MiRonTop: mining microRNAs targets across large scale gene expression studies

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ABSTRACT

Summary: Current challenges in microRNA (miRNA) research are to improve the identification of in vivo mRNA targets and clarify the complex interplay existing between a specific miRNA and multiple biological networks. MiRonTop is an online java web tool that integrates DNA microarrays or high-throughput sequencing data to identify the potential implication of miRNAs on a specific biological system. It allows a rapid characterization of the most pertinent miRNA targets according to several existing miRNA target prediction approaches. It also provides useful representations of the enrichment scores according to the position of the target site along the 3′-UTR, where the contribution of the sites located in the vicinity of the stop codon and of the polyA tail can be clearly highlighted. It provides different graphs of miRNA enrichment associated with up- or down-regulated transcripts and different summary tables about selections of miRNA targets and their functional annotations by Gene Ontology.

Availability: http://www.microrarray.fr:8080/MiRonTop/index

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1 INTRODUCTION

MiRNAs appear capable to block translation and also stimulate mRNA degradation. Several mechanisms of miRNA action, including block of cap binding or elongation, increased ribosome drop-off rate or increased de-adenylation, have already been proposed (Guo et al., 2010). It remains, however, that target recognition follows a complex set of rules dominated by the existence of a perfect match between 6 and 8 nt located in the 3′-UTR (called the ‘seed’) and the target mRNA (Grimson et al., 2007). The ability of miRNAs to interact with many targets and the possibility for some of them to share a same target increase tremendously the complexity of many biological networks. In that context, an experimental validation of in silico predictions appears quite necessary. Many available target prediction softwares combine a search for a perfect seed match between miRNA and 3′-untranslated region (3′-UTR) and a conservation of the interaction across species. In fact, comparison of results obtained with several of these computational tools show significant divergences. MiRonTop aims at detecting influence of miRNAs on gene expression profiles by analyzing potential overrepresentation of specific miRNAs targets across an experimental gene set. Other tools have been recently described to explore miRNA influence on datasets (Alexiou et al., 2010; Antonov et al., 2009; van Dongen et al., 2008). MiRonTop provides several novel and complementary information including (i) the possibility to screen and compare miRNA signatures using different target prediction tools; (ii) options to calculate enrichment scores according to the spatial distribution of predicted targets sites along the transcript, since true sites may be preferentially located in close vicinity of stop codons and polyadenylation sites (Chi et al., 2009; Majoros and Ohler, 2007); and (iii) access to Gene Ontology functional annotations of the different gene lists. Overall, these different modes of representation increase the sensitivity of the research in order to define a set of either mRNA or miRNAs associated with the selected experiment.

2 IMPLEMENTATION

2.1 Description of the interface

MiRonTop takes as input the results of a global mRNA expression data. An acceptable format corresponds to a tab-delimited text file containing a unique identifier (HGNC gene symbol, NCBI gene ID, Affymetrix or Agilent probe identifiers), a signal intensity, a sequence (CDS). Predictions can be tuned with several adjustable parameters defining expressed genes (signal intensity ≥ cut-off1) and statistically significant genes (expressed genes having |log2 ratio| ≥ cut-off2 and P-value ≤ cut-off3 or odds ratio ≥ cut-off4). The three cut-offs, selected by the end-user, are used to define two sets: the down-regulated gene set (where enrichment for mRNA targets of up-regulated miRNAs should be detected) and the up-regulated gene set (where mRNA targets for down-regulated miRNAs are expected). For each miRNA considered, an enrichment of its predicted targets according to one selected prediction tool is defined for the ‘Down’ and the ‘Up’ gene sets, significance...
being evaluated using the hypergeometric law. The interface then displays for each miRNA the relationship between a Benjamini and Hochberg-adjusted hypergeometric P-value and the fold enrichment, relative to the Down and Up gene sets. In this representation, miRNAs having the largest influence on ‘Down’ or ‘Up’ gene set (green or red circles, respectively) are located in the top right quadrant of the plot. A possibility is given to highlight the effect of a specific miRNA (large square). The interface also provides a summary table. Functional annotations of the different gene lists can be accessed through several hyperlinks that show the results of Gene Ontology enrichment analyses. Additional information provided for each miRNA corresponds to the expression levels of the transcripts encoded by the same host gene. Each table can be easily downloaded for further analysis.

2.2 Database resources

Genes set references are based on Refseq v14/06/2010. Gene Ontology annotations are linked to Refseq genes through NCBI Gene IDs. The set of miRNAs corresponds to release 15 of Mirbase (Griffiths-Jones, 2006). The interface accommodates several miRNA prediction softwares: Targetscan v5.1 (Lewis et al., 2003), MicroCosm Targets v5 (Griffiths-Jones et al., 2008), Miranda-sept.2008 (Enright et al., 2003), MicroCosm Targets v5 (Griffiths-Jones et al., 2008), Miranda-sept.2008 (Enright et al., 2003) and PicTar (Krek et al., 2005) and exact search of miRNA seeds 2–7, 1–8 and 2–8 into the CDS of the reference set of genes. The set of miRNAs corresponds to release 15 of Mirbase (Griffiths-Jones, 2006). The interface accommodates several miRNA prediction softwares: Targetscan v5.1 (Lewis et al., 2003), MicroCosm Targets v5 (Griffiths-Jones et al., 2008), Miranda-sept.2008 (Enright et al., 2003), MicroCosm Targets v5 (Griffiths-Jones et al., 2008), Miranda-sept.2008 (Enright et al., 2003) and PicTar (Krek et al., 2005) and exact search of miRNA seeds 2–7, 1–8 and 2–8 into the CDS of the reference set of genes.

2.3 Example of dataset exploration

Figure 1 summarizes the results of a typical experiment where miR-34a, miR-34b* and miR-34c-5p were individually overexpressed into HeLa cells. Expression data have been publicly deposited in Gene Expression Omnibus (reference GSE15957). Corresponding top tables are also accessible through MiRonTop web portal. Cut-offs were equal to 8 for the log signal, 0.5 for the log ratio (miR-34a,b*,c-5p versus controls) and 0 for the odds ratio. Large variations in the − log(adjusted P-value), which depicts the significance of the enrichment, were noticed for miR-34abc (large black square) when different zones of the transcripts were analyzed. From this representation and in agreement with Majeros and Ohler, the most specific profiles were observed for the regions of the 3′-UTR adjacent to the stop codon, and, to a lesser extent, to the polyA tail. Low enrichment was noticed for the CDS and in the middle of the 3′-UTR. This was consistent with Hafner et al. showing that CDS sites were not as functionally active as 3′-UTR sites (Hafner et al., 2010). Similar relationships were made for about 10 distinct miRNAs, regardless of the target prediction software that was used (data not shown).

3 CONCLUSION

MiRonTop is an online application allowing the detection of miRNAs that significantly affect gene expression at a large scale. The flexibility of the interface allows a fine-tuning of the ‘Down’ and ‘Up’ gene sets according to three parameters that correspond to the level of expression, the extent of differential expression and a significance index. Such a selection allows experimenters to easily explore a large-scale experiment and get a summary report of the different predictions made by several miRNA target identification softwares. Recent investigations on other datasets have indicated that miRonTop is not only useful in experiments of miRNA overexpression/knock-down but can also detect more subtle miRNA activities in a physiological context. In the future, MiRonTop will be easily updated in order to integrate any additional predictions made publicly available, such as experimentally validated pairs of miRNA and mRNA that could now be provided by recent experimental approaches (Chi et al., 2009; Hafner et al., 2010).

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