Genome analysis

Don’t use a cannon to kill the...miRNA mosquito

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Supervised machine-learning approaches are frequently applied on biological data to learn a regression or classification model, whether used for prediction, classification or for gaining an understanding on the biological process that has generated the data (Larranaga et al., 2006). Arguably however, it is sometimes the case that sophisticated and complicated methods are employed, published and advocated as advances without a comparison even against the simplest baseline methods. We consider a baseline method as the simplest method that an expert analyst can conceive within a few minutes of consideration of the problem and does not require any engineering or scientific ingenuity or novelty. A baseline can take the form of comparing against predicting by the mean of the outcome on a known dataset, without use of any special predicting variables, or comparing against random guessing. Because of this lack of comparison, the added-value of the sophisticated methods—if any—is not quantified; it remains unknown whether the extra effort for implementing or applying it is worth. A false perception about the difficulty of the problem may be created.

We now present an example of the above argument on the problem of identifying the position of miRNA mature molecules on their precursor RNA molecules, which typically have a hairpin-like secondary structure. In the cell, the miRNA precursor is first cut into a complex of two substring sequences (strands) with high complementarity called the ‘5‘-strand’ and the ‘3‘-strand’. The complex is called the miRNA:miRNA* duplex defined by its four corners denoted as k₅₅, k₅₃, k₃₅ and k₃₃ corresponding to the 5‘-strand 5‘-end, 5‘-strand 3‘-end, 3‘-strand 5‘-end and 3‘-strand 3‘-end positions, respectively (Fig. 1). The two strands are then separated and either one or both become a functional miRNA. The task is to predict the positions k₅₅, k₅₃, k₃₅ and k₃₃ given the sequence of a miRNA-hairpin molecule. Note that a hairpin corresponds to the sequence of the ‘Stem-loop’ structure as provided by miRBase, which does not necessarily represent the exact pre-miRNA sequence but consists of the latter extended with some flanking nucleotides. Solving the problem can suggest novel miRNAs within suspected miRNA-hairpin sequences, to guide miRNA discovery, as well as provide intuition regarding the mechanisms regulating the miRNA biogenesis.

The first tool that addressed this prediction task was ProMiR (Nam et al., 2005) which did not use any baseline method. Subsequent studies used the previous methods as baselines without considering the simplest possible method. What is the baseline ‘straw man’ on this problem? Arguably, it is making a prediction based on the mean position of each corner k₅₅, k₅₃, k₃₅ and k₃₃ as estimated from a training set of known miRNA duplexes and their precursor sequences. We call this method the Simple Geometric Locator (SGL) obviously providing a constant predicted position on any hairpin independent of the input sequence. An important detail to address is to define the reference point for measuring the mean position since miRNA precursors have various lengths. We chose the terminal loop tip as the reference point as it does not depend on the length of the pre-miRNA-flanking regions included in the hairpin sequence (see Supplementary Material, file 1 for details).

The set of ‘cannons’ to compare against SGL form four of the state-of-the-art tools for the task, namely MatureBayes [we note that MatureBayes did compare against the SGL in Gkritzou (2009). Unfortunately, the reference point used for the SGL was the beginning of the flanking regions, whose length is arbitrarily chosen before miRNA precursors are inserted in the MiRBase; hence, the performance of the SGL was found inferior in that work] (Gkritzou et al., 2010), MiRPara (Wu et al., 2011), MaturePred (Xuan et al., 2011) and the most recent MiRdup (Leclercq et al., 2013) published in respectable venues such as PLoS ONE, BMC Bioinformatics and Nucleic Acids Research. These tools employ machine-learning algorithms such as the Naïve Bayes Classifier, Support Vector Machines and the random forest classifier. They also employ complex raw and constructed features that include the nucleotide sequence, the secondary structure, number of loops and bulges, matches or mismatches for each nucleotide and others.

In our comparison, ‘prediction error’ on the task for each corner (end) is measured as the End Absolute Error (EAE): the absolute error of the predicted minus the true position (in nucleotides) for a specific duplex end (see Supplementary Material, file 1 for an example). To measure ‘prediction accuracy’, we define as ‘correct’ a prediction with error less or equal to a number x, i.e. EAE ≤ x. Then, the prediction accuracy for an error bound (tolerance) of at most x, denoted as Accu(x), is the percentage of correct predictions in the test set. For example, if a

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Fig. 1. Prediction performance per corner. Performance accuracies are estimated using the EAE for up to 5 nt. In each subplot, the y-axis shows the prediction accuracy of the Simple Geometric Locator (in %) for the same error tolerance. Lines comparing against the Simple Geometric Locator correspond to MatureBayes (magenta), MaturePred (red), MiRPara (cyan) and MiRdup (green). In addition, upward triangle, diamond, downward triangle, circle, right pointed triangle and square correspond to errors $<0, 1, 2, 3, 4, 5$ nt, respectively. Statistically significant results are indicated with filled symbols.

As evident from the figure, SGL clearly and statistically significantly outperforms MaturePred (red) in predicting any of the four duplex corners for all error bounds and MatureBayes (magenta) for error bounds up to 3–4 nt. On the 5'-strand SGL and MiRdup (green) achieve similar accuracies for absolute error of at most 0 and 1 nt with MiRdup slightly improving for larger error bounds; MiRPara (cyan) on the 5'-strand is better by only 2–5%. In the 3'-strand, MiRdup and MiRPara exhibit an overall better performance than SGL. However, when focusing on the accuracy with zero tolerance Accu(0), i.e. the percentage of duplexes identified on their exact position corresponding to the first point of each line, only MiRPara shows significantly better results with the difference in performance ranging from 4–11% (see Fig. 1 and Supplementary Table S1).

CONCLUSION

Comparing against the simplest possible method as a baseline in data analysis is an important step of the analysis. Foregoing this step may result in unnecessary effort and energy spent in code developing, publishing and evaluations by future researchers, unnecessary use of computationally expensive methods and a false impression about their benefits and added value they provide in a given task. As an example we show that using the mean positions in predicting the four corners of a miRNA duplex complex outperforms some state-of-the-art methods and is on par with the rest when trying to predict the exact location of the duplex with zero tolerance.

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REFERENCES


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