For this example, we will look at data from donor CAP256 34 weeks after infection, from <u>Doria-Rose et al. J. Virol 2015</u>. This is the first time point at which the CAP256-VRC26 antibody lineage was detected. We will use the first 12 members of this lineage (available from <u>GenBank</u>).

Start by creating a working folder and downloading the data: schrammca@ai-hpcn095:-\$ mkdir cap256-week34H/ schrammca@ai-hpcn095:-\$ cd cap256-week34H/ schrammca@ai-hpcn095:-\$ cd cap256-week34H/ schrammca@ai-hpcn095:-/cap256-week34H\$ fastq-dump SRR2126754 Written 518852 spots for SRR2126754 ow, run module 1 to annotate the V, D, and J genes and collapse duplicate reades: chrammca@ai-hpcn095:-/cap256-week34H\$ sonar blast_V --fasta SRR2126754.fastq --locus H --derep --threads 8 Tue Feb 12 12:10:26 2019 -- SONAR b'' run with command: /nethome/schrammca/sonar/annotate/1.1-blast_V.py --fasta SRR2126754.fastq --locus H --derep --threads 8 vsearch v2.8.1_linux_x86_64, 251.8GB RAM, 16 cores https://github.com/torognes/vsearch Reading file /nethome/schrammca/cap256-week34H/work/internal/tempForDerep.fa 100% 204399756 nt in 518852 seqs, min 40, max 1196, avg 394 Dereplicating 100% orting 100% Softing 100% 260705 unique sequences, avg cluster 2.0, median 1, max 767 Writing output file 100% Writing uc file, first part 100% Writing uc file, second part 100% 10122 processed, 10000 good; starting file cap256-week34H 002 20688 processed, 20000 good; starting file cap256-week34H 003 32287 processed, 30000 good; starting file cap256-week34H 004 44519 processed, 40000 good; starting file cap256-week34H 006 56559 processed, 50000 good; starting file cap256-week34H 006 2000 good; starting file cap256-week34H 006 56559 processed, 50000 good; starting file cap256-week34H_006 68344 processed, 70000 good; starting file cap256-week34H_007 79953 processed, 70000 good; starting file cap256-week34H_008 91476 processed, 80000 good; starting file cap256-week34H_009 102816 processed, 80000 good; starting file cap256-week34H_011 128164 processed, 100000 good; starting file cap256-week34H_011 125401 processed, 120000 good; starting file cap256-week34H_012 136561 processed, 120000 good; starting file cap256-week34H_012 136563 processed, 120000 good; starting file cap256-week34H_018 158730 processed, 130000 good; starting file cap256-week34H_018 168730 processed, 150000 good; starting file cap256-week34H_018 1680827 processed, 150000 good; starting file cap256-week34H_018 202894 processed, 170000 good; starting file cap256-week34H_018 213898 processed, 180000 good; starting file cap256-week34H_018 235968 processed, 200000 good; starting file cap256-week34H_021 249256 processed, 220000 good; starting file cap256-week34H_021 Starting blast of /nethome/schrammca/cap256-week34H/work/annotate/vgene/cap256-week34H_001.fasta against /nethome/schrammca/sonar/germDB/IgHV.fa... Starting blast of /nethome/schrammca/cap256-week34H/work/annotate/vgene/cap256-week34H_002.fasta against /nethome/schrammca/sonar/germDB/IgHV.fa... Starting blast of /nethome/schrammca/cap256-week34H/work/annotate/vgene/cap256-week34H_003.fasta against /nethome/schrammca/sonar/germDB/IgHV.fa... Starting blast of /nethome/schrammca/cap256-week34H/work/annotate/vgene/cap256-week34H_004.fasta against /nethome/schrammca/sonar/germDB/IgHV.fa... Starting blast of /nethome/schrammca/cap256-week34H/work/annotate/vgene/cap256-week34H_005.fasta against /nethome/schrammca/sonar/germDB/IgHV.fa... Starting blast of /nethome/schrammca/cap256-week34H/work/annotate/vgene/cap256-week34H_005.fasta against /nethome/schrammca/sonar/germbB/IgHV.fa... Starting blast of /nethome/schrammca/cap256-week34H/work/annotate/vgene/cap256-week34H_007.fasta against /nethome/schrammca/sonar/germbB/IgHV.fa... Starting blast of /nethome/schrammca/cap256-week34H/work/annotate/vgene/cap256-week34H_007.fasta against /nethome/schrammca/sonar/germbB/IgHV.fa... Starting blast of /nethome/schrammca/cap256-week34H/work/annotate/vgene/cap256-week34H_008.fasta against /nethome/schrammca/sonar/germbB/IgHV.fa... Starting blast of /nethome/schrammca/cap255-week34H/work/annotate/vgene/cap256-week34H_009.fasta against /nethome/schrammca/sonar/germbB/IgHV.fa... Starting blast of /nethome/schrammca/cap255-week34H/work/annotate/vgene/cap256-week34H_010.fasta against /nethome/schrammca/sonar/germbB/IgHV.fa... Starting blast of /nethome/schrammca/cap256-week34H/work/annotate/vgene/cap256-week34H_012.fasta against /nethome/schrammca/sonar/germbB/IgHV.fa... Starting blast of /nethome/schrammca/cap256-week34H/work/annotate/vgene/cap256-week34H_012.fasta against /nethome/schrammca/sonar/germbB/IgHV.fa... Starting blast of /nethome/schrammca/cap256-week34H/work/annotate/vgene/cap256-week34H_013.fasta against /nethome/schrammca/sonar/germbB/IgHV.fa... Starting blast of /nethome/schrammca/cap256-week34H/work/annotate/vgene/cap256-week34H_013.fasta against /nethome/schrammca/sonar/germbB/IgHV.fa... Starting blast of /nethome/schrammca/cap256-week34H/work/annotate/vgene/cap256-week34H_015.fasta against /nethome/schrammc schrammca@ai-hpcn095:~/cap256-week34H\$ sonar blast J --threads 8 Tue Feb 12 12:22:26 2019 -- SONAR b'' run with command: /nethome/schrammca/sonar/annotate/1.2-blast J.py --threads 8 curating 5'end and strand... 10000 done, 9992 good... 20000 done, 19975 good... 30000 done, 19975 good... 30000 done, 39918 good... 40000 done, 39918 good... 50000 done, 49874 good... 60000 done, 59838 good... 70000 done, 69804 good... 80000 done, 79774 good... 100000 done, 89741 good... 100000 done, 99714 good... 110000 done, 109677 good... 120000 done, 199646 good... 140000 done, 129616 good... 140000 done, 139591 good... curating 5'end and strand.... 130000 done, 199549 good... 150000 done, 199549 good... 150000 done, 199549 good... 170000 done, 199479 good... 17

```
Starting blast of /nethome/schrammca/cap256-week34H/work/annotate/jgene/cap256-week34H_009.fasta against /nethome/schrammca/sonar/germDB/IgHC_CH1.fa...
Starting blast of /nethome/schrammca/cap256-week34H/work/annotate/jgene/cap256-week34H_010.fasta against /nethome/schrammca/sonar/germDB/IgHC_CH1.fa...
Starting blast of /nethome/schrammca/cap256-week34H/work/annotate/jgene/cap256-week34H_012.fasta against /nethome/schrammca/sonar/germDB/IgHC_CH1.fa...
Starting blast of /nethome/schrammca/cap256-week34H/work/annotate/jgene/cap256-week34H_012.fasta against /nethome/schrammca/sonar/germDB/IgHC_CH1.fa...
Starting blast of /nethome/schrammca/cap256-week34H/work/annotate/jgene/cap256-week34H_013.fasta against /nethome/schrammca/sonar/germDB/IgHC_CH1.fa...
Starting blast of /nethome/schrammca/cap256-week34H/work/annotate/jgene/cap256-week34H_015.fasta against /nethome/schrammca/cap256-week34H_016.fasta against /nethome/schrammca/cap256-week34H_016
      schrammca@ai-hpcn095:~/cap256-week34H$ sonar finalize
     Tue Feb 12 12:26:25 2019 -- SONAR b'' run with command: /nethome/schrammca/sonar/annotate/1.3-finalize_assignments.py
 Internation of International Content of Intern
      Total raw reads: 260702
      Correct Length: 228881
              assigned: 228790
           assigned: 223022
       CDR3 assigned: 221892
      In-frame junction: 192304
No indels: 142657
     Continuous ORF with no stop codons: 139448
       schrammca@ai-hpcn095:~/cap256-week34H$ sonar cluster Sequences --id .97 --min2 2
     Tue Feb 12 12:36:51 2019 -- SONAR b'' run with command:
/nethome/schrammca/sonar/annotate/1.4-cluster_sequences.py --id .97 --min2 2
     vsearch v2.8.1_linux_x86_64, 251.8GB RAM, 16 cores https://github.com/torognes/vsearch
  Reading file temp.fa 100% 51161824 nt in 139448 seqs, min 127, max 445, avg 367 Dereplicating 100% Sorting 100% 90756 unique sequences, avg cluster 4.2, median 1, max 4215 Writing output file 100% Writing uc file, first part 100% Writing uc file, second part 100%
     vsearch v2.8.1_linux_x86_64, 251.8GB RAM, 16 cores https://github.com/torognes/vsearch
     Reading file temp dedup.fa 100%
33294745 nt in 90756 seqs, min 127, max 445, avg 367
     33294745 nt in 90756 seqs,
Masking 100%
Sorting by abundance 100%
Counting k-mers 100%
Clustering 100%
Sorting clusters 100%
Writing clusters 100%
Clusters: 42826 Size min 1
     Clusters: 42826 Size min 1, max 7156, avg 2.1
Singletons: 29330, 32.3% of seqs, 68.5% of clusters
```

```
Let's look at different ways to divide the bulk data into lineages:
schrammca@ai-hpcn095:~/cap256-week34H$ sonar id-div -a CAP256-VRC26.01-12H.fa -t 8
   Tue Feb 12 12:58:11 2019 -- SONAR b'' run with command:
/nethome/schrammca/sonar/lineage/2.1-calculate_id-div.py -a CAP256-VRC26.01-12H.fa -t 8
Starting work on /nethome/schrammca/cap256-week34H/work/lineage/align/align00000.fa...
Starting work on /nethome/schrammca/cap256-week34H/work/lineage/align/align00000.fa...
Starting work on /nethome/schrammca/cap256-week34H/work/lineage/align/align00000.fa...
Starting work on /nethome/schrammca/cap256-week34H/work/lineage/align/align000003.fa...
Starting work on /nethome/schrammca/cap256-week34H/work/lineage/align/align000003.fa...
Starting work on /nethome/schrammca/cap256-week34H/work/lineage/align/align000005.fa...
Starting work on /nethome/schrammca/cap256-week34H/work/lineage/align/align000005.fa...
Starting work on /nethome/schrammca/cap256-week34H/work/lineage/align/align000007.fa...
Starting work on /nethome/schrammca/cap256-week34H/work/lineage/align/align000009.fa...
Starting work on /nethome/schrammca/cap256-week34H/work/lineage/align/align00001.fa...
Starting work on /nethome/schrammca/cap256-week34H/work/lineage/align/align000012.fa...
Starting work on /nethome/schrammca/cap256-week34H/work/lineage/align/align000013.fa...
```

```
Loading required package: misc3d

Left click to accept, right click to re-select region

Left click to accept, right click to re-select region

[1] "Saved 129 transcript IDs to output/tables/islandSeqs"

There were 50 or more warnings (use warnings() to see the first 50)
```

Another option is a phylogenetic based analysis: sehrammeaRcogsworth:/nethome/schrammca/cap256-week34H\$ sonar intradonor --n CAP256-VRC26.01-12H.fa --v IGHV3-30*18 --threads 8 -f loading sequence info from CAP256-VRC26.01-12H.fa... loading sequence info from /nethome/schrammca/sonar/germDB/IgHV.fa... Tue Feb 19 16:47:54 2019 -- SONAR b'v2.0.0-133-g091cbc1-dirty' run with command: /nethome/schrammca/sonar/lineage/2.3-intradonor_analysis.py --n CAP256-VRC26.01-12H.fa --v 'IGHV3-30*18' --threads 8 -f Inethone's chrammary and analysis from scratch... Iodding reads from /nethone/schrammar/acp56-week34Hyoutpi/sequences/nuclectide/cap56-week34HygoodVJ unique.fs assigned to IOHVJ-30... 2028 loaded... 2028 16:47:54 - Starting a new analysis from scratch... loading reads from /nethome/schrammca/cap256-week34H/output/sequences/nucleotide/cap256-week34H_goodVJ_unique.fa assigned to IGHV3-30... Always sanity check your results: In this case, a quick scan of the CDR3 sequences shows that we have failed to pick up any lineage members using CAP256-VRC26.01 and CAP256-VRC26.08 as our anchors in this analysis! We can also do unseeded lineage analysis: cap256-week34H\$ sonar groups -v 'IGHV3-30*18' -j 'IGHJ3*01' -t 8 Fri Feb 22 09:25:26 2019 -- SONAR b'v2.0.0-133-g091cbcl-dirty' run with command: /nethome/schrammca/sonar/lineage/2.4-cluster into groups.py -v 'IGHV3-30*18' -j 'IGHJ3*01' -t 8 Processing chunk #1... Processing chunk #5... Processing chunk #4... Processing chunk #6... Processing chunk #2... minseqlength 15: 3 sequences discarded. minseqlength 15: 1 sequence discarded. minseqlength 15: 1 sequence discarded. minseqlength 15: 1 sequence discarded. Let's make this into a longitudinal analysis, by adding data from two other time points: 48 weeks after infection (SRR1057707). I've downloaded each of those to their own directories and processed them as above. We're going to use the islands identified by 2.1/2.1 in each case. schrammca@cogsworth:/nethome/schrammca/cap256-week34H\$ sonar getfasta -l output/tables/islandSeqs.txt \ -f output/sequences/nucleotide/cap256-week34H_goodVJ_unique.fa -o output/sequences/nucleotide/cap256-week34H_islandSeqs.fa Fri Feb 22 13:36:39 2019 -- SONAR b'v2.0.0-133-g09lcbcl-dirty' run with command: /nethome/schrammca/sonar/utilities/getFastaFromList.py -l output/tables/islandSeqs.txt -f output/sequences/nucleotide/ cap256-week34H_goodVJ_unique.fa -o output/sequences/nucleotide/cap256-week34H_islandSeqs.fa schrammca@cogsworth:/nethome/schrammca/cap256-week34H\$ cd .. schrammca@cogsworthi/nethome/schrammca\$ mkdir cap256-longitudinal schrammca@cogsworthi/nethome/schrammca\$ oap256-longitudinal/ schrammca@cogsworthi/nethome/schrammca\$ cap256-longitudinal/ schrammca@cogsworthi/nethome/schrammca/cap256-longitudinal| schrammca@cogsworthi/nethome/schrammca/cap256-longitudinal5 sonar merge time --seqs ../cap256-week34H/output/sequences/nucleotide/cap256-week34H_islandSeqs.fa --labels w34 \ --seqs ../cap256-week48H/output/sequences/nucleotide/cap256-week48H_islandSeqs.fa\ --labels w48 --seqs ../cap256-week59H/output/sequences/nucleotide/cap256-week59H_islandSeqs.fa --labels w59 Tue Feb 19 17:15:23 2019 -- SONAR v2.0.0-133-g091cbcl-dirty run with command: /nethome/schrammca/sonar/phylogeny/3.1-merge_timepoints.pl --seqs ../cap256-week34H/output/sequences/nucleotide/cap256-week34H_islandSeqs.fa --labels w34 --seqs ../cap256-week48H/output/sequences/nucleotide/cap256-week59H_islandSeqs.fa --labels w59 utput/sequences/nucleotide/cap256-week59H_islandSeqs.fa --labels w59 /nethome/schrammca/sonar/third-party/vsearch -derep fulllength work/phylo/all seqs.fa -uc work/phylo/uc vsearch v2.8.1_linux_x86_64, 31.1GB RAM, 8 cores https://github.com/torognes/vsearch Reading file work/phylo/all seqs.fa 100% 128379 nt in 297 seqs, min 391, max 436, avg 432 Dereplicating 100% Sorting 100% 297 unique sequences, avg cluster 1.0, median 1, max 1 Writing uc file, first part 100% Writing uc file, second part 100%

```
Finally, let's construct a tree. For best results, I've added the VH3-30 germline sequence and our 12 native mAbs to the collected sequences from all 3 time points, aligned them, and manually validated the alignment. These steps can be automated using the -v and --natives flags to 3.2, but the automatic alignments get really messed up if there are a lot of insertions or deletions. This step takes several hours, given the amount of data (~300 sequences); use the --quick argument to skip slower optimizations.

schrammca@cogsworth:/nethome/schrammca/cap256-longitudinal$ sonar igphyml -i output/sequences/nucleotide/cap256-longitudinal-collected_aligned.fa -f
Thu Feb 21 10:22:01 2019 -- SONAR b'v2.0.0-133-g091cbc1-dirty' run with command: /nethome/schrammca/sonar/phylogeny/3.2-run_IgPhyML.py -i output/sequences/nucleotide/cap256-longitudinal-collected_aligned.fa -f
 Looks like a fasta file..
                                                                    CURRENT SETTINGS
 . Sequence filename: infile
```

```
Number of taxa: 310
Sequence length: 150
Data type: Codon
Alphabet size: 61
Genetic code: Standard
Sequence format: Interleaved
Number of data sets: 1
Number of bootstrapped data sets: 0
Model type: GY+WH*F
Equilibrium frequencies model: Empirical CF3x4
Prop. of invariable sites: 0.00
Number of subst. rate categs: 1
Number of dn/ds rate categs: 1
Number of dn/ds rate categs: 1
Sequence formation strategy: Multi-variate (BFGS)
Tree topology search: SPRS
Starting tree: BioNJ+ GYECMKO7
Add random input tree: No
Optimise branch lengths: Yes
Optimise subst. model params: Yes
Matrix exponential: Eigenvalue problem
Run ID: gy94
Random seed: 1211583751
  Number of taxa: 310
Sequence length: 150
  Run ID: 9994
Random seed: 1211583751
Code optimization: BLAS, LAPACK and OpenMP
Version: 1.0 201703.16
  WARNING: sequences are not compressed !
150 patterns found (out of a total of 150 sites).
0 sites without polymorphism (0.00%).
Computing pairwise distances...USING KOSI07
FYI: Using single partitioned GY94 for initial tree.
omputing pairwise distances...100.00% concluded.
  Calculation of pairwise distances completed in 103 sec.
Building BioNJ tree...
WARNING: this analysis will use at least 151 Mo of memory space...
Maximizing likelihood (using SPR moves)...
                                                                                             -24367.8806] [Initial Tree
-24089.8525] [ts/tv ratio
-24089.8525] [dn/ds ratio
-23802.5294] [Branch lengtl
                                                                                                                                                                     [ts/tv ratio
[dn/ds ratio
[Branch lengths
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][0.76 ]
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113 sec)
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Use sonar tree to create a figure-ready plot of the tree!