SOFTWARE TOOL ARTICLE

**miRcomp-Shiny:** Interactive assessment of qPCR-based microRNA quantification and quality control algorithms [version 1; referees: awaiting peer review]

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Abstract

The miRcomp-Shiny web application allows interactive performance assessments and comparisons of qPCR-based microRNA expression and quality estimation methods using a benchmark data set. This work is motivated by two distinct use cases: (1) selection of methodology and quality thresholds for use analyzing one’s own data, and (2) comparison of novel expression estimation algorithms with currently-available methodology. The miRcomp-Shiny application is implemented in the R/Shiny language and can be installed on any operating system on which R can be installed. It is made freely available as part of the miRcomp package (version 1.3.3 and later) available through the Bioconductor project at: http://bioconductor.org/packages/miRcomp. The web application is hosted at https://laurenkemperman.shinyapps.io/mircomp/. A detailed description of how to use the web application is available at: http://lkemperm.github.io/miRcomp_shiny_app

This article is included in the Bioconductor gateway.
Introduction
Quantitative real-time PCR (qPCR) is one of the most widely used methods to measure the expression of a target transcript. A variety of algorithms have been developed to estimate expression from qPCR fluorescence measurements. The vast majority of these algorithms were developed and tested using gene expression data\(^1\); however, they are now routinely applied to qPCR-based microRNA expression measurements. To evaluate the performance of these methods on microRNA data, we developed a benchmark data set and a collection of statistical assessments\(^2\). These and other recent assessments\(^3\) highlight the need to develop qPCR quantification and quality assessment methodology specifically tailored to microRNA expression platforms.

Current methods to estimate expression from raw qPCR amplification data have been developed in a variety of programming languages (e.g. R, Python, SAS) and may be restricted to a particular operating system (e.g. Windows, Mac OS, Unix/Linux)\(^4\). Furthermore, these algorithms often return different data structures, complicating comparisons between methods. The miRcomp-Shiny web application provides a unified assessment environment that is platform independent and takes simple expression and quality matrices as input. This approach removes barriers to usage and facilitates the comparison of methods.

Methods
Implementation
We have developed a Shiny (http://shiny.rstudio.com/) interface to the miRcomp R package (version \(\geq 1.3.3\)). Currently, six of the most widely-used algorithms to estimate miRNA expression and sample quality are included in the miRcomp-Shiny app. Each method provides both expression estimates and quality metrics. Assessments can be performed on individual algorithms, or two available algorithms can be compared. Researchers can also upload the results from their own method to be assessed. As new methods are developed and tested, we will continue to add these methods to miRcomp-Shiny. The development of a repository of qPCR-based miRNA expression estimation algorithms will be a valuable resource for researchers seeking to develop new methodology or comparison existing algorithms across a wide variety of assessment criteria.

Advantages of an interactive interface to the miRcomp package
The web application framework in R (Shiny) has enabled us to make several aspects of the miRcomp package more interactive than they were previously and facilitate comparisons that would have been difficult to make in R. Below we describe two common use cases that motivated the development of miRcomp-Shiny.

Methodology and quality threshold selection
When selecting methodology to analyze a data set, miRcomp-Shiny can be used to evaluate the performance of existing methods based on the benchmark data. The results of these evaluations can be used to guide the selection of an expression estimation algorithm and quality threshold based on the assessments most relevant to the user’s experiment. Additionally, one can examine the effect of changing quality thresholds on the performance of each method. The result of changes in the quality threshold are then displayed immediately for each assessment. This is particularly useful when selecting a quality threshold for one’s own data.

Comparison of novel algorithms with current methods
Another use case is comparison of a new method to an existing method. By providing current methods for comparison, researchers do not have to implement these algorithms themselves, which is often a substantial bottleneck in the development and assessment of novel algorithms. Additionally, we will continue to add new methods to miRcomp-Shiny. This will produce a richer set of available methods in a single location to guide comparisons. The success of this approach has been demonstrated by the affycomp webtool\(^5\).

Operation
Installation. To access miRcomp-Shiny locally, the miRcomp R package and all required dependencies can be installed from Bioconductor with the following commands:

\[
\text{source('http://bioconductor.org/biocLite.R')}
\]

\[
\text{biocLite('miRcomp')}
\]

To access miRcomp-Shiny remotely, simply go to:

https://laurenkemperman.shinyapps.io/mircomp/

Input. The miRcomp-Shiny app takes one or two quantification methods as input. These methods can be selected from the drop-down menus on the left panel (Figure 1). Alternatively, the user can upload the results of their own method by selecting the custom option from the menu. If the custom option is selected, the user is prompted to upload a matrix of quality values (qc) and a matrix of expression estimates (ct). Once the method or methods have been selected, each assessment plot contains additional assessment-specific options below the plotting window (Figure 1).

Output. The miRcomp-Shiny app produces five plots to assess the performance of the method or methods selected: Limit of Detection, Accuracy, Precision, Quality Assessment, and Titration Response.

Use cases
The assessments performed by miRcomp-Shiny are based on a benchmark data set available at: http://bioconductor.org/packages/miRcompData/

Users wishing to assess quantification and quality control metrics beyond those currently implemented, can run any algorithm on those data and upload the resulting matrices of quality values (qc) and expression estimates (ct). Examples of these matrices are included as R data objects in the miRcomp package for each of the currently implemented methods.
Figure 1. The miRcomp-Shiny interface is organized into two panels: on the left the user selects one or more methods to compare and on the right the user selects an assessment tab. The results of several methods are available or the user can upload the results of their own method (as shown above). After selecting one or more methods, the user can examine the 5 assessment tabs: limit of detection, accuracy, precision, quality assessment, and titration response. Each tab has its own options shown at the bottom of the pane.
Summary
The success of the miRcomp Shiny web application and software will depend on new methods being developed and the application being used to test them. We have already begun encouraging people to use the package, and hope that readers will do the same. Widespread use of these tools will lead to improvements across all benchmarks in microRNA expression estimation, and the accessibility of the web application makes that possible.

Software availability
Software available from: https://laurenkemperman.shinyapps.io/mircomp/
Source code available from: http://bioconductor.org/packages/miRcomp
Archived source code as at time of publication: http://doi.org/10.5281/zenodo.1049074
Software license: GPL-3

References

Author contributions
L.K. developed the software under the supervision of M.N.M. All authors wrote and approved the final manuscript.

Competing interests
No competing interests were disclosed.

Grant information
This work was supported by the National Institutes of Health grant to M.N.M (R00-HG006853).
The authors confirm that the funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Acknowledgements
We thank Dr. Marc Halushka for his helpful comments on this manuscript. The OpenArray chips were run by the staff at the Genetic Resources Core Facility, Johns Hopkins Institute of Genetic Medicine, Baltimore, MD.
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