

Actividad 4

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Procedure

These are the steps we followed in order to identify the phylogenetic tree of these variants of the SARS-CoV-2 virus:

- Collected all the sequences (5 variants + the reference genome).
- Merged all the `.fastas` in a single file.
- Used the Clustal Omega tool (<https://www.ebi.ac.uk/Tools/msa/clustalo/>) to align the sequences.

Our results can be found in <https://www.ebi.ac.uk/Tools/services/web/toolresult.ebi?jobId=clustalo-I20210425-021750-0190-3504822-p2m> and <https://www.ebi.ac.uk/Tools/services/web/toolresult.ebi?jobId=clustalo-I20210423-005428-0562-5987948-p1m> (will only be available for a limited time).

- Used the aligned sequences to calculate the “Pairwise Distances from DNA Sequences”.
- Used these results to build the tree with “Neighbor-Joining Tree Estimation”.
- **NOTE:** We repeated the process with the sequences provided by the activity, and another analysis for the sequences we gathered in the previous activity.

Dependencies

```
suppressMessages(library(seqinr))  
suppressMessages(library(Biostrings))  
suppressMessages(library(ape))  
suppressMessages(library(ggplot2))  
suppressMessages(library(ggtree))
```

Analysis 1

```
accessions <- c(
  "JX869059", "AY508724", "MN908947", "AY390556",
  "AY278489", "MN985325", "AY485277", "MT292571"
)

read.file <- function(loc) {
  my_data <- readChar(loc, file.info(loc)$size)
  cat(my_data)
}

plot.colors = c(
  "-" = "#000000",
  "a" = "#106BFF",
  "t" = "#FC2B2D",
  "g" = "#30D33B",
  "c" = "#FECF0F",
  "n" = "#FD8D0E"
)
```

Before alignment

```
all.seq = read.dna("./virus/MERGED.fasta", format = "fasta")
all.seq

## 8 DNA sequences in binary format stored in a list.
##
## Mean sequence length: 29834.5
##   Shortest sequence: 29732
##   Longest sequence: 30119
##
## Labels:
## AY278489.2 SARS coronavirus GD01, complete genome
## AY390556.1 SARS coronavirus GZ02, complete genome
## AY485277.1 SARS coronavirus Sino1-11, complete genome
## AY508724.1 SARS coronavirus NS-1, complete genome
## JX869059.2 Human betacoronavirus 2c EMC/2012, complete genom...
## MN908947.3 Severe acute respiratory syndrome coronavirus 2 i...
## ...
##
## Base composition:
##   a   c   g   t
## 0.287 0.194 0.204 0.315
## (Total: 238.68 kb)
```

Results of sequence alignment (showing only first 233)

```
read.file("./virus/aligned-first-places.txt")
```

```
## CLUSTAL O(1.2.4) multiple sequence alignment
##
##
## JX869059.2      -----GATTTAAGTGAATAGCTTGGCTATCTCACTTCCCCTCGTTCTCTTGCAGAACT 53
## MT292571.1      -----AGATCT 6
## MN908947.3      ATTAAAGGTTTATACCTTCCCAGGTAACAAACCAACCAACTTTCGATCTCTTGTAGATCT 60
## MN985325.1      ATTAAAGGTTTATACCTTCCCAGGTAACAAACCAACCAACTTTCGATCTCTTGTAGATCT 60
## AY485277.1      ATATTAGGTTTTTACCTACCCAGG--AAAAGCCAACCAAC-CTCGATCTCTTGTAGATCT 57
## AY508724.1      -----TACCCAGG--AAAAGCCAACCAAC-CTCGATCTCTTGTAGATCT 41
## AY278489.2      -----TACCCAGG--AAAAGCCAACCAAC-CTCGATCTCTTGTAGATCT 41
## AY390556.1      ATATTAGGTTTTTACCTACCCAGG--AAAAGCCAACCAAC-CTCGATCTCTTGTAGATCT 57
##
##
## JX869059.2      TTGATTTTAACGAACCTTAAATAAAAAGCCCTGTTGTTTAGCGTATCGTTGCACTTGTCTGG 113
## MT292571.1      GTTCTCTAAACGAACCTTAAAAATCTGTGTGGCTGT----- 41
## MN908947.3      GTTCTCTAAACGAACCTTAAAAATCTGTGTGGCTGT----- 95
## MN985325.1      GTTCTCTAAACGAACCTTAAAAATCTGTGTGGCTGT----- 95
## AY485277.1      GTTCTCTAAACGAACCTTAAAAATCTGTGTAGCTGT----- 92
## AY508724.1      GTTCTCTAAACGAACCTTAAAAATCTGTGTAGCTGT----- 76
## AY278489.2      GTTCTCTAAACGAACCTTAAAAATCTGTGTAGCTGT----- 76
## AY390556.1      GTTCTCTAAACGAACCTTAAAAATCTGTGTAGCTGT----- 92
##
##      * * * ***** ** * * * *
##
## JX869059.2      TGGGATTGTGGCATTAAATTTGCCTGCTCATCTAGGCAGTGGACATATGCTCAACACTGGG 173
## MT292571.1      -----CACTCGGCTGCATGCTTAGTGCACTCACGCAG 73
## MN908947.3      -----CACTCGGCTGCATGCTTAGTGCACTCACGCAG 127
## MN985325.1      -----CACTCGGCTGCATGCTTAGTGCACTCACGCAG 127
## AY485277.1      -----CGCTCGGCTGCATGCCTAGTGACACCTACGCAG 124
## AY508724.1      -----CGCTCGGCTGCATGCCTAGTGACACCTACGCAG 108
## AY278489.2      -----CGCTCGGCTGCATGCCTAGTGACACCTACGCAG 108
## AY390556.1      -----CGCTCGGCTGCATGCCTAGTGACACCTACGCAG 124
##
##
## JX869059.2      TATAATTCTAATTGAATACTATTTTTCAGTTAGAGCGTCGTGTCTCTTGTACGTCTCGGT 233
## MT292571.1      TATAATTAATAACTAA--TTACTGTCGTTGACAGGACACGAGTAACCTCGTCTATCTTCTG 131
## MN908947.3      TATAATTAATAACTAA--TTACTGTCGTTGACAGGACACGAGTAACCTCGTCTATCTTCTG 185
## MN985325.1      TATAATTAATAACTAA--TTACTGTCGTTGACAGGACACGAGTAACCTCGTCTATCTTCTG 185
## AY485277.1      TATAACAATAATAAAATTTTACTGTCGTTGACAAGAAACGAGTAACCTCGTCCCTCTTCTG 184
## AY508724.1      TATAACAATAATAAAATTTTACTGTCGTTGACAAGAAACGAGTAACCTCGTCCCTCTTCTG 168
## AY278489.2      TATAACAATAATAAAATTTTACTGTCGTTGACAAGAAACGAGTAACCTCGTCCCTCTTCTG 168
## AY390556.1      TATAACAATAATAAAATTTTACTGTCGTTGACAAGAAACGAGTAACCTCGTCCCTCTTCTG 184
##
##      ***** * ** ** * * * * * ** ** ** ** **
```

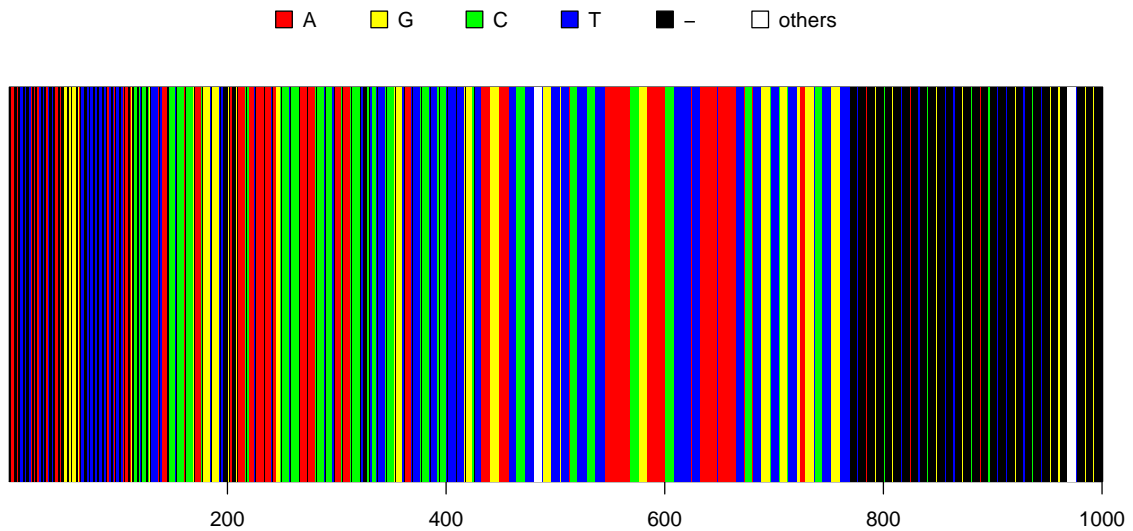
After alignment

```
clust <- read.alignment(  
  "./virus/clustalo-I20210425-021750-0190-3504822-p2m.clustal_num",  
  format = "clustal", forceToLower = TRUE,  
)  
dna <- as.DNABin(clust)  
dna
```

```
## 8 DNA sequences in binary format stored in a matrix.  
##  
## All sequences of same length: 33165  
##  
## Labels:  
## JX869059.2  
## MT292571.1  
## MN908947.3  
## MN985325.1  
## AY485277.1  
## AY508724.1  
## ...  
##  
## Base composition:  
##      a      c      g      t  
## 0.287 0.194 0.204 0.315  
## (Total: 265.32 kb)
```

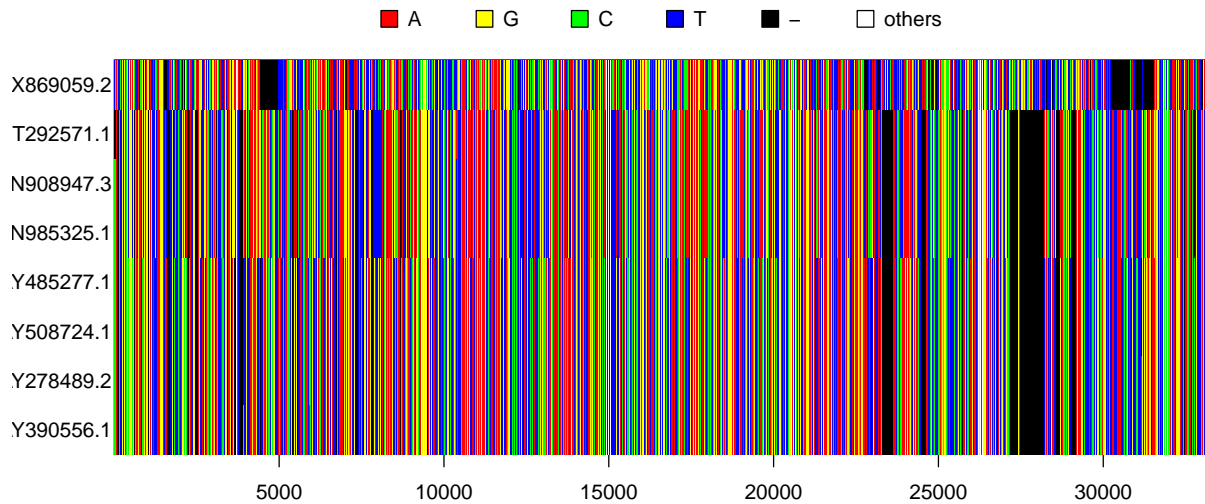
Alignment of the first 1,000 bases

```
image.DNABin(dna[1:1000])
```



Complete alignment

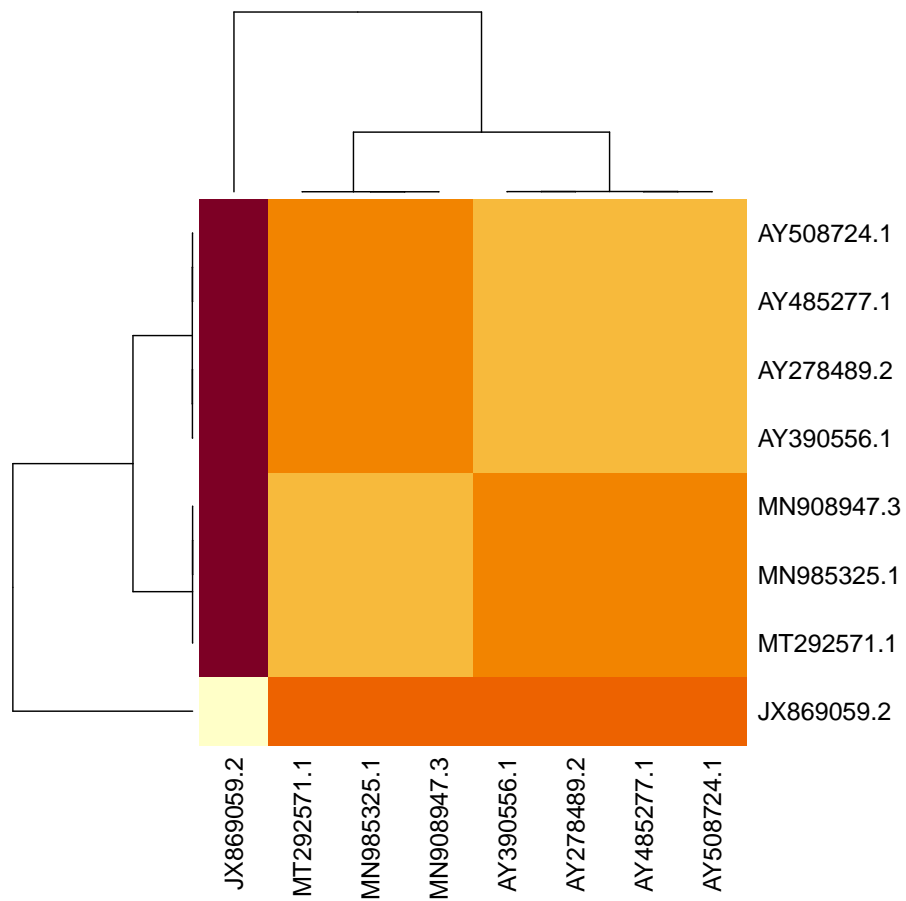
```
image.DNABin(dna)
```



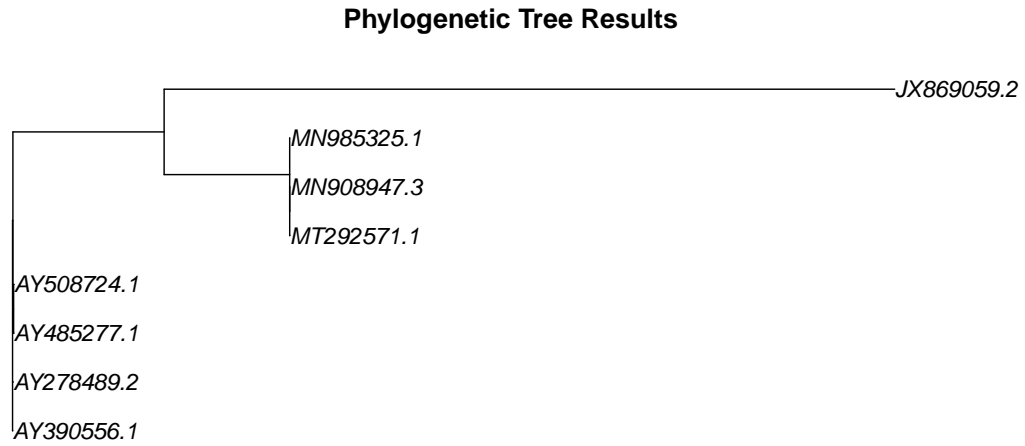
Difference matrix (between sequences)

```
D <- dist.dna(dna)
D.mat <- as.matrix(D)

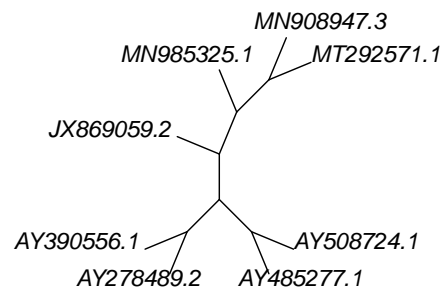
heatmap(D.mat, margins = c(10, 10))
```



```
tree <- nj(D)
plot.phylo(tree, main = "Phylogenetic Tree Results")
```



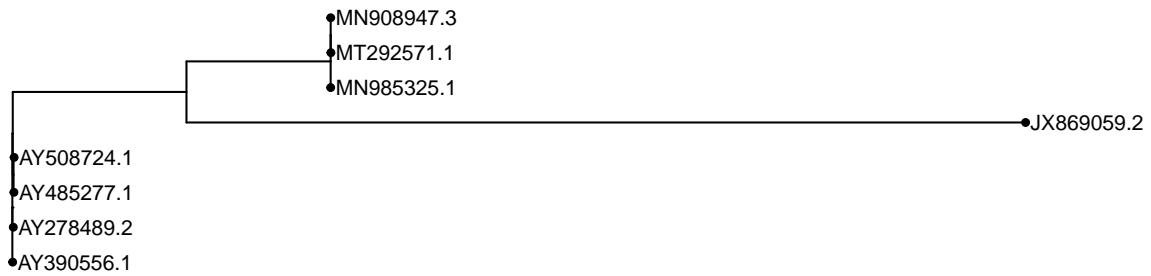
```
plot.phylo(
  unroot(tree),
  type = "unrooted",
  no.margin = TRUE,
  use.edge.length = FALSE ## not to scale in order to avoid overlaps
)
```



Using ggplot2

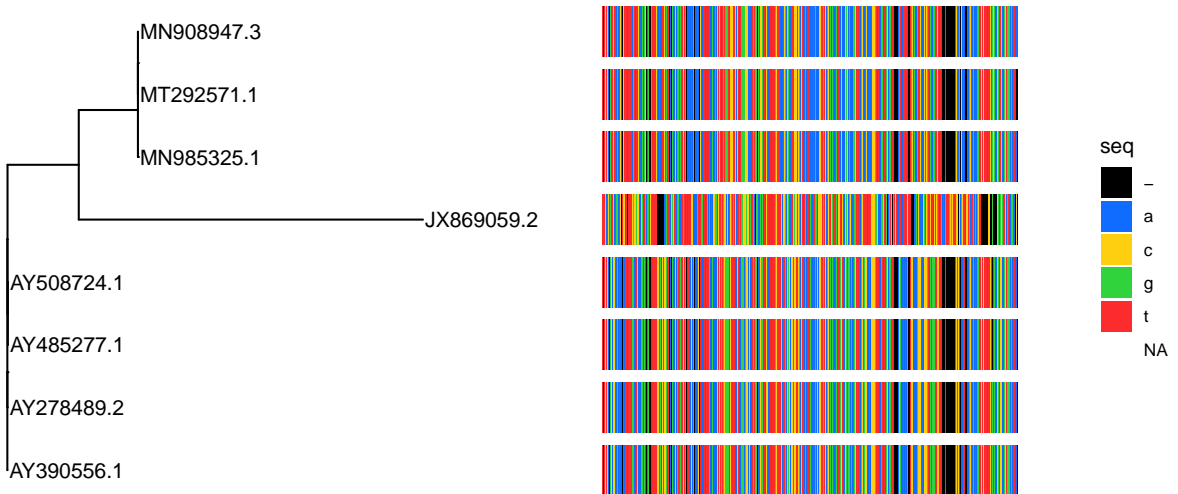
```
ggtree(tree) + ggtitle("Phylogenetic Tree Results Using ggplot") +  
  geom_tippoint() + geom_tiplab() + xlim(0, 0.8)
```

Phylogenetic Tree Results Using ggplot



```
msaplot(p=ggtree(tree), fasta = dna, color=plot.colors, offset=0.3) +  
  ggtitle("Tree + Sequence Alignment") +  
  geom_tiplab()
```

Tree + Sequence Alignment



Analysis 2: Different variants of coronavirus

```
accessions <- c(
  "MN908947.3" = "Reference genome",
  "MW913791.1" = "B.1.1.7",
  "FR990267.1" = "B.1.351",
  "FR990291.1" = "P.1",
  "MW453109.1" = "B.1.427",
  "MW778462.1" = "B.1.429"
)
```

Before alignment

```
all.seq = read.dna(
  "../SARS-CoV-2 Sequences (Sample)/SARS-CoV-2 MERGED.fasta", format = "fasta"
)
all.seq
```

```
## 6 DNA sequences in binary format stored in a list.
##
## Mean sequence length: 29833.33
##   Shortest sequence: 29692
##   Longest sequence: 29903
##
## Labels:
## MN908947.3 Severe acute respiratory syndrome coronavirus 2 i...
## MW913791.1 Severe acute respiratory syndrome coronavirus 2 i...
## FR990267.1 Severe acute respiratory syndrome coronavirus 2 i...
## FR990291.1 Severe acute respiratory syndrome coronavirus 2 i...
## MW453109.1 Severe acute respiratory syndrome coronavirus 2 i...
## MW778462.1 Severe acute respiratory syndrome coronavirus 2 i...
##
## Base composition:
##   a      c      g      t
## 0.299 0.184 0.196 0.322
## (Total: 179 kb)
```

Results of sequence alignment (showing only first 360)

```
read.file("../SARS-CoV-2 Sequences (Sample)/aligned-first-360-places.txt")
```

```
## CLUSTAL O(1.2.4) multiple sequence alignment
##
## MW913791.1      -----AGATCT 6
## FR990267.1      -----actttcgatctctttagatct 22
## MW453109.1      -----CCAACTTCGATCTCTTGTAGATCT 25
## FR990291.1      attaaaggtttataccttcccaggttaacaaaccaaccaactttcgatctctttagatct 60
## MN908947.3      ATTAAAGGTTTATACCTTCCCAGGTAACAAACCAACCAACTTTCGATCTCTTGTAGATCT 60
## MW778462.1      -----TTTATACCTTCCCAGGTAACAAACCAACCAACTTTCGATCTCTTGTAGATCT 52
##
##
## MW913791.1      GTTCTCTAAACGAACCTTTAAATCTGTGTGGCTGTCACTCGGCTGCATGCTTAGTGCACT 66
## FR990267.1      gttctctaaacgaactttaaaatctgtgtggctgtcactcggtgcatgcttagtgact 82
## MW453109.1      GTTCTCTAAACGAACCTTTAAATCTGTGTGGCTGTCACTCGGCTGCATGCTTAGTGCACT 85
## FR990291.1      gttctctaaacgaactttaaaatctgtgtggctgtcactcggtgcatgcttagtgact 120
## MN908947.3      GTTCTCTAAACGAACCTTTAAATCTGTGTGGCTGTCACTCGGCTGCATGCTTAGTGCACT 120
## MW778462.1      GTTCTCTAAACGAACCTTTAAATCTGTGTGGCTGTCACTCGGCTGCATGCTTAGTGCACT 112
##
## *****
##
## MW913791.1      CACGCAGTATAATTAATAACTAATTACTGTCGTTGACAGGACACGAGTAACCTCGTCTATC 126
## FR990267.1      cacgcagtataattaataactaattactgtcgttgacaggacacgagtaactcttctatc 142
## MW453109.1      CACGCAGTATAATTAATAACTAATTACTGTCGTTGACAGGACACGAGTAACCTCGTCTATC 145
## FR990291.1      cacgcagtataattaataactaattactgtcgttgacaggacacgagtaactcttctatc 180
## MN908947.3      CACGCAGTATAATTAATAACTAATTACTGTCGTTGACAGGACACGAGTAACCTCGTCTATC 180
## MW778462.1      CACGCAGTATAATTAATAACTAATTACTGTCGTTGACAGGACACGAGTAACCTCGTCTATC 172
##
## *****
##
## MW913791.1      TTCTGCAGGCTGCTTACGGTTTCGTCGCGTGTGACGCCGATCATCAGCACATCTAGGTTT 186
## FR990267.1      ttctgcaggctgcttacggtttcgtcggtgttgacgccgatcatcagcacatctaggttt 202
## MW453109.1      TTCTGCAGGCTGCTTACGGTTTCGTCGCGTGTGACGCCGATCATCAGCACATCTAGGTTT 205
## FR990291.1      ttctgcaggctgcttacggtttcgtcggtgttgacgccgatcatcagcacatctaggttt 240
## MN908947.3      TTCTGCAGGCTGCTTACGGTTTCGTCGCGTGTGACGCCGATCATCAGCACATCTAGGTTT 240
## MW778462.1      TTCTGCAGGCTGCTTACGGTTTCGTCGCGTGTGACGCCGATCATCAGCACATCTAGGTTT 232
##
## *****
##
## MW913791.1      TGTCCGGGTGTGACCGAAAGGTAAGATGGAGAGCCTTGTCCCTGGTTTCAACGAGAAAAAC 246
## FR990267.1      tgtccgggtgtgaccgaaaggtaagatggagagccttgtccctggtttcaacgagaaaaac 262
## MW453109.1      TGTCCGGGTGTGACCGAAAGGTAAGATGGAGAGCCTTGTCCCTGGTTTCAACGAGAAAAAC 265
## FR990291.1      tgtccgggtgtgaccgaaaggtaagatggagagccttgtccctggtttcaacgagaaaaac 300
## MN908947.3      CGTCCGGGTGTGACCGAAAGGTAAGATGGAGAGCCTTGTCCCTGGTTTCAACGAGAAAAAC 300
## MW778462.1      TGTCCGGGTGTGACCGAAAGGTAAGATGGAGAGCCTTGTCCCTGGTTTCAACGAGAAAAAC 292
##
## *****
##
## MW913791.1      ACACGTCCAACCTCAGTTTGCCTGTTTTACAGGTTTCGCGACGTGCTCGTACGTGGCTTTGG 306
## FR990267.1      acacgtccaactcagtttgcctgttttacaggttcgcgacgtgctcgtagctggctttgg 322
## MW453109.1      ACACGTCCAACCTCAGTTTGCCTGTTTTACAGGTTTCGCGACGTGCTCGTACGTGGCTTTGG 325
## FR990291.1      acacgtccaactcagtttgcctgttttacaggttcgcgacgtgctcgtagctggctttgg 360
## MN908947.3      ACACGTCCAACCTCAGTTTGCCTGTTTTACAGGTTTCGCGACGTGCTCGTACGTGGCTTTGG 360
## MW778462.1      ACACGTCCAACCTCAGTTTGCCTGTTTTACAGGTTTCGCGACGTGCTCGTACGTGGCTTTGG 352
##
## *****
```

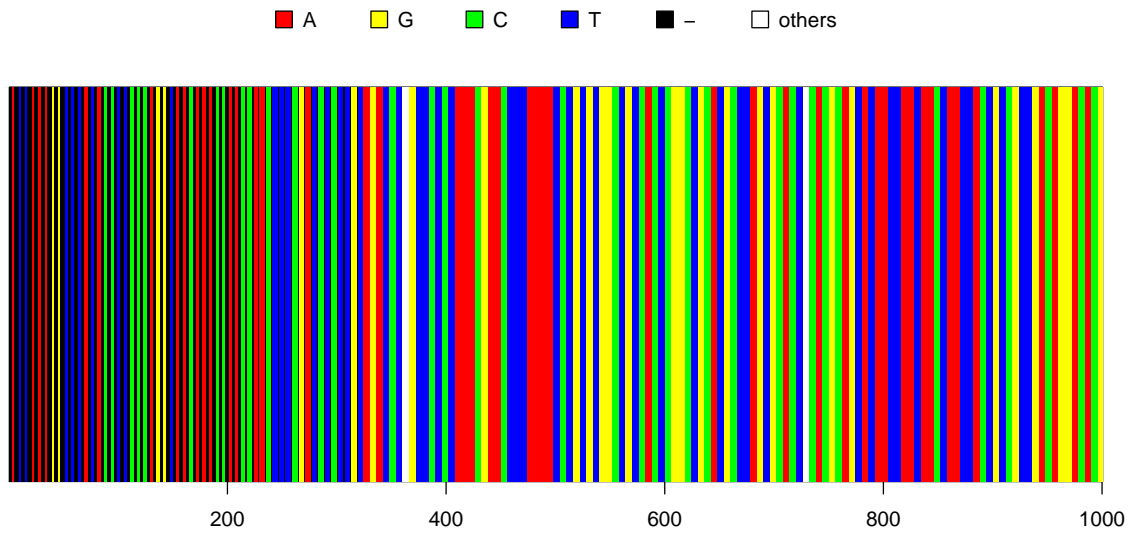
After alignment

```
clust <- read.alignment(  
  "../SARS-CoV-2 Sequences (Sample)/clustalo-I20210423-005428-0562-5987948-p1m.clustal_num",  
  format = "clustal", forceToLower = TRUE,  
)  
dna <- as.DNABin(clust)  
dna
```

```
## 6 DNA sequences in binary format stored in a matrix.  
##  
## All sequences of same length: 30406  
##  
## Labels:  
## MW913791.1  
## FR990267.1  
## MW453109.1  
## FR990291.1  
## MN908947.3  
## MW778462.1  
##  
## Base composition:  
##      a      c      g      t  
## 0.299 0.184 0.196 0.322  
## (Total: 182.44 kb)
```

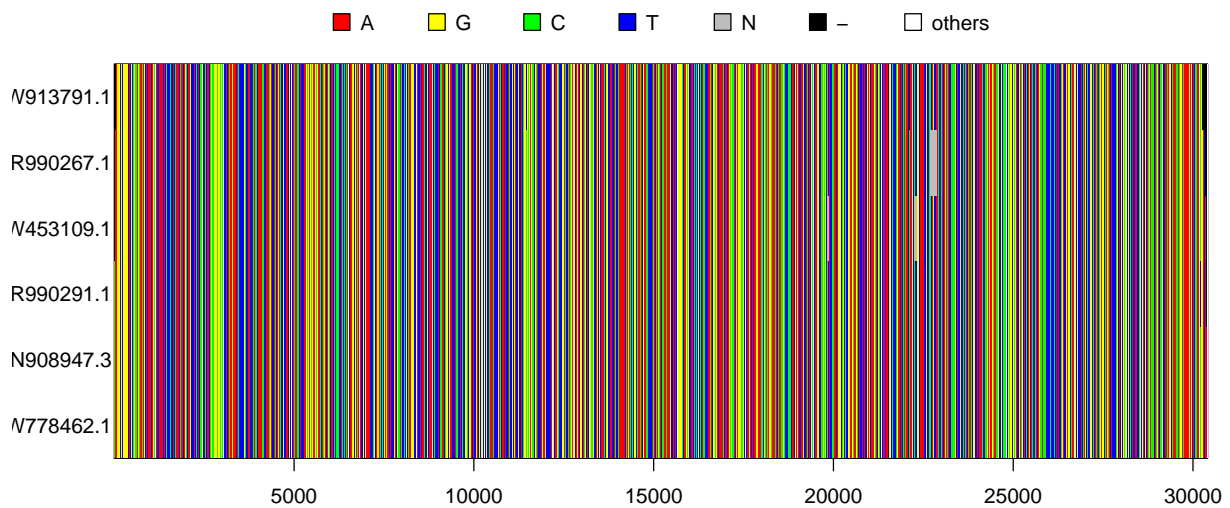
Alignment of the first 1,000 bases

```
image.DNABin(dna[1:1000])
```



Complete alignment

```
image.DNABin(dna)
```

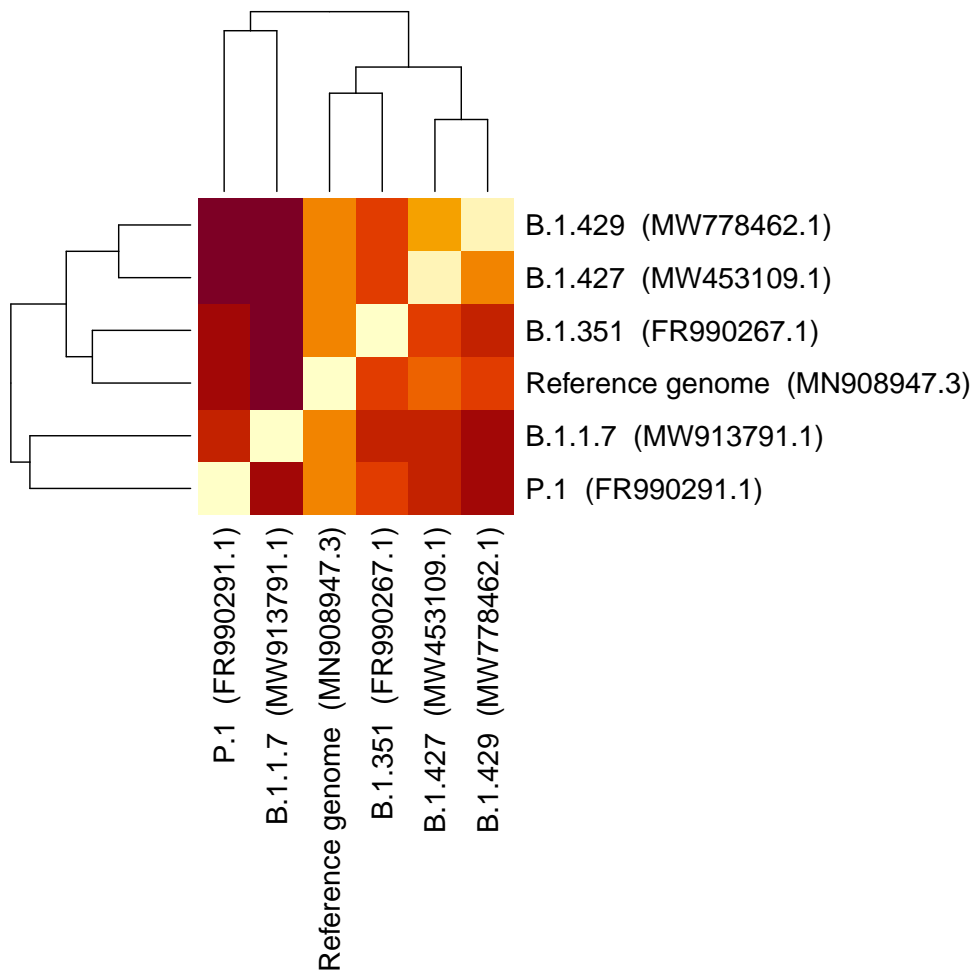


Difference matrix (between sequences)

```
D <- dist.dna(dna, model = "TN93")
D.mat <- as.matrix(D)

rows <- sapply(row.names(D.mat), function(i) { paste(accessions[[i]], " (", i, ")", sep = "") })
cols <- sapply(colnames(D.mat), function(i) { paste(accessions[[i]], " (", i, ")", sep = "") })

heatmap(D.mat, margins = c(20, 20), labRow = rows, labCol = cols)
```

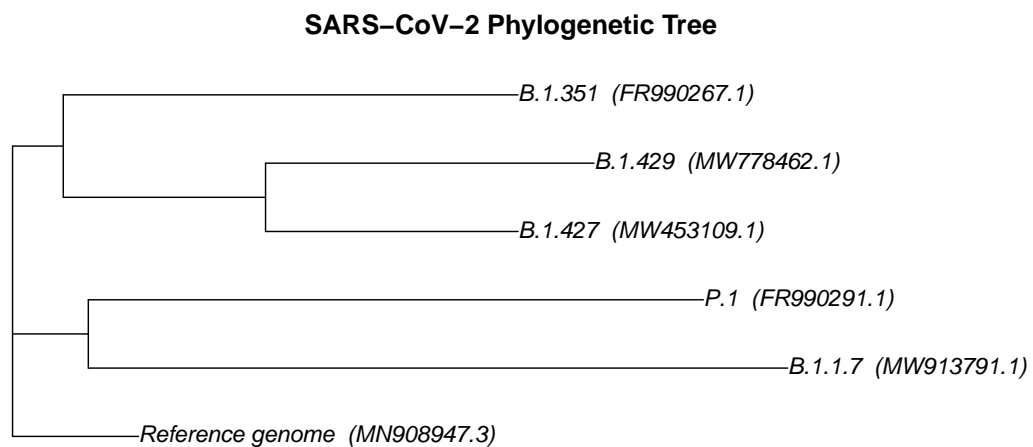


```

tree <- nj(D)
labs <- sapply(tree$tip.label, function(i) { paste(accessions[[i]], " (", i, ")", sep = "") })
tree$tip.label <- labs;

plot.phylo(tree, main = "SARS-CoV-2 Phylogenetic Tree")

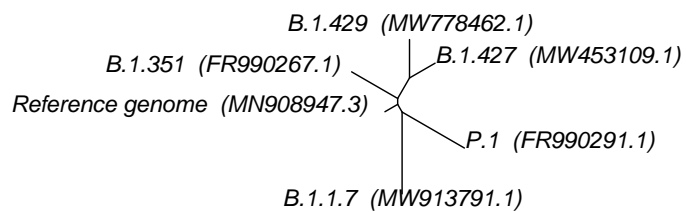
```



```

plot.phylo(unroot(tree), type = "unrooted", no.margin = TRUE)

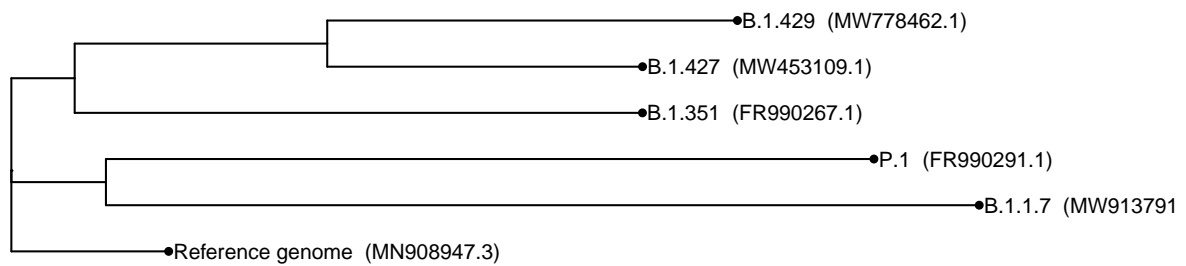
```



Using ggplot2

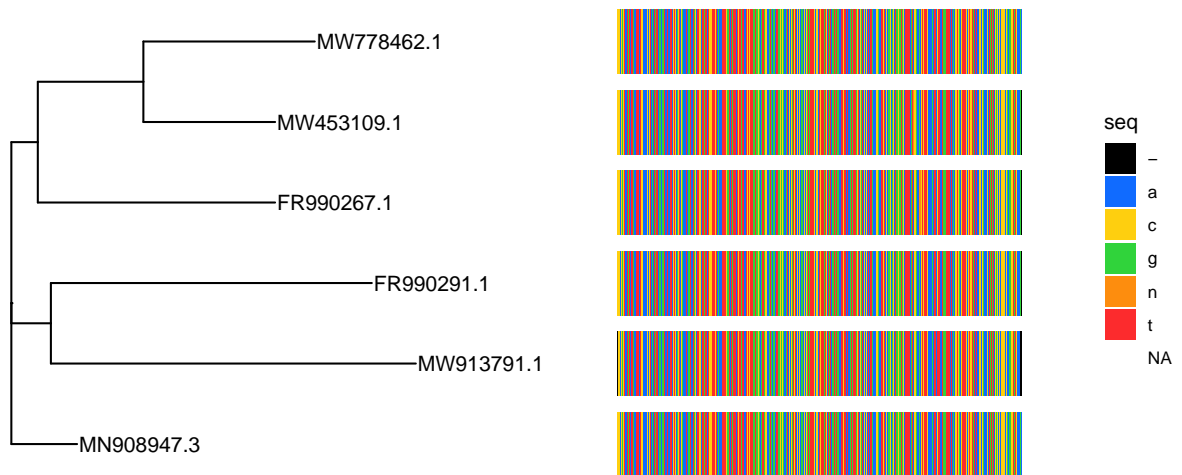
```
ggtree(tree) + ggtitle("SARS-CoV-2 Phylogenetic Tree Using ggplot") +  
  geom_tippoint() + geom_tiplab() + xlim(0, 0.0012)
```

SARS-CoV-2 Phylogenetic Tree Using ggplot



```
tree$tip.label <- names(tree$tip.label)  
msaplot(p=ggtree(tree), fasta = dna, color=plot.colors, offset = 0.0005) +  
  ggtitle("Tree + Sequence Alignment") +  
  geom_tiplab()
```

Tree + Sequence Alignment



Key learnings

- In the first analysis, some samples of SARS-CoV-2 resemble each other a lot. They were probably from an outbreak in the same region in similar times.
 - This is illustrated by the branches in the tree from the same node that have similar lengths.
- In the second analysis, it is shown how the different classified variants of the virus are related to each other:
 - The Reference Genome from Wu Han is *most likely* their ancestor.
 - The other variants are *newer*, as they are farther in the tree.
 - Our research had previously stated that the B.1.427 and B.1.429 variants were closely related.

References

- *Module 24: An Intro to Phylogenetic Tree Construction in R*. Retrieved on April 22, 2021, from <https://fuzzyatelin.github.io/bioanth-stats/module-24/module-24.html>
- *Pairwise Distances from DNA Sequences*. Retrieved on April 22, 2021, from <https://www.rdocumentation.org/packages/ape/versions/5.4-1/topics/dist.dna>
- *Tajimas D for sequences of different length*. Retrieved on April 22, 2021, from <https://stackoverflow.com/questions/33301632/tajimas-d-for-sequences-of-different-length>
- *Visualizing and Annotating Phylogenetic Trees with R+ggtree*. Retrieved on April 23, 2021, from <https://4va.github.io/biodatasci/r-ggtree.html>