

SARS-CoV-2 phylodynamics

Alignment of dataset sequences

```
echo ""
echo ""
echo "SARS-CoV-2 phylodynamic analysis"
echo ""
echo ""

echo ""
echo ""
echo "Alignment of sequences"

singularity exec /mnt/cive/csalazar/software/nextalign.sif nextalign \
-i derep_retrived_clean2_dataset1_headers.fasta \
-r /mnt/cive/csalazar/references/gisaid_hcov-19_2021_11_22_20.fasta \
-d nextalign_dataset1_clean2_fixed \
-j 32 > nextalign.log 2>&1

if [ -d "nextalign_dataset1_clean2_fixed" ]; then
echo "Alignment DONE!";
else
echo "Alignment FAILED!!";
fi
```

Construct initial tree topology using IQ-TREE

```
echo ""
echo ""
echo "ML tree"

conda activate nextstrain
cd nextalign_dataset1_clean2_fixed/
iqtree -s derep_retrived_clean2_dataset1_headers.aligned.fasta -T AUTO -m GTR+G > IQTree.log 2>&1

if [ -f "derep_retrived_clean2_dataset1_headers.aligned.fasta.treefile" ]; then
echo "ML phylogeny DONE!";
else
echo "ML phylogeny FAILED!!";
fi
```

Check molecular clock

Root-to-tip distance was also assessed using TempEst tool

```
echo ""
echo ""
```

```

echo "Check the molecular clock-like behavior"

treetime clock --tree *.fasta.treefile \
--dates ../fx_final_metadata-dataset1.tsv \
--aln *.aligned.fasta \
--outdir clock_results_filter3 --clock-filter 3 > treetime_clock_filter3.log 2>&1

if [ -d "clock_results_filter3" ]; then
echo "Temporal signal assessment DONE!";
else
echo "Temporal signal assessment FAILED!!";
fi

```

Create time-scaled phylogeny using the joint method of TreeTime

```

echo ""
echo ""
echo "Create time-scaled phylogeny using the joint model"

treetime --tree *.fasta.treefile \
--dates ../fx_final_metadata-dataset1.tsv \
--aln *.aligned.fasta \
--outdir treetime_out_joint_clean2 \
--reroot oldest \
--plot-tree dated_tree.png \
--plot-rtt rtt.png > treetime_joint.log 2>&1

if [ -d "treetime_out_joint_clean2" ]; then
echo "Time-scaled phylogeny using the joint model DONE!";
else
echo "Time-scaled phylogeny using the joint model FAILED!!";
fi

```

Ancestral state reconstruction

```

echo ""
echo ""
echo "Ancestral state reconstruction"

treetime ancestral --aln *.aligned.fasta \
--tree treetime_out_joint_clean2/timetree.nexus \
--outdir treetime_out_joint_clean2/ancestral_results > treetime_ancestral.log 2>&1

if [ -d "treetime_out_joint_clean2/ancestral_results" ]; then
echo "Ancestral state reconstruction DONE!";
else
echo "Ancestral state reconstruction FAILED!!";
fi

```

Transition between geographical locations

```

echo ""
echo ""
echo "Transition between geographical regions estimation"

```

```

treetime migration --tree treetime_out_joint_clean2/ancestral_results/annotated_tree.nexus \
--states ../fx_final_metadata-dataset1.tsv \
--attribute location \
--confidence \
--outdir treetime_out_joint/joint_migration_region \
--missing-data MD > treetime_migration_region.log 2>&1

if [ -d "treetime_out_joint/joint_migration_region" ]; then
echo "Migration using Region DONE!";
else
echo "Migration using Region FAILED!!";
fi

echo ""
echo ""
echo "Transition between geographical subregions estimation"

treetime migration --tree treetime_out_joint_clean2/ancestral_results/annotated_tree.nexus \
--states ../fx_final_metadata-dataset1.tsv \
--attribute subregion \
--confidence \
--outdir treetime_out_joint/joint_migration_region \
--missing-data MD > treetime_migration_subregion.log 2>&1

if [ -d "treetime_out_joint/joint_migration_region" ]; then
echo "Migration using subregion DONE!";
else
echo "Migration using subregion FAILED!!";
fi

```