Pyro-Align: Sample-Align based Multiple Alignment system for Pyrosequencing Reads of Large Number

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1 Introduction

Pyro-Align is a multiple alignment program specifically designed for pyrosequencing reads of huge number. Multiple sequence alignment is shown to be NP-hard [1] and heuristics are designed for approximate solutions. Multiple sequence alignment of pyrosequencing reads is complex mainly because of 2 factors. One being the huge number of reads, making the use of traditional heuristics, that scale very poorly for large number, unsuitable. The second reason is that the alignment cannot be performed arbitrarily, because the position of the reads with respect to the original genome is important and has to be taken into account.

Before we indulge in the details of the algorithms itself, a short note on Domain Decomposition and Sample-Align-D algorithm would be useful.

2 Sample-Align-D and Domain Decomposition

Sample-Align-D [2] algorithm, is a domain decomposition based technique that is designed for multiple alignment of biological sequences on multiprocessor platforms. The domain decomposition based technique, in addition to yielding better quality, gives enormous advantage in terms of execution time and memory requirements. The proposed strategy allows to decrease the time complexity of any known heuristic of $O(N)^x$ complexity by a factor of $O(1/p)^x$, where $N$ is the problem size in terms of number and length of sequences, $x$ depends on the underlying heuristic approach, and $p$ is the number of processing nodes. We have presented the decomposition strategy as a parallel computing solution. However, the super-linear speed-ups on multiple processors suggest that the use of the sampling based decomposition strategy on a single processor systems...
would also be able to deliver significant time and space advantages and is thus used in Pyro-Align. For complete description of the technique the author refers the reader to [3].

3 Pyro-Align ALGORITHM

I won’t give a complete description of the algorithm here but I will try to explain the major components of the system. Hopefully a short summary would help explain the workings of the algorithm in an abstract manner.

The algorithm can be divided into 4 major components:

1. Overlapping alignment
2. Clustering
3. Pairwise alignments
4. Profile-profile alignments

Each of these components are explained below;

3.1 Overlapping Alignment

The first step is to determine where the reads are positioned with respect to the reference genome in question. This step is necessary to ensure that the reads are in the right position with respect to the reference before an actual alignment is executed. Had this step been omitted, there are number of alignments that would be correct but would be inaccurate if analyzed in the global context. A read that is not constricted in terms of position, may give the same score (SP score) for the multiple alignment but would be incorrect in context of the reference. To accomplish the task of ‘placing’ the reads in the correct context with respect to the reference genome we execute overlapping alignment for each read with the reference.

An overlapping alignment ignores the start and end gaps. The overlapping alignment is also known as semi-global alignment because the sequences are globally aligned but the start and end gaps are ignored. Here the start gaps are the gaps that occur before the first 'character' in the sequence and end gaps are that occur after the last character. The overlap alignment can be obtained by modifications to the Needleman-Wunsch algorithm. The alignment thus obtained will potentially provide information of the overlap between the reference genome and the respective read.

The modification to the basic algorithm is now described. Let the two sequences to be aligned be x and y and $A(i, j)$ present the score of the optimal alignment. Since, we would not like to penalise the starting gaps, we modify the dynamic programming matrix by initialising the first row and first column to be zero. The gaps at the end are also not to be penalised. Let $A(i, j)$ represent the optimal score of $x_1, \ldots, x_i$ and $y_1, \ldots, y_j$. Then $A(m, j)$ is the score that represents optimally aligning $x$ with $y_1, \ldots, j$. The optimal alignment therefore, is now detected as the maximum value on the last row or column. Therefore the best score is $A(i, j) = max_{k,l}(A(k, n), A(m, l))$ and the alignment can be obtained by tracking the path from $A(i, j)$ to $A(0, 0)$.

After each read is semi-globally aligned with the reference genome, we obtain set of leading and trailing gaps, with the first character after the gaps is the starting position for the read with
respect to the reference genome. The information for these alignments are stored in hashtables that are further used for processing in the clustering.

3.2 Clustering

The method followed by most multiple alignments is that a quick similarity measure is done using k-mer counting or some other heuristic mechanism. Thereafter a distance matrix is computed from the pairwise similarities and a tree is constructed from the distance matrix using UPGMA or neighboring joining. The progressive alignment is thus built, following the branching order of the tree, giving a multiple alignment. The briefly explained procedure for multiple alignment, the two steps of distance matrix and tree construction require \( O(N^2) \) time each. For huge number of reads, as in our case this similarity measure is not feasible. Therefore, a method was required which would give us the similarity measure but would be linear in terms of time complexity.

To reduce the complexity of the algorithm, we exploit the information that we already know. We know the fact that the reads are coming from the same reference or nearly same reference. This in turn means that the reads which start from the same or near same ‘starting’ point with respect to the reference genome are likely to be similar to each other. Therefore, we already have the clustering information or the ‘guide tree’ from the first step of the algorithm. Our guide tree, or the order in which sequences will be aligned in the progressive alignment is from the starting position of the reads from the first stage. Of course the decomposition of the reads (the subtree of the profiles that we built) doesn’t render the reads in the same order as in traditional progressive alignment, but nevertheless the order is more or less the same when the profiles of these reads are aligned.

3.3 Pairwise Alignment

The original version of the algorithm, required to align the clusters obtained from the second step, using multiple alignment clustalw system. Our experimentation however, suggested that the pairwise alignment of the reads with same ordering information also give reasonable results. Therefore pairwise local alignment using smithwater is executed on these reads (the ordering is still the same as discussed in section 3.2). After this stage, the reads are aligned in pairs such that we have \( N/2 \) pair of aligned reads.

3.4 Profile-Profile Alignments

Profile-profile alignments are used to re-align two or more existing alignments. It is useful for two reasons; one being that the user may want to add sequences gradually, and second being that the user may want to keep one high quality profile fixed and keep on adding sequences aligned to that fixed profile [4]. We will take advantage of both of these properties in our domain decomposition. In this stage of the algorithm, the \( N/2 \) pair of aligned reads have to be combined to get a multiple alignment. The profiles of these reads can be aligned sequentially, one by one, from the clustering information acquired in the first stage. However, we have shown in [3] that the decomposition of the profiles gives a fair amount of time advantages even on single processors. Therefore a hierarchical model similar to [2] is implemented in the algorithm. The model requires that instead of combining the profiles in a sequential manner (one by one), a binary tree is built such that the profiles that are aligned are the leaves of the tree. Currently, the tree is followed for upto 100
clusters (pairs of 2 reads), after which the profiles are merged in a sequential manner. The reason is to ensure reasonable quality for large number of reads and is extensively discussed in [3]. Profile sum of pairs (PSP) is the function used in Clustalw [4], Mafft [5] and Muscle [6] to maximize Sum of Pairs (SP) score, which in turn maximizes the Alignment score such that the columns in the profiles are preserved, as depicted in Fig. 1.

Figure 1: Two profiles (X and Y) are aligned under the columns constrains, producing profile Z

In order to apply pair-wise alignment functions to profiles, a scoring function must be defined, similar to the substitution methods defined for pair-wise alignments. One of the most commonly used profile functions is the sequence-weighted sum of substitution matrix scores for each pair of amino acid letters. Let $i$ and $j$ be the amino acid, $p_i$ the background probability of $i$, $p_{ij}$ the joint probability of $i$ and $j$ aligned to each other, $S_{ij}$ the substitution matrix being used, $f^x_i$ the observed frequency of $i$ in column $x$ of the first profile, $xG$ the observed frequency of gaps in that column. The same attributes are assumed for the profile $y$. Then PSP score can be defined as in [7] and [6]:

$$S_{ij} = \log(p_{ij}/p_ip_j)$$ (1)

$$PSP^{xy} = \sum_i \sum_j f^x_if^y_j \log(p_{ij}/p_ip_j)$$ (2)

For our purposes, we will take advantage of PSP functions based on 200 PAM matrix [8] and the 240 PAM VTML matrix [9]. Some multiple alignment methods implement different scoring
functions such as Log expectation (LE) functions, but for our purposes PSP scoring suffices. Profile functions have evolved to be quite complex and good discussion on these can be found at [6] and [10]. We use the profile functions from the clustalw system.

### 3.5 Complexity Analysis and Results

The complexity presented in the section are only approximate. A more rigourous time and space complexity analysis is still required. N is the number of reads, L the average length of the read and \( L_g \) the length of the genome.

The breakdown of time complexity for the algorithm is presented below:

1. \( O(N \times L^2) \)
2. \( O(N \times L) \)
3. \( O(N \times L^2) \)
4. \( O(N \log N \times L_g^2) \)

The timing for the algorithm is also verified by the experiments conducted. The time required for aligning 2000 reads on a desktop computer (Intel dual quad-core Xeon(R) each running at 2.66GHz with 16GB of RAM and 4096Kb cache size and Linux Redhat with kernel 2.6.18-92.1.10.e15) using sequential clustalw was observed to be around 6 hours and 36 minutes. The time observed for decomposition based Pyro-Align was around 13 minutes.

### 4 Installations & Running Instructions

Please refer to the README file in the distribution of Pyro-Align.

### References

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