Bioinfomics days : introduction to variation graphs

Benjamin Linard

Introduction to variation graphs (VG)

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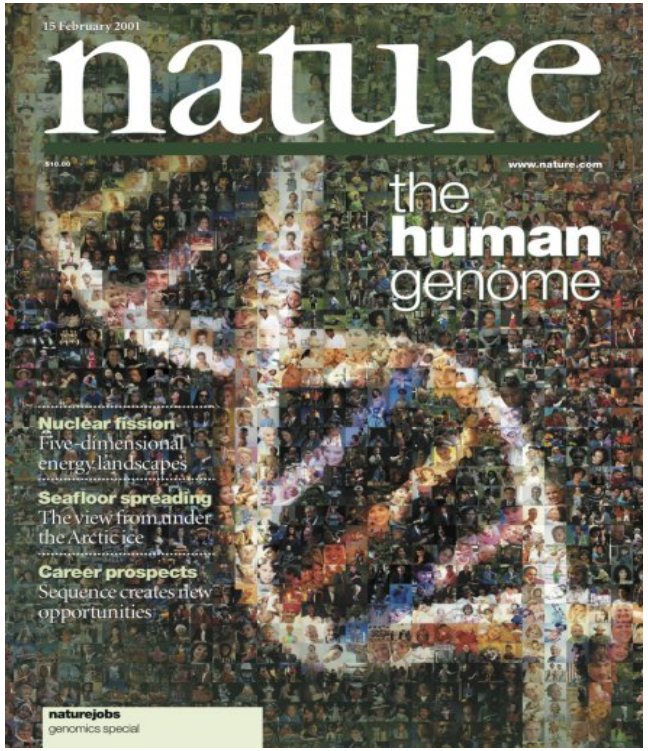
I. Context



2001

1st « complete » genome

2010

First overview of genome variations diversity

2023

Paradigm shift
Analysis contextualised with full known genome diversity

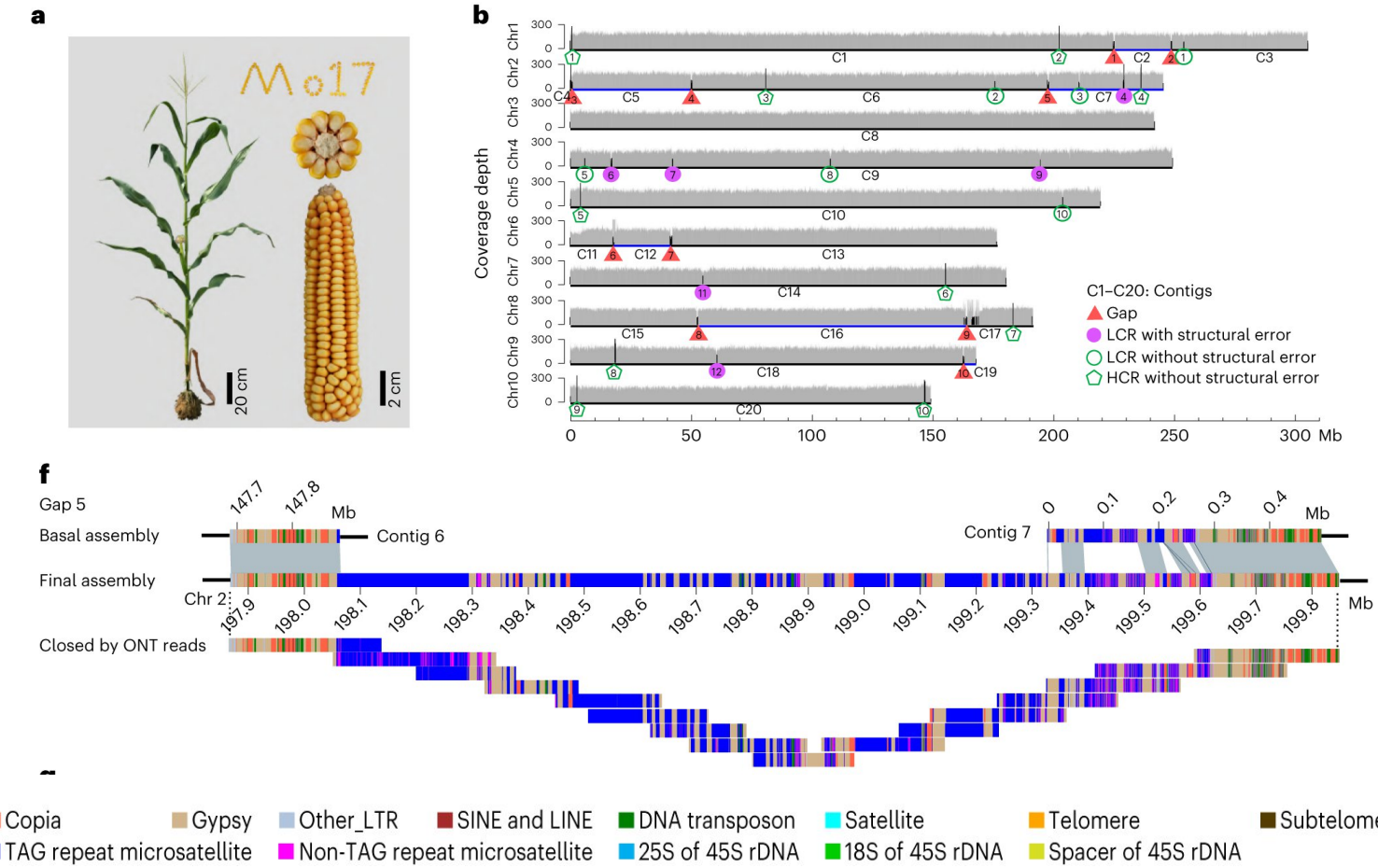
Why now ?

Recent advances
in genome sequencing.

- Many long read technologies (Hi-Fi, ONT, HiFi, 10x)
- Hi-C : 3D data improves long-distance scaffolding
- Efficient hybrid assemblers: Hifiasm, CANU, 3DDNA …

Haplotype-resolved, telomere to telomere, full genome assemblies !

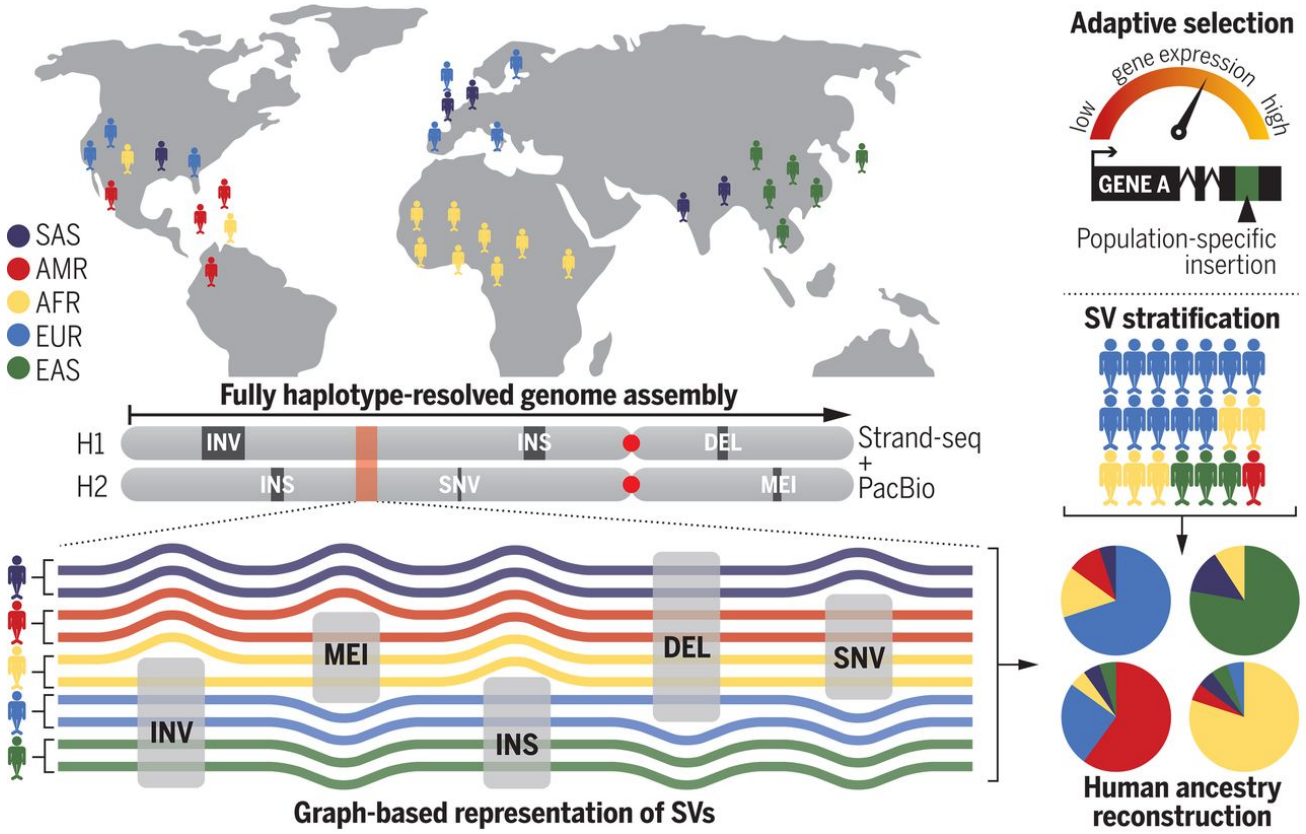
Chen et al, june 2023.
"The 2,178.6 Mb T2T Mo17 genome with a base accuracy of over 99.99% unveiled the structural features of all repetitive regions of the genome."



I. Context

Paradigm change : genomic analyses can be contextualised with all known genome diversity

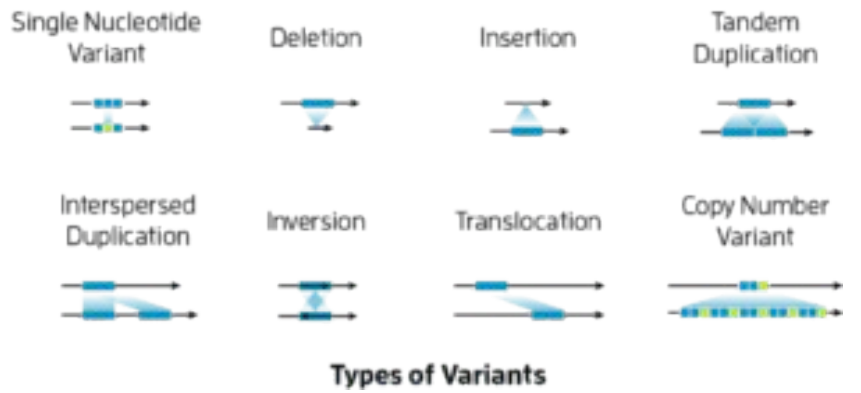
- *"The current version of the reference genome (GRCh38) is estimated to miss up to 10% of our species genetic information" (SS Sherman RM, 2020)*



P Ebert et al. *Science*, 2021

I. Context : structural variations (SV)

- **A major step:**
  better access to genome
  Structural variants (SV)

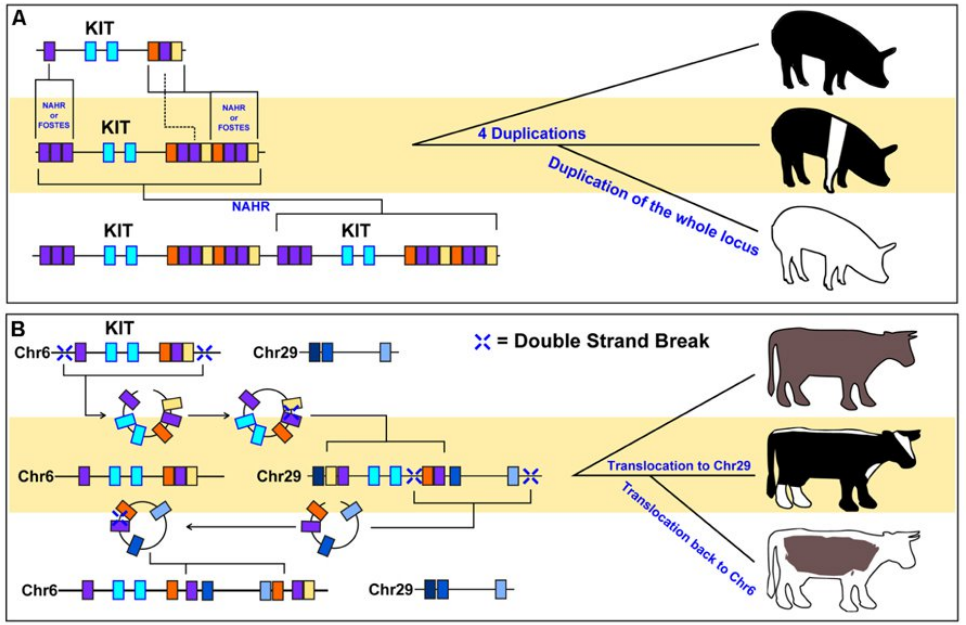


- Unexplored impact of SVs

Dosage effects

Positionnal effects

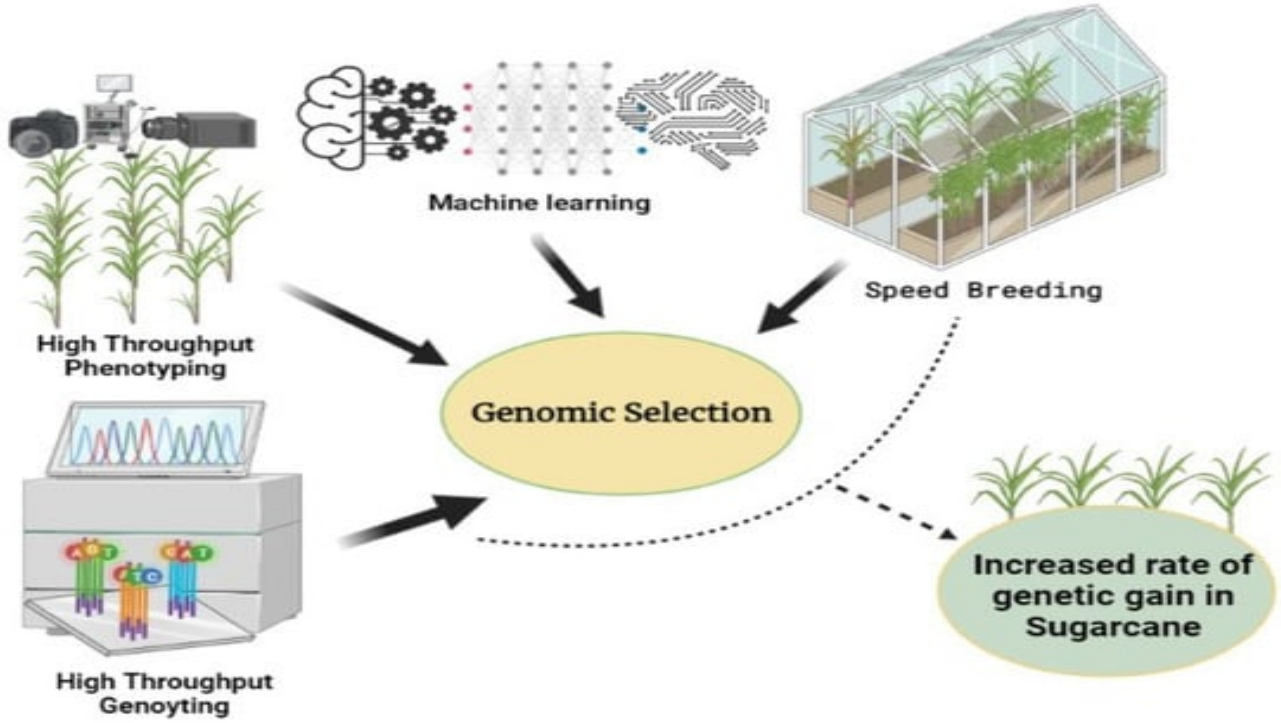
Disruptions: Gene or regulation



Bickhart DM & Liu GE, 2014

I. Context : agronomy and selection

- **New context for data generation**
  - HiFi & Hi-C technologies
  - « telomere to telomere » genomes
  - Assembly is becoming a routine step
- **New opportunities for genetics**



Sandhu et al, 2022. Plants

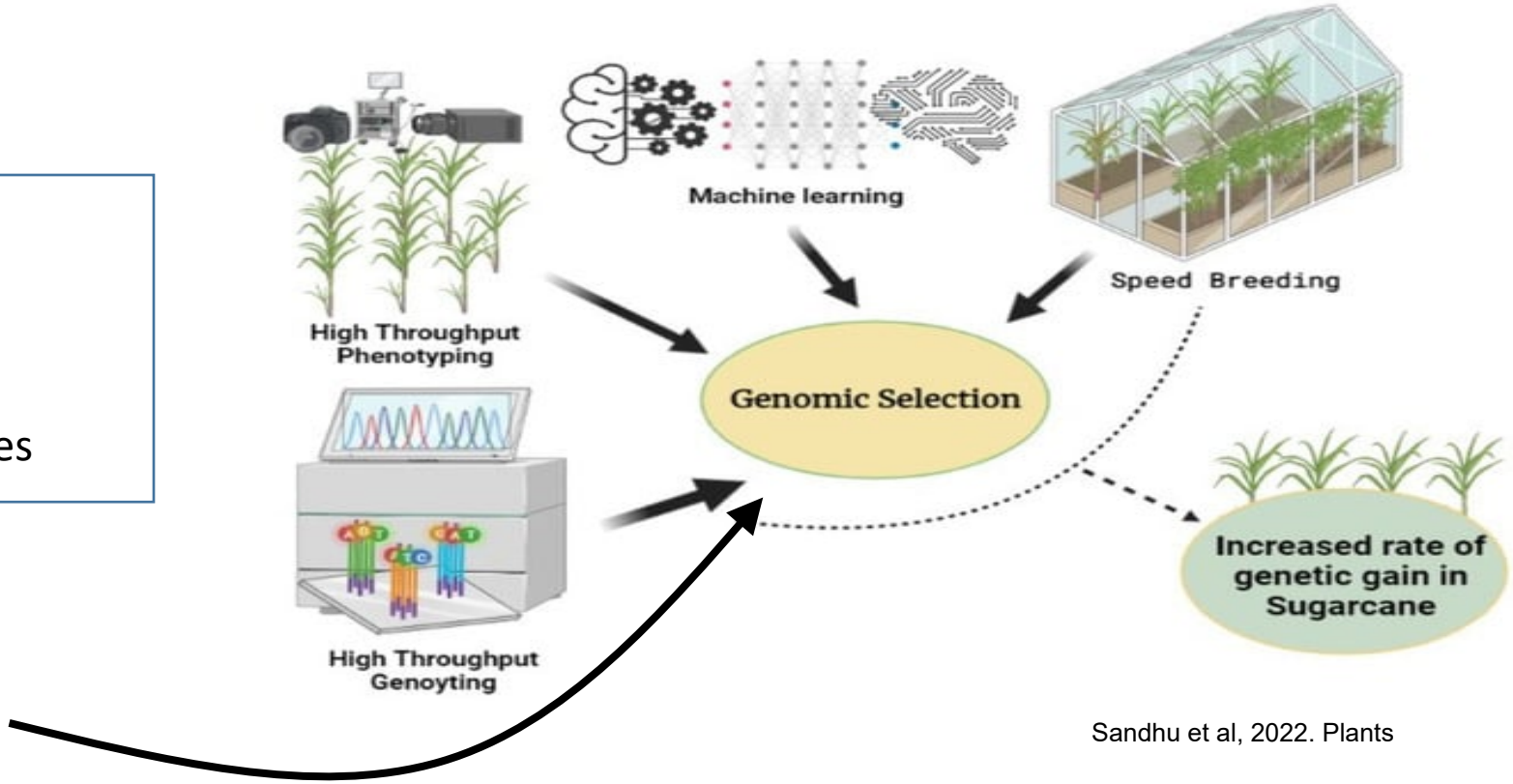
I. Context : agronomy and selection

- **New context for data generation**
  - HiFi & Hi-C technologies
  - « telomere to telomere » genomes
  - Assembly is becoming a routine step

- **New opportunities for genetics**

- Complete genomes
  => SV extraction and analysis
- Many individuals
  => variant frequencies and histories

Genomic diversity of the species



Sandhu et al, 2022. Plants

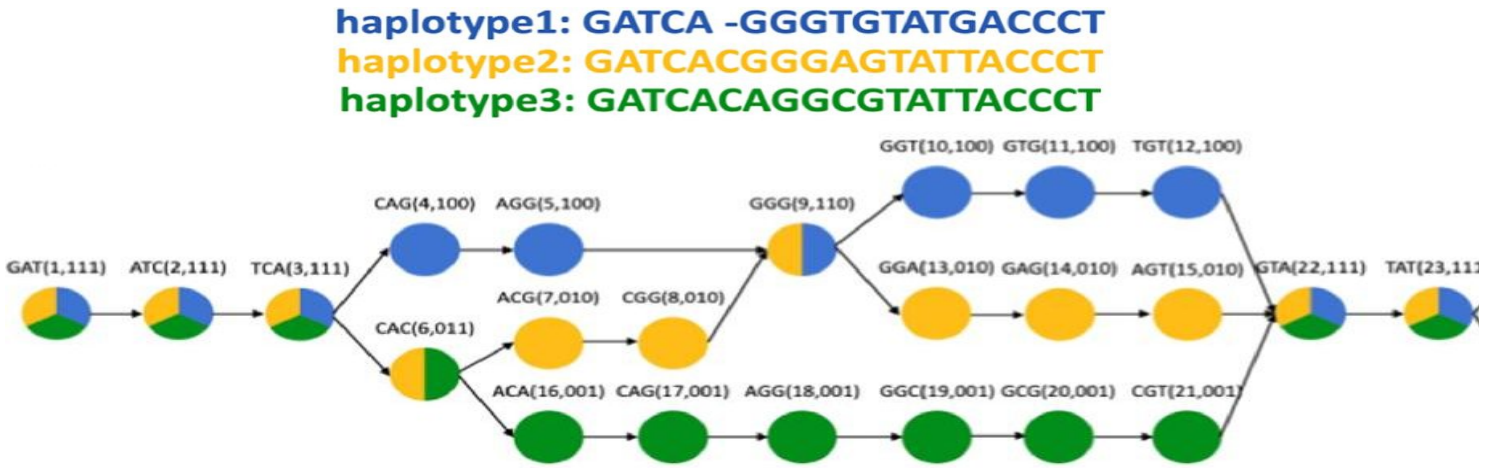
II. Models

- **Modelling genome diversity with a graph**

De Bruijn graphs
(compacted/ colored)

Microbiomes
(mainly)

(>1000 small genomes)

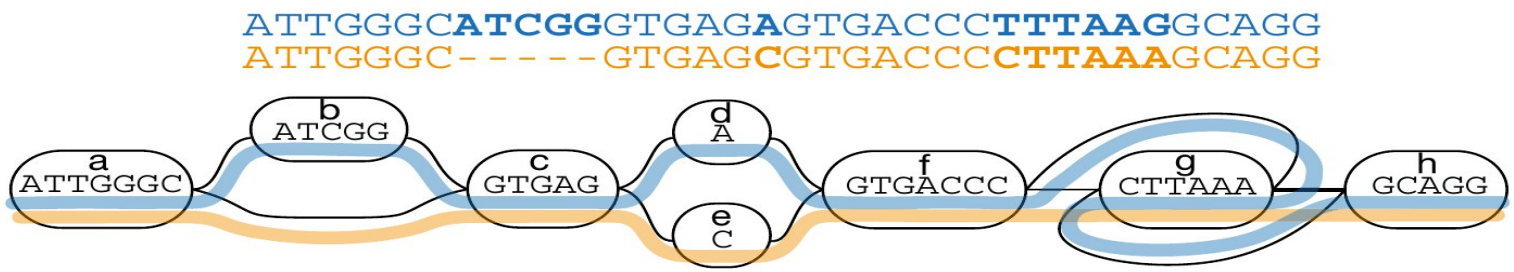


Guo, J. *et al.* (2021)

Variation graphs

Eukaryote genomes
(mainly)

(10 to 100 large genomes)



II. VG model : avoiding reference biaises

(a)

```
Ref.  ACGGTTAAGGGCGATCG--CTCGTTTT
      ACGGTTAAG--CGATCG--CTCGTTTT
      ACCGTTAA----GATCGAACTCG----
      ACCGTTAAGGGCGATCGAA----TTTT
```

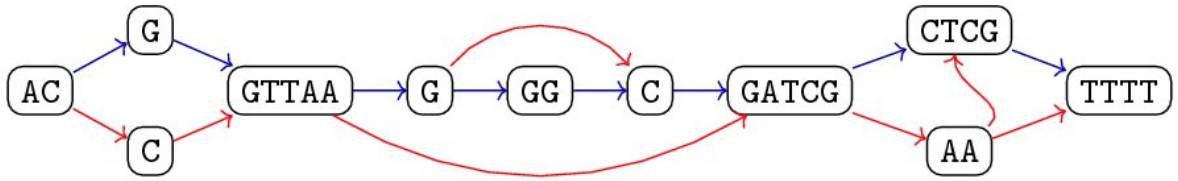
Reads:

```
ACCGTTAAGCGA
TCGAATTTT
```

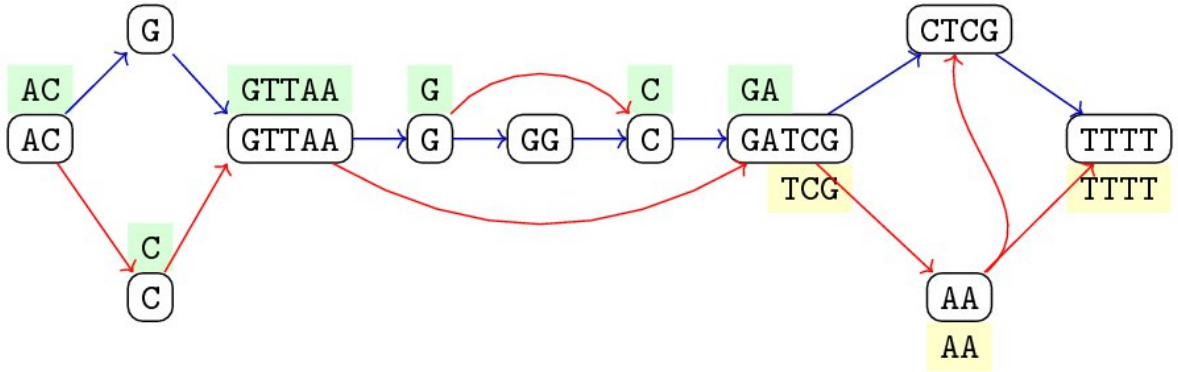
(c)

```
ACCGTTAAGCGA
ACGGTTAAGGGCGATCGCTCGTTTT
            TCGAA--TTTT
```

(b)



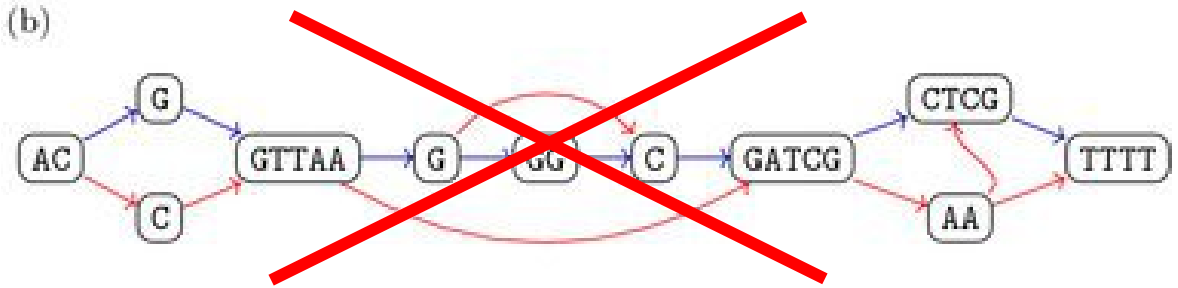
(d)



JA Baaijens et al, 2022

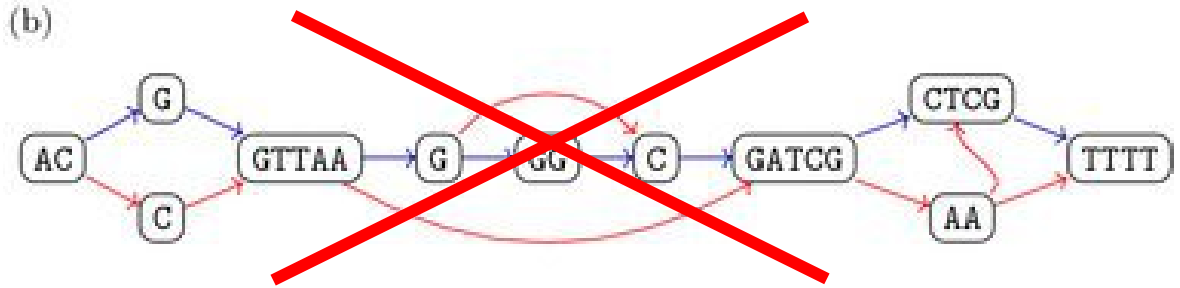
II. VG model

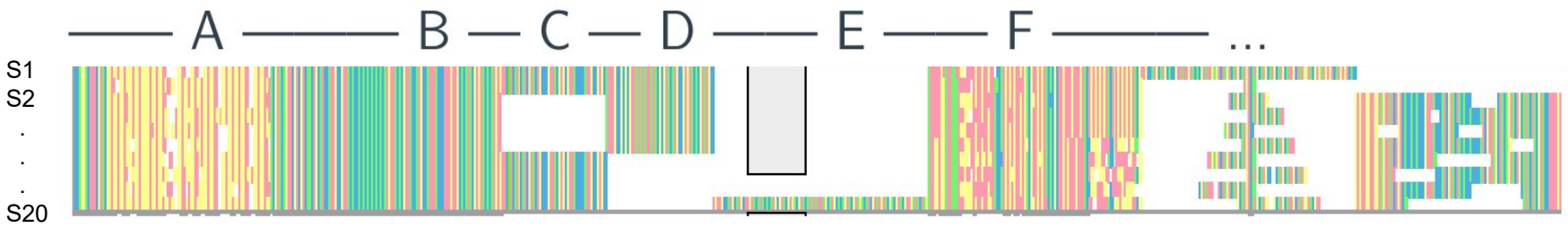
- **In practice ...**

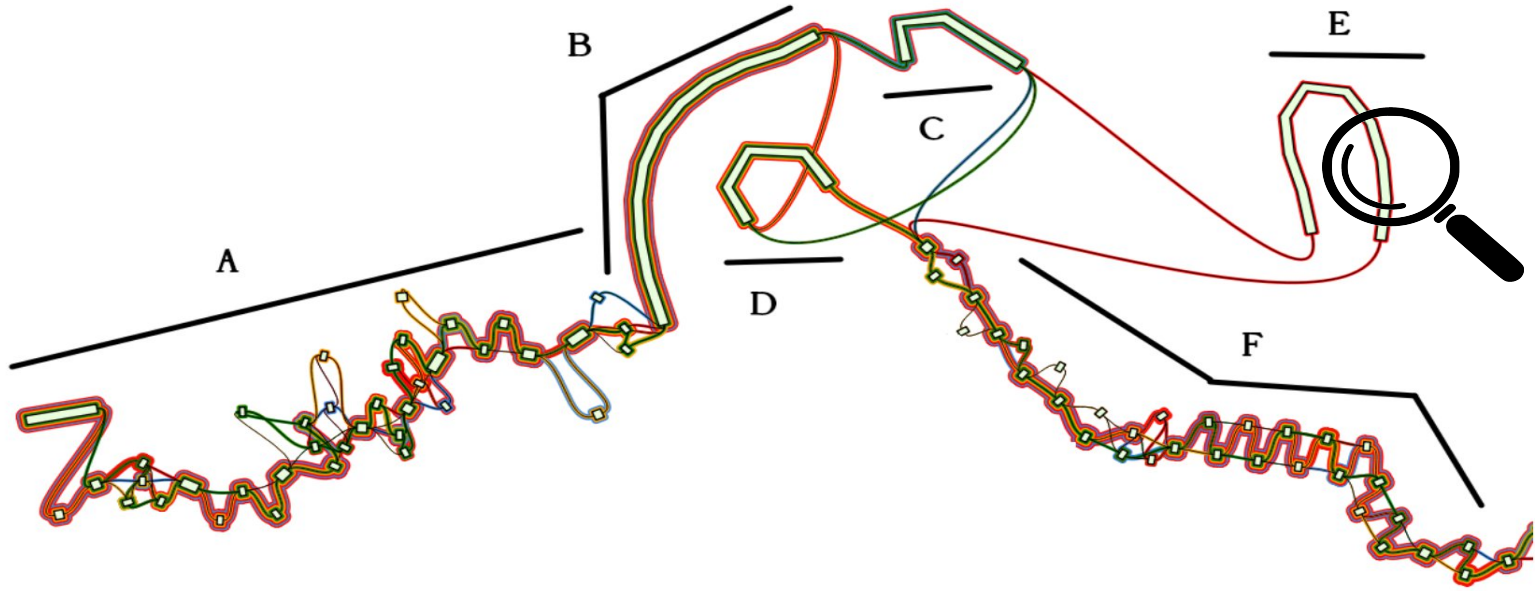


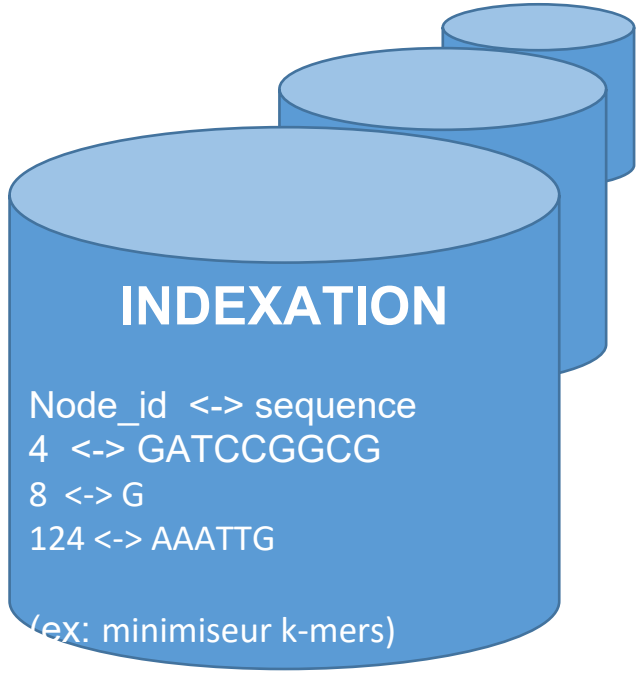
II. VG model

- **In practice ...**



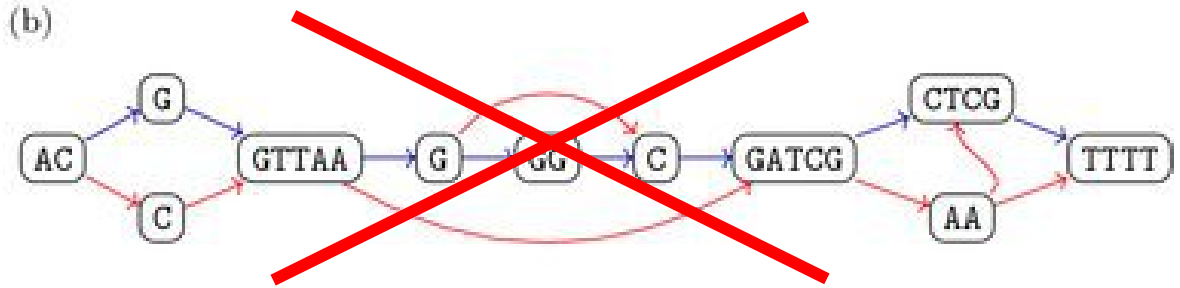


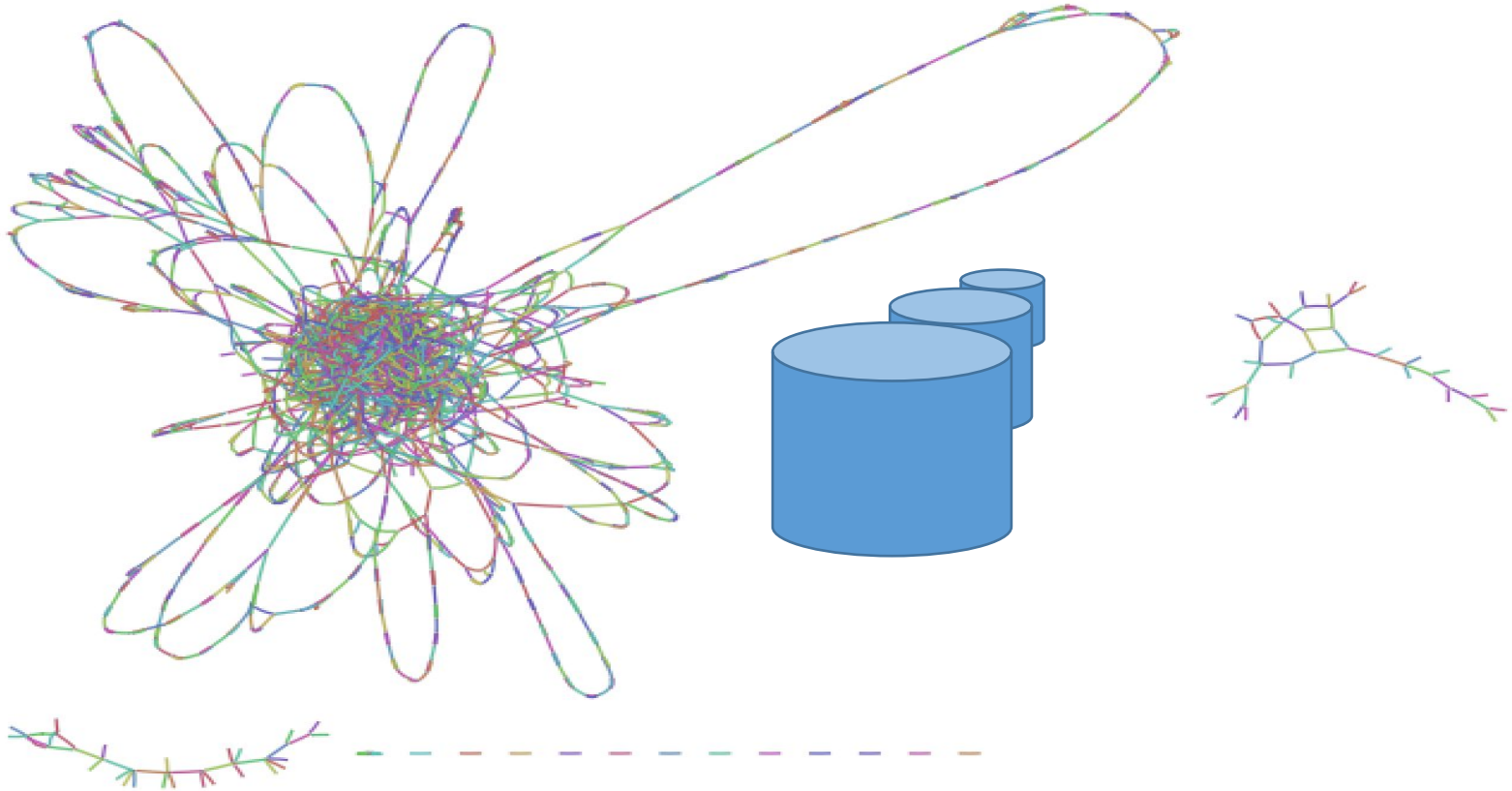




II. VG model

- **In practice ...**





II. VG models : challenges

- **Challenges related to the model and its exploitation**

Graph dimensions :

- 12 bovines assemblies, $>10^7$ nodes (Leonard AS et al, 2023)
- problems related to memory / indexation
- nœud : associé de 1 à *n* kilobases

Topology :

- potentially nodes with high degree
- complexi bubbles in some regions
- cycles (many algorithms are not polynomial anymore)

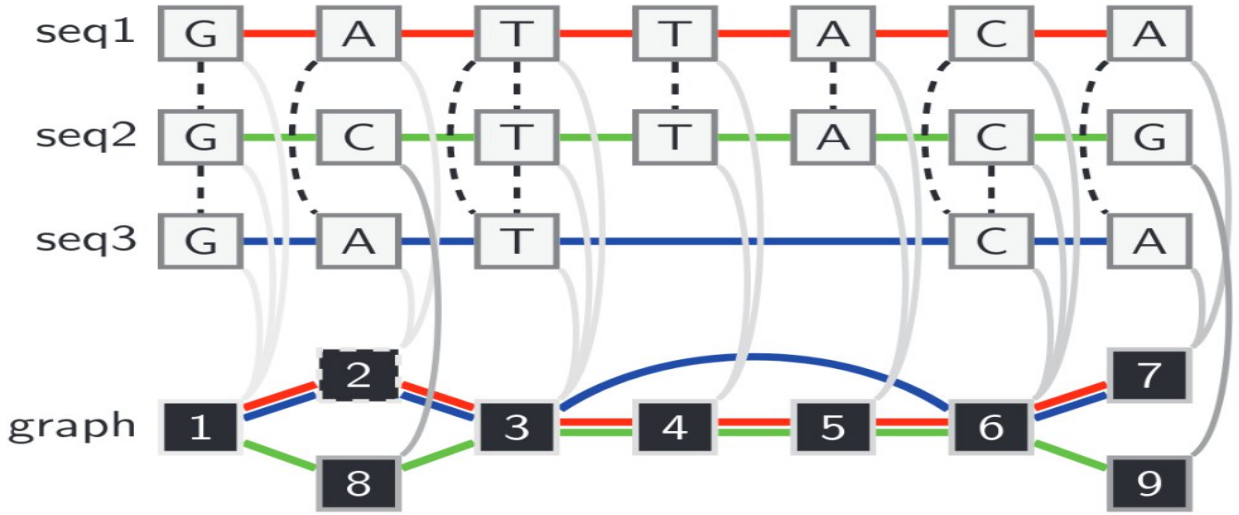
Applications :

- Up to which level of genetic diversity : how many haplotypes ? beyond the species level ?
- Adapting bioinformtic tools to this new representation

.

III. Construction : global approach

- **Construction of a variation graph**
  - Inputs : N a set of high-quality assemblies, pairwised aligned
    A the set of per-character pairwise homology (alignments)
  - Outputs : An oriented graph (string graph) $G = \{V, E, P\}$
    where P , is the set of path representing the integrated assemblies
  - Aim : G includes N and every paiwise relationships described by the genome alignments
    (G cannot contain more information than in A)



Garrisson et al, 2023

paths
seq1 : [1,2,3,4,5,6,7]
seq2 : [1,8,3,4,5,6,7]
seq3: [1,2,3,6,7]

III. Construction : available tools

In its own category:

- Variation Graph (VG) (Garrisson et al, 2018)

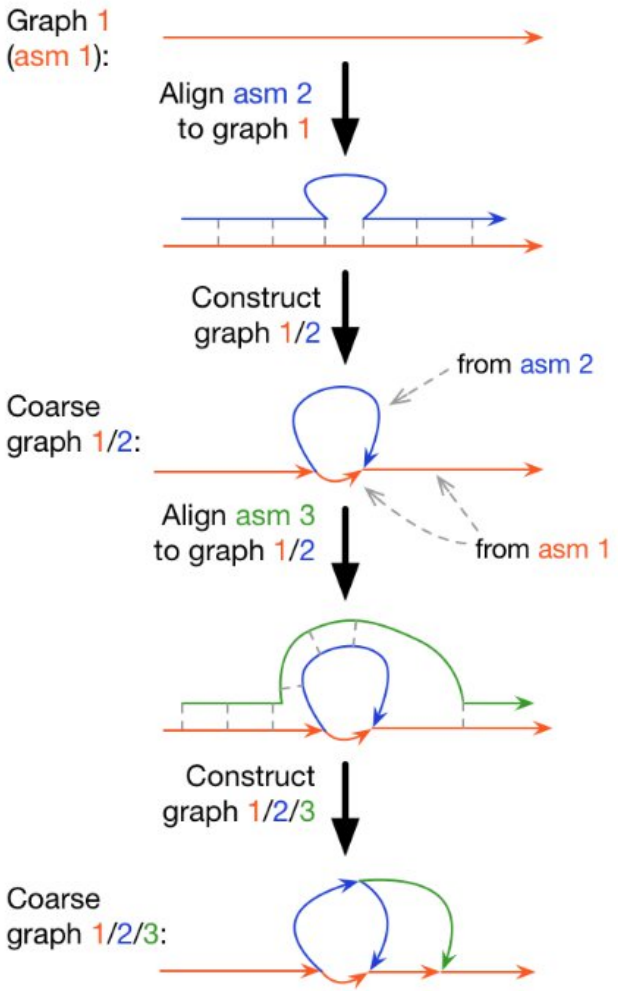
Genome alignment, graph construction, Post-processes.

- Minigraph (MG) (Li et al, 2020)
- Minigraph-Cactus (MGC) (Hickey et al, 2023)
- PanGenome Graph Builder (PGGB) (Garrisson et al, 2023)

III. Construction : VG

- INPUT:
  - A complete reference genome (fasta)
  - Sets of variants, as VCF files relative to this reference.
  - Or multiple sequence alignments (fasta, clustal)
- INCREMENTAL CONSTRUCTION:
  - Each variant described in the VCF adds a topological change in the graph.
  - Every variant is relative to the reference.
  - Excepted reference, assemblies not compulsory (ex: BED>VCFs from chip-seq)
- TOY EXAMPLE: `gtpb.github.io/CPANG18/pages/toy_examples`

III. Construction : Minigraph

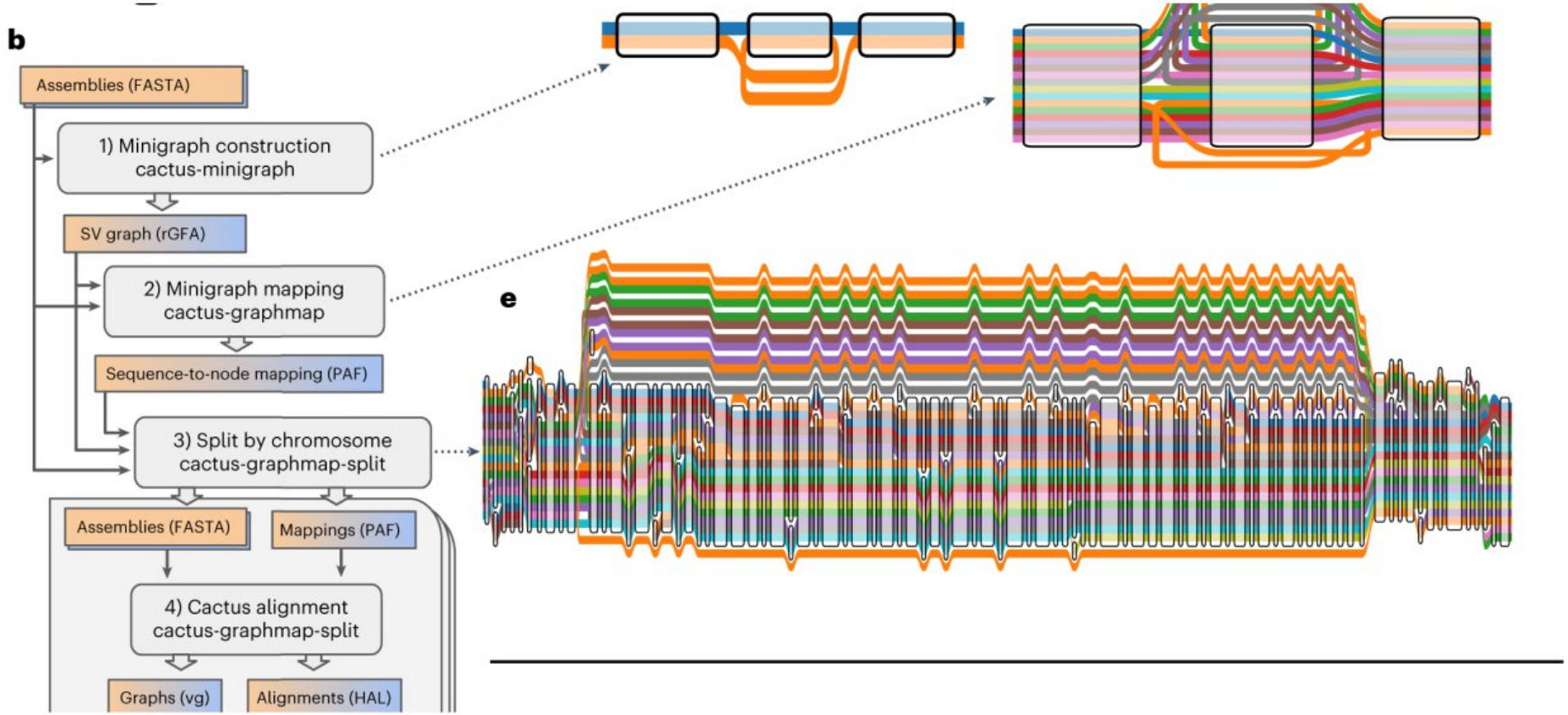


- INPUT:
  - A complete reference genome (fasta)
  - A set of other haplotypes (fasta)
- INCREMENTAL CONSTRUCTION:
  - Haplo 2 mapped to ref: output $\rightarrow G_{[ref,2]}$
  - Haplo 3 mapped to $G_{[ref,2]}$: output $\rightarrow G_{[ref,2,3]}$
  - ... etc ...

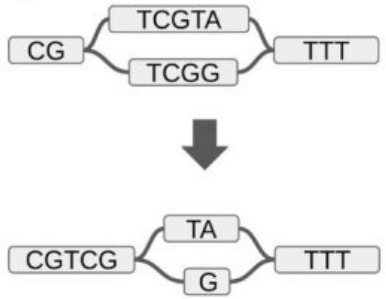
III. Construction : Minigraph-cactus

- INPUT:
  - At least 1, complete, high-quality reference genome (fasta)
  - A set of assemblies for other individuals (fasta)
  - (Optionnal) A tree describing the relationship between individuals
- INCREMENTAL CONSTRUCTION:
  1. Minigraph launched to get high-level SV graph
  2. Assemblies re-aligned to this backbone with Progressive-Cactus $\rightarrow$ adds nodes up to SNP level
  3. Post-processes to simplify/modify the graph.
- WARNING:
  - Minigraph min SV length is FIXED (programmatically, 50bp, 09/2023)

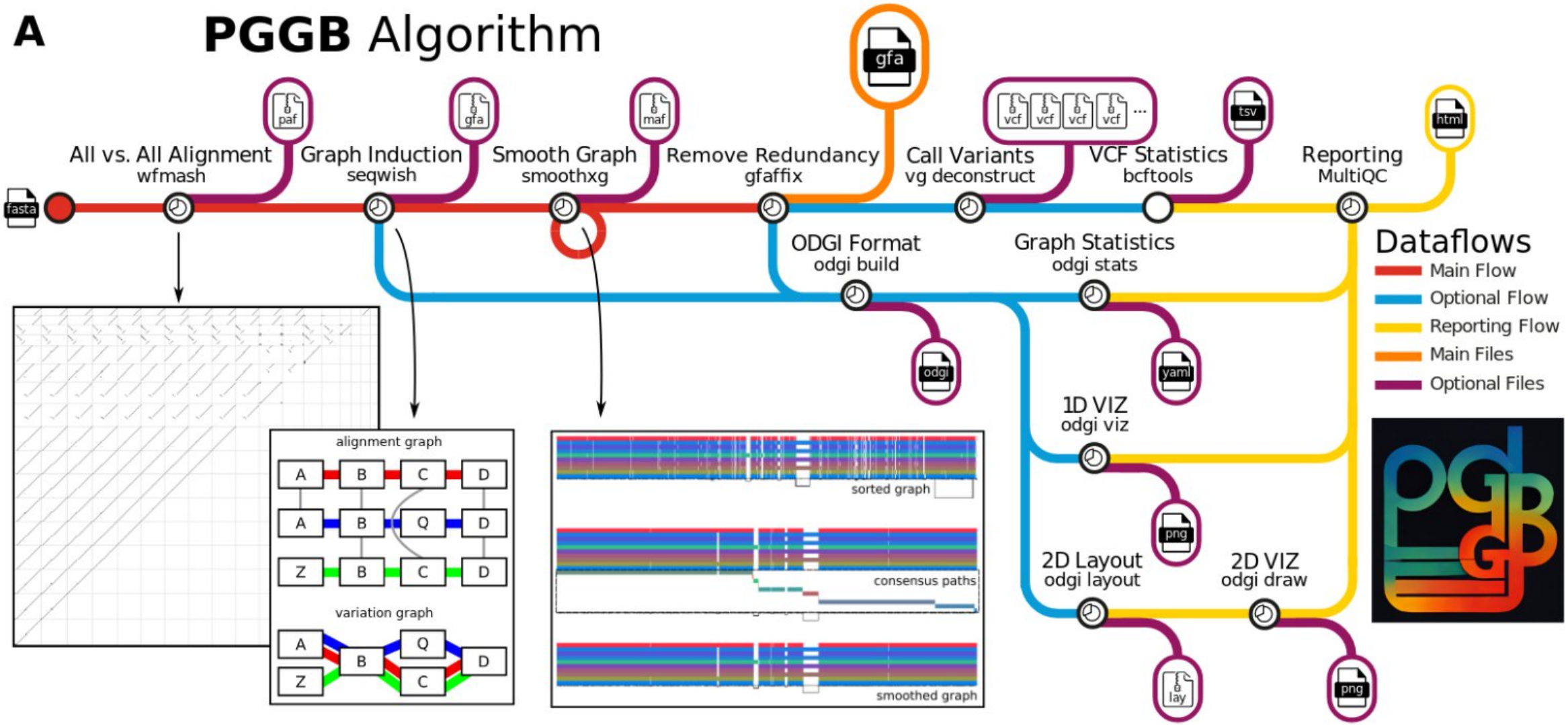
III. Construction : Minigraph-cactus



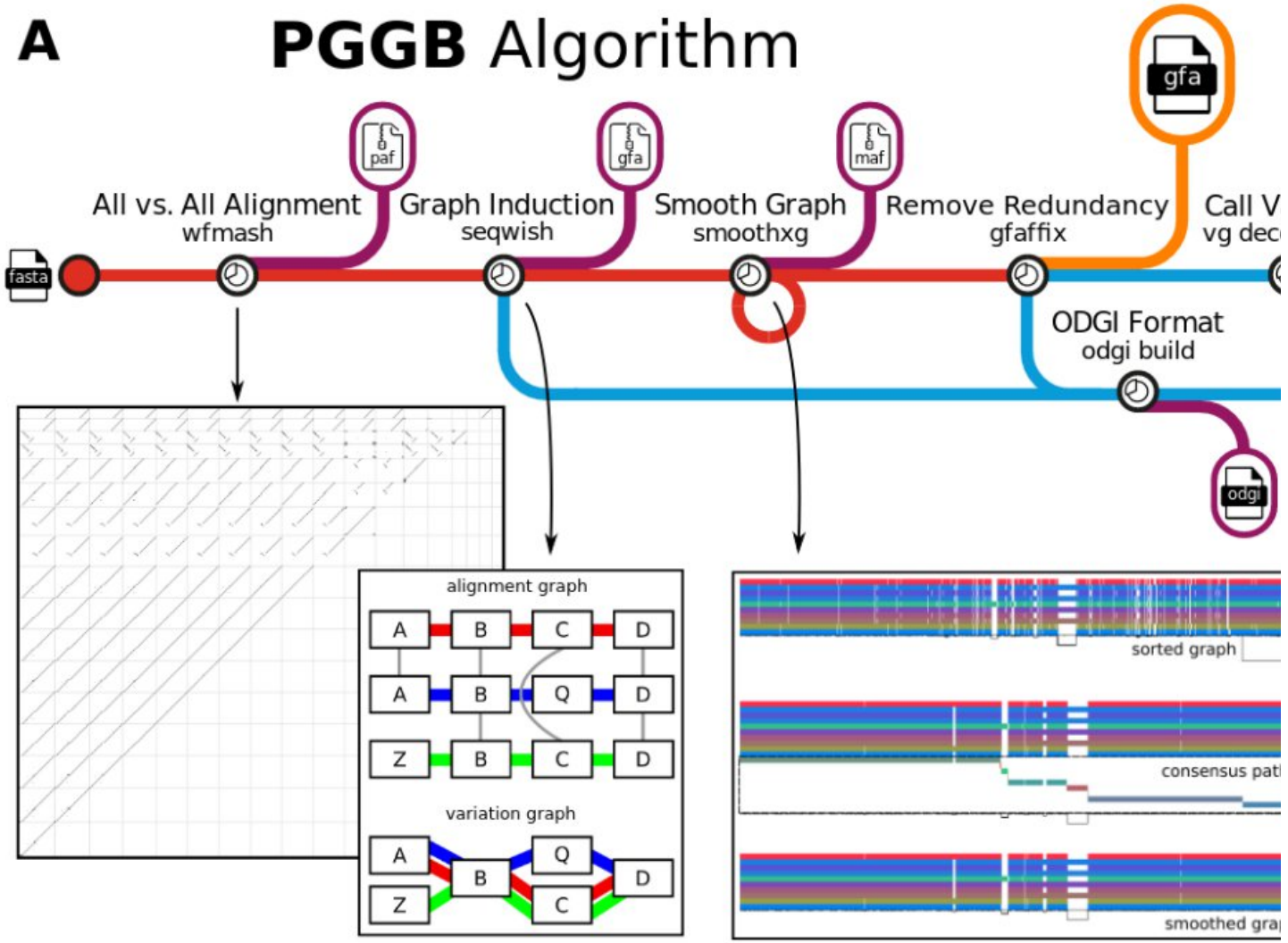
Post-process example: GFAffix tool



III. Construction : PGGB



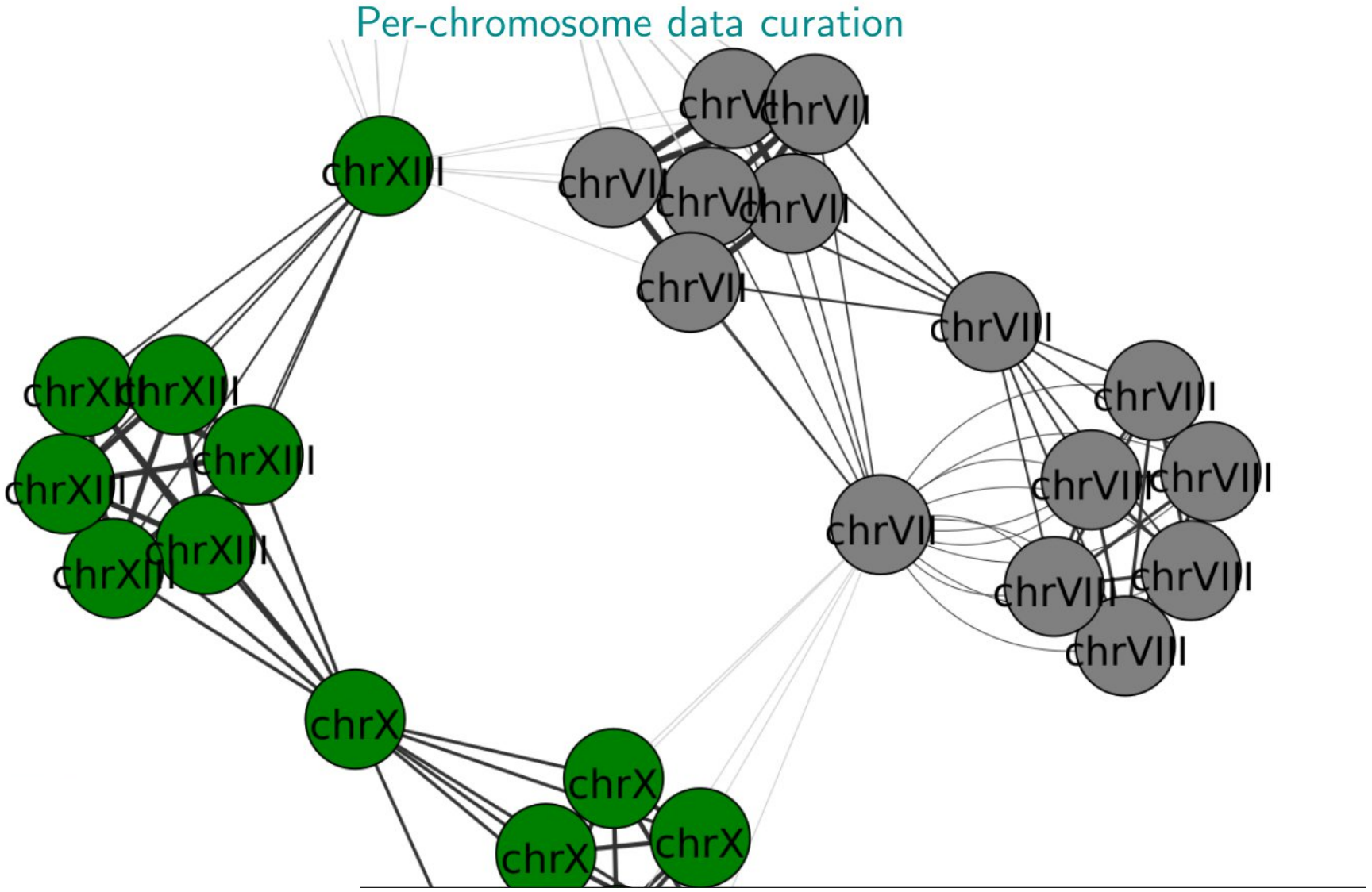
III. Construction : PGGB



III. Construction : PGGB

- INPUT:
  - A set of assemblies, one per chromosome.
- PROCESSUS:
  1. Paiwise alignments for all assemblies with wfmash (Guarracino et al, 2023)
  2. Graph induction with Seqwish (Garrisson et al, 2023)
  3. Graph "smoothing" with smoothxg (not published, not documented)
  4. Post-processes with GFAffix, ODGI ...
- COMMENTS:
  - Similar to MGC, PGGB sets lots of default parameters for you.
  - But logs are more clear, launched subprocesses are logged ...
  - Each step can be run independantly and then parameters changed manually.
  - Documentation / tutorials are not always clear.
  - Smoothxg is not published, but does MANY things.

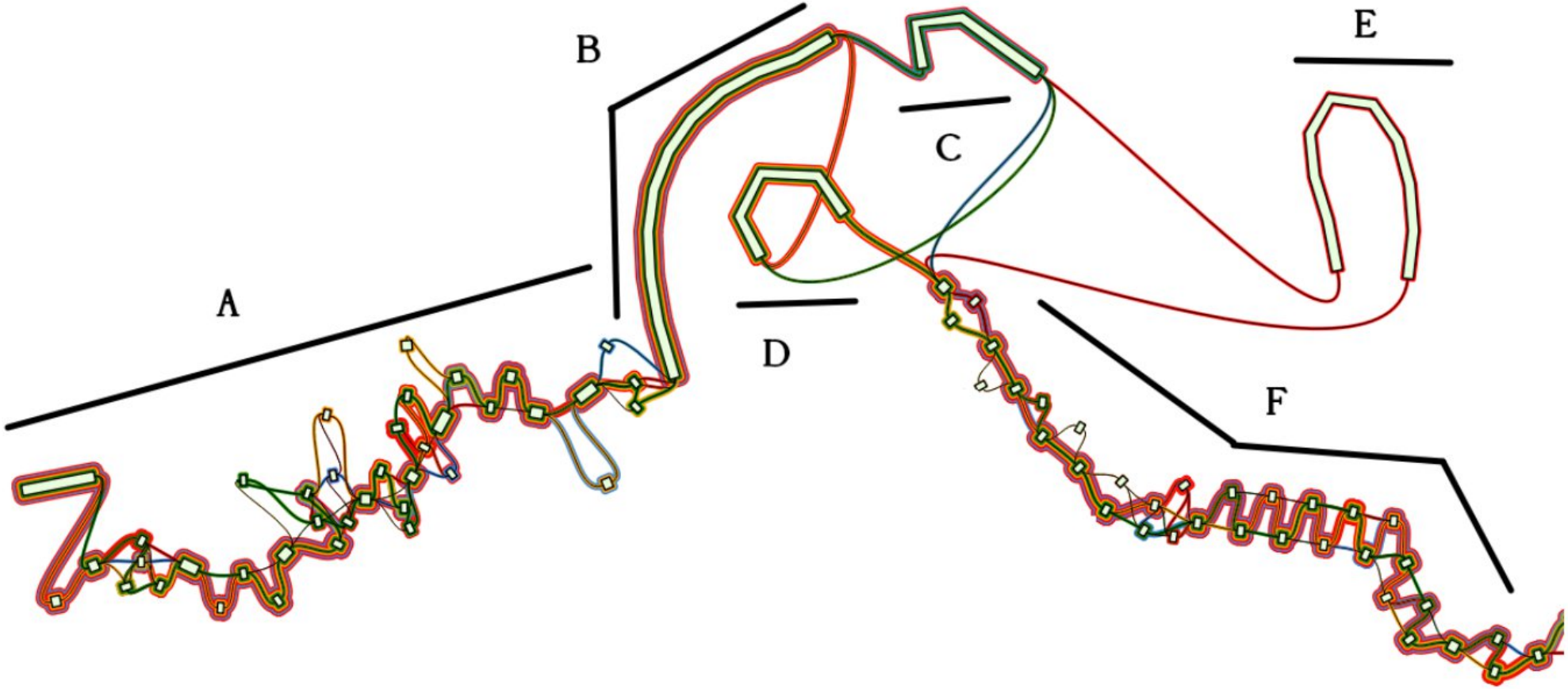
III. Construction : PGGB



III. Construction : PGGB

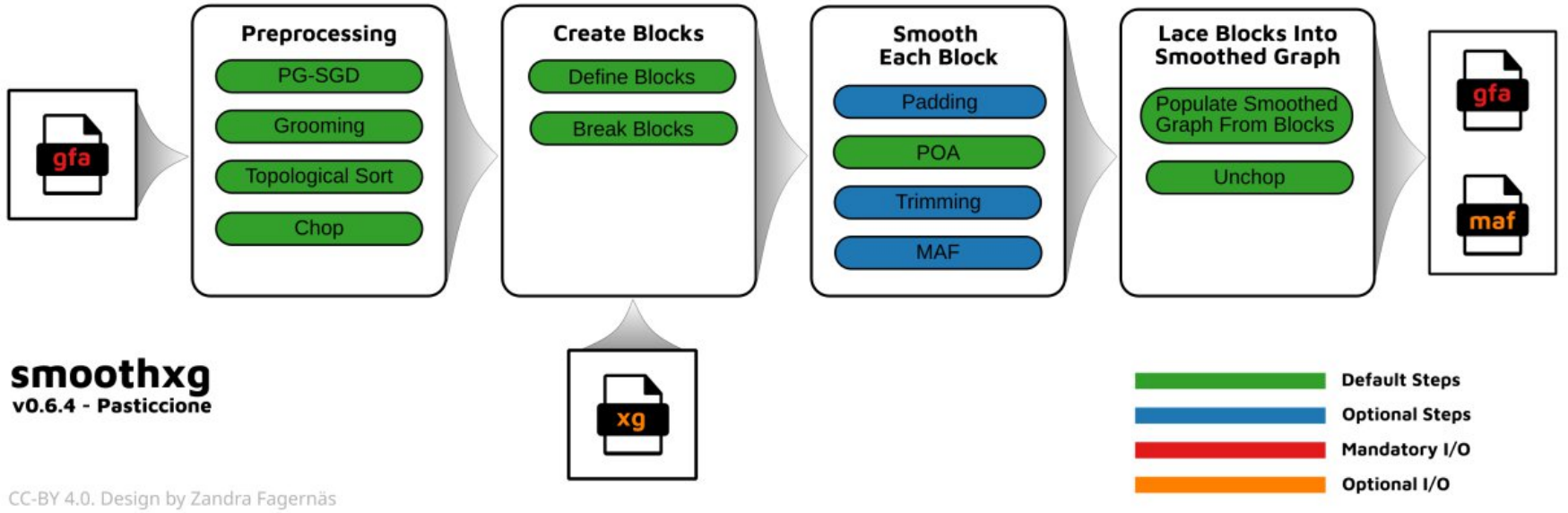
Seqwish output IS the expected graph

But this is NOT
the final PGGB graph.



III. Construction : PGGB

Smoothxg role described recently in Garrisson et al, 2024. Nat Methods.



Note: Graph sorting then POA, which is similar to minigraph-cactus.

III. Construction : fundamental difference

Impact of genome order

Reminder: The tree generated (or given) to Minigraph & Minigraph-Cactus guides the iterative assembly alignement. (Figure from seqwish paper)

III. Construction

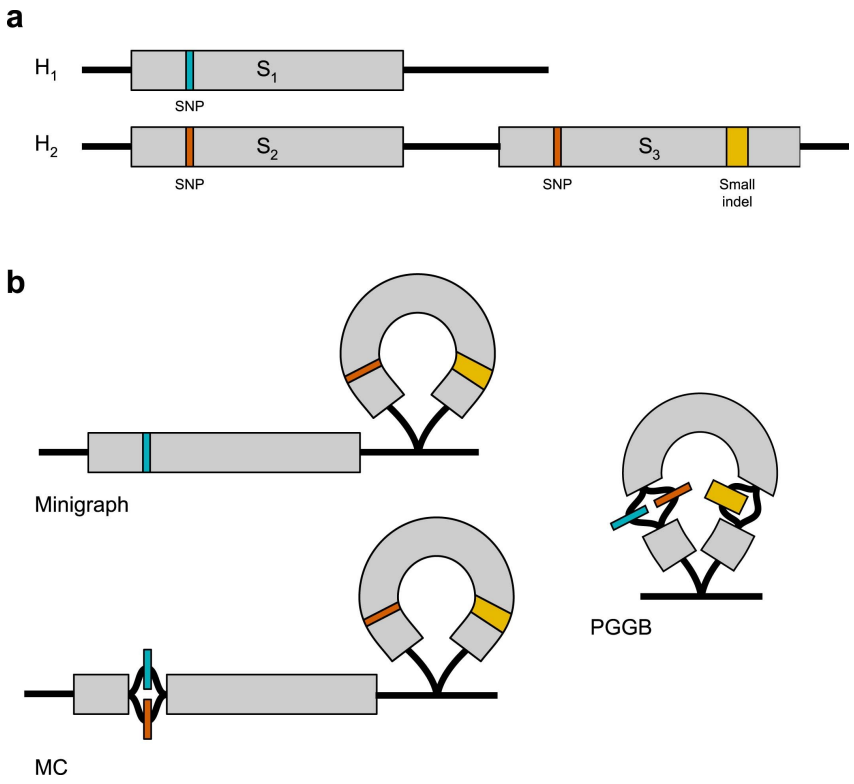
- Andreace et al 2023, https://doi.org/10.1186/s13059-023-03098-2

Metric	Bifrost	pggb	Minigraph-Cactus	Minigraph	mdbg
1) Construction speed	●●○	●○○	●○○	●●○	●●●
2) Variations	●●●	●●●	●●●	●●○	●●○
3) Scalablilty	●●●	●○○	●○○	●●○	●●●
4) Editability	●●●	●●○	●○○	●●○	●○○
5) Stability	●●●	●○○	●○○	●●○	●●●
6) Accessibility by downstream applications	●○○	●●●	●●●	●●○	●○○
7) Haplotype compression performance	●●○	●●●	●●●	●○○	●○○
8) Ease of visualization	●○○	●●○	●●○	●●●	●●●
9) Loci visualization and interpretability	●○○	●●○	●●●	●●○	●○○
10) Metadata and annotation	●●○	●●●	●●○	●○○	●○○
11) Compatibility with a linear reference coordinates	●○○	●●●	●●●	●●○	●○○

III. Construction : challenges

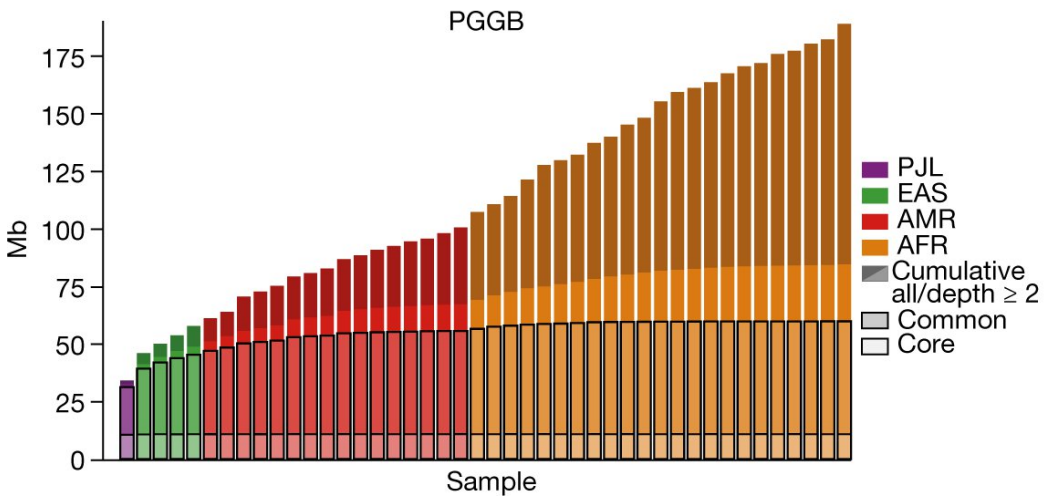
- Integrating every genomic variation from 10s to 100s of long genomes is not trivial !
  - Today 4 methods (from 2 research groups)
  - For 12 bovine assemblies (Leonard AS et al, 2023)
    - Graphs are huge !
    - Computational requirements can be huge !

Parameter	Unit	minigraph	cactus	pggb
Nodes	N	427,012	198,431,246	179,575,371
Edges	N	606,926	272,102,708	245,150,846
Node length	bp	2,598,811,581	3,041,026,095	3,012,039,323
Path steps	N	3,358,976	1,621,936,527	1,442,793,659
Repetitive sequence	bp	1,107,501,421	1,361,489,638	1,415,552,890
Centromeric sequence	bp	2,939,789	291,982,193	255,091,362
CPU time	h	14[a]	226[b]	3,559
Max memory	GiB	7	54	46
GFA file size	GB	2.6	26.1	23.7

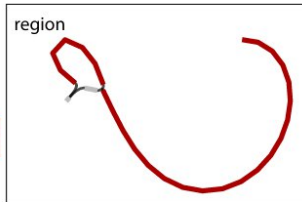
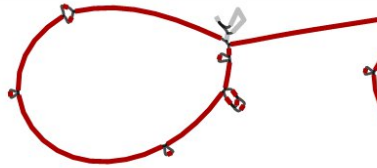
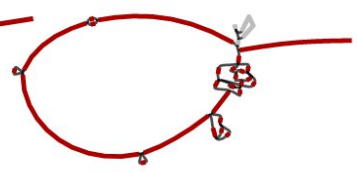
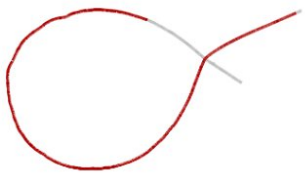


III. Construction : challenges

- A balance : variation length / variant frequency / # integrable genomes
  - All methods discard “some” variations to reduce graph size or complexity.
  - 50 human haplotypes, graph size still continue to increase. (many rare variants)



Andreace et al, 2023

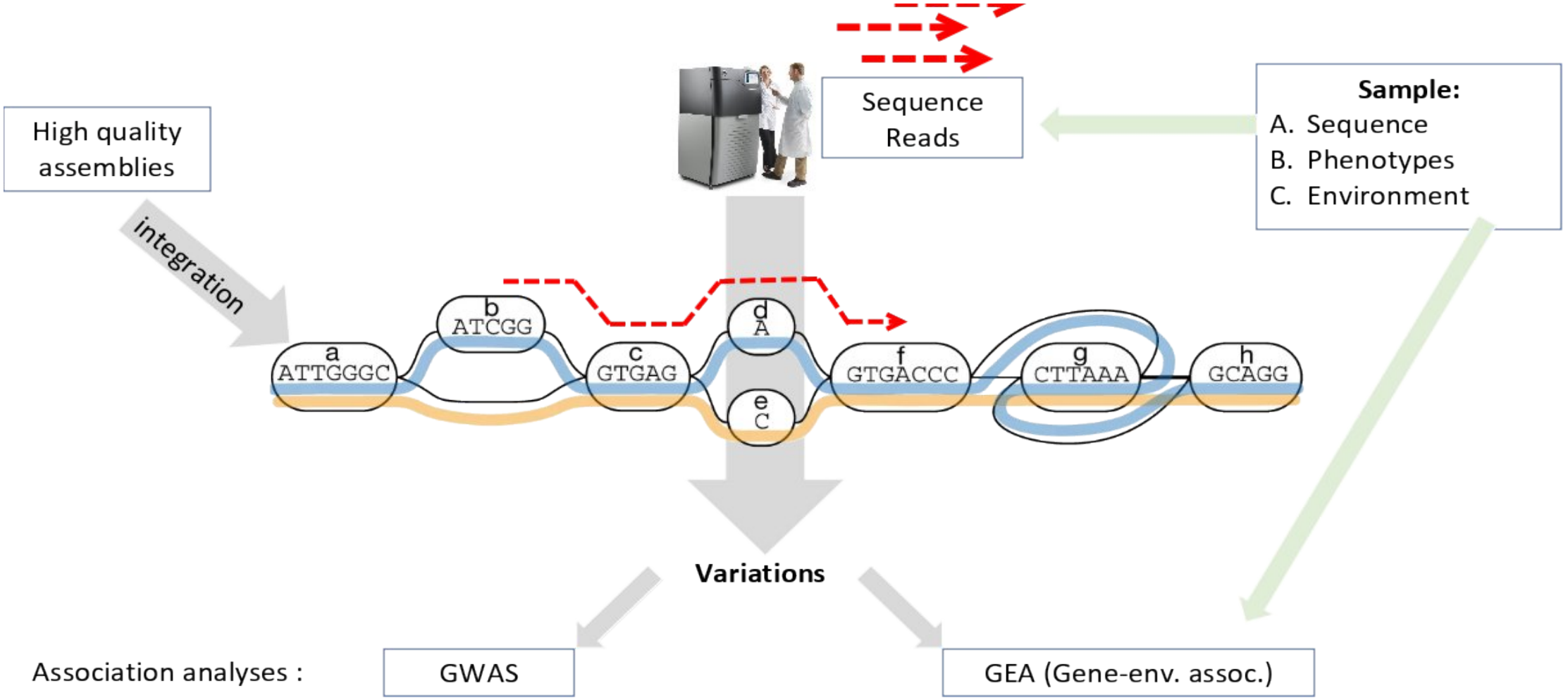
Tool	Metric	2 haplo.	10 haplo.	104 haplo.
MINIGRAPH	region			
	nodes	4	19	29
	edges	1	26	40
MINIGRAPH CACTUS	region			
	nodes	842	3543	--
	edges	1127	4016	--
PGGB	region			
	nodes	2	3824	--
	edges	0	5160	--

III. Construction

- Building a variation graph will not be smooth. Keep clam, this is expected.
- Methods and tools evolve rapidly and are not stable.
- Today: choose between Minigraph / Minigraph-Cactus depending on targeted scale.
- PGGB should improve soon...
- Many tools for graph postprocesses: impact poorly evaluated today.
- Pipelines are self sufficient, but hard to tune.
- Current default parameters were optimized for human. Impacts on non-primate metazoans, plants, fungi pangenome graphs ?

IV. Mapping and variant calling

- **Today, main application is variant calling, genotyping.**



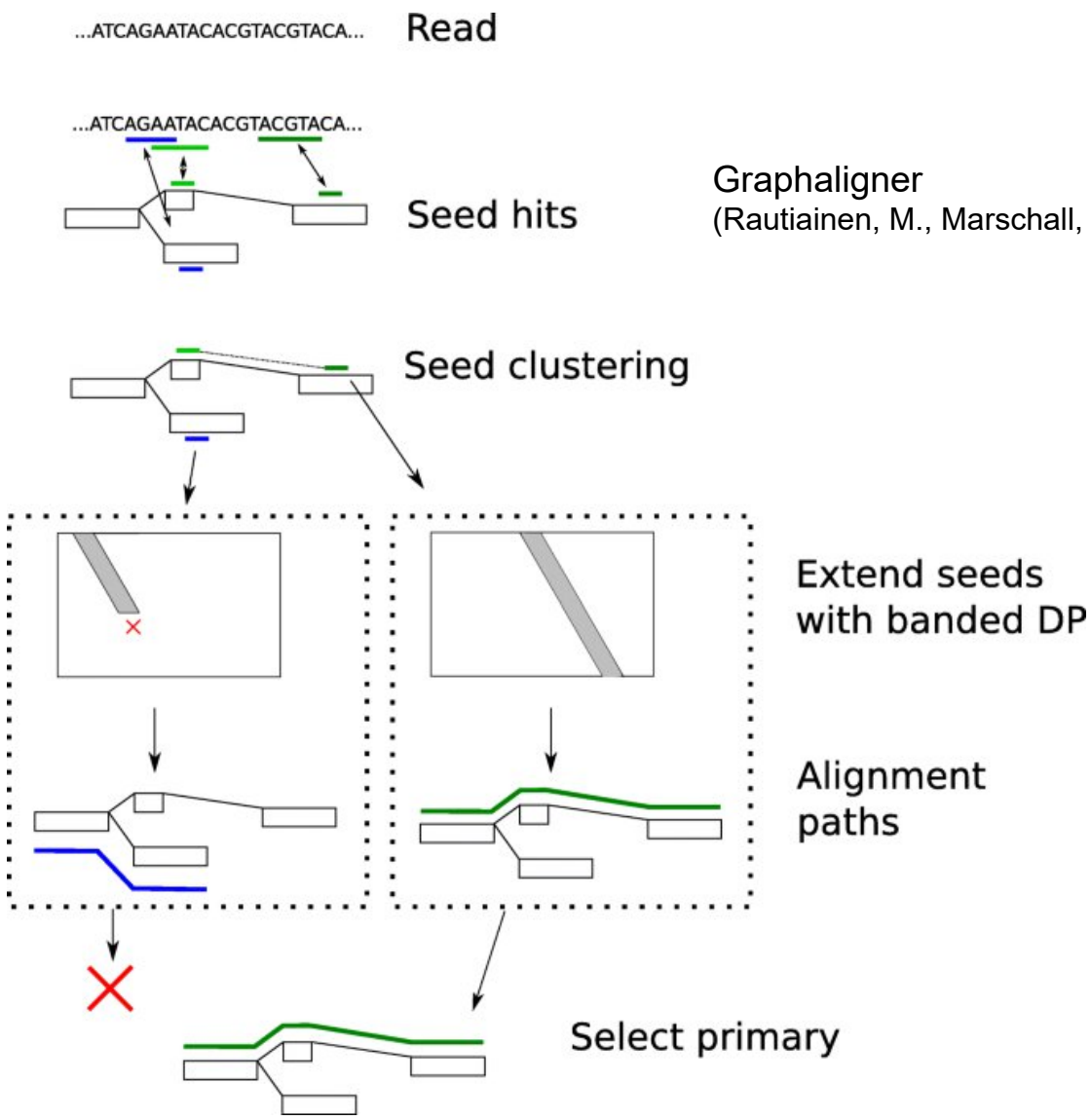
IV. Mapping and variant calling

- “seed and extend” approach

1. Pre-requisite => graph k-mers indexation (seeds indexation)

2. Mapping itself :

    1. **Find anchors** between query and graph nodes (k-mer seeds)
    2. **From these anchor, select a path in the graph**
    3. Then, do a “classic” **alignment** between the query sequence and selected path

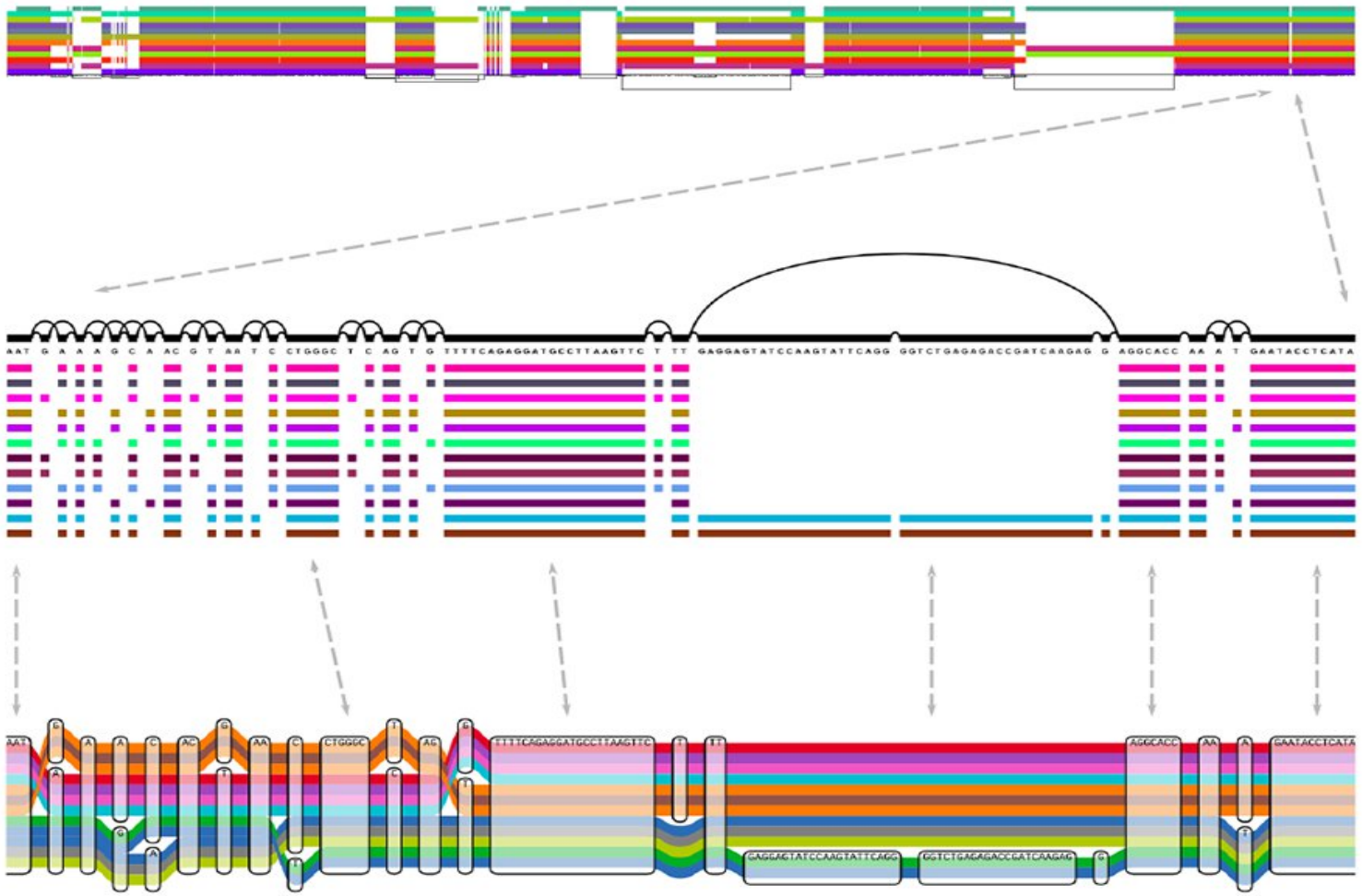


Graphaligner
(Rautiainen, M., Marschall, T, 2020)

V. Visualisation

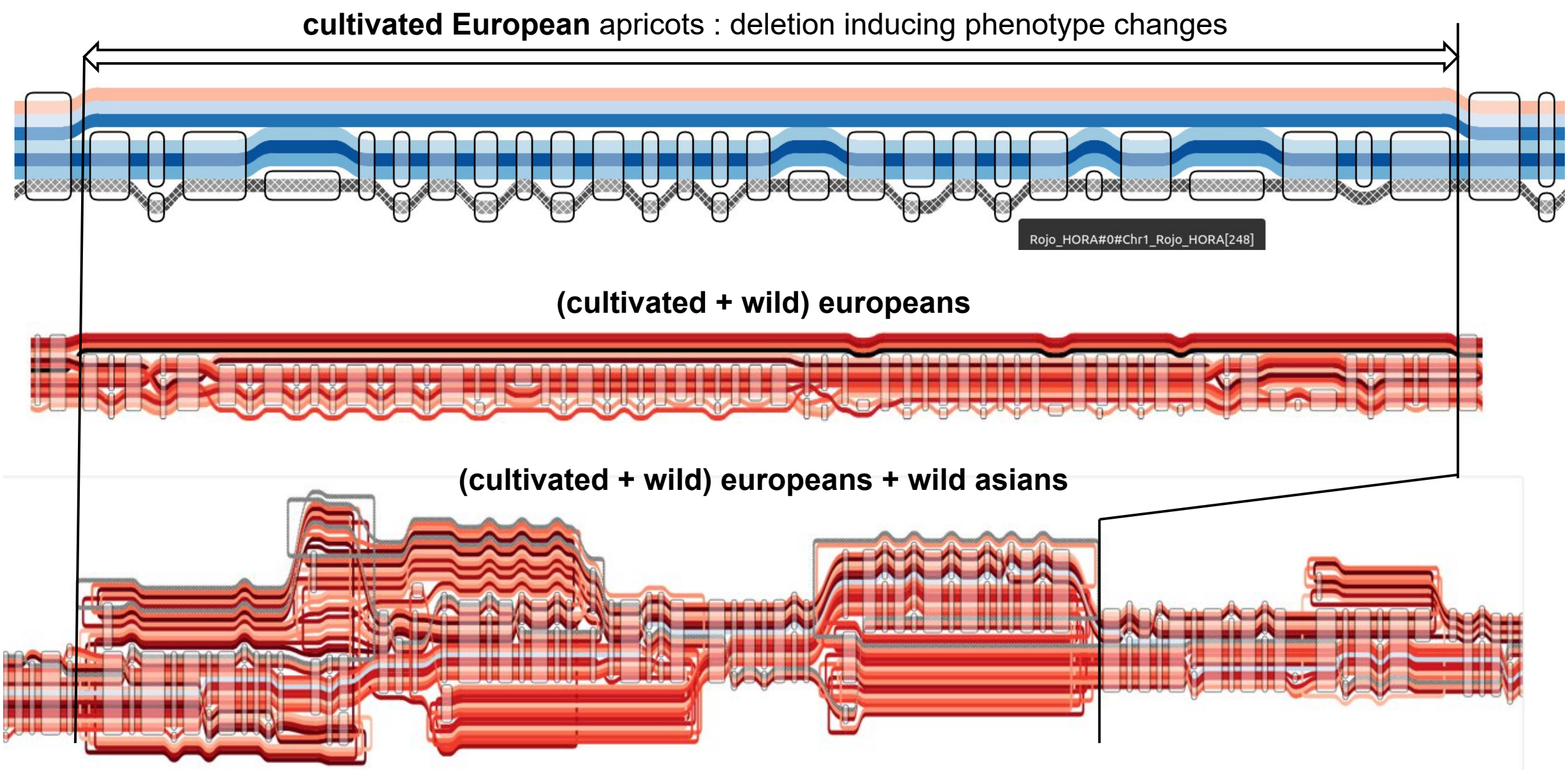
- Likely the most limited aspect of VG tools today :
  - How to switch from coordinate-based to graph-based representation ?
  - How to make sense of huge & complex topologies in 2D ?

ODGI
(Guarracino et al, · 2022)



SequenceTubeMap
(unpublished)

V. Visualisation

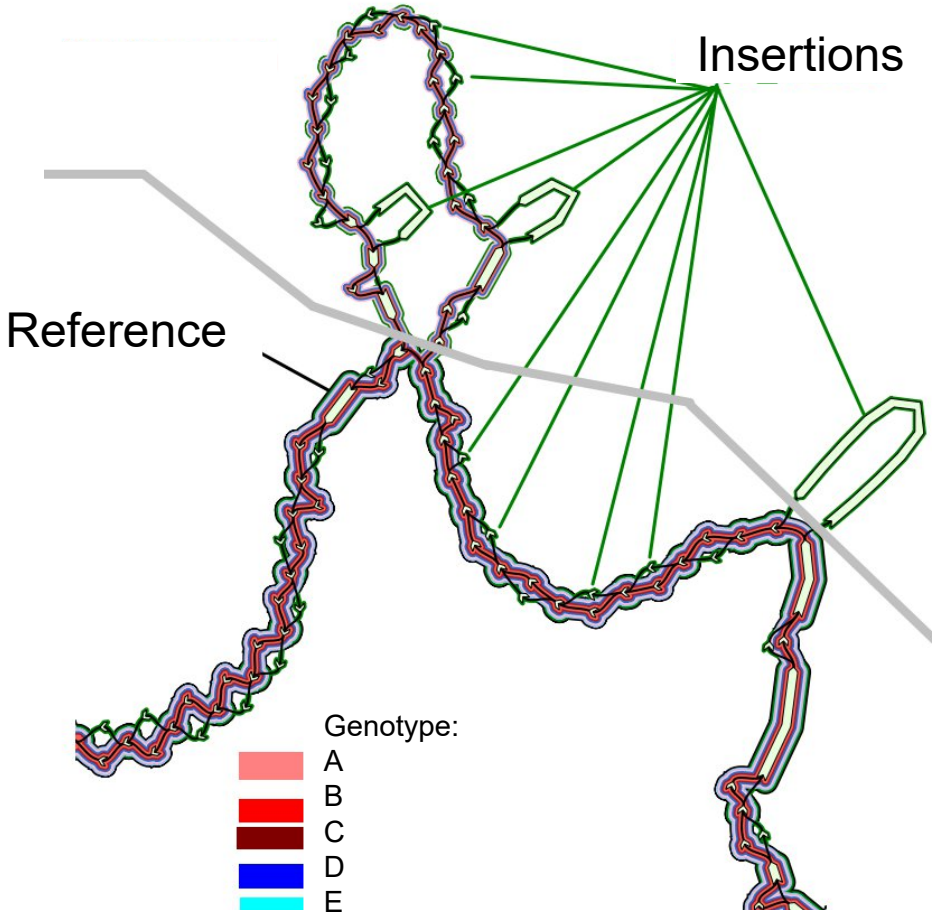


Courtesy of Ismael Blanchard, Véronique Decroocq.INRAE, BFP, Bordeaux

V. Visualisation

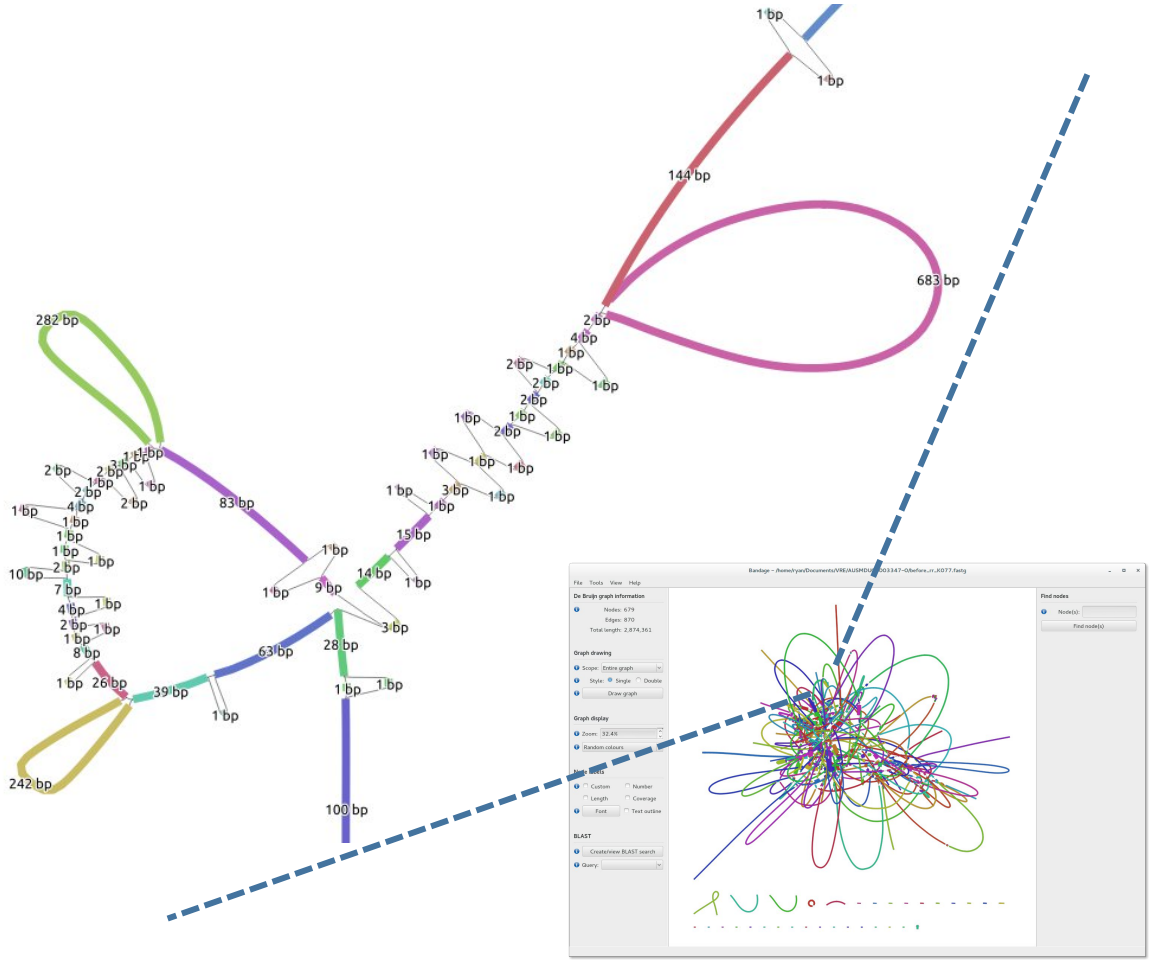
gfaviz (Gonnella et al, 2018)

+ customizable path annotations
+ adapted to figures generation
– limited to very small subgraphs



Bandage (Wick et al, 2015)

+ manipulation of very large graphs
– paths cannot be displayed



Thank you for your attention.

A monthly-evolving pangenome graph. ;) (https://www.pangenome.eu/)