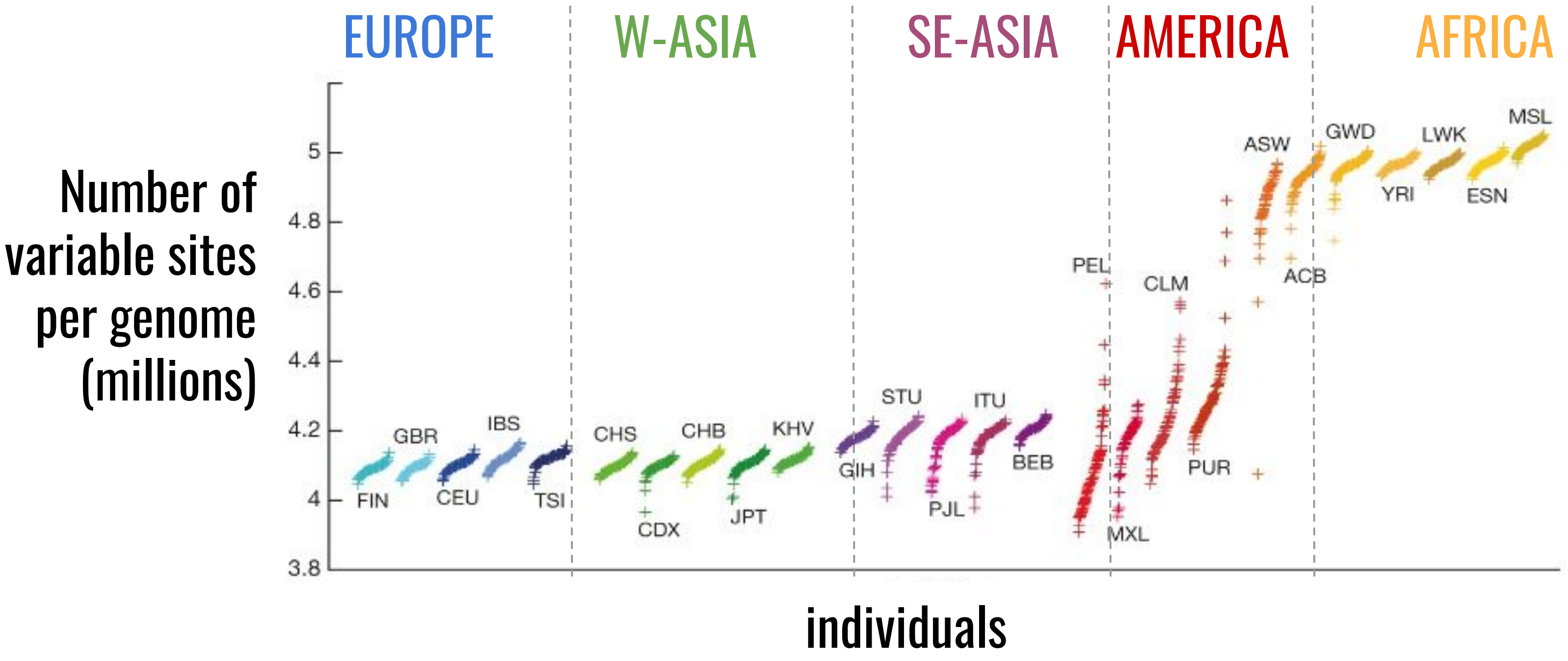
Population Genomics Analyses on pangenome graphs

Flavia Villani

Consiglio Nazionale delle Ricerche | Istituto di Genetica e Biofisica "Adriano Buzzati-Traverso" | Napoli

NETTAB / BBCC 2020 Meeting
November 16-18, 2020
Department of Agricultural Sciences, University of Naples “Federico II”, Portici, Naples, Italy.

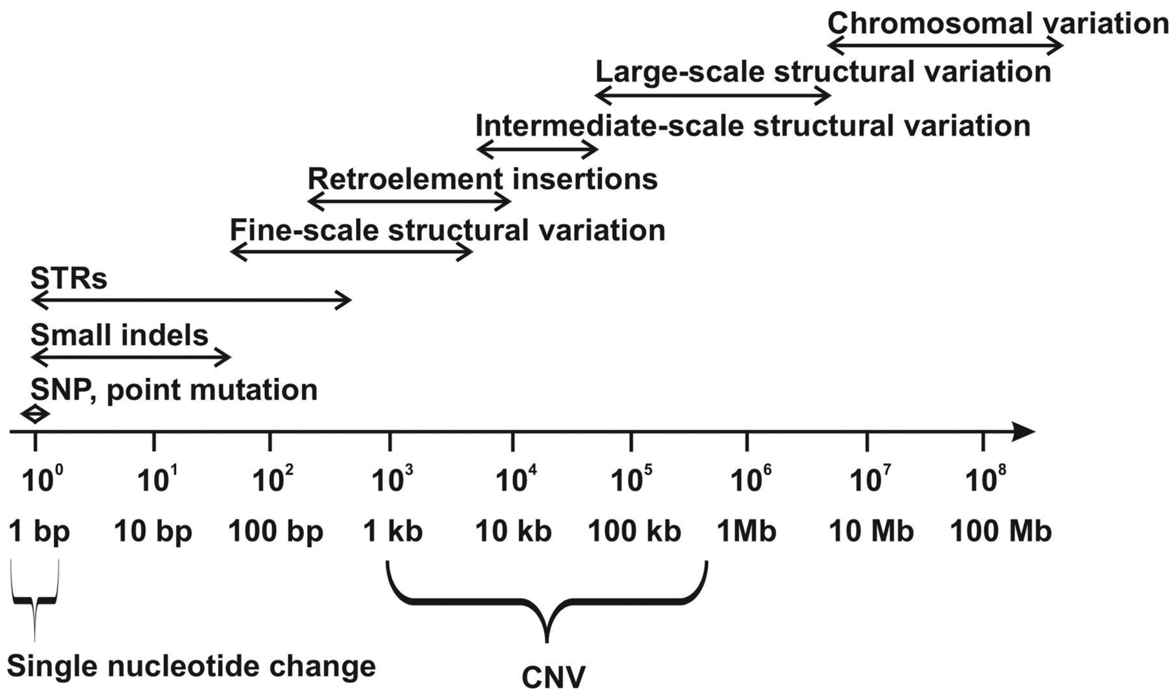
Population Genetics



A Auton et al. Nature 526, 68-74 (2015) doi:10.1038/nature15393

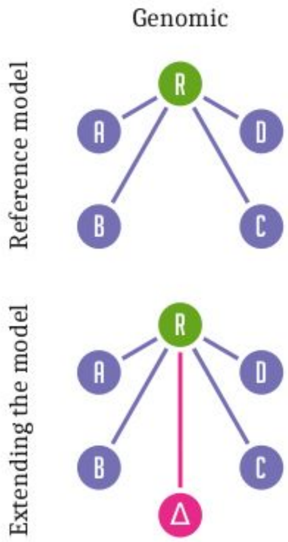
4.3 M differences on average between two individuals

Pangenomics approach for identification structural variants



Rebecca L Pollex and Robert A Hegele. “Copy number variation in the human genome and its implications for cardiovascular disease”. In: Circulation 115.24 (2007),pp. 3130–3138

Genomic *versus* pangenomic



Eizenga, Jordan & Novak, Adam & Sibbesen, Jonas & Heumos, Simon & Ghaffaari, Ali & Hickey, Glenn & Chang, Xian & Seaman, Josiah & Rounthwaite, Robin & Ebler, Jana & Rautiainen, Mikko & Garg, Shilpa & Paten, Benedict & Marschall, Tobias & Sirén, Jouni & Garrison, Erik. (2020). Pangenome Graphs. Annual Review of Genomics and Human Genetics.

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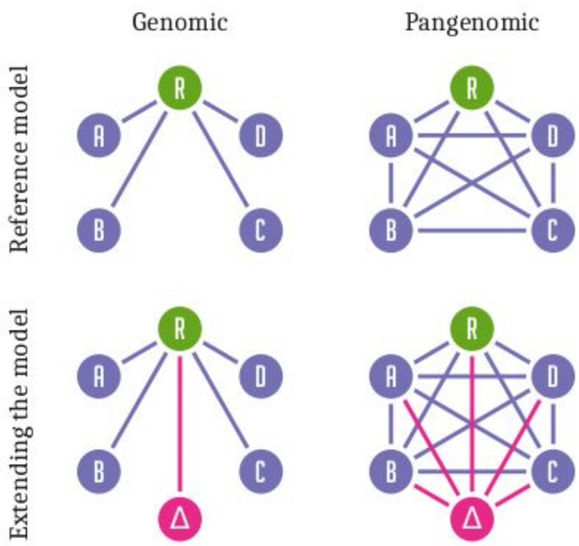

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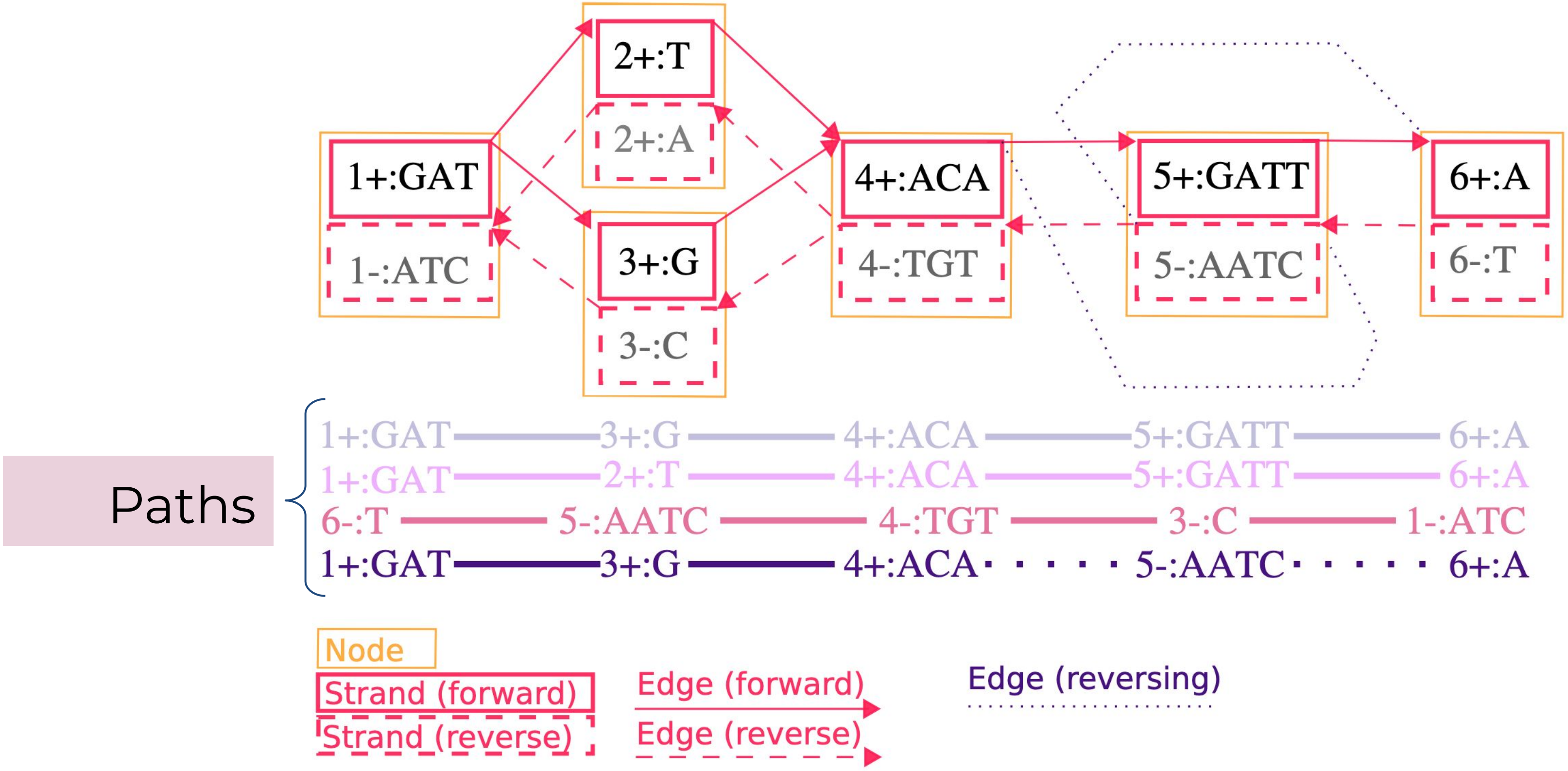
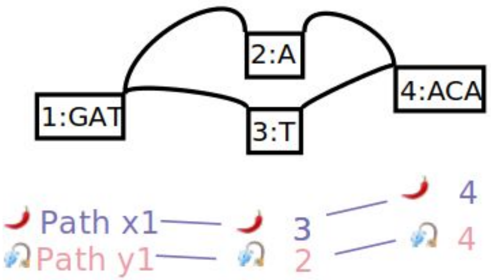
Graphic representation of a pangenome



Eizenga J. M*., Novak A. M.*, Kobayashi E., Villani F., Cisar C., Heumos S., Hickey G., Colonna V., Paten B. & Garrison E. (2020). Efficient dynamic variation graphs. ***Bioinformatics Oxford.***

Genetic variants in the linear and graphical model

Pangenome (GFA)



Genomics (VCF)

#CHROM	POS	REF	ALT
x	4	A	T

Genomics standard analyses are based on linear representation of genomes

Goal

To develop a library of functions (vgpop) for population genetic analysis on pangenomic models

Library vgpop

Parsing pangenome	Population genetics	Format conversion	Application
bubblepop	num_sequences	gfa2vcf	Simulated data
	num_segregatingsites		
	allele_frequencies	seqgen2gfa+vcf	Real data: - HLA - Sars-Cov2
	fst		

https://github.com/Flavia95/VGpop

Library vgpop

Parsing pangenome

- bubblepop

Population genetics

- num_sequences
- num_segregatingsites
- allele_frequencies
- fst

Format conversion

- gfa2vcf
- seqgen2gfa+vcf

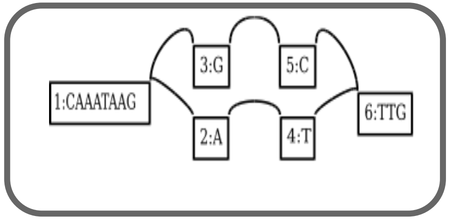
Application

- Simulated data
- Real data:
  - HLA
  - Sars-Cov2

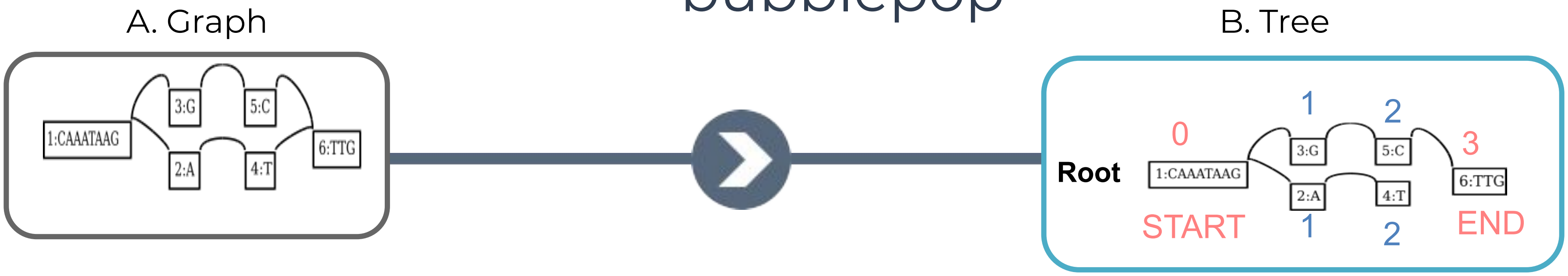
https://github.com/Flavia95/VGpop

bubblepop

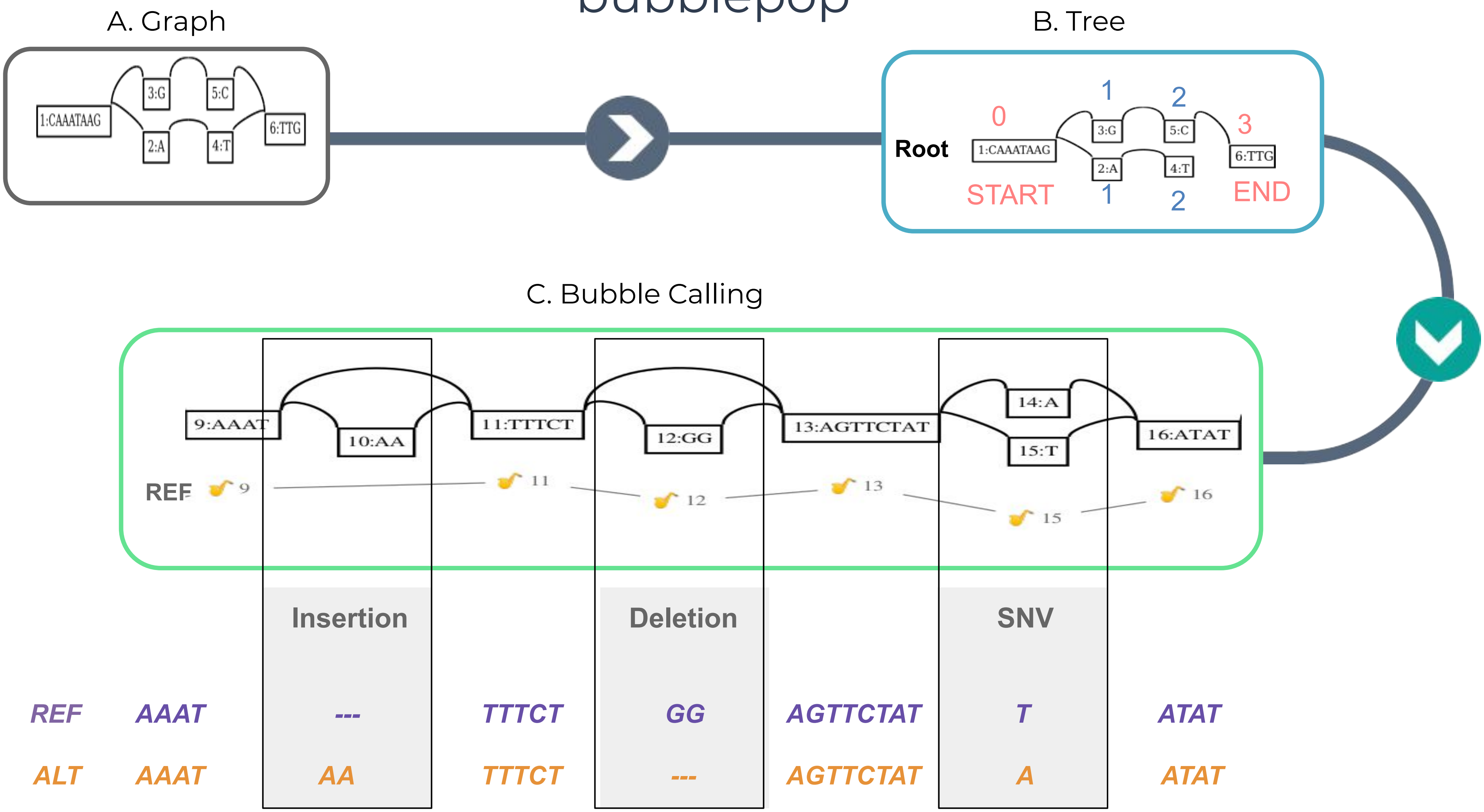
A. Graph



bubblepop


A. Graph
1:CAAATAAG
3:G
5:C
2:A
4:T
6:TTG
B. Tree
Root
0
1:CAAATAAG
START
1
3:G
2
5:C
3
6:TTG
END
2:A
4:T
1
2

bubblepop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0
1:CAAATAAG
START
1
3:G
2:A
1
2
5:C
4:T
2
3
6:TTG
END
C. Bubble Calling
9:AAAT
10:AA
11:TTTCT
12:GG
13:AGTTCTAT
14:A
15:T
16:ATAT
REF
9
11
12
13
15
16
Insertion
Deletion
SNV
REF AAAT --- TTTCT GG AGTTCTAT T ATAT
ALT AAAT AA TTTCT --- AGTTCTAT A ATAT

	pos1	pos2	pos3	pos4
PATH1	T	T	T	T
PATH2	A	G	A	T
PATH3	T	A	T	A
PATH4	A	T	A	A

Library vgpop

Parsing pangenome	Population genetics	Format conversion	Application
bubblepop	num_sequences num_segregatingsites allele_frequencies fst	gfa2vcf seqgen2gfa+vcf	Simulated data Real data: - HLA - Sars-Cov2

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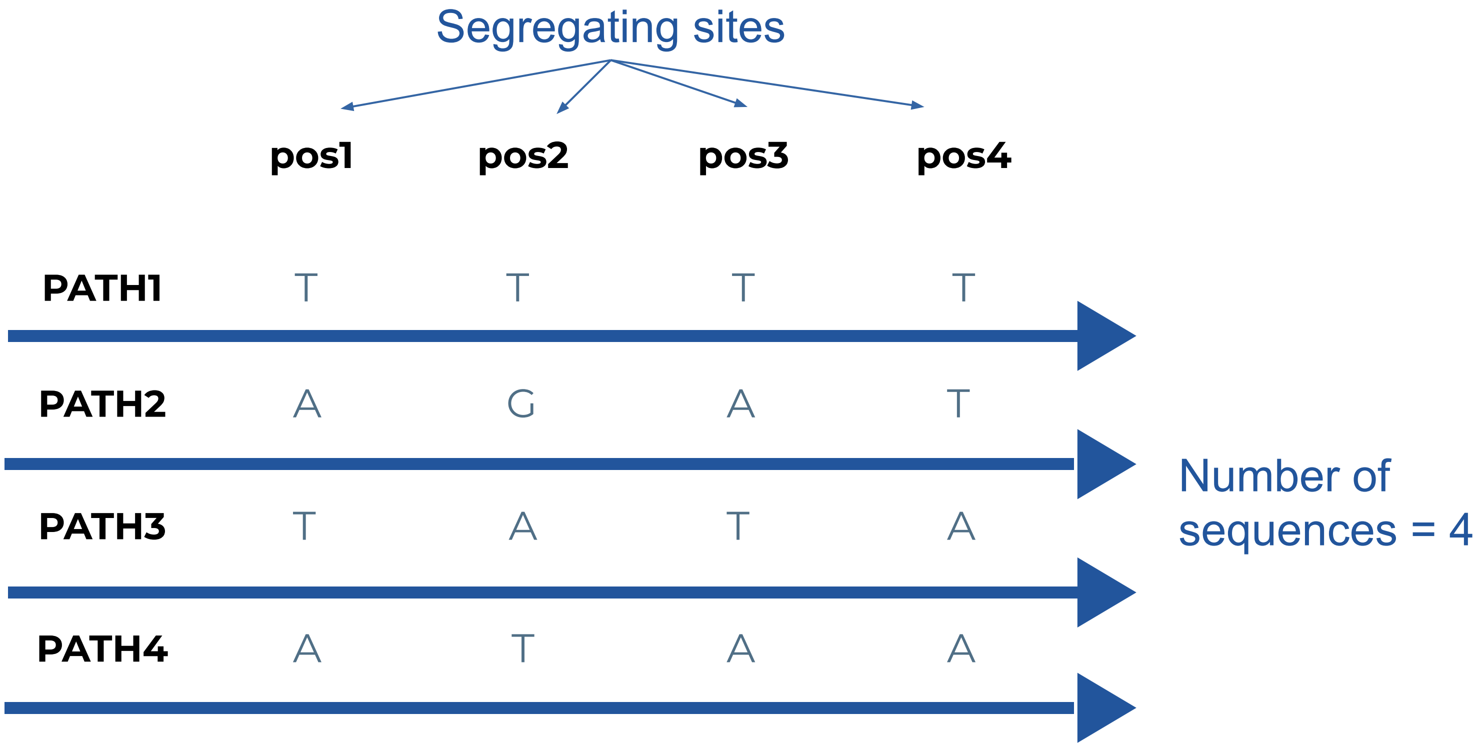
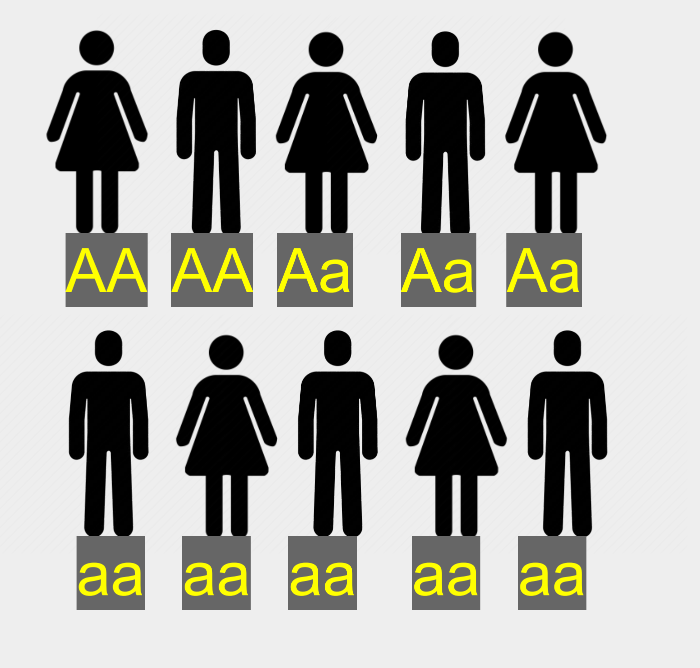
Segregation sites and sequences



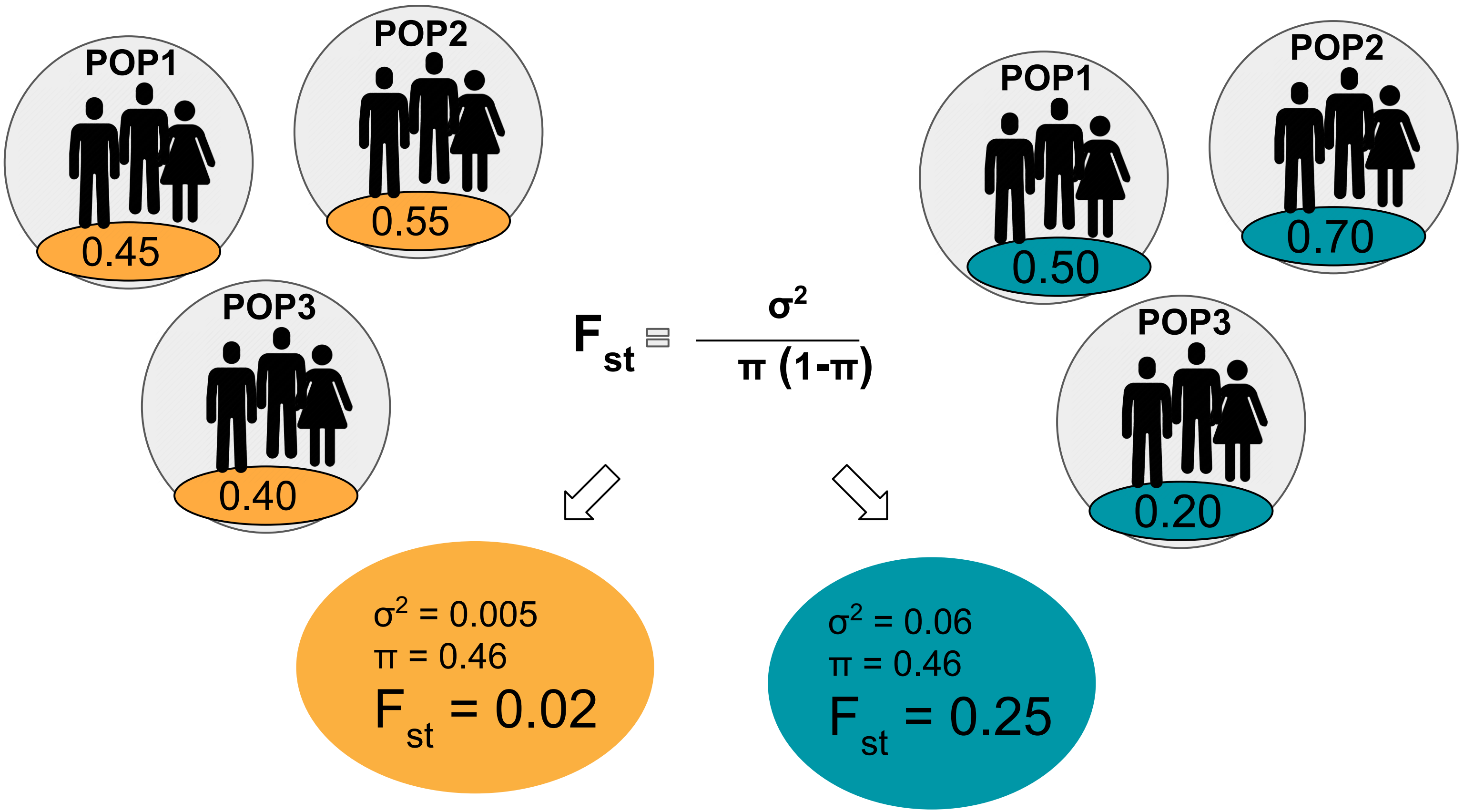
Allele frequencies

2N = 20 chromosomes
(APLOID)



ALLELE	**A**	**a**
ALLELE COUNTS	$n_A = 7$	$n_a = 13$
ALLELE FREQUENCIES	$f_A = \frac{n_A}{2N} = 0.35$	$f_a = \frac{n_a}{2N} = 0.65$

Wright's fixation index (F_{st})



Barbujani, G., & Colonna, V. (2010). Human genome diversity: frequently asked questions. *Trends in Genetics*, *26*(7), 285-295.

Library vgpop

Parsing pangenome

- bubblepop

Population genetics

- num_sequences
- num_segregatingsites
- allele_frequencies
- fst

Format conversion

- gfa2vcf
- seqgen2gfa+vcf

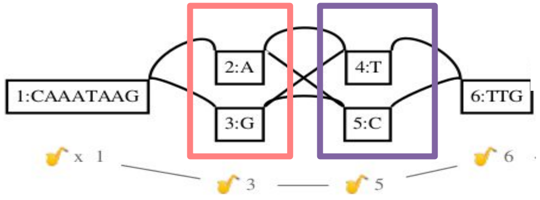
Application

- Simulated data
- Real data:
  - HLA
  - Sars-Cov2

https://github.com/Flavia95/VGpop

Format conversion

Pangenomic model (GFA)



gfa2vcf

Linear model (VCF)

#CHROM	POS	ID	REF	ALT	INFO
x	9	.	G	A	TYPE=snv
x	10	.	C	T	TYPE=snv

Simulation sequences (Seq-Gen)

2 10
Taxon1 ATCTTTGTAG
Taxon2 ATCCTAGTAG

seqgen2gfa+vcf

Pangenomic model (GFA)

H	VN:Z:1			
S	1	CACTA		
S	2	ATTA		
L	1	+	2	+ 0M
P	x	1+,2+	0M	

Linear model (VCF)

#CHROM	POS	ID	REF	ALT	INFO
x	2	.	G	A	TYPE=snv
x	3	.	C	T	TYPE=snv

Implementation of vgpop in Rust

Rust is a programming language focused on performance and safety.

- Great ***ecosystem*** (Cargo, crates.io, docs.rs).
- Much ***safer*** than C++ while having a similar ***speed.***
- Friendly and helpful ***community.***
- Used in many open source projects, such as **Firefox.**

https://www.rust-lang.org/

Francesco Porto
Gianluca Della Vedova

https://github.com/HopedWall/rs-gfatovcf

Library vgpop

Parsing pangenome

- bubblepop

Population genetics

- num_sequences
- num_segregatingsites
- allele_frequencies
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Format conversion

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Application

- Simulated data
- Real data:
  - HLA
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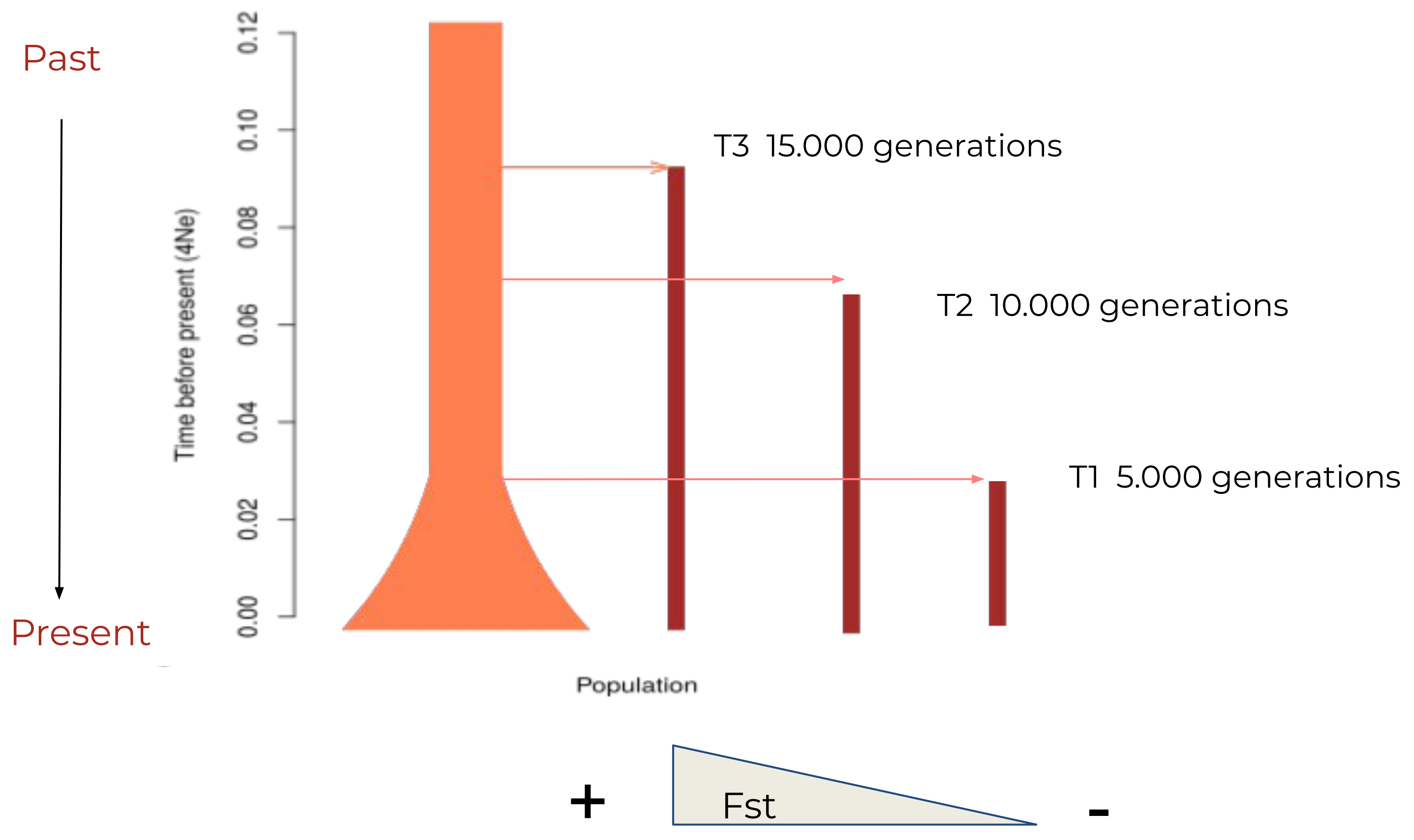
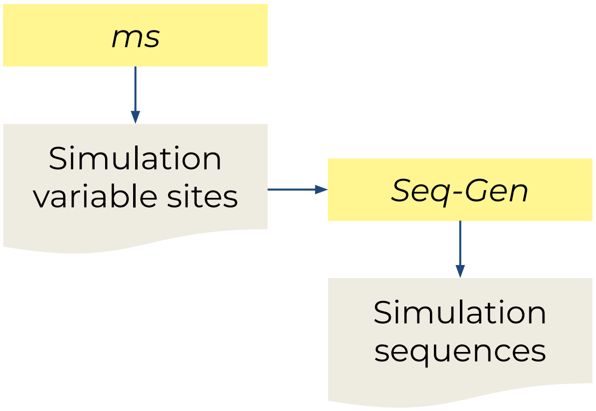
https://github.com/Flavia95/VGpop

F_{st} on simulated data

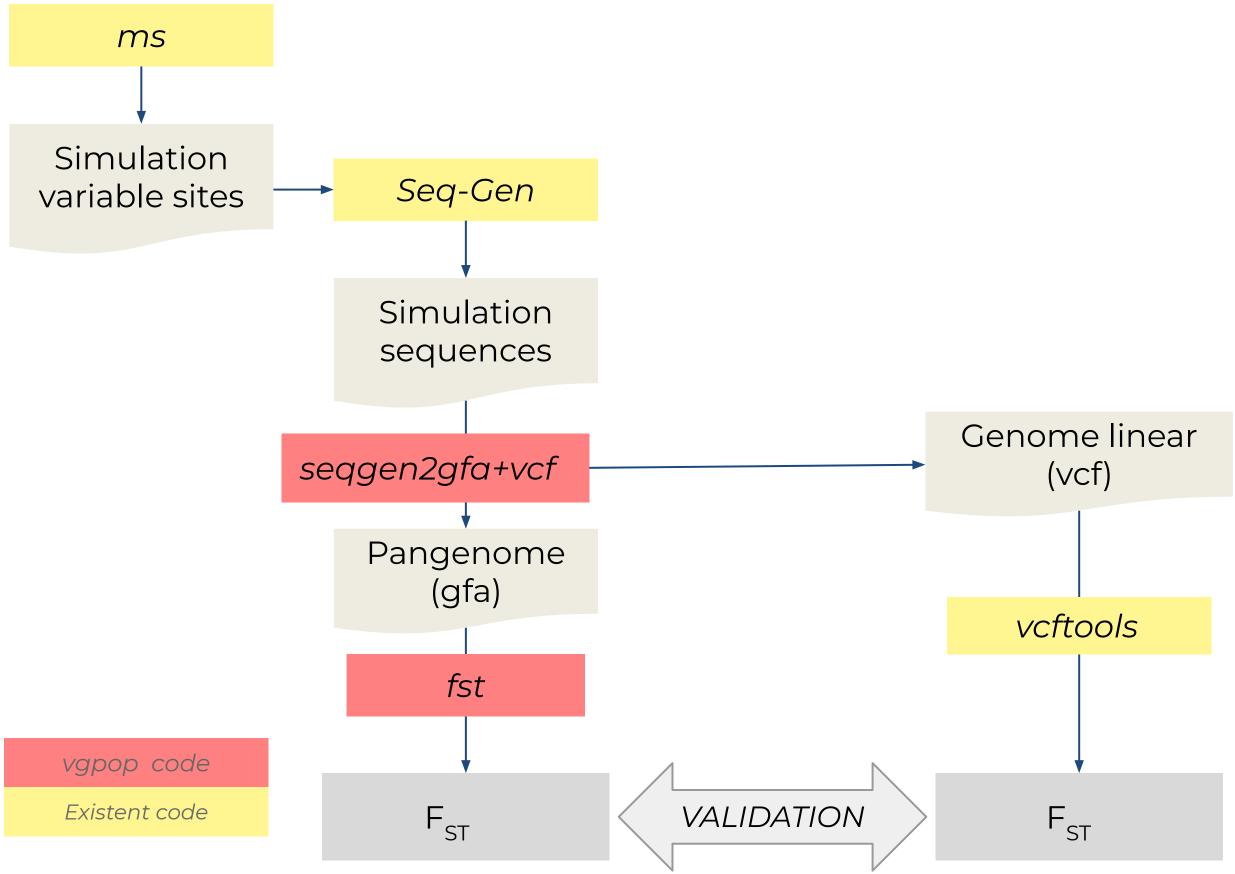


Workflow


ms
Simulation variable sites
Seq-Gen
Simulation sequences

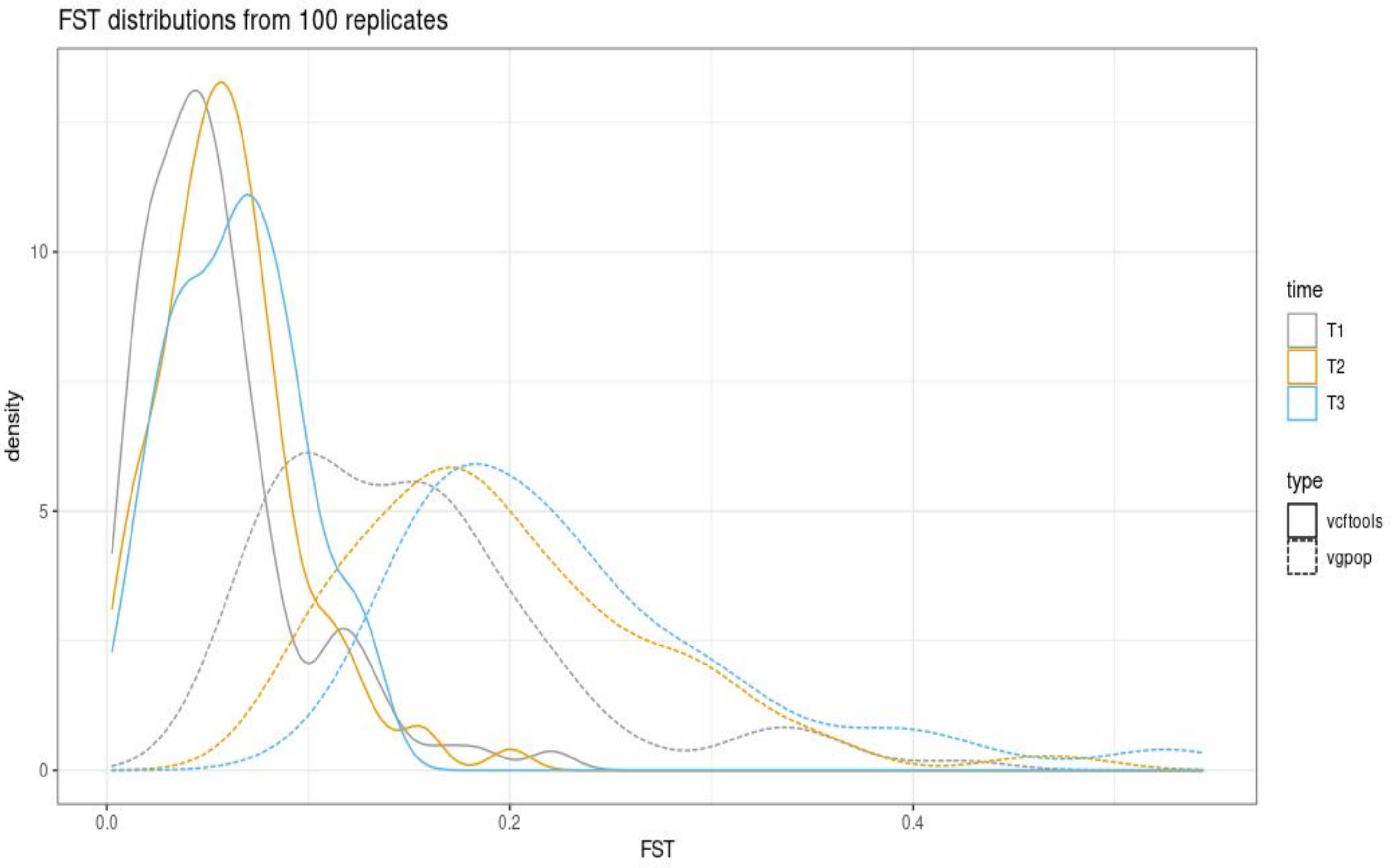

vgpop code
Existent code

Workflow



ms
Simulation variable sites
Seq-Gen
Simulation sequences
seqgen2gfa+vcf
Genome linear (vcf)
Pangenome (gfa)
vcftools
fst
vgpop code
Existent code
F_{ST}
VALIDATION
F_{ST}

F_{ST} on 100 replicate use *vgpop e vcftools*



allele_freq
fst

Library vgpop

Parsing pangenome

- bubblepop

Population genetics

- num_sequences
- num_segregatingsites
- allele_frequencies
- fst

Format conversion

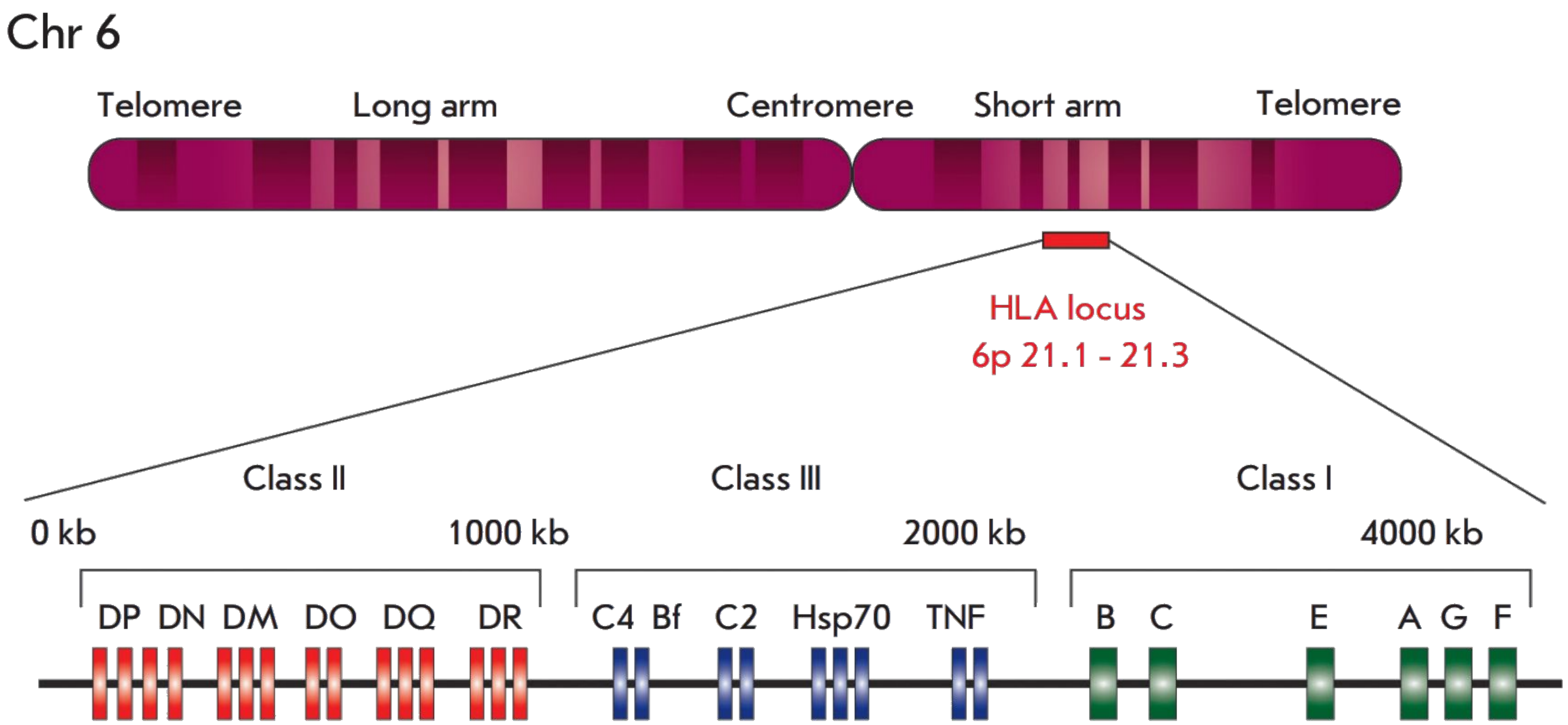
- gfa2vcf
- seqgen2gfa+vcf

Application

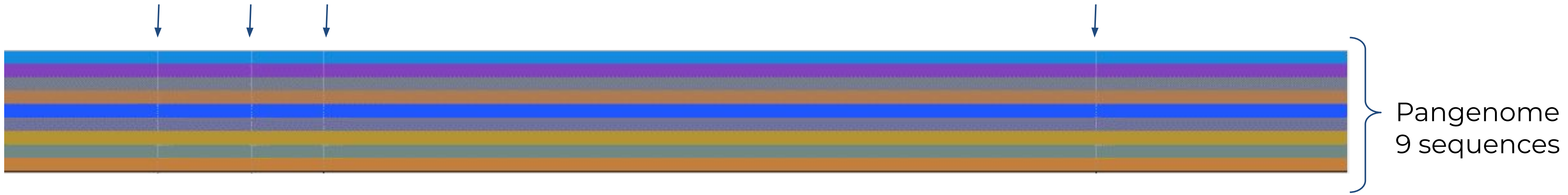
- Simulated data
- Real data:
  - HLA
  - Sars-Cov2

https://github.com/Flavia95/VGpop

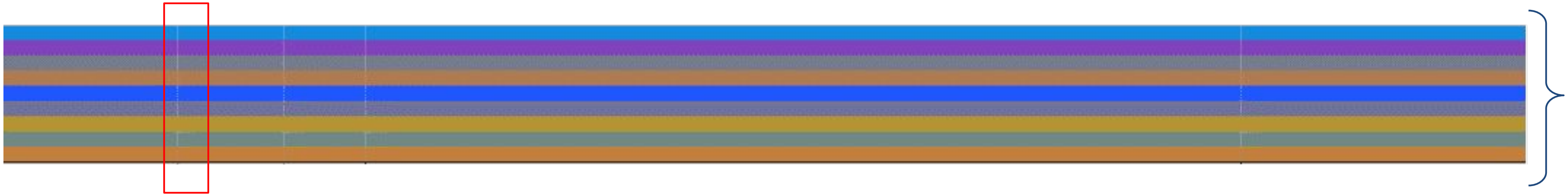
Allele frequencies on HLA



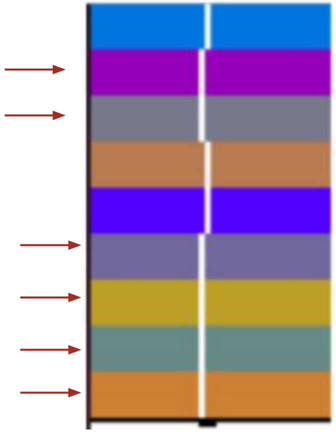
Gene HLA-E



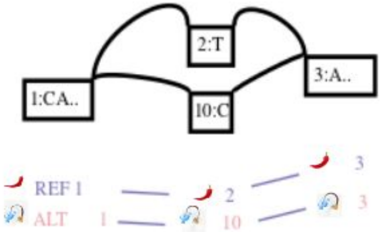
Gene HLA-E



Pangenome
9 sequences



REF



$$\text{Freq} = \frac{6}{9}$$

GENE	PANGENOME	POSITION	REF	ALT	FREQ
HLA-E	HLAE-3133	551	T	C	0.67

Variant discovery in HLA with rust implementation

- From 12 sequences
- Size: 163416 nucleotides
- Run time: ~0.1s
- Variants found: 7505

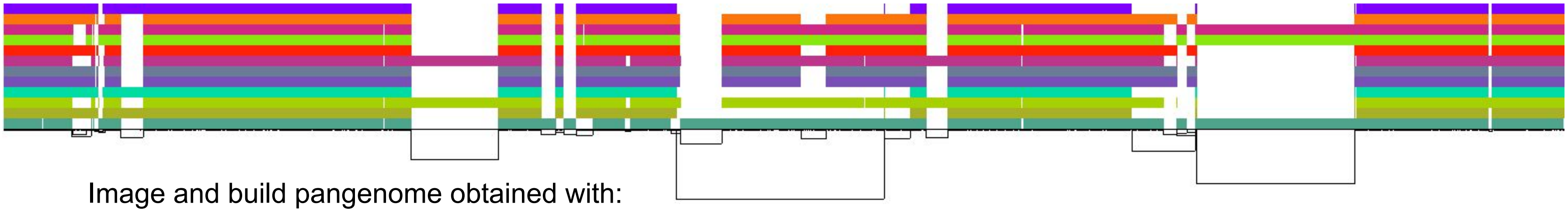
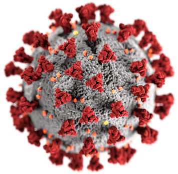


Image and build pangenome obtained with:
https://github.com/pangenome/pggb

Code available at:
https://github.com/HopedWall/rs-gfatovcf

Variant discovery in Sars-Cov2 with rust implementation

- From 15127 genomes
- 1.2 Gbytes
- 78571 fragments
- Run time: ~16m
- Variants found: 294626



COVID-19
PubSeq

Data available at
http://covid19.genenetwork.org/

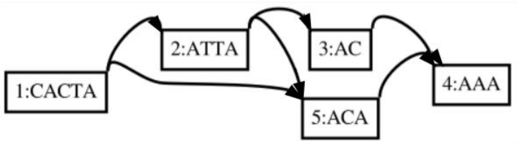
Andrea Guarracino
Pjotr Prins

Conclusion and next steps

vgpop

Software for population genetics analyses on pangenomes

Rust

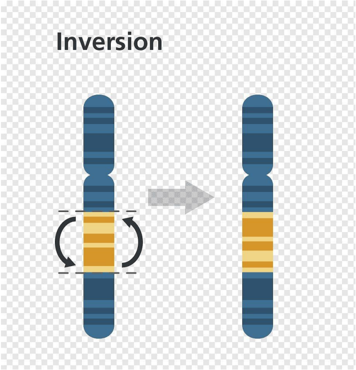


Adding parallel computing to increase performances

https://crates.io/crates/gfautil

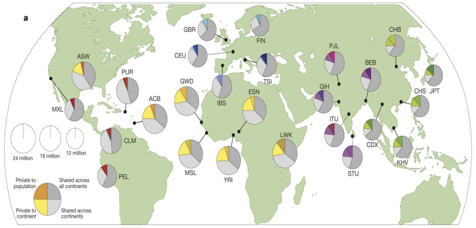
Structural variation

Little considered in the standard population genetics analysis



Population genomics analyses

Based on haplotype and on the differentiation of frequencies between populations



IGB-CNR (US)
Vincenza Colonna
Silvia Buonaiuto
Gianluca Damaggio
Giuliana D'Angelo

University of Milano Bicocca (Italy)
Francesco Porto
Gianluca Della Vedova

University of Rome Tor Vergata (Italy)
Andrea Guarracino

Department of Genetics, Genomics and Informatics (UTHSC)
Pjotr Prins
Robert W. Williams
Christian Fischer

UCSC (US)
Erik Garrison

THANKS FOR YOUR ATTENTION!