Name:	Ms. Prarthi Hrishit Kothari
Class:	M. Sc. Bioinformatics (Part I)
Roll Number:	115
Course:	M. Sc. Bioinformatics
Department:	Department of Bioinformatics
Paper:	Mandatory Paper I
Paper Name and Code:	Fundamental of Biology & Bioinformatics (GNKPSBI1501)
Academic Year:	2023-24

SGCP's
Guru Nanak Khalsa College
of Arts, Science & Commerce (Autonomous)

DEPARTMENT OF BIOINFORMATICS

CERTIFICATE

This is to certify that Ms. Prarthi Hrishit Kothari (Roll No: 115) of M.Sc. Bioinformatics (Part I) has satisfactorily completed the practical for Mandatory Paper 1: Fundamental of Biology & Bioinformatics (GNKPSBI1501) for Semester I course prescribed by the University of Mumbai during the academic year 2023-2024.

TEACHER-IN-CHARGE	**HEAD OF THE DEPARTMENT**	**EXTERNAL EXAMINER**
(Mrs. Aparna Patil Kose)	(Dr. Gursimran Kaur Uppal)	

INDEX

Sr. No.	Experiment	Date	Page No.	Sign
1.	2-D Separation of Plant Pigments using Paper Chromatography	09/09/23		
2.	Estimation of Vitamin C using UV-VIS Spectrophotometer	27/09/23		
3.	Thin Layer Chromatography to determine “curcumin” content of Turmeric sample	04/10/23		
4.	Biochemical Estimation of RNA using orcinol method	03/11/23		
5.	Biochemical Estimation of DNA using DPA method	04/11/23		
6.	Introduction to Sequence Alignment tools:	01/11/23		
6(A)	To study and explore similar sequences of the protein ‘Albumin’ (UniProt ID: P02768) by using Basic Local Alignment Search Tool (BLAST).	01/11/23		
6(B)	To study protein sequence similarity by exploring the FASTA tool for the query ‘Maltose’ (UniProt ID: P68187).	01/11/23		
6(C)	To explore the PSI BLAST tool for the further study of the query ‘Leucine’ (UniProt ID: Q8IX15).	01/11/23		
6(D)	To perform an iterative blast for query Flavodoxin (UniProt ID: P53554) by exploring Pattern Hit Initiated BLAST (PHI-BLAST) Tool.	01/11/23		
6(E)	To explore and compare the protein sequences of ‘Myosin’ from two organisms *Gallus gallus* (UniProt ID: Q90623) and *Mus musculus* (UniProt ID: F8VQB6) by performing global pairwise sequence alignment using EMBOSS Needle Tool.	01/11/23		
6(F)	To explore and compare the protein sequences of ‘Collagen’ in two organisms, Rattus norvegicus (UniProt ID: P05539) and Homo sapiens (UniProt ID: P08572), by performing local pairwise sequence alignment using the EMBOSS Water tool.	01/11/23		

Date: 09/09/2023

EXPERIMENT 1
PAPER CHROMATOGRAPHY

AIM:

To separate plant pigments using paper chromatography

THEORY:

Chromatography is an analytical technique commonly used for separation, this technique was invented by Russian M.S. Tswett in 1903 and he is also regarded as the father of chromatography. Chromatography is a word literally translated from its Greek roots *chroma* (colour) and *graphein* (writing) in chromatography there are 3 components, mixture, mobile phase and stationary phase the substance which is to be separated is known as sample or mixture the mixture is dissolved in a fluid called the mobile phase, which carries it through a second substance called the stationary phase.

The different components of the mixture travel through the stationary phase at different speeds, causing them to separate from one another. The nature of the specific mobile and stationary phases determines which substances travel more quickly or slowly, and is how they are separated. These different travel times are termed retention time**.** By altering the mobile phase, the stationary phase, and/or the factor determining speed of travel, a wide variety of chromatographic methods have been created, each serving a different purpose and ideal for different mixtures. Chromatography is used in downstream processing to effectively purify the biological products like proteins pharmaceuticals, diagnostic compounds

There are different types of chromatography techniques which include paper chromatography, gas chromatography, liquid chromatography, HPLC. Paper chromatography is a technique that involves placing a dot or line of sample solution onto a strip of chromatography paper. The paper is placed in a jar containing a shallow layer of solvent and sealed. As the solvent rises through the paper, it meets the sample mixture, which starts it travel up the paper with the solvent. The pigments are separated on the paper based on the differential solubility. *Rf* value (Retention factor) is the rate at which the pigments in the mixture travels along the solvent. It is used to identify particular pigment in the mixture during chromatographic separation. Thus, after separation, the *Rf* value is calculated for each pigment.

$$Retention\ factor\ (Rf) = \frac{Distance\ travelled\ by\ the\ solute\ from\ the\ point\ of\ application}{Distance\ travelled\ by\ solvent}$$

PRINCIPLE:

Paper chromatography is based on the principle of differential solubility. It is a type of adsorption/partition chromatography. The stationary phase in this technique is a piece of filter paper, and the mobile phase is a liquid solvent. The components of the mixture to be separated migrate at different rates can appear at spots at different points on the paper. The solvent moves up the paper by capillary action, and as it moves, it carries the different components of the mixture along with it.

REQUIREMENTS:

1. **Sample:** Fresh vegetable leaves (spinach leaves).

2. **Chemicals**: Magnesium Carbonate – (pinch).
    i. Acetone (one drop)
    ii. Petroleum Ether (16ml).
    iii. Diethyl ether (4ml).

3. **Glassware:** Clean and dry glass pipette- 10ml
    i. Clean and dry glass measuring cylinder-50ml
    ii. Clean and dry glass measuring cylinder- 10ml
    iii. Clean and dry glass beaker- 250 ml
    iv. Falcon tube- 45ml
4. **Miscellaneous:** Whatman filter paper no.1 strip (10*2.5cm), capillary tube, muslin cloth, scale, pencil.

PREPARATION:

Preparation of the sample:

1. Crush the sample (spinach leaves) by using mortar and pestle.
2. Add a pinch of Magnesium carbonate to the mixture.
3. Add one drop of acetone to the mixture and mix thoroughly.

Preparation of mobile phase:

1. Take a Falcon tube add 16ml of petroleum ether.
2. Add 4ml of diethyl ether in the falcon tube
3. Add 1 drop of acetone to the mixture and mix it thoroughly.

PROCEDURE:

1. Take few spinach leaves wash and clean them.

2. Add those leaves in mortal pestle and crush them.

3. Add a pinch of magnesium carbonate and acetone into the mixture and then filter it using a muslin cloth.

4. Take a Whatman's filter paper cut them into 10cm by 2.5cm rectangle and mark a line with pencil 1.5cm (sample application) from bottom and 7 cm. (solvent front).

5. Using a capillary tube, spot the sample in the centre of the line (sample application) 3 to 4 times.

6. Add the mobile phase in the Camag Twin Turf chamber and keep it closed for 5-10 minutes for saturation.

7. After drying, place the filter paper (slanted position) into the chamber containing the mobile phase for 5 minutes.

8. Once the mobile phase has reached the solvent front remove the filter paper and then measure the Rf values of respective pigments using the formula.

OBSERVATION:

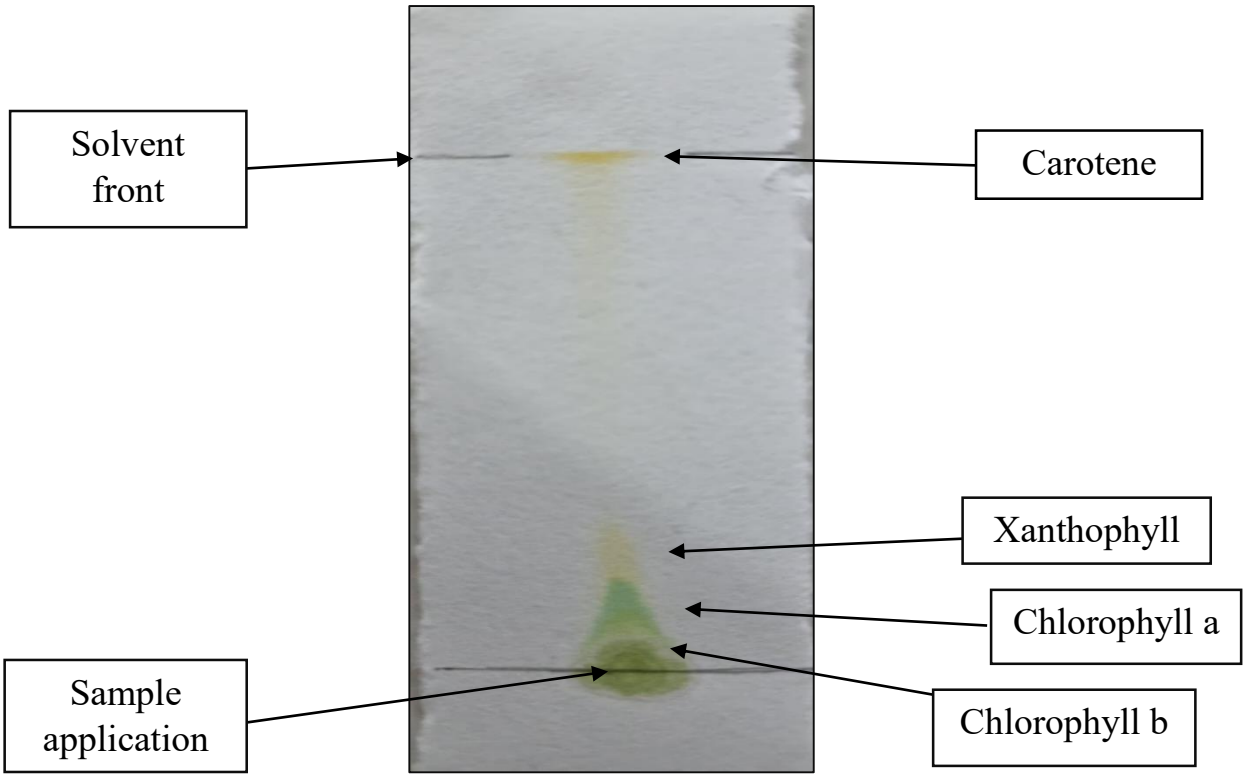


Figure 1: Paper Chromatography

OBSERVATION TABLE:

Sr. No.	Plant pigment	Distance travelled by solute (cm)	Distance travelled by solvent (cm)	*Rf* value
1.	Carotene (Dark yellow)	6.7	7.2	0.93
2.	Xanthophyll (Light Yellow)	2.1	7.2	0.29
3.	Chlorophyll a (Dark Green)	1.4	7.2	0.19
4.	Chlorophyll b (Light Green)	0.5	7.2	0.069

CALCULATION:

Distance travelled by solute from the origin line:

a) Carotene – 6.7
b) Xanthophyll – 2.1
c) Chlorophyll a – 1.4
d) Chlorophyll b – 0.5

$$Retention\ factor\ (Rf) = \frac{Distance\ travelled\ by\ the\ solute\ from\ the\ point\ of\ application}{Distance\ travelled\ by\ solvent}$$

a) For carotene, Rf = 6.7/7.2
= 0.93

b) For Xanthophyll, Rf = 2.1/7.2
= 0.29

c) For Chlorophyll a, Rf = 1.4/7.2
= 0.19

d) For Chlorophyll b, Rf = 0.5/7.2
= 0.069

RESULTS:

Paper chromatography was performed and plant pigments were separated using spinach as sample, the sample was spotted on to the filter paper and chromatography was performed, pigments were separated in the form of coloured bands , dark yellow band was of Carotene pigment, light yellow was of Xanthophyll, dark green band was of Chlorophyll a and light green band represented as Chlorophyll b. *Rf* values for each pigments were calculated and were 0.93 for carotene 0.29 for Xanthophyll 0.19 for chlorophyll a and 0.069 for chlorophyll b.

CONCLUSION:

Paper chromatography is an analytical technique used for separation of various components. The separation takes place based on rates of migration and the solvent flows in upwards direction due to capillary action. Here the plant pigments like carotene, xanthophyll, chlorophyll a, chlorophyll b were separated using spinach as a sample.

Date: 27/09/23

EXPERIMENT 2
ESTIMATION OF VITAMIN C USING UV-VIS SPECTROPHOTOMETER

AIM:

To estimate Vitamin C content of "Celin" tablet using UV-VIS spectrophotometer.

THEORY:

Vitamin C is as essential water-soluble vitamin also known as Ascorbic acid. It is known for its antioxidant properties and its role in various physiological processes. As a cofactor in numerous enzymatic reactions, it plays a crucial role in collagen synthesis, wound healing, and the absorption of non-heme iron. As a potential antioxidant, it protects cells from oxidative damage by scavenging free radicals. Its redox properties enable it to donate electrons, contributing to its antioxidant capacity. However, vitamin C is sensitive to environmental factors such as heat, light and oxygen, making its stability an important consideration in pharmaceutical formulations.

Celin tablets are pharmaceutical formulations designed to provide a standardized and convenient dosage of Vitamin C. Typically containing 500mg of ascorbic acid as the active ingredient, these tablets serve as dietary supplements to address vitamin C deficiencies and support overall health. In addition to ascorbic acid, Celin tablets may contain various excipients and binders necessary for tablet formulation. Quality control measures ensure the tablets' composition, integrity, and accurate dosage to meet regulatory standards and ensure their efficacy.

UV spectrophotometry is an analytical technique that utilizes the interaction between ultraviolet light and matter for quantitative analysis. UV spectrophotometers operate on the principle of measuring the absorbance of light by a substance in solution. The instruments typically consist of a light source emitting UV radiation, a monochromator to isolate a specific wavelength, a sample compartment, and a detector to measure the intensity of the transmitted or absorbed light. This analytical tool is widely used in pharmaceutical analysis, including the quantification of vitamin C in formulations like Celin tablets. "UV Probe" is a multifunctional, easy to use software supplied as standard with Shimadzu UV-VIS Spectrophotometers.

The spectrophotometer operates in spectrum mode. In this mode, the instrument scans a range of wavelengths to generate an absorbance spectrum of sample. The spectrum aids in identifying the wavelength at which vitamin C exhibits maximum absorbance, ensuring the subsequent photometric measurement is conducted at the most sensitive and specific wavelength. Whereas in the photometric mode of UV spectrophotometer, the instrument measures the absorbance of the sample solution at a specific wavelength chosen based on the maximum absorbance of vitamin C. This mode provides a direct and accurate assessment of the concentration of

ascorbic acid in the sample.A double-beam UV spectrophotometer involves the simultaneous measurement of the absorbance of two light beams: one passing through the sample and the other through a reference solution. The instrument starts by splitting the light from a single source into these two beams. The sample and reference beams then pass through their respective cells,typically made of quartz to allow UV transmission. As the beam exits the cells, they are directed to a detector, which measures the intensity of each beam. The absorbance of the sample is determined by comparing its intensity to that of the reference.

PRINCPLE:

UV spectrophotometry is a technique that uses light absorption to measure the concentration of an analyte in solution. Spectrophotometer works on the principle of Beer-Lambert's Law. Beer's law was stated by August Beer which states that concentration and absorbance are directly proportional to each other. While, Johann Heinrich Lambert stated Lambert law. It states that absorbance and path length are directly proportional. Therefore, Beer-Lambert law states that, for a given material sample path length and concentration of the sample are directly proportional to the absorbance of the light; which is expressed as follows:

$$A = \varepsilon.b.c$$

where;

A = Absorbance of the Solution
ε = Molar Absorptivity of the Analyte
b = Path Length (distance the light travels through the solution)
c = Concentration of the Analyte

REQUIREMENTS:

Sr. No.	Requirements	Particulars	Quantity
1.	Sample:	Celin Tablets	1 packet
2.	Glassware:	50mL measuring cylinder	2
		10 mL pipette	2
		Round bottom flask	2
		Beakers	2
3.	Miscellaneous:	Weighing machine	1
		Spatula	1
		UV-VIS Spectrophotometer	1
		Graph paper	1

METHODOLOGY:

A. STANDARD PREPARATION:

1. Stock Preparation:

- Take 500mg of the Vitamin C tablet and dissolve in 500mL of water (1000ppm).

2. Working Stock Preparation:

- Make a 500ppm solution by taking, 125mL of 1000ppm and add distilled water to make a total volume of 250mL.
- Make a 250ppm solution by taking, 125mL of 500ppm and add distilled water to make a total volume of 250mL.
- Make a 100ppm solution by taking, 200mL of 250ppm and add distilled water to make a total volume of 500mL.

3. Dilution table:

- Working stock = 100ppm
- Range (R) = 5-50ppm
- Diluent= Distilled Water

Sr. No.	Standard Concentration (ppm)	Volume of stock (mL)	Volume of diluent (mL)	Total volume (mL)
1.	5	5	95	↑ 100mL ↓
2.	10	10	90	
3.	20	20	80	
4.	30	30	70	
5.	40	40	60	
6.	50	50	50	
7.	Unknown	-	-	

B. PROTOCOL:

1. Formulate standard dilutions as mentioned on the dilution table and prepare the spectrophotometer for use.
2. Initialize the spectrophotometer and refrain from pressing any button.
3. Select windows Start>Program>Shimadzu>UVProbe (software)> enter the username-password.
4. Select Window>Spectrum or click the Spectrum button.
5. Ensure the module is activated and click on the "Connect" icon.
6. Put the cuvettes into the respective sockets.
7. Scan to get the maximum wavelength in the spectrum mode.
8. Click on "Auto Zero".
9. Press "start" to initiate scan. (It scans from 400nm to 200nm)
10. After the maximum absorbance is calculated put the wavelength, calibration and measurement parameters into the method wizard and save.
11. Click "Auto zero".
12. Input the Sample ID, concentration and click on "Read".
13. Click "Okay" to the dialog box.
14. Do the same for all the standards prepared.
15. Input the unknown ID in the respective table and click on the "concentration" column while taking the readings.
16. To save the data, select File>Save As. Save the file as .spc extension in your folder.

OBSERVATIONS:

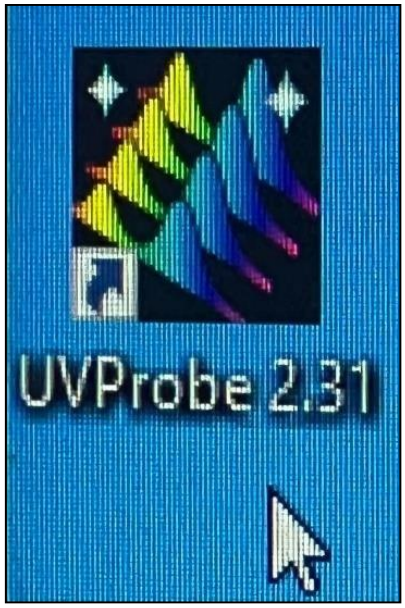


Figure 1: UVProbe Software Icon

Connect to the Spectrophotometer machine

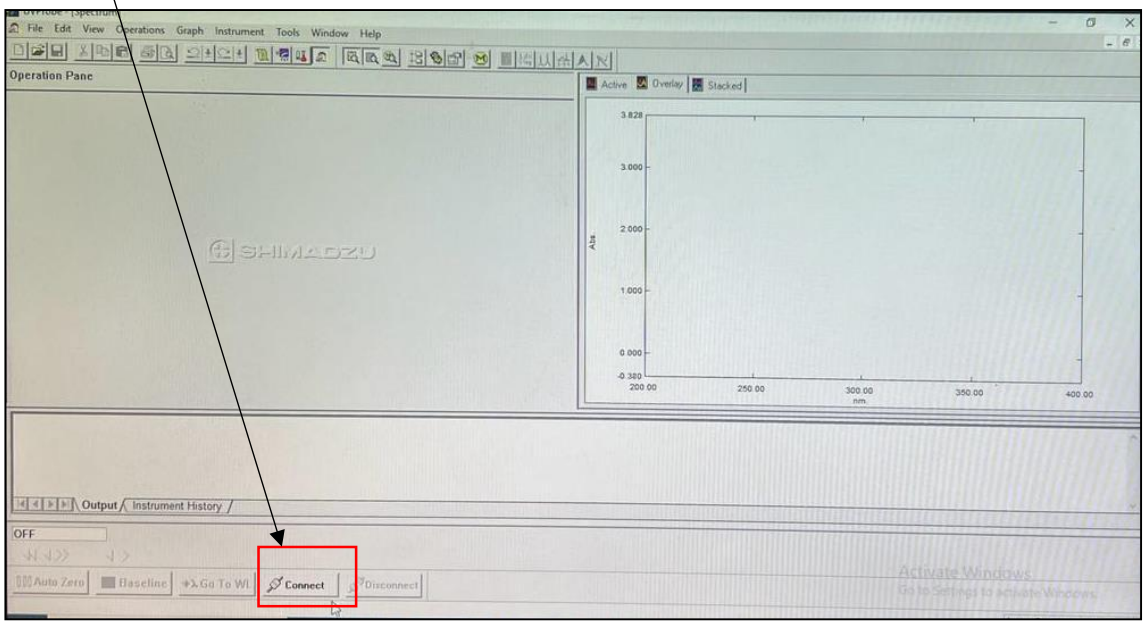


Figure 2: UVProbe Software homepage

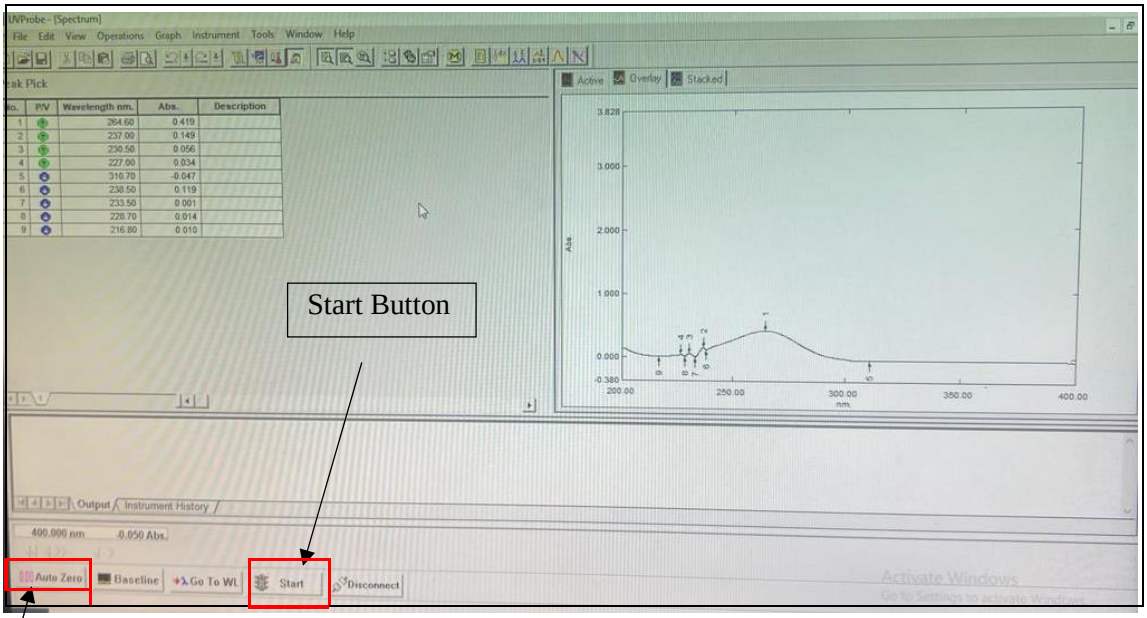


Figure 3: Spectrum scan, finding the maximum wavelength.

Auto Zero button

Peak Pick

No.	P/V	Wavelength nm.	Abs.	Description
1	↑	264.60	0.419	
2	↑	237.00	0.149	
3	↑	230.50	0.056	
4	↑	227.00	0.034	
5	↓	310.70	-0.047	
6	↓	238.50	0.119	
7	↓	233.50	0.001	
8	↓	228.70	0.014	
9	↓	216.80	0.010	

Figure 4: Wavelength table for finding maximum

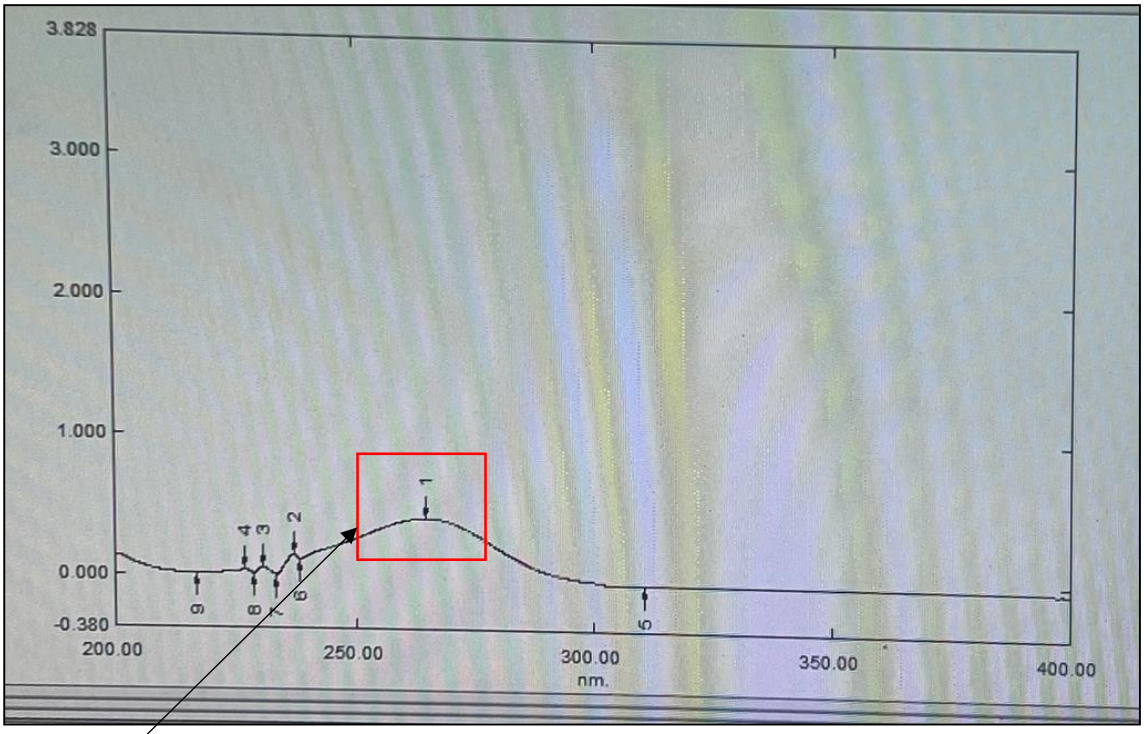


Figure 5: Maximum absorbance graph plotted by the software.

Peak for maximum absorbance (265nm)

Adding maximum wavelength

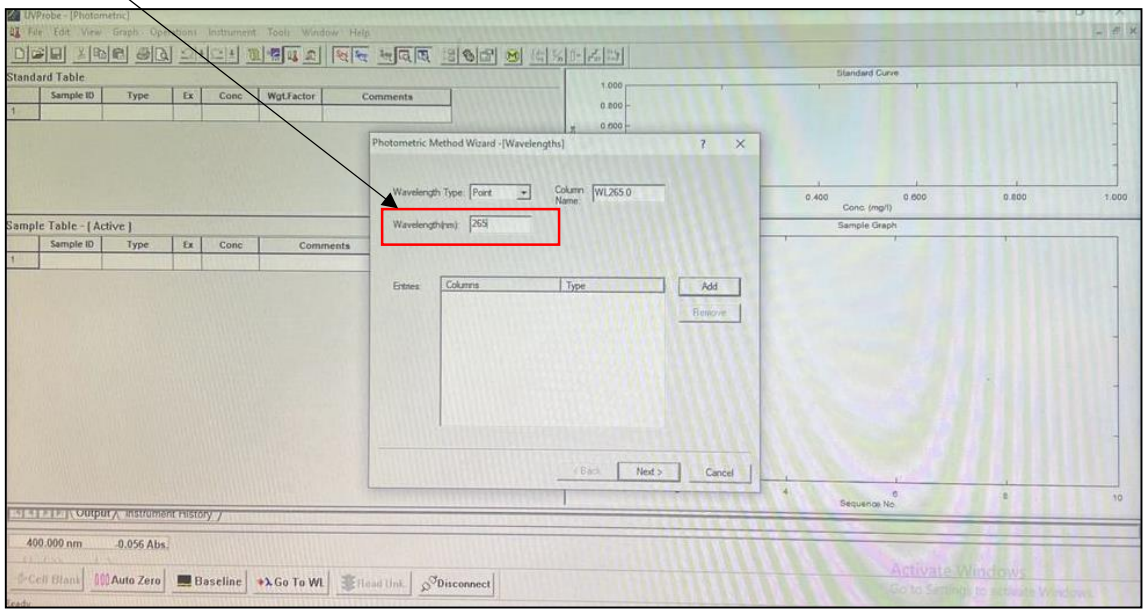


Figure 6: Adding columns with maximum wavelength.

Standard Table

Method Wizard

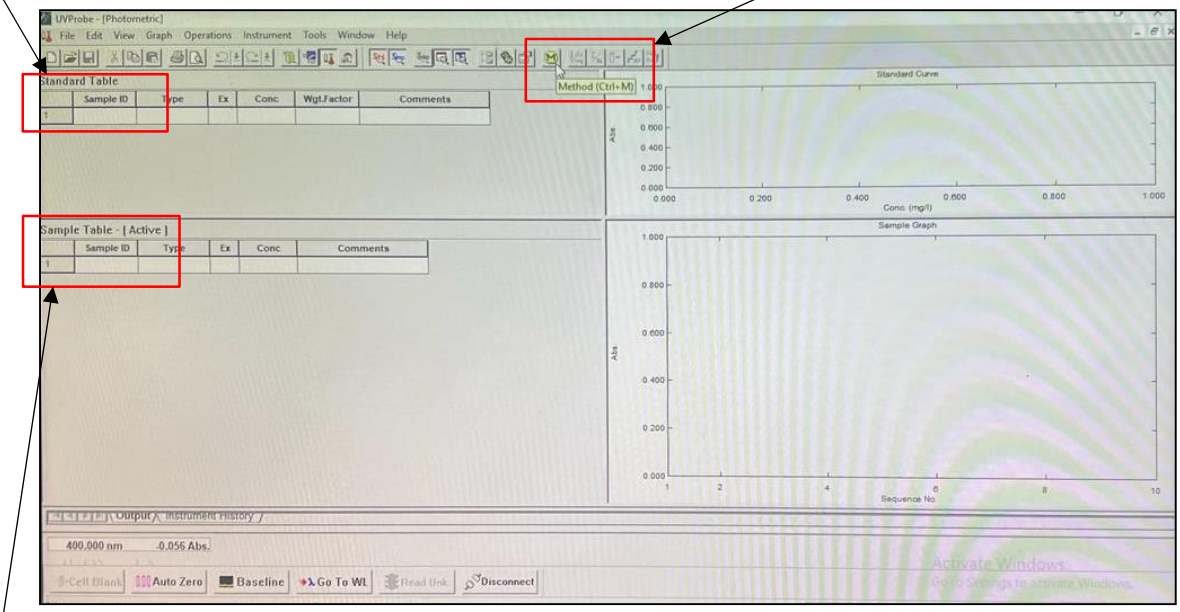
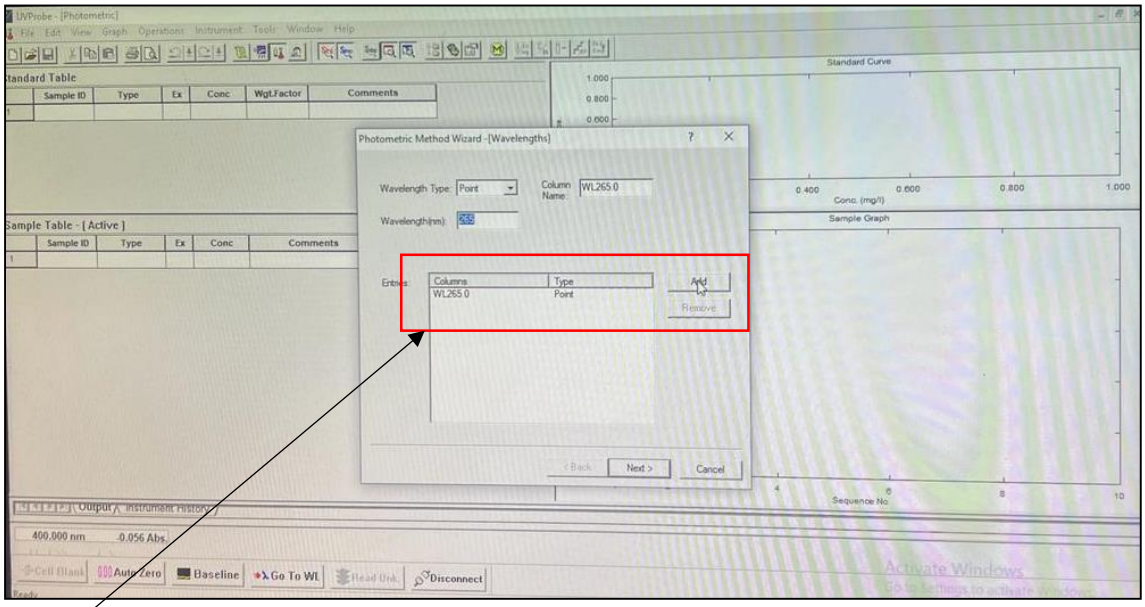


Figure 7: Standard table and Sample table added.

Sample Table



Adding λ max column

Figure 8: Photometric Wizard (Wavelength)

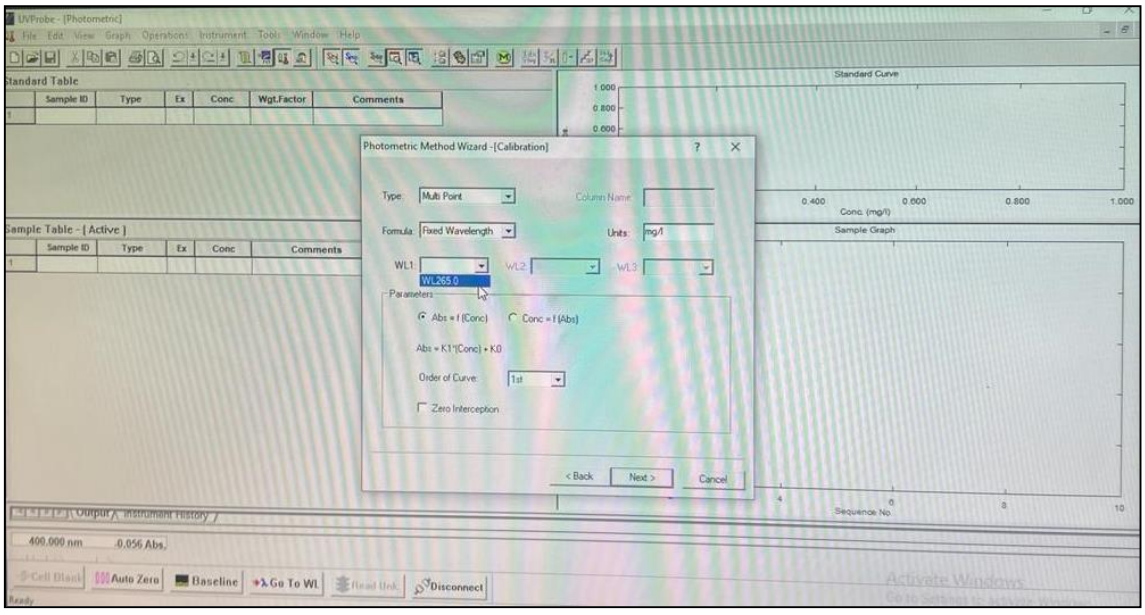


Figure 9: Photometric Wizard (Calibration)

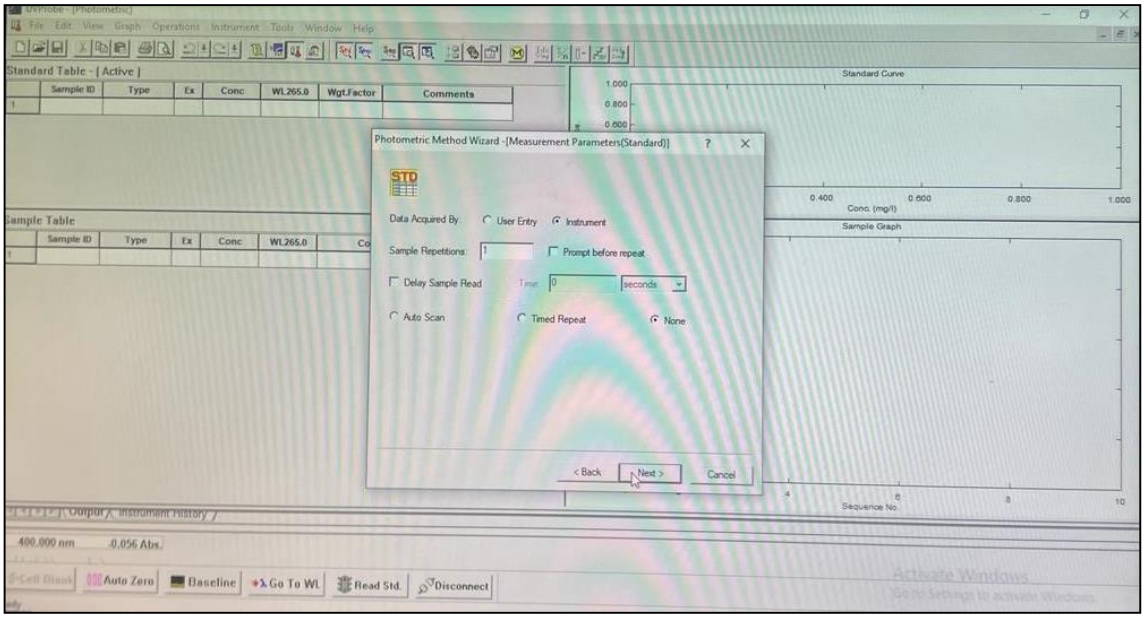


Figure 10: Photometric Method Wizard (Measurement Parameters)

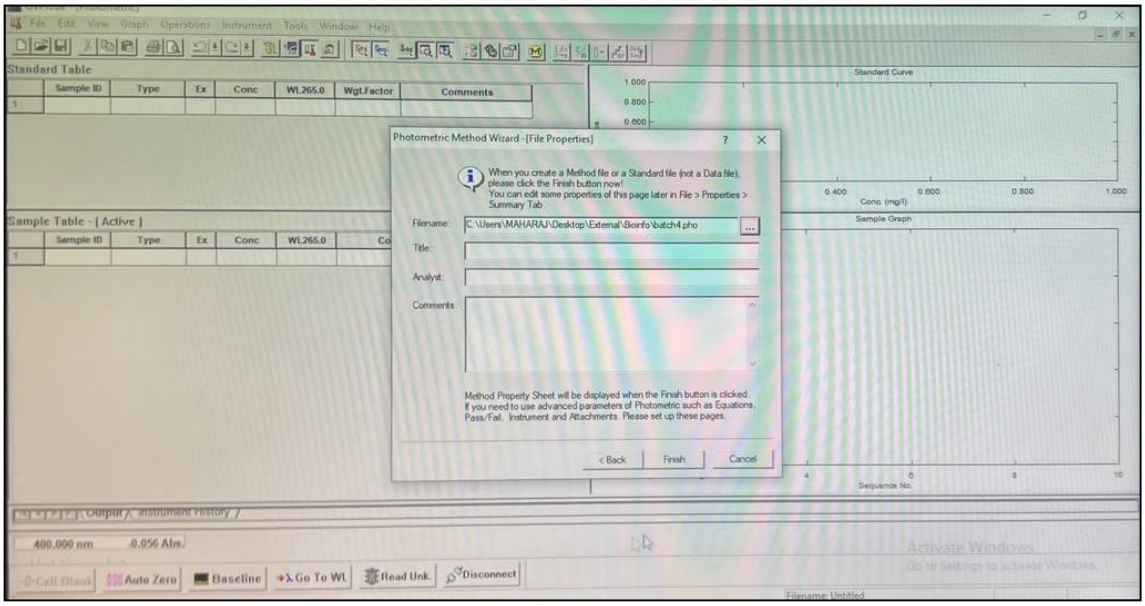


Figure 11: Photometric Method Wizard (File Properties)

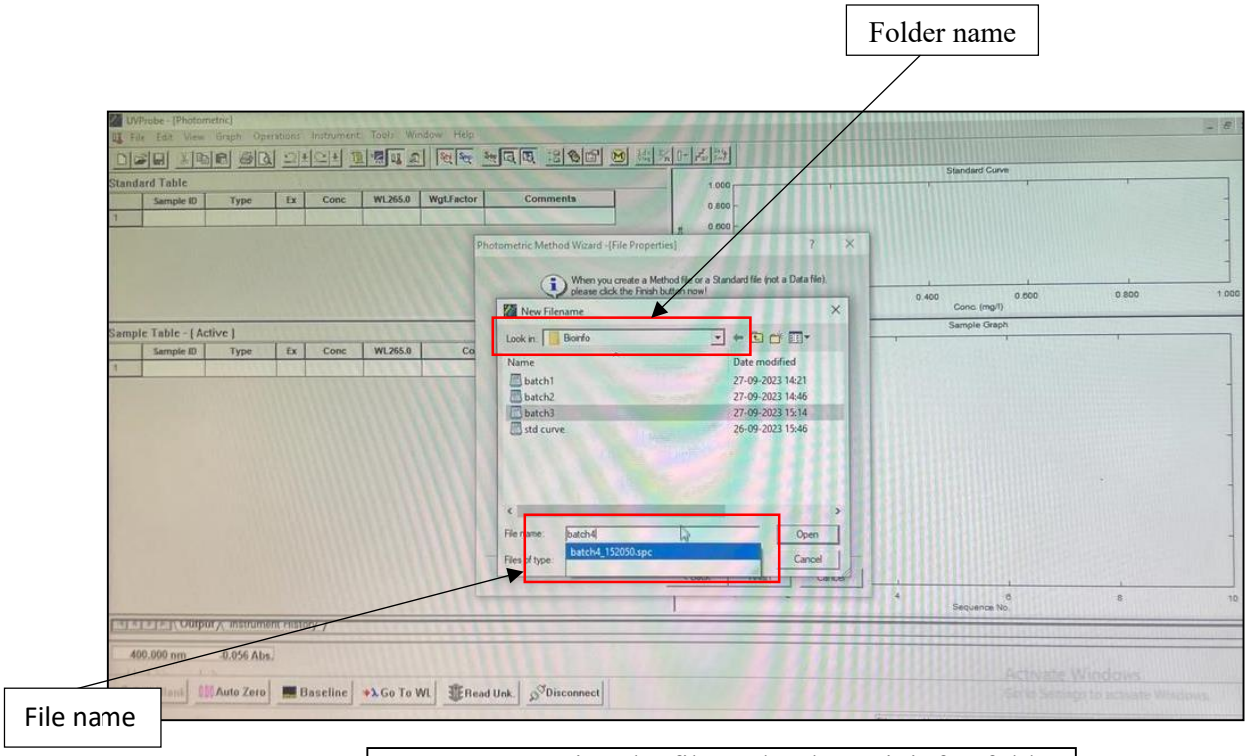
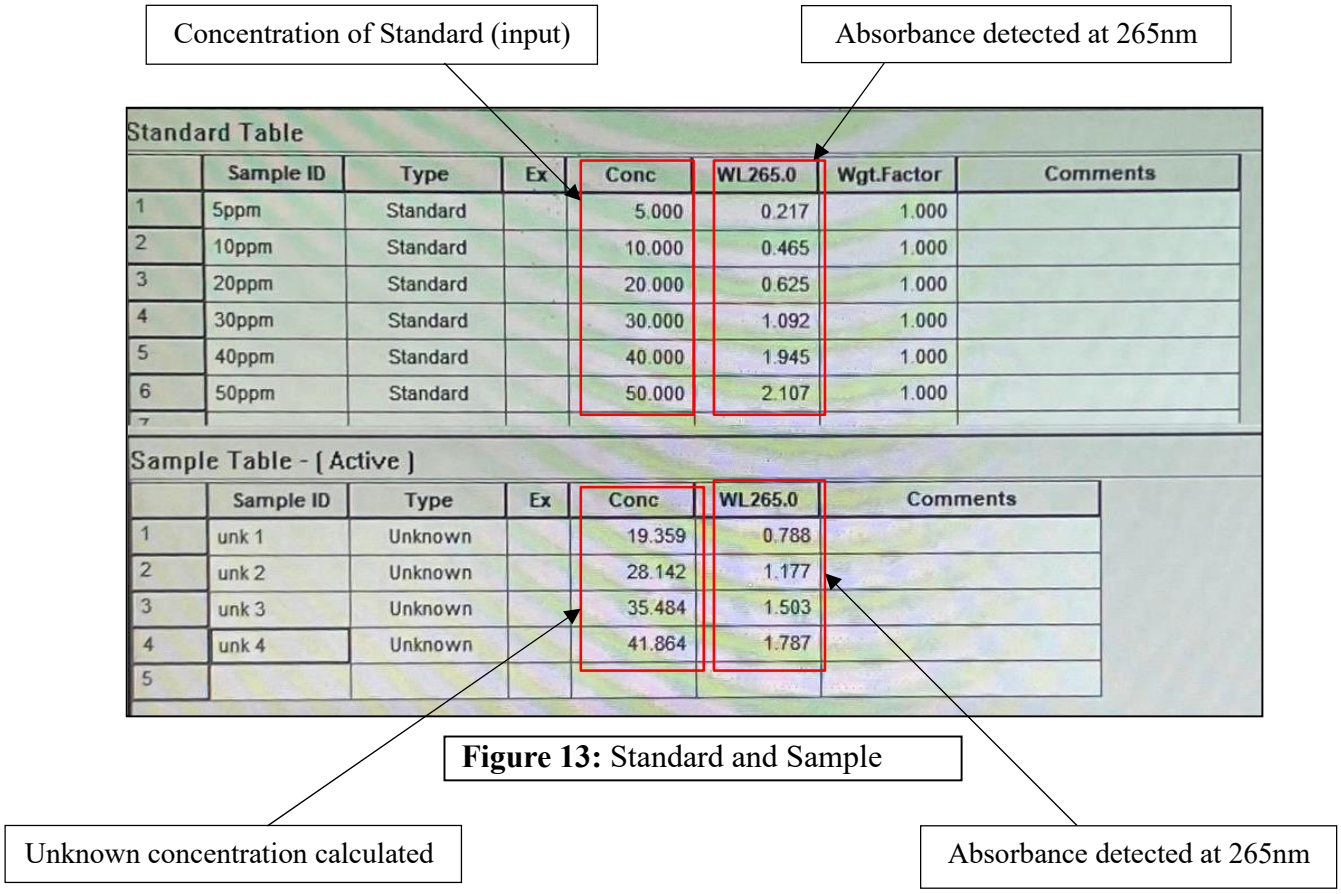


Figure 12: Saving the file under the "Bioinfo" folder.



Standard Table

	Sample ID	Type	Ex	Conc	WL265.0	Wgt.Factor	Comments
1	5ppm	Standard		5.000	0.217	1.000	
2	10ppm	Standard		10.000	0.465	1.000	
3	20ppm	Standard		20.000	0.625	1.000	
4	30ppm	Standard		30.000	1.092	1.000	
5	40ppm	Standard		40.000	1.945	1.000	
6	50ppm	Standard		50.000	2.107	1.000	

Sample Table - [Active]

	Sample ID	Type	Ex	Conc	WL265.0	Comments
1	unk 1	Unknown		19.359	0.788	
2	unk 2	Unknown		28.142	1.177	
3	unk 3	Unknown		35.484	1.503	
4	unk 4	Unknown		41.864	1.787	
5						

Figure 13: Standard and Sample

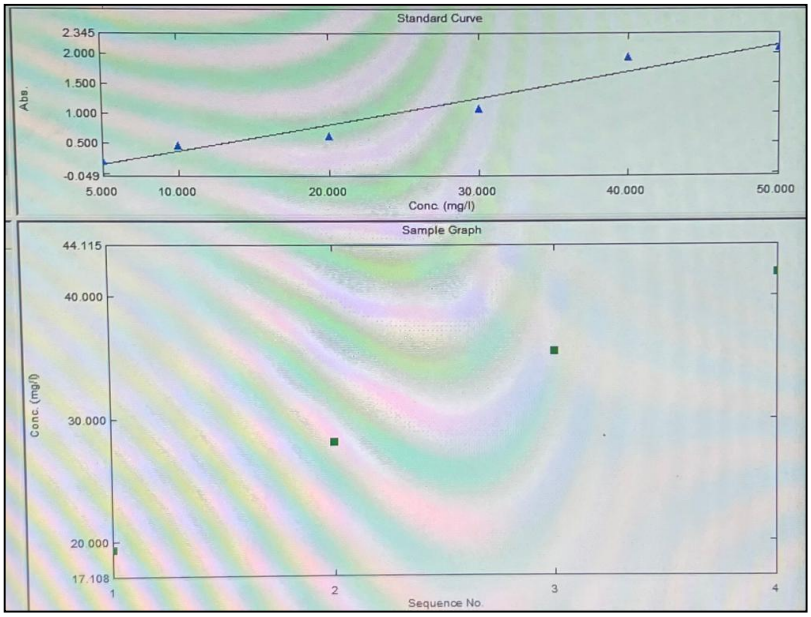


Figure 14: Standard Curve and Sample Graph

OBSERVATION TABLE:

For Standard:

Sr. No.	Concentration (ppm)	Absorbance at 265nm
1.	5	0.27
2.	10	0.58
3.	20	0.97
4.	30	1.3
5.	40	2.0
6.	50	2.2

For Sample:

Sample	Concentration (ppm)	Absorbance at 265nm
Unknown 1	17.36	0.87

CALCULATION:

1. Calculation by formula:

Formula:

$$\%\text{Assay} = \frac{\text{Sample Absorbance x Standard dilution x Average weight x purity}}{\text{Standard Absorbance x Sample dilution x 100 x Label Claim (L.C)}} \times 100$$

(Average weight = 599mg; Purity= 98.9; L.C. = 500mg)

$$\%Assay = \frac{0.865}{0.97} \times \frac{\left[\frac{500.25}{500} \times \frac{125}{250} \times \frac{125}{250} \times \frac{200}{500} \times \frac{20}{100}\right]}{600 \times 125 \times 125 \times 200 \times 15} \times \frac{599}{100} \times \frac{98.9}{500} \times 100$$

$$\%Assay = \frac{0.865 \times 0.2 \times 599 \times 98.9}{0.975 \times 1.2 \times 0.15 \times 500}$$

$$\%Assay = \frac{10248.7}{87.75}$$

$$\%Assay = 116\%$$

2. Calculation by graph:

RESULTS:

Vitamin C content of a 500mg Celin Tablet was estimated using a UV-VIS Spectrophotometer. Absorbance of standard Vitamin C and Unknown was estimated using spectroscopy and a graph was plotted with Absorbance on y-axis and Concentration on X-axis. The graph was interpolated to find the unknown concentration; which was found to be 18ppm. Using the %assay formula, the Vitamin C content was calculated to be 116%

CONCLUSION:

Vitamin C, or ascorbic acid, is essential for collagen synthesis, boosting the immune system, and acting as an antioxidant. Found in fruits and vegetables, its deficiency can lead to scurvy, while adequate intake supports overall health. Estimating vitamin C via UV-Visible spectroscopy involves measuring absorbance at a specific wavelength. Vitamin C, exhibits absorption around 265 nm. A standard concentration v/s Absorbance curve was prepared to interpolate the unknown concentration of vitamin C in the sample. The concentration was found to be 18ppm. Using the found sample absorbance, Percentage Assay analysis was carried out, which indicates that the measured concentration of Vitamin C in the sample corresponds to a certain percentage of the label claim (500mg). The value was calculated to be 116%. This suggests that the sample contains the declared amount of Vitamin C. The UV-Vis spectrophotometer proved to be a valuable tool for the accurate determination of vitamin C content in the tested tablets.

Date: 04/10/2023

EXPERIMENT 3
TLC (THIN LAYER CHROMATOGRAPHY)

AIM:

Thin Layer Chromatography analysis of curcumin content in turmeric sample.

THEORY:

Chromatography is an important biophysical technique that enables the separation, identification, and purification of the components of a mixture for qualitative and quantitative analysis.

In this physical method of separation, the components to be separated are distributed between two phases, one of which is stationary (stationary phase) while the other (the mobile phase) moves in a definite direction. Depending upon the stationary phase and mobile phase chosen, they can be of different types. TLC (Thin Layer Chromatography) is an analytical tool widely used because of its simplicity, relative low cost, high sensitivity, and speed of separation.

TLC consists of three steps - spotting, development, and visualization. Development consists of placing the bottom of the TLC plate into a shallow pool of a development solvent, which then travels up the plate by capillary action. As the solvent travels up the plate, it moves over the original spot. A competition is set up between the silica gel plate and the development solvent for the spotted material. The very polar silica gel tries to hold the spot in its original place and the solvent tries to move the spot along with it as it travels up the plate. The outcome depends upon a balance among three polarities - that of the plate, the development solvent and the spot material. If the development solvent is polar enough, the spot will move some distance from its original location. Different components in the original spot, having different polarities, will move different distances from the original spot location and show up as separate spots. When the solvent has travelled almost to the top of the plate, the plate is removed, the solvent front marked with a pencil, and the solvent allowed to evaporate.

TLC system components consists of:

1. **TLC plates,** preferably ready made with a stationary phase: These are stable and chemically inert plates, where a thin layer of stationary phase is applied on its whole surface layer. The stationary phase on the plates is of uniform thickness and is in a fine particle size.
2. **TLC chamber-** This is used for the development of TLC plate. The chamber maintains a uniform environment inside for proper development of spots. It also prevents the evaporation of solvents, and keeps the process dust free.
3. **Mobile phase-** This comprises of a solvent or solvent mixture The mobile phase used should be particulate-free and of the highest purity for proper development of TLC spots. The solvents recommended are chemically inert with the sample, a stationary phase.

PRINCIPLE:

TLC is based on the classic chromatography principle where mixture components are separated between a fixed stationary phase and a liquid mobile phase by differential affinities between the two phases.

The retention factor (R_f) is used to measure the movement of compounds along the TLC plate. R_f is defined as the distance travelled by an individual component divided by the total distance travelled by the solvent.

$$R_f = \frac{\textit{distance travelled by component}}{\textit{distance travelled by solvent}}$$

In general, the stronger a compound binds to the stationary phase adsorbent, the slower it migrates up the TLC plate. As TLC adsorbents are typically polar, non-polar compounds tend to travel more rapidly up the plate, resulting in a higher R_f values, whereas polar compounds tend to move slowly and have lower R_f values.

REQUIREMENTS:

1. Chemicals: Chloroform and methanol.
2. Glassware: Pipettes (1ml, 5ml & 10ml), twin trough chamber, capillaries and stoppered test tubes.
3. Miscellaneous: TLC Silica Gel 60 F254 Plate, forcep, pencil, scale, vortex instrument, filter paper, eppendorf tubes.
4. Sample & Standard: Turmeric sample and curcumin standard

PROCEDURE:

1. Preparation of mobile phase (solvent): Take 19ml of chloroform and 1ml of methanol in stoppered test tube with the help of pipettes. Mix the solvents by using vortex or shake the test tube.
2. Preparation of sample and standard solution: Weigh 0.5g of sample and standard in different eppendorf tube and add 5ml of methanol in each tube. Sonicate for 05 minutes.
3. Saturation of twin trough chamber: Place filter paper (saturation pad) inside chamber and pour prepared mobile phase on top of filter paper. Immediately close the chamber with lid. Leave chamber undisturbed for 20minutes.
4. Spotting of sample and standard: Before spotting, mark application point (1cm above from bottom) and solvent front (7 cm above from application point) by pencil. Finely spot sample and standard spot (at some space in between) using pointed capillary.
5. Run the plate: Place plate in chamber carefully (facing filter paper) with the help of forcep and allow it to run till solvent front. Remove plate with forcep after complete run.
6. Encircle three spots for both sample and standard using pencil. Measure the distance of all three spots from center to application point for both sample and standard.
7. Calculate Retention factor (Rf) by using the formula.

OBSERVATION:

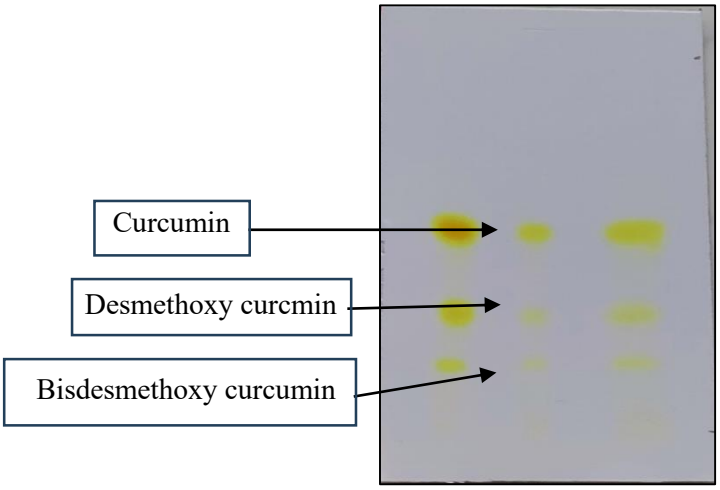


Figure 1: Separation of Turmeric components of TLC plate

OBSERVATION TABLE:

Spot no.	Standard	Distance travelled by compound(cm)	Distance travelled by solvent(cm)	Rf
1.	Bisdesmethoxy curcumin	2.2 cm	8 cm	0.27
2.	Desmethoxy curcumin	3.2 cm	8 cm	0.4
3.	Curcumin	4.8 cm	8 cm	0.6

Spot no.	Sample	Distance travelled by compound(cm)	Distance travelled by solvent(cm)	Rf
1.	Bisdesmethoxy curcumin	2.1 cm	8 cm	0.26
2.	Desmethoxy curcumin	3.2 cm	8 cm	0.4
3.	Curcumin	4.7 cm	8 cm	0.58

CALCULATIONS:

Standard:

1. For Bisdesmethoxy curcumin,
   Rf = 2.2/8 = 0.27

2. For Desmethoxy curcumin,
   Rf = 3.2/8 = 0.4

3. For Curcumin,
   Rf = 4.8/8 = 0.6

Sample:

1. For Bisdesmethoxy curcumin,
   Rf = 2.1/8 = 0.26

2. For Desmethoxy curcumin,
   Rf = 3.2/8 = 0.40

3. For Curcumin,
   Rf = 4.7/8 = 0.58

RESULT:

TLC technique was performed with turmeric as a sample and curcuminoids as standard. Mobile phase composition was chloroform: methanol (19:1) (V/V). Three separate spots were obtained for three components of turmeric namely Curcumin, Desmethoxy curcumin and Bisdesmethoxy curcumin at a distance of 2.1, 3.2 and 4.7 cm respectively. The Rf value of Curcumin, Desmethoxy curcumin and Bisdesmethoxy curcumin in the turmeric sample were found to be 0.26, 0.40 and 0.58 respectively.

CONCLUSION:

Thin Layer Chromatography (TLC) of turmeric effectively separated its components based on their differential migration on the TLC plate. The distinct spots observed allowed for the calculation of Rf values, providing insight into the relative polarities of the compounds. This analytical technique proved valuable for identifying and characterizing components within complex mixtures, contributing to a deeper understanding of the composition of turmeric extract.

DATE:04/11/2023

EXPERIMENT 4
ESTIMATION OF RNA BY ORCINOL

AIM:

To estimate the concentration of RNA by orcinol reaction.

THEORY:

RNA, a fundamental biomolecule, plays a pivotal role in cellular processes, serving as the intermediary between DNA and protein synthesis. Accurate quantification of RNA is crucial for understanding gene expression dynamics, cellular responses, and various biological phenomena. Among the numerous methods available for RNA quantification, the orcinol method stands out as a colorimetric approach based on the reaction between orcinol and ribose, a component of RNA.

This experiment aims to employ the orcinol method to estimate the concentration of RNA in a given sample. The method relies on the formation of a colored complex, the intensity of which is proportional to the amount of ribose released from RNA during acid hydrolysis. By measuring the absorbance of this complex at a specific wavelength, a standard curve can be constructed, allowing for the quantitative determination of RNA concentration in unknown samples.

Significance:

Accurate RNA quantification is fundamental to molecular biology and biochemistry research. The orcinol method offers advantages such as simplicity, sensitivity, and cost-effectiveness. Understanding the principles and applications of this method contributes to a researcher's toolkit for studying gene expression, RNA purification, and other molecular biology experiments.

Objective:

1. To estimate the concentration of RNA in a given sample using the orcinol method.
2. To construct a standard curve correlating RNA concentration with absorbance.
3. To apply the method in a controlled experiment and assess its reliability and precision.

Principle:

The principle of estimating RNA by orcinol involves the reaction of orcinol with ribose sugar present in RNA. In the presence of sulfuric acid, orcinol reacts with ribose to form a colored complex. The intensity of the color produced is proportional to the amount of ribose, and consequently, RNA in the sample. This colorimetric method is commonly used for the quantification of RNA in biochemical and molecular biology experiments.

Reaction:

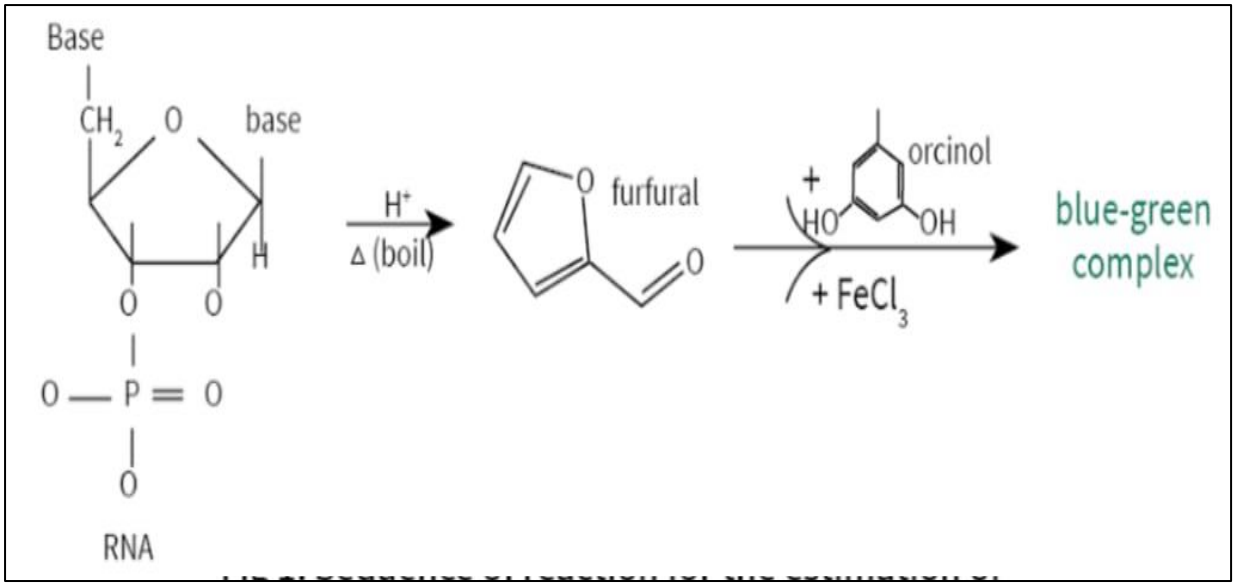


Figure 1: Reaction of RNA with orcinol reagent

REQUIREMENTS:

The estimation of RNA by orcinol requires specific reagents and equipment. Here are the key requirements:

1. **RNA Sample:** A biological sample containing RNA. This could be extracted RNA from cells, tissues, or other sources.
2. **Orcinol Reagent:** A solution containing orcinol and sulfuric acid. Orcinol is the compound that reacts with ribose to form the colored complex.
3. **Ribose Standard:** Known concentrations of ribose to generate a standard curve. This curve is used for the quantitative determination of RNA concentration based on absorbance readings.
4. **Spectrophotometer:** A spectrophotometer is needed to measure the absorbance of the colored solution at a specific wavelength.
5. **Incubation Setup:** Facilities for incubating the reaction mixture under specified conditions. This may include a water bath or an incubator.
6. **Laboratory Glassware:** Standard laboratory glassware for handling and mixing reagents, as well as for preparing standard solutions.
7. **Pipettes and Pipette Tips:** Accurate pipettes for precise measurement and transfer of liquid volumes.

Preparation of Reagents:

1. Standard RNA Solution: 25mg std RNA in 25ml std measuring flask and Perchloric acid till mark.
2. Working RNA Solution: 10ml of std ,RNA solution with home distilled water.
3. Orcinol Reagent:
    a. Fecl3 o.1g in 100ml HCL.
    b. Orcinol 0.6g in 10ml ethanol
    c. Reagent A + Reagent B 3.5ml + Mixture

PROCEDURE:

1. The 7 Test-rubes grease free were taken and oven dried.
2. After that the Standard RNA solution was added in all the test tube except the last one unknown sample added).
3. Then the distilled water was added in all the tubes except the last one.
4. After that orcinol reagent (2ml) was added to all the tubes and allowed to incubate for 20min in boiling water bath.
5. Cool the Tubes, and Absorbance at 660nm was taken.
6. The graph of Absorbance v/s concentration was plotted.

OBSERVATION:

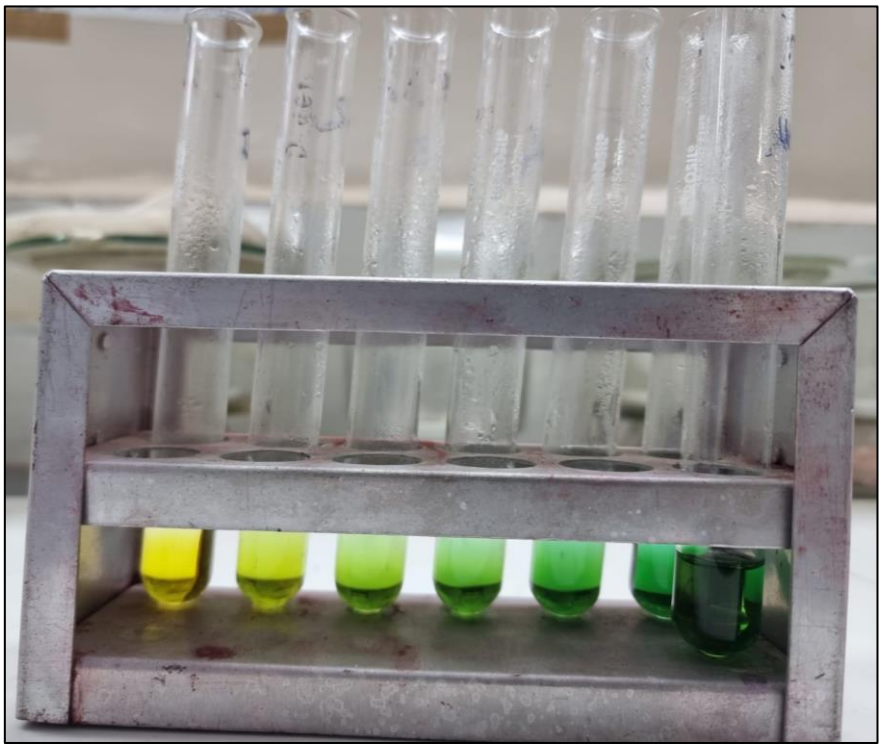


Figure 2: Solution of Estimation of RNA by Orcinol method

OBSERVATION TABLE:

Volume of standard (200 µg/ml) RNA	Volume of distilled water (ml)	Concentration of RNA (µg)	Volume of Orcinol reagent (ml)	Incubate in boiling water bath for 20 Min & Cool	O.D (optical density)
0.0	1	00	2		0.0
0.2	0.8	40	2		0.17
0.4	0.6	80	2		0.36
0.6	0.4	120	2		0.48
0.8	0.2	160	2		0.74
1.0	0.0	200	2		0.96
Unknown	0.0		2		0.82

CALCULATION:

By Calculation

$$\frac{\text{O.D of Std}}{\text{Concentration of Std}} = \frac{\text{O.D of Unknown}}{\text{Concentration of unknown}}$$

$$\therefore \frac{0.96}{200} = \frac{0.82}{?}$$

∴ Concentration of unknown = 170.83 µg/ml

GRAPH:

By Graph = 90 µg/ml

RESULTS:

The concentration of RNA estimated is 90 µg/ml (by graph) and 170.83 µg/ml (by Calculation).

CONCLUSION:

The orcinol method successfully estimated the concentration of RNA in the unknown sample. The standard curve exhibited a strong linear relationship between RNA concentration and absorbance. The unknown concentration of RNA was found to be 90 µg/ml (by graph) and 170.83 µg/ml (by calculation) using orcinol.

Date: 04/11/23

EXPERIMENT 5
ESTIMATION OF DNA BY DIPHENYLAMINE REACTION

AIM:

To determine the concentration of DNA sample using diphenylamine method.

THEORY:

DNA is deoxyribose nucleic acid that carries the genetic information in a cell and is capable of self-replication and synthesis of RNA. DNA consists of two long chains of nucleotides twisted into a double helix and joined by hydrogen bonds between the complementary bases adenine and thymine or guanine and cytosine. DNA acts as a genetic material and carries the genetic information. The sequence of nucleotides determines individual hereditary characteristics.

Nucleotides are the building blocks of all nucleic acids. Nucleotides have a distinctive structure composed of three components covalently bound together: Nitrogen-containing "base" - either a pyrimidine (one ring) or purine (two rings), five-carbon sugar - ribose or deoxyribose, and a phosphate group. The combination of a base and sugar is called a nucleoside. Nucleotides also exist in activated forms containing two or three phosphates, called nucleotide diphosphates or triphosphates respectively. If the sugar in a nucleotide is deoxyribose, the nucleotide is called a deoxynucleotide; if the sugar is ribose, the term ribonucleotide is used.

The bases of DNA are heterocyclic (carbon and nitrogen-containing) aromatic rings. Adenine (A) and guanine (G) are purines, bicyclic structures (two rings), whereas cytosine (C), thymine (T) are monocyclic pyrimidines. DNA is an important biological molecule, which can be isolated from various sources. To characterize the DNA sample, it is often necessary to determine its concentration. The concentration of a DNA sample can be determined using diphenylamine method using a colorimeter or a spectrophotometer. In this method a set of standards (where the concentration of DNA is known) is used and the concentration of the unknown sample is then determined by comparing it with the standards.

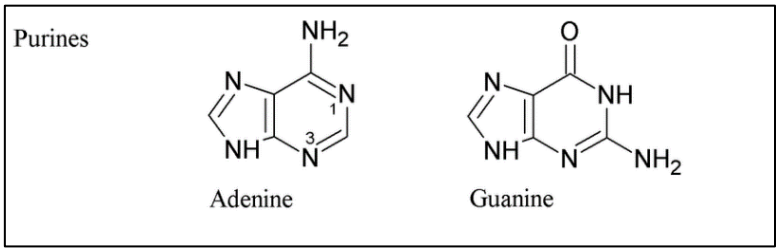


Figure 1.1: Purines present in DNA.

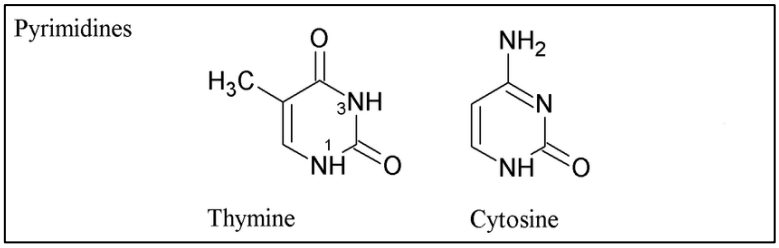


Figure 1.2: Pyrimidines present in DNA

PRINCIPLE:

The deoxyribose in DNA in the presence of acid forms β-hydroxylevulinaldehyde which reacts with diphenylamine to give a blue colour with a sharp absorption maximum at 595nm. In DNA, only the deoxyribose of the purine nucleotides react, so that the value obtained represents half of the total deoxyribose present. The amount of blue coloured complex is proportional to the concentration of DNA.

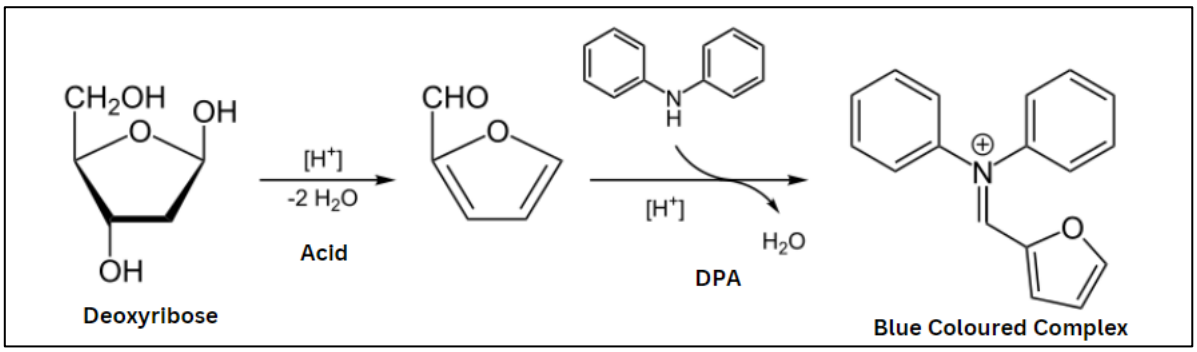


Figure 2: Reaction of Diphenylamine with DNA.

REQUIREMENTS:

1. Instruments:

- Colorimeter
- Water bath

2. Reagents:

- Standard DNA solution (0.25mg/ml)
- Diphenylamine reagent (DPA)
- Glacial acetic acid
- Conc. H_2SO_4
- Distilled water
- Phosphate buffer saline (PBS)
- Test Sample of DNA

3. Glassware and Miscellaneous:

- Test tubes
- Pipettes
- Graduated cylinder

PREPARATION OF REAGENT:

1. Standard DNA solution (1mg/ml): Dissolve 25 mg of DNA in 25 ml of Phosphate buffer.
2. Phosphate Buffer: Take 4 g of Sodium Chloride (NaCl) and add 0.1 g of Potassium Chloride(KCL). Add 0.72 g of Disodium Hydrogen Phosphate (Na_2HPO_4). To this add 0.12 g of Potassium dihydrogen phosphate (KH_2PO_4) and 400 ml of distilled water. Adjust the pH to 7.4 by adding Hydrochloric Acid (HCl) and then make up the volume to 100 ml by adding distilled water.
3. Diphenylamine solution: Dissolve 1 g of diphenylamine in 100 ml of glacial acetic acid. Add 2.5 ml of conc. H2SO4.

PROCEDURE:

1. Pipette out 0.0, 0.2, 0.4, 0.6, 0.8 and 1 ml of working standard in to the series of labelled test tubes.
2. Pipette out 1 ml of the given sample in another test tube.
3. Make up the volume to 1 ml in all the test tubes. A tube with 1 ml of distilled water serves as the blank.
4. Now add 2 ml of DPA reagent to all the test tubes including the test tubes labelled 'blank' and 'unknown'.
5. Mix the contents of the tubes by vortexing / shaking the tubes and incubate on a boiling water bath for 10 min.
6. Then cool the contents and record the absorbance at 595 nm against blank.
7. Then plot the standard curve by taking concentration of DNA along X-axis and absorbance at 660 nm along Y-axis.
8. Then from this standard curve calculate the concentration of DNA in the given sample.

OBSERVATION:

Figure 3: Color gradation of test tubes after incubation

OBSERVATION TABLE:

Sr no.	Stock (µg/ml)	Diluent (PBS)(µl)	Concentration (µg/ml)	Total Volume (ml)	DPA (ml)		O.D (660 nm)
1.	0.0	2.0	-	2 ml	4 ml	Incubate in boiling water bath for 20 mins and then cool at room temperature	0.0
2.	0.4	1.6	200				0.07
3.	0.8	1.2	400				0.14
4.	1.2	0.8	600				0.24
5.	1.6	0.4	800				0.34
6.	2.0	0.0	1000				0.37
7.	Unknown	-	-				0.22

CALCULATION:

$$\frac{O.D\ of\ std}{Conc.of\ std} = \frac{O.D\ of\ Unknown}{Conc.\ of\ Unknown}$$

$$\frac{0.24}{600} = \frac{0.22}{x}$$

= **550 µg/ml**

By calculation concentration of the given unknown DNA sample is 550 µg /ml.

GRAPH:

By graph concentration of the given unknown DNA sample is 530 µg /ml.

RESULT:

The concentration of DNA present in the given unknown sample is found to be 530µg /ml by graph and 550 µg/ml by calculation.

CONCLUSION:

The Diphenylamine (DPA) method successfully estimated the concentration of RNA in the unknown sample. The standard curve exhibited a strong linear relationship between DNA concentration and absorbance. The calculated concentration in the unknown sample was found to be 550 µg/ml.

DATE: 01/11/2023

WEBLEM 6
INTRODUCTION TO SEQUENCE ALIGNMENT TOOLS

INTRODUCTION:

Alignment of biological sequences is a fundamental task in bioinformatics. It involves identifying regions of similarity between two or more sequences, which can then be used to infer functional, structural, or evolutionary relationships. Sequence alignment is the problem of comparing biological sequences by searching for a series of nucleotides or amino acids that appear in the same order in the input sequences, possibly introducing gaps into them. When there are two sequences, it is called pairwise sequence alignment; otherwise, it is called multiple sequence alignment (MSA). Global alignment is to find the best match between the entire sequences.

Most MSA methods are based on one of the two pairwise alignment algorithms: the optimal algorithm proposed by Needleman and Wunsch (NW) for global alignment, and the improvement to the NW algorithm proposed by Smith and Waterman (SW) to obtain the local alignment. Various algorithms are employed for sequence alignment, two prominent ones being the Needleman-Wunsch algorithm and the Smith-Waterman algorithm.

The Needleman-Wunsch algorithm performs global alignment, comparing entire sequences, while the Smith-Waterman algorithm is utilized for local alignment, identifying regions of similarity within sequences. These algorithms form the backbone of sequence alignment studies and are accessible through powerful bioinformatics tools available under EMBOSS (European Molecular Biology Open Software Suite). Both algorithms are composed of three phases: initialization, distance matrix computation and trace back. Nevertheless, they differ in their applied techniques at each phase. There are many different techniques used in sequence alignment methods, such as heuristic algorithms, and dynamic programming. Although they ensure the best alignment, dynamic programming methods (such as Needleman-Wunsch and Smith-Waterman) can be computationally demanding for longer sequences. For big datasets, heuristic approaches frequently yield near-optimal alignments, by favoring optimality for of speed and efficiency.

Among the widely used tools and methods, BLAST (Basic Local Alignment Search Tool) and FASTA (Fast Alignment Search Tool) are pivotal in bioinformatics. BLAST uses heuristic methods for comparing sequences quickly and efficiently against large databases, allowing rapid identification of homologous sequences. FASTA combines heuristic methods with probability models to perform quick sequence alignments and similarity searches. These tools are used by researchers in a wide range of fields to identify homologous sequences, infer evolutionary relationships, identify functional and structural motifs, and design primers and probes.

Pairwise Alignment Tools

Pairwise alignment tools are typically used to identify regions of similarity between two sequences of unknown evolutionary relationship. They work by comparing the two sequences and identifying regions of identical or similar characters. Gaps are inserted between the

characters of the two sequences so that the identical or similar characters are aligned in successive columns.

BLAST:

BLAST (Basic Local Alignment Search Tool) is a family of sequence alignment algorithms and programs designed to search for regions of similarity between biological sequences. It is used to search for homologous sequences in a database of known sequences, which can be used to identify genes, infer evolutionary relationships, and design primers and probes. It works by comparing a query sequence to a database of sequences using a heuristic approach. This means that it does not search the entire database for matches, but instead uses a number of shortcuts to identify potential matches. The first step in BLAST is to break the query sequence into short segments, called words. The length of the words depends on the type of sequence being searched (e.g., DNA or protein). BLAST then searches the database for sequences that contain the same words as the query sequence. If a match is found, BLAST extends the alignment in both directions to find the longest possible alignment. BLAST calculates a score for each alignment, which is based on the similarity of the two sequences and the presence of gaps. The higher the score, the more similar the two sequences are. BLAST then reports the alignments with the highest scores.

Types of BLAST:

There are five types (variants) of BLAST that are differentiated based on the type of sequence (DNA or protein) of the query and database sequences.

1. **BLASTN** compares a nucleotide query sequence to a nucleotide sequence database.
2. **BLASTP** compares a protein query sequence to a protein sequence database.
3. **BLASTX** compares a nucleotide query sequence to a protein sequence database by translating the query sequence into its six possible reading frames and aligning them with the protein sequences.
4. **TBLASTN** compares a protein query sequence to a nucleotide sequence database by translating the nucleotide sequences in all six reading frames and aligning them with the protein sequence.
5. **TBLASTX** compares a nucleotide query sequence to a nucleotide sequence database by translating the query sequence in all six reading frames and aligning them with the nucleotide sequences.

FASTA:

FASTA (Fast Alignment Search Tool) is a sequence alignment algorithm and program that is used to search for regions of similarity between biological sequences. It works by first building a hash table of the query sequence. The hash table is a data structure that allows FASTA to quickly find all of the sequences in the database that contain the same words as the query sequence. It then aligns the query sequence to each of the matching sequences in the database to find the longest possible alignment. It calculates a score for each alignment, which is based on the similarity of the two sequences and the presence of gaps. The higher the score, the more similar the two sequences are. It then reports the alignments with the highest scores. It is often used in conjunction with BLAST to identify and analyze homologous sequences. FASTA is

also used to design primers and probes for PCR and other molecular biology techniques.

PSI-BLAST:

PSI-BLAST (Position-Specific Iterative BLAST) is a sequence alignment tool that uses a position-specific scoring matrix (PSSM) to search for distant homologs in protein sequences. It is particularly well-suited for identifying homologs that have diverged significantly from their known relatives. It works by first running a regular BLAST search of the protein sequence database using the query sequence. This produces a list of initial hits. It then constructs a PSSM from the alignments of the initial hits. The PSSM is a statistical model that describes the probability of each amino acid at each position in the alignment. PSI-BLAST then uses the PSSM to search the protein sequence database again. This produces a list of new hits. It then repeats this process, using the PSSM from the previous iteration to search for new hits. PSI-BLAST continues to iterate until the PSSM no longer changes or until a certain number of iterations have been reached. PSI-BLAST then reports the alignments with the highest scores.

PHI-BLAST:

PHI-BLAST (Phylogenetically Inconsistent BLAST) is a sequence alignment tool that uses a probabilistic model to search for distant homologs in protein sequences. It is particularly well-suited for identifying homologs that have diverged significantly from their known relatives. It works by first building a phylogenetic tree of the known homologs of the query sequence. It then uses this tree to generate a position-specific scoring matrix (PSSM) for each node in the tree. The PSSM is a statistical model that describes the probability of each amino acid at each position in the alignment. It then searches the database of protein sequences for sequences that match the PSSMs at the nodes of the phylogenetic tree. It does this by calculating a score for each alignment based on the similarity of the sequences and the PSSM. The higher the score, the more similar the sequences are and the more likely they are to be homologous. It then reports the alignments with the highest scores. It also reports the probability that each alignment is a true homolog. This probability is based on the score of the alignment, the PSSM of the node in the phylogenetic tree, and the phylogenetic relationships between the sequences in the alignment. PHI-BLAST is a powerful tool for identifying distant homologs. It is used by researchers in a wide range of fields, including genetics, genomics, proteomics, and molecular biology.

EMBOSS Needle:

EMBOSS Needle is a pairwise sequence alignment tool that uses the Needleman- Wunsch algorithm to produce global alignments. A global alignment is an alignment that aligns the entire length of both sequences. It works by comparing the two sequences and identifying regions of identical or similar characters. Gaps are inserted between the characters of the two sequences so that the identical or similar characters are aligned in successive columns. It calculates a score for each alignment, which is based on the similarity of the two sequences and the presence of gaps. The higher the score, the more similar the two sequences are. It then reports the alignment with the highest score. EMBOSS Needle is a powerful tool for aligning biological sequences and it is particularly well-suited for aligning sequences of known evolutionary relationship or sequences with low levels of divergence.

EMBOSS Water:

EMBOSS Water is a pairwise alignment tool that uses the Smith-Waterman algorithm to produce local alignments. This means that only the most similar regions of the two sequences are aligned. It is a good choice for aligning sequences of unknown evolutionary relationship or sequences with high levels of divergence. It works by comparing the two sequences and identifying regions of identical or similar characters. Gaps are inserted between the characters of the two sequences so that the identical or similar characters are aligned in successive columns. It then calculates a score for each alignment, which is based on the similarity of the two sequences and the presence of gaps. The higher the score, the more similar the two sequences are. It then reports the alignment with the highest score. It is a powerful tool for aligning biological sequences. It is often used in conjunction with other alignment tools, such as BLAST and FASTA, to identify and analyze homologous sequences. EMBOSS Water is also used to design primers and probes for PCR and other molecular biology techniques.

REFERENCES:

1. Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. Journal of Molecular Biology, 215(3), 403–410. https://doi.org/10.1016/s0022-2836(05)80360-2
2. Needleman, S. B., & Wunsch, C. D. (1970). A general method applicable to the search for similarities in the amino acid sequence of two proteins. Journal of Molecular Biology, 48(3), 443–453. https://doi.org/10.1016/0022-2836(70)90057-4
3. Bhagwat, M., & Aravind, L. (2007). PSI-BLAST Tutorial. In Methods in molecular biology (pp. 177–186). https://doi.org/10.1007/978-1-59745-514-5_10
4. Sievers, F., Wilm, A., Dineen, D., Gibson, T. J., Karplus, K., Li, W., López, R., McWilliam, H., Remmert, M., Söding, J., Thompson, J., & Higgins, D. G. (2011). Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. Molecular Systems Biology, 7(1). https://doi.org/10.1038/msb.2011.75

DATE: 01/11/2023

WEBLEM 6(A)
BASIC LOCAL ALIGNMENT SEARCH TOOL (BLAST)
(URL: https://blast.ncbi.nlm.nih.gov)

AIM:

To study and explore similar sequences of the protein albumin (UniProt ID: P02768) by using Basic Local Alignment Search Tool (BLAST).

INTRODUCTION:

BLAST (Basic Local Alignment Search Tool) is an algorithm and program for comparing primary biological sequence information, such as the amino-acid sequences of proteins or the nucleotides of DNA and/or RNA sequences. A BLAST search enables a researcher to compare a subject protein or nucleotide sequence (called a query) with a library or database of sequences, and identify database sequences that resemble the query sequence above a certain threshold. BLAST (Basic Local Alignment Search Tool) has become the defacto standard in search and alignment tools [Altschul et al., 1990]. The BLAST algorithm works by finding a short, or local, region of high similarity between two sequences, and then extending this match out from this starting point to both the left and the right. A score is assigned to the match. The score will increase as more residues are found to match and will decrease if there are gaps in the alignment. Alignments with a score that exceeds a certain threshold are reported in the output.

BLAST searches for high scoring sequence alignments between the query sequence and the existing sequences in the database using a heuristic approach that approximates the Smith-Waterman algorithm.

BLAST tool can be used to identify unknown sequences by comparing them with known sequences in a database which helps in predicting the functions of proteins or genes which can be used in phylogenetic analysis as well as in identifying functionally conserved domains within proteins which is important for predicting the functions of proteins.

Albumin:

Albumin is a family of globular proteins, with the most common members being the serum albumins. All proteins within the albumin family are water-soluble, moderately soluble in concentrated salt solutions, and susceptible to heat denaturation. Albumins are commonly present in blood plasma and distinguish themselves from other blood proteins by their lack of glycosylation. Compounds containing albumins are termed albuminoids. Several blood transport proteins share an evolutionary relationship within the albumin family, including serum albumin, alpha-fetoprotein, vitamin D-binding protein, and afamin. This family is exclusively found in vertebrates. In a broader sense, the term "albumins" may refer to other proteins that coagulate under specific conditions.

METHODOLOGY:

1. Open the Homepage of the UniProt database and search for the query of Albumin protein.
2. Select one entry from the results for *Homo sapiens* (UniProt ID: P02768) and download its FASTA sequence in canonical format.
3. Open the homepage of BLAST and select Protein BLAST, i.e., BLASTP.
4. Paste the FASTA sequence in 'Enter Query Sequence' box.
5. Set the desired parameters.
6. Run the BLAST.

OBSERVATIONS:

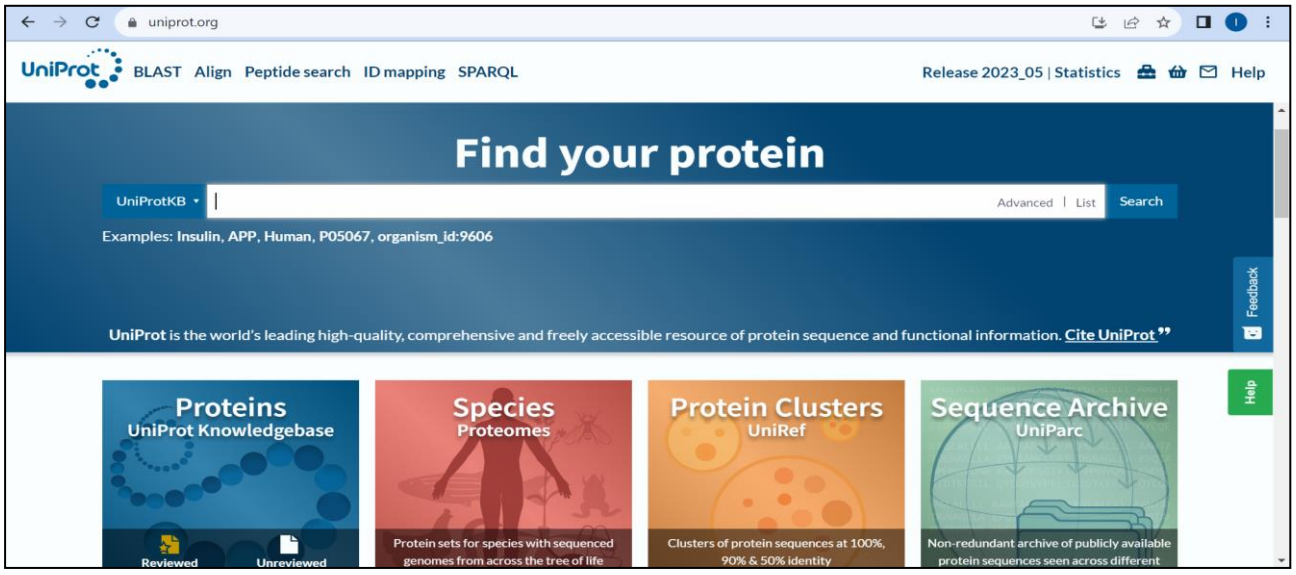


Figure 1: Homepage of the UniProt Database

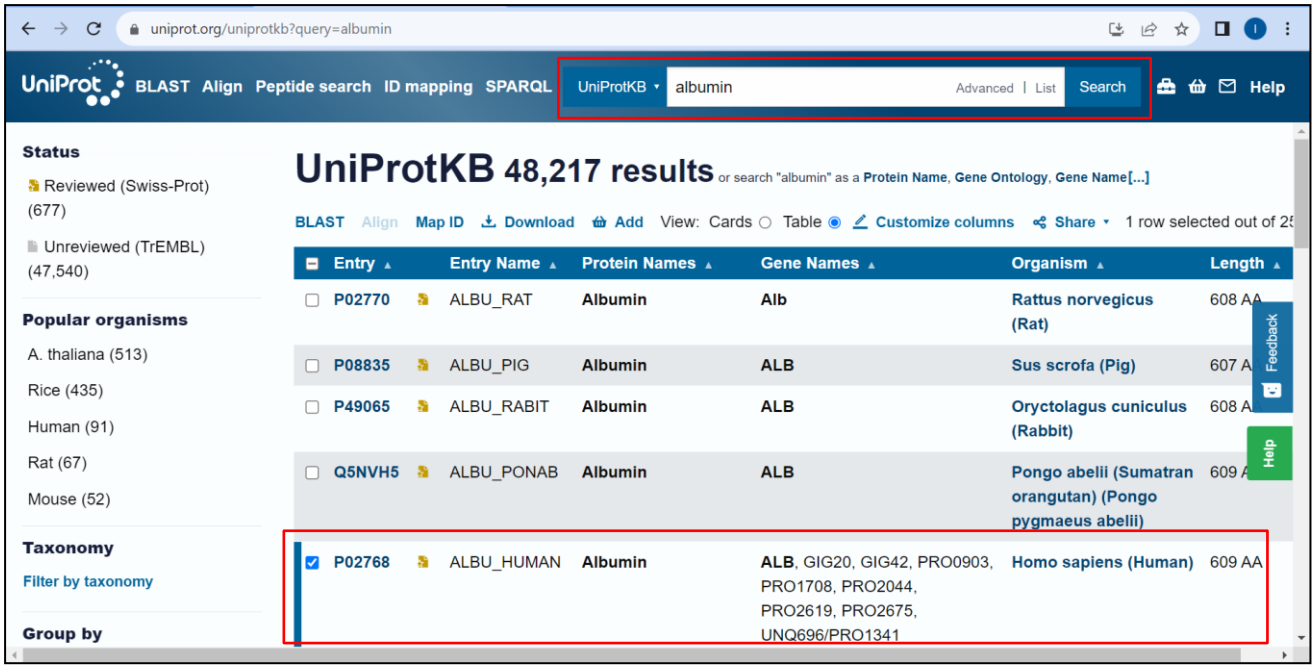


Figure 2: Searching for the query albumin and selecting (UniProt ID: P02768)

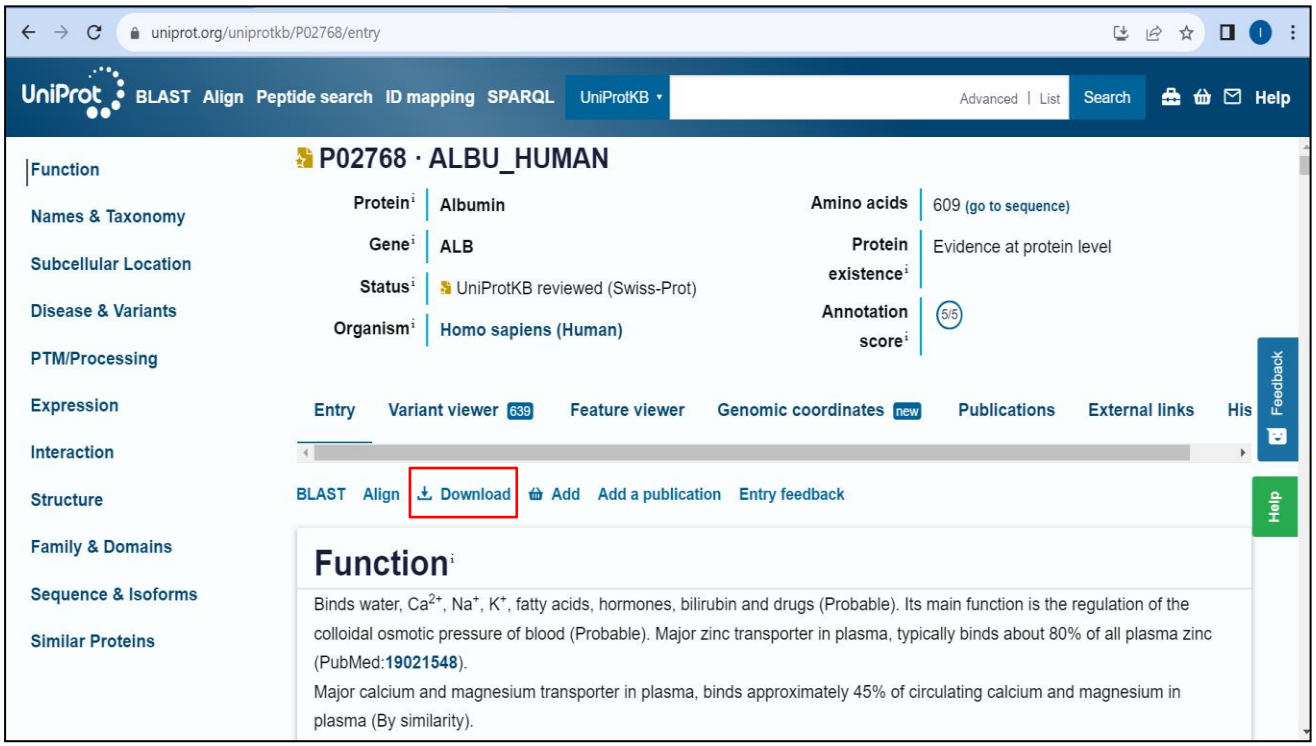


Figure 3: Download option for retrieving FASTA sequence

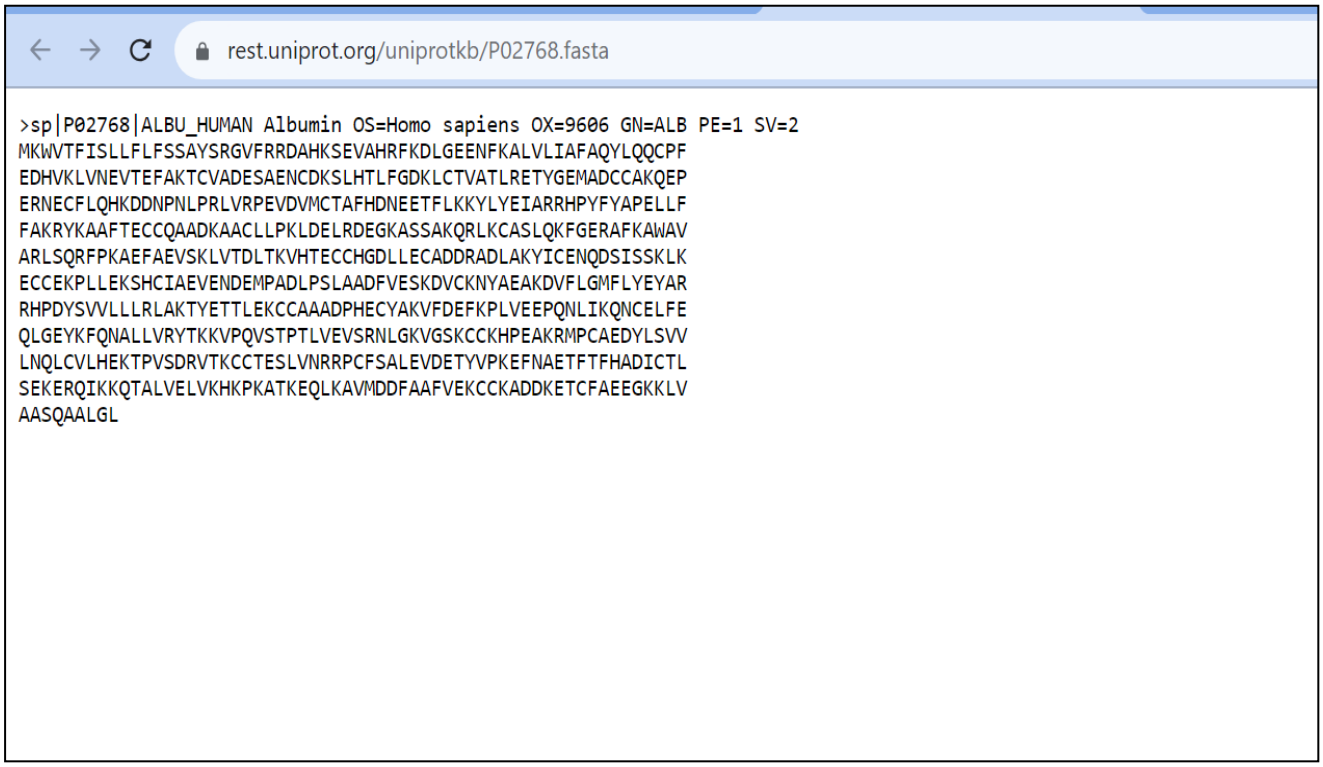


Figure 4: FASTA sequence in canonical format

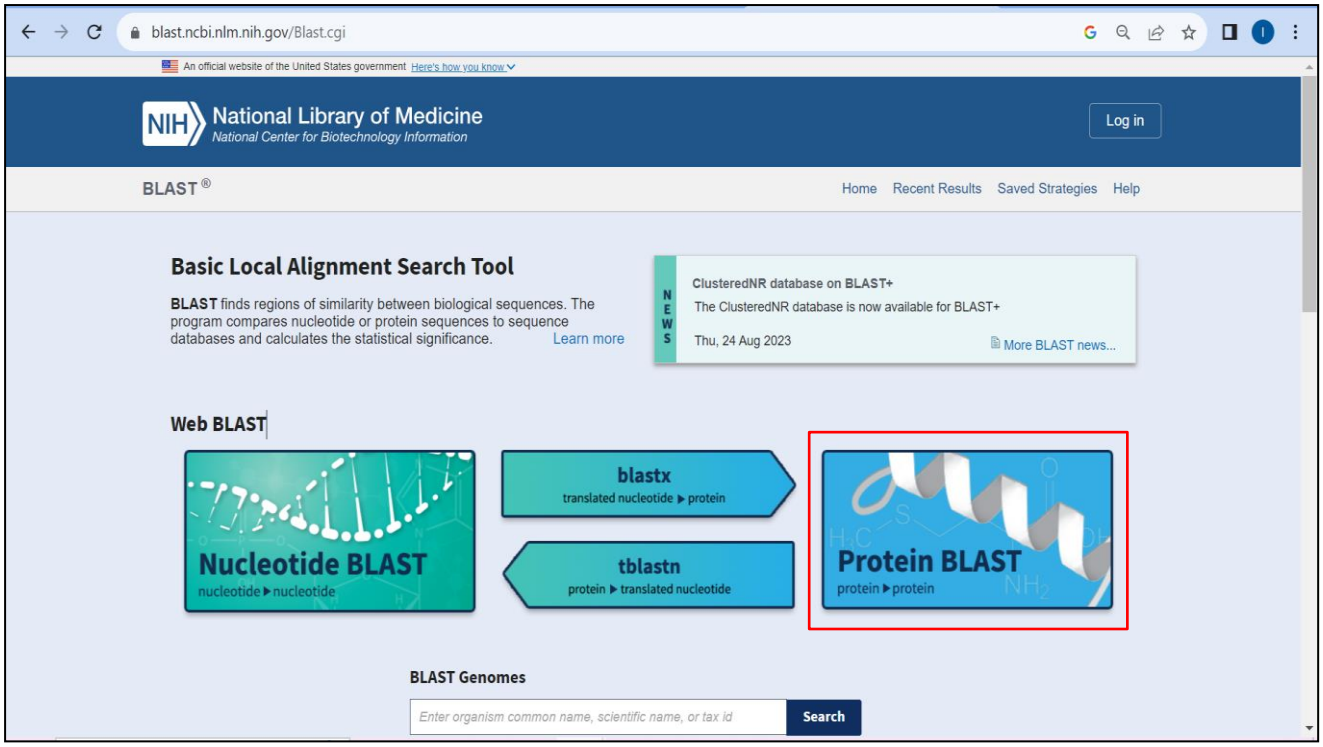


Figure 5: Homepage of Basic Local Alignment Search Tool (BLAST)

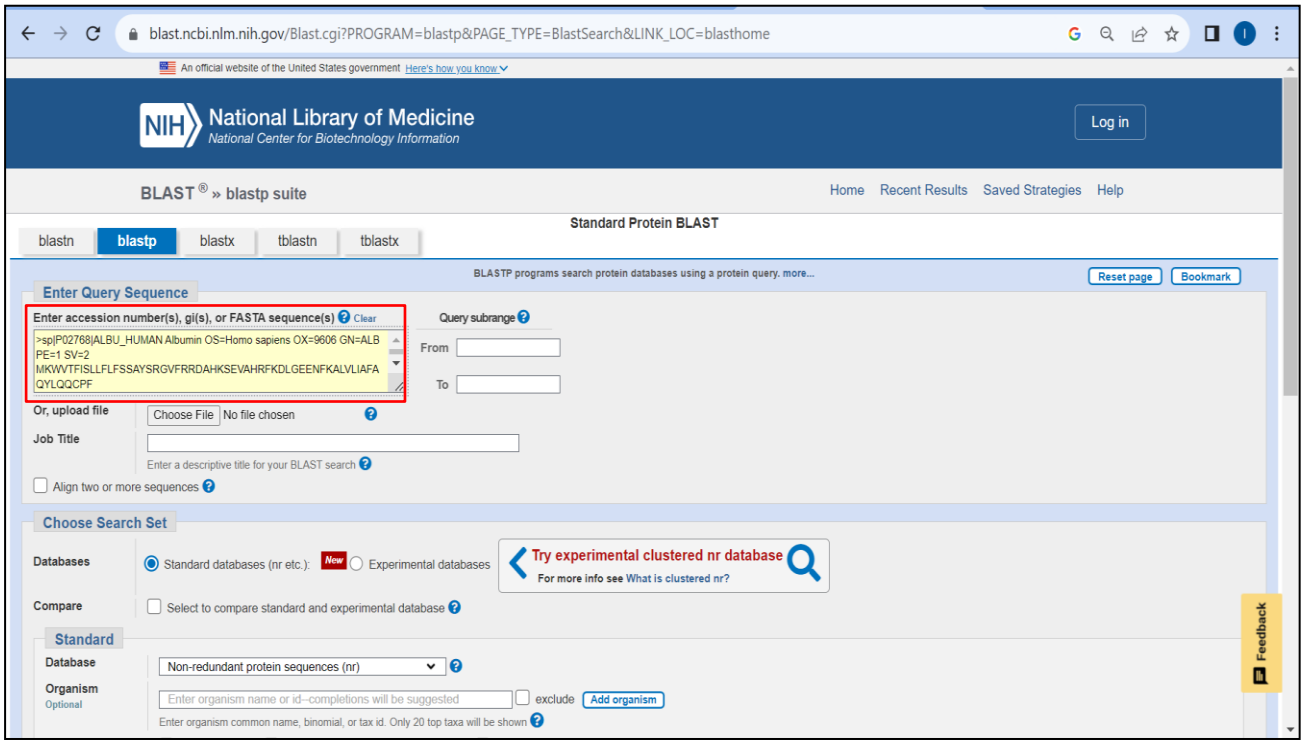


Figure 6: FASTA sequence pasted in 'Enter Query Sequence' box

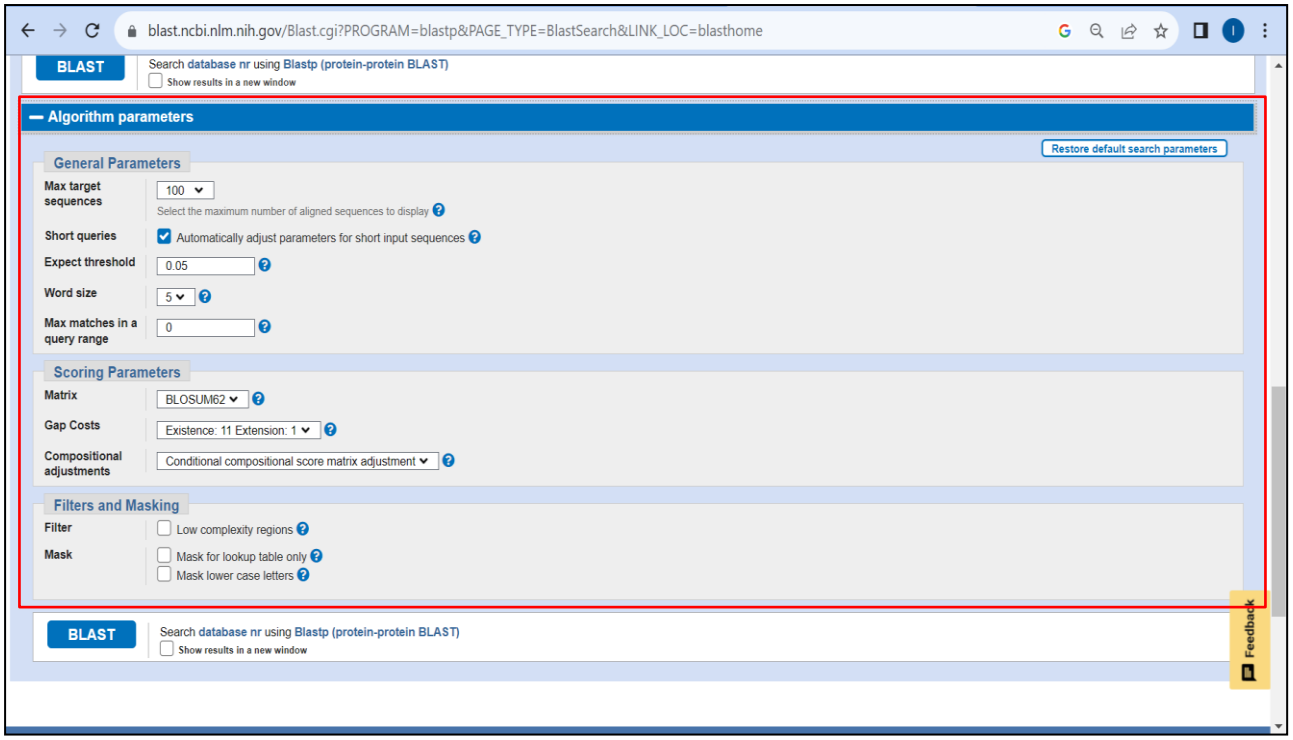


Figure 7: Setting the Algorithm parameters

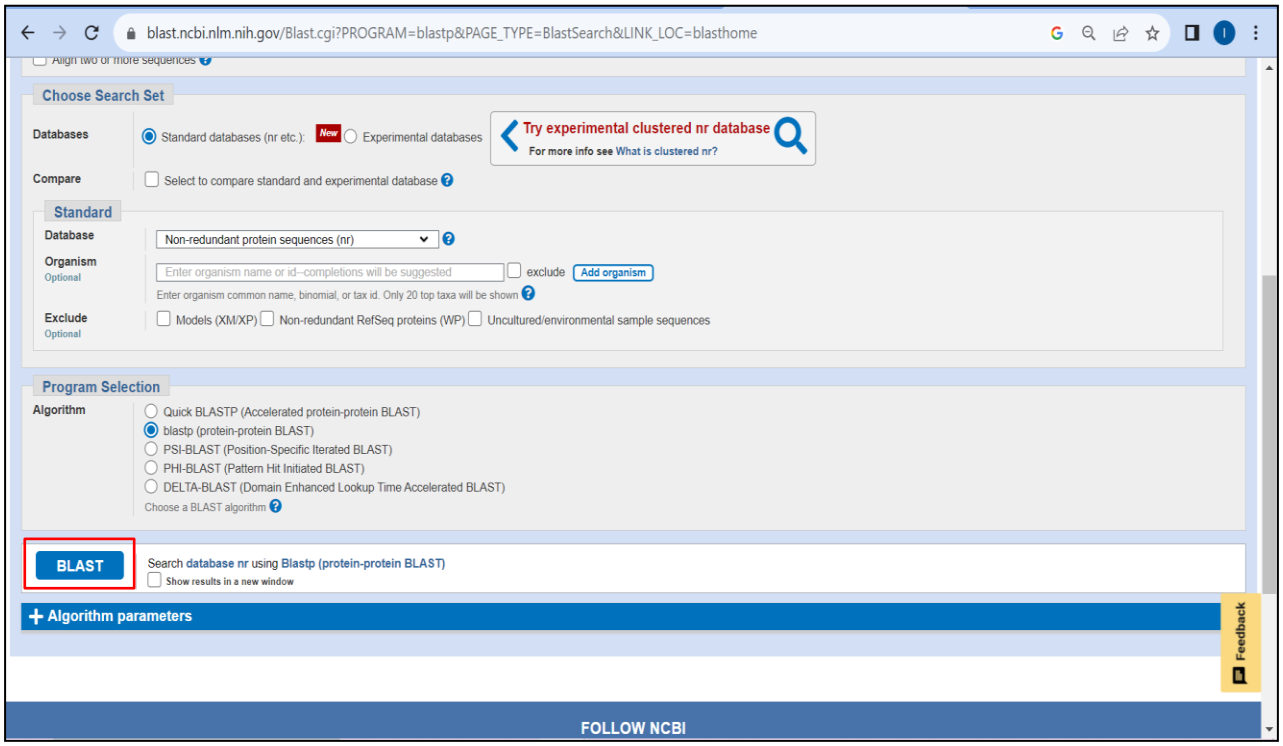


Figure 8: Running BLAST

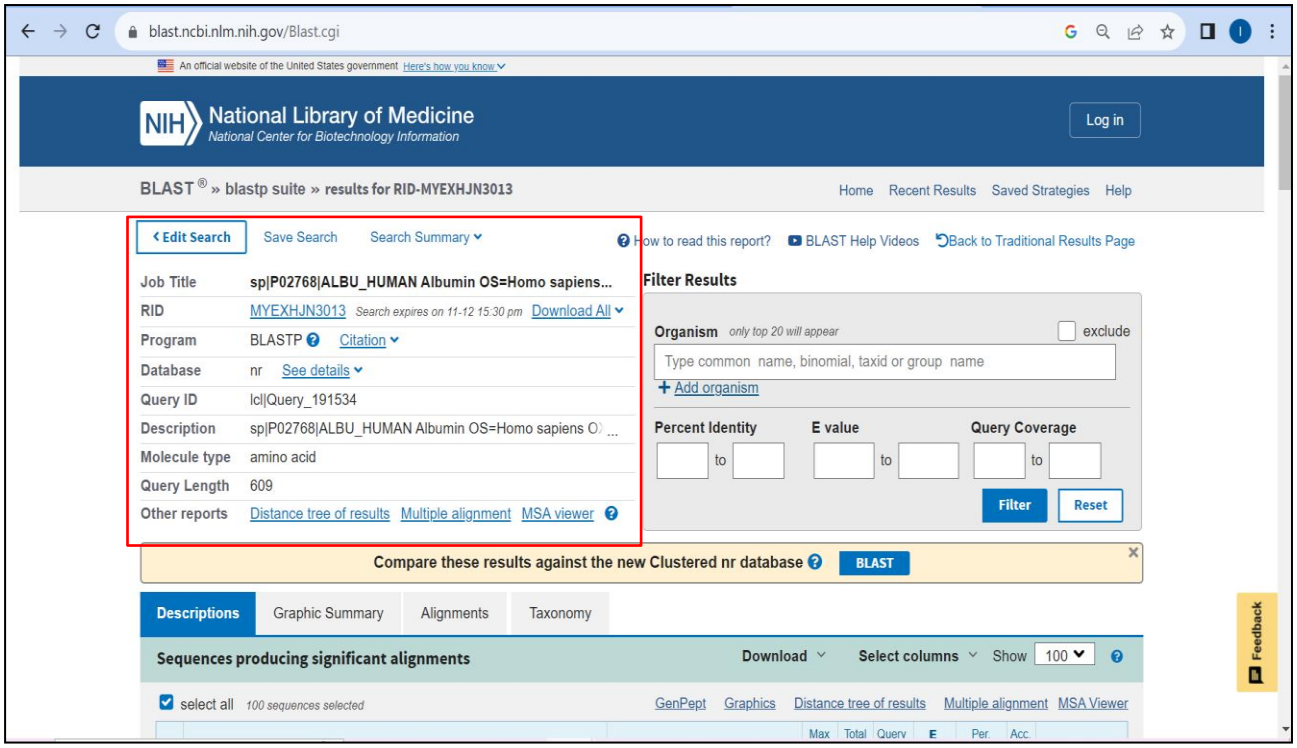
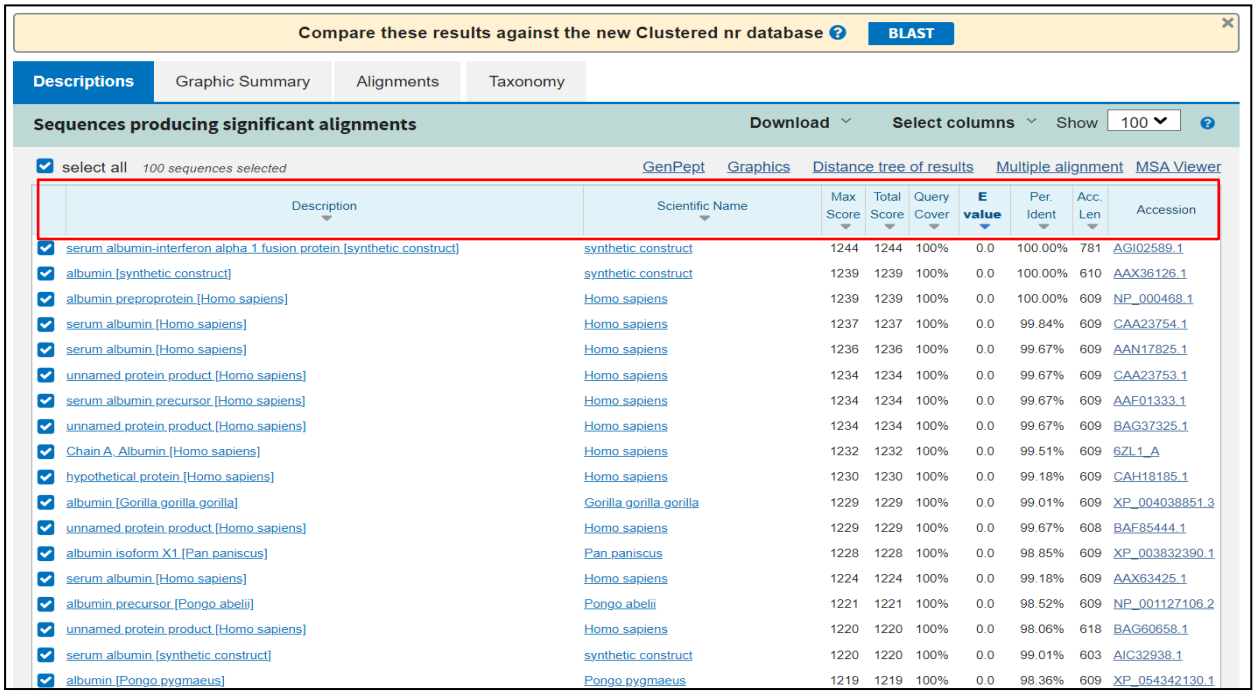


Figure 9: Results for the query, Header Section (UniProt ID: P02768)



Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
serum albumin-interferon alpha 1 fusion protein [synthetic construct]	synthetic construct	1244	1244	100%	0.0	100.00%	781	AGI02589.1
albumin [synthetic construct]	synthetic construct	1239	1239	100%	0.0	100.00%	610	AAX36126.1
albumin preproprotein [Homo sapiens]	Homo sapiens	1239	1239	100%	0.0	100.00%	609	NP_000468.1
serum albumin [Homo sapiens]	Homo sapiens	1237	1237	100%	0.0	99.84%	609	CAA23754.1
serum albumin [Homo sapiens]	Homo sapiens	1236	1236	100%	0.0	99.67%	609	AAN17825.1
unnamed protein product [Homo sapiens]	Homo sapiens	1234	1234	100%	0.0	99.67%	609	CAA23753.1
serum albumin precursor [Homo sapiens]	Homo sapiens	1234	1234	100%	0.0	99.67%	609	AAF01333.1
unnamed protein product [Homo sapiens]	Homo sapiens	1234	1234	100%	0.0	99.67%	609	BAG37325.1
Chain A, Albumin [Homo sapiens]	Homo sapiens	1232	1232	100%	0.0	99.51%	609	6ZL1_A
hypothetical protein [Homo sapiens]	Homo sapiens	1230	1230	100%	0.0	99.18%	609	CAH18185.1
albumin [Gorilla gorilla gorilla]	Gorilla gorilla gorilla	1229	1229	100%	0.0	99.01%	609	XP_004038851.3
unnamed protein product [Homo sapiens]	Homo sapiens	1229	1229	100%	0.0	99.67%	608	BAF85444.1
albumin isoform X1 [Pan paniscus]	Pan paniscus	1228	1228	100%	0.0	98.85%	609	XP_003832390.1
serum albumin [Homo sapiens]	Homo sapiens	1224	1224	100%	0.0	99.18%	609	AAX63425.1
albumin precursor [Pongo abelii]	Pongo abelii	1221	1221	100%	0.0	98.52%	609	NP_001127106.2
unnamed protein product [Homo sapiens]	Homo sapiens	1220	1220	100%	0.0	98.06%	618	BAG60658.1
serum albumin [synthetic construct]	synthetic construct	1220	1220	100%	0.0	99.01%	603	AIC32938.1
albumin [Pongo pygmaeus]	Pongo pygmaeus	1219	1219	100%	0.0	98.36%	609	XP_054342130.1

Figure 10: Result for Description Section

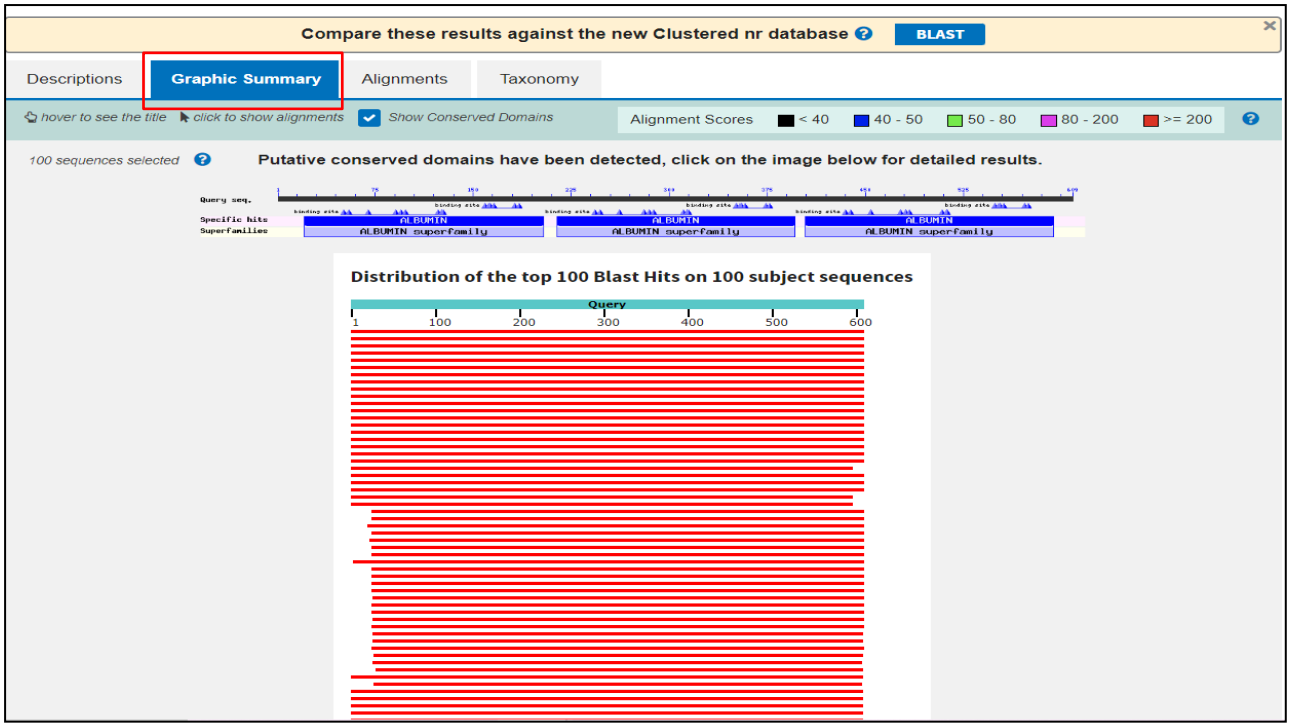


Figure 11: Result for Graphic Summary Section

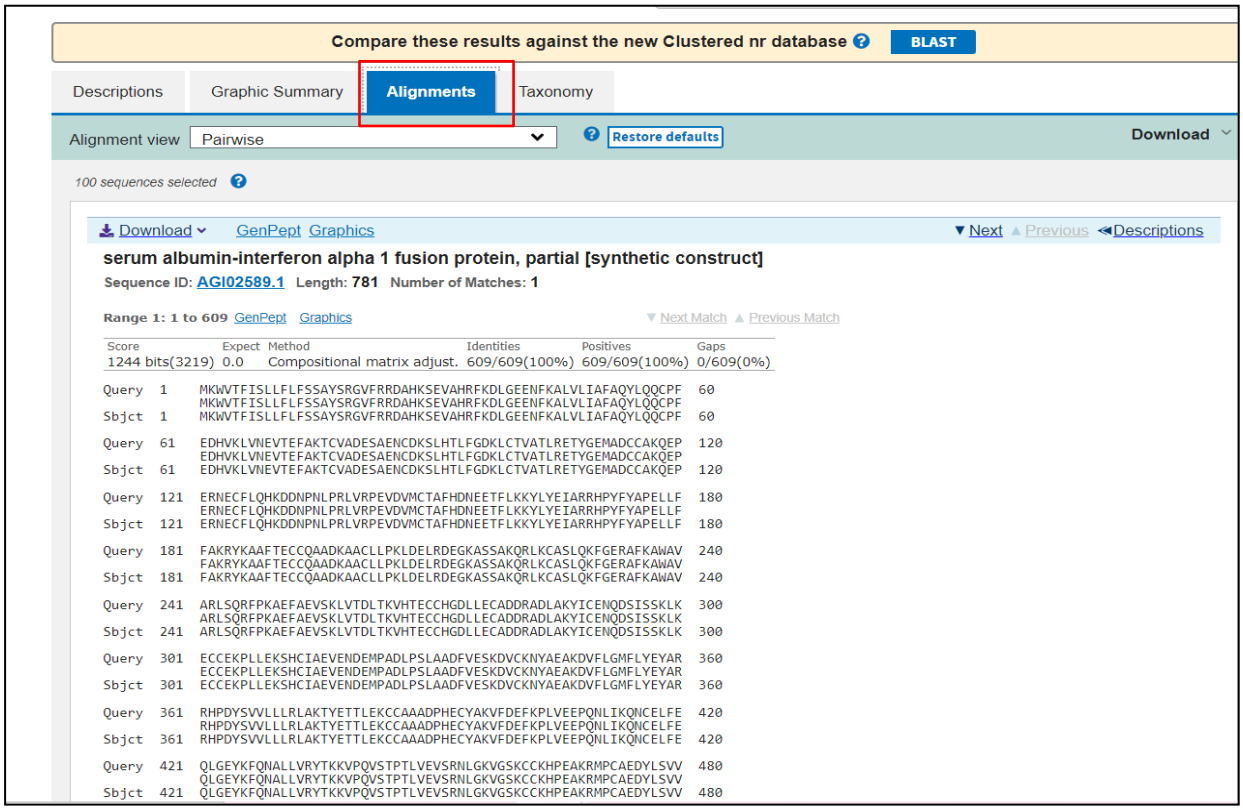


Figure 12: Result for Alignment Section

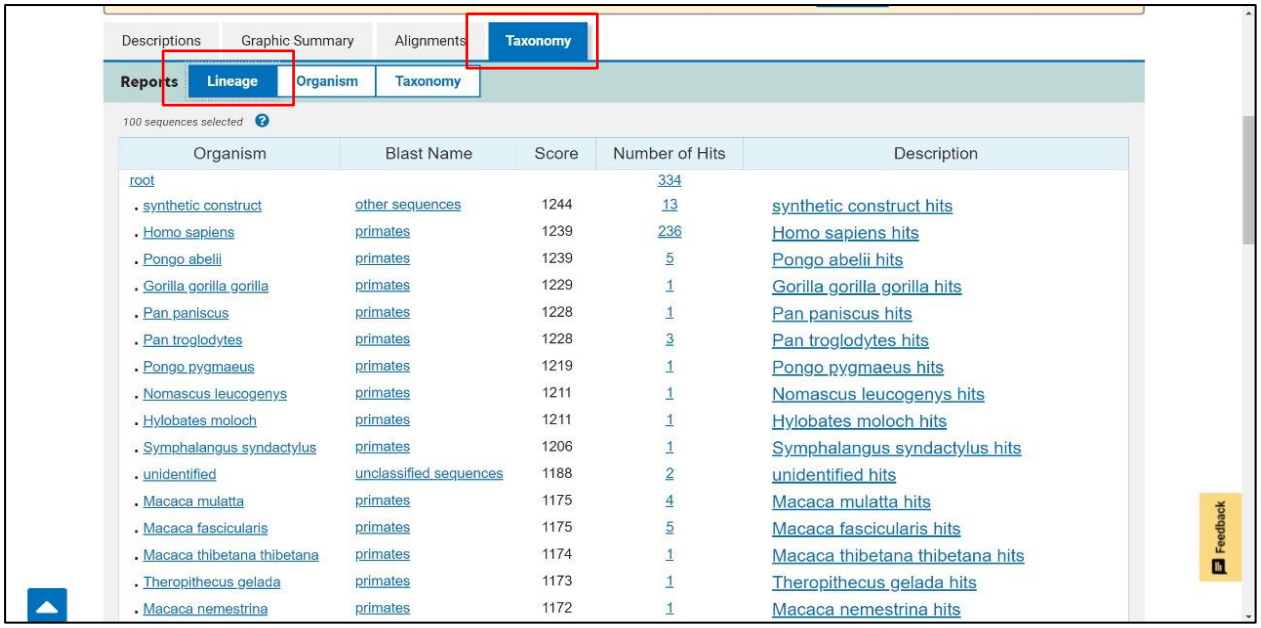


Figure 13: Result for Taxonomy Section based on Lineage

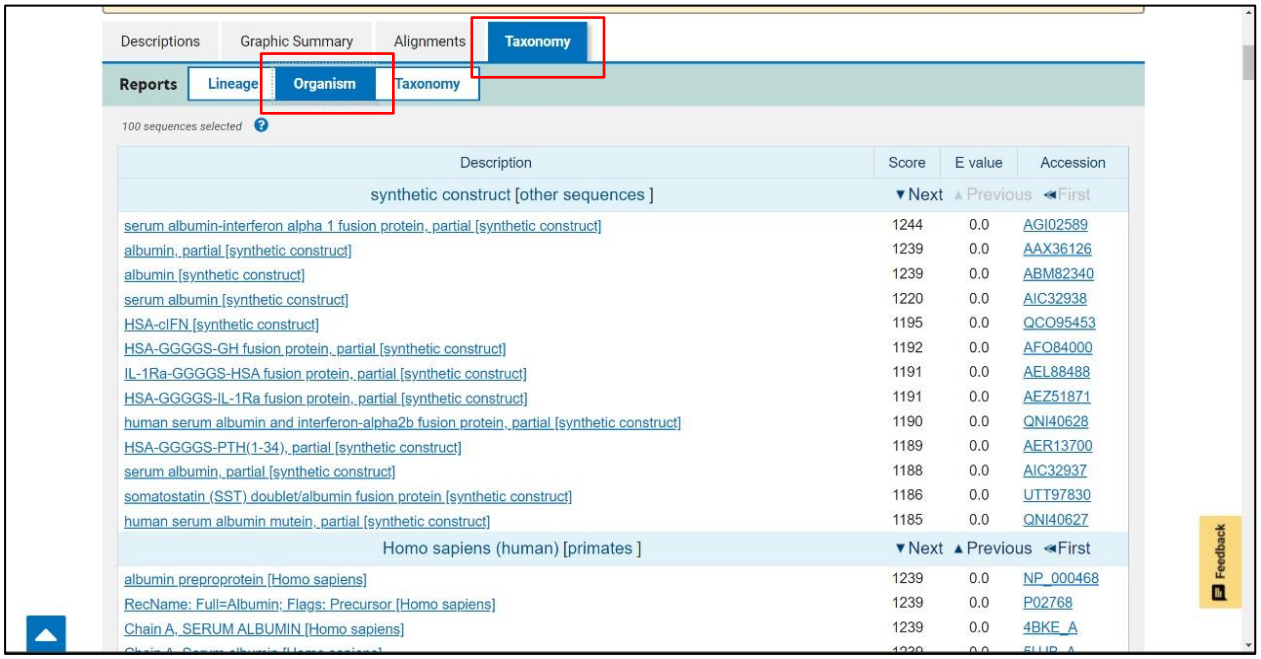


Figure 13a: Result for Taxonomy Section based on Organism

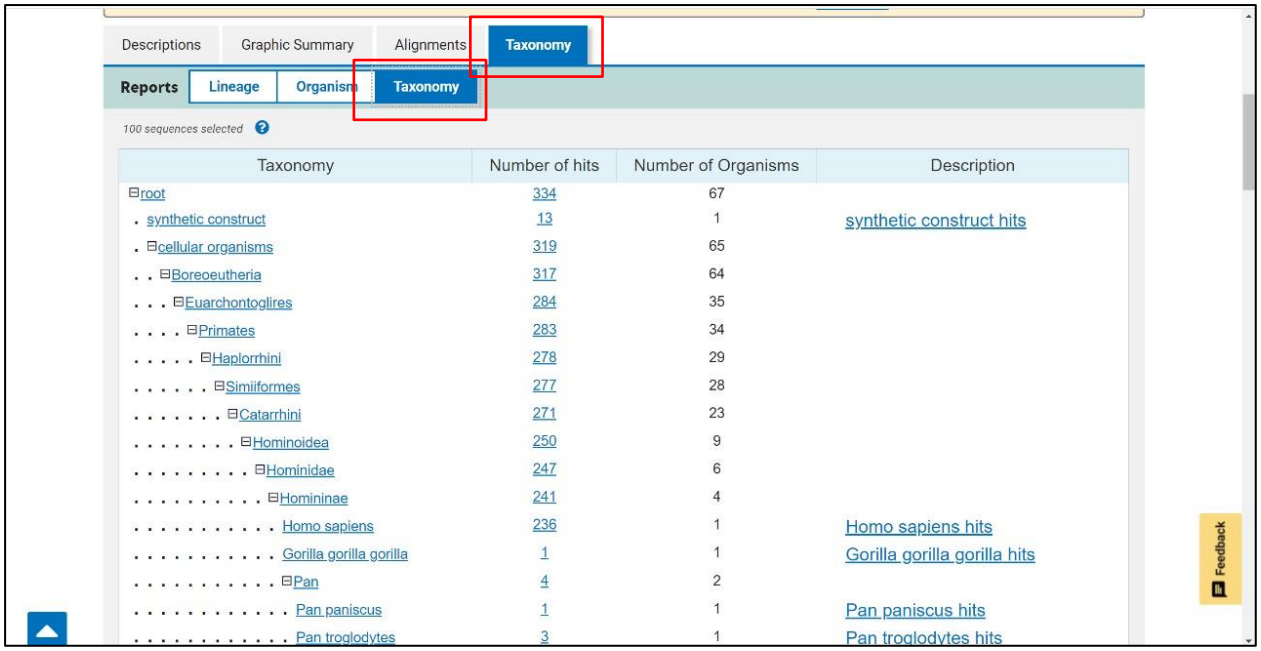


Figure 13b: Result for Taxonomy Section based on Taxonomy

RESULTS:

The Basic Local Alignment Search Tool (BLAST) was used to explore the protein sequences similar to the protein sequence of albumin (UniProt ID: P02768). The query sequence is found 100% identical to three sequence entries.

Sequence Title	Organism	Max Score	Total Score	E Value	Percentage Identity	Accession ID
serum albumin-interferon alpha 1 fusion protein	Synthetic construct	1244	1244	0.0	100.0%	AGI02589.1
albumin	Synthetic construct	1239	1239	0.0	100.0%	AAX36126.1
albumin preproprotein	*Homo Sapiens*	1239	1239	0.0	100.0%	NP_000468.1

CONCLUSION:

The protein sequences similar to the protein sequence of albumin (UniProt ID: P02768) were studied by exploring the Basic Local Alignment Search Tool (BLAST).

REFERENCES:

1. Xiong, J. (2006). *Essential Bioinformatics*. Cambridge: Cambridge University Press. https://doi.org/10.1017/CBO9780511806087
2. S. Sugio, A. Kashima, S. Mochizuki, M. Noda, K. Kobayashi, Crystal structure of human serum albumin at 2.5 Å resolution, *Protein Engineering, Design and Selection*, Volume 12, Issue 6, June 1999, Pages 439–446, https://doi.org/10.1093/protein/12.6.439

3. He, X., Carter, D. Atomic structure and chemistry of human serum albumin. *Nature* 358, 209–215 (1992). https://doi.org/10.1038/358209a0

DATE: 01/11/2023

WEBLEM 6(B)
FASTA TOOL
(URL: https://www.ebi.ac.uk/Tools/sss/fasta/)

AIM:

To study protein sequence similarity by exploring FASTA tool for the query maltose (UniProt ID: P68187).

INTRODUCTION:

FASTA tool was originally developed for comparing protein sequences. FASTA is a text-based format for representing nucleotide or amino acid sequences. It's used in bioinformatics and biochemistry. FASTA is an abbreviation for "Fast-All". FASTA is a sequence alignment tool that takes nucleotide or protein sequences as input and compares it with existing databases. It was the first database similarity search tool developed, preceding the development of BLAST. The FASTA format allows for sequence names and comments to precede the sequences. Nucleotides or amino acids are represented using single-letter codes. For example, A => adenosine, C => cytidine, G => guanine, T => thymidine, and N => A/G/C/T (any). The original program was referred to as FASTP. It quickly became a popular tool for sequence alignment and database searching. The program has been continually updated and improved.

There are now different FASTA programs available, each used for different types of sequence searches:

1. **FASTA** compares a DNA query sequence against a database of DNA sequences or a protein query sequence against a database of protein sequences using the FASTA algorithm.
2. **SSEARCH** performs protein-protein or DNA-DNA comparisons using the SmithWaterman algorithm.
3. **GGSEARCH/GLSEARCH** works using a global alignment algorithm (GGSEARCH) or a combination of global and local alignment algorithms (GLSEARCH) to compare protein and nucleotide sequences.
4. **FASTX/FASTY** compares a DNA sequence and a database of protein sequences by translating the DNA sequence into three frames and allowing gaps and frameshifts.
5. **TFASTX/TFASTY** compares a protein sequence and a database of DNA sequences. The DNA sequence is translated in six frames – three in the forward direction and three in the reverse direction.
6. **FASTF/TFASTF** compares mixed peptide sequences against a protein (FASTF) or translated DNA (TFASTF) databases.
7. **FASTS/TFASTS** compares a set of short peptide fragments against the protein (FASTS) or translated DNA (TFASTS) databases.

1. **How FASTA Works**

   FASTA works by comparing a query sequence to a database of sequences to identify similar matches. The program uses a heuristic algorithm to quickly search the database and identify the most significant matches.

2. **The working mechanism of FASTA is described in the following steps:**

   Step 1: Identifying Regions

   The first step is identifying regions with high similarity by creating a lookup table for the query sequence. This step is also called hashing step. To create the lookup table, the query sequence is first broken down into smaller words known as k-tuples (ktup).

   Step 2: Re-Scoring

   In the second step, the ten best diagonals are rescored using suitable scoring matrices. For protein, BLOSUM50 or PAM matrix is used; for DNA sequences, the identity matrix is used. A subregion with the highest score is identified for each of the rescanned diagonal regions.

   Step 3: Joining Threshold

   Next, a score cutoff or the joining threshold is applied that excludes segments unlikely to be part of the final alignment. The library sequences are ranked based on their Initial scores.

   Step 4: Final Alignment

   Finally, the gapped alignment is refined to produce the final alignment. This is done by using the banded Smith-Waterman algorithm, which is a dynamic programming algorithm that calculates the optimal score (opt) for alignment.

Maltose:

Maltose-binding protein (MBP) is a part of the maltose/maltodextrin system of Escherichia coli, which is responsible for the uptake and efficient catabolism of maltodextrins. It is a complex regulatory and transport system involving many proteins and protein complexes. MBP has an approximate molecular mass of 42.5 kilodaltons.

METHODOLOGY:

1. The protein FASTA (canonical) sequence for the desired protein for the query of ‘Maltose’ (UniProt ID: P68187) was retrieved from the UniProt Database.
2. Open the homepage of EBI – FASTA tool. Select the desired Protein Database and paste the retrieved FASTA (canonical) sequence of Maltose (UniProt ID: P68187) in the query box of the EBI – FASTA tool.
3. Set the desired parameters and select the ‘SUBMIT’ option to submit the query to the tool.
4. The results were shown in different tabs, namely, Submission Information, Tool Output, Graphic Output, Functional Forecasts, and Summary Table.
5. Interpret the results obtained.

OBSERVATIONS:

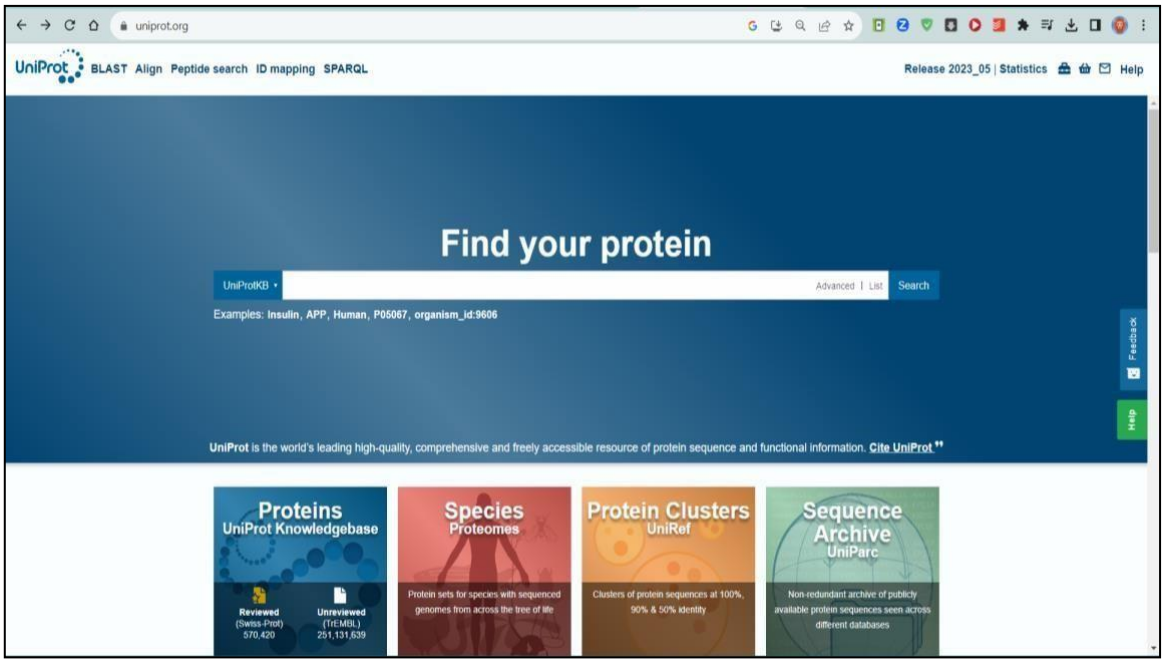


Figure 1: Homepage of the UniProt Database

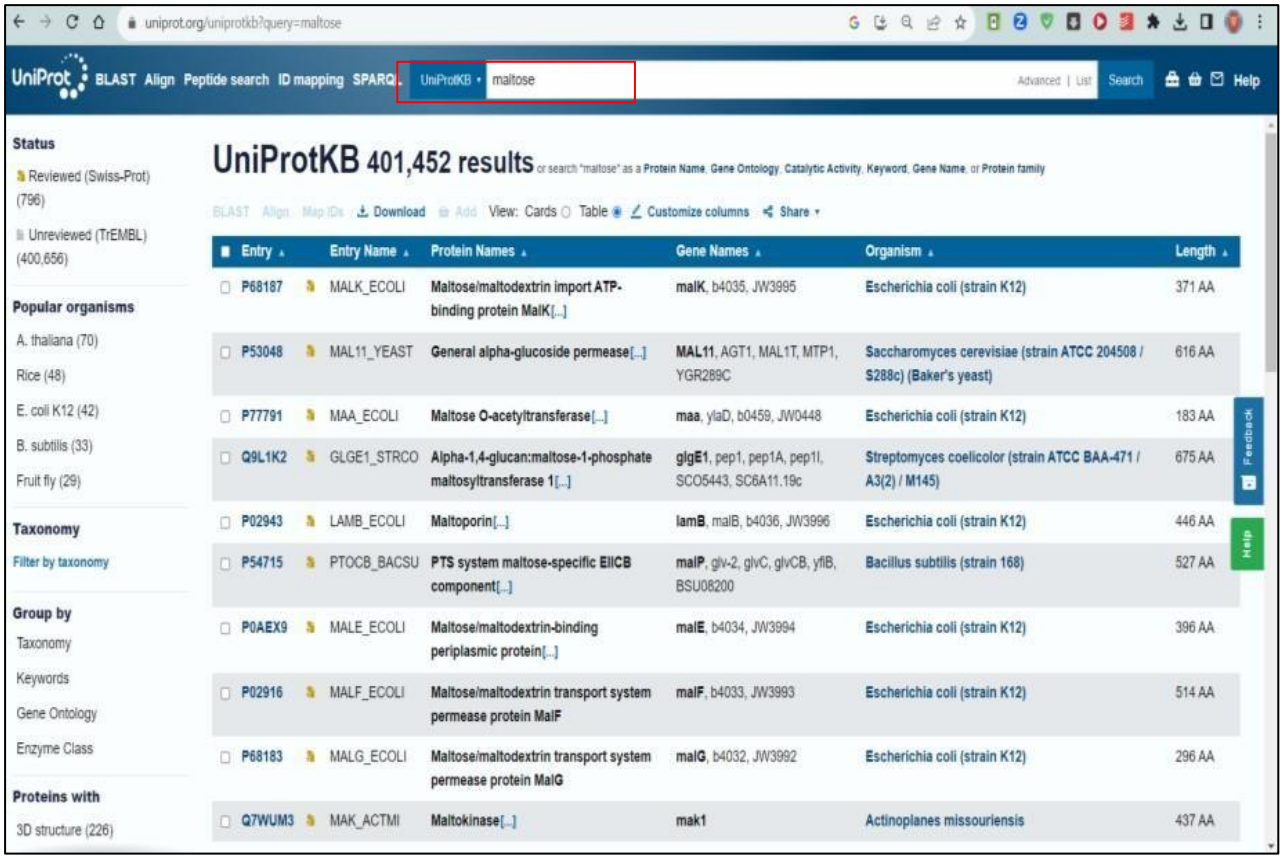


Figure 2: Searching for query maltose protein.

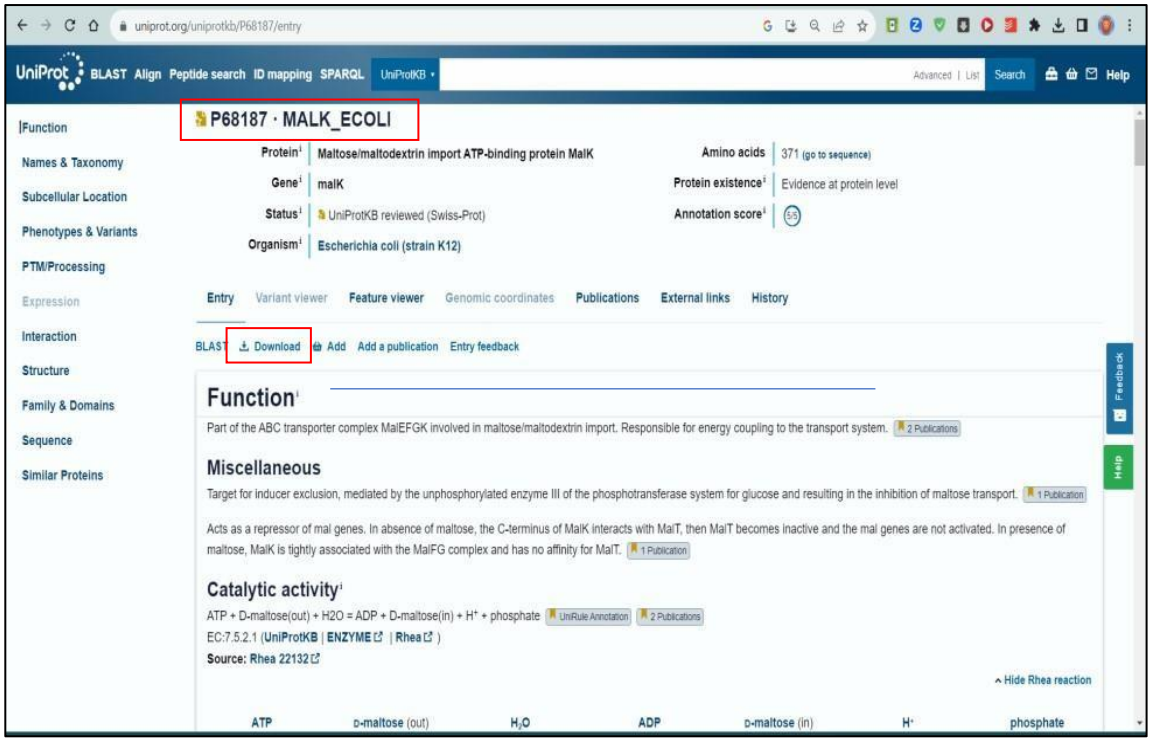


Figure 3: 'Download' option for retrieving the FASTA sequence of the protein

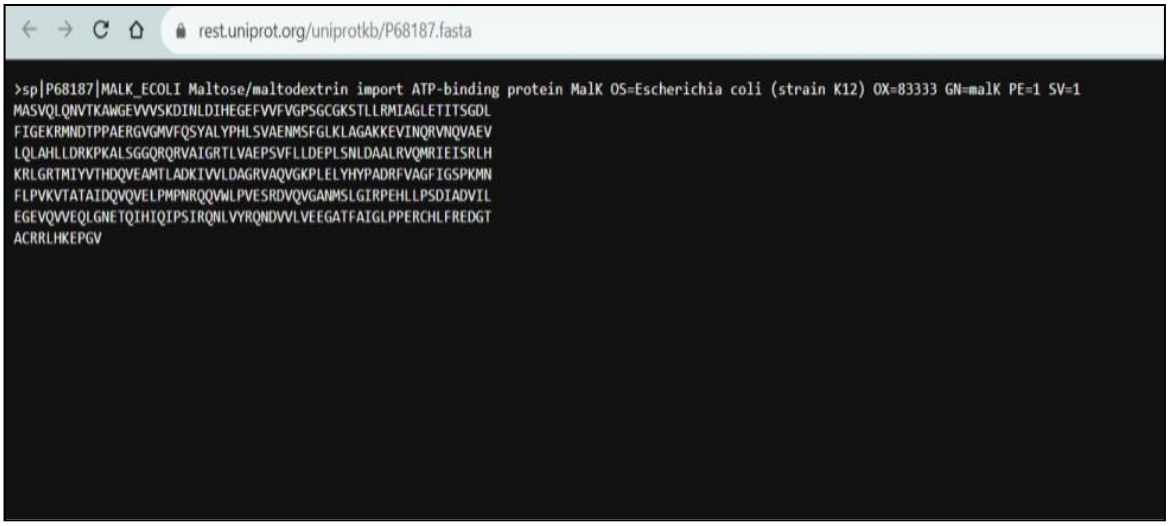


Figure 4: FASTA sequence of maltose protein.

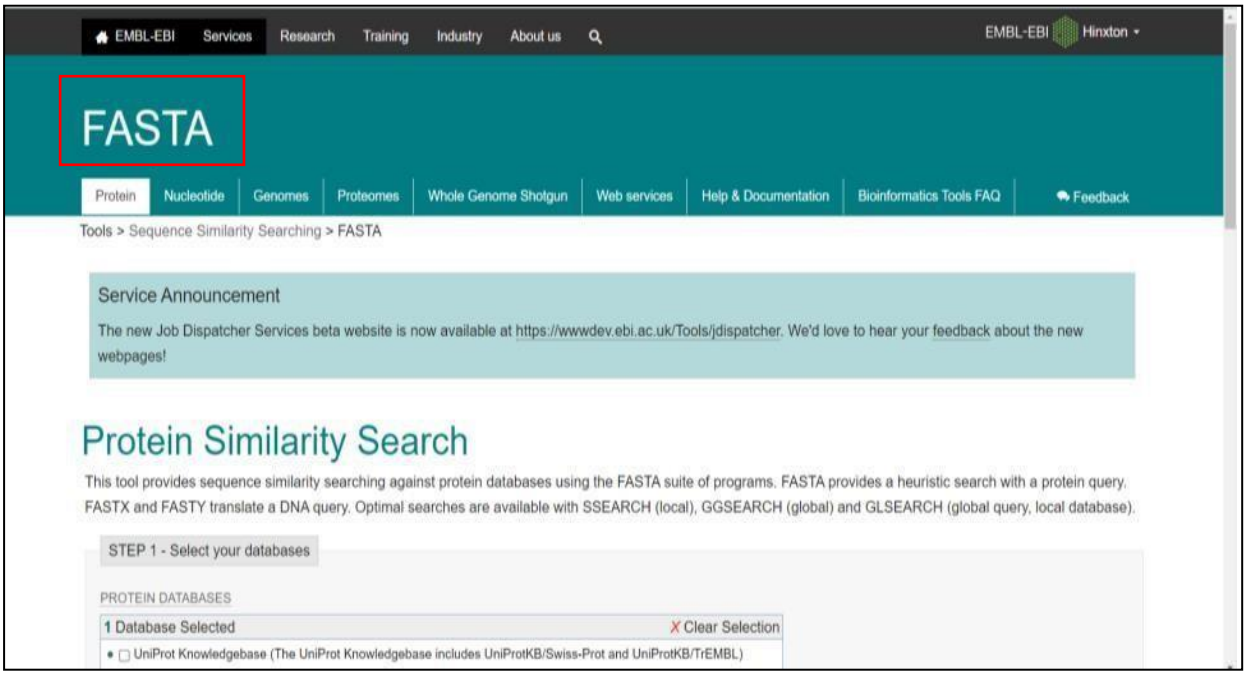


Figure 5: Homepage of FASTA tool.

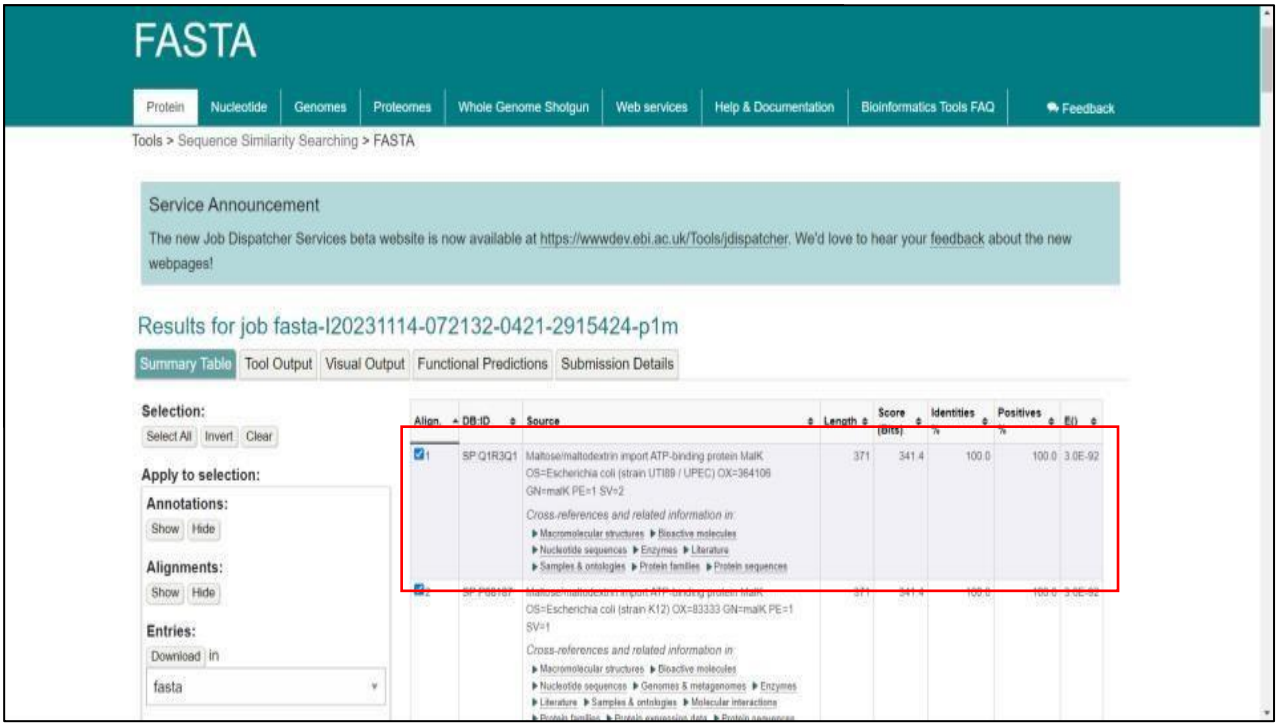


Figure 6: Searching sequence protein in FASTA tool.

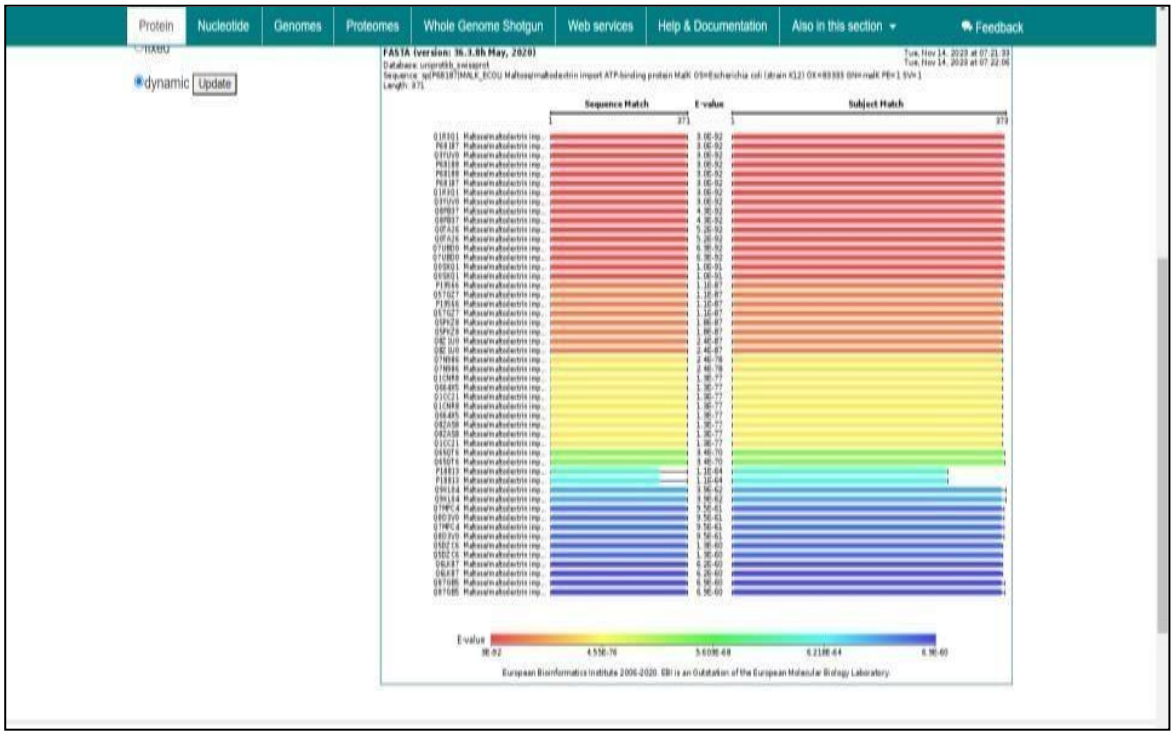


Figure 7: Visual output of maltose protein sequence.

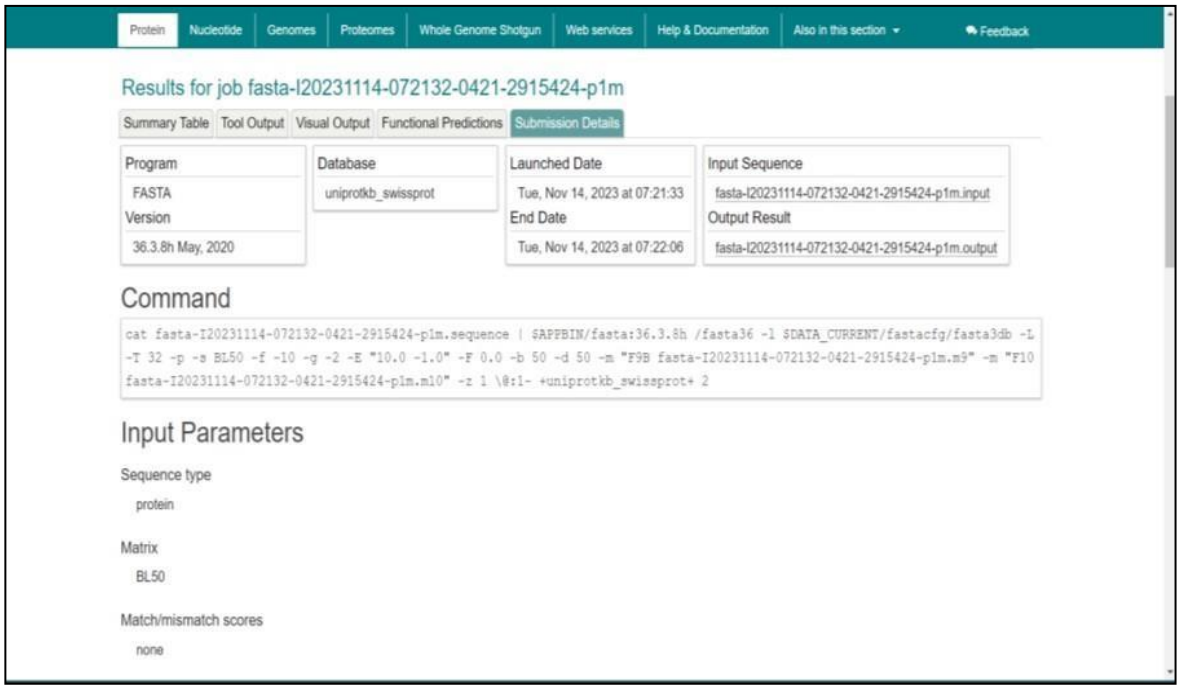


Figure 8: Submission details of maltose protein on FASTA tool.

RESULTS:

The EBI – FASTA tool was used to explore the sequences similar to the sequence of maltose (UniProt ID: P02768). The query sequence is found 100% identities & 100% positives to maltose sequence entries found in two organisms, viz., *Escherichia coli* and *Shigella sonnei*, with E Value of 5.2e-98 and sequence length of 371.

CONCLUSION:

FASTA is a versatile bioinformatics tool primarily used for storing, searching and comparing biological sequence data. It's commonly employed for tasks like sequence alignment, similarity searches and database comparisons. Sequence similarity was searched and studiedfor the Query 'Maltose' (UniProt ID: P68187) using the FASTA program.

REFERENCES:

1. Kryukov K, Ueda MT, Nakagawa S, Imanishi T (July 2020). "Sequence Compression Benchmark (SCB) database—A comprehensive evaluation of reference-free compressors for FASTA-formatted sequences". GigaScience. 9 (7): giaa072. https://doi.org/10.1093/gigascience/giaa072
2. Andrew Lloyd, Bioinformatics: A Practical Guide to the Analysis of Genes and Proteins (Methods of Biochemical Analysis, 43), Briefings in Bioinformatics, Volume 2, Issue 4, December 2001, Pages 407–408, https://doi.org/10.1093/bib/2.4.407
3. Pratas D, Hosseini M, Pinho A (2017). "Cryfa: a tool to compact and encrypt FASTA files". 11th International Conference on Practical Applications of Computational Biology & Bioinformatics (PACBB). Advances in Intelligent Systems and Computing. Vol. 616. Springer. Pp. 305–312. Doi:10.1007/978-3-319-60816-7_37. https://link.springer.com/book/10.1007/978-3-319-60816-7

DATE: 01/11/2023

WEBLEM 6(C)
PROTEIN- SPECIFIC ITERATED BLAST (PSI BLAST)
(URL: https://blast.ncbi.nlm.nih.gov/Blast.cgi)

AIM:

To explore the PSI BLAST tool to search putative homologs for query "Leucine" (UniProt ID: Q8IX15).

INTRODUCTION:

PSI-BLAST (Position-Specific Iterative Basic Local Alignment Search Tool) derives a position-specific scoring matrix (PSSM) or profile from the multiple sequence alignment of sequences detected above a given score threshold using protein–protein BLAST. This PSSM is used to further search the database for new matches and is updated for subsequent iterations with these newly detected sequences. Thus, PSI-BLAST provides a means of detecting distant relationships between proteins. BLAST (Basic Local Alignment Search Tool) is a sequence similarity search method, in which a query protein or nucleotide sequence is compared to nucleotide or protein sequences in a target database to identify regions of local alignment and report those alignments that score a given score threshold. Position-Specific Iterative (PSI)-BLAST is a protein sequence profile search method that builds off the alignments generated by a run of the BLASTp program. The first iteration of a PSI-BLAST search is identical to a run of BLASTp program. It then generates a multiple alignment of the highest scoring pairs of the BLASTp run above a certain preset score or *e*-value threshold and calculates a profile or a position-specific score matrix (PSSM) from the multiple alignment.

The PSSM captures the conservation pattern in alignment and stores it as a matrix of scores for each position in the alignment-highly conserved positions receive high scores and weakly conserved positions receive scores near zero. This profile is used in place of the original substitution matrix for a further search of the database to detect sequences that match the conservation pattern specified by the PSSM. The newly detected sequences from this second round of the search, which are above the specified score (*e*-value) threshold is again added to alignment the profile is refined for another round of searching. This process is iteratively continued until desired or until convergence, i.e., the state where no new sequences are detected above the defined threshold. The iterative profile generation process makes PSI-BLAST far more capable of detecting distant sequence similarities than a single query alone in BLASTp, because it combines the underlying conservation information from a range of related sequence into a single score matrix. In the evolution, three-dimensional (3D) structures of proteins may be conserved even after considerable erosion of their sequence similarity. PSI-BLAST has been demonstrated to be useful in detecting such relationships via sequence searches, which were previously only detected through direct comparison of the 3D structures. Here, we discuss practical aspects of using PSI-BLST and provide a tutorial on how to uncover distant relationships between proteins and use them to reach biological meaningful conclusions.

Significance:

1. PSI-BLAST is most conveniently used on the internet with the help of the graphical user interface provided by the PSI-BLAST search page on National Centre for Biotechnology Information (NCBI).
2. The PSI-BLAST page may be customized by the user in terms of automated or semiautomated or "two-page formatting" and other parameters modified as desired. This page can then be saved as permanent internet bookmark for repeated use on futureoccasions.
3. As a rule of the thumb, beginners are advised to use the profile-inclusion threshold of expect (*e*)-value = 0.005 for their analysis. However, a user familiar with globular domains and compositional bias may use the inclusion threshold of 0.01 for inclusion in the profile, if a sequence does not have any major compositionally biased segments.
4. A pair of protein sequences can either be homologous (sharing a common evolutionary ancestor) or nonhomologous (evolutionarily unrelated).
    a. It should be noted that PSI-BLAST does not offer a direct binary decision on whether two sequences are related or not. However, the *e*-value obtained for a PSI-BLAST alignment can be used as a guide for this purpose.
5. As a heuristic it may be assumed that any compositionally unbiased query, encompassing a globular domain in a protein, giving a hit with *e*-value = <0.01 is likelyto be an indication of a homologous relationship. However, a user must carefully evaluate such alignments case-by-case because there can occasionally be false- positives.
6. A user may set the number of alignments and hits view as at least 1000 if searching the nonredundant (nr) database of NCBI, because of the large number hits obtained due tothe current size of the database. PSI-BLAST may also be downloaded and run as a standalone program for Windows or UNIX-type operating systems.
    a. However, in this case the various parameters need to be specified using the set of command-line flags for the program. An advantage of using the standalone version is the ability to use alignments as queries to generate a starting PSSM or saving and reusing the profile generated by a run of PSI-BLAST.

Leucine:

Leucine (symbol **Leu** or **L**) is essential amino acid that is used in the biosynthesis of proteins. Leucine is an α-amino acid, meaning it contains an α-amino group (which is in the protonated $-NH_3^+$ form under biological conditions), an α-carboxylic acid group (which is in the deprotonated $-COO^-$ form under biological conditions), and a side chain isobutyl group, making it a non-polar aliphatic amino acid. It is essential in humans, meaning the body cannot synthesize it: it must be obtained from the diet. Human dietary sources are foods that contain protein, such as meats, dairy products, soy products, and beans and other legumes. It is encoded by the codons UUA, UUG, CUU, CUC, CUA, and CUG.

Like valine and isoleucine, leucine is a branched-chain amino acid. The primary metabolic end products of leucine metabolism are acetyl-CoA and acetoacetate; consequently, it is one of the two exclusively ketogenic amino acids, with lysine being the other. It is the most important ketogenic amino acid in humans.

L-leucine is the L-enantiomer of leucine. It has a role as a plant metabolite, an Escherichia coli metabolite, a Saccharomyces cerevisiae metabolite, a human metabolite, an algal metabolite

and a mouse metabolite. It is a pyruvate family amino acid, a proteinogenic amino acid, a leucine and a L-alpha-amino acid. It is a conjugate base of a L-leucinium. It is a conjugate acid of a L-leucinate. It is an enantiomer of a D-leucine. It is a tautomer of a L-leucine zwitterion.

METHODOLOGY:

1. Go to the website of BLAST tool.
2. Click protein blast as protein is more conserved than nucleotide.
3. Go on UniProt portal.
4. Search for query ‘Leucine’.
5. From shown results select UniProt ID: ‘Q8IX15’ entry.
6. Download the sequence in FASTA (Canonical) format.
7. Copy the sequence and paste under BLASTp suite.
8. Select Protein Data Bank (PDB) database under standard and program algorithm parameter as psi-blast with threshold 0.001.
9. Click BLAST to run the query.
10. Click Run to observe 2nd iterated and continue till 5 iterations.

OBSERVATIONS:

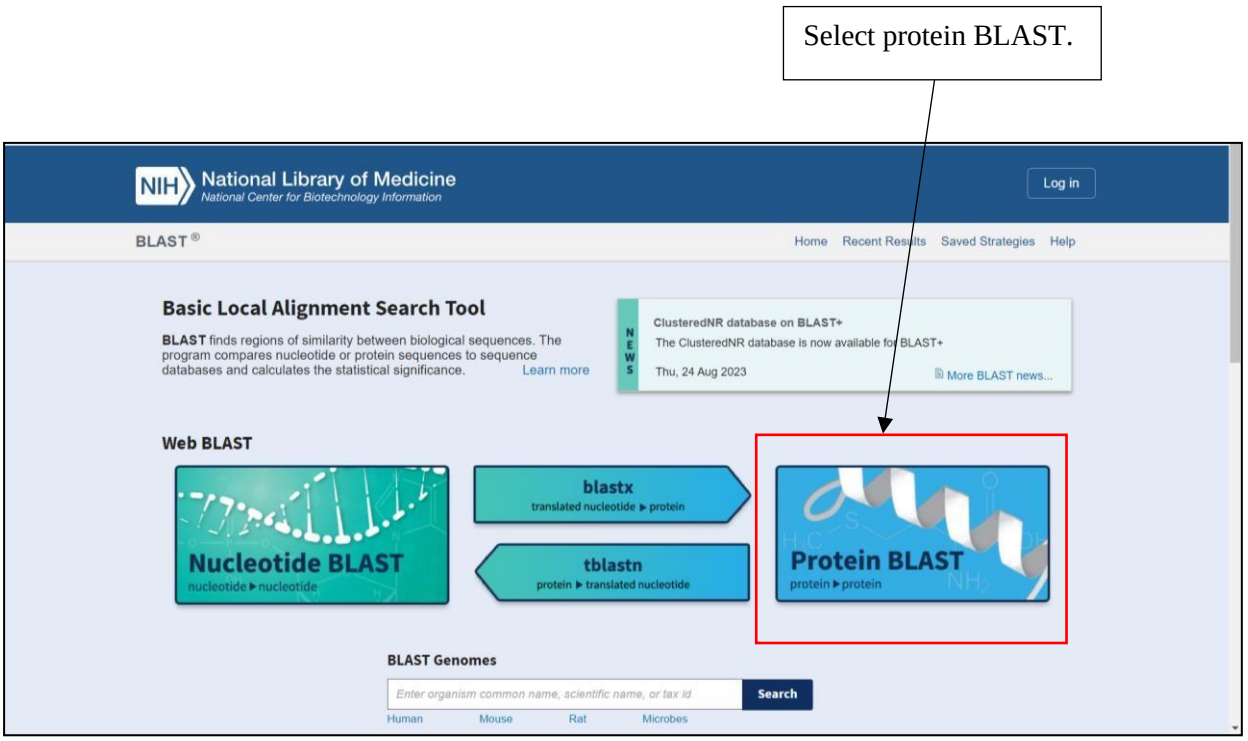


Figure 1: Homepage of BLAST

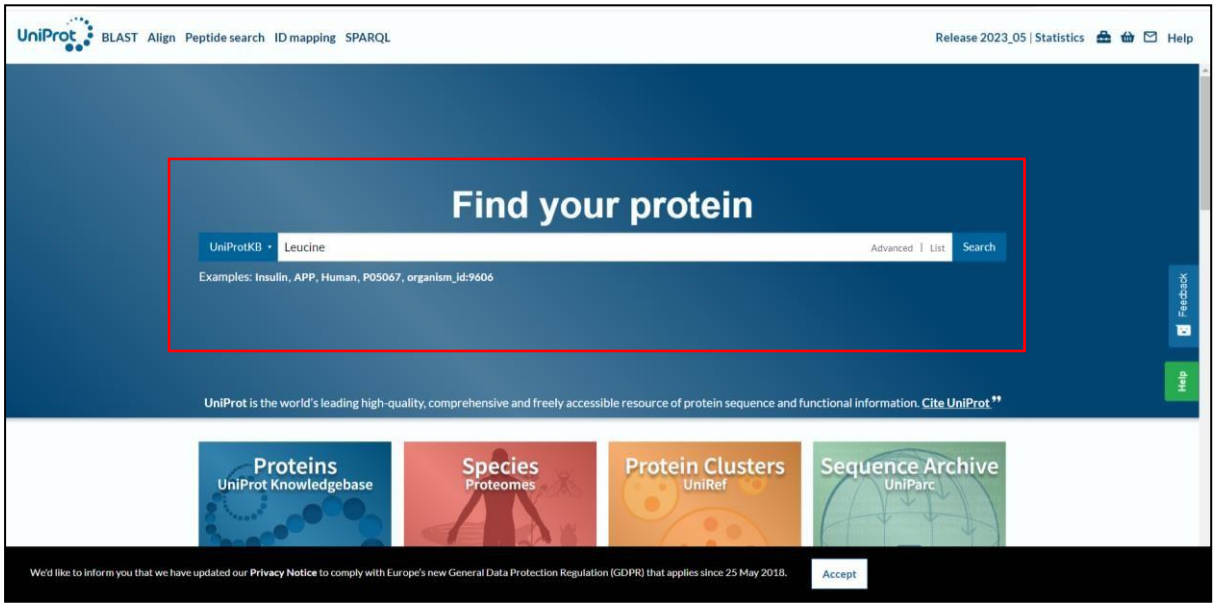


Figure 2: Query search in UniProt portal

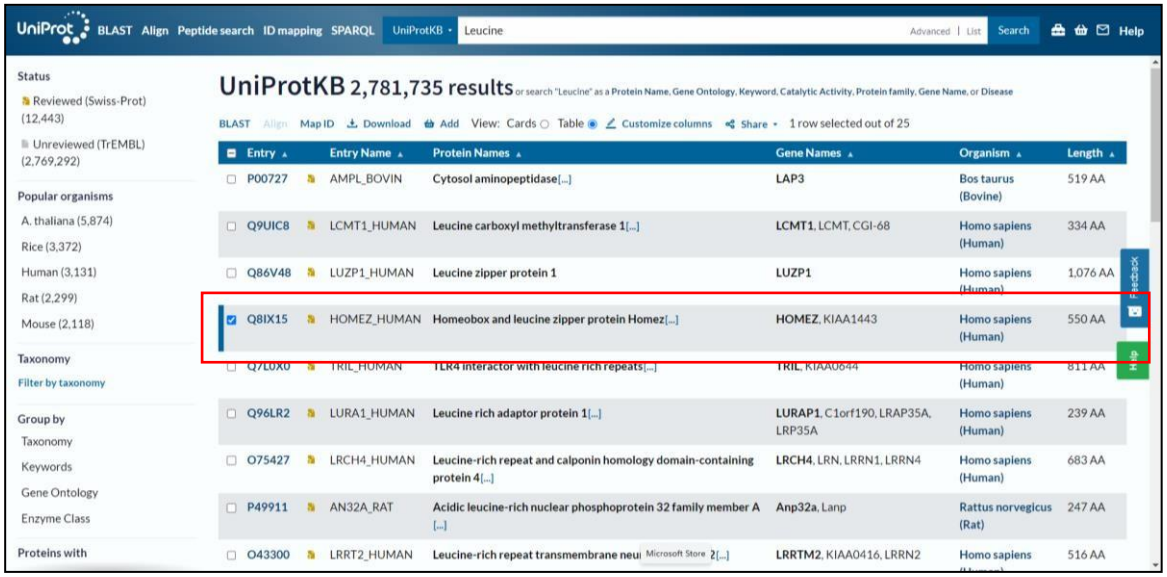


Figure 2a: Select desired organism

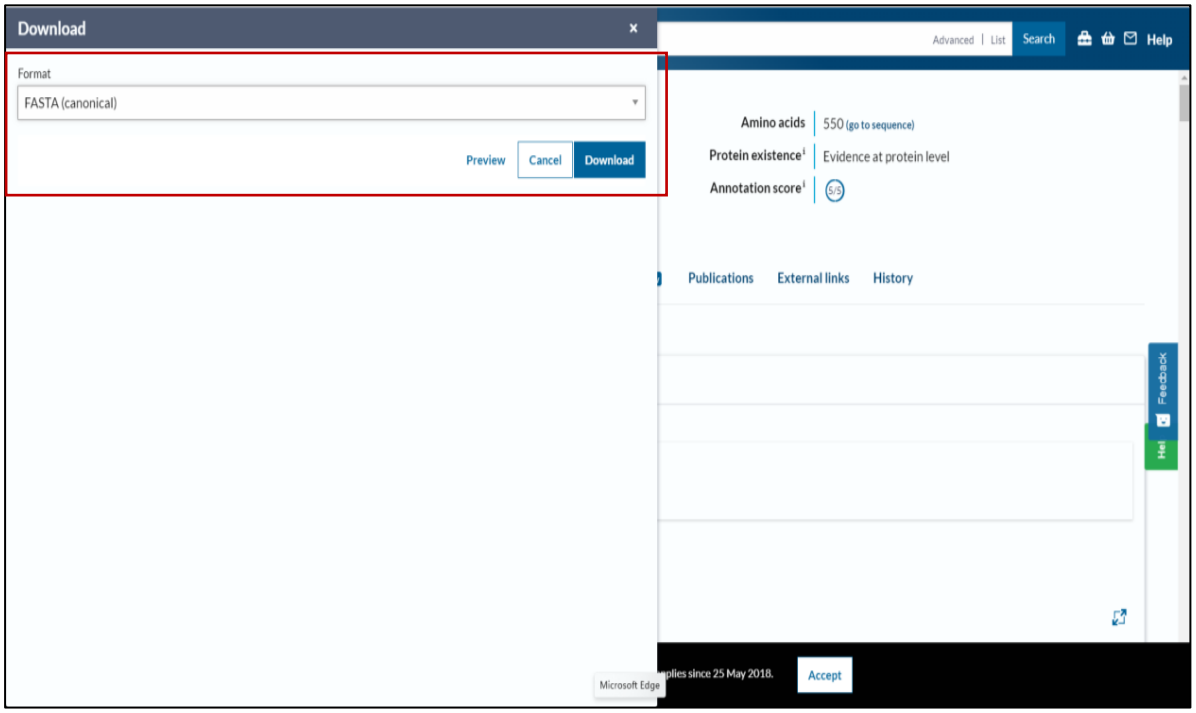


Figure 2b: Download sequence in FASTA (Canonical) format

```
>sp|Q8IX15|HOMEZ_HUMAN Homeobox and leucine zipper protein Homez OS=Homo sapiens OX=9606 GN=HOMEZ PE=1 SV=2
MVRGWEPPPGLDCAISEGHKSEGTMPPNKEASGLSSSPAGLICLPPISEELQLVWTQAAQ
TSELDSNEHLLKTFSYFPYPSLADIALLCLRYGLQMEKVKTWFMAQRLRCGISWSSEEIE
ETRARVVYRRDQLHFKSLLSFTHHAGRPPEEVPPPPVPAPEQVGIGIGPPTLSKPTQTKG
LKVEPEEPSQMPPLPQSHQKLKESLMTPGSGAFPYQSDFWQHLQSSGLSKEQAGRGPNQS
HGIGTASWNHSTTVPQPQARDKPPPIALIASSCKEESASSVTPSSSSTSSSFQVLANGAT
AASKPLQPLGCVPQSVSPSEQALPPHLEPAWPQGLRHNSVPGRVGPTEYLSPDMQRQRKT
KRKTKEQLAILKSFFLQCQWARREDYQKLEQITGLPRPEIIQWFGDTRYALKHGQLKWFR
DNAVPGAPSFQDPAIPTPPPSTRSLNERAETPPLPIPPPPPDIQPLERYWAAHQQLRETD
IPQLSQASRLSTQQVLDWFDSRLPQPAEVVVCLDEEEEEEEEELPEDDEEEEEEEEDDD
DDDDDVIIQD
```

Figure 2c: Copying the sequence

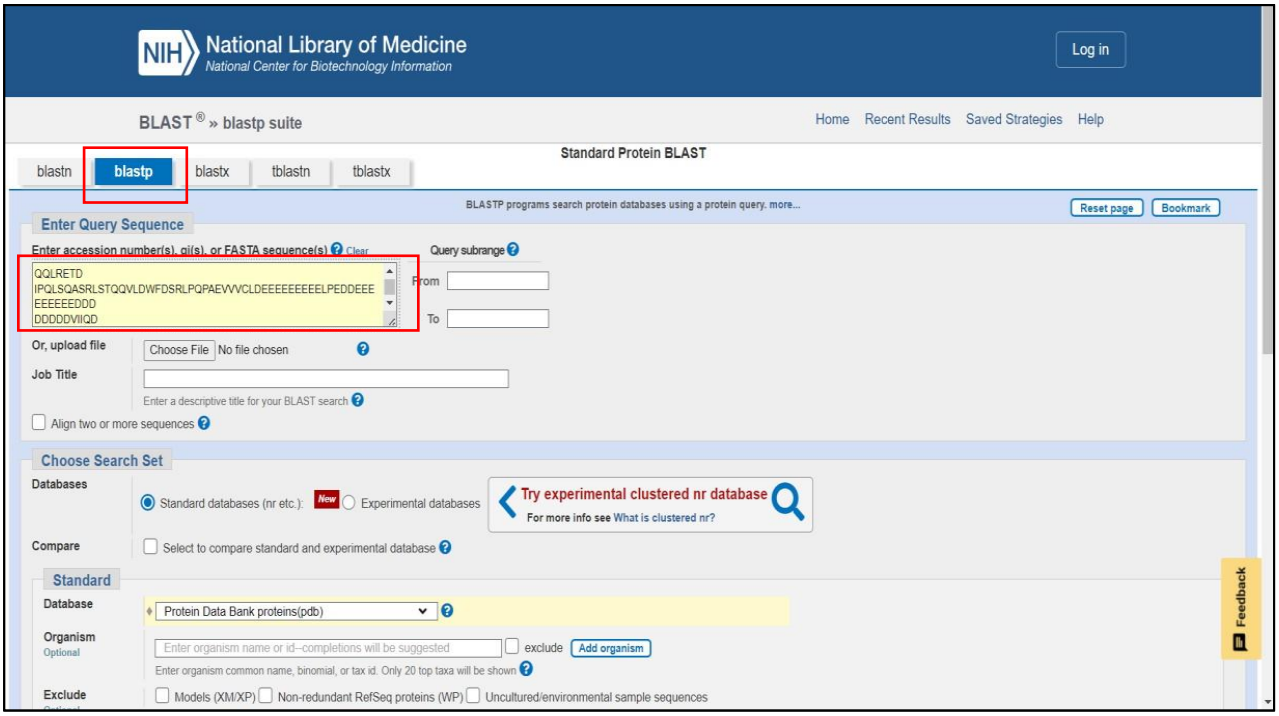


Figure 3: Pasting the sequence in BLASTp format

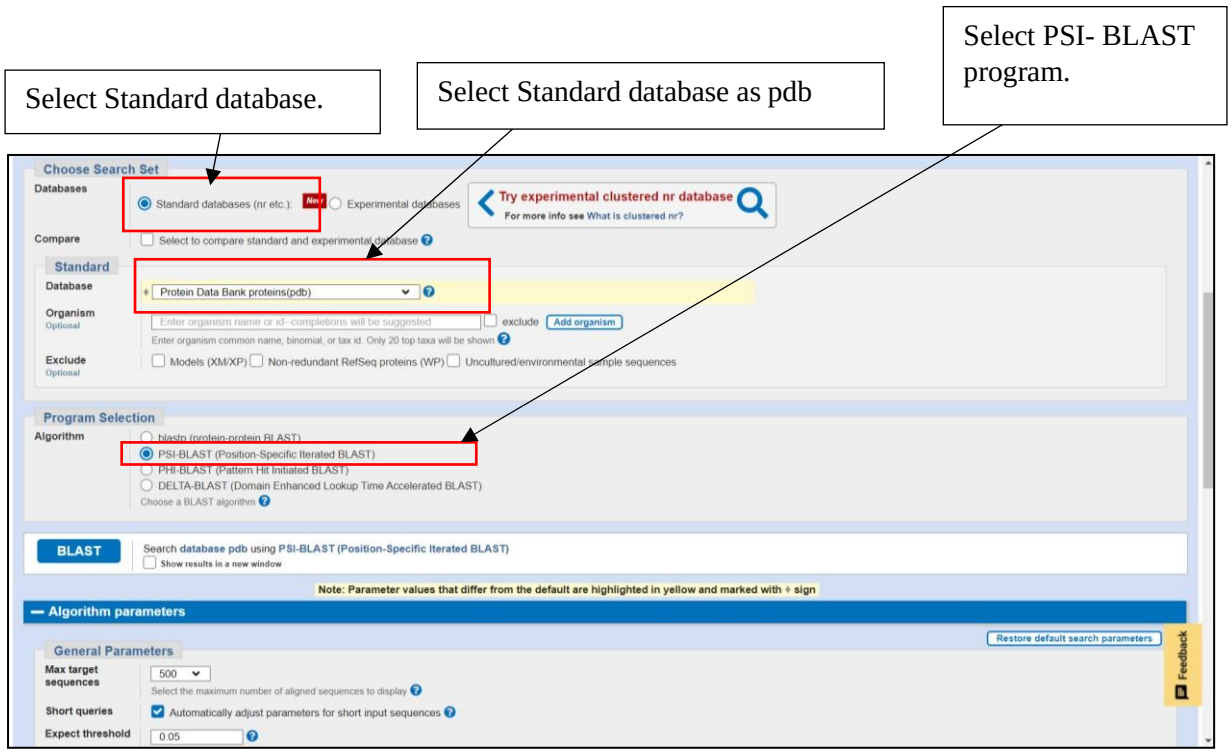


Figure 4: Selecting Standard database as pdb and program selection as PSI- BLAST

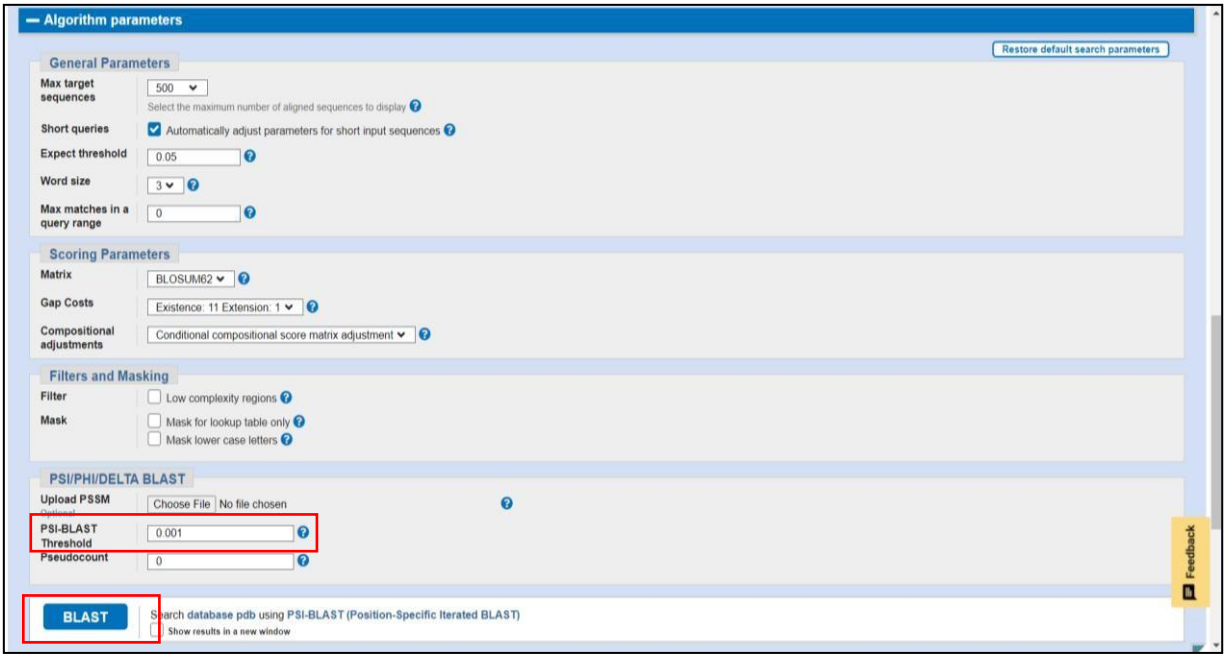


Figure 5: Keeping PSI-BLAST threshold as 0.001 and running PSI - BLAST

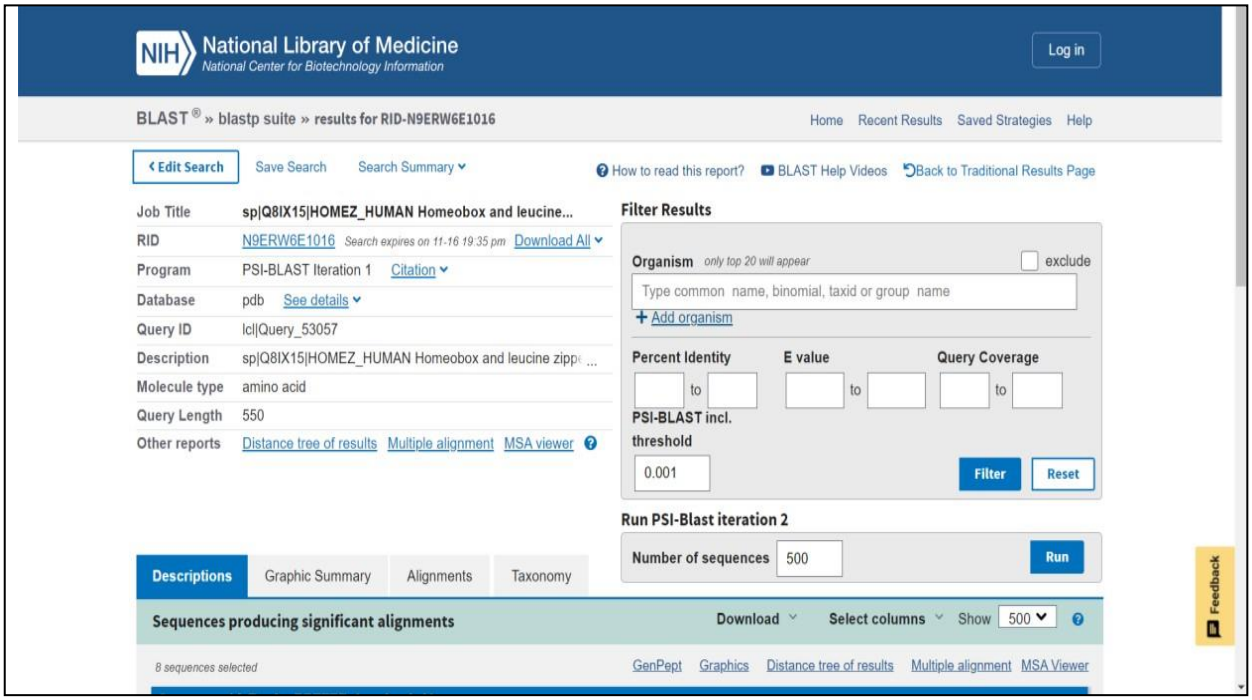


Figure 6: Result shown for UniProt ID: Q8IX15 in BLASTp

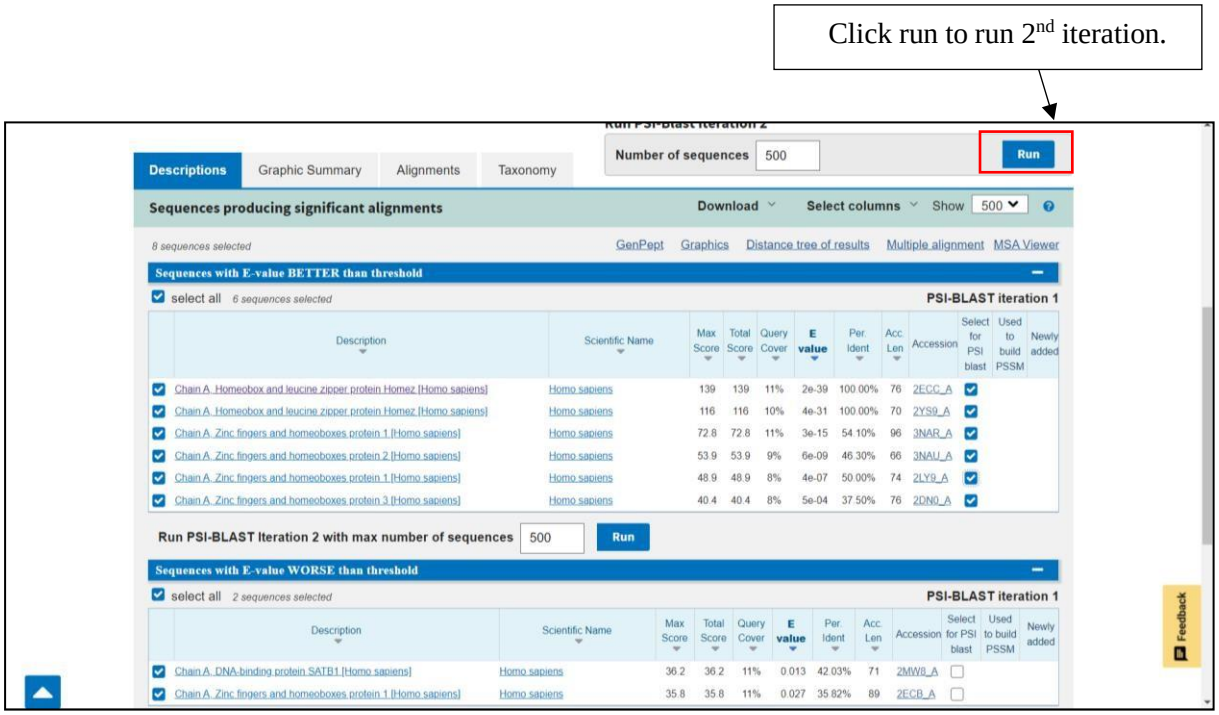


Figure 6a: Result shown for sequence with E- value better and worse than threshold

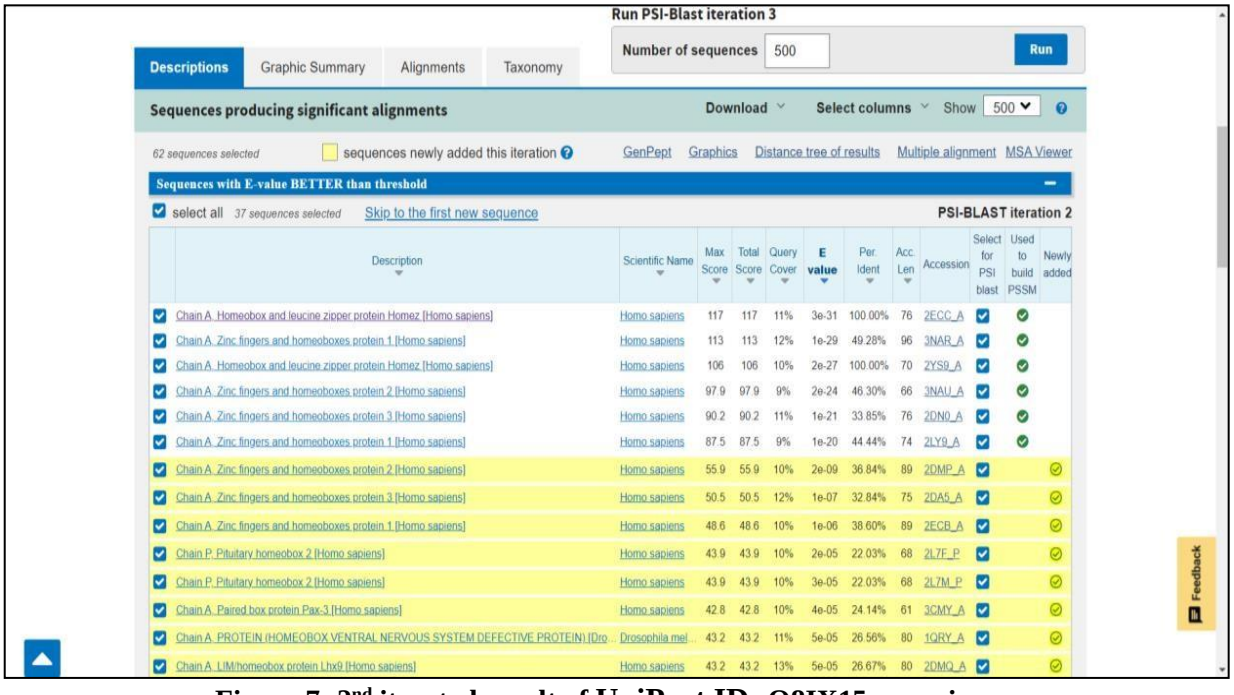


Figure 7: 2nd iterated result of UniProt ID: Q8IX15 organism

RESULTS:

PSI BLAST was explored using query 'Leucine' (Q8IX15) in order to get putative homologs. The first iteration showed 8 new putative sequences and the addition of new sequences was carried till 5th iteration, but then the process if halted as further iteration would drop the result accuracy and the iteration showed that new putative homologs are available for query 'Leucine'.

CONCLUSION:

PSI-BLAST (Position-Specific Iterative Basic Local Alignment Search Tool) derives a position-specific scoring matrix (PSSM) or profile from the multiple sequence alignment of sequences detected above a given score threshold using protein–protein BLAST. This PSSM is used to further search the database for new matches and is updated for subsequent iterations with these newly detected sequences. Thus, PSI-BLAST provides a means of detecting distant relationships between proteins. PSI-BLAST (Position specific iterative – BLAST) algorithm program was used to view and explore best iterated results for query 'Leucine' (UniProt ID: Q8IX15).

REFERENCES:

1. Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. Journal of Molecular Biology, 215(3), 403–410.
2. PSI-BLAST. (n.d.). National Institutes of Health. https://www.ncbi.nlm.nih.gov/books/NBK2590/
3. Pruitt KD, Tatusova T, Ostell JM. McEntyre J, Ostell J, editors. The Reference Sequence (RefSeq) Project. National Library of Medicine (US), NCBI; Bethesda, MD: The NCBI Handbook. 2005 Chapter 18.
4. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. (1997 September 01) *Nucleic acids research* 25 (17) :3389-3402
5. Zhang, L., Li, F., Guo, Q., Duan, Y., Wang, W., Zhong, Y., Yang, Y., & Yin, Y. (2020). Leucine Supplementation: A Novel Strategy for Modulating Lipid Metabolism and Energy Homeostasis. *Nutrients*, *12*(5), 1299. https://doi.org/10.3390/nu12051299

DATE: 01/11/2023

WEBLEM 6(D)
PATTERN HIT INITIATED BLAST (PHI-BLAST) TOOL
(URL: https://blast.ncbi.nlm.nih.gov)

AIM:

To perform iterative blast for query 'Flavodoxin' protein (UniProt ID: P53554) by exploring Pattern Hit Initiated BLAST (PHI-BLAST) Tool.

INTRODUCTION:

Pattern Hit Initiated BLAST (PHI-BLAST) Tool, represents a variant of the BLAST algorithm employed for searching a protein database to identify other instances of a specific pattern occurring at least once within the input sequence. It facilitates the alignment and construction of the Position-Specific Scoring Matrix (PSSM) around a motif present in the query sequence. PHI-BLAST was developed by Stephen Altschul, Warren Gish, Webb Miller, Eugene Myers, and David J. Lipmann at the National Institutes of Health (NIH).

PHI-BLAST finds application in the analysis of various protein sequences, including CED4-like cell death regulators, HS90-type ATPase domains, archaeal tRNA nucleotidyltransferases, and archaeal proteins. It is utilized to identify protein sequences containing a specific pattern specified by the user and similar to the query sequence.

Compared to other BLAST tools, PHI-BLAST offers advantages such as increased speed and the ability for the user to express a rigid pattern occurrence requirement. This feature aids in reducing the number of hits that solely contain the pattern but lack true homology to the query sequence. However, PHI-BLAST may have a potential disadvantage in that it might be less sensitive than PSI-BLAST for detecting remote homologs. Additionally, the use of a specific pattern may restrict the search scope, potentially causing the omission of homologs lacking the specified pattern.

Flavodoxin:

Flavodoxins are small, soluble, electron-transfer proteins. Flavodoxins contains flavin mononucleotide as prosthetic group. The structure of flavodoxin is characterized by a five-stranded parallel beta sheet, surrounded by five alpha helices. They have been isolated from prokaryotes, cyanobacteria, and some eukaryotic algae. It functions in various metabolic processes, including photosynthesis, nitrogen and fatty acid metabolism. Flavodoxin is also involved in the detoxification of reactive oxygen species. The protein is reduced by flavodoxin reductase and transfers electrons to various redox enzymes. The semiquinone conformation of flavodoxin is stabilized by a hydrogen bond to the N-5 position of flavin, and a common tryptophan residue near the binding site aids in lowering SQ reactivity. The hydroquinone form is forced into a planar conformation, destabilizing it.

METHODOLOGY:

1. Open the homepage of UniProt database and search for the query ‘Flavodoxin’ protein.
2. Select any one entry from the results e.g., *Bacillus subtilis (strain 168)* **(**UniProt ID: P53554) and download its FASTA sequence in canonical format.
3. Open the homepage of BLAST and click on protein BLAST.
4. Paste the FASTA sequence in ‘Enter query sequence’ box and in program selection click on PHI-BLAST option.
5. Open the homepage of PROSITE database and search for the query ‘Flavodoxin’ protein.
6. Enter the FASTA sequence in ‘Quick Scan mode of ScanProsite’ box and scan it.
7. Copy the decoded pattern and paste it in the pattern in ‘Enter a PHI pattern’ box on PHI-BLAST portal and set the desired algorithm parameters.
8. Run the PHI-BLAST.
9. After each iteration, the new sequences are added to the results. These new sequences are highlighted using yellow color.
10. Run the PHI-BLAST iteration for 3-5 times, post which it starts generating garbage results, due to the decrease in sensitivity.
11. Interpret the results obtained.

OBSERVATIONS:

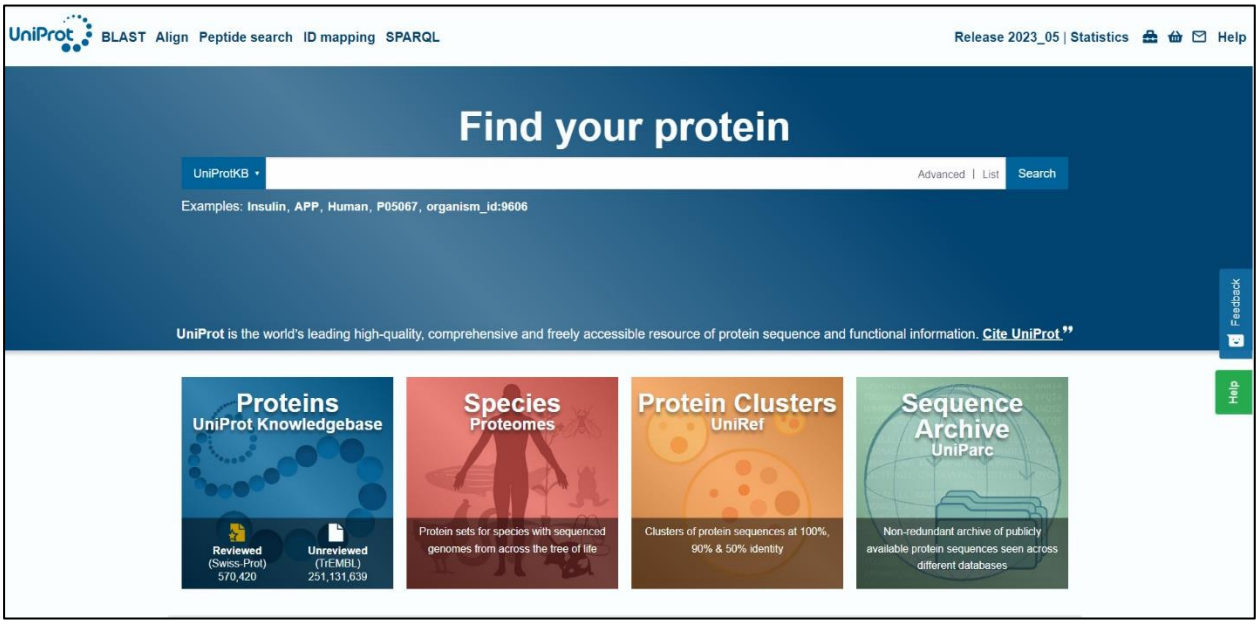


Figure 1: Homepage of the UniProt database

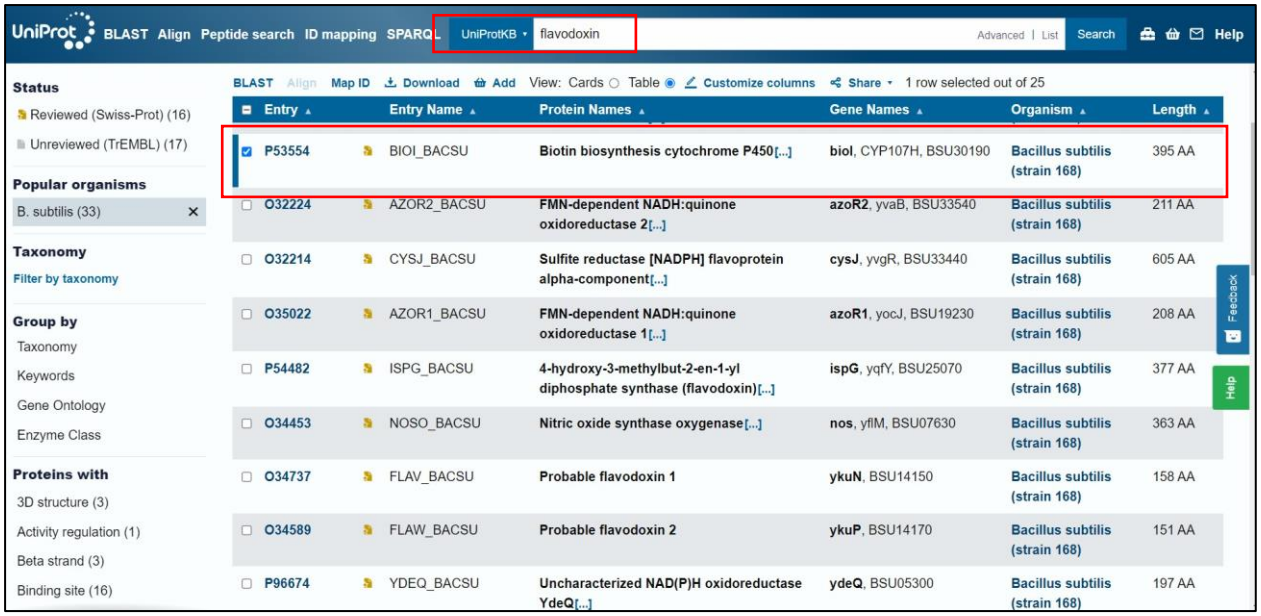


Figure 2: Query search for 'Flavodoxin' protein

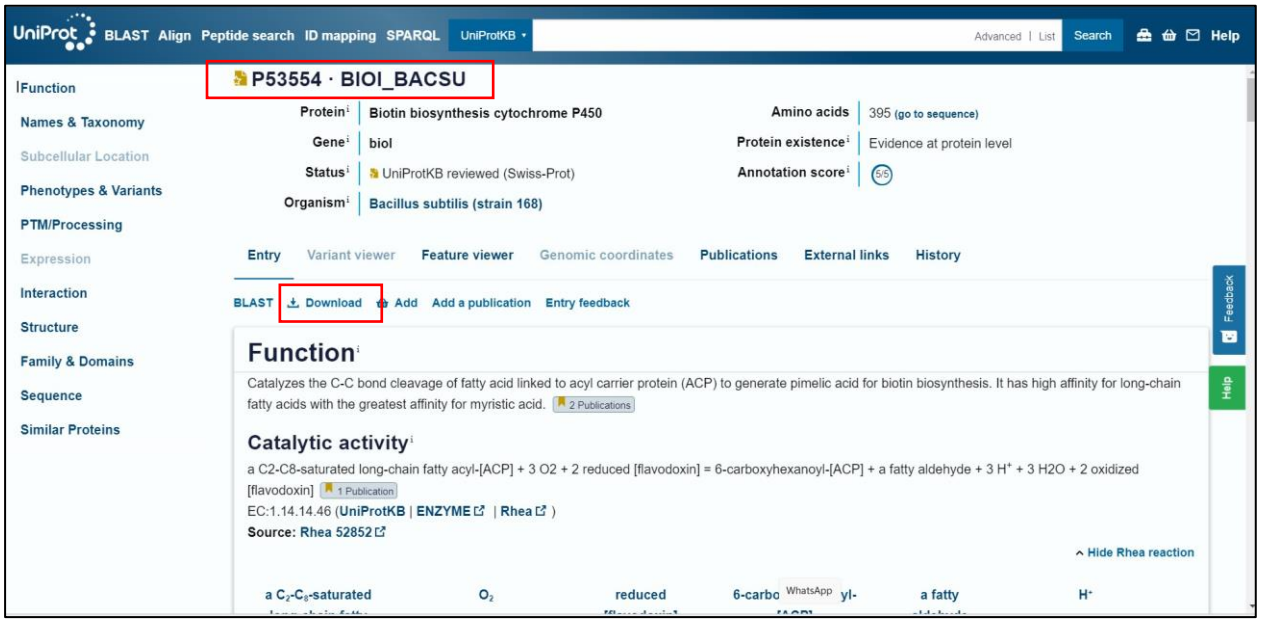


Figure 2a: Downloading the FASTA sequence for selected UniProt ID: P53554

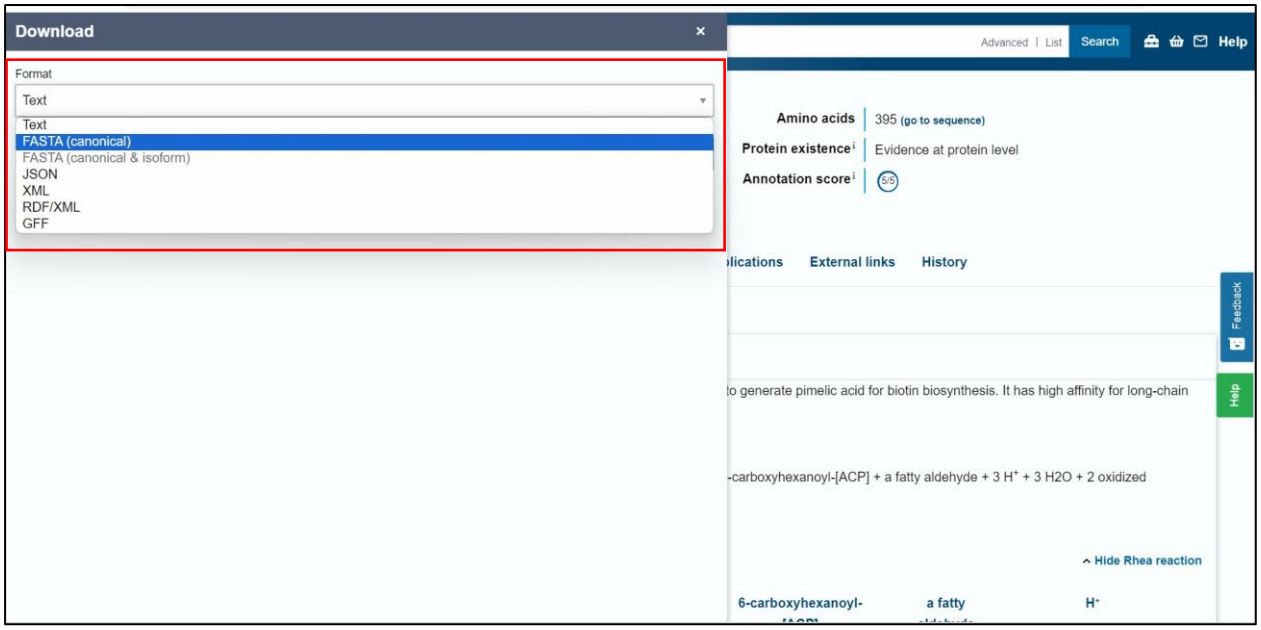


Figure 2b: Downloading the FASTA sequence in canonical format

```
>sp|P53554|BIOI_BACSU Biotin biosynthesis cytochrome P450 OS=Bacillus subtilis (strain 168) OX=224308 GN=bioI PE=1 SV=1
MTIASSTASSEFLKNPYSFYDTLRAVHPIYKGSFLKYPGWYVTGYEETAAILKDARFKVR
TPLPESSTKYQDLSHVQNQMMLFQNQPDHRRLRTLASGAFTPRTTESYQPYIIETVHHLL
DQVQGKKKMEVISDFAFPLASFVIANIIGVPEEDREQLKEWAASLIQTIDFTRSRKALTE
GNIMAVQAMAYFKELIQKRKRHPQQDMISMLLKGREKDKLTEEEAASTCILLAIAGHETT
VNLISNSVLCLLQHPEQLLKLRENPDLIGTAVEECLRYESPTQMTARVASEDIDICGVTI
RQGEQVYLLLGAANRDPSIFTNPDVFDITRSPNPHLSFGHGHHVCLGSSLARLEAQIAIN
TLLQRMPSLNLADFEWRYRPLFGFRALEELPVTFE
```

Figure 2c: View of the downloaded FASTA sequence

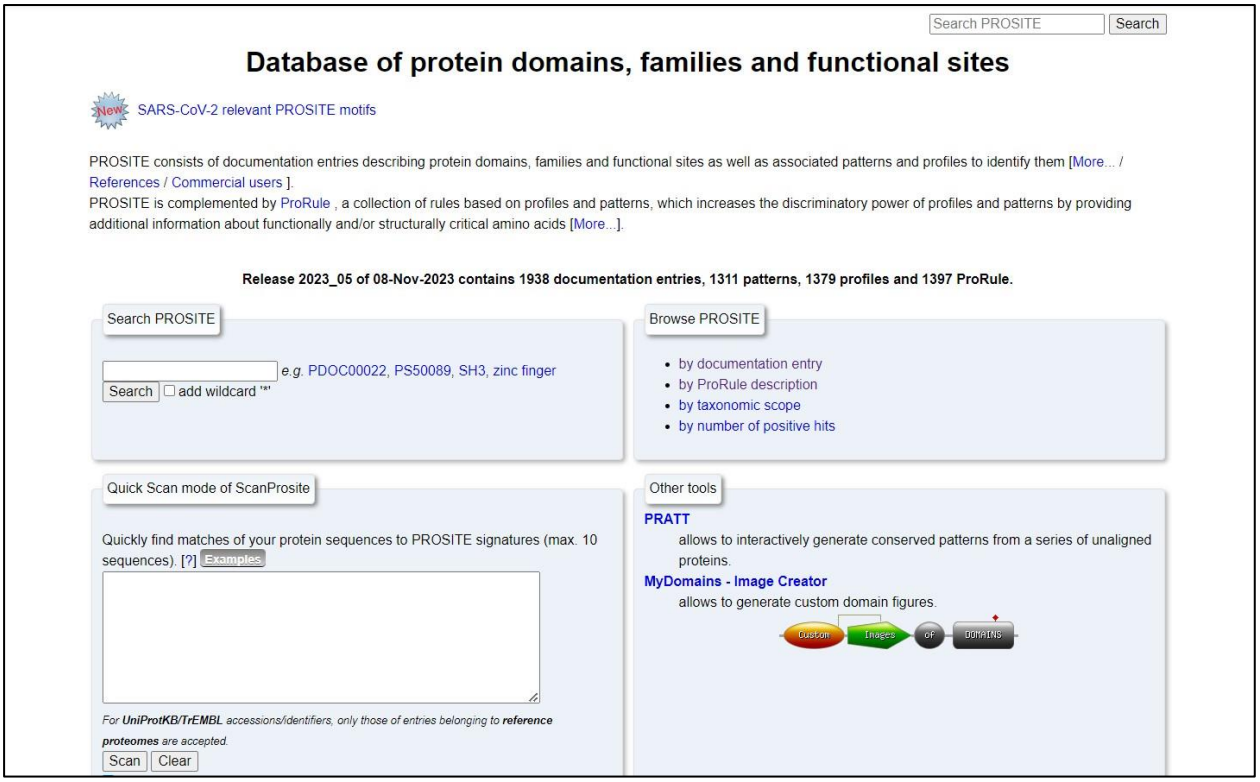


Figure 3: Homepage of PROSITE Database

Figure 3a: Paste the downloaded FASTA sequence for pattern

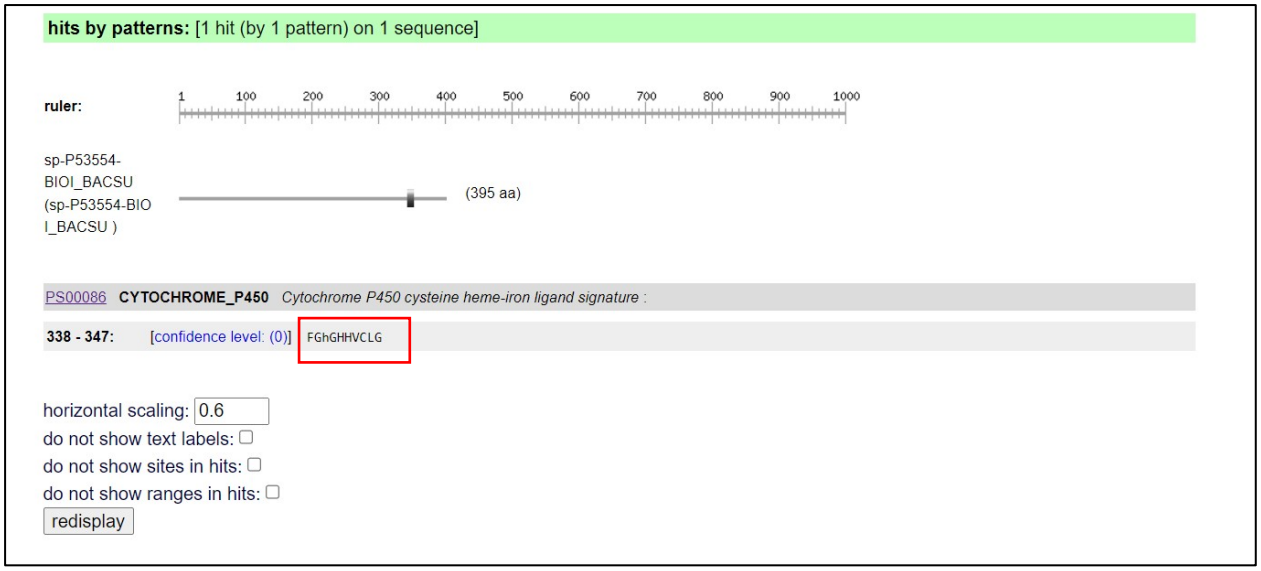


Fig 3b: Results page for the Quick Scan of ScanProSite using the sequence and retrieving the decoded sequence

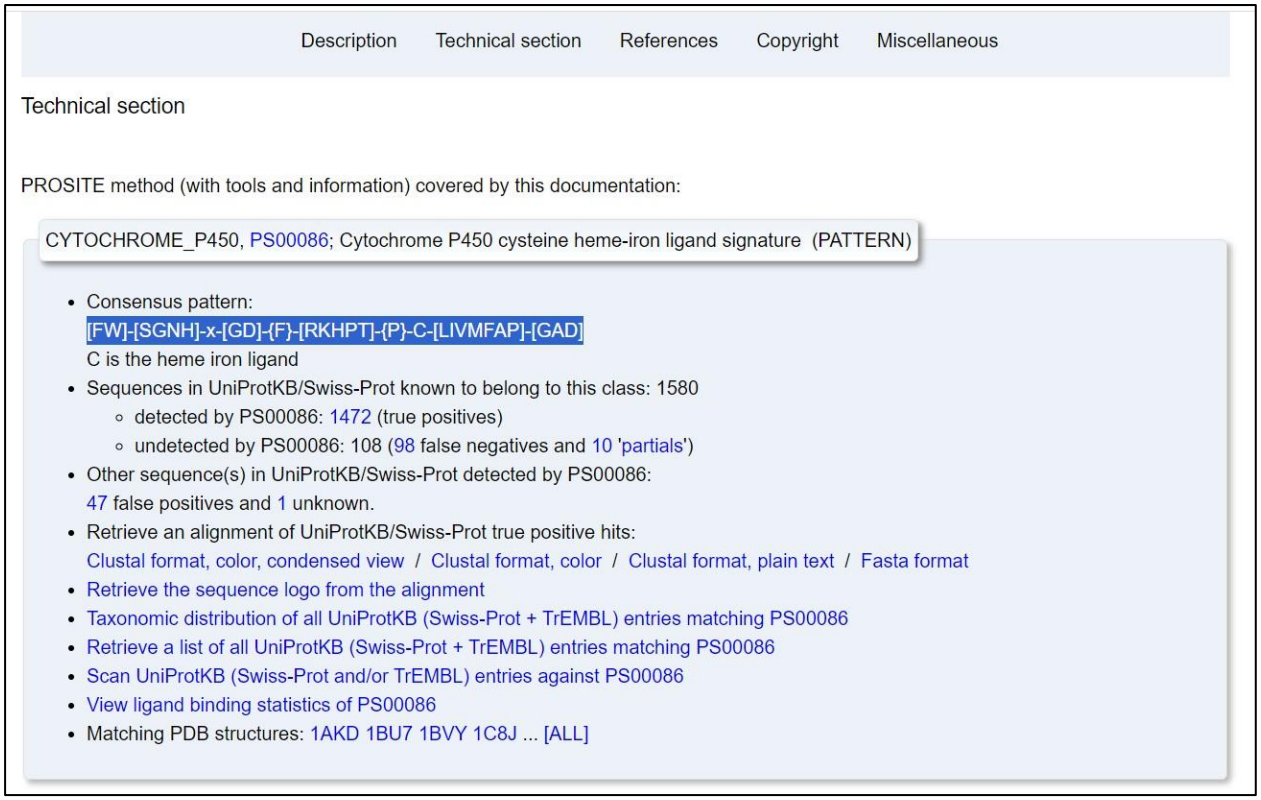


Figure 3c: Consensus pattern for the FASTA sequence

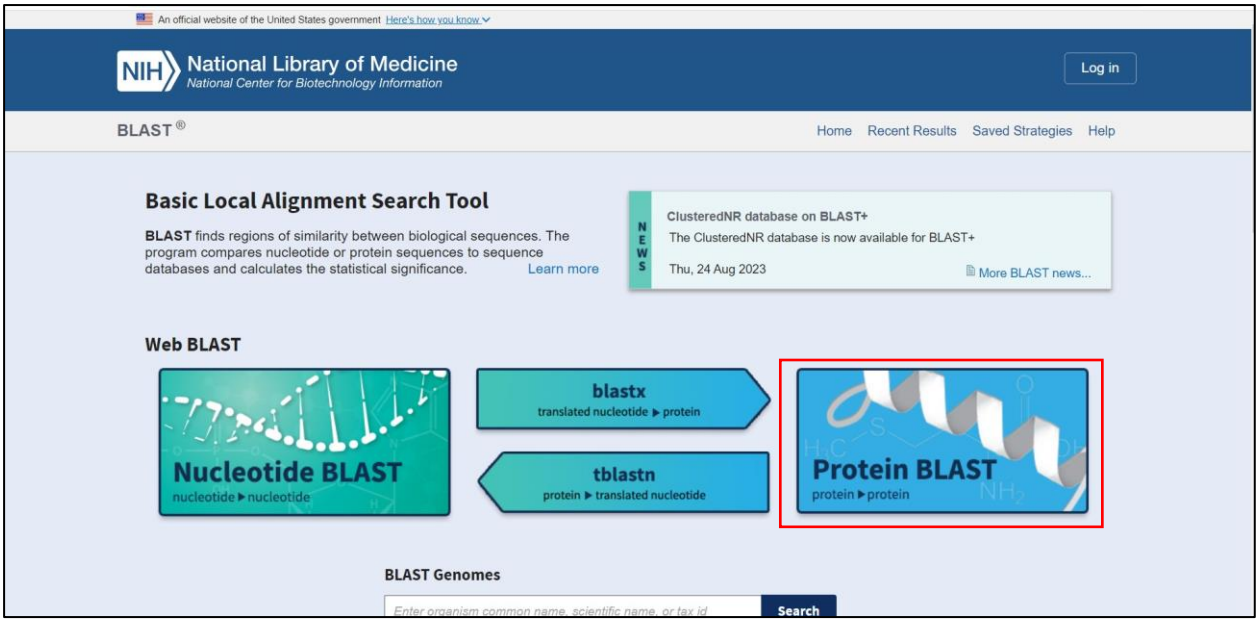


Figure 4: Homepage of Basic Local Alignment Search Tool (BLAST)

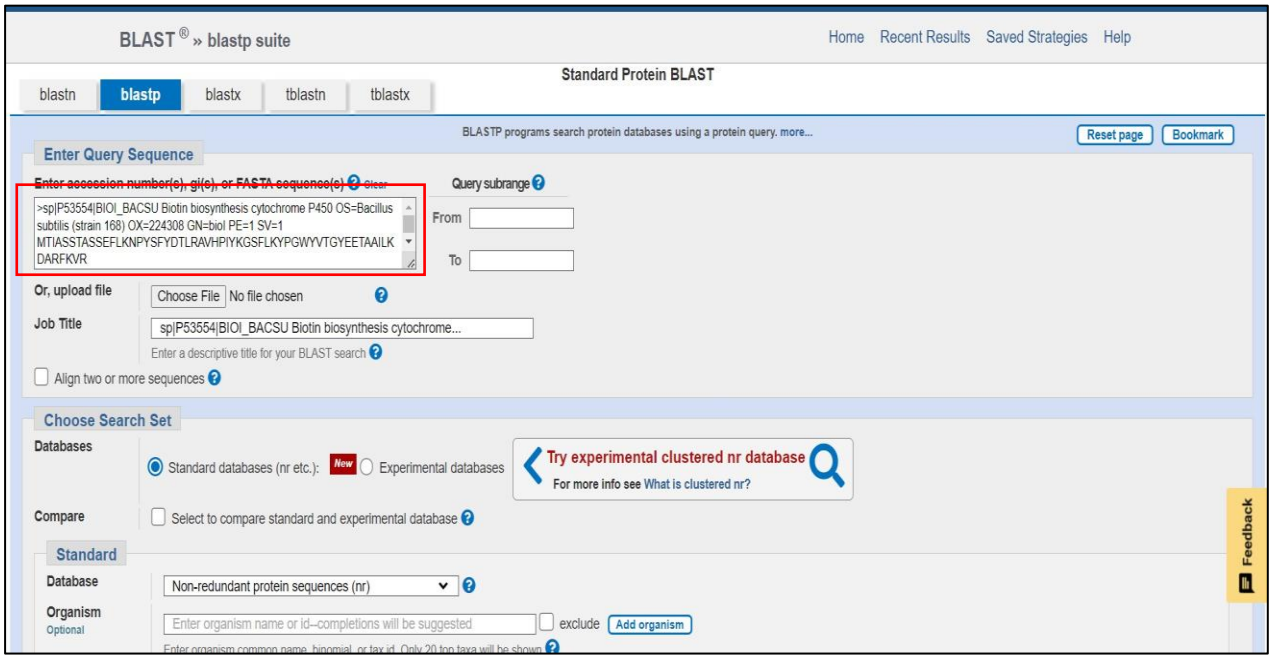


Figure 5: Pasting the FASTA sequence in 'Enter query sequence' box

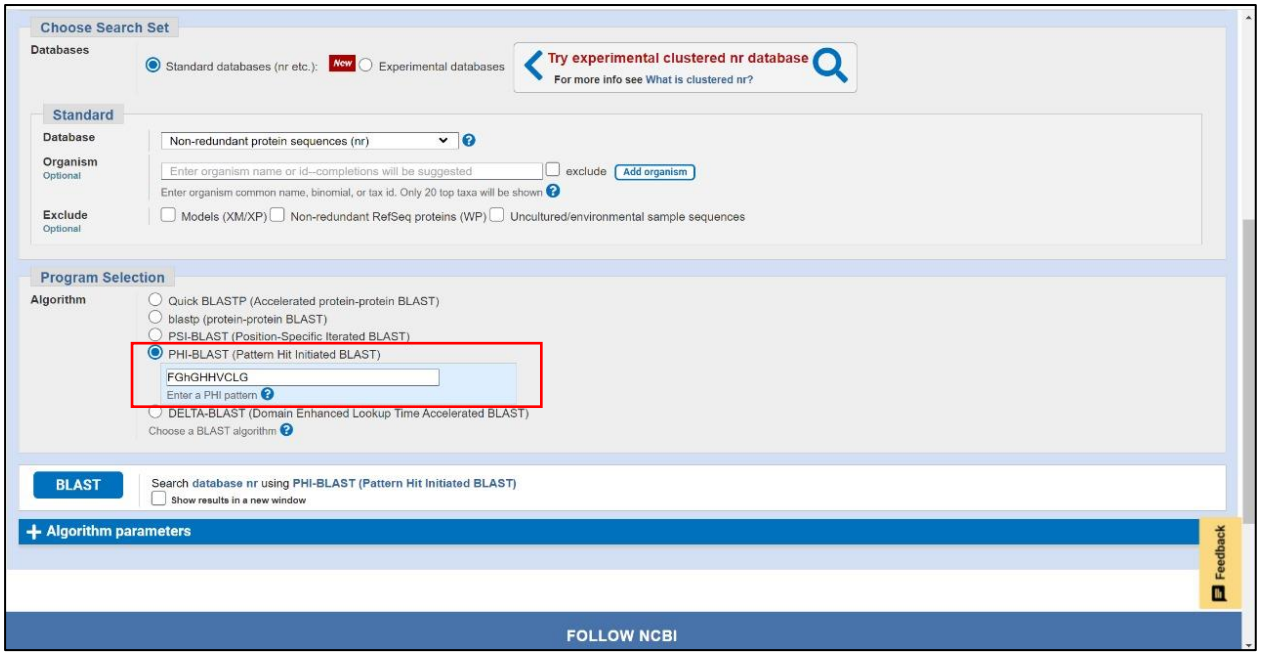


Fig 5a: Paste the decoded pattern from ProSite in 'Enter a PHI pattern' box

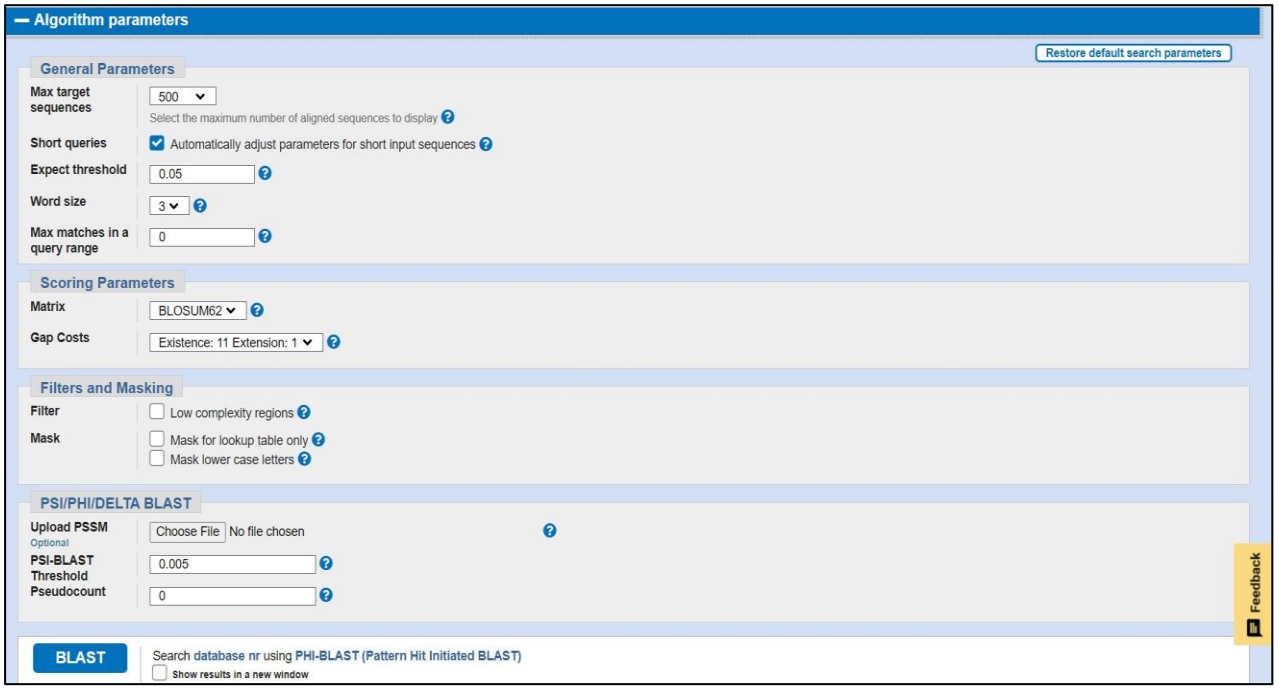


Figure 5b: Setting the parameters for running BLAST Tool

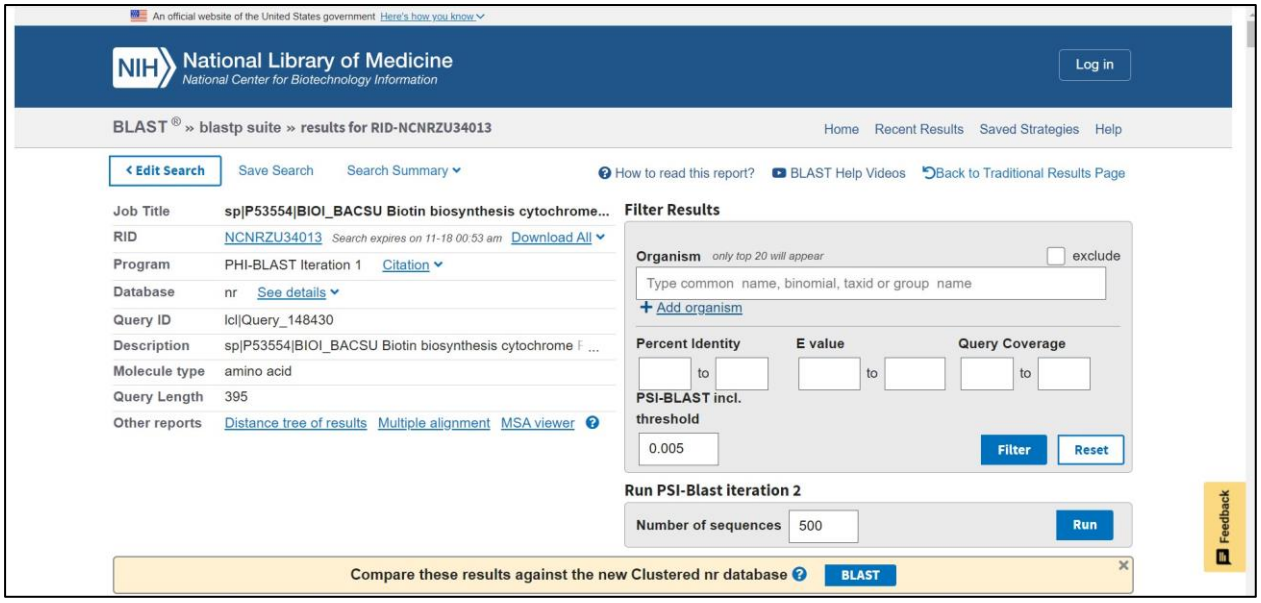
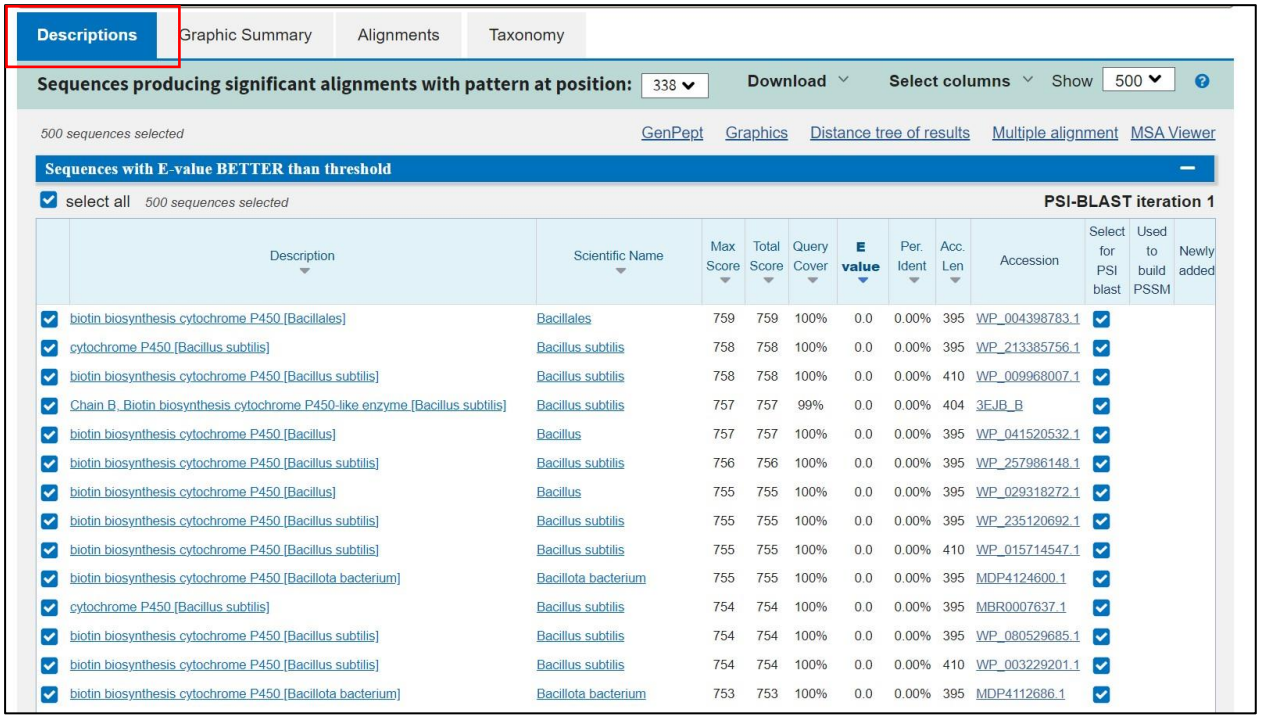


Figure 6: Results obtained after running BLAST tool



Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession	Select for PSI blast	Used to build PSSM	Newly added
biotin biosynthesis cytochrome P450 [Bacillales]	Bacillales	759	759	100%	0.0	0.00%	395	WP_004398783.1	☑		
cytochrome P450 [Bacillus subtilis]	Bacillus subtilis	758	758	100%	0.0	0.00%	395	WP_213385756.1	☑		
biotin biosynthesis cytochrome P450 [Bacillus subtilis]	Bacillus subtilis	758	758	100%	0.0	0.00%	410	WP_009968007.1	☑		
Chain B, Biotin biosynthesis cytochrome P450-like enzyme [Bacillus subtilis]	Bacillus subtilis	757	757	99%	0.0	0.00%	404	3EJB_B	☑		
biotin biosynthesis cytochrome P450 [Bacillus]	Bacillus	757	757	100%	0.0	0.00%	395	WP_041520532.1	☑		
biotin biosynthesis cytochrome P450 [Bacillus subtilis]	Bacillus subtilis	756	756	100%	0.0	0.00%	395	WP_257986148.1	☑		
biotin biosynthesis cytochrome P450 [Bacillus]	Bacillus	755	755	100%	0.0	0.00%	395	WP_029318272.1	☑		
biotin biosynthesis cytochrome P450 [Bacillus subtilis]	Bacillus subtilis	755	755	100%	0.0	0.00%	395	WP_235120692.1	☑		
biotin biosynthesis cytochrome P450 [Bacillus subtilis]	Bacillus subtilis	755	755	100%	0.0	0.00%	410	WP_015714547.1	☑		
biotin biosynthesis cytochrome P450 [Bacillota bacterium]	Bacillota bacterium	755	755	100%	0.0	0.00%	395	MDP4124600.1	☑		
cytochrome P450 [Bacillus subtilis]	Bacillus subtilis	754	754	100%	0.0	0.00%	395	MBR0007637.1	☑		
biotin biosynthesis cytochrome P450 [Bacillus subtilis]	Bacillus subtilis	754	754	100%	0.0	0.00%	395	WP_080529685.1	☑		
biotin biosynthesis cytochrome P450 [Bacillus subtilis]	Bacillus subtilis	754	754	100%	0.0	0.00%	410	WP_003229201.1	☑		
biotin biosynthesis cytochrome P450 [Bacillota bacterium]	Bacillota bacterium	753	753	100%	0.0	0.00%	395	MDP4112686.1	☑		

Figure 7: Result for Description section of query

Figure 8: Result for Graphic Summary section

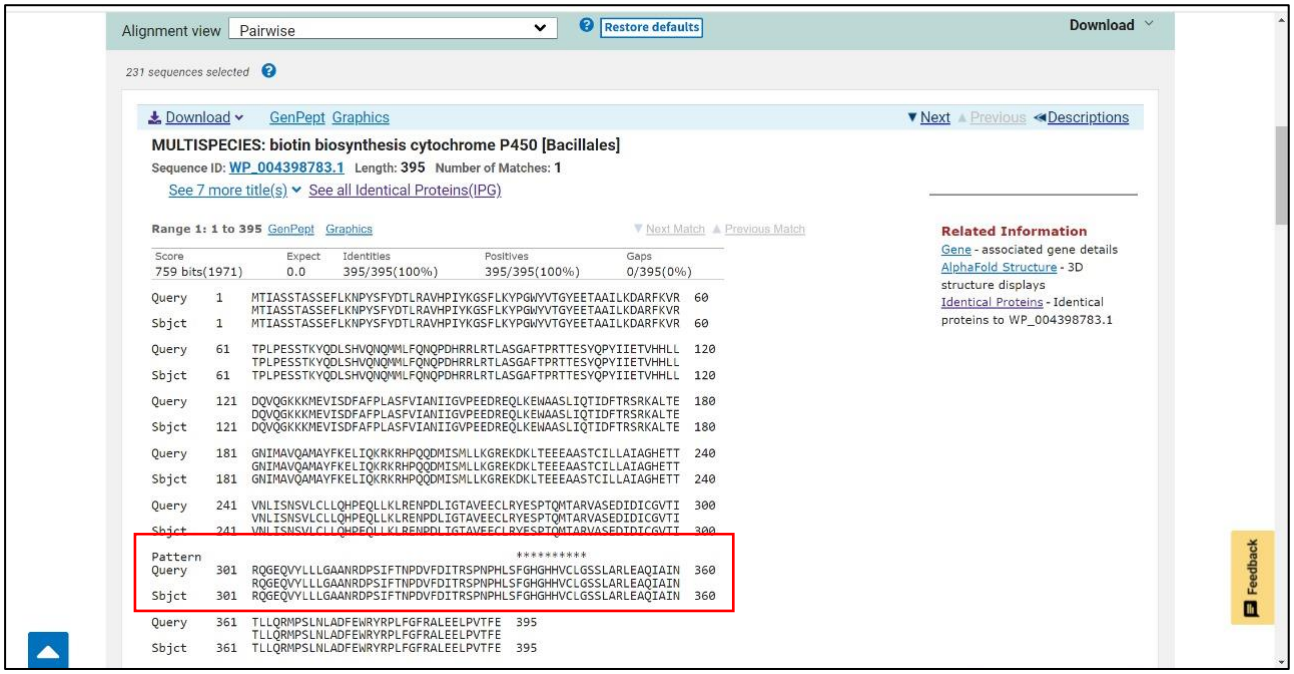


Figure 9: Result for Alignment Section

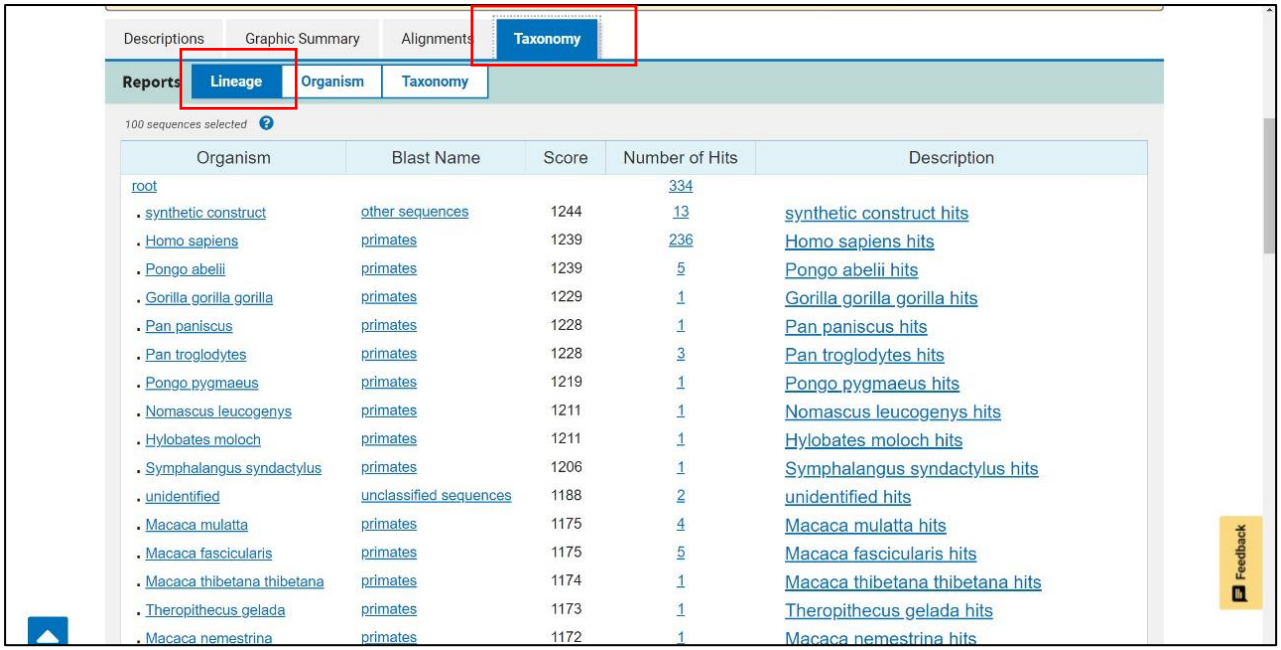


Figure 10: Result for Taxonomy section based on 'Lineage'

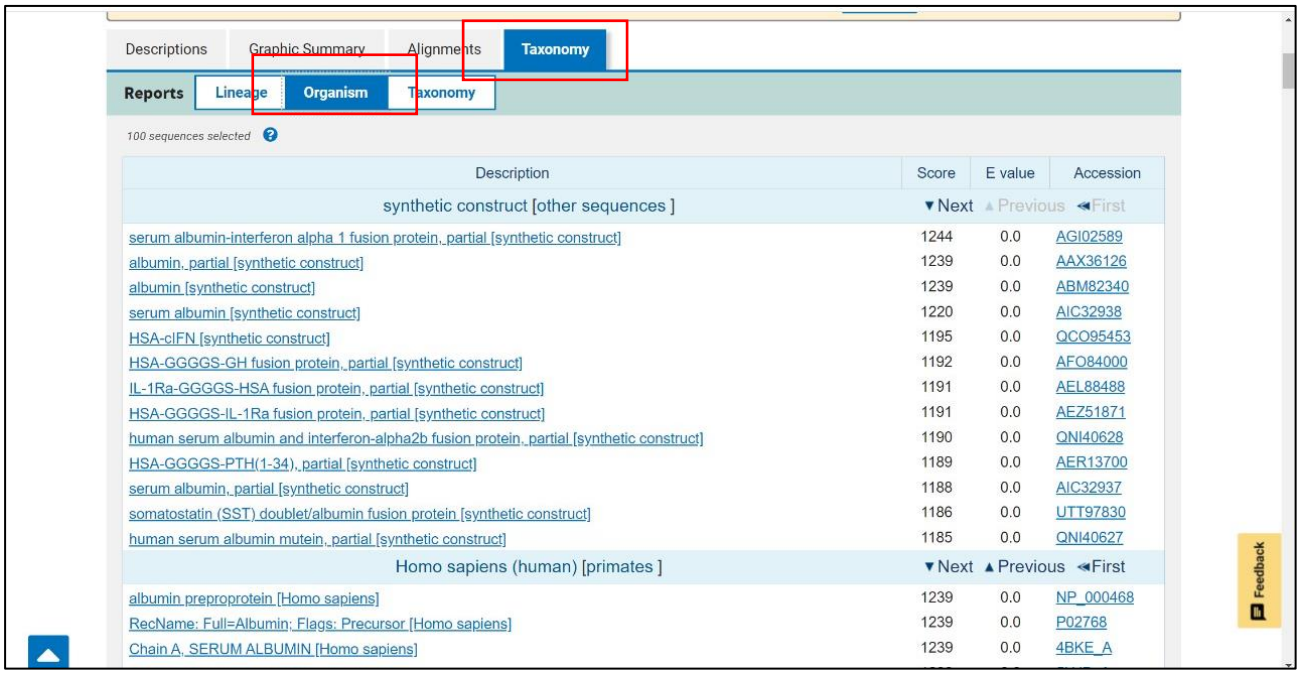


Figure 11: Result for Taxonomy section based on 'Organism'

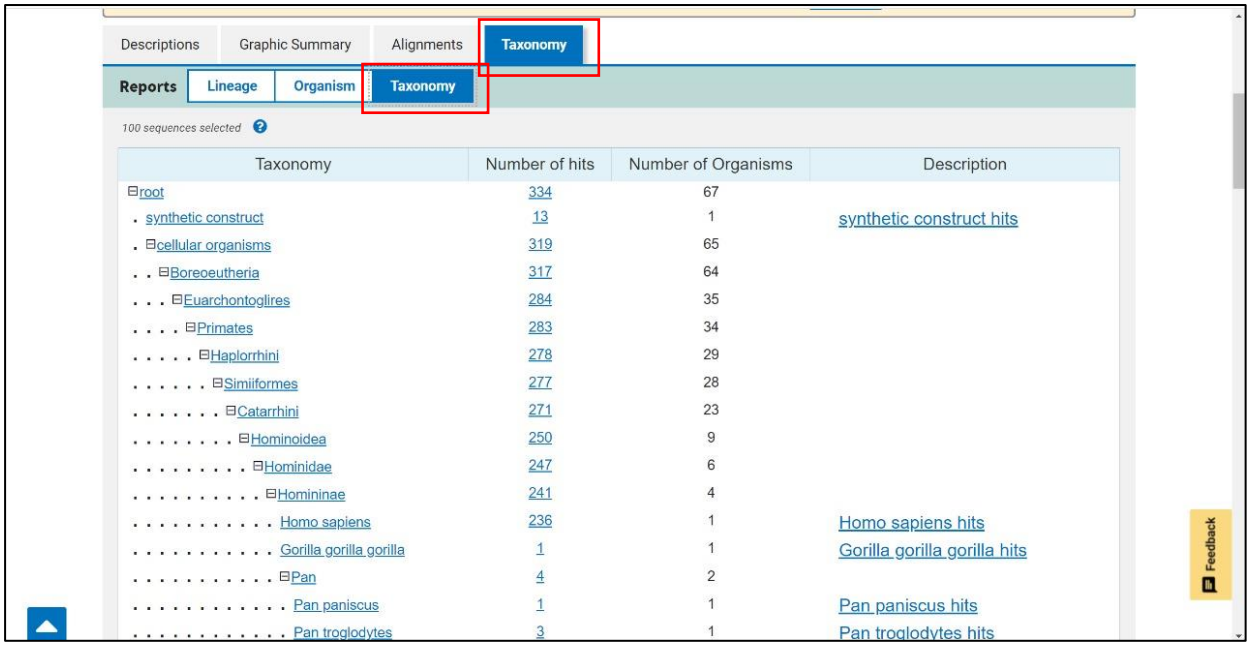


Figure 12: Result for Taxonomy section based on 'Taxonomy'

RESULTS:

Pattern-Hit Initiated BLAST (PHI-BLAST) tool is a variant of the Basic Local Alignment Search Tool (BLAST) algorithm, specifically designed for detecting distant relationships between protein sequences and identifying domains of potential functional significance within sequences. The tool was used to studied query where it is able to detect the pattern in the organisms which confirms the identification of remote homologs or conserved domains for the query protein sequences.

CONCLUSION:

PHI-BLAST is widely used in bioinformatics, particularly for analyzing protein sequences to identify conserved domains, motifs, or functional signatures. It aids in understanding evolutionary relationships between proteins and assists in annotating sequences with functional information based on conserved patterns. Its ability to focus the alignment and construction of the PSSM around a motif provides a valuable approach for researchers and bioinformaticians working in the field of protein analysis.

REFERENCES:

1. ResearchGate. (2023). BLAST Algorithm. https://www.reseatchgate.net/publication/230503487
2. Zheng Zhang, Webb Miller, Alejandro A. Schäffer, Thomas L. Madden, David J. Lipman, Eugene V. Koonin, Stephen F. Altschul, Protein sequence similarity searches using patterns as seeds, Nucleic Acids Research, Volume 26, Issue 17, 1 September 1998, Pages 3986–3990, https://doi.org/10.1093/nar/26.17.3986
3. Sancho J. Flavodoxins: sequence, folding, binding, function and beyond. Cell Mol Life Sci. 2006 Apr;63(7-8):855-64. doi: 10.1007/s00018-005-5514-4. PMID: 16465441. https://pubmed.ncbi.nlm.nih.gov/16465441

DATE: 01/11/23

WEBLEM 6(E)
EMBOSS NEEDLE – GLOBAL PAIRWISE SEQUENCE ALIGNMENT
(URL: https://www.ebi.ac.uk/Tools/psa/emboss_needle/)

AIM:

To explore and compare the protein sequences of 'Myosin' from two organisms *Gallus gallus* (UniProt ID: Q90623) and *Mus musculus* (UniProt ID: F8VQB6) by performing global pairwise sequence alignment using EMBOSS Needle Tool.

INTRODUCTION:

The European Molecular Biology Open Software Suite, or EMBOSS, is a part of the European Bioinformatics Institute (EBI). One of the prominent tools of EMBOSS is EMBOSS Needle, which is based on the Needleman-Wunsch algorithm. The Needleman-Wunsch algorithm was developed by Saul B. Needleman and Christian D. Wunsch in 1970 for global sequence alignment. It works on the principle of dividing the large problem into a series of smaller problems and uses the solutions to the smaller problems to find an optimal solution to the larger problem, assigning a score to every possible alignment and finding all possible alignments having the highest score.

The unique feature of the EMBOSS Needle tool is that it finds the alignment with the maximum possible score where the score of an alignment is equal to the sum of the matches taken from the scoring matrix, minus penalties arising from opening and extending gaps in the aligned sequences. The substitution matrix and gap opening and extension penalties are user-specified. A penalty is subtracted from the score for each gap opened (Gap insertion penalty) and a penalty is subtracted from the score for the extension of the inserted gaps (Gap extension penalty). Typically, the cost of extending a gap is set to be 5-10 times lower than the cost for opening a gap.

Penalty for a gap of n positions is calculated using the following formula:

Gap at n^{th} position = gap opening penalty + (n - 1) * gap extension penalty

Myosin:

Myosin is a motor protein with a primary role in muscle contraction, interacting with actin filaments to generate force and movement. Beyond muscles, myosin participates in cell motility, cell division, intracellular transport, and maintenance of cell shape, making it a crucial component in various cellular processes. The need to analyze myosin with the EMBOSS Needle tool arises from the diverse functions of myosin, which contribute to the dynamic behavior and structural integrity of cells. By analyzing the sequence and structure of myosin, researchers can gain insights into its mechanisms and interactions, which can help develop a deeper understanding of its role in various cellular processes and potentially lead to new therapeutic strategies for muscle and non-muscle related disorders.

METHODOLOGY:

1. Open the UniProt database and search for the query of ‘Myosin’.
2. From the results page, open the proteins of interest. Here, *Gallus gallus* (UniProt ID: Q90623) and *Mus musculus* (UniProt ID: F8VQB6).
3. Download the myosin protein sequences of both the organisms in FASTA file format.
4. Open the homepage of EMBOSS Needle tool and paste the sequences in the query box and set the desired parameters. Select the ‘SUBMIT’ to submit the query.
5. The results page of EMBOSS Needle tool displays the Alignment, Submission Details and View Alignment File. Interpret the results.

OBSERVATIONS:

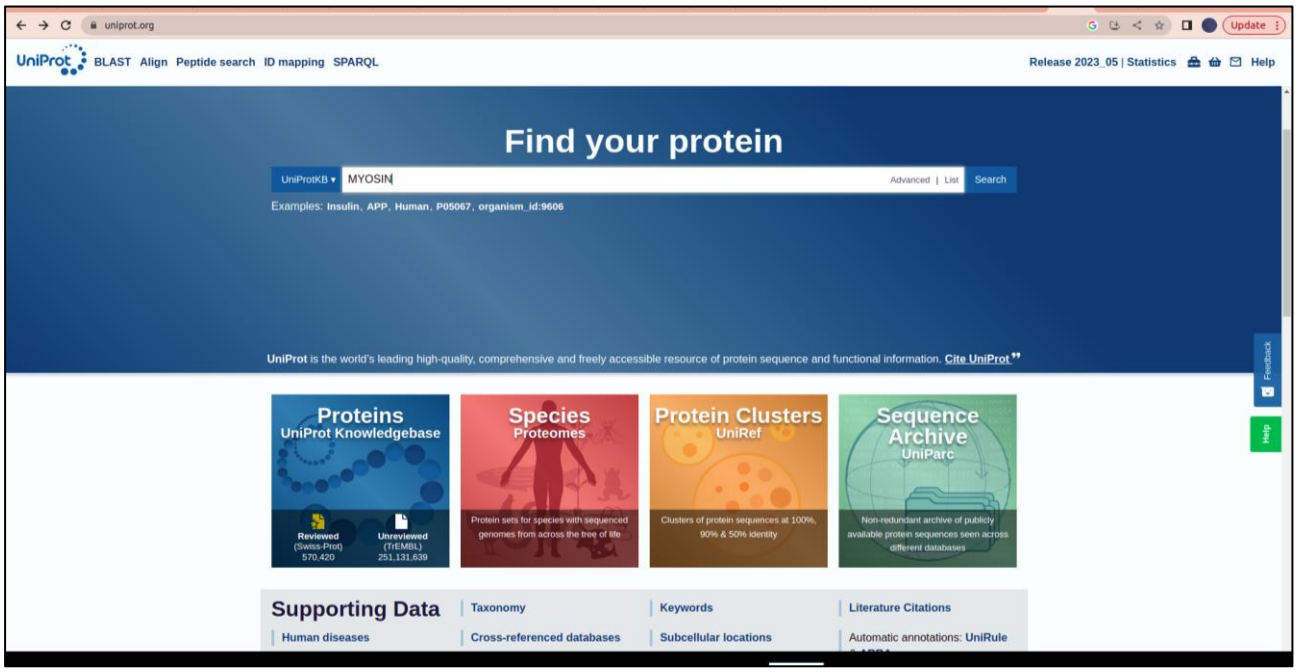


Figure 1: Homepage of the UniProt Database

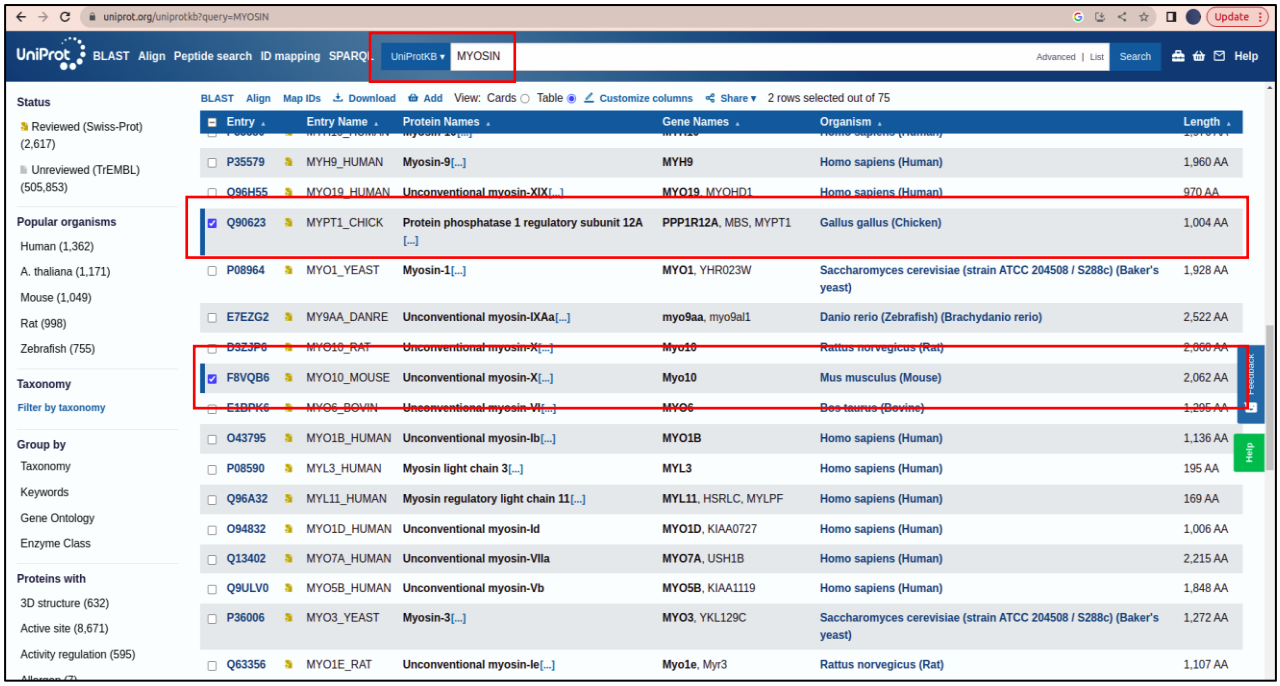


Figure 2: Results page of the UniProt Database for the query of Myosin with selected entries

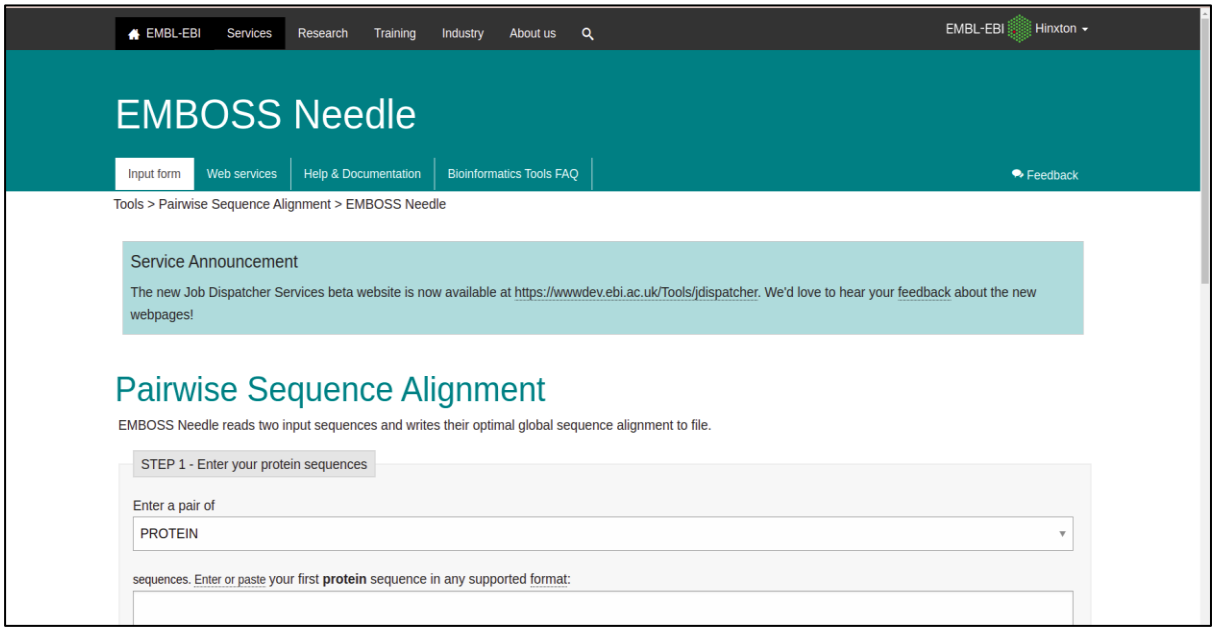


Figure 3: Homepage of EMBOSS Needle Tool

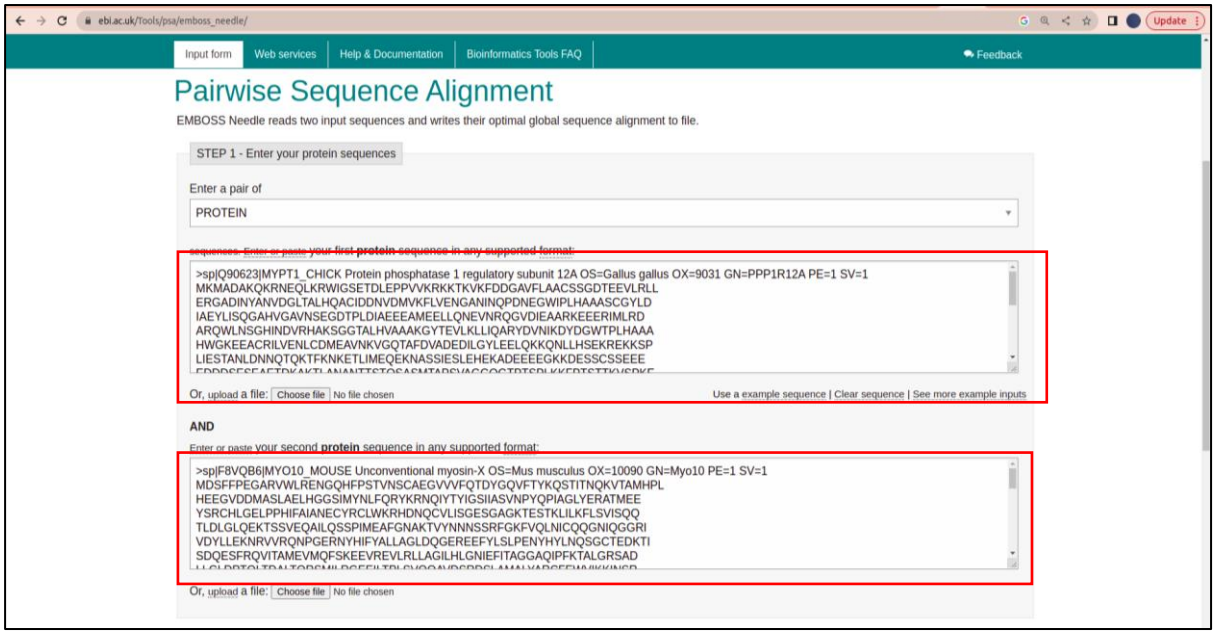


Figure 4: Submission of the protein sequences retrieved from the UniProt Database in the EMBOSS Needle Tool

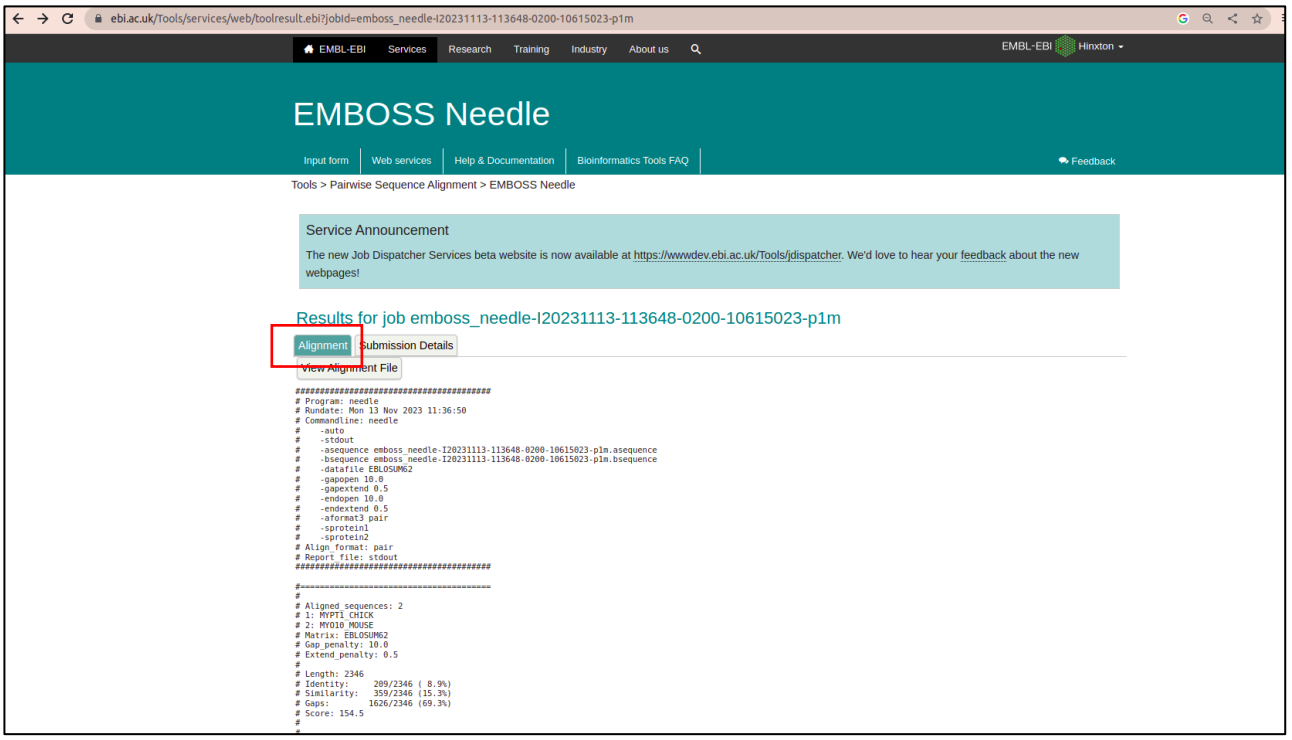
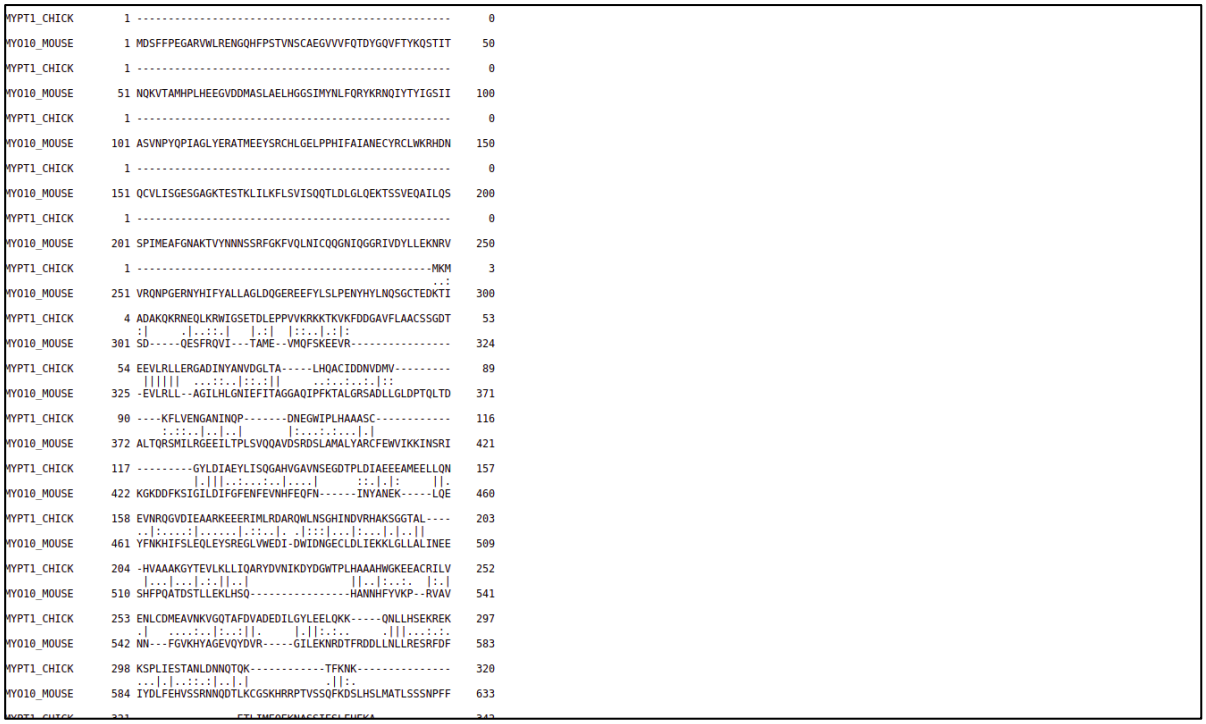


Figure 5: Results page of the submitted query with Alignment option



```
MYPT1_CHICK       1 --------------------------------------------------      0
MYO10_MOUSE       1 MDSFFPEGARVWLRENGQHFPSTVNSCAEGVVVFQTDYGQVFTYKQSTIT     50
MYPT1_CHICK       1 --------------------------------------------------      0
MYO10_MOUSE      51 NQKVTAMHPLHEEGVDDMASLAELHGGSIMYNLFQRYKRNQIYTYIGSII    100
MYPT1_CHICK       1 --------------------------------------------------      0
MYO10_MOUSE     101 ASVNPYQPIAGLYERATMEEYSRCHLGELPPHIFAIANECYRCLWKRHDN    150
MYPT1_CHICK       1 --------------------------------------------------      0
MYO10_MOUSE     151 QCVLISGESGAGKTESTKLILKFLSVISQQTLDLGLQEKTSSVEQAILQS    200
MYPT1_CHICK       1 --------------------------------------------------      0
MYO10_MOUSE     201 SPIMEAFGNAKTVYNNNSSRFGKFVQLNICQQGNIQGGRIVDYLLEKNRV    250
MYPT1_CHICK       1 ------------------------------------------------MKM      3
                                                                   ..:
MYO10_MOUSE     251 VRQNPGERNYHIFYALLAGLDQGEREEFYLSLPENYHYLNQSGCTEDKTI    300
MYPT1_CHICK       4 ADAKQKRNEQLKRWIGSETDLEPPVVKRKKTKVKFDDGAVFLAACSSGDT     53
                    :|     .|..::.|   |.:|  |:::.|.:|:
MYO10_MOUSE     301 SD-----QESFRQVI---TAME--VMQFSKEEVR---------------     324
MYPT1_CHICK      54 EEVLRLLERGADINYANVDGLTA-----LHQACIDDNVDMV---------      89
                    ||||||  ...:::..|::.:||     ...:..:...:.|::
MYO10_MOUSE     325 -EVLRLL--AGILHLGNIEFITAGGAQIPFKTALGRSADLLGLDPTQLTD    371
MYPT1_CHICK      90 ----KFLVENGANINQP-------DNEGWIPLHAAASC------------     116
                        :.::..|..|..|       |:...:.:...|.|
MYO10_MOUSE     372 ALTQRSMILRGEEILTPLSVQQAVDSRDSLAMALYARCFEWVIKKINSRI    421
MYPT1_CHICK     117 ---------GYLDIAEYLISQGAHVGAVNSEGDTPLDIAEEEAMEELLQN    157
                            |.|||..:.:...:|....|       ::.|.|:      ||.
MYO10_MOUSE     422 KGKDDFKSIGILDIFGFENFEVNHFEQFN------INYANEK-----LQE    460
MYPT1_CHICK     158 EVNRQGVDIEAARKEEERIMLRDARQWLNSGHINDVRHAKSGGTAL----    203
                    ..|:....:|.....|.::..|. .|:::|...|:...|.|..||
MYO10_MOUSE     461 YFNKHIFSLEQLEYSREGLVWEDI-DWIDNGECLDLIEKKLGLLALINEE    509
MYPT1_CHICK     204 -HVAAAKGYTEVLKLLIQARYDVNIKDYDGWTPLHAAAHWGKEEACRILV    252
                     |...|...|.:.||..|                ||..|:.:.:  |:.|
MYO10_MOUSE     510 SHFPQATDSTLLEKLHSQ---------------HANNHFYVKP--RVAV    541
MYPT1_CHICK     253 ENLCDMEAVNKVGQTAFDVADEDILGYLEELQKK-----QNLLHSEKREK    297
                    .|   ....:..|:..:||.     |.||:.:..     .|||...:.:.
MYO10_MOUSE     542 NN---FGVKHYAGEVQYDVR-----GILEKNRDTFRDDLLNLLRESRFDF    583
MYPT1_CHICK     298 KSPLIESTANLDNNQTQK-----------TFKNK--------------      320
                    ...|.|..::.:|..|.|           .||:.
MYO10_MOUSE     584 IYDLFEHVSSRNNQDTLKCGSKHRRPTVSSQFKDSLHSLMATLSSSNPFF    633
```

Figure 5a: Results page of the submitted query with Alignment option

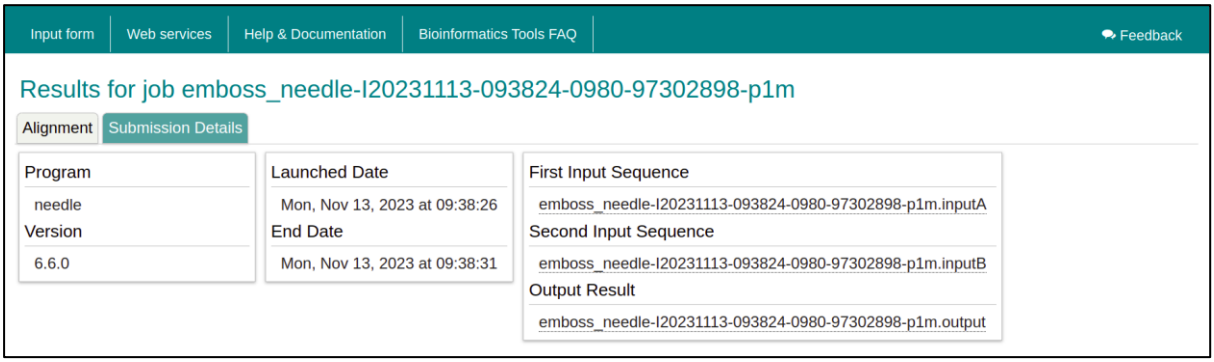


Figure 6: View of submission details

RESULTS:

By exploring global pairwise sequence alignment using the EMBOSS Needle tool, the results were observed and studied for the protein query 'Myosin' in organisms *Gallus gallus* (UniProt ID: Q90623) and *Mus musculus* (UniProt ID: F8VQB6). It was found that in the pairwise alignment of the two organisms, they were not identical upon comparison, as the sequence identity is only 8.9%.

Length	2346
Identity	209/2346 (8.9%)
Similarity	359/2346 (15.3%)
Gaps	1626/2346 (69.3%)
Score	154.5

CONCLUSION:

EMBOSS Needle tool, for Global Pairwise Sequence Alignment, was explored by comparative study of protein 'Myosin' of two different organisms, namely, *Gallus gallus* (UniProt ID: Q90623) and *Mus musculus* (UniProt ID: F8VQB6).

REFERENCES:

1. Needleman, S. B. and Wunsch, C. D. (1970*) J. Mol. Biol*. 48, 443-453. https://www.bioinformatics.nl/cgi-bin/emboss/help/needle
2. Robert S. Adelstein, James R. Sellers, in *Biochemistry of Smooth Muscle Contraction*, 1996. https://doi.org/10.1016/B978-0-12-801387-8.00003-X

DATE: 01/11/23

WEBLEM 6(F)

EMBOSS WATER – LOCAL PAIRWISE SEQUENCE ALIGNMENT

(URL: https://www.ebi.ac.uk/Tools/psa/emboss_water/)

AIM:

To explore and compare the protein sequences of 'collagen' in two organisms, *Rattus norvegicus* (UniProt ID: P05539) and *Homo sapiens* (UniProt ID: P08572), by performing local pairwise sequence alignment using the EMBOSS Water tool.

INTRODUCTION:

The European Molecular Biology Open Software Suite, or EMBOSS, is a part of the European Bioinformatics Institute (EBI). One of the prominent tools of EMBOSS is EMBOSS Water, which is based on the Smith-Waterman algorithm. Smith-Waterman algorithm was developed by Temple F. Smith and Michael S. Waterman in 1981 and is used for local sequence alignment, which finds the best subsequence match between two sequences by comparing all possible pairs of subsequences. The unique aspect of the EMBOSS Water tool is that it uses a speed-accelerated version of the Smith-Waterman method to determine the local alignment of a sequence with one or more other sequences. By examining every potential alignment and choosing the best one, dynamic programming techniques guarantee the best possible local alignment. To do this, a scoring matrix with values for each potential residue or nucleotide match is incorporated.

The EMBOSS Water tool employs a modified Smith-Waterman algorithm with speed enhancements to compute the local alignment of one or more sequences. Users have the flexibility to specify the gap insertion penalty, gap extension penalty, and substitution matrix for calculating alignments. The output is a standard EMBOSS alignment file. Identity refers to the percentage of identical matches between two sequences over the entire reported aligned region, inclusive of any length gaps. Similarly, similarity represents the percentage of matches between the two sequences over the length of the reported aligned region, considering any gaps.

Collagen:

The most prevalent protein in the body, collagen, is found in various connective tissues such as the skin, tendons, bones, and ligaments. Its inherent stiffness and resistance to stretching contribute significantly to providing structural support within the extracellular space of connective tissues. Understanding collagen's structure, function, and its implications in various diseases and conditions, including autoimmune disorders like rheumatoid arthritis, lupus, dermatomyositis, and scleroderma, is crucial. These conditions can adversely affect collagen, highlighting the importance of in-depth research.

The EMBOSS Water tool serves as a valuable resource in this pursuit. It is a pairwise sequence alignment program designed to determine the local alignment of one or more sequences. The tool utilizes a modified version of the Smith-Waterman technique, offering faster results for

researchers. By employing the EMBOSS Water tool to analyze collagen, researchers can gain deeper insights into its molecular makeup and its role in health and disease.

METHODOLOGY:

1. Open the UniProt database and search for the query of ‘Collagen’.
2. From the results page, open the proteins of interest. Here, *Rattus norvegicus* (UniProt ID: P05539) and *Homo sapiens* (UniProt ID: P08572).
3. Download the collagen protein sequences of both the organisms in FASTA canonical file format.
4. Open the homepage of EMBOSS Water tool and paste the sequences in the query box and set the desired parameters. Select the ‘SUBMIT’ to submit the query.
5. The results page of EMBOSS Water tool displays the Alignment, Submission Details and View Alignment File. Interpret the results.

OBSERVATIONS:

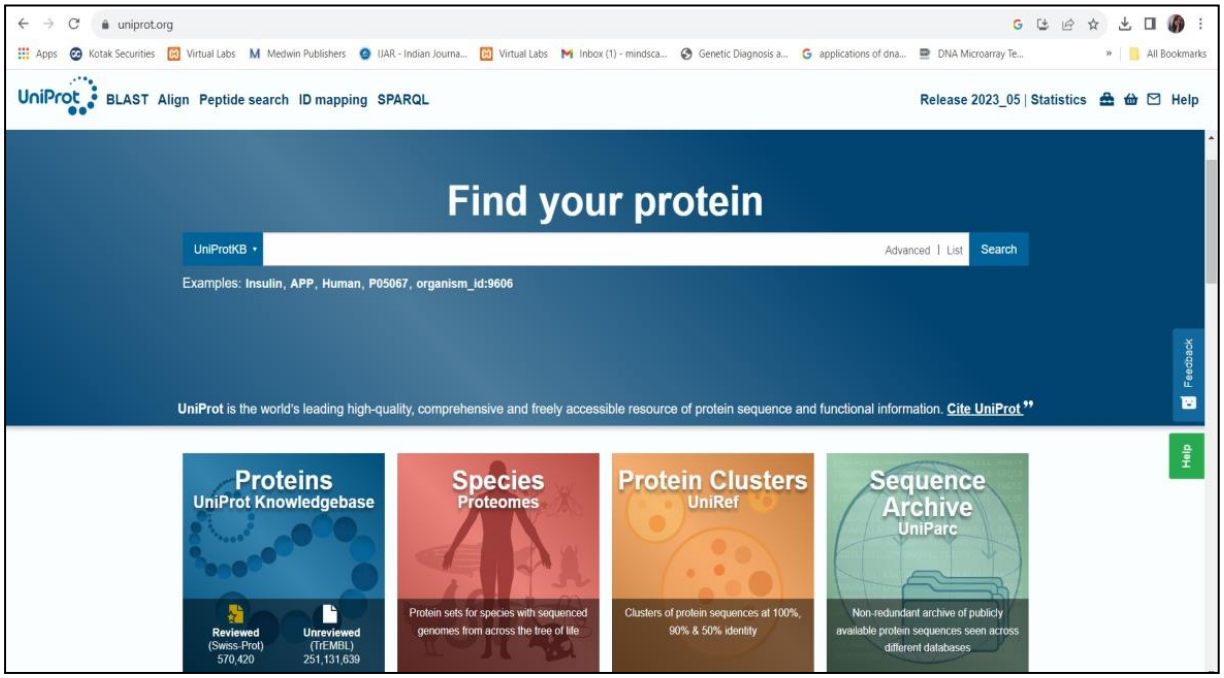


Figure 1: Homepage of the UniProt database

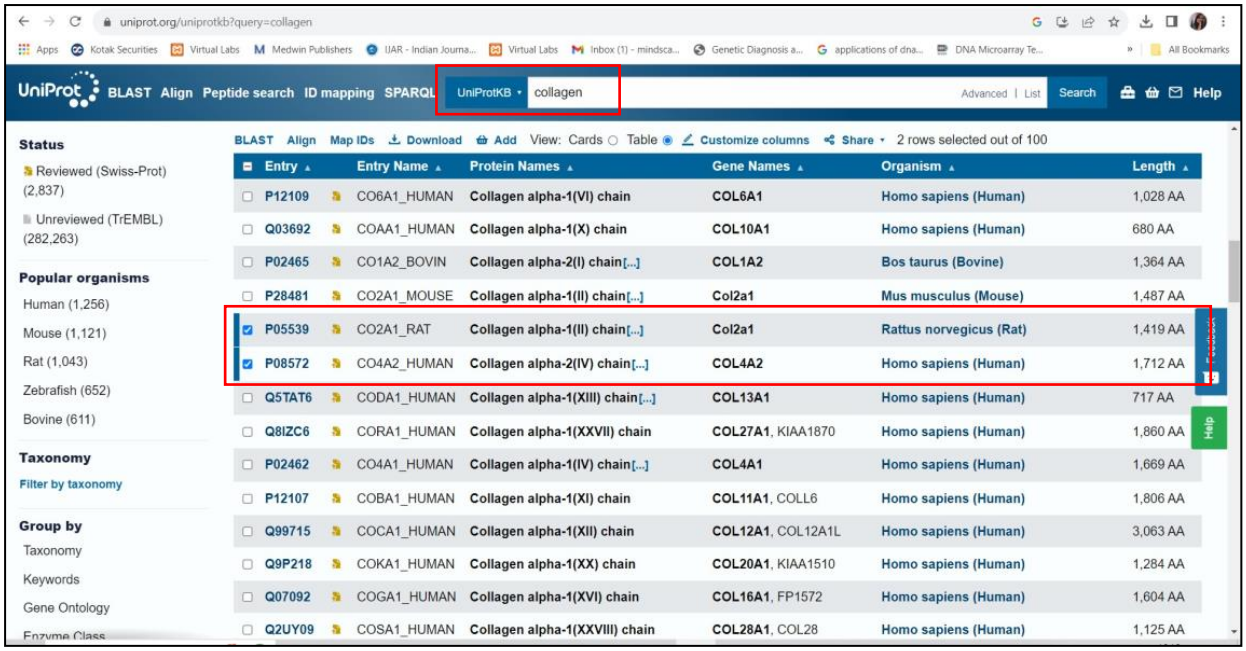


Figure 2: Results page of the UniProt Database for the query of collagen with selected entries

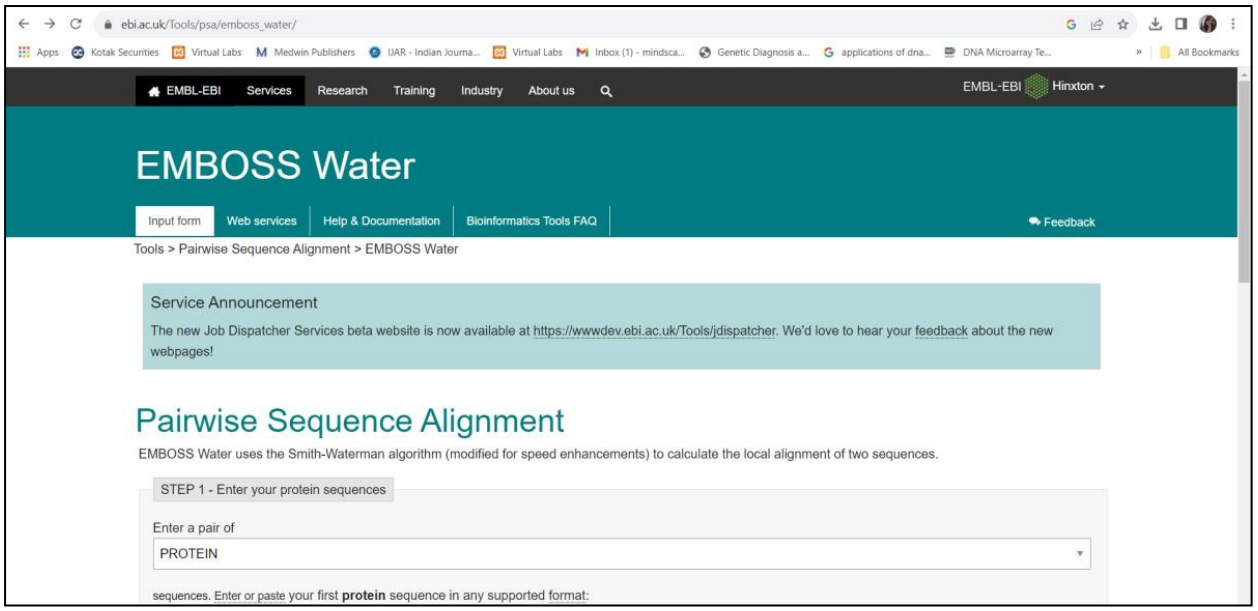


Figure 3: Homepage of EMBOSS Water Tool

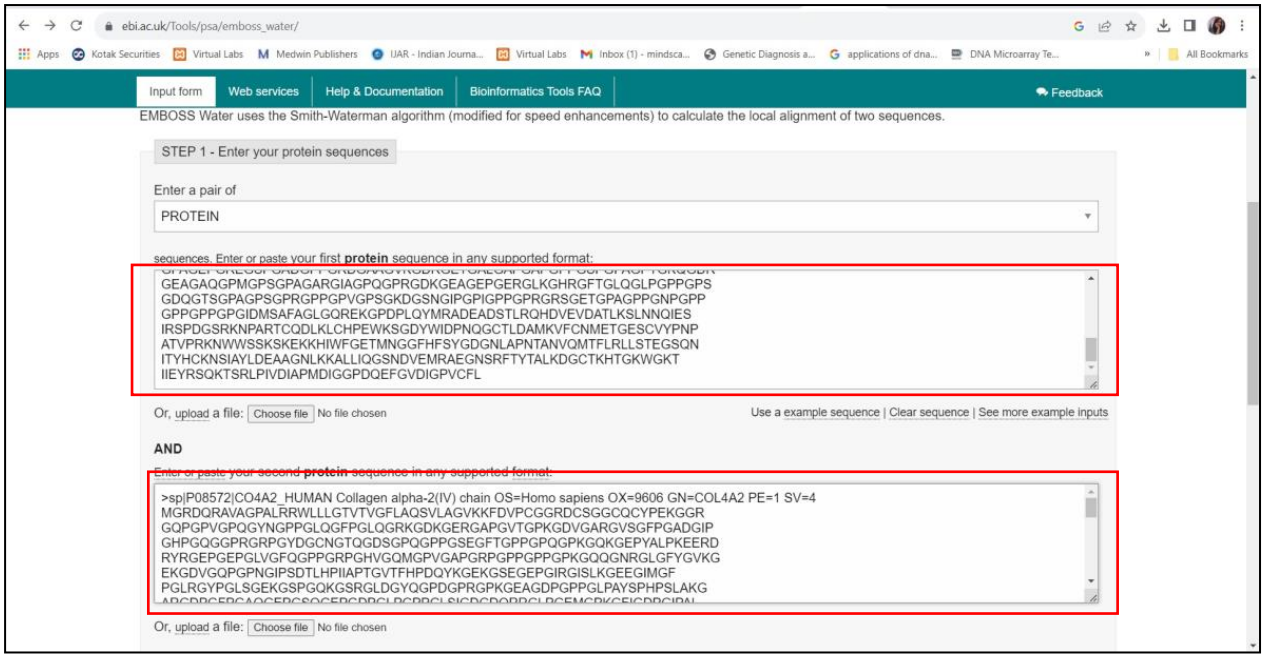


Figure 4: Submission of the protein sequences retrieved from the UniProt Database in the EMBOSS Water Tool

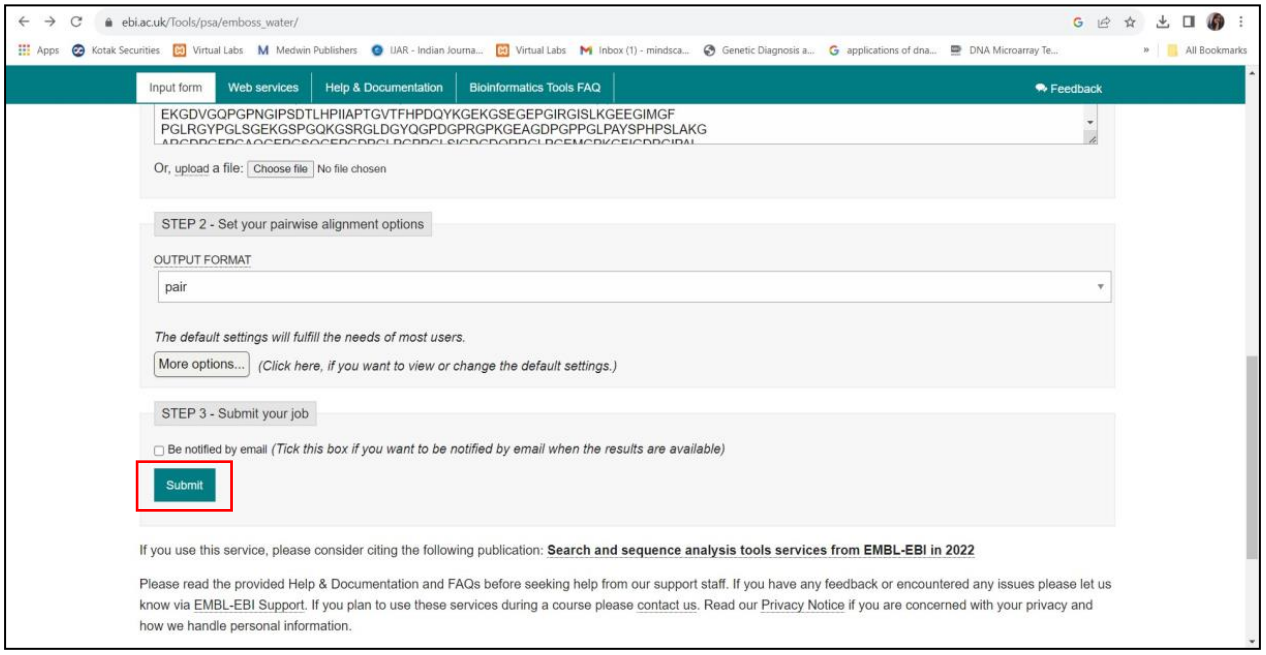


Figure 5: Submission of the query to the EMBOSS Water Tool

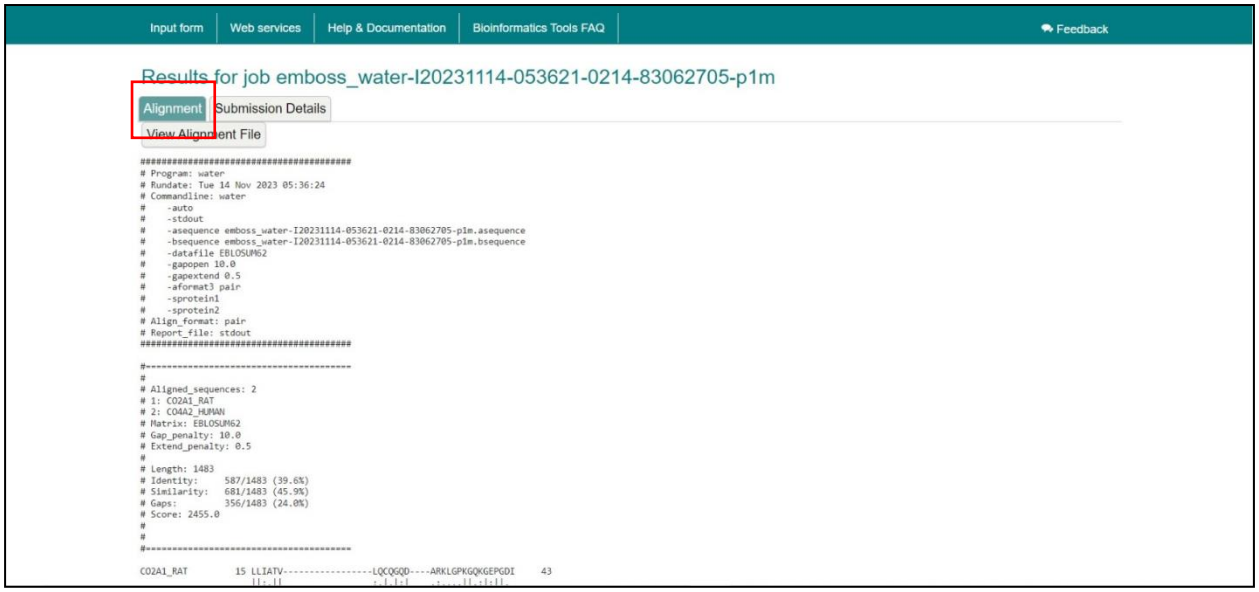


Figure 6: Results page of the submitted query with Alignment option

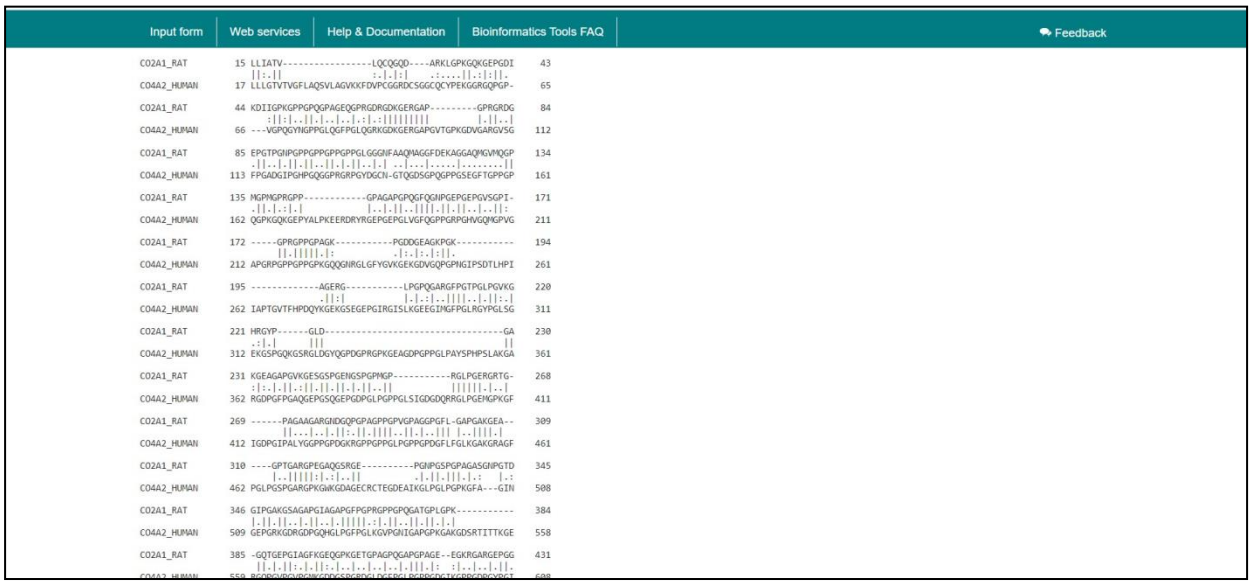


Figure 6a: Results page of the submitted query with Alignment option

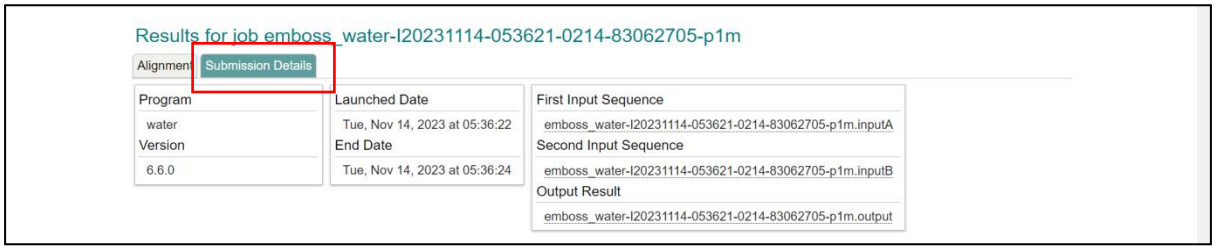


Figure 7: View of Submission details

RESULTS:

By exploring local pairwise sequence alignment using EMBOSS Water Tool, the results were observed and studied for query for protein query 'collagen' for organism *Rattus norvegicus* (UniProt ID: P05539) and *Homo sapiens* (UniProt ID: P08572) and it was observed that the local pairwise sequence alignments of the two organisms were found to be identical upon comparison, as the sequence identity is 39.6%.

Length	1483
Identity	587/14683 (39.6%)
Similarity	681/1483 (45.9%)
Gaps	356/1483 (69.3%)
Score	2455.0

CONCLUSION:

EMBOSS Water tool, for Local Pairwise Sequence Alignment, was explored by comparative study of protein collagen of two different organisms, namely, *Rattus norvegicus* (UniProt ID: P05539) and *Homo sapiens* (UniProt ID: P08572).

REFERENCES:

1. Smith TF, Waterman MS (1981) *J. Mol. Biol* 147(1). https://emboss.sourceforge.net/apps/release/6.6/emboss/apps/water.html
2. H. Jawad, R.A. Brown, in *Comprehensive Biotechnology*, 2011. https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/collagen