FRANCE GÉNOMIQUE

Institut Pasteur Genopole

“Finding variants in bacterial genomes: application to the analysis of epidemic or endemic clones”

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Objective

- Compare automatically a large number of genomes
- Minimize the number of false positive and false negative
- Minimize human curration of the data
- Visualization of the polymorphisms

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First generation pipeline – whole genome phylogeny

Objective: discovering the reason for the emergence of *Streptococcus agalactiae* neonatal infections in the 1960s

- **Sequencing of 230 isolates**
- **Use of different reference genome**
- **Filtering low quality reads**
- **Aligner: BWA**
- **SNA calling: SAMtools MPILEUP and varFilter**
- **Velvet assembly of the 230 genomes**

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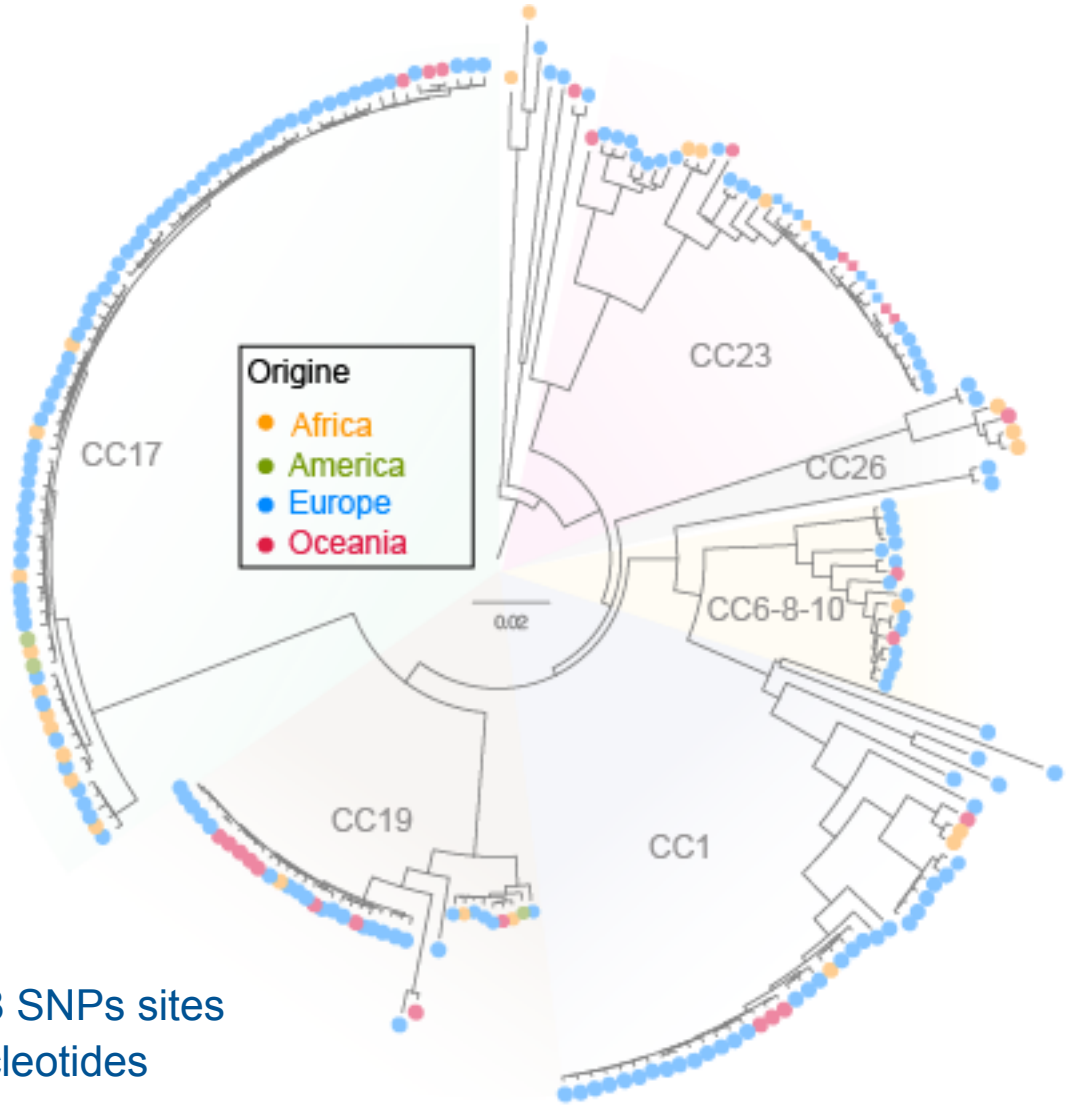
Phylogeny of *Streptococcus agalactiae* isolates



Tree based on 40,898 SNPs sites
among 1,384,073 nucleotides

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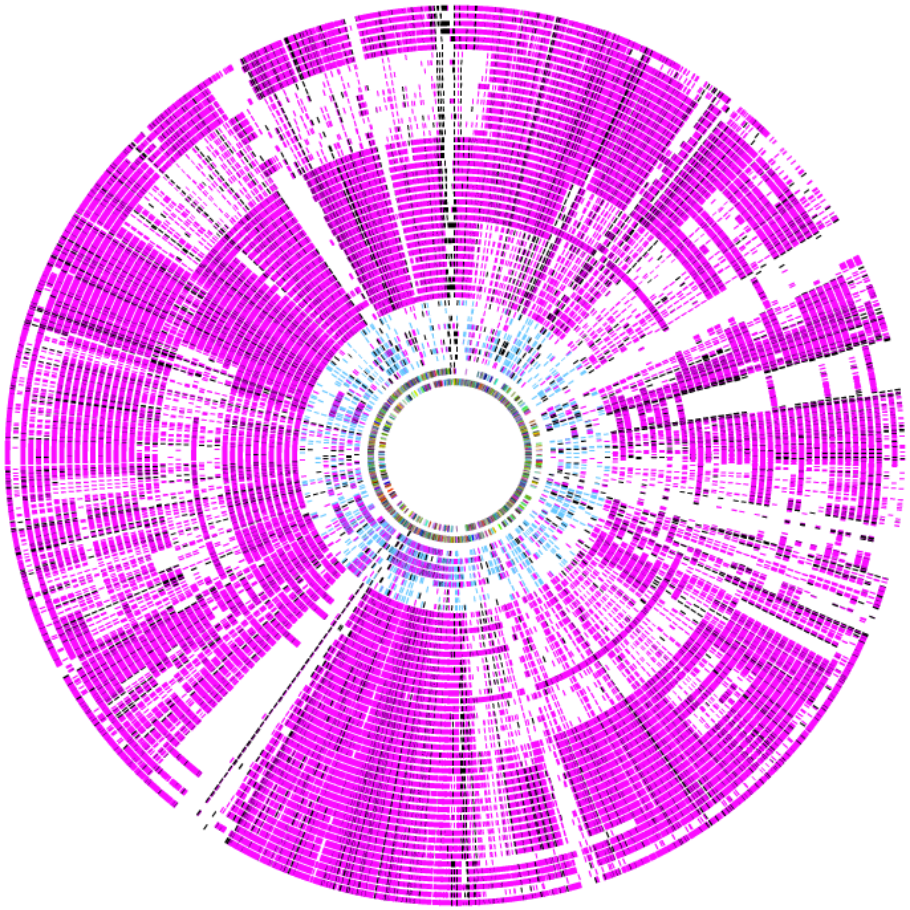
SNP distribution in 63 representative isolates



⇒ All parts of the genome have recombined

⇒ A true phylogeny of the species is not feasible

⇒ Analysis of the 6 CC with specific reference genomes

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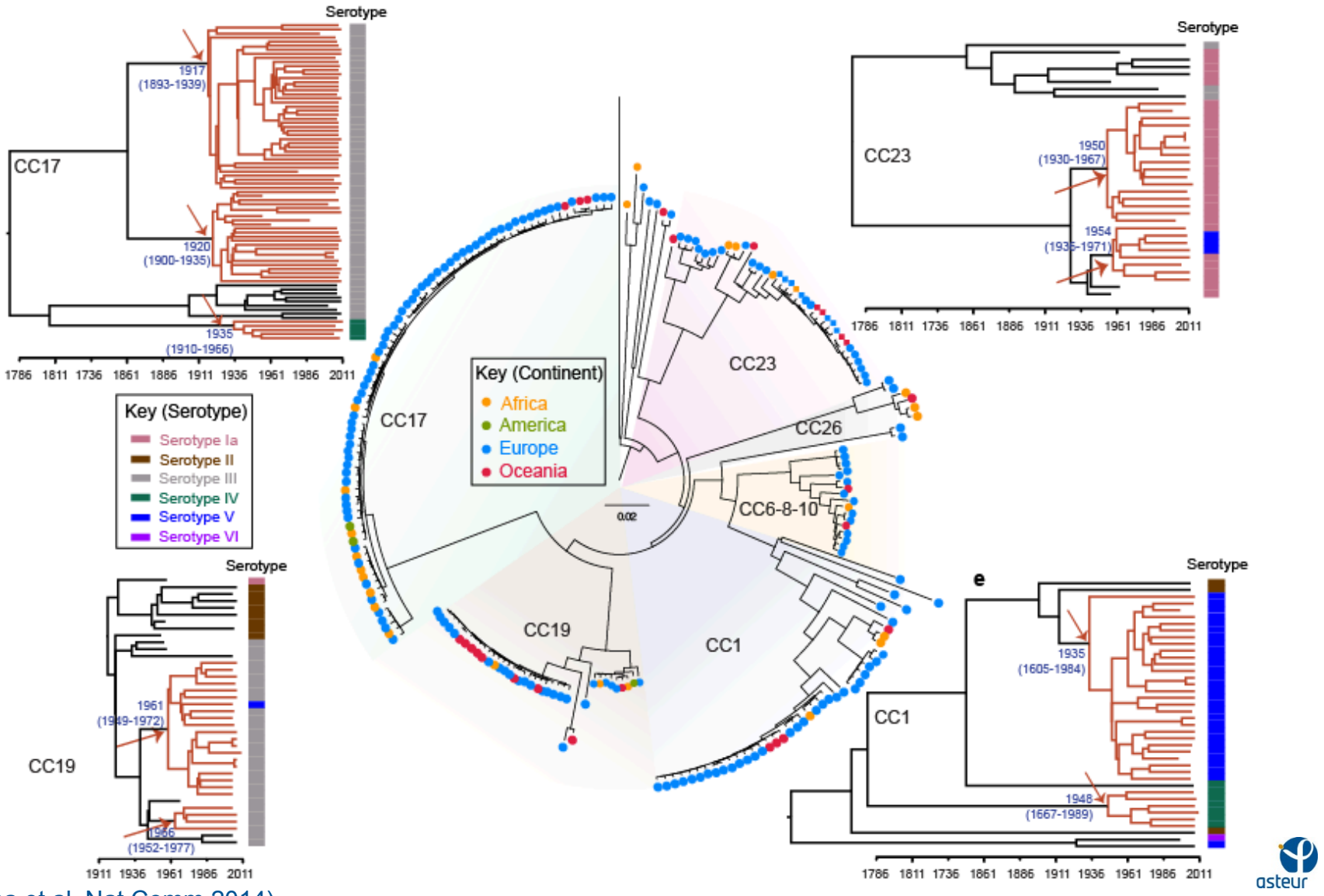
Understanding the emergence of *S. agalactiae* neonatal infections



Da Cunha et al. Nat Comm 2014)

Variant calling workflow

Comparing every strain to a reference sequence :


```
@HISEQ:376:C3JL4ACXX:2:1101:3736:2085 1:N:0:CGTACG
AAGANCTCCAGAGCCTTACAAAGGTAAAGGTATTCGTTACCAAGGTGAATACGTTCGCCGTAAAGAAGGGAAAACTGGTAAATAATAGATAAACTCTAAAG
+
@@@B#2=BFBH<FDGIGEH@E>GECFGEIG*?CEFHFHEIDCHGGDDFGGHHGDFEGGIIGB82=7>@B'.;?CCACCC@A:@>AA>CCD@3,>CCC@###

31 31 31 33 2 17 28 33 37 […] 29 32 32 29 34 34 35 31 18 11 29 34 34 34 31 2 2 2
```

*Criscuolo A, Brisse S : **AlienTrimmer : A tool to quickly and accurately trim off multiple short contaminant sequences from high-throughput sequencing reads.** *Genomics* 102 (2013) 500-506

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+
@@@B#2=BFBH<FDGIGEH@E>GECFGEIG*?CEFHFHEIDCHGGDDFGGHHGDFEGGIIGB82=7>@B'.;?CCACCC@A:@>AA>CCD@3,>CCC@###

31 31 31 33 2 17 28 33 37 […] 29 32 32 29 34 34 35 31 18 11 29 34 34 34 31 2 2 2
```

```
@HISEQ:376:C3JL4ACXX:2:1101:3736:2085 1:N:0:CGTACG
AAGANCTCCAGAGCCTTACAAAGGTAAAGGTATTCGTTACCAAGGTGAATACGTTCGCCGTAAAGAAGGGAAAACTGGTAAATAATAGATA
+
@@@B#2=BFBH<FDGIGEH@E>GECFGEIG*?CEFHFHEIDCHGGDDFGGHHGDFEGGIIGB82=7>@B'.;?CCACCC@A:@>AA>CCD@
```

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Variant calling workflow

Comparing every strain to a reference sequence :

*Li H : **Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM.** *arXiv:1303.3997v2*

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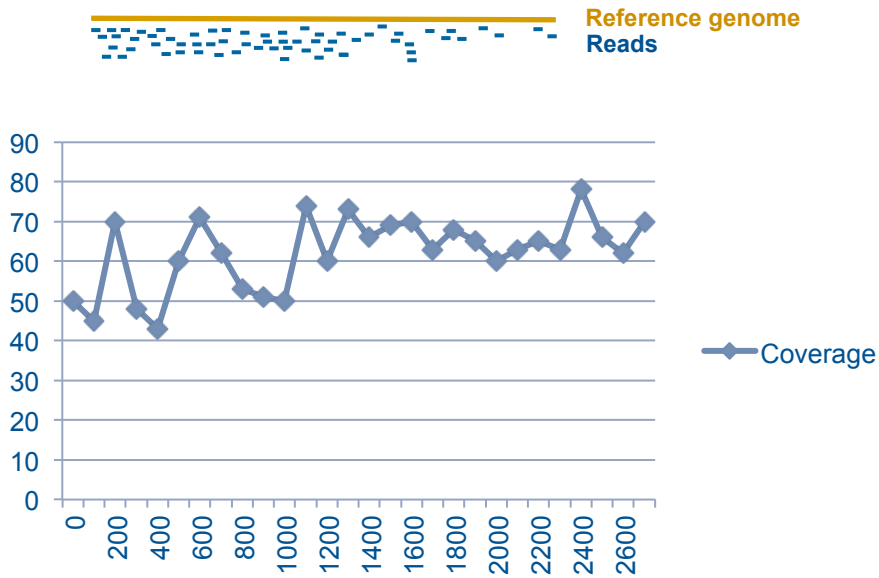
Variant calling workflow

- **Comparing every strain to a reference sequence :**



*Li H : **Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM.** *arXiv:1303.3997v2*

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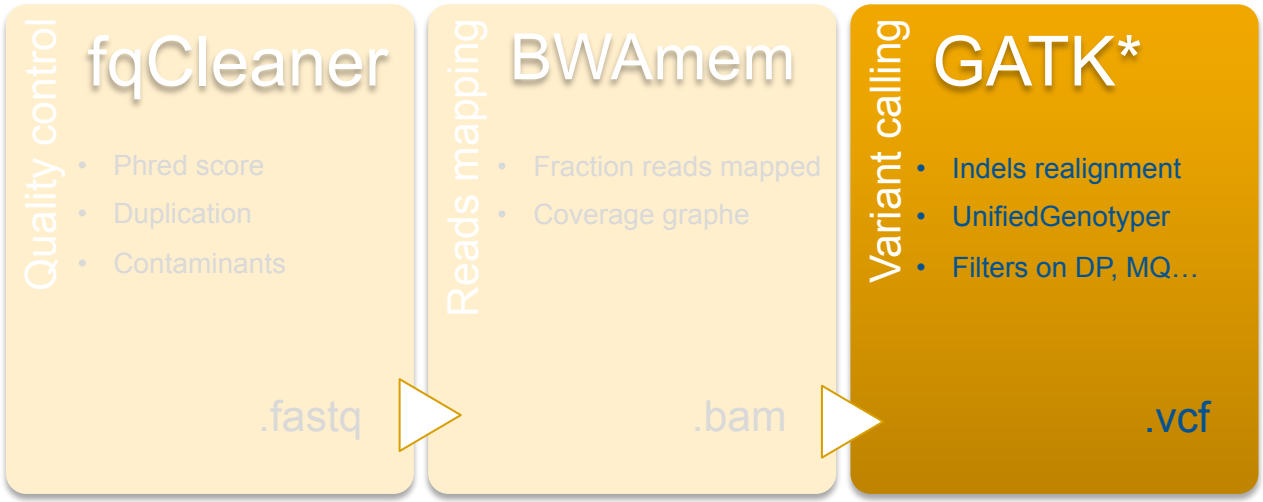
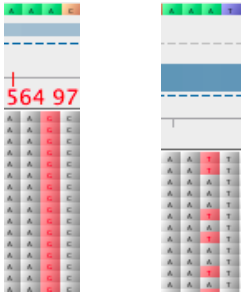
Variant calling workflow

Comparing every strain to a reference sequence :





Are these positions SNPs ?

- Reads coverage
- Strand bias
- Mapping quality
- Found in another strain
- …

*DePristo M, *et. al* : **A framework for variation discovery and genotyping using next-generation DNA sequencing data.** *Nature Genetics* (2011) **43**:491-498

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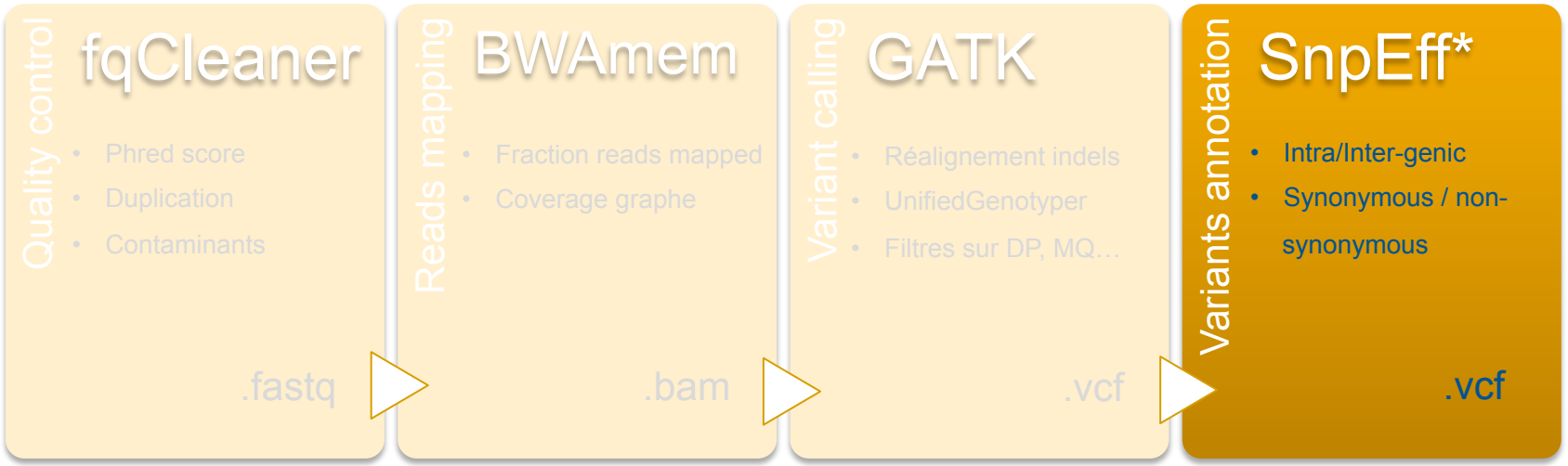
Variant calling workflow

- **Comparing every strain to a reference sequence :**



*Cingolani P, *et. al* : **A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of Drosophila melanogaster strain w1118; iso-2; iso-3.** *Fly* 2012 Apr-Jun;**6**(2):80-92

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Validation of the pipeline on 512 *bacterial isolates*

Sequenced at the Genopole (PF1)

- 67 strains from the French NRC (1x100bp, Hiseq 2000)
- 6 old (~10-15yo) american strains (1x150bp, Miseq)

HiSeq 2000

From the Short Reads Archive database

- 403 american strains*, (2x100bp, Hiseq 2000)
- 36 american strains**, (2x100bp Genome Analyzer IIx)

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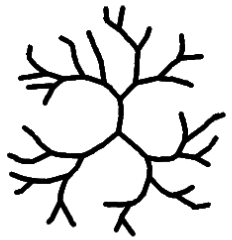
Building a phylogeny

- **>14000 polymorphic positions :**
  - **~ 50-100 SNPs for most strains**
  - **~ 200-700 SNPs for some american strains**
  - **2200 SNPs for the outgroup**

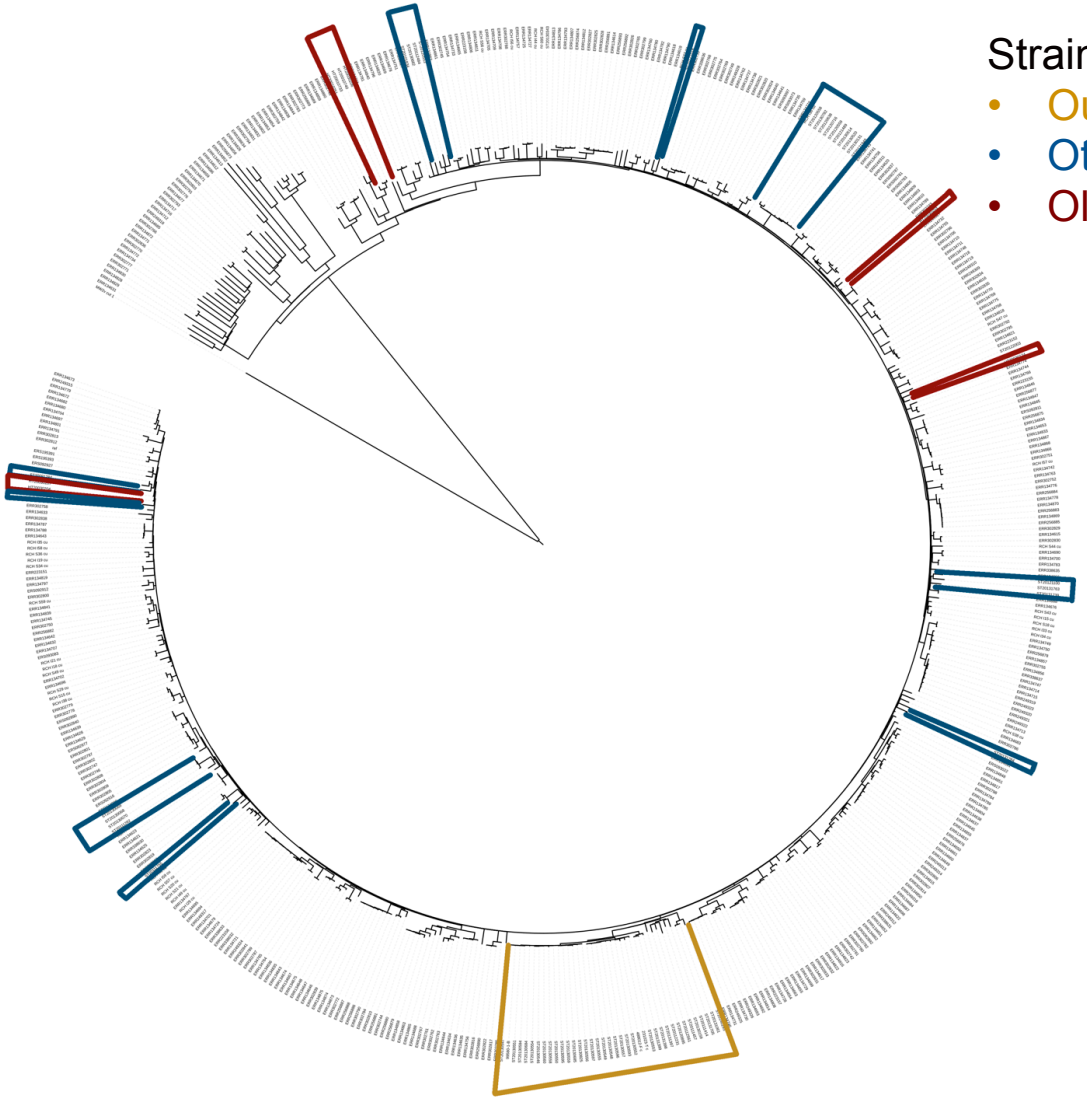
- **Additionnal filters :**
  - Known plasmids and repeated regions were removed
  - Cluster of isolates are remove - In one isolate, SNPs must be distant from each other (e. g. 100 bp)

- **Phylogeny was built using PhyML***



*Guindon S, *et. al* : **New Algorithms and Methods to Estimate Maximum-Likelihood Phylogenies: Assessing the Performance of PhyML 3.0.** *Systematic Biology*, **59**(3):307-21, 2010.

All strains phylogeny



Strains sequenced at IP

- Outbreak
- Other recent french cases
- Old american cases

All strains phylogeny

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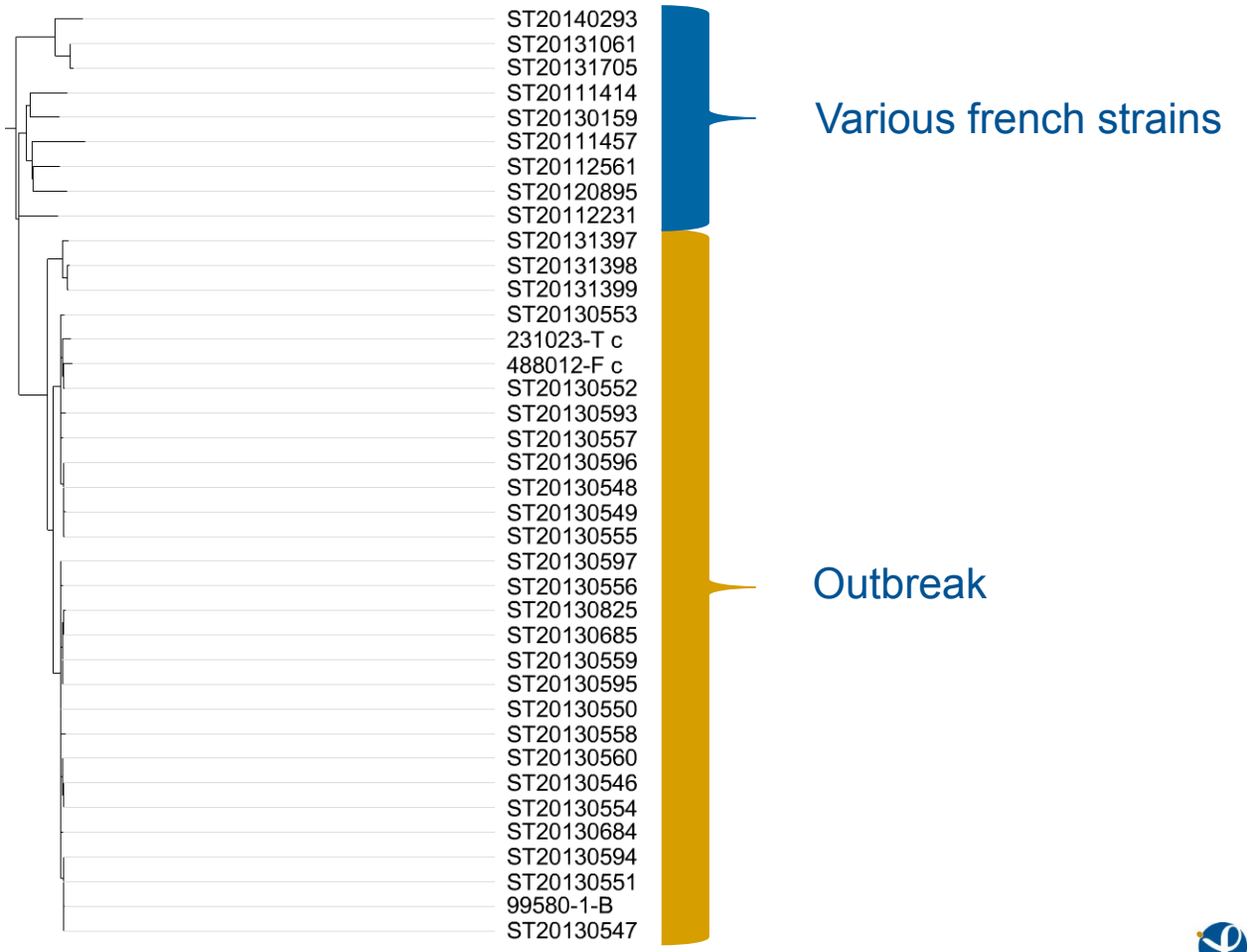
French subtrees

The hospital outbreak strains form a monophyletic group



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In vivo evolution

- **Objective comparing S. agalactiae strains isolated from the infantand from their mother.**
- **Sequencing of 47 isolates from 19 pairs (mother / infant)**
- **Disease or carriage associated isolates**
- **Identify polymorphisms linked to the transition from comensal to pathogen**

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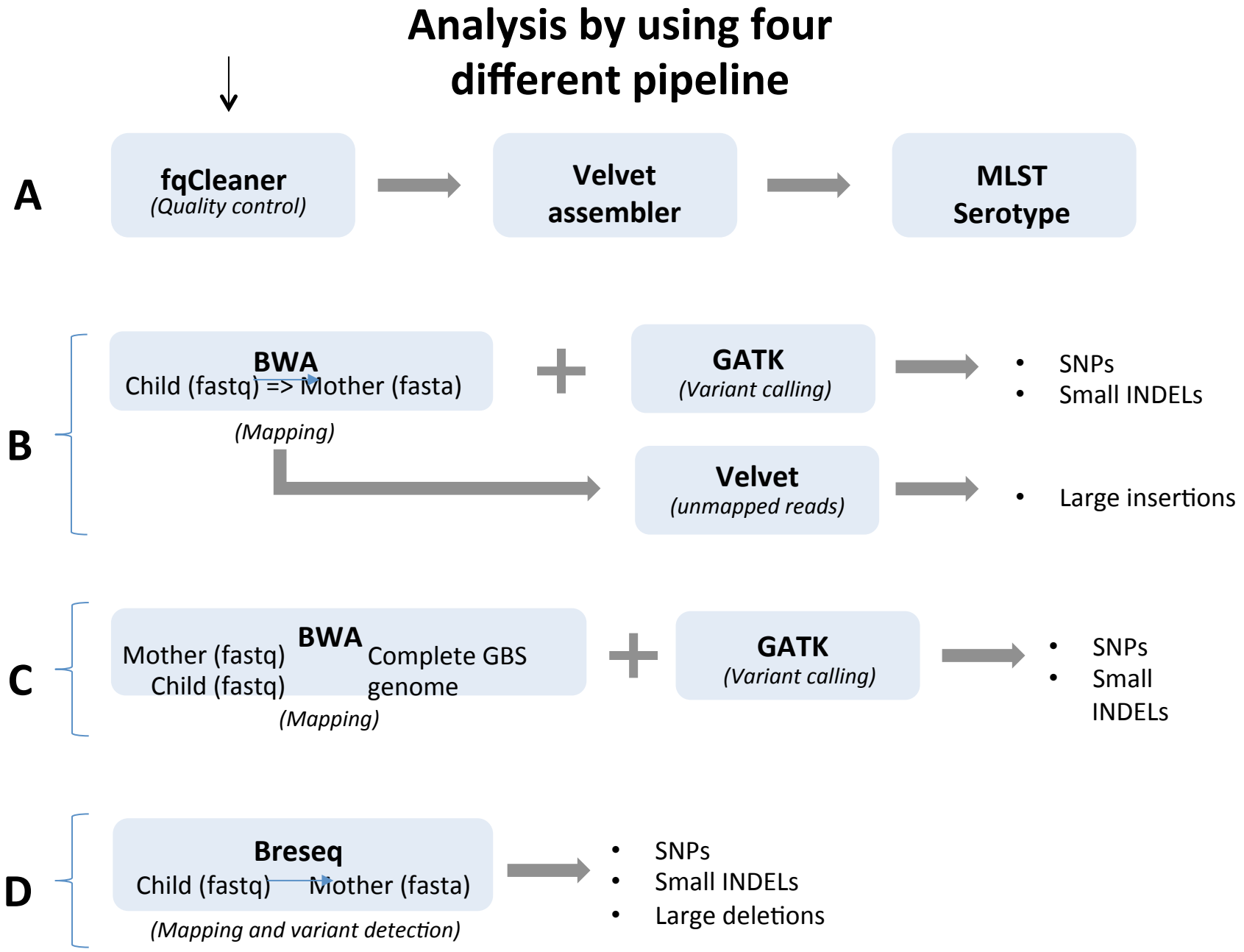
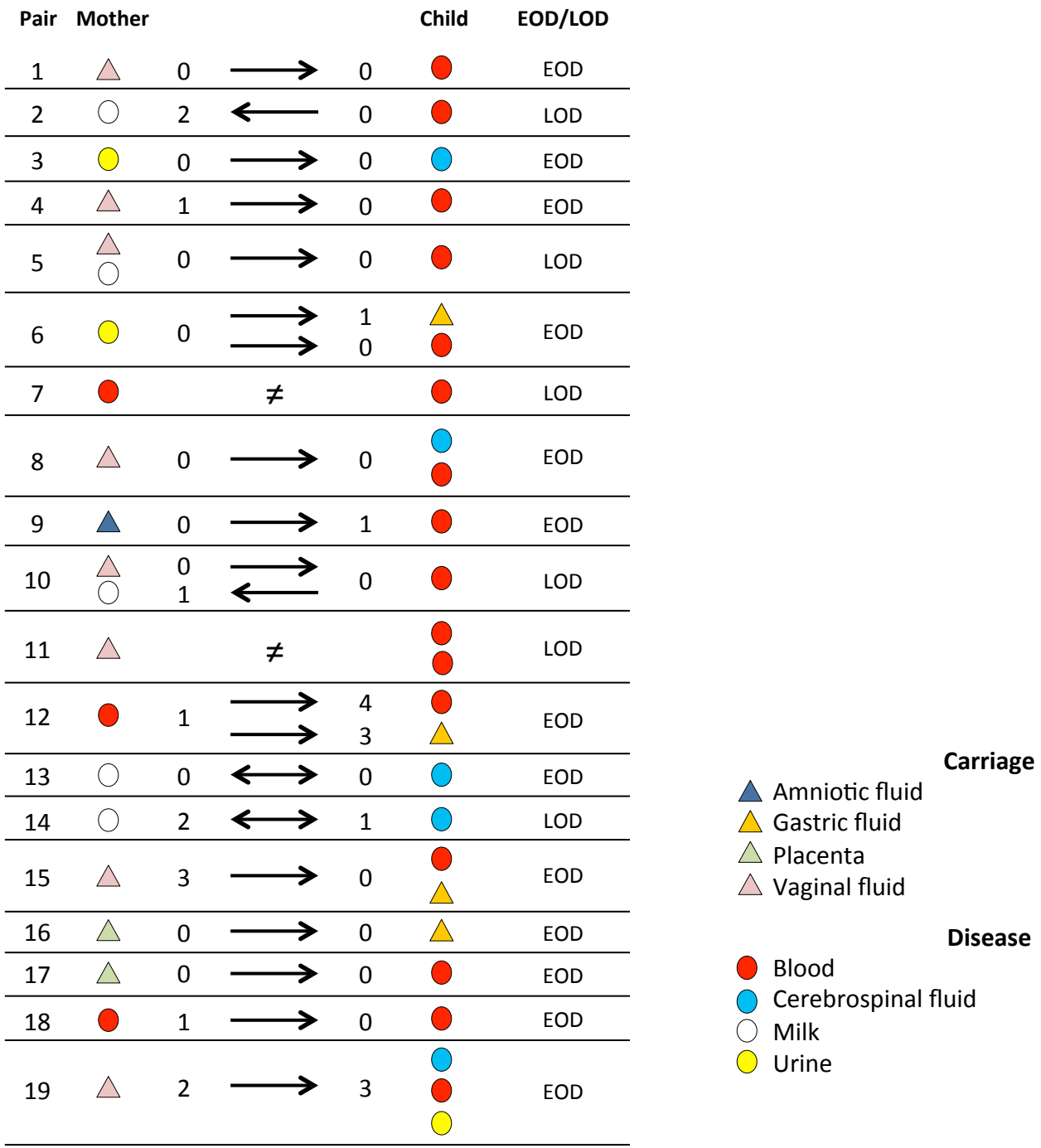

Analysis by using four different pipeline
A
fqCleaner
(Quality control)
Velvet assembler
MLST Serotype
B
BWA
Child (fastq) => Mother (fasta)
(Mapping)
GATK
(Variant calling)
SNPs
Small INDELs
Velvet
(unmapped reads)
Large insertions
C
Mother (fastq)
Child (fastq)
BWA
Complete GBS genome
(Mapping)
GATK
(Variant calling)
SNPs
Small INDELs
D
Breseq
Child (fastq) Mother (fasta)
(Mapping and variant detection)
SNPs
Small INDELs
Large deletions


Pair
Mother
Child
EOD/LOD
1
0
0
EOD
2
2
0
LOD
3
0
0
EOD
4
1
0
EOD
5
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LOD
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EOD
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0
EOD
17
0
0
EOD
18
1
0
EOD
19
2
3
EOD
Carriage
Amniotic fluid
Gastric fluid
Placenta
Vaginal fluid
Disease
Blood
Cerebrospinal fluid
Milk
Urine

Visualisation with SynTView

SynTView*

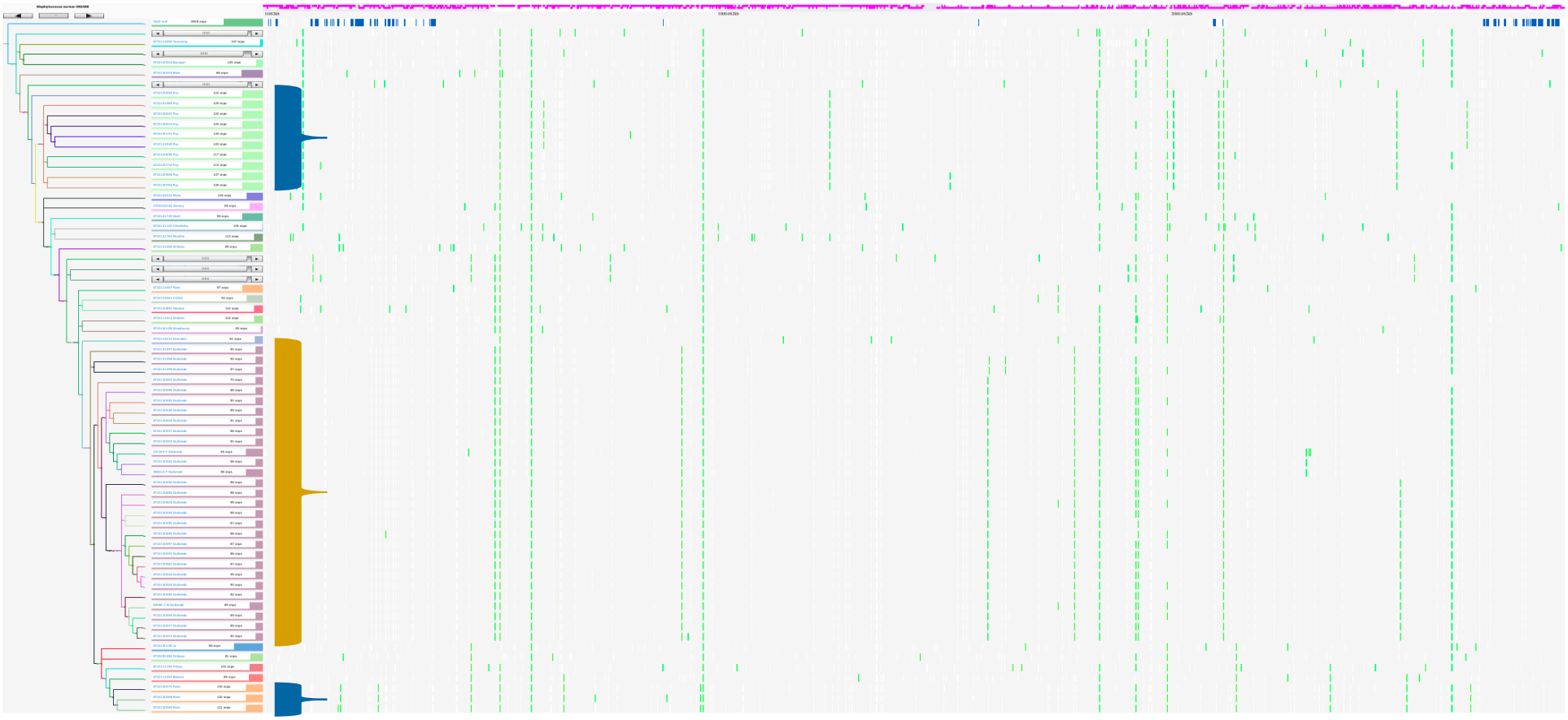
- Genome browser with multiple view (synteny, circular view, SNPs…)
- Polymorphisms and short indels visualisation for n strains versus a reference chromosome
- Integration of metadata : virulence, date of isolation…
- Ordering of strains with a phylogenetic tree
- genopole.pasteur.fr/SynTView/

SynTView 2

- Developped in collaboration with Genostar (Wallgene)
- Reference as contigs is now supported
- Better interface

*Lechat P, Souche E, Moszer I **: SynTView — an interactive multi-view genome browser for next-generation comparative microorganism genomics.** *BMC Bioinformatics* 2013, **14**:277

Analysing French strains in SynTView



Insertions and deletions

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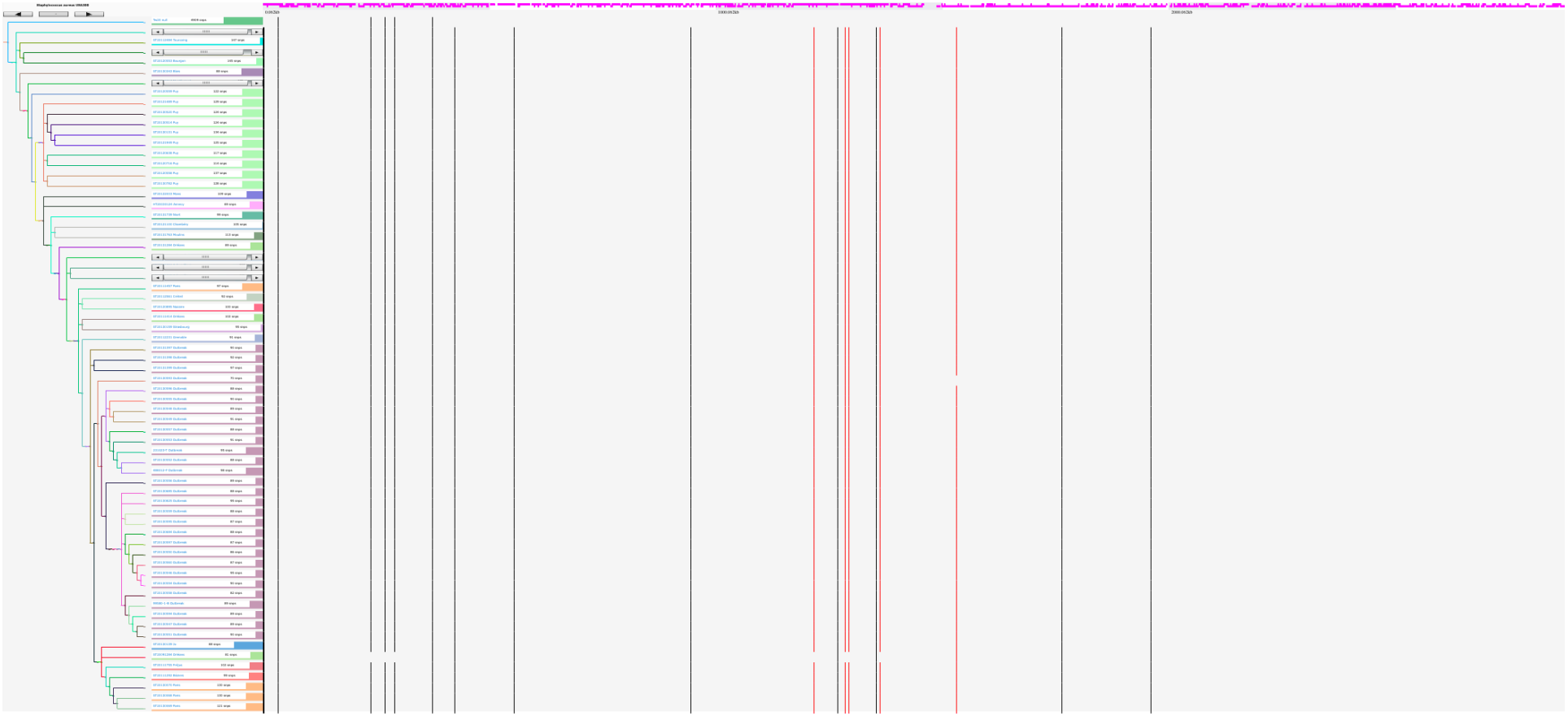
Analysing French strains in SynTView



SNPs shared by at least 67 strains

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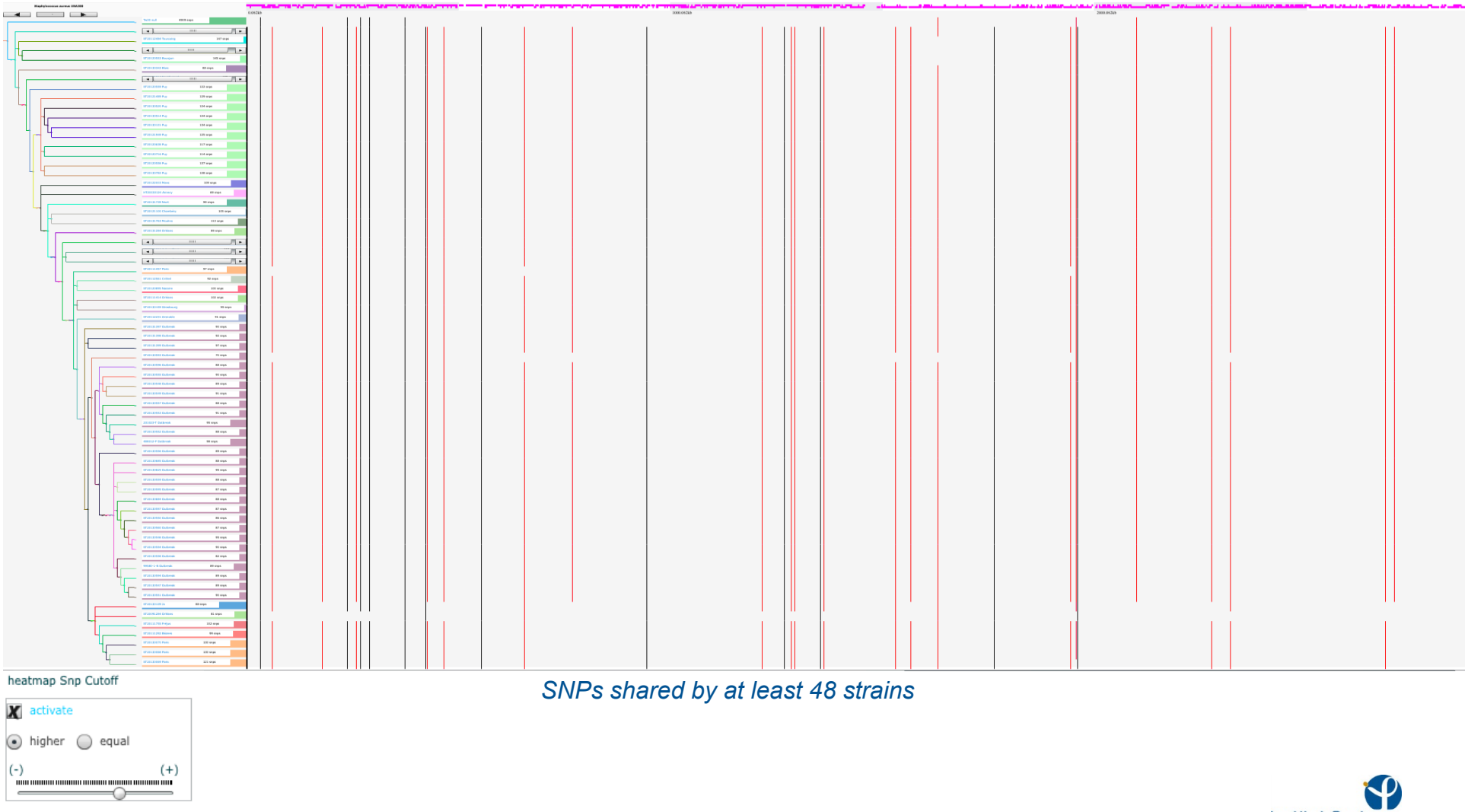
Analysing French strains in SynTView



SNPs shared by at least 48 strains

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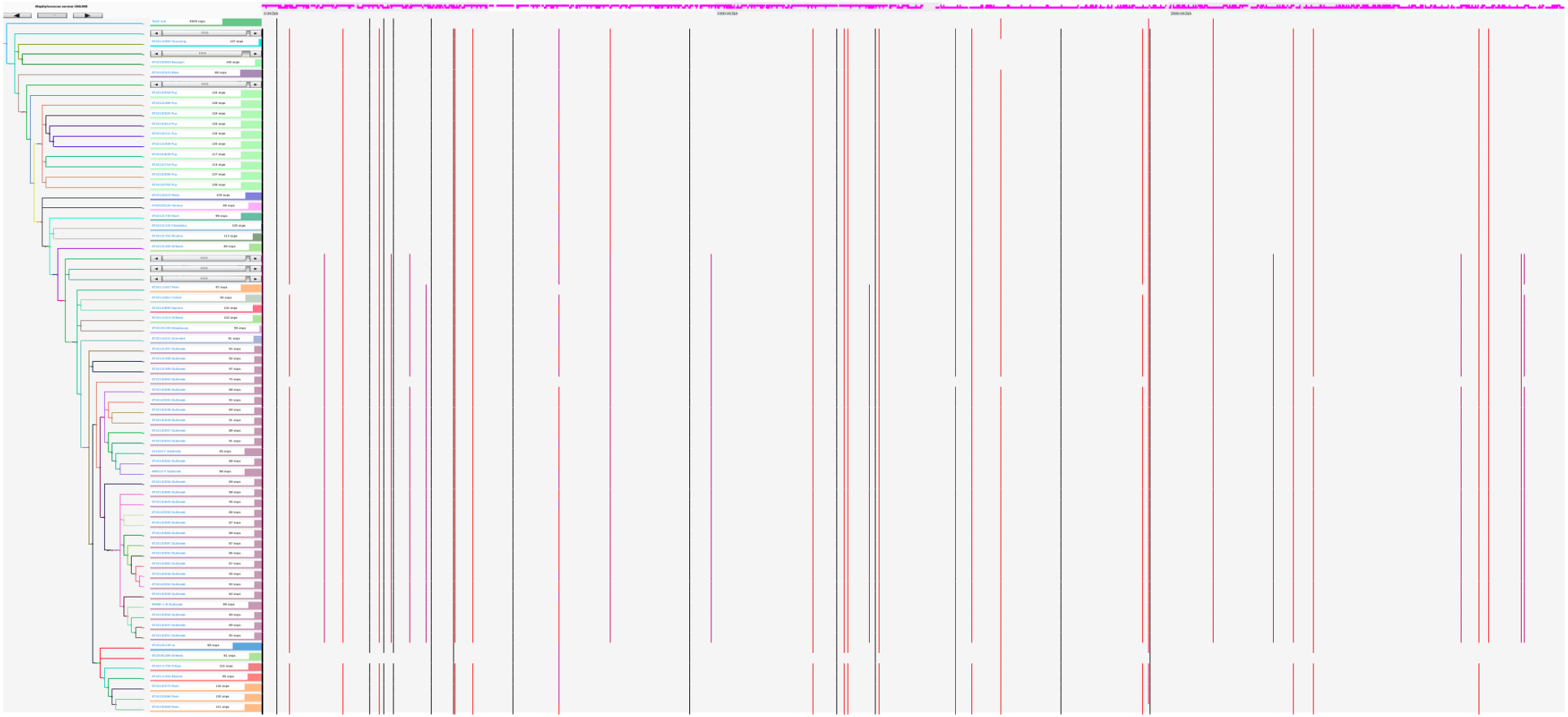
Analysing French strains in SynTView



heatmap Snp Cutoff

activate

higher equal

(-) (+)

SNPs shared by at least 32 strains

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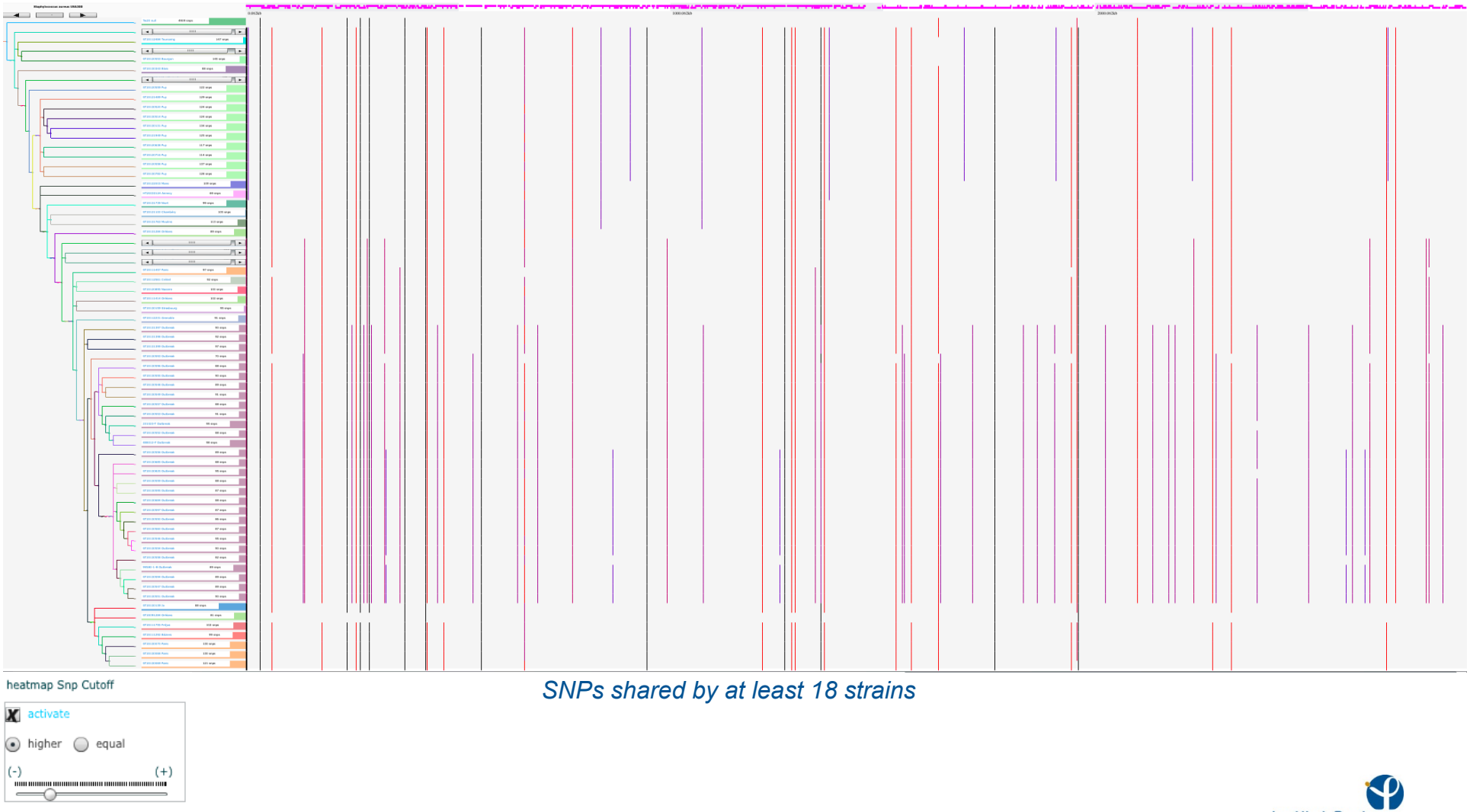
Analysing French strains in SynTView



SNPs shared by at least 18 strains

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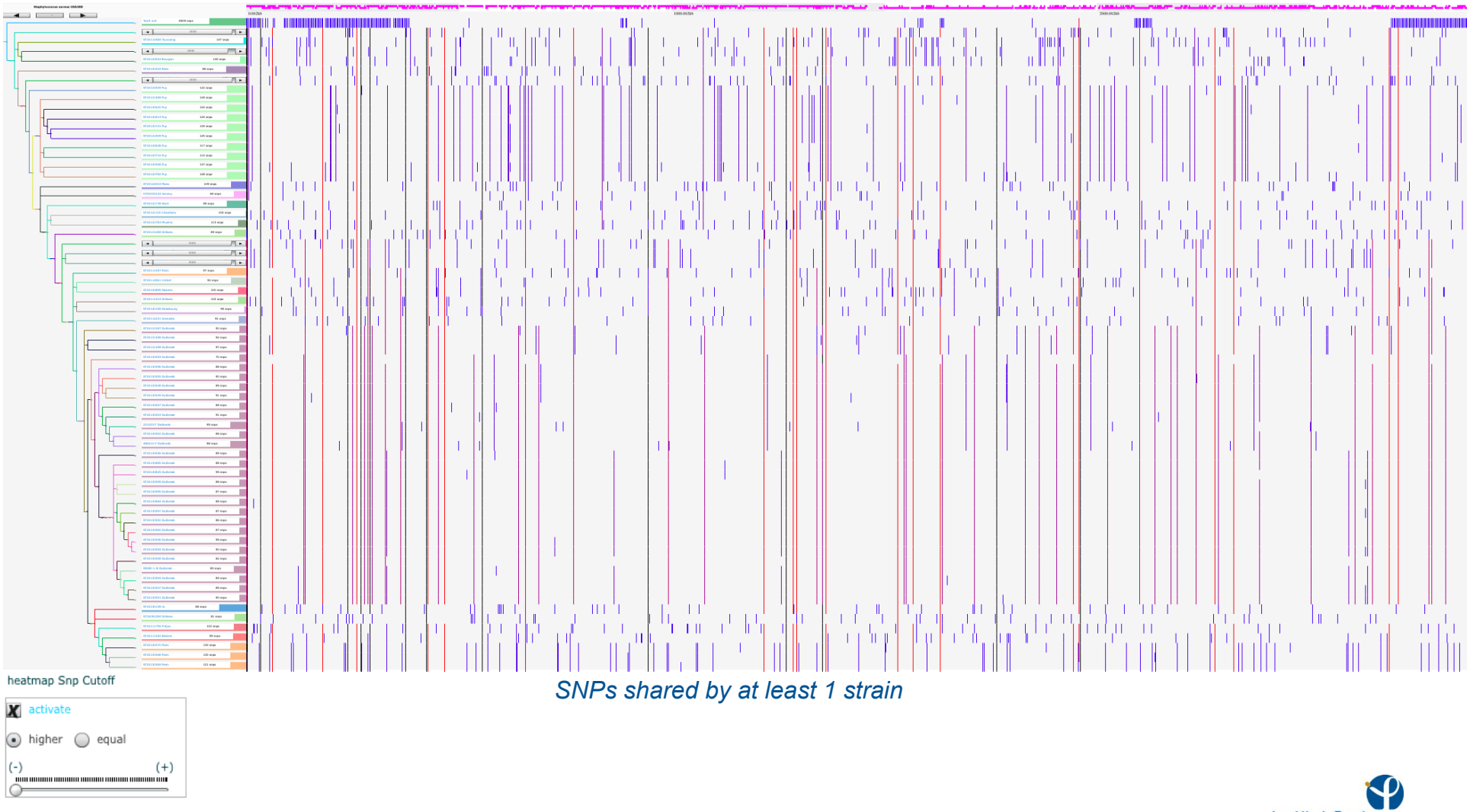
Analysing French strains in SynTView



SNPs shared by at least 1 strain

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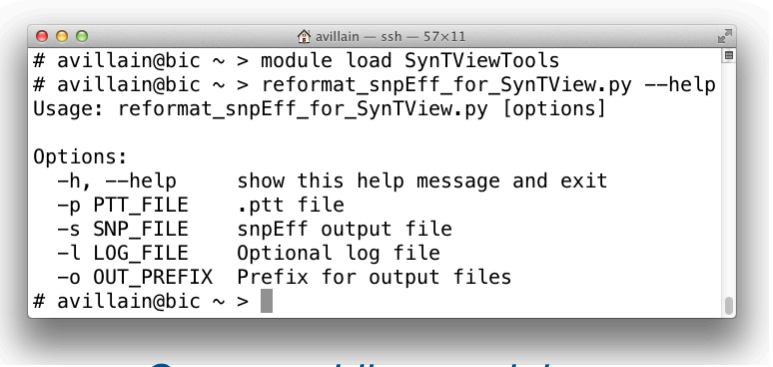
Set up a SynTView website

Conversion to SynTView formats :

- Reduce the size of files loaded on the site
- Easy to use scripts available :
  - **On central-bio or bic@pasteur.fr**



Command-line module

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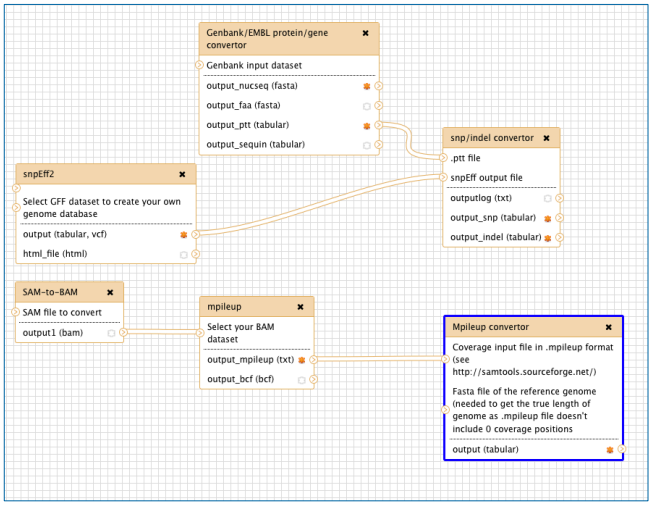

Galaxy workflow

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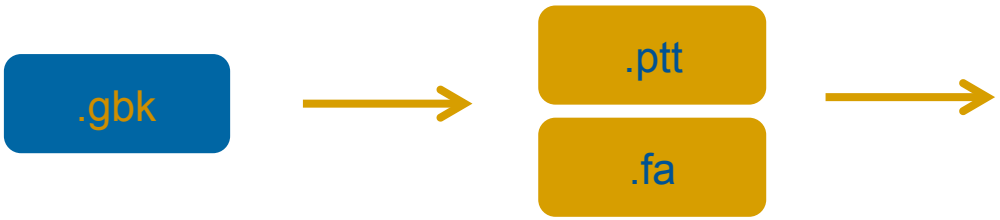
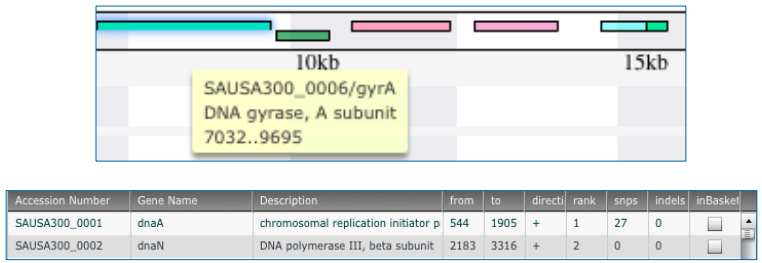
Steps :

- Get coding sequences information





ORFs and genes list

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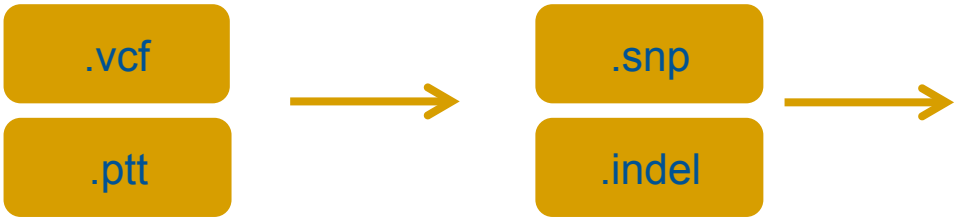
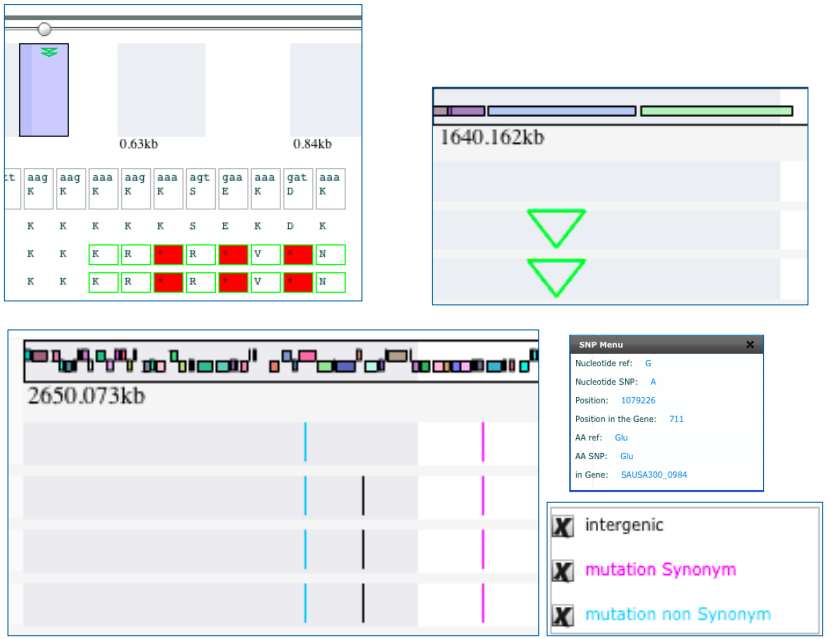
Steps :

- Get coding sequences information
- Convert variant file





SNPs and short indels visualisation

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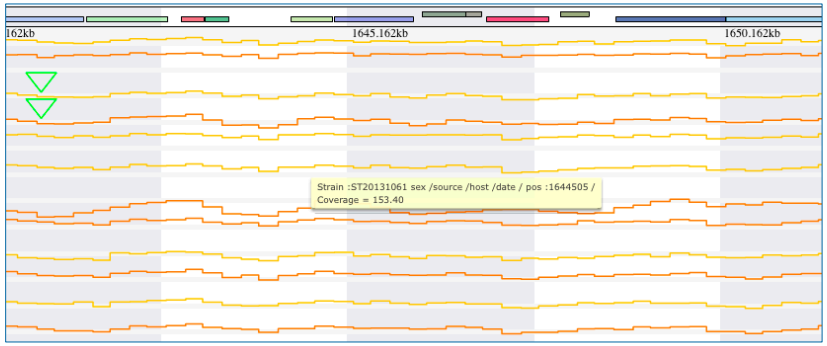
  - **In galaxy@pasteur.fr toolshed**

Steps :

- Get coding sequences information
- Convert variant file
- Add coverage track

.mpileup → .coverage →



Coverage along genome

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Conclusion and perspective

- The pipeline has been validated on the 500 bacterial isolates
- SynTView is particularly suited for the exploration of results
- Workflows for SNPs detection and results integration into SynTView are available on the servers and Galaxy
- Current work on detection of large indels and CNVs
- Implementation of tools for detecting recombination events and homoplasy.
- SynTView 2 is in active development and constantly improving

Thank you for your attention

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