

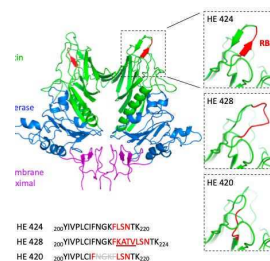
Sep 23, 2022

Version 3

Phylogenetic Analysis of Complete Bovine Coronavirus Genome Sequences V.3

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Protocol status: Working

We use this protocol and it's working

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Protocol Integer ID: 70350

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Abstract

Bovine coronavirus (BCoV) has spilled over to many species, including humans, where the host range variant coronavirus OC43 is endemic. Balance of the opposing activities of the surface spike (S) and hemagglutinin esterase (HE) glycoproteins control virion avidity which is critical for interspecies transmission and host adaptation. Here, 78 genomes were sequenced directly from clinical samples collected between 2013 and 2022 from cattle in 12 states, primarily in the Midwestern U.S. Relatively little genetic diversity was observed, with genomes having >98% nucleotide identity. Eleven isolates collected between 2020 and 2022 from four states (Nebraska, Colorado, California, and Wisconsin) contained a 12-nucleotide insertion in the receptor-binding domain (RBD) of the HE gene identical to one recently reported in China, and a single genome from Nebraska collected in 2020 contained a novel 12-nucleotide deletion in the HE gene RBD. Isogenic HE proteins containing either the insertion or deletion in the HE RBD maintained esterase activity and the ability to bind bovine submaxillary mucin, a substrate enriched in the receptor 9-*O*-acetylated-sialic acid, despite modeling that predicted structural changes in the HE R3 loop critical for receptor binding. The emergence of BCoV with structural variants in the RBD raises the possibility of further interspecies transmission.

Software

1

Software

MAFFT

NAME

Kazutaka Katoh

DEVELOPER

<https://mafft.cbrc.jp/alignment/software/>

SOURCE LINK

2

Software

RAxML

NAME

UBUNTU Linux ` 20.4

OS

Alexandros Stamatakis

DEVELOPER

<https://github.com/stamatak/standard-RAxML>

REPOSITORY

<https://cme.h-its.org/exelixis/web/software/raxml/>

SOURCE LINK

3



Software

FAST --- Fast Analysis of Sequences Toolbox NAME

UBUNTU Linux 22 LTS

OS

Travis Lawrence

DEVELOPER

[Github](#)

REPOSITORY

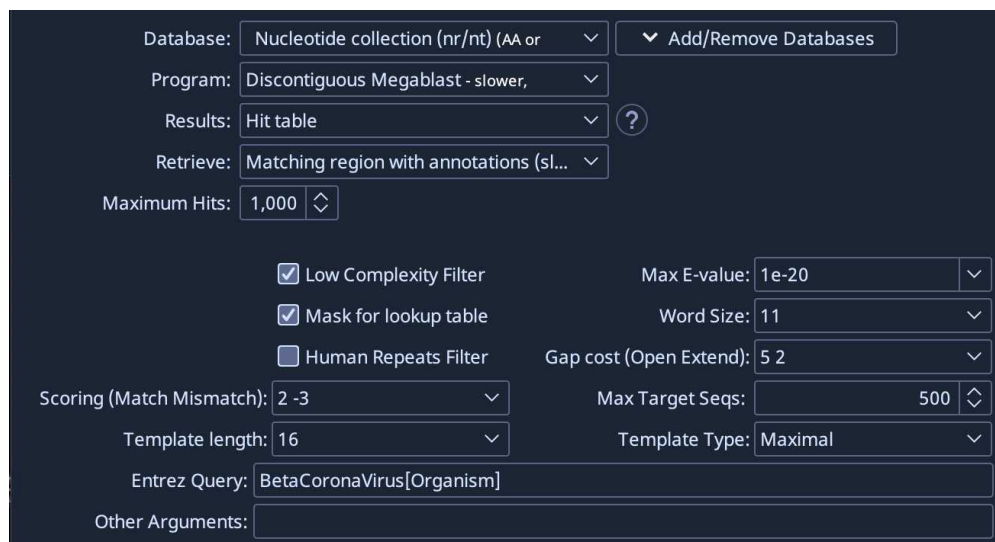
<https://github.com/tlawrence3/FAST>

SOURCE LINK

BLAST

- 4 Extract consensus HE sequence from the MAFFT aligned 192 whole genomes (see below) using Geneious

Discontiguous MegaBLAST input:  HE_realigned_consensus_sequenc...



Discontiguous MegaBLAST of BCoV HE consensus sequence with E-value of E-20 against all BetaCoronavirus sequences in the GenBank nt database

Output:  BetaCoronaVirus_HE_BlastMatch_B... This list of 844 subject sequences (hits) was reduced to 714 by requiring that each hit covered at least 98.4% (1255 bp) of query HE sequence resulting in the following FASTA file :



BetaCoronaVirus_HE_GE_1255_BP_....

This file was filtered for sequences with "bovine coronavirus" in the FASTA description field yielding :



345_Sequences_Bovine_HE_only.fa...

MAFFT Alignment

5 Align using MAFFT v7.450

The sequences were aligned using the MAFFT accuracy methods ---globalpair as defined in the mafft manual page below

Note

DESCRIPTION

MAFFT is a multiple sequence alignment program for unix-like operating systems. It offers a range of multiple alignment methods.

Accuracy-oriented methods:

- L-INS-i (probably most accurate; recommended for <200 sequences; iterative refinement method incorporating local pairwise alignment information):

mafft --localpair --maxiterate 1000input [> output]

linsi input [> output]

- G-INS-i (suitable for sequences of similar lengths; recommended for <200 sequences; iterative refinement method incorporating global pairwise alignment information):

mafft--globalpair--maxiterate1000input [> output]

ginsi input [> output]

Keep in mind ...

1. The commands below generate files that contain both STDOUT (mafft output to the "terminal") as well as the multifasta alignment file (.afa). The STDOUT is found at the beginning of the file and the alignments follow the STDOUT
2. All .afa_out files were split in a text editor into two separate .out and .afa files for downstream analysis.
3. The input FASTA file headers contain description information; this header information should be stripped out to facilitate readable, compact leaf names/identifiers. Use the following command to strip out the description text.

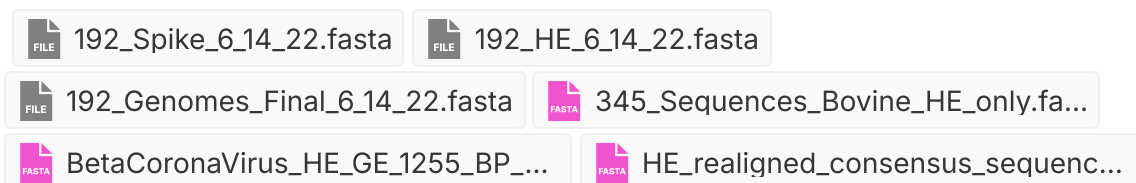
Command

Strip out description text from multifasta alignment file (UBUNTU Linux)

sed to strip out the description in the header from FASTA

```
sed '/^>/ s/ .*//' input_alignment_multifasta_file.afa >  
output_alignment_multifasta_file.SEQID_Only.afa
```

5.1 The input FASTA files are:



The spike and HE genes were extracted from the annotated genomes into the file of fasta sequences above. There are duplicated sequences within 192_HE_6_22.fasta as well as within 192_Spike_6_14_22.fasta. The genome sequences within 192_Genomes_Final_6_14_22.fasta are unique.

The spike and HE FASTA sequence files were deduplicated with **fassort** & **fasuniq** from the FAST Analysis of Sequences Toolbox. The unique sequences present in multiple genomes will be designated with a sequence identifier comprised of a concatenation of the sequence identifiers used in the multiple genomes separated by a "_" or ":". For

example, the HE fasta sequence id IWT-11:SHG-6:TCG-21:TCG-23:TCG-22 represents the identical HE fasta sequences IWT-11, SHG-6, TCG-21, TCG-23 and TCG-22.

Command

Create a file of unique HE FASTA sequences. The unique sequences present in multiple genomes will be designated with a sequence identifier comprised of a concatenation of the sequence identifiers used in the multiple genomes, separated by a double underscore "__" (UBUNTU Linux)

Create file of unique spike FASTA sequences

```
fassort -s 192_HE_6_14_22.fasta | fasuniq --concat='__' >
unique_HE_6_14_22.fasta
```



unique_HE_6_14_22.fasta

Command

Create a file of unique Spike FASTA sequences. The unique sequences present in multiple genomes will be designated with a sequence identifier comprised of a concatenation of the sequence identifiers used in the multiple genomes, separated by a double underscore "__" (UBUNTU Linux)

Create file of unique Spike FASTA sequences

```
fassort -s 192_Spike_6_14_22.fasta | fasuniq --concat='__' >
unique_Spike_6_14_22.fasta
```



unique_Spike_6_14_22.fasta

Command

new command name

```
fassort -s 345_Sequences_Bovine_HE_only.fasta | fasuniq --concat='__'
> unique_345_Sequences_Bovine_HE_only.fasta
```



unique_345_Sequences_Bovine_HE...

Command

new command name

```
fassort -s  
BetaCoronaVirus_HE_GE_1255_BP_BlastMatch_Bovine_HE_Consensus.fasta |  
fasuniq --concat="__" >  
unique_BetaCoronaVirus_HE_GE_1255_BP_BlastMatch_Bovine_HE_Consensus.fasta
```



unique_BetaCoronaVirus_HE_GE_12...

- 5.2 Remove description text (all text after sequence id) in HE , Spike, and Genomes fasta files.

Command

sed to strip out the description in the header from FASTA (UBUNTU Linux)

```
sed '/^>/ s/ .*//' unique_HE_6_14_22.fasta >  
unique_HE_6_14_22.SEQID_Only.fasta
```

output :



unique_HE_6_14_22.SEQID_Only.fasta

**Command**

sed to strip out the description in the header from FASTA (UBUNTU Linux)

```
sed '/^>/ s/ .*//' unique_HE_6_14_22.fasta >  
unique_HE_6_14_22.SEQID_Only.fasta
```

output:  unique_Spike_6_14_22.SEQID_Only.f...

Command

sed to strip out the description in the header from FASTA (UBUNTU Linux)

```
sed '/^>/ s/ .*//' 192_Genomes_Final_6_14_22.fasta >  
192_Genomes_Final_6_14_22.SEQID_Only.fasta
```

output:  192_Genomes_Final_6_14_22.SEQID...

Command

new command name

```
sed '/^>/ s/ .*//' unique_345_Sequences_Bovine_HE_only.fasta >  
unique_345_Sequences_Bovine_HE_only.SEQID_Only.fasta
```



unique_345_Sequences_Bovine_HE...

Command

new command name

```
sed '/^>/ s/ .*//'  
unique_BetaCoronaVirus_HE_GE_1255_BP_BlastMatch_Bovine_HE_Consensus.fasta >  
unique_BetaCoronaVirus_HE_GE_1255_BP_BlastMatch_Bovine_HE_Consensus_Se  
qID_Only.fasta
```



unique_BetaCoronaVirus_HE_GE_12...

5.3 Align 192 **BCV** genomes using the default **PAM (JTT)** 200 substitution matrix

**Command****new command name**

```
nohup mafft --thread 25 --globalpair --maxiterate 1000 --jtt 200 --  
reorder 192_Genomes_Final_6_14_22.SEQID_Only.fasta >  
192_Genomes_Final_6_14_22.SEQID_Only.jtt_200.globalpair.afa_out &
```

outputs :

 192_Genomes_Final_6_14_22.jtt_20...

This was split into

 192_Genomes_Final_6_14_22.jtt_20...

&

 192_Genomes_Final_6_14_22.jtt_20...**5.4 Align 192 BCV genomes using the PAM (JTT) 100 substitution matrix****Command****new command name**

```
nohup mafft --thread 30 --globalpair --maxiterate 1000 --jtt 100 --  
reorder 192_Genomes_Final_6_14_22.SEQID_Only.fasta >  
192_Genomes_Final_6_14_22.jtt_100.globalpair.SEQID_Only.afa_out &
```

outputs :

 192_Genomes_Final_6_14_22.jtt_100...

This was split into

 192_Genomes_Final_6_14_22.jtt_100...

&

 192_Genomes_Final_6_14_22.jtt_100...

5.5 Align **SPIKE** using the default **PAM (JTT) 200** substitution matrix

Command

new command name

```
nohup mafft --thread 15 --globalpair --maxiterate 1000 --jtt 200 --  
reorder unique_Spike_6_14_22.SEQID_Only.fasta >  
unique_Spike_6_14_22.SEQID_Only.jtt_200.globalpair.afa_out &
```

Outputs :

 unique_Spike_6_14_22.SEQID_Only.j...

This was split into

 unique_Spike_6_14_22.SEQID_Only.j...

&

 unique_Spike_6_14_22.SEQID_Only.j...

5.6 Align **SPIKE** using the **PAM (JTT) 100** substitution matrix

Command

new command name

```
nohup mafft --thread 15 --globalpair --maxiterate 1000 --jtt 100 --  
reorder unique_Spike_6_14_22.SEQID_Only.fasta >  
unique_Spike_6_14_22.SEQID_Only.jtt_100.globalpair.afa_out &
```

Outputs :

 unique_Spike_6_14_22.SEQID_Only.j...

 unique_Spike_6_14_22.SEQID_Only.j...

This was split into

&

 unique_Spike_6_14_22.SEQID_Only.j...

5.7 Align **HE** using the default **PAM (JTT) 200** substitution matrix

Command

new command name

```
nohup mafft --thread 15 --globalpair --maxiterate 1000 --jtt 200 --reorder unique_HE_6_14_22.SEQID_Only.fasta > unique_HE_6_14_22.SEQID_Only.jtt_200.globalpair.afa_out
```

 unique_HE_6_14_22.SEQID_Only.jtt_...

Outputs :

 unique_HE_6_14_22.SEQID_Only.jtt_...

This was split into

&

 unique_HE_6_14_22.SEQID_Only.jtt_...

5.8 Align **HE** using the **PAM (JTT) 100** substitution matrix

Command

new command name

```
nohup mafft --thread 15 --globalpair --maxiterate 1000 --jtt 100 --reorder unique_HE_6_14_22.SEQID_Only.fasta > unique_HE_6_14_22.SEQID_Only.jtt_100.globalpair.afa_out
```

Output:  unique_HE_6_14_22.SEQID_Only.jtt_...

This was split into:  unique_HE_6_14_22.SEQID_Only.jtt_... &  unique_HE_6_14_22.SEQID_Only.jtt_...

5.9 Align unique BCV HE sequences the **PAM (JTT) 100** substitution matrix

Command

new command name

```
nohup mafft --thread 25 --globalpair --maxiterate 1000 --jtt 100 --
reorder unique_345_Sequences_Bovine_HE_only.SEQID_Only.fasta>
unique_345_Sequences_Bovine_HE_only.SEQID_Only.jtt_100.globalpair.afa_
out &
```

Output:  unique_345_Sequences_Bovine_HE...

Was split into  unique_345_Sequences_Bovine_HE... &  unique_345_Sequences_Bovine_HE...



Command

new command name

```
nohup mafft --thread 15 --globalpair --maxiterate 1000 --jtt 100 --reorder  
unique_BetaCoronaVirus_HE_GE_1255_BP_BlastMatch_Bovine_HE_Consensus_Se  
qID_Only.fasta >  
unique_BetaCoronaVirus_HE_GE_1255_BP_BlastMatch_Bovine_HE_Consensus_Se  
qID_Only.jtt_100.globalpair.afa_out
```



unique_BetaCoronaVirus_HE_GE_12...



unique_BetaCoronaVirus_HE_GE_12...



unique_BetaCoronaVirus_HE_GE_12...

Tree Building

6 RaXML Tee Building

Notes below are cut from the [RaXML manual](#) to put the command line parameters used in context

Note

"This is a how-to, which describes how RAxML should best be used for a simple real-world biological analysis, given an example alignment named ex_al"

Now, if you want to run a full analysis, i.e., BS and ML search type:

```
raxmlHPC -f a -x 12345 -p 12345 -# 100 m GTRGAMMA -s ex_al -n TEST
```

This will first conduct a BS search and once that is done a search for the best-scoring ML tree.

Such a program run will return the bootstrapped trees (RAxML_bootstrap.TEST), the best scoring ML tree (RAxML_bestTree.TEST), and the BS support values drawn on the best-scoring tree as node labels (RAxML_bipartitions.TEST) as well as, more correctly since support values refer to branches as branch labels (RAxML_bipartitionsBranchLabels.TEST).

Finally, note that by increasing the number of BS replicates via `-#` you will also make the ML thorough since for ML optimization every 5th BS tree is used as a starting point to for ML trees.

raxmlHPC reports 192_Genomes_Final_6_14_22.jtt_100.globalpair.SEQID_Only.afa has 3438 DNA alignment patterns

Note

Thus, if you run RAxML with 32 instead of 1 thread this does not mean that it will automatically become 32 times faster, it may actually even become slower. As I already mentioned, the parallel efficiency, that is, with how many threads/cores you can still execute it efficiently in parallel depends on the alignment length, or to be more precise on the number of distinct patterns in your alignment.

This number is printed by RAxML to the terminal and into the RAxML_info.runID file" and look like this:

Alignment has 70 distinct alignment patterns

As a rule of thumb **I'd use one core/thread per 500 DNA site patterns**, i.e., if you have less, than it's probably better to just use the sequential version. Single-gene DNA alignments with around 1000 sites can be analyzed with 2 or at most 4 threads. Thus, the more patterns your alignment has, the more threads/cores you can use efficiently.



Given the directions above from the [RaXML manual](#), use 8 threads for raxmlHPC tree building

Command

Run raxmlHPC command using nohup on 'nix platform to protect from premature job termination in case of remote connection loss ... and run in background with & at end of command line. Perform a 2000 bootstrap search followed by a search for the best-scoring maximum likelihood tree.

```
-T 8 ( use 8 threads)
-f a
-x 12345
-p 12345
-m GTRGAMMA
-N 2000
```

new command name

```
nohup raxmlHPC -T 8 -f a -x 12345 -p 12345 -N 2000 -m GTRGAMMA -s
192_Genomes_Final_6_14_22.jtt_100.globalpair.SEQID_Only.afa -n
192_Genomes_Final_6_14_22.jtt_100.globalpair.SEQID_Only.T8.N2000 >
192_Genomes_Final_6_14_22.jtt_100.globalpair.SEQID_Only.T8.N2000.out &
```

Output :



RAxML_bootstrap.192_Genomes_Fi...



192_Genomes_Final_6_14_22.jtt_100...



RAxML_info.192_Genomes_Final_6_...



RAxML_bipartitionsBranchLabels.1...



RAxML_bipartitions.192_Genomes_...



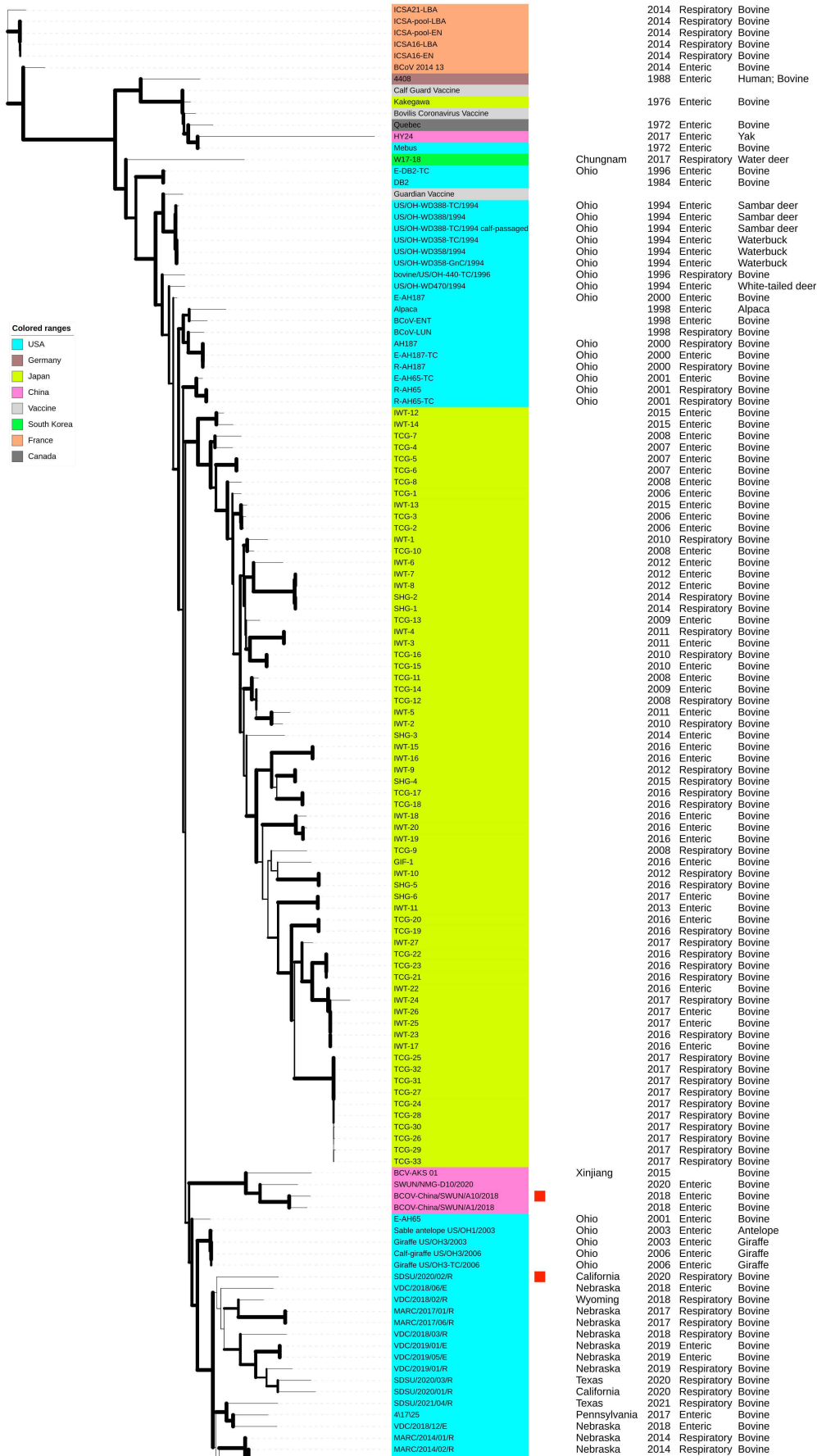
RAxML_bestTree.192_Genomes_Fin...

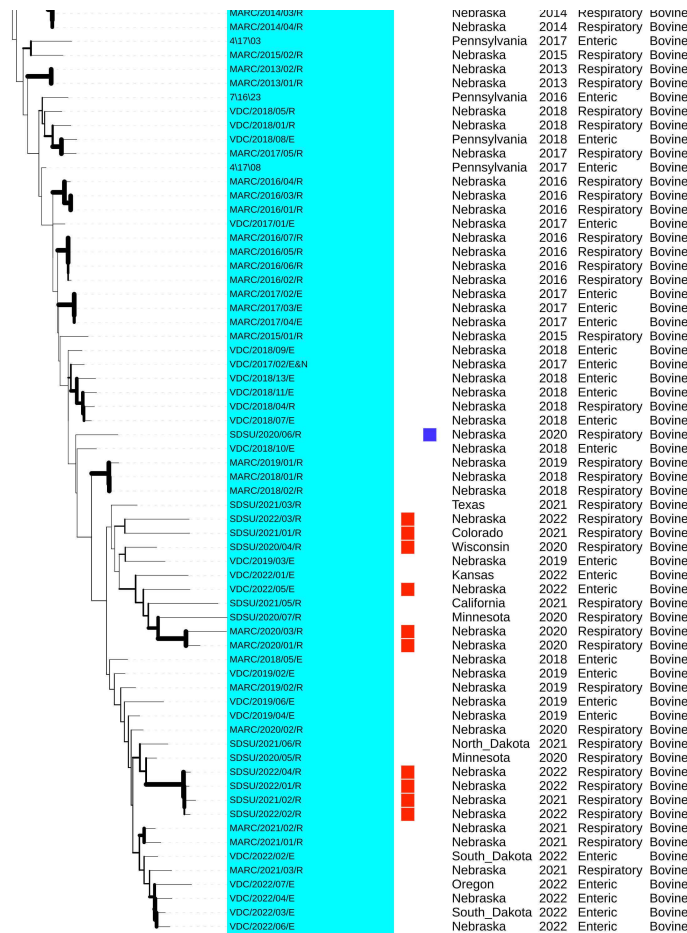


Use

RAxML_bipartitionsBranchLabels.192_Genomes_Final_6_14_22.jtt_100.globalpair.SEQID_Only.T8.N2000 file to create phylogenetic tree with bootstrap support values provided as branch labels.

Visualize this tree of **192 Bovine coronavirus genomes, MAFFT Global Pair Alignment, PAM 100** in the **Interactive Tree of Life**





RAxML tree of 192 bovine coronavirus genomes. Bootstrap support values are proportional to the line thickness of the branches. Isolates with HE deletions are denoted with a filled red square while the single isolate with an HE insertion is denoted with a filled blue square. Tree metrics are best investigated in the [IToL tree](#).

The pdf suitable for download is  RaXML tree of 192 livestock corona...

7 Following the same approach with the 192 BCoV genomes other RaXML Trees were generated for spike and HE and are included here

 RAxML_bipartitionsBranchLabels.u...

 RAxML_bipartitionsBranchLabels.u...

 RAxML_bipartitionsBranchLabels.u...

 RAxML_bipartitionsBranchLabels.u...

 RAxML_bipartitionsBranchLabels.u...



unique_BetaCoronaVirus_HE_GE_12...



RAxML_bipartitionsBranchLabels.u...