

User Guide

1. **Upload FASTA File** – Ensure the file has 3 or more sequences.

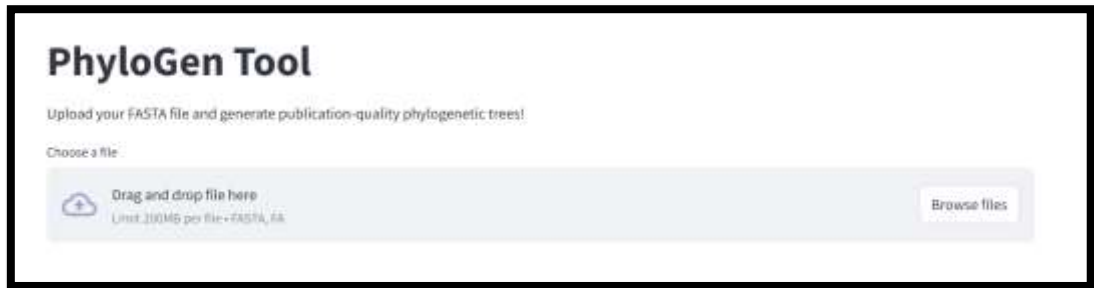


Fig1. Choose a multi-fasta file

2. **Preview Sequences** - Sequences IDs and Description can be displayed.



Fig2. Display sequence information in table

3. **Select Sequence type** - DNA or Protein.

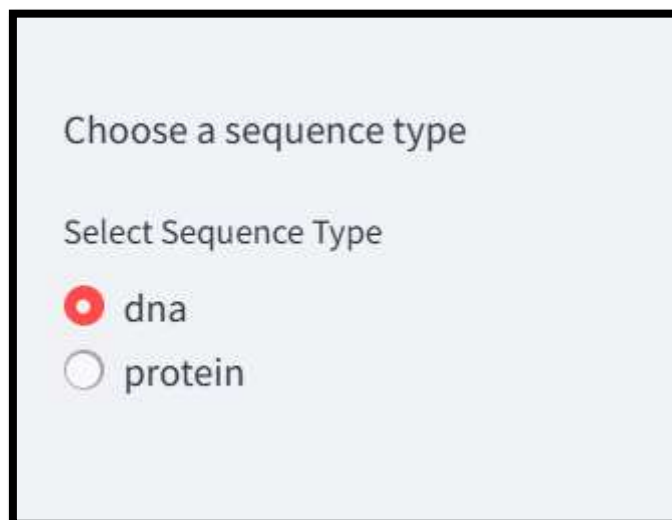


Fig3. Choose the type of data

4. Select Alignment Tool

- Choose from **MAFFT**, **MUSCLE**, **ClustalW** or **TCoffee**.
- Set gap penalties and substitution models
- Choose tree construction method:
 - ~ Distance-based: **Neighbour Joining**, **UPGMA**
 - ~ Character-based: **Maximum Parsimony**, **Maximum Likelihood**

The screenshot shows the 'STEP 2: Processing Options' window. It is divided into two main sections: 'Alignment Settings' and 'Tree Construction'. In 'Alignment Settings', the 'Alignment Algorithm' is set to 'mafft'. The 'Gap Open Penalty (base/1.0)' is set to '2.70' and the 'Gap Extension Penalty (base/1.0)' is set to '1.80'. In 'Tree Construction', the 'Construction Method' is set to 'Distance based', the 'Distance based' method is set to 'Neighbour Joining', and the 'Substitution Model' is set to 'megsblast'. A red 'Run Full Analysis' button is located at the bottom left.

Fig4. Set alignment options, gap penalties, tree construction method and substitution model

5. View & Download – Preview and download results.

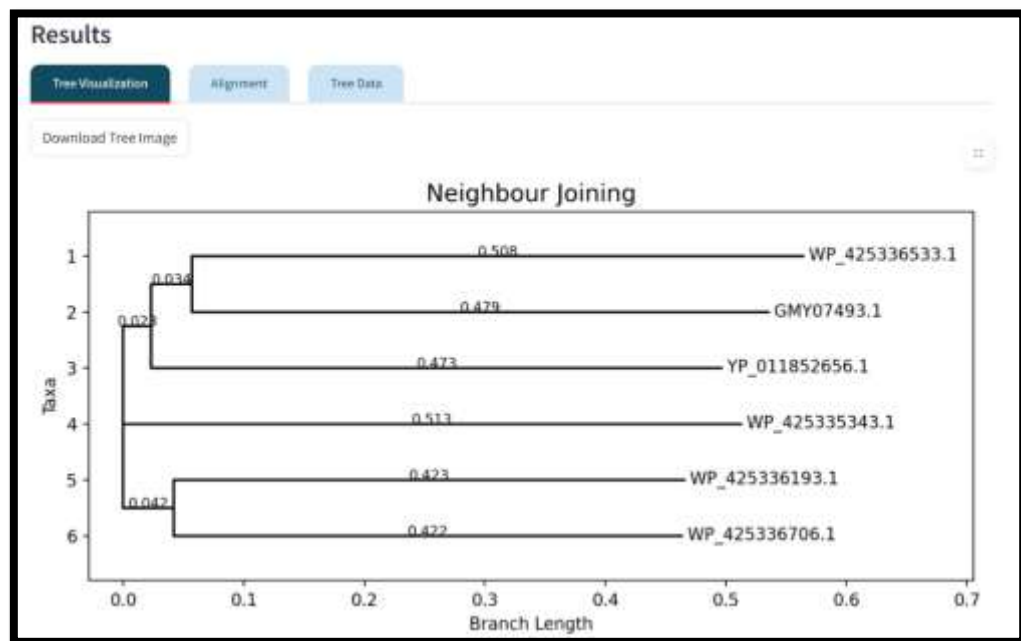


Fig5. Download phylogenetic tree as PNG

Results

Tree Visualization **Alignment** Tree Data

Download Alignment

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>YP_011852656.1 hypothetical protein [Phage vB_SabS_5ds2]
MKLMFSELTLLDEFLGFVTERGFINVDPQTLEVLQSIQNKTKTOLEDL-WRPLSELTPLM
MKIVKNKETGEEREEMVKELANSYS-----P-----ESLVMHDD-----
---EPETL-MTAHYIMRLP-----
--
>MP_425335343.1 DUF1963 domain-containing protein [Rhodoblastus acidophilus]
RHHGVK-----LKLTPATSLDTGDSMLGGNPLPPPFEM-----
-----P-----HVEGKPLHFLGQID
CSALPAEI-WGGLGPDQVPACGV-ADGGDRRFPRAGIGQAAATGRLTSGSSINGVIVSS
VM
>QMY87493.1 UPF0369 protein C6orf57 [Fagus cranata]
MASRIL-----FSSKSPLSSTPKLVFNATRSOP-----
-----MTRSLSNVSTRLKCSATQQPQP-----HQESPWKEDTDAIK
-----EDL-ETKEEEDGGEHVKATGEIGGPRGPEPTRFGDMERNGR-----S
DF
>MP_425336786.1 recombinase family protein [Sphingomonas molluscorum]
MTNPIRA-----ARYVLRVSTEEQOLERQEAIVAGARAA-----
-----GYVVAAYVREKASGAR---PDR---P-----ELIHMIEDL-----
---QPGEV-VLAEKIDRISRLPL-PEERLVAAIRAKGARLAVPGSWTC-----L
IS
>MP_425336533.1 GFA family protein [Synechococcus elongatus]
NGTCHCSR-----KVGASTIVFYKREAFSLVSGADAITTYKAEP-----

```

Fig6. Download alignment as FASTA

Results

Tree Visualization Alignment **Tree Data**

Download Newick

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(((MP_425336533.1:0.58752,QMY87493.1:0.47865)Inner2:0.83363,YP_011852656.1:0.47346)Inner3:0.82338,MP_425335343.1:0.51274,

```

Fig7. Download tree data in Newick format