**BIOINFORMATICS APPLICATIONS NOTE**

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**Gene expression**

**shortran: a pipeline for small RNA-seq data analysis**

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ABSTRACT

Summary: High-throughput sequencing currently generates a wealth of small RNA (sRNA) data, making data mining a topical issue. Processing of these large data sets is inherently multidimensional as length, abundance, sequence composition, and genomic location all hold clues to sRNA function. Analysis can be challenging because the formulation and testing of complex hypotheses requires combined use of visualization, annotation and abundance profiling. To allow flexible generation and querying of these disparate types of information, we have developed the shortran pipeline for analysis of plant or animal short RNA sequencing data. It comprises nine modules and produces both graphical and MySQL format output.

Availability: shortran is freely available and can be downloaded from http://users-mb.au.dk/pmgrp/shortran/

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Supplementary information: Supplementary data are available at Bioinformatics online.

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1 INTRODUCTION

One of the challenges in bioinformatics is to create tools with sufficient flexibility to enable formulation and testing of complex biological hypotheses while remaining technologically simple and user friendly. Useful programs for processing small ribonucleic acid (sRNA)-seq data exist, such as the UEA (University of East Anglia) sRNA toolkit (Moxon et al., 2008), wapRNA (Zhao et al., 2011) and Dario (Fasold et al., 2011). However, during our analysis of large-scale sRNA-seq datasets, two issues hampered our progress when using the existing tools. One was restrictions on data upload sizes and parameter adjustment options for web-based tools and the other was a lack of options for easy integration and combined querying of sRNA expression and annotation data. To help address these issues, we developed the shortran pipeline for sRNA-seq analysis. It is a command-line Python-based software package consisting of nine modules that can be used independently. The pipeline output includes graphical summaries, text files that can be easily parsed and automatic construction of a MySQL database, which allows simultaneous querying of expression and annotation data. Its features are compared with those of the existing pipelines in Supplementary Table S1. shortran is aimed at a broad group of users with only basic bioinformatics training, requiring only little experience with command line and database environments for successful use.

2 METHODS

2.1 Analysis pipeline summary

For maximum flexibility, the pipeline consists of independent modules. They include pre-processing (Module 1), annotation (Modules 2–6), quantitative analysis (Modules 7–8) and database querying (Module 9) (Fig. 1).

2.2 Data pre-processing

The data pre-processing implemented in Module 1 enables the user to quickly focus on the most relevant sequences. The first steps are adapter trimming of the raw sequencing files using FASTX (http://hannonlab.cshl.edu/fastx_toolkit/), followed by an optional k-mer correction using ECHO (Kao et al., 2011). Subsequently, read information is summarized in an expression profile table, which is then used as a basis for filtering by abundance and inter-library variation. Finally, Bowtie (Langmead et al., 2009) is used for filtering based on similarity to sequences in a user-provided multi-fasta file. Module 1 also generates a number of graphical data summaries for checking library sizes and length distributions.

2.3 Annotation

The mapping and annotation modules feed their results into the MySQL output database, where the annotation data is appended to the profile tables, allowing simultaneous querying of both expression data and annotations. The annotation process starts with mapping filtered reads to a reference genome using Bowtie (Langmead et al., 2009) (Module 2). For convenient visualization, the genomic mapping positions are combined with expression profiles in .igv files (http://www.broadinstitute.org/igv/). As a first step in sRNA categorization, loci characterized by multiple, closely spaced, sRNAs are defined as clusters (Module 3). To predict potential plant and animal miRNAs and plant ta-sRNAs, we have included support for mirDeep-P and mirDeep2 (Friedländer et al., 2012; Yang and Li, 2011) (Module 4), and a modified version of Chen’s algorithm (Chen et al., 2007) (Module 5). As a quality control for miRNA prediction, the results are mapped and compared with conserved miRNAs. Finally, by using Bowtie to map sRNAs against user-supplied sequence files and directly transferring the resulting annotations to the MySQL database, we provide a simple way of adding multiple sets of similarity-based annotations (Module 6).

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high variation scores (Supplementary Table S5 and of conserved and putative novel miRNAs that displayed study, miRNA prediction by Module 4 identified a number not affect the sequence composition of nucleotides 1–3 (Supplementary Table S3, Supplementary Fig. S3) but did variation score cutoff elevated the correlation coefficients ignored it in the subsequent analyses. Application of the vari-

dation score cutoff elevated the correlation coefficients (Supplementary Table S3, Supplementary Fig. S3) but did not affect the sequence composition of nucleotides 1–3 (Supplementary Table S4). In accordance with the original study, miRNA prediction by Module 4 identified a number of conserved and putative novel miRNAs that displayed high variation scores (Supplementary Table S5 and Supplementary Table S7 Q1). The graphical data summaries produced by Module 1 showed that the stele and endodermis libraries were strongly enriched for 19-mers compared with the cortex, epidermis and columella samples (Supplementary Figs S4 and S5). To determine the precise nature of the en-

richment, we queried the shortran MySQL output database (Supplementary Table S6), filtering out 19-mer sRNAs at least three times enriched in stele and endodermis (Supplementary Table S7, Q2). Both construction and querying of the database were computationally light (Supplementary Table S8). All the resulting 69 sRNAs were assigned to the same genomic clusters by Module 3, and Module 6 mapped them all to Glycine transfer ribonucleic acids (tRNAs) with TCC anticodons (Supplementary Table S9 and Fig. S6). Using further queries (Supplementary Table S7 Q3–Q10), we found that the Gly tRNA derived sRNAs fully explained the 19-mer enrichment in the stele and endodermis libraries, and we showed that the enrichment was not limited to the most abundant Gly TTC-associated sRNA (Supplementary Table S10, lines 1–10). This dominant role of Gly tRNAs in sRNA gen-

eration agrees with previous observations (Hsieh et al., 2009). Interestingly, the remaining 19-mer tRNA-derived sRNAs were most abundant in columella cells (Supplementary Table S10, lines 11–12). This enrichment was caused exclusively by sRNAs derived from Arg CCT tRNAs (Supplementary Table S10, lines 11–12). This enrichment was not limited to the most abun-
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icance tRNA-associated sRNAs.

4 CONCLUSION

Shortran provides an efficient and user-friendly tool for flexible analysis of sRNA-seq data. Using shortran, we were able to quickly generate and combine annotation and profiling information from a complex sRNA-seq experiment. We could then easily mine the data using MySQL queries to identify specific sRNAs for experimental follow-up.

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REFERENCES


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