Bespoke Strain-Level Analysis of Bacterial Genomes

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Introduction

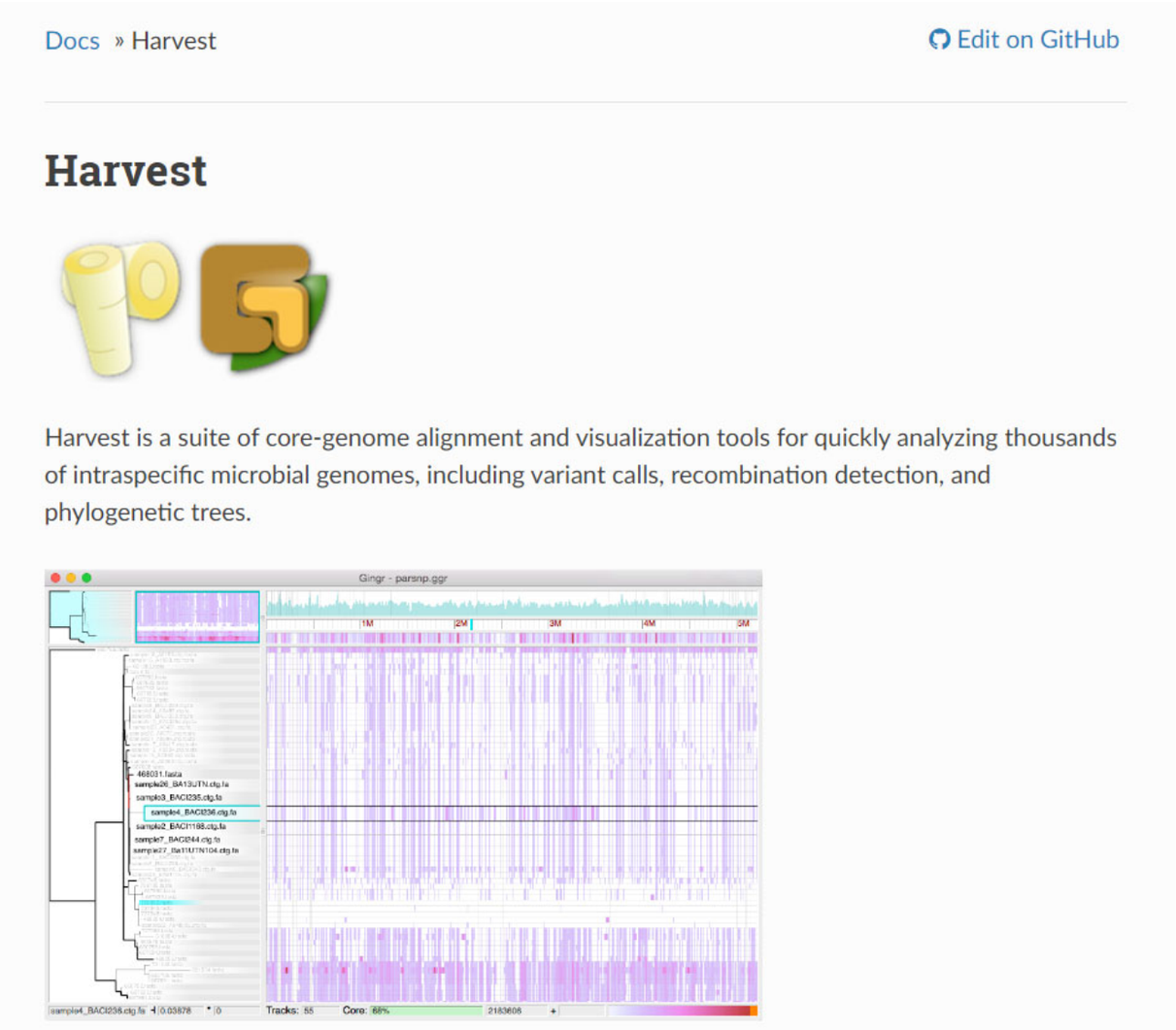
- What does “Strain” mean?
  - Particular SNP?
  - Multiple particular SNPs?
  - Presence/Absence of certain genes?
  - Phenotype?
  - *Other?*
- How do we compare strains?
  - Multiple Genome Alignment
  - Pangenome Analysis

Whole-Genome Alignment

- Idea: align specifically the *shared (“core”)* portion of several genomes.
- Use these aligned segments to identify phylogenetic relationships, etc…
- Visualize what exactly is similar and different…

Tools:

- Parsnp
- Mauve
- SibeliaZ
- (others…)



Whole Genome Alignment: Quick How-To with Parsnp

- Get *assembled* genomes from individual organisms
  - Isolates are nice, MAGs will do
  - Contigs are fine for this, doesn't have to be complete
  - Helps to have at least 1 high-quality, annotated reference genome
  - Useful to run QUAST to QC the assembly
- Run Parsnp:

```
contig_repo=./parsnp_contigs
parsnp_out=./parsnp_output_13
ref_genbank=./ref_assembly_GCF_008121495/Ref_ATCC_29149.gbff

parsnp -g $ref_genbank -d $contig_repo -p 15 -o $parsnp_out
```

Annotated Reference Genome (.gbff format)

Folder with 1 fasta file for each assembly (containing all contigs)
...OR...
File with a newline-separated list of assembly fasta files (full paths).

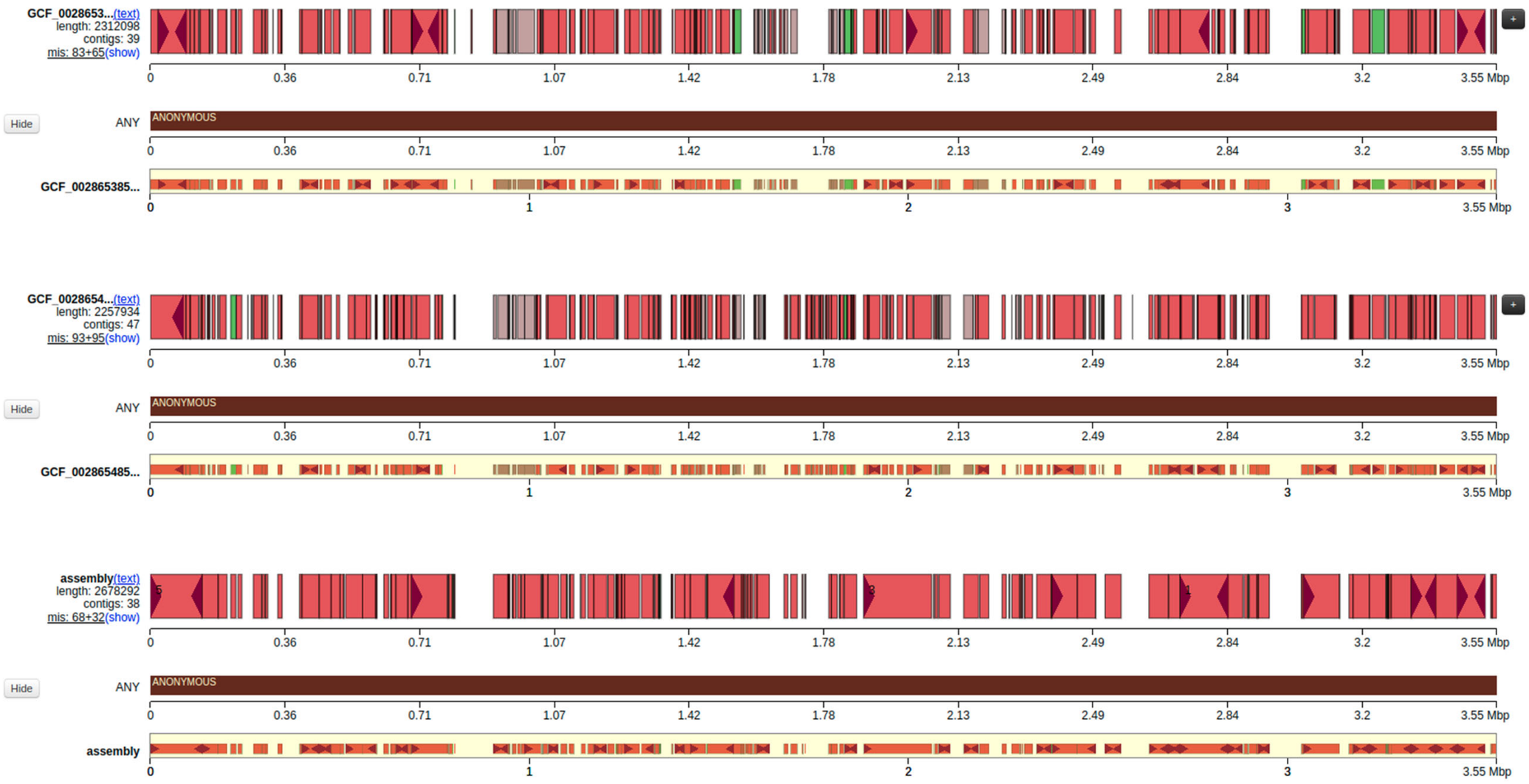
 # processors

Output folder

Interlude #1: QC-ing an Assembly with QUAST

What can we learn?

- Assembly Quality Issues?
- Issues with Reference?



Notes:

The top two assemblies are SPAdes assemblies done by the original authors of the R. Gnavus paper (citation later).

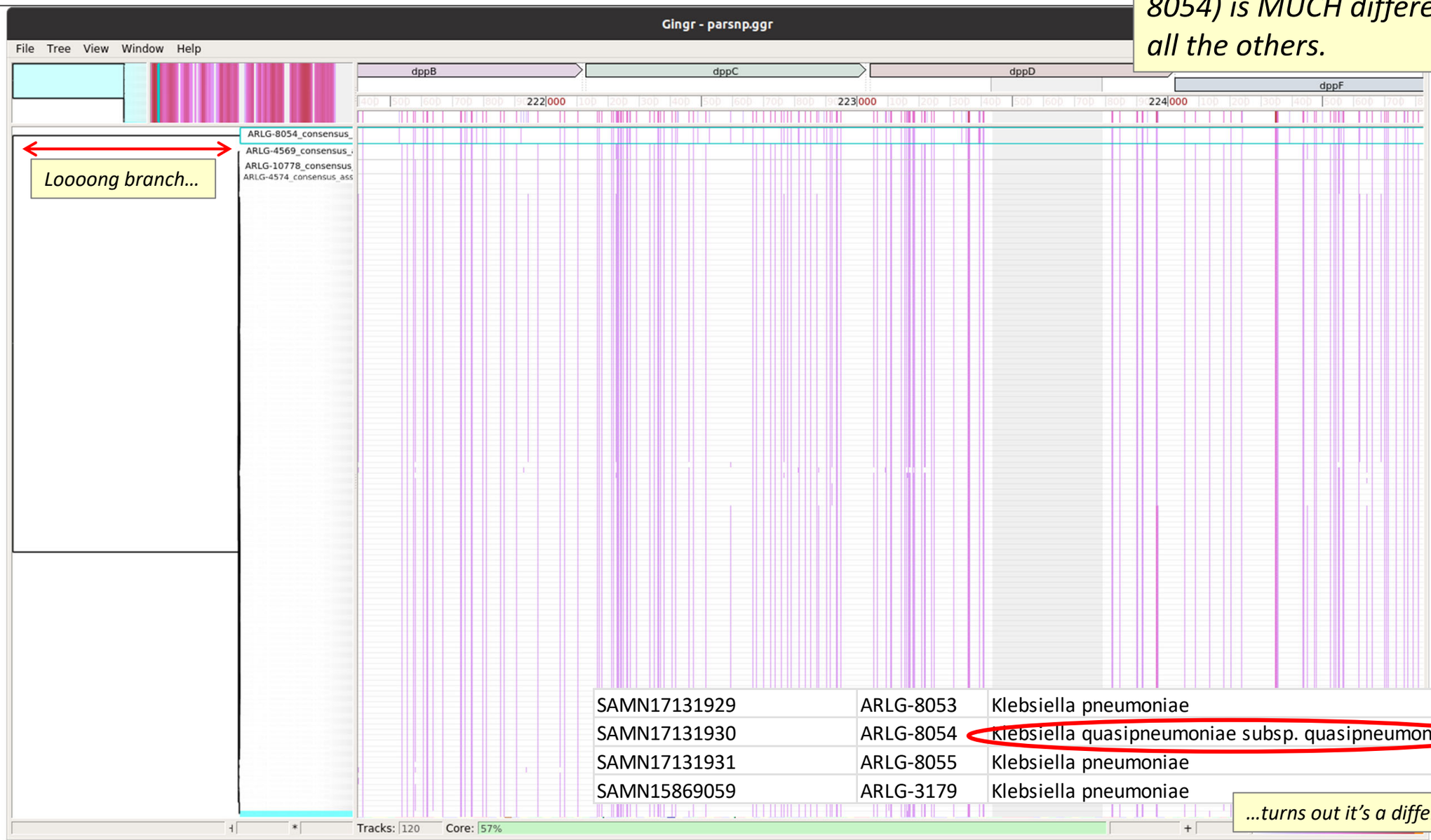
The bottom is a Unicycler assembly from the same reads.

Case Study #3: *Klebsiella pneumoniae* (Shropshire, et al., 2022)

- 119 Carbapenam-resistant *Kp* isolates (95 from Houston Hospitals)
- Hybrid short/long-read assemblies
- Focus of study was to show spread of two separate “clonal groups”

Case Study #3: Observations…

***Observation #1**:*
One of these genomes (ARLG-8054) is MUCH different from all the others.

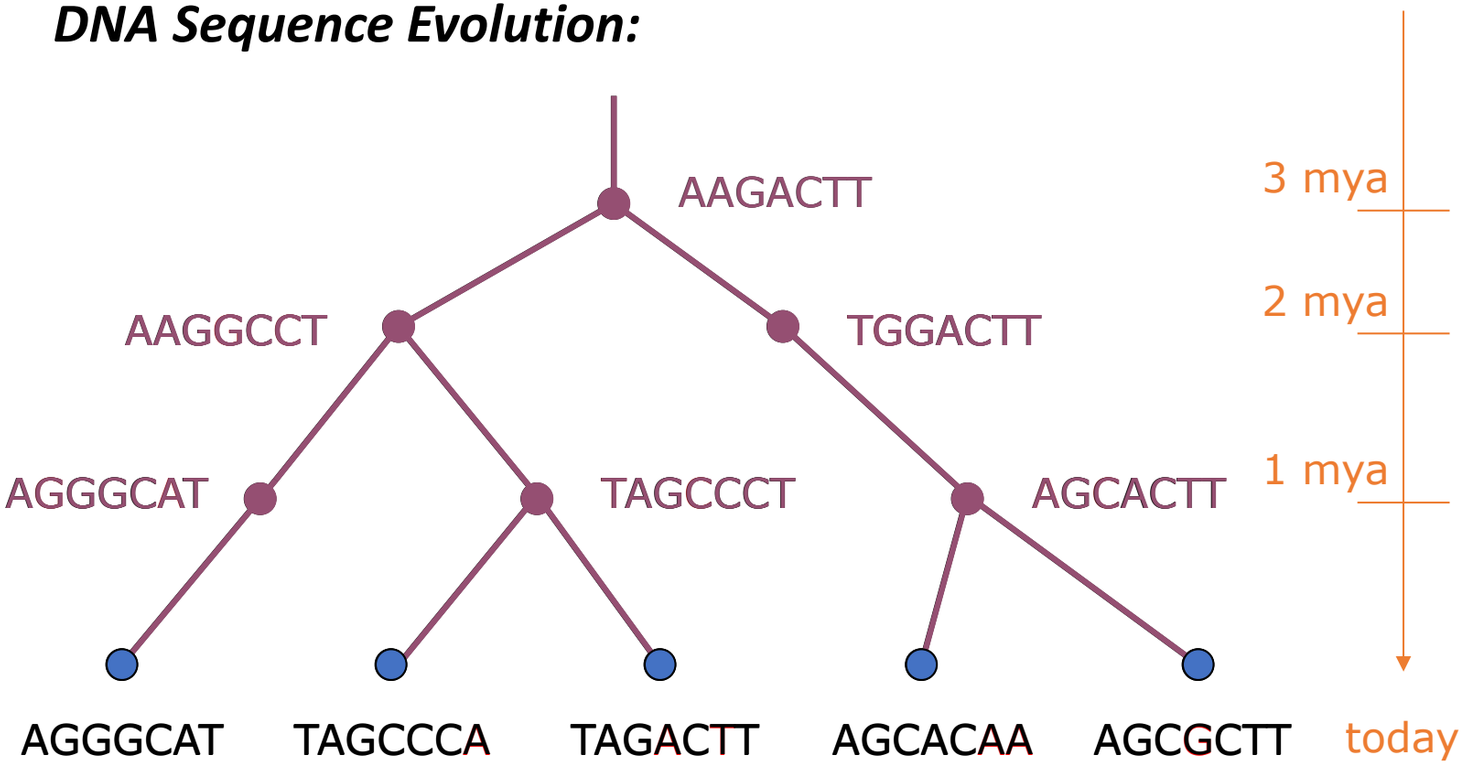


SAMN17131929	ARLG-8053	Klebsiella pneumoniae	TX
SAMN17131930	ARLG-8054	Klebsiella quasipneumoniae subsp. quasipneumoniae	TX
SAMN17131931	ARLG-8055	Klebsiella pneumoniae	TX
SAMN15869059	ARLG-3179	Klebsiella pneumoniae	MI

…turns out it's a different species

Interlude #2: Brief Intro to Molecular Phylogenetics

DNA Sequence Evolution:



Notes:

- insertions and deletions also occur randomly on branches (not shown)
- In statistical terms, the extant sequences are the **observed data**, while the tree shape, tree topology and mutation rates are the **unknown model parameters** to be estimated.

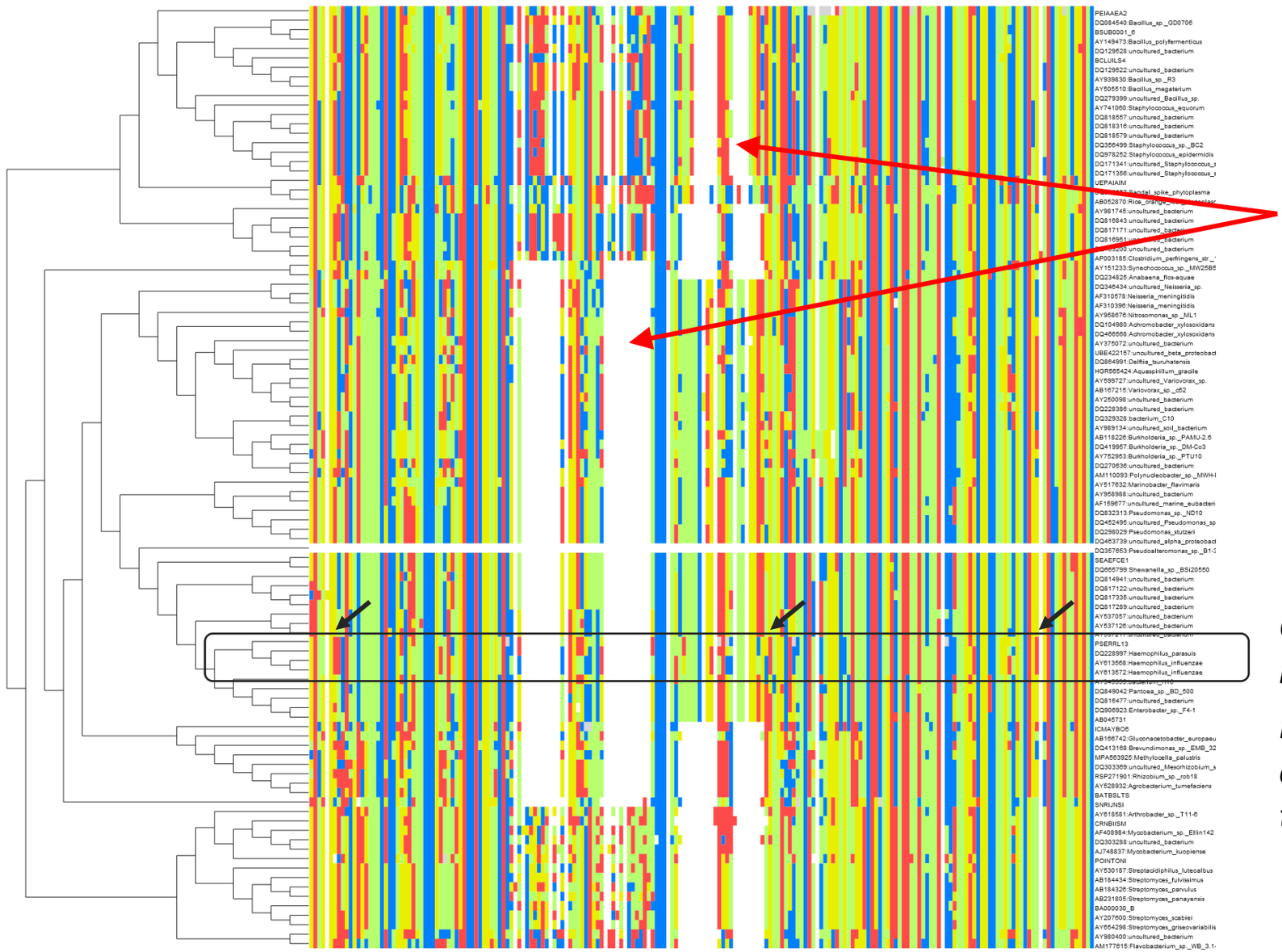
Two Separate Problems (both NP-Hard):

1. Identify which groups of characters share a common ancestor. (Multiple Sequence Alignment)
2. Find the maximum likelihood tree and model parameters (ML Tree Estimation)

*mya = million years ago

*Sequence Animation Credit: Prof. Tandy Warnow

Interlude #2: Multiple Sequence Alignment (Example)



White indicates a “gap” in the alignment, a.k.a. an insertion or deletion (indel)

Conserved mutation patterns indicate evolutionary closeness.

Phylogeny estimation algorithms use this to build a tree…

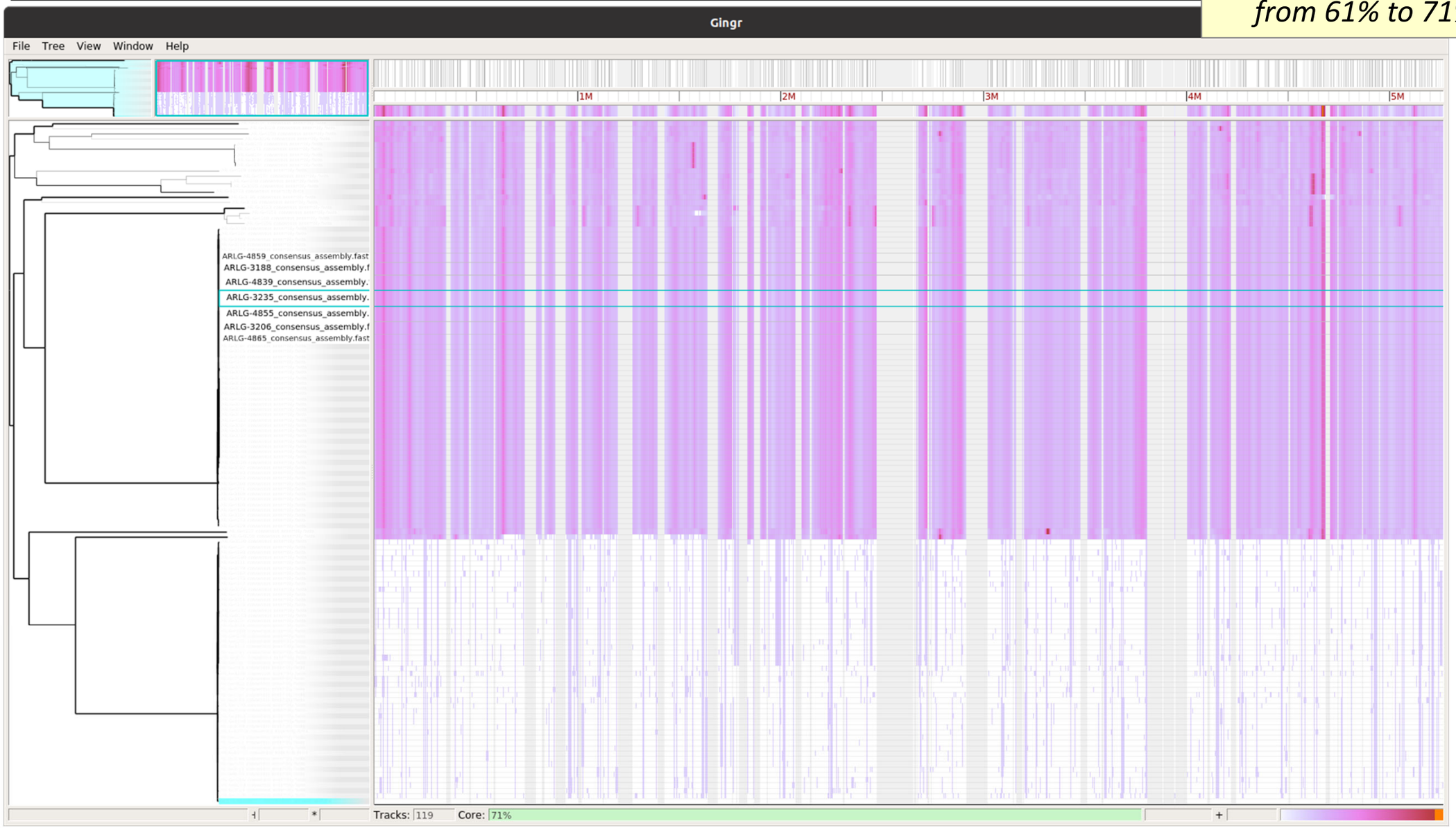
Legend:

A	T
C	G

Case Study #3: Excluding ARLG-8054

Observation #2:

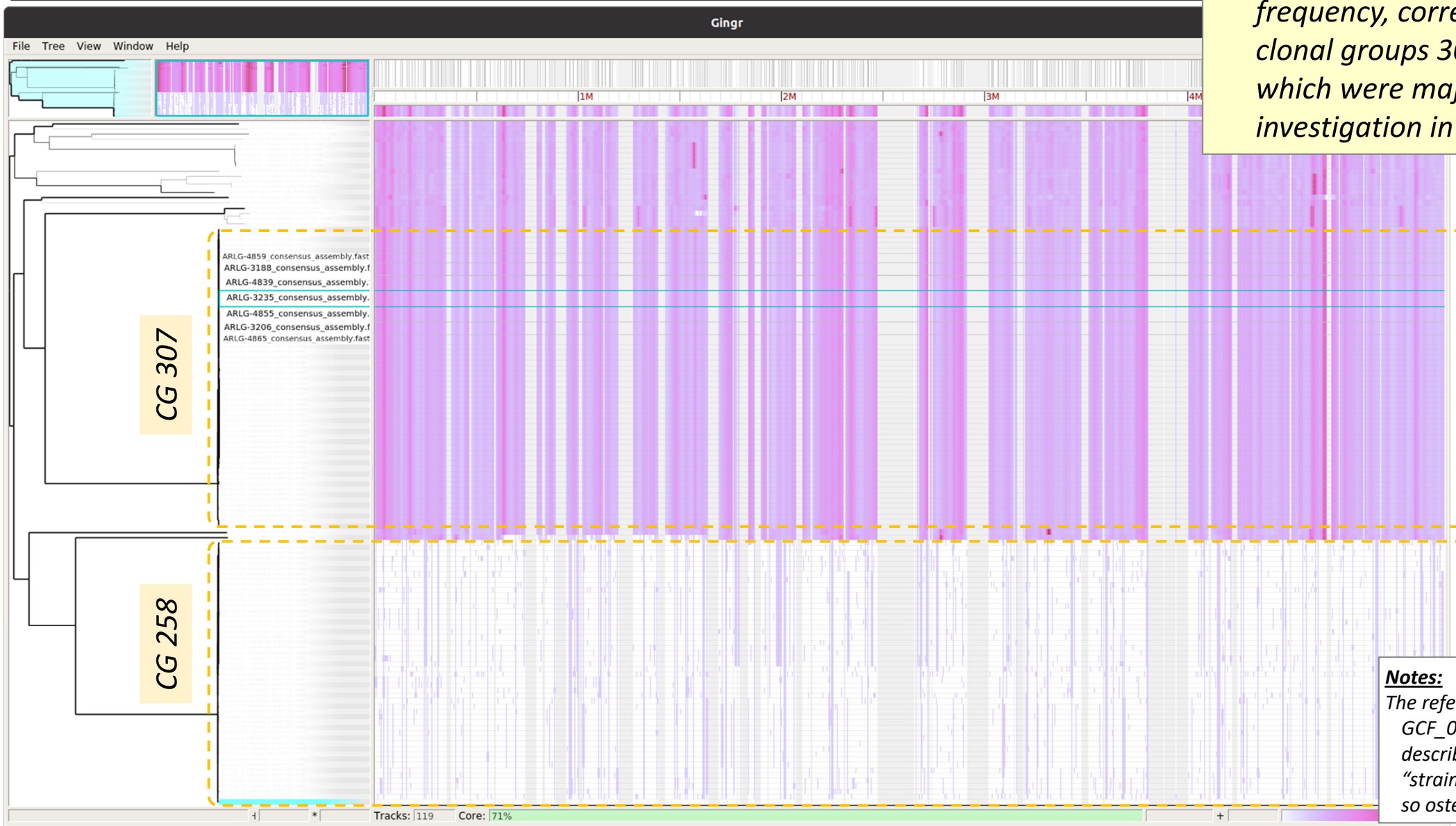
- *Without 8054, core % goes from 61% to 71%*



Case Study #3: Excluding ARLG-8054

Observation #3:

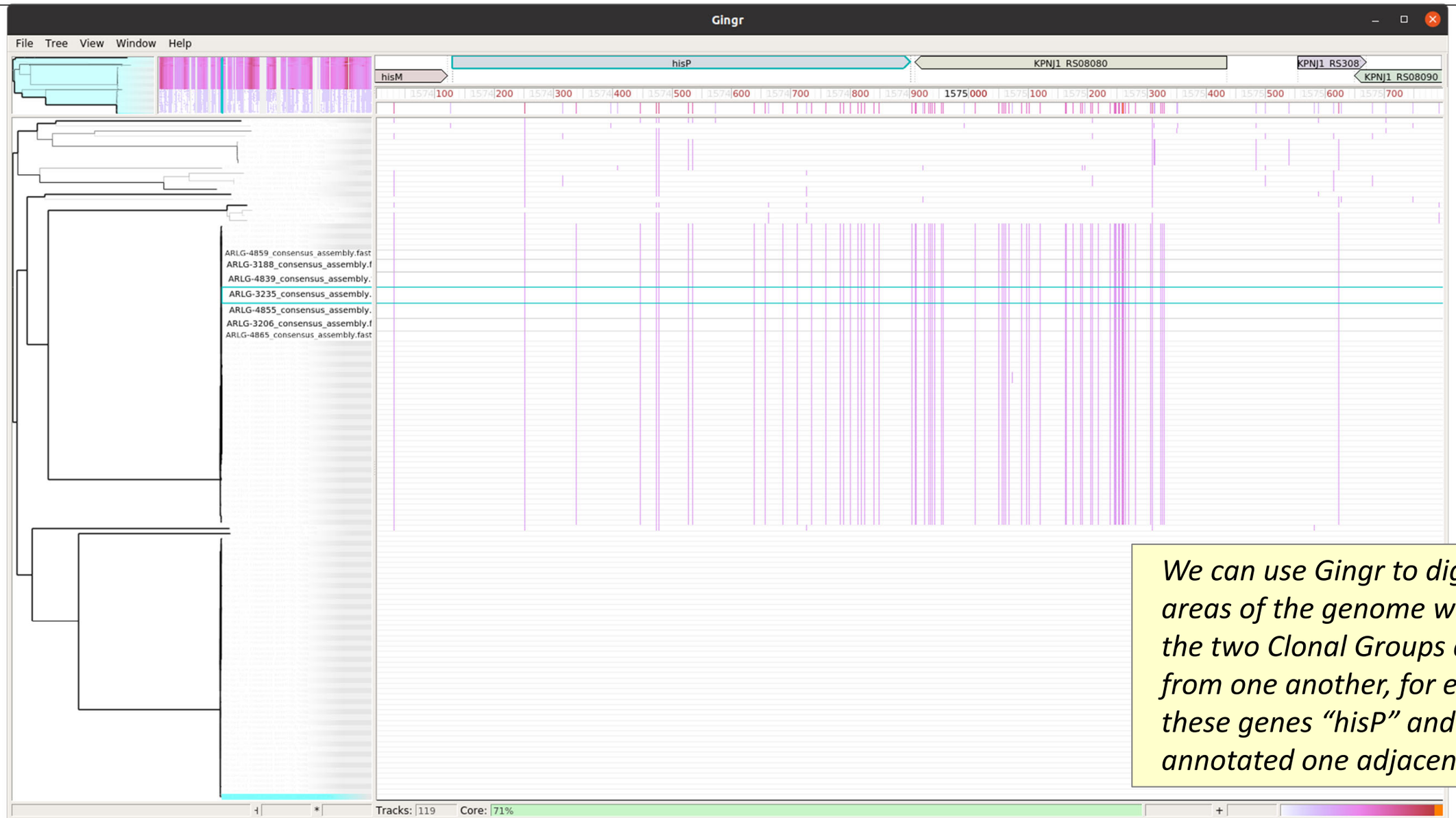
- *2 Clades with highest frequency, corresponding to clonal groups 307, 258 which were major targets of investigation in this paper.*



Notes:

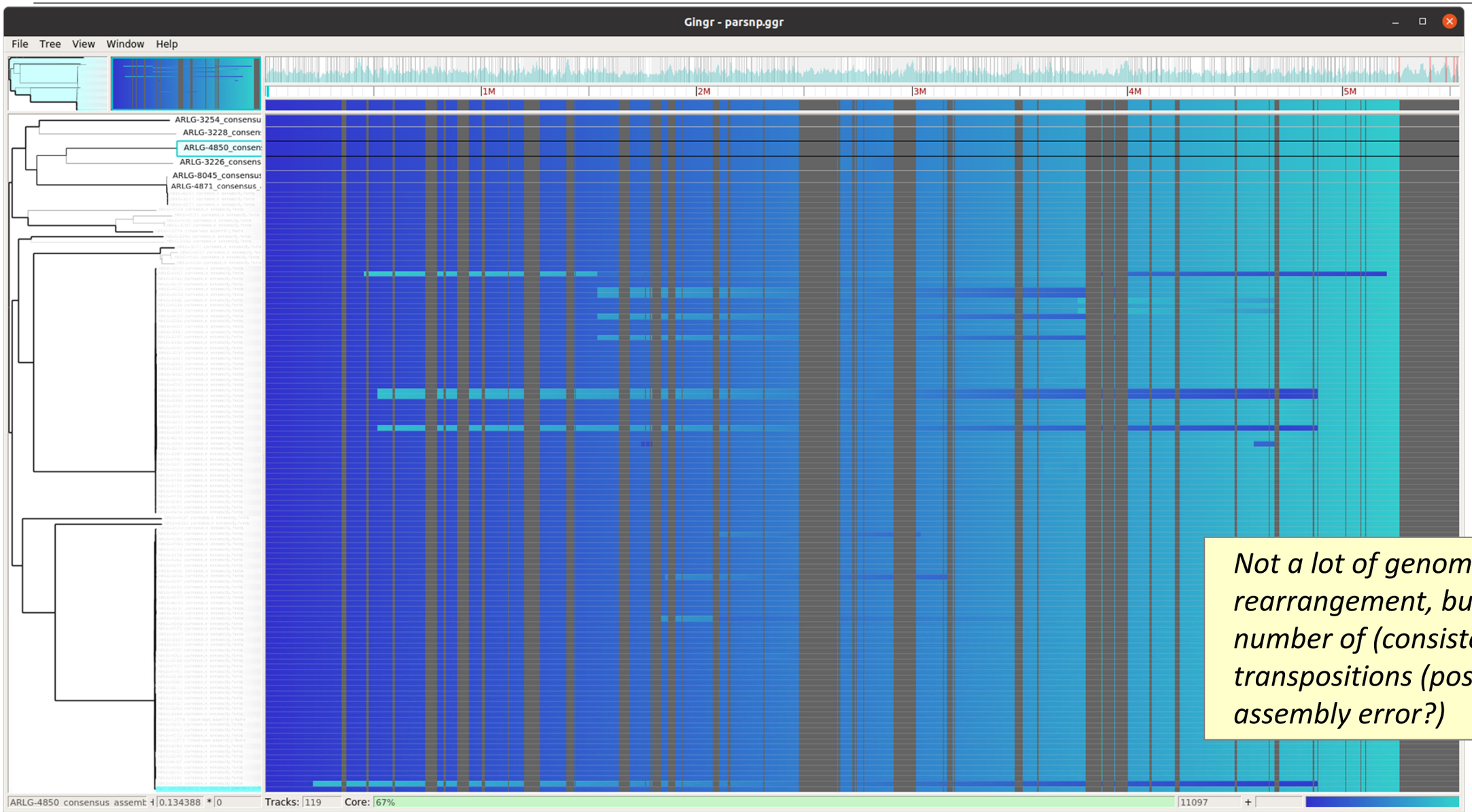
The reference genome here is GCF_000598005.1, described as "strain=30660/NJST258_1", so ostensibly ST258.

Case Study #3: Digging In…



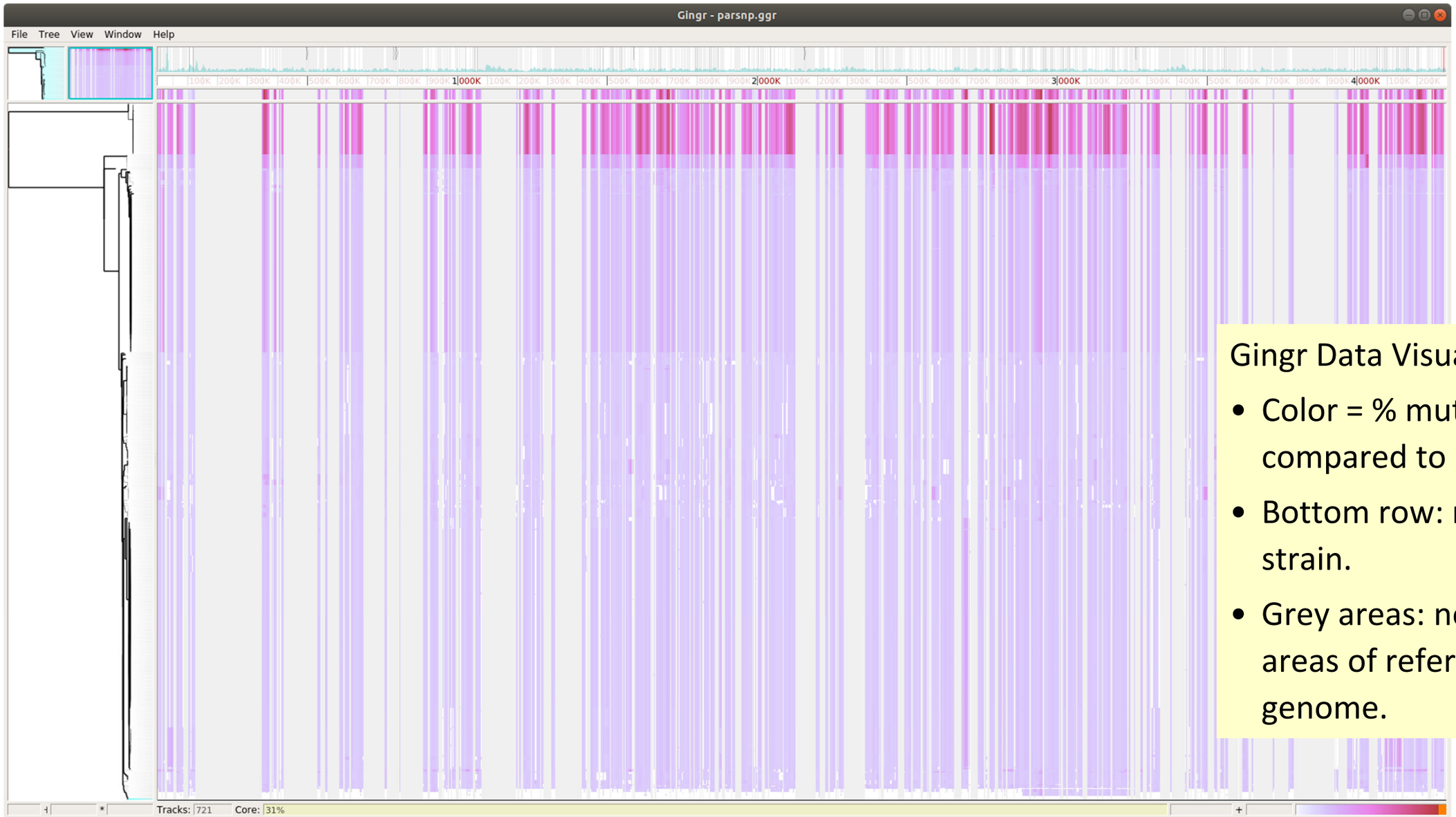
We can use Gingr to dig into areas of the genome where the two Clonal Groups differ from one another, for example these genes "hisP" and an un-annotated one adjacent to it.

Case Study #3: Synteny Comparison



Not a lot of genome rearrangement, but a small number of (consistent) large transpositions (possible assembly error?)

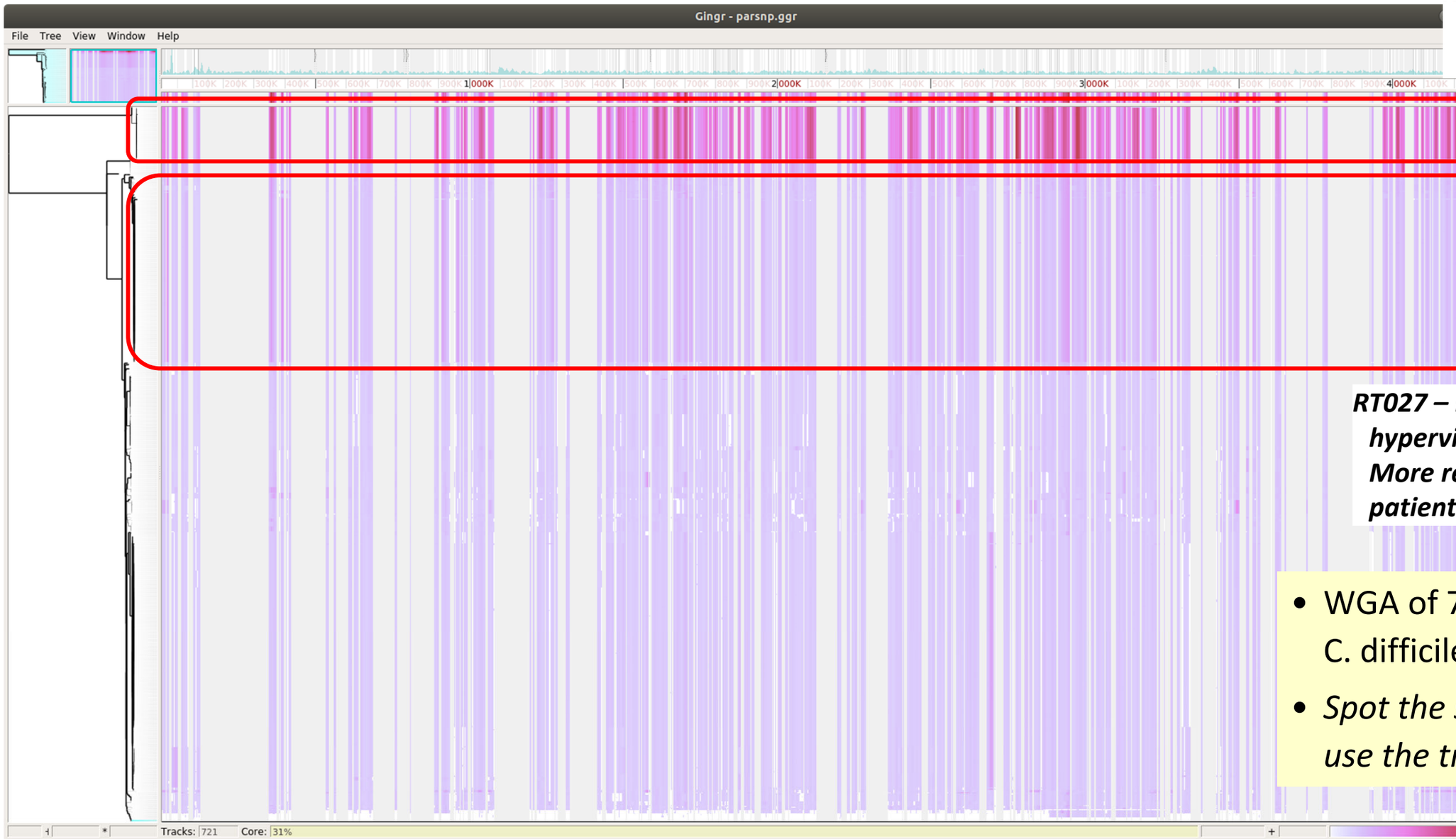
Case-Study #1: *C. difficile* Genomes



Gingr Data Visualization:

- Color = % mutation compared to reference
- Bottom row: reference strain.
- Grey areas: non “core” areas of reference genome.

Case-Study: *C. difficile* Genomes

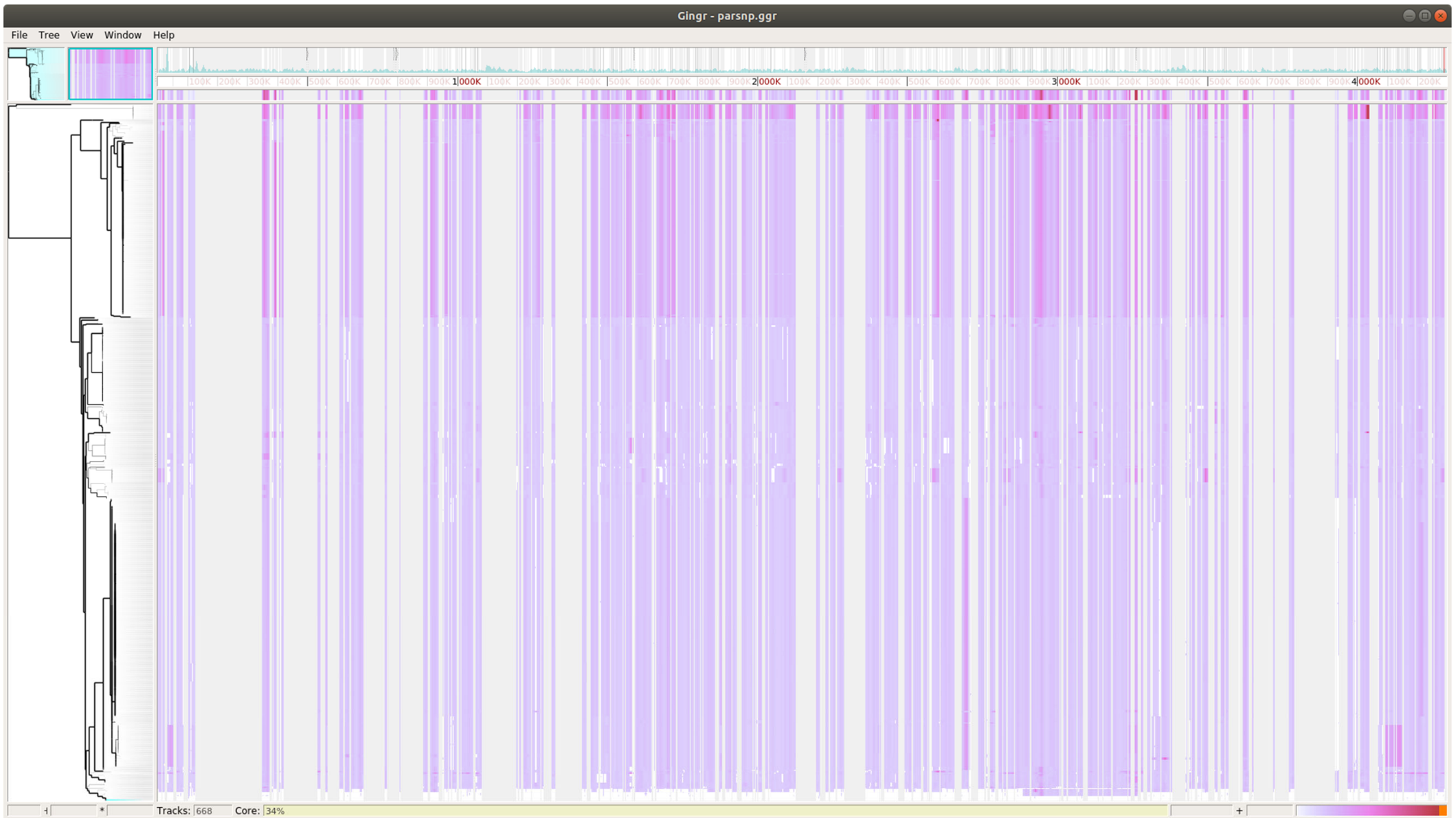


RT078 – Originated in animal host, crossed over

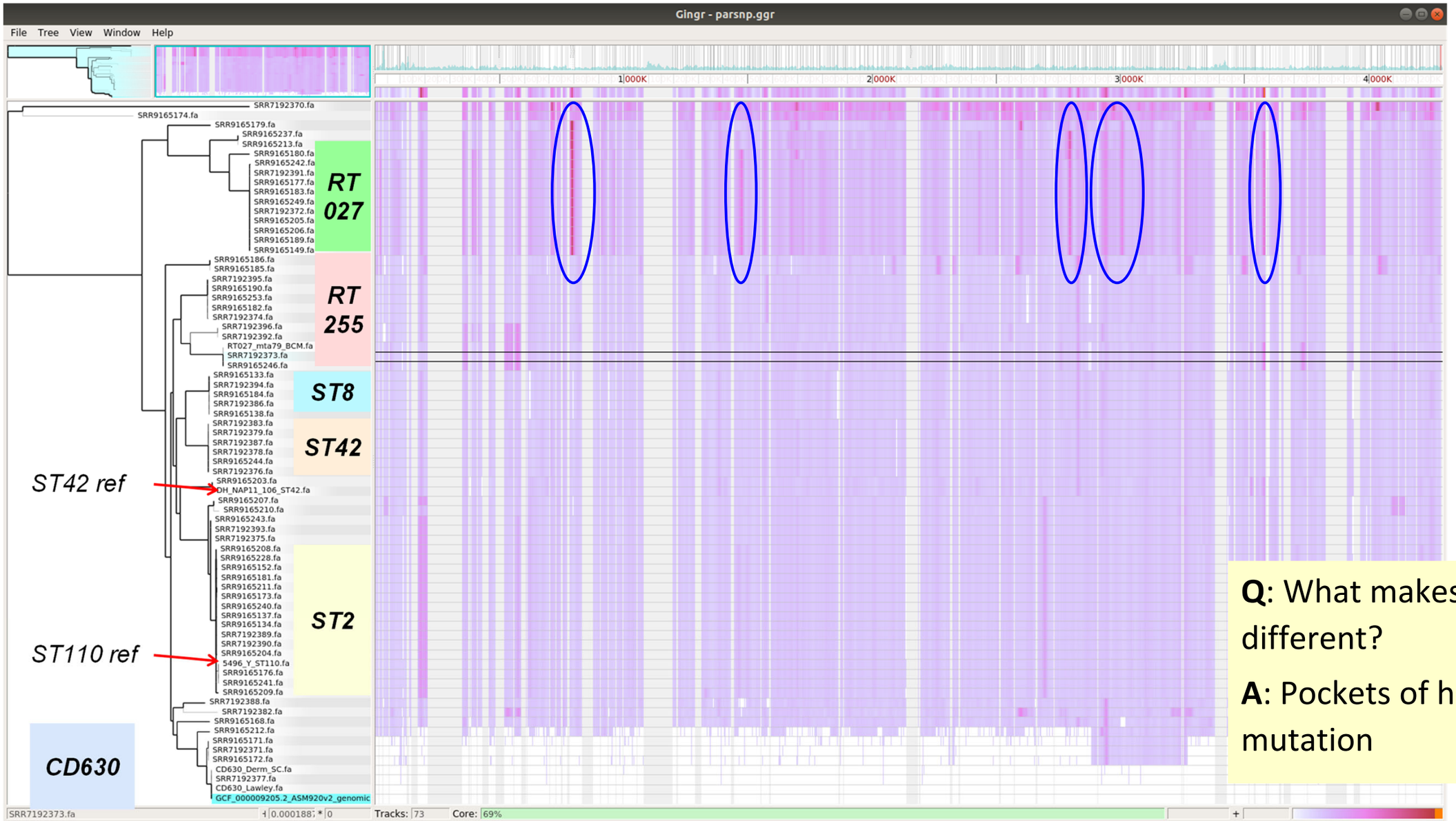
RT027 – Known hypervirulent strain. More recurrent, nastier patient outcomes.

- WGA of 720 assembled C. difficile genomes
- *Spot the strains… (hint: use the tree)*

Case-Study: *C. difficile* Genomes (excluding RT078 samples)



Subset of Genomes w/ST annotation



Q: What makes RT027 different?

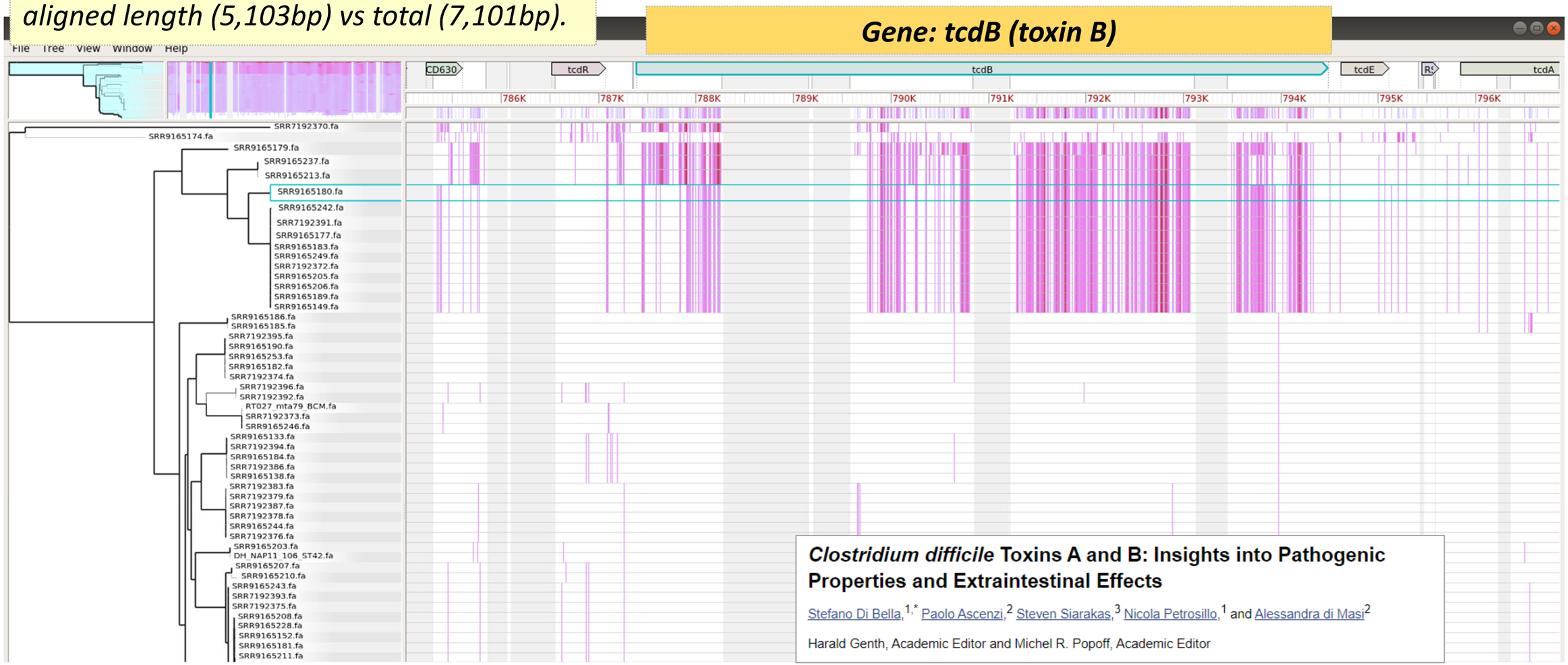
A: Pockets of heavy mutation

Digging Deeper…

***Note**: not all of the tcdB gene was aligned by Parsnp, so this table represents the aligned length (5,103bp) vs total (7,101bp).*

- *This particular region is precisely the coding locus for Toxin B.*
- *RT027 carries a variant tcdB gene with altered function that contributes to its virulence.*

Gene: tcdB (toxin B)



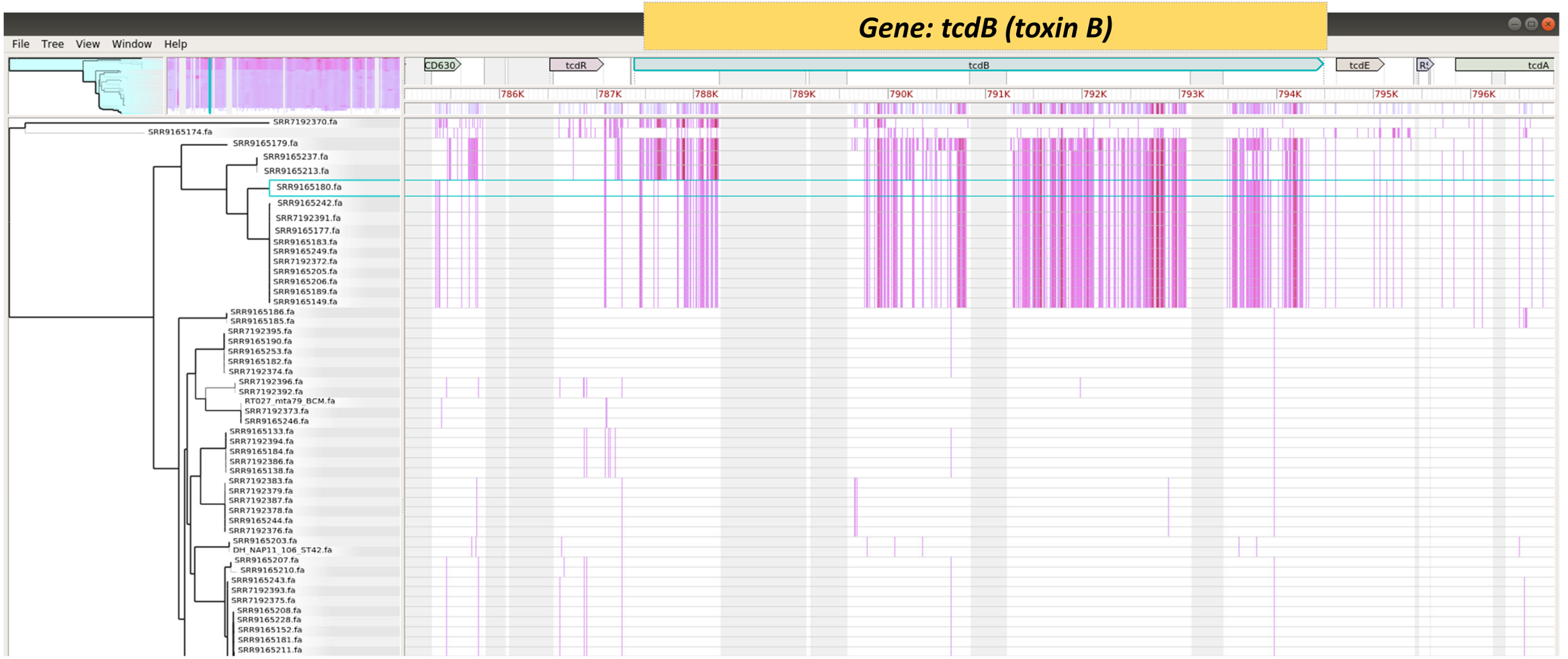
***Clostridium difficile* Toxins A and B: Insights into Pathogenic Properties and Extraintestinal Effects**

Stefano Di Bella,[1,*] Paolo Ascenzi,[2] Steven Siarakas,[3] Nicola Petrosillo,[1] and Alessandra di Masi[2]

Harald Genth, Academic Editor and Michel R. Popoff, Academic Editor

Remark…

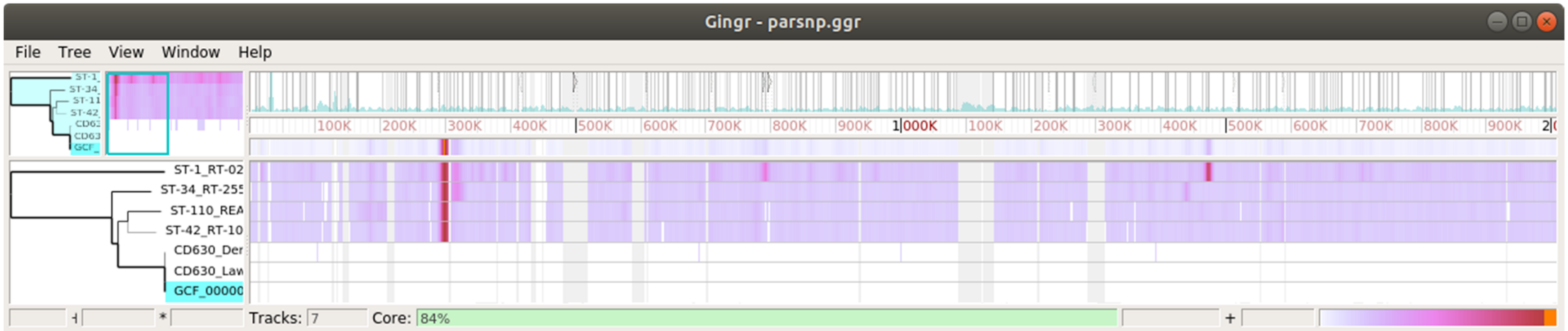
Gene-level phylogenetic signal largely matches up with whole-genome phylogeny…



Comparing Reference Genomes for Some Strains

***Note**: RT027 is in the top row. CD630 is a lab strain used as a common reference.*

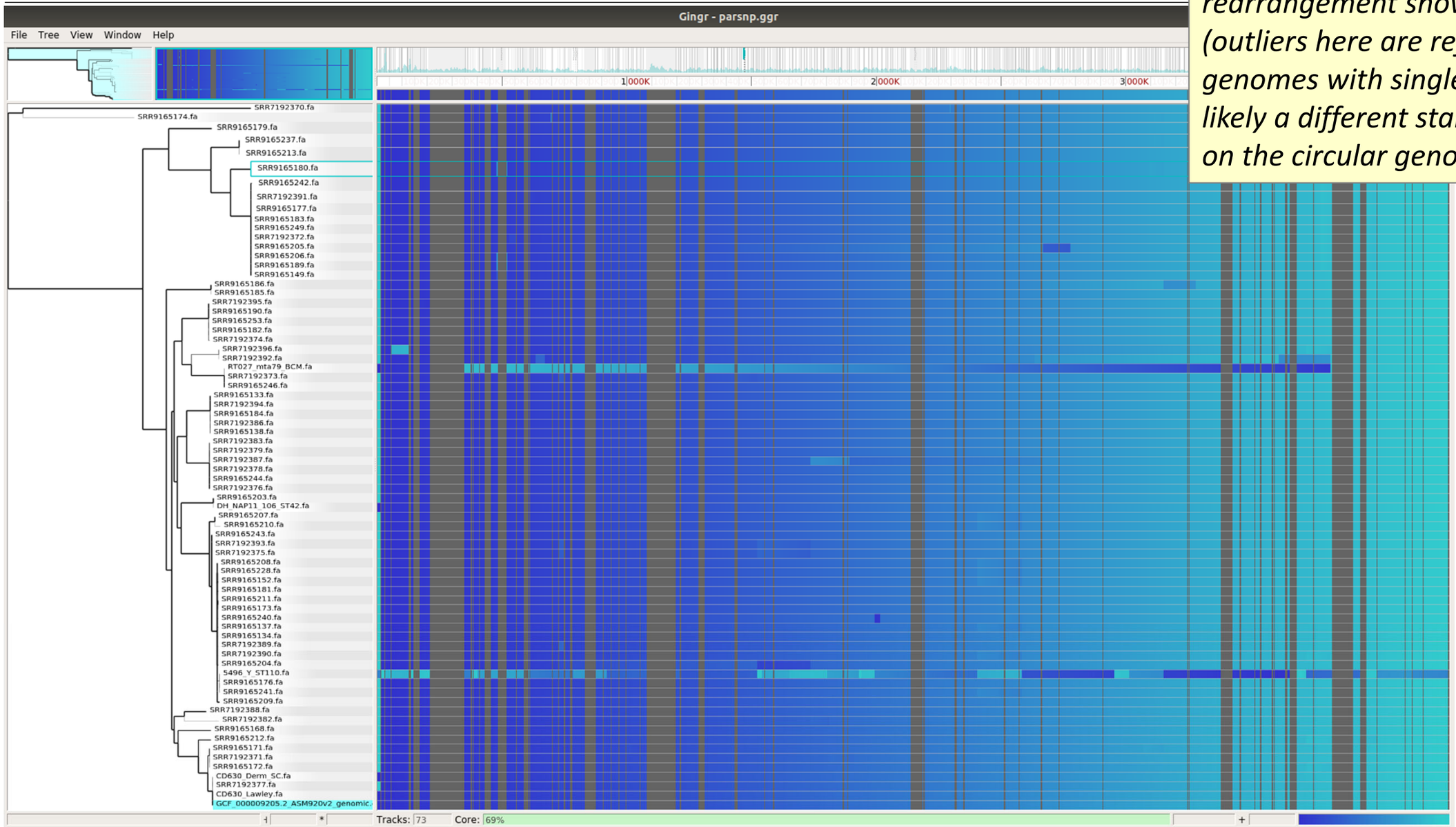
Segment 1
(positions 0-2mbp)



Segment 2
(positions 2-4mbp)

Here the mutation pockets are much clearer.

Synteny Comparison: *C. difficile* Isolates



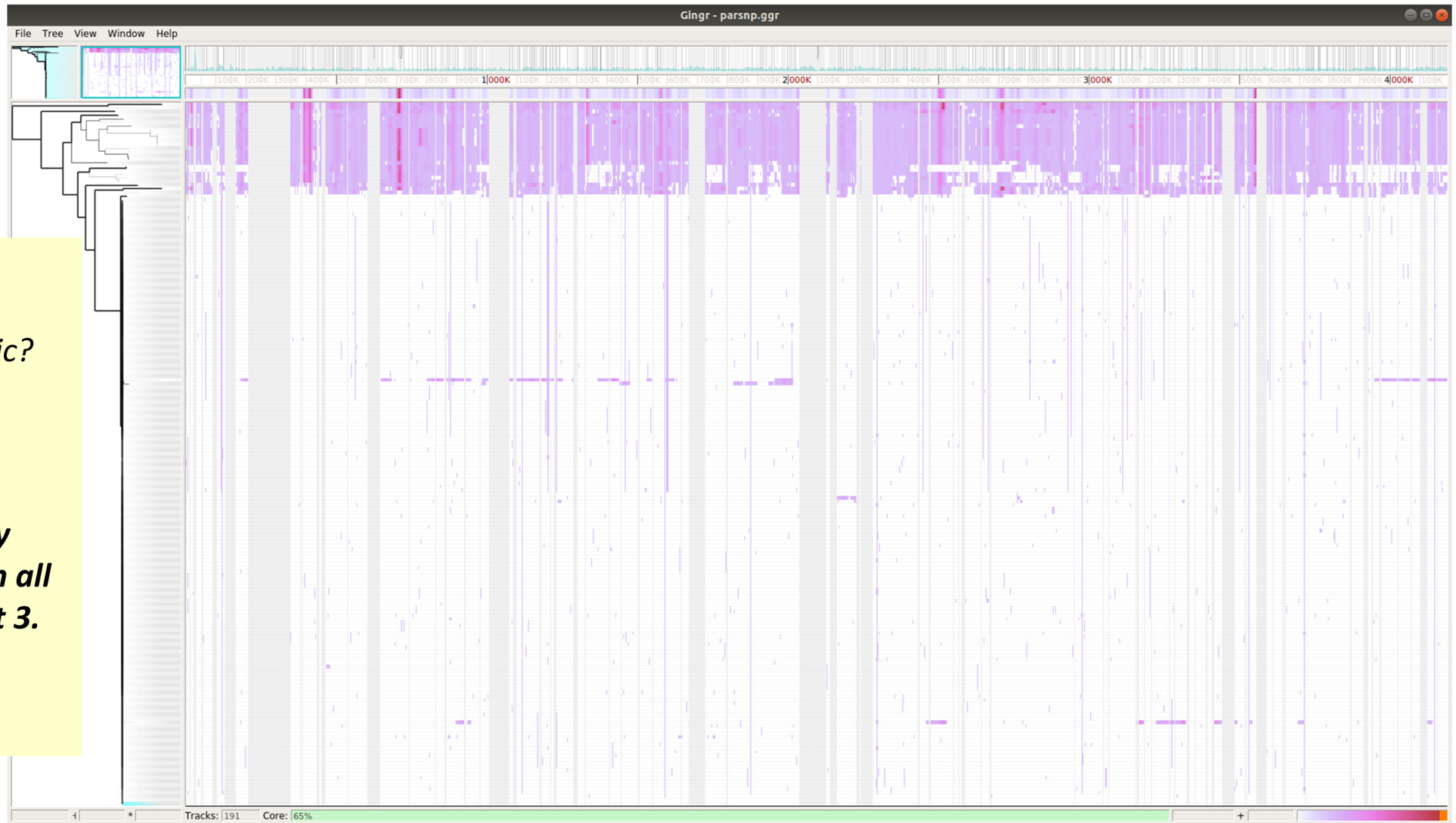
For C. diff, even across a huge number of isolates, very little rearrangement shows up (outliers here are reference genomes with single contig, likely a different starting point on the circular genome.)

Alignment of RT027 isolates (and near relatives) to RT027 ref.

Q: *Does the RT027 Reference match the genomes from the clinic?*

A: *…Yes*

- ***Very little to see, very high match level with all RT027 isolates except 3.***



Case Study #2: *R. gnavus* Isolates from IBD Patients

14 Genomes:

- Reference: ATCC 29149 (RefSeq GCF_008121495)
- ATCC 29149 *de novo* assembly (by me)
- ATCC 35913 (GenBank GCA_900036035)
- 12 Genomes from Hall et al. (2017) (table at right)

RJX1118*	Stool from infant treated with antibiotics
RJX1119*	Stool from infant treated with antibiotics
RJX1120*	Biopsy from IBD patient
RJX1121*	Biopsy from IBD patient
RJX1122*	Biopsy from IBD patient
RJX1123*	Biopsy from IBD patient
RJX1124*	Biopsy from IBD patient
RJX1125*	Biopsy from IBD patient
RJX1126*	Biopsy from IBD patient
RJX1127*	Biopsy from IBD patient
RJX1128*	Stool from IBD patient

Another Case Study: *R. gnavus* Isolates from IBD Pat

Game 1 : Spot the 2 Genomes from Infant Stool (non-IBD)

Another Case Study: *R. gnavus* Isolates from IBD Pat

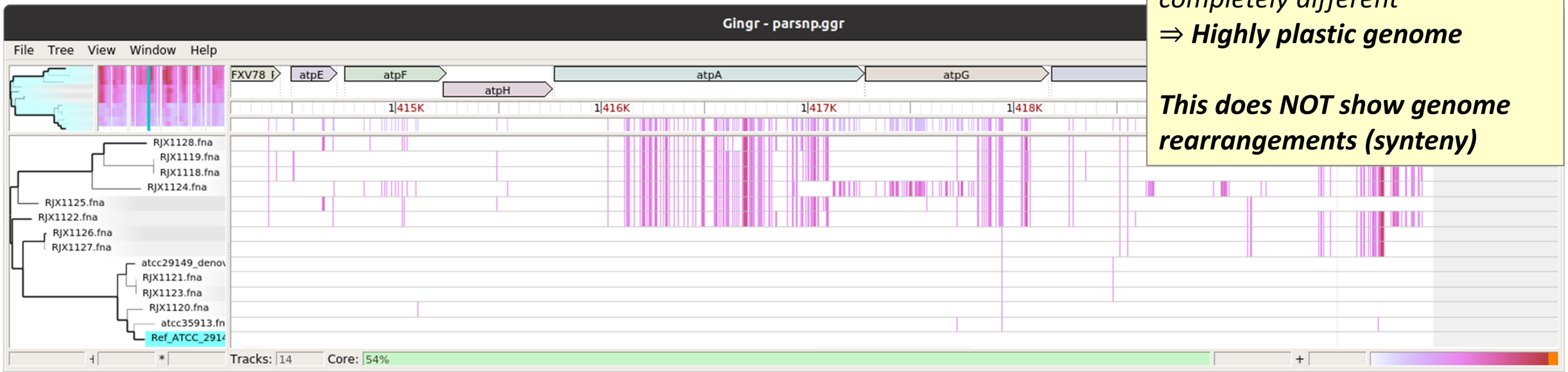
Game 1 : Spot the 2 Genomes from Infant Stool (non-IBD)

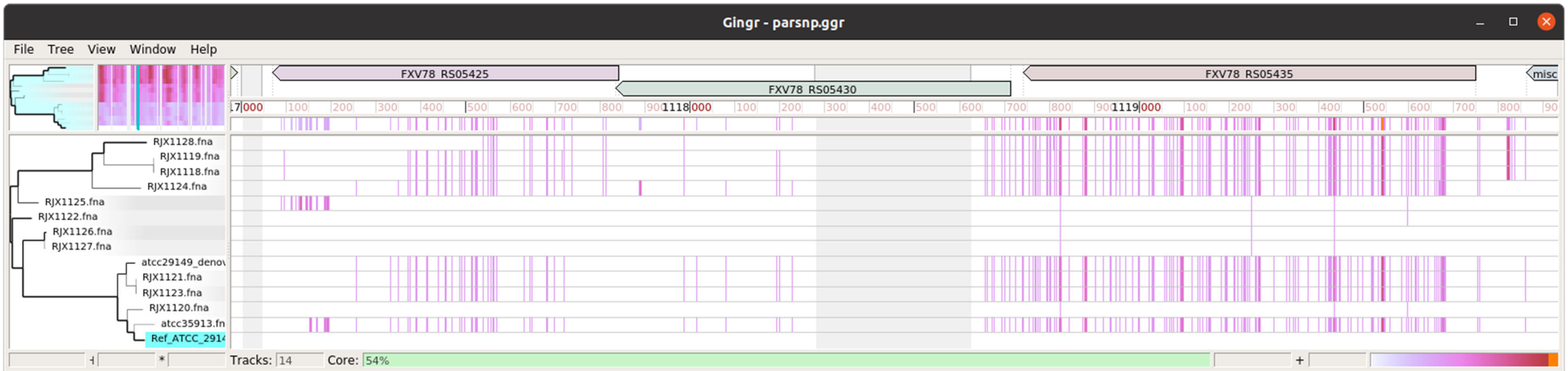
Game 2 : Spot the 2nd ATCC 29149 gnome (supposedly the same as the reference)

R. gnavus strain-level phylogenetic signal is a mess

Depending on the operon, the phylogenetic appearance is completely different
⇒ ***Highly plastic genome***

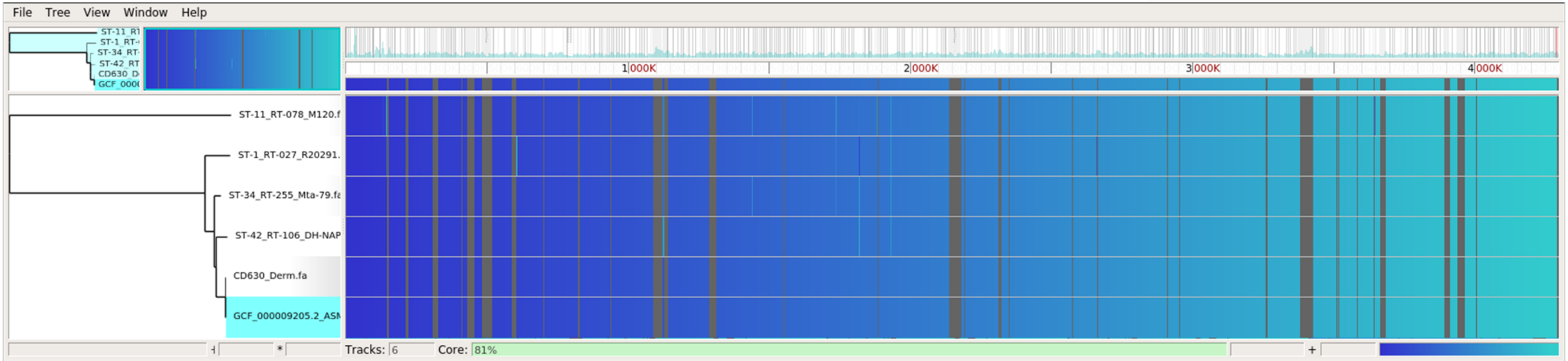
This does NOT show genome rearrangements (synteny)

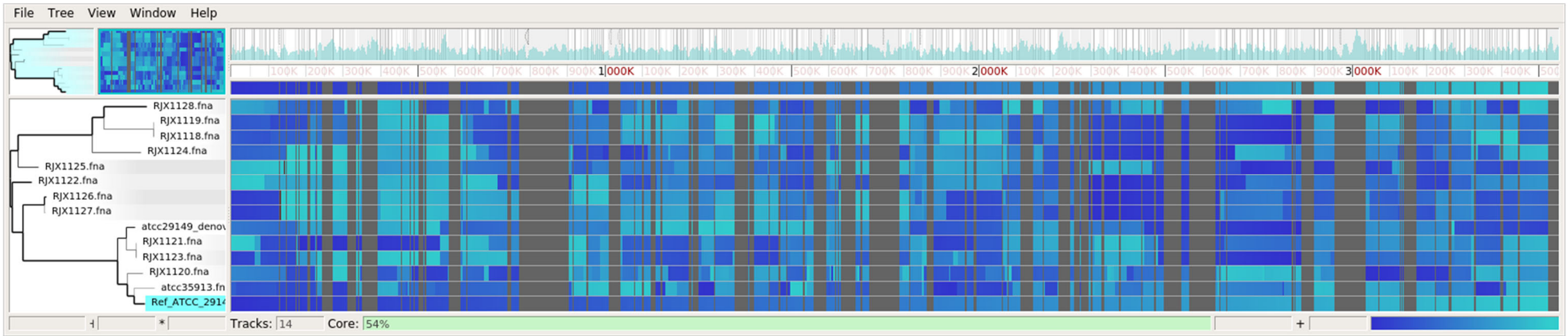




Synteny Comparison: *R. gnavus* & *C. difficile*

These two organisms have very different types of genome plasticity.





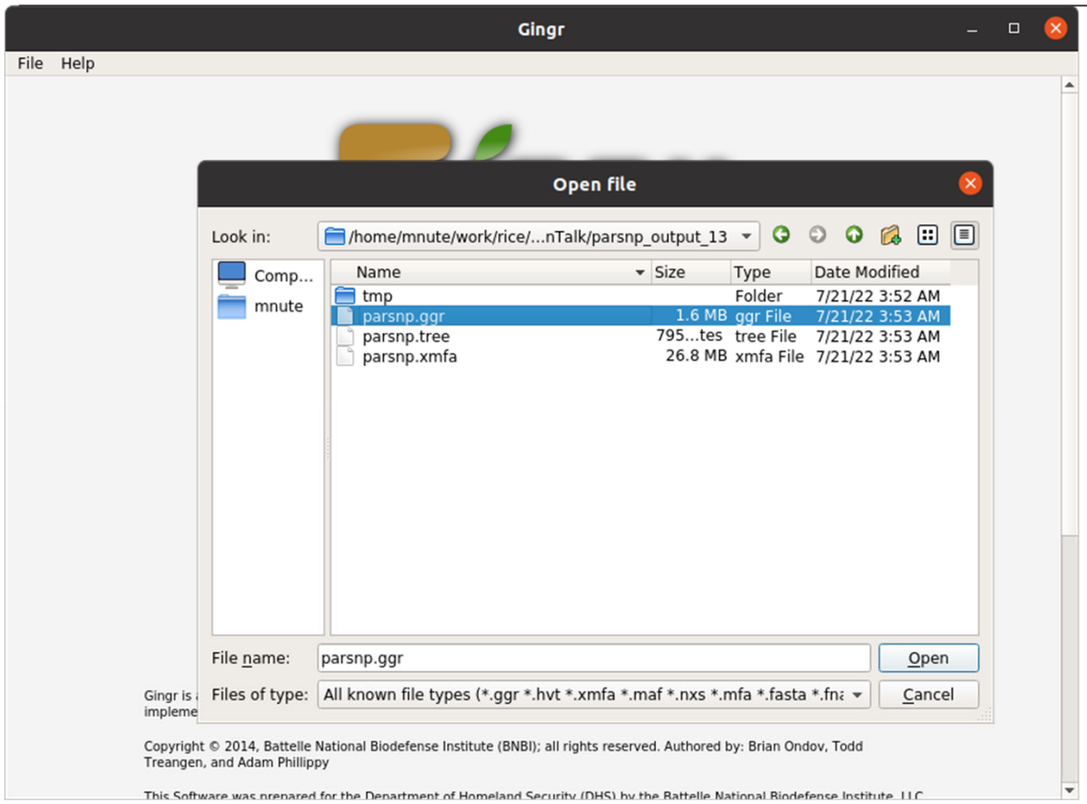
Conclusions

- Whole-genome alignment will give a detailed comparison specifically of the *core* genome
  - Maybe also auxiliary genes (*pan*-genome)
- Visualization can get you up close and personal with the data
  - (This statement applies to almost everything, not just genomes)
- Strains can differ from one another in weird ways.
  - Selective mutation at points of interest
  - Gene gain/loss depending on environment
  - Genome-wide phylogenetic signal vs. Locus-specific signal
  - Etc…?

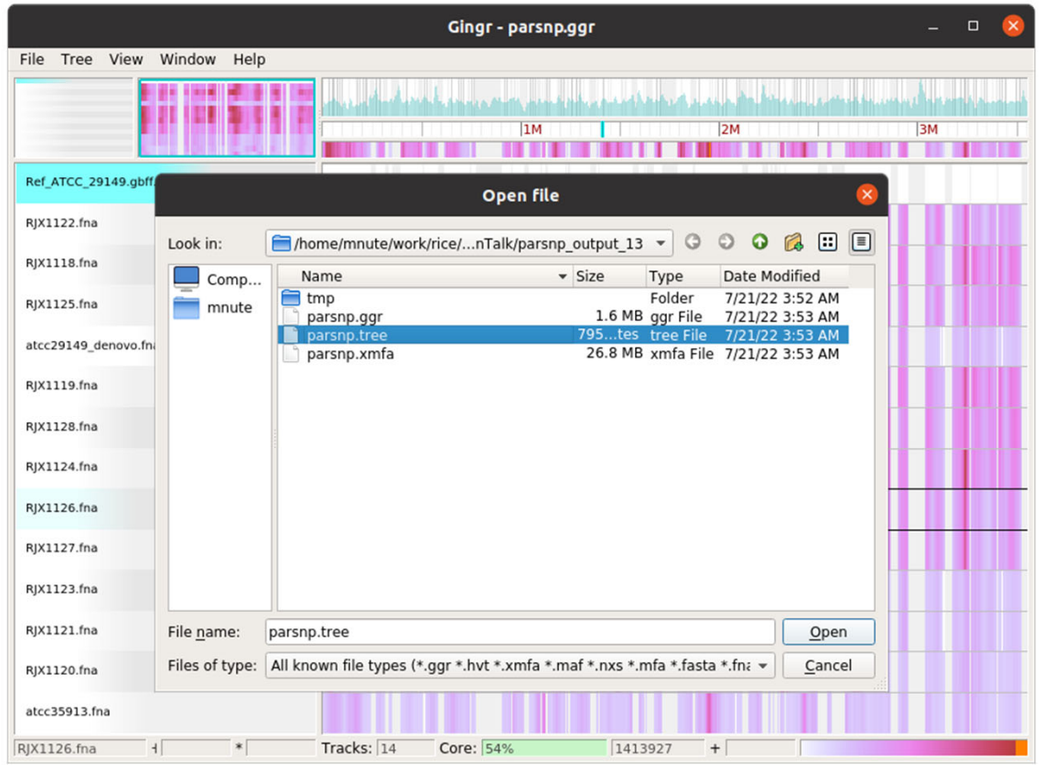
Special Thanks To:

- The Treangen Lab (Rice)
  - Todd Treangen
  - Bryce Killie
  - Kristen Curry
  - Nick Sapoval
  - Yunxi Liu
  - Yilei Fu
  - Advait Balaji
- The Savidge Lab (Baylor College of Medicine)
  - Qinglong Wu
  - Charlie Seto
- Taylor Reiter (for the *R. gnavus* idea)

Appendix: Quick How-to with Gingr (1 of 2)



*1.) Open the *.ggr file created in the parsnp output folder.*



*2.) Once it is open, go back to the “Open” dialogue and open the *.tree file in the same folder.*

Appendix: Quick How-to with Gingr (2 of 2)

3.) This will give you the standard Gingr view. Other options to re-root the tree or to switch to Synteny view are available under the "Tree" and "View" menus.