

## MAFFT

For fasta format:

```
mafft --6merpair --addfragments sars-cov-2.fas reference_sars_cov_2.fas > out_aln.fas
```

For clustal format

```
mafft --clustalout --6merpair --addfragments sars-cov-2.fas reference_sars_cov_2.fas >
```

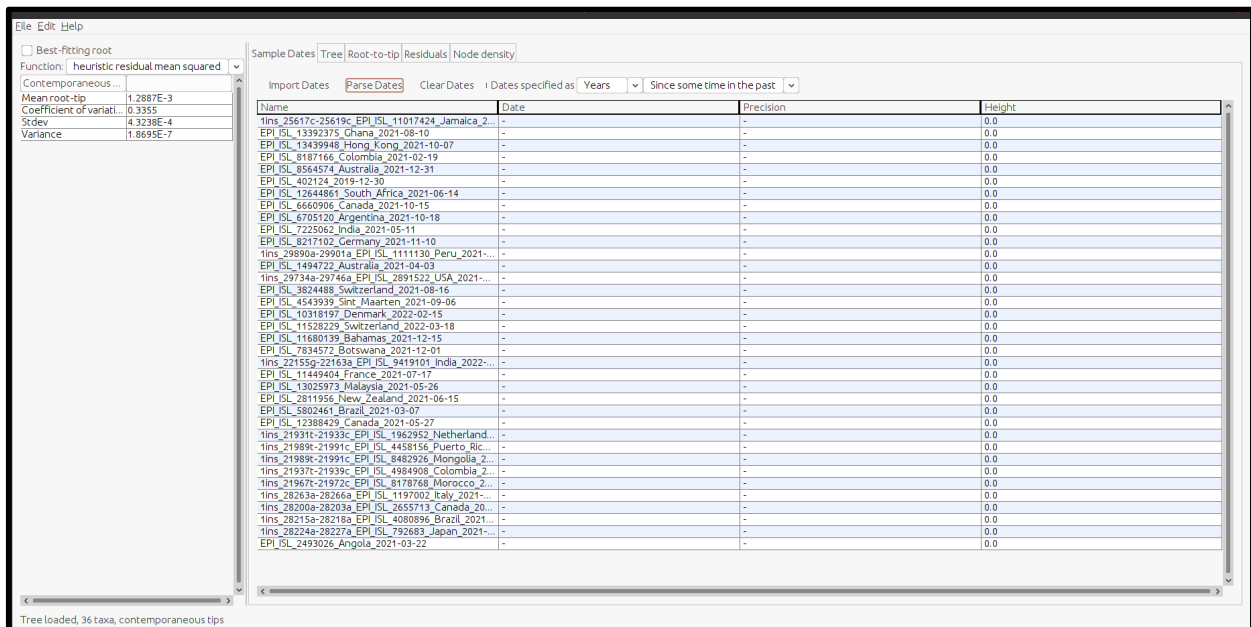
For identifying number of conserved nucleotides:

```
grep -o "*" out_aln | wc -l
```

## IQTREE

```
iqtree -s sars-cov-2_aln.fas -B 1000
```

## TempEst

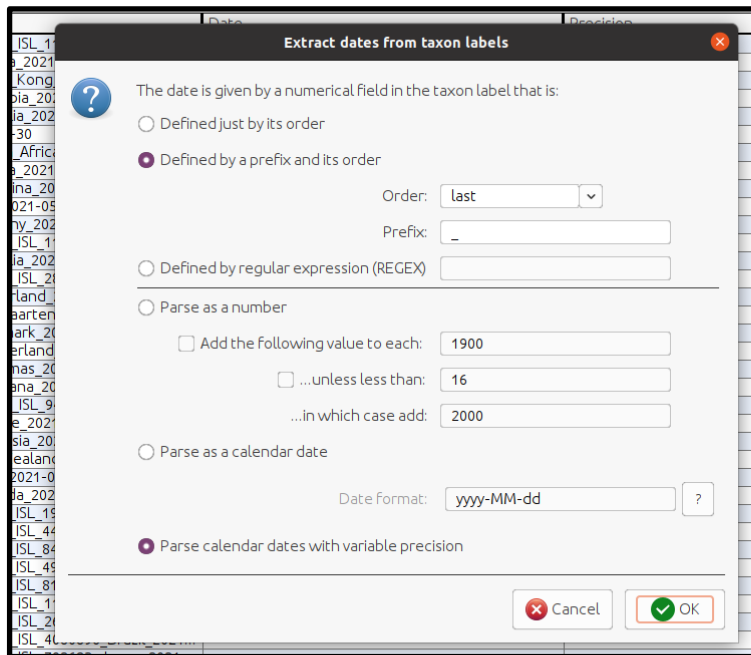


The screenshot displays the TempEst software interface. The main window shows a table of sample dates and their corresponding precision and height values. The table has four columns: Name, Date, Precision, and Height. The data is as follows:

Name	Date	Precision	Height
*ins_25617c-25619c_EPI_ISL_11017424_Jamaica_2...	-	-	0.0
EPI_ISL_13392375_Chana_2021-08-10	-	-	0.0
EPI_ISL_13439948_Hong_Kong_2021-10-07	-	-	0.0
EPI_ISL_8187166_Colombia_2021-02-19	-	-	0.0
EPI_ISL_8564574_Australia_2021-12-31	-	-	0.0
EPI_ISL_402124_2019-12-30	-	-	0.0
EPI_ISL_12644861_South_Africa_2021-06-14	-	-	0.0
EPI_ISL_6660906_Canada_2021-10-15	-	-	0.0
EPI_ISL_6705120_Argentina_2021-10-18	-	-	0.0
EPI_ISL_7225062_India_2021-05-11	-	-	0.0
EPI_ISL_8217102_Germany_2021-11-10	-	-	0.0
*ins_29890a-29901a_EPI_ISL_1111130_Peru_2021-...	-	-	0.0
EPI_ISL_1494722_Australia_2021-04-03	-	-	0.0
*ins_29734a-29745a_EPI_ISL_2891522_USA_2021-...	-	-	0.0
EPI_ISL_3824488_Switzerland_2021-08-16	-	-	0.0
EPI_ISL_4543939_Sint_Maarten_2021-09-06	-	-	0.0
EPI_ISL_10318197_Denmark_2021-02-15	-	-	0.0
EPI_ISL_11528229_Switzerland_2021-03-18	-	-	0.0
EPI_ISL_11680139_Bahamas_2021-12-15	-	-	0.0
EPI_ISL_7834572_Botswana_2021-12-01	-	-	0.0
*ins_22155g-22163a_EPI_ISL_9419101_India_2022-...	-	-	0.0
EPI_ISL_11449404_France_2021-07-17	-	-	0.0
EPI_ISL_13025973_Malaysia_2021-05-26	-	-	0.0
EPI_ISL_2811956_New_Zealand_2021-06-15	-	-	0.0
EPI_ISL_5802461_Brazil_2021-03-07	-	-	0.0
EPI_ISL_12388429_Canada_2021-05-27	-	-	0.0
*ins_21931t-21933c_EPI_ISL_1962952_Netherland...	-	-	0.0
*ins_21989t-21991c_EPI_ISL_4458156_Puerto_Ric...	-	-	0.0
*ins_21989t-21991c_EPI_ISL_8482926_Mongolia_2...	-	-	0.0
*ins_21937t-21939c_EPI_ISL_4984988_Colombia_2...	-	-	0.0
*ins_21967t-21972c_EPI_ISL_8178768_Morocco_2...	-	-	0.0
*ins_28263a-28266a_EPI_ISL_1197002_Italy_2021-...	-	-	0.0
*ins_28200a-28203a_EPI_ISL_2655713_Canada_20...	-	-	0.0
*ins_28215a-28218a_EPI_ISL_4090896_Brazil_2021...	-	-	0.0
*ins_28224a-28227a_EPI_ISL_792683_Japan_2021-...	-	-	0.0
EPI_ISL_2493026_Angola_2021-03-22	-	-	0.0

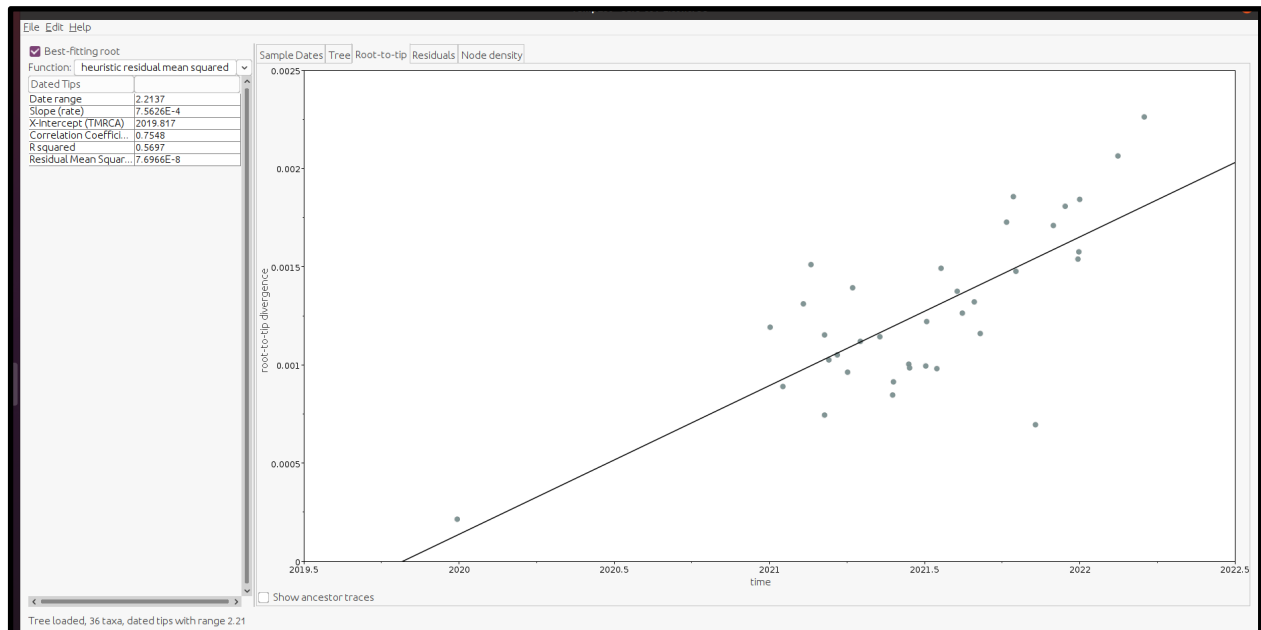
Select "Parse Dates" tab

For example, identifiers are in following format: where "\_" is used as a separator and last field is date of isolation **EPI\_ISL\_402124\_2019-12-30**



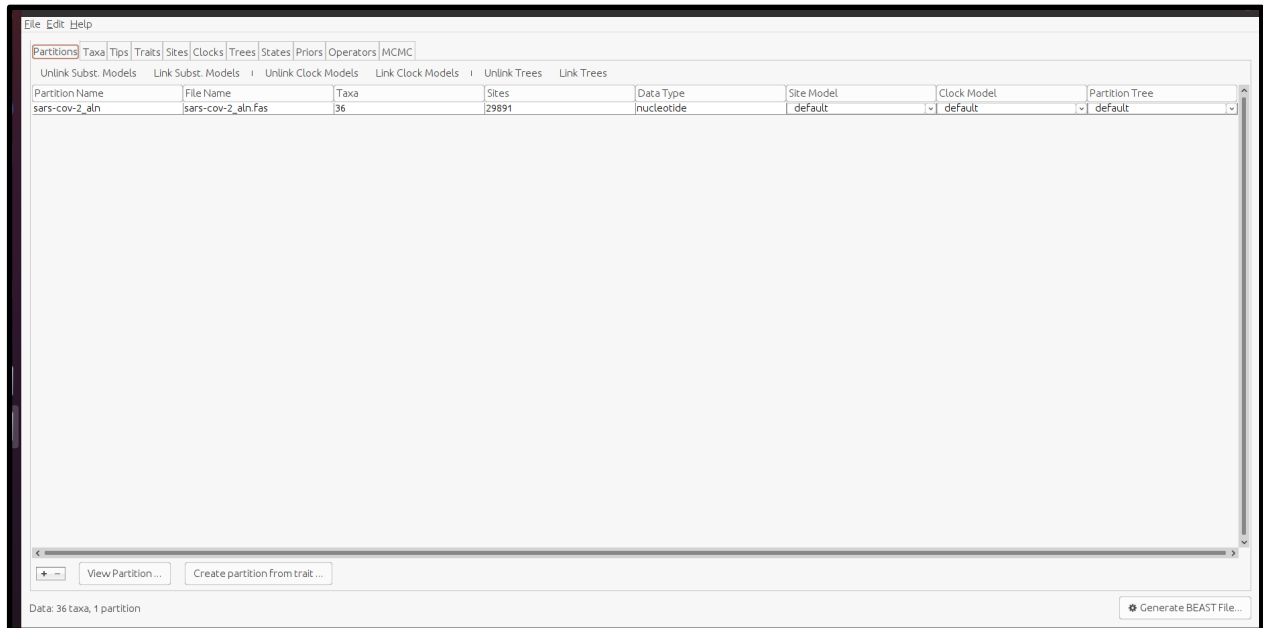
Check the **“Best-fitting root”** option (top left) and view the graph in **“Root-to-tip”** tab to understand clock behaviour

The value of **“Slope(Rate)”** is to be noted and used for BEAST run

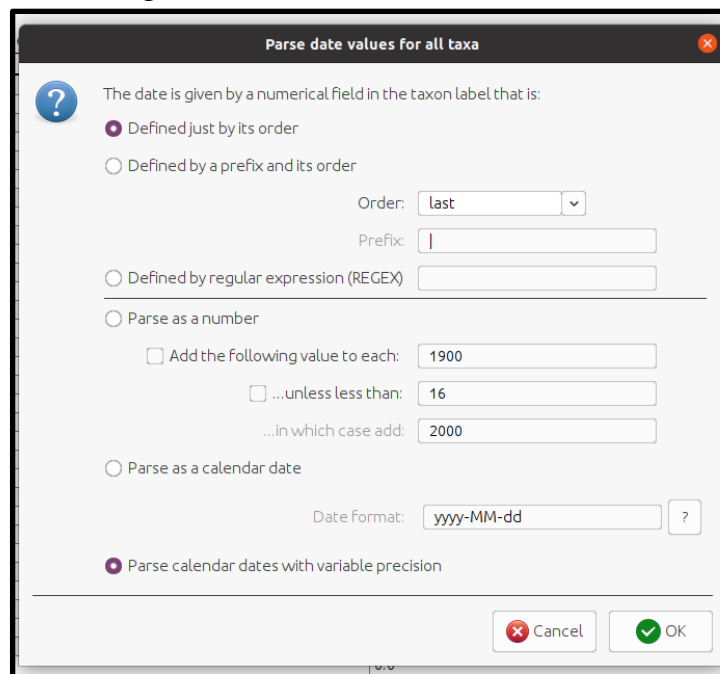


## BeauTi

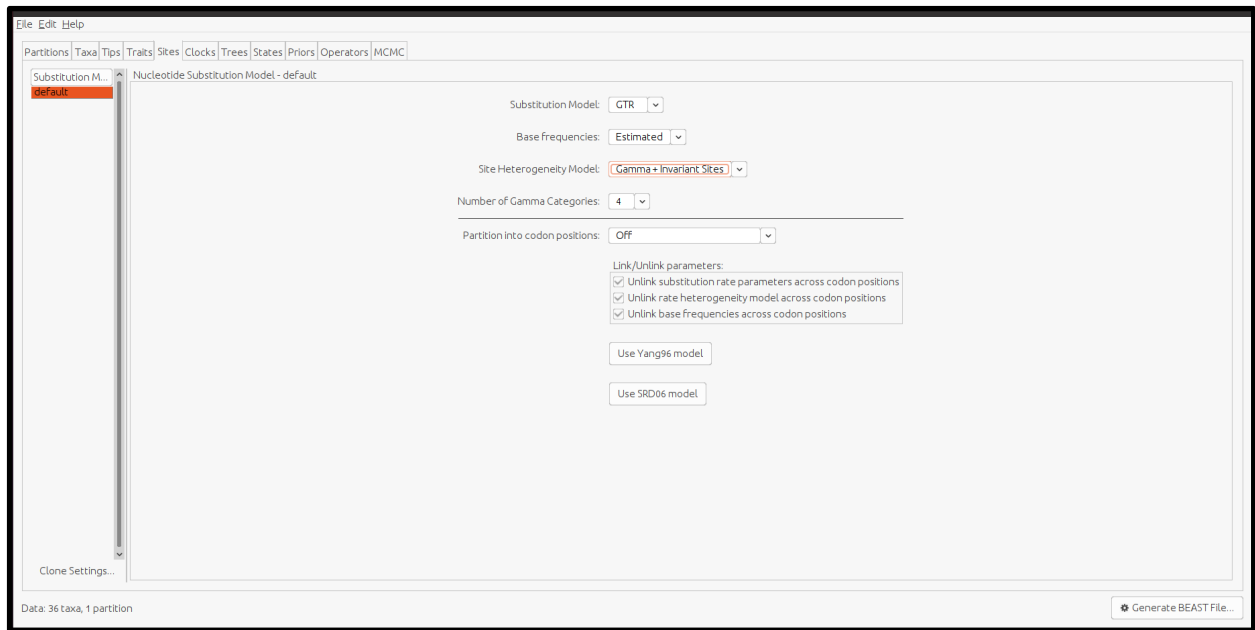
From File menu, load the alignment file (fasta format) using “Import data” option



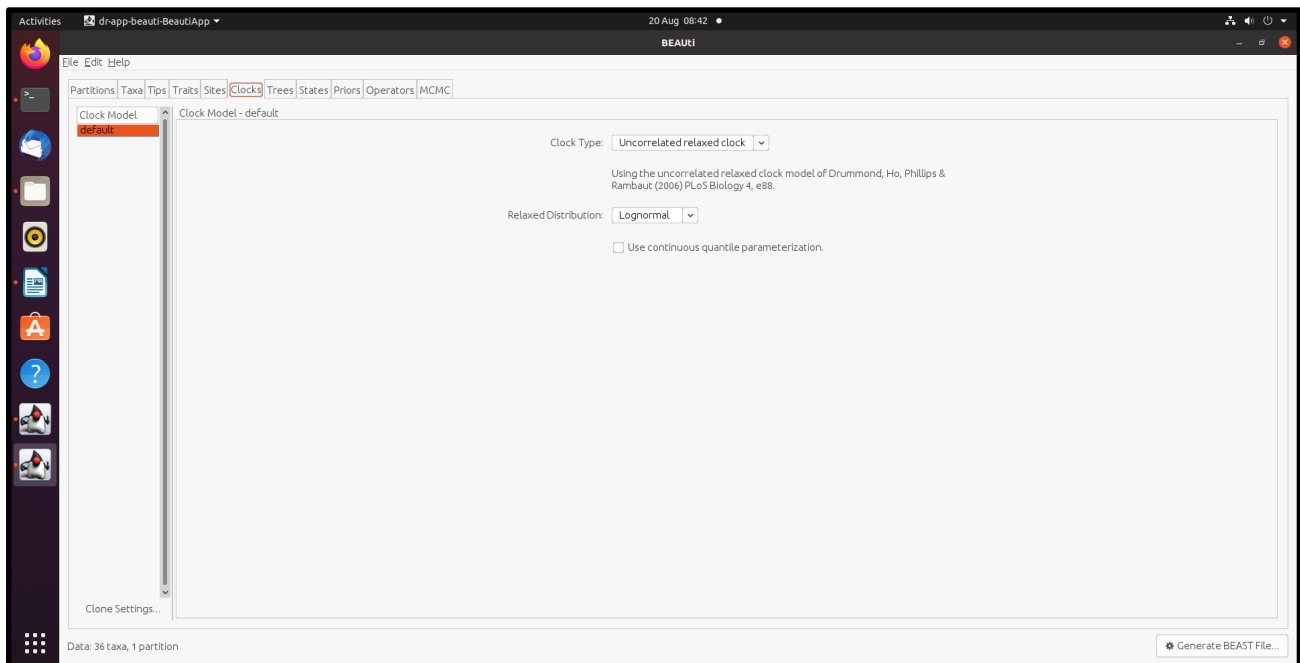
From “Tips” tab → Select “Use Tip dates” → Parse dates



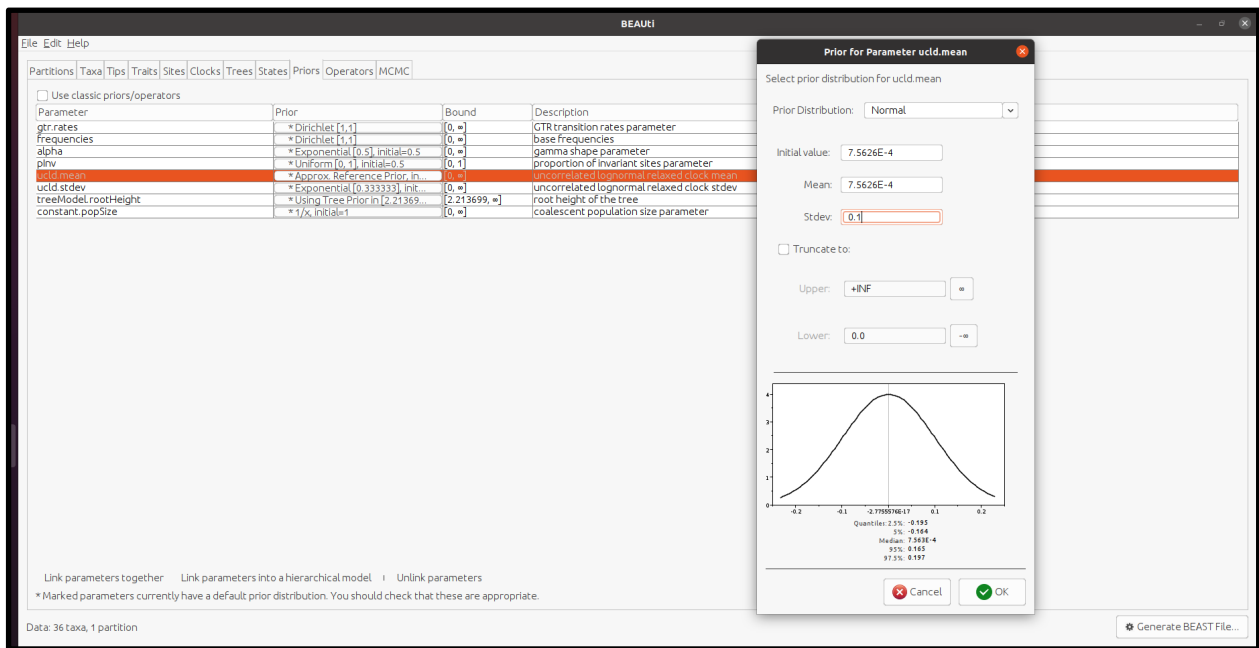
From “Sites tab” → Substitution Model: GTR; Base frequencies: Estimated; Site Heterogeneity model: Gamma + Invariant Sites



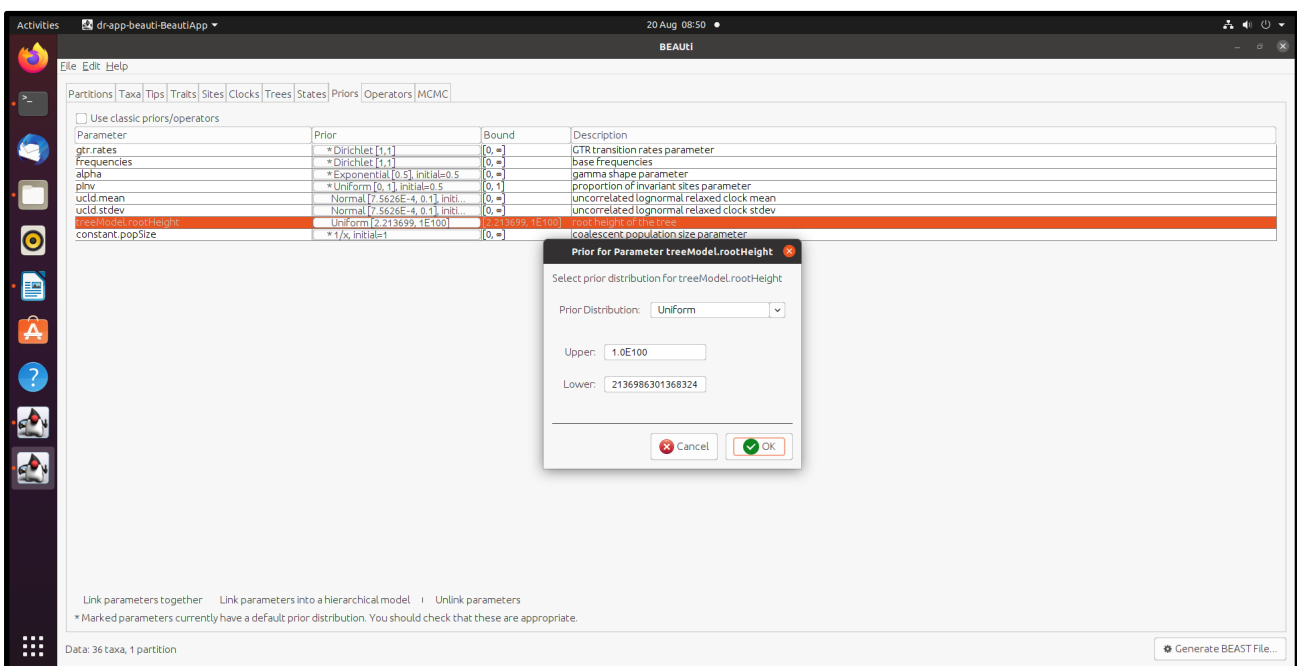
From “Clocks” tab → Clock Type: Uncorrelated relaxed clock; Distribution: Lognormal



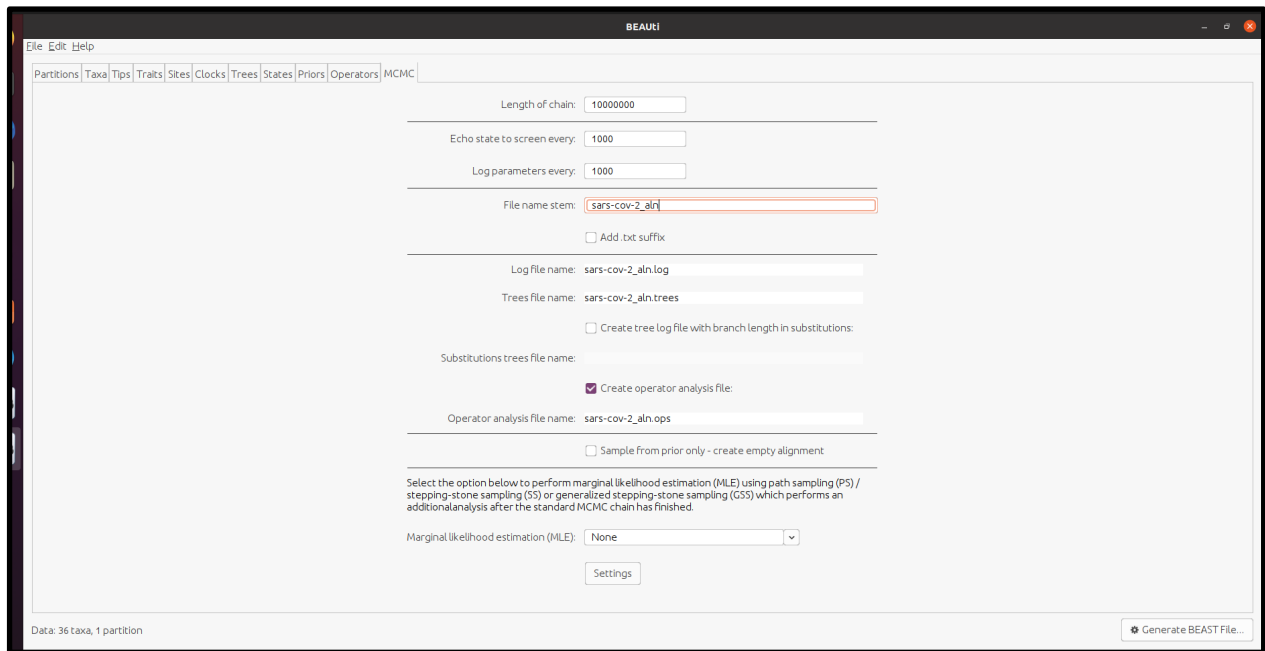
From “Priors tab” → Select “**uclid.mean**” and use the following values; repeat same for “**uclid.stdev**”



From “Priors tab” → Select “**treeModel.rootHeight**” and set “Prior Distribution” to **uniform**



From “MCMC” tab, use default parameters → Select “Generate BEAST File” (bottom right)



The output file is saved as “.xml” file

## BEAST

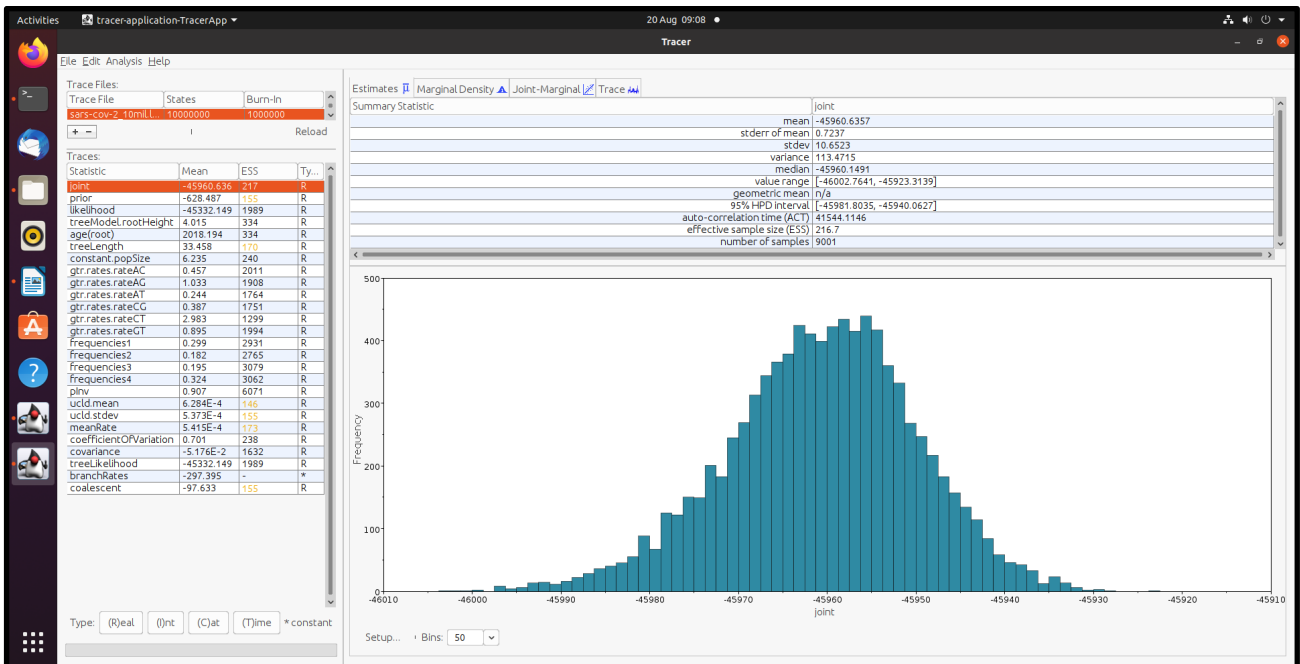
Choose the “.xml” file generated using BeauTi, run BEAST with all default options



The output files generated are: .log and .trees

## Tracer

Upload the log file generated using BEAST (select “+” symbol on left panel)



## Treannotator

Set “Burnin (as states)”: 1000; posterior probability limit: 0.8

“Input Tree file”: “.trees” file generated by BEAST

generate a blank out file

“Output File”: Select the blank file generated earlier

The screenshot shows the TreeAnnotator v1.10.4 Prerelease #bc6cbd9 dialog box. The 'Specify the burnin as the number of states' option is selected. The 'Burnin (as states)' field is set to 1000. The 'Posterior probability limit' field is set to 0.8. The 'Target tree type' is set to 'Maximum clade credibility tree'. The 'Node heights' are set to 'Median heights'. The 'Target Tree File' is 'not selected'. The 'Input Tree File' is 'sars-cov-2.trees.txt'. The 'Output File' is 'out.tree'. There are 'Run' and 'Quit' buttons at the bottom.

# Figtree

Set the following options from Control panel in Figtree (left panel)

- 1) Time Scale → Scale by factor → Offset by: 2022 (date of isolation of latest entry)
- 2) Node labels → Display → Node Ages
- 3) Scale Axis → Reverse Axis

Save the tree file

