

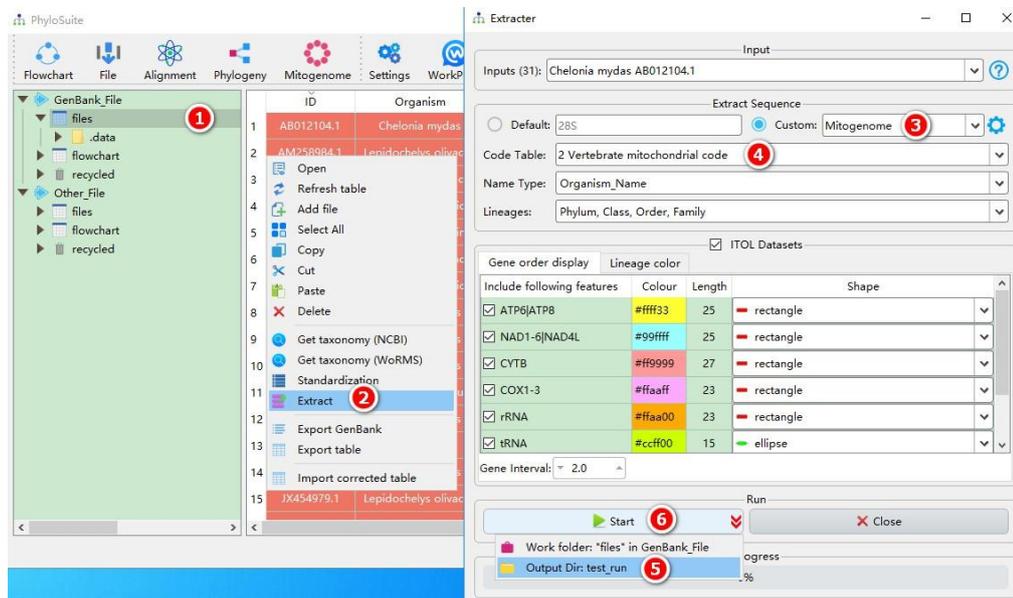
Test run

url: <https://dongzhang0725.github.io/dongzhang0725.github.io/example/>

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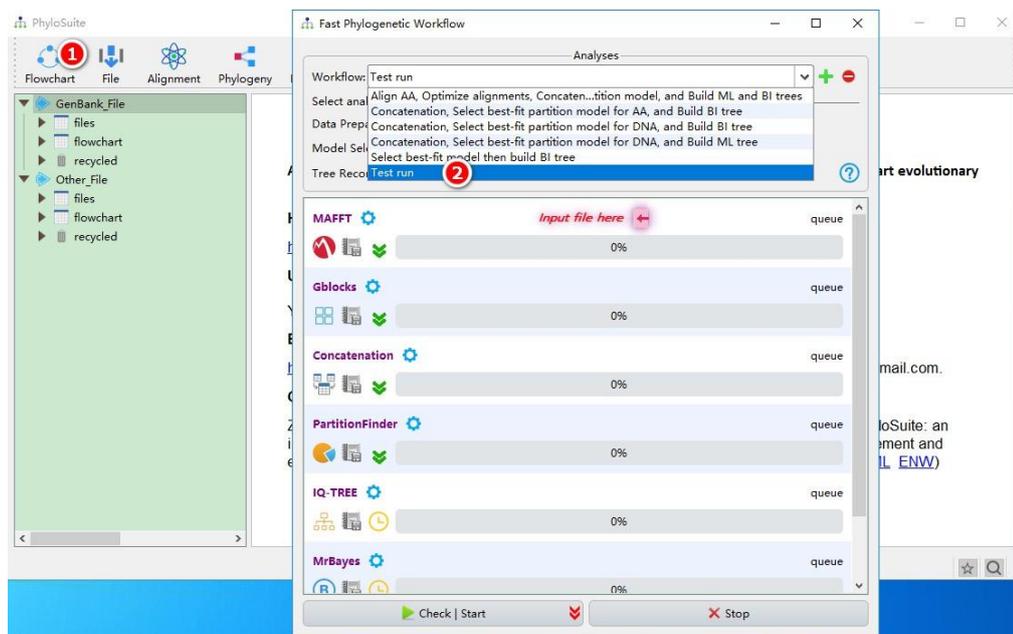
4. Select **2 Vertebrate mitochondrial code**;
5. Click to rename the output dir name as **test_run**;
6. Click **Start** to extract.



3.3 Phylogenetic workflow

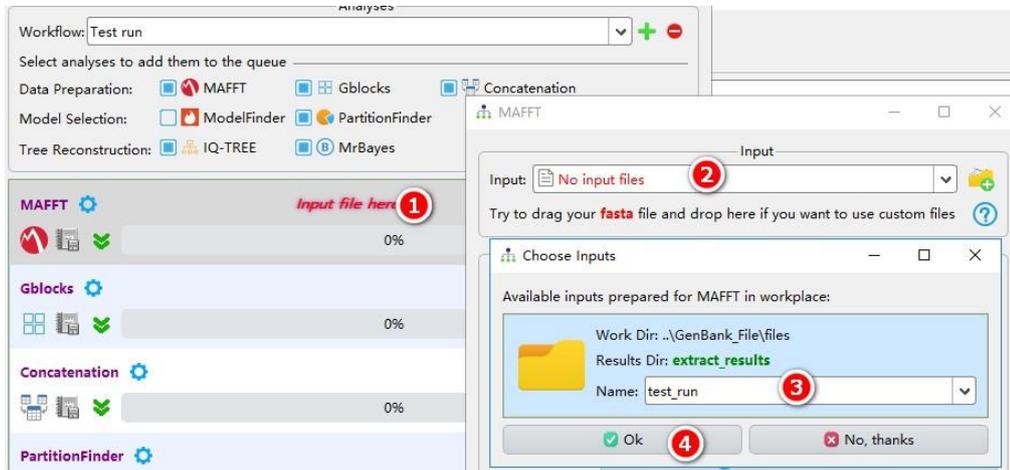
3.3.1 Choose workflow

1. Click **Flowchart** in the menu bar;
2. Choose **Test run** in the **Workflow** menu;

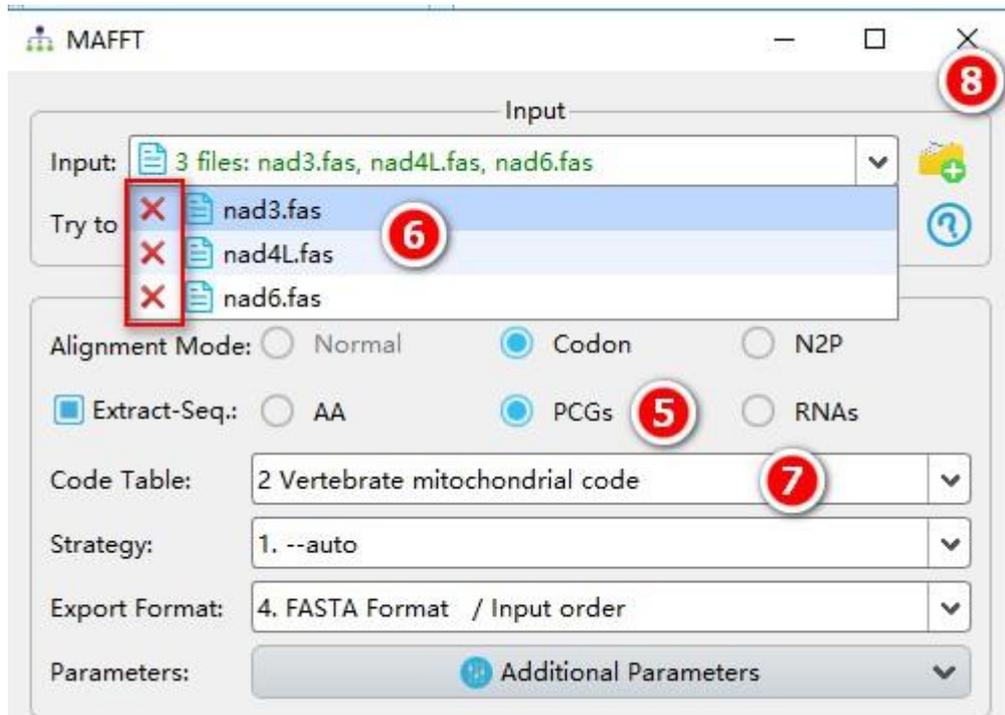


3.3.2 Input file and parameter setting

1. Only the first program needs an input file, so click the red **Input file here** to open the MAFFT program window;
2. Click the file input box of MAFFT to view the automatically prepared input files (you may opt to use a different file via **No, thanks**);
3. Select the results that you extracted in section 3.2 (**test_run** in **extract_results**);
4. Click **Ok** to import.

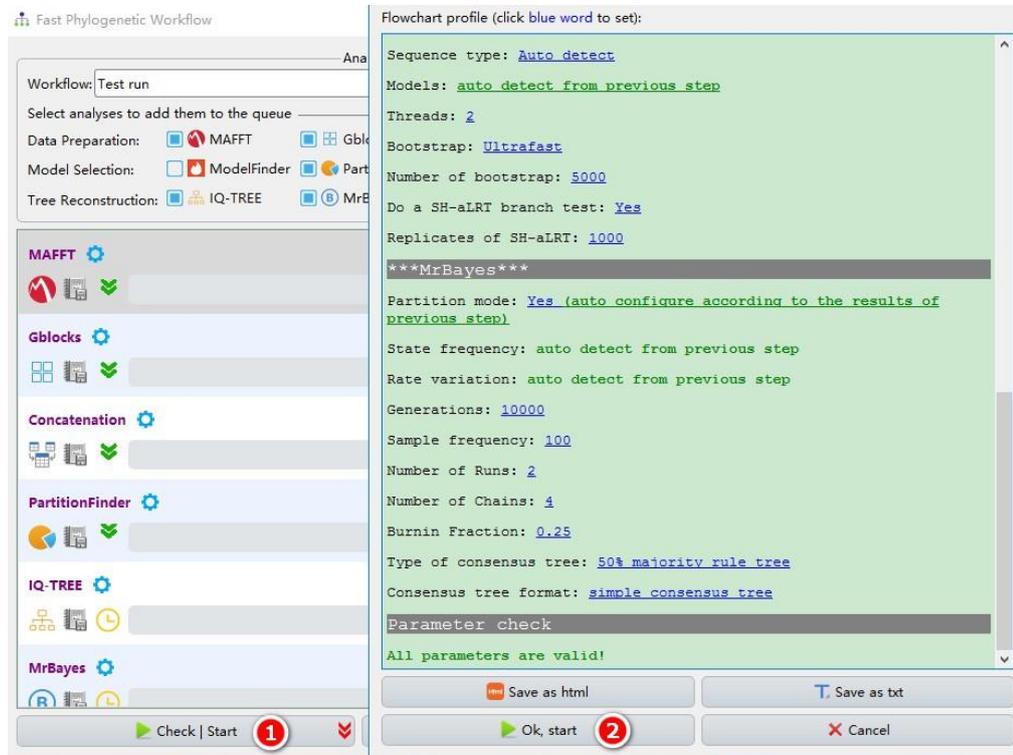


5. Tick **Extract-Seq** option and then tick **PCGs**, and the nucleotide sequences of protein-coding genes will be imported automatically;
6. To speed up the test, you may pop-up the files menu and remove all files but 'nad3.fas', 'nad4L.fas' and 'nad6.fas' files (using the **remove button** [red x sign]);
7. Select **2 Vertebrate mitochondrial code**;
8. Just close the window to save the imported files and parameters.



3.3.3 Start workflow

1. Click the **Check | Start** button, and the parameter summary window will pop up, allowing you to check and modify the parameters;
2. Click **Ok, start** to start the workflow.



3.3.4 Get workflow results

When all the tasks are finished, click **Ok** to view the results, double-click the folders in the **list of results** box to open the corresponding results:

The screenshot displays the PhyloSuite interface with six task progress bars, all showing 100% completion. A central dialog box titled 'Flowchart' contains a green checkmark and the text 'All the tasks finished!' with an 'OK' button. At the bottom, there are 'Running...' and 'Stop' buttons.

The screenshot shows the PhyloSuite desktop application. The top menu bar includes 'Flowchart', 'File', 'Alignment', 'Phylogeny', 'Mitogenome', 'Settings', 'WorkPlace', and 'About'. The main window is titled 'Name: Test run' and contains a log of the analysis process. A callout box highlights the 'list of results' section at the bottom, which contains folders for MAFFT, Gblocks, Concatenation, PartitionFinder, MrBayes, and IQ-TREE. The text 'Click these folder will open the corresponding results' is overlaid on this section.

Materials & methods with references

PhyloSuite (Zhang et al., 2018) was used to conduct, manage and streamline the analyses with the help of several plug-in programs:

3 sequences were aligned in batches with **MAFFT** (Katoh and Standley, 2013) using '--auto' strategy and codon alignment mode. Ambiguously aligned fragments of 3 alignments were removed in batches using **Gblocks** (Talavera and Castresana, 2007) with the following parameter settings: minimum number of sequences for a conserved/flank position (16/16), maximum number of contiguous non-conserved positions (5), minimum length of a block (10), allowed gap positions (with half). Best partitioning scheme and evolutionary models for 3 pre-defined partitions were selected using **PartitionFinder2** (Lanfear et al., 2017), with all algorithm and AICc criterion. Bayesian Inference phylogenies were inferred using **MrBayes 3.2.6** (Ronquist et al., 2012) under partition model (2 parallel runs, 10000 generations), in which the the initial 25% of sampled data were discarded as burn-in. Maximum likelihood phylogenies were inferred using **IQ-TREE** (Nguyen et al., 2015) under the model for 5000 ultrafast (Minh et al., 2013) bootstraps, as well as the Shimodaira-Hasegawa-like approximate likelihood-ratio test (Guindon et al., 2010).

References

Zhang, D., Gao, F., Li, W.X., Jakovljić, I., Zou, H., Zhang, J., and Wang, G.T. (2018). PhyloSuite: an integrated and scalable desktop platform for streamlined molecular sequence data management and evolutionary phylogenetics studies. *bioRxiv*, doi: 10.1101/489088.

Katoh, K., Standley, D.M., 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol. Biol. Evol.* 30, 772-780.

Talavera, G., Castresana, J., 2007. Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. *Syst. Biol.* 56, 564-577.

Lanfear, R., Frandsen, P. B., Wright, A. M., Senfeld, T., Calcott, B. (2016) PartitionFinder 2: new methods for selecting partitioned models of evolution formolecular and morphological phylogenetic analyses. *Molecular biology and evolution*. DOI: dx.doi.org/10.1093/molbev/msw260

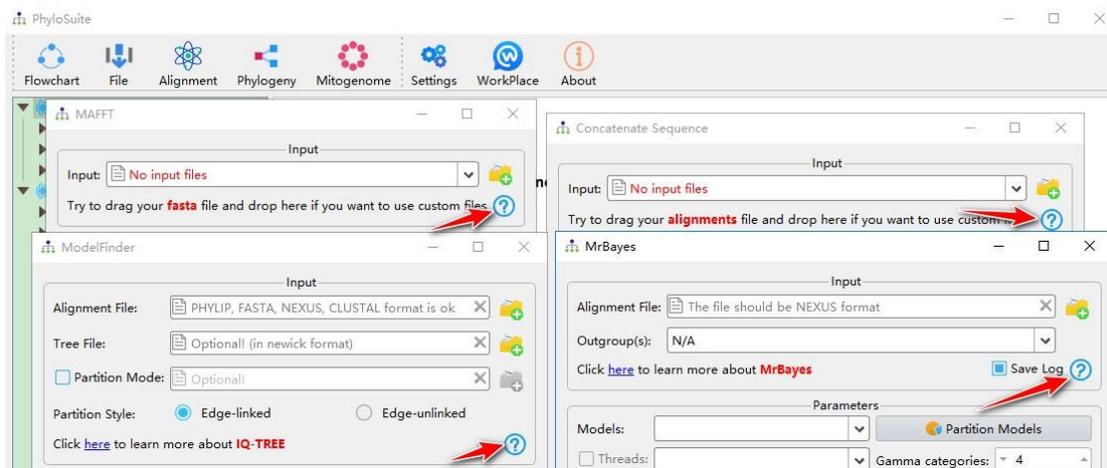
Scroll to the bottom of the results, and you will see that the whole workflow takes around 3 minutes (CPU: AMD Ryzen 7 1700; RAM: 8 G; OS: Windows 10 64 bit).

```
Flowchart start at: 2019-05-09 11:06:19.172226
Flowchart finish at: 2019-05-09 11:09:11.346319
Total time used for Flowchart: 0:02:52.174093
Time used for MAFFT: 0:00:05.153051
Time used for Gblocks: 0:00:00.133002
Time used for Concatenation: 0:00:00.050000
Time used for PartitionFinder: 0:02:26.088031
Time used for MrBayes: 0:00:11.684009
Time used for IQ-TREE: 0:00:20.614005
```

Note: to make this 'test run' very fast, I set a very low number of generations for MrBayes (10000) and IQ-TREE ultrafast bootstrap (5000).

4. Test run of each function

You may view a brief demo and/or test for each function via the inbuilt question mark button. For example:



5. Recommended reading

Other demo tutorials can be found [here](#).