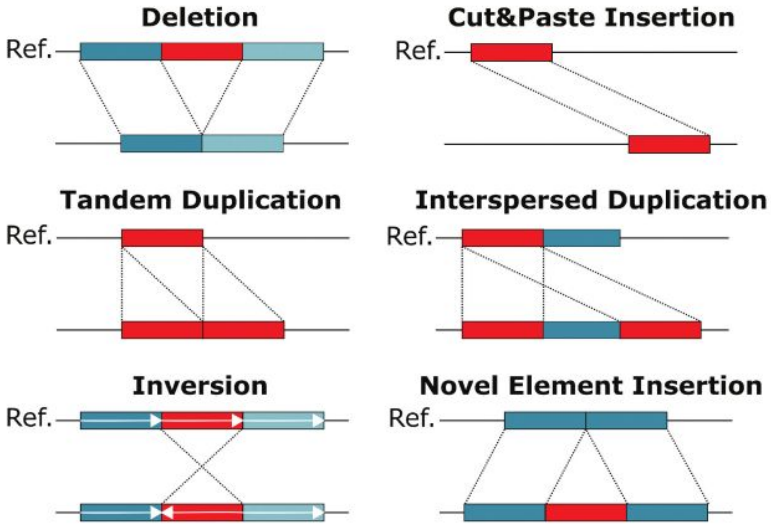
SVJedi-graph: using a variation graph to improve structural variant genotyping with long reads

Sandra ROMAIN [1], Claire Lemaitre [1]

SeqBIM 2022

INRIA, GenScale team, Rennes, France [1]

Structural variants

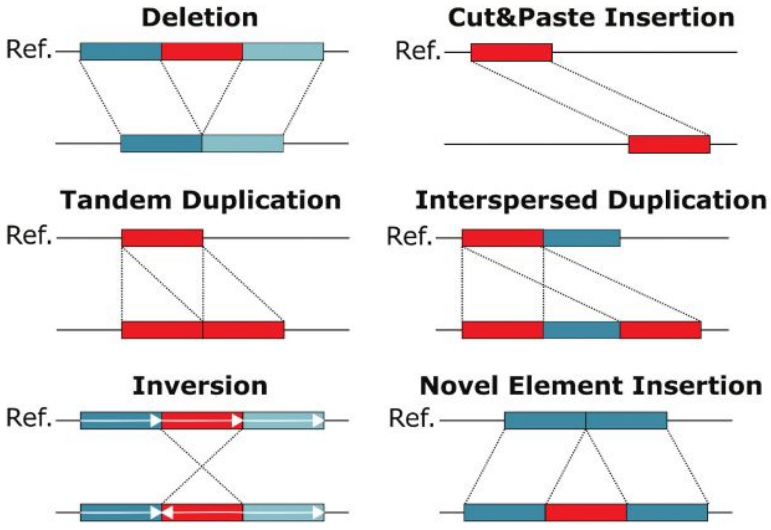


Heller and Vingron, 2019

Defined:

as	rearrangements ≥ 50 bp
relatively to	a reference genome
by	breakpoints sequence

Structural variants



Heller and Vingron, 2019

Defined:

as rearrangements ≥ 50 bp

relatively to a reference genome

by
- breakpoints
- sequence

Impact:

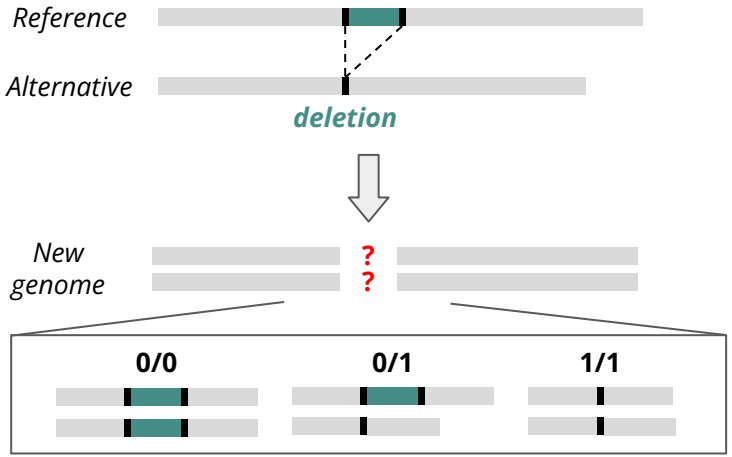
depends on genomic context

can lead to
- diseases
- polymorphism in agronomic key traits

Genotyping structural variants

- After SV identification
  - type
  - position
  - sequence for insertions

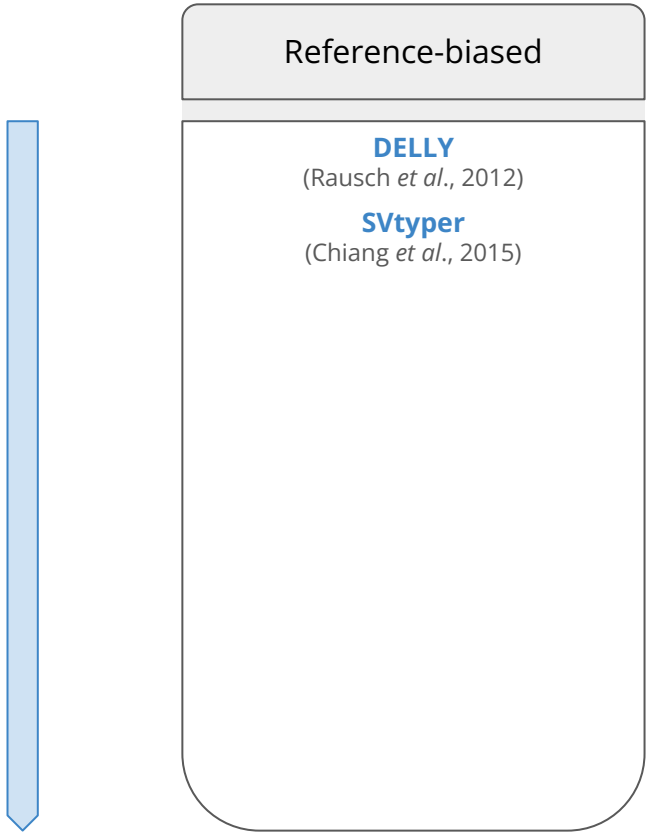
- Presence of the SVs on the haplotypes ?



Approach: Reads ⟺ Reference genome + SV description

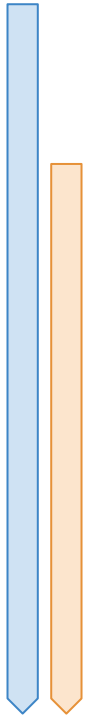
State of the art

- Short reads



State of the art

- Short reads
- Long reads



Reference-biased

DELLY
(Rausch *et al*., 2012)

SVtyper
(Chiang *et al*., 2015)

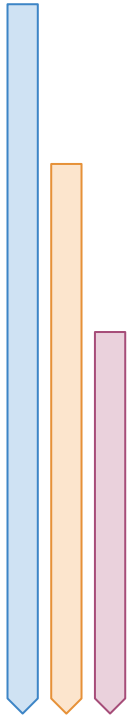
svviz2
(https://svviz2.readthedocs.io/en/latest/, 2017)

Sniffles
(Sedlazeck *et al*., 2018)

Sniffles2
(Smolka *et al*., 2022)

State of the art

- Short reads
- Long reads
- Reads compared both to the reference and alternative SV sequences



Reference-biased	Non reference-biased
DELLY (Rausch *et al.*, 2012)	
SVtyper (Chiang *et al.*, 2015)	
svviz2 (https://svviz2.readthedocs.io/en/latest/, 2017)	
Sniffles (Sedlazeck *et al.*, 2018)	
	Paragraph (Chen *et al.*, 2019)
	GraphTyper2 (Eggertsson *et al.*, 2019)
	SVJedi (Lecompte *et al.*, 2020)
	Giraffe (VG toolkit) (Sirén *et al.*, 2021)
	Pangenie (Ebler *et al.*, 2022)
Sniffles2 (Smolka *et al.*, 2022)	

State of the art

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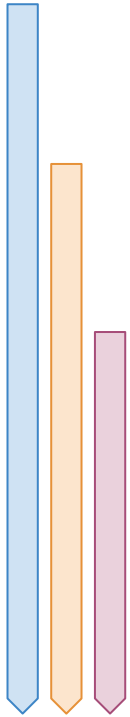

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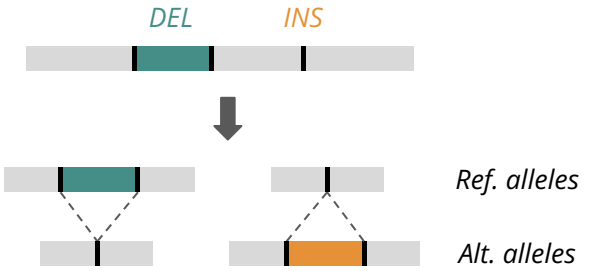


Principle: Representing both alleles for each SV in linear reference

→ *Avoid reference bias*

Tool	Genotyping accuracy	Genotyping rate	Time
SVJedi	**92.2**	90.3	**2h25m**
Sniffles -Ivcf	82.0	**99.8**	17h16m
svviz2	65.9	**100**	5days

from Lecompte et al., 2020

→ on a curated GIAB dataset (HG002 Tier 1 SVs)

Rate: % of SVs genotyped / all SVs

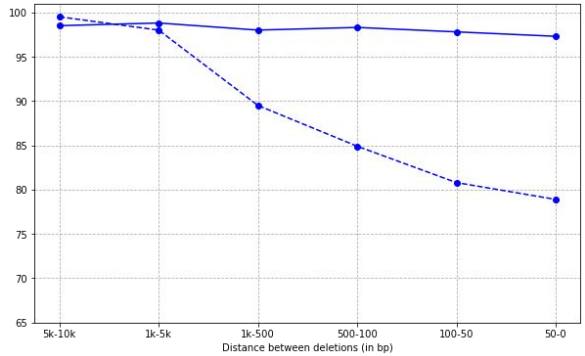
Accuracy: % of SVs accurately genotyped / genotyped SVs

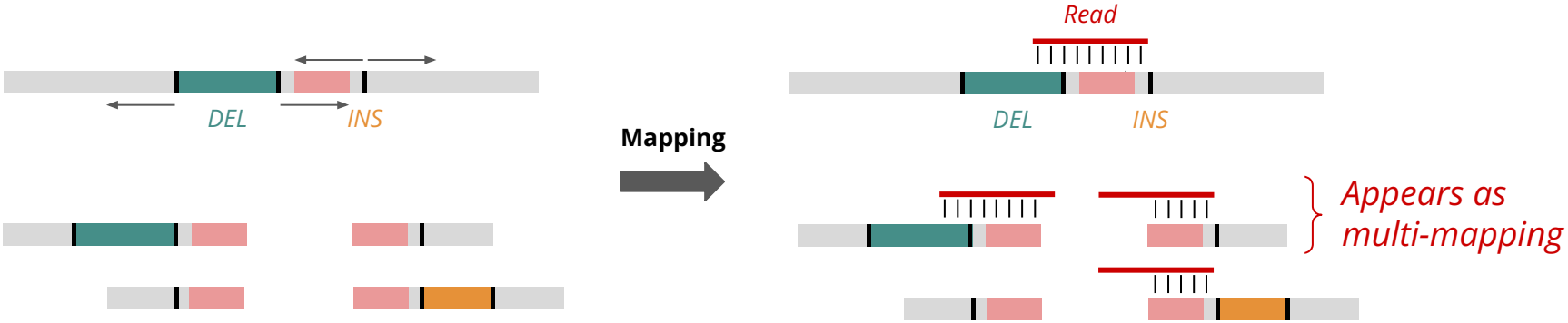
https://github.com/llecompte/SVJedi

SVJedi (Lecompte *et al*., 2020)

Limitation: Drop of genotyping rate with close/overlapping SVs

→ ⚠ *Sequence redundancy*





https://github.com/llecompte/SVJedi

Our contribution: SVJedi-graph

Long read SV genotyper using a variation graph representation

➢ Improve close SV genotyping by using a variation graph

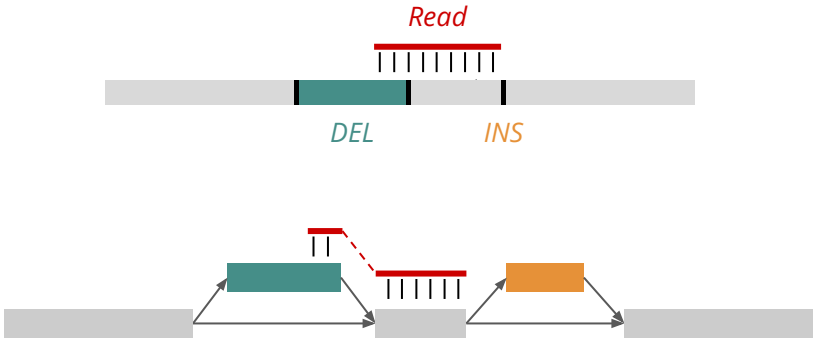
- nodes labelled with non-overlapping sequences
- bubble = variation
- a path in the graph = haplotype

➢ Represent the whole genome sequence

Our contribution: SVJedi-graph

Long read SV genotyper using a variation graph representation

➢ Improve close SV genotyping by using a variation graph

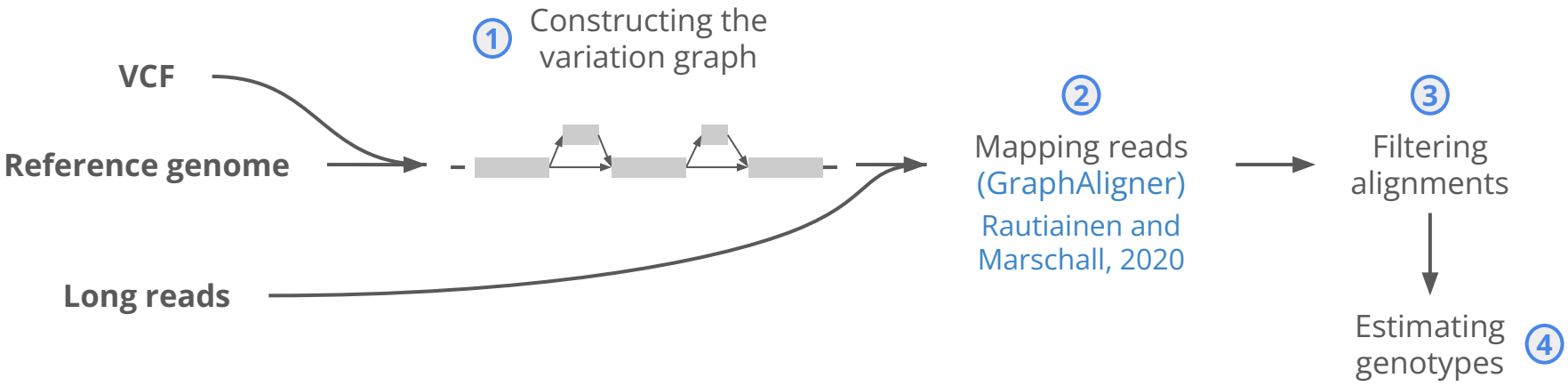


Only mapped once on the graph

Method

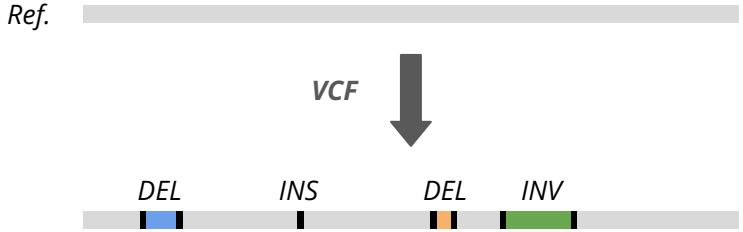
Overview of SVJedi-graph

Input: reference genome, SV set, long reads



Output: genotyped SV set

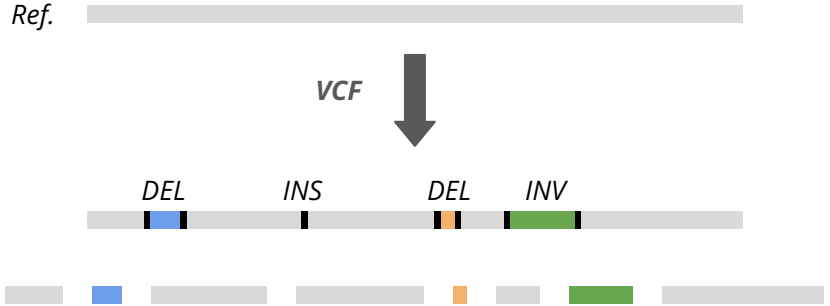
(1) Constructing the variation graph



For each chromosome:

1. List & sort breakpoint positions

(1) Constructing the variation graph

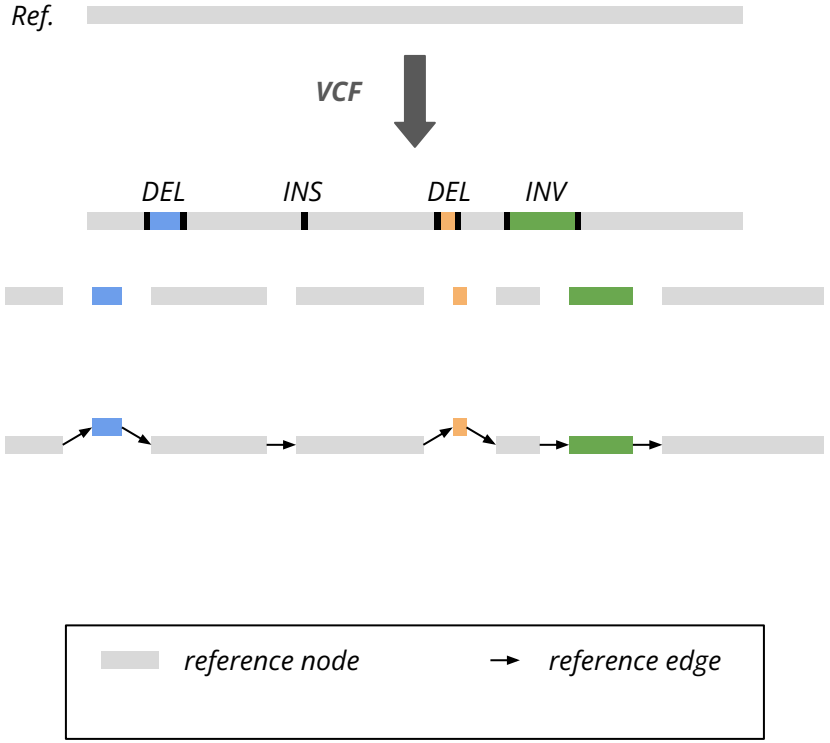


For each chromosome:

1. List & sort breakpoint positions
2. Split sequence at each breakpoint

1 fragment = 1 node

(1) Constructing the variation graph

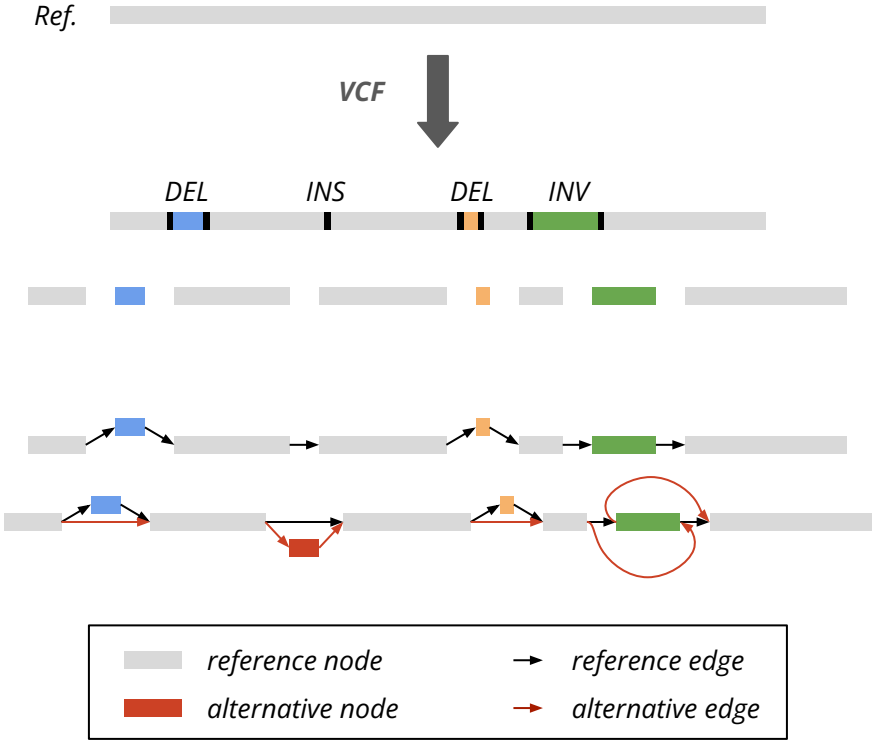


For each chromosome:

1. List & sort breakpoint positions
2. Split sequence at each breakpoint

   1 fragment = 1 node
3. Add reference edges

(1) Constructing the variation graph



For each chromosome:

1. List & sort breakpoint positions
2. Split sequence at each breakpoint

   1 fragment = 1 node
3. Add reference edges
4. Add alternative edges

   + alternative nodes for insertions

(2)(3) Mapping the reads and filtering the alignments

Mapping: GraphAligner (Rautiainen and Marschall, 2020)

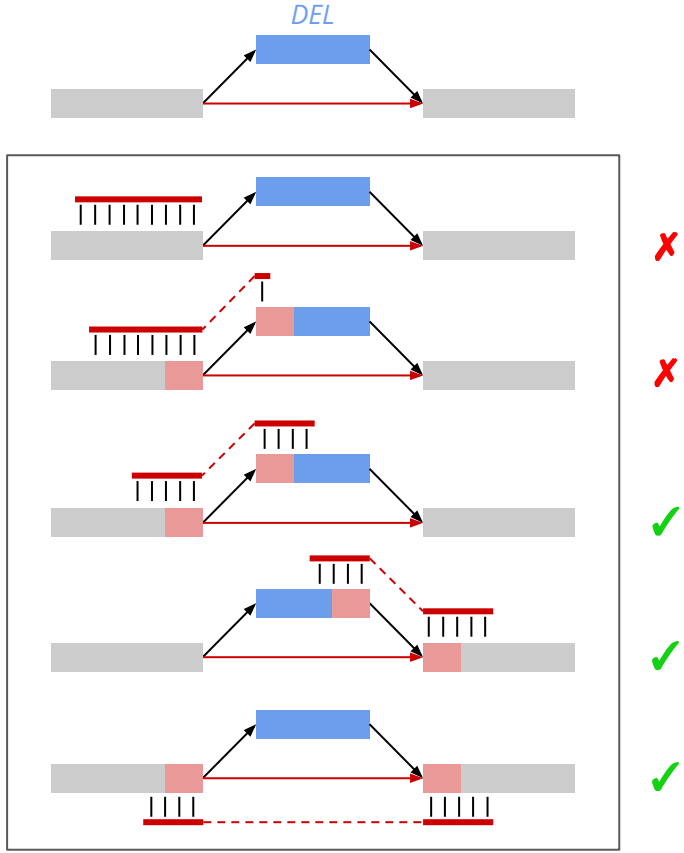
Alignments filters:

➢ Number of nodes in the alignment path ≥ 2

→ *Filtering alignments to analyze*

➢ Breakpoints overlap ≥ d_{over} *(100 bp)*

→ *Confidence in supported allele*



(4) Predicting the genotype

- Count supporting reads for each allele
- Normalize by breakpoint number
- Compute likelihood for each genotype

Reused from SVJedi (Lecompte et al., 2020)

Evaluation on simulated datasets

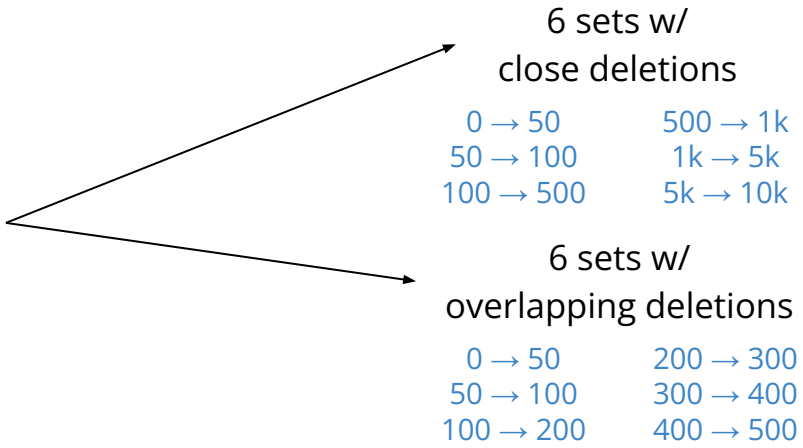
The simulated datasets

Reference: human chromosome 1 (GRCh37.p13)

SV sets generation:

- ➢ 1,000 deletions from dbVar
- ➢ + close/overlapping deletions

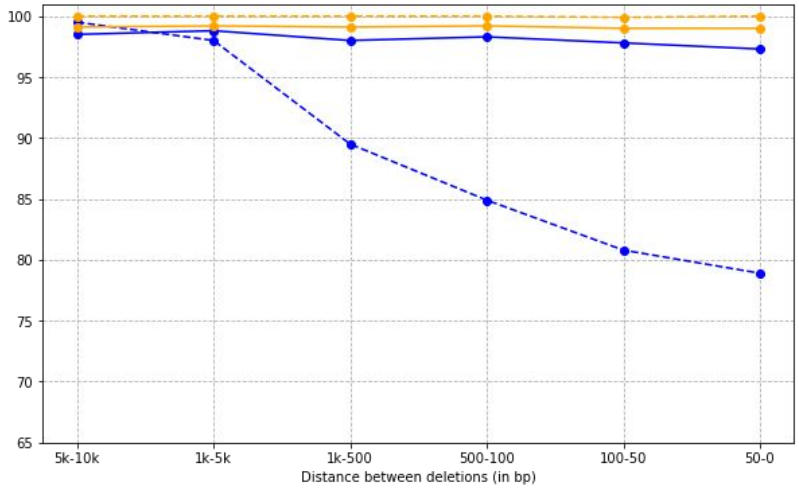
  (⅓ 0/0 - ⅓ 0/1 - ⅓ 1/1)



Reads simulation: PacBio, 16 % error rate (SimLoRD)

The simulated datasets - Results (SVJedi-graph)

Close deletions



genotyping accuracy

genotyping rate

SVJedi

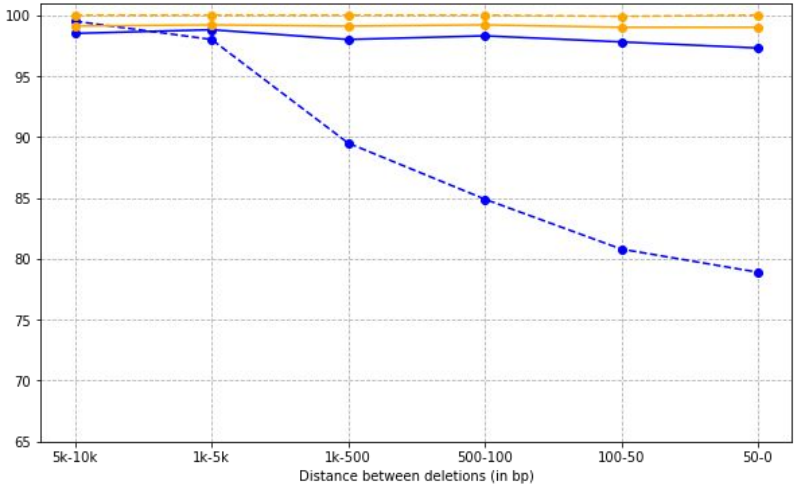
SVJedi-graph

Rate: % of SVs genotyped / all SVs

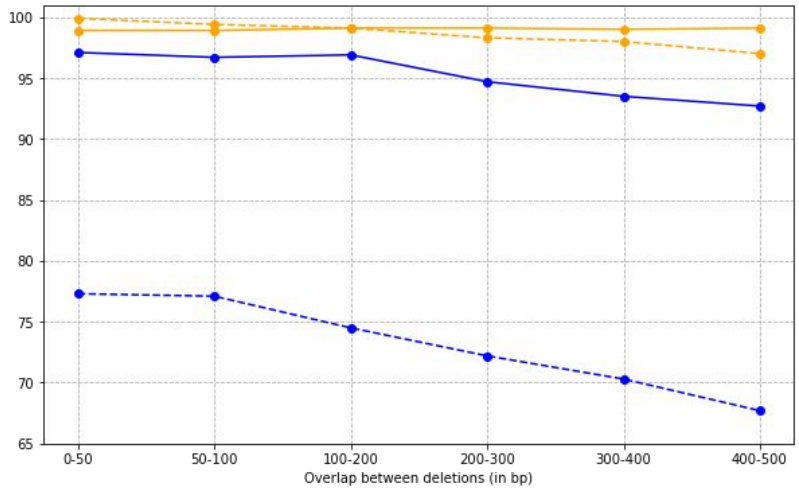
Accuracy: % of SVs accurately genotyped / genotyped SVs

The simulated datasets - Results (SVJedi-graph)

Close deletions



Overlapping deletions



genotyping accuracy

genotyping rate

SVJedi

SVJedi-graph

➢ **Recovery of genotyping rate**

Evaluation on real dataset

The GIAB dataset *(a clean and curated set)*

Reference: human reference genome (GRCh37.p13)

Reads: 40X CLR PacBio reads from HG002 (GIAB dataset)

SV set: HG002 Tier 1 (Zook *et al.*, 2019) → ***clean and curated set***

- ➢ 5,464 deletions
- ➢ 7,281 insertions

} *with ground truth genotypes*

The GIAB dataset - Results

Reference: human reference genome (GRCh37.p13)

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svviz2	65.9	**100**	5days
SVJedi-graph	**91.2**	**100**	15h28m

→ *Time cost of mapping on graph*

A more realistic dataset generated from GIAB data

Reference: human reference genome (GRCh37.p13)

Reads: 70X CLR PacBio reads from HG002 (GIAB dataset)

SV calling: Sniffles (Sedlazeck *et al*., 2018) with GIAB mapping results

- ➢ 7,922 deletions
- ➢ 9,529 insertions
- ➢ 202 inversions

Dataset	all SVs	“close” SVs
GIAB clean set	12,721	581 (4.6%)
Raw SV calling set	**17,624**	**2,205 (12.5%)**

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Dataset	all SVs	“close” SVs
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	Genotyping rate
SVJedi	51 %
SVJedi-graph	**98 %**

No accuracy

Concluding remarks

Implemented in python

Availability:


https://github.com/SandraLouise/SVJedi-graph


https://anaconda.org/bioconda/svjedi-graph

Work in progress:

Evaluation on a more difficult GIAB SV dataset

Improve read mapping time

Genotyping translocations

Acknowledgements

Access to computing cluster

And my PhD supervisors: Claire Lemaitre and Fabrice Legeai

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Genotype likelihood (SVJedi & SVJedi-graph)

$$\ell(0/0) = (1 - err)^{c_0^*} \times err^{c_1} \times C^{c_0^*}_{c_0^*+c_1}$$

$$\ell(1/1) = err^{c_0^*} \times (1 - err)^{c_1} \times C^{c_0^*}_{c_0^*+c_1}$$

$$\ell(0/1) = \left(\tfrac{1}{2}\right)^{c_0^*+c_1} \times C^{c_0^*}_{c_0^*+c_1}$$

from SAMtools (Danecek et al., 2021)

- C_0 read count on allele 0
- C_1 read count on allele 1
- err sequencing error