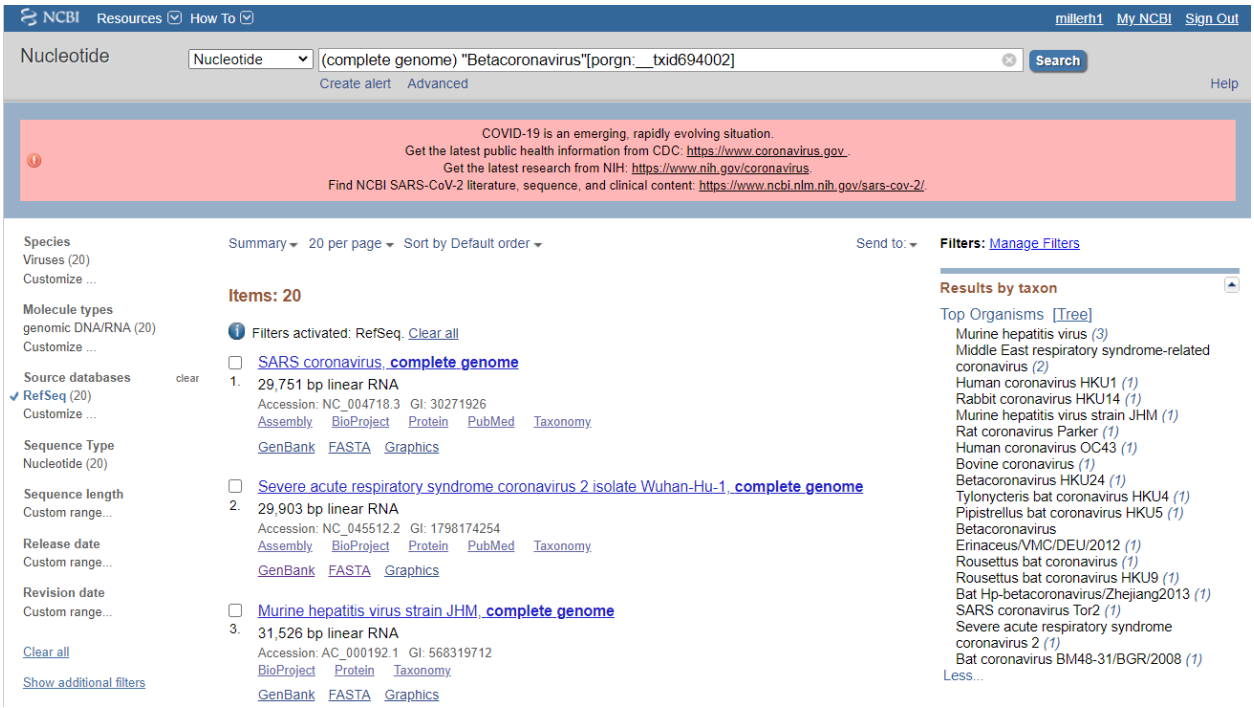
Phylogenetic analysis of SARS-CoV-2

SARS-CoV-2 is a novel coronavirus which emerged from Wuhan, China in late 2019. While much is known about SARS-CoV-2, it is still unclear how this virus differs from other coronaviruses. To address this gap in knowledge I will:

1. Obtain SARS-CoV-2 sequence submitted to the NCBI along with other coronaviruses
2. Use multiple sequence alignment to compare them
3. Calculate phylogenetic distance and visualize the results

Obtaining coronavirus sequences

I started by finding the taxonomy ID number for the *betacoronavirus* genus using the NCBI taxonomy browser. I then used that taxonomy ID to search for all the betacoronavirus genus RefSeq genomes available from NCBI. I selected the option to download these sequences as a `.fasta` file.



Using multiple sequence alignment to compare coronavirus genomes

Multiple sequence alignment (MSA) is a fundamental bioinformatics technique which involves comparisons of multiple gene sequences. To perform MSA, I used the `msa` package.

Initial alignment of CoV genomes

I began my analysis by reading the sequences from the `.fasta` file with the `readDNAStringSet` command.

```
suppressPackageStartupMessages(library(msa))
sequences <- readDNAStringSet("sequences.fasta")
sequences
```

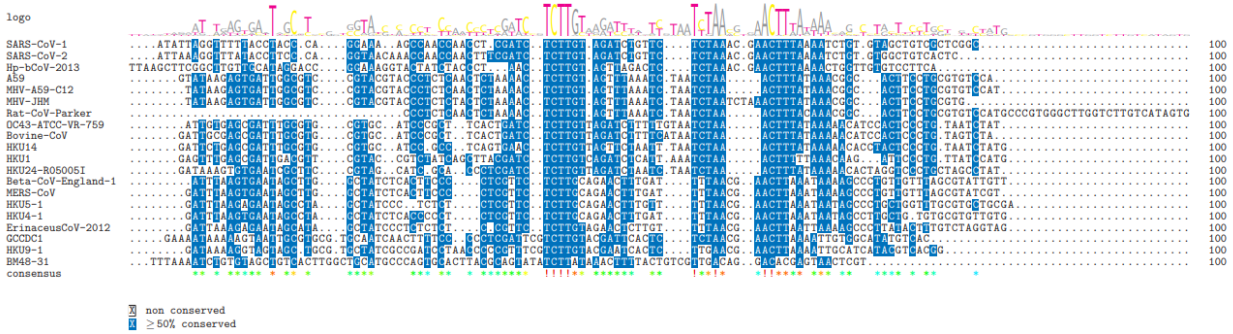
```
## DNAStringSet object of length 20:
##      width seq                                               names
##  [1] 29751 ATATTAGGTTTTTACCTACCCAG...AAAAAAAAAAAAAAAAAAAAAA NC_004718.3 SARS ...
##  [2] 29903 ATTAAAGGTTTATACCTTCCCAG...AAAAAAAAAAAAAAAAAAAAAA NC_045512.2 Sever...
##  [3] 31526 TATAAGAGTGATTGGCGTCCGTA...GAATCACAAAAAAAAAAAAAAA AC_000192.1 Murin...
##  [4] 29926 GAGTTTGAGCGATTGACGTTCGT...TATAGCCTTTTGGAGGAATTAC NC_006577.2 Human...
##  [5] 31335 GTATAAGAGTGATTGGCGTCCGT...CATGGCCAATTGGAAGAATCAC NC_048217.1 Murin...
##  ...   ... ...
## [16] 29114 GATAAAAGGTAGTAGCTGCGTGC...ATTTGCAAAAAAAAAAAAAAAA NC_009021.1 Bat c...
## [17] 30482 GATTTAAGAGAATAGCCTAGCTA...ATTTGCAAAAAAAAAAAAAAAA NC_009020.1 Bat c...
## [18] 30286 GATTTAAGTGAATAGCCTAGCTA...ATTAACAAAAAAAAAAAAAAAA NC_009019.1 Bat c...
## [19] 31028 GATTGCGAGCGATTTGCGTGCGT...TATGGCCAATTGGAAGAATCAC NC_003045.1 Bovin...
## [20] 31357 TATAAGAGTGATTGGCGTCCGTA...AAAAAAAAAAAAAAAAAAAAAA NC_001846.1 Mouse...
```

Because these sequences are derived from NCBI, they are labeled in an official and unreadable way. We can add some human-readable names to help with visualizing the results:

```
names(sequences) <- c("SARS-CoV-1", "SARS-CoV-2", "MHV-JHM", "HKU1", "A59",
                      "OC43-ATCC-VR-759", "ErinaceusCoV-2012", "Beta-CoV-England-1", "GCCDC1", "HKU24
                      "Hp-bCoV-2013", "MERS-CoV", "HKU14", "BM48-31", "Rat-CoV-Parker",
                      "HKU9-1", "HKU5-1", "HKU4-1", "Bovine-CoV", "MHV-A59-C12")
```

It isn't possible to easily visualize a multi-Kb full-genome MSA, but a small subset is easier to interpret. I started the analysis process by subsetting the first 100 base pairs of each sequence and aligning them with `msa`. Finally I rendered the alignment results as a PDF:

```
sequences_small <- subseq(sequences, 1, 100)
dev.null <- capture.output({ # This wrapper prevents print statements
  small_alignment <- msa(sequences_small)
})
msaPrettyPrint(small_alignment, output = "pdf", askForOverwrite = FALSE,
               file = "misc/short_alignment.pdf", paperWidth = 22)
```



Full-length MSA of CoV genomes

The full-length MSA took around 45 minutes to finish. To avoid losing my results and having to run the alignment again, I saved the output of the `msa` command as an `.rda` file using the `save` command.

I also included a little bit of `if...else` logic such that if the alignment has already been run, the file will be loaded with the `load` command and alignment will not be run again.

```
if (! file.exists("alignment_results.rda")) {
  alignment <- msa(sequences)
  save(alignment, file = "alignment_results.rda")
} else {
  load("alignment_results.rda")
}
```

MSA creates a 'consensus sequence' that represents conservation of sequence similarity between our genomes. Consensus sequences contain special characters which are coded based on MSA results. A useful guide is found here.

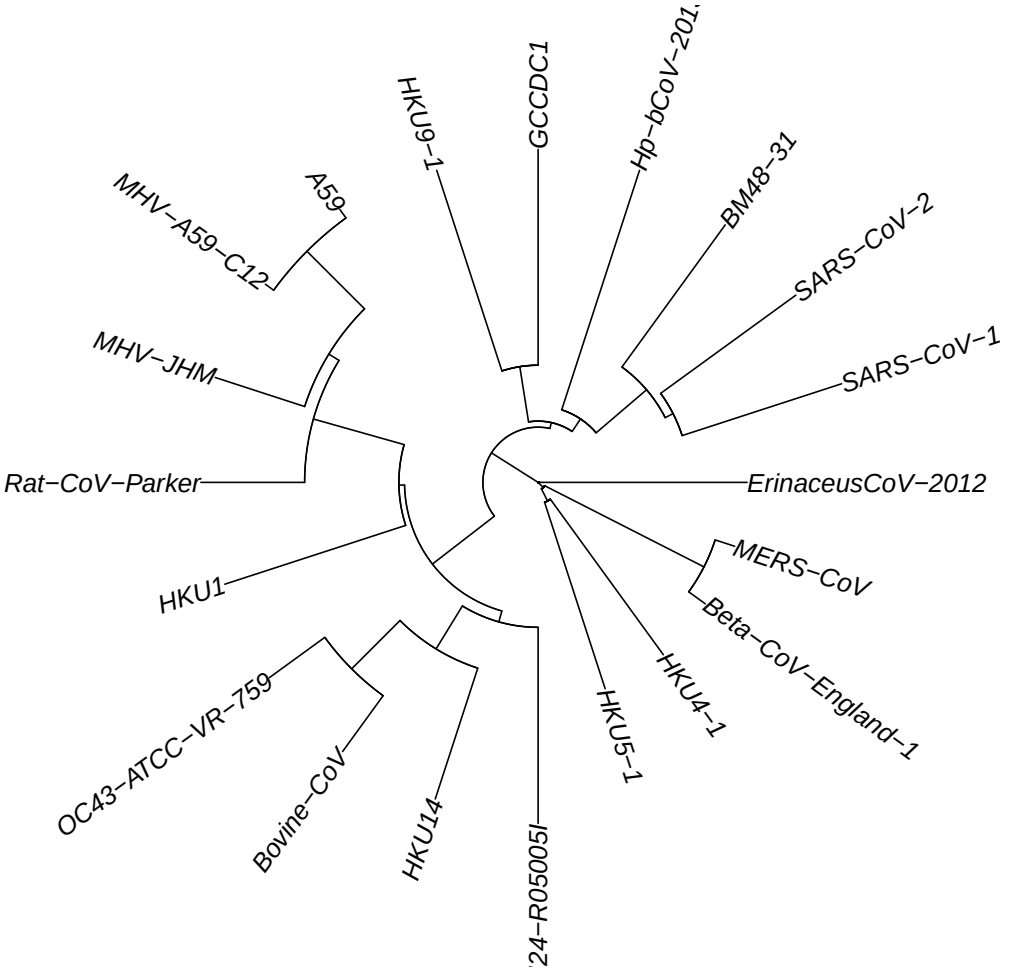
```
paste0("Consensus sequence: ", substr(consensusString(alignment), 500, 1000))
```

```
## [1] "Consensus sequence: TVKYBKYSTTSYWRMWYYRK-WRRADKGMKKYRTKMMGYMTSYYMYARKMCSTSRTRTTTWYSTSRWTRARRRK-
```

Phylogenetic comparison of CoV genomes

Finally, I used the **seqinr** and **ape** packages to calculate phylogenetic distance between our betacoronavirus species and visualize the results as a dendrogram.

```
suppressPackageStartupMessages(library(seqinr))
suppressPackageStartupMessages(library(ape))
alignment_seqinr <- msaConvert(alignment, type="seqinr::alignment")
d <- dist.alignment(alignment_seqinr, "identity")
tree <- nj(d)
plot.phylo(tree, type = "fan")
```



As expected, we can see that SARS-CoV-2 is most similar to SARS-CoV-1. Interestingly, both SARS strains are also similar to BM48-31, a bat CoV originating from Bulgaria. Previous studies showed that BM48-31 did not have SARS-CoV-1 surface antigens. However, there were unanticipated similarities in other protein domains:

> However, the receptor binding domain of SARS CoV showed higher similarity with that of BtCoV/BM48-31/Bulgaria/2008 than with that of any Chinese bat-borne CoV. Critical spike domains 472 and 487 were identical and similar, respectively.

Drexler, J. F., Gloza-Rausch, F., Glende, J., Corman, V. M., Muth, D., Goettsche, M., Seebens, A., Niedrig, M., Pfefferle, S., Yordanov, S., Zhelyazkov, L., Hermanns, U., Vallo, P., Lukashev, A., Müller, M. A., Deng, H., Herrler, G., & Drosten, C. (2010). Genomic Characterization of Severe Acute Respiratory Syndrome-Related Coronavirus in European Bats and Classification of Coronaviruses Based on Partial RNA-Dependent RNA Polymerase Gene Sequences. Journal of Virology. https://doi.org/10.1128/jvi.00650-10

Conclusion

In this short project, I searched the NCBI database for the genomes of betacoronaviruses, used `msa` to perform multiple sequence alignment, and then used `ape` and `seqinr` to calculate phylogenetic distance and visualize the results as a dendrogram.

I found that MSA-based analysis is robust for calculating phylogenetic distance, evidenced by the similarity between SARS-CoV-1 and SARS-CoV-2 on the dendrogram. I also found that SARS CoV strains show unanticipated similarity with BM48-31. While China is the source of both SARS strains, BM48-31 originates from Bulgaria. Despite this great geographical distance, SARS-CoV-1 and BM48-31 share nearly identical spike protein domains 472 and 487. This unlikely similarity could have important implications for our understanding of coronavirus genetics and the evolutionary changes which may lead to future outbreaks.