

*Modernizing

nextstrain/**lassa**

Nextstrain build for Lassa virus



- Bedford Lab Meeting -

Jennifer Chang, Ph.D.

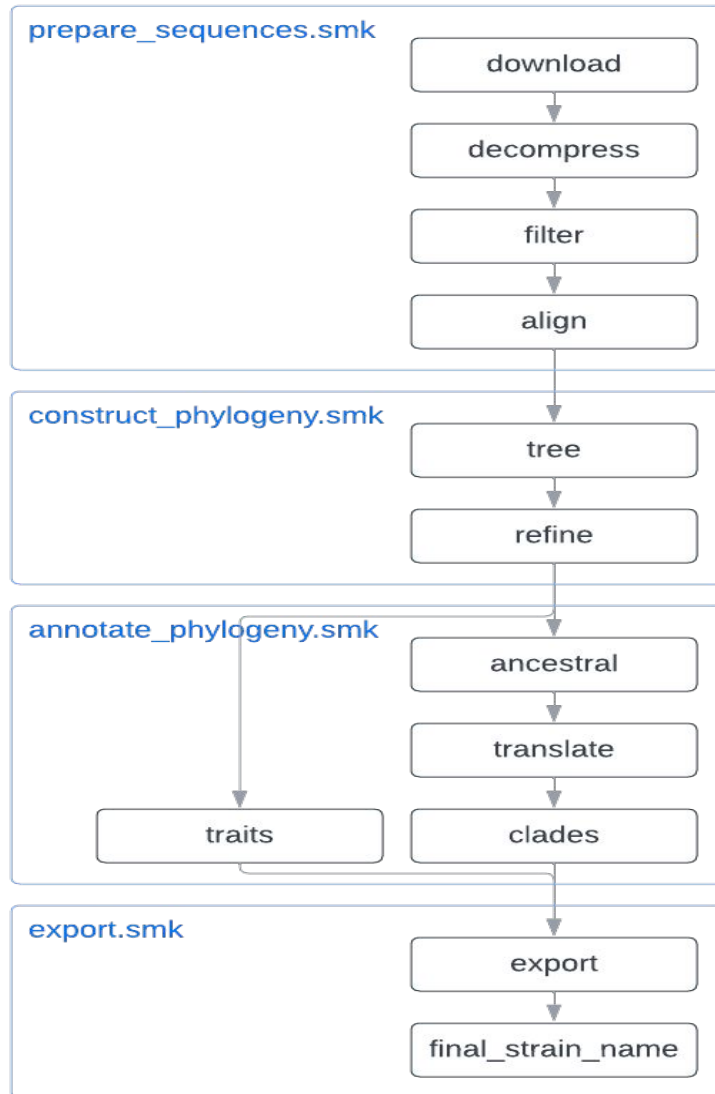
Bioinformatic Analyst III

Fred Hutchinson Cancer Center

Outline

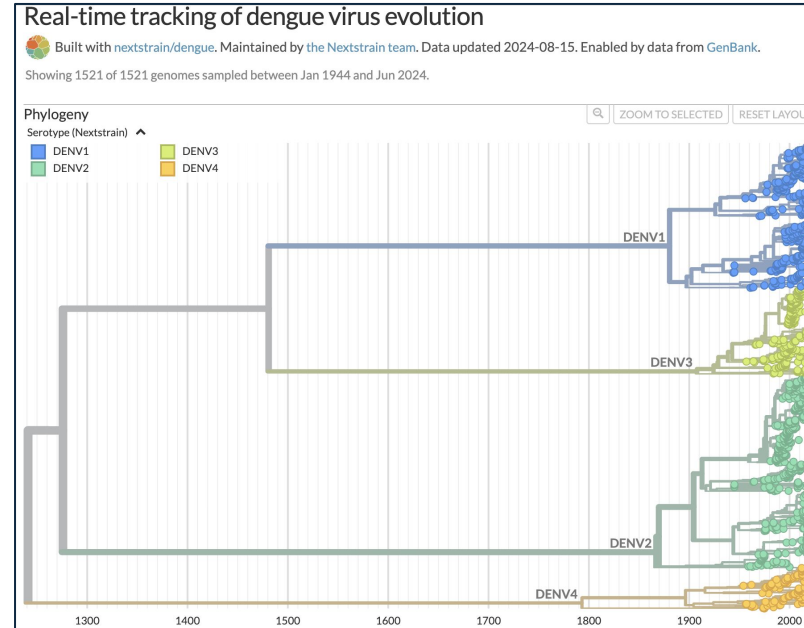
- **Lessons learned from modernizing Dengue workflows**
- About Lassa Virus
- Organizing meetings with Subject Matter Experts and feedback
- Thinking about external contributions
- Next steps

Lessons learned from modernizing Dengue



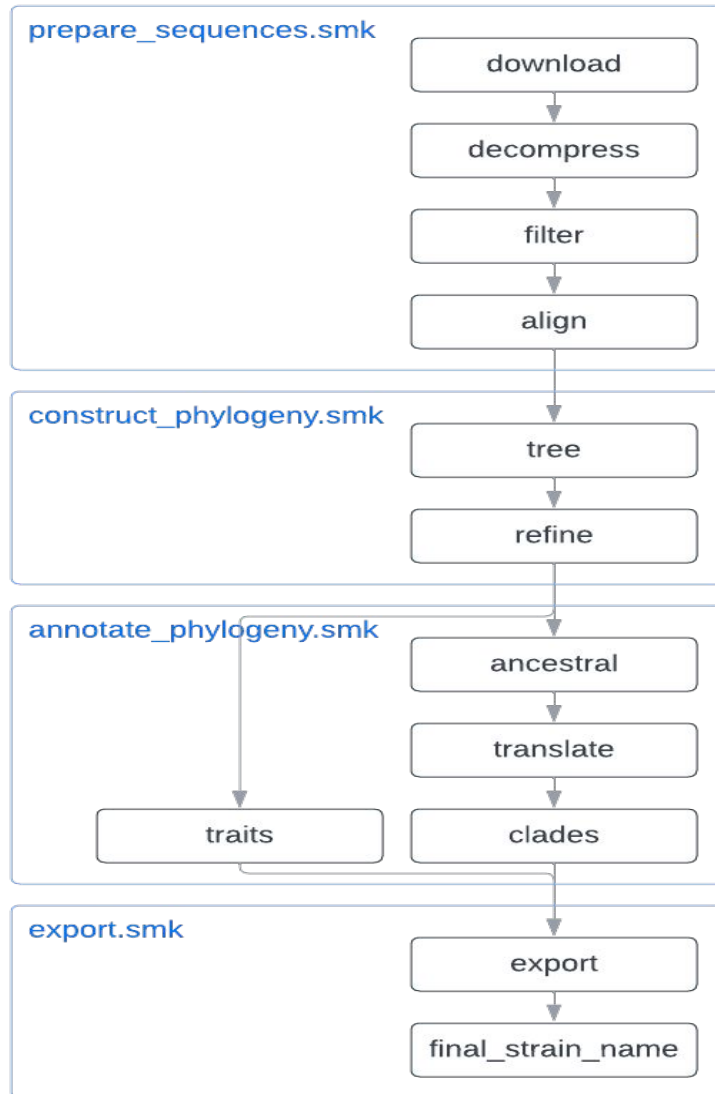
<https://github.com/nextstrain/dengue>

- Learned how to configure the **ingest** workflow
- Learned how to configure the **phylogenetic** workflow
- Learned how to create **gene** trees
- Learned some ways to generate a **Nextclade dataset**



Pipeline is organized according to the [GitHub: nextstrain/pathogen-repo-guide](https://github.com/nextstrain/pathogen-repo-guide)

Lessons learned from modernizing Dengue



<https://github.com/nextstrain/dengue>

- Learned how to configure the **ingest** workflow
- Learned how to configure the **phylogenetic** workflow
- Learned how to create **gene** trees
- Learned some ways to generate a **Nextclade dataset**
- Realized too late that the dengue serotype and genotype system is not particularly clear and therefore it became very difficult to "validate the accuracy of the dataset"
- Realized that working directly with one or more Subject-Matter-Experts (SMEs) could have flagged these and other issues faster than I can read the dengue literature

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Lessons learned from modernizing Dengue

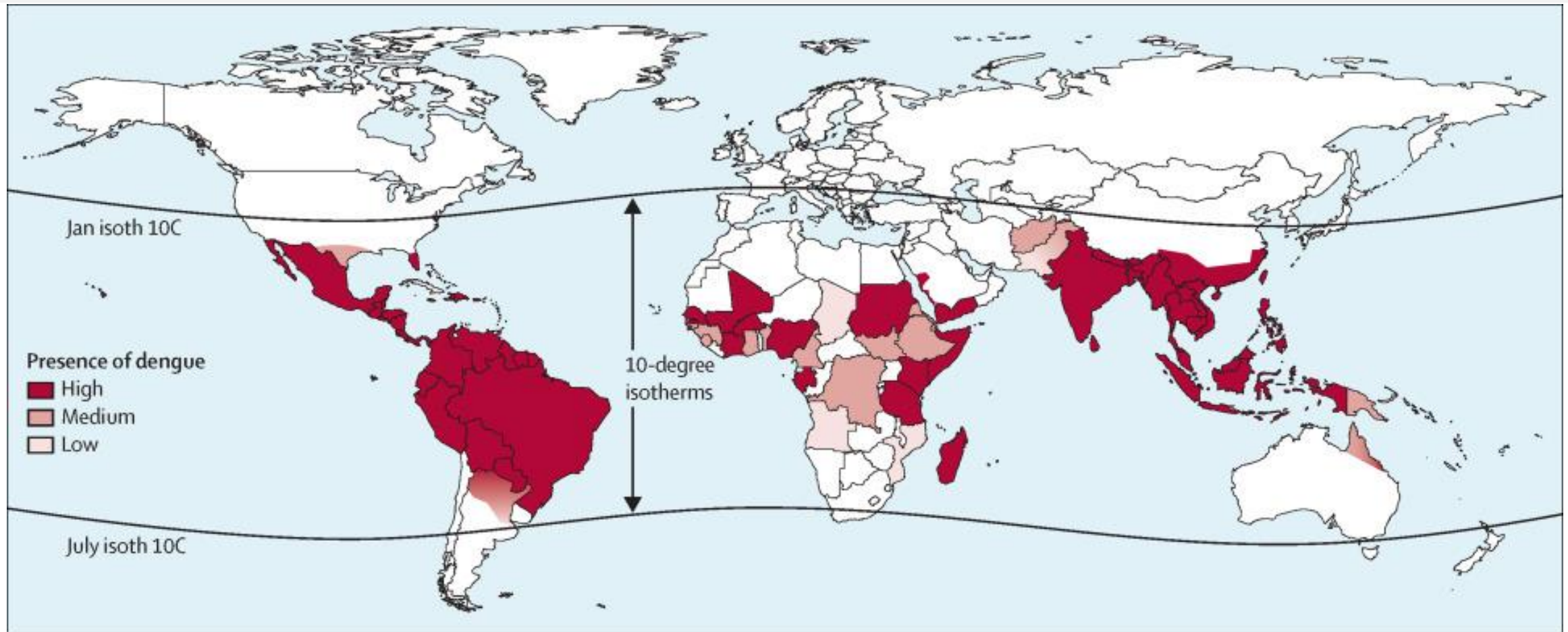
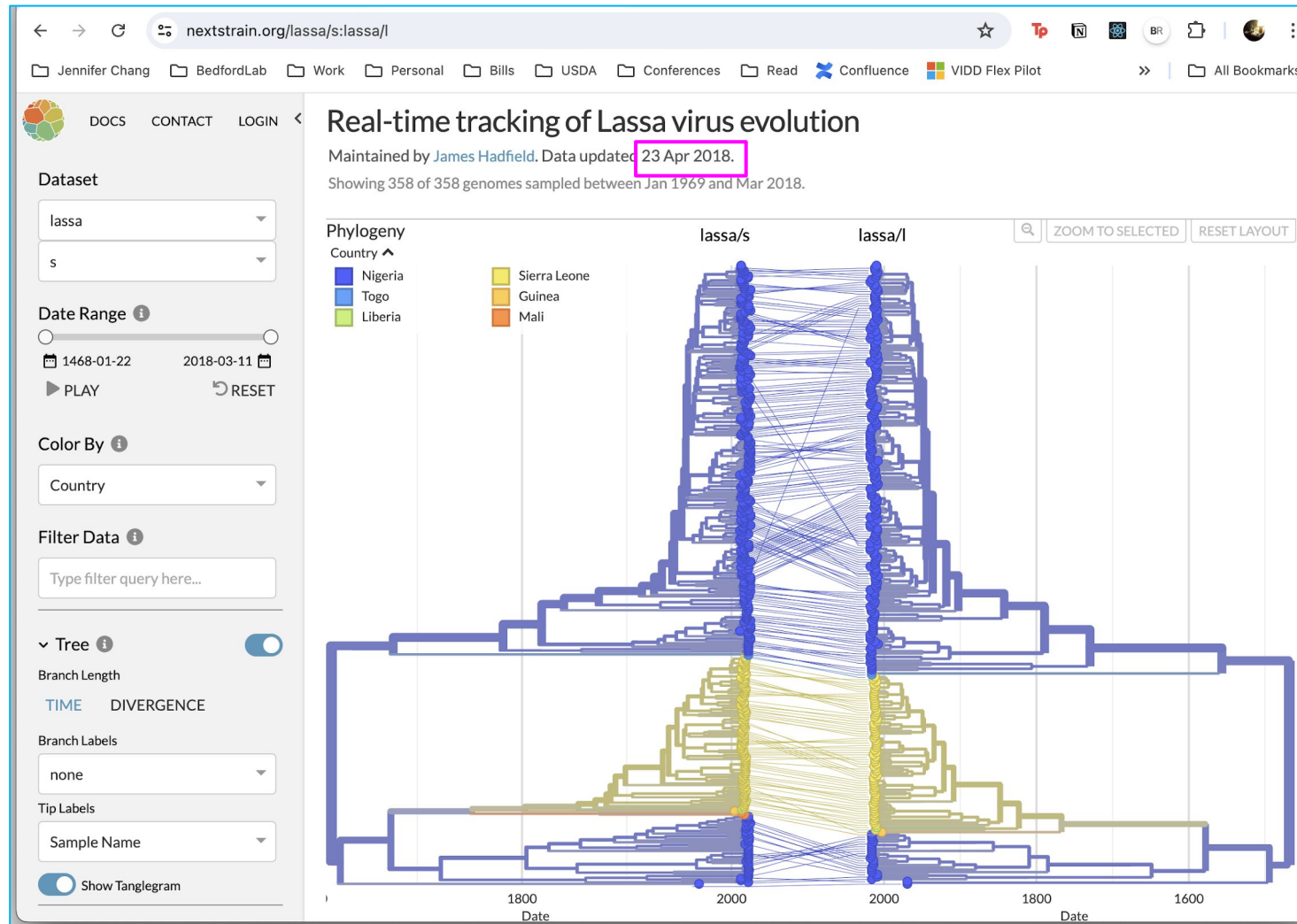


Figure 1: The global dengue burden, 2014 (Guzman and Harris, The Lancet, 2015)

Outline

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Modernizing the Lassa build



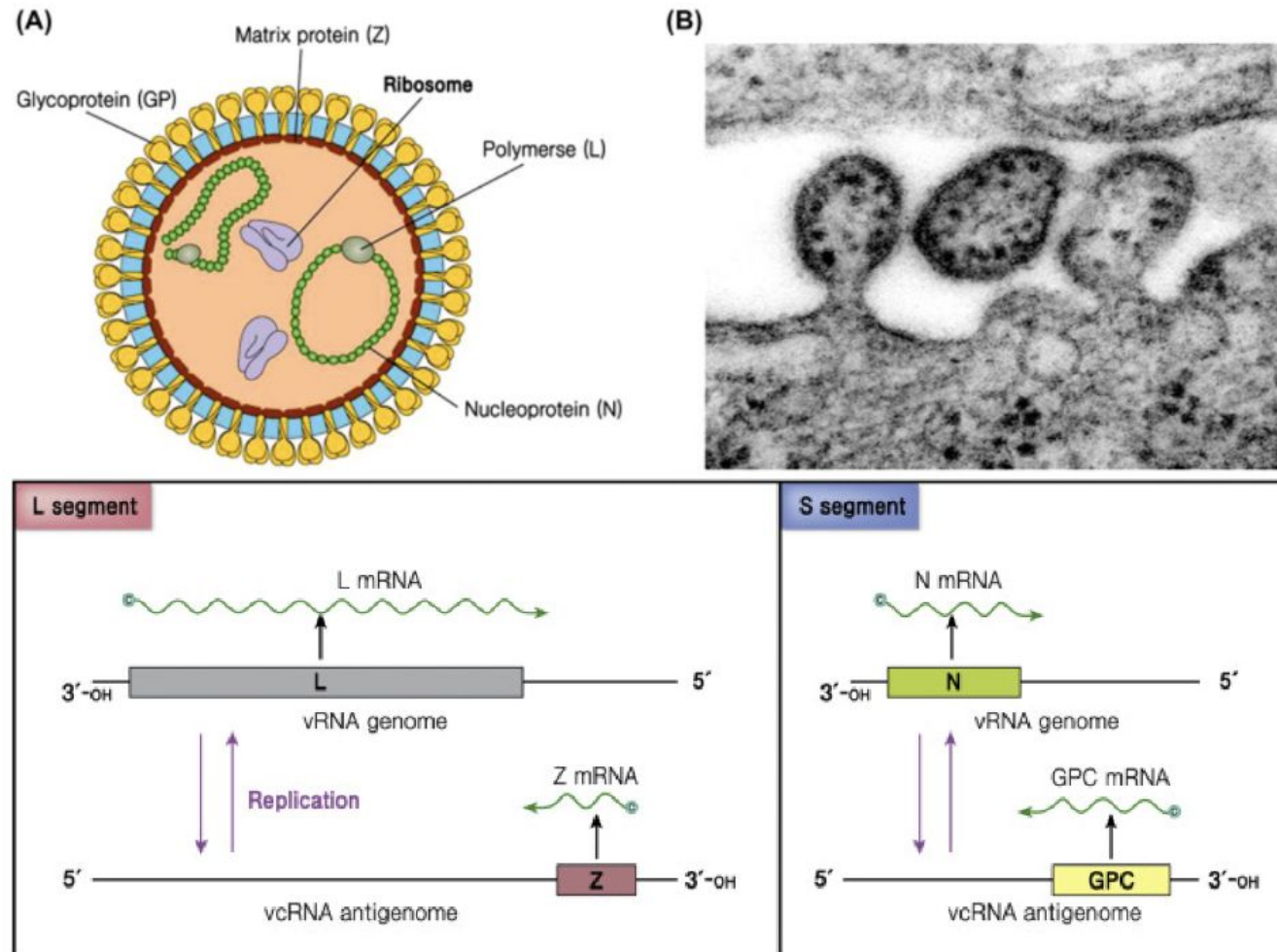
- Update the dataset
- Adhere to the pathogen-repo-guide
- Move and refactor 'phylogenetic' workflows into subdirectories
- Connect GitHub action automation
- Have a team of SME (subject-matter-experts) to check the validity of the site and make suggestions
- Do not "scoop" people's work, provide a general analysis

About Lassa Virus

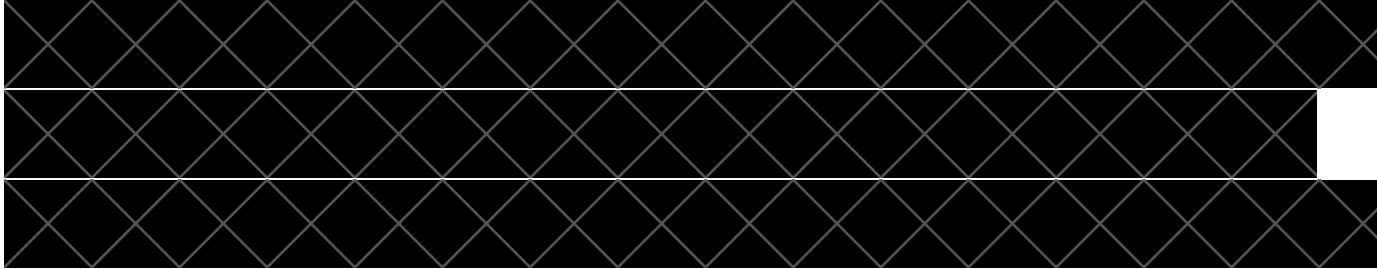
- Biosafety Level 4 (BSL-4)
- Causes severe disease with high mortality rates (15-20% in hospitalized patients)
- Spread by rats found in parts of West Africa although person-to-person transmission can occur
- No licensed vaccine
- Treatment options limited
- Stable as an aerosol, remains infectious for several days outside host

Background

Lassa has two segments "L" and "S" from Chapter 16 of "Molecular Virology of Human Pathogenic Viruses" by Wang-Shick Ryu



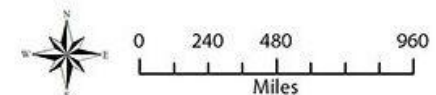
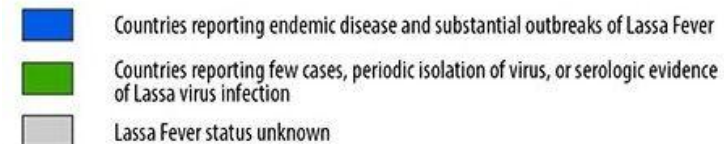
List of potential SMEs



- Other suggested contacts?



LASSA FEVER DISTRIBUTION MAP



<https://www.cdc.gov/lassa-fever/about/index.html>

Outline

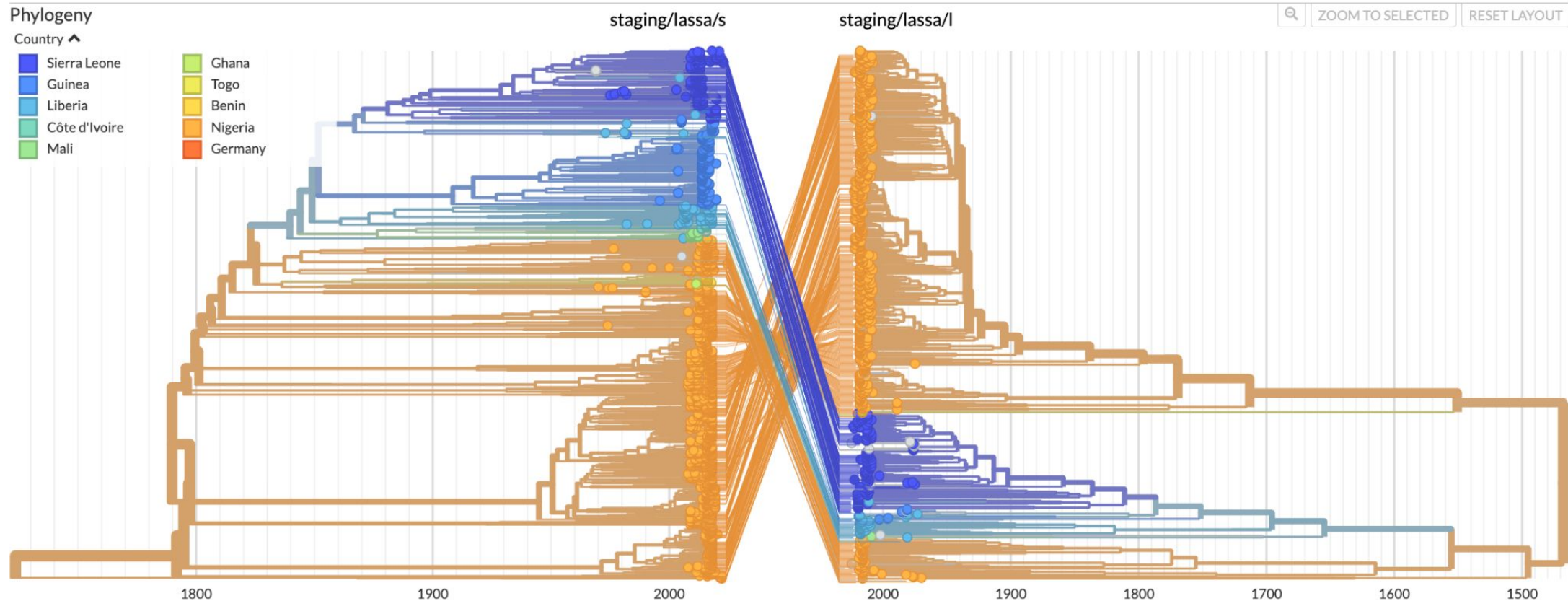
- Lessons learned from modernizing Dengue workflows
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Update the Lassa trees

Real-time tracking of Lassa virus evolution

Maintained by the [Nextstrain team](#). Data updated 2024-08-02. Enabled by data from [GenBank](#).

Showing 1138 of 1138 genomes sampled between Dec 1968 and Aug 2024.

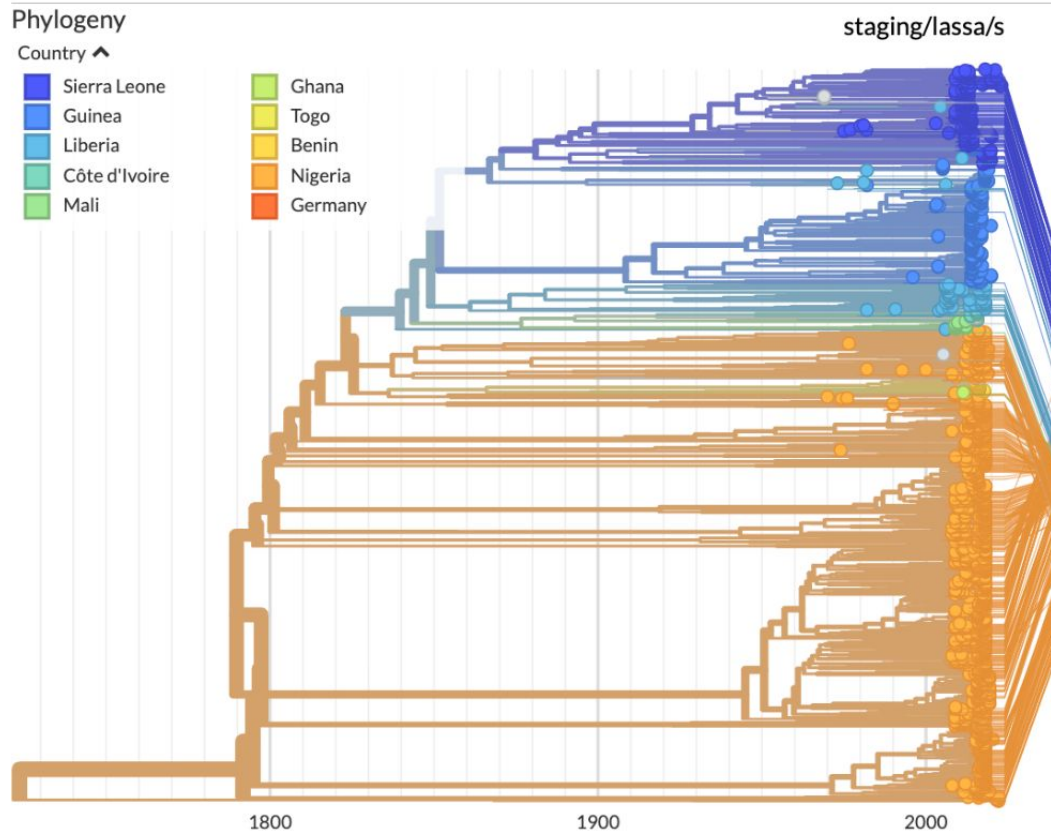


- <https://next.nextstrain.org/staging/lassa/l>

Update the Lassa trees

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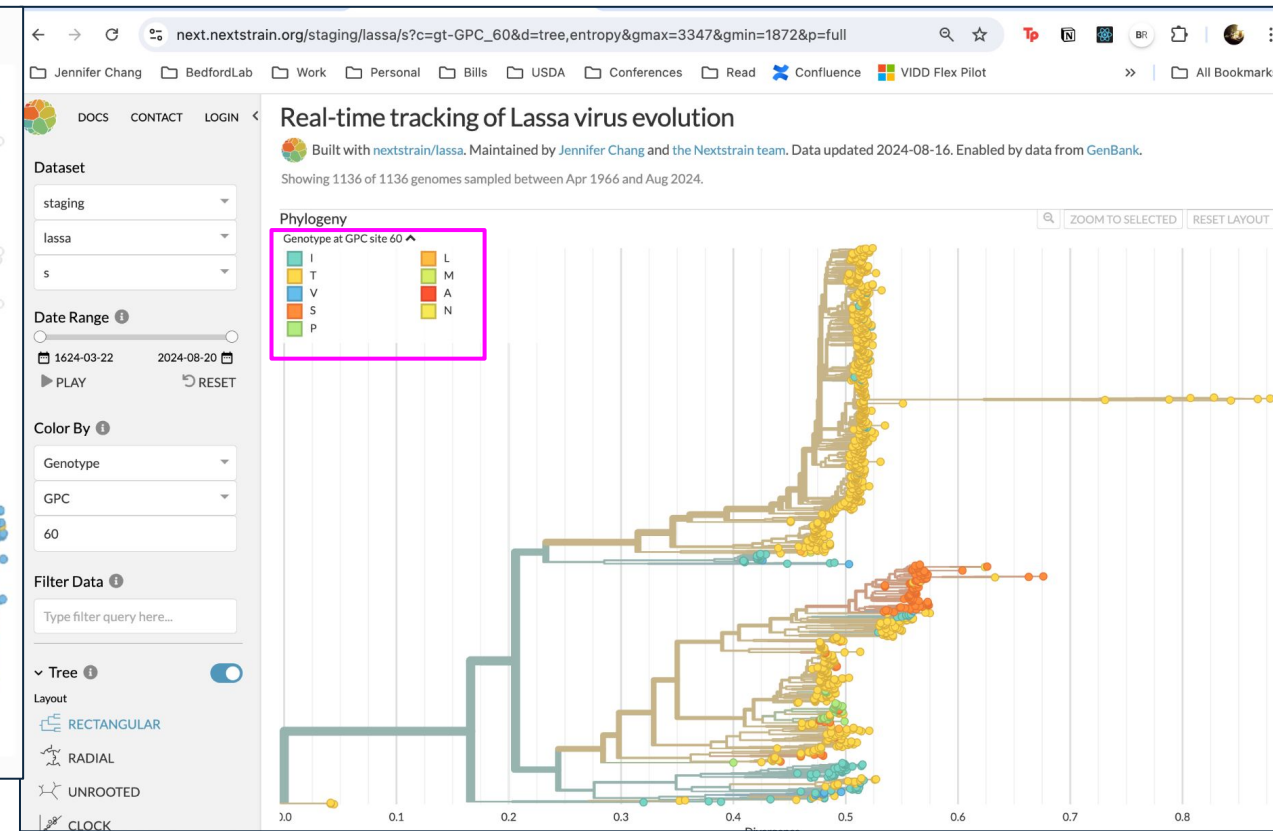
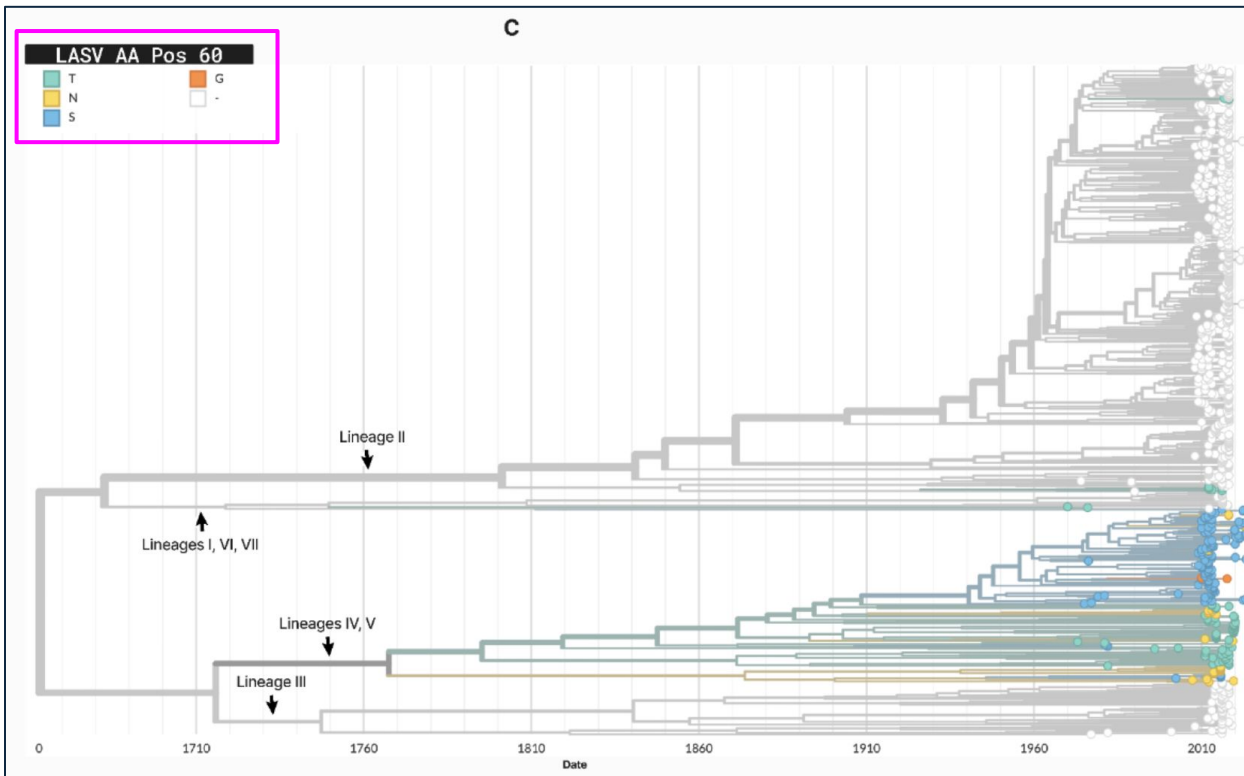


Questions for the Subject Matter Experts (SMEs)

1. Are there any obvious issues with the trees?
2. Should we extract a smaller region than the L and S segments?
3. Does Lassa have vaccine strains that need annotation on the tree?
4. Are there recommended host categories for tree annotation?
5. Can we link the L and S segment by methods other than strain name?

• <https://next.nextstrain.org/staging/lassa/1>

Amino acid position 60 in the GPC



[Daudu et al, preprint, biorxiv](#)

Our phylogenetic trees

"disrupt the functioning of all known anti-GPC antibodies ([Robinson et al., 2016](#))."

A.A. pos. 60 in the GPC - fix codon alignment

The image displays two side-by-side screenshots of the Lassa alignment viewer interface, showing sequence alignments for different datasets. Both windows have tabs for Alignment View, Distances, Text View, Lineage, and Info. The left window is titled 'final_passed_sequences_manual_curated (Alignment) - Lassa' and the right window is titled 'gpc_with_gff_outframepenalty_test (Alignment) - Lassa'.

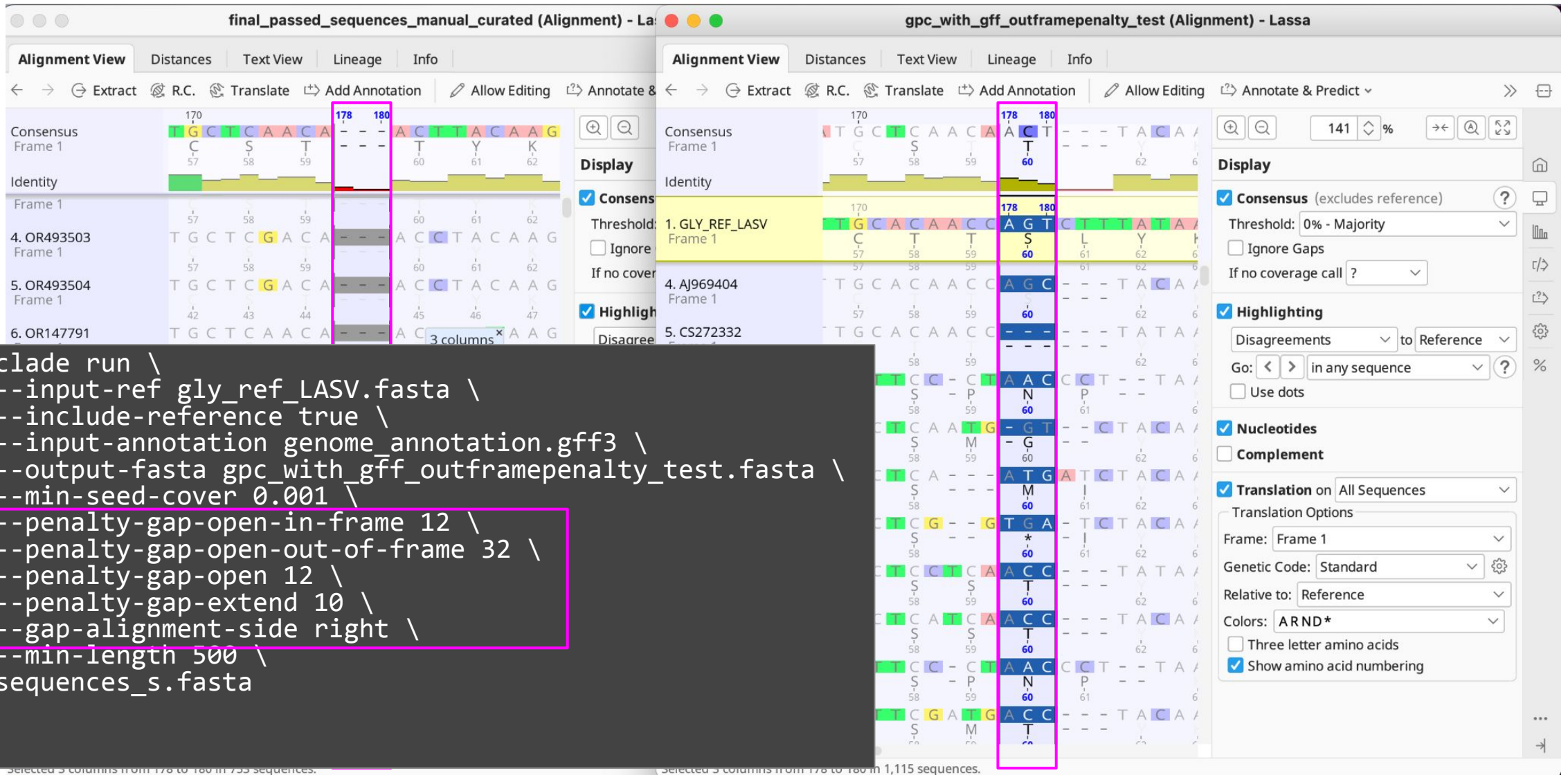
Both windows show a consensus sequence at the top, followed by individual sequence alignments. The left window shows 14 sequences (OR493503 to OR041682) and the right window shows 13 sequences (GLY_REF_LASV to AF333969). The sequences are aligned in Frame 1. The left window shows a consensus sequence with positions 170, 178, and 180 highlighted. The right window shows a consensus sequence with positions 170, 178, and 180 highlighted. The right window also shows a 'Display' panel on the right side with various settings.

In both windows, a vertical pink box highlights a specific region of the alignment, corresponding to positions 178 to 180. In the left window, this region is labeled '3 columns' and 'Selected 3 columns from 178 to 180 in 753 sequences.' In the right window, this region is labeled 'Selected 3 columns from 178 to 180 in 1,115 sequences.'

The right window's 'Display' panel includes the following settings:

- Consensus** (excludes reference): Threshold: 0% - Majority, Ignore Gaps: ☐ If no coverage call: ?
- Highlighting**: Disagreements to Reference, Go: < > in any sequence, Use dots: ☐
- Nucleotides**: ☐ Complement
- Translation** on: All Sequences, Translation Options: Frame: Frame 1, Genetic Code: Standard, Relative to: Reference, Colors: ARND*, ☐ Three letter amino acids, ☒ Show amino acid numbering

A.A. pos. 60 in the GPC - fix codon alignment



nextclade run \

- input-ref gly_ref_LASV.fasta \
- include-reference true \
- input-annotation genome_annotation.gff3 \
- output-fasta gpc_with_gff_outframepenalty_test.fasta \
- min-seed-cover 0.001 \
- penalty-gap-open-in-frame 12 \
- penalty-gap-open-out-of-frame 32 \
- penalty-gap-open 12 \
- penalty-gap-extend 10 \
- gap-alignment-side right \
- min-length 500 \

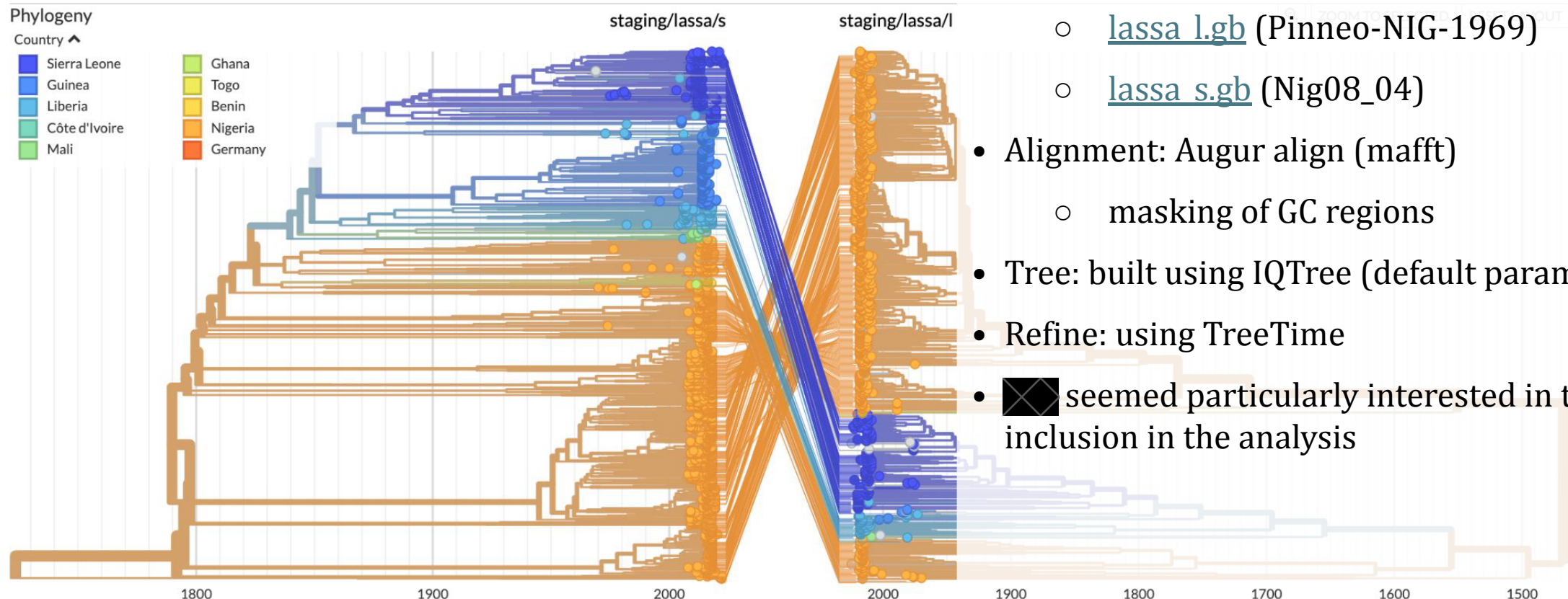
sequences_s.fasta

Questions

Real-time tracking of Lassa virus evolution

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Showing 1138 of 1138 genomes sampled between Dec 1968 and Aug 2024.



More details:

- Reference: based on
 - [lassa l.gb](#) (Pinneo-NIG-1969)
 - [lassa s.gb](#) (Nig08_04)
- Alignment: Augur align (mafft)
 - masking of GC regions
- Tree: built using IQTree (default params)
- Refine: using TreeTime
-  seemed particularly interested in the criteria for inclusion in the analysis

• <https://next.nextstrain.org/staging/lassa/l>

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Levels of commitment - external

- Option 1: Active Code Contributor

- Responsibilities:

- Participate actively in the GitHub repository
 - Draft and submit GitHub issues, PRs, and reviews

- Potential challenges:

- Adhering to or adjusting Nextstrain GitHub contribution best-practices

- Support:

- I am available to guide and support people through these challenges

- Option 2: Reviewer

- Responsibilities:

- Review live Lassa builds and provide feedback
 - Optionally submit GitHub issues or emails to flag any obvious errors

- Engagement:

- You will be emailed or pinged for reviews

- Option 3: SME Contributor

- Responsibilities:

- Regularly summarize recent Lassa virus research papers in presentations to the code contributors
 - Help brainstorm and suggest new features for the public build

Active Code Contributor

- Option 1: Active Contributor
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 - Support:
 - I am available to guide and support people through these challenges
- I can put together some slides on:
 - the pathogen repo guide
 - github commit internal practices
- We can collaborative submit and go through the PR process

Reviewer

- Option 2: Reviewer

- Responsibilities:

- Review live Lassa builds and provide feedback
 - Optionally submit GitHub issues or emails to flag any obvious errors

- Engagement:

- You will be emailed or pinged for reviews

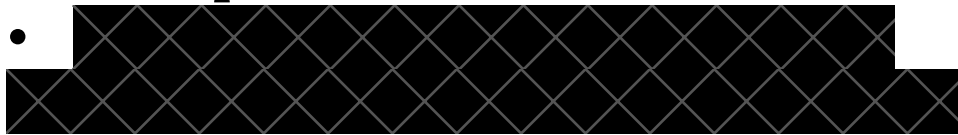
- I will email out an update with the live build
- If possible, get response if the trees look acceptable or not within a week (or set email that you are on vacation)

SME Contributor

- Option 3: SME Contributor
 - Responsibilities:
 - Regularly summarize recent Lassa virus research papers in presentations to the code contributors
 - Help brainstorm and suggest new features for the public build
- If you'd be willing to compile and share a powerpoint summarizing the literature
- Schedule a later meeting to go through the slides

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Outline

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- Thinking about external contributions
- **Next steps**
 - Creating slides and onboarding for an Active Code Contributor
 - Test run with [REDACTED] to contribute to the Lassa Repo
 - Use slides and onboarding for [REDACTED] work with [REDACTED]

Nextstrain GitHub Standards

Why adhere to a pathogen repo guide?

Nextstrain GitHub Practices

Consistency and Reproducibility

Nextstrain's focus on pathogen genomics requires a high degree of consistency in data analysis workflows. By implementing best practices, particularly in Snakemake workflows, we ensure:

- Reproducible analysis across different datasets and pathogens
- Uniform coding standards that facilitate easier code review and maintenance
- Consistent file structures and naming conventions

Continuous Improvement and Adaptability

The field of pathogen genomics is rapidly evolving, and Nextstrain's best practice aims to collaboratively adapt and maintain high quality by:

- Regular review and updates to best practices to incorporate new tools and methodologies
- Some flexibility to adapt workflows for different pathogens and analysis requirements

Nextstrain GitHub Practices

Consistency and Reproducibility

To ensure consistency, try to make sure all pathogen repos adhere to the pathogen-repo-guide

- <https://github.com/nextstrain/pathogen-repo-guide>
- Mostly this means following the file structure

Continuous Improvement and Adaptability

A high degree of comments on PR

- Regular review and updates to best practices to incorporate new tools and methodologies
- Some flexibility to adapt workflows for different pathogens and analysis requirements

Steps for adding features

- Create a GitHub Issue
 - this is where we discuss potential solutions
- Create a Fork or PR linked to the Issue
 - this is where we implement potential solutions
 - this often turns into a long dialogue on various aspects of the solution
 - can trigger the creation of other github issues/PRs
 - is not guaranteed to be merged in
- If approved, use GitHub rebase to clean up commits on PR
 - this cleans up github commit history
 - this helps incorporate changes that have already been merged into the repository
- Pick merge or squash merge
 - use a "merge" commit if there are multiple changes that we want to preserve history
 - use squash merge if there is one small or minor change

HackMD to coordinate with Richard Daudu

The screenshot shows a HackMD document titled "Lassa Notes" with a "To Do" list. The list is organized under the heading "GPC Tree".

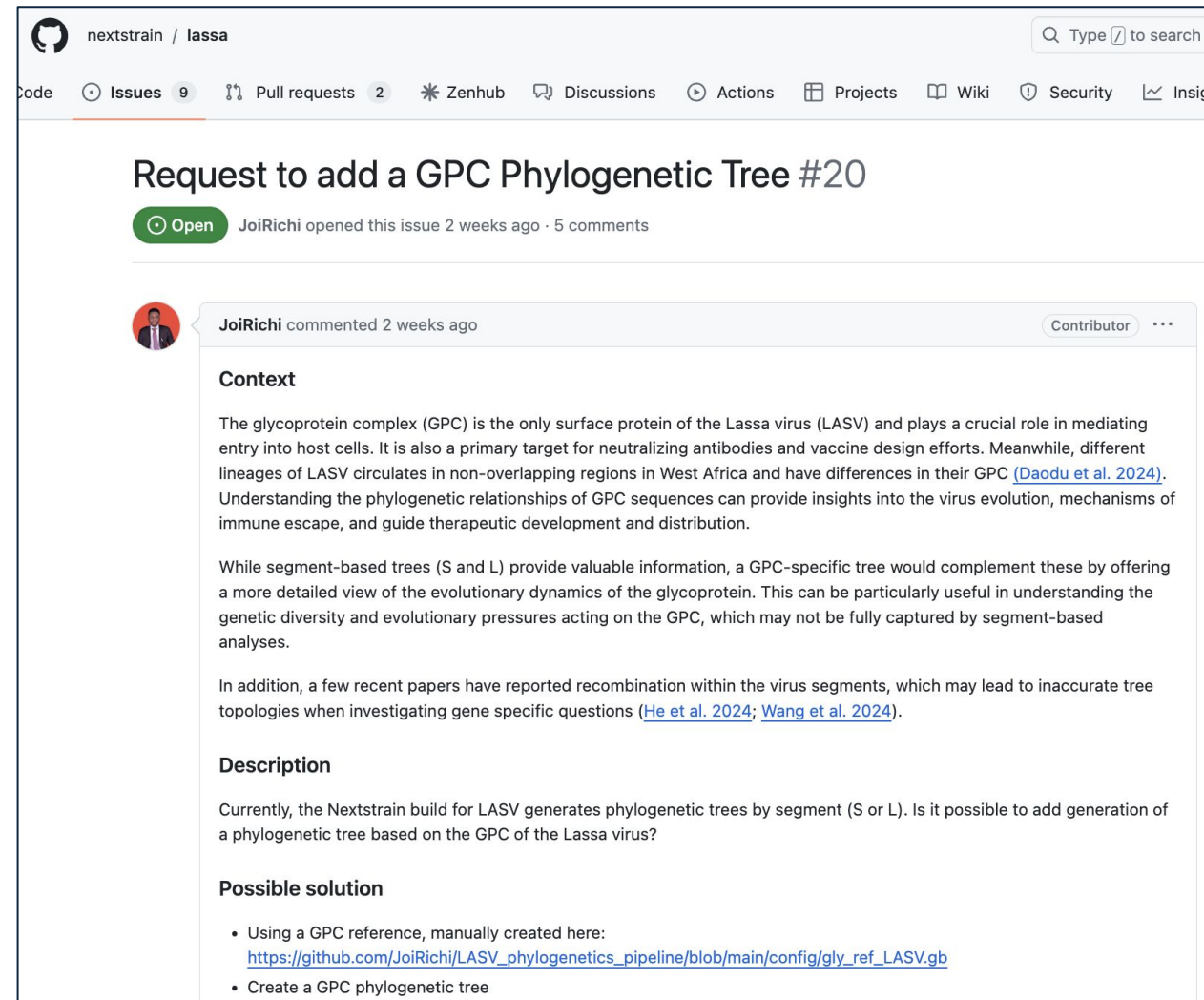
To Do

GPC Tree

- ☒ **Richard** - Submit a Github Issue requesting a GCP Tree
 - 3 subunits, important to know the subunits for different functions
 - signal maturation of the gpc
 - <https://github.com/nextstrain/lassa>
- ☒ **Richard** - Create a fork: ~~Or create new branch add-gcp-tree~~
- ☒ **Richard** - Add reference GenBank for GCP to repo (perhaps in `shared` and `phylogenetic/defaults`) and submit a PR
- ☒ **Jennifer** - Get clarification on reference genbanks for different parts of the build
 - L segment: `Pinneo-NIG-1969` [lassa_l.gb](#)
 - `Pinneo-NIG-1969` [lassa_l.gb](#)
 - lineage 1, has a deletion around GPC 62
 - might cause nextclade v3 to cut this region out of the regular sequences during L and S assignment
 - manually fix the alignment (alignment parameters)
 - S segment: `Nig08_04` [lassa_s.gb](#)
 - `Nig08_04` [lassa_s.gb](#)
 - GPC region: `Josiah` strain [gly_ref_LASV.gb](#)
 - `Josiah` strain [gly_ref_LASV.gb](#)
 - Got clarification to official move to 'Josiah' strain for reference and PR merged with <https://github.com/nextstrain/lassa/pull/23>
- ☐ Add nextclade v3 rules to pull out GCP in `ingest` (to follow segment pattern) or justify adding it in `phylogenetic`

HackMD to coordinate with Richard Daudu

Submit a GitHub Issue



The screenshot shows a GitHub issue page for the repository 'nextstrain / lassa'. The issue title is 'Request to add a GPC Phylogenetic Tree #20', opened by JoiRichi 2 weeks ago with 5 comments. The issue is marked as 'Open'. A comment from JoiRichi, dated 2 weeks ago, contains the following text:

Context

The glycoprotein complex (GPC) is the only surface protein of the Lassa virus (LASV) and plays a crucial role in mediating entry into host cells. It is also a primary target for neutralizing antibodies and vaccine design efforts. Meanwhile, different lineages of LASV circulates in non-overlapping regions in West Africa and have differences in their GPC (Daodu et al. 2024). Understanding the phylogenetic relationships of GPC sequences can provide insights into the virus evolution, mechanisms of immune escape, and guide therapeutic development and distribution.

While segment-based trees (S and L) provide valuable information, a GPC-specific tree would complement these by offering a more detailed view of the evolutionary dynamics of the glycoprotein. This can be particularly useful in understanding the genetic diversity and evolutionary pressures acting on the GPC, which may not be fully captured by segment-based analyses.

In addition, a few recent papers have reported recombination within the virus segments, which may lead to inaccurate tree topologies when investigating gene specific questions (He et al. 2024; Wang et al. 2024).

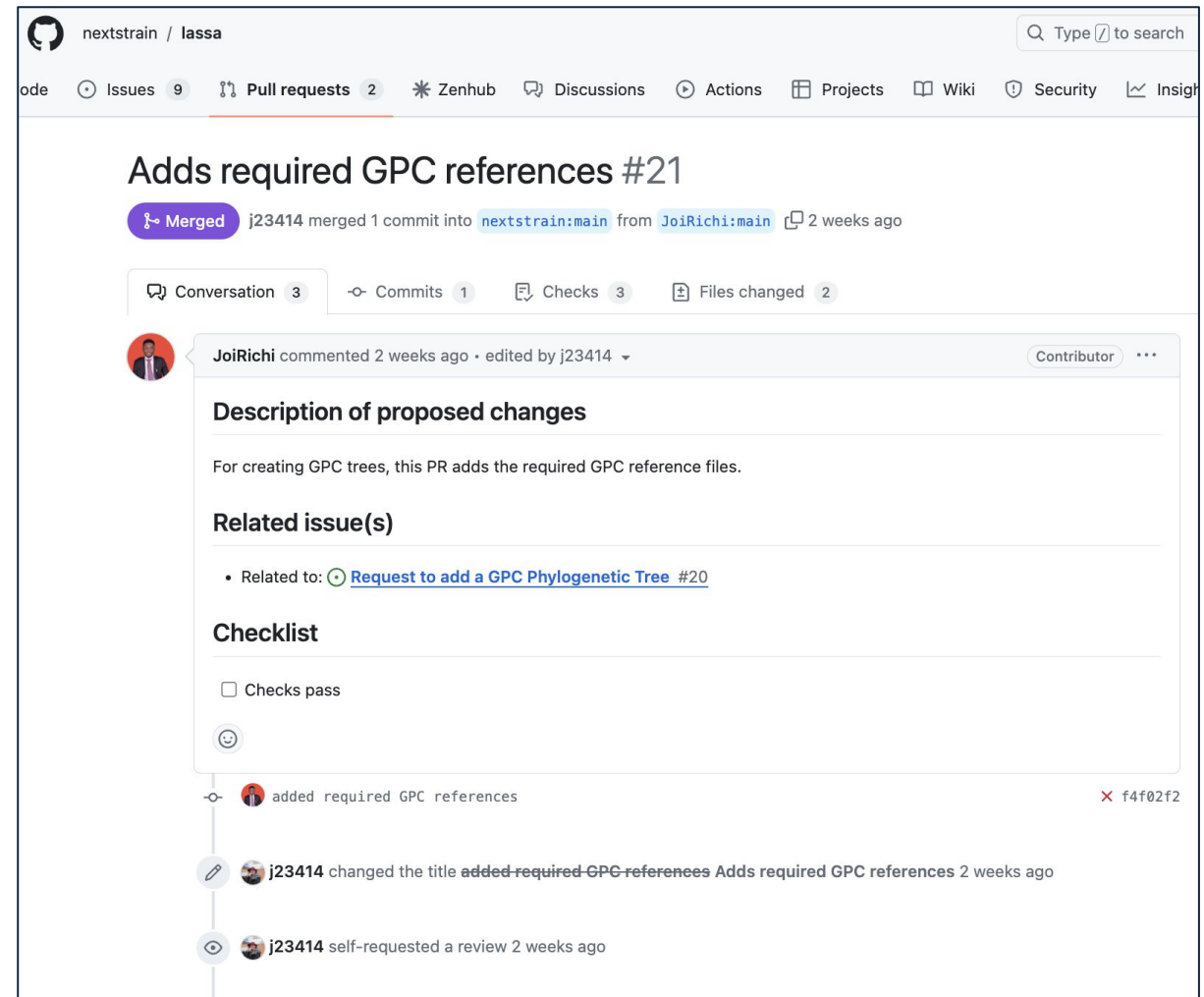
Description

Currently, the Nextstrain build for LASV generates phylogenetic trees by segment (S or L). Is it possible to add generation of a phylogenetic tree based on the GPC of the Lassa virus?

Possible solution

- Using a GPC reference, manually created here: https://github.com/JoiRichi/LASV_phylogenetics_pipeline/blob/main/config/gly_ref_LASV.gb
- Create a GPC phylogenetic tree

Submit a GitHub PR



The screenshot shows a GitHub pull request page for the repository 'nextstrain / lassa'. The PR title is 'Adds required GPC references #21', merged by j23414 2 weeks ago. The PR is marked as 'Merged'. A comment from JoiRichi, dated 2 weeks ago and edited by j23414, contains the following text:

Description of proposed changes

For creating GPC trees, this PR adds the required GPC reference files.

Related issue(s)

- Related to: [Request to add a GPC Phylogenetic Tree #20](#)

Checklist

- ☐ Checks pass

The PR history shows the following actions:

- j23414 added required GPC references (commit f4f02f2)
- j23414 changed the title ~~added required GPC references~~ to **Adds required GPC references** 2 weeks ago
- j23414 self-requested a review 2 weeks ago

Acknowledgements

- Nextstrain Team
- Bedford Lab
- SMEs:

- Richard Daudu

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