. If plotting by fold change, transcripts with infinite fold changes will not be graphed, but the user may choose to include them in the data viewing table.

After managing data (uploading data, specifying fields, and selecting categories), users can use the VIEW DATA feature which displays select data in a table format with search and sorting options. This feature allows users to preview their data before downloading.

Finally, the user-curated dataset can be downloaded as a tab-delimited text file for further analysis, such as pathway analysis. The user may choose which columns from the dataset to include in the file.

3 USAGE EXAMPLE

DEACT was tested using RNA-Seq data from Friend Leukemia Virus Integration 1 (FLI1) research. Specifically, two independent RNA-Seq studies were analyzed with DEACT: (1) gain-of-function (GOF) and (2) loss-of-function by knockdown (LOF) experiments in MDA MB231, an aggressive human breast cancer cell line. FLI1 belongs to the ETS family of transcription factors, which are associated with key cellular processes dysregulated in cancer cells (Watson, 2010). Although aberrant expression of FLI1 has been observed in hematological cancers, it was recently found to also be dysregulated in breast cancer, an epithelial-derived cancer (Scheiber et al., 2014).

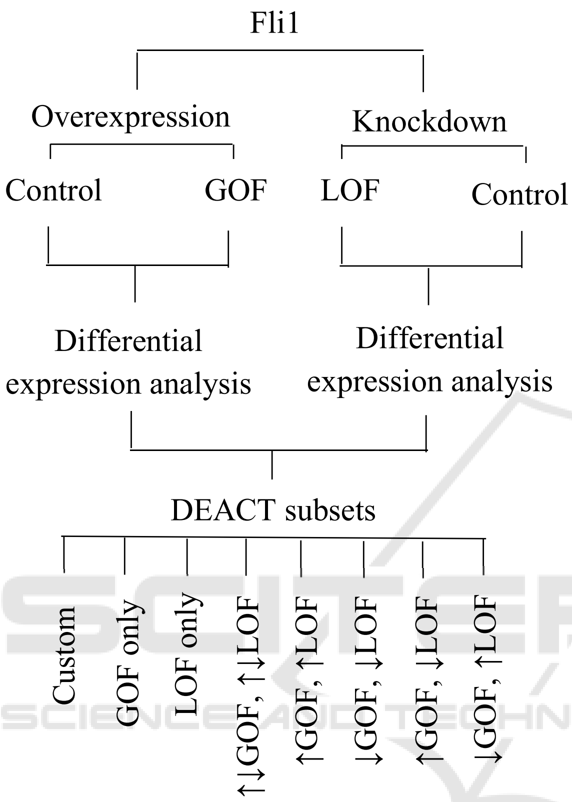

Figure 3: Diagram of the FLI1 loss- (LOF) and gain-of-function (GOF) studies. The quantified differential expression data from both were subsetted by DEACT into categories with possible biological relevance.

An shRNA constructed in lentiviruses was used in the FLI1 LOF experiment, while adenovirus was used in the FLI1 GOF experiment (Figure 3). Three biological replicates each were prepared for the LOF, GOF, and lentival and adenoviral controls. RNA was extracted and sequenced using the Illumina HiSeq2500. Sequenced reads were filtered for quality, processed, mapped, and quantified using the Tuxedo Suite (Bioinformatics, 2011; Bolger and Giorgi, 2014; Trapnell et al., 2012). Probability values were corrected for the false discovery rate. CummeRbund was used to prepare a file for DEACT from CuffDiff data from each experiment.

Table 1: Genes categorized by regulation direction across two FLI1 expression studies. DEACT identified significantly dysregulated transcripts.

Gain-of-Function FLI1	Loss-of-Function	Genes
↑↓		3318
	↑↓	1305
↑↓	↑↓	956
↑	↑	271
↓	↓	199
↓	↑	223
↑	↓	263

DEACT was able to visualize and subset data from both studies. DEACT categorized significant (α = 0.05) genes affected in each FLI1 experiment (Table 1). This information may prompt preliminary biological conclusions from the data. For example, take the 271 genes that were up-regulated and the 199 that were down-regulated in both conditions. From these subsets, it can be concluded that of genes that were affected by both conditions, 470 (49%) were regulated in the same direction. This result is counter-intuitive; GOF and LOF studies are rarely performed for the same gene in part because their results are expected to be non-informative due to an expected contrast. The subsets that show contraregulation between the gain-of-function and the loss-of-function may be of particular interest to researchers because expression of these genes may be dependent upon expression of FLI1. Similarly, pathway analysis of these subsets may show which cellular functions are correlated with FLI1 expression.

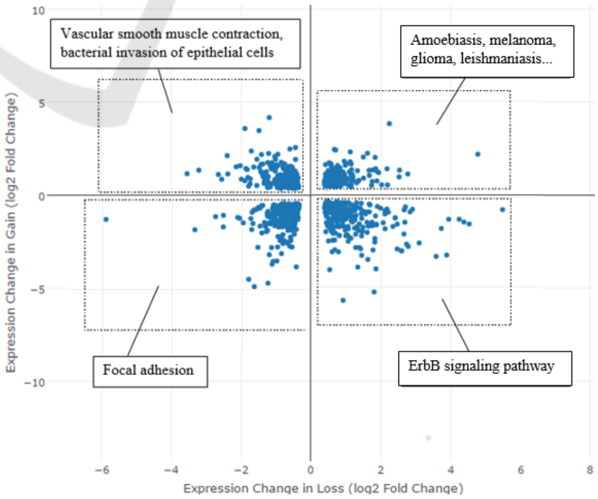

Figure 4: A visual representation of pathway analysis by subset. Small subsets from Table 1 are denoted with significant metabolic pathways related to those genes.

The genes categorized into the last four rows of Table 1 were downloaded as four subsets. These se-

Table 2: Pathway categorized by regulation direction across two FLI1 expression studies.

Gain-of-Function FLI1	Loss-of-Function	Pathways
↑↓		153
	↑↓	64
↑↓	↑↓	69
↑	↑	33
↓	↓	35
↓	↑	35
↑	↓	33

lections represent the possible regulation patterns for genes affected by both conditions. Each subset, as well as the full dataset, were subjected to pathway analysis using iPathwayGuide (Draghici et al., 2007). Each subset is labeled with its significant (α = 0.05) pathways (Figure 4).

The top 100 pathways for genes up-regulated in GOF, down-regulated in GOF, and so on for LOF were identified and similarly categorized in Table 2. These tables demonstrate how subsets of genes may translate into subsets of pathways. For example, of the original 400 pathways, 33 (8.25%) involve genes up-regulated in GOF and down-regulated in LOF. Closer investigation is necessary to determine if each pathway as a whole was up- or down-regulated. Pathways that are up-regulated in one condition and down-regulated by the other are often of particular interest, as these may indicate which cellular processes are directly correlated with a condition.

4 DISCUSSION

DEACT's interactive user interface for rapid visualization and categorization of expression data is intuitive for researchers with little or no programming experience. It supports any two complementary studies and can compare biological replicates or contrasting experimental conditions. DEACT automatically categorizes significant data points by their response to each condition. These practically relevant categories may be downloaded as subsets for further study, for example, in a file format accepted by iPathwayGuide and other programs. Alternatively, unique subsets can be selected directly from the user interface. The responsive user interface allows users to instantly identify and select any set of genes, thus achieving a level of engagement that neither scripts nor traditional plots offer.

The simple, interactive design makes DEACT an effective collaboration tool for research laboratories. Unlike biclustering, DEACT can be effectively used to interpret small datasets and does not require the additional time or training required to optimize a biclustering algorithm. Instead, it is built to quickly answer preliminary questions about new RNA-seq data to prompt downstream analyses and encourage a flow of discussion. With DEACT, researchers may easily create highly customizable datasets to fit any question, however specific. In the future, pathway analysis may be integrated with DEACT in order to visualize cellular responses on the pathway level as well as the gene level. This feature may be incorporated into the user interface, as shown in Figure 3.

DEACT adds to a growing set of meta-analytical tools for RNA-Seq data. With a tool like DEACT, non-computationally trained researchers can mine their data for novel insights on gene expression and function. Such meta-analyses not only augment our understanding of cellular processes, but they have the potential to lead to novel life-saving therapeutics.

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