

Gene expression

Riborex: fast and flexible identification of differential translation from Ribo-seq data

Wenzheng Li¹, Weili Wang¹, Philip J. Uren¹, Luiz O. F. Penalva^{2,3} and Andrew D. Smith^{1,*}

¹Molecular and Computational Biology, Division of Biological Sciences, University of Southern California, Los Angeles, CA, USA, ²Department of Cellular and Structural Biology and ³Children's Cancer Research Institute, University of Texas Health Science Center at San Antonio, San Antonio, TX, USA

*To whom correspondence should be addressed.

Associate Editor: Bonnie Berger

Received on July 21, 2016; revised on January 21, 2017; editorial decision on January 23, 2017; accepted on January 26, 2017

Abstract

Motivation: Global analysis of translation regulation has recently been enabled by the development of Ribosome Profiling, or Ribo-seq, technology. This approach provides maps of ribosome activity for each expressed gene in a given biological sample. Measurements of translation efficiency are generated when Ribo-seq data is analyzed in combination with matched RNA-seq gene expression profiles. Existing computational methods for identifying genes with differential translation across samples are based on sound principles, but require users to choose between accuracy and speed.

Results: We present Riborex, a computational tool for mapping genome-wide differences in translation efficiency. Riborex shares a similar mathematical structure with existing methods, but has a simplified implementation. Riborex directly leverages established RNA-seq analysis frameworks for all parameter estimation, providing users with a choice among robust engines for these computations. The result is a method that is dramatically faster than available methods without sacrificing accuracy.

Availability and Implementation: <https://github.com/smithlabcode/riborex>

Contact: andrewds@usc.edu

Supplementary information: [Supplementary data](#) are available at *Bioinformatics* online.

1 Introduction

Regulation of translation has been shown to play an essential role in many biological processes, and misregulation of translation is associated with disease states (Bazzini *et al.*, 2012; Lu *et al.*, 2007). The recent development of Ribosome Profiling technology, also called Ribo-seq, now enables accurate global analysis of translation (Ingolia *et al.*, 2009). Ribo-seq involves deep sequencing of 'ribosome-protected fragments' (RPFs) which are presumed to exist in proportion to the fraction of total translation that is associated with a given gene. RPFs can also provide information on the modes by which cells regulate translation, and to date Ribo-seq has been applied in a broad variety of biological contexts (Ingolia, 2016).

Often the goal of a Ribo-seq experiment is to generate genome-wide maps of translation efficiency. The number of RPFs associated with a given gene or transcript is determined both by gene-specific translation activity and mRNA abundance. Without accounting for background mRNA levels, one cannot distinguish differences associated with translation from those arising via regulation of other process (*e.g.* transcription). Ribo-seq data is therefore usually accompanied by RNA-seq conducted in a matched biological sample. The key analysis problem then becomes identifying genes whose difference in RPF abundance cannot be explained by differences in background mRNA abundance. Several methods were developed for this purpose, including anota (Larsson *et al.*, 2011), Babel (Olshen

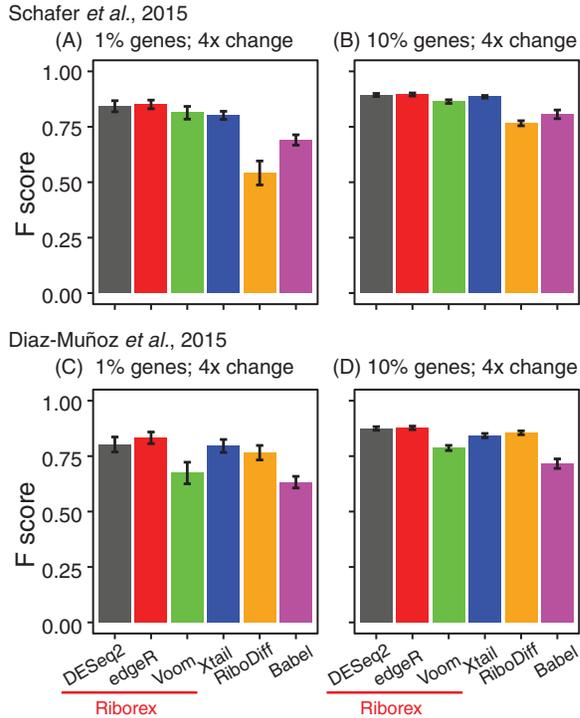


Fig. 1. Accuracy identifying differentially translated genes in simulated data (see supp. info.) comparing Riborex (using DESeq2, edgeR and Voom (Law et al., 2014)), Xtail, RiboDiff and Babel. Values are averages of 100 simulations and error bars indicate standard deviation of F scores. (A) and (B) Results based on rat heart data (Schafer et al., 2015) with 1% and 10% implanted true differentially translated genes, respectively, and simulated 4-fold change in translation efficiency. (C) and (D) Results from simulations based on mouse HuR data (Diaz-Muñoz et al., 2015)

et al., 2013), RiboDiff (Zhong et al., 2016) and Xtail (Xiao et al., 2016). These methods differ in details of parameter estimation and implementation, but are all founded on a similar model for the differential translation problem.

We developed Riborex as a simple method to identify genes exhibiting differential translation from Ribo-seq data. The name Riborex is derived from the objective of analyzing Ribo-seq ratios with expression. Similar to previous approaches, ours involves modeling a natural dependence of translation on mRNA levels as a generalized linear model (GLM). Unlike previous methods, we directly leverage existing model-based frameworks for gene expression analysis, using them to simultaneously estimate all parameters of our model. As a consequence, Riborex is significantly faster than all existing approaches, supports general experimental designs, and employs robust and mature software implementations for the underlying statistical calculations.

2 Approach

Established frameworks for RNA-seq data analysis, e.g. edgeR (Robinson et al., 2010) and DESeq2 (Love et al., 2014), model the read count y_{gi} from gene g in sample i as following a negative binomial distribution. Expected counts satisfy

$$\log(\mathbb{E}(y_{gi})) = \log(\lambda_{gi}) + \log(N_i) = \mathbf{x}_j^T \boldsymbol{\beta}_g + \log(N_i),$$

where N_i is the total counted reads, \mathbf{x}_j is a covariate vector associated with the treatment condition j , and $\boldsymbol{\beta}_g$ is the corresponding

vector of gene-specific coefficients. The expected proportion λ_{gi} of reads from gene g may then be regarded as the expression level of gene g in condition j . The negative binomial distribution includes a dispersion parameter for each gene. The need to consider dispersion parameter is clear, but how best to estimate dispersion remains an active area of research. Our strategy for detecting differentially translated genes models read counts r_{gi} from Ribo-seq in a similar way:

$$\begin{aligned} \log(\mathbb{E}(r_{gi})) &= \mathbf{x}_j^T \boldsymbol{\alpha}_g + \log(R_i) + \log \lambda_{gi} \\ &= \mathbf{x}_j^T (\boldsymbol{\alpha}_g + \boldsymbol{\beta}_g) + \log(R_i), \end{aligned}$$

where R_i is the total counted reads for sample i . The parameter $\boldsymbol{\alpha}_g$ may be thought of as representing how the translation of g differs from its background expression level. One interpretation is that any regulation specifically at the level of translation will be captured by the coefficients vector $\boldsymbol{\alpha}_g$. Identifying differential translation between conditions then amounts to testing contrasts concerning $\boldsymbol{\alpha}$. We combine RNA-seq and Ribo-seq read counts by constructing a specific design matrix (details in supp. info.). Then all coefficients $\boldsymbol{\alpha}$ and $\boldsymbol{\beta}$ can be fit simultaneously in existing RNA-seq data analysis frameworks (i.e. the ‘engine’ used by Riborex). The dispersion for each gene is estimated using an approach specific to the engine used.

3 Results

We evaluated Riborex in comparison with existing methods using a simulation approach based on modifying real data to introduce differences and examining accuracy in identifying those differences. We compared Riborex with a representative set of existing methods: Xtail (Xiao et al., 2016), RiboDiff (Zhong et al., 2016) and Babel (Olshen et al., 2013), acknowledging that more comprehensive comparisons already exist in the literature (Xiao et al., 2016). Briefly, from a published dataset with 5 replicates (Schafer et al., 2015), we randomly selected 4 and divided those into two groups of two replicates. We randomly sampled 1% of $\sim 15k$ expressed genes and assigned them a fold change of 4 in both RNA-seq and Ribo-seq data; these, along with unchanged genes, are considered true negatives. As positive control, we randomly sampled 1% of genes and assigned a different fold change to RNA-seq and Ribo-seq to introduce 4-fold change in translation efficiency (details in supp. info.). Riborex, Xtail, RiboDiff and Babel were applied to this semi-simulated data. We set a FDR cutoff at 0.05 and calculated the F scores for different methods. We repeated the simulations with larger control sets (10% of genes) and also on a second dataset (Diaz-Muñoz et al., 2015). The results of 100 such simulations are summarized in Figure 1. The methods give extremely similar performance, with Riborex (using all three engines) and Xtail having a slight advantage over RiboDiff and Babel. Repeating the simulation with relatively small differences ($2\times$) in translation efficiency yielded decreased accuracy for most methods (details in supp. info.).

We measured running time of each method based on 4 datasets differing in numbers of genes (mouse vs. human) and numbers of replicates (data in supp. info.) (Bennett et al., 2016; Hsieh et al., 2012; Schafer et al., 2015; Zur et al., 2016). Riborex finishes in seconds; all other methods take substantially longer, with Xtail requiring > 4 hours (Table 1).

In summary, Riborex is implemented as an open source R package, and has accuracy on par with the most accurate existing methods, but is significantly faster.

Table 1. Running time of Riborex (using DESeq2, edgeR and Voom as engine), Xtail, RiboDiff and Babel on four published Ribo-seq datasets

Dataset	Riborex engine					
	DESeq2	edgeR	Voom	Xtail	RiboDiff	Babel
MSI2	39 s	12 s	7 s	4.34 h	29.60 min	23.53 min
Hela S3	49 s	16 s	7 s	5.31 h	32.55 min	25.47 min
mTOR	23 s	8 s	4 s	4.16 h	34.07 min	15.27 min
Liver	56 s	19 s	8 s	5.69 h	26.22 min	54.30 min

Funding

This work has been supported by NIH grant R01 HG006015.

Conflict of Interest: none declared.

References

- Bazzini, A.A. *et al.* (2012) Ribosome profiling shows that miR-430 reduces translation before causing mRNA decay in zebrafish. *Science*, **336**, 233–237.
- Bennett, C.G. *et al.* (2016) Genome-wide analysis of Musashi-2 targets reveals novel functions in governing epithelial cell migration. *Nucleic Acids Res.*, **1**, gkw207.
- Diaz-Muñoz, M.D. *et al.* (2015) The RNA-binding protein HuR is essential for the B cell antibody response. *Nat. Immunol.*, **16**, 415–425.
- Hsieh, A.C. *et al.* (2012) The translational landscape of mTOR signalling steers cancer initiation and metastasis. *Nature*, **485**, 55–61.
- Ingolia, N.T. (2016) Ribosome footprint profiling of translation throughout the genome. *Cell*, **165**, 22–33.
- Ingolia, N.T. *et al.* (2009) Genome-wide analysis in vivo of translation with nucleotide resolution using ribosome profiling. *Science*, **324**, 218–223.
- Larsson, O. *et al.* (2011) anota: analysis of differential translation in genome-wide studies. *Bioinformatics*, **27**, 1440–1441.
- Law, C.W. *et al.* (2014) Voom: precision weights unlock linear model analysis tools for RNA-seq read counts. *Genome Biol.*, **15**, R29.
- Love, M.I. *et al.* (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.*, **15**, 1–21.
- Lu, P. *et al.* (2007) Absolute protein expression profiling estimates the relative contributions of transcriptional and translational regulation. *Nat. Biotechnol.*, **25**, 117–124.
- Olshen, A.B. *et al.* (2013) Assessing gene-level translational control from ribosome profiling. *Bioinformatics*, **29**, 2995–3002.
- Robinson, M.D. *et al.* (2010) edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics*, **26**, 139–140.
- Schafer, S. *et al.* (2015) Translational regulation shapes the molecular landscape of complex disease phenotypes. *Nat. Commun.*, **6**, 7200.
- Xiao, Z. *et al.* (2016) Genome-wide assessment of differential translations with ribosome profiling data. *Nat. Commun.*, **7**, 11194.
- Zhong, Y. *et al.* (2016) RiboDiff: detecting changes of translation efficiency from ribosome footprints. *bioRxiv*, **1**, 017111.
- Zur, H. *et al.* (2016) Complementary post transcriptional regulatory information is detected by punch-p and ribosome profiling. *Sci. Rep.*, **6**, 21635.