Bioinformatics for COVID19

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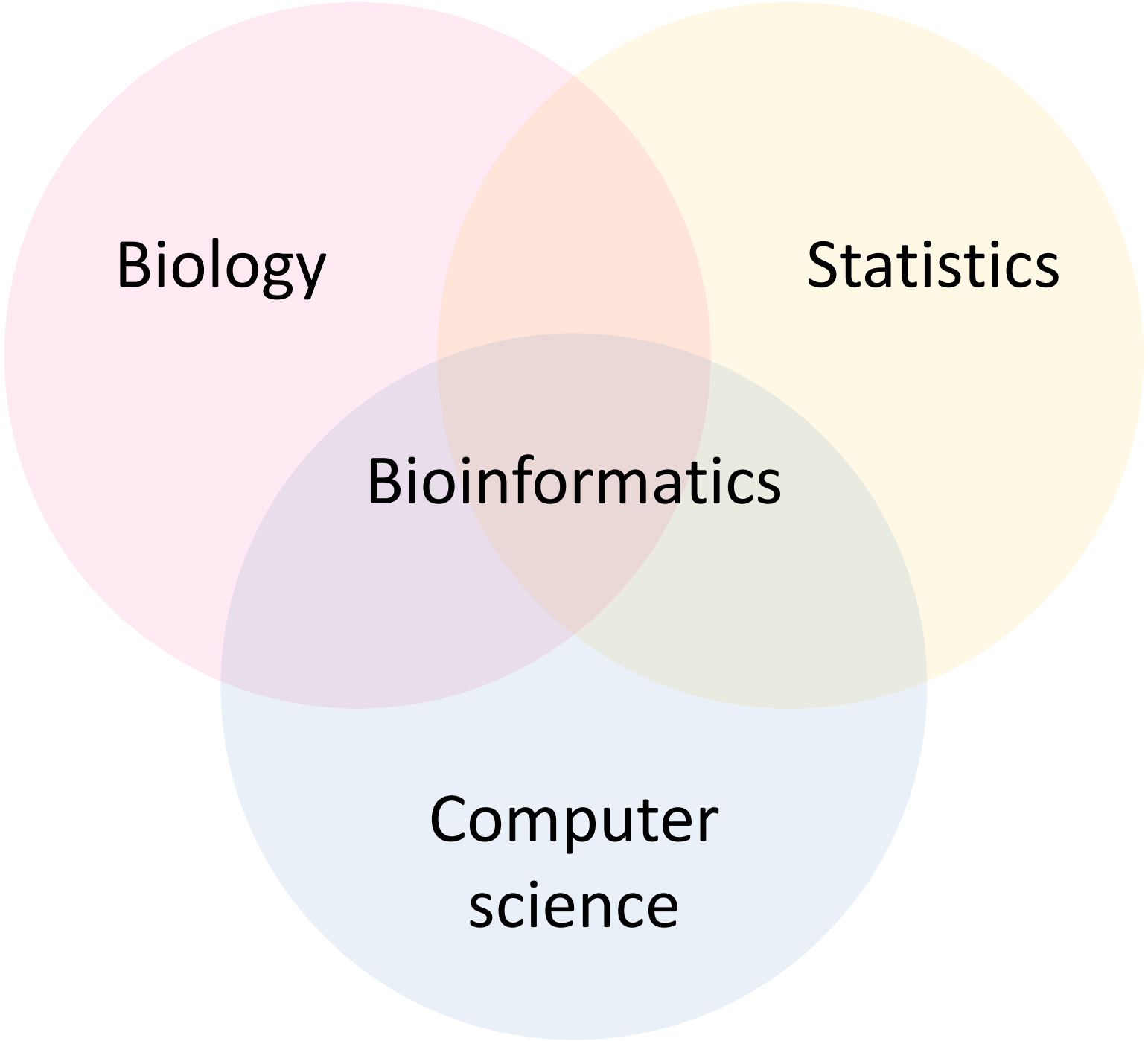
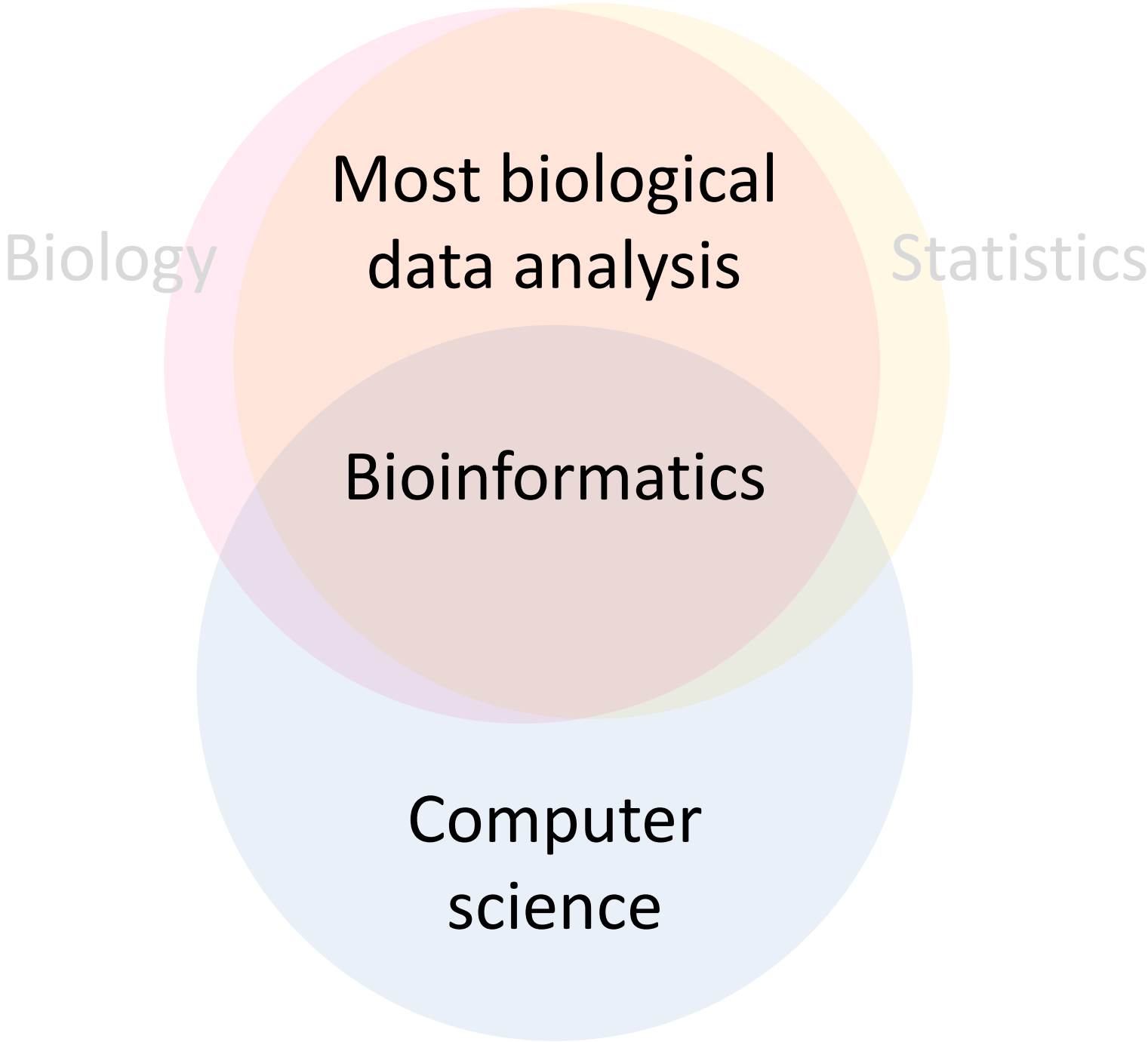
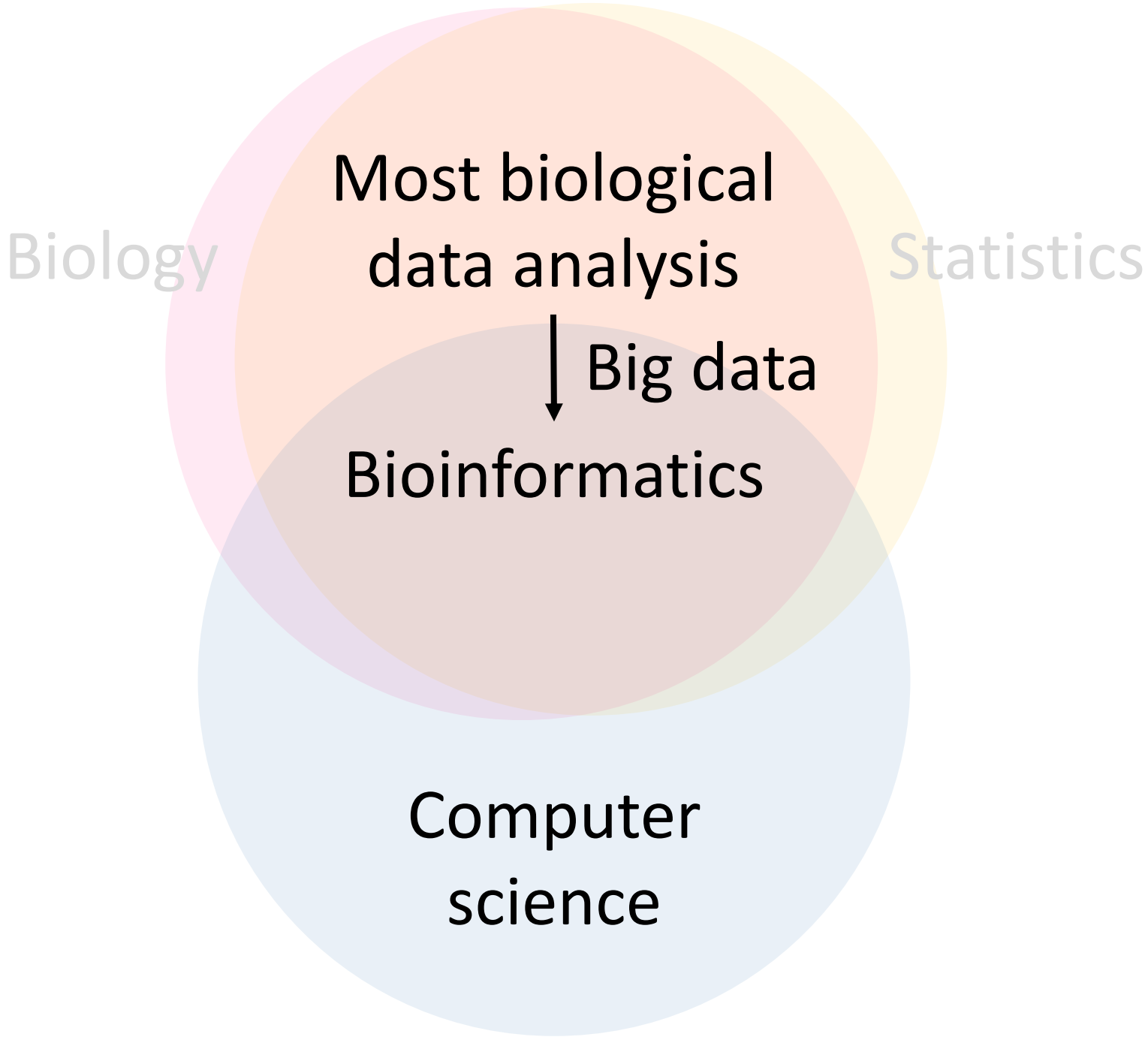

Biology
Statistics
Bioinformatics
Computer
science


Biology
Most biological data analysis
Statistics
Bioinformatics
Computer science


Biology
Statistics
Most biological data analysis
Big data
Bioinformatics
Computer science

Goal

Build a bioinformatic pipeline to efficiently and reproducibly analyze Respiratory Pathogen ID/AMR Panel sequences

Specific aims

- Generate COVID19 consensus genomes for upload to public database
- Track variants of interest and variants of concern
- Track co-infections
- Create phylogenetic trees to
  - Track local variants
  - Identify local outbreaks
- Create reproducible, informative reports

Building a pipeline: Consider the data

Respiratory Pathogen ID/AMR Panel (RPIP)

- Targeted sequencing of
  - All genes in SARS-CoV-2
  - All genes in influenza A/B
  - Identifying genes for 180 bacteria, 50 fungi, 40 viruses
  - Identifying genes for 1200 antimicrobial resistance markers

- Sequences from MiniSeq
  - 7 million (mid)
  - 20 million (rapid)
  - 22 million (high)

Raw data

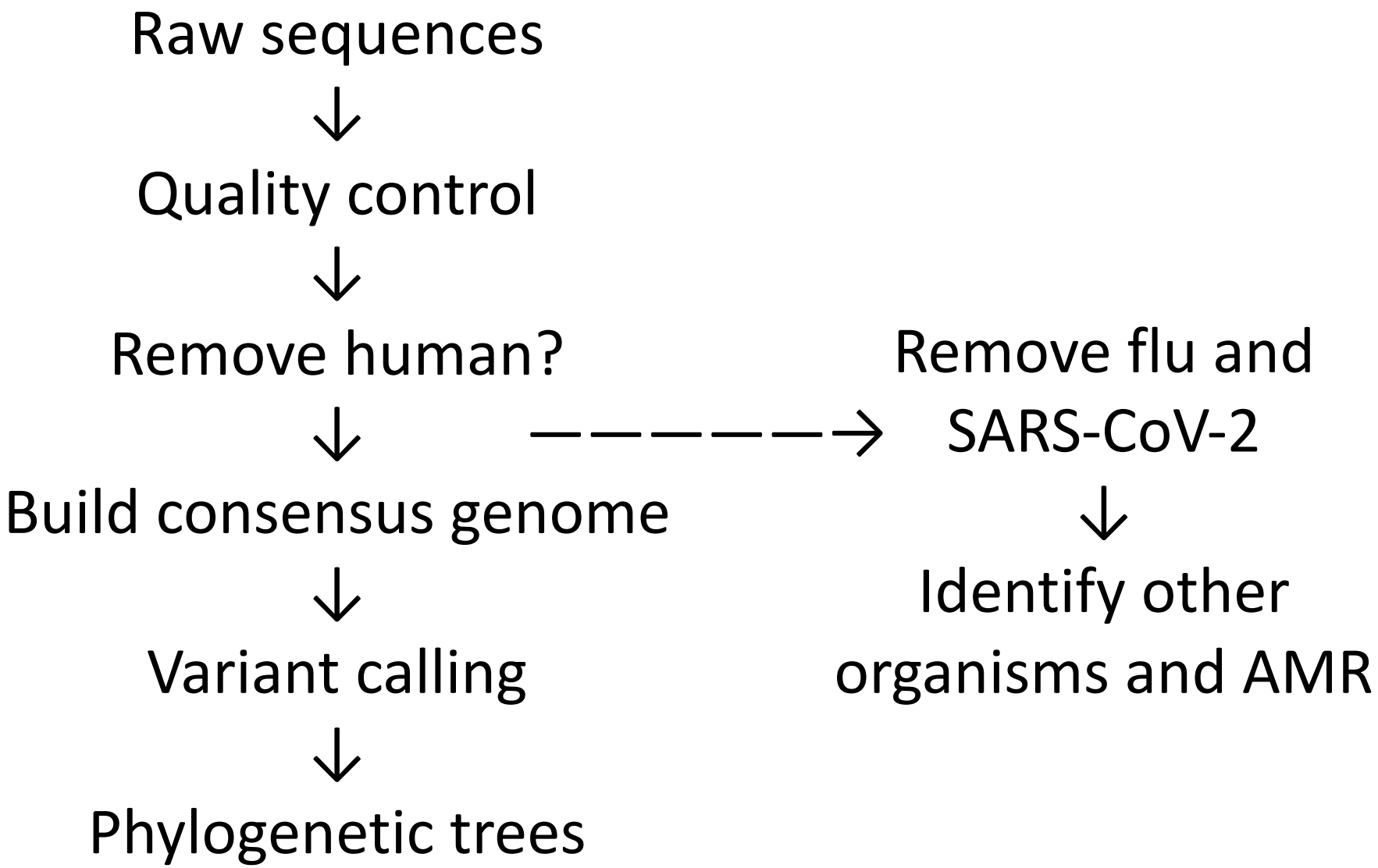
.fastq

`@SRR001666.1`	Unique ID
`GGGTGATGGCCGCTGCCGATGGCGTCAAATCCCAC`	Sequence
`+SRR001666.1`	+Unique ID repeated
`IIIIIIIIIIIIIIIIIIIIIIIIIIIIII9IG9IC`	Quality scores

.fasta

`@SRR001666.1`	Unique ID
`GGGTGATGGCCGCTGCCGATGGCGTCAAATCCCAC`	Sequence

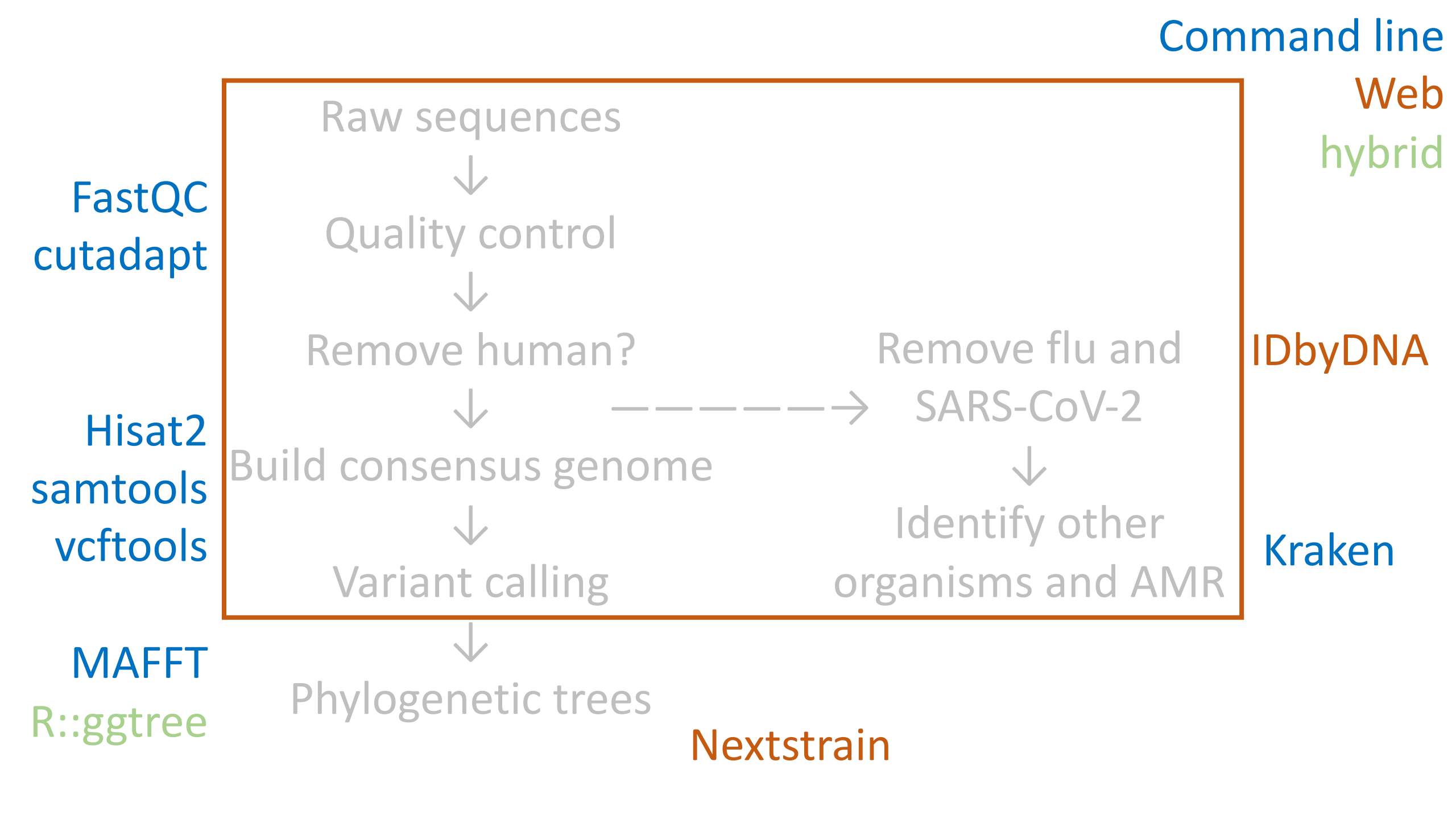
Building a pipeline: Outline a workflow


Raw sequences
↓
Quality control
↓
Remove human?
↓
Build consensus genome
↓
Variant calling
↓
Phylogenetic trees
Remove flu and
SARS-CoV-2
↓
Identify other
organisms and AMR

Building a pipeline: Research software

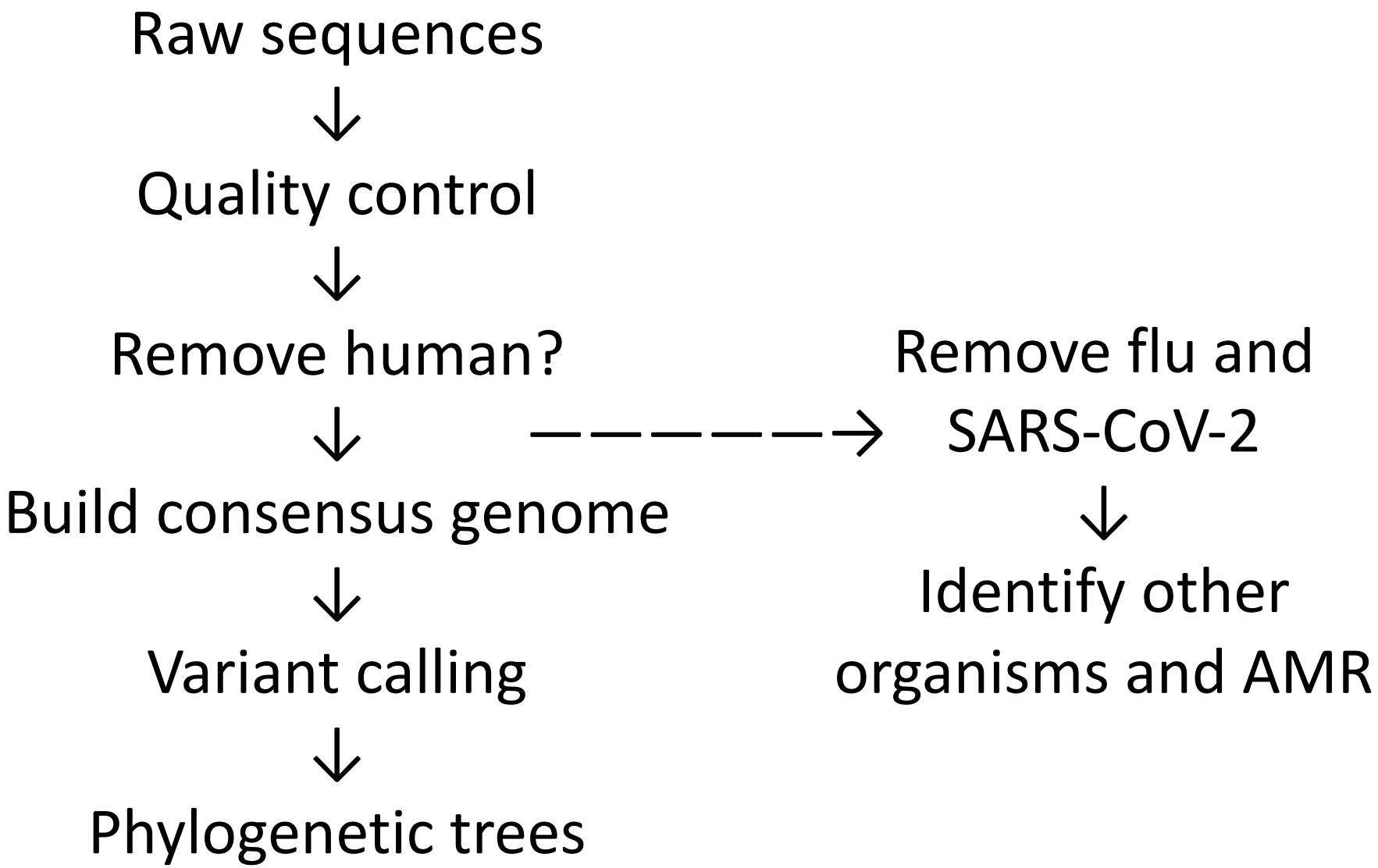
Software considerations

0. Usefulness
   - Does it do what you need it to do?
1. Documentation
   - Manuals, tutorials, GitHub
   - Open-source or black box
2. Cost
   - Ownership vs annual membership vs cost-per-use
3. Platform
   - Local (Windows, Mac, Linux), cloud, web
   - Command line, graphical user interface (GUI), hybrid


Command line
Web
hybrid
FastQC
cutadapt
Raw sequences
Quality control
Remove human?
IDbyDNA
Hisat2
samtools
vcftools
Remove flu and
SARS-CoV-2
Build consensus genome
Identify other
organisms and AMR
Kraken
Variant calling
MAFFT
R::ggtree
Phylogenetic trees
Nextstrain

Specific aims

- Generate COVID19 consensus genomes for upload to public database
- Track variants of interest and variants of concern
  - How often does it update the global database?
- Track co-infections
- Create phylogenetic trees to
  - Track local variants
  - Identify local outbreaks
- Create reproducible, informative reports
  - How will software updates impact results?


Raw sequences
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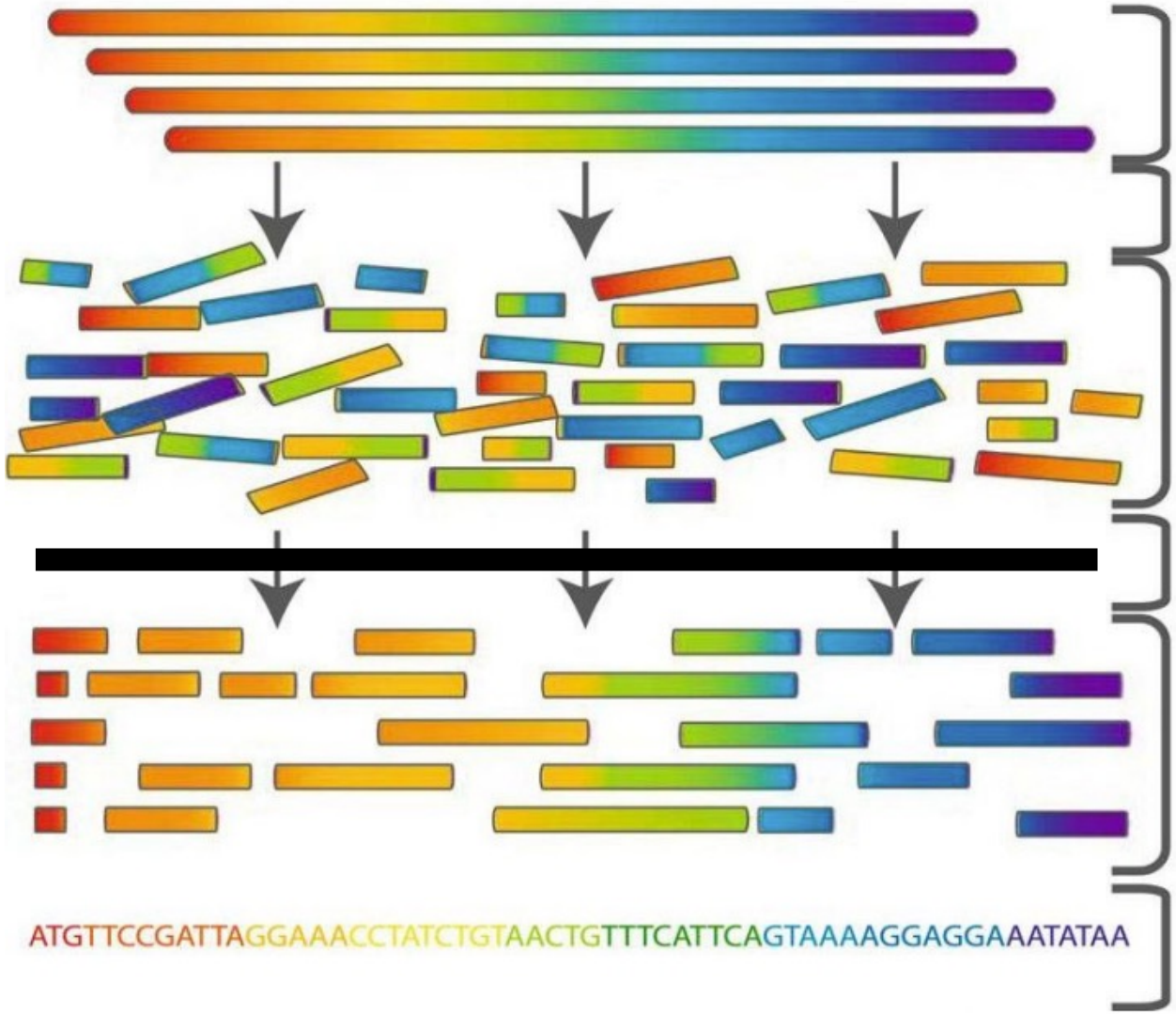
Building a consensus genome



DNA in sample

Shear / amplify

Sequenced reads

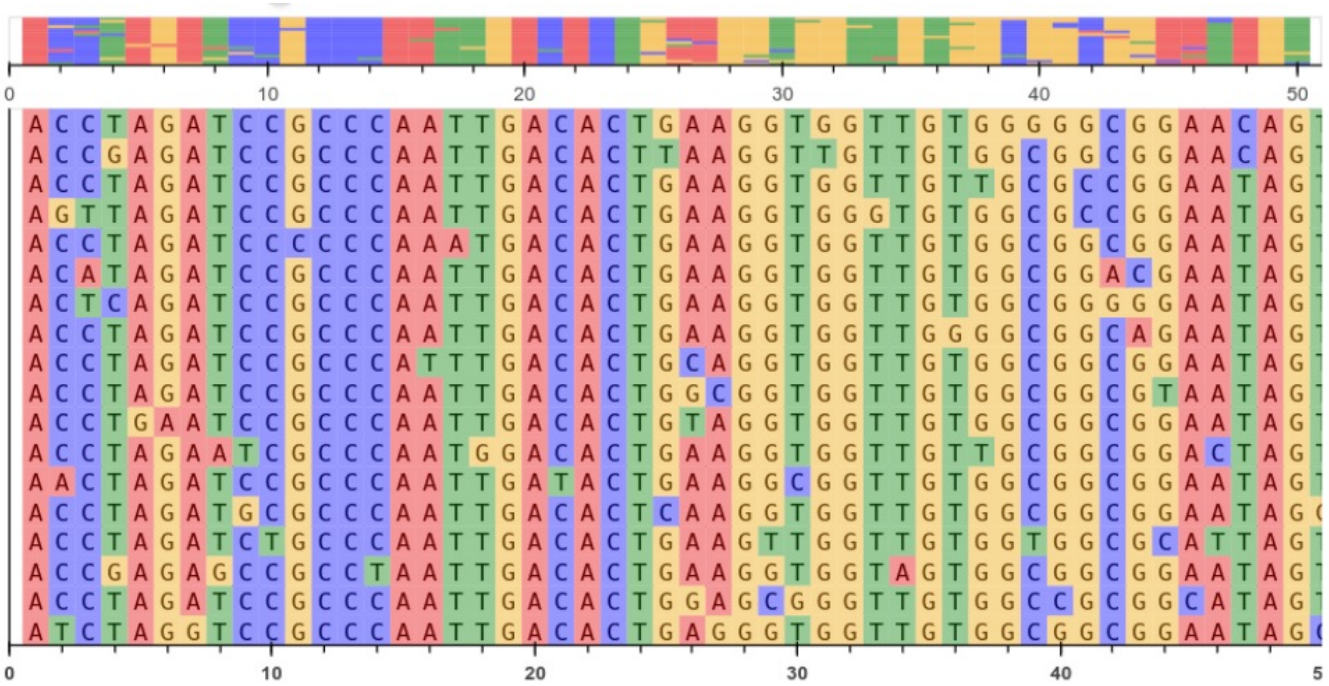
Reference genome

Reconstruct ~~*de novo*~~

Consensus

Torsten Seemann

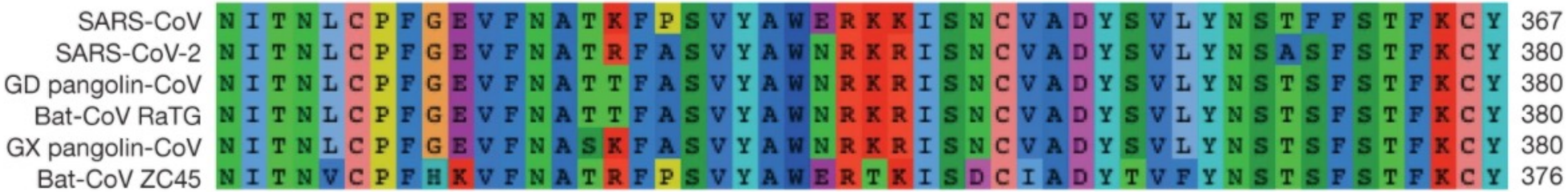
Errors in consensus



- Real or sequencing error? → PhiX control DNA
- If it's real…
  - IUPAC codes for uncertainty → R = A/G, N = ACTG, etc
  - Mixed population

Variant calling

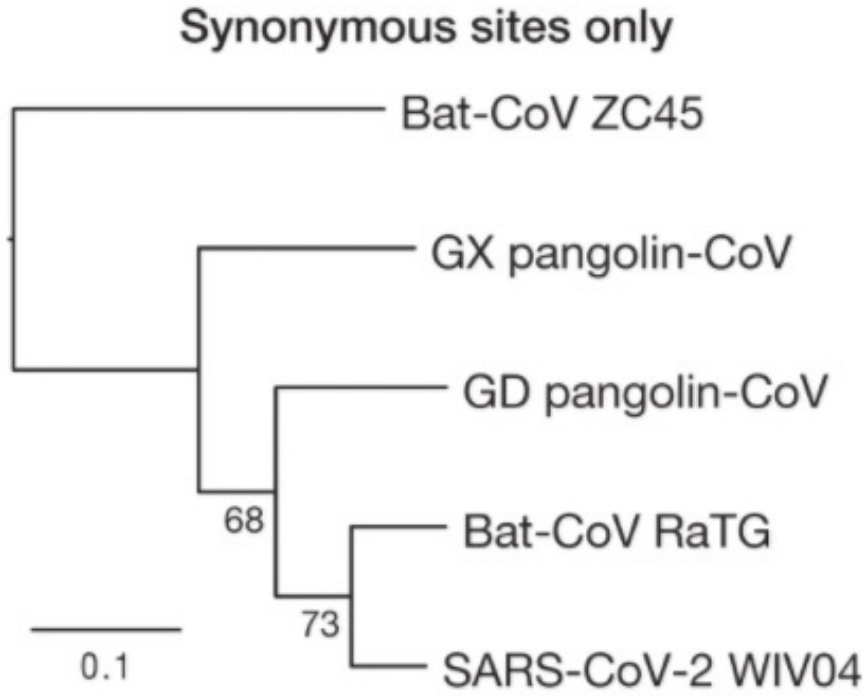
- Compare to database with variants of interest/concern
- Usually translated alignment



Lam et al 2020 doi: 10.1038/s41586-020-2169-0

Phylogenetic tree

- Based on translated alignment
- Groups more similar “species”



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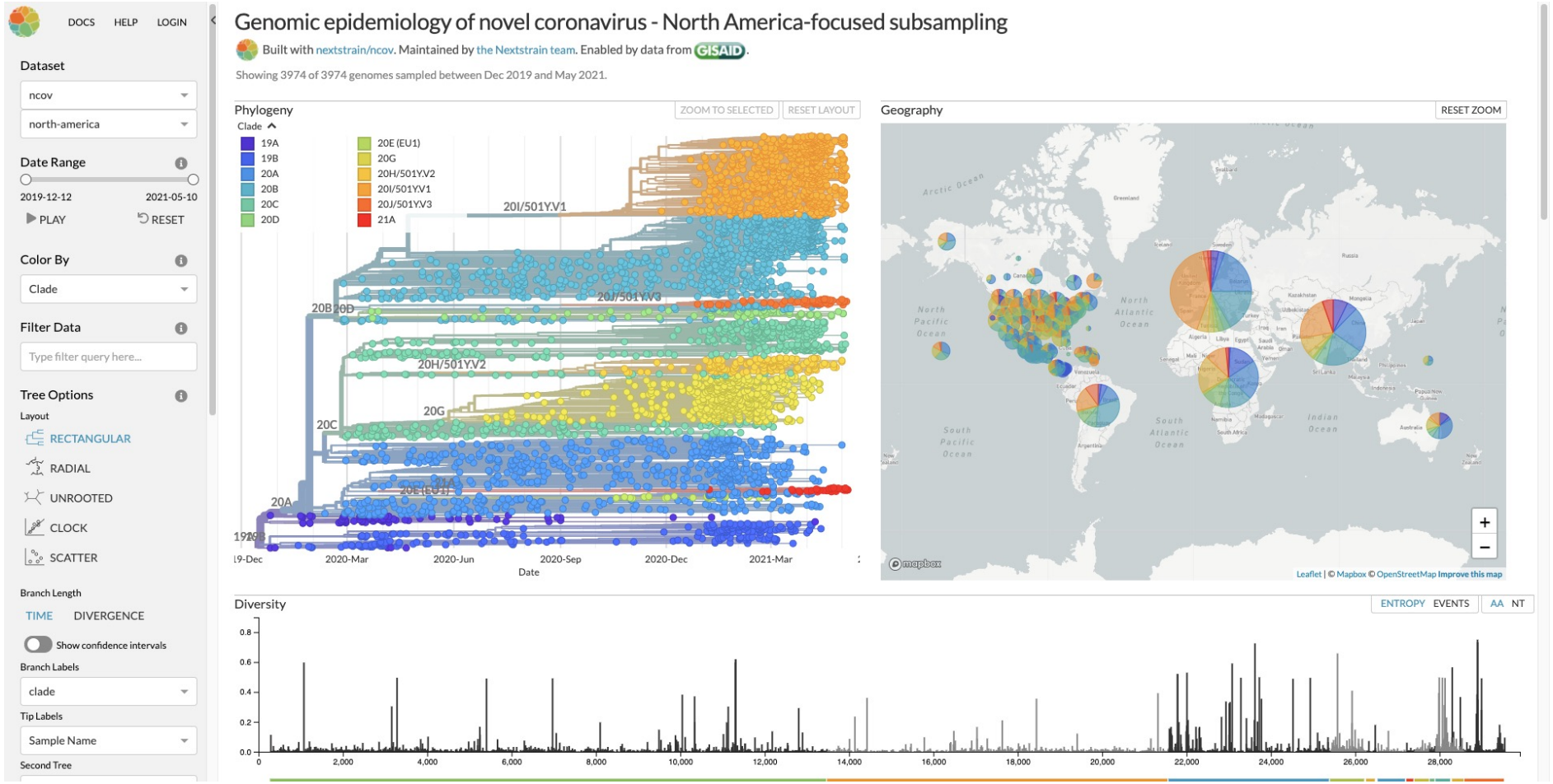
NextStrain



https://nextstrain.org/ncov/north-america

Building a pipeline: Putting it all together

Pipeline wrappers

- Custom executable script
  - Short input in command line like `my_pipeline.sh data.fastq`
  - Download data + databases and run locally
- Web-based workflows (Terra Bio, Shiny apps)
  - Run executable script in the cloud
  - Data + databases can also be stored in the cloud
- Hybrid with IDbyDNA
  - Most steps on web
  - Download consensus sequence
  - Trees run locally, web, or cloud

Next steps

- Decide on desired pipeline format (platform, wrapper, cost, etc)
- Build and test pipeline on pilot data

Addtl resources

- NextStrain https://nextstrain.org/
- Shiny apps https://shiny.rstudio.com/gallery/
- Terra Bio https://terra.bio/
  - Example workflow usage https://www.youtube.com/watch?v=HObb_J9fPc0&t=604s