# Visualization methods for RNA-sequencing data

Summary:    This is the summary for this paper.

Key words:    Data visualization; Exploratory data analysis; Interactive graphics; RNA-sequencing; Statistical graphics

## 1. Introduction

RNA-sequencing (RNA-seq) uses next-generation sequencing (NGS) to estimate the quantity of RNA in biological samples at given timepoints. In recent years, decreasing cost and increasing throughput has rendered RNA-seq an attractive alternative to transcriptome profiling. Prior to RNA-seq, gene expression studies were performed with microarray techniques, which required prior knowledge of reference sequences. RNA-seq does not have this limitation, and has enabled a new range of applications such as transcriptome de novo assembly (Grabherr et al., 2011; Robertson et al., 2010) and detection of alternative splicing processes (Anders, Reyes, and Huber, 2012; Pan et al., 2008). Coupled with its high resolution and sensitivity, RNA-seq will likely revolutionize our understanding of the intricacies of eukaryotic transcriptomes (Wang, Gerstein, and Snyder, 2009; Zhao et al., 2014).

Gene expression data is multivariate data, and its basic form is a matrix containing mapped read counts for $n$ rows of genes and $p$ columns of samples. These mapped read counts provide estimations of the gene expression levels across samples. Researchers typically conduct RNA-seq studies to identify differentially expressed genes (DEGs) between treatment groups. In most popular RNA-seq analysis packages, this objective is approached with models, such as the negative binomial model (Anders and Huber, 2010; Trapnell et al., 2013; Trapnell et al., 2012; Robinson et al., 2010) and linear regression models (Law et al., 2014).

Initially, it was widely claimed that RNA-seq produced unbiased data that did not require sophisticated normalization (Wang et al., 2009; Morin et al., 2008; Marioni et al., 2008). However, numerous studies have since revealed that RNA-seq data is replete with biases and that accurate detection of DEGs is not a negligible task. Biases that complicate the analysis of RNA-seq data include nucleotide-specific and read-position specific biases (Hansen et al., 2010), biases related to the lengths of genes and sequencing depths (Oshlack, Robinson, and Young, 2010; Robinson and Oshlack, 2010), biases related to the number of replica-

tions (Schurch et al., 2016), biases introduced during library preparation (McIntyre et al., 2011), biases related to alternative gene isoforms and overlapping senseantisense transcripts (Trapnell et al., 2013), and confounding issues arising from the combination of technical and biological biases (Bullard et al., 2010).

## 2. Model

### 2.1 *First Model Subsection*

The Cox model (Cox, 1972) is one of the most widely used statistical models. Hastie, Tibshirani, and Friedman (2001) is an example of a citation to a work with three authors. The first time you reference one of these in the text, use all the authors names. However, in all subsequent references, just use Hastie et al. (2001). Works with four or more authors are always referenced in the text using "et al." All authors names should appear in the bibliography for all entries.

### 2.2 *Second Model Subsection*

Please use a recent issue of *Biometrics* as a guide to the style for citations and bibliography entries, and follow that style exactly!!

## 3. Inference

Please see the file `biomsample.tex` for fancy examples of making tables. Here is a very simple one. Use `table` for tables that are narrow enough to fit in one column of the typeset journl; use `table*` for tables that need to span two columns. For figures, use of `figure` and `figure*` is analogous.

[Table 1 about here.]

You can experiment with fancier tables than Table 1.

We can get bold symbols using \bmath, for example, $\boldsymbol{\alpha}_i$.

## 4. Discussion

Put your final comments here.

SUPPLEMENTARY MATERIALS

Web Appendix A, referenced in Section 2, is available with this paper at the Biometrics website on Wiley Online Library.

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### Appendix

### *Title of appendix*

Put your short appendix here. Remember, longer appendices are possible when presented as Supplementary Web Material. Please review and follow the journal policy for this material, available under Instructions for Authors at `http://www.biometrics.tibs.org`.

**Table 1**
*This is a simple table.*

| Culture/Library prep. | Library prep. protocol | Growth condition | Flow-cell |
|---|---|---|---|
| Y1 | Protocol 1 | YPD | 428R1 |
| Y1 | Protocol 1 | YPD | 4328B |
| Y2 | Protocol 1 | YPD | 428R1 |
| Y2 | Protocol 1 | YPD | 4328B |
| Y7 | Protocol 1 | YPD | 428R1 |
| Y7 | Protocol 1 | YPD | 4328B |
| Y4 | Protocol 2 | YPD | 61MKN |
| Y4 | Protocol 2 | YPD | 61MKN |