

## Lab 1: Reading and Visualizing DNA Sequences: Sequence Software Scanner

### The value of studying bioinformatics

- Identification
- Taxonomy
- Evolution

### The steps of molecular identification:

Extraction, PCR, Electrophoresis, Sequencing and Bioinformatics Analysis

### The choice of the 16S rRNA gene for molecular identification

- The stability of the gene ends allows the synthesis of universal primers.
- The large database available on the internet is useful for comparison.
- It is a universal gene present in all living beings.
- It contains stable regions with a low rate of change and unstable regions with a high rate of change of high evolution.

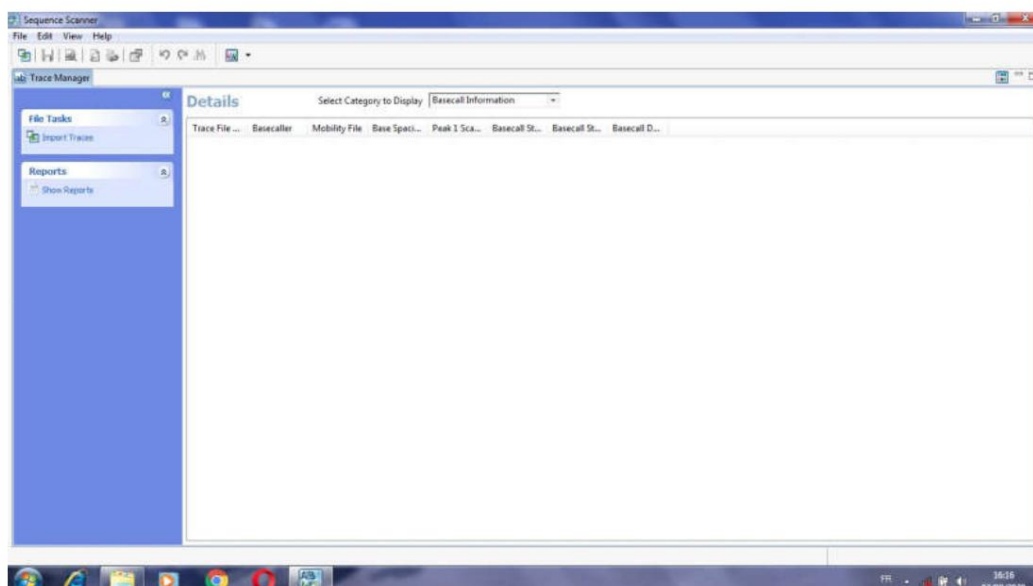
### Alternatives to the 16S rRNA gene in molecular identification?

- Tufa gene
- Genes coding for: Enzyme, toxin, receptor, hormone.

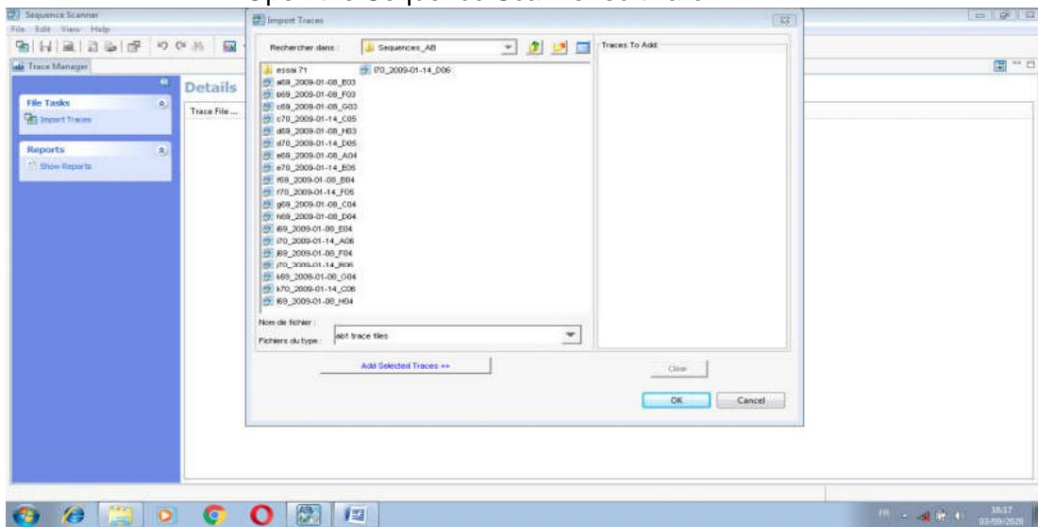
### The role of the Sequence Scanner software

The Sequence Scanner software (Applied Biosystems) allows you to read and view the files

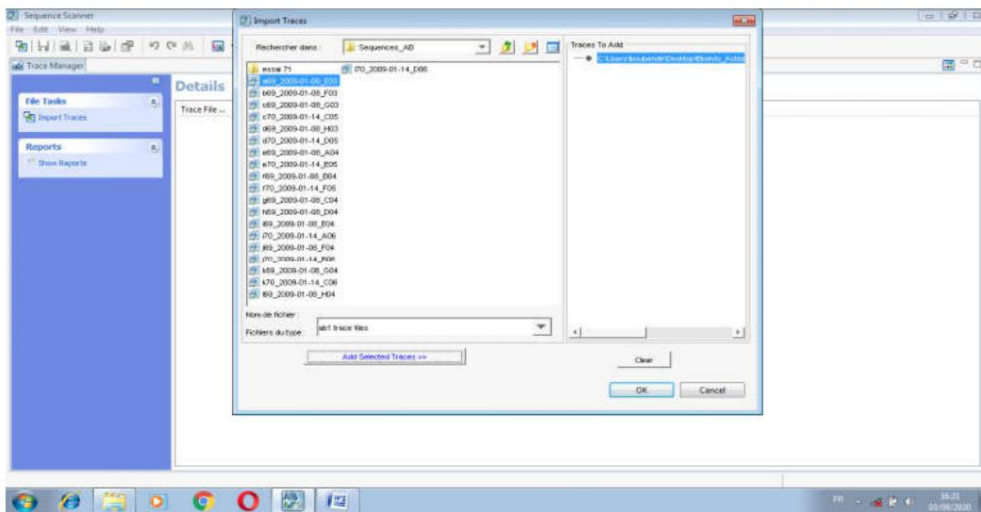
AB of the sequencer, its manipulation is carried out according to the following steps:



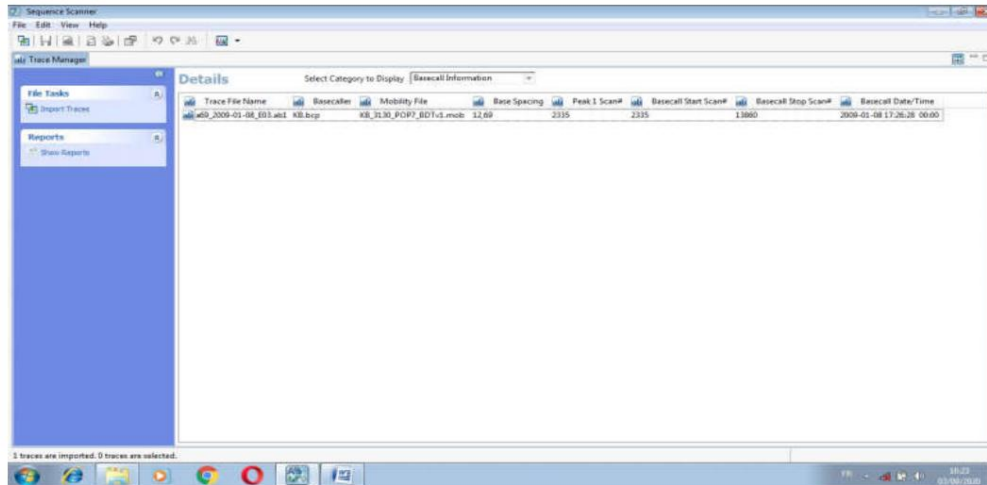
Open the Sequence Scanner software.



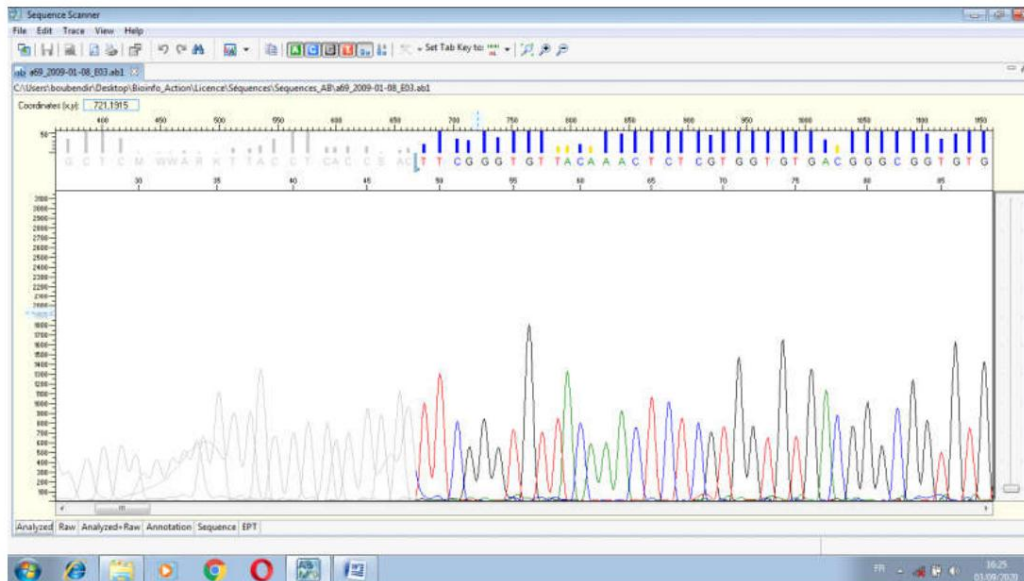
Click on Import Traces (top left) to search the AB files on your PC.



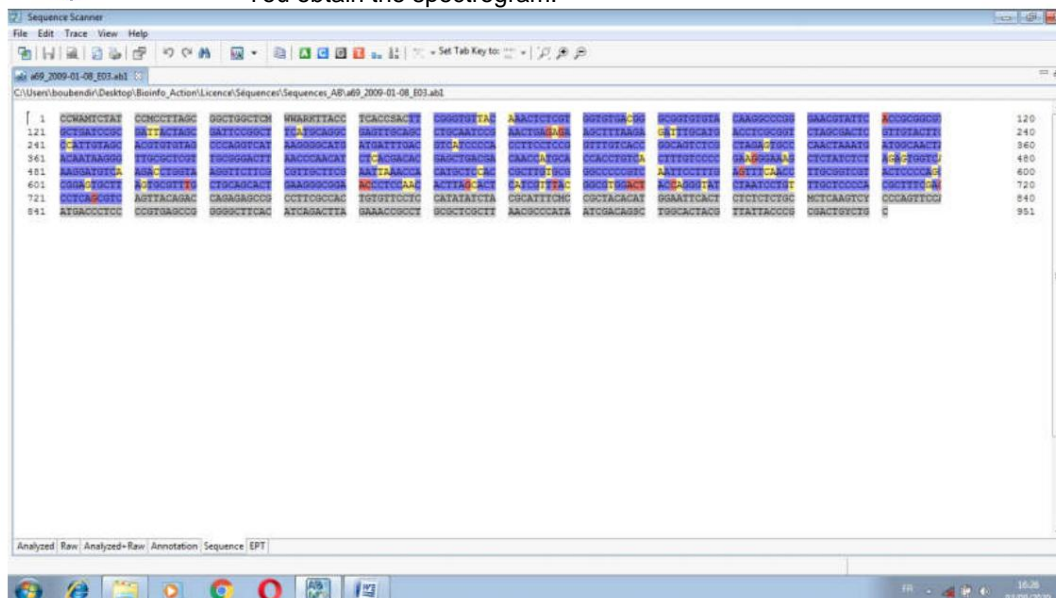
Select an AB file and click on Add Selected Traces (at the bottom) to introduce it into the software, then click on OK.



File AB is ready to be read. Double-click to open it.



You obtain the spectrogram.





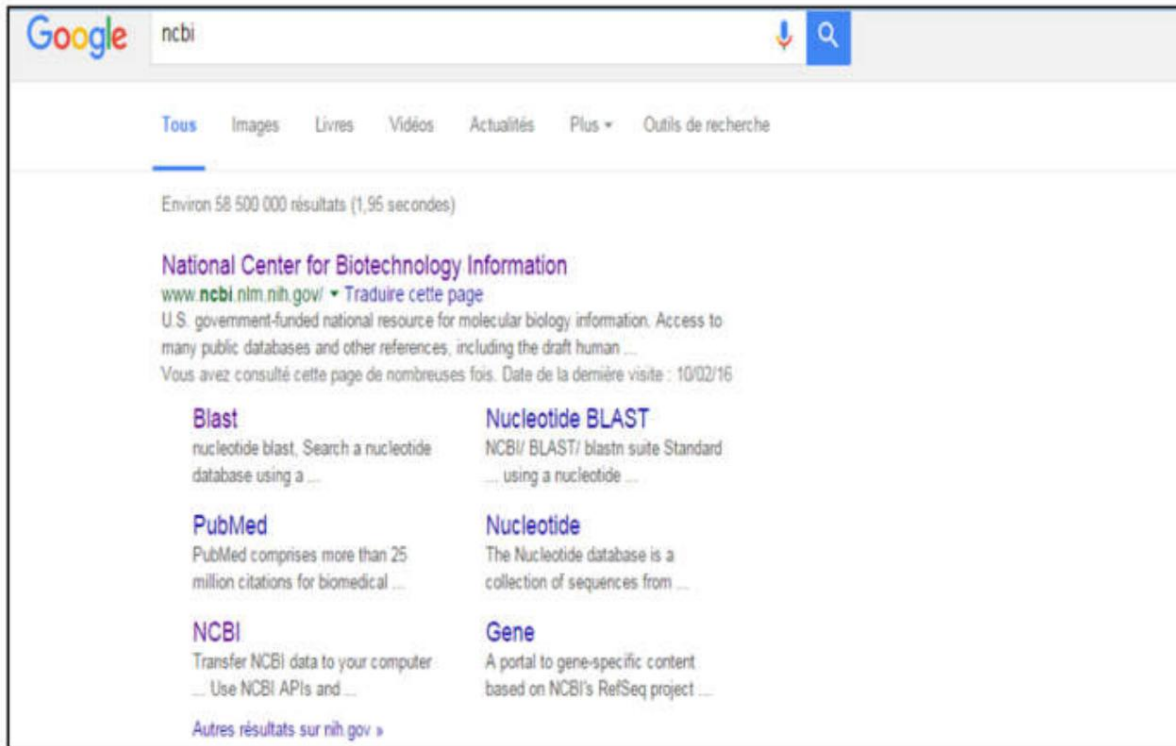
Click on Sequence to view your DNA details.

## Lab 2: Searching for the alignment of 16S rRNA gene sequences on NCBI

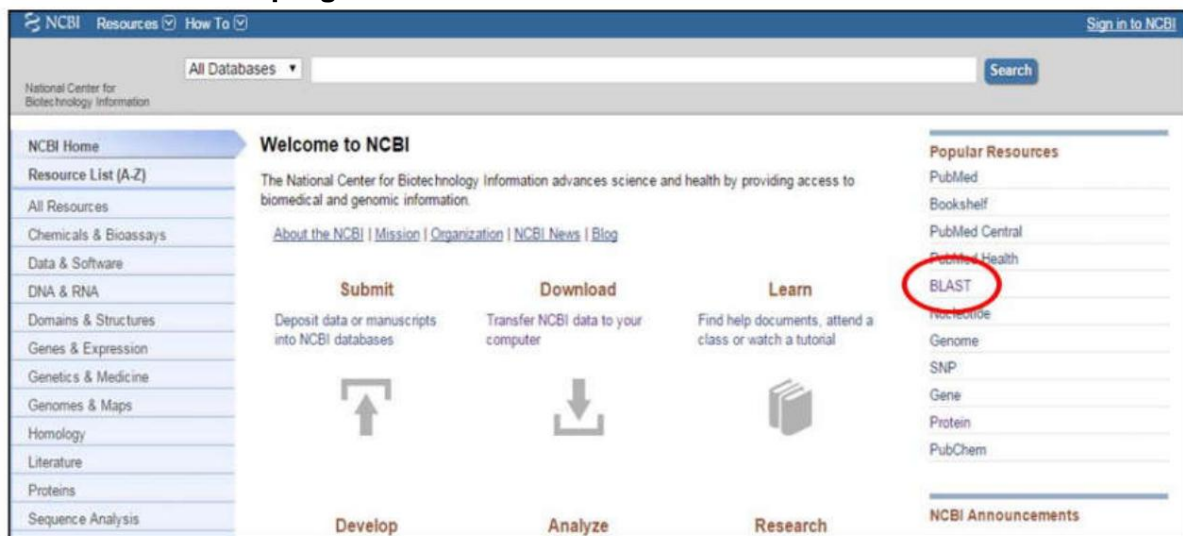
### Work stages

- Opening the NCBI link on the internet using the search engine  
Google
- Selection of the BLAST program •  
Selection of the BLASTn nucleotide tool •
- Insertion of the DNA sequence or the Access Number on Gene Bank and activation of  
the BLAST tool • Reading of the list  
of Alignment results • Reading of the details of the Alignment  
results • Collection of information on the individual by the  
access number on Gene Bank: Author, affiliation, publication, sequence, etc.

1. **Opening the NCBI link on the internet using the search engine  
Google search**



## 2. BLAST program selection



## 3. BLASTn nucleotide tool selection

**Basic Local Alignment Search Tool**

BLAST finds regions of similarity between biological sequences. The program compares nucleotide or protein sequences to sequence databases and calculates the statistical significance. [Learn more](#)

**NEWS**  
**Magic-BLAST 1.2.0 released**  
 A new version of the BLAST RNA-seq mapping tool is now available.  
 Mon, 27 Feb 2017 14:00:00 EST [More BLAST news...](#)

**Web BLAST**

**Nucleotide BLAST**  
 nucleotide → nucleotide

**blastx**  
 translated nucleotide → protein

**tblastn**  
 protein → translated nucleotide

**Protein BLAST**  
 protein → protein

4. **Inserting the DNA sequence or the Access Number on Gene Bank and BLAST tool activation**

BLAST® Basic Local Alignment Search Tool

Home Recent Results Saved Strategies Help

My NCBI (Register)

NCBI/BLAST/blastn suite **Standard Nucleotide BLAST**

blastn blastp blastx tblastn tblastx

BLASTn programs search nucleotide databases using a nucleotide query. [more...](#) [Reset page](#) [Bookmark](#)

Enter Query Sequence

Enter accession number(s), g(s), or FASTA sequence(s)

Clear Query subrange

From

To

Or, upload file  Aucun fichier choisi

Job Title

Enter a descriptive title for your BLAST search

Align two or more sequences

Choose Search Set

Database:  Human genomic + transcript  Mouse genomic + transcript  Others (nr etc.)

Nucleotide collection (nr/nt)

Limit by  Organism  BioProjectID  WGS Project

5. **Reading the list of alignment results**

Sequences producing significant alignments:

Select: [All](#) [None](#) Selected: 0

Alignments [Download](#) [GapBank](#) [Graphics](#) [Distance tree of results](#)

Description	Max score	Total score	Query cover	E value	Ident	Accession
<input type="checkbox"/> <a href="#">Chryseobacterium indologenes partial 16S rRNA gene, isolate 6</a>	1328	1328	100%	0.0	100%	<a href="#">HF678414.1</a>
<input type="checkbox"/> <a href="#">Chryseobacterium indologenes partial 16S rRNA gene, isolate 12</a>	1323	1323	100%	0.0	99%	<a href="#">HF678419.1</a>
<input type="checkbox"/> <a href="#">Chryseobacterium indologenes partial 16S rRNA gene, isolate 3</a>	1323	1323	100%	0.0	99%	<a href="#">HF678415.1</a>
<input type="checkbox"/> <a href="#">Bacterium 14S134.16S ribosomal RNA gene, partial sequence</a>	1317	1317	100%	0.0	99%	<a href="#">KC734365.1</a>
<input type="checkbox"/> <a href="#">Bacterium 14S132.16S ribosomal RNA gene, partial sequence</a>	1317	1317	100%	0.0	99%	<a href="#">KC734363.1</a>
<input type="checkbox"/> <a href="#">Chryseobacterium enrichment culture clone RA-M137.16S ribosomal RNA gene, partial sequence</a>	1317	1317	100%	0.0	99%	<a href="#">J0083171.1</a>

6. **Reading the detailed results of the Alignment**

Download - GenBank Graphics

Chryseobacterium indologenes partial 16S rRNA gene, isolate 6  
Sequence ID: [em|HF678414.1](#) Length: 719 Number of Matches: 1

Range 1: 1 to 719 [Graphics](#) [Graphics](#)

Score	Expect	Identities	Gaps	Strand
1328 bits(719)	0.0	719/719(100%)	0/719(0%)	Plus/Minus

Query 1 TGCSCGATTACTAGCGATTCCAGCTTCATAGAGTCGAGTTGCGAGACTCCGAATCCGAACCTG 60  
 Sbjct 719 TGCSCGATTACTAGCGATTCCAGCTTCATAGAGTCGAGTTGCGAGACTCCGAATCCGAACCTG 660

Query 61 AGACCGGCTTCGAGATTTGCATCAGTCGCTGTGTAGCTGCCCTCTGTACCGCCATTG 120  
 Sbjct 659 AGACCGGCTTCGAGATTTGCATCAGTCGCTGTGTAGCTGCCCTCTGTACCGCCATTG 600

Query 121 TATTACGTGTGTGGCCCAAGGCGTAAGGBCCGTGTGATTTGACGTCAATCCCACTTCC 180  
 Sbjct 599 TATTACGTGTGTGGCCCAAGGCGTAAGGBCCGTGTGATTTGACGTCAATCCCACTTCC 540

Query 181 TCTCTACTTGCSTAGGCACTCACTAGAGTCCCAACTTAATGATGGCACTAGTACCA 240  
 Sbjct 539 TCTCTACTTGCSTAGGCACTCACTAGAGTCCCAACTTAATGATGGCACTAGTACCA 480

Query 241 GGGTTGGCTCGTTGCAGGACTTAACCTAACCTCACGGACAGCTGACGACAACCA 300  
 Sbjct 479 GGGTTGGCTCGTTGCAGGACTTAACCTAACCTCACGGACAGCTGACGACAACCA 420

Query 301 TGCAGCACCTTGAAAAATGTCGAGAAAAAGTCTATTTCTAAACTGTGATTTCCCATTT 360  
 Sbjct 419 TGCAGCACCTTGAAAAATGTCGAGAAAAAGTCTATTTCTAAACTGTGATTTCCCATTT 360

7. **Gathering information about the individual using their access number on Gene Bank: Author, affiliation, publication, sequence, etc.**

Chryseobacterium indologenes partial 16S rRNA gene, isolate 6

GenBank: HF678414.1

[FASTA](#) [Graphics](#)

[Go to:](#) ☺

LOCUS HF678414 719 bp DNA linear BCT 21-FEB-2013

DEFINITION Chryseobacterium indologenes partial 16S rRNA gene, isolate 6.

ACCESSION HF678414

VERSION HF678414.1 GI:452084714

KEYWORDS -

SOURCE Chryseobacterium indologenes

ORGANISM [Chryseobacterium indologenes](#)  
 Bacteria; Bacteroidetes; Flavobacteriia; Flavobacteriales;  
 Flavobacteriaceae; Chryseobacterium.

REFERENCE 1

AUTHORS Boubendir, A.

TITLE Analyse et prevalence du risque infectieux de Listeria monocytogenes dans les laits crus recoltés dans deux regions a climat different (Zone semi-aride et le Nord-Est algeriens) : Modelisation spatiale de la diversite floristique

JOURNAL Thesis (2012) Constantine 1 University, Algeria

REFERENCE 2 (bases 1 to 719)

AUTHORS Hamidechi, A.

TITLE Direct Submission

JOURNAL Submitted (11-FEB-2013) Constantine University, Constantine, Route de Ain El-Bey, 25000, ALGERIA

FEATURES

source 1..719  
 /organism="Chryseobacterium indologenes"  
 /mol\_type="genomic DNA"  
 /isolate="6"  
 /isolation\_source="raw milk"  
 /db\_xref="taxon:253"

Customize view

Analyze this sequence

Run BLAST

Pick Primers

Highlight Sequence Features

Find in this Sequence

Related information

Taxonomy

LinkOut to external resources

Ribosomal Database Project II  
 [Ribosomal Database Project II]

SILVA SSU Database  
 [SILVA]

Recent activity

Turn Off Clear

Chryseobacterium indologenes partial 16S rRNA gene, isolate 6 Nucleotide

Nucleotide Sequence (719 letters) BLAST

### Practical Session 3: Introduction to Phylogenetic Analysis: MEGA6 Software

The MEGA 06 software is used in phylogenetic analysis; it allows for:

- Multiple alignment.
- The distance matrix.
- The phylogenetic tree.

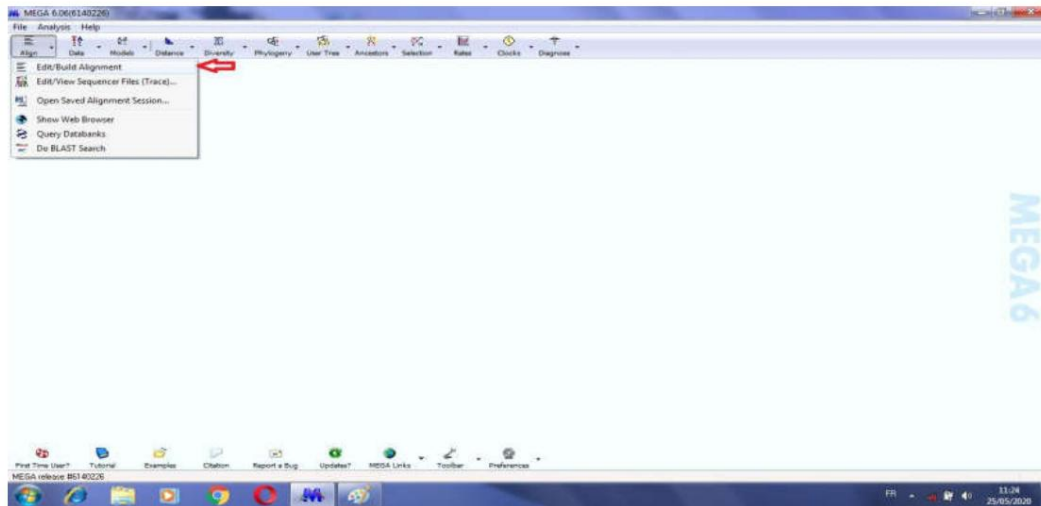
#### The value of comparing DNA sequences in Multiple Alignment

-Stable regions: comparison of distant species.

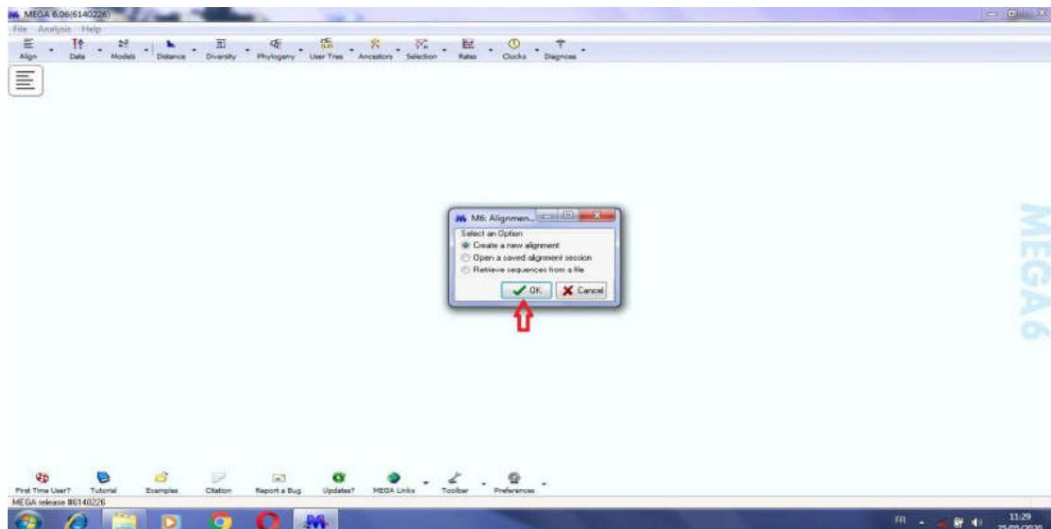
-Unstable regions: comparison of closely related species.

This software is operated according to the following steps:

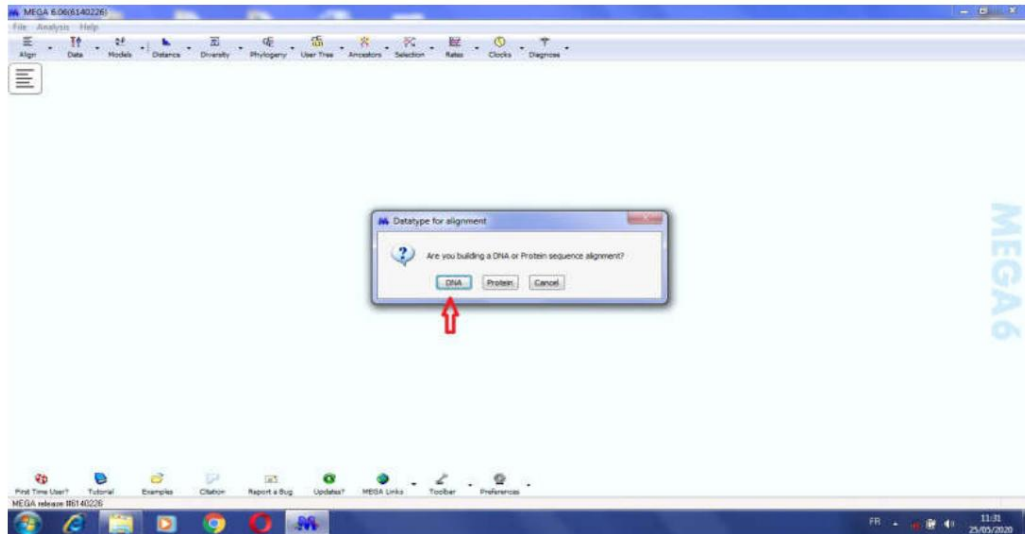
1. **Multiple Alignment: Alignment Explorer**



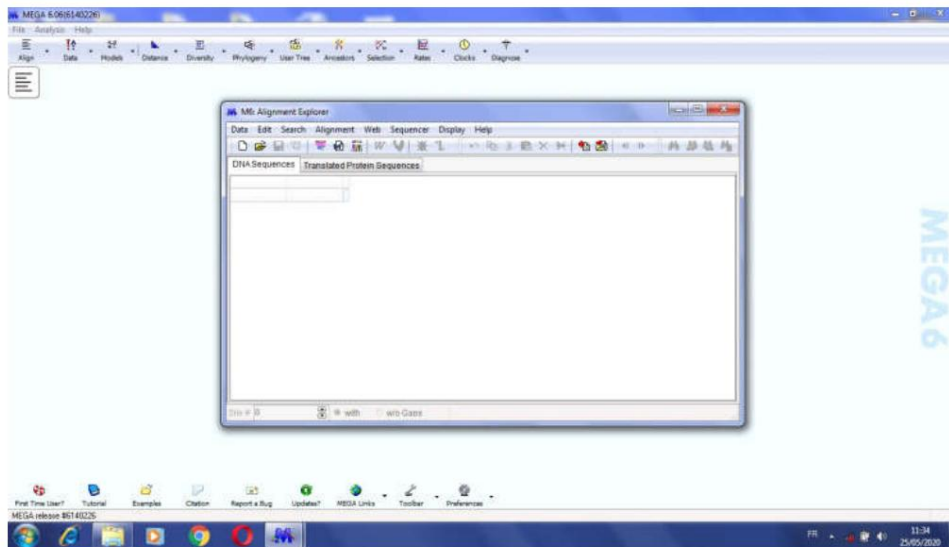
ÿ Open the Alignment Explorer program and click on Edit/Build Alignment.



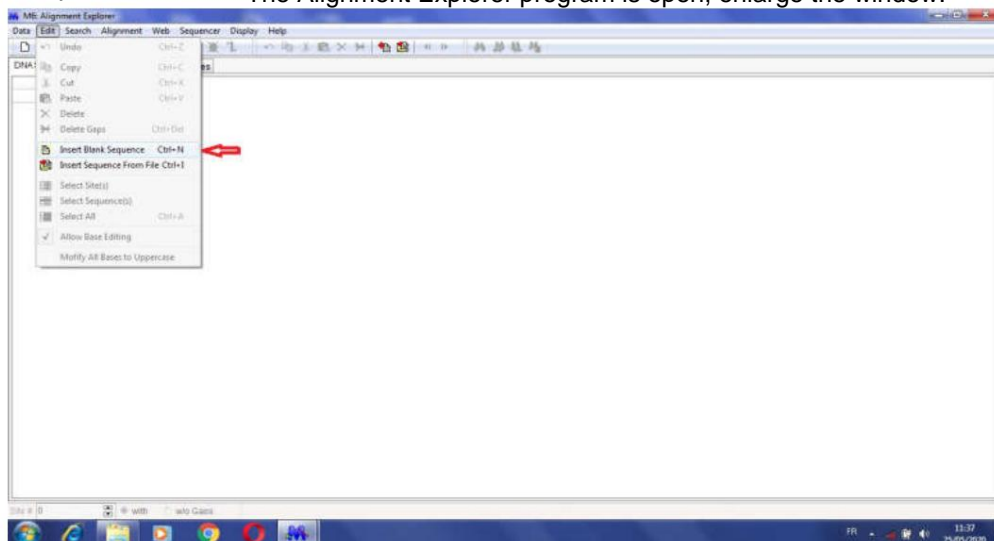
ÿ Click OK to confirm the creation of a new alignment.



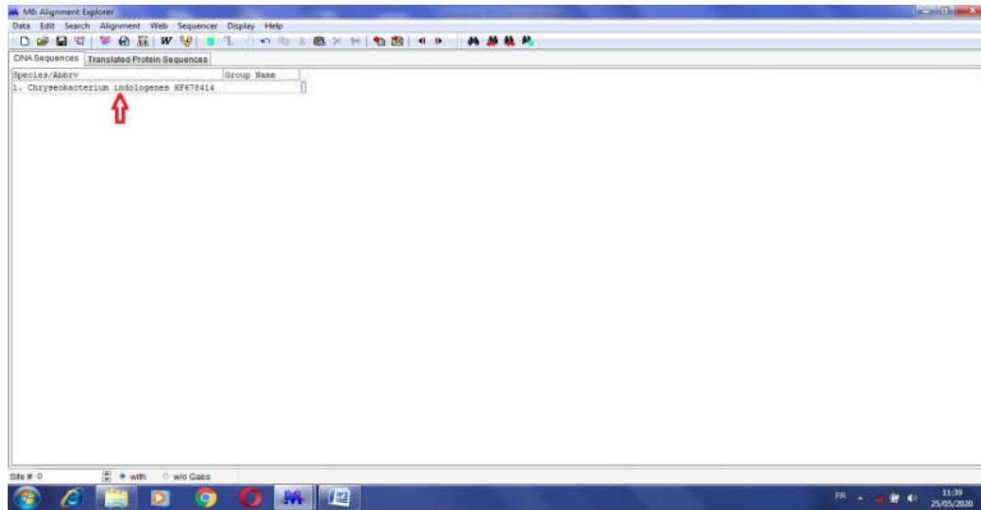
ÿ Confirm your analysis substrate: DNA or Protein.



ÿ The Alignment Explorer program is open, enlarge the window.



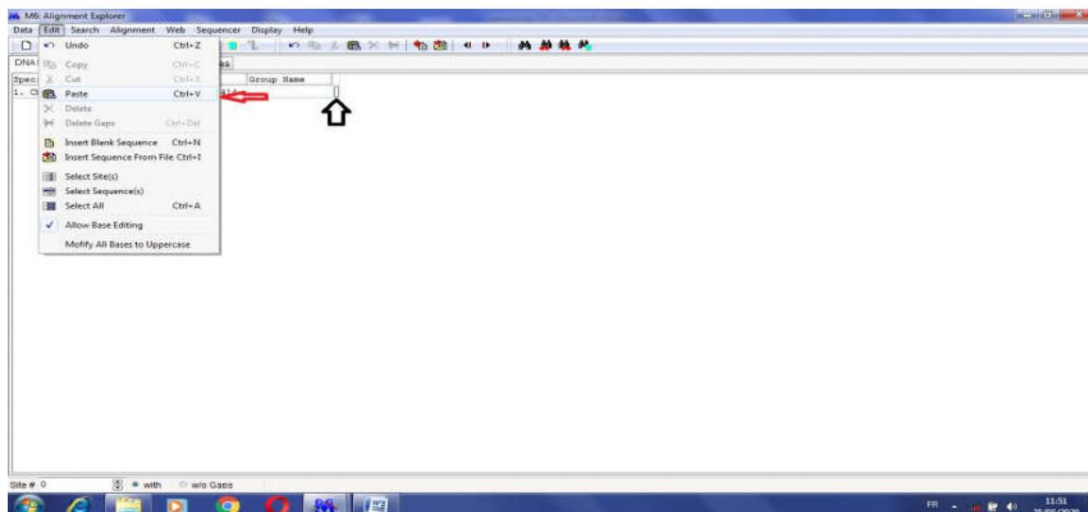
ÿ Click on Edit\_Insert Blank Sequence.



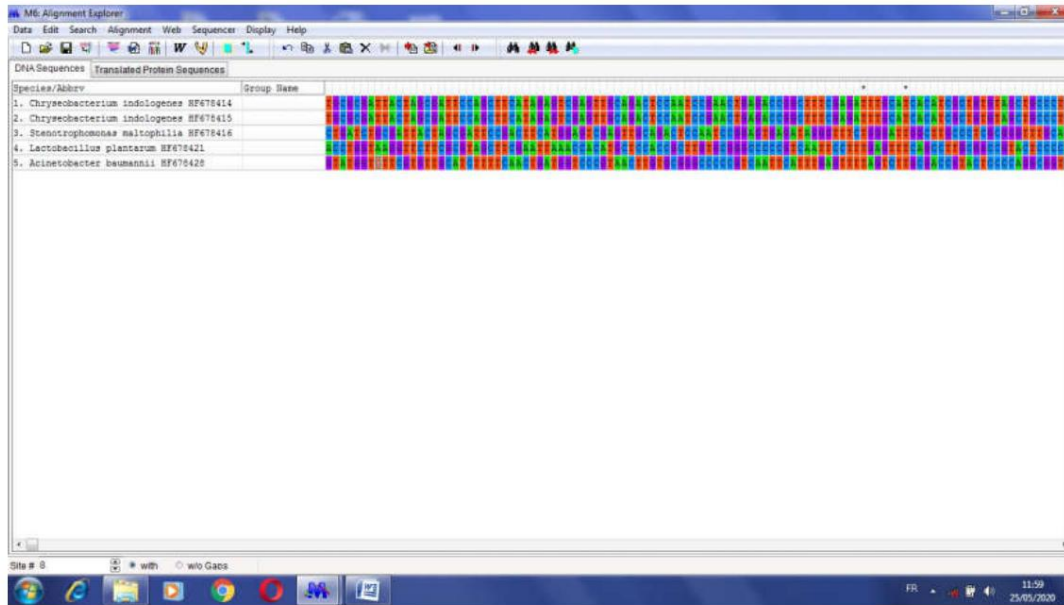
ÿ Enter the species name and its access number on Gene Bank.



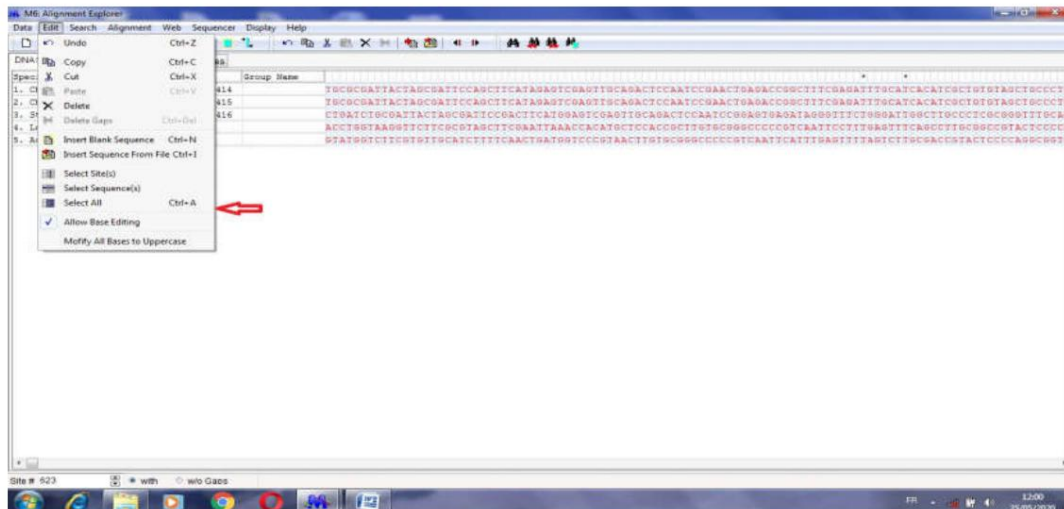
ÿ Select and copy your sequence to analyze (prepared beforehand).



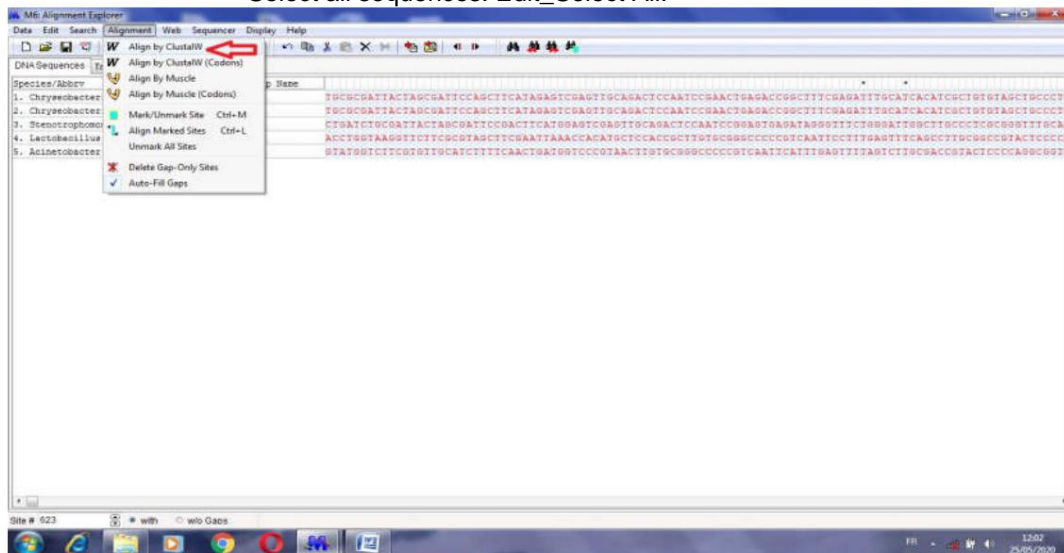
ÿ Click on Edit\_Paste to insert the sequence in the specified location.



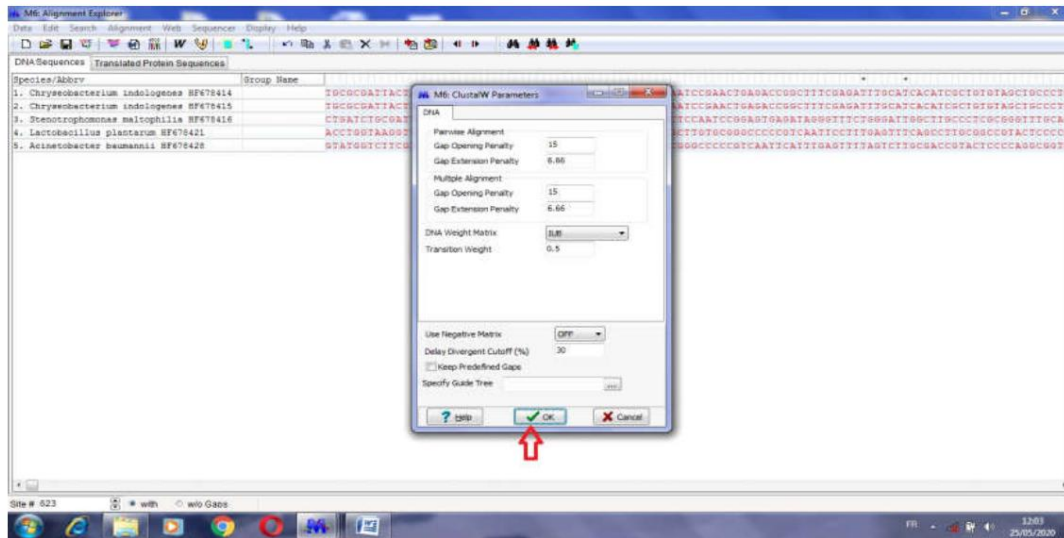
ÿ Do the same to insert the other sequences.



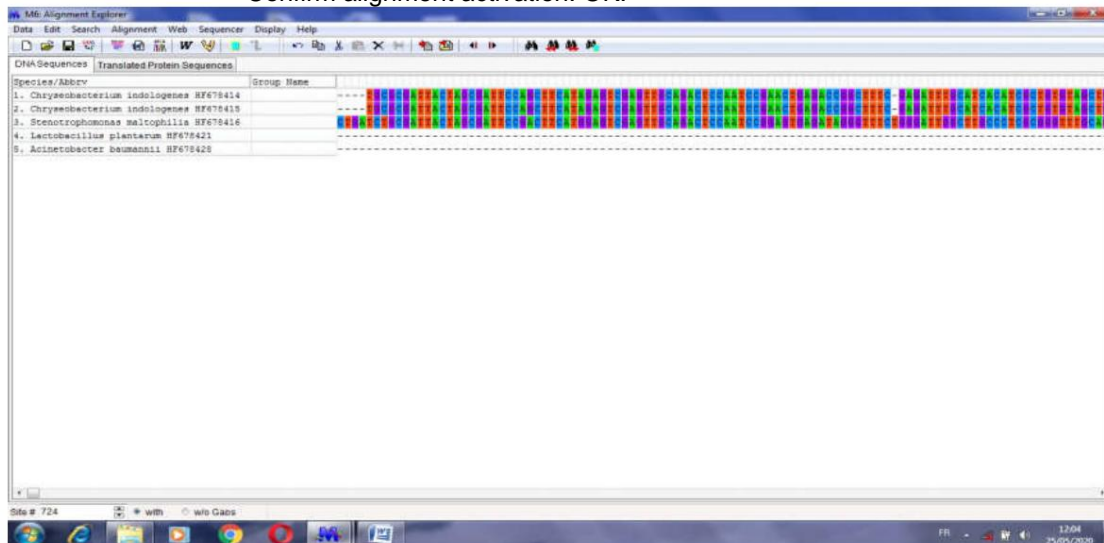
ÿ Select all sequences: Edit\_Select All.



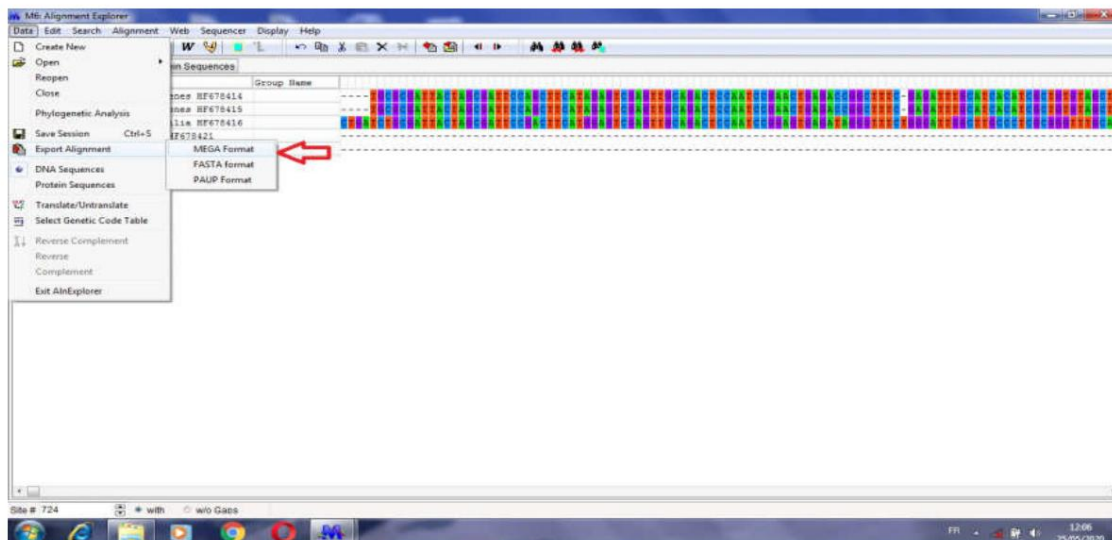
ÿ Enable alignment: Alignment\_Align by ClustalW.



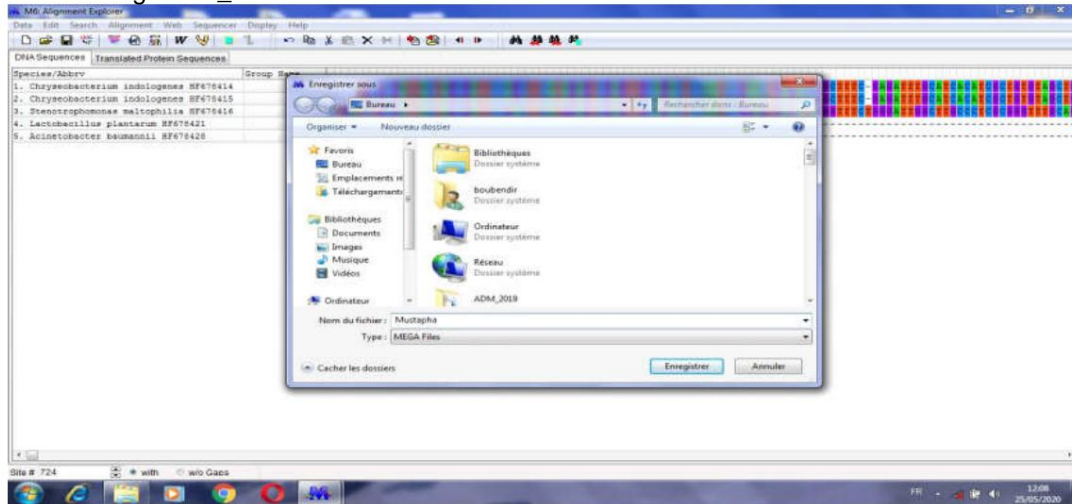
ÿ Confirm alignment activation: OK.



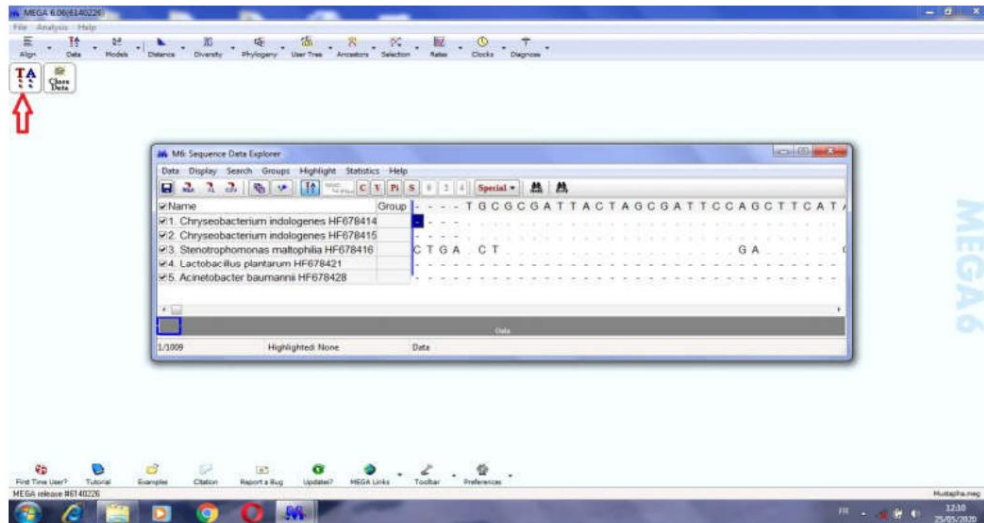
ÿ The appearance of the GAPS confirms the achievement of the alignment.



ÿ Save the alignment in MEGA format: Data\_Export  
Alignment\_MEGAFormat.

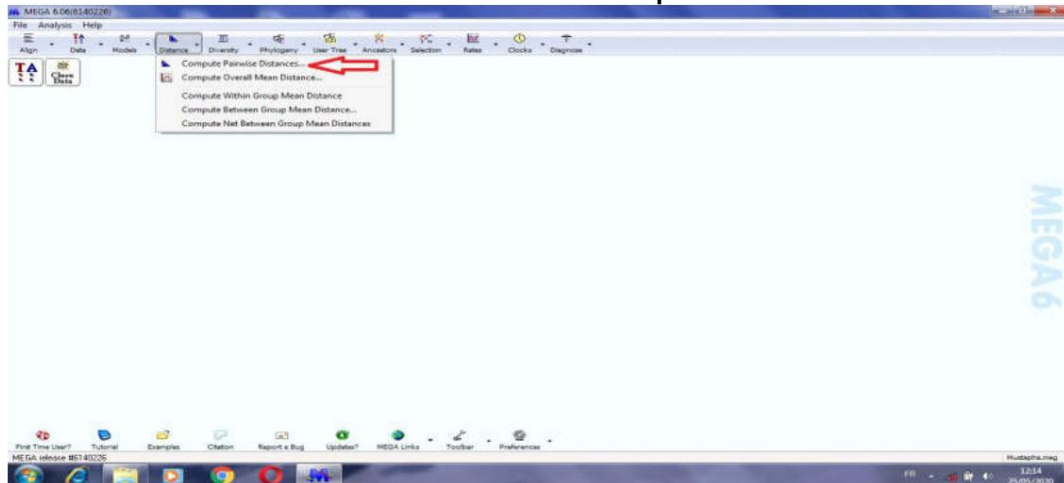


ÿ Name the file MEGA and save it.

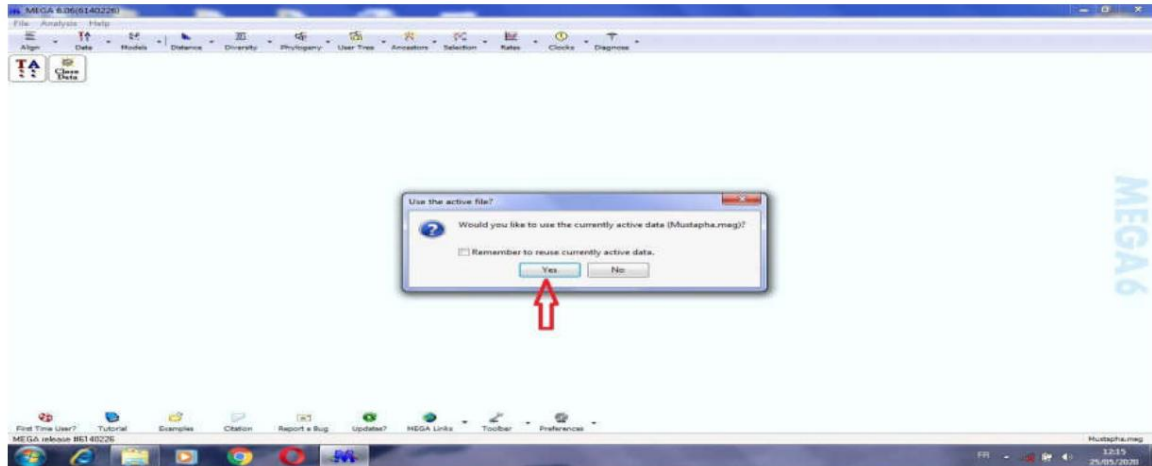


ÿ Finally, open the MEGA file and view your alignment.

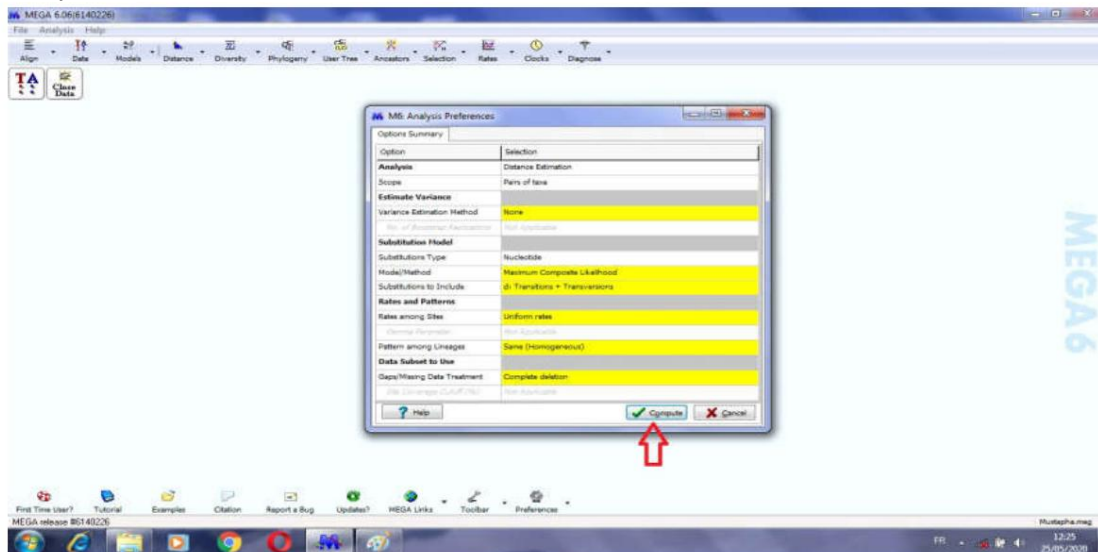
## 2. The distance matrix: Marix Ditances Explorer



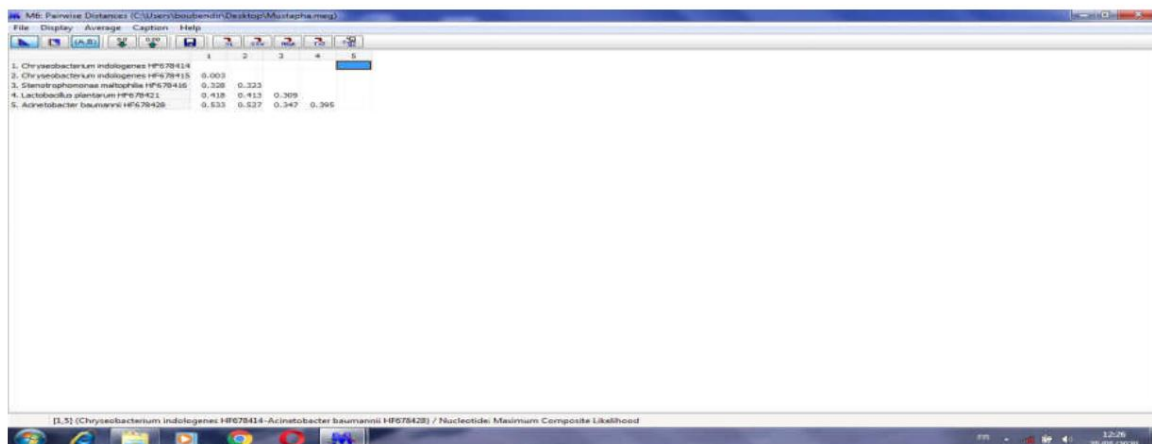
ÿ Activate the Matrix Distance Explorer program and choose the ComputePairwise Distance action.



ÿ Confirm use of active data: Yes.

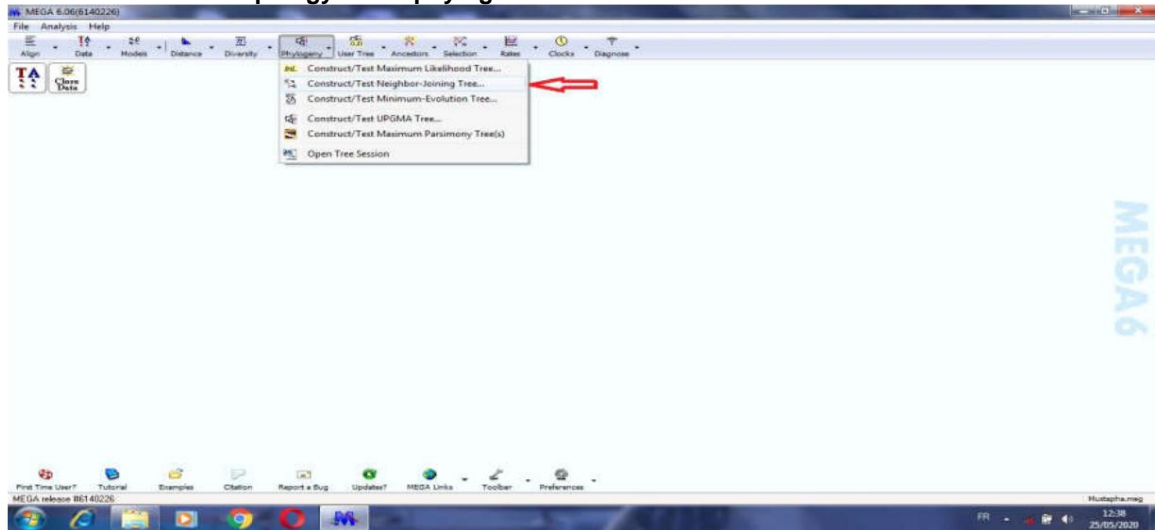


ÿ Click on Compute to launch the distance matrix.

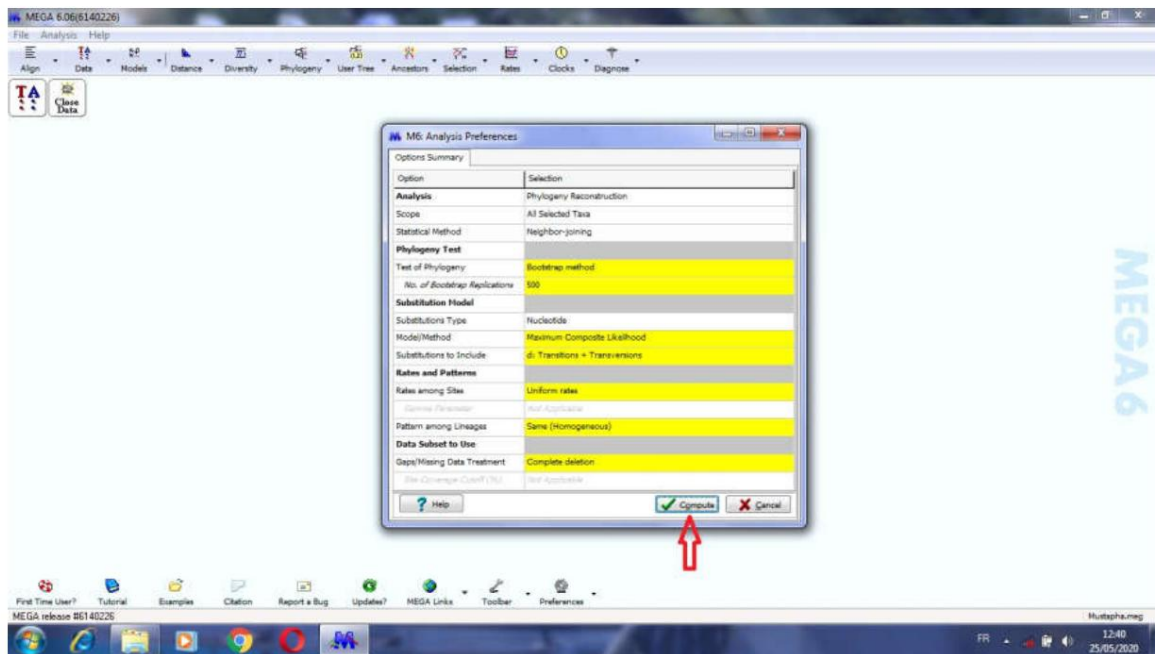


ÿ Finally, you obtain the distance matrix.

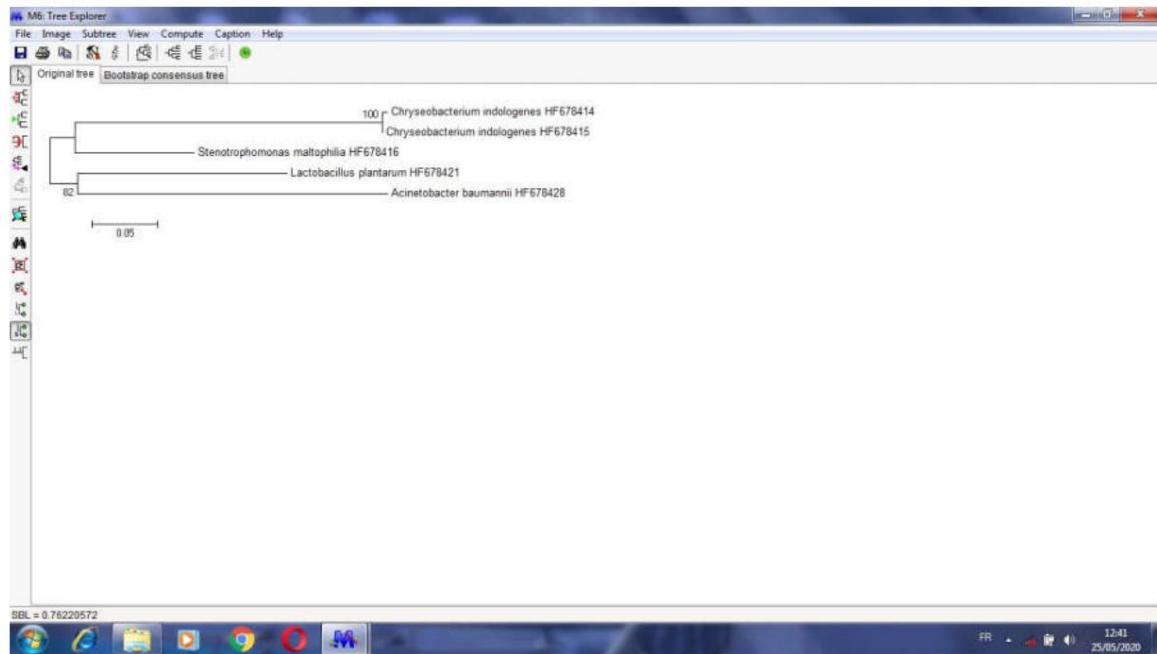
### 3. The topology of the phylogenetic tree



ÿ Activate the Tree Explorer program and select the method of constructing the NJ tree or others.



ÿ Start the build with the bootstrap test.



Finally , you have the phylogenetic tree