International Bioinformatics Workshop on Molecular Epidemiology and Phylodynamics

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Multiple Sequence Alignment

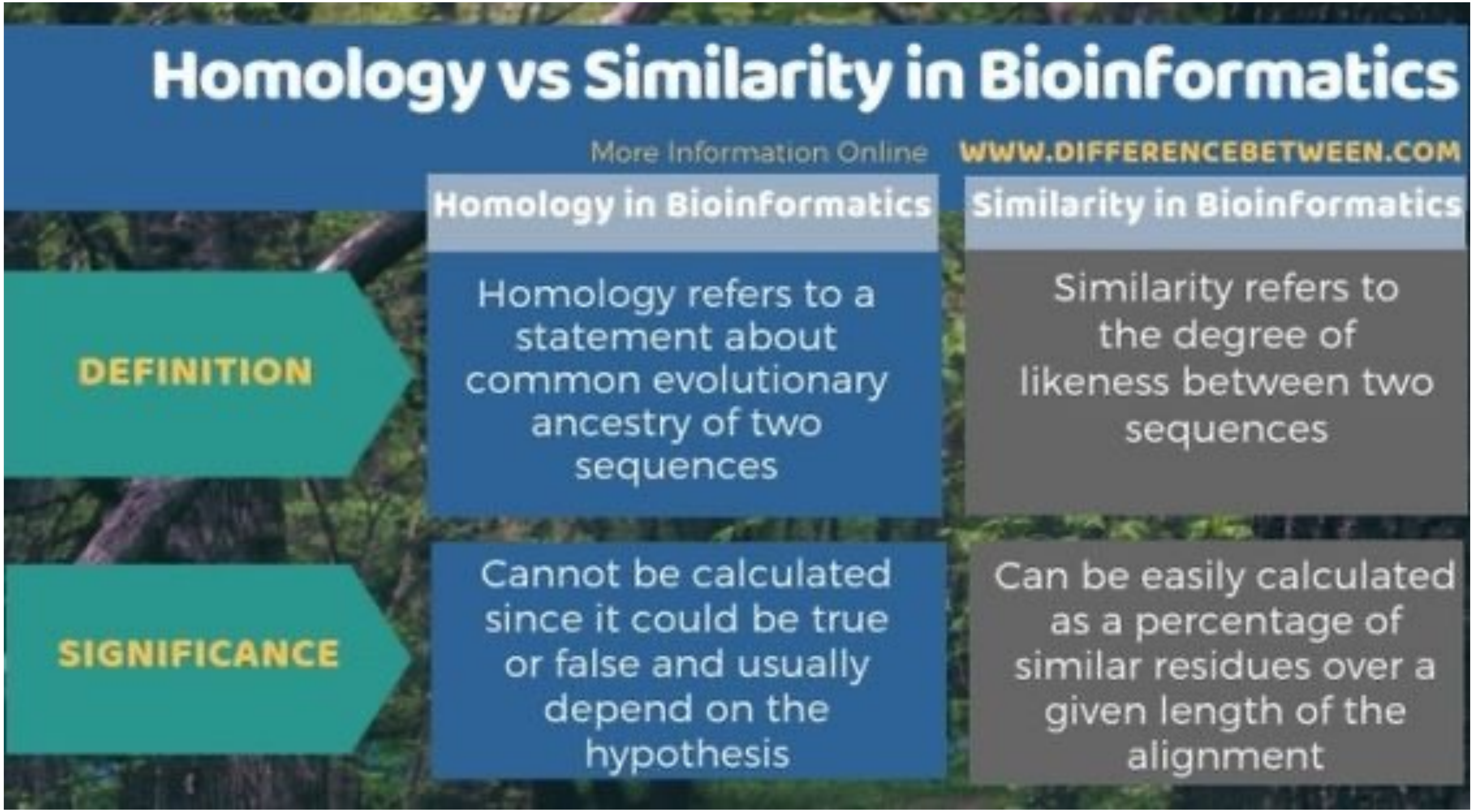
Learning Objectives

- Understand the algorithms behind multiple sequence alignment.
- Perform pairwise alignment and multiple sequence alignments using tools
  - EMBOSS (needle and water)
  - MAFFT

Outline

1. Homology v.s. Similarity
2. Global Alignment v.s. Local Alignment
3. Pairwise Alignment v.s. Multiple Sequence Alignment
4. Alignment methods
    - Longest common subsequence
    - Time Complexity
5. Sum of pairs for evaluating an alignment
6. Hands-on session

Homology v.s. Similarity



Homology vs Similarity in Bioinformatics

More Information Online WWW.DIFFERENCEBETWEEN.COM

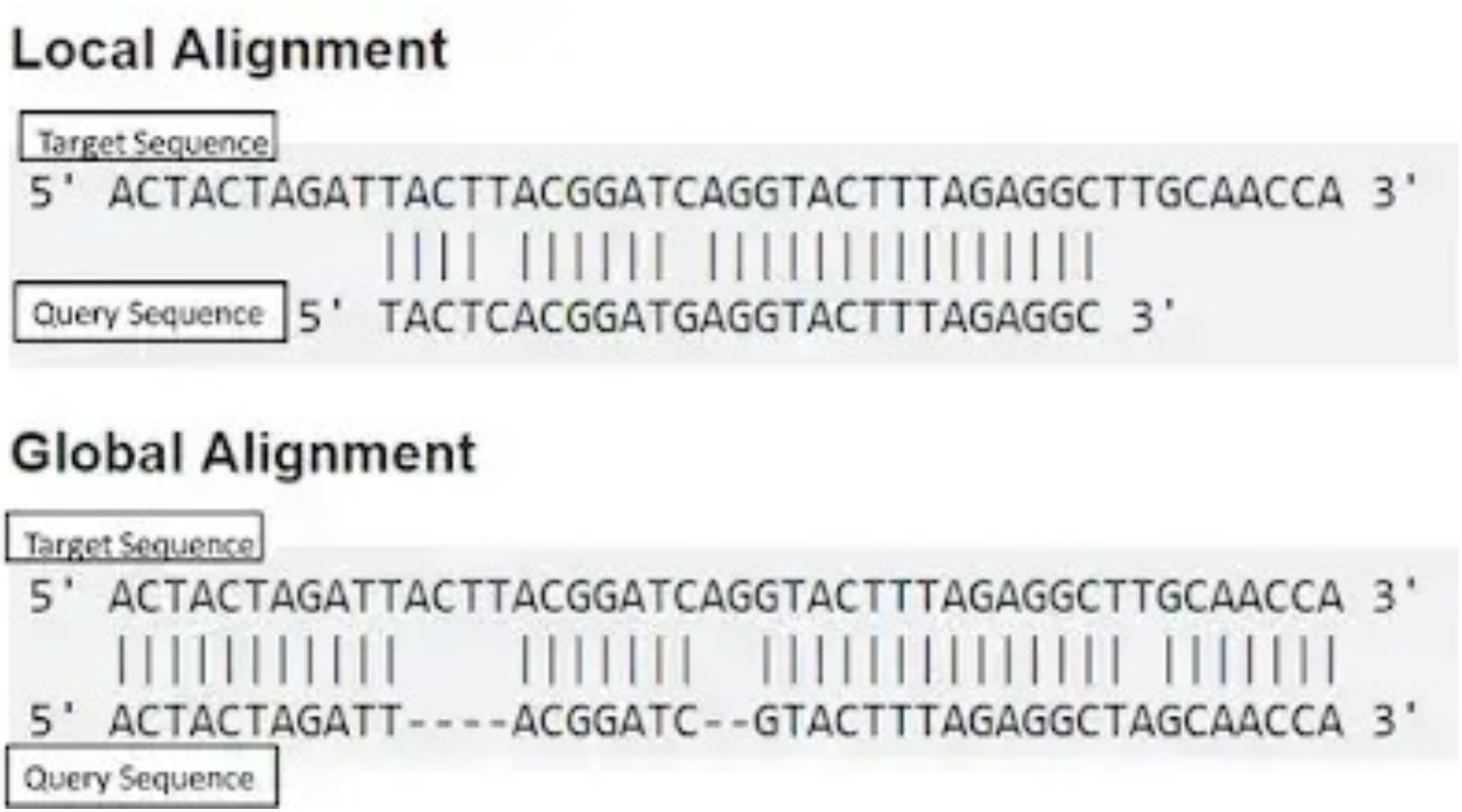
	Homology in Bioinformatics	Similarity in Bioinformatics
DEFINITION	Homology refers to a statement about common evolutionary ancestry of two sequences	Similarity refers to the degree of likeness between two sequences
SIGNIFICANCE	Cannot be calculated since it could be true or false and usually depend on the hypothesis	Can be easily calculated as a percentage of similar residues over a given length of the alignment

https://pin.it/76TSRgW

Global Alignment v.s. local Alignment

Global Alignment	Local Alignmennt
Purpose: Align the entire sequences	Find local regions with high similarity
Contains all nucletoides (or amino acids)	Align a substring of query to a substring of target
If two sequences are quite similar	Find stretches of sequences without considering the rest of sequence
Suitalbe for aligning two closely related sequences	Suitable for aligning more divergent sequences
Needleman-Wunsch algorithm (EMBOSS neelde)	Smith-Waterman algorithm (EMBOSS water)

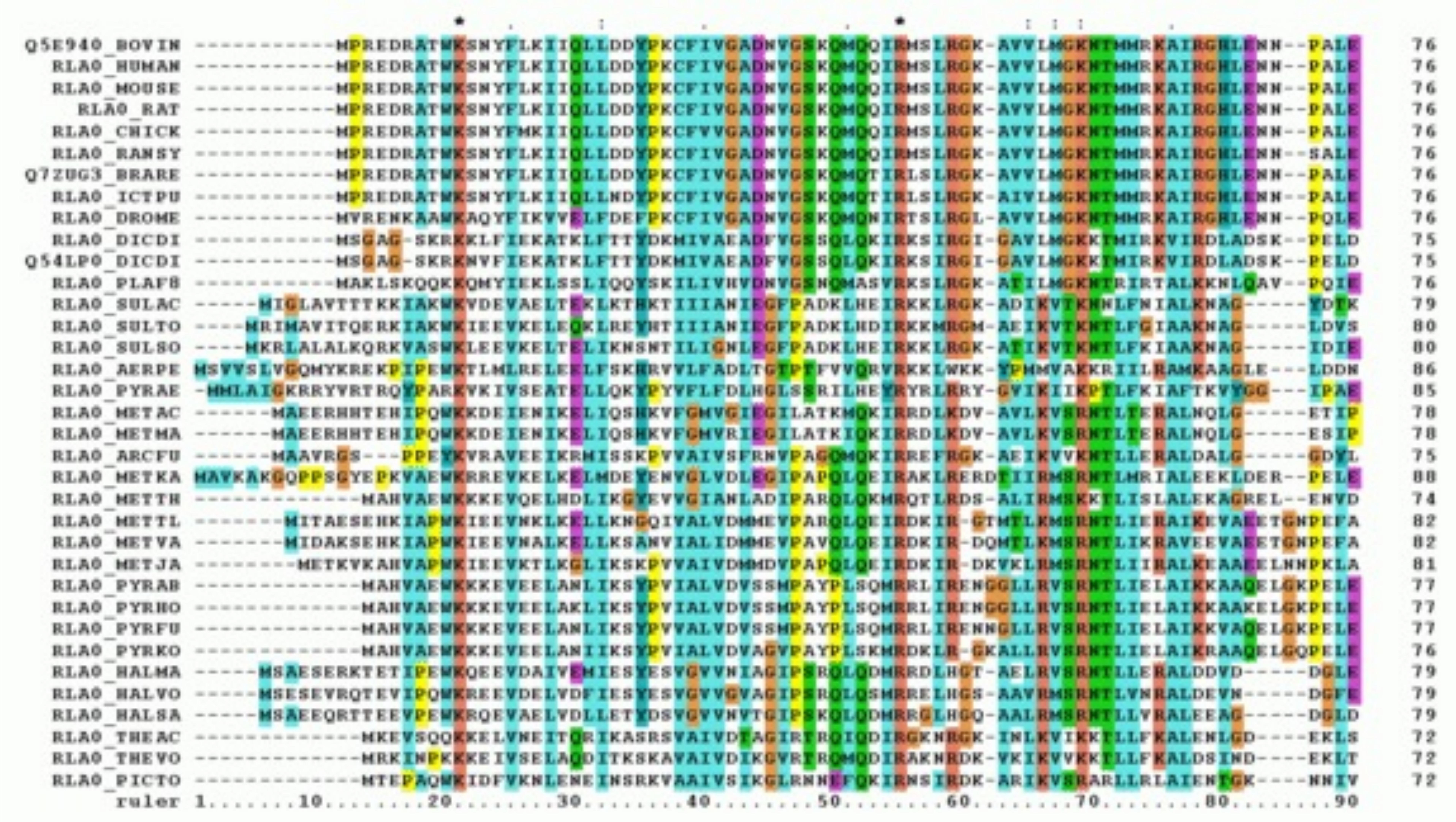
https://www.majordifferences.com/2016/05/difference-between-global-and-local.html#.VzizhtJ96M8



```
Local Alignment

Target Sequence
5' ACTACTAGATTACTTACGGATCAGGTACTTTAGAGGCTTGCAACCA 3'
             |||| |||||| |||||||||||||||
Query Sequence
          5' TACTCACGGATGAGGTACTTTAGAGGC 3'

Global Alignment

Target Sequence
5' ACTACTAGATTACTTACGGATCAGGTACTTTAGAGGCTTGCAACCA 3'
   |||||||||||    |||||||  |||||||||||||| |||||||
5' ACTACTAGATT----ACGGATC--GTACTTTAGAGGCTAGCAACCA 3'
Query Sequence
```

https://www.majordifferences.com/2016/05/difference-between-global-and-local.html#.VzizhtJ96M8

Multiple Sequence Alignment



```
                                 *   .      :       .          .   *         : : :      .
Q5E940_BOVIN -----------MPREDRATWKSNYFLKIIQLLDDYPKCFIVGADNVGSKQMQQIRMSLRGK-AVVLMGKNTMMRKAIRGHLENN--PALE 76
  RLA0_HUMAN -----------MPREDRATWKSNYFLKIIQLLDDYPKCFIVGADNVGSKQMQQIRMSLRGK-AVVLMGKNTMMRKAIRGHLENN--PALE 76
  RLA0_MOUSE -----------MPREDRATWKSNYFLKIIQLLDDYPKCFIVGADNVGSKQMQQIRMSLRGK-AVVLMGKNTMMRKAIRGHLENN--PALE 76
    RLA0_RAT -----------MPREDRATWKSNYFLKIIQLLDDYPKCFIVGADNVGSKQMQQIRMSLRGK-AVVLMGKNTMMRKAIRGHLENN--PALE 76
  RLA0_CHICK -----------MPREDRATWKSNYFMKIIQLLDDYPKCFVVGADNVGSKQMQQIRMSLRGK-AVVLMGKNTMMRKAIRGHLENN--PALE 76
  RLA0_RANSY -----------MPREDRATWKSNYFLKIIQLLDDYPKCFIVGADNVGSKQMQQIRMSLRGK-AVVLMGKNTMMRKAIRGHLENN--SALE 76
Q7ZUG3_BRARE -----------MPREDRATWKSNYFLKIIQLLDDYPKCFIVGADNVGSKQMQTIRLSLRGK-AVVLMGKNTMMRKAIRGHLENN--PALE 76
  RLA0_ICTPU -----------MPREDRATWKSNYFLKIIQLLNDYPKCFIVGADNVGSKQMQTIRLSLRGK-AIVLMGKNTMMRKAIRGHLENN--PALE 76
  RLA0_DROME -----------MVRENKAAWKAQYFIKVVELFDEFPKCFIVGADNVGSKQMQNIRTSLRGL-AVVLMGKNTMMRKAIRGHLENN--PQLE 76
  RLA0_DICDI -----------MSGAG-SKRKKLFIEKATKLFTTYDKMIVAEADFVGSSQLQKIRKSIRGI-GAVLMGKKTMIRKVIRDLADSK--PELD 75
Q54LP0_DICDI -----------MSGAG-SKRKNVFIEKATKLFTTYDKMIVAEADFVGSSQLQKIRKSIRGI-GAVLMGKKTMIRKVIRDLADSK--PELD 75
  RLA0_PLAF8 -----------MAKLSKQQKKQMYIEKLSSLIQQYSKILIVHVDNVGSNQMASVRKSLRGK-ATILMGKNTRIRTALKKNLQAV--PQIE 76
  RLA0_SULAC -----MIGLAVTTTKKIAKWKVDEVAELTEKLKTHKTIIIANIEGFPADKLHEIRKKLRGK-ADIKVTKNNLFNIALKNAG-----YDTK 79
  RLA0_SULTO ----MRIMAVITQERKIAKWKIEEVKELEQKLREYHTIIIANIEGFPADKLHDIRKKMRGM-AEIKVTKNTLFGIAAKNAG-----LDVS 80
  RLA0_SULSO ----MKRLALALKQRKVASWKLEEVKELTELIKNSNTILIGNLEGFPADKLHEIRKKLRGK-ATIKVTKNTLFKIAAKNAG-----IDIE 80
  RLA0_AERPE MSVVSLVGQMYKREKPIPEWKTLMLRELEELFSKRRVVLFADLTGTPTFVVQRVRKKLWKK-YPHMVAKKRIILRAMKAAGLE---LDDN 86
  RLA0_PYRAE -MMLAIGKRRYVRTRQYPARKVKIVSEATELLQKYPYVFLFDLHGLSSRILHEYRYRLRRY-GVIKIIKPTLFKIAFTKVYGG---IPAE 85
  RLA0_METAC ------MAEERHHTEHIPQWKKDEIENIKELIQSHKVFGMVGIEGILATKMQKIRRDLKDV-AVLKVSRNTLTERALNQLG-----ETIP 78
  RLA0_METMA ------MAEERHHTEHIPQWKKDEIENIKELIQSHKVFGMVRIEGILATKIQKIRRDLKDV-AVLKVSRNTLTERALNQLG-----ESIP 78
  RLA0_ARCFU ------MAAVRGS---PPEYKVRAVEEIKRMISSKPVVAIVSFRNVPAGQMQKIRREFRGK-AEIKVVKNTLLERALDALG-----GDYL 75
  RLA0_METKA MAVKAKGQPPSGYEPKVAEWKRREVKELKELMDEYENVGLVDLEGIPAPQLQEIRAKLRERDTIIRMSRNTLMRIALEEKLDER--PELE 88
  RLA0_METTH -------------MAHVAEWKKKEVQELHDLIKGYEVVGIANLADIPARQLQKMRQTLRDS-ALIRMSKKTLISLALEKAGREL--ENVD 74
  RLA0_METTL -------MITAESEHKIAPWKIEEVNKLKELLKNGQIVALVDMMEVPARQLQEIRDKIR-GTMTLKMSRNTLIERAIKEVAEETGNPEFA 82
  RLA0_METVA -------MIDAKSEHKIAPWKIEEVNALKELLKSANVIALIDMMEVPAVQLQEIRDKIR-DQMTLKMSRNTLIKRAVEEVAEETGNPEFA 82
  RLA0_METJA --------METKVKAHVAPWKIEEVKTLKGLIKSKPVVAIVDMMDVPAPQLQEIRDKIR-DKVKLRMSRNTLIIRALKEAAEELNNPKLA 81
  RLA0_PYRAB -------------MAHVAEWKKKEVEELANLIKSYPVIALVDVSSMPAYPLSQMRRLIRENGGLLRVSRNTLIELAIKKAAQELGKPELE 77
  RLA0_PYRHO -------------MAHVAEWKKKEVEELAKLIKSYPVIALVDVSSMPAYPLSQMRRLIRENGGLLRVSRNTLIELAIKKAAKELGKPELE 77
  RLA0_PYRFU -------------MAHVAEWKKKEVEELANLIKSYPVVALVDVSSMPAYPLSQMRRLIRENNGLLRVSRNTLIELAIKKVAQELGKPELE 77
  RLA0_PYRKO -------------MAHVAEWKKKEVEELANIIKSYPVIALVDVAGVPAYPLSKMRDKLR-GKALLRVSRNTLIELAIKRAAQELGQPELE 76
  RLA0_HALMA -----MSAESERKTETIPEWKQEEVDAIVEMIESYESVGVVNIAGIPSRQLQDMRRDLHGT-AELRVSRNTLLERALDDVD-----DGLE 79
  RLA0_HALVO -----MSESEVRQTEVIPQWKREEVDELVDFIESYESVGVVGVAGIPSRQLQSMRRELHGS-AAVRMSRNTLVNRALDEVN-----DGFE 79
  RLA0_HALSA -----MSAEEQRTTEEVPEWKRQEVAELVDLLETYDSVGVVNVTGIPSKQLQDMRRGLHGQ-AALRMSRNTLLVRALEEAG-----DGLD 79
  RLA0_THEAC -------------MKEVSQQKKELVNEITQRIKASRSVAIVDTAGIRTRQIQDIRGKNRGK-INLKVIKKTLLFKALENLGD----EKLS 72
  RLA0_THEVO -------------MRKINPKKKEIVSELAQDITKSKAVAIVDIKGVRTRQMQDIRAKNRDK-VKIKVVKKTLLFKALDSIND----EKLT 72
  RLA0_PICTO -------------MTEPAQWKIDFVKNLENEINSRKVAAIVSIKGLRNNEFQKIRNSIRDK-ARIKVSRARLLRLAIENTGK----NNIV 72
       ruler 1........10........20........30........40........50........60........70........80........90
```

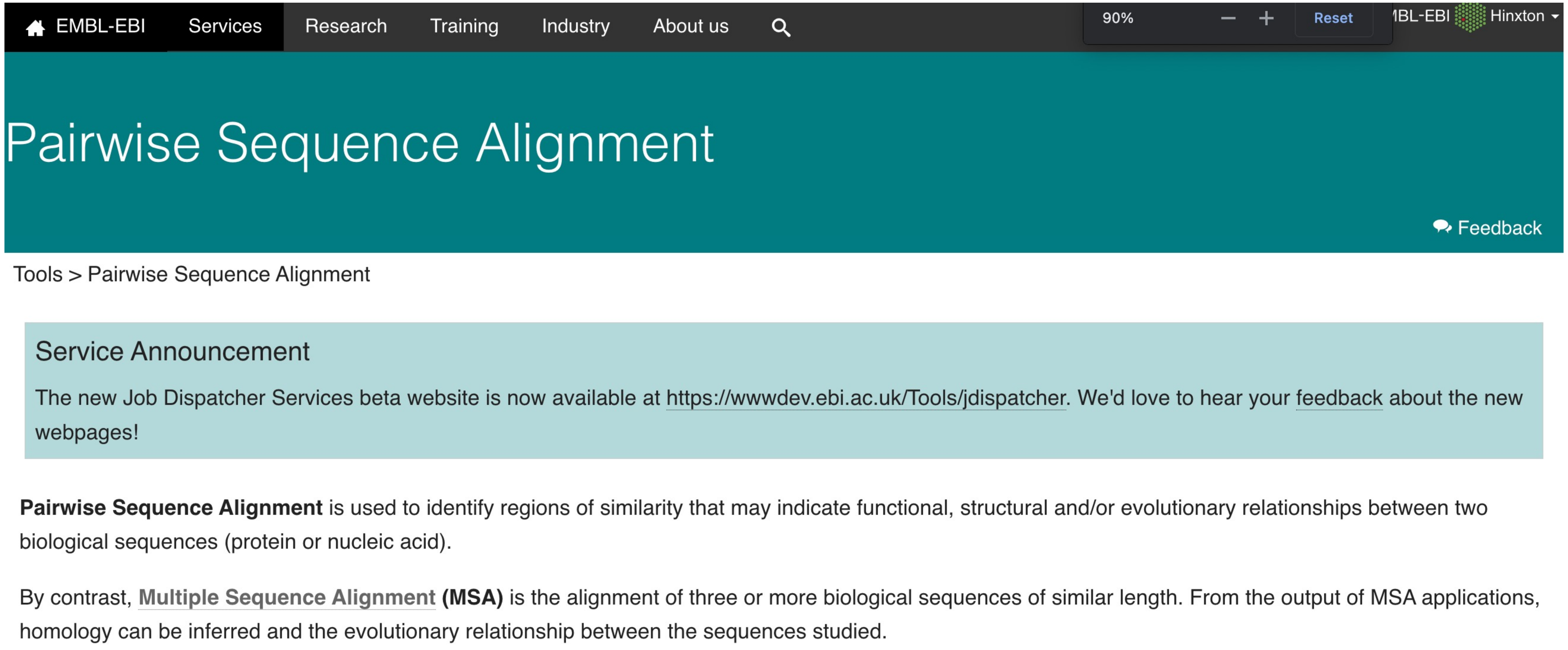
https://en.wikipedia.org/wiki/File:RPLP0_90_ClustalW_aln.gif

Pairwise Alignment v.s. Multiple Sequence Alignment

Pairwise Alignment	Multiple Sequence Alignmennt
two sequences	Three or more
Global or local alignment	Global
Comparatively simple algorithm	Complex sophisticated algorithm
Needleman-Wunsch algorithm (EMBOSS neelde), Smith-Waterman algorithm (EMBOSS water), and so on	Progressive alignment construction, Iterative methods, and so on
Applications: (1) find out conserved regions between two sequences; (2) similarity searches in a database	Applications: (1) find out regions of variability or conservation in a family; (2) similarity searches between a newly sequence and an existing gene family; (3) phylogenetic analysis

Online tools

https://www.ebi.ac.uk/Tools/psa/; https://www.ebi.ac.uk/Tools/msa/



Pairwise Sequence Alignment

Tools > Pairwise Sequence Alignment

Service Announcement

The new Job Dispatcher Services beta website is now available at https://wwwdev.ebi.ac.uk/Tools/jdispatcher. We'd love to hear your feedback about the new webpages!

Pairwise Sequence Alignment is used to identify regions of similarity that may indicate functional, structural and/or evolutionary relationships between two biological sequences (protein or nucleic acid).

By contrast, **Multiple Sequence Alignment** **(MSA)** is the alignment of three or more biological sequences of similar length. From the output of MSA applications, homology can be inferred and the evolutionary relationship between the sequences studied.

Global Alignment

Global alignment tools create an end-to-end alignment of the sequences to be aligned.

Needle (EMBOSS)

EMBOSS Needle creates an optimal global alignment of two sequences using the Needleman-Wunsch algorithm.

Launch Needle

Stretcher (EMBOSS)

EMBOSS Stretcher uses a modification of the Needleman-Wunsch algorithm that allows larger sequences to be globally aligned.

Launch Stretcher

GGSEARCH2SEQ

GGSEARCH2SEQ finds an optimal global alignment using the Needleman-Wunsch algorithm.

Launch ggsearch2seq

Local Alignment

Local alignment tools find one, or more, alignments describing the most similar region(s) within the sequences to be aligned. They are can align protein and nucleotide sequences.

Water (EMBOSS)

EMBOSS Water uses the Smith-Waterman algorithm (modified for speed enhancements) to calculate the local alignment of two sequences.

Launch Water

Matcher (EMBOSS)

EMBOSS Matcher identifies local similarities between two sequences using a rigorous algorithm based on the LALIGN application.

Launch Matcher

LALIGN

LALIGN finds internal duplications by calculating non-intersecting local alignments of protein or DNA sequences.

Launch LALIGN

SSEARCH2SEQ

SSEARCH2SEQ finds an optimal local alignment using the Smith-Waterman algorithm.

Launch ssearch2seq

Genomic Alignment

Genomic alignment tools concentrate on DNA (or to DNA) alignments while accounting for characteristics present in genomic data.

GeneWise

GeneWise compares a protein sequence to a genomic DNA sequence, allowing for introns and frameshifting errors.

Launch GeneWise

The tools described on this page are provided using **Search and sequence analysis tools services from EMBL-EBI in 2022**

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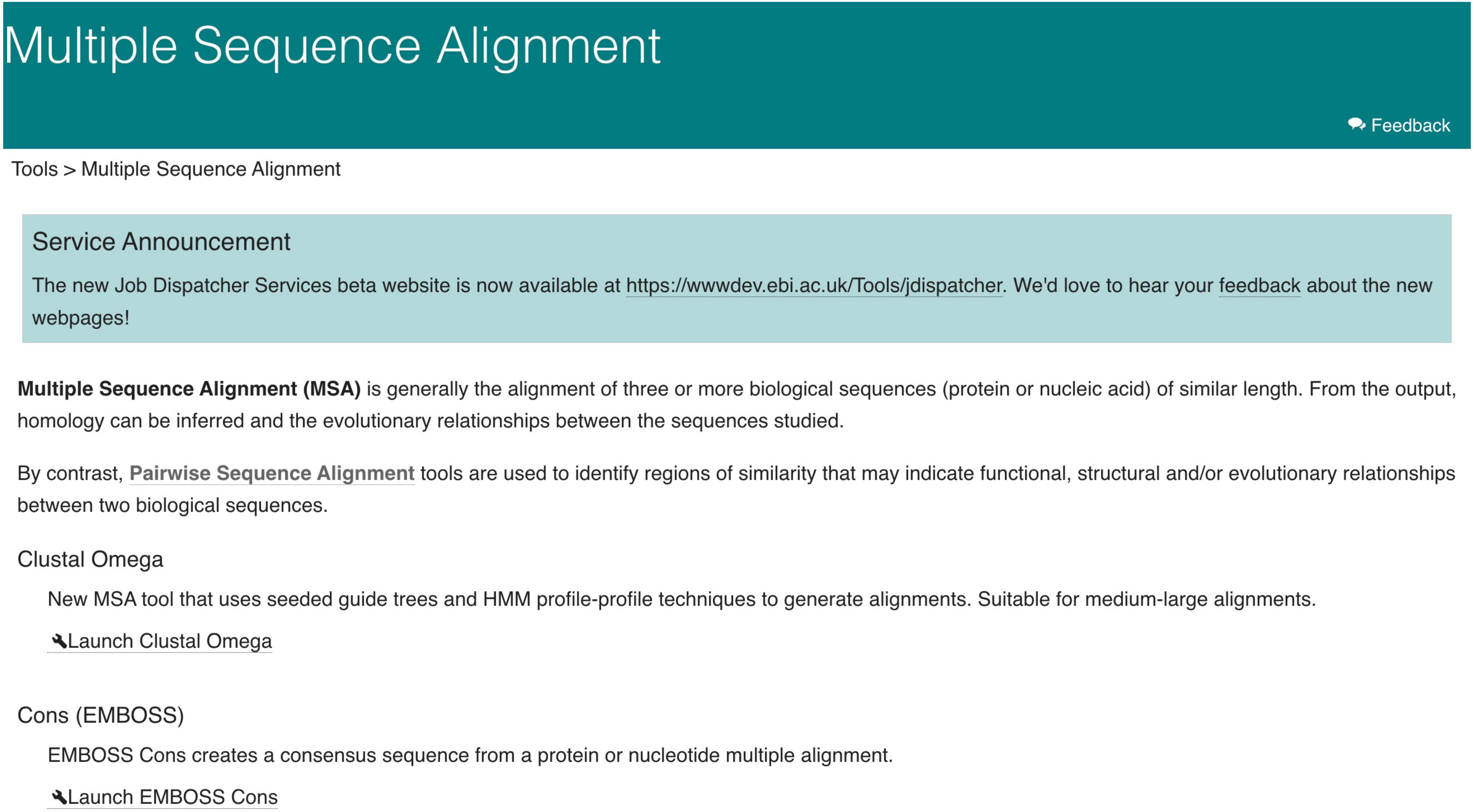

Multiple Sequence Alignment

Tools > Multiple Sequence Alignment

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Multiple Sequence Alignment (MSA) is generally the alignment of three or more biological sequences (protein or nucleic acid) of similar length. From the output, homology can be inferred and the evolutionary relationships between the sequences studied.

By contrast, **Pairwise Sequence Alignment** tools are used to identify regions of similarity that may indicate functional, structural and/or evolutionary relationships between two biological sequences.

Clustal Omega

New MSA tool that uses seeded guide trees and HMM profile-profile techniques to generate alignments. Suitable for medium-large alignments.

Launch Clustal Omega

Cons (EMBOSS)

EMBOSS Cons creates a consensus sequence from a protein or nucleotide multiple alignment.

Launch EMBOSS Cons

Clustal Omega

New MSA tool that uses seeded guide trees and HMM profile-profile techniques to generate alignments. Suitable for medium-large alignments.

🔧Launch Clustal Omega

Cons (EMBOSS)

EMBOSS Cons creates a consensus sequence from a protein or nucleotide multiple alignment.

🔧Launch EMBOSS Cons

Kalign

Very fast MSA tool that concentrates on local regions. Suitable for large alignments.

🔧Launch Kalign

MAFFT

MSA tool that uses Fast Fourier Transforms. Suitable for medium-large alignments.

🔧Launch MAFFT

MUSCLE

Accurate MSA tool, especially good with proteins. Suitable for medium alignments.

🔧Launch MUSCLE

MView

Transform a Sequence Similarity Search result into a Multiple Sequence Alignment or reformat a Multiple Sequence Alignment using the MView program.

Launch MView

T-Coffee

Consistency-based MSA tool that attempts to mitigate the pitfalls of progressive alignment methods. Suitable for small alignments.

Launch T-Coffee

WebPRANK

The EBI has a new phylogeny-aware multiple sequence alignment program which makes use of evolutionary information to help place insertions and deletions.
Try it out at WebPRANK.

Alignment methods

Longest Common Subsequence

To find a longest common subsequence between two strings.

```
 S1: TAGTCACG

 S2: AGACTGTC

LCS: AGACG
```

Dynamic Programming

a computer programming technique where an algorithmic problem is **first broken down into sub-problems, the results are saved, and then the sub-problems are optimized to find the overall solution** — which usually has to do with **finding the maximum and minimum range of the algorithmic query.**

https://www.spiceworks.com/tech/devops/articles/what-is-dynamic-programming/

Dynamic programming:

$$c_{i,j} = \max \begin{cases} c_{i-1,j-1} + 1 & if\ a_i = b_j \\ c_{i-1,j} + 0 & if\ a_i \neq b_j \\ c_{i,j-1} + 0 & if\ a_i \neq b_j \end{cases}$$

Step 1

	S2	A	G	A	C	T	G	T	C
S1	0	0	0	0	0	0	0	0	0
T	0								
A	0								
G	0								
T	0								
C	0								
A	0								
C	0								
G	0								

Step 2

	S2	A	G	A	C	T	G	T	C
S1	0	0	0	0	0	0	0	0	0
T	0	0	0	0	0	1	1	1	1
A	0								
G	0								
T	0								
C	0								
A	0								
C	0								
G	0								

Dynamic programming:

$$c_{i,j} = \max \begin{cases} c_{i-1,j-1} + 1 & if\ a_i = b_j \\ c_{i-1,j} + 0 & if\ a_i \neq b_j \\ c_{i,j-1} + 0 & if\ a_i \neq b_j \end{cases}$$

Step 3

	S2	A	G	A	C	T	G	T	C
S1	0	0	0	0	0	0	0	0	0
T	0	0	0	0	0	1	1	1	1
A	0	1	1	1	1	1	1	1	1
G	0								
T	0								
C	0								
A	0								
C	0								
G	0								

Step 4

	S2	A	G	A	C	T	G	T	C
S1	0	0	0	0	0	0	0	0	0
T	0	0	0	0	0	1	1	1	1
A	0	1	1	1	1	1	1	1	1
G	0	1	2	2	2	2	2	2	2
T	0								
C	0								
A	0								
C	0								
G	0								

Dynamic programming:

$$c_{i,j} = \max\begin{cases} c_{i-1,j-1} + 1 & \text{if } a_i = b_j \\ c_{i-1,j} + 0 & \text{if } a_i \neq b_j \\ c_{i,j-1} + 0 & \text{if } a_i \neq b_j \end{cases}$$

Step 5

	S2	A	G	A	C	T	G	T	C
S1	0	0	0	0	0	0	0	0	0
T	0	0	0	0	0	1	1	1	1
A	0	1	1	1	1	1	1	1	1
G	0	1	2	2	2	2	2	2	2
T	0	1	2	2	2	3	3	3	3
C	0								
A	0								
C	0								
G	0								

…

Dynamic programming:

$$c_{i,j} = \max\begin{cases} c_{i-1,j-1}+1 & if\ a_i = b_j \\ c_{i-1,j}+0 & if\ a_i \neq b_j \\ c_{i,j-1}+0 & if\ a_i \neq b_j \end{cases}$$

Final

	S2	A	G	A	C	T	G	T	C
S1	0	0	0	0	0	0	0	0	0
T	**0**	0	0	0	0	1	1	1	1
A	0	**1**	1	1	1	1	1	1	1
G	0	1	**2**	2	2	2	2	2	2
T	0	1	**2**	2	2	3	3	3	3
C	0	1	**2**	2	3	3	3	3	4
A	0	1	2	**3**	3	3	3	3	4
C	0	1	2	3	**4**	**4**	4	4	4
G	0	1	2	3	4	4	**5**	**5**	**5**

S1:TAGTCACG

S2:AGACTGTC

LCS:AGACG

Alignment

TAGTCAC-G--

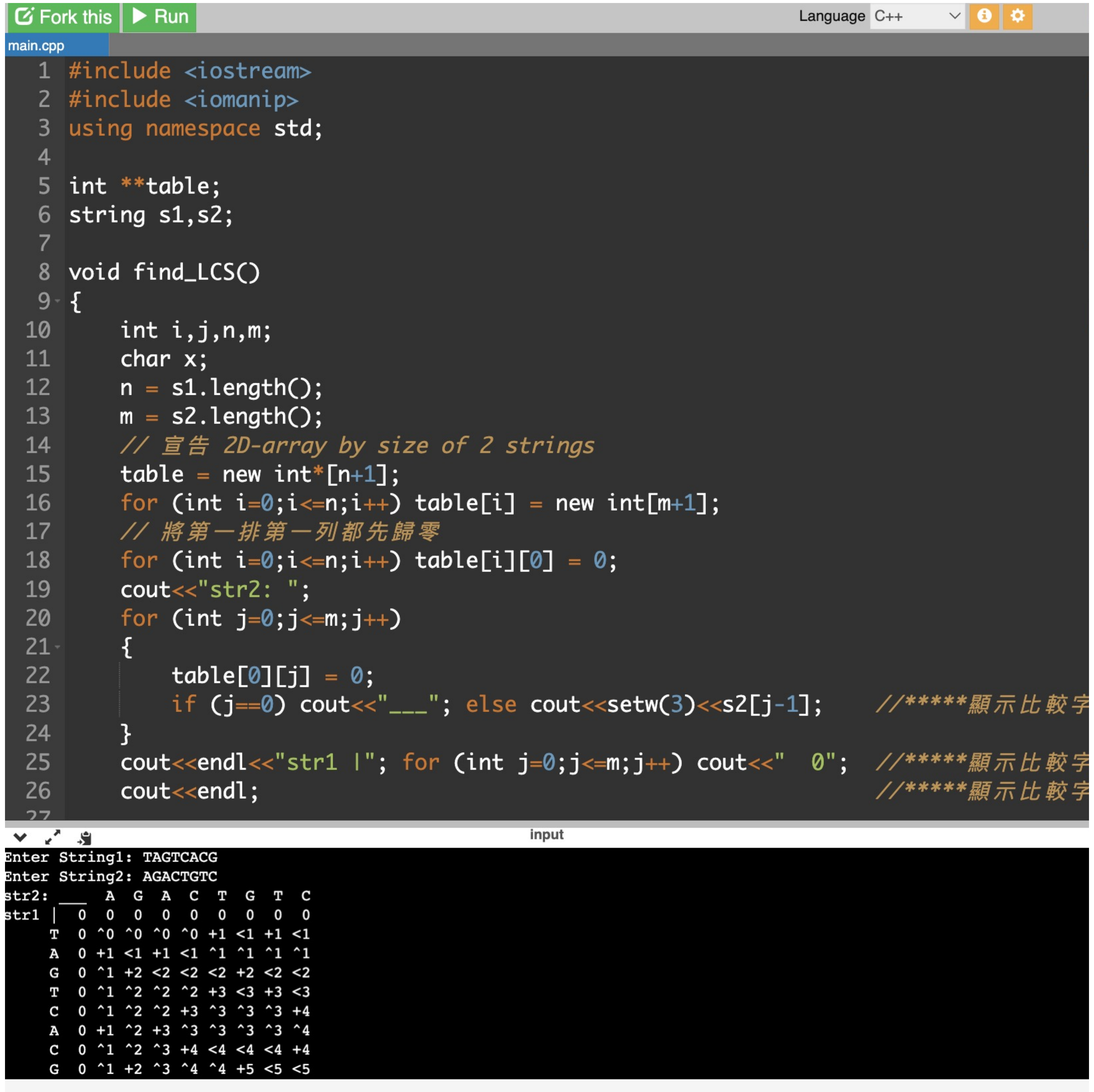
-AG--ACTGTC

...

Equation

$$c_{i,j}=\max\begin{cases} c_{i-1,j-1}+1 & \mathit{if}\ a_i=b_j \\ c_{i-1,j}+0 & \mathit{if}\ a_i\neq b_j \\ c_{i,j-1}+0 & \mathit{if}\ a_i\neq b_j \end{cases}$$

Go to this website and input sequences



```cpp
#include <iostream>
#include <iomanip>
using namespace std;

int **table;
string s1,s2;

void find_LCS()
{
    int i,j,n,m;
    char x;
    n = s1.length();
    m = s2.length();
    // 宣告 2D-array by size of 2 strings
    table = new int*[n+1];
    for (int i=0;i<=n;i++) table[i] = new int[m+1];
    // 將第一排第一列都先歸零
    for (int i=0;i<=n;i++) table[i][0] = 0;
    cout<<"str2: ";
    for (int j=0;j<=m;j++)
    {
        table[0][j] = 0;
        if (j==0) cout<<"___"; else cout<<setw(3)<<s2[j-1];    //*****顯示比較字
    }
    cout<<endl<<"str1 |"; for (int j=0;j<=m;j++) cout<<"  0";  //*****顯示比較字
    cout<<endl;                                                 //*****顯示比較字
```

```
Enter String1: TAGTCACG
Enter String2: AGACTGTC
str2: ___  A  G  A  C  T  G  T  C
str1 |  0  0  0  0  0  0  0  0  0
     T  0 ^0 ^0 ^0 ^0 +1 <1 +1 <1
     A  0 +1 <1 +1 <1 ^1 ^1 ^1 ^1
     G  0 ^1 +2 <2 <2 <2 +2 <2 <2
     T  0 ^1 ^2 ^2 ^2 +3 <3 +3 <3
     C  0 ^1 ^2 ^2 +3 ^3 ^3 ^3 +4
     A  0 +1 ^2 +3 ^3 ^3 ^3 ^3 ^4
     C  0 ^1 ^2 ^3 +4 <4 <4 <4 +4
     G  0 ^1 +2 ^3 ^4 ^4 +5 <5 <5
```

https://www.onlinegdb.com/Hkx1yiw2v

Time Complexity

$$c_{i,j} = \max \begin{cases} c_{i-1,j-1} + 1 & if\ a_i = b_j \\ c_{i-1,j} + 0 & if\ a_i \neq b_j \\ c_{i,j-1} + 0 & if\ a_i \neq b_j \end{cases}$$

The number of sequences is *k*

- K-dimensional table
- Each dimension is sequence length
- Each entry depends on 2^k-1 adjacent entries
- Complexity: $O(2^k n^k)$
- NP (nondeterministic polynomial time)-complete problem

Evaluating an alignment: Sum-of-pairs

Alignment #1

$$\sum_{i<j} scoring(S_i, S_j)$$

```
s1 = ATTCGAT
s2 = -TT-GAG
s3 = AT--GCT
```

- $s(S_i$ and $S_j) = 1$ when match
- $s(S_i$ and $S_j) = –1$ when mismatch
- $s(S_i$ and $S_j) = –2$ when gap

For the alignment, the pairwise alignment score of position 7:

$score(s_1,s_2) = -1$

$score(s_2,s_3) = -1$

$score(s_1,s_3) = 1 \Rightarrow$ SP score = –1

Alignment #2

$$\sum_{i<j} scoring(S_i, S_j)$$

s_1 = ATTCGAT-

s_2 = -TT-GA-G

s_3 = AT--GCT-

- s(S_i and S_j) = 1 when match
- s(S_i and S_j) = –1 when mismatch
- s(S_i and S_j) = –2 when gap

For the alignment, the pairwise alignment score of position 7 and 8:

score(s_1,s_2) = -2-2

score(s_2,s_3) = -2+1

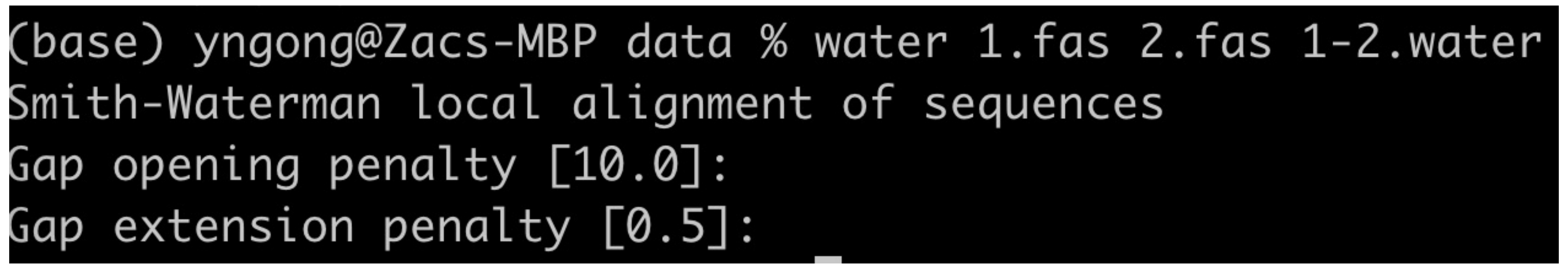
score(s_1,s_3) = 1+1 ⇒ SP score = -3

Hands-on session

```
>1
ACTACTAGATTACTTACGGATCAGGTACTTTAGAGGCTTGCAACCA

>2
TACTCACGGATGAGGTACTTTAGAGGC
```

1. Copy and save as two sequence files (1.fas and 2.fas) (using Sublime app)
2. Install EMBOSS by using conda (https://anaconda.org/bioconda/emboss)
3. Perfrom Needle and Water to compare global and local alignment
   ```
   $ water 1.fas 2.fas 1-2.water
   $ needle 1.fas 2.fas 1-2.needle
   ```



```
(base) yngong@Zacs-MBP data % water 1.fas 2.fas 1-2.water
Smith-Waterman local alignment of sequences
Gap opening penalty [10.0]:
Gap extension penalty [0.5]:
```

Hit “Enter” to use the default setting of penalty.