# **SCIENCE 2**

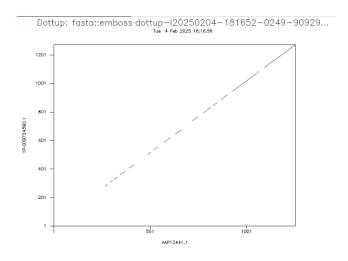
# **TUTORIAL-1**

Name: Varun Gupta Roll No: 2023101108

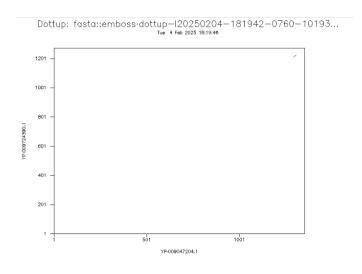
# **Question 1**

# **Dottup**

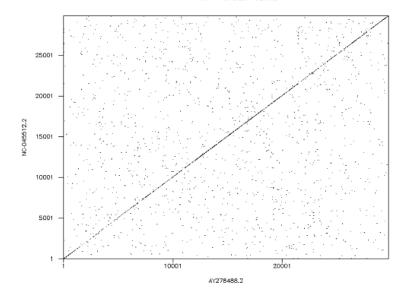
# SARS-CoV2 vs SARS-CoV (Protein)



# SARS-Cov2 vs MERS-CoV (Protein)

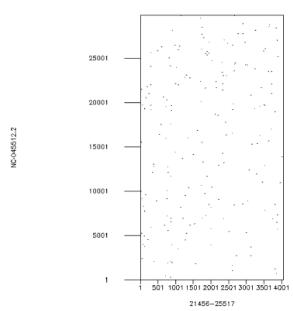


Dottup: fasta::emboss-dottup-I20250204-182149-0076-29332... Tue 4 Feb 2025 16:21:52



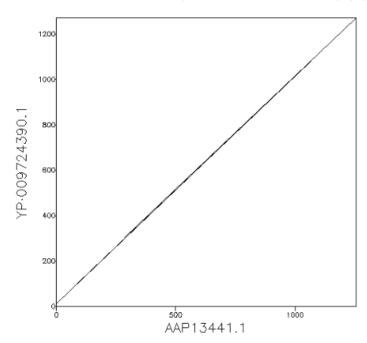
# SARS-Cov2 vs MERS-CoV (DNA)

Dottup: fasta::emboss-dottup—120250204—182446—0962—53553... Tue 4 Feb 2025 18:24:52



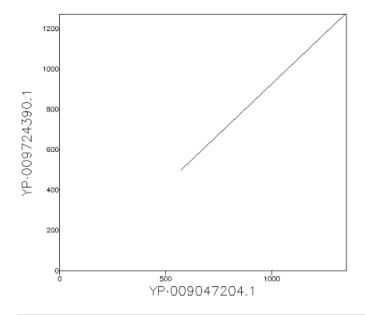
## SARS-CoV2 vs SARS-CoV (Protein)

Dotmatcher: fasta::emboss·dotmatcher-|20250204-182324-08... (windowsize = 500, threshold = 100.00 04/02/25)

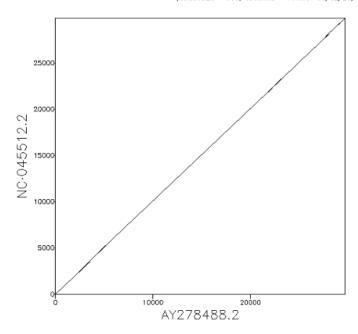


# SARS-Cov2 vs MERS-CoV (Protein)

Dotmatcher: fasta::emboss-dotmatcher-120250204-182519-09... (Windowsize = 500, threshold = 100.00 04/02/25)

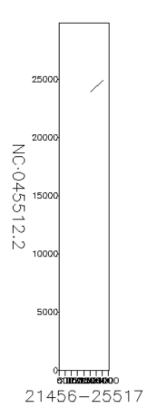


Dotmatcher: fasta::emboss·dotmatcher-|20250204-183213-07... (windowsize = 500, threshold = 100.00 04/02/25)



# SARS-Cov2 vs MERS-CoV (DNA)

Dotmatcher: fasta::emboss·dotmatcher-120250204-184741-06... (windowsize = 500, threshold = 100.00 04/02/25)



 SARS-CoV-2 is more similar to SARS-CoV (2003) than to MERS-CoV (2012). The dot plots reveal a strong, continuous diagonal pattern between SARS-CoV-2 and SARS-CoV, indicating high sequence similarity. In contrast, the plots comparing SARS-CoV-2 and MERS-CoV show fewer matching points and a lack of a continuous diagonal, suggesting a more distant relationship. Dottup results confirm an almost continuous alignment with small gaps between SARS-CoV-2 and SARS-CoV, while no such diagonal is observed for SARS-CoV-2 and MERS-CoV. Similarly, Dotmatcher results show a continuous diagonal alignment for SARS-CoV-2 and SARS-CoV, but only scattered matches toward the latter part of the sequence with MERS-CoV.

- 2. It is easier to identify similarities using protein sequences than DNA sequences. Protein sequence dot plots are clearer and more continuous, as seen in the 7th plot (Dotmatcher between protein sequences of SARS-CoV-2 and SARS-CoV), which shows a continuous diagonal line compared to the slightly broken diagonal in the 3rd plot (DNA sequences). DNA sequence plots are more fragmented due to silent mutations (nucleotide changes that do not alter amino acids) and codon redundancy, where multiple codons code for the same amino acid, causing variations in DNA without affecting the resulting protein.
- 3. In Dottup, the K-tuple represents the word size, which is set to 10. In Dotmatcher, the window size is 500, and the threshold value is 100.

# **Question 2**

#### Part A

(i) At the DNA level for SARS-CoV-2 and SARS-CoV:

Percentage identity: 73.3%Percentage similarity: 73.3%

At the protein level for two proteins:

Percentage identity: 76.4%Percentage similarity: 87.0%

The higher percentage similarity at the protein level is due to the use of the BLOSUM62 matrix, which accounts for not only exact matches but also biochemical similarities between amino acids. In contrast, nucleotide comparisons are binary—bases either match or they don't—resulting in identical percentage values for identity and similarity. This highlights that proteins provide a more detailed measure of sequence similarity, making them more effective for comparing sequences than nucleotides.

(ii) Sequence identity strictly counts the exact matches between the bases of two sequences without accounting for gaps or penalties. For sequences of different lengths, the analysis is based on the shorter sequence.

In contrast, sequence similarity is a broader metric that measures the overall resemblance between two sequences. It considers both matches and mismatches and evaluates how well the sequences align. Unlike identity, similarity uses a more flexible and nuanced approach, making it less rigid in its assessment.

(iii) Global alignment aligns the entire length of both sequences from start to end, making it ideal for sequences of similar lengths. In contrast, local alignment focuses on specific regions of similarity, making it suitable for identifying conserved segments within longer sequences.

Global alignment is performed using Emboss Needle, while local alignment is done using Emboss Water. As shown, the similarity and identity scores from both Needle and Water aligners are very close (73.3% and 72.8%), indicating that global and local alignments yield comparable results in this case.

(iv) EMBOSS WATER (Pairwise Alignment)

#### SARS-CoV2, SARS-CoV (Protein)

```
ныныныныныныныныныныныныныны
# Program: water
# Rundate: Tue 4 Feb 2025 18:31:47
# Commandline: water
     -auto
     -stdout
     -asequence emboss_water-I20250204-183144-0276-3181322-p1m.asequence
     -bsequence emboss water-I20250204-183144-0276-3181322-p1m.bsequence
    -datafile EBLOSUM62
     -gapopen 10.0
     -gapextend 0.5
     -aformat3 pair
     -sprotein1
     -sprotein2
# Align_format: pair
# Report_file: stdout
# Aligned_sequences: 2
# 1: YP_009724390.1
# 2: AAP13441.1
# Matrix: EBLOSUM62
# Gap_penalty: 10.0
# Extend_penalty: 0.5
#
# Length: 1277
**-n+i+v: 975/1277 (76.4%)
# Similarity: 1111/1277 (87.0%)
--,+2// (87.0%)

" Gaps: 26/1277 ( 2.0%)

# Score: 5230.0
```

```
# Rundate: Tue 4 Feb 2025 18:40:11
# Commandline: water
    -asequence emboss_water-I20250204-183901-0647-27915578-p1m.asequence
    -bsequence emboss_water-I20250204-183901-0647-27915578-p1m.bsequence
    -datafile EDNAFULL
    -gapopen 10.0
    -gapextend 0.5
    -aformat3 pair
    -snucleotide1
    -snucleotide2
# Align_format: pair
# Report_file: stdout
ннинининининининининининининининин
# Aligned_sequences: 2
# 1: NC_045512.2
# 2: AY278488.2
# Matrix: EDNAFULL
# Gap_penalty: 10.0
# Extend_penalty: 0.5
# Length: 30425
# Identity: 24132/30425 (79.3%)
# Similarity: 24132/30425 (79.3%)
# Gaps:
             1257/30425 ( 4.1%)
# Score: 95783.5
```

### **EMBOSS NEEDLE (Pairwise Alignment)**

## SARS-CoV2, SARS-CoV (Protein)

```
# Program: needle
# Rundate: Tue 4 Feb 2025 18:39:39
# Commandline: needle
     -stdout
     -asequence emboss_needle-I20250204-183936-0008-88772737-p1m.asequence
     -bsequence emboss needle-I20250204-183936-0008-88772737-p1m.bsequence
     -datafile EBLOSUM62
     -gapopen 10.0
     -endopen 10.0
     -endextend 0.5
     -aformat3 pair
     -sprotein2
# Align_format: pair
# Report file: stdout
# Aligned_sequences: 2
# 1: YP_009724390.1
# 2: AAP13441.1
# Matrix: EBLOSUM62
# Gap_penalty: 10.0
# Extend_penalty: 0.5
# Length: 1277
# Identity: 975/1277 (76.4%)
# Similarity: 1111/1277 (87.0%)
# Gaps: 26/1277 ( 2.0%)
# Score: 5230.0
```

```
инанинанинанинанинанинанинанин
# Program: needle
# Rundate: Tue 4 Feb 2025 18:45:28
# Commandline: needle
     -auto
     -asequence emboss needle-I20250204-184434-0664-53518741-p1m.asequence
     -bsequence emboss_needle-I20250204-184434-0664-53518741-p1m.bsequence
     -datafile EDNAFULL
     -gapextend 0.5
     -endopen 10.0
     -endextend 0.5
     -snucleotide1
# Align_format: pair
# Report_file: stdout
# Aligned_sequences: 2
# 1: NC_045512.2
# 2: AY278488.2
# Matrix: EDNAFULL
# Gap penalty: 10.0
# Extend_penalty: 0.5
# Length: 30481
# Identity: 24125/30481 (79.1%)
# Similarity: 24125/30481 (79.1%)
# Gaps: 1334/30481 (4.4%)
# Gaps: 13
# Score: 95783.5
```

### For protein alignments:

Matrix: BLOSUM62Gap penalty: 10Extend penalty: 0.5

# For DNA alignments:

Matrix: DNAfullGap penalty: 10Extend penalty: 0.5

#### Part B

**EMBOSS WATER (Pairwise Alignment)** 

# SARS-Cov2 vs MERS-CoV (Protein)

```
# Program: water
# Rundate: Tue 4 Feb 2025 18:46:05
# Commandline: water
    -auto
    -stdout
    -asequence emboss_water-I20250204-184558-0828-55458133-p1m.asequence
   -bsequence emboss_water-I20250204-184558-0828-55458133-p1m.bsequence
    -datafile EBLOSUM62
    -gapopen 10.0
    -gapextend 0.5
    -aformat3 pair
   -sprotein1
-sprotein2
# Align_format: pair
# Report_file: stdout
# Aligned_sequences: 2
# 1: YP_009724390.1
# 2: YP_009047204.1
# Matrix: EBLOSUM62
# Gap_penalty: 10.0
# Extend_penalty: 0.5
# Length: 1440
# Identity: 433/1440 (30.1%)
# Similarity: 655/1440 (45.5%)
# Gaps:
              276/1440 (19.2%)
# Score: 1568.5
```

SARS-Cov2 vs MERS-CoV (DNA)

```
# Program: water
# Rundate: Tue 4 Feb 2025 18:50:58
# Commandline: water
     -stdout
     -asequence emboss_water-I20250204-185049-0200-91566408-p1m.asequence
    -bsequence emboss water-I20250204-185049-0200-91566408-p1m.bsequence
    -gapopen 10.0
     -gapextend 0.5
     -aformat3 pair
     -snucleotide1
     -snucleotide2
# Align_format: pair
# Report file: stdout
# Aligned_sequences: 2
# 1: NC_045512.2
# 2: 21456-25517
# Matrix: EDNAFULL
# Gap_penalty: 10.0
# Extend_penalty: 0.5
# Length: 6807
# Identity: 2779/6807 (40.8%)
# Similarity: 2779/6807 (40.8%)
               3258/6807 (47.9%)
# Gaps:
# Score: 4835.0
```

#### **EMBOSS NEEDLE (Pairwise Alignment)**

#### SARS-Cov2 vs MERS-CoV (Protein)

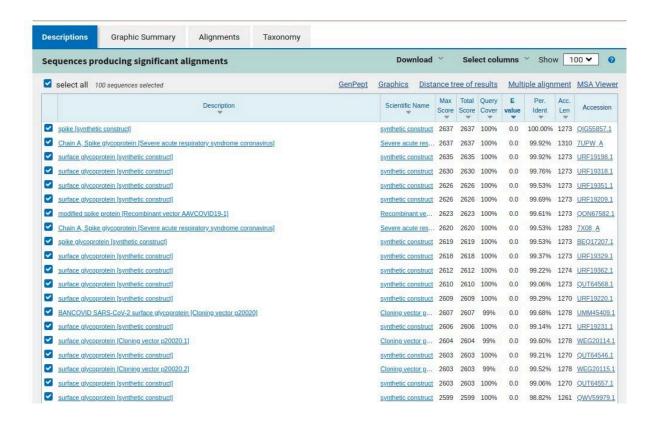
```
# Rundate: Tue 4 Feb 2025 18:50:54
# Commandline: needle
    -stdout
    -asequence emboss_needle-I20250204-185049-0136-56216348-p1m.asequence
    -bsequence emboss_needle-I20250204-185049-0136-56216348-p1m.bsequence
    -datafile EBLOSUM62
    -gapopen 10.0
    -gapextend 0.5
    -endopen 10.0
    -endextend 0.5
    -sprotein1
    -sprotein2
# Align_format: pair
# Report file: stdout
# 1: YP_009724390.1
# 2: YP_009047204.1
# Matrix: EBLOSUM62
# Gap_penalty: 10.0
# Extend_penalty: 0.5
# Length: 1454
# Identity: 434/1454 (29.8%)
# Similarity: 658/1454 (45.3%)
```

```
# Program: needle
# Rundate: Tue 4 Feb 2025 20:02:42
# Commandline: needle
   -asequence /var/lib/emboss-explorer/output/135364/.asequence
   -bsequence /var/lib/emboss-explorer/output/135364/.bsequence
   -noendweight
#
   -brief
  -outfile outfile
   -aformat3 srspair
# Align_format: srspair
# Report file: outfile
# Aligned sequences: 2
# 1: 21456-25517
# 2: 21563-25384
# Matrix: EDNAFULL
# Gap_penalty: 10.0
# Extend_penalty: 0.5
# Length: 4867
# Identity: 2330/4867 (47.9%)
# Similarity: 2330/4867 (47.9%)
# Gaps: 1850/4867 (38.0%)
# Score: 4414.5
```

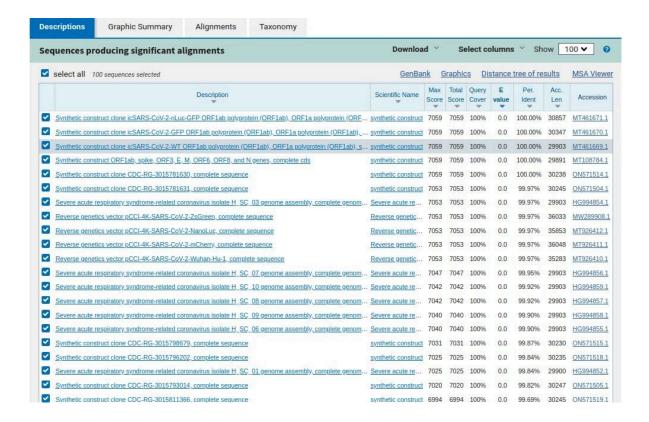
- (i) Proteins with a percentage similarity exceeding 30% are considered homologous. According to the Emboss Water results, the similarity percentage between SARS-CoV-2 and MERS-CoV is 51%. Thus, it can be concluded that SARS-CoV-2 and MERS-CoV are homologous.
- (ii) The conclusions are based on protein sequence alignments performed using Emboss Water.

## **Question 3**

#### BLAST Protein Search of SARS-Cov2



#### **BLAST Nucleotide Search of SARS-Cov2**



(i) Based on the BLASTp (protein) search, the closest homolog is the Spike glycoprotein (Chain A) from Severe Acute Respiratory Syndrome Coronavirus. Similarly, the BLASTn (nucleotide) search identifies the closest homolog as the genome assembly of the Severe Acute Respiratory Syndrome-related coronavirus isolate H SC 03.

#### (ii) Protein Alignment:

Percentage Identity: 99.92%

Length: 1310Score: 2637E-value: 0.0

#### **Nucleotide Alignment:**

Percentage Identity: 99.97%

Length: 30,245Score: 7053E-value: 0.0

Both alignments show high identity with highly significant E-values (0.0).

(iii) The Spike glycoprotein of SARS-CoV stands out as the top hit in the BLAST search results, indicating it as the closest homolog to the query sequence. The percentage identity and similarity values obtained from this search align closely with those generated by the 'water' alignment tool, further supporting the similarity between the sequences. The images below illustrate these alignment results.

```
# Program: water
# Rundate: Tue 4 Feb 2025 16:56:54
# Commandline: water
     -auto
#
     -stdout
    -asequence /var/lib/emboss-explorer/output/493580/.asequence
-bsequence /var/lib/emboss-explorer/output/493580/.bsequence
#
#
    -datafile EDNAFULL
#
#
     -gapopen 10.0
#
    -gapextend 0.5
    -aformat3 pair
#
#
    -snucleotide1
     -snucleotide2
# Align_format: pair
# Report_file: stdout
# Aligned sequences: 2
# 1: 21563-25384
# 2: HG994854.1
# Matrix: EDNAFULL
# Gap penalty: 10.0
# Extend penalty: 0.5
# Length: 3822
# Identity: 3821/3822 (100.0%)
# Similarity: 3821/3822 (100.0%)
                2/3822 ( 1.0%)
# Gaps:
# Score: 19101.0
#
```

#### **Protein**

```
**************************************
# Program: water
# Rundate: Tue 4 Feb 2025 16:56:54
# Commandline: water
    -auto
    -stdout
#
    -asequence emboss_water-120240405-175911-0682-16077024-pim.asequence
#
    -bsequence emboss_water-120240405-175911-0682-16077024-pim.bsequence
    -datafile EBLOSUM62
-gapopen 10.0
#
#
    -gapextend 0.5
#
#
     -aformat3 pair
    -sprotein1
#
     -sprotein2
#
# Align format: pair
# Report file: stdout
#
# Aligned_sequences: 2
# 1: YP_009724390.1
# 2: 7UPWA
# Matrix: EBLOSUM62
# Gap_penalty: 10.0
 Extend penalty: 0.5
# Length: 1273
# Identity:
                1272/1273 (99.9%)
# Similarity: 1272/1273 (99.9%)
# Gaps:
                1/1273 ( 0.9%)
# Score: 6715.0
```

The consistency between the results highlights the effectiveness and precision of the **BLAST** search algorithm in sequence alignment. Unlike **Water**, where sequences are directly input for comparison, **BLAST** performs a search across the entire database. The agreement in the results reinforces the reliability and accuracy of the **BLAST** search method in identifying homologous sequences.

(iv)



When searching for SARS-CoV-2 in BLAST, a hit for bat SARS coronavirus is found. Below are the alignment metrics for the spike glycoprotein of SARS-CoV-2 and the spike glycoprotein of bat SARS coronavirus:

Percentage Similarity: NA
Percentage Identity: 98.43%
Length of Alignment: 1269

Score: 2593E-value: 0.0

# **Question 4**

#### **Database Statistics:**

UniProtKB (Protein Database):

o Entries: 248,805,733

o Total amino acids: 87,574,368,369

o Reference

• GenBank (Nucleotide Database):

Total bases: 2,570,711,588,044Total sequences: 249,060,436

o Reference

# **Dynamic Programming (DP) Search:**

- **Search Complexity**: O(m \* n), where **m** is the query sequence length and **n** is the target sequence length.
- **Processing speed**: 10 million matrix cells per second, implying a time of 10^-7 seconds per cell.

#### (i) Search Time Estimates:

- UniProt (Protein Database):
  - o Bases: 1000
  - Amino acids: ~333 (since each amino acid is represented by 3 bases)
  - **Total cells**: ~2.92 × 10<sup>13</sup>
  - Calculation Time: ~2.92 × 10<sup>6</sup> seconds
- GenBank (Nucleotide Database):
  - o Total cells: 2,570,711,588,044,000
  - Calculation Time: ~257,071,158.8044 seconds

#### (ii) Space Complexity for Chromosomes:

- Human Chromosome 1:
  - Size: 249 Mbp
  - o Query Sequence Length: 1000
  - Space Complexity: ~2.49 × 10¹¹ bits
- Mouse Chromosome 1:
  - Size: 195 Mbp
  - o Query Sequence Length: 1000
  - Space Complexity: ~1.95 × 10<sup>11</sup> bits