Empirical Transition Probability Indexing
Sparse-Coding Belief Propagation (ETPI-SCoBeP)

Genome Sequence Alignment

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Abstract

The advance in human genome sequencing technology has significantly reduced the cost of data
generation and overwhelms the computing capability of sequence analysis. Efficiency, efficacy and
scalability remain challenging in sequence alignment, which is an important and foundational operation
for genome data analysis. In this paper, we propose a two stage approach to tackle this problem. In
the preprocessing step, we match blocks of reference and target sequences based on the similarities
between their empirical transition probability distributions using belief propagation. We then conduct a
refined match using our recently published SCoBeP technique. Our experimental results demonstrated
robustness in nucleotide sequence alignment and our results are competitive to those of the SOAP aligner
and the BWA algorithm. Moreover, compared to SCoBeP alignment, the proposed technique can handle
sequences of much longer lengths.

I. INTRODUCTION

In bioinformatics, sequence alignment is an important way to identify similar regions that
might be associated with similar functional and structural relationship between sequences. With
the quick growth of genomic data, it is important to develop effective sequence alignment

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techniques that are scalable. The past decade has witnessed the development of many sequence alignment technologies. Cancers are caused by the collection of genomic sequence changes [1]. Therefore, alignment and analyses of cancer genome sequences provide basics to understand cancer biology, diagnosis and therapy.

In general, pairwise sequence alignment methods can be classified into local and global approaches. The global alignment attempts to find the best match between two strings with similar lengths through global optimization. In contrast, the local alignment is usually used to identify regions of similarity between a short query and a longer sequence. Global alignments [2]–[5] are less prone to demonstrating false homology as each letter of one sequence is constrained to being aligned to only one letter of the other. Local alignments [6]–[9], on the other hand, can cope with rearrangements between non-syntenic, orthologous sequences by identifying similar regions in sequences; this, however, comes at the expense of a higher false positive rate due to the inability of local aligners to take into account overall conservation maps [10].

A lot of efforts have been made to improve the efficiency and efficacy of sequence alignments. The ClustalW program proposed by Thompson and Larkin [11], [12] uses a multi-stage mechanism to weight and to align sub-sequences based on sequence divergences. In addition, sequence annealing technique incrementally builds sequence alignment one at a time by checking whether a single match is consistent with a partial multiple alignments [13]. Darling et al. proposed a hidden Markov model that uses a sum-of-pairs breakpoint score to facilitate the detection of rearrangement breakpoints, when genomes have unequal gene content [14]. Mummer is a highly efficient suffix tree based matching tool for whole genome alignment, as well as incomplete genomes [15].

Researchers also proposed heuristics to accelerate sequence alignment. For example, the bounded sparse dynamic programming (BSDP) is used to support rapid approximation of exhaustive alignment in [16]. Another heuristic-driven approach, namely FastTree, is a tree-based method that stores profiles of internal nodes in a tree, such that candidate joins can be quickly identified. FastTree is also scalable for handling alignments over 10,000 sequences [17], [18].

Maximum-likelihood based approaches like PhyML and RAxML-VI-HPC have been developed as well. PhyML [19] used a hill-climbing algorithm that adjusts tree topology and branch length at each tree modification iteration. RAxML-VI-HPC [20], which stands for randomized accelerated maximum likelihood for high performance computing, takes advantages of a parallel
program to support large-scale genome alignment.

In this paper, we propose a novel alignment method that uses sparse coding [21] and empirical transition probability to tackle the scalability challenge. Thanks to the sparse representation, our mechanism can handle long sequences with reduced memory footprint. We also leverage belief propagation to combine local and neighboring information of candidate nucleotides into consideration and generate matching scores to determine the best match. The rest of this paper is structured as follows. Section II introduces our proposed method. Section III presents our results, including the comparison against SOAP aligner [22] and BWA [23]. Finally, we draw our conclusions in Section IV.

II. PROPOSED METHOD

In this section, we present our genome indexing and alignment framework in detail, where the proposed method includes three steps: indexing, index matching, and sequence matching. In this paper, we refer to “reference sequence” as the base-line sequence and try to align a “read sequence” against the base-line sequence.

A. Indexing

The current genome indexing methods generate huge indices before performing the actual alignment to decrease the alignment time [24], [25]. The indexing process can be very time-consuming. In contrast, our proposed indexing technique provides a faster and light-weight alternative for index generation, which is similar to the big data retrieval systems that were proposed [26]–[28]. These indices can reduce the search space and provide an estimation of the read sequence locations in the reference sequence. The proposed genome indexing technique models a nucleotide sequence as a graph by counting the transitions between each pair of nucleotides. To be more specific, as shown in Fig. 1, we consider a graph with four states according to the different types of nucleotides and sixteen vertices according to all possible transitions between nucleotides. We read the first nucleotide of the sequence and treat it as the initial state. Then, we move from one state to the other state by scanning the next nucleotide repeatedly till the end of the sequence. Afterwards, we calculate the number of nucleotide transitions where we count how many times we pass one vertex in the graph and store them in...
a $4 \times 4$ matrix. Finally, we normalize the resulting matrix as follows:

$$
I = \begin{pmatrix}
A & C & G & T \\
A & k_{aa} & k_{ac} & k_{ag} & k_{at} \\
C & k_{ca} & k_{cc} & k_{cg} & k_{ct} \\
G & k_{ga} & k_{gc} & k_{gg} & k_{gt} \\
T & k_{ta} & k_{tc} & k_{tg} & k_{tt}
\end{pmatrix} \times \frac{1}{\sum_{s,w \in \{a,c,g,t\}} k_{sw}}
$$

(1)

where $k_{sw}$ is the number that has the $S$-type nucleotide immediately before the $W$-type nucleotide.

If the length of a sequence is larger than a given threshold i.e., $h$, we divide it into subsequences with maximum length of $h$, where each subsequence will have $o$ nucleotides overlap with their neighbors. We set $o \geq \frac{h}{2}$ so that each pair of nucleotides can be counted at least twice. For each subsequence $i$, we count the transition of the nucleotides from the start of the subsequence till its end to reveal the number of different nucleotides that reside beside each other. In Fig. 2, an input sequence with $h = 250$ is used to demonstrate the proposed indexing process, where $I_i$ is the calculated index for the input sequence based on the transition graph shown on the left hand side. Finally, we normalize the transition matrix, which will be used to find the approximate location of each subsequence in the next step.

B. Index matching

The index matching step is designed to find similar indices based on global information of the sequence. We define a symmetric distance function between two index matrices $I$ and $J$ as follows: $D_{MSE}(I, J) = \| I - J \|_F$, where $\| \cdot \|_F$ is the Frobenius norm of the matrix.

After generating the indices of the reference sequence and the read sequence, the $D_{MSE}$ distances to all reference sequence indices are calculated, where the top $t$ most similar indices in terms of $D_{MSE}$ are chosen as candidate indices. To find the best matched index, we resort to belief propagation (BP) on a factor graph. In this paper, we provide a concise review about the BP algorithm on factor graph for the proposed algorithm. Interested readers can check our earlier publications in [29]–[31] for more details about the factor graph design and the BP algorithm.

We apply BP to the factor graph of the test sequence with $n$ candidate nucleotides as the prior knowledge. BP updates the probability of candidate nucleotides based on the probabilities of their neighbors.
Fig. 1: The transition diagram between nucleotides. $k_{sw}$ is the number of appearance of the $W$-type nucleotide immediately after the $S$-type nucleotide where $s, w \in \{a, c, g, t\}$.

Then, the candidate index numbers are fed to a factor graph and the corresponding $D_{MSE}$ of each of candidates is employed to calculate the initial probability (prior probability) of each candidate. Then, message passing (i.e., forward and backward) algorithm is applied to calculate the best match indices. The correspond subsequences of these indices is used in the next step.

C. Sequence matching

The sequence matching step is based on sparse coding and BP algorithm. In this step, we use the subsequences that were selected in the previous step to generate an over-complete dictionary. Then, for each nucleotide in the read sequence, we pick $n$ candidate nucleotides using sparse coding. By applying belief propagation to a factor graph, we can obtain the best match for each nucleotide in the read sequence. A detailed description about the sequence matching can be found in our recent publications [31], [32]. A summary of the main procedure for our proposed alignment method is shown in Algorithm 1.
Input sequence = \{aaaccaagggcagttgacagccactgcactccagcctagaacagagttagcactctcgaacaacaaacaacaaaaaactacacactttttgacttgac\}

\[
I_i = \begin{pmatrix}
45 & 20 & 18 & 14 \\
26 & 8 & 0 & 16 \\
13 & 12 & 3 & 8 \\
12 & 11 & 15 & 28
\end{pmatrix} \times \frac{1}{249}
\]

\[
= \begin{pmatrix}
0.1807 & 0.0803 & 0.0723 & 0.0562 \\
0.1044 & 0.0321 & 0.0 & 0.0643 \\
0.0522 & 0.0482 & 0.0120 & 0.0321 \\
0.0482 & 0.0442 & 0.0602 & 0.1124
\end{pmatrix}
\]

Fig. 2: An example of the indexing procedure for a small sample subsequence.

D. Implementation details:

- \(I_i = \text{MakeIndex}(x_i)\) fills the state matrix \(I_i\) using the relationship of nucleotides in the subsequence \(x_i\). The subsequence \(x_i\) is scanned through all its nucleotides and the corresponding counts will be stored into the state matrix \(I_i\). For example, \(k_{cg}\) in \(I_i\) in (2) shows how many times the nucleotide \(C\) will be identified, which is next to the nucleotide \(G\) in the subsequence \(x_i\). Note that each subsequence \(x_i\) has a separate state matrix \(I_i\), where \(i\) is the subsequence index.

\[
I_i = \begin{pmatrix}
A & C & G & T \\
A & k_{aa} & k_{ac} & k_{ag} & k_{at} \\
C & k_{ca} & k_{cc} & k_{cg} & k_{ct} \\
G & k_{ga} & k_{gc} & k_{gg} & k_{gt} \\
T & k_{ta} & k_{tc} & k_{tg} & k_{tt}
\end{pmatrix}
\]

- \([c_j, \rho_j] = \text{FindCandidates}(J_j, I, k)\) identifies \(k\) candidate state matrices that are highly similar to the test state matrix \(J_j\) in \(I\) and stores their indices in vector \(c_j\) and their probabilities in vector \(\rho_j\). Note that the approach will compute the Mean Square Error (MSE) of the test state matrix \(J_j\) with each possible \(I_i\) of the reference state matrices and select \(I_i\) that has the smallest MSEs.
Algorithm 1 Proposed nucleotide sequence alignment algorithm for estimating the location of the input sequence

**Inputs:** a reference sequence $\mathcal{X} \in \mathbb{R}^M$, a test sequence $\mathcal{Y} \in \mathbb{R}^N$, number of the candidate state matrix $k$, number of the candidate points $n$

**Initialize:** a $4 \times 4$ state matrix $I$ storing the numbers of nucleotide states (2), nucleotide overlap $v$

**Fill the reference state matrix $I$:** For each subsequence $x_i \in \mathcal{X}$ with $v$ nucleotide overlap in each direction perform:
- $I_i = \text{MakeIndex}(x_i)$

**Fill the test state matrix $J$:** For each subsequence $y_j \in \mathcal{Y}$ with $v$ nucleotide overlap in each direction perform:
- $J_j = \text{MakeIndex}(y_j)$
- $[c_j, \rho_j] = \text{FindCandidates}(J_j, I, k)$

**Refine the candidate state matrix:**
- $\hat{\rho} = \text{BP}(c, \rho)$

**Find the correspond nucleotide in the reference sequence $\mathcal{X}$:** For each subsequence $y_j \in \mathcal{Y}$ with $v$ nucleotide overlap in each direction perform:
- $z_j = \text{FindBestSubsequence}(\mathcal{X}, y_j, \theta, n)$ (see [31], [32] for more details)

**Output:** the estimated version of aligned sequence $Z$

- $\hat{\rho} = \text{BP}(c, \rho)$ models the problem by a factor graph and applies belief propagation [33] to update probability $\rho$. The updated probability $\hat{\rho}$ can be used to align the reference state matrix index onto the test state matrix index. In our case, we assign a variable node for each test state matrix index and connect each pair of neighboring state matrix indices with a factor node. Also, we introduce one extra factor node to take care of the prior knowledge obtained in the MSE step for each test state matrix index (for more details, see [32]).
- $z_j = \text{FindBestSubsequence}(\mathcal{X}, y_j, \theta, n)$ finds the corresponding location for a nucleotide $y_j \in \mathcal{Y}$. In this step, the reference nucleotide sequence $\mathcal{X}$ and the test nucleotide sequence $\mathcal{Y}$ are converted into two integer sequences. Then, an over-complete dictionary is built with all
Fig. 3: The results of proposed method for non-collinear nucleotide sequence alignment. a) comparison among alignment results of the ground truth, 1D-SCoBeP [31] and the proposed method. b) zoomed of the black square in figure 3–a to show the gap between the proposed method, ground truth and 1D-SCoBeP [31] on the jump point. The x-axis and y-axis are the index numbers of the original genome sequences and the shuffled genome sequences, respectively.

subsequences in the $X$. We then apply sparse coding followed by using Belief Propagation (BP) to identify the best matches. (see [31], [32] for more details) Note that we used non-overlapped subsequences to build the dictionary. This change decreases the memory usage and the accuracy of the proposed algorithm in compare to 1D-SCoBeP [31], but it increases the speed of our alignment algorithm.

III. EXPERIMENTAL RESULTS

We designed our experiments based on work in [14] to evaluate the proposed method for aligning the nucleotide sequences and to compare it with SOAP aligner [22], BWA [23] and 1D-SCoBeP [31]. We considered the problem of aligning a sequence of human nucleotides from the National Center for Biotechnology Information (NCBI) [34] and Cancer Genomics Hub (CGHub) [35].

To evaluate the performance of our approach, we conducted two sets of tests on the nucleotide sequences. In the first set, we selected fifty short sub-sequences of human genomes and then
used SOAP aligner, BWA, 1D-SCoBeP and the proposed method to find the location of selected sub-sequence nucleotide in the human chromosome. All of four algorithms successfully passed this test. We created twenty shuffled sub-sequences of the reference sequence as follows: for each read sequence $R$, we cut it into five pieces $p_1, p_2, p_3, p_4$ and $p_5$. Then we switched $p_2$ with $p_4$. Therefore, we converted a read sequence $\hat{R} = [p_1, p_2, p_3, p_4, p_5]$ into a new read sequence $\hat{R} = [p_1, p_4, p_3, p_2, p_5]$.

Fig. 3 shows the result of the 1D-SCoBeP and the proposed method show a better performance with a gap of 100 to 120 nucleotides away from the ground truth. Since we were using non-overlapped subsequences for the dictionary generation, the gap between the proposed method and the ground truth was larger than these reported in 1D-SCoBeP [31]. In our experiments, the following parameters were used: the number of candidate points $n$ is set to be 3, the sparsity factor $k = 3$ and the dictionary column size $a = 200$.

To evaluate the robustness of the proposed method, we generate indices for long human genome sequences (i.e. $5 \times 10^8$ nucleotides) where $h = 10000$ and $o = 5000$. Moreover, we synthesized insertion, deletion and mutation (i.e., indel) in these sequences. For indel rate, we picked $10^5$ number of subsequences with size of $10^4$ nucleotides. Then, we randomly modified a
Fig. 5: The percentage of successful alignments in present of 0.5% to 1.5% indels. The **green line** is the percentage successful alignments where the rate of the indels are changing with step equal 0.05% between 0.7% and 1.3%. The **blue line** is the percentage successful alignments where the rate of the indels are changing with step equal 0.1% between 0.5% and 1.5% and the **red line** is the same as the Fig. 4. Each point represents $10^5$ random site selection with same indels rate. Note that the genome sequences used in this studies were obtained from [35] and [34].

We investigated the impact of small indel rate in the range from 0.5% to 1.5% in Fig. 5. In this figure, we showed accuracy of 1% indels in red for the data set used in 4 as reference. To verify our result, we repeat the experiments with different indel steps and different read locations and present the results in green and blue, respectively. Note that each point in this figure was obtained from the evaluation over $10^5$ read sequences. There are slight variation among the curves due
to statistical deviation. The summary of the indel rate accuracy was shown in Table I.

The computational complexity of proposed is mainly determined by the following three steps: 1) indexing 2) index matching 3) extracting sub-sequence nucleotides as features and constructing the dictionary, 4) finding candidate nucleotides via sparse coding, and 5) applying BP. Assume the size of the read and reference sequences are $N$ and $M$ nucleotides, respectively. The required time for create indexes is $O(M + N)$, because we have to scan whole read and reference sequences. The number of reference sequence indexes is $I_M = \frac{M}{h} + \frac{2aM}{h^2} = O\left(\frac{M}{h}\right)$ and similarly, $I_N = O\left(\frac{N}{h}\right)$. Therefore, the time for the index match matching is $O\left(\frac{M}{h}\right) \times O\left(\frac{N}{h}\right) = O\left(\frac{MN}{h^2}\right)$.

After index matching step, the size of search space reduces from $M$ to $\bar{M} = I_s \times h$ where $I_s$ is the number selected indexes $I_s$ and $h$ is the size of each index. The required time of feature extraction will be $O(a(\bar{M} + N))$, where $a$ is the size of the vector of extracted features for each nucleotide. The dictionary construction step involves the normalization of each column, which requires $O(a\bar{M})$ amount of time. Thus the total time complexity of the first step is $O(a(\bar{M} + N))$.

In the next step, the time complexity of Subspace Pursuit (SP) is $O\left(\log(f)a\bar{M}\right)$ [36], where $f$ is the number of iterations for searching the sparse vector. Since we have to repeat the process to find candidate points for all $N$ feature vectors, the time complexity of finding candidate points by SP is $O\left(\log(f)a\bar{M}N\right)$. Then, the time complexity of Belief Propagation in our factor graph is $O(\nu n^2\bar{M})$, where $\nu$ is the number iterations before converging and $n$ is the number of candidates in each variable node. Finally, the time complexity of proposed method will be $O\left(MN + \log(f)a\bar{M}N + \nu n^2\bar{M}\right)$.

IV. Conclusion

In this paper, we proposed a sparse coding and BP based method for indexing and alignment genome sequences. The proposed method builds a transition matrix based on the neighboring nucleotides of an input sequence and then reduces the search space by selecting the top $K$ most similar subsequences based on their distances. The proposed algorithm selects candidate nucleotides by using sparse coding with an over-completed dictionary, which was constructed from the nucleotides of reference sequence in the indexing step. BP algorithm is then applied to select the best matches. Through experimental results, we showed that the proposed algorithm are comparable to SOAP aligner [22], BWA [23] and 1D-SCoBeP [31] in terms of the alignment accuracy. In addition, the proposed method is robust to insertions, deletions, and mutations in the
genome sequences when comparing with SOAP aligner and BWA. Finally, the proposed method is able to process much longer sequences than our previous 1D-SCoBeP approach.
REFERENCES


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