Pairwise Sequence Alignment Assignment

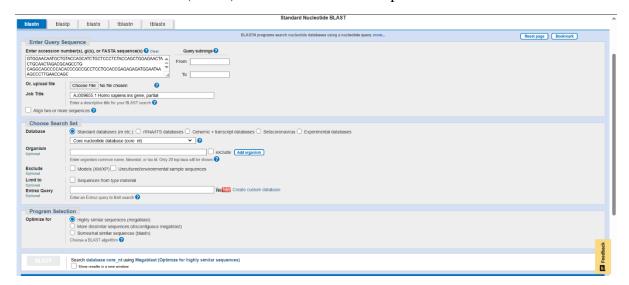
Task 1: Choose a nucleotide or protein sequence from GenBank.

Chosen sequence – Human Insulin (Accession Number- AJ009655.1)



Task 2: Perform BLASTN (for nucleotides) or BLASTP (for proteins) against the NCBI database

Standard nucleotide blast (blastn) of Human Insulin was performed



blastn Report

Descriptions	Graphic Summary	Alignments	Taxonomy									
Sequences producing significant alignments					Downlo	ad ~	s	elect (column	s Y Sh	now 1	0 🗸
select all 0	sequences selected					ank		ics [tree of re	esults	MSA View
		Description			Scientific Name	Max Score		Query Cover	E value	Per. Ident	Acc. Len	Accession
Homo sapiens	ins gene, partial				Homo sapiens	2573	2573	100%	0.0	100.00%	1393	AJ009655.1
Human gene fo	r preproinsulin, from chromoso	ome 11. Includes a high	nly polymorphic region	pstream from the ins	Homo sapiens	2567	2567	100%	0.0	99.93%	4992	V00565.1
Homo sapiens tyrosine hydroxylase (TH) gene. 3' end. insulin (INS) gene, complete cds; insulin-like growth facto			Homo sapiens	2567	2567	100%	0.0	99.93%	12565	L15440.1		
Homo saplens	insulin (INS) gene, complete c	:ds			Homo sapiens	2567	2567	100%	0.0	99.93%	4969	AH002844.2
Homo sapiens	INS-IGF2 readthrough (INS-IG	GF2), RefSeqGene on c	chromosome 11		Homo sapiens	2545	2545	100%	0.0	99.64%	39098	NG_050578
Homo sapiens	chromosome 11, clone RP11-8	889117, complete seque	ence		Homo sapiens	2545	2545	100%	0.0	99.64%	170027	AC132217.1
Homo sapiens	insulin isoform U2 (INS) mRN/	A, complete cds, alterna	atively spliced		Homo sapiens	2545	2545	100%	0.0	99.64%	1707	MT335688.1
Homo sapiens	insulin (INS), RefSegGene on	chromosome 11			Homo sapiens	2545	2545	100%	0.0	99.64%	8416	NG_007114
Human alpha-ty	ype insulin gene and 5' flankin	g polymorphic region			Homo sapiens	2545	2545	100%	0.0	99.64%	3943	M10039.1
Homo sapiens	haplotype ICa tyrosine hydrox	vlase (TH) gene, partia	I sequence: insulin (IN) gene, complete cds	. Homo sapiens	2540	2540	100%	0.0	99.57%	7496	AH012037.2

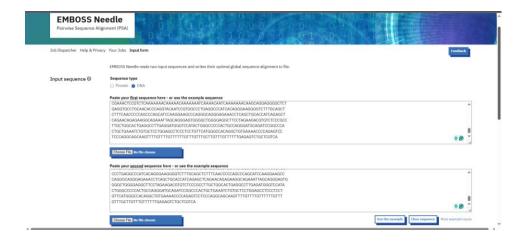
Task 3: Interpret the alignment score, E-value, and percent identity.

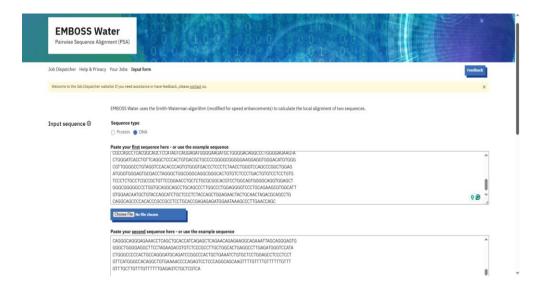
Accession	Alignment	E- value	Percentage	Length(Query
Number	Score		Identity	length- 1393)
<u>V00565.1</u>	2567	0	99.93%	4992
<u>L15440.1</u>	2567	0	99.93%	12565
AH002844.2	2567	0	99.93%	4969
NG_050578.1	2545	0	99.64%	39098
AC132217.15	2545	0	99.64%	170027

Among the top five hits, the first entry is the best match because it has a low E-value, a high alignment score, and a high percentage identity. Additionally, its target sequence length is more similar to the query compared to other hits with longer sequence lengths.

Task 4: Compare results with Needleman-Wunsch (global) and Smith-Waterman (local) algorithms (using EMBOSS)

Approach 1: Query is aligned with top 5 hits one by one in EMBOSS Needle and EMBOSS Water





```
# Aligned_sequences: 2
# 1: AJ009655.1
                                  # Aligned_sequences: 2
                                  # 1: AJ009655.1
# 2: L15440.1
                                  # 2: L15440.1
# Matrix: EDNAFULL
                                  # Matrix: EDNAFULL
# Gap_penalty: 10.0
                                  # Gap_penalty: 10.0
# Extend_penalty: 0.5
                                  # Extend_penalty: 0.5
# Length: 1393
                                  # Length: 12565
# Identity: 1392/1393 (99.9%)
                                  # Identity: 1392/12565 (11.1%)
# Similarity: 1392/1393 (99.9%)
                                  # Similarity: 1392/12565 (11.1%)
# Gaps: 0/1393 ( 0.0%)
                                  # Gaps: 11172/12565 (88.9%)
# Score: 6956.0
                                  # Score: 6956.0
#
```

Local Alignment

Global Alignment

L15440.1	4351 CCCAGCTCTGCAGCAGGGAGGACGTGGCTGGGCTCGTGAAGCATGTGGGG	4400
AJ009655.1	118 GTGAGCCCAGGGGCCCCAAGGCAGGGCACCTGGCCTTCAGCCTGCCT	167
L15440.1	4401 GTGAGCCCAGGGGCCCCAAGGCAGGGCACCTGGCCTTCAGCCTGCCT	4450
AJ009655.1	168 CCCTGCCTGTCTCCCAGATCACTGTCCTTCTGCCATGGCCCTGTGGATGC	217
L15440.1	4451 CCCTGCCTGTCTCCCAGATCACTGTCCTTCTGCCATGGCCCTGTGGATGC	4500
AJ009655.1	218 GCCTCCTGCCCCTGCTGGCGCTGCTGGCCCTCTGGGGACCTGACCCAGCC	267
L15440.1	4501 GCCTCCTGCCCCTGCTGGCGCTGCTGGCCCTCTGGGGACCTGACCCAGCC	4550
AJ009655.1	268 GCAGCCTTTGTGAACCAACACCTGTGCGGCTCACACCTGGTGGAAGCTCT	317
L15440.1	4551 GCAGCCTTTGTGAACCAACACCTGTGCGGCTCACACCTGGTGGAAGCTCT	4600
AJ009655.1	318 CTACCTAGTGTGCGGGGAACGAGGCTTCTTCTACACACCCCAAGACCCGCC	367
L15440.1	4601 CTACCTAGTGTGCGGGGAACGAGGCTTCTTCTACACACCCCAAGACCCGCC	4650
AJ009655.1	368 GGGAGGCAGAGGACCTGCAGGGTGAGCCAACCGCCCATTGCTGCCCCTGG	417
L15440.1	4651 GGGAGGCAGAGGACCTGCAGGGTGAGCCAACCGCCCATTGCTGCCCCTGG	4700
AJ009655.1	418 CCGCCCCAGCCACCCCTGCTCCTGGCGCTCCCACCCAGCATGGGCAGA	467
L15440.1	4701 CCGCCCCAGCCACCCCTGCTCCTGGCGCTCCCACCCAGCATGGGCAGA	4750
AJ009655.1	468 AGGGGGCAGGAGGCTGCCACCCAGCAGGGGGTCAGGTGCACTTTTTTAAA	517
L15440.1	4751 AGGGGCAGGAGGCTGCCACCCAGCAGGGGGTCAGGTGCACTTTTTTAAA	4800
AJ009655.1	518 AAGAAGTTCTCTTGGTCACGTCCTAAAAGTGACCAGCTCCCTGTGGCCCA	567
L15440.1	4801 AAGAAGTTCTCTTGGTCACGTCCTAAAAGTGACCAGCTCCCTGTGGCCCA	4850
A3000/FF 4	F/O OTOACAATOTOACOOTOACOAOCOTOTTOCOCTOTOCOCAACCOCOACATAC	747

```
emboss_water-I20250330-055215-0537-18184253-p1m - Notepad
File Edit Format View Help
# Length: 1393
# Identity: 1392/1393 (99.9%)
# Similarity: 1392/1393 (99.9%)
# Gaps: 0/1393 (0.0%)
 Score: 6956.0
AJ009655.1
             1 AGCAGGTCTGTTCCAAGGGCCTTTGCGTCAGGTTGGGCTCAGGGTTCCAGG
                                                         50
           L15440.1
           AJ009655.1
                                                        100
L15440.1
                                                       4383
AJ009655.1
            150
           L15440.1
                                                       4433
           AJ009655.1
L15440.1
                                                       4483
AJ009655.1
           201 CATGGCCCTGTGGATGCGCCTCCTGCCCCTGCTGGCGCTGCTGGCCCTCT
                                                        250
           4484 CATGGCCCTGTGGATGCGCCCTCTGCCCCTGCTGGCGCTGCTGGCCCTCT
L15440.1
                                                       4533
AJ009655.1
           L15440.1
                                                       4583
AJ009655.1
            301 CACCTGGTGGAAGCTCTCTACCTAGTGTGCGGGGAACGAGGCTTCTTCTA
                                                        350
           4584 CACCTGGTGGAAGCTCTCTACCTAGTGTGCGGGGAACGAGGCTTCTTCTA
L15440.1
                                                       4633
AJ009655.1
            351 CACACCCAAGACCCGCCGGGAGGCAGAGGACCTGCAGGGTGAGCCAACCG
                                                        400
           4634 CACACCCAAGACCCGCCGGGAGGCAGAGGACCTGCAGGGTGAGCCAACCG
L15440.1
```

```
mboss_water-I20250330-060025-0873-21332805-p1m - Notepad
File Edit Format View Help
# Aligned_sequences: 2
# 1: AJ009655.1
# 2: AH002844.2
# Matrix: EDNAFULL
 Gap_penalty: 10.0
Extend_penalty: 0.5
 Length: 1393
 Identity: 1392/1393 (99.9%)
Similarity: 1392/1393 (99.9%)
Gaps: 0/1393 (0.0%)
#
 Gaps:
 Score: 6956.0
#
          AJ009655.1
                                                          50
AH002844.2
                                                         2272
            51 GTGGCTGGACCCCAGGCCCAGCTGTGCAGCAGGAGGACGTGGCTGGGC
AJ009655.1
                                                         100
          2273 GTGGCTGGACCCCAGGCCCCAGCTCTGCAGCAGGAGGACGTGGCTGGGC
AH002844.2
                                                         2322
AJ009655.1
            150
            AH002844.2
                                                         2372
AJ009655.1
            2373 CCTTCAGCCTGCCTCAGCCCTGCTCTCCCAGATCACTGTCCTTCTGC
AH002844.2
            AJ009655.1
AH002844.2
                                                         2472
AJ009655.1
            251 GGGGACCTGACCCAGCCGCAGCCTTTGTGAACCAACACCTGTGCGGCTCA
                                                          300
                AH002844.2
            2473 GGGGACCTGACCCAGCCGCAGCCTTTGTGAACCAACACCTGTGCGGCTCA
                                                         2522
mboss water-120250330-060314-0001-26192335-p1m - Notepad
File Edit Format View Help
# Aligned sequences: 2
 1: AJ009655.1
2: NG_050578.1
Matrix: EDNAFULL
 Gap_penalty: 10.0
 Extend_penalty: 0.5
#
 Length: 1394
Identity:
 Identity: 1389/1394 (99.6%)
Similarity: 1389/1394 (99.6%)
Gaps: 1/1394 (0.1%)
#
#
 Score: 6919.0
           AJ009655.1
                                                           50
NG_050578.1
                                                          5087
            AJ009655.1
                                                          100
NG 050578.1
                                                          5137
AJ009655.1
            150
                NG 050578.1
            5187
AJ009655.1
             151 CCTTCAGCCTGCCTCAGCCCTGTCTCCCAGATCACTGTCCTTCTGC
                                                          200
            NG 050578.1
                                                          5237
AJ009655.1
                                                          250
            NG 050578.1
AJ009655.1
            5288 GGGGACCTGACCCAGCCGCAGCCTTTGTGAACCAACACCTGTGCGGCTCA
NG_050578.1
                                                          5337
AJ009655.1
             301 CACCTGGTGGAAGCTCTCTACCTAGTGTGCGGGGAACGAGGCTTCTTCTA
                                                           350
```

```
mboss_water-I20250330-060731-0076-88674023-p1m - Notepad
File Edit Format View Help
# Aligned_sequences: 2
# 1: AJ009655.1
# 2: AC132217.15
# Matrix: EDNAFULL
# Gap_penalty: 10.0
# Extend_penalty: 0.5
 Length: 1394
 Identity: 1389/1394 (99.6%)
Similarity: 1389/1394 (99.6%)
              1/1394 ( 0.1%)
 Score: 6919.0
AJ009655.1
               {\tt 1} {\tt AGCAGGTCTGTTCCAAGGGCCTTTGCGTCAGGTTGCGCTCAGGGTTCCAGG}
          AC132217.15
                                                             86505
                                                              100
AJ009655.1
              51 GTGGCTGGACCCCAGGCCCCAGCTGTGCAGCAGGAGGACGTGGCTGGGC
           AC132217.15
                                                             86555
              101 TCGTGAAGCATGTGGGGGTGAGCCCAGGGGCCCCAAGGCAGGGCACCTGG
AJ009655.1
AC132217.15
            86605
            200
AJ009655.1
AC132217.15
                                                             86655
              201 CATGGCCCTGTGGATGCGCCTCCTGCCCCTGCTGGCGCTGCTGGCCCTCT
AJ009655.1
                                                              250
AC132217.15
            86656 CATGGCCCTGTGGATGCGCCTCCTGCCCCTGCTGGCGCTGCTGGCCCTCT
                                                             86705
AJ009655.1
              251 GGGGACCTGACCCAGCCGCAGCCTTTGTGAACCAACACCTGTGCGGCTCA
                                                              300
                 86706 GGGGACCTGACCCAGCCGCAGCCTTTGTGAACCAACACCTGTGCGGCTCA
AC132217.15
                                                             86755
```

Query with	Global Alignment(Smith				Local Alignment (Needleman			
Targets-	Waterman)			Wunsch)				
	Gaps	Identity%	Length	Score	Gaps	Identity%	Length	Score
(<u>V00565.1</u>)	72.1%	27.9%	4992	6956	0%	99.9%	1393	6956
<u>L15440.1</u>	88.9%	11.1%	12565	6956	0%	99.9%	1393	6956
AH002844.2	72.0%	28.0%	4969	6956	0%	99.9%	1393	6956
NG_050578.1	96.4%	3.6%	39098	6919	0.1%	99.6%	1394	6919
AC132217.15	99.2%	0.8%	170027	6919	0.1%	99.6%	1394	6919

Table 1: Comparison of Results obtained when query was aligned with first 5 hits of blast using global and local alignment

From the table 1, the following comparison can be made:-

	Global Alignment(Needleman Wunsch)	Local Alignment (Smith - Waterman)
Function	Aligns entire sequence	Covers only the sequence that matches
Gaps	More Gaps are added in Global Alignment	Fewer gaps are added
Length of sequence	Length of sequence in Global Alignment is greater	Length of sequence in Global Alignment is less
Use	To identify closely related species	To identify conserved regions, and functional domains
Identity	Has lower identity and similarity percentage	Has higher identity and similarity percentage

Approach 2: All 5 sequences are added as query in EMBOSS Clustal Omega

File Edit Format	View Help	
/00565.1	AGGTGGGCTCAGGGTTCCAGGGTGGCTGGACCCCAGGCCCCAGCTCTGCAGCAG	2305
AC132217.15	GGGTGGCTGCTCCTGCACCCTCTGCAGACAGCTACCTCACCCTCACGTCT	31726
NG_050578.1	GGAGGACGTGGCTGGGCTCGTGAAGCATGTGGGGGTGAGCCCAGGGGCCCCAAGGCAGGG	5180
H002844.2	GGAGGACGTGGCTCGTGAAGCATGTGGGGGTGAGCCCAGGGGCCCCAAGGCAGGG	2365
15440.1	GGAGGACGTGGCTGGGCTCGTGAAGCATGTGGGGGTGAGCCCAGGGGCCCCAAGGCAGGG	4426
J009655.1	GGAGGACGTGGCTGGGCTCGTGAAGCATGTGGGGGTGAGCCCAGGGGCCCCAAGGCAGGG	143
/00565.1	GGAGGACGTGGCTCGTGAAGCATGTGGGGGTGAGCCCCAGGGCCCCAAGGCAGGG ** ****** *** ** * * * * * * * * * *	2365
AC132217.15	CCCCAAGCCCTGGTCACACATTGGCCTGTCCACTTGAGGGCCTCGCGGGCTTCTGAAAA	31786
NG_050578.1	CACCTGGCCTTCAGCCTCAGCCCTGCCTGTCTCCCAGATCACTGTCCTTCTGCCAT	5240
H002844.2	CACCTGGCCTTCAGCCTCAGCCCTGCCTGTCTCCCAGATCACTGTCCTTCTGCCAT	2425
15440.1	CACCTGGCCTTCAGCCTCAGCCCTGCCTGTCTCCCAGATCACTGTCCTTCTGCCAT	4486
J009655.1	CACCTGGCCTTCAGCCTCAGCCCTGCCTGTCTCCCAGATCACTGTCCTTCTGCCAT	203
/00565.1	CACCTGGCCTTCAGCCTGAGCCCTGCCTGTCTCCCAGATCACTGTCCTTCTGCCAT * ** *** *	2425
AC132217.15	CTCACTTGTCTGGGACTTGCTCCCGGTCCCCCTCACCTACCCCACAGTGGCCGTCCTGG	31846
NG_050578.1	GGCCCTGTGGATGCGCCTCCTGCCCCTGCTGGCGCTGCTGGCCCTCTGGGGACCTGACCC	5300
H002844.2	GGCCCTGTGGATGCGCCTCCTGCCCCTGCTGGCGCTGCTGGCCCTCTGGGGACCTGACCC	2485
15440.1	GGCCCTGTGGATGCGCCTCCTGCCCCTGCTGGCGCTGCTGGCCCTCTGGGGACCTGACCC	4546
J009655.1	GGCCCTGTGGATGCGCCTCCTGCCCCTGCTGGCGCTGCTGGCCCTCTGGGGACCTGACCC	263
00565.1	GGCCCTGTGGATGCGCCTCCTGCCCCTGCTGCGCGCTGCTGGCCCTCTGGGGACCTGACCC * **	2485
AC132217.15	TCCCCCTTCCCCTATCCCCCAGCGCCCGTCCCGGTCCCCCCTCACCTACCCCCCC	31901
NG_050578.1	AGCCGCAGCCTTTGTGAACCAACACCTGTGCGGCTCACACCTGGTGGAAGCTCTCTACCT	5360
H002844.2	AGCCGCAGCCTTTGTGAACCAACACCTGTGCGGCTCACACCTGGTGGAAGCTCTCTACCT	2545
15440.1	AGCCGCAGCCTTTGTGAACCAACACCTGTGCGGCTCACACCTGGTGGAAGCTCTCTACCT	4606
NJ009655.1	AGCCGCAGCCTTTGTGAACCAACACCTGTGCGGCTCACACCTGGTGGAAGCTCTCTACCT	323
/00565.1	AGCCGCAGCCTTTGTGAACCAACACCTGTGCGGCTCACACCTGGTGGAAGCTCTCTACCT ** * * * * * * * * * * * * * * * * * *	2545
AC132217.15	AGCACCCGTCCCAGTCCCCTTCACCTACTCCCCAGCGGCCTTCCGGGTCCCCTCTCACCT	31961
NG_050578.1	AGTGTGCGGGGAACGAGGCTTCTTCTACACACCCAAGACCCGCCGGGAGGCAGAGGACCT	5420
H002844.2	AGTGTGCGGGGAACGAGGCTTCTTCTACACACCCAAGACCCGCCGGGAGGCAGAGGACCT	2605
15440.1	AGTGTGCGGGGAACGAGGCTTCTTCTACACACCCAAGACCCGCCGGGAGGCAGAGGACCT	4666
AJ009655.1	AGTGTGCGGGGAACGAGGCTTCTTCTACACACCCAAGACCCGCCGGGAGGCAGAGGACCT	383
/00565.1	AGTGTGCGGGGAACGAGGCTTCTTCTACACACCCAAGACCCGCCGGGAGGCAGAGGACCT	2605

The following identity matrix from EMBOSS Clustal Omega was extracted to identify percentage identity

	1	2	3	4	5
1: NG_050578.1	100.00	88.66	99.28	<mark>99.71</mark>	98.53
2: AH002844.2	88.66	100.00	89.41	<mark>99.93</mark>	89.91
3: L15440.1	99.28	89.41	100.00	<mark>99.93</mark>	99.08
4: AJ009655.1	99.71	99.93	99.93	100.00	99.93
5: V00565.1	98.53	89.91	99.08	<mark>99.93</mark>	100.00

The highlighted part (yellow)is the alignment of query insulin with different targets using Clustal Omega

Query -	Clustal Omega	Percentage Identity
AJ009655.1	Percentage Identity of	observed in Smith
	queries with	Waterman alogorithm
	AJ009655.1	_
NG_050578.1	99.71	99.6%
AH002844.2	99.93	99.9%
L15440.1	99.93	99.9%
V00565.1	99.93	99.9%

Table 3:Percentage Identity obtained from ClustalW and Smith Waterman Algorithm

Conclusion:

Hence the comparison showed that results obtained from both approaches are similar

Task 5: Discuss the significance of high vs. low E-values.

Criteria	Low E value	High E values
Significance	Alignment is homologous	Alignment not biologically
		significant
Seen in	Related species, conserved	Unrelated sequences
	gene	
Meaning	Strong Match	Weak match
Example	1e-10, value close to 0,0	Above 1