MUSCLE

a multiple sequence alignment method with reduced time and space complexity

Robert C. Edgar

presented by Chase Jenkins
November 13, 2008

Edgar, R.C. (2004) MUSCLE: a multiple sequence alignment method with reduced time and space complexity.
BMC Bioinformatics 5(1): 113.

Overview

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Overview

- MUSCLE
  - "Multiple sequence comparison by log-expectation"
- Previous paper introducing MUSCLE
  - Edgar, Robert C. (2004), MUSCLE: multiple sequence alignment with high accuracy and high throughput, Nucleic Acids Research 32(5), 1792-97
  - Gave an overview of MUSCLE and showed its excellent performance against four alignment benchmarks

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Overview

- This paper
  - More in-depth discussion and analysis of the MUSCLE algorithm
  - Describes techniques to improve the MUSCLE algorithm's biological accuracy and/or computational complexity
  - Introduces "MUSCLE-fast" for high performance uses

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Overview

- Results
  - Speed and accuracy compared with:
    - CLUSTALW
    - Progressive POA
    - MAFFT script FFTNS1
      - *"fastest previously published program known to the author"*

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Overview

- Results
  - Comparison made using four benchmarks:
    - BAliBASE
    - SABmark
    - SMART
    - The author's own benchmark, PREFAB

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Overview

- Results
  - Three variants of MUSCLE tested:
    - MUSCLE (default)
      - Most accurate
    - MUSCLE-fast
      - High throughput
    - MUSCLE-prog
      - A compromise between the two
    - A more detailed description of these will be given later

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Overview

- Conclusions
  - *"MUSCLE offers a range of options that provide improved speed and/or alignment accuracy compared with currently available programs."*
  - MUSCLE is freely available
    - http://www.drive5.com/muscle

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Overview

- Multiple sequence alignment (MSA) algorithms have two characteristics of primary importance to the user (as with most algorithms):
  - Accuracy
    - Specifically, biological accuracy
  - Computational complexity
    - Running time
    - Space requirements

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Implementation

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Implementation

- Basic idea
  - Bring together the concepts of sequence alignment and phylogenetic tree construction
  - The basic strategy is similar to that used by PRRP and MAFFT
    - Build a progressive alignment
    - Apply "horizontal refinement"
      - Extract pairs of profiles from the progressive alignment and re-align them
      - Keep the new alignment only if it improves some objective score

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Basic Algorithm Elements

Implementation

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Basic Algorithm Elements

- Similarity measures
  - A measure calculated for a pair of sequences that estimates their degree of evolutionary divergence
    - Operates under the assumption that the sequences are related

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Basic Algorithm Elements

- Similarity measures
  - Two types of similarity measures are used by MUSCLE:
    - The fractional identity, *D*, calculated from a global alignment of the two sequences
    - *k*-mer (subsequences of length *k*) counting
      - Related sequences should have more common subsequences than unrelated sequences
      - Generally faster than performing a global alignment
      - Relies on *k* not being too large, and the sequences not being too divergent
  - Author has previously shown that *k*-mer counting correlates well with fractional identity

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Basic Algorithm Elements

- Distance measures
  - The distance measure between two sequences, *A* and *B*, should be additive
    - *d(A, B) = d(A, C) + d(C, B)* for any sequences *A*, *B*, *C* that are related
  - The ideal distance measure is the "mutation distance" – the number of mutations that occurred on the evolutionary path between the two sequences

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Basic Algorithm Elements

- Distance measures
  - The fractional identity, *D*, is often used as a similarity measure. For closely related sequences, *1 - D* is then a good approximation of the mutation distance.
    - As we've discussed, *1 - D* would be an exact measure of the mutation distance under the *infinite site model*
    - As sequences diverge, however, there is a higher probability for multiple mutations at a single site, therefore MUSCLE uses the Kimura distance as an estimate of the mutation distance:

$$d_{\text{Kimura}} = -\log_e (1 - D - D^2/5)$$

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Basic Algorithm Elements

- Distance measures
  - When a *k*-mer similarity measure is used, the following equation is used for the distance measure:

$$d_{\text{kmer}} = 1 - F.$$

where *F* is the *k*-mer similarity measure.

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Basic Algorithm Elements

- Tree construction
  - Given a triangular distance matrix, a binary tree is constructed by clustering the matrix
    - Clustering is performed using either UPGMA or neighbor-joining
      - Given two clusters (subtrees), the distance to a third cluster is calculated using a method similar to that used by MAFFT, where a weighted mix of the average distance and minimum distance is used

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Basic Algorithm Elements

- Progressive alignment
  - In the first two stages of the algorithm, a progressive alignment is constructed for the sequences.
  - This requires a rooted binary tree where each sequence is a leaf node of the tree.
    - Tree is constructed by calculating the distance measures, placing them in a triangular matrix, then clustering the matrix, as previously mentioned.
  - The branching order of the tree is followed from the leaf nodes up to the root. At each internal node, a profile-profile alignment is used to align the existing two subtree alignments, and that new alignment is assigned to that internal node.
  - Progressing in this fashion, an MSA is obtained at the root of the tree.

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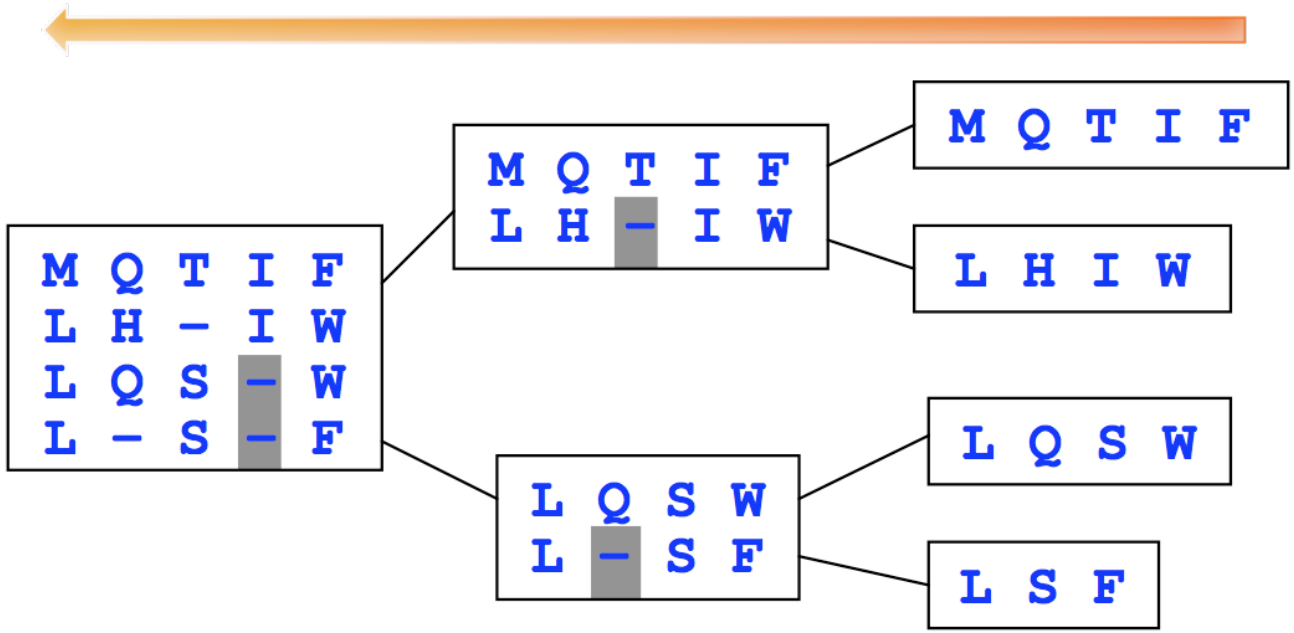
Basic Algorithm Elements

- Progressive alignment



Implementation

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Basic Algorithm Elements

- Objective score
  - In its third stage, the MUSCLE algorithm iteratively attempts to maximize an objective score. The scoring method used by MUSCLE is the **SP (sum-of-pairs) score**.
    - This is the sum of the alignment scores of each pair of sequences.
    - The alignment score of an individual sequence pair is simply the sum of the scores of each aligned residue using a substitution matrix, plus affine gap penalties

$$g + \lambda e$$

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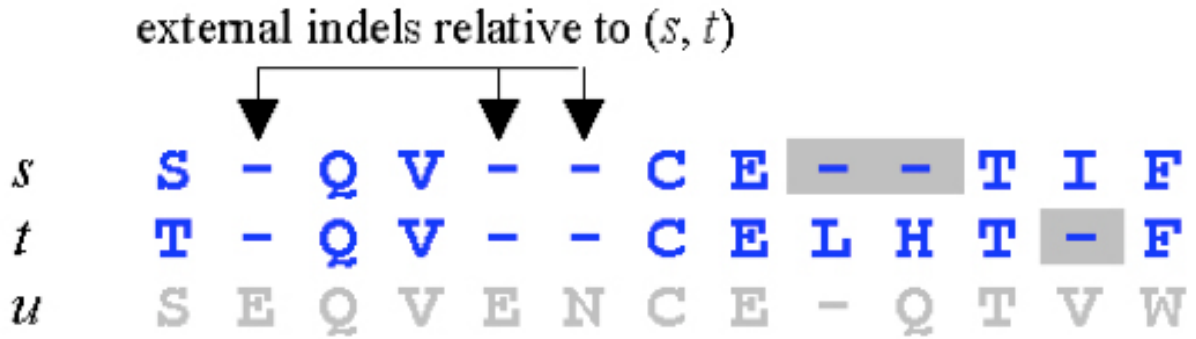
Basic Algorithm Elements

- Objective score
  - The contribution of gap penalties to the SP for a single pair of sequences is calculated by discarding all indels common to both sequences, then calculating an affine gap penalty for the two sequences



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Algorithm Overview

Implementation

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Algorithm Overview

- Three-stage algorithm
  - Stage 1: Draft progressive alignment built
  - Stage 2: Improved progressive alignment built
  - Stage 3: Iterative refinement performed
- An MSA is available at the completion of each stage

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Algorithm Overview

- **Stage 1**: Draft progressive alignment built
  - Steps:
    - *Similarity measure*
    - *Distance estimate*
    - *Tree construction*
    - *Progressive alignment*

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Algorithm Overview

- **Stage 1**: Draft progressive alignment built
  - *Similarity measure*
    - A measure of the similarity of each pair of sequences is computed either:
      - Using *k-mer* counting, or
      - By constructing a global alignment of the two sequences and calculating *D*, the "fractional identity"

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Algorithm Overview

- **Stage 1**: Draft progressive alignment built
  - *Distance estimate*
    - A triangular distance matrix is computed from the pair-wise similarity measures

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Algorithm Overview

- **Stage 1**: Draft progressive alignment built
  - *Tree construction*
    - A tree is constructed from the distance matrix using either:
      - UPGMA, or
      - Neighbor-joining

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Algorithm Overview

- **Stage 1**: Draft progressive alignment built
  - *Progressive alignment*
    - A progressive alignment is built by following the branching order of the constructed tree
    - This gives an MSA of all input sequences at the root of the tree

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Algorithm Overview

- **Stage 2**: Improved progressive alignment built
  - This stage is iterative.
  - Steps:
    - *Similarity measure*
    - *Distance estimate & tree construction*
    - *Tree comparison*
    - *Progressive alignment*

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Algorithm Overview

- **Stage 2**: Improved progressive alignment built
  - *Similarity measure*
    - A measure of the similarity of each pair of sequences is again computed
    - This time, however, the similarity measure is computed using the fractional identity of the two sequences as aligned in the current MSA

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Algorithm Overview

- **Stage 2**: Improved progressive alignment built
  - *Distance estimate & tree construction*
    - A tree is constructed by calculating a Kimura distance matrix and applying a clustering method to the matrix
      - again, either UPGMA or neighbor-joining

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Algorithm Overview

- **Stage 2**: Improved progressive alignment built
  - *Tree comparison*
    - The previous tree (either the tree from Stage 1 or the tree from the previous execution of Stage 2) is compared to the new tree and the internal nodes which change the branching order are identified.
    - If the number of changed internal nodes has not decreased, the tree has been "fully" improved and iteration ceases.

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Algorithm Overview

- **Stage 2**: Improved progressive alignment built
  - *Progressive alignment*
    - A new progressive alignment is built based on the previous alignment and the changed tree nodes
    - The previous alignment is retained for each subtree where there are no changes in the branching order.
      - New alignments are computed only for the changed nodes (if there are any)

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Algorithm Overview

- **Stage 2**: Improved progressive alignment built
  - *Progressive alignment*
    - When the alignment at the tree's root completes, the algorithm can:
      - Terminate
      - Return to the first step of Stage 2
      - Move on to Stage 3

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Algorithm Overview

- **Stage 3**: Iterative refinement performed
  - This stage is also iterative.
  - Iterative refinement is performed using partitioning of the tree.
  - Steps:
    - *Choice of bipartition*
    - *Profile extraction*
    - *Re-alignment*
    - *Acceptance / Rejection*

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Algorithm Overview

- **Stage 3**: Iterative refinement performed
  - *Choice of bipartition*
    - An edge is deleted from the tree, partitioning the tree into two disjoint subtrees
    - For iteration, edges are visited beginning farthest away from the root, progressing closer to the root.

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Algorithm Overview

- **Stage 3**: Iterative refinement performed
  - *Profile extraction*
    - The profile (MSA) of each of the two partitions is extracted from the overall MSA
    - Columns containing no residues (that is, only indels/gaps) are discarded

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Algorithm Overview

- **Stage 3**: Iterative refinement performed
  - *Re-alignment*
    - The two profiles obtained in the previous step are aligned using profile-profile alignment

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Algorithm Overview

- **Stage 3**: Iterative refinement performed
  - *Acceptance / Rejection*
    - The **SP score** of the MSA derived from the new profile-profile alignment is calculated.
      - If the score is greater than the previous alignment's score, the new MSA is kept.
      - Otherwise, it is discarded.

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Algorithm Overview

- **Stage 3**: Iterative refinement performed
  - *Acceptance / Rejection*
    - If either:
      - All edges have been visited
      - A user-defined number of maximum iterations has been reached

    then the algorithm terminates, otherwise it returns to the first step in Stage 3.

"*Visiting edges in order of decreasing distance from the root has the effect of first re-aligning individual sequences, then closely related groups of sequences.*"

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Additional Algorithm Elements

Implementation

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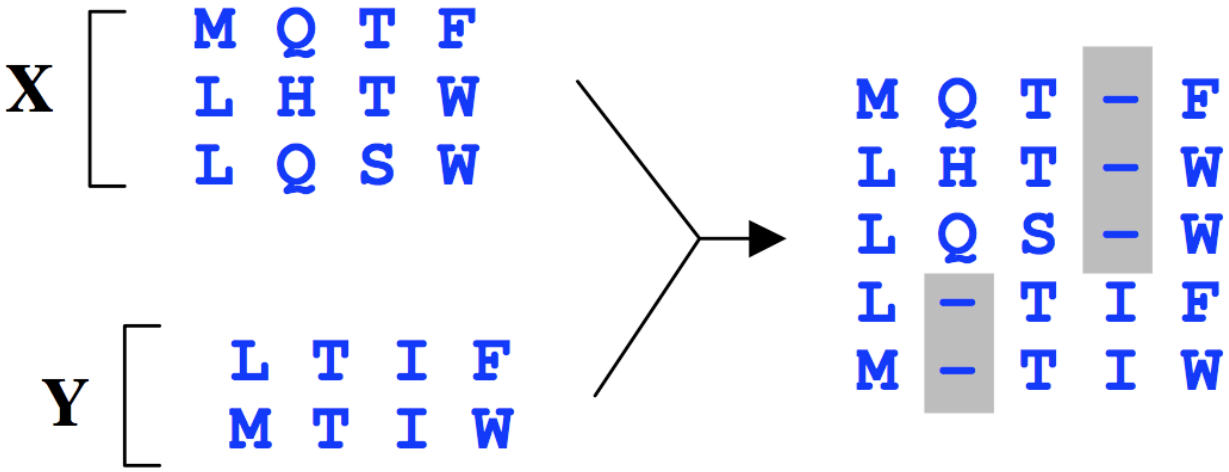
Additional Algorithm Elements

- Profile functions
  - To align profiles, a scoring function must be defined for a pair of profile positions (MSA columns)
    - Analogous to a substitution matrix for residues



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Additional Algorithm Elements

- Profile functions
  - PSP is the method used by CLUSTALW and MAFFT for this purpose, and it is also used by MUSCLE, as based on PAM 200 and VTML 240
  - Additionally, MUSCLE uses LE – log-expectation, a modified version of log-average (LA), which uses probabilities computed from VTML 240

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Additional Algorithm Elements

- Tree comparison
  - In progressive alignment, two trees with the same branching order and the same sequences at their leaves produce identical alignments
  - When a new tree is constructed in Stage 2, the subtrees that are unchanged do not have to have their alignments re-computed.
    - Progressive alignments only have to be computed for the changed subtrees, yielding an alignment at the root that would be identical to the alignment constructed without retaining previous alignments

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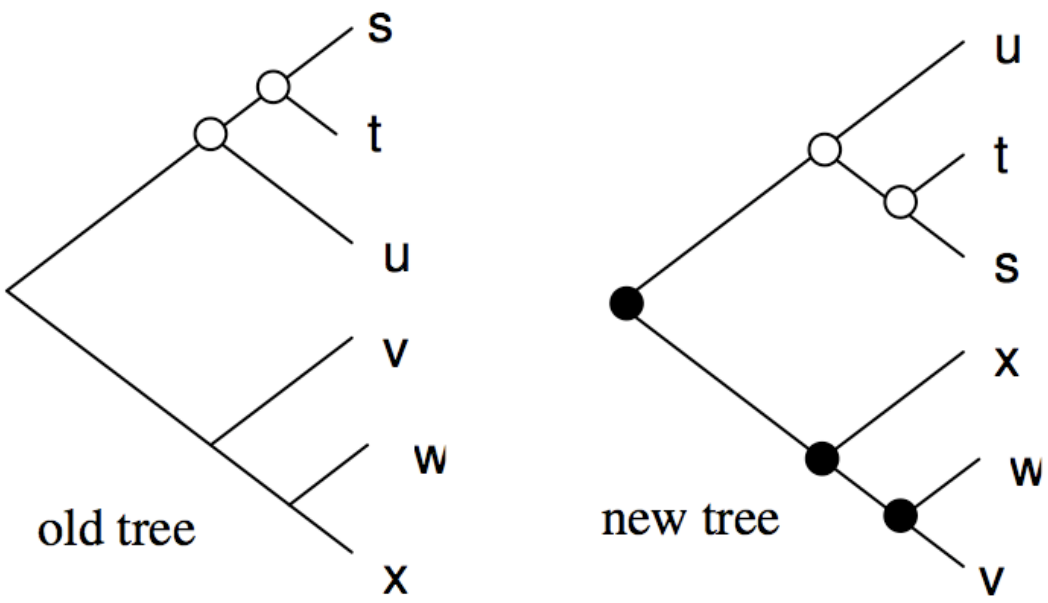
Additional Algorithm Elements

- Tree comparison



Implementation

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Additional Algorithm Elements

- Sequence weighting
  - Sequences should be weighted to correct for biased sampling from a family of related proteins
  - No consensus on how this is best done
  - MUSCLE allows multiple methods of sequence weighting:
    - None (sequences not weighted)
    - Henikoff
    - PSI-BLAST
    - CLUSTALW's GSC
    - The three-way method

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Additional Algorithm Elements

- Sequence weighting
  - Author found weighting to yield a small improvement in accuracy against the benchmarks (1% against BAliBASE)
    - Little difference between the weighting methods (excluding none)
    - CLUSTALW's GSC reduces the algorithm's complexity, so it is used by default

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Defaults, optimizations, and complexity

Implementation

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Defaults, optimizations, and complexity

- There are a variety of options in MUSCLE for improving accuracy or improving complexity
- The algorithm's default options provide the most accurate results, as tested against the four benchmarks

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Results

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Results

- MUSCLE provides a number of options that trade-off accuracy and time-space complexity
- Three sets of options were tested:
  - Full **MUSCLE**, including all three stages with default options
  - **MUSCLE-prog**: Stages 1 and 2 only with default options
  - **MUSCLE-fast**: Stage 1 only using the fastest options

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Results

Table 2: Complexity of MUSCLE. Here we show the big-O asymptotic complexity of the elements of MUSCLE as a function of *L*, the typical sequence length, and *N*, the number of sequences, retaining the highest-order terms in *N* with *L* fixed and vice versa.

Step	O(Space)	O(Time)
K-mer distance matrix	$N^2 + L$	N^2L
UPGMA	N^2	N^2
Progressive (one iteration)	$L_P^2 = NL + L^2$	$L_P^2 = N^2 + L^2$
Progressive (root alignment)	$NL_P = N^2 + NL$	$NL_P \log N = N^2 \log N + NL \log N$
Progressive (*N* iterations + root)	$N^2 + NL + L^2$	$N^3 + NL^2$
Refinement (one edge)	$NL_P + L_P^2 = N^2 + L^2$	$N^2L_P + L_P^2 = N^3 + L^2$
Refinement (*N* edges)	$N^2 + L^2$	$N^4 + NL^2$
TOTAL	$N^2 + L^2$	$N^4 + NL^2$

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Results

Table 3: Accuracy scores. The average accuracy, measured by the Q score, is reported for each method on each set of reference alignments.

Method	PREFAB	BAliBASE	SABmark	SMART
MUSCLE	0.648	0.896	0.430	0.856
MUSCLE-prog	0.634	0.883	0.427	0.837
FFTNS1	0.619	0.844	0.376	0.815
MUSCLE-fast	0.616	0.849	0.396	0.813
CLUSTALW	0.588	0.860	0.404	0.823
POA-blast	0.577	0.839	0.352	0.788
POA	0.576	0.834	0.280	0.797

Note that the methods are ranked from highest to lowest scores *using the author's benchmark, PREFAB*

The full MUSCLE algorithm, as well as MUSCLE-prog, still outperform the other methods for all four benchmarks for accuracy

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Results

Table 4: CPU times. The total CPU time required to create all alignments in each test set, measured in seconds on a 2.5 GHz Pentium 4 desktop computer.

Method	PREFAB	BAliBASE	SABmark	SMART
MUSCLE-fast	540	11	45	30
FFTNS1	720	16	70	46
MUSCLE-prog	3,000	52	429	180
MUSCLE	11,000	81	1,500	560
POA-blast	11,000	90	290	670
CLUSTALW	15,000	160	210	480
POA	24,000	130	380	880

Note, again, that the methods are ranked from highest to lowest scores *using the author's benchmark, PREFAB*

MUSCLE-fast outperforms all the others for speed in all four benchmarks, however, other methods (e.g. CLUSTALW) outperform the full MUSCLE algorithm in two of the four benchmarks – of particular note is SABmark, where CLUSTALW significantly outperforms MUSCLE, while their accuracy scores are quite similar (see previous slide)

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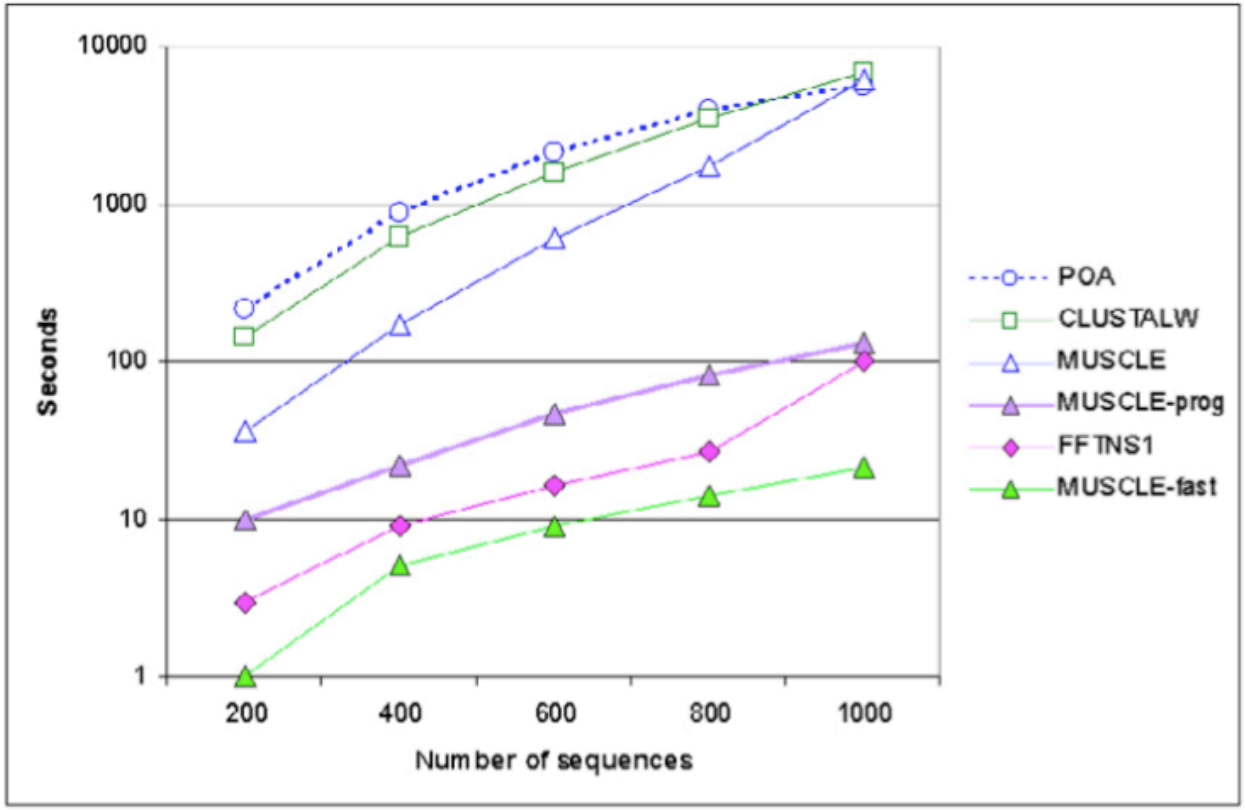
Results



Execution time vs. number of input sequences

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Conclusions

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Conclusions

- *"MUSCLE demonstrates improvements in accuracy and reductions in computational complexity…"*
  - **My thoughts**:
    - We must note that there is still typically a trade-off between the two, as the data shows.
    - MUSCLE's various options make it a very powerful tool, and its accuracy at its default settings add to its strength.
    - However, even the benchmarks demonstrate that other methods, such as CLUSTALW, can rival its processing time, and MUSCLE's high throughput configuration, MUSCLE-fast, is outperformed in accuracy by half of the other methods.

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