Student Worksheet

Fungi Diversity

For 10th grade students





LIVEWORKSHEETS

STUDENT WORKSHEET

Theme	:	**Fungi**
Sub-theme	:	**Diversity and Role of Fungi**
Class	:	**X**
Semester	:	**.....**
Group	:	**.....**
Group Members	:	**1.....**
		2.....
		3.....
		4.....
		5.....

Learning Outcomes

- To learn how to use MEGA software for conducting phylogenetic analysis based on fungal ITS rDNA sequences.
- To identify fungal species present in the intestinal tract of Sinyonya common carp (*Cyprinus carpio*) through morphological and molecular data.
- To retrieve and utilize reference sequences from the NCBI GenBank database for bioinformatics analysis.
- To perform multiple sequence alignment to determine sequence similarity and homology among fungal species.
- To construct and interpret a phylogenetic tree to understand the evolutionary relationships between fungal isolates and reference species.

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ACTIVITY

A. Introduction

Answer the following questions!

Question	Expected Answer
What do you know about fungi?	
What types of fungi do you know?	
What are the roles of fungi that you know?	
In your opinion, can fungi provide benefits to living organisms?	
What is a phylogenetic tree?	
What do you know about NCBI?	
What do you know about MEGA?	

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Now, let's watch a video about Fungi!

A. What are Fungi?

https://www.youtube.com/watch?v=0HK8y6k2j94

B. Role of Fungi

https://www.youtube.com/watch?v=laUPud5PVhU

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B. Investigation

In Pandeglang, Banten, there is a high-value local fish species known as the Sinyonya strain of common carp (*Cyprinus carpio*), characterized by its golden-yellow scales and distinctive narrow eyes. Within its digestive tract lives a diverse community of fungi; some provide benefits, such as producing digestive enzymes or secondary metabolites, while others may act as pathogens.

A researcher isolated fungi from the intestinal organs (stomach and intestines) of the Sinyonya carp through a series of steps, including sample collection, cultivation on PDA medium with antibiotics, colony purification, macroscopic and microscopic identification (using the slide culture method and Lactophenol Cotton Blue staining).

The fungal identified included:

- ***Saccharomyces cerevisiae*** – Yeast widely used in fermentation and with probiotic potential.
- ***Debaryomyces hansenii*** – Halotolerant yeast found in various habitats, potentially enhancing fish immunity.
- ***Candida tropicalis*** – Fungus that can aid in mineral absorption but may act as an opportunistic pathogen.
- ***Trichosporon asahii*** – Yeast with blastoconidia and pseudohyphae, some strains are opportunistic pathogens.
- ***Rhodotorula mucilaginosa*** – Known for its red-orange pigmented colonies, capable of producing bioactive compounds.
- ***Aspergillus niger*** – Mold that produces digestive enzymes and antimicrobial secondary metabolites but may also produce mycotoxins.
- ***Penicillium digitatum*** – Mold producing various enzymes and metabolites, with some species producing antibiotics but also possible mycotoxins.

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Let's help researcher build phylogenetic tree!

To confirm the taxonomic identity and determine the phylogenetic relationships between species, the researcher performed phylogenetic analysis based on ITS rDNA sequences. Let's help the researcher build the phylogenetic tree by using ITS rDNA sequence data from the identified fungi and reference sequences from **NCBI**, performing multiple sequence alignment, and constructing the phylogenetic tree in **MEGA**.

Fungal Sample

- *Saccharomyces cerevisiae* ITS rDNA
- *Debaryomyces hansenii* ITS rDNA
- *Candida tropicalis* ITS rDNA
- *Trichosporon asahii* ITS rDNA
- *Rhodotorula mucilaginosa* ITS rDNA
- *Aspergillus niger* ITS rDNA
- *Penicillium digitatum* ITS rDNA

Fungal Sample Sequence
Accessible via the link or barcode below!
http://bit.ly/4mtitmF

Scan Here!

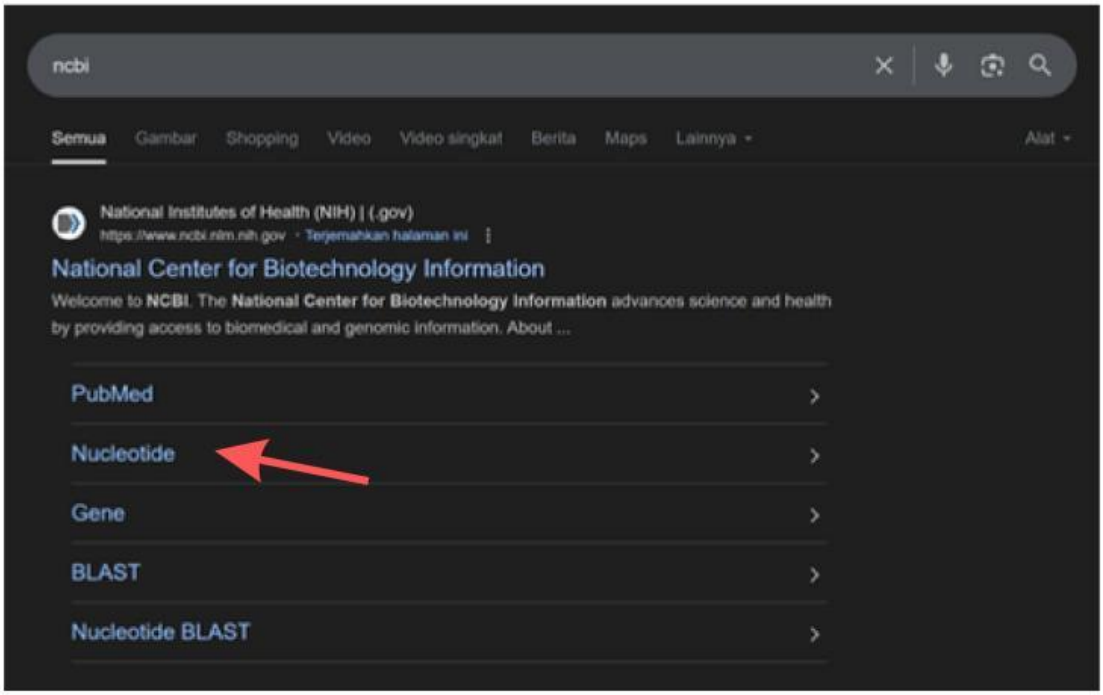


Let's start building a phylogenetic tree with the following steps!

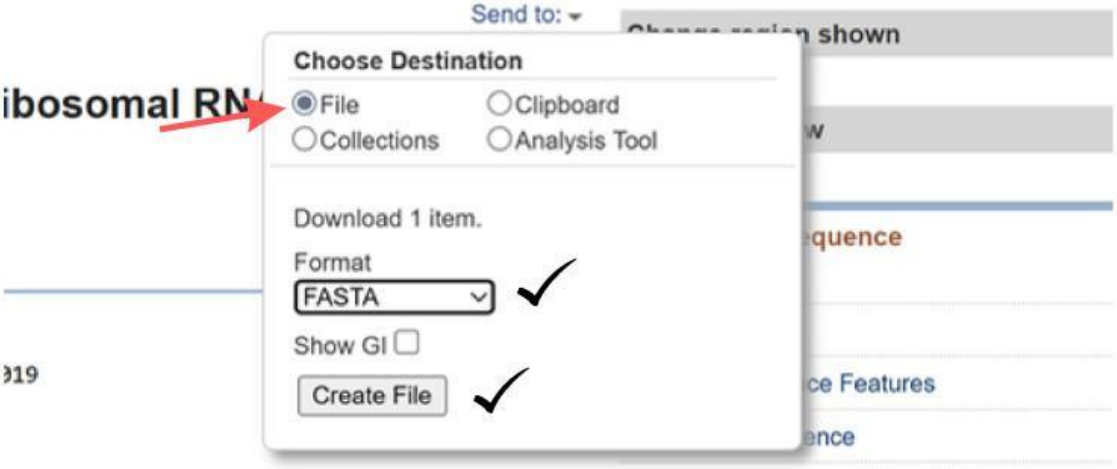
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Obtaining Sequence Data from NCBI

- Go to **NCBI** website, click **Nucleotide**.



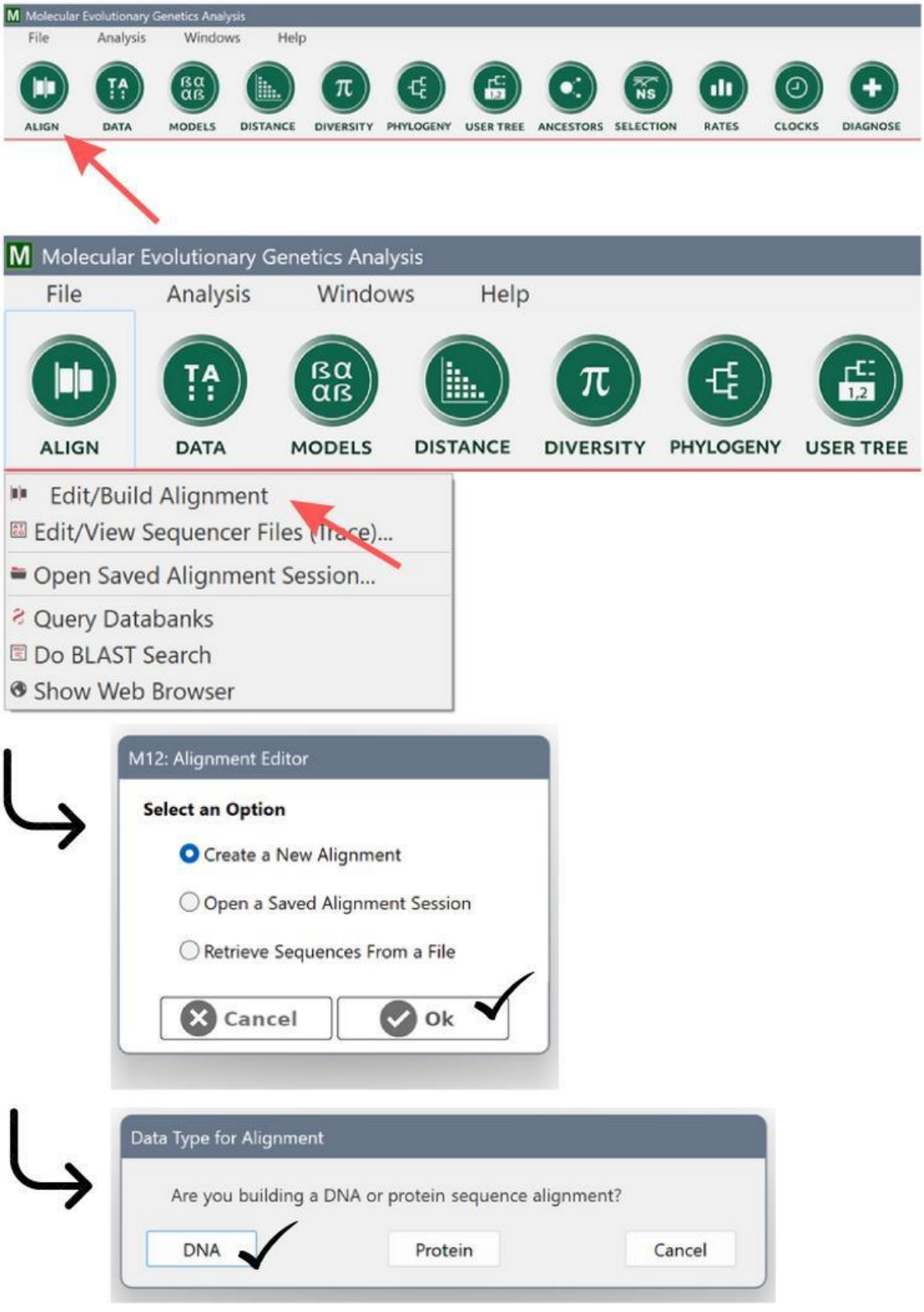
- Type the target species name, for example:
  - *Saccharomyces cerevisiae ITS rDNA*
  - *Debaryomyces hansenii ITS rDNA*
  - *Candida tropicalis ITS rDNA*
  - *Trichosporon asahii ITS rDNA*
  - *Rhodotorula mucilaginosa ITS rDNA*
  - *Aspergillus niger ITS rDNA*
  - *Penicillium digitatum ITS rDNA*



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Creating Alignment in MEGA

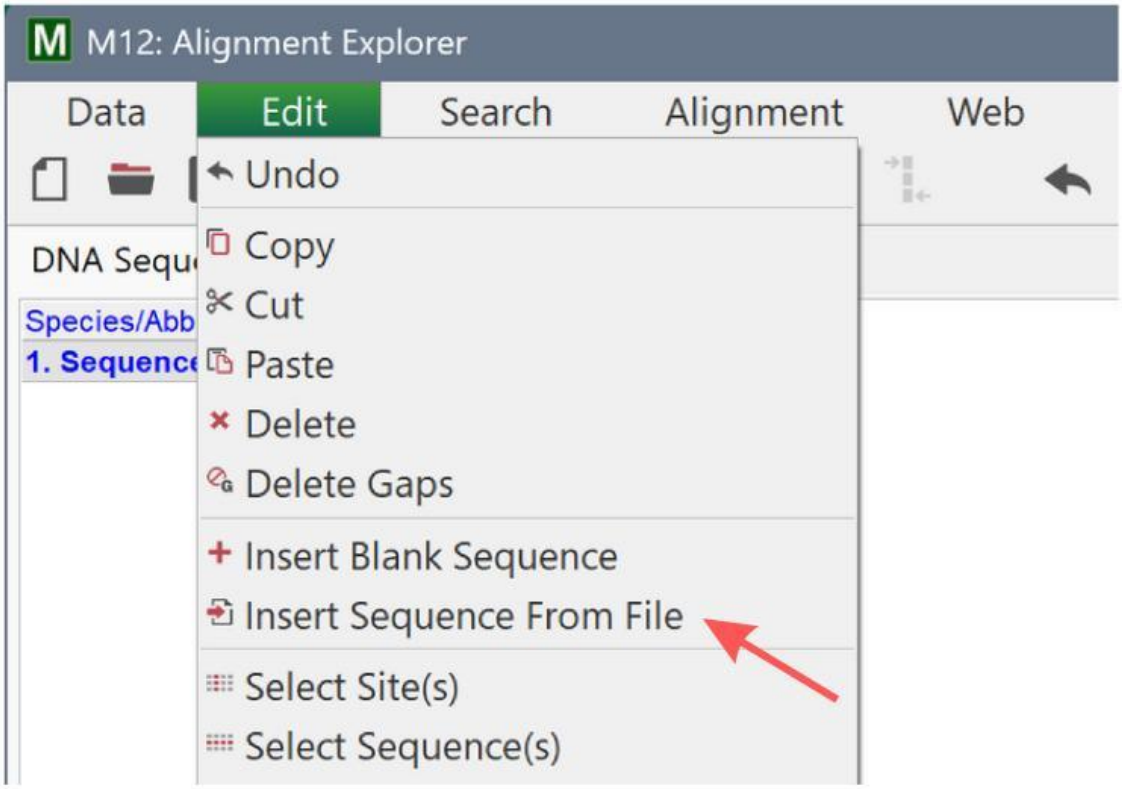
- Open MEGA → Align → Edit/Build Alignment → Create New Alignment → select DNA.

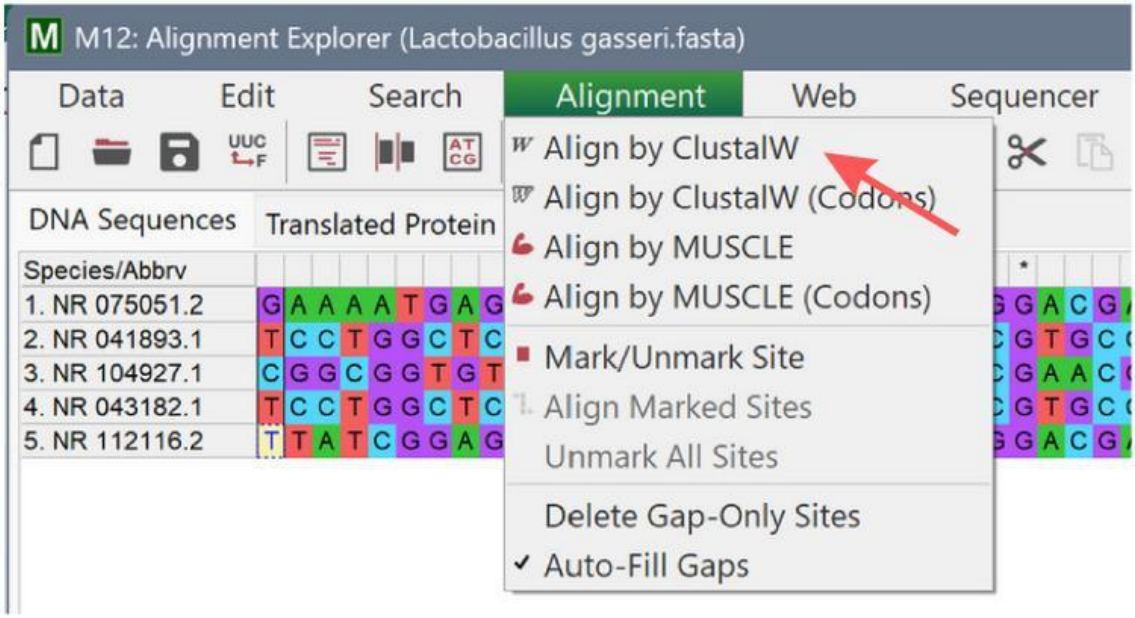


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Creating Alignment in MEGA

- Import the combined .fasta file. → Perform alignment using ClustalW → Save the aligned file as .meg.

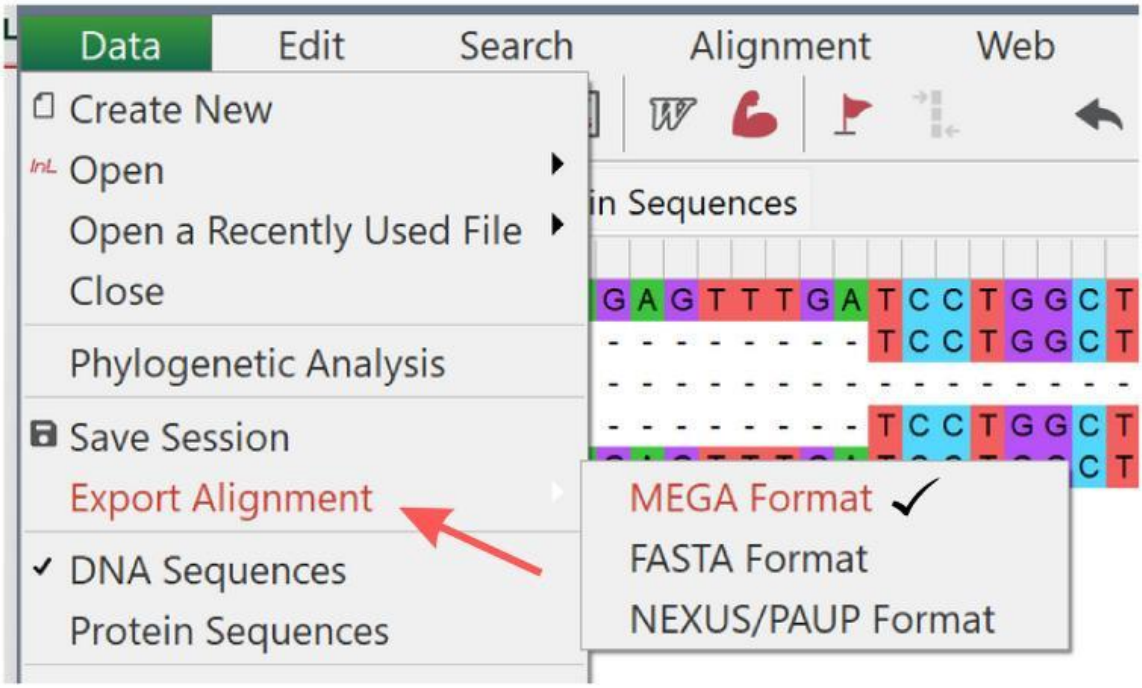




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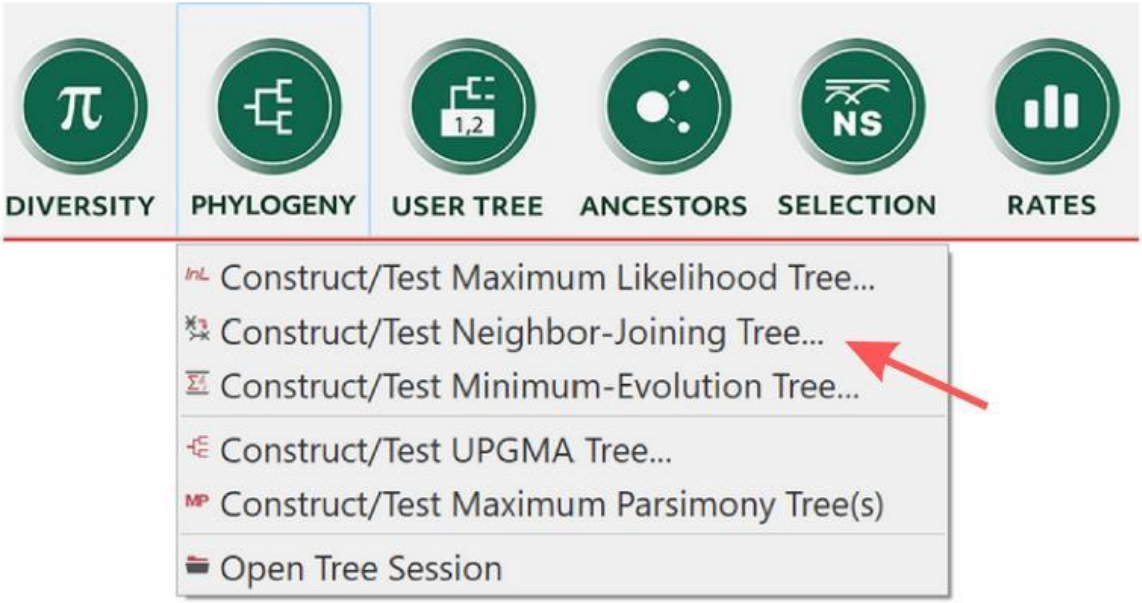
Creating Alignment in MEGA

- Save the aligned file as .meg.



Building the Phylogenetic Tree

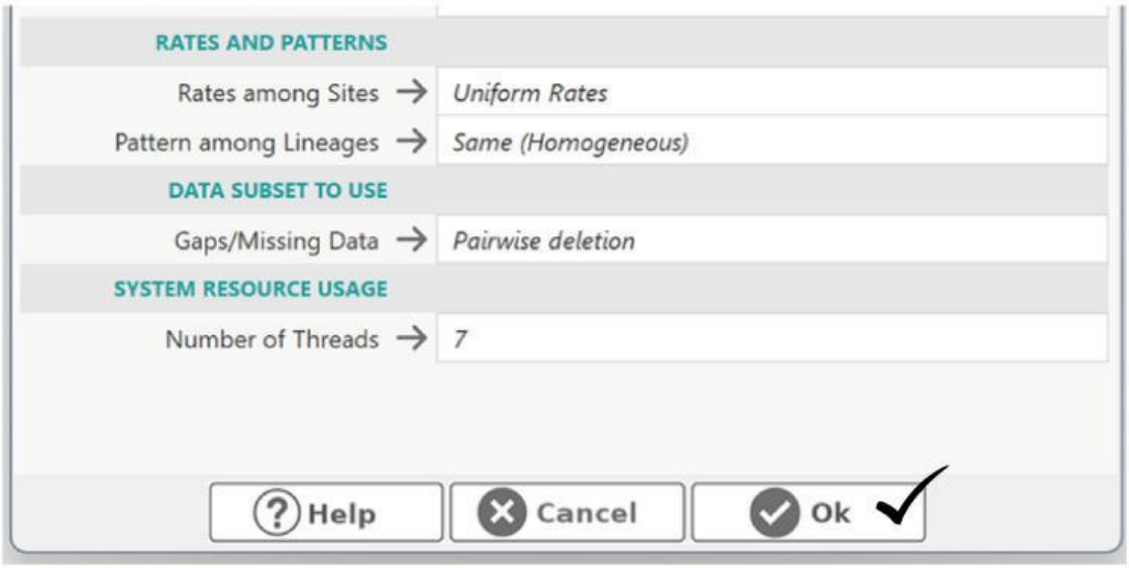
- From the aligned file, select Phylogeny → Construct/Test Neighbor-Joining Tree.



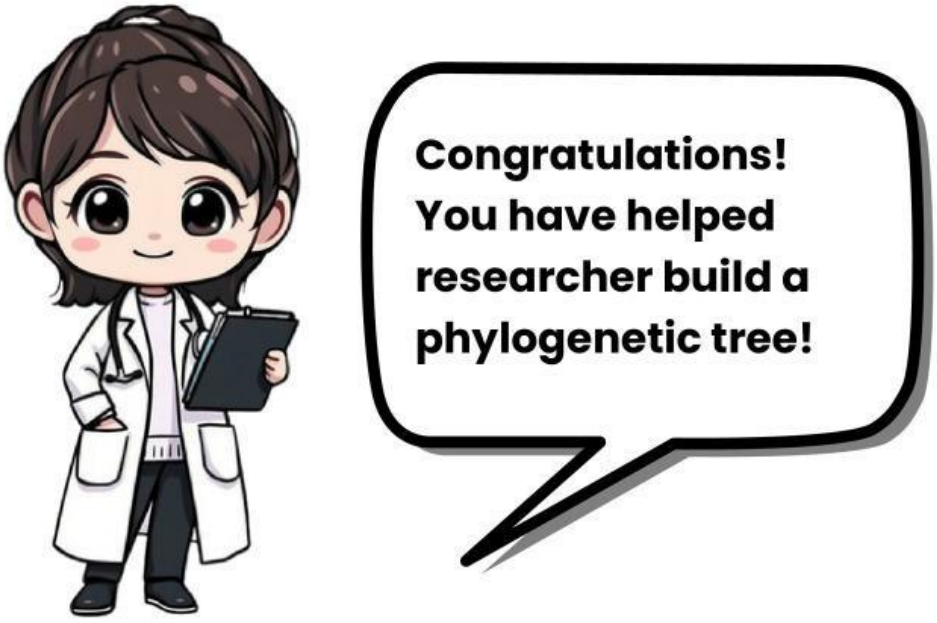
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Building the Phylogenetic Tree

- Choose a nucleotide substitution model, such as Tamura-Nei model.
- Enable the Bootstrap test (e.g., 1000 replications) to assess branch reliability.
- Run the analysis and wait for the tree to be generated.



- After clicking OK, Mega will construct a phylogenetic tree based on the Fasta file that has been entered, and the phylogenetic tree will be complete.



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Now, let's answer the questions below!

Based on the phylogenetic tree analysis you have created, answer the questions below

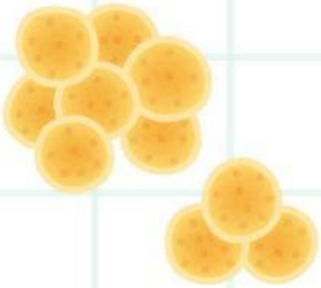
Question	Expected Answer
Does the fungal sample from the research show a close relationship with beneficial yeast species such as Saccharomyces cerevisiae? Explain your answer.	
Based on the phylogenetic tree, is the fungal sample more closely related to Saccharomyces cerevisiae or Debaryomyces hansenii? Provide evidence from your analysis.	
According to your analysis, to which genus does the fungal sample belong? Explain the morphological and molecular characteristics that support this identification.	
Explain how phylogenetic tree analysis can help researchers confirm the identity of fungi isolated from the intestine of Sinyonya common carp (Cyprinus carpio)	

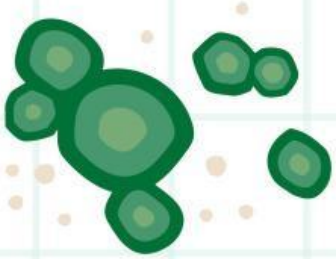
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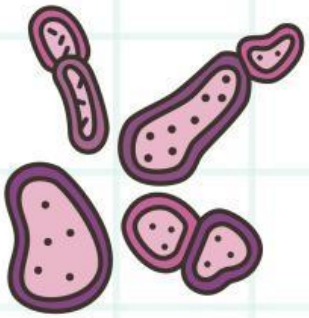
Please answer the questions below.

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Congratulations!
You have completed today's lesson!





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