9 Multiple Match Refinement and T-Coffee

In this section we describe how to extend the match refinement to the multiple case and then use *T-Coffee* to heuristically compute a multiple trace.

This exposition is based on the following sources, which are all recommended reading:

1. Notredame, Higgins, Heringa: T-Coffee, a Novel Method for Fast and Accurate Multiple Sequence Alignment, Journal of Molecular Biology, 2000, Vol 302, pages 205-217.
2. Rausch, Emde, Weese, Döring, Notredame, Reinert: Segment-based multiple sequence alignment, ECCB 2008, Cagliari

9 Multiple Match refinement

We start by extending the pairwise match refinement to the multiple case. The result of the multple match refinement naturally induces an input graph for the *multiple trace problem* which is NP-hard but could be solved with ILP based techniques.

However, in practice we can resort to more efficient, but heuristic methods. In the second part of the lecture we will describe the TCoffee algorithm which is basically a heuristic for the multiple trace problem (although originally not advertised as such).

9.1 Multiple Match refinement

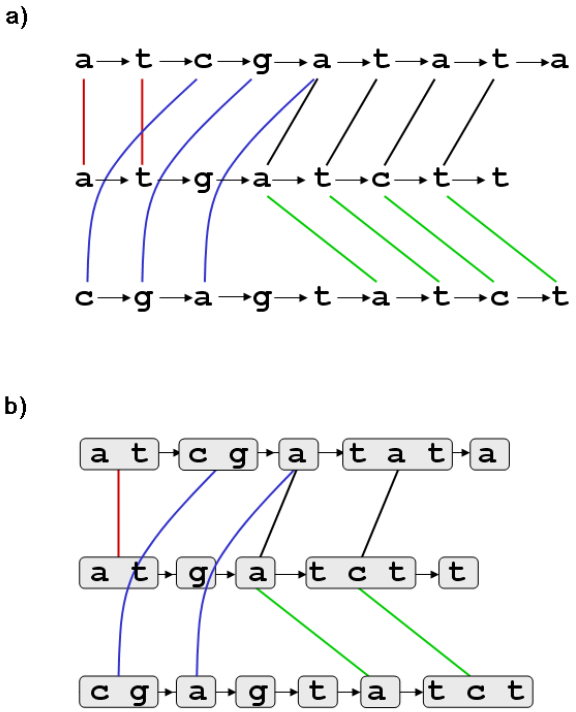
Lets start by extending the definitons from the pairwise case to the multiple case.

Definition 1. Let $\mathcal{S} = \{S^1, S^2, ..., S^n\}$ be a set of n sequences with $S^p = s^p_1 s^p_2, ..., s^p_{|S^p|}$, $p \in \{1, 2, ..., n\}$. A *segment match* $M = (S^p_{ij}, S^q_{kl})$ is an alignment between the two *segments* $S^p_{ij} = s^p_{i+1} s^p_{i+2} ... s^p_j$ and $S^q_{kl} = s^q_{k+1} s^q_{k+2} ... s^q_l$ with $p, q \in \{1, 2, ..., n\}$ and $p \neq q$ and $0 \leq i \leq j \leq |S^p|$ and $0 \leq k \leq l \leq |S^q|$.

The only change is that we consider more than two sequences. The other definitions are the same except that we consider now matches between all pairs of sequences. We refrain from giving them here again.

As in the pairwise case, the *segment match refinement* algorithm takes as input a set of segment matches $\mathcal{M}$ containing pairwise segment matches for a set of sequences $\mathcal{S} = \{S^1, S^2, ..., S^n\}$ together with a set of projection maps $\sum$. The output is the *minimal resolved refinement* $\mathcal{M}'$ of $\mathcal{M}$.

For example the four depicted segment matches in the next figure (a) would be refined into a total of seven segment matches in the figure (b). The figure already shows the corresponding input alignment graph for the trace problem. One can clearly see the reduction in problem size compared to the non segment match alignment graph version.



Pseudocode 1 describes the algorithm as a depth first search (as opposed to the breadth first description in the pairwise case). For each $S^i \in \mathcal{S}$, a node set V^i is maintained. In the beginning we have $V^i = S^i$-*support* of $\mathcal{M}$ for all $i \in \{1, 2, ..., n\}$. After the refinement process, V^i contains the S^i-*support* of $\mathcal{M}'$. Also, a set of edges E is maintained that stores all projections that have been made.

The segment matches are processed one by one. We follow each chain of projections of each left and each right position until we encounter a position h^i that is already contained in the corresponding set V^i.

If a position h^i is already contained in V^i, then h^i has either been refined previously - therefore it does not have to be treated again - or it is a left or right position of one of the original matches and will be refined, when processing this match.

In the end, every position contained in each V^i is a result of a chain of projections stemming from a position on an original match $M \in \mathcal{M}$. Therefore, no superfluous cuts are made. Each projection made is recorded in the edge set E. From this edge set, we compute the refinement of each $M \in \mathcal{M}$. The union of all refinements is the minimal resolved refinement $\mathcal{M}'$.

Pseudocode 1 Multiple Segment Match Refinement

function REFINE($\mathcal{M}, \Sigma, \mathcal{S} = \{S^1, S^2, ..., S^n\}$)
  $\mathcal{V} = \{V^1, V^2, ..., V^n\}$
  $\forall p \in \{1, 2, ..., n\} : V^p = S^p - support$ of $\mathcal{M}$
  $E = \emptyset$
  E.insert(M, i, k)
  E.insert(M, j, l)
  $cut(\mathcal{M}, \Sigma, \mathcal{V}, E, p, i)$
  $cut(\mathcal{M}, \Sigma, \mathcal{V}, E, p, j)$
  $cut(\mathcal{M}, \Sigma, \mathcal{V}, E, q, k)$
  $cut(\mathcal{M}, \Sigma, \mathcal{V}, E, q, l)$
  lexicographically order edges in E using
  (M, i, k) and $(M, i, -k)$ for direct and reverse matches,respectively
  $\mathcal{M}' = \{(S^p_{ij}, S^q_{kl}) | (M, i, k)$ and (M', j, l) are consecutive and $M = M'\}$
end function

function CUT($\mathcal{M}, \Sigma, \mathcal{V}, E, p, h$)
  for each $M = (S^p_{ij}, S^q_{kl})$ or $M = (S^q_{kl}, S^p_{ij}) \in \mathcal{M}$ with $i < h < j$ **do**
    $h' = \sigma^{S^p}_M(h)$
    E.insert(M, h, h') or E.insert(M, h', h) depending on the sequence order in M
    if $(h' \notin V^q)$ **then**
      V^q.insert(h')

```
 7:             cut(M, V, Σ, E, q, h')
 8:         end if
 9:     end for
10: end function
```

9.2 Avoiding fragmentation

Remeber the worst cas escenario for the pairiwise segment match refinement. In the example two segments matches were cut into a refined set oconsiting of singel character matches.

In order to prevent this scenario one can use a heuristic to stop the refinement. This heuristic takes as input the parameter α that specifies the minimal allowed segment length. Cuts that would result in a segment length smaller than α are simply not made. Instead the pro jection edge is *bent* toward the closest realized cut, thereby introducing gaps into the segment match

9.3 T-Coffee

We now discuss a simple multiple alignment algorithm that outperforms ClustalW on certain data sets, namely T-Coffee. T-Coffee stands for **T**ree based **C**onsistency **O**bjective **F**unction **F**or alignm**E**nt **E**valuation. Its basic idea consists of combining global and local sequence information.

It uses the same progressive alignment method as ClustalW but tries to rectify the greedy choices made by incorporating information from *all* pairs of sequences.

The basic steps of T-Coffee are:

1. Generate *primary* libraries of alignments.
2. Derive library *weights*.
3. *Combine* libraries into single primary library.
4. *Extend* the library.
5. Use the extended library for progressive alignment.

9.4 Generating primary libraries

A primary library in T-Coffee contains a set of pairwise alignments and could be derived using any method. By default two libraries are generated:

1. A library of all global pairwise alignments using ClustalW.
2. A library of the ten top-scoring local alignments using Lalign from the FASTA package.

All these alignments contain information that is more or less reliable. Hence T-Coffee combines them to produce more reliable alignments.

9.5 Derive library weights

Each library is weighted with the percent identity, a measure which is known to be a reasonable indicator when aligning sequences with more than 30% identity.

We give now an example for computing a global primary library and weighting it. Assume we are given the four strings:

```
s1 = GARFIELD THE LAST FAT CAT
s2 = GARFIELD THE FAST CAT
s3 = GARFIELD THE VERY FAST CAT
s4 = THE FAT CAT
```

The regular progressive alignment method would produce the following alignment:

```
a1 = GARFIELD THE LAST FA-T CAT
a2 = GARFIELD THE FAST CA-T
a3 = GARFIELD THE VERY FAST CAT
a4 = -------- THE ---- FA-T CAT
```

Obviously the third CAT is not correctly aligned. T-Coffee would first produce the following global, primary library.

The percent identity is computed on *matching* positions. (Ignore the blanks, they are inserted for better readibility.)

```
a1=GARFIELD THE LAST FAT CAT        a2=GARFIELD THE ---- FAST CAT
a2=GARFIELD THE FAST CAT ---        a3=GARFIELD THE VERY FAST CAT
     weight=88                           weight=100

a1=GARFIELD THE LAST FA-T CAT       a2=GARFIELD THE FAST CAT
a3=GARFIELD THE VERY FAST CAT       a4=-------- THE FA-T CAT
     weight=80                           weight=100

a1=GARFIELD THE LAST FAT CAT        a3=GARFIELD THE VERY FAST CAT
a4=-------- THE ---- FAT CAT        a4=-------- THE ---- FA-T CAT
     weight=100                          weight=100
```

9.6 Combine libraries

If there is more than one primary library available, they are combined by merging any pair that is duplicated between the two libraries. The combined pair has as a new weight the sum of the two individual weights.

After combining the primary libraries into a single primary library, this library is extended by incorporating consistency information.

9.7 Extending the primary library

Finding the highest weighted, consistent subsets of pairwise contraints is known as the *maximum weight trace* problem and is by itself NP-hard.

Hence T-Coffee employs a heuristic strategy with the goal to compute weights that reflect the information contained in the whole library. To do so it employs a *triplet* approach. This works as follows for each pair of sequences s_i, s_j.

1. Align s_i and s_j *through* another sequence s_l, $1 \leq l \neq i, j \leq k$. For each column c of the alignment $A(s_i, s_j)$ that contains no gap character, we set the initial weight of the match $A[i, c] \leftrightarrow A[j, c]$ to the primary library's weight of the alignment of the x-th character of s_i with the y-th character of s_j. The weight of columns containing gap characters is set to 0.
2. Take the minimum weight of the primary library alignments between s_i, s_l and s_l, s_j if the x-th character of s_i is aligned to the z-th character of s_l and the z-th character of s_l is aligned to the y-th of s_j. The weight is added to the inital weight of the match $x \leftrightarrow y$.

Lets go back to our example. We want to extend the primary library for the pair s_1, s_2. Note that the primary library has uniform weights since we did not combine more than one.

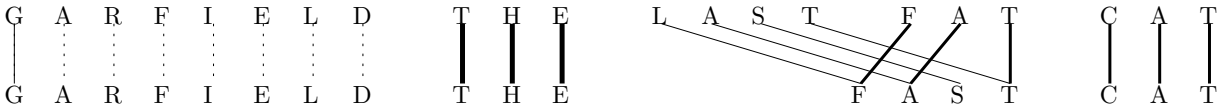
```
a1=GARFIELD THE LAST FAT CAT
   ******** ***  ***  **        weight = 88
a2=GARFIELD THE FAST CAT ---

a1=GARFIELD THE LAST FA-T CAT
   ******** ***       ** * ***
```

```
a3=GARFIELD THE VERY FAST CAT  weight = 80
   ******** ***       **** ***
a2=GARFIELD THE ---- FAST CAT

a1=GARFIELD THE LAST FA-T CAT
            ***       ** * ***
a4=         THE       FA-T CAT  weight = 100
            ***       ** * ***
a2=GARFIELD THE       FAST CAT
```

We combine the above extensions into the weighting scheme for the pair of sequences s_1, s_2.



weight 168 weight 268 weight 88 weight 180 weight 180

Finally we use the above weighting scheme to compute the pairwise alignment using a dynamic programming algorithm without gap penalties. All the information is already incorporated into the libraries. In our case the best pairwise alignment would be:

```
a1=GARFIELD THE LAST FA-T CAT
a2=GARFIELD THE ---- FAST CAT
```

This will correct the error in the inital alignment.

9.8 Computing the progressive alignment

In order to compute the progressive alignment we compute the distance matrix using the extended alignment library as computed above. This again is used to compute a guide tree using the neighbor joining method.

The gaps introduced in the first alignment are fixed and cannot be shifted later.

When aligning two groups of sequences (containing possibly only one sequence) the *average* score from the extended libraries is used for each column.

9.9 Combining TCoffee with the multiple srm

The just presented, original T-Coffee uses *libraries* which are simply alignment graphs where each vertex corresponds to a single residue or nucleotide. Similar to T-Coffee's library, the alignment graph contains alignment information about a set of sequences $\mathcal{S} = \{S^0, S^1, ..., S^{n-1}\}$.

Given such an initial alignment graph, we apply the triplet extension introduced in T-Coffee. Whereas T-Coffee ensures consistency on pairs of characters we ensure consistency on pairs of vertices.

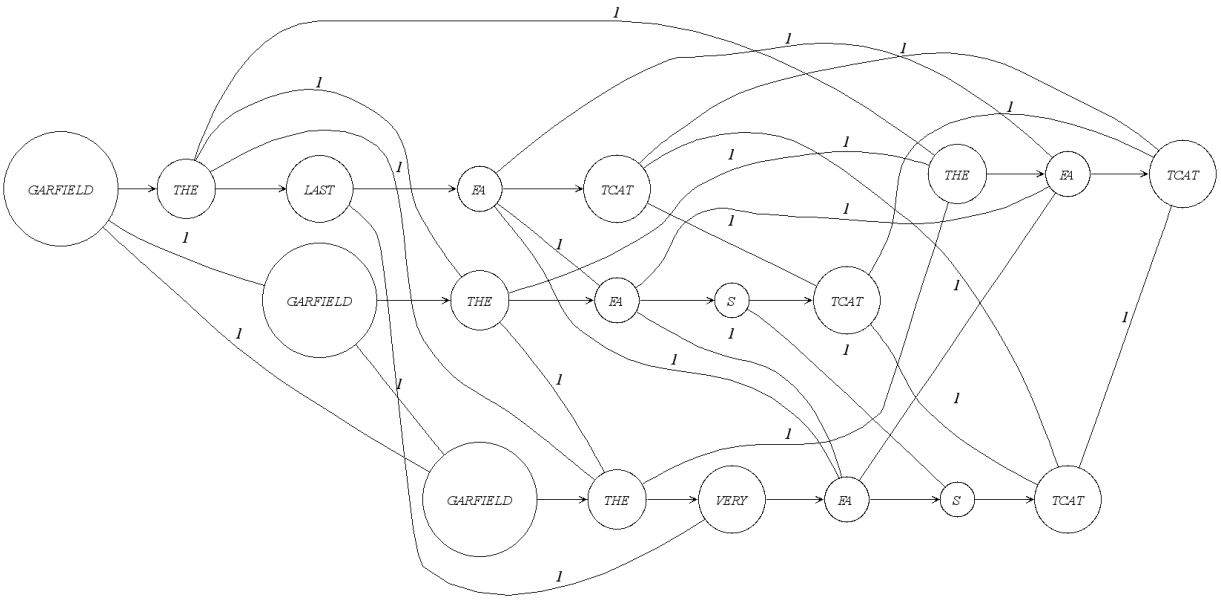
9.10 Combining TCoffee with the multiple srm

Similarly, a progressive alignment is possible on graphs, i.e., a pairwise alignment simply aligns two strings of vertices instead of sequence characters.

A profile in terms of an alignment graph is a string where each position has a set of vertices. Thus, given a guide tree we can perform a progressive alignment on an alignment graph of multiple sequences in the same manner as aligning the sequences themselves.

The approach is, however, more generic because a single vertex can be any group of characters, e.g., a large segment, a gene, or just a single character.

9.11 Combining TCoffee with the multiple srm



9.12 Combining TCoffee with the multiple srm

The advantage of our method is that the input can be *any* set of segment matches. To illustrate it, we explain two different mechanisms to generate segment matches.

For protein alignments and short DNA sequences the standard match generation algorithm takes a set of sequences and builds all pairwise global and local alignments using the dynamic programming algorithms of Gotoh or Waterman and Eggert. We compute the dynamic programming matrix column-wise and save the traceback pointers in a reduced alphabet that can be efficiently stored. This enables us to use these algorithms for fairly long sequences, e.g., a set of adenovirus genomes.

However, for very long (genomic) sequences algorithms using quadratic space are impossible to use and space-efficient dynamic programming algorithms become too inefficient. For these problem instances we either use the enhanced suffix arrays to compute maximal unique matches or external tools such as BLAST.

9.13 Combining TCoffee with the multiple srm

Aligner	= 6	≥ 5	≥ 4	≥ 3	Avg. identity	CPU Time (s)
DIALIGN-T	7888	12161	18187	27690	48%	1259
SeqAn::T-Coffee	12795	18525	25147	32396	63%	1751
MAFFT*	12450	18011	24624	32084	62%	118
MUSCLE*	50	817	5257	21849	38%	673
SeqAn::T-Coffee*	**12911**	**20078**	**27011**	**33147**	**65**%	328

Alignment of 6 adenoviruses: Running time and alignment quality of an alignment of 6 adenoviruses. The number of columns with at least 6, 5, 4, and 3 identical characters are reported together with the average identity.

As it can be seen the combined match refinement and TCoffee approach outperforms the currently best programs in the field.

9.14 Summary

You should know:

- Pairwise and multiple match refinement are efficient methods to preprocess overlapping segment matches.
- In the worst case, segment match refinement can refine a set of input matches to single base matches.
- TCoffee is a consistency based alignment algorithm that computes heuristically a multiple trace.
- The combination of multiple match refinement and TCoffee results in a versatile method to compare sequences based on a set of input segment matches.