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 >

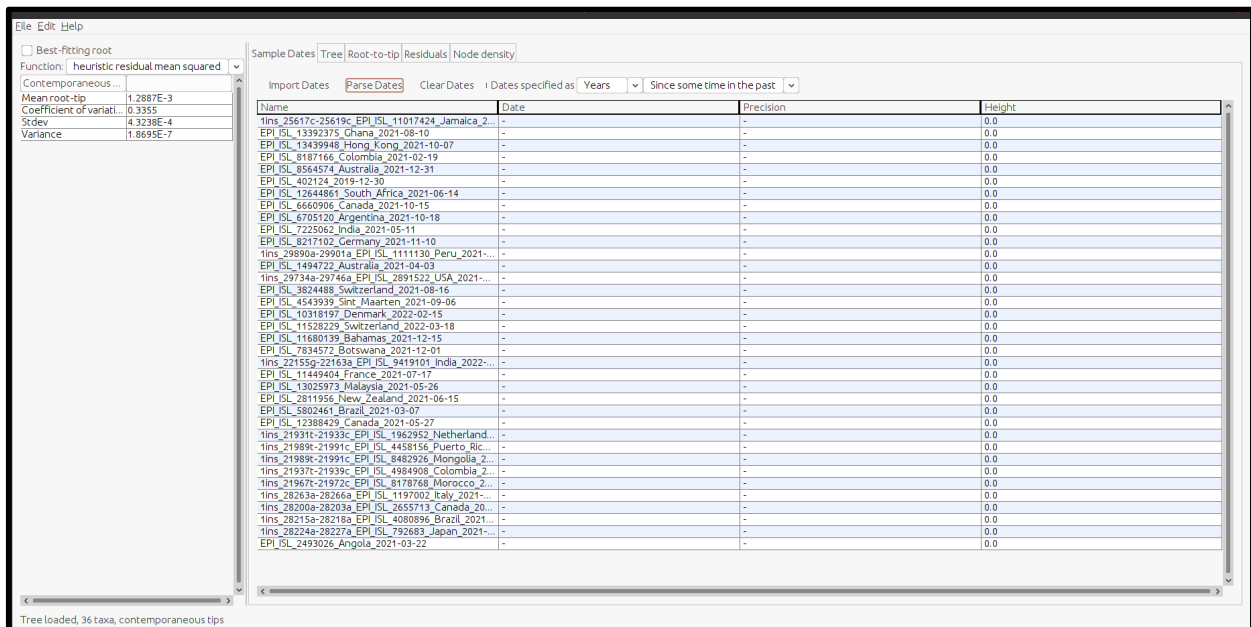For identifying number of conserved nucleotides:

grep -o "*" out_aln | wc -l

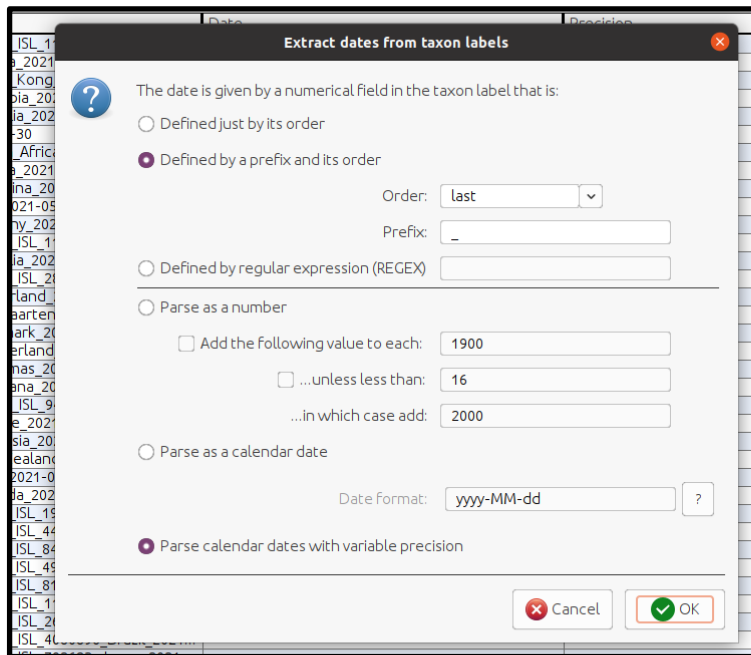## IQTREE

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

TempEst
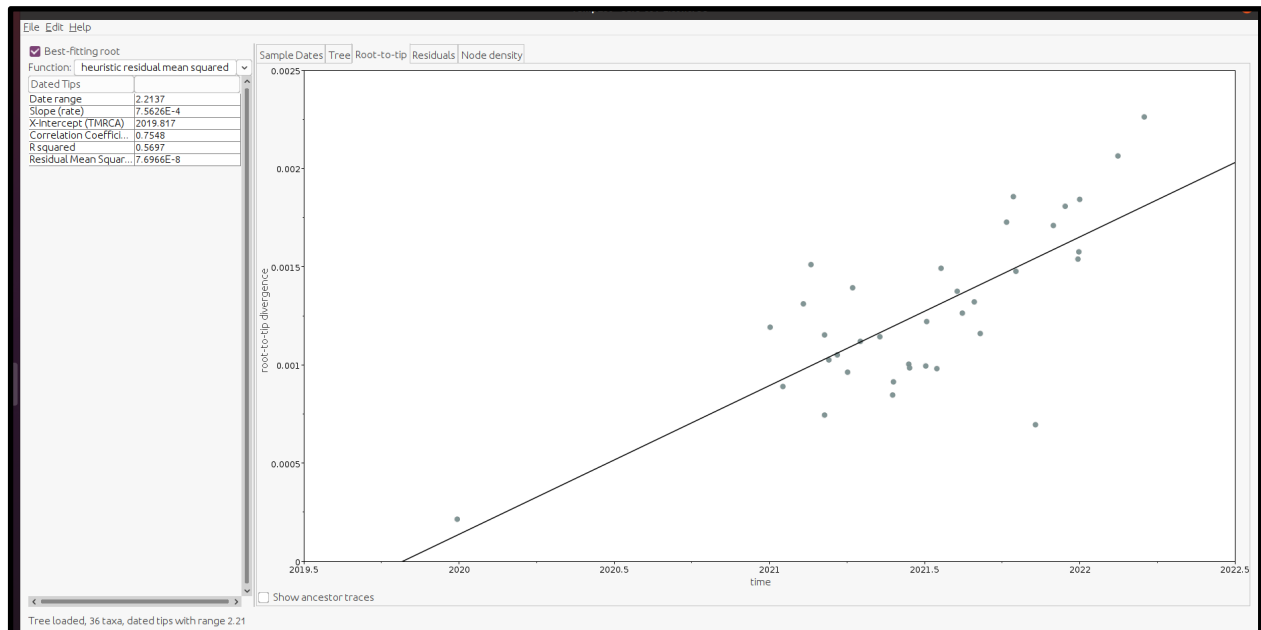

Select "Parse Dates" tab

For example, identifiers are in following format: where "_" is used as a seperator and last field is date of isolation **EPI_ISL_402124_2019-12-30**
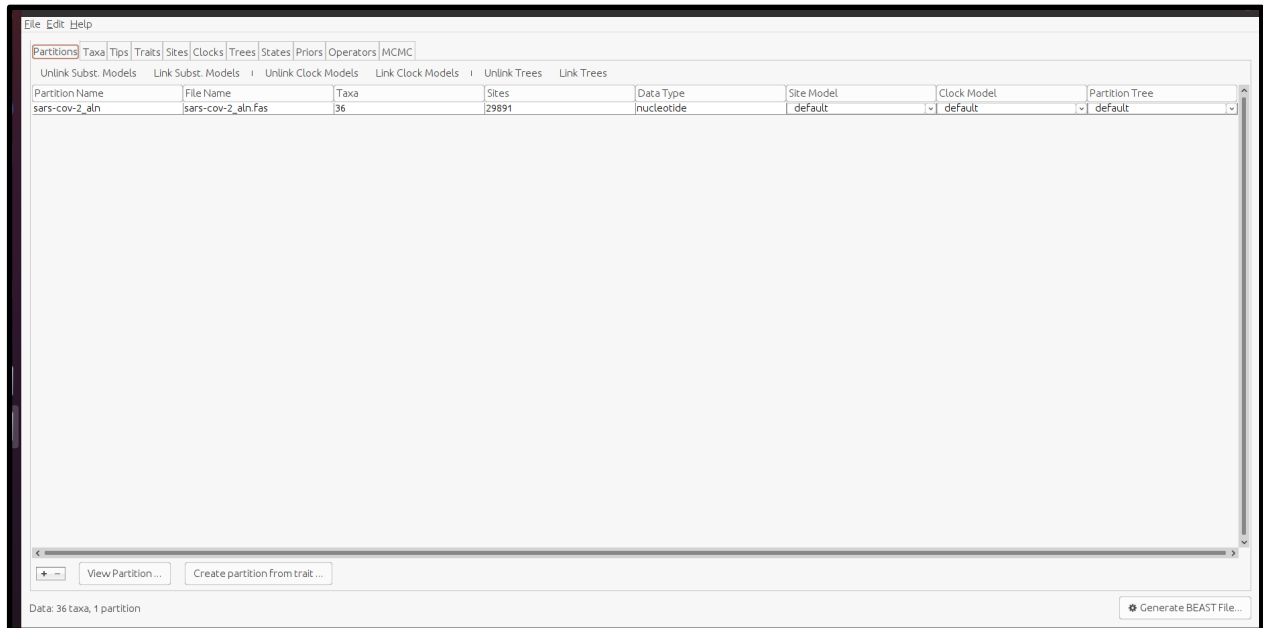
Check the "**Best-fitting root**" option (top left) and view the graph in "**Root-to-tip**" tab to understand clock behaviour

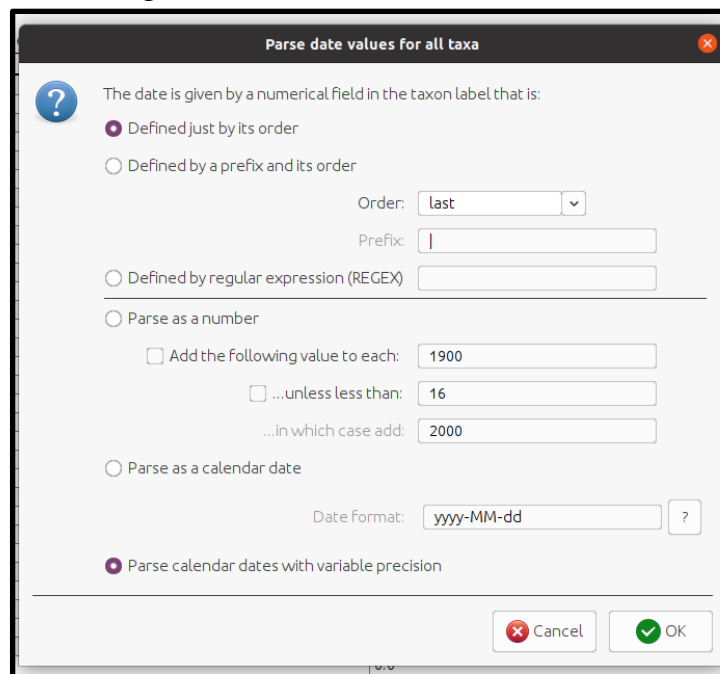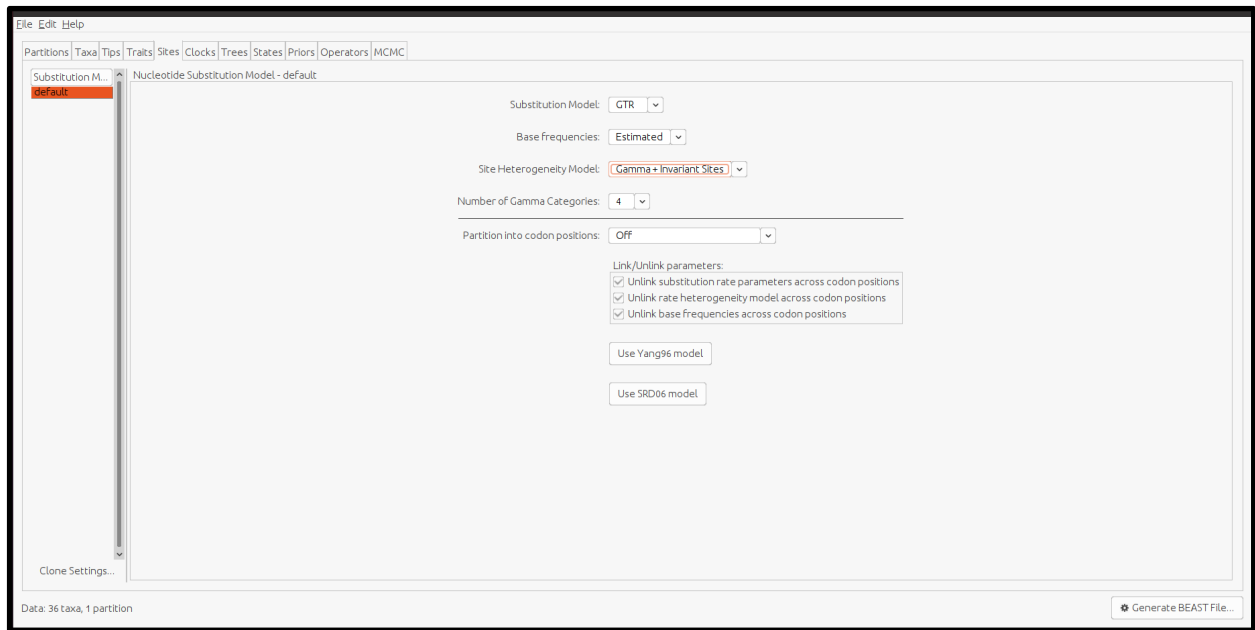The value of "**Slope(Rate)**" is to be noted and used for BEAST run

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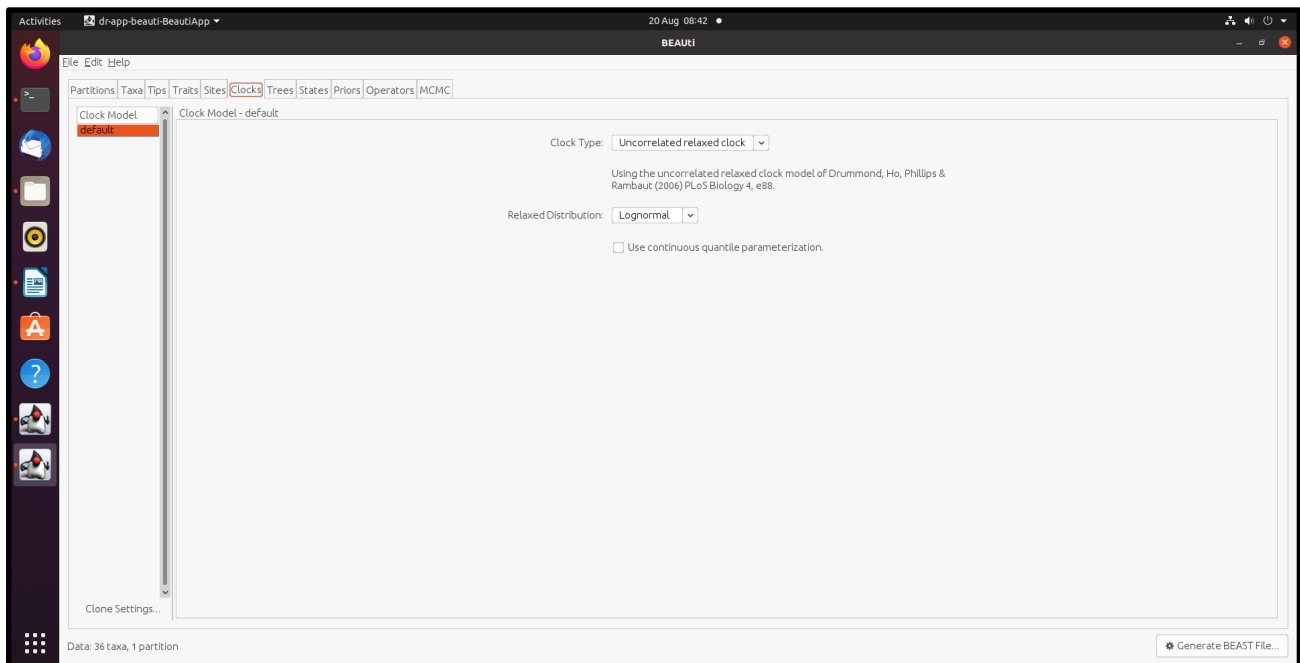From File menu, load the alignment file (fasta format) using "Import data" option

From "Tips" tab → Select "Use Tip dates" → Parse dates

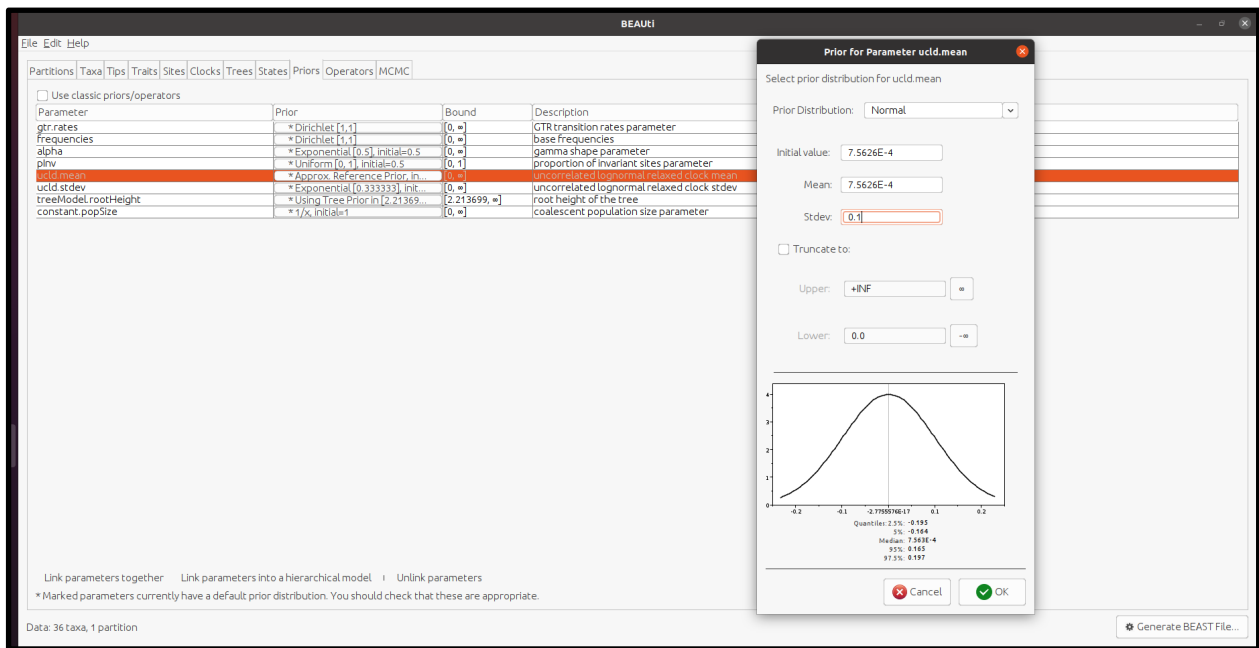From "Sites tab" → Substitution Model: GTR; Base frequencies: Estimated; Site Heterogenity model: Gamma + Invariant Sites
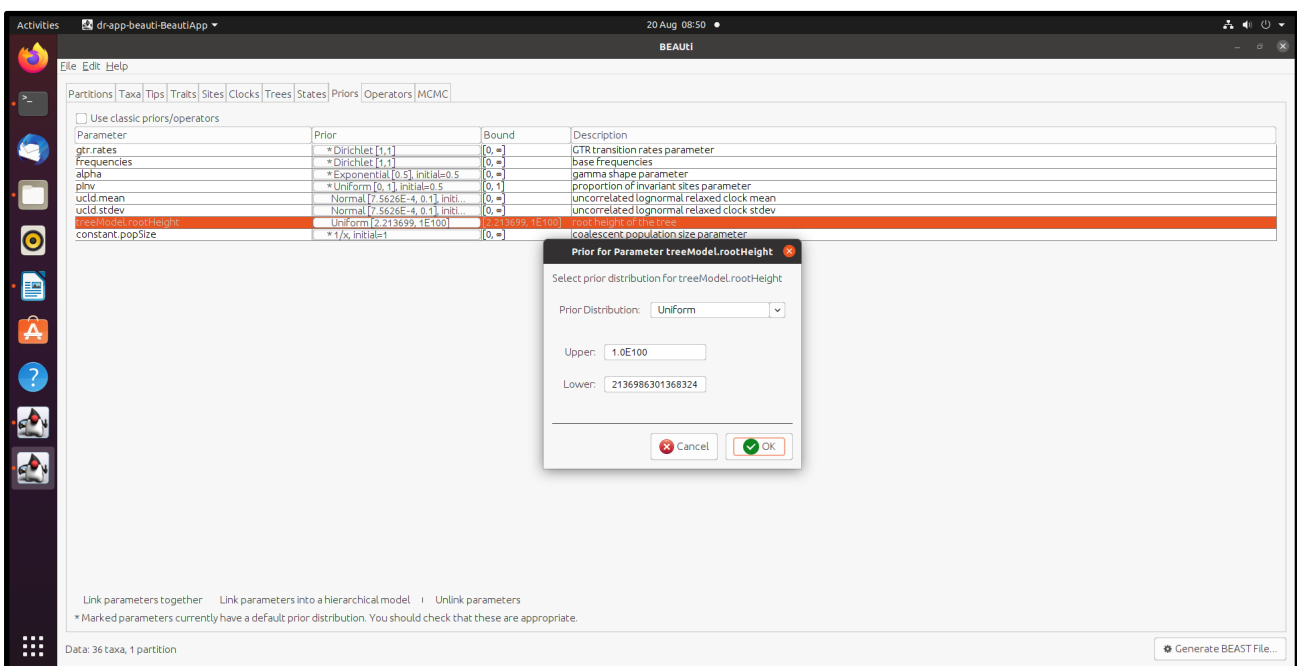
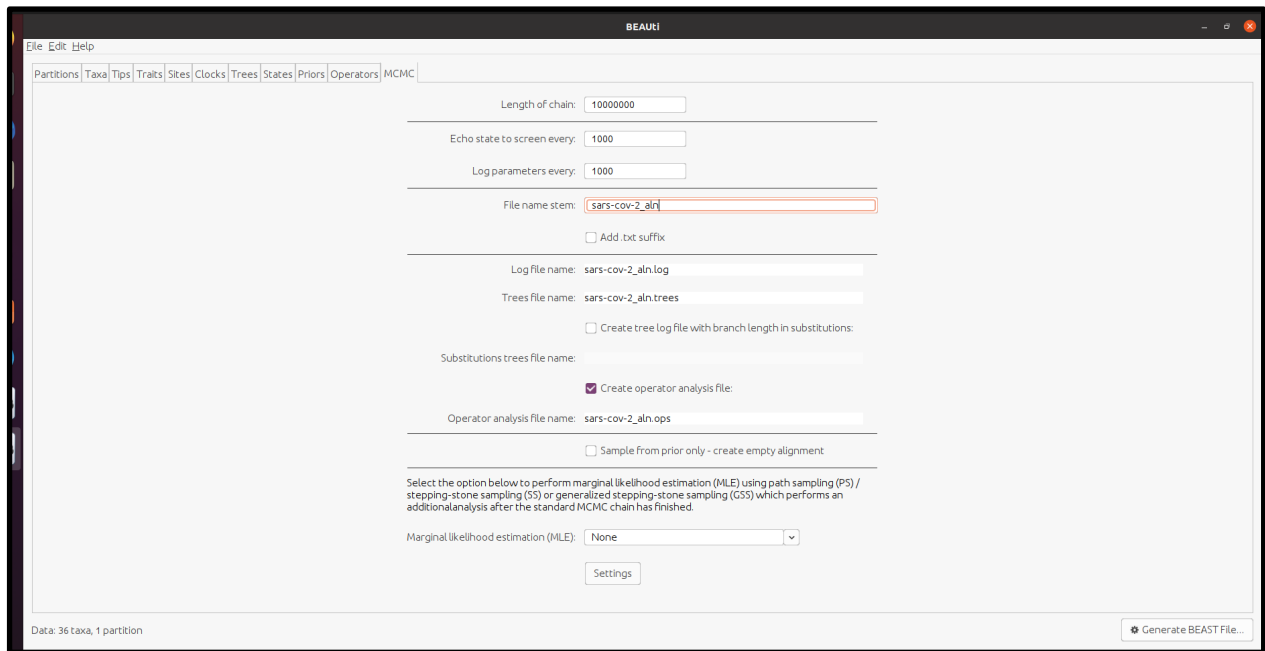From "Clocks" tab → Clock Type: Uncorrelated relaxed clock; Distribution: Lognormal

From "Priors tab" → Select "**ucld.mean**" and use the following values; repeat same for "u**cld.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)

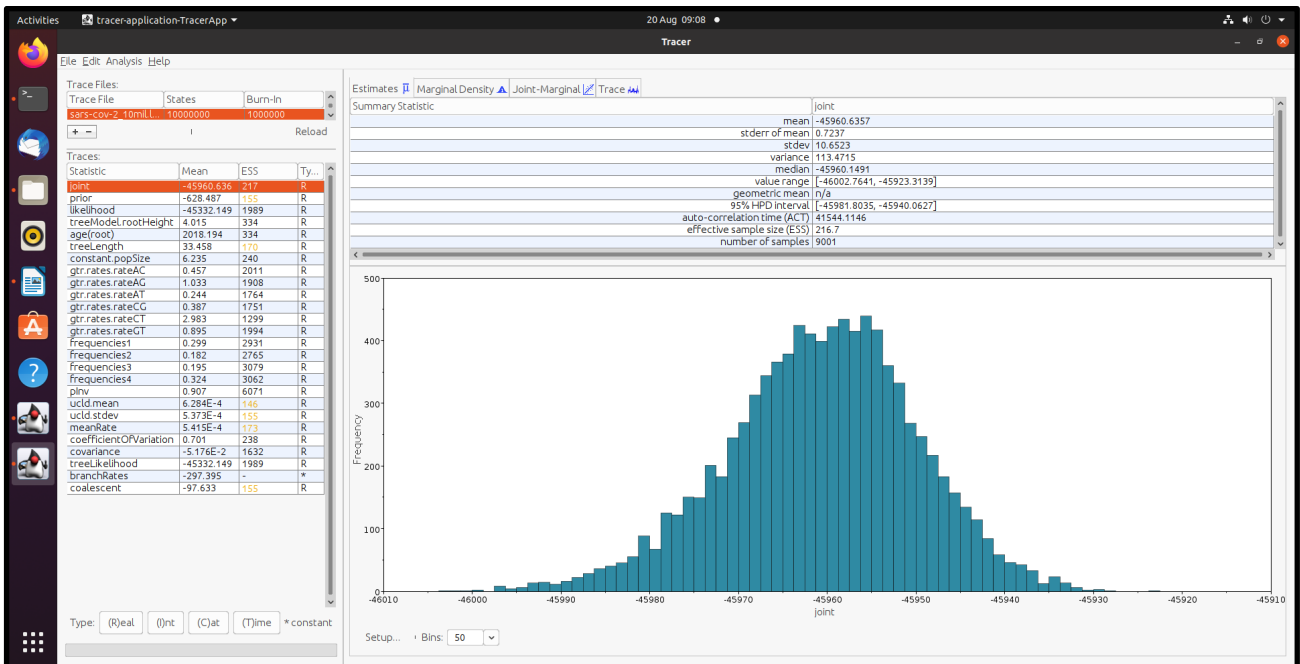The output file is saved as ".xml" file

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)

Treeannotator
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

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