



BHARATHIDASAN UNIVERSITY

**Tiruchirappalli- 620024,
Tamil Nadu, India.**

Programme : M.Sc., Biomedical Science

Course Title : Bioinformatics

Course Code : BM35S1BI

Unit-III

TOPIC: MULTIPLE SEQUENCE ALIGNMENT

- **CLSTALW**
- **TCOFFEE**

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Guest Lecturer

Department of Biomedical Science

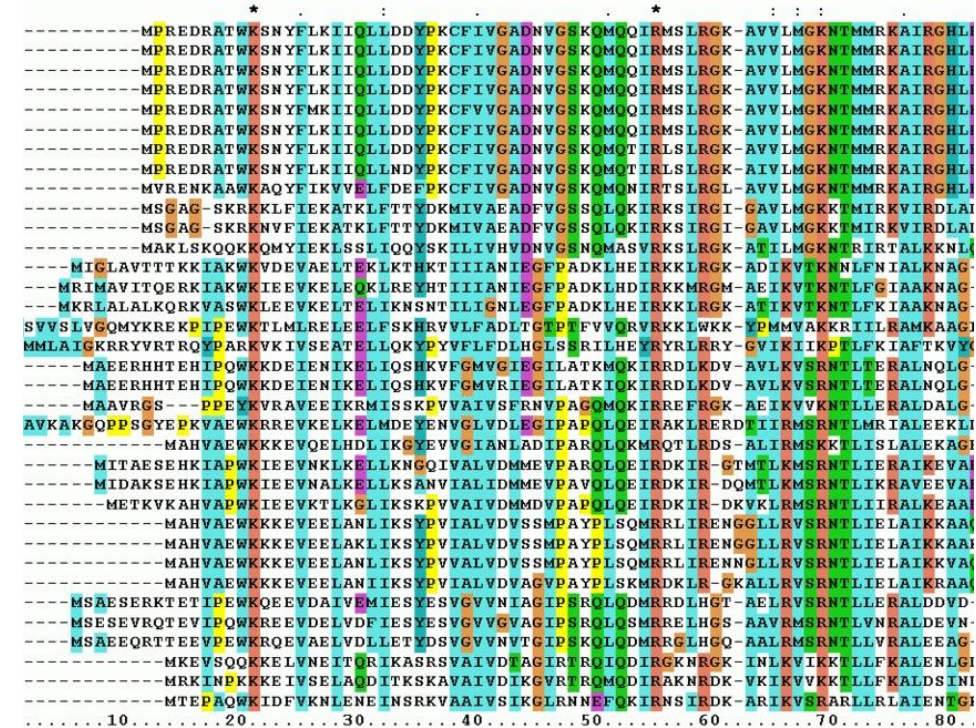
MULTIPLE SEQUENCE ALIGNMENT

- **CLSTALW**
- **TCOFFEE**

,

MULTIPLE SEQUENCE ALIGNMENT

- In bioinformatics, a sequence alignment is a way of **arranging the sequences** of DNA, RNA or protein to identify regions of similarity
- The reason to perform sequence alignment, is **to find out the regions of similarity**, which refers to functional equivalence and evolutionary relationship between sequences.
- Now, Multiple sequence alignment is basically an alignment of **more than two** sequences



Approaches

To carry out MSA , with dynamic programming, multidimensional matrix is needed- the amount of computing time and computer memory it requires **increases exponentially** as the number of sequence increases. So it cannot be applied for more than 10 sequences.

Hence , Heuristic approaches which engage pairwise dynamic programming algorithms are most often used.

It falls into three categories:

1. Progressive alignment type(ex- Clustal tool)
2. Iterative alignment type
3. Block- based alignment type

STEPS INVOLVED IN MULTIPLE SEQUENCE ALIGNMENT

1. Align the new sequence to each of the previous sequence
2. Create a distance matrix / function for each sequence pair
3. Create a phylogenetic guide tree from the matrices placing the sequences at the terminal nodes.
4. Use the guide tree to determine the next sequence to be added to the alignment.

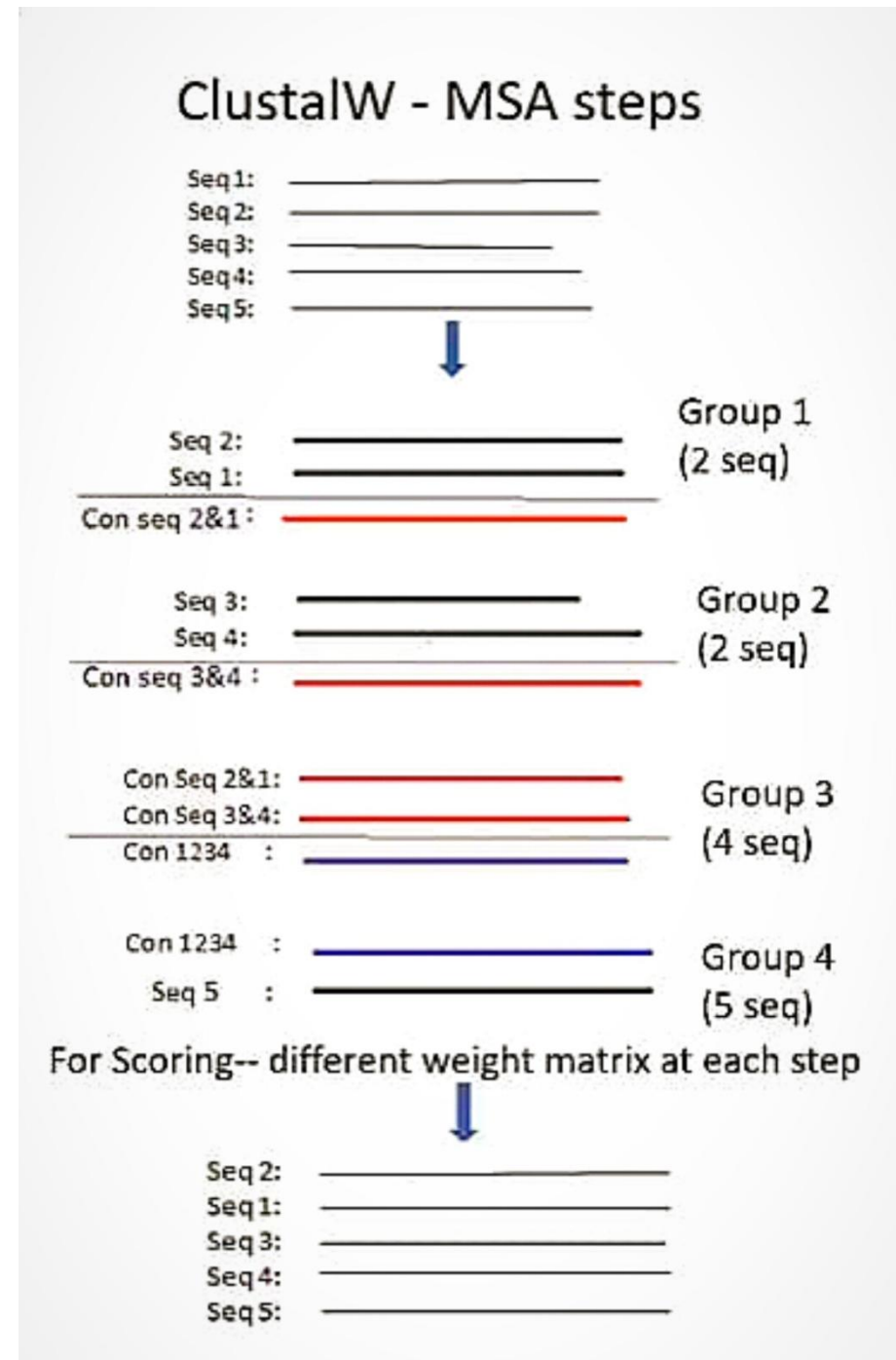
TOOLS OF MSA

- Clustal W
- Clustal W2
- Clustal Omega
- MAFFT
- MUSCLE
- M View
- T- Coffee
- Web PRANK
- MACAW

ClustalW

- CLUSTALW (eg. <https://www.genome.jp/tools-bin/clustalw>) is a progressive MSA program, which follows a heuristic approach.
- ClustalW is produced by **Julie D. Thompson, Toby Gibson** of European Molecules Biology Laboratory.
- ClustalW can create multiple alignment, manipulate existing alignment, do profile analysis and create phylogenetic trees.
- Alignment can be done by 2 methods;
 1. Slow/accurate
 2. Fast/approximate
- 'W' stands for 'weighted' (sequences are weighted differently).

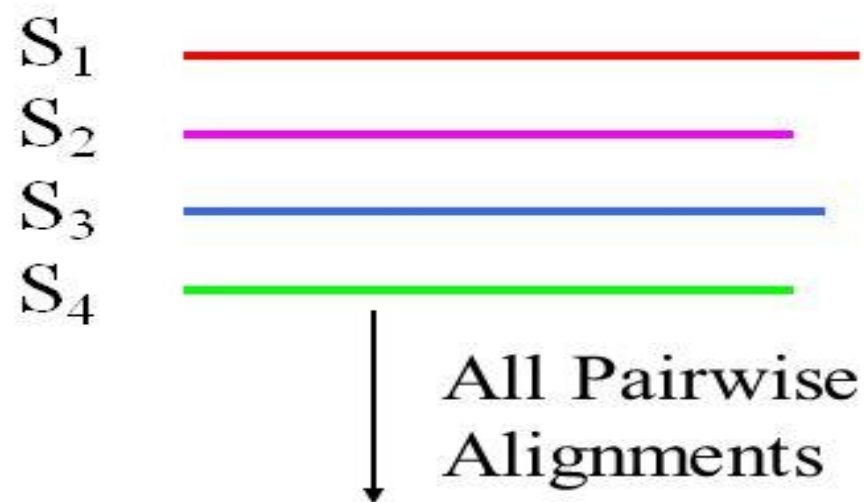
- The Clustal software align sequence using a heuristic that progressively builds a multiple sequence alignment from series of pairwise alignment.
- This method works by analysing the sequence as a whole, then utilizing the **UPGMA /Neighbor-joining** methods to generate a distance matrix.
- A guide tree is then calculated from the scores of the sequences in the matrix, then progressively align the sequence in order of similarity
- Essentially, Clustal creates multiple sequence alignment through three main steps:



The ClustalW Algorithm

- Step 1 : Determined all pairwise alignment between sequence and determine degree of similarity between each pair.
- Step 2 : Construct a similarity tree.
- Step 3 : Combine the alignment starting from the most closely related group to the most distantly related groups using the “once a gap always a gap” rule.

ClustalW steps



Similarity Matrix

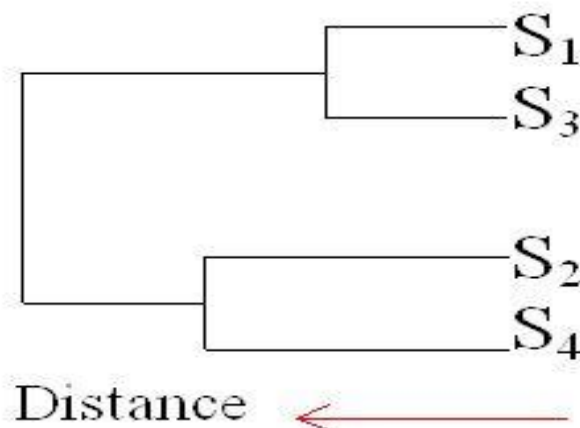
	S_1	S_2	S_3	S_4
S_1		4	9	4
S_2			4	7
S_3				4
S_4				

Cluster Analysis

Multiple Alignment Step:

1. Aligning S_1 and S_3
2. Aligning S_2 and S_4
3. Aligning (S_1, S_3) with (S_2, S_4) .

Dendrogram



From Higgins(1991) and Thompson(1994).



Multiple Sequence Alignment by CLUSTALW

ETE3

MAFFT

CLUSTALW

PRRN

[Help](#)

General Setting Parameters:

Output Format: ▾

Pairwise Alignment: FAST/APPROXIMATE SLOW/ACCURATE

Enter your **sequences** (with labels) below (copy & paste): PROTEIN DNA

Support Formats: FASTA (Pearson), NBRF/PIR, EMBL/Swiss Prot, GDE, CLUSTAL, and GCG/MSF

Or give the file name containing your query

No file chosen

Filter by:

- Reviewed (2,304) Swiss-Prot
- Unreviewed (72,000) TrEMBL
- Popular organisms
 - Human (216)
 - A. thaliana (74)
 - Fruit fly (60)
 - Mouse (52)
 - S. cerevisiae (42)
- Other organisms Go

- Search terms
- Filter "ace" as:
- author (1)
 - disease (1)
 - gene name (2,529)
 - organism (18,049)
 - protein name (409)

BLAST Align Download Add to basket Columns

Entry	Entry name	Protein names	Gene names	Organism	Length
<input type="checkbox"/> Q9BYF1	ACE2_HUMAN	Angiotensin-converting enzyme 2	ACE2 UNQ868/PRO1885	Homo sapiens (Human)	805
<input type="checkbox"/> Q8R0I0	ACE2_MOUSE	Angiotensin-converting enzyme 2	Ace2	Mus musculus (Mouse)	805
<input type="checkbox"/> P09470	ACE_MOUSE	Angiotensin-converting enzyme	Ace Dcp1	Mus musculus (Mouse)	1,312
<input type="checkbox"/> Q5EGZ1	ACE2_RAT	Angiotensin-converting enzyme 2	Ace2	Rattus norvegicus (Rat)	805
<input type="checkbox"/> P47820	ACE_RAT	Angiotensin-converting enzyme	Ace Dcp1	Rattus norvegicus (Rat)	1,313
<input type="checkbox"/> Q56H28	ACE2_FELCA	Angiotensin-converting enzyme 2	ACE2	Felis catus (Cat) (Felis silvestris catus)	805
<input type="checkbox"/> Q50JES	ACE_MESAU	Angiotensin-converting enzyme	Ace Dcp1	Mesocricetus auratus (Golden hamster)	1,314
<input type="checkbox"/> Q5RFN1	ACE2_PONAB	Angiotensin-converting enzyme 2	ACE2	Pongo abelii (Sumatran orangutan) (Pongo pygmaeus abelii)	805
<input type="checkbox"/> Q56NL1	ACE2_PAGLA	Angiotensin-converting enzyme 2	ACE2	Paguma larvata (Masked palm civet)	805
<input type="checkbox"/> Q58DD0	ACE2_BOVIN	Angiotensin-converting enzyme 2	ACE2	Bos taurus (Bovine)	804
<input type="checkbox"/> P12821	ACE_HUMAN	Angiotensin-converting enzyme	ACE DCP, DCP1	Homo sapiens (Human)	1,306
<input type="checkbox"/> Q9GLN7	ACE_PANTR	Angiotensin-converting enzyme	ACE DCP1	Pan troglodytes (Chimpanzee)	1,304
<input type="checkbox"/> P21192	ACE2_YEAST	Metallothionein expression activato...	ACE2 YLR131C, L3123, (9606.10)	Saccharomyces cerevisiae (strain ATCC 204508 / S288c) (Baker's yeast)	770

More Detail Parameters...

Pairwise Alignment Parameters:

For FAST/APPROXIMATE:

K-tuple(word) size: , Window size: , Gap Penalty:
Number of Top Diagonals: , Scoring Method:

For SLOW/ACCURATE:

Gap Open Penalty: , Gap Extension Penalty:
Select Weight Matrix:

(Note that only parameters for the algorithm specified by the above "Pairwise Alignment" are valid.)

Multiple Alignment Parameters:

Gap Open Penalty: , Gap Extension Penalty:

Weight Transition: YES (Value:) , NO

Hydrophilic Residues for Proteins:

Hydrophilic Gaps: YES NO

Select Weight Matrix:

Type additional options (delimited by whitespaces) below:

(-options for help)

Pairwise Alignment Parameters:

For FAST/APPROXIMATE:

K-tuple(word) size: , Window size: , Gap Penalty:

Number of Top Diagonals: , Scoring Method:

For SLOW/ACCURATE:

Gap Open Penalty: , Gap Extension Penalty:

Select Weight Matrix:

Wilbur & Lipman algorithm

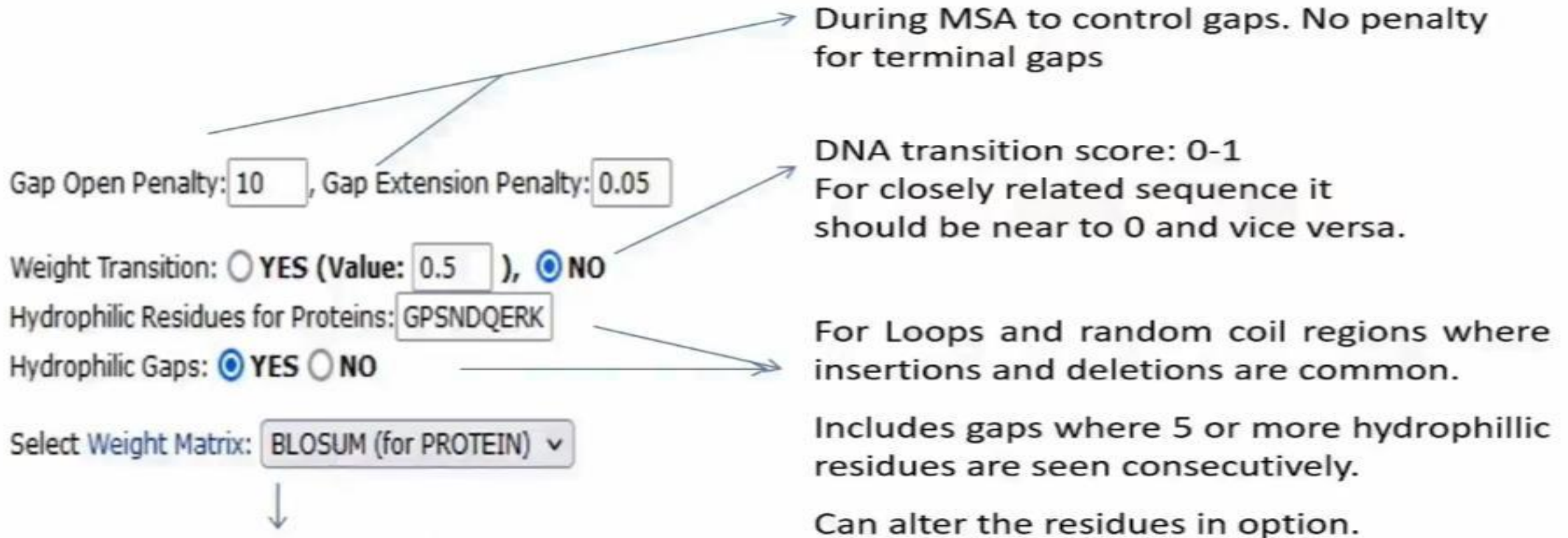
(Heuristic approach)

Dynamic programming algorithm

Choose this for less number of short sequences.



Multiple alignment parameters



For proteins- series of matrices are used depending upon the similarity of sequences aligned at each step.

For DNA –Single matrix is used.

Result

CLUSTALW Result

[clustalw.aln][clustalw.dnd][readme]

Select tree menu ▾ Exec

CLUSTAL 2.1 Multiple Sequence Alignments

Sequence type explicitly set to Protein
Sequence format is Pearson

Sequence 1:	ACE2_HUMAN	805	aa
Sequence 2:	ACE2_MOUSE	805	aa
Sequence 3:	ACE2_RAT	805	aa
Sequence 4:	ACE2_CAT	805	aa
Sequence 5:	ACE2_CIVET	805	aa
Sequence 6:	ACE2_ORANGUTAN	805	aa
Sequence 7:	AGE2_COW	804	aa

Start of Pairwise alignments
Aligning...

Sequences (1:2)	Aligned.	Score: 82.1118
Sequences (1:3)	Aligned.	Score: 82.4845
Sequences (1:4)	Aligned.	Score: 85.2174
Sequences (1:5)	Aligned.	Score: 83.4783
Sequences (1:6)	Aligned.	Score: 98.1366

Sequence 6: ACE2_ORANGUTAN 805 aa
Sequence 7: ACE2_COW 804 aa
Start of Pairwise alignments
Aligning...

Sequences (1:2) Aligned. Score: 82.1118
Sequences (1:3) Aligned. Score: 82.4845
Sequences (1:4) Aligned. Score: 85.2174
Sequences (1:5) Aligned. Score: 83.4783
Sequences (1:6) Aligned. Score: 98.1366
Sequences (1:7) Aligned. Score: 80.8458
Sequences (2:3) Aligned. Score: 90.4348
Sequences (2:4) Aligned. Score: 81.7391
Sequences (2:5) Aligned. Score: 81.6149
Sequences (2:6) Aligned. Score: 81.7391
Sequences (2:7) Aligned. Score: 80.2239
Sequences (3:4) Aligned. Score: 81.6149
Sequences (3:5) Aligned. Score: 81.118
Sequences (3:6) Aligned. Score: 81.9876
Sequences (3:7) Aligned. Score: 80.0995
Sequences (4:5) Aligned. Score: 93.2919
Sequences (4:6) Aligned. Score: 84.9689
Sequences (4:7) Aligned. Score: 82.9602
Sequences (5:6) Aligned. Score: 83.2298
Sequences (5:7) Aligned. Score: 81.592
Sequences (6:7) Aligned. Score: 81.0945
Guide tree file created: [clustalw.dnd]

There are 6 groups
Start of Multiple Alignment

Aligning...
Group 1: Sequences: 2 Score:13303
Group 2: Sequences: 2 Score:13025
Group 3: Sequences: 4 Score:12290
Group 4: Sequences: 2 Score:12748
Group 5: Sequences: 6 Score:12194
Group 6: Sequences: 7 Score:12107
Alignment Score 92252

CLUSTAL-Alignment file created [clustalw.aln]

clustalw.aln

CLUSTAL 2.1 multiple sequence alignment

CLUSTAL 2.1 Multiple Sequence Alignments

Sequence type explicitly set to Protein

Sequence format is Pearson

Sequence 1: ACE2_HUMAN 805 aa
 Sequence 2: ACE2_MOUSE 805 aa
 Sequence 3: ACE2_RAT 805 aa
 Sequence 4: ACE2_CAT 805 aa
 Sequence 5: ACE2_CIVET 805 aa
 Sequence 6: ACE2_ORANGUTAN 805 aa
 Sequence 7: ACE2_COW 804 aa

Start of Pairwise alignments

Aligning...

Sequences (1:2) Aligned. Score: 82.1118
 Sequences (1:3) Aligned. Score: 82.4845
 Sequences (1:4) Aligned. Score: 85.2174
 Sequences (1:5) Aligned. Score: 83.4783
 Sequences (1:6) Aligned. Score: 98.1366
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 Guide tree file created: [clustalw.dnd]

There are 6 groups

Start of Multiple Alignment

Aligning...

Group 1: Sequences: 2 Score:13303

Group 2: Sequences: 2 Score:13025

guidetree.docx - Word Abhishek Mudaliar AM

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Calibri (Body) 11

1 Normal 1 No Spac... Heading 1

clustalw.dnd

```
(
(
(
ACE2_HUMAN:0.00820,
ACE2_ORANGUTAN:0.01043)
:0.07143,
(
ACE2_CAT:0.02784,
ACE2_CIVET:0.03924)
:0.04347)
:0.00480,
(
ACE2_MOUSE:0.04728,
ACE2_RAT:0.04837)
:0.05047,
ACE2_COW:0.10008);
```

CLUSTALW Result

genome.jp/tools-bin/clustalw

Sequence 6: ACE2_ORANGUTAN 805 aa
 Sequence 7: ACE2_COW 804 aa
 Start of Pairwise alignments
 Aligning...

Sequences (1:2) Aligned. Score: 82.1118
 Sequences (1:3) Aligned. Score: 82.4845
 Sequences (1:4) Aligned. Score: 85.2174
 Sequences (1:5) Aligned. Score: 83.4783
 Sequences (1:6) Aligned. Score: 98.1366
 Sequences (1:7) Aligned. Score: 80.8458
 Sequences (2:3) Aligned. Score: 90.4348
 Sequences (2:4) Aligned. Score: 81.7391
 Sequences (2:5) Aligned. Score: 81.6149
 Sequences (2:6) Aligned. Score: 81.7391
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 Guide tree file created: [clustalw.dnd]

There are 6 groups
 Start of Multiple Alignment

Aligning...

Group 1: Sequences:	2	Score:13303
Group 2: Sequences:	2	Score:13025
Group 3: Sequences:	4	Score:12290
Group 4: Sequences:	2	Score:12748
Group 5: Sequences:	6	Score:12194
Group 6: Sequences:	7	Score:12107

Alignment Score 92252

CLUSTAL-Alignment file created [clustalw.aln]

clustalw.aln

CLUSTAL 2.1 multiple sequence alignment

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Calibri (Body) 11

1 Normal 1 No Spac... Heading 1

clustalw.dnd

```
(
(
(
ACE2_HUMAN:0.00820,
ACE2_ORANGUTAN:0.01043)
:0.07143,
(
ACE2_CAT:0.02784,
ACE2_CIVET:0.03924)
:0.04347)
:0.00480,
(
ACE2_MOUSE:0.04728,
ACE2_RAT:0.04837)
:0.05047,
ACE2_COW:0.10008);
```

clustalw:aln

CLUSTAL 2.1 multiple sequence alignment

```
ACE2_HUMAN      MSSSSWLLLSLVAVTAAQSTIEEQAKTFLDKFNHEAEEDLYQSSSLASWNYNTNITEENIQ
ACE2_ORANGUTAN  MSGSSWLLLSLVAVTAAQSTIEEQAKTFLDKFNHEAEEDLYQSSSLASWNYNTNITEENIQ
ACE2_CAT        MSGSFWLLLSFAALTAAQSTTEELAKTFLEKFNHEAEELSYQSSSLASWNYNTNITDENIQ
ACE2_CIVET      MSGSFWLLLSFAALTAAQSTTEELAKTFLETFNIEAQELSYQSSVASWNYNTNITDENAK
ACE2_MOUSE     MSSSSWLLLSLVAVTAAQSLTEENAKTFLNFNQEAEDLYQSSSLASWNYNTNITEENIQ
ACE2_RAT       MSSSCWLLLSLVAVTAAQSLIEEKAESFLNKFNQEAEDLYQSSSLASWNYNTNITEENIQ
ACE2_COW       MTGSFWLLLSLVAVTAAQSTTEEQAKTFLEKFNHEAEEDLYQSSSLASWNYNTNITDENIQ
*:.: * *****: .: : : : * * * * * : : : * * * * * : : : * * * * * : : : *
```

```
ACE2_HUMAN      NMNINAGDKWSAFLKEQSTLAQMYPLQETQNLTVKLLQLQALQQNGSSVLSADKSKRLNITIL
ACE2_ORANGUTAN  NMNINAGDKWSAFLKEQSTLAQMYPLQETQNLTVKLLQLQALQQNGSSVLSADKSKRLNITIL
ACE2_CAT        KMNEAGAKWSAFYEEQSKLAKTYPLAETHIITVVKRQLQALQQSGSSVLSADKSQRLNITIL
ACE2_CIVET      NMNEAGAKWSAYYEEQSKLAQTYPLAETQDAKIKRQLQALQQSGSSVLSADKSQRLNITIL
ACE2_MOUSE     KMSEAAAKWSAFYEEQSKTAQSFSLQEIQTPIIKRQLQALQQSGSSALSADKHKQLNITIL
ACE2_RAT       KMNEAAAKWSAFYEEQSTIAQNFSLQETQNIATIKRQLKALQQSGSSALSPDKHKQLNITIL
ACE2_COW       KMNEARAKWSAFYEEQSRMAKYSLEETQNLTKRQLKALQHSQTSALSADKSKRLNITIL
: * : * * * * : : * * * * * : : * * * * * : : * * * * * : : * * * * * : : *
```

```
ACE2_HUMAN      NTMSTIYSTGKVCNPNPQECLELLEPGLNEIMANSLDYNERLWANESWRSEVQKLRPLY
ACE2_ORANGUTAN  NTMSTIYSTGKVCNPNPQECLELLEPGLNEIMANSLDYNERLWANESWRSEVQKLRPLY
ACE2_CAT        NAMSTIYSTGKACNPNPQECLELLEPGLDDIMENSKDYNERLWANEGWRAEVGKLRPLY
ACE2_CIVET      NAMSTIYSTGKACNPNPQECLELLEPGLDRIIMENSKDYNERLWANEGWRAEVGKLRPLY
ACE2_MOUSE     NTMSTIYSTGKVCNPNPQECLELLEPGLDEIMATSTDYNSRLWANEGWRAEVGKLRPLY
ACE2_RAT       NTMSTIYSTGKVCNPNPQECLELLEPGLDEIMATSTDYNRRLWANEGWRAEVGKLRPLY
ACE2_COW       NKMSTIYSTGKVLDPN-TQECLELLEPGLDDIMENSRDYNRRLWANEGWRAEVGKLRPLY
* * * * * : : * * * * * : : * * * * * : : * * * * * : : * * * * * : : *
```

```
ACE2_HUMAN      EEYVVLKNEMARANHYEDYGDYWRGDYEVNIGVDGYDYSRGQLIEDVEHTFEEIKPLYEHL
ACE2_ORANGUTAN  EEYVVLKNEMARANHYEDYGDYWRGDYEVNIGVDSYDYSRGQLIEDVEHTFEEIKPLYEHL
ACE2_CAT        EEYVALKNEMARANHYEDYGDYWRGDYEEENTDGYNYRSQLIKDVEHTFTQIKPLYQHL
ACE2_CIVET      EEYVALKNEMARANHYEDYGDYWRGDYEEENTDGYNYRSQLIKQVEDTFEQIKPLYQHL
ACE2_MOUSE     EEYVVLKNEMARANHYEDYGDYWRGDYEAEGADGYNYRNRQLIEDVERTFAEIKPLYEHL
ACE2_RAT       EEYVVLKNEMARANHYEDYGDYWRGDYEAEGVEGYNYRNRQLIEDVENTFKEIKPLYEQL
ACE2_COW       EEYVVLKNEMARANHYEDYGDYWRGDYEVAGDGYDYSRDQLHKDVERTFAEIKPLYEQL
* * * * * : : * * * * * : : * * * * * : : * * * * * : : * * * * * : : *
```

```
ACE2_HUMAN      HAYVRAKLHNIAYPSYISPTGCLPAHLLGDMWGRFWTNLYSLVVPFGQKPHIDVTDAMVDQ
ACE2_ORANGUTAN  HAYVRAKLHNIAYPSYISPTGCLPAHLLGDMWGRFWTNLYSLVVPFGQKPHIDVTDAMVDQ
ACE2_CAT        HAYVRAKLHDTYPSRISPTGCLPAHLLGDMWGRFWTNLYPLVVPFGQKPHIDVTDAMVQ
ACE2_CIVET      HAYVRAKLHDTYPSRISPTGCLPAHLLGDMWGRFWTNLYPLVVPFGQKPHIDVTDAMVQ
ACE2_MOUSE     HAYVRRKLDHTYPSYISPTGCLPAHLLGDMWGRFWTNLYPLVVPFQKPHIDVTDAMVQ
ACE2_RAT       HAYVRTKLHEVYPSYISPTGCLPAHLLGDMWGRFWTNLYPLTTPFLQKPHIDVTDAMVQ
ACE2_COW       HAYVRAKLHHTYPSYISPTGCLPAHLLGDMWGRFWTNLYSLVVPFEHKPSIDVTEKMFNQ
* * * * * : : * * * * * : : * * * * * : : * * * * * : : * * * * * : : *
```

- ‘ * ‘ indicates positions which have a single **fully conserved residue**
- ‘ : ‘ indicates that one of the following ‘**strong**’ groups is fully conserved

ClustalW output - Symbol

STA

NEQK

NHQK

NDEQ

QHRK

MILV

MILF

HY

FYW

Example:

```

YASIDISKGENNPGFQNTDDVQTSF
YASIDISKGENNPGFQNTDDVQTSF
YASVDLSKGENNPGFQHADDVQTSF
YASVDLNKGENNPGFQHADDVQTSF
YDSMDIGKGESNAGFQNSDDAQTSE
YDSMDIGKGESNAGFQNSDDAQTSE
YGSVDLNKGENNSGFQNI DDVQTSL
*  *:*:.***.*.***: **.***:
```

Cont,

- ‘ indicates that one of the following ‘weaker’ groups is fully conserved

CSA

ATV

SAG

STNK

STPA

SGND

SNDEQK

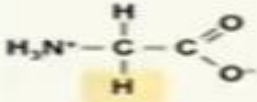
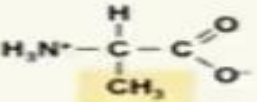
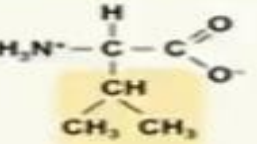
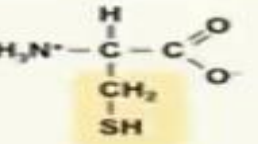
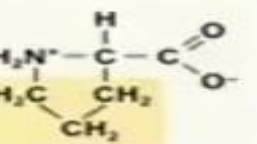
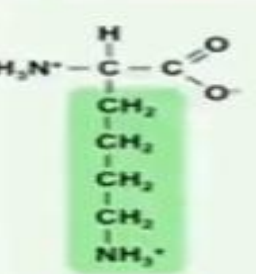
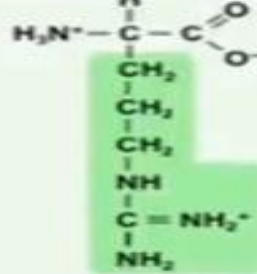
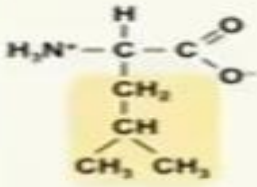
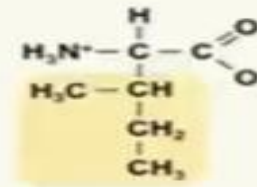
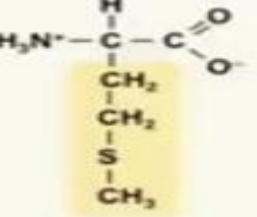
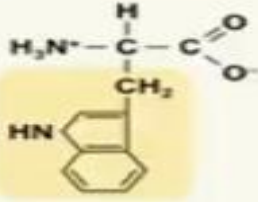
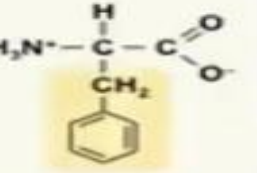
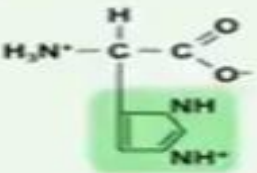
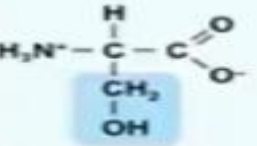
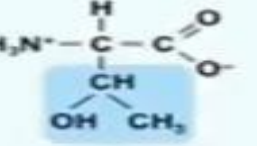
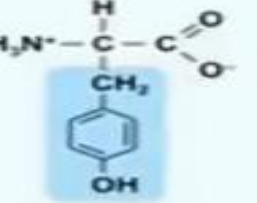
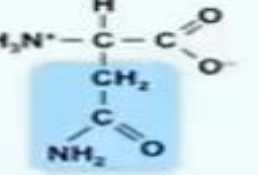
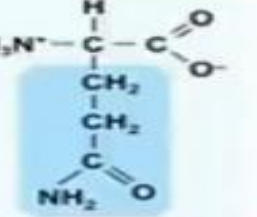
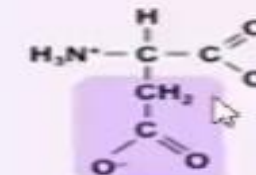
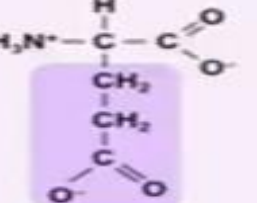
NDEQRK

NEQHRK

FVLIM

HFY

Amino acid Substitutions

Hydrophobic amino acid (Non-Polar)					Basic (+ charge), Polar	
 <p>Glycine (Gly / G)</p>	 <p>Alanine (Ala / A)</p>	 <p>Valine (Val / V)</p>	 <p>Cysteine (Cys / C)</p>	 <p>Proline (Pro / P)</p>	 <p>Lysine (Lys / K)</p>	 <p>Arginine (Arg / R)</p>
 <p>Leucine (Leu / L)</p>	 <p>Isoleucine (Ile / I)</p>	 <p>Methionine (Met / M)</p>	 <p>Tryptophan (Trp / W)</p>	 <p>Phenylalanine (Phe / F)</p>	 <p>Histidine (His / H)</p>	
Less polar					Acidic (- charge), Polar	
 <p>Serine (Ser / S)</p>	 <p>Threonine (Thr / T)</p>	 <p>Tyrosine (Tyr / Y)</p>	 <p>Asparagine (Asn / N)</p>	 <p>Glutamine (Gln / Q)</p>	 <p>Aspartic Acid (Asp / D)</p>	 <p>Glutamic Acid (Glu / E)</p>

1. Aliphatic, aromatic
2. Size

3. Acidic, Basic (charged), Neutral (uncharged)
4. Hydrophobic (non-polar), hydrophilic (polar)

T-COFFEE

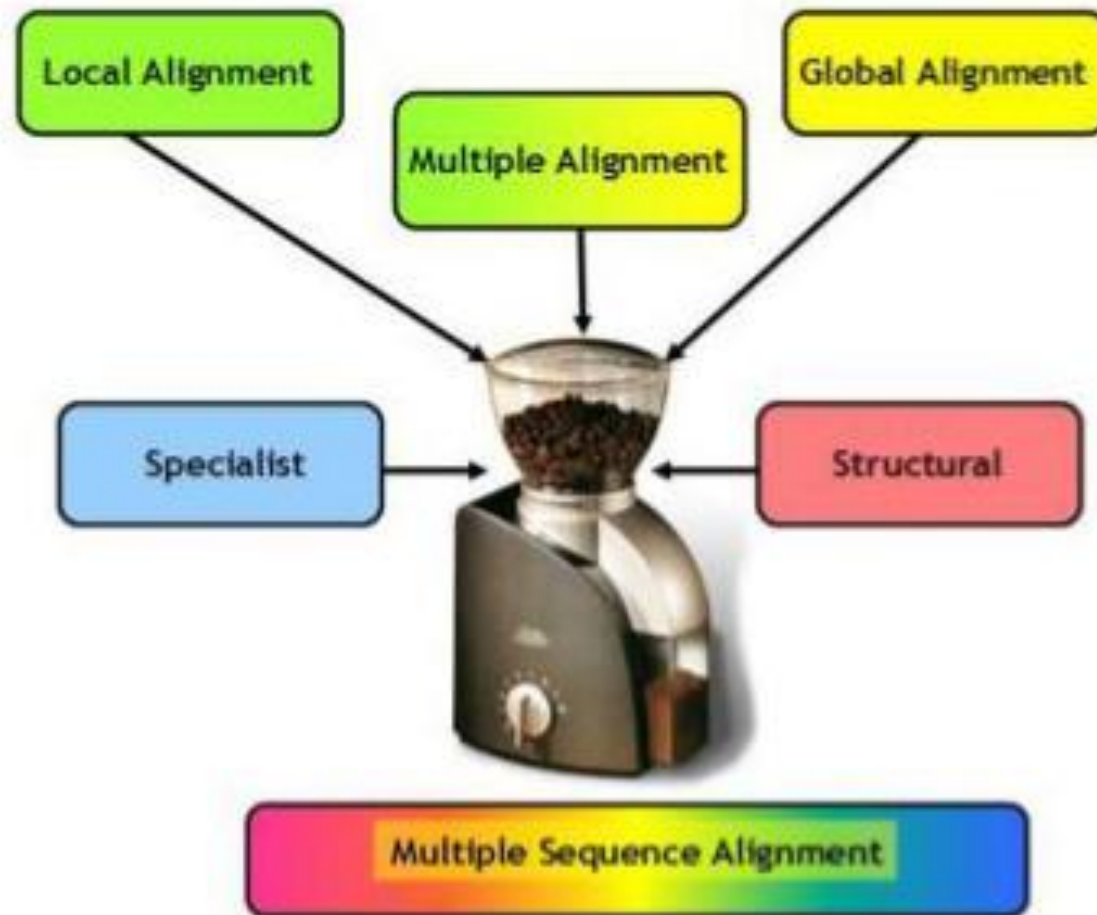
- T-COFFEE (Tree –based Consistency Objectives Function For Alignment Evaluation) is a multiple sequence alignment software using a progressive approach.
- It generates a **library of pairwise alignment** to guide the multiple sequence alignment.
- It has advanced features to evaluate the quality of alignments and capacity for identifying occurrence of motifs.
- The main characteristic of T-coffee is that it will combine results obtained with several alignment methods. This tool can align up to **500 sequences** or a maximum file size of **1 MB**.
- T coffee integrates different Pair-wise alignment techniques and combines different multiple alignment methods and sequence alignment methods and plug in user knowledge

ALGORITHMS

How coffee works?

- T-Coffee alignment utilizes heterogeneous data sources and provide simple and flexible means of generating multiple alignment.
- Create a library of pairwise alignment for each possible pairs of sequences and compare each pair of aligned residues in the MSA to its counterpart in the library.
- T-coffee can complete multiple alignment using a library that was generated using a mixture of local and global pair-wise alignment.
- Weight alignment by the percentage of identical residues.
- Residues that consistently match up, end up with very high weight, this is what is meant by CONSISTENCY-BASED SCORING.

The overall consistency score is equal to the **number of pairs that occurs in both MSA and the library, divided by the total no. Of pairs in MSA.**



T- Coffee and Consistency.....

1. With T-coffee we pre-process a data set of all pair-wise alignment between the Sequences.
2. This provides us with a library of alignment information that can be used to guide the progressive alignment.
3. Intermediate alignments are based not only on the sequence to be aligned next, and also on how all of the sequences align with each other.

T-Coffee

[Input form](#)[Web services](#)[Help & Documentation](#)[Bioinformatics Tools FAQ](#)[Feedback](#)[Share](#)

Tools > Multiple Sequence Alignment > T-Coffee

Multiple Sequence Alignment

T-Coffee is a multiple sequence alignment program. Its main characteristic is that it will allow you to combine results obtained with several alignment methods.

Important note: This tool can align up to 500 sequences or a maximum file size of 1 MB.

STEP 1 - Enter your input sequences

Enter or paste a set of

PROTEIN

sequences in any supported format:

>Seq1

>Seq5

```
MEEPQSDPSVEPPLSQETFSDLWKLLPENNVLSPLPSQAVDDLLSPPDIAQWFIEDPGPDEAPRMSEAASPVDPAPAAP  
IPAAPAPAPSWPLSSSVPSQKTYQGSYGFRLGFLHSGTAKSVTCTYSPALNKMFCQLAKTCPVQLWVDSTPPPGRVVRAM  
AIYKQSQHMTEVRRRCPPHHERCSDSDGLAPPQHLIRVEGNLRVEYLDDRNTFRHSVWVPYEPPEVGSDCTTIHYNMCNS  
SCMGGMNRRLPILTIITLEDSSGNLLGRNSFEVRCACPRDRRTEENFRKKGEPHHELPPGSTKRALPNNTSSSPQPKK  
KPLDGEYFTLQDQTSFQKENC
```

Or upload a file: No file chosen[Use a example sequence](#) | [Clear sequence](#) | [See more example inputs](#)

STEP 2 - Set your Parameters

OUTPUT FORMAT:

HTML

The default settings will fulfill the needs of most users.

 (Click here, if you want to view or change the default settings.)

STEP 3 - Submit your job

 Be notified by email *(Tick this box if you want to be notified by email when the results are available)*

Tools > Multiple Sequence Alignment > T-Coffee

Your job is currently running... please be patient

The result of your job will appear in this browser window.

Job ID: [tcoffee-l20220819-113257-0191-82986554-p1m](#)

Please note the following

- You may press Shift+Refresh or Reload on your browser at any time to check if results are ready.
- You may bookmark this page to view your results later if you wish.
- Results are stored for 7 days.



Services

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- By name (A-Z)
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Waiting for www.ebi.ac.uk...

T-Coffee

Tools > Multiple Sequence Alignment > T-Coffee

Results for job tcffee-l20220819-113257-0191-82986554-p1m

- Alignments**
 - Result Summary
 - Guide Tree
 - Phylogenetic Tree
 - Results Viewers
 - Submission Details
- Download Alignment File

T-COFFEE, Version_13.45.0.4846264 (2020-10-15 17:52:11 - Revision 5becd5d - Build 620)
 Cedric Notredame
 CPU TIME:0 sec.
 SCORE=655

```

*
BAD AVG GOOD
*
AC105292.3 1867 : 54
AE014298.5 9585 : 68
CP023329.1 9479 : 68
CP023335.1 9479 : 68
NM_001272455.2 : 68
cons : 65

AC105292.3 1867 GA----TTTGCTT-CTCTGTTGTTTGGTTCA-ATCGTCT--TTAT
AE014298.5 9585 ATGCCATTTGTGGACCCCTCAGCGTCGCACATATACACGCCATAT
CP023329.1 9479 ATGCCATTTGTGGACCCCTCAGCGTCGCACATATACACGCCATAT
CP023335.1 9479 ATGCCATTTGTGGACCCCTCAGCGTCGCACATATACACGCCATAT
NM_001272455.2 ATGCCATTTGTGGACCCCTCAGCGTCGCACATATACACGCCATAT

cons ***** * * * * * * * * * *

AC105292.3 1867 TTTGCTTCACTGTTGACAAACATTCCATTT-GTTTATATCATTTA
AE014298.5 9585 C-TCCAACCATGCCGCCCAAAACCGATTTTCAGTTTTTTGACCTT
>GATTTTCAGTTTTTTGACCTT

```

CPU TIME:0 sec.

SCORE=655

*

BAD AVG GOOD

*

AC105292.3_1867	:	54
AE014298.5_9585	:	68
CP023329.1_9479	:	68
CP023335.1_9479	:	68
NM_001272455.2_	:	68
cons	:	65

AC105292.3_1867	GA----	TTTGCTT-CTCTGTTGTTTGGTTCA-ATCGTCT--TTAT
AE014298.5_9585	ATGCCATTTGTGGACCCCTCAGCGTCGCACATATAACACGCCATAT	
CP023329.1_9479	ATGCCATTTGTGGACCCCTCAGCGTCGCACATATAACACGCCATAT	
CP023335.1_9479	ATGCCATTTGTGGACCCCTCAGCGTCGCACATATAACACGCCATAT	
NM_001272455.2_	ATGCCATTTGTGGACCCCTCAGCGTCGCACATATAACACGCCATAT	
cons		*** * * * * * * * * * *

AC105292.3_1867	TTTGCTTCACTGTTGACAAACATTCCATTT-GTTTATATCATTTA	
AE014298.5_9585	C-TCCAACCATGCCGCCCCAAAACCGATTTTCAGTTTTTGACCTT	
CP023329.1_9479	C-TCCAACCATGCCGCCCCAAAACCGATTTTCAGTTTTTGACCTT	
CP023335.1_9479	C-TCCAACCATGCCGCCCCAAAACCGATTTTCAGTTTTTGACCTT	
NM_001272455.2_	C-TCCAACCATGCCGCCCCAAAACCGATTTTCAGTTTTTGACCTT	
cons		* * * * * * * * * * * * * * *

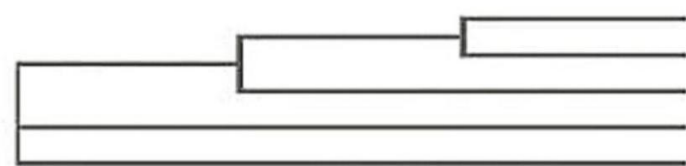
Alignments Result Summary Guide Tree **Phylogenetic Tree** Results Viewers Submission Details

Download Phylogenetic Tree Data

Phylogenetic Tree

This is a Neighbour-joining tree without distance corrections.

Branch length: Cladogram Real



AC105292.3_18679-22817 0.52693
 AE014298.5_9585675-9589813 0
 CP023329.1_9479705-9483843 0
 CP023335.1_9479704-9483842 0
 NM_001272455.2_1-4139 0

Tree Data



```
(
(
(
AC105292.3_18679-22817:0.52693,
AE014298.5_9585675-9589813:0.00000)
:0.00000,
CP023329.1_9479705-9483843:0.00000)
:0.00000,
CP023335.1_9479704-9483842:0.00000,
NM_001272455.2_1-4139:0.00000);
```

Applications of MSA

- Detecting **similarities** between sequences(closely or distinctly related).
- Detecting **conserved regions** or motifs in sequences.
- Detecting of **structural homologies**.
- Thus, assisting the improved prediction of **secondary and tertiary structures of proteins**.
- An important step for **phylogenetic analysis**.
- Useful in designing experiments to test and modify the function of specific proteins and also in predicting the function and structure of proteins, and in **identifying new members of protein families**.

References

1. Statistics for Bioinformatics

-Julie Dawn Thompson

2. Multiple Sequence Alignment methods

-David J.Russell

3. Bioinformatics for DUMMIES (2nd Edition)

-Jean – Micha Claverie, Cedric Notredame



THANK YOU