HybPhyloMaker ‘rapid instructions’ (June 2026)

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1. Log in to skirit (skirit.ics.muni.cz aka brno2) MetaCentrum frontend

```
ssh metausername@skirit.ics.muni.cz
```

2. Clone HybPhyloMaker from GitHub and move HybSeqSource folder to home folder, download reference

```
git clone https://github.com/tomas-fer/HybPhyloMaker
```

```
mv HybPhyloMaker/HybSeqSource/ .
```

```
cd HybSeqSource
```

```
wget https://raw.githubusercontent.com/tomas-fer/HybSeqCourse/master/curcuma_HybSeqProbes_first958.fa
```

```
cd ../HybPhyloMaker
```

3. Copy test dataset (Hlavatá et al. 2023. Molecular Phylogenetics and Evolution 178, 107666)

```
cp -r /storage/projects2/hybseqcourse/Amomum .
```

OR (in case the command above is not working)

```
mkdir Amomum
```

```
cd Amomum
```

```
wget https://botany.natur.cuni.cz/fer/hybseqcourse/10rawreads.zip
```

```
unzip 10rawreads
```

```
rm 10rawreads.zip
```

```
cd ..
```

4. Edit file settings.cfg (Ctrl+W to search, Ctrl+O to save changes, Ctrl+X to exit editor)

```
nano settings.cfg
```

```
location=1
server=brno2
data=HybPhyloMaker/Amomum
OUTGROUP="Zingiber-officinale_S242"
mlbs=yes
combine=yes
MISSINGPERCENT=75
SPECIESPRESENCE=40
probes=curcuma_HybSeqProbes_first958.fa
raxmlperjob=1
phypartsbs=50
```

5. Prepare pseudoreference

```
qsub HybPhyloMaker0b_preparereference.sh
```

6. Install appropriate R packages (and wait until this and previous jobs are finished)

(HybPhyloMaker0b_preparereference.sh – need to be finished prior to running HybPhyloMaker2a_readmappingParallel.sh)

(HybPhyloMaker0c_Rsetup_MetaCentrum.sh – need to be finished prior to running HybPhyloMaker5_missingdataremoval.sh)

```
qsub HybPhyloMaker0c_Rsetup_MetaCentrum.sh #(this runs for ca. 45-60mins)
```

7. Filter the raw data

```
qsub HybPhyloMaker1a_rawprocessParallel.sh
```

8. Run this submitter created by the previous job (this script submits 35 jobs)

```
cd Amomum/20filtered/ && ./submitRawProcessJobs.sh && cd ../..
```

9. Run the summary script (after all jobs are finished)

```
qsub HybPhyloMaker1a2_rawprocessParallel_summary.sh
```

10. Map reads to pseudoreference

```
qsub HybPhyloMaker2a_readmappingParallel.sh
```

11. Run this submitter created by the previous job (this script submits 35 jobs)

```
cd Amomum/exons/21mapped_bwa/ && ./submitMappingJobs.sh && cd ../../..
```

12. Run the summary script (after all jobs are finished)

```
qsub HybPhyloMaker2a2_readmappingParallel_summary.sh
```

13. Generate PSLX files

```
qsub HybPhyloMaker3_generatepslx.sh
```

14. Copy PSLX files

```
mkdir pslx_Amomum2026
```

```
cp Amomum/exons/50pslx/*.pslx pslx_Amomum2026
```

15. Edit file settings.cfg

```
nano settings.cfg
```

```
otherpslx=HybPhyloMaker/pslx_Amomum2026
```

16. Generate exon assemblies

```
qsub HybPhyloMaker4a_processpslx.sh
```

17. Filter missing data

```
qsub HybPhyloMaker5_missingdataremoval.sh
```

18. Generate RAxML trees

```
qsub HybPhyloMaker6a_RAxML_for_selected.sh
```

19. Run this submitter created by the previous job (this script submits 246 jobs)

```
cd Amomum/exons/72trees75_40/RAxML/ && ./submitRAxMLjobs.sh && cd ../../../..
```

20. Run the summary script (after all jobs are finished)

```
qsub HybPhyloMaker6a2_RAxML_trees_summary.sh
```

21. Create tree files

```
qsub HybPhyloMaker7_roottrees.sh
```

22. Create diverse species trees (run all four, no need to wait…)

```
qsub HybPhyloMaker8a_astral.sh
```

```
qsub HybPhyloMaker8a2_astral4.sh
```

```
qsub HybPhyloMaker8b_astrid.sh
```

```
qsub HybPhyloMaker8c_mrl.sh
```

```
qsub HybPhyloMaker8e_concatenatedFastTree.sh
```

```
qsub HybPhyloMaker8f_concatenatedExaML.sh
```

23. After all jobs are finished run these additional scripts

```
qsub HybPhyloMaker8h_neighbourNetwork.sh
```

```
qsub HybPhyloMaker8k_quartetsampling.sh
```

```
qsub HybPhyloMaker8n_SNPheatmap.sh
```

```
qsub HybPhyloMaker11_phyparts.sh
```

```
qsub HybPhyloMaker15_combinetrees.sh
```

24. Edit file settings.cfg

```
nano ../HybSeqSource/configuration.txt
```

```
numsites = 330944

mrca = root Zingiber-officinale_S242 Amomum-biphyllum_S311

min = root 20.6

max = root 20.6
```

25. Date ASTRAL-IV (astral4) tree using treePL

```
qsub HybPhyloMaker14_treePL.sh
```

26. Make a subselection (19 samples as clade representatives), download excludelist.txt and set folder name

```
cp /storage/projects2/hybseqcourse/excludelist.txt ../HybSeqSource
```

```
nano settings.cfg
```

selection=forNetwork

OUTGROUP="Aframomum-melegueta_S70"

27. Run script to filter alignments

```
qsub HybPhyloMaker12_excludesamples.sh
```

28. Filter alignments, create gene trees and ASTRAL tree (run one by one)

```
qsub HybPhyloMaker5_missingdataremoval.sh
```

```
qsub HybPhyloMaker6a_RAxML_for_selected.sh
```

```
cd Amomum/exons/forNetwork/72trees75_40/RAxML/ && ./submitRAxMLjobs.sh && cd ../../../../..
```

```
qsub HybPhyloMaker6a2_RAxML_trees_summary.sh
```

```
qsub HybPhyloMaker7_roottrees.sh
```

```
qsub HybPhyloMaker8a_astral.sh
```

29. Install julia packages (necessary for network plotting)

```
qsub HybPhyloMaker0d_julia_setup_MetaCentrum.sh
```

30. Run PhyloNet analyses (takes approx. 8-16 hours)

```
qsub HybPhyloMaker8m_PhyloNet.sh
```

```
cd Amomum/exons/forNetwork/72trees75_40/RAxML/species_trees/PhyloNet &&
./submitPhyloNetjobs.sh && cd ../../../../../../..
```

```
qsub HybPhyloMaker8m2_PhyloNet_summary.sh
```