Measurement of genome-wide RNA synthesis and decay rates with Dynamic Transcriptome Analysis (DTA)

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ABSTRACT

Summary: Standard transcriptomics measures total cellular RNA levels. Our understanding of gene regulation would be greatly improved if we could measure RNA synthesis and decay rates on a genome-wide level. To that end, the Dynamic Transcriptome Analysis (DTA) method has been developed. DTA combines metabolic RNA labeling with standard transcriptomics to measure RNA synthesis and decay rates in a precise and non-perturbing manner. Here, we present the open source R/Bioconductor software package DTA. It implements all required bioinformatics steps that allow the accurate absolute quantification and comparison of RNA turnover.

Availability: DTA is part of R/Bioconductor. To download and install DTA refer to http://bioconductor.org/packages/2.10/bioc/html/DTA.html

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1 INTRODUCTION

Total RNA levels are a consequence of RNA synthesis and decay. These individual contributions can be monitored by Dynamic Transcriptome Analysis (DTA) [Aumann et al. 2011; Friedel et al. 2010, Miller et al. 2011]. DTA requires culturing cells in the presence of a labeling substrate (e.g. 4sU or 4tU). During a short, non-perturbing RNA labeling pulse, cells incorporate 4tUTP into newly transcribed RNA instead of uridine. This setup yields three types of RNA fractions: total cellular RNA, newly transcribed labeled RNA and pre-existing unlabeled RNA. The quantification of these fractions on microarrays or by RNA-seq is used to estimate RNA synthesis and decay rates on a genome-wide scale, assuming exponential decay. For each RNA g, the synthesis rate \( \lambda_g \) and the decay rate \( \mu_g \) are estimated from the equations

\[
\frac{e^{-\mu_g t} + \alpha}{T_g} = 1 - F_g - \frac{U_g}{T_g} = \frac{U_g}{T_g} + \frac{\mu_g}{\lambda_g + \alpha}, \quad \alpha = \log(2) / CCL,
\]

where \( t \) is the labeling duration, CCL the cell cycle length, and \( L_g, U_g, T_g \) are the measurements of the labeled, unlabeled and total RNA fractions, respectively. \( \alpha \) can be set to 0 in the case of primary cells (e.g. macrophages). It has been shown that DTA has higher sensitivity and higher temporal resolution in detecting gene regulatory changes than standard transcriptomics.

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2 IMPLEMENTATION AND AVAILABILITY

DTA is implemented in R (version \( \geq 2.14 \)) and is part of Bioconductor (version \( \geq 2.10 \)). For a more detailed explanation of the required objects, we refer to the package vignette. In addition to this, the number of uridines in each transcript is needed for

3 USAGE AND APPLICATION

DTA can be used for genome-wide synthesis and decay rate estimation under all kinds of perturbations and for all kinds of organisms. The rate extraction procedure can be easily accessed via the core function DTA.estimate. Its input consists of a matrix containing the normalized measurements of the total, labeled and unlabeled RNA fractions (datamat) and a description of the experimental design (pheno.mat). For a more detailed explanation of the required objects, we refer to the package vignette. In addition to this, the number of uridines in each transcript is needed for

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of quality control plots, if $W_t = DTA.estimate(Wt.phenomat, Sc.datamat, Sc.datamat = DTA.map.it(cDTA.datamat, cDTA.datamat = DTA.normalize(Raw.datamat, LtoTratio = 0.05, check = FALSE)), Sc.tnumber, Sc.ensg.reliable, ccl = 93.5, check = TRUE)

> r = Sc.ensg.reliable
> x = log2(Pol$6$"dr"[r]/Wt$6$"dr"[r])
> y = log2(Pol$6$"sr"[r]/Wt$6$"sr"[r])
> heatscatter(x,y,xlim=c(-4,1),ylim=c(-4,1))

4 CONCLUSION
The DTA package delivers straightforward methods to estimate RNA synthesis and decay rates from pre-processed microarray or RNA-Seq measurements that are obtained via the DTA/cDTA protocol. The DTA package fulfills the high standard of the Bioconductor platform, regarding documentation and usability. It can therefore be easily incorporated in R scripts for pre-processing and further statistical analysis of the results can readily be carried out by other methods within the R/Bioconductor programming environment.

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REFERENCES