

# **Bioinformatics**

**CS300**

**Chap 3**

**Sequence Alignment  
with ClustalW**

**Fall 2021**

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# What is Sequence Alignment?

- Sequence alignment is a way of arranging the sequence of genetic material (DNA, RNA or protein) to identify regions of similarity that may be a consequence of functional, structural or evolutionary relationships between the sequences.

How much of this gene  
can be found in  
the other sequence?





# Needleman-Wunsch Algorithm Background

- **Global Alignment:** Used to determine which parts of a sequence (inside the sequence) are shared (common) with another sequence.
- Developed by Saul B. Needleman and Christian D. Wunsch in 1970.
- Dynamic programming to find optimal solution for matching the characters of the two sequences.

# Types of Alignment

<i>A</i>	<i>T</i>	<i>C</i>	<i>G</i>	<i>A</i>	<i>A</i>	<i>C</i>	<i>T</i>	<i>G</i>	<i>G</i>	<i>C</i>	<i>C</i>	–	–
.	.			.									
<i>T</i>	<i>A</i>	<i>C</i>	<i>G</i>	<i>C</i>	<i>A</i>	<i>C</i>	<i>T</i>	–	–	<i>C</i>	<i>C</i>	<i>A</i>	<i>A</i>

(a) Global alignment example

–	<i>A</i>	<i>T</i>	<i>C</i>	<i>G</i>	<i>A</i>	<i>A</i>	<i>C</i>	<i>T</i>	<i>G</i>	<i>G</i>	<i>C</i>	<i>C</i>	–	–
					.									
<i>T</i>	<i>A</i>	–	<i>C</i>	<i>G</i>	<i>C</i>	<i>A</i>	<i>C</i>	<i>T</i>	–	–	<i>C</i>	<i>C</i>	<i>A</i>	<i>A</i>

(b) Semi-global alignment example

<i>A</i>	<i>T</i>	<i>C</i>	<i>G</i>	<i>A</i>	<i>A</i>	<i>C</i>	<i>T</i>	<i>G</i>	<i>G</i>	<i>C</i>	<i>C</i>		
				.									
<i>T</i>	<i>A</i>	<i>C</i>	<i>G</i>	<i>C</i>	<i>A</i>	<i>C</i>	<i>T</i>	–	–	<i>C</i>	<i>C</i>	<i>A</i>	<i>A</i>

(c) Local alignment example



# Ex: Pairwise Alignment

## Alignment of a gene from two closely related viruses

Hemagglutinin gene from virus A: ATGAACGCAATACTCGTAGTT...

||||| ||||| |||||

Hemagglutinin gene from virus B: ATGAAGGCAATACTAGTAGTT...

Few Mismatches



## Alignment of a gene from two distantly related viruses

Hemagglutinin gene from virus A: ATGAACGCAATACTCGTAGTT...

||| ||| ||| |||| | |

Hemagglutinin gene from virus C: ATGCACGAAATGCTCGGACCT...

Lots of Mismatches





# What is Global Sequence Alignment?

- We search for matches, matches and gaps between two sequences to determine their **relatedness**.
- (\*) indicate matches or similar nucleotides (bases) along sequence
- Here, the sequences may have a common ancestor

ACGTACT  
ACTACGT  
\*\* \*

ACGTAC-T  
AC-TACGT  
\*\* \*\*\* \*

ACGTACT- - - -  
- - - -ACTACGT  
\*\*\*\*



# Ex: Comparing DNA

- We compare DNA samples from several different organisms.

		850		860		870		880								
<i>Gallus_gallus</i> /1-2533	CT	CAG	AAAA	CT	GCTTT	AAAT	GAA	GCC	CAT	CCA	GCA	GCTT	GG	AG	GG	GC
<i>Mus_musculus</i> /1-2491	CT	GGG	AAAA	CT	GTTTT	AAAT	CAA	GCT	AT	TTT	ACA	AGCTT	GG	AG	GG	AC
<i>Rattus_norvegicus</i> /1-2601	CT	GGG	AAAA	CT	GTTTT	AAAT	CAA	GCT	AT	ATT	ACA	AGCTT	GG	AG	GG	AC
<i>Dasypus_novemcinctus</i> /1-2306	CT	GGG	AAAA	CT	GCTTT	AAAT	CAA	GCT	AT	TTT	GCA	AACTT	GG	AG	GG	AC
<i>Loxodonta_africana</i> /1-2443	CT	GGG	AAAG	CT	GCTTT	GAAT	CAA	A	CT	GT	TTT	T	CAA	CTT	GG	AGGGGC
<i>Oryctolagus_cuniculus</i> /1-2522	CT	GGG	AAAA	CT	GCTTT	AAAT	CAA	GCT	GT	ATT	GCA	AACTT	GG	AG	GG	AC
<i>Equus_caballus</i> /1-2583	CT	GGG	AAAA	CT	GCTTT	AAAT	CAA	GCT	GT	ATT	GCA	AACTT	GG	AG	GG	AC
<i>Gorilla_gorilla</i> /1-4513	CT	GGG	AAAA	CT	GCTTT	AAAT	CAA	GCT	AT	ATT	GCA	AACTT	GG	AG	GG	AT
<i>homo_sapiens</i> /1-4639	CT	GGG	AAAA	CT	GCTTT	AAAT	CAA	GCT	AT	ATT	GCA	AACTT	GG	AG	GG	AT
<i>Macaca_mulatta</i> /1-2393	CT	GGG	AAAA	CT	GCTTT	AAAT	CAA	GCT	AT	ATT	GCA	AACTT	GG	AG	GG	AT
<i>Bos_taurus</i> /1-2527	CT	GGG	AAAA	CT	GCTTT	AAGT	CAT	GCC	CAT	ATT	GCA	AACTT	GG	AG	GG	AC
<i>Tursiops_truncatus</i> /1-2513	CT	GGG	AAAA	CT	GCTTT	AAGT	CAA	GCT	GT	ATT	GCA	AACTT	GG	AG	GG	AC
<i>Canis_lupus_familiaris</i> /1-2513	CT	GGG	AAAA	CT	GCTTT	AAAT	CAA	GCT	AT	TTT	GCA	AACTT	GG	AG	GG	AC
<i>Felis_catus</i> /1-2884	CT	GGG	AAAA	CT	GCTTT	AAAT	CAA	GCT	AT	ATT	GCA	AACTT	GG	AG	GG	AC

Consensus

CTGGGAAACTGCTTTAAATCAAGCTATATTGCAACTTGGAGGAC

# Ex: Comparing Protein

- We compare protein samples from several different organisms.

	*																																*	
Human	W	N	Q	S	T	A	R	W	L	R	R	L	V	F	Q	H	S	R	A	W	P	L	L	Q	T	F	A	F	S	A	W	W	H	G
Pig	W	N	H	S	T	A	Q	W	L	R	R	L	V	F	Q	Q	G	R	T	W	P	L	L	Q	T	F	V	F	S	A	W	W	H	G
Cow	W	N	Q	S	T	A	R	W	L	R	R	L	V	F	Q	Q	R	R	T	W	P	L	L	Q	T	F	L	F	S	A	W	W	H	G
Dog	W	N	Q	S	T	A	R	W	L	R	R	L	V	F	Q	Q	R	R	T	W	P	L	L	Q	T	F	L	F	S	A	W	W	H	G
Rat	W	N	R	S	T	A	Q	W	L	K	R	L	V	F	Q	R	S	R	R	W	P	V	L	Q	T	F	A	F	S	A	W	W	H	G
Mouse	W	N	R	S	T	A	L	W	L	R	R	L	V	F	R	K	S	R	R	W	P	L	L	Q	T	F	A	F	S	A	W	W	H	G
Chicken	W	N	R	S	T	S	L	W	L	R	R	L	V	F	Q	R	C	P	V	Q	P	L	L	A	T	F	A	F	S	A	W	W	H	G
Zebrafish	W	N	Q	T	T	V	D	W	L	R	K	I	V	F	N	R	T	S	R	S	P	L	F	M	T	F	G	F	S	A	L	W	H	G



- The common code have same nucleotides but there are still breaks in these common regions.

		*	:	*	:	:			
Q5E940_BOVIN	-----MPREDRATWKS	NYFLKIIQLDDYPKC	FI	VGADNVGSKOMQ	QIRMSLRGK-AVVL	MGKNTMMRKAIRGHLENN--PALE	76		
RLA0_HUMAN	-----MPREDRATWKS	NYFLKIIQLDDYPKC	FI	VGADNVGSKOMQ	QIRMSLRGK-AVVL	MGKNTMMRKAIRGHLENN--PALE	76		
RLA0_MOUSE	-----MPREDRATWKS	NYFLKIIQLDDYPKC	FI	VGADNVGSKOMQ	QIRMSLRGK-AVVL	MGKNTMMRKAIRGHLENN--PALE	76		
RLA0_RAT	-----MPREDRATWKS	NYFLKIIQLDDYPKC	FI	VGADNVGSKOMQ	QIRMSLRGK-AVVL	MGKNTMMRKAIRGHLENN--PALE	76		
RLA0_CHICK	-----MPREDRATWKS	NYFMKIIQLDDYPKC	FF	VGADNVGSKOMQ	QIRMSLRGK-AVVL	MGKNTMMRKAIRGHLENN--PALE	76		
RLA0_RANSY	-----MPREDRATWKS	NYFLKIIQLDDYPKC	FI	VGADNVGSKOMQ	QIRMSLRGK-AVVL	MGKNTMMRKAIRGHLENN--SALE	76		
Q7ZUG3_BRARE	-----MPREDRATWKS	NYFLKIIQLDDYPKC	FI	VGADNVGSKOMQ	TIRLSLRGK-AVVL	MGKNTMMRKAIRGHLENN--PALE	76		
RLA0 ICTPU	-----MPREDRATWKS	NYFLKIIQLDNDYPKC	FI	VGADNVGSKOMQ	TIRLSLRGK-AIVL	MGKNTMMRKAIRGHLENN--PALE	76		
RLA0_DROME	-----MVRENKA	AWKAQYFIKVVELFDE	FPKC	FI	VGADNVGSKOMQ	NIRTSLRGK-AVVL	MGKNTMMRKAIRGHLENN--PQLE	76	
RLA0 DICDI	-----MSGAG-SKR	KKLFIE	AKLFTTYDKMIVA	EADNVGSSOLQ	KIRKSIIRGI-GAVL	MGKKTMIKKVIRDLDASK--PELD	75		
Q54LP0 DICDI	-----MSGAG-SKR	KNVFI	EATKLFTTYDKMIVA	EADNVGSSOLQ	KIRKSIIRGI-GAVL	MGKKTMIKKVIRDLDASK--PELD	75		
RLA0_PLAF8	-----MAKLSKQ	QKKQMYIEKLSSLIQ	QYSKILIVHVDNVGS	NQMASVRKSLRGK-ATIL	MGKNTIRIRTALKKNLQAV--PQIE	76			
RLA0_SULAC	----MIGLAVTTT	KKIAKWVDEVAELTE	KLTKHTITIANIEGF	PADKLHEIRKKLRGK-ADIK	VTKNLNFNIALKNAG----YDTK	79			
RLA0_SULTO	----MRIMAVITQ	ERKIAWKIEEVKELEOK	LREYHTITIANIEGF	PADKLHDIRKKMRGM-AEIK	VTKNLTLFGIAAKNAG----LDVS	80			
RLA0_SULSO	----MKRLALALQ	KRVASWGLEEVKELTEL	IKNSNTILIGNLEGFP	ADKLHEIRKKLRGK-ATIK	VTKNLTLFKIAAKNAG----IDIE	80			
RLA0_AERPE	MSVVS	LVGQMYKREKPIPEW	KTMLRELEELFSKHRYV	FLDLTGPTIFVVQRV	KKLWKK-YPMV	VAKKRILFLAMKAAGLE---LDDN	86		
RLA0_PYRAE	-MMLA	IGKRRYVRTQYIPAR	KVI	SEATELKQYPPYVFLDL	HGLSSRI	LHERYYRLARY-GVIKI	KPTL FKFIAFTKVYGG---IPA E	85	
RLA0_METAC	-----MAEERH	HTEHIPQWKDE IENIKEL	IQSHKVF	GMV G IE GLATKM Q IR RD LKDV -	AVLKVS RNTLT ER AL N QL G ---	ETIP	78		
RLA0_METMA	-----MAEERH	HTEHIPQWKDE IENIKEL	IQSHKVF	GMVRIEGILATKIQ	KIRRD LKDV - AVLKVS RNTLT ER AL N QL G ---	ESIP	78		
RLA0_ARCFU	-----MAAVRGS --	PPEYKVRAVEEIKRMISSK	PVVAIVSFRNPAGOMQ	KIRREFRGK-AEIKV	VKNLT LER AL D A LG ----	GDYL	75		
RLA0_METKA	MAYKAKGQPPSGYE	PKVAEWKRREV	KELKELMDEYEN	VG	LV	DL	EGIPAPOLQEIRAKLRERDTIIRMSRNTLMRIA	LEEKLDER--PELE	88
RLA0_METHH	-----MAHVAE	WKKKEVQELHDLIKG	YEVVGIANLADIPAR	LOKMRQTLRDS-ALIR	MSKKT LIS LALEAKREL--ENVD	74			
RLA0_METTL	-----MITAESE	HKIAPWKIEEYNKLKELLNGQ	IVALDMMEVPAROLOE	IRDKIR-CTMTLK	SRNTLIERAKEVAEETGNPEFA	82			
RLA0_METVA	-----MIDAKSE	HKIAPWKIEEYNKLKELLKS	ANVALIDMM	EVPAVOLQEIRDKIR-DQMTLK	SRNTLIKRAEEVAEETGNPEFA	82			
RLA0_METJA	-----METKVKA	HVAPWKIEEVTCLKGLIKS	KEPVVAIVDMMDVPAPOLQE	IRDKIR-DKVKLR	MSRNTLIIRALKEAAEELN	NP	PKLA	81	
RLA0_PYRAB	-----MAHVAE	WKKKEVEELANLIKSYPI	VALVDVSSMPAYPLSQMRRL	IRENGLLRVS	RNTLIELAIKKA	AAQELGKPELE	77		
RLA0_PYRHU	-----MAHVAE	WKKKEVEELAKLIKSYPI	VALVDVSSMPAYPLSQMRRL	IRENGLLRVS	RNTLIELAIKKA	AAQELGKPELE	77		
RLA0_PYRFU	-----MAHVAE	WKKKEVEELANLIKSYPI	VALVDVSSMPAYPLSQMRRL	IRENGLLRVS	RNTLIELAIKKA	AAQELGKPELE	77		
RLA0_PYRKO	-----MAHVAE	WKKKEVEELANIKSYP	IVALVDVAGVPAYPLSKMRDKLR-GK	ALLRVS	RNTLIELAIKRAAQELGQ	PELE	76		
RLA0_HALMA	-----MSAESER	KTETIPEWKQEEVDVAIVMES	YESVG	VNVTGIPSR	OLQDMRDLHGT-AELRVS	RNTLIERALDDVD---DGLE	79		
RLA0_HALVO	-----MSESEVR	QTEVIPQWKREEVDDELVD	FIESYESVG	VVG	VAGIPSR	OLQSMRRELHGS-AAVRMS	RNTLVN	RALDEVN---DGFE	79
RLA0_HALSA	-----MSAEEQRT	TEEVPEWKQEAELVDLLET	YDSVG	VNVTGIPSK	OLQDMRRLHGO-AALRMS	RNTLLV	RALEEAG----DGLD	79	
RLA0_THEAC	-----MKEVSQ	KKELVNETORIKASRSVAIVDTAG	IRTRQIQDIRGKNRGK-INLK	VIKKTLLFKALENLGD---EKLS	72				
RLA0_THEVO	-----MRKINP	KKKEIVSELADITKSKAVAIVDTKG	VRTROMODIRAKNRDK-VKIK	VVKKTLLFKALDSIND---EKLT	72				
RLA0_PICTO	-----MTEPAQ	WKIDFVKNLENEINSRKVAAIVSIG	KLRNNEFOKIRNSIRDK-ARIK	VSRA	RLRLAIENTGK----NNIV	72			
ruler	1.....10.....20.....30.....40.....50.....60.....70.....80.....90								



# Terms

- Alignment is divided up into sub problems
- Solutions are scored; the best solutions for char by char comparison are kept in the overall solution.
- **Match** – bases of each sequence at position ARE same
- **Mismatch** – bases of each sequence at position are NOT same
- **Gap** – bases are not the same, some insert or deletion may have occurred.

AGGCTATCACCTGACCTCCAGGCCGATGCCC  
TAGCTATCACGACCGCGGTCGATTGCCCCGAC

–AGGCTATCACCTGACCTCCAGGCCGA––TGCCC––  
TAG–CTATCAC––GACCGC––GGTCGATTGCCCCGAC





# Terms

- **Homology** – Two or more sequences have a common ancestor
- **Similarity** – Two sequences are similar in terms of base arrangements. Note: this similarity does not refer to any specific evolutionary process; the sequences show *similarity* as they are compared.
- **Conserved regions** – Regions in code which are very similar (or the same) across a wide group of organisms. Having code which has not changed, in light of mutations, in all the organisms suggests that the region have been maintained by natural selection (and may serve an important function.)
- **DNA Coding Regions** – Contains code that is more likely to make protein, often less likely to change genetically. Mutations in these areas may cause danger.
- **DNA NonCoding Regions** – Contains DNA that does not necessarily code for protein, but may serve in gene regulation, such as the binding or recognition sites of ribosomes and transcription factors. May still be be conserved within a genome.



ALLEGHENY  
COLLEGE

# Bring the Tool!



**Up Next!**

# Clustal Omega Multiple Sequence Alignment

## Clustal Omega

Input form

Web services

Help & Documentation

Also in this section ▼

Feedback

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Tools > Multiple Sequence Alignment > Clustal Omega

## Multiple Sequence Alignment

Clustal Omega is a new multiple sequence alignment program that uses seeded guide trees and HMM profile-profile techniques to generate alignments between **three or more** sequences. For the alignment of two sequences please instead use our [pairwise sequence alignment tools](#).

Link:

<https://www.ebi.ac.uk/Tools/msa/clustalo/>

# Clustal Omega Multiple Sequence Alignment

Enter DNA  
sequences  
in this **FASTA**  
format.

## STEP 1 - Enter your input sequences

Enter or paste a set of

DNA

sequences in any supported format:

```
>seq_1
ATGCATGCATGCATGC
>seq_2
ATGCATGCATGC AAAA
>seq_3
ATGCATGC AAAAAAAAAA
```

Or, upload a file:  No file chosen

## STEP 2 - Set your parameters

OUTPUT FORMAT

ClustalW with character counts

*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)*

Submit your job with  
the *Character  
Counts* option.

# Clustal Omega Results

## Clustal Omega

Input form | Web services | Help & Documentation | Also in this section ▾ | Feedback | Share

Tools > Multiple Sequence Alignment > Clustal Omega

Your job is currently running... please be patient

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

Job ID: [clustalo-l20210321-210208-0237-5254380-p2m](#)

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.

Depending the number of seqs, you may have to wait...

The '\*'s denote same bases across sequences.

## Results for job clustalo-l20210321-210208-0237-5254380-p2m

Alignments | Result Summary | Guide Tree | Phylogenetic Tree | Results Viewers

Submission Details

Download Alignment File

seq_3	ATGCATGCAAAAAAA	16
seq_1	ATGCATGCATGCATGC	16
seq_2	ATGCATGCATGCAAAA	16
	***** *	

# Percent Identity Matrix

## How similar are the seqs?

Results for job clustalo-l20210321-210208-0237-5254380-p2m

Alignments Result Summary Guide Tree Phylogenetic Tree Results Viewers

Submission Details

Download Alignment File

seq_3	ATGCATGCAAAAAAA	16
seq_1	ATGCATGCATGCATGC	16
seq_2	ATGCATGCATGCAAAA	16
	***** *	

Find percent  
Identity results here

How similar are  
the sequences?.

```
#
#
# Percent Identity Matrix - created by Clustal2.1
#
#
```

	Seq_3	Seq_1	Seq_2
1: seq_3	100.00	62.50	81.25
2: seq_1	62.50	100.00	81.25
3: seq_2	81.25	81.25	100.00



# Percent Identity Matrix

## How similar are the seqs?

Results for job clustalo-l20210321-210208-0237-5254380-p2m

Alignments Result Summary Guide Tree Phylogenetic Tree Results Viewers

Submission Details

Download Alignment File

seq_3	ATGCATGCAAAAAAA	16
seq_1	ATGCATGCATGCATGC	16
seq_2	ATGCATGCATGCAAAA	16
	***** *	

Find similarities  
as trees here

View similarity  
in a tree from.

Phylogram

Branch length: ☒ Cladogram ☐ Real



seq\_3 0.15625  
seq\_1 0.09375  
seq\_2 0.09375

Guide Tree

Phylogram

Branch length: ☐ Cladogram ☒ Real



seq\_3 0.15625  
seq\_1 0.09375  
seq\_2 0.09375

Guide Tree

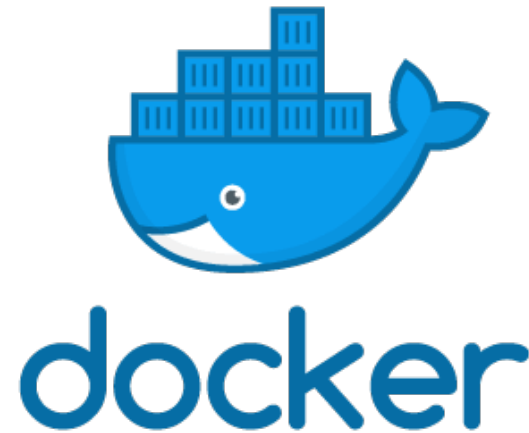


Please rebuild  
your container!!

# New Dockerfile

(for Docker  
Desktop)

Adds a tool called  
ClustalW for  
sequence  
comparison.



```
FROM ubuntu:20.04
```

```
RUN apt-get update && apt-get -y  
install git htop vim python3 python3-pip  
clustalw
```

```
RUN \  
pip3 install --upgrade pip \  
pip install bioPython
```

```
WORKDIR /root
```

```
CMD ["bash"]
```



# ClustalW On Your Own Machine

Enter sequences in a FASTA format:

```
>seq_1
ATGCATGCATGCATGC
>seq_2
ATGCATGCATGCAAAA
>seq_3
ATGCATGCAAAAAAAAA
```

```
root@ea16b8965382:~# clustalw samples.fasta
```

```
CLUSTAL 2.1 Multiple Sequence Alignments
```

```
Sequence format is Pearson
```

```
Sequence 1: seq_1      16 bp
```

```
Sequence 2: seq_2      16 bp
```

```
Sequence 3: seq_3      16 bp
```

```
Start of Pairwise alignments
```

```
Aligning...
```

```
Sequences (1:2) Aligned. Score: 81
```

```
Sequences (1:3) Aligned. Score: 56
```

```
Sequences (2:3) Aligned. Score: 75
```

```
Guide tree file created: [samples.dnd]
```

```
There are 2 groups
```

```
Start of Multiple Alignment
```

```
Aligning...
```

```
Group 1: Sequences: 2      Score:256
```

```
Group 2: Sequences: 3      Score:214
```

```
Alignment Score 236
```

```
CLUSTAL-Alignment file created [samples.aln]
```