Getting started with Nextstrain

COVID-19 Genomic Epidemiology Toolkit: Module 3.1

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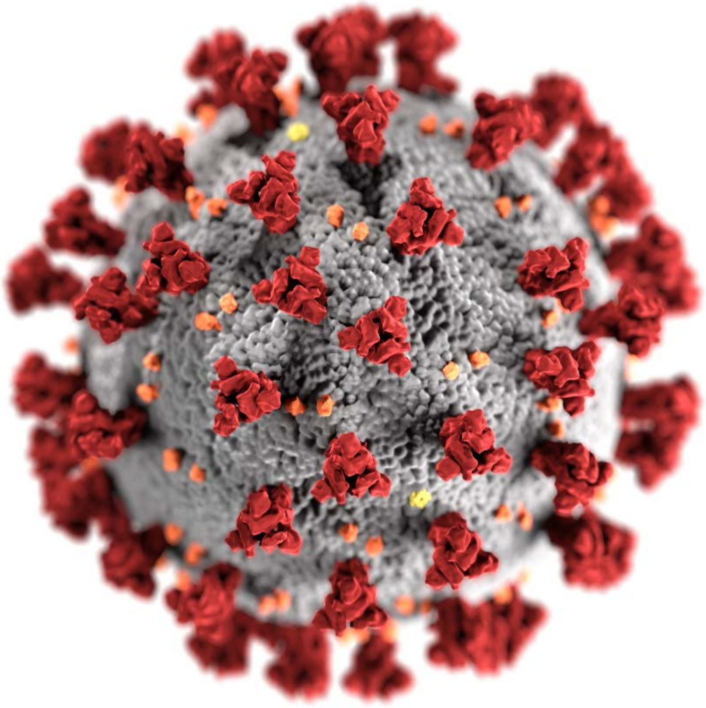


cdc.gov/coronavirus

Toolkit map

Part 1: Introduction

1.1 What is genomic epidemiology?

1.2 The SARS-CoV-2 genome

1.3 How to read phylogenetic trees

Part 2: Case Studies

2.1 SARS-CoV-2 sequencing in Arizona

2.2 Healthcare cluster transmission

2.3 Community Transmission

Part 3: Implementation

3.1 Getting started with Nextstrain

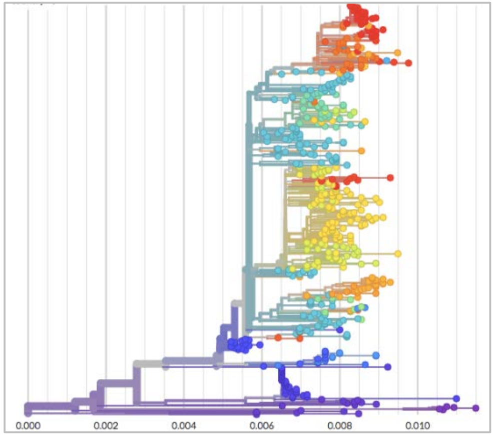
3.2 Getting started with MicrobeTrace

3.3 Linking epidemiologic data

What is Nextstrain?

- Open-source project to harness the power of pathogen genome data
- Powerful analytics and interactive visualizations
- Designed to aid epidemiological understanding, improve outbreak response, and provide real-time snapshots of evolving pathogen populations

- Learn (a lot) more at
  - https://nextstrain.org
  - https://docs.nextstrain.org
  - 🐦 @nextstrain



Nextstrain: Default view

Phylogeny

Map

Genome

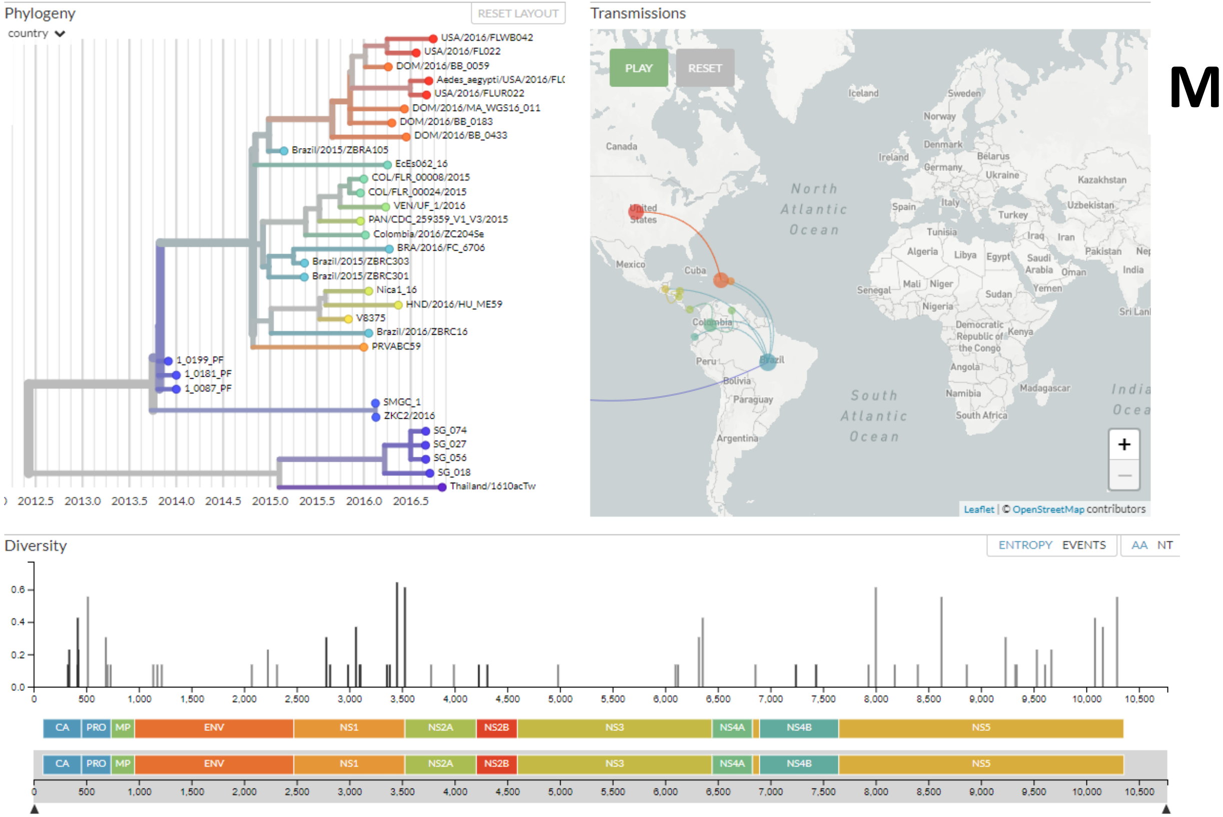
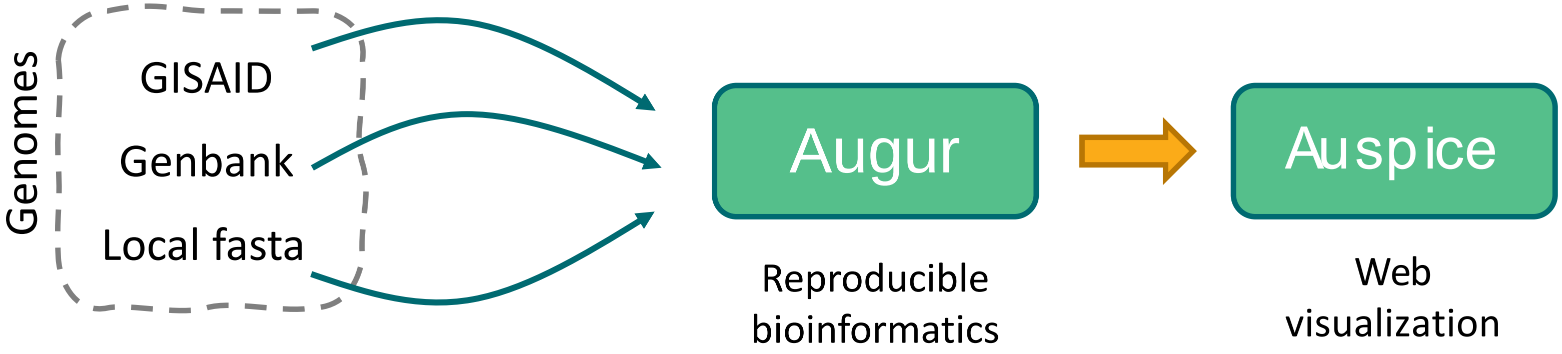


Image from Trevor Bedford Group: nextstrain.org

Nextstrain architecture

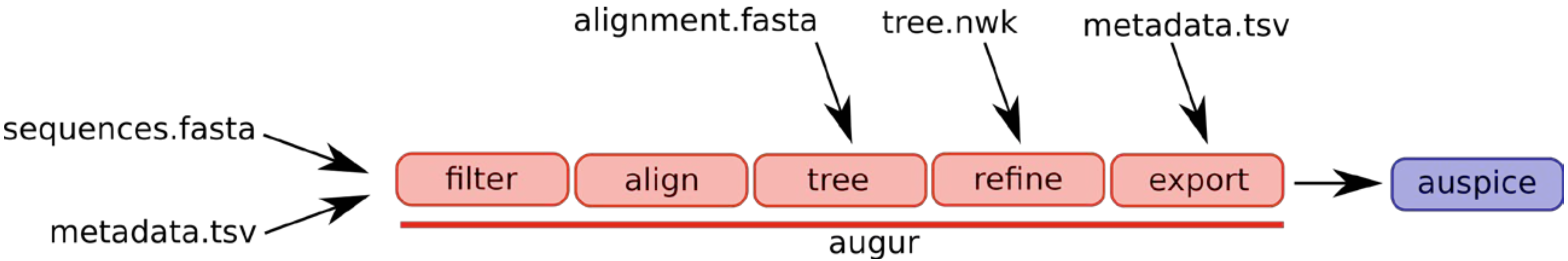
Two goals, two components

1. Rapid and flexible phylodynamic analysis (*Augur*)
2. Interactive visualization (*Auspice*)



More details at: nextstrain.org/docs/getting-started/introduction

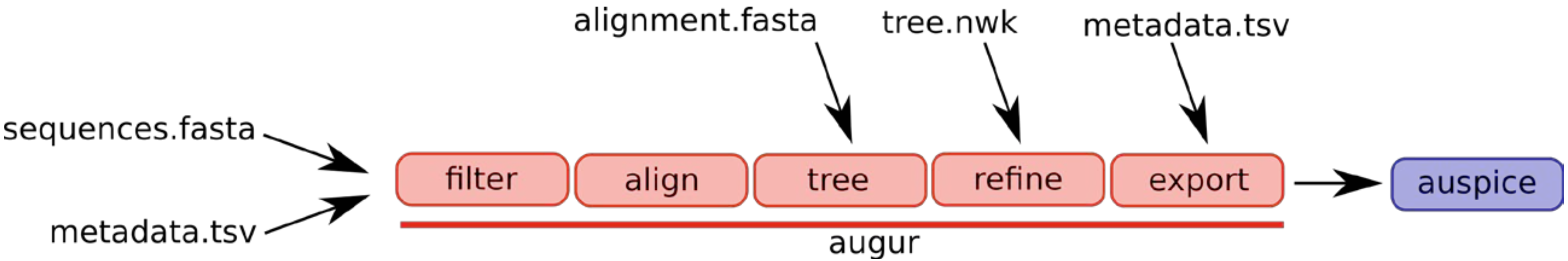
Augur: what does it do?



- Input data
  - sequences.fasta
  - metadata.tsv
  - *Can also import a tree if already constructed (like a Bayesian tree)*
- Visualization data for Auspice
  - Colors, lat_longs, reference genome

Adapted from Nathan Grubaugh nextstrain.org

Augur: what does it do?

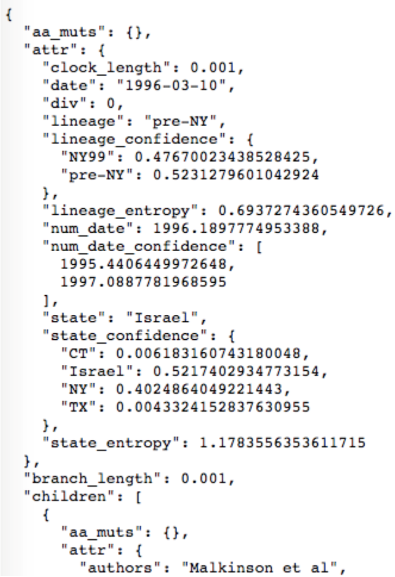


- What augur does
  - Prepare pathogen sequences and metadata
  - Align sequences
  - Construct a phylogeny from aligned sequences
  - Annotate the phylogeny with inferred ancestral pathogen dates, sequences, and traits
  - Export the annotated phylogeny and corresponding metadata into auspice-readable text file (JSON)

Adapted from Nathan Grubaugh nextstrain.org

Auspice: what does it do?

- Interactive web-app for tree visualization
  - Translates data text files from augur into trees



```
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  "aa_muts": {},
  "attr": {
    "clock_length": 0.001,
    "date": "1996-03-10",
    "div": 0,
    "lineage": "pre-NY",
    "lineage_confidence": {
      "NY99": 0.47670023438528425,
      "pre-NY": 0.5231279601042924
    },
    "lineage_entropy": 0.6937274360549726,
    "num_date": 1996.1897774953388,
    "num_date_confidence": [
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      1997.0887781968595
    ],
    "state": "Israel",
    "state_confidence": {
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      "Israel": 0.5217402934773154,
      "NY": 0.4024864049221443,
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      "attr": {
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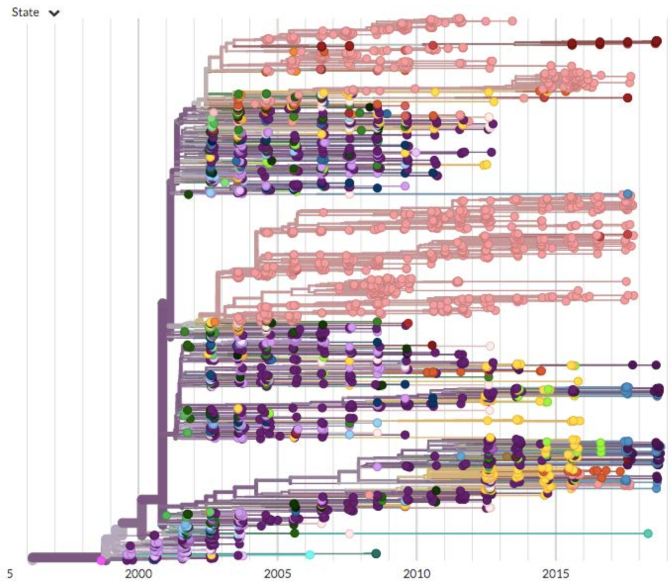


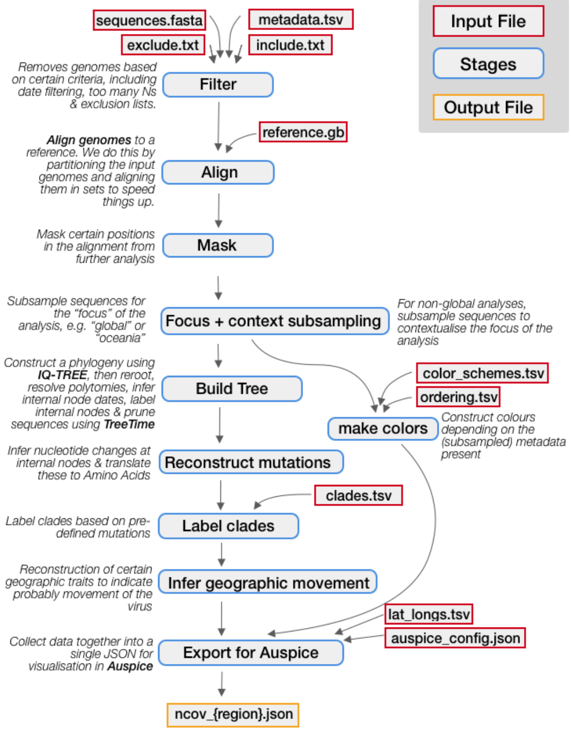
Adapted from Nathan Grubaugh nextstrain.org

What is a Nextstrain ‘build’ ?

- ***Set of commands, parameters, and input files to reproducibly execute bioinformatic analyses and generate an output file for visualization***

- Allows user to frequently run several different analysis workflows or datasets, for example:
  1. Just your lab’s data, from your jurisdiction
  2. Your data AND data from public repositories
  3. Data from your jurisdiction AND neighboring counties/states/etc.

- Nextstrain’s focus on providing a *real-time* snapshot of evolving pathogen populations necessitates a reproducible analysis that can be rerun when new sequences are available



Removes genomes based on certain criteria, including date filtering, too many Ns & exclusion lists.

***Align genomes** to a reference. We do this by partitioning the input genomes and aligning them in sets to speed things up.*

Mask certain positions in the alignment from further analysis

Subsample sequences for the “focus” of the analysis, e.g. “global” or “oceania”

For non-global analyses, subsample sequences to contextualise the focus of the analysis

*Construct a phylogeny using **IQ-TREE**, then reroot, resolve polytomies, infer internal node dates, label internal nodes & prune sequences using **TreeTime***

Construct colours depending on the (subsampled) metadata present

Infer nucleotide changes at internal nodes & translate these to Amino Acids

Label clades based on pre-defined mutations

Reconstruction of certain geographic traits to indicate probably movement of the virus

*Collect data together into a single JSON for visualisation in **Auspice***

https://docs.nextstrain.org/en/latest/tutorials/SARS-CoV-2/steps/orientation-workflow.html

Nextstrain documentation

- **A Getting Started Guide to the Genomic Epidemiology of SARS-CoV-2**
  - Template and tutorial walks through the process of running a basic phylogenetic analysis on SARS-CoV-2 data, specifically to enable Departments of Public Health to start using Nextstrain to understand their SARS-CoV-2 genomic data
  - https://docs.nextstrain.org/en/latest/tutorials/SARS-CoV-2/steps/index.html#a-getting-started-guide-to-the-genomic-epidemiology-of-sars-cov-2

Analysis:

1. Setup and installation
2. Preparing your data
3. Orientation: analysis workflow
4. Orientation: which files should I touch?
5. Running & troubleshooting
6. Customizing your analysis
7. Customizing your visualization

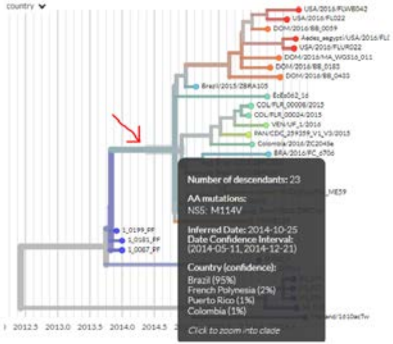
Visualization and interpretation:

1. Options for visualizing and sharing results
2. Interpreting your results
3. Writing a narrative to highlight key findings

Nextstrain documentation

- **Interacting with auspice, the visualization web application**
  - Guides through the default phylogeny, map, and genome panels
  - https://neherlab.org/201901_krisp_auspice.html

Node details



Number of descendants: 23

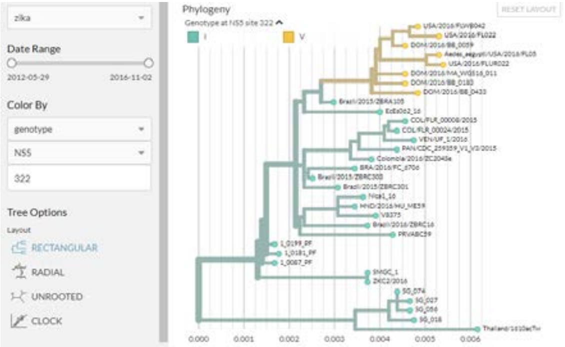
AA mutations:
NS5: M114V

Inferred Date: 2014-10-25
Date Confidence Interval:
(2014-05-11, 2014-12-21)

Country (confidence):
Brazil (95%)
French Polynesia (2%)
Puerto Rico (1%)
Colombia (1%)

Click to zoom into clade

Highlight variants



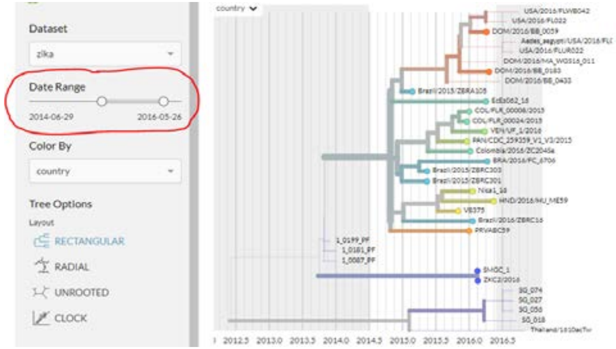
zika

Date Range
2012-05-29 2016-11-02

Color By
genotype
NS5
322

Tree Options

Select date ranges



Dataset
zika

Date Range
2014-06-29 2016-05-26

Color By
country

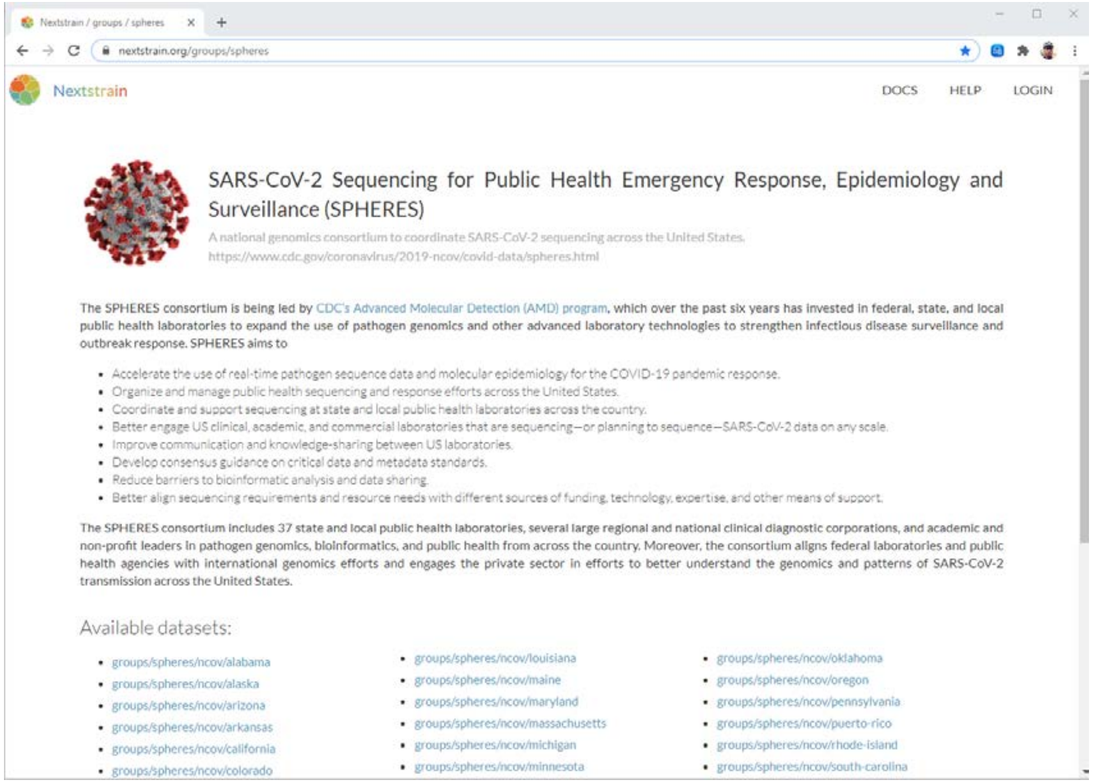
Tree Options

Images from nextstrain.org

SARS-CoV-2 Sequencing for Public Health Emergency Response, Epidemiology and Surveillance

SPHERES

nextstrain.org/groups/spheres



SARS-CoV-2 Sequencing for Public Health Emergency Response, Epidemiology and Surveillance (SPHERES)

A national genomics consortium to coordinate SARS-CoV-2 sequencing across the United States.
https://www.cdc.gov/coronavirus/2019-ncov/covid-data/spheres.html

The SPHERES consortium is being led by CDC's Advanced Molecular Detection (AMD) program, which over the past six years has invested in federal, state, and local public health laboratories to expand the use of pathogen genomics and other advanced laboratory technologies to strengthen infectious disease surveillance and outbreak response. SPHERES aims to

- Accelerate the use of real-time pathogen sequence data and molecular epidemiology for the COVID-19 pandemic response.
- Organize and manage public health sequencing and response efforts across the United States.
- Coordinate and support sequencing at state and local public health laboratories across the country.
- Better engage US clinical, academic, and commercial laboratories that are sequencing—or planning to sequence—SARS-CoV-2 data on any scale.
- Improve communication and knowledge-sharing between US laboratories.
- Develop consensus guidance on critical data and metadata standards.
- Reduce barriers to bioinformatic analysis and data sharing.
- Better align sequencing requirements and resource needs with different sources of funding, technology, expertise, and other means of support.

The SPHERES consortium includes 37 state and local public health laboratories, several large regional and national clinical diagnostic corporations, and academic and non-profit leaders in pathogen genomics, bioinformatics, and public health from across the country. Moreover, the consortium aligns federal laboratories and public health agencies with international genomics efforts and engages the private sector in efforts to better understand the genomics and patterns of SARS-CoV-2 transmission across the United States.

Available datasets:

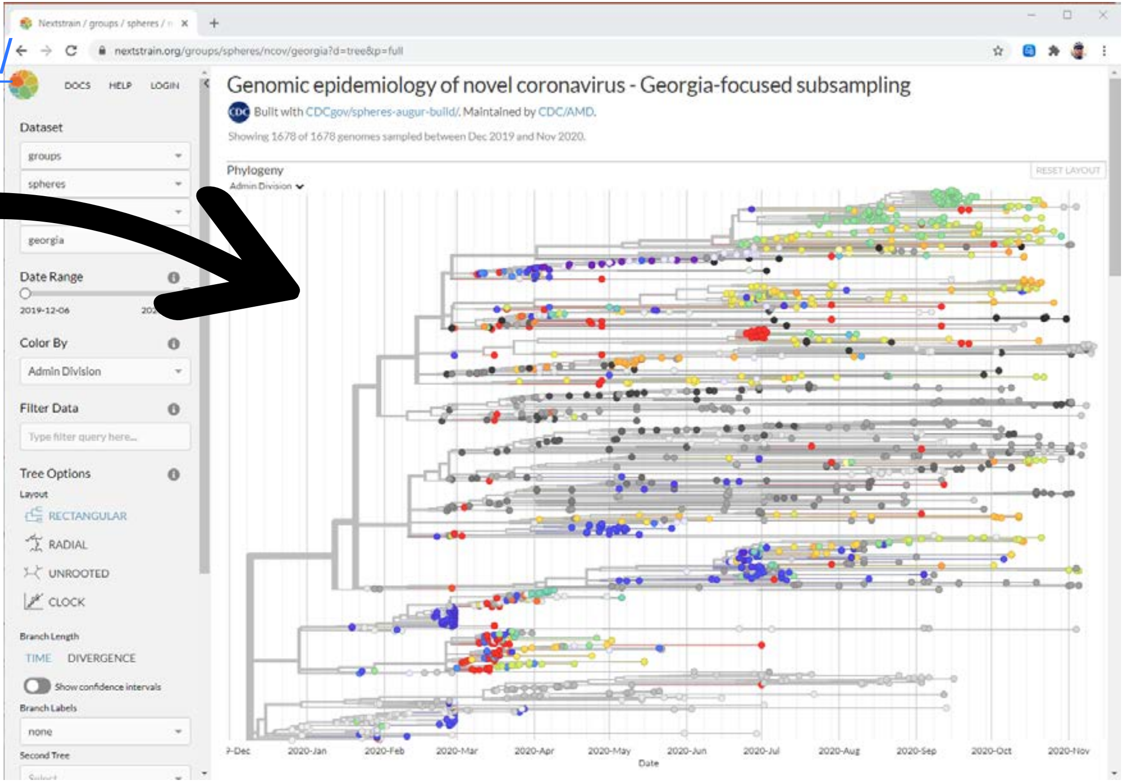
- groups/spheres/ncov/alabama
- groups/spheres/ncov/alaska
- groups/spheres/ncov/arizona
- groups/spheres/ncov/arkansas
- groups/spheres/ncov/california
- groups/spheres/ncov/colorado
- groups/spheres/ncov/louisiana
- groups/spheres/ncov/maine
- groups/spheres/ncov/maryland
- groups/spheres/ncov/massachusetts
- groups/spheres/ncov/michigan
- groups/spheres/ncov/minnesota
- groups/spheres/ncov/oklahoma
- groups/spheres/ncov/oregon
- groups/spheres/ncov/pennsylvania
- groups/spheres/ncov/puerto-rico
- groups/spheres/ncov/rhode-island
- groups/spheres/ncov/south-carolina

Drag-n-drop metadata

nextstrain.org/groups/spheres /ncov/georgia

metadata.tsv

Strains	Cluster
Isolate-1	Hospital
Isolate-2	Hospital
Isolate-3	University
…	…



nextstrain.org/groups/spheres/ncov/georgia?d=tree&p=full

Genomic epidemiology of novel coronavirus - Georgia-focused subsampling

Built with CDCgov/spheres-augur-build/. Maintained by CDC/AMD.

Showing 1678 of 1678 genomes sampled between Dec 2019 and Nov 2020.

Dataset: groups, spheres, georgia

Date Range: 2019-12-06

Color By: Admin Division

Filter Data: Type filter query here...

Layout: RECTANGULAR

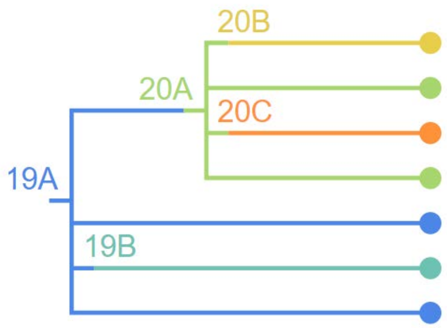
Branch Length: TIME

Branch Labels: none

Second Tree: Select

What about Nextclade?

- Web-browser tool to quickly analyze your SARS-CoV-2 genome:
  - Identify mutations compared to a reference used by Nextstrain
  - Assign your sequences to major clades
  - Quality check of your sequence data



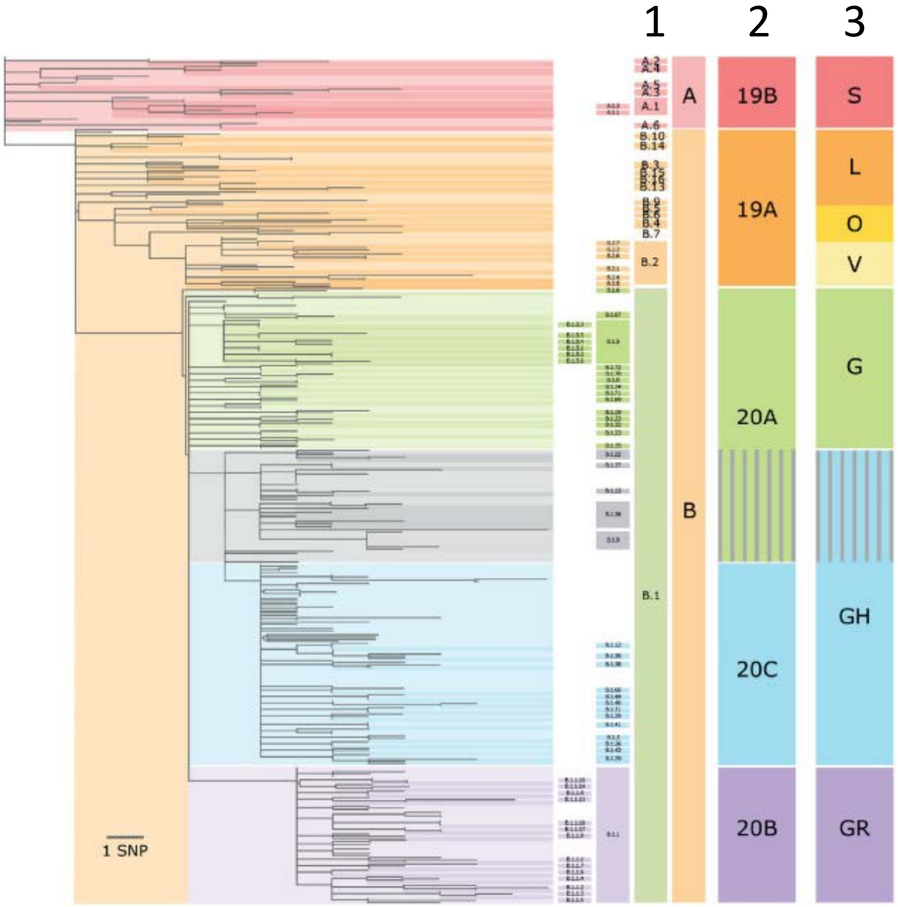
- Drag-and-drop a sequence file or paste sequences into the text box
- All analyses happen in your browser, never leaving your computer

Images from https://clades.nextstrain.org/

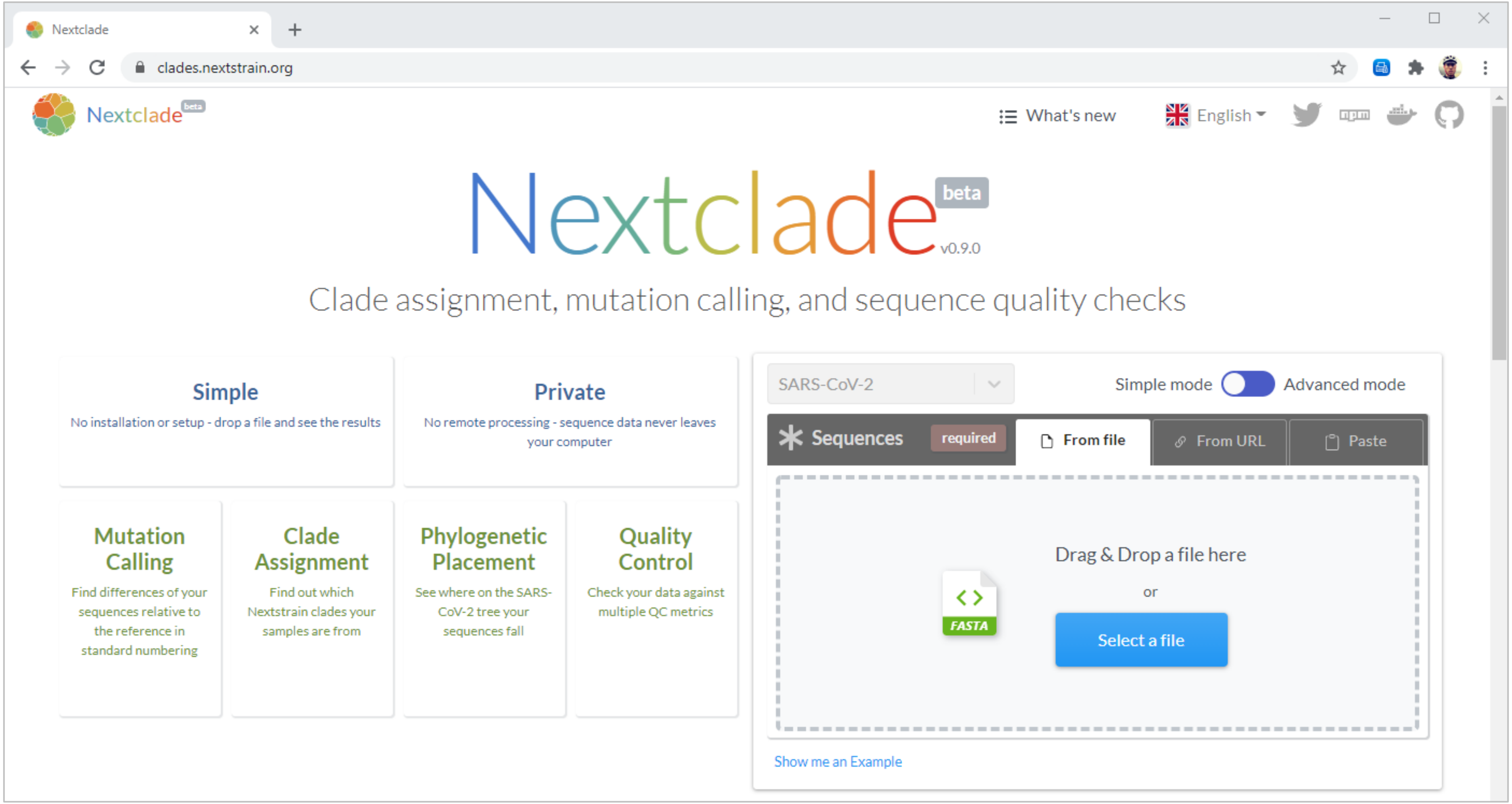
SARS-CoV-2 clades:

Term for clades:

1. Pangolin Lineages
   - cov-lineages.org
2. Clades by Nextstrain ****
   - nextstrain.org
3. Clades by GISAID
   - gisaid.org



Adapted from Alm et al. 2020



Nextclade beta v0.9.0

Clade assignment, mutation calling, and sequence quality checks

Simple

No installation or setup - drop a file and see the results

Private

No remote processing - sequence data never leaves your computer

Mutation Calling

Find differences of your sequences relative to the reference in standard numbering

Clade Assignment

Find out which Nextstrain clades your samples are from

Phylogenetic Placement

See where on the SARS-CoV-2 tree your sequences fall

Quality Control

Check your data against multiple QC metrics

https://clades.nextstrain.org/

Summary

- Nextstrain is a powerful tool to analyze pathogen genomic data and aid epidemiological understanding
- Design focus on *real-time* snapshots of evolving pathogen populations through reproducible analysis (augur)
- Features interactive web application for visualization (auspice)
- Widely used to monitor SARS-CoV-2 genome sequences
- TONS of documentation and tutorials at https://nextstrain.org

Acknowledgements

Nextstrain development is lead by

- Trevor Bedford, Fred Hutchinson Cancer Research Center
- Richard Neher, Universität Basel

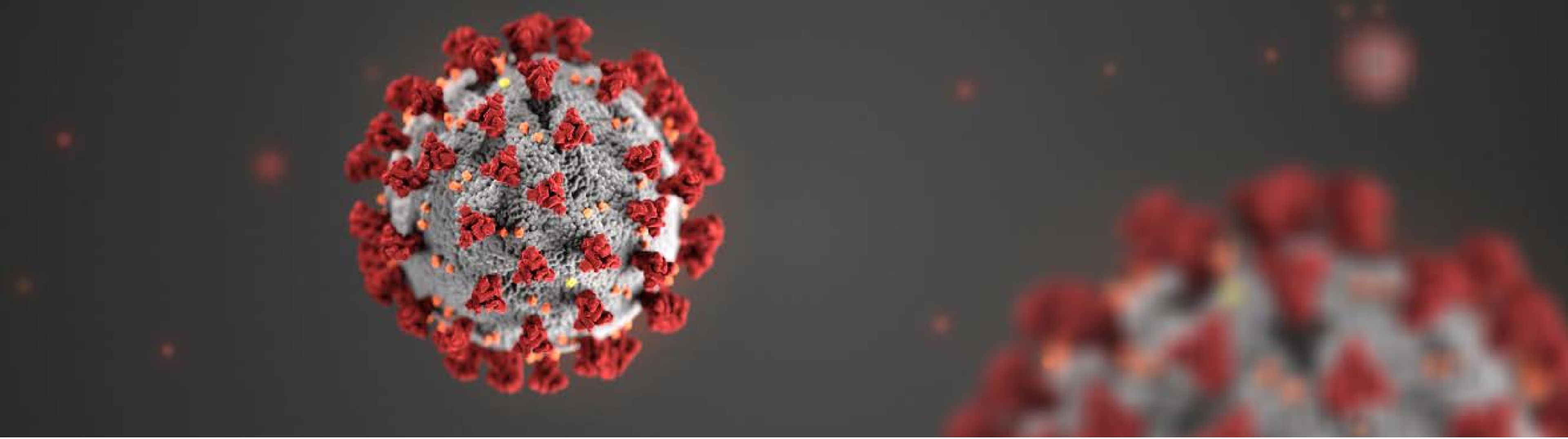
Nextstrain application to SARS-CoV-2 is lead by

- James Hadfield
- Emma Hodcroft

Learn more

- Next modules
  - 3.2 Getting started with MicrobeTrace
  - 3.3 Linking epidemiologic data

- COVID-19 Genomic Epidemiology Toolkit
  - Find further reading
  - Subscribe to receive updates on new modules as they are released
  - go.usa.gov/xAbMw



For more information, contact CDC
1-800-CDC-INFO (232-4636)
TTY: 1-888-232-6348 www.cdc.gov

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