CentroidHomfold-LAST: accurate prediction of RNA secondary structure using automatically collected homologous sequences

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ABSTRACT
Although secondary structure predictions of an individual RNA sequence have been widely used in a number of sequence analyses of RNAs, accuracy is still limited. Recently, we proposed a method (called ‘CentroidHomfold’), which includes information about homologous sequences into the prediction of the secondary structure of the target sequence, and showed that it substantially improved the performance of secondary structure predictions. CentroidHomfold, however, forces users to prepare homologous sequences of the target sequence. We have developed a Web application (CentroidHomfold-LAST) that predicts the secondary structure of the target sequence using automatically collected homologous sequences. LAST, which is a fast and sensitive local aligner, and CentroidHomfold are employed in the Web application. Computational experiments with a commonly-used data set indicated that CentroidHomfold-LAST substantially outperformed conventional secondary structure predictions including CentroidFold and RNAfold.

INTRODUCTION
Secondary structure prediction of a single RNA sequence is a classical and fundamental problem in bioinformatics which has been widely used in research. The importance of accurate predictions of RNA secondary structure has increased because of the recent discoveries of functional non-coding RNAs and the relation between secondary structures and function (1). There are a number of studies of RNA secondary structure prediction (2–5). The most popular method is to predict the minimum free energy (MFE) structure, and this was used in Mfold (5), RNAfold (6) and RNAstructure (7). Another successful approach is to maximize the expected accuracy (MEA). In this approach, the entire distribution of possible structures is considered [see also (8) for the usefulness of MEA]. The MEA-based estimator used in CONTRAfold (2) and the γ-centroid estimator used in CentroidFold (3) use this method. Although several state-of-the-art algorithms have been proposed, the accuracy of secondary structure prediction seems to have peaked, and it is important to seek ways to improve secondary structure predictions.

In many cases, homologous sequences of the target RNA are available and using those sequences can improve the accuracy of secondary structure predictions. For example, it is well-known that common secondary structures improve the accuracy (9–11). However, what we would like to estimate is not a common secondary structure but a secondary structure for the specific target RNA sequence. Motivated by this idea, Hamada et al. (12) have proposed a new algorithm, called CentroidHomfold, which predicts a secondary structure of the target sequence by considering their homologous sequences. Although CentroidHomfold achieves much better accuracy than conventional secondary structure predictions, a drawback is that homologous sequences of the target RNA sequence must be prepared (which seems to be a hard task for most users).

We have, therefore, developed a Web application that predicts the secondary structure of a specific target sequence by employing ‘automatically’ collected homologous sequences. (The architecture is shown in Figure 1). To collect homologous sequences of the target sequence...
automatically, we used LAST, which is a sensitive and fast aligner (13,14), and a database of known RNA sequences derived from Rfam (15). Computational experiments in which a commonly-used data set was used (S151-Rfam data set; (2)) indicated that CentroidHomfold–LAST substantially outperformed conventional secondary structure predictors, such as CentroidFold (3), RNAfold (16), Simfold (17) and Sfold (18). A standalone pipeline is also available from our Web site.

MATERIALS AND METHODS

CentroidHomfold

CentroidHomfold, which was originally proposed by Hamada et al. (12), takes a target RNA sequence and its homologous sequences as the input. Then, it estimates a secondary structure of the target sequence using the information in the homologous sequences.

Basically, CentroidHomfold adopts the $\gamma$-centroid estimator (3), which is based on the MEA with respect to base pairs. As a probability distribution of secondary structures for the target sequence, CentroidHomfold employs a marginalized and averaged distribution of probability distributions of structural alignments (cf. the Sankoff model (19)) between the target sequence and each of the homologous sequences (see Supplementary Figure S1).

However, it requires huge computational effort to consider the probability distributions of the structural alignments. CentroidHomfold, therefore, factorizes the distributions into three parts: (i) the distribution of the secondary structures of the target sequence (given by the McCaskill model (20), for example), (ii) the distribution of the pairwise alignments between the target sequence and each homologous sequence (given by the CONTRAlign model (21), for example) and (iii) the distributions of the secondary structures of each homologous sequence (see Figure 2). By this factorization, the computational cost becomes much smaller than for the non-approximated method, so CentroidHomfold is

**Figure 1.** Architecture of the CentroidHomfold Web application (a.k.a. CentroidHomfold-LAST). The input is an RNA sequence and the output is a secondary structure of the input sequence. The Web application uses a pre-compiled database of known RNA sequences in order to find homologous sequences of the input sequence.

**Figure 2.** Illustration of the CentroidHomfold algorithm proposed in Hamada et al. (12), where $t$ is a target RNA sequence and $H = \{h_1, h_2, h_3, h_4\}$ is a set of homologous sequences of $t$. CentroidHomfold considers all the secondary structures of the target and homologous sequences, as well as all the pairwise alignments between the target sequence and each homologous sequence. This algorithm can be viewed as an approximation of the $\gamma$-centroid estimator (3) with a marginalized and averaged distribution of distributions of structural alignments between the target sequence and each of homologous sequences. See also Supplementary Figure S1.
sufficiently practical for predicting the secondary structures of long RNA sequences. More precisely, computational cost with respect to time for CentroidHomfold scales as $O(nL^2)$, where $n$ is the number of homologous sequences and $L$ is the (maximum) length of those sequences (12).

In the current version of CentroidHomfold (in CentroidFold package version 0.0.9), the McCaskill model (implemented in the Vienna RNA package), the CONTRAfold model (implemented in CONTRAfold version 2.02) and the BL model (22) (the McCaskill model with Boltzmann likelihood parameters: http://www.cs.ubc.ca/labs/beta/Projects/RNA-Params/data/parameters_BLstar_Vienna.txt) can be employed as a probability distribution for secondary structures (cf. ‘Model (secondary structure)’ in Table 1). The ProbCons model (23) and the CONTRAAlign model (21) can be used for the probability distribution of pairwise alignments (cf. ‘Model (alignment)’ in Table 1).

The parameter $\gamma$ in CentroidHomfold (‘Gamma’ in Table 1) adjusts the balance between the sensitivity and the positive predictive value (PPV) of a predicted secondary structure. Larger $\gamma$ produces more base pairs in the prediction.

**Pseudo base-pairing probabilities in CentroidHomfold**

In CentroidHomfold, the following pseudo base-pairing probability $p_{ij}$ of a base pair $(x_i, x_j)$ of a target RNA sequence $x$ is computed:

$$p_{ij} = \alpha p_i^{(x,x)} + \frac{1-\alpha}{|H|} \sum_{k \in H} \sum_{h \in H} p_k^{(a,x,h)} p_{ij}^{(a,x,h)} p_{hl}^{(x,h)}.$$  \hspace{1cm} (1)

Here, $\alpha \in [0, 1]$ is a weighting parameter, $p_i^{(x,x)} = p_i^{3}(\theta_i^{3} = 1|x)$ is a base-pairing probability and $p_k^{(a,x,h)} = p_k^{al}(\theta_k^{al} = 1|x, h)$ is an aligned base probability, where $p_i^{3}(\cdot|x)$ and $p_k^{al}(\cdot|x, h)$ denote the probability distribution of secondary structures of $x$ and the probability distribution of pairwise alignments between $x$ and $h$, respectively. It is easily seen that $p_{ij} \in [0, 1]$ (12). This (pseudo) probability is used as the reliability of a base pair in a graphical representation of an input RNA sequence in the Web application (cf. Figure 3B).

**LAST**

LAST (13,14) finds similar regions between sequences. LAST is sensitive and faster, because it uses variable-length (spaced) seeds realized by a suffix array. In LAST, the default settings and parameters, which are well-optimized for DNA and RNA sequences, were used (13). (We used LAST-128, downloaded from http://last .cbrc.jp, in this study.)

For a given (target) RNA sequence, a given database of RNA sequences and an $E$-value $e$ (i.e. the expected number of alignments for a random database), a score threshold of significant alignments is determined using a method proposed by Sheeolin et al. (24). (The method is implemented in the lastex software in the LAST package.) It should be emphasized that this method enables us to choose a threshold for each input RNA sequence adaptively.

**Known RNA sequences**

To find homologous sequences of a specific RNA sequence, we utilized six databases of known RNA sequences, both of which were taken from the Rfam database (version 10.0) (15): (i) the sequences covered by Rfam full alignments, filtered to $<X\%$ identity (Rfam.full.X); (ii) the sequences covered by Rfam seed alignments, filtered to $<X\%$ identity (Rfam.seed.X). The cd-hit-est program (25) is employed to filter redundant sequences in both databases. Note that Rfam.full.99 includes a large number of predicted RNA sequences. See Supplementary Table S1 for a summary of the data.

**Benchmark settings**

In our computational experiments, we used the S-151 data set (2), which consists of 151 RNA sequences (each of which belongs to a different RNA family) with their reliable reference secondary structures. The smallest length, the largest length and average length of sequences in the data set is 23, 568 and 136.3, respectively. This data set has been used in several previous studies of RNA secondary structure prediction (2,3,17).
As performance measures, we used the sensitivity (SEN) and PPV: $\text{SEN} = \frac{\text{TP}}{\text{TP} + \text{FN}}$ and $\text{PPV} = \frac{\text{TP}}{\text{TP} + \text{FP}}$ where TP, FP and FN are the numbers of true positive base pairs, false positive base pairs and false negative base pairs, respectively.

RESULTS AND DISCUSSION

CentroidHomfold-LAST Web application

Figure 1 shows the architecture of the CentroidHomfold Web application (CentroidHomfold–LAST pipeline) with default parameters (see the ‘Default’ column in Table 1). We used several databases to provide known RNA sequences (see Supplementary Table S1 and the ‘Materials and Methods’ section for details; users can select a database using the ‘Database’ option in Table 1). It should be emphasized that each database contains only information about RNA sequences and does not give any common secondary structures or secondary structures of particular RNA sequences. We prepared a compiled database of each data set, using lastdb with default parameters. For a given input sequence, homologous sequences with a given $E$-value were found using LAST. The $E$-value, $e$, is the expected number of chance alignments and a score threshold for significant alignment is automatically computed from $e$ for each input RNA sequence (see ‘Materials and Methods’ section). (The parameter $e$ corresponds to ‘$E$-value’ in Table 1.) When the number of homologous sequences collected by LAST is large, a subset of those sequences are chosen by selecting the top-$n$ sequences. (The parameter $n$ corresponds to ‘No. of homologous’ in Table 1.) We then run CentroidHomfold with those automatically collected homologous sequences. Finally, CentroidHomfold can output ‘non-canonical’ base pairs in a predicted secondary structure, non-canonical base pairs were removed from the set of base pairs in the predicted secondary structure.

We implemented the above pipeline in a Web application (http://www.ncrna.org/centroidhomfold), which has an interface that is quite similar to the CentroidFold Web application (26) (Figure 3). The prediction results are shown in two ways: the input sequence with standard base pair notation (Figure 3B, ‘Seq/Fold’), and a popular secondary structure graph (Figure 3B, ‘Graph’). A PDF version of the graph and a FASTA file of homologous sequences (used in CentroidHomfold) can be downloaded by clicking on a link. The colors of base pairs in the graphical representation indicate pseudo base-pairing probabilities for each base pair (See ‘Materials and Methods’ section for details). Users can download the PDF file of secondary structure and homologous sequences used in CentroidHomfold.

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Figure 3. (A) A screen-shot of the Web interface of a CentroidHomfold-LAST pipeline (http://www.ncrna.org/centroidhomfold), which is similar to the CentroidFold Web application (26). Users can paste RNA sequence in the text area and push the ‘Execute CentroidHomfold’ button. Then, the secondary structure of the input is returned immediately (B). By expanding the ‘Advanced options’ control, the parameters in Table 1 are adjustable. (B) An example of output from the Web application. The colors of base pairs in the graphical representation indicate pseudo base-pairing probabilities for each base pair (See ‘Materials and Methods’ section for details). Users can download the PDF file of secondary structure and homologous sequences used in CentroidHomfold.
CentroidHomfold–LAST substantially outperforms conventional secondary structure predictors

Figure 4 indicates that CentroidHomfold–LAST has substantially improved accuracy compared with the conventional secondary structure predictors CentroidFold (3), Sfold (18), Simfold (17) and RNAfold (6). Figure 5 shows an example of the predictions of the CentroidHomfold–LAST (this work) and the CentroidFold Web application (26) (http://www.ncrna.org/centroidfold/) for a typical tRNA sequence. In this example, CentroidHomfold–LAST successfully predicted a ‘clover-leaf’ secondary structure while CentroidFold failed.

It is also interesting that using Rfam.full (which contains a large number of predicted RNA sequences) gave slightly better performances than using Rfam.seed (which consists of only manually-curated sequences). This suggests that tentative RNA sequences included in Rfam.full improve the accuracy.

In order to confirm robustness of CentroidHomfold–LAST, we conducted the experiments with various values for the parameters \( n \) and \( e \), where \( n \) is the maximum number of homologous sequences and \( e \) is a threshold \( E \)-value for homologous searches (Supplementary Table S2). This figure indicates that CentroidHomfold–LAST is robust to changes in \( n \) and \( e \), although a smaller \( n \) does yield a slightly worse performance.

We also conducted experiments using various probabilistic models (Supplementary Figure S3): the ProbCons model (23) and the CONTRAlign model (21) for probability distribution of pairwise alignments; the CONTRAfold model (2) and the BL model (22) for probability distributions of secondary structures. Supplementary Figure S3 indicates that using the BL model resulted in better performance than using the CONTRAfold model (while using the ProbCons model and the CONTRAlign model lead to similar performances). Moreover, using the BL model is faster than using the CONTRAfold model (Supplementary Table S2). Therefore, the BL model is employed as the default setting in our Web application.

One drawback of CentroidHomfold–LAST is that it is slower than conventional algorithms for secondary structure prediction. It takes 451 (Rfam.full.99) and 267 (Rfam.seed.99) s to compute secondary structures of 151 RNA sequences using CentroidHomfold–LAST (Table 2), while it takes only 30 s using CentroidFold (3). This is because our method computes base-paring probability matrices for all the input sequences (target and

Figure 4. Performance of CentroidHomfold–LAST with default settings (\( n = 30 \) and \( e = 0.01 \)), compared to the conventional secondary structure predictors CentroidFold, RNAfold, Sfold and SimFold. The axes are the PPV and Sensitivity with respect to base pairs in secondary structures. We used 17 values of the \( \gamma \) parameter \( \gamma \in \{2^k : -5 \leq k \leq 10, k \in \mathbb{Z} \cup \{0\}\} \) in order to draw the sensitivity–PPV curves for CentroidHomfold–LAST.

Figure 5. Example of a secondary structure predicted by the CentroidFold Web application (26), http://www.ncrna.org/centroidfold/ (A) and the CentroidHomfold–LAST Web application (B). A typical tRNA sequence GAGGUCUUAGCUUAUUAAGCAAUUGAUUGCAUUCAUA GAUGUAGGAUGAAGUCUUCAGUCCUUA (L07095.1/5018-5086) was used.
homologous sequences) and all the alignment probability matrices between the target sequence and each of homologous sequences.

Another drawback of the Web application is that users can only employ database based on the Rfam databases (Supplementary Table S1) and the length of the input sequence is limited to 500. A standalone pipeline system is therefore available from http://www.ncrna.org/software/centroidhomfold/pipeline/, in which there is no restriction for the length of the input RNA sequence and an original (user defined) RNA database can be employed for the candidates of homologous sequences. In our future work, we plan to develop an interface that enables users to upload RNA sequences for the database in the Web application, and provide more databases derived from, for example, the UCSC genome browser.

CONCLUSION

By combining CentroidHomfold (12) and LAST (13), we have developed a novel Web application (CentroidHomfold–LAST pipeline) that predicts the secondary structure of an input RNA sequence using automatically collected homologous sequences of the input sequence. Benchmark experiments with a commonly used data set indicated that CentroidHomfold–LAST substantially outperformed conventional secondary structure predictors (e.g. RNAfold and CentroidFold). The Web application is freely available from http://www.ncrna.org/centroidhomfold/.

Table 2. Total computational time in seconds

<table>
<thead>
<tr>
<th></th>
<th>Rfam.full.99</th>
<th>Rfam.seed.99</th>
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<tbody>
<tr>
<td>Lastal</td>
<td>62</td>
<td>14</td>
</tr>
<tr>
<td>Select homologous from lastal results</td>
<td>54</td>
<td>22</td>
</tr>
<tr>
<td>CentroidHomfold</td>
<td>334</td>
<td>229</td>
</tr>
<tr>
<td>Total</td>
<td>451</td>
<td>267</td>
</tr>
</tbody>
</table>

Each value indicates the total computational time for predicting secondary structures for 151 RNA sequences (17 values of γ were used in order to draw the performance curves in Figure 4). The row ‘Lastal’ includes a score threshold calculation (by using lastex) and a lastal search of a given database. The row ‘Select homologous from lastal results’ includes collection of actual RNA sequences in the database using Lastal results. We used an Intel(R) Xeon(R) CPU X5550 machine with a Linux OS.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

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