# Lecture 5: Multiple sequence alignment

## Introduction to Computational Biology

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(with some additions by Martin Vingron)

# Why do we need multiple sequence alignment

Pairwise sequence alignment for more distantly related sequences is not reliable

- it depends on gap penalties, scoring function and other details

- There may be many alignments with the same score – which is right?

- Discovering conserved motifs in a protein family

# NCBI

## Conserved Domains

| HOME | SEARCH | SITE MAP | Entrez | CDD | Structure | Protein | Help |
|------|--------|----------|--------|-----|-----------|---------|------|

### pfam00173: Cyt-b5

Cytochrome b5-like Heme/Steroid binding domain. This family includes heme binding domains from a diverse range of proteins. This family also includes proteins that bind to steroids. The family includes progesterone receptors. Many members of this subfamily are membrane anchored by an N-terminal transmembrane alpha helix. This family also includes a domain in some chitin synthases. There is no known ligand for this domain in the chitin synthases.

**➕ Links**

**➕ Statistics**

**➕ Structure View**

**Other Related Conserved Domains** ⊡

`COG4892`

## Sequence Alignment

| Reformat | Format: Hypertext ▾ | Row Display: up to 10 ▾ | Color Bits: 2.0 bits ▾ | Type Selection: the most diverse members ▾ |
|----------|---------------------|-------------------------|------------------------|---------------------------------------------|

```
                        10        20        30        40        50        60        70        80
                ....*....|....*....|....*....|....*....|....*....|....*....|....*....|....*....|
1LTD_A             5  KISPAEVAKHN--KPDDCWVVINGYVYDLTR-FLPNHPGG--------------QDVIKFNAGKDVTAIF---------- 57
gi 75050696       62  DFTPAELRRFDgvQDPRILMAINGKVFDVTKgRKFYGPEG--------------PYGVFAGRDASRGLatfcldkeal 125
gi 75024827       64  DMTVEELRKYDgvKNEHILFGLNGTIYDVTR-GKGFYGPG--------------KAYGTLAGHDATRALgtmdqnavss 127
gi 91206848     1290  YVRRADMENLL--LDGSRCIILAGYVCDLSG-YNCESETL--------------RSVLDSGLGKDLTAEMs--------- 1343
gi 74739702     1209  LIRKADLENHN--KDGGFWTVIDGKVYDIKD-FQTQSLTG--------------NSILAQFAGEDPVVAL---------- 1261
gi 91206849     1210  LIRKADLENHN--KDGGFWTVIDGKVYDIKD-FQTQSLTG--------------NSILAQFAGEDPVVAL---------- 1262
gi 74582634      303  YYNWTDI--HE---PGTSLMVFNGNVLDLSR-LRYLTPNIplpiq-----iaqiVGPGSAFIGRDATYWLs--------- 362
gi 5921760       407  YFTWADIRNNS-----RNLFVYSGNVLDLDL-LFWFNRDQvniprrfeelrdknNAANRAIRGRDATRTF---------- 470
gi 44889038      372  YFTWDDIKNSS-----RNLVVYSGHVLDLDL-LHWFNDTQvtyparfkelrdknTAGNQAIRGRDITHAF---------- 435
gi 122065155     402  QVSLQWNNVTD---PARNLAVYRGSVLDLNR-LNNLTTGLsypel-----ydtlKRRNDSWAGRDVTSAV---------- 462

                        90       100       110
                ....*....|....*....|....*....|....*..
```

# **Multiple alignment as generalization of pairwise alignment**

$S^1, S^2, \ldots, S^k$ a set of sequences over the same alphabet

As for the pair-wise alignment, the goal is to find alignment that maximizes some scoring function:

M Q P I L LP

M L R – L- P

M P V I L KP

How to score such multiple alignment?

# **Sum of pairs (SP) score**

Example consider all pairs of letters in each column and add the scores:

$$\text{SP-score}\begin{pmatrix} A \\ V \\ V \\ - \end{pmatrix} =$$

score(A,V)+score(V,V)+score(V,-)+score(A,-)+score(A,V)

k sequences gives k(k-1)/2 summands.

Individual pair-scores may come, e.g., from the Dayhoff matrix.

Gap scoring: Score(-,-) = 0

# Sum of pairs is not the perfect scoring system

No theoretical justification for the score.

- In the example below identical pairs are scored 1 and different 0.

```
A   A   A   A
A   A   A   A
A   A   A   A
A   A   A   I
A   A   I   I
A   I   I   I
------------------------
15  10  7   6
```

# **Entropy based score (minimum)**

$$-\sum_{j}(c_j/C) \log (c_j/C)$$

$c_j$- number of occurrence of amino-acid j in the column

C – number of symbols in the column

```
A  A   A   A   A
A  A   A   A   I
A  A   A   A   K
A  A   A   I   L
A  A   I   I   S
A  I   I   I   W
----------------------
0  .44  .65  .69  1.79
```

(in the example natural ln)

# Dynamic programming solution for multiple alignment

Recall recurrence for multiple alignment:

$$\text{Align}(S^1_i, S^2_j) = \max \begin{cases} \text{Align}(S^1_{i-1}, S^2_{j-1}) + s(a_i, a_j) \\ \text{Align}(S^1_{i-1}, S^2_j)\ -g \\ \text{Align}(S^1_i, S^2_{j-1})\ -g \end{cases}$$

For multiple alignment, under maximization we have all possible combinations of matches and gaps on the last position

For k sequences dynamic programming table will have size $n^k$

# Recurrence for 3 sequences

$$\text{Align}(S^1_i, S^2_j, S^3_k) = \max \begin{cases} \text{Align}(S^1_{i-1}, S^2_{j-1}, S^3_{k-1}) + s(a_i, a_j, a_k) \\ \text{Align}(S^1_{i-1}, S^2_j, S^3_{k-1}) + s(a_i, -, a_k) \\ \text{Align}(S^1_i, S^2_{j-1}, S^3_{k-1}) + s(-, a_j, a_k) \\ \text{Align}(S^1_{i-1}, S^2_{j-1}, S^3_k) + s(a_i, a_j, -) \\ \text{Align}(S^1_i, S^2_j, S^3_{k-1}) + s(a_i, -, -) \\ \text{Align}(S^1_i, S^2_{j-1}, S^3_k) + s(-, a_j, -) \\ \text{Align}(S^1_{i-1}, S^2_j, S^3_k) + s(-, -, a_k) \end{cases}$$

optimal

(0,0,0)

(n,n,n)
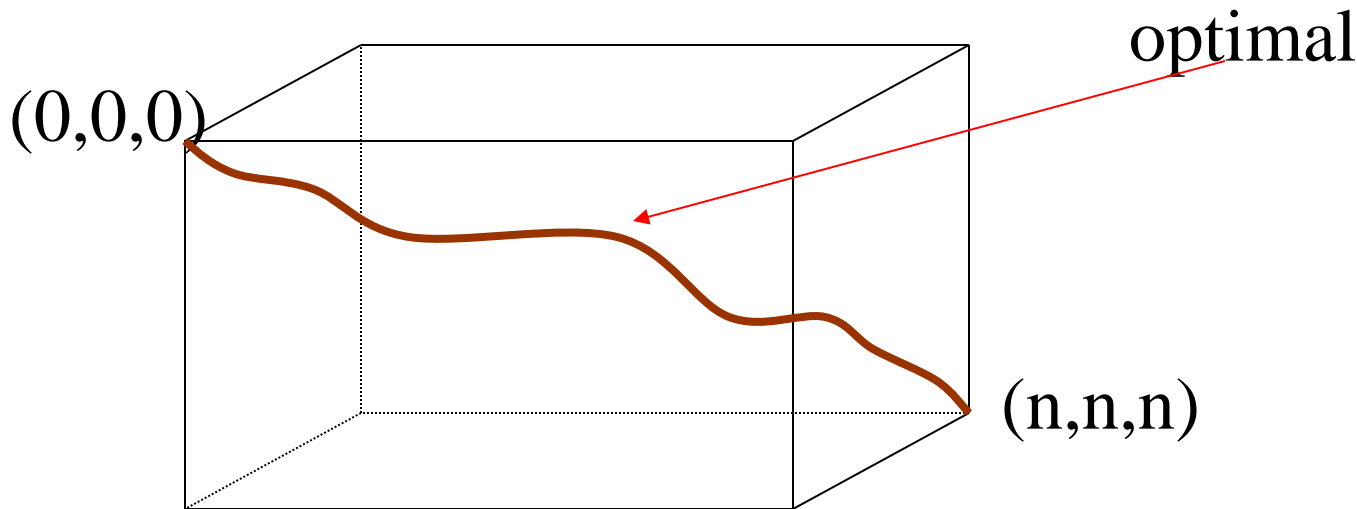
# **In dynamic programming approach running time grows exponentialy with the number of sequences**

- Two sequences $O(n^2)$
- Three sequences $O(n^3)$
- k sequences $O(n^k)$

Some approaches to accelerate computation:

- Use only part of the dynamic programming table centered along the diagonal (like banded alignment)
- Branch and bound
- Use heuristic solutions

# Understanding the problem better …

- The pairwise optimal alignments may not work in the context of the multiple alignment
- The pair-projections will score (a bit?) worse than the optimal pair alignment

# Heuristic approaches to multiple sequence alignment

- Heuristic methods:

  - Star alignment
  - Progressive alignment methods
    CLUSTALW
    T-Coffee
    MUSCLE
    SATé
  - Heuristic variants of Dynamic Programming Approach
  - Genetic algorithms
  - Gibbs sampler
  - Branch and bound

# Star alignment - using pairwise alignment for heuristic multiple alignment

- Choose one sequence to be the center
- Align all pair-wise sequences with the center
- Merge the alignments: use the center as reference.
- Rule "once a gap always a gap"

```
ACT       ACT   A-CT      ACT
TCT      -C T   ATCT      ACT
```

First merging:          Second merging      third merging

```
ACT              A-CT              A-CT
TCT              T-CT              T-CT
-CT              - -CT             --CT
                 ATCT              ATCT

                                   A-CT
```
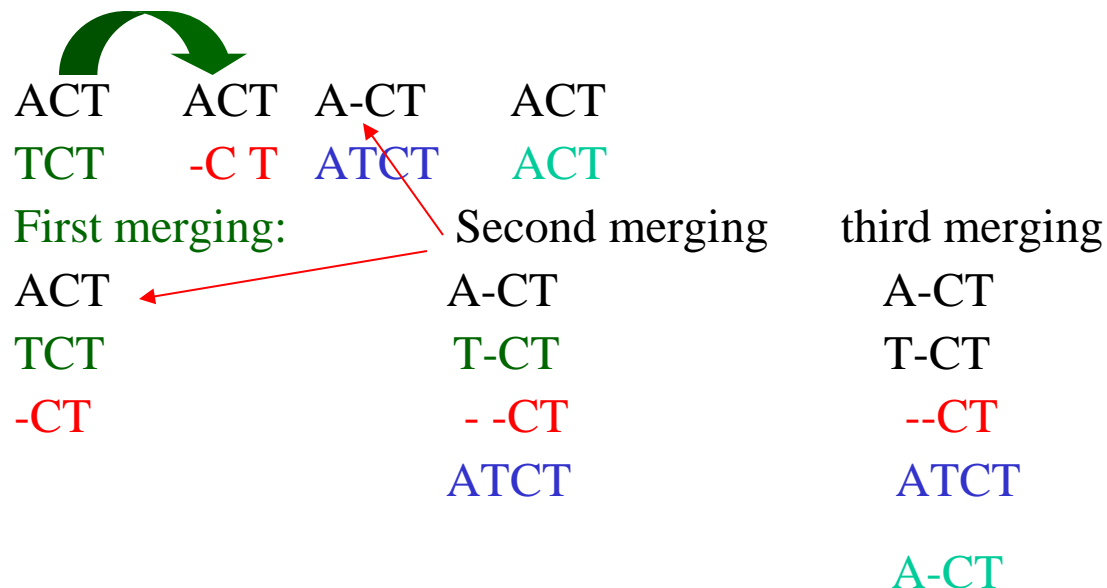
# **Merging the sequences in stair alignment :**

- Use the center as the "guide" sequence
- Add iteratively each pair-wise alignment to the multiple alignment
- Go column by column:
  - If there is no gap neither in the guide sequence in the multiple alignment nor in the merged alignment (or both have gaps) simply put the letter paired with the guide sequence into the appropriate column (all steps of the first merge are of this type.
  - If pair-wise alignment produced a gap in the guide sequence, force the gap on the whole column of already aligned sequences (compare second merge)
  - If there us a gap in added sequence but not in the guide sequences, keep the gap in the added sequence

# Larger example

```
ATTGCCATT
ATGGCCATT


ATTGCCATT--
ATC-CAATTTT
                                              ATTGCCATT--
                                              ATGGCCATT--
                                              ATC-CAATTTT
                                              ATCTTC-TT--
ATTGCCATT                                     ACTGACC----
ATCTTC-TT


ATTGCCATT
ACTGACC
```

# Two ways of choosing the center

1.  Try all possibilities and choose the resulting alignment that gives highest score; or

2.  Take sequence $S_c$ that maximizes

$$\sum_{i \text{ different than } c} \text{pairwise-score}(S_c, S_i)$$

(need to compute all pairwise alignments)

# Star alignment as 2-approximation of SOP alignment

- Assume a metric distance setup
- Then the score of the optimal (over all center choices) star-alignment is a 2-approximation for the SOP alignment

# Bounding the search space

- Carillo-Lipman bound – MSA program

# **Progressive alignment**

- Idea:
  - First align pair(s) of most closely related sequences
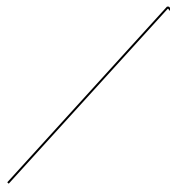  - Then interactively align the alignments to obtain an alignment for larger number of sequences

- T<span style="color:red">CT</span>

- -<span style="color:red">CT</span>

A -<span style="color:red">CT</span>

AT<span style="color:red">CT</span>

A - CT
ATCT
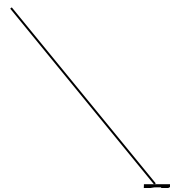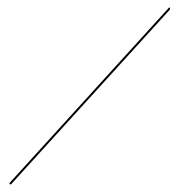
TCT
- CT
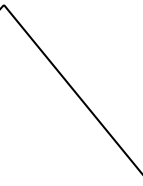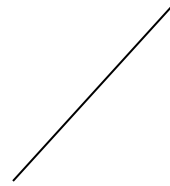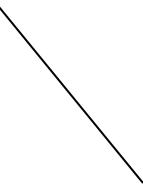
ACT     ATCT

TCT     CT

# **Aligning alignments**

Dynamic programming where a column in each alignment is treated as sequence element

| A | I | K | A |
|---|---|---|---|
| A | L | - | A |

| A | L |   | A |
|---|---|---|---|
| V | L | - | A |

Score of a match – score for the composite column

| A A | I L | K - | A A |

| | 0 | 0 | 0 | 0 | 0 |
|---|---|---|---|---|---|
| A V | 0 | | | | |
| L L | 0 | | | | |
| A A | 0 | | | | |

gap

gap

score for column
with I L L L

Gaps:

 as for sequences

Match for position (i,j):

 Alignment score for the
  column composed from
  colum i in the first
  sequence and column j in

 the second sequence

# Deciding on the order to merge the alignment

- You want to make most similar sequences first – you are less likely to miss-align them.

- After you align more sequences the alignment works like a profile and you know which columns are to be conserved in a given family – this helps in correct alignment of more distant family members

# CLUSTALW

1. Perform all pair pairwise alignments

2. Use the alignment score to produce distance based phylogenic tree *(phylogenic tree constructed methods will be presented later in class)*

3. Align sequences in the order defined by the tree: from the leaves towards the root.

   (Initially this involves alignment of sequences and later alignment of alignments.)

# Problems with CLUSTAL W and other "progressive alignments"

- Dependence of the initial pair-wise sequence alignment.

- Propagating errors form initial alignments.

# Example

*This and next figures examples are from T-coffee paper:*
*Noterdame, Higgins, Heringa, JMB 2000, 302 205-217*

a)Regular Progressive Alignment Strategy
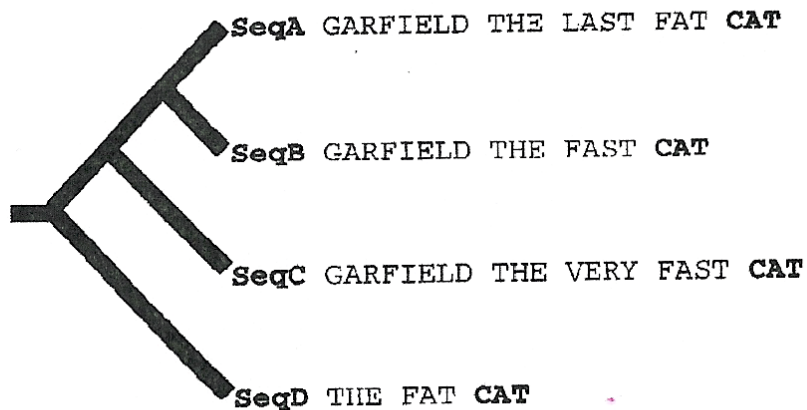
SeqA GARFIELD THE LAST FAT **CAT**

SeqB GARFIELD THE FAST **CAT**

SeqC GARFIELD THE VERY FAST **CAT**

SeqD THE FAT **CAT**

→

```
SeqA GARFIELD THE LAST FA-T CAT
SeqB GARFIELD THE FAST CA-T ---
SeqC GARFIELD THE VERY FAST CAT
SeqD ------- THE ---- FA-T CAT
```

# T-Coffee (Tree-Based Consistency Objective Function for alignment Evaluation)

- ## Construct a library of pair-wise alignments
  - In library each alignment is represented as a list of pair-wise residue matches (e.g.res.x sequence A is aligned with res. y of sequence B)
  - The weight of each alignment corresponds to percent identity (per aligned residua)

b)Primary Library

```
SeqA GARFIELD THE LAST FAT CAT      Prim. Weight = 88
SeqB GARFIELD THE FAST CAT ---
```

```
SeqB GARFIELD THE ---- FAST CAT     Prim Weight = 100
SeqC GARFIELD THE VERY FAST CAT
```

```
SeqA GARFIELD THE LAST FA-T CAT     Prim. Weight = 77
SeqC GARFIELD THE VERY FAST CAT
```

```
SeqB GARFIELD THE FAST CAT          Prim. Weight = 100
SeqD ------- THE FA-T CAT
```

```
SeqA GARFIELD THE LAST FAT CAT      Prim. Weight =100
SeqD ------- THE ---- FAT CAT
```
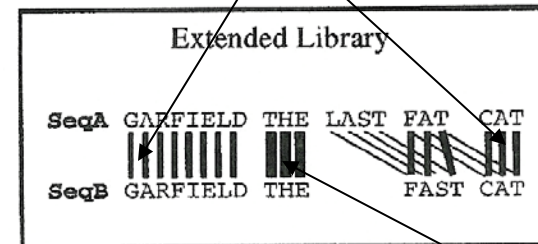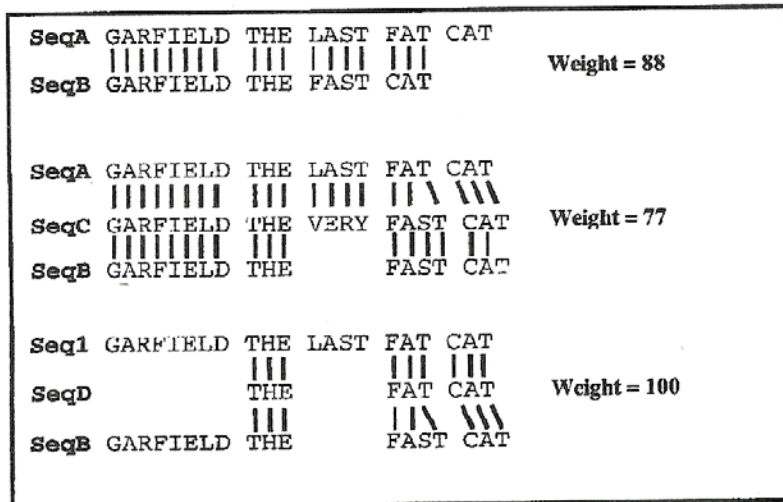
```
SeqC GARFIELD THE VERY FAST CAT     Prim. Weight = 100
SeqD ------- THE ---- FA-T CAT
```

# T-coffee continued

- Consistency alignment: for every pair-wise alignments (A,B) consider alignment with third sequence C. What would be the alignment "through" third sequence A-C-B

- Sum-up the weights over all possible choices if C to get "~~extended library~~"

c)Extended Library for seq1 and seq2



Consistent with 2 alignments

Consistent with 3 alignments (higher score for much)

# **Last step of T-coffee**

- Do progressive alignment using the tree but using the weights from extended library for scoring the alignment.

(e.g. "A" in FAST will have higher score with "A" in FAT and lower with "A" in LAST.)

# T-coffee summary

- More accurate than CLUSTALW
- Slower (significantly) the CLUSTALW but much faster than MSA and can handle more sequences.

# A newer consistency based approach

## ProbCons: Probabilistic consistency-based multiple sequence alignment

Chuong B. Do,[1] Mahathi S.P. Mahabhashyam,[1] Michael Brudno,[1] and Serafim Batzoglou[1,2]

[1]*Department of Computer Science, Stanford University, Stanford, California 94305, USA*

To study gene evolution across a wide range of organisms, biologists need accurate tools for multiple sequence alignment of protein families. Obtaining accurate alignments, however, is a difficult computational problem because of not only the high computational cost but also the lack of proper objective functions for measuring alignment quality. In this paper, we introduce *probabilistic consistency*, a novel scoring function for multiple sequence comparisons. We present ProbCons, a practical tool for progressive protein multiple sequence alignment based on probabilistic consistency, and evaluate its performance on several standard alignment benchmark data sets. On the BAliBASE, SABmark, and PREFAB benchmark alignment databases, ProbCons achieves statistically significant improvement over other leading methods while maintaining practical speed. ProbCons is publicly available as a Web resource.

[Supplemental material is available online at www.genome.org. Source code and executables are available as public domain software at http://probcons.stanford.edu.]

Genome research 2005

# MUSCLE

**Robert C. Edgar\*** Nucleic Acids Research,
2004, Vol. 32, No. 5 **1792-1797**

**MUSCLE: multiple sequence alignment
with high accuracy and high throughput**

# MUSCLE idea

- Build quick approximate sequence similarity tree – without pair-wise alignment but compute distances by computing the number of short "hits" (short gapless matching) between any pair of sequences.
- Compute MSA using the tree.
- Compute pair-wise distances from MSA and new tree
- Re-compute MSA using new tree
- Refine the alignment by iteratively partitioning the sequence into two groups and merging the aligning multiple alignment from the two groups

**Figure 2. This diagram summarizes the flow of the MUSCLE algorithm.**

Nucleic Acids Research
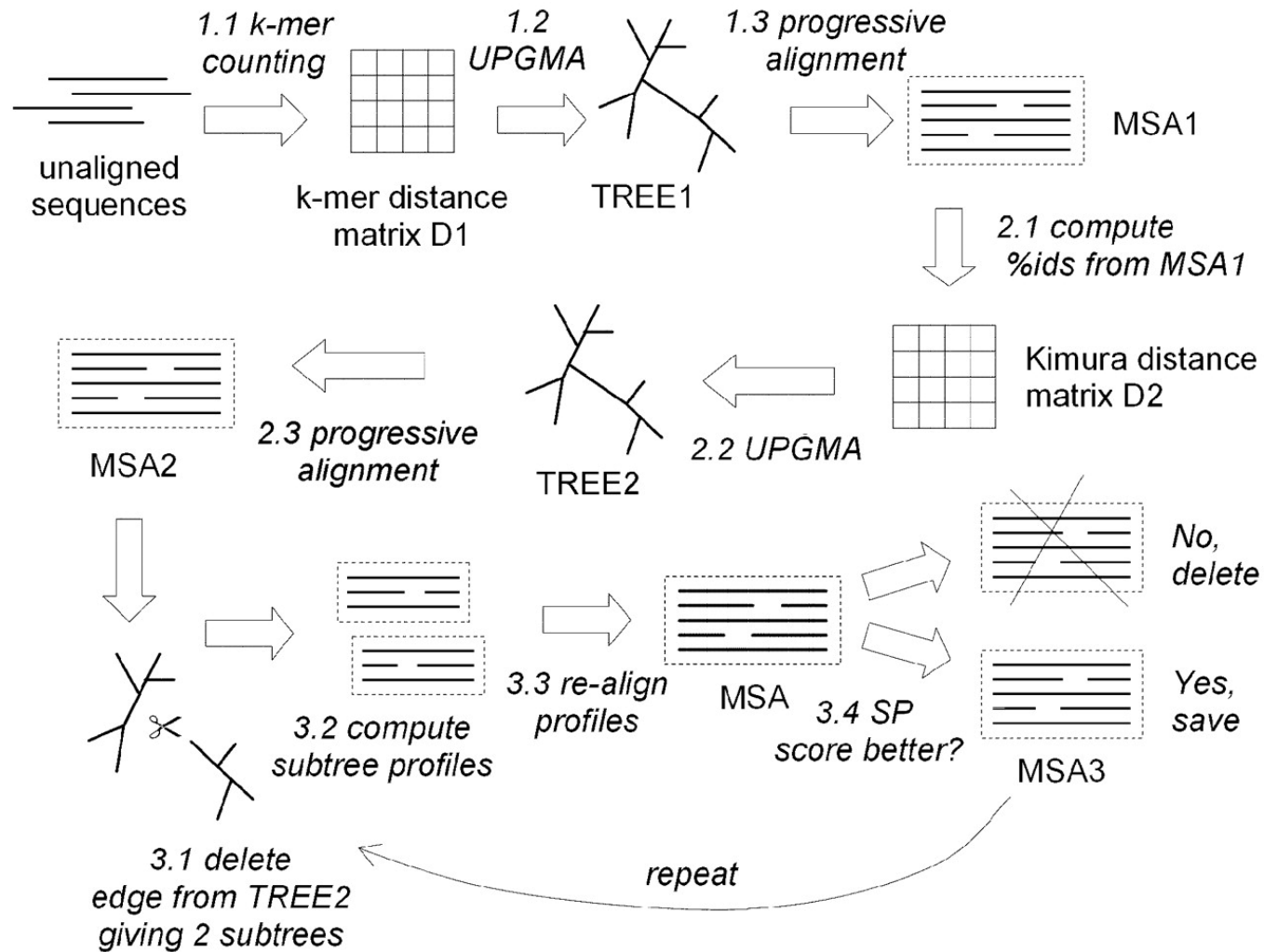
# **Where the speed-up comes from**

- Finding all short hits is fast due because  we can use methods like hashing

- ClustalW computed n(n-1)/2 pairwise alignments while given a tree one needs to do only n-1 alignments

# Simultaneous Alignment and Tree Estimation (SATé)

… is a software package for constructing phylogenies and alignments from sequence data. It is particularly useful for large datasets (hundreds to thousands of sequences), where it has been shown to produce more accurate alignments and trees than previous methods. (From the web page of Tandy Warnow)
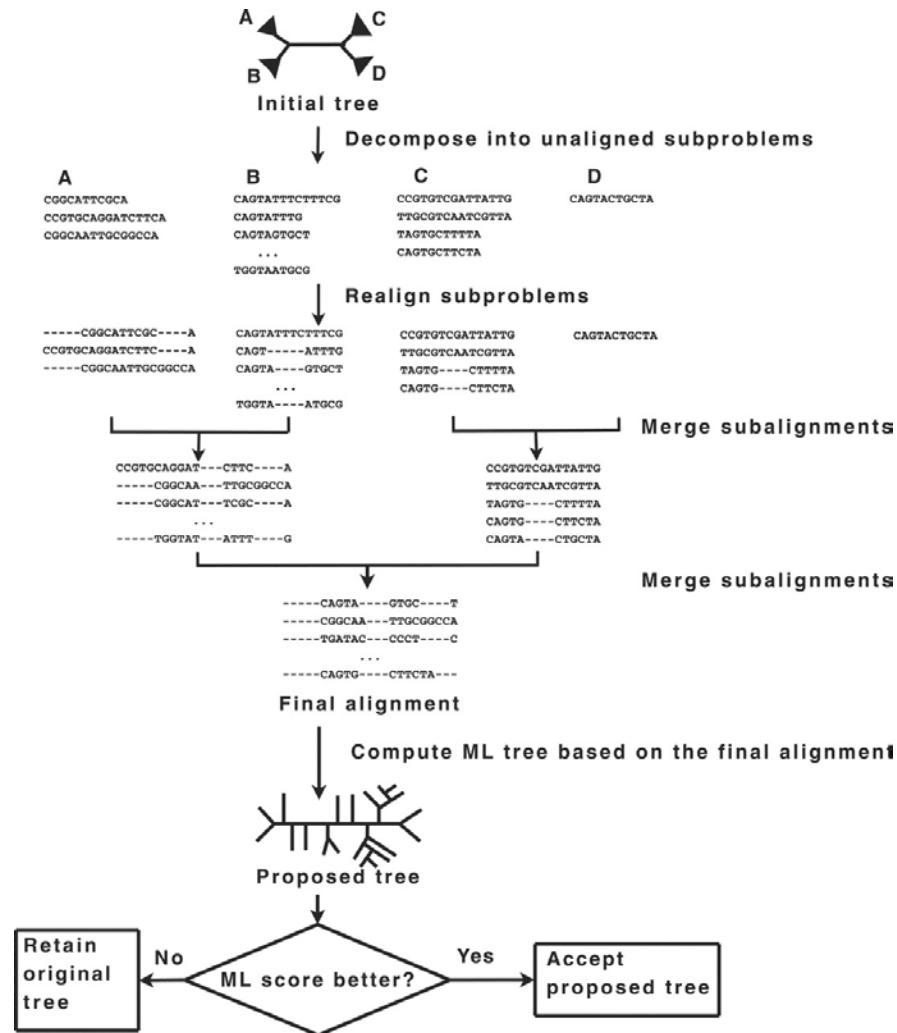
Successor program: PASTA … faster and more accurate, and can analyze larger datasets (up to 200,000 sequences). (also from the web site)

•**Fig. 1 SATé's second stage.**
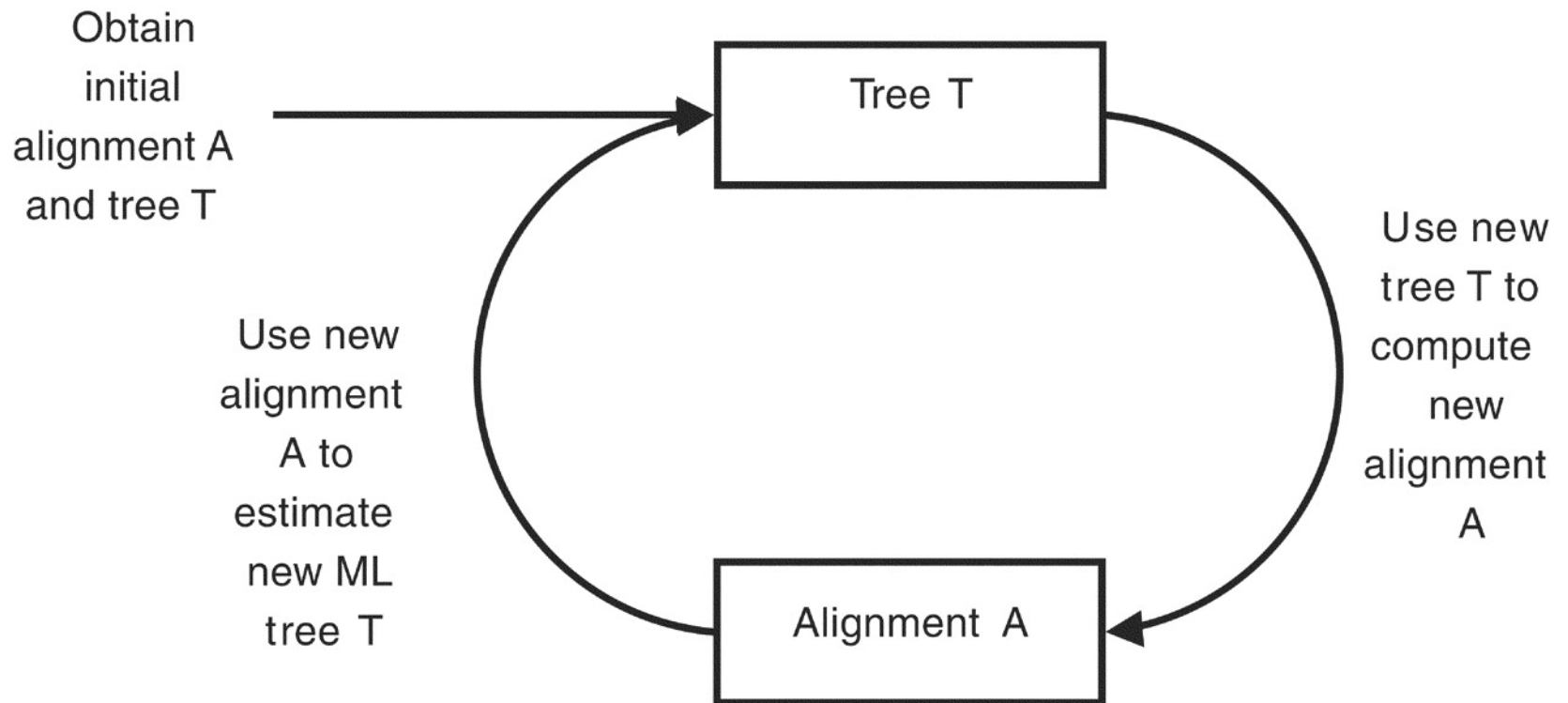
**•Fig. 2 SATé's divide-and-conquer strategy, illustrated with a CT-2 decomposition.**

# Refining multiple sequence alignment

- Given – multiple alignment of sequences
- Goal improve the alignment
- One of several methods:
  - Choose a random sentence
  - Remove from the alignment (n-1 sequences left)
  - Align the removed sequence to the n-1 remaining sequences.
  - Repeat
- Alternatively – (MUSCLE approach) the alignment set can be subdivided into two subsets, the alignment of the subsets recomputed and alignment aligned

# **Evaluating an MSA**

- Based on alignment of structures
  (e.g. BaliBase test set)
- Simulation: simulate random evolutionary changes
- Testing for correct alignment of annotated functional residues