Genotyping structural variation in variation graphs with the vg toolkit

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Authors

Glenn Hickey[1,©], David Heller[1,©], Jean Monlong[1,©], Adam Novak[1], Benedict Paten[1,†]

© — These authors contributed equally to this work

† — To whom correspondence should be addressed: bpaten@ucsc.edu

1. UC Santa Cruz Genomics Institute, University of California, Santa Cruz, California, USA

Abstract

Variation graphs have been proposed to represent human genomes and improve on different aspects of genomics analysis, such as read mapping and variant calling. Structural variants (SVs) have been associated with diseases but they remain under-studied due to their complexity and certain technological challenges. Still, thousands of SVs have been characterized and better catalogs are now being generated thanks to new technologies. There is now an opportunity to integrate previously neglected SVs into the unified framework of variation graphs. We first show that the vg toolkit is capable of genotyping insertions, deletions and inversions, even in the presence of small errors in the variant definition. We then benchmarked vg across three high-quality sequence-resolved SV catalogs generated by recent studies. vg was compared to state-of-the-art SV genotypers using simulated and real Illumina short reads. On real data, vg produced the best genotype predictions systematically in all datasets. Finally, we found that better graphs could be constructed directly from de novo assembly alignment. We experimented with assemblies from 12 yeast strains and showed that SV genotyping was improved compared to graphs built from intermediate SV catalogs in the VCF format. Our results demonstrate the power of variation graphs for SV genotyping. Beyond single nucleotide variants and short insertions/deletions, the vg toolkit now includes SV in its unified variant calling framework and provides a natural solution to integrate high-quality SV catalogs and assemblies.

Introduction

Structural variation (SV) represents genomic mutation involving 50 bp or more and can take several forms, such as for example deletions, insertions, inversions, or translocations. SVs have long been associated with developmental disorders, cancer and other complex diseases and phenotypes[1]. However, SVs have been under-studied for technological reasons and due to their complexity as compared to other types of genomic variation. Although whole-genome sequencing (WGS) made it possible to assess virtually any type of structural variation, many challenges remain. SV-supporting reads are generally difficult to map to reference genomes, in part because most SVs are larger than the sequencing reads. Repeated sequences in the genome often confuse read mapping algorithms, which can produce mappings that seem to support an SV. In practice, large-scale projects had to combine several methods to achieve better accuracy. This methodology has been used to compile catalogs with tens of thousands of SVs in humans[2,3]. Overall, SV detection from short-read sequencing remains laborious and of lower accuracy than small variant detection. This explains why these variants and their impact have been under-studied as compared to single-nucleotide variants (SNVs) and small insertions/deletions (indels).

Over the last few years, exciting developments in sequencing technologies and library preparation have made it possible to produce long reads or retrieve long-range information over kilobases of sequence. This is particularly useful for SV detection and de novo assembly. Several recent studies using long-read or linked-read sequencing have produced large catalogs of structural variation, the majority of which was novel and sequence-resolved[4,5,6,7,8]. These technologies are also enabling the production of high-quality de novo genome assemblies[4,9], and large blocks of haplotype-resolved sequences[10]. Such technical advances promise to expand the amount of known genomic variation in humans in the near future. However, their cost prohibits their use in large-scale studies that require hundreds or thousands of samples, such as disease association studies.

At the same time, the reference genome is evolving from a linear reference to a graph-based reference that incorporates known genomic variation[11,12,13]. By including variants in the graph, both read mapping and variant calling become variant-aware and benefit in term of accuracy and sensitivity[12]. In addition, different variant types are called simultaneously by a unified framework. vg was the first openly available tool that scaled to multi-gigabase genomes and provides read mapping, variant calling and haplotype modeling[12]. In vg, graphs can be built from both variant catalogs in the VCF format or assembly alignment. Other genome graph implementations have also been used specifically to genotype variants. Using a sliding-window approach, GraphTyper realigns reads to a graph build from known SNVs and short indels[14]. BayesTyper build graphs of both short variants and SVs, and genotypes variants based on the khmer distribution of sequencing reads[15]. Here again, the graph-based approaches showed clear advantages over standard methods that use the linear reference.

Other SV genotyping approaches typically compare read mapping to the reference genome and to a sequence modified with the SV. For example SMRT-SV was designed to genotype SVs identified on PacBio reads[5]. SVTyper uses paired-end mapping and split-read mapping information and can genotype deletions, duplications, inversions and translocations[16]. Delly provides a genotyping feature in addition to its discovery mode and can genotype all types of SVs although the VCF needs special formatting for some[17]. SMRT-SV2 is a machine learning tool that was trained to genotype SVs from the alignment of read to the reference genome augmented with SV-containing sequences as alternate contigs[8].

We show that the unified variant calling framework implemented in vg is capable of genotyping deletions, insertions and inversions. We compare vg with state-of-the-art SV genotypers: SVTyper[16], Delly[17], BayesTyper[15] and SMRT-SV2[8]. On simulation, vg is robust to small errors in the breakpoint location and outperforms most other methods on shallow sequencing experiments. Starting from SVs discovered in recent long-read sequencing studies[18,19,20,8], we evaluated the genotyping accuracy when using simulated or real Illumina reads. Across all three datasets that we tested, vg is the best performing SV genotyper on real short-read data for all SV types and sizes. Going further, we show that building graphs from the alignment of de novo assemblies leads to better genotyping performance.

Results

Structural variation in vg

In addition to SNV and short indels, vg can handle large deletions, insertions and inversions (Figure 1a). As a proof-of-concept we simulated genomes and different types of SVs with a size distribution matching real SVs[18]. We compared vg against SVTyper, Delly and BayesTyper across different level of sequencing depth. Some errors were also added at the breakpoints to investigate their effect on genotyping (see Methods). The results are shown in Figure 1b. When using the correct breakpoints, vg tied with Delly as the best genotyper for deletions, and with BayesTyper as the best genotyper for insertions. For inversions, vg was the second best genotyper after BayesTyper. The differences between the methods were the most visible at lower sequencing depth. In the presence of 1-10 bp errors in the breakpoint location, the performance of Delly and BayesTyper dropped significantly. The dramatic drop for BayesTyper can be explained by its khmer-based approach that requires exact SV definition. In contrast, vg was only slightly affected by the presence of errors in the input VCF (Figure 1b). For vg, the F1 scores for all SV types decreased no more than of 0.07 point. Overall, these results show that vg is capable of genotyping SVs and is robust to errors in the input VCF.
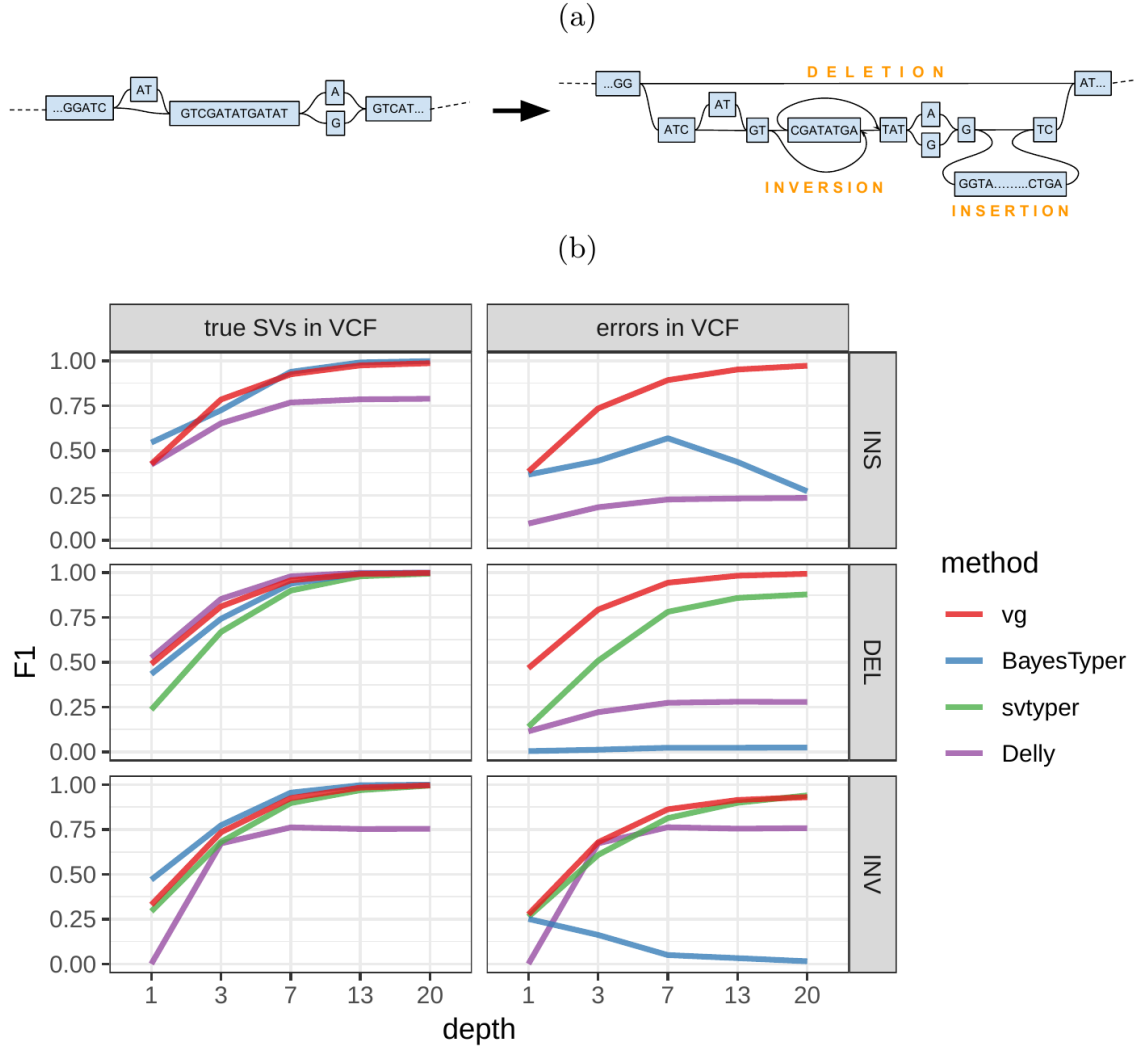
Figure 1: **Structural variation in vg.** a) Adding large deletions and insertions in a variation graph. b) Simulation experiment. For each experiment (method, depth and input VCF with/without errors), the maximum F1 was picked when using different quality thresholds, and is reported on the y-axis.
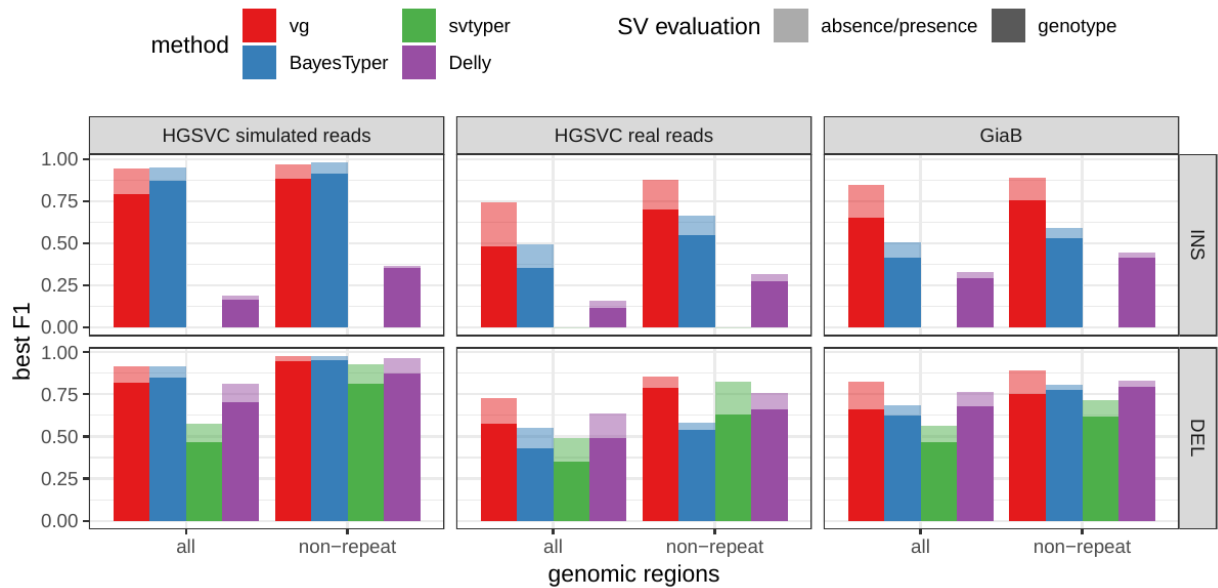
HGSVC dataset

The Human Genome Structural Variation Consoritum (HGSVC) generated a high-quality SV catalog of three samples, obtained using a consensus from different sequencing, phasing and variant caling technologies[18]. The three samples come from different human populations: a han Chinese individual (HG00514), a Puerto-Rican individual (HG00733), and a Yoruban Nigerian individual (NA19240). These SVs were used to construct a graph with vg and as input for the other genotypers. SVs were genotyped from short reads and compared with the original catalog (see Methods).

First, by simulating reads for HG00514, we compared the different methods in the ideal situation where the SV catalog is correct and matches exactly the SVs supported by the reads. While vg outperformed Delly and SVTyper, BayesTyper showed the best F1 score and precision-recall trade-

off (Figures 2 and S1, Table S1). When restricting the comparisons to regions not identified as tandem repeats or segmental duplications, the genotyping predictions were significantly better for all methods, with vg almost as good as BayesTyper on deletions (F1 of 0.944 vs 0.955). We observed similar results when evaluating the absence/presence of a SV instead of the exact genotype (Figures 2 and S2). Overall, both graph-based methods, vg and BayesTyper, outperformed the two other methods tested.

We then repeated the analysis using real Illumina reads from HG00514, to benchmark the methods on a more realistic experiment. Here vg clearly outperformed other approach, most likely because of its graph-based strategy and robustness to errors in the SV catalog (Figures 2 and S3). In non-repeat regions and across the whole genome, the F1 scores and precision-recall curves were higher for vg compared to other methods. For example, for deletions in non-repeat regions, the F1 score for vg was 0.801 while the second best method, Delly, had a F1 score of 0.692. We observed similar results when evaluating the absence/presence of a SV instead of the exact genotype (Figures 2 and S4).

Figure 2: **Structural variants from the HGSVC and Genome in a Bottle datasets**. HGSVC: Simulated and real reads were used to genotype SVs and compared with the high-quality calls from Chaisson et al.[18]. Reads were simulated from the HG00514 individual. Using real reads, the three HG00514, HG00733, and NA19240 individuals were tested. GiaB: Real reads from the HG002 individual were used to genotype SVs and compared with the high-quality calls from the Genome in a Bottle consortium.[19,20]. Maximum F1 score for each method (color), across the whole genome or focusing on non-repeat regions (x-axis). The calling and genotyping evaluation are shown with different shades.

Other long-read datasets

The Genome in a Bottle (GiaB) consortium is currently producing a high-quality SV catalog for a Ashkenazim individual (HG002)[19,20]. Dozens of SV callers and datasets from short, long and linked reads were used to produce this set of SVs. vg performed similarly on this dataset than in the HGSVC dataset, with a F1 score of 0.75 for both insertions and deletions in non-repeat regions (Figures 2, S5 and S6, and Table S2). As before, other methods produced lower F1 scores in most cases, although Delly and BayesTyper predicted better genotype for deletions in non-repeat regions.

A recent study by Audano et al. generated a SV catalog using long-read sequencing across 15 individuals [8]. These variants were then genotyped from short reads across 440 individuals using SMRT-SV2, a machine-learning genotyper implemented for this study. We first called SVs from the pseudo-diploid genome and reads used to train SMRT-SV2 and constructed by merging datasets from two haploid cell lines[8]. The absence/presence predictions from vg were systematically better than SMRT-SV2 for both SV types but SMRT-SV2 produced better genotypes for deletions (see Figures 3, S7 and S10, and Table S3). Using publicly available Illumina reads, we then genotyped SVs in 3 of the 15 individuals that were used for discovery in Audano et al.[8]. Compared to SMRT-SV2, vg had a better precision-recall curve and a higher F1 for both insertions and deletions (Figures 3 and S11, and Table S4). Of note, Audano et al. had identified 217 sequence-resolved inversions. vg correctly predicted the presence of around 14% of the inversions present in the three samples (Table S4). Inversions are often complex, harboring additional variation that makes their characterization and genotyping challenging.
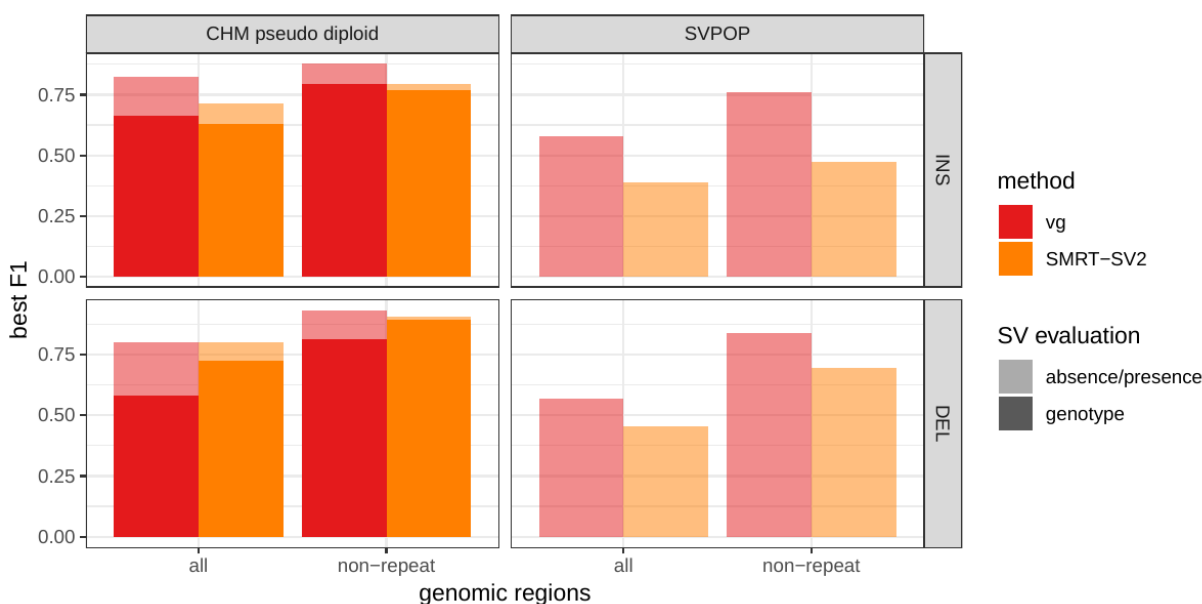
Figure 3: **Structural variants from Audano et al.[8]**. The pseudo-diploid genome built from CHM cell lines was used originally used to train SMRT-SV2 in Audano et al.[8]. The SVPOP panel shows the combined results for the HG5014, HG00733 and NA19240 individuals, 3 of the 15 individuals used to generate the high-quality SV catalog in Audano et al.[8]. Maximum F1 score for each method (color), across the whole genome or focusing on non-repeat regions (x-axis). The calling and genotyping evaluation are shown with different shades.

Breakpoint fine-tuning

Maybe better in discussion.

In addition to genotyping, vg can be used in "call" mode and use an augmentation step to modify the graph based on the read alignment. On the simulated SVs from Figure 1b, this approach was able to correct the many errors in the input VCF. The breakpoints were accurately fine-tuned for 93.8% of the insertions (Figure S12a and Table S5). For deletions, 78.1% of the variants were corrected when only one breakpoint had an error. In situations where both breakpoints of the deletions were incorrect, only 18.6% were correctly fine-tuned, and only when the amount of error was small (Figure S12b). The breakpoints of less than 20% of the inversions could be corrected. Across all SV types, the size of the variant didn't affect the ability to fine-tune the breakpoints throught graph augmentation (Figure S12c).

Genotyping SV using vg and de novo assemblies

We investigated whether genome graphs derived from genome-genome alignments yield advantages for SV genotyping. To this end, we analyzed public sequencing datasets for 12 yeast strains from two clades (S. cerevisiae and S. paradoxus) [21]. From these datasets, we generated two different types of genome graphs. The first graph type (in the following called *construct graph*) was created from a linear reference genome of the S.c. S288C strain and a set of SVs relative to

this reference strain in VCF format. We compiled the SV set using the output of three methods for SV detection from genome assemblies: Assemblytics [22], AsmVar [23] and paftools [24]. All three methods were run to detect SVs between the reference strain S.c. S288C and each of the other 11 strains. Merging the results from the three methods and the 11 strains provided us with a high-sensitivity set of SVs occuring in the two yeast clades. We used this set to construct the *construct graph*. The second graph (in the following called *cactus graph*) was derived from a multiple genome alignment of all 12 strains using our Cactus tool [25]. While the *construct graph* is still mainly linear and highly dependent on the reference genome, the cactus graph is completely unbiased in that regard.
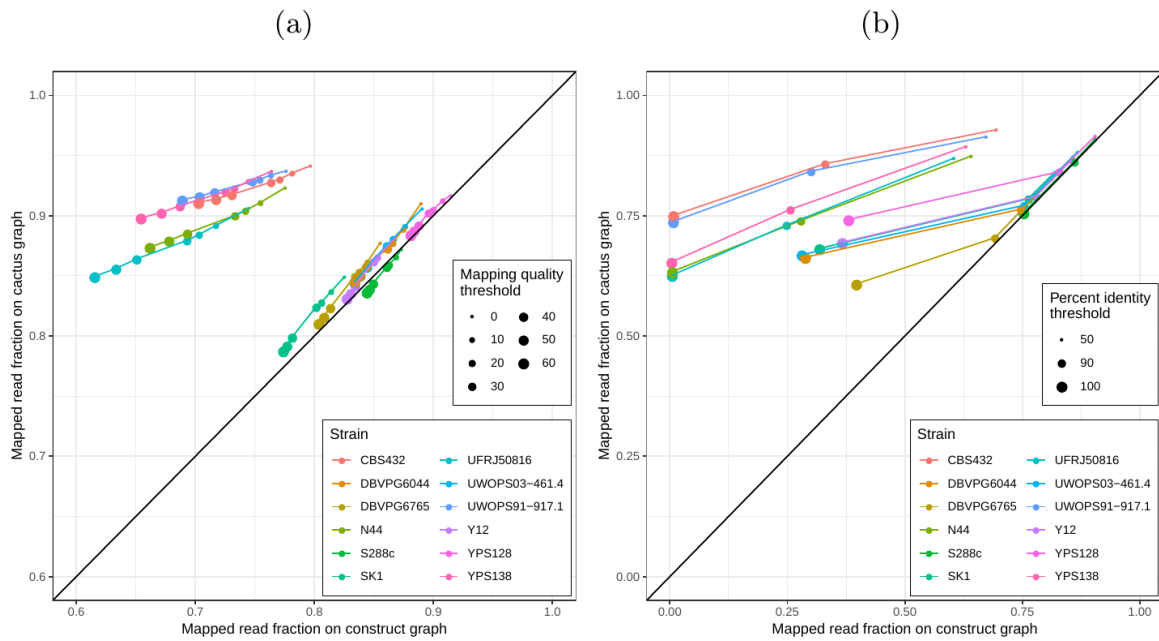
Figure 4: **Mapping comparison.** The fraction of reads mapped to the cactus graph (y-axis) and the construct graph (x-axis) are compared. a) Stratified by mapping quality threshold. b) Stratified by percent identity threshold.

In a first step, we tested our hypothesis that the *cactus graph* has higher mappability due to its better representation of sequence diversity among the yeast strains. Figure 4a shows the fraction of Illumina reads from the 12 strains that was mapped with a mapping quality above a certain threshold to the *cactus graph* and to the *construct graph*. Generally, more reads were mapped to the *cactus graph* than to the *construct graph* regardless of the chosen mapping quality threshold. Only for the reference strain S.c. S288C, both graphs exhibited similar mappability. This suggests that not the higher sequence content in the *cactus graph* alone (XX Mb compared to XX Mb in the *construct graph*) drives the improvement in mappability. Instead, our measurements suggest that genetic distance to the reference strain increases the advantage of the *cactus graph* over the *construct graph*. Consequently. the benefit of the *cactus graph* is largest for strains in the S. paradoxus clade and smaller for reads from strains in the S. cerevisiae clade.

When we explored the mapping identity of the short reads on the graphs, we observed a similar trend (see Figure 4b). For strains in the S. paradoxus clade, the *cactus graph* enabled substantially more mappings with high percent identity than the *construct graph*. With strains in the S. cerevisiae clade, the difference was smaller, at least for a percent identity threshold up to 90%. When comparing read fractions with perfect identity (i.e. percent identity threshold = 100%), the *cactus graph* clearly outperforms the *construct graph* on 11 out of 12 samples. The only exception again is the reference strain S288C.
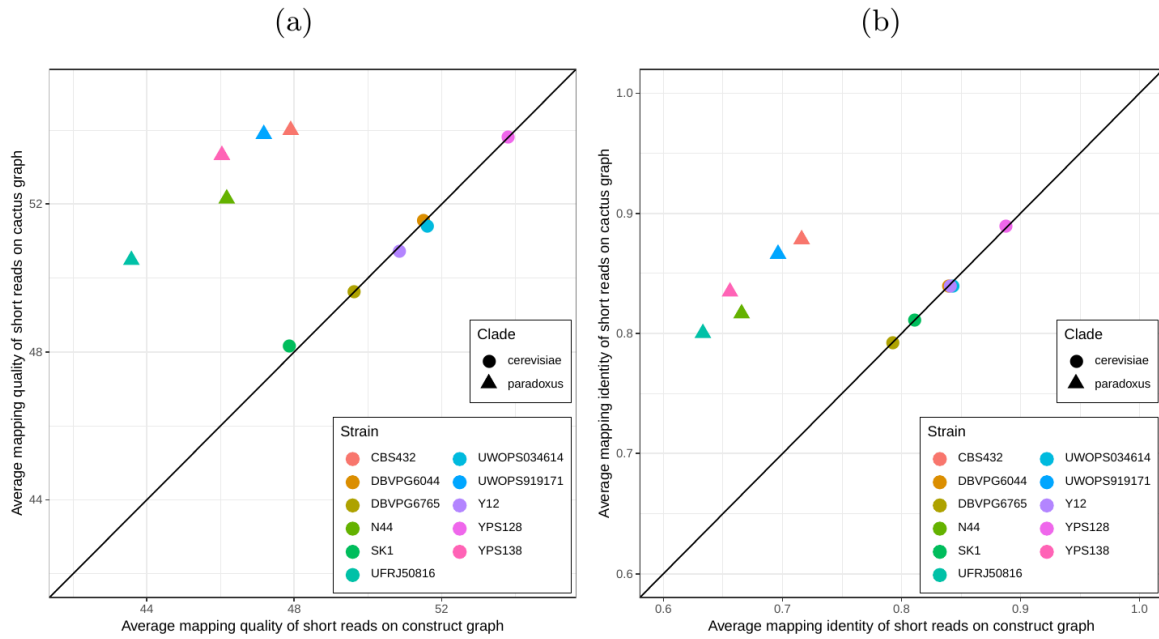
Figure 5: **SV genotyping comparison.** a) Average mapping quality of short reads mapped to the *cactus graph* (y-axis) and *construct graph* (x-axis). b) Average mapping identity of short reads mapped to the *cactus graph* (y-axis) and *construct graph* (x-axis). Colors and shapes represent the 11 non-reference strains and two clades, respectively

Next, we compared the SV genotype performance of both graphs. We mapped short reads from the 11 non-reference strains to both graphs and called variants using vg's variant calling module. To compare the callsets from both graphs, we generated a sample graph for each callset using the reference genome and the callset. Each sample graph is a graph representation of the respective callset. If a given callset is correct, we would expect that reads from the same sample can be mapped confidently and with high identity to the corresponding sample graph. Therefore, we compared the average mapping identity of the short reads on both types of sample graphs (see Figure 5b). Similar to the results of our mapping analysis above, the *cactus graph* clearly outperformed the *construct graph* for strains in the S. paradoxus clade. With strains in the S. cerevisiae clade, both graphs were on a par.

This trend was confirmed when we looked at two our measures, average mapping quality and average alignment score (see Figures 5a and S14).

Methods

Simulation experiment

HGSVC

(Whole-genome) Simulation

The phasing information in the HGSVC VCF was used to extract two haplotypes for sample HG00514, and 30X pairend-end reads were simulated using vg sim. The reads were used to call VCFs then compared back to the original HGSVC calls.

When restricting the comparisons to regions not identified as tandem repeats or segmental duplications in the Genome Browser (Table S1).

(Whole-genome) Real reads

Illumina reads were downloaded from …

De novo assembly alignment from 12 yeast strains

Discussion

Potential topics for the discussion.

Performance across datasets

Although vg was overall the best genotyper in our benchmarks, other methods were superior in some datasets and some situations. Some of these differences might be explained by the quality of the input SV catalog. The GiaB catalog is more curated and, specifically for deletions in non-repeat regions, Delly and BayesTyper were better at predicting genotypes compared to vg. This might be because the breakpoint resolution for this type of SV in these regions is better in this dataset compared to the HGSVC dataset which was derived mostly from long-read sequencing. Similarly, SMRT-SV2 performs better for deletions in the pseudo-diploid genome constructed from two high quality genome assembly of CHM cell lines.

Providing a resource to be used by large-scale sequencing project

As a result of this study we provide a variation graph containing XX millions of SNVs and indels from the 1000 Genomes Project as well as XX thousands of SVs derived from long-read sequencing. This variation graph could serve as a richer reference for large scale projects that use short-read sequencing. For instance, more and more large-scale projects are sequencing the genomes of thousands or hundreds of thousands of individuals, e.g the Pancancer Analysis of Whole Genomes, the Genomics England initiative, and the TOPMed consortium(REFS). These large WGS studies will provide a deeper look into the mechanism of common diseases and, in some cases, will be used directly in a clinical setting. Clinicians and researchers are eager to use these growing WGS resources to interrogate the importance of SVs in disease at a scale never achieved before, either to get a more complete picture of the genetic factors of a disease or to produce a more comprehensive clinical report. As sequencing reaches the clinic, whole-genome sequencing will become routine for many patients. Clinicians will rely on variant calling and interpretation for diagnosis and treatment. For variant interpretation in particular, a comprehensive and unified characterization of the genomic variation will be extremely valuable.

Easier to use

Some methods require additional information or special VCF formatting [26]. SVTyper was designed to use VCFs created by Lumpy. The genotyping module from Delly was implemented for variants found by its discovery module. SMRT-SV requires a VCF with information about XXX. Nebula, a new khmer-based genotyper, requires reads from a sample containing the SV during khmer selection[27]. In contrast, vg can take as input either explicit or symbolic VCFs, as well as assembly alignment.

Assemblies are the future

Our results suggest that constructing a graph from de novo assembly alignment is more representative of the sequencing reads and leads to better SV genotyping. De novo assemblies for human are becoming more and more common, for example from optimized mate-pair libraries[28] or long-read sequencing[28]. For an optimal representation of the genomic variation, we expect the future graphs to include information from the alignment of numerous de novo assemblies. Aligning assembled contigs to existing variation graphs, like to ones created from SVs catalogs, is still experimental but could generate a genome graph augmented with both existing variant databases and new high-quality assemblies.

Future improvements in vg

The vg toolkit is in active development. Read mapping is an area of constant improvement, both in term of computational efficiency and accuracy. For example, haplotype information can be modeled

in variation graph and, in the future, assist read mapping and variant calling. These upcoming developments will directly benefit SV genotyping with vg.

Limitations

Copy number variants (CNVs) are currently represented as deletions or insertions. For this reason duplications are represented as additional sequence rather than encoded as a loop in the graph. While this is sufficient to represent single copy changes, such as deletions or single tandem duplications, CNVs with multi-copies states are not addressed by the current implementation. The genotyping algorithm would need to be extended to model copy number in order to assess these variants.

Near-breakpoint resolution is necessary. Simulations have shown that SV genotyping with vg is robust to errors up to 10 bp in breakpoint location.

The genotyping evaluation of invertions is limited by the lack of existing gold-standards. We showed that vg is capable of genotyping simple inversions from simulation or the few discovered in the SV catalog from Audano et al.[8]. However most inversions are complex and involve small insertions/deletions around their breakpoints(REF). While these complex variants are difficult to represent in the VCF format, they would naturally be represented through the alignment of de novo assemblies. For example, in our experiment with yeast assemblies, we identified XX variants that can be considered complex inversion as they contain at least XX inverted bases.

Supplementary Material

Table S1: Genotyping evaluation on the HGSVC dataset. Precision, recall and F1 score for the call set with the best F1 score. The numbers in parenthesis corresponds to the results in non-repeat regions.

Experiment	Method	Type	Precision	Recall	F1
Simulated reads	vg	INS	0.795 (0.885)	0.796 (0.883)	0.795 (0.884)
		DEL	0.869 (0.971)	0.771 (0.92)	0.817 (0.945)
	BayesTyper	INS	0.91 (0.935)	0.835 (0.9)	0.871 (0.917)
		DEL	0.898 (0.981)	0.806 (0.929)	0.849 (0.954)
	svtyper	DEL	0.809 (0.876)	0.328 (0.754)	0.467 (0.81)
	Delly	INS	0.767 (0.866)	0.093 (0.225)	0.166 (0.358)
		DEL	0.696 (0.903)	0.707 (0.846)	0.701 (0.874)
Real reads	vg	INS	0.431 (0.683)	0.541 (0.726)	0.48 (0.704)
		DEL	0.65 (0.886)	0.519 (0.708)	0.577 (0.787)
	BayesTyper	INS	0.601 (0.747)	0.254 (0.433)	0.357 (0.549)
		DEL	0.627 (0.91)	0.325 (0.381)	0.428 (0.537)
	svtyper	DEL	0.661 (0.733)	0.236 (0.551)	0.348 (0.629)
	Delly	INS	0.516 (0.621)	0.068 (0.176)	0.12 (0.275)

Experiment	Method	Type	Precision	Recall	F1
		DEL	0.55 (0.838)	0.445 (0.547)	0.492 (0.662)

Table S2: Genotyping evaluation on the Genome in a Bottle dataset. Precision, recall and F1 score for the call set with the best F1 score. The numbers in parenthesis corresponds to the results in non-repeat regions.

Method	Type	Precision	Recall	F1
vg	INS	0.658 (0.774)	0.646 (0.735)	0.652 (0.754)
	DEL	0.68 (0.768)	0.643 (0.735)	0.661 (0.751)
BayesTyper	INS	0.776 (0.879)	0.286 (0.379)	0.418 (0.53)
	DEL	0.808 (0.886)	0.512 (0.696)	0.627 (0.779)
svtyper	DEL	0.742 (0.818)	0.342 (0.496)	0.468 (0.618)
Delly	INS	0.822 (0.894)	0.177 (0.268)	0.291 (0.412)
	DEL	0.722 (0.822)	0.645 (0.768)	0.681 (0.794)

Table S3: Genotyping evaluation on the pseudo-diploid genome built from CHM cell lines in Audano et al.[8].

Method	Region	Type	Precision	Recall	F1
vg	all	INS	0.665	0.661	0.663
		DEL	0.688	0.500	0.579
	non-repeat	INS	0.806	0.784	0.795
		DEL	0.869	0.762	0.812
SMRT-SV2	all	INS	0.757	0.536	0.628
		DEL	0.848	0.630	0.723
	non-repeat	INS	0.880	0.680	0.767
		DEL	0.971	0.824	0.891

Table S4: Calling evaluation on the SVPOP dataset. Combined results for the HG5014, HG00733 and NA19240 individuals, 3 of the 15 individuals used to generate the high-quality SV catalog in Audano et al.[8].

| Method	Region	Type	TP	FP	FN	Precision	Recall	F1
vg	all	INS	25838	22042	15772	0.540	0.621	0.577
		DEL	14545	6824	15425	0.681	0.485	0.567
		INV	27	26	173	0.509	0.135	0.213
	non-repeat	INS	8051	3258	1817	0.712	0.816	0.760
		DEL	3769	623	818	0.858	0.822	0.840
		INV	19	12	75	0.613	0.202	0.304
SMRT-SV2	all	INS	16270	26031	25340	0.385	0.391	0.388
		DEL	11793	10106	18177	0.539	0.393	0.455
	non-repeat	INS	4483	4659	5385	0.490	0.454	0.472
		DEL	2928	930	1659	0.759	0.638	0.693

Table S5: Breakpoint fine-tuning using augmentation through "vg call". For deletions and inversions, either one or both breakpoints were shifted to introduce errors in the input VCF. For insertions, the insertion location and sequence contained errors.

SV type	Error type	Breakpoint	Variant	Proportion	Mean size (bp)	Mean error (bp)
DEL	one end	incorrect	220	0.219	422.655	6.095
		fine-tuned	784	0.781	670.518	5.430
	both ends	incorrect	811	0.814	826.070	6.275
		fine-tuned	185	0.186	586.676	2.232
INS	location/ seq	incorrect	123	0.062	428.724	6.667
		fine-tuned	1877	0.938	440.043	6.439
INV	one end	incorrect	868	0.835	762.673	5.161
		fine-tuned	172	0.165	130.244	5.884
	both ends	incorrect	950	0.992	556.274	5.624
		fine-tuned	8	0.008	200.000	1.375
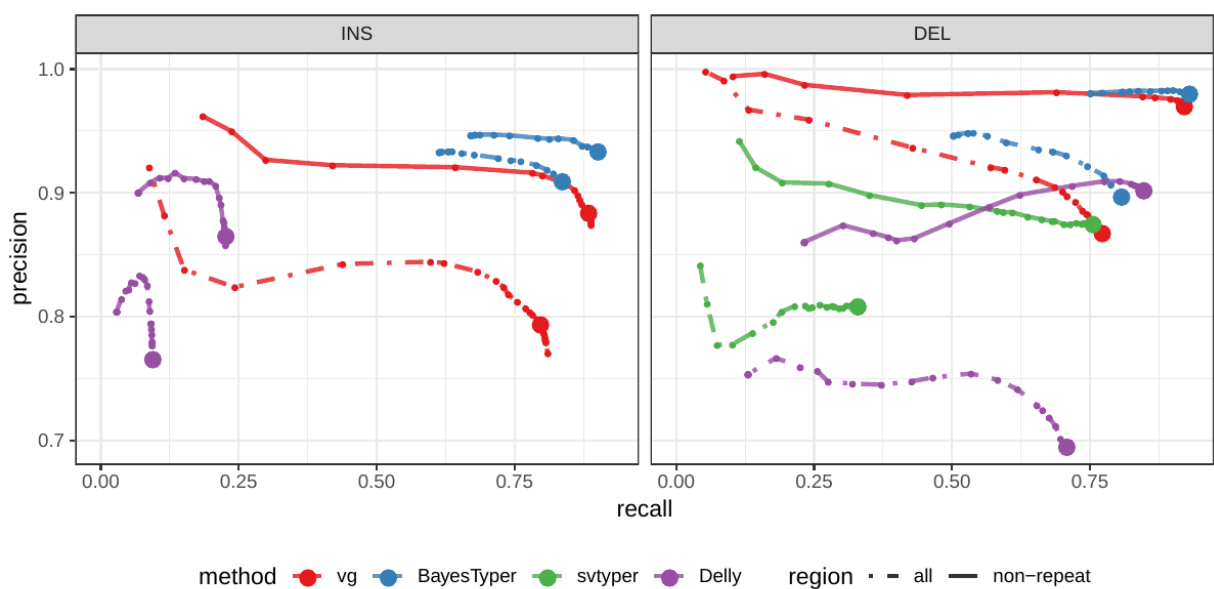
Figure S1: **Structural variants from the HGSVC dataset**. Genotyping evaluation for simulated reads.
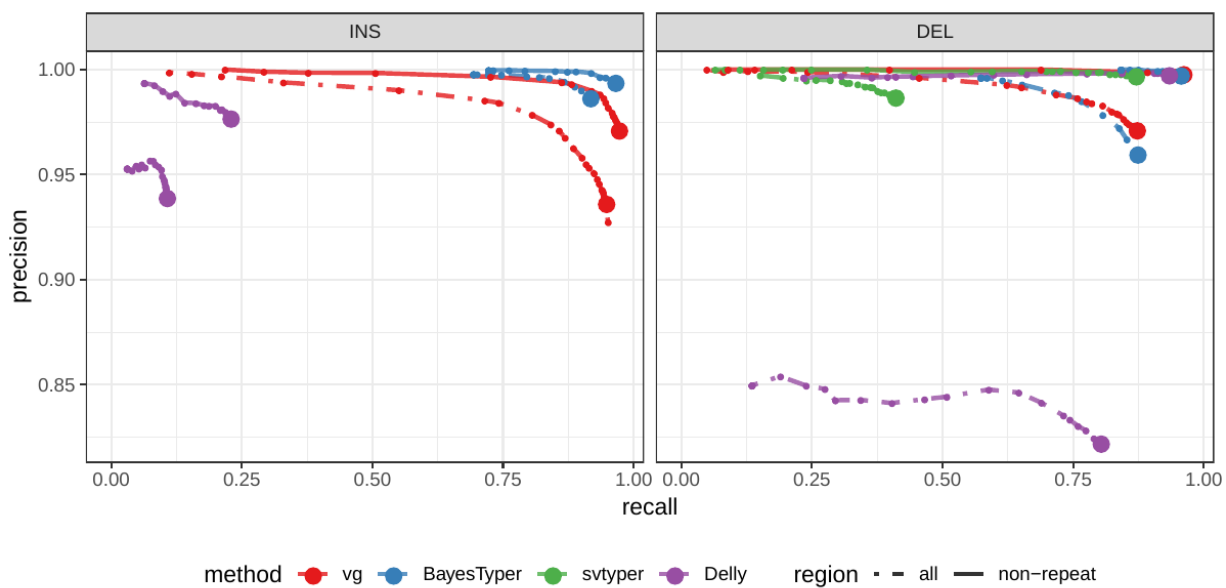
Figure S2: **Structural variants from the HGSVC dataset**. Calling evaluation for simulated reads.
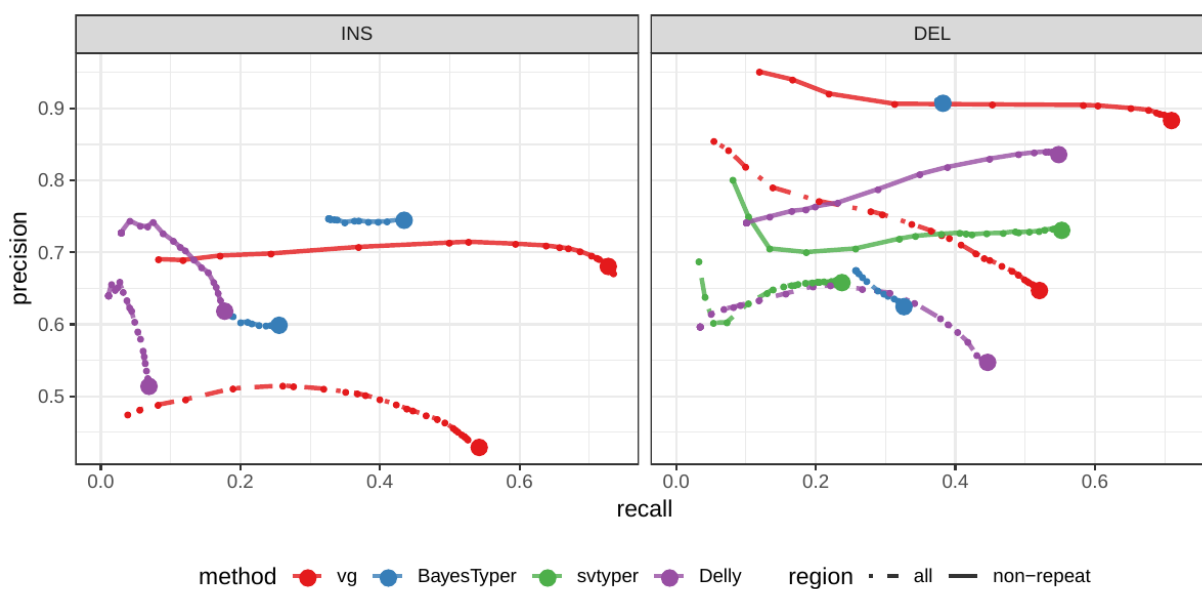
Figure S3: **Structural variants from the HGSVC dataset**. Genotyping evaluation for real reads.
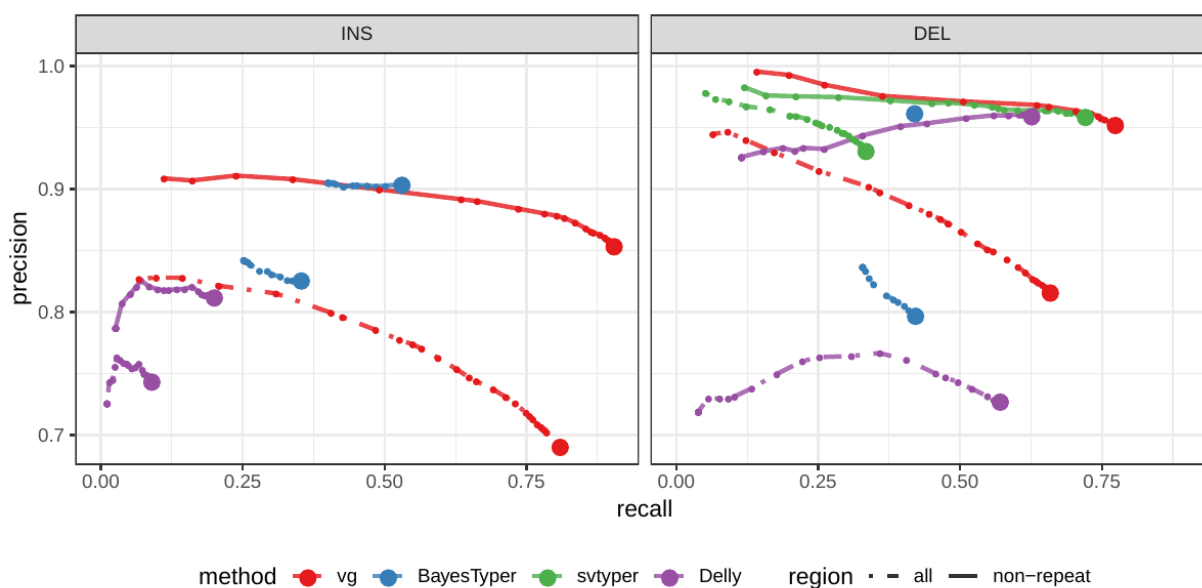
Figure S4: **Structural variants from the HGSVC dataset**. Calling evaluation for real reads.
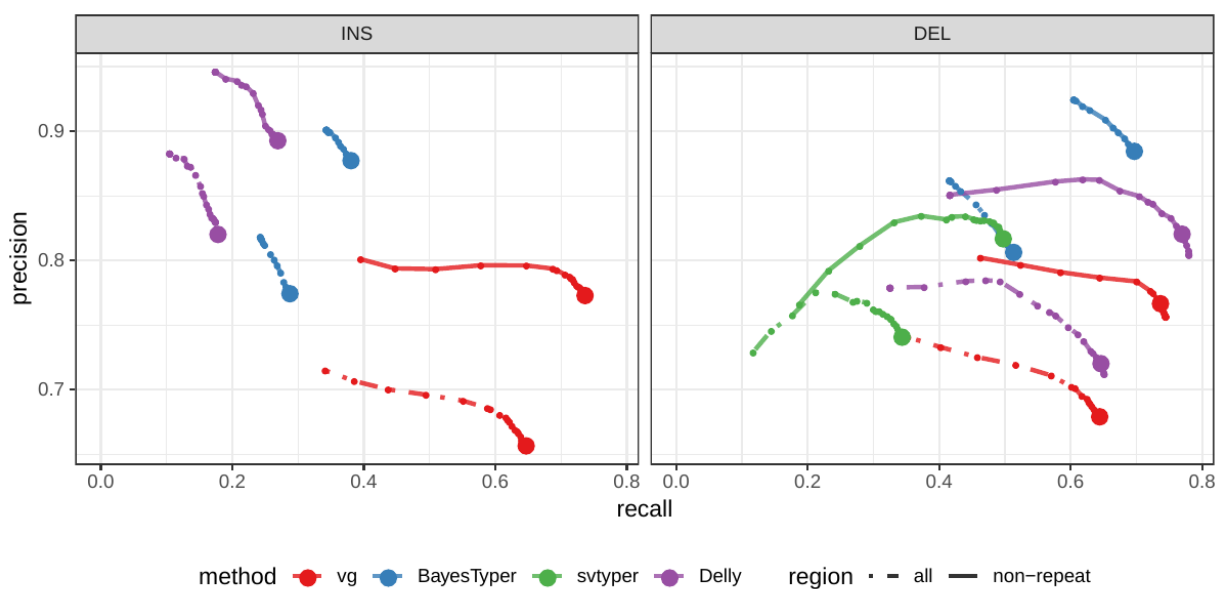
Figure S5: **Structural variants from the Genome in a Bottle dataset**. Genotyping evaluation.
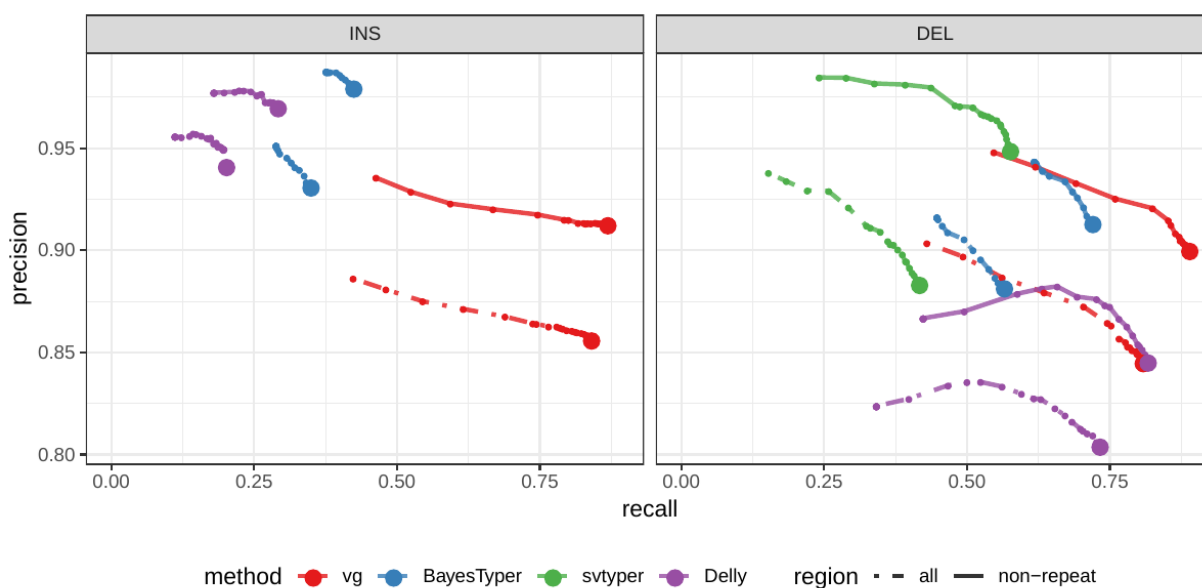
Figure S6: **Structural variants from the Genome in a Bottle dataset**. Genotyping evaluation.
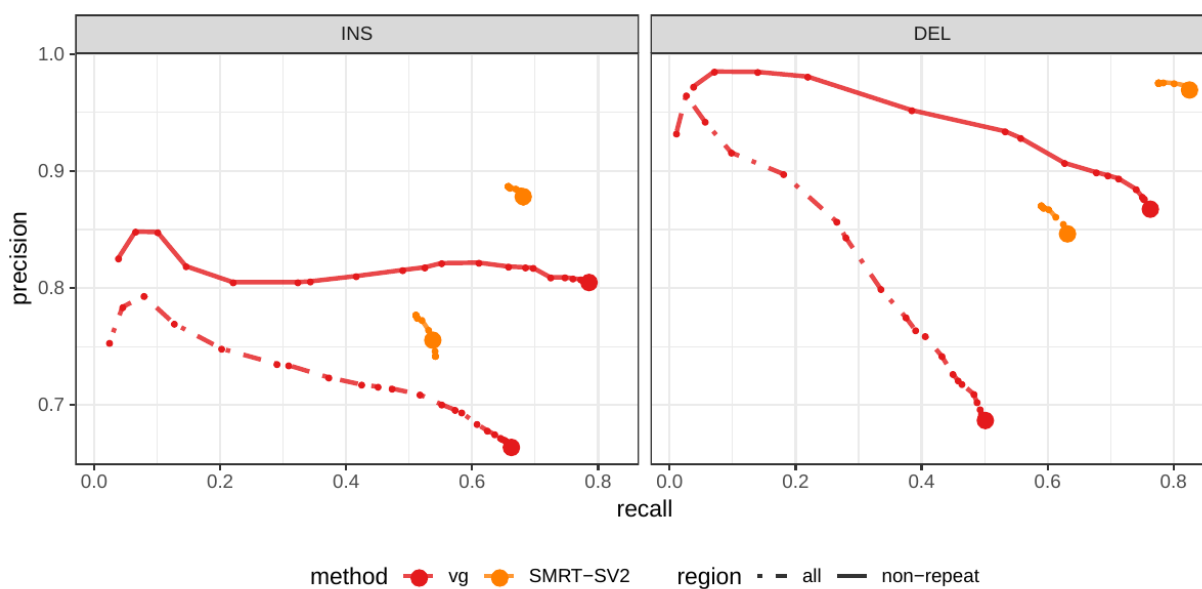
Figure S7: **Structural variants from the CHM pseudo-diploid dataset**. Genotyping evaluation.
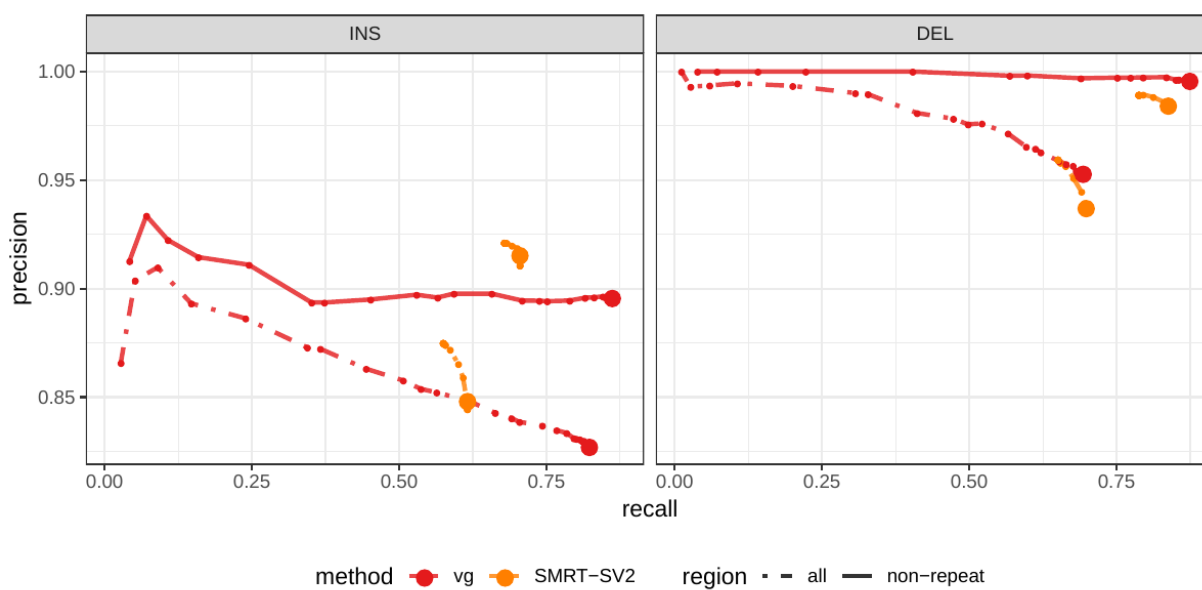
Figure S10: **Structural variants from the CHM pseudo-diploid dataset**. Calling evaluation.
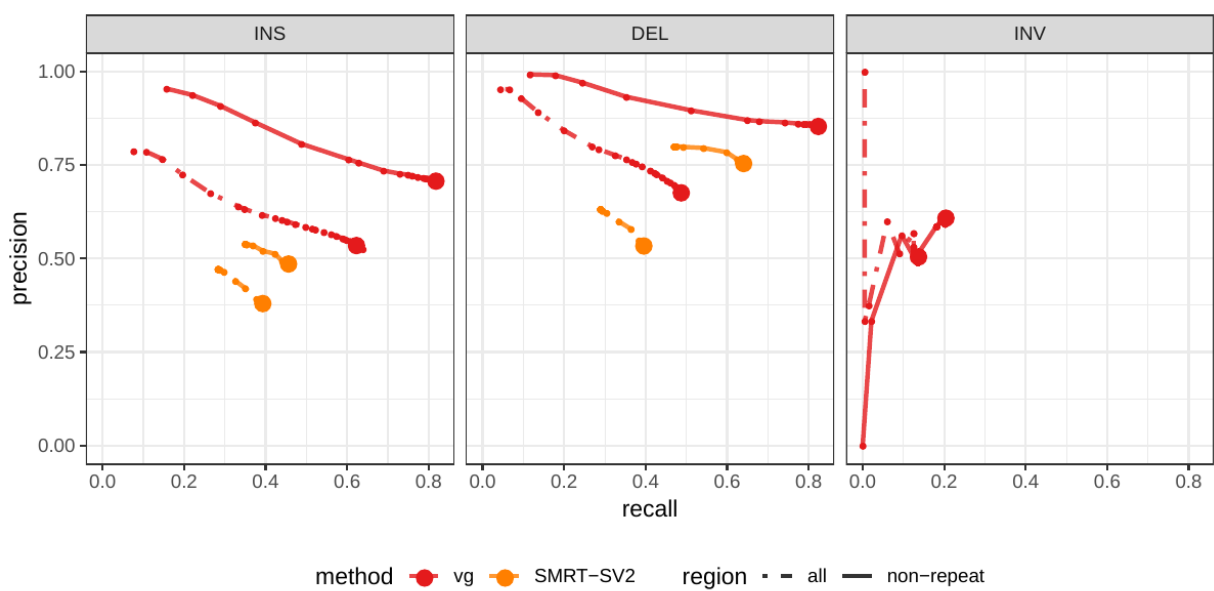
Figure S11: **Structural variants from the SVPOP dataset**. Calling evaluation.
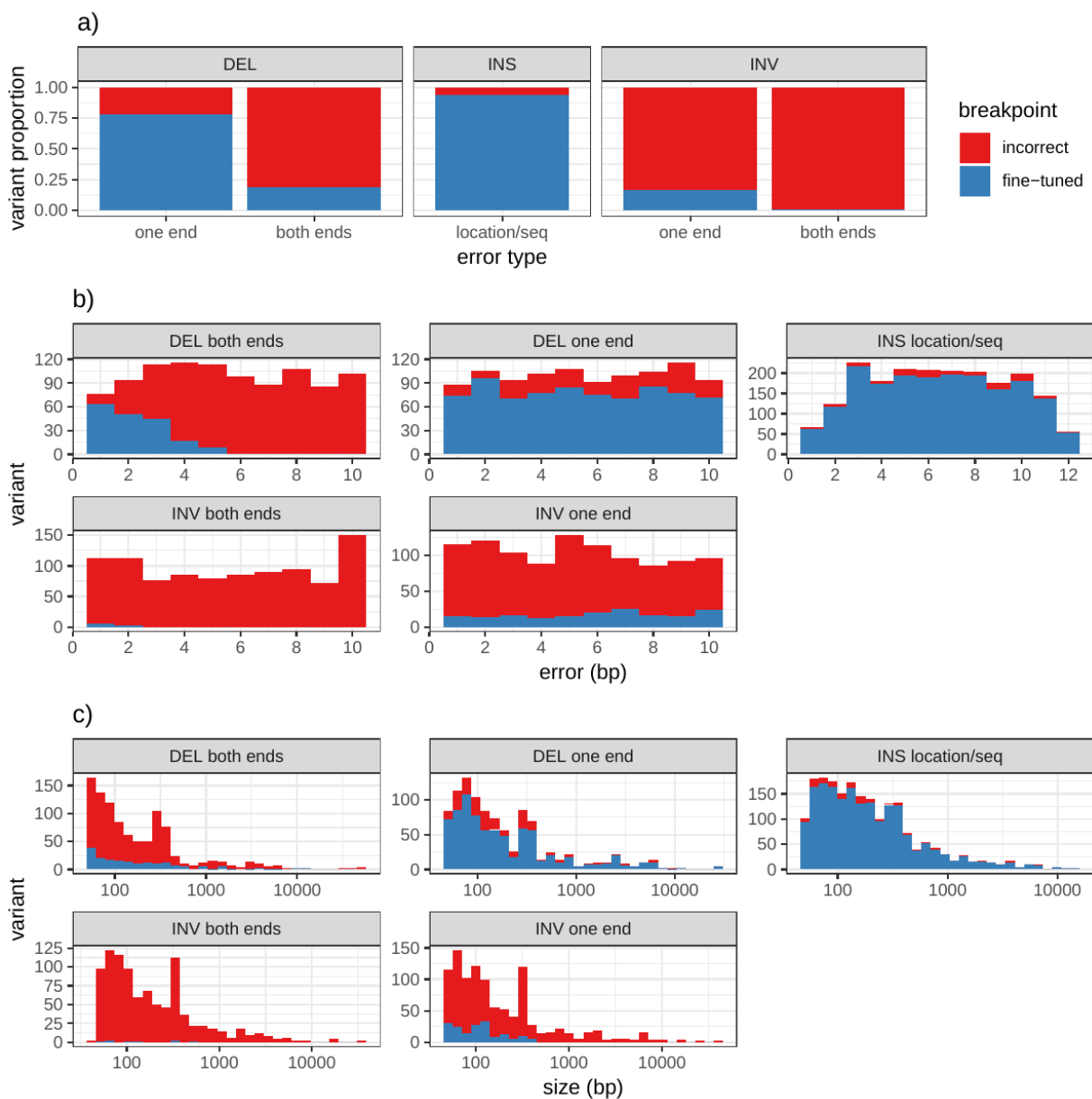
Figure S12: **Breakpoint fine-tuning using augmentation through "vg call".**. For deletions and inversions, either one or both breakpoints were shifted to introduce errors in the input VCF. For insertions, the insertion location and sequence contained errors. a) Proportion of variant for which breakpoints could be fine-tuned. b) Distribution of the amount of errors that could be corrected or not. c) Distribution of the size of the variants whose breakpoints could be fine-tuned or not.
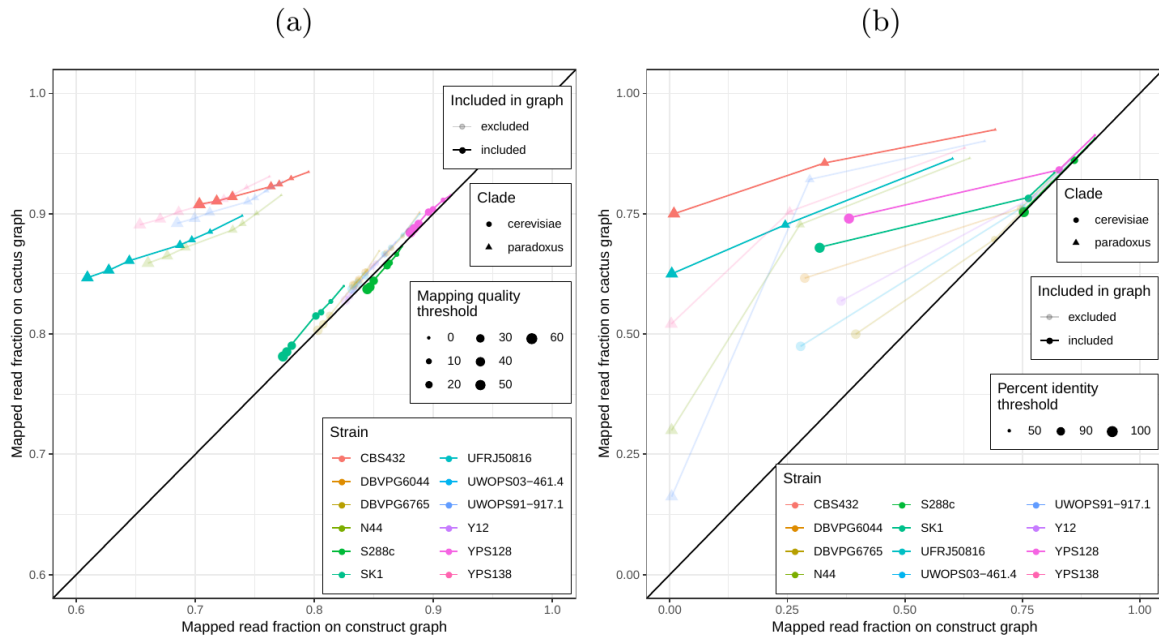
Figure S13: **Mapping comparison on graphs of five strains.** The fraction of reads mapped to the cactus graph (y-axis) and the construct graph (x-axis) are compared. a) Stratified by mapping quality threshold. b) Stratified by percent identity threshold.
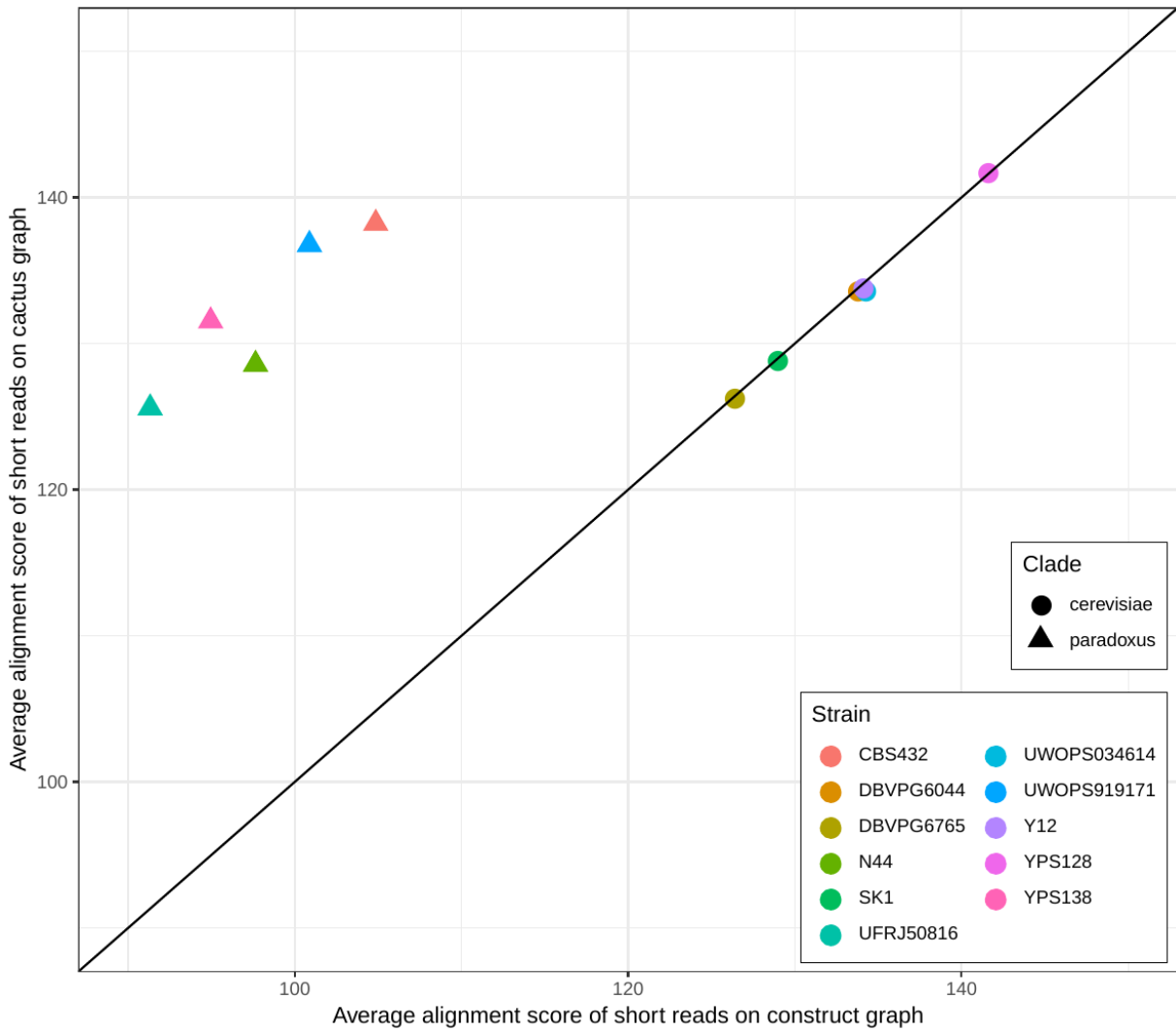
Figure S14: **SV genotyping comparison.** Average alignment score of short reads mapped to the *cactus graph* (y-axis) and *construct graph* (x-axis) is compared. Colors and shapes represent the 11 non-reference strains and two clades, respectively

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