Sequence analysis

The UEA sRNA workbench: a suite of tools for analysing and visualizing next generation sequencing microRNA and small RNA datasets

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ABSTRACT

Summary: RNA silencing is a complex, highly conserved mechanism mediated by small RNAs (sRNAs), such as microRNAs (miRNAs), that is known to be involved in a diverse set of biological functions including development, pathogen control, genome maintenance and response to environmental change. Advances in next generation sequencing technologies are producing increasingly large numbers of sRNA reads per sample at a fraction of the cost of previous methods. However, many bioinformatics tools do not scale accordingly, are cumbersome, or require extensive support from bioinformatics experts. Therefore, researchers need user-friendly, robust tools, capable of not only processing large sRNA datasets in a reasonable time frame but also presenting the results in an intuitive fashion and visualizing sRNA genomic features. Herein, we present the UEA sRNA workbench, a suite of tools that is a successor to the web-based UEA sRNA Toolkit, but in downloadable format and with several enhanced and additional features.

Availability: The program and help pages are available at http://srna-workbench.cmp.uea.ac.uk.

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1 INTRODUCTION

RNA silencing is a complex, highly conserved mechanism mediated by small RNAs (sRNAs) (Chapman and Carrington, 2007). sRNAs, such as micro RNAs (miRNAs), are typically 20–24 nt in length and act as guide molecules to regulate gene expression. Next generation sequencing (NGS) technologies have become the de facto way to generate sRNA datasets. These datasets often come from several samples, each consisting of millions of distinct reads. Therefore, there is an increasing need for computational tools for exploring and analysing such data.

Several tools have been developed for NGS sRNA analysis (see Li et al. 2012 for a recent overview of tools for miRNA analysis). These are typically command-line driven, focused on a specific application (e.g. miRNA detection) and often require installation of supporting software packages. In addition, many of them lack tools for visualizing results.

Herein, we present the UEA sRNA workbench, a suite of interactive Java-based sRNA analysis tools, which provides an easy-to-use, well-documented platform to create workflows for processing sRNA NGS data. The workbench is a successor to the web-based UEA sRNA Toolkit (Moxon et al. 2008), which, since its establishment in 2009, has received an average of 12 000 page views per month and processed over 5400 sRNA datasets. As well as being downloadable, the new workbench provides many improved features compared with the toolkit, with new tools for visualizing data and processing multiple datasets. In addition, the workbench is limited only by local memory whereas the toolkit had strict dataseize limits due to the nature of its web-based interfaces. The workbench can also be command-line driven to allow users to easily plug it into existing pipelines (Studholme, 2011).

2 DESCRIPTION OF THE WORKBENCH

2.1 Helper tools

• Adaptor remover removes adaptor fragments from raw short read sequence data and outputs data to FASTA format.

• Filter produces a filtered version of an sRNA dataset, controlled by several user-defined criteria, including sequence length, abundance, complexity, transfer and ribosomal RNA removal.

• Sequence alignment provides a graphical front-end for the PatMaN (Prüfer et al. 2008) sequence alignment tool.

2.2 Computational analysis tools

• miRCat (miRNA categorization) predicts mature miRNAs and their precursors from an sRNA dataset and a genome.
ta-siRNA prediction of a TAS gene. The locus is visualized using VisSR (visualization of sRNAs). After SiLoCo predicts the miR164 locus in four sRNA samples (including the sample used in (a)), output is sent to VisSR for visualization. Reads of lengths 19, 20–21 and 22–23 are coloured pink, red and green, respectively.

### 2.3 Visualization tools

- **Hairpin annotation** generates a secondary structure from an RNA sequence and highlights regions of interest using RNAplot (Hofacker et al. 1994). This tool can be activated from miRCat to display predicted miRNA precursors with both the miRNA and the miRNA* highlighted (Fig. 1c). Alternatively, a user can input long and up to 14 short sequences to generate a secondary structure. For each displayed miRNA precursor, the minimum free energy of the structure is also reported.

- **VisSR (visualization of sRNAs)** Visualization of sRNA features can be used to gain insight into their likely biogenesis or function. To do this, VisSR uses components from GenoViz (Helt et al. 2009) to generate a visual representation of sRNAs and user-imported genomic features. Arrows are used to depict the strand to which a short read maps to, colour is used to represent the size class of the short read and thickness illustrates abundance. VisSR can be activated from various tools to display predicted sRNA loci with each sample displayed in separate tiers (Fig. 1d), phased sRNAs for both strands in separate tiers and all unphased 21nt sRNAs in a further tier (Fig. 1e), and predicted miRNAs with miRNA* if present with the precursor as it appears on the genome. Output is generated in an image format, suitable for publication.

### 3. DISCUSSION

The relative low cost of NGS has generated an abundance of sRNA data necessitating a demand for dedicated bioinformatics support. The sRNA workbench enables biologists to gain an understanding of the sRNAome in plants and animals through the efficient processing of large NGS sRNA datasets, complemented with easily accessible visualization tools. It is hoped that these tools will enable biologists to shed light on the key molecular pathways involved in RNA silencing.

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### REFERENCES


The UEA sRNA workbench


