From chip to SNP in a zip with Parsnp

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Homeland Security

Science and Technology

Scaling genome alignment up to 10K genomes and beyond

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Acknowledgement

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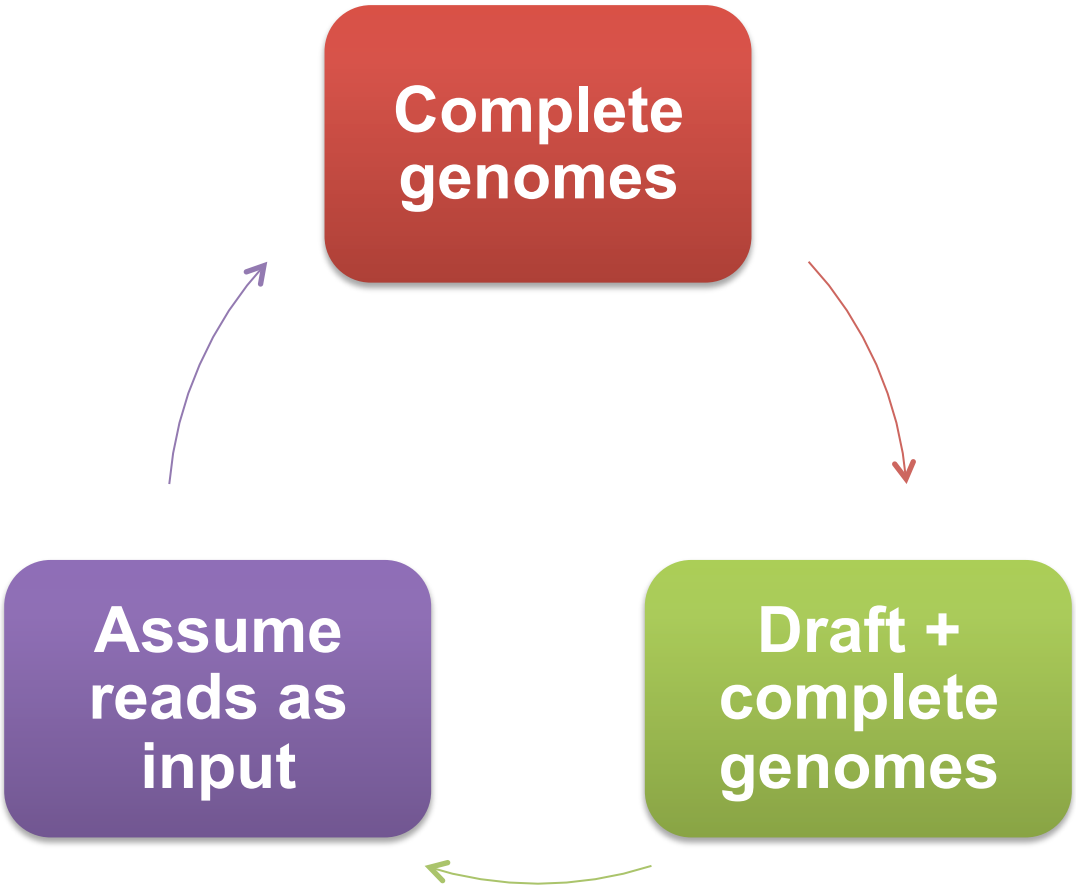
Historical perspective: expected input



Genome revolution

- **Enabled by:**
  - **Long reads + correction methods:**
    - **PacBio, 5-6kbp+ reads**
    - **Routinely generating single contig assemblies**
  - **Affordable sequencing:**
    - **MiSeq, 2x300bp**
    - **HiSeq, 2x150bp**
  - **Push-button assembly**
- **Soon to be a major factor:**
  - **Oxford Nanopore**

Outline

① **From chip to assembly**

② From assembly to SNP

High-confidence assembly

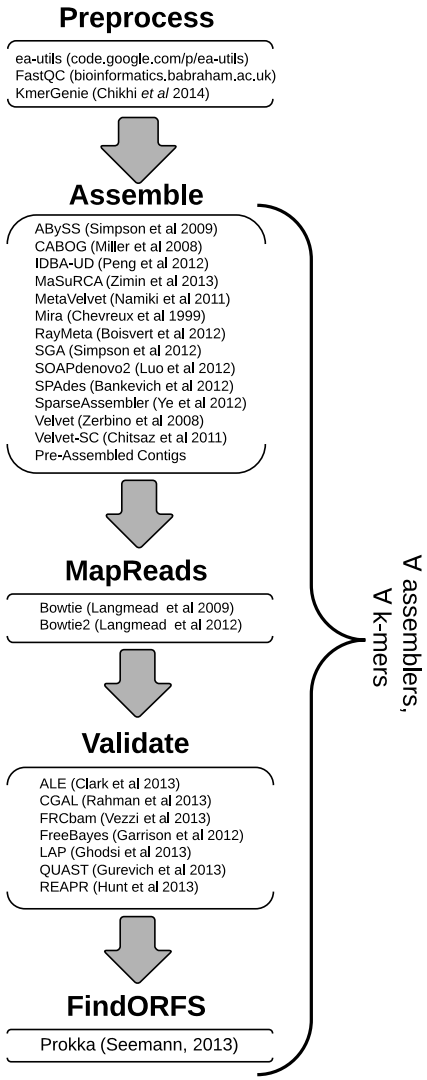
– **Assembly hard, validation easy**
  - Assembly is chaotic
  - Assembly as a hypothesis

– **Ensemble assembly** and validation
  - Each run is an assembly competition
  - Tolerant of tool failures
  - Can beat expert users

:metAMOS:

a metagenomic assembly pipeline for AMOS

- **iMetAMOS: automated sample analysis**
  - Promote and record assembly best practices
    - e.g. Everything is a metagenome
  - Reproducible workflows
  - Low startup cost (frozen binary)



Koren, S., Treangen, T. J., Hill, C. M., Pop, M., & Phillippy, A. M. (2014)
Automated ensemble assembly and validation of microbial genomes. *BMC Bioinformatics*.

GAGE in a box

- GAGE-B *Rhodobacter sphaeroides* MiSeq dataset

Assembler	GAGE-B rank	GAGE-B N50	GAGE-B CN50	iMetAMOS rank	iMetAMOS N50	iMetAMOS CN50
ABySS	5	21,441	21,307	5	38,322	36,101
MIRA	6	15,792	15,190	4	52,034	46,977
MaSuRCA	1	130,714	119,839	1	163,762	139,231
SGA	7	9,108	9,055	7	3,657	3,655
SOAPdenovo2	3	33,491	32,605	3	87,036	65,337
SPAdes	2	118,093	89,065	2	118,214	81,505
Velvet	4	23,979	23,230	6	19,652	19,355

Ensemble Assembly Validation

:metAMOS:

Treangen TJ, Koren S, et. al. Genome Biol. 2013 Jan 15;14(1):R2. PMID: 23320958.

- Preprocess OK
- Assemble OK
- MapReads OK
- Validate OK
- FindORFS OK
- FindRepeats SKIPPED
- Scaffold SKIPPED
- FindScaffoldORFS OK
- Abundance OK
- Annotate OK
- FunctionalAnnotation OK
- Propagate SKIPPED
- Classify OK
- Browse Results OK

Pipeline summary
Run commands

Version: v1.3 Candied Bacon
workflows: core,isolate
Created: 06 Nov 2013, 5:29PM

Selected assembler: spades.47
Selected reference: Mycobacterium_tuberculosis_EAI5_NITR206_uid202218
Sample may have contaminants, only 97.57% contigs/reads >100bp assigned to Mycobacterium. Check Annotate output.

Assembly	Lap	Ale	Cgal	Snp	Frcbam	Orf	N50	Reapr
SPADES.47	-8.528025	-172424658.572994	-1205000000.000000	797	N/A	5109	38,016	N/A
VELVET.47	-8.768155	-226035245.216896	-1050360000.000000	217	N/A	4471	6,610	N/A
MASURCA.47	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

14,627,217	Reads
3,916	Contigs
3,916	Scaffolds
5,109	ORFs
0	Motifs

QUAST report

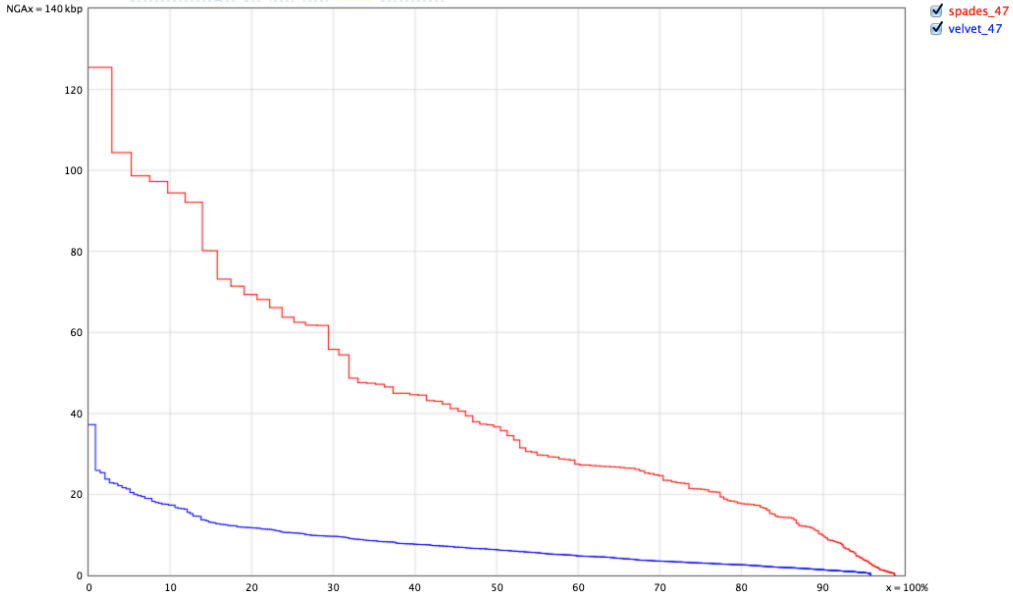
12 November 2013, Tuesday, 17:20:46

All statistics are based on contigs of size >= 500 bp, unless otherwise noted (e.g., "# contigs (>= 0 bp)" and "Total length (>= 0 bp)" include all contigs.)

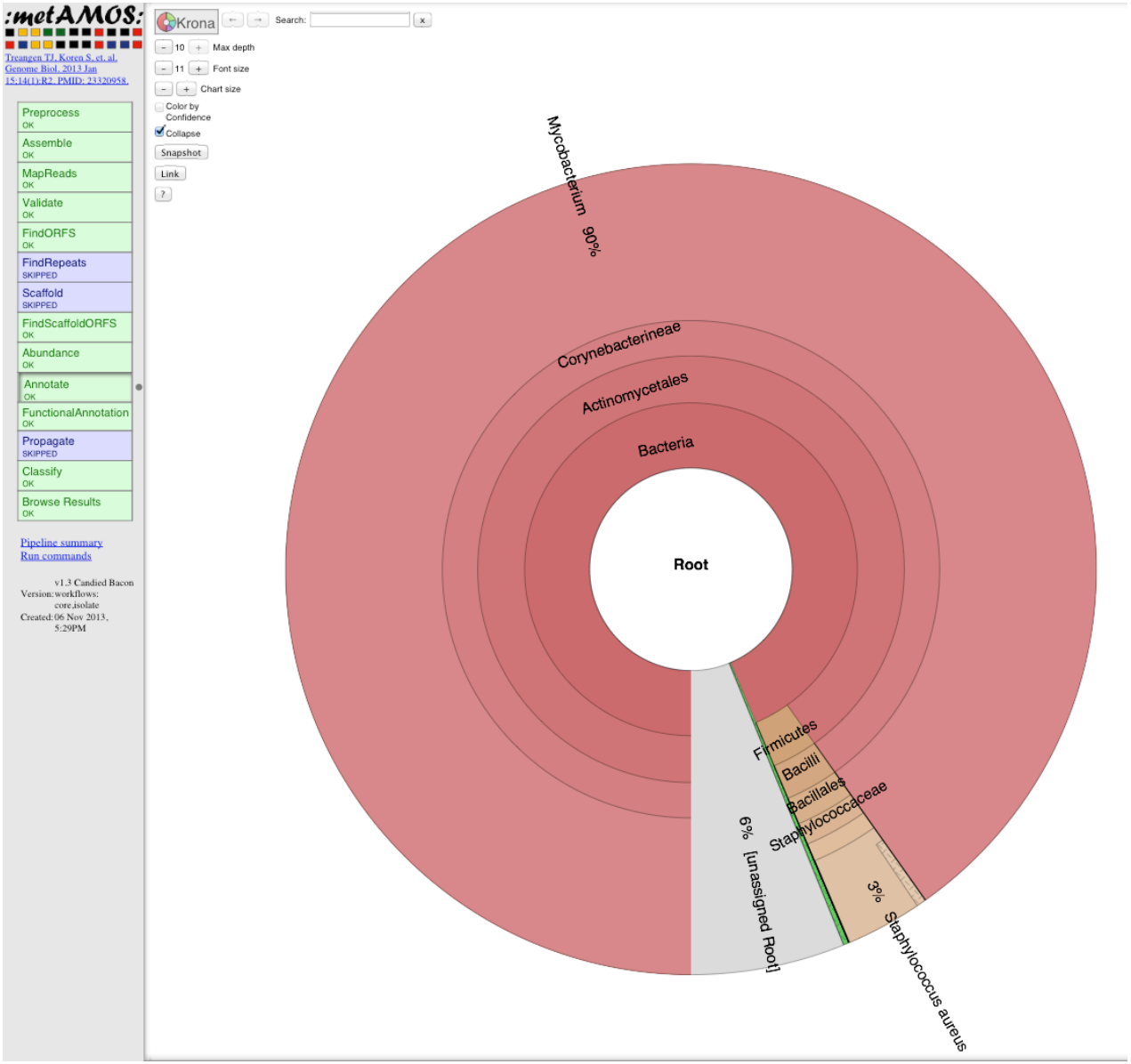
Short report worst.....best

Statistics without reference	spades_47	velvet_47
# contigs	1627	1045
# contigs (>= 0 bp)	1627	1045
# contigs (>= 1000 bp)	531	874
Largest contig	125 443	37 235
Total length	5 541 211	4 248 713
Total length (>= 0 bp)	5 541 211	4 248 713
Total length (>= 1000 bp)	4 792 027	4 120 067
N50	27 266	6656
N75	5902	3379
L50	52	195
L75	137	418
GC (%)	58.52	65.27
Misassemblies		
# misassemblies	16	8
# relocations	16	8
# translocations	0	0
# inversions	0	0
# misassembled contigs	13	7
Misassembled contigs length	475 706	65 772
# local misassemblies	31	19
Unaligned		
# fully unaligned contigs	1359	9
Fully unaligned length	1 173 848	15 804
# partially unaligned contigs	12	16
# with misassembly	1	0
# both parts are significant	3	6
Partially unaligned length	15 870	16 039
Mismatches		
# mismatches	2509	2407
# indels	1303	1058
Indels length	1979	1581
# mismatches per 100 kbp	58.81	57.57
# indels per 100 kbp	30.54	25.31
# short indels (<= 5 bp)	1276	1033
# long indels (> 5 bp)	27	25
# N's	0	0
# N's per 100 kbp	0	0
Genome statistics		
Genome fraction (%)	97.292	95.23
Duplication ratio	1.022	1.009
Largest alignment	125 443	37 235
NG50	37 942	6526
NG75	23 073	3136

Plots: Cumulative length Nx NAx NGx NGAx GC content



ENA TB Contamination



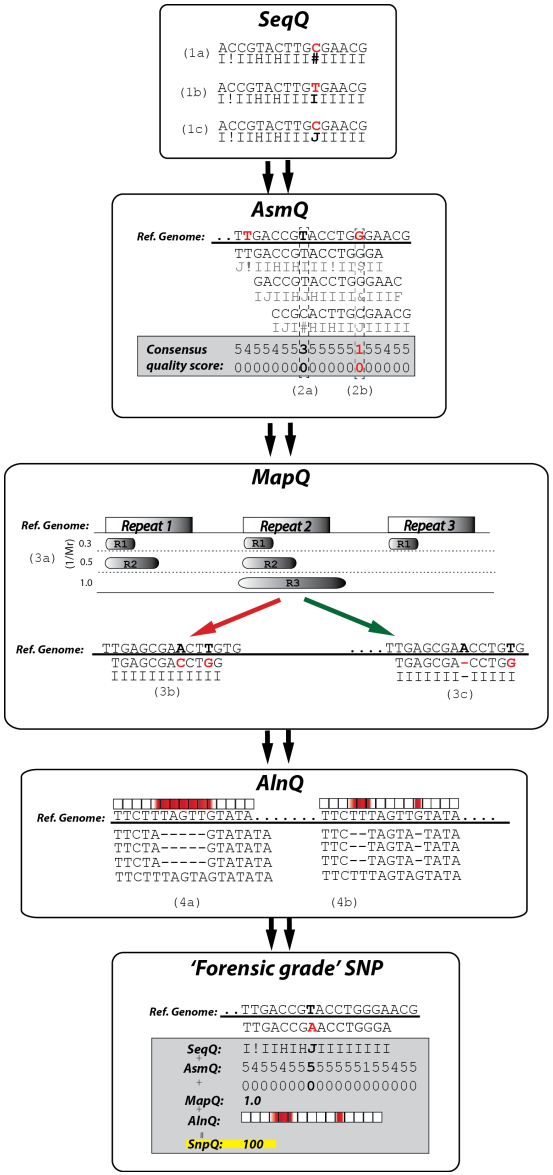
3% of samples

Outline

① From chip to assembly

② **From assembly to SNP**

High-confidence SNPs for outbreak analysis



How can we identify SNPs?

- **K-mers**
  - **No assembly required**
  - **Typically 21-31 length sequence with a wobble position in middle**
  - **Missing context, good for broad brush analysis**

How can we identify SNPs?

- **Read-mapping**
  - Many excellent tools for mapping reads to a reference genome
    - BWA, Bowtie-2, NUCmer, etc
  - No assembly required
  - Can have issues with multi-mapping & Indels
  - Primary input shifting back to genomes
  - Multiple alignment is not directly available

How can we identify SNPs?

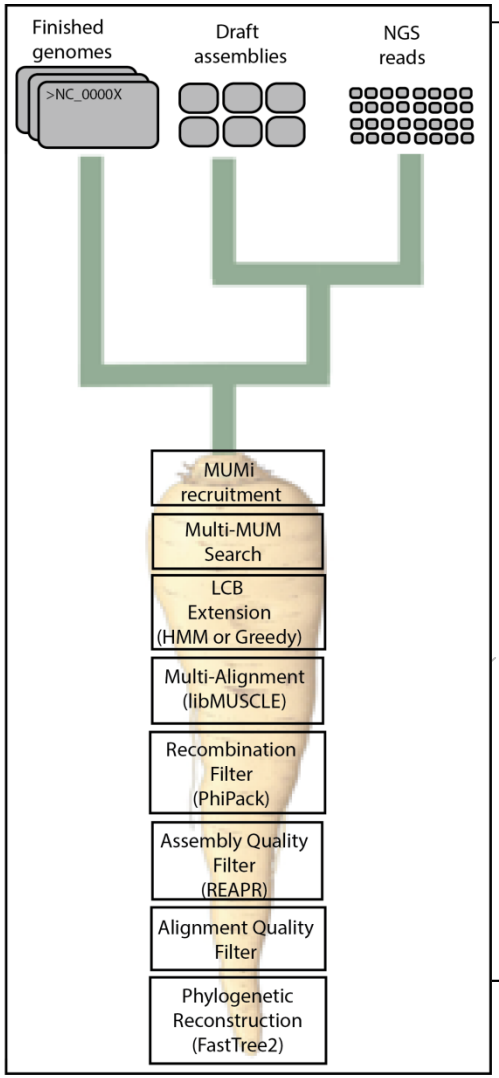
- **Whole genome alignment**
  - 1 to 1 relationship for each and every base pair
  - Strategy is typically all pairs of genomes
  - Exponential increase in runtime, not feasible to align many

How can we identify SNPs?

- **Core genome alignment**
  - 1 to 1 relationship for each and every base pair, within commonly conserved regions
  - Eliminates subset problem, focuses on well defined subproblem of genome alignment
  - Useful for phylogenetic reconstruction and rapid analyses of outbreaks
- **Parsnp built around core genome detection**

ParSNP

1. NGS reads, draft assemblies, and/or finished genomes as input
2. Near-neighbor genomes are recruited
3. **Efficient Multi-MUM search to identify locally collinear blocks**



4. Multi-Alignment of LCBs with Muscle
5. HMM-based LCB extension
6. Apply quality filters
7. Phylogenetic reconstruction with FastTree2
8. Done !

400 600 800 1000

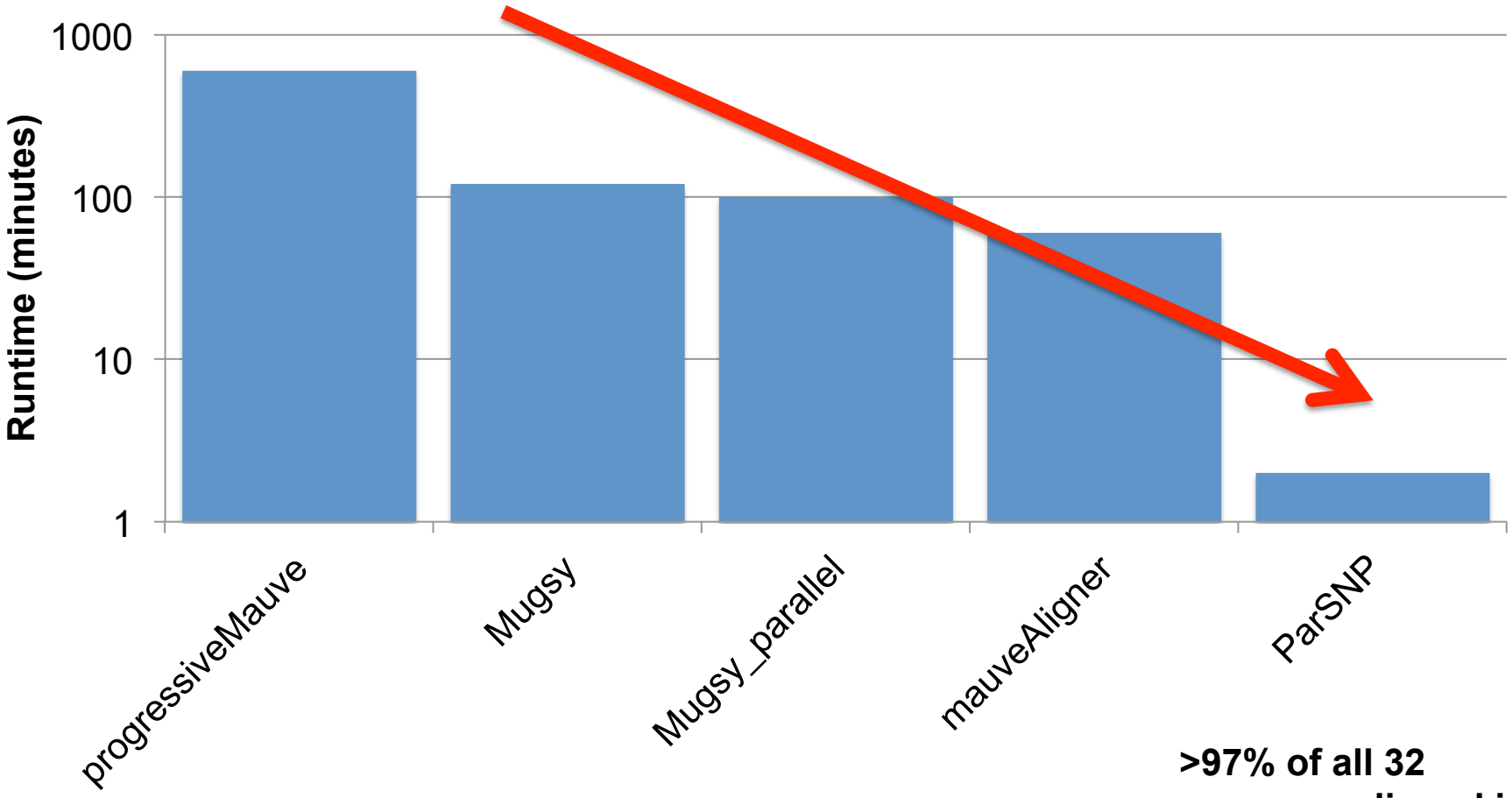
# of genomes

OK, but..

- is it efficient?

Run time performance

(32 simulated E. coli W3110 genomes)

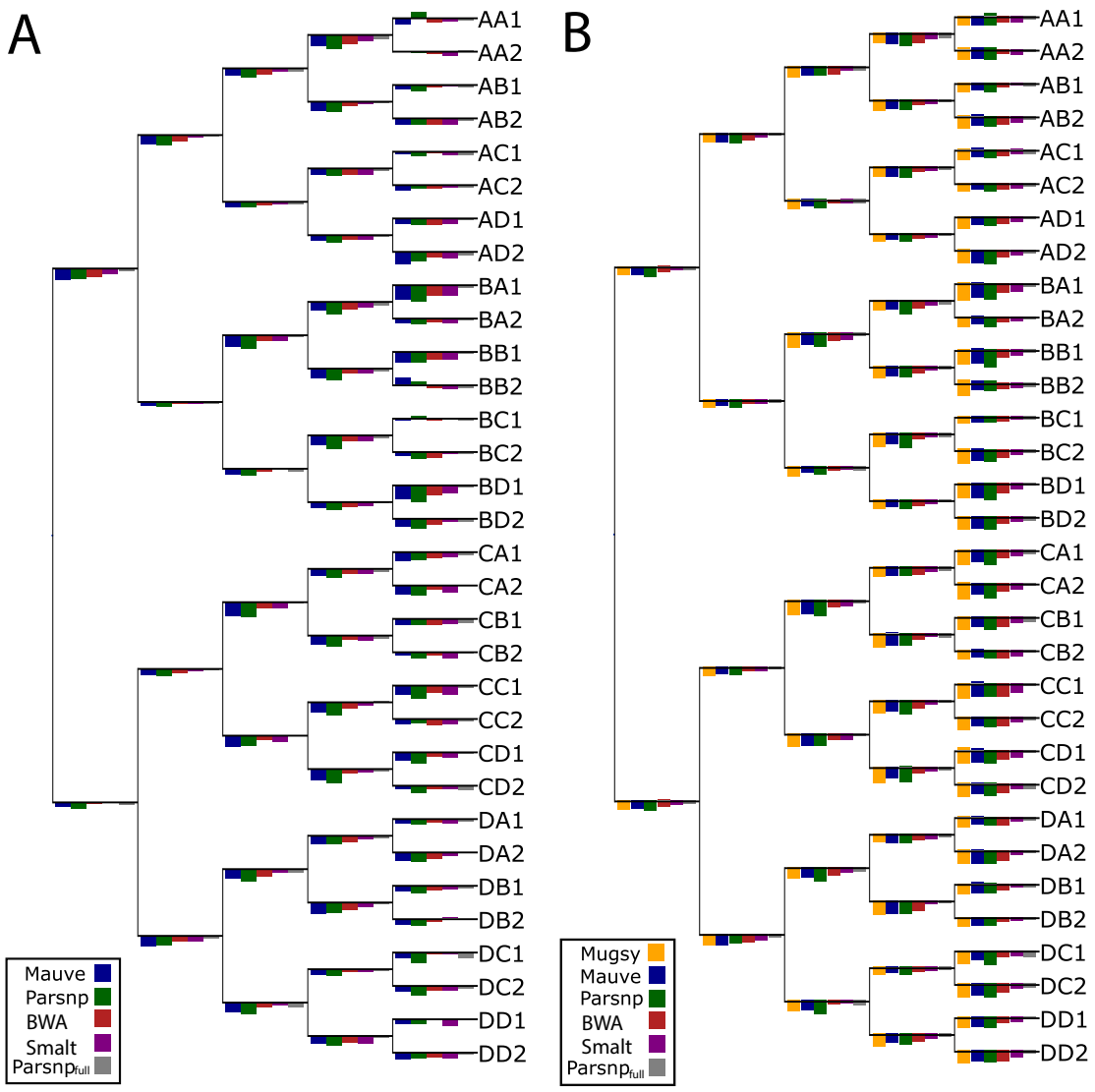


>97% of all 32 genomes aligned in less than 3 minutes!

OK, but..

- is it accurate?

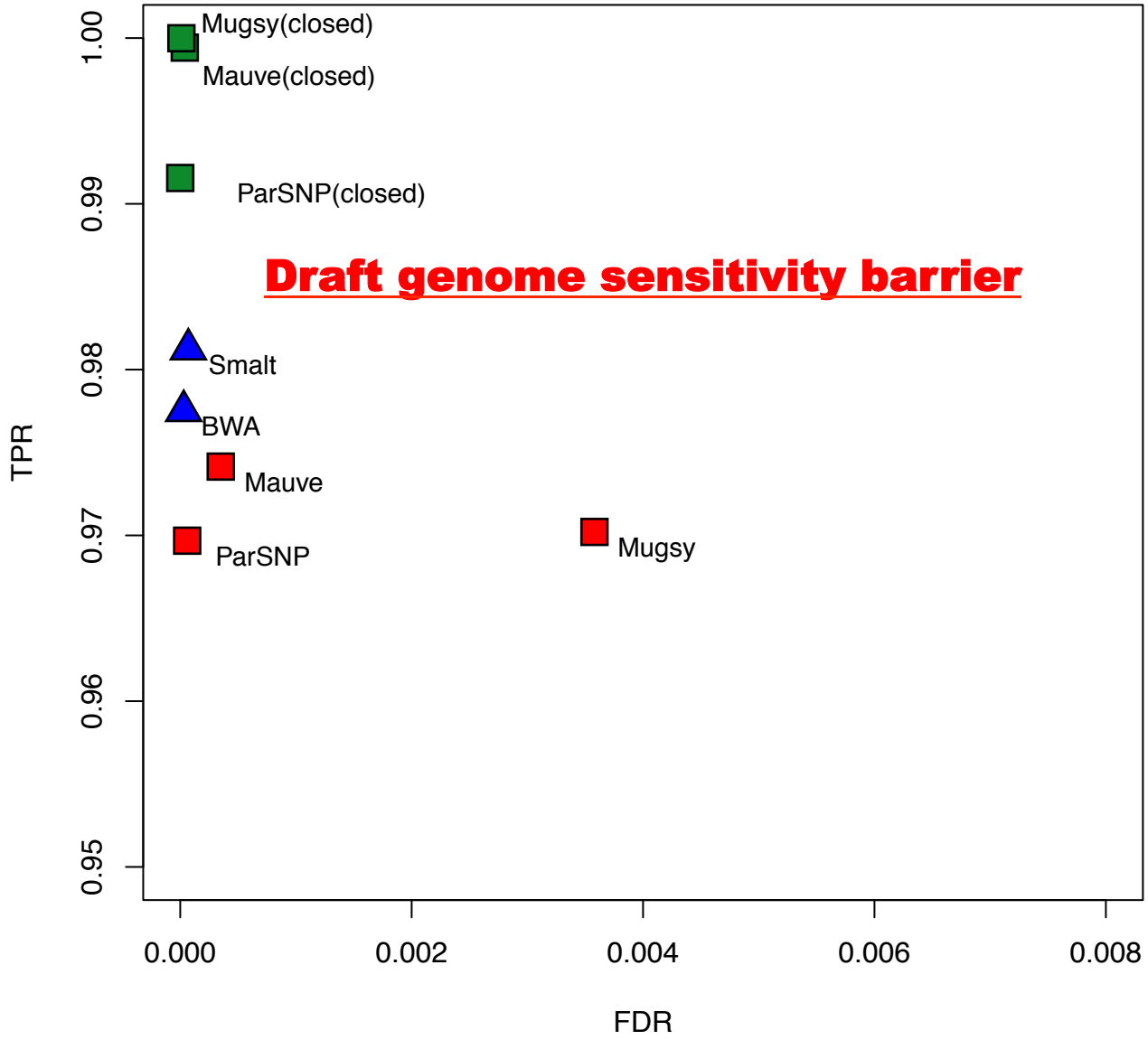
Branch SNP/length performance



OK, but..

- is it sensitive?

Sensitive, high precision SNP calls



. kSNP

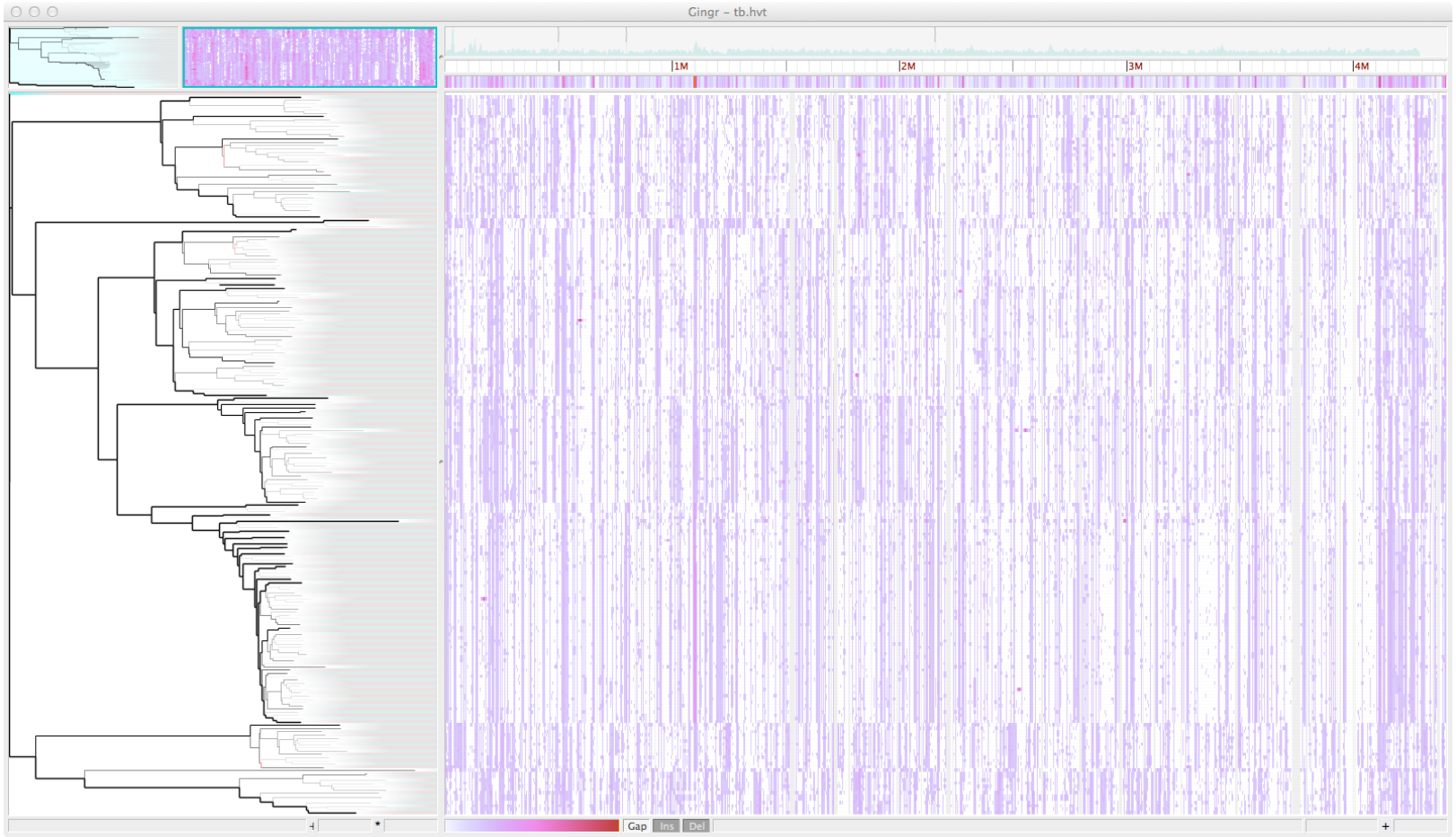
From simulated to real

- **Mycobacterium tuberculosis, "Out of Africa" study involving 200+ isolates (Comas et al 2013)**

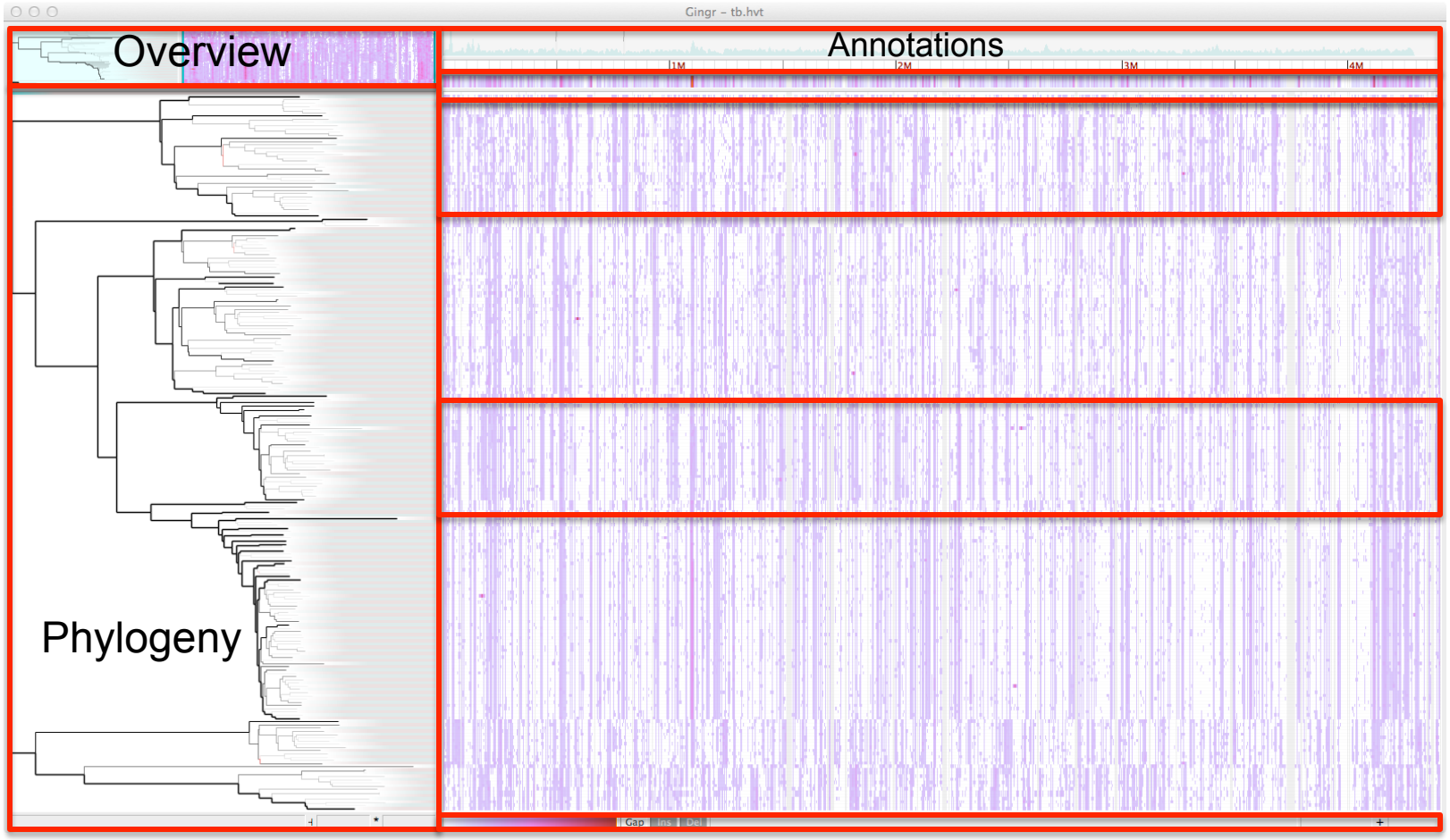
- **Downloaded all data associated with publication**
  - **Data ranged from 51bp SE reads to 100bp PE reads**

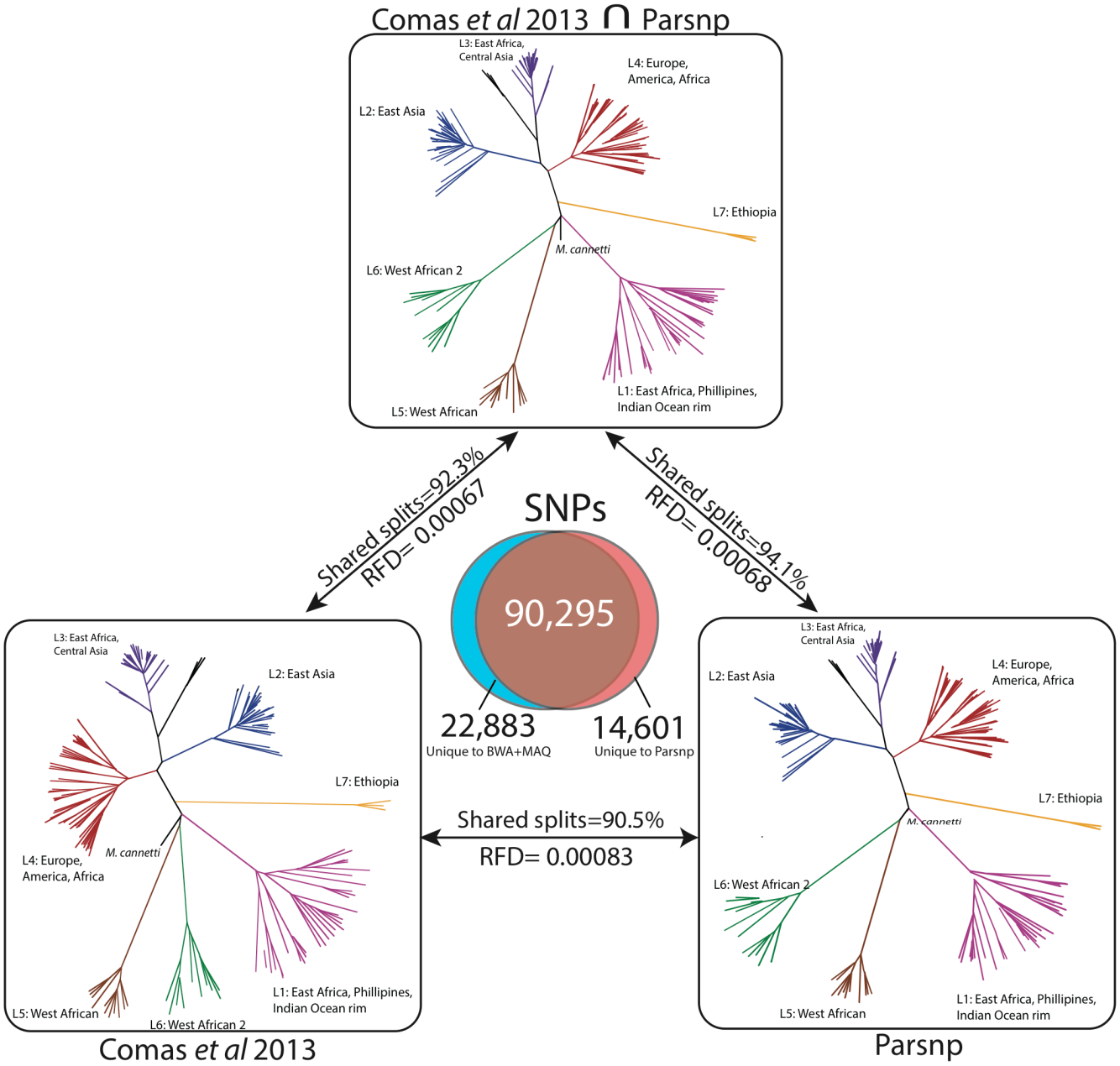
- **Questions to answer:**
  - **How does our phylogeny compare to the Comas et al study?**
  - **How sensitive are we in this "worst case" scenario?**

Gingr view of MTB alignment

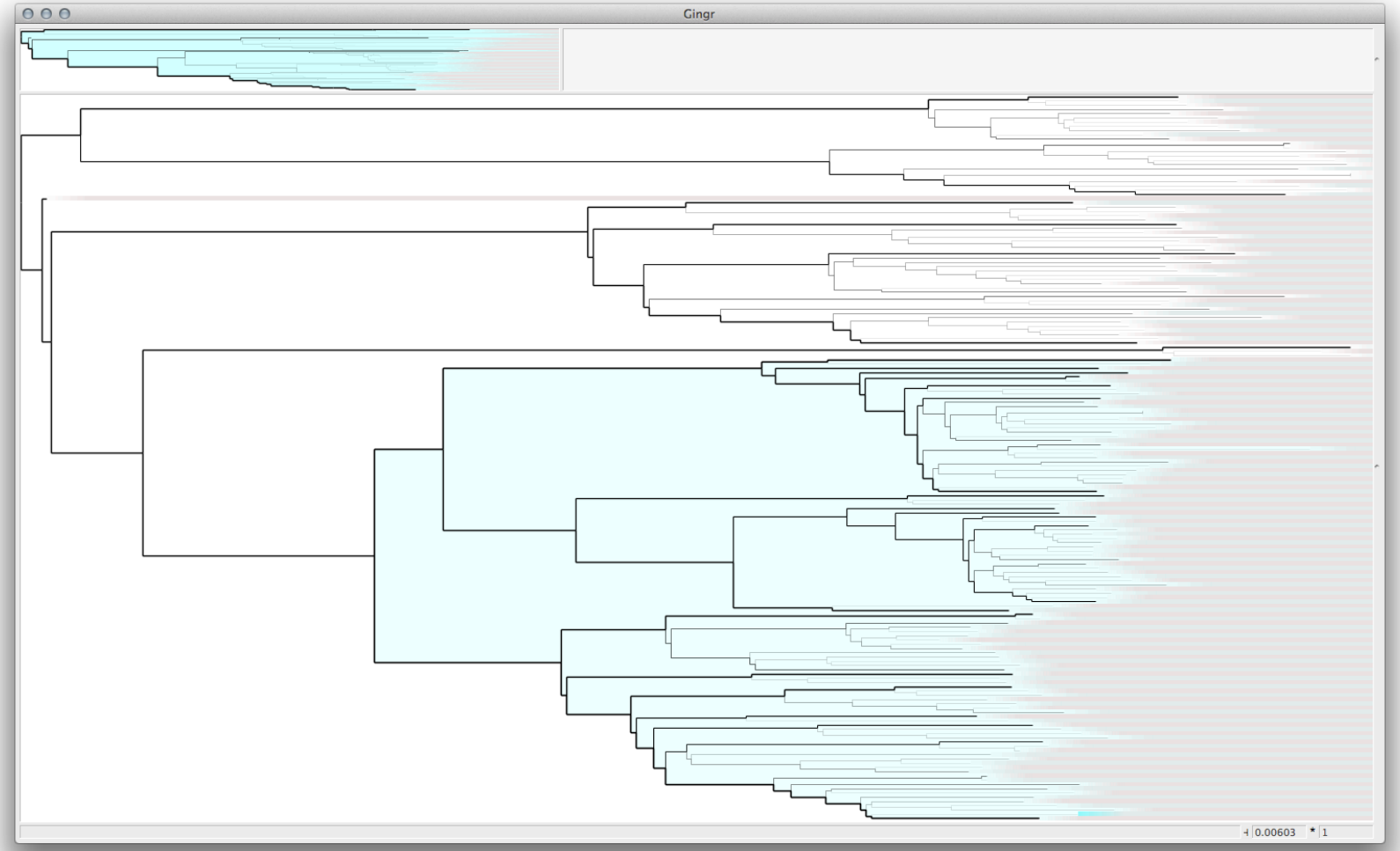


Gingr view of MTB alignment




Comas et al 2013 ∩ Parsnp
L3: East Africa, Central Asia
L4: Europe, America, Africa
L2: East Asia
L7: Ethiopia
M. cannetti
L6: West African 2
L5: West African
L1: East Africa, Phillipines, Indian Ocean rim
Shared splits=92.3%
RFD= 0.00067
Shared splits=94.1%
RFD= 0.00068
SNPs
90,295
22,883
Unique to BWA+MAQ
14,601
Unique to Parsnp
Shared splits=90.5%
RFD= 0.00083
Comas et al 2013
Parsnp

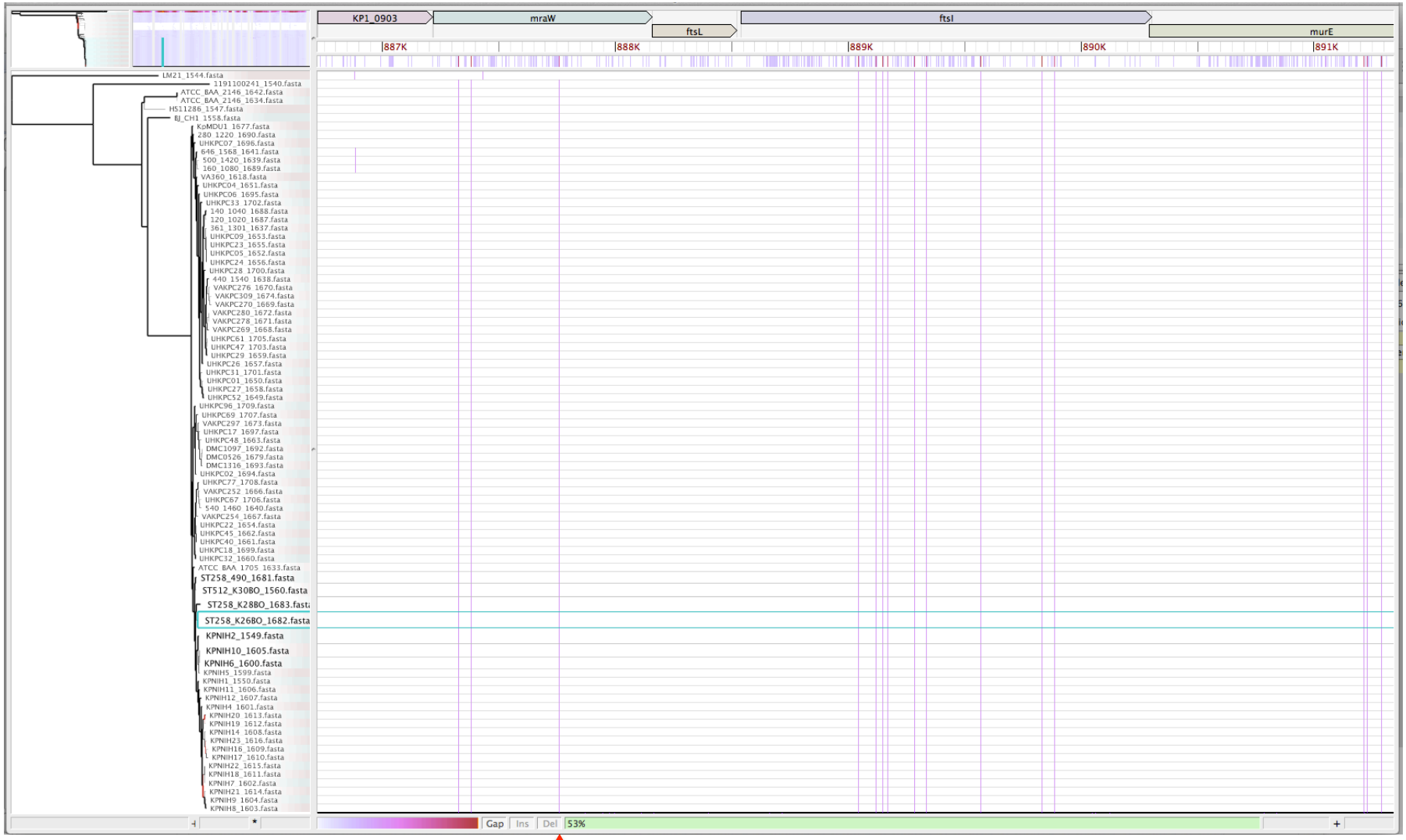
SNPs unique to each method



Gingr interactive viewer

159 *Klebsiella pneumoniae genomes*

At the gene level



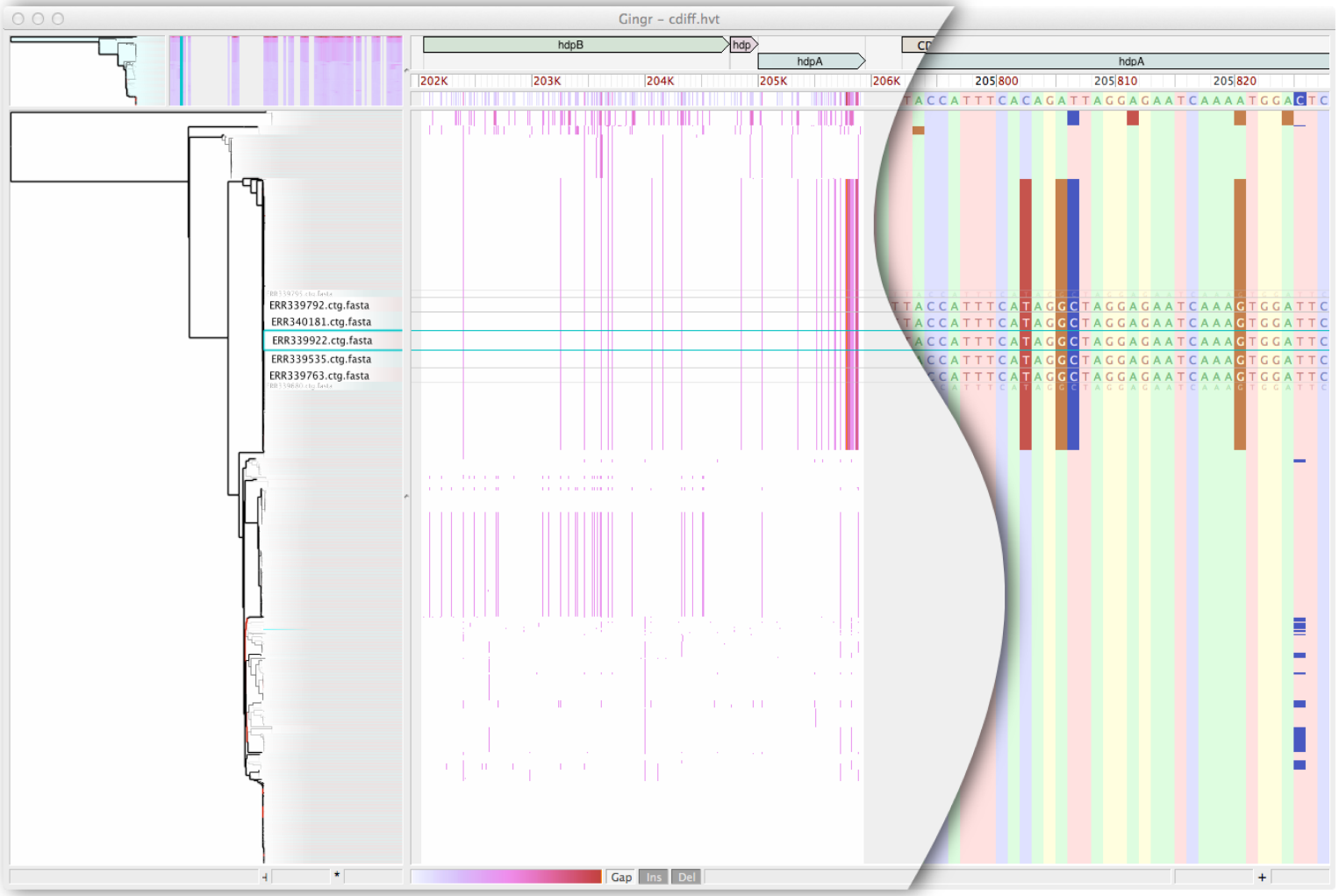
Inspecting individual SNPs (*mraW* gene)

P. difficile

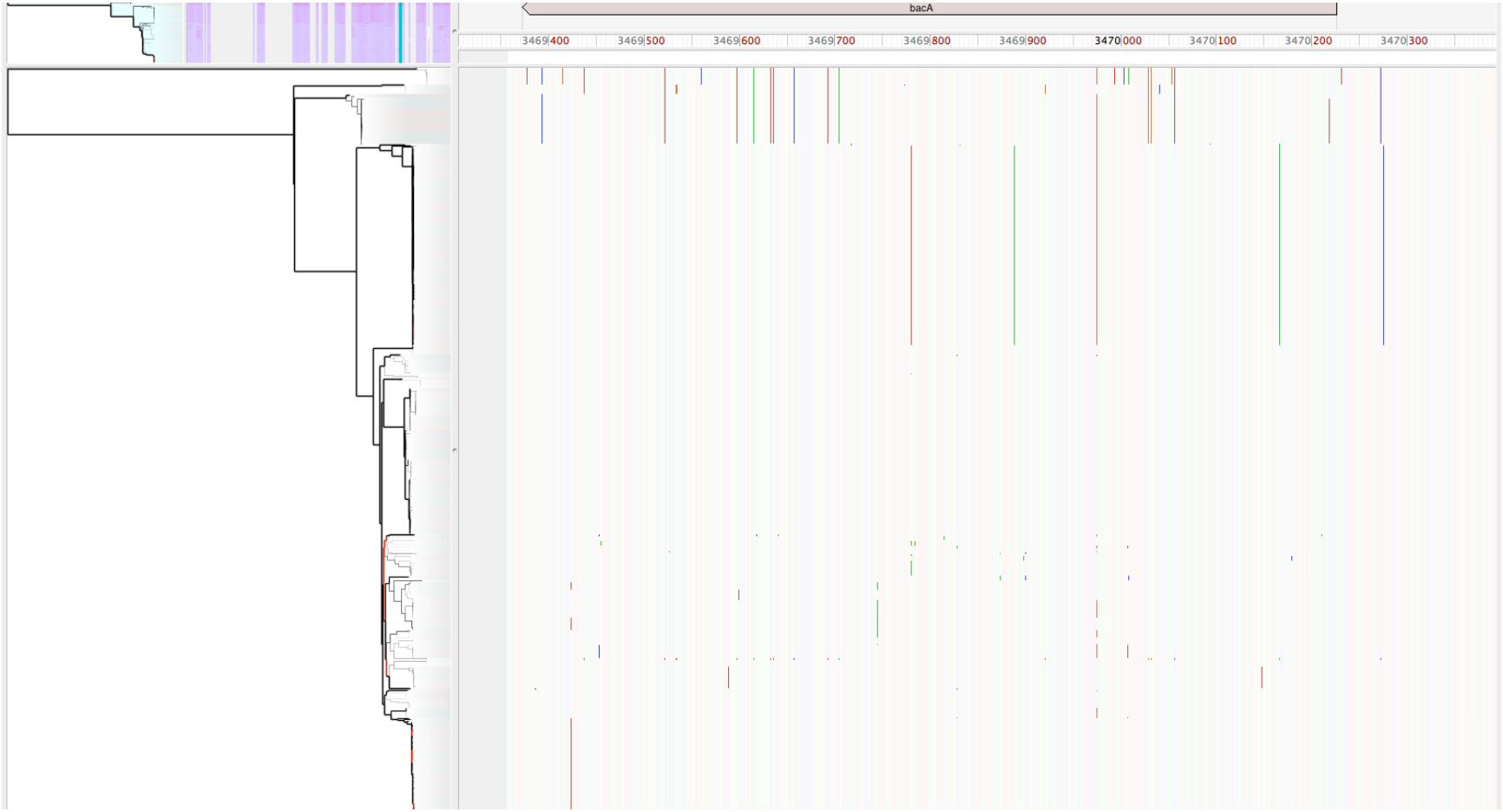
- ***Clostridium difficile → Peptoclostridium difficile***
  - ***Yitin et al, Environ Micro, 2013***

- **Study published in NEJM, downloaded sequencing data**
  - **Eyre et al NEJM, 2013**

Phylogeny, annotation & nucleotiodes



BacA gene conservation



Conclusions

① **iMetAMOS for automated assembly of MiSeq data, PBcR, PacBio data for closed genomes**

② **Align hundreds of closely-related microbial strains in *minutes***

- **32 *E. coli* genomes in 4 minutes on 8 cores**
- **200 *K. pneumoniae genomes in 20 minutes on 8 cores***
- ***800 P. dificile* genomes in 40 minutes on 32 cores**

③ **Interactive interface for simultaneous visualization of:**

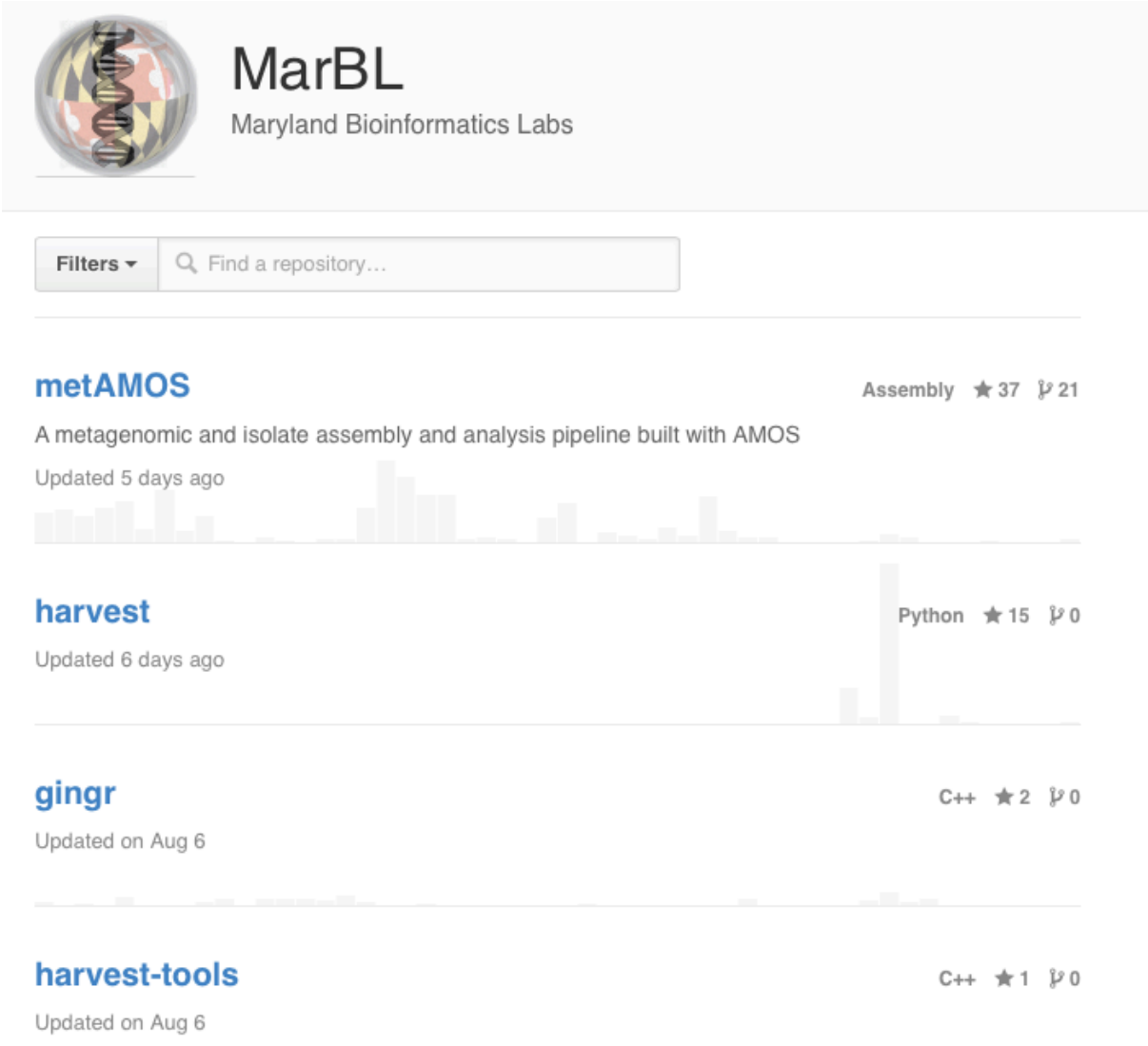
- ***core genomes***
- ***SNPs***
- ***clade phylogeny***

④ **Assembly + multiple alignment as a sustainable path to core genome SNP typing:**

- **825 genomes → 1500 GB (reads) → 3.3 GB (asms) → 0.13 GB (aln)**

Software download

- github.com/marbl



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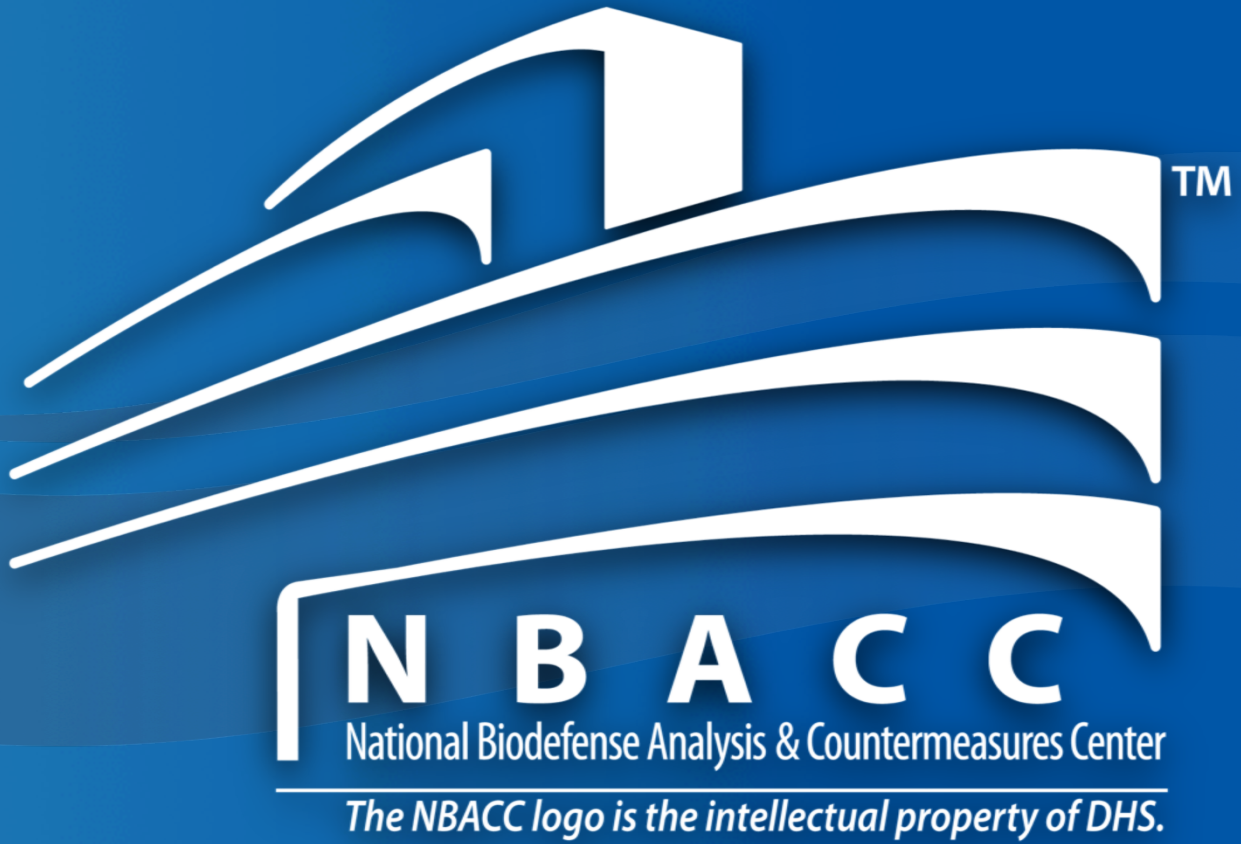
  - Adam Phillippy
  - Brian Ondov (→Gingr!)
  - Sergey Koren

- Centre for Public Health Research (CSSIP), Valencia, Spain
  - Iñaki Comas (MTB data)

Questions?



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