

# Genotyping structural variation in variation graphs with the vg toolkit

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## Authors

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# Abstract

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# Introduction

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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. Although whole-genome sequencing (WGS) made it possible to assess virtually any type of structural variation, many challenges remain. In particular, SV-supporting reads are difficult to map to reference genomes. Multi-mapping, caused by widespread repeated sequences in the genome, is another issue because it often resembles SV-supporting signal. As a result, many SV detection algorithms have been developed and multiple methods must usually be combined to minimize false positives. Several large-scale projects used this ensemble approach, cataloging tens of thousands of SV in humans[1,2]. SV detection from short-read sequencing remains laborious and of lower accuracy, explaining 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 made it possible to produce long reads or retrieve long-range information over kilobases of sequence. These approaches are maturing to the point where it is feasible to analyze the human genome. This multi-kbp information is particularly useful for SV detection and de novo assembly. In the last few years, several studies using long-read or linked-read sequencing have produced large catalogs of structural variation, the majority of which were novel and sequence-resolved[3,4,5,6,7]. These technologies are also enabling high-quality de novo genome assemblies to be produced[3, 8], as well as large blocks of haplotype-resolved sequences[9]. These technological advances promise to expand the amount of known genomic variation in humans in the near future.

In parallel, the reference genome is evolving from a linear reference to a graph-based reference that contains known genomic variation[10,11,12]. By having variants in the graph, mapping rates are increased and variants are more uniformly covered, including indels and variants in complex regions[11]. Both the mapping and variant calling become variant-aware and benefit in terms of accuracy and sensitivity. In addition, different variant types are called simultaneously by a unified framework. Graphs have also been used locally, i.e. to call variants at the region level. GraphTyper[13] and BayesTyper[14] both construct variation graphs of small regions and use them for variant genotyping. Here again, the graph-approach showed clear advantages over standard approaches that use the linear reference. Other SV genotyping approaches compare read mapping in the reference genome and a sequence modified with the SV. For example SMRT-SV was designed to genotype SVs identified on PacBio reads[4], SVTyper uses paired-end mapping and split-read mapping information[15], and Delly provides a genotyping feature in addition to its discovery mode[16].

# Results

## Structural variation in vg

In addition to SNV and short indels, vg can handle large deletions and insertions (and inversion?) (Figure 1). As a proof-of-concept we simulated genomes and SVs of varying sizes. Some errors were added at the breakpoints to investigate their effect on genotyping. In all simulations, vg performed better than SVtyper[15] and Delly[16] (Figure 2). The recall was particularly higher than other methods at low sequencing depth. vg was also more robust to errors around the breakpoints, performing almost as well as in the absence of errors.

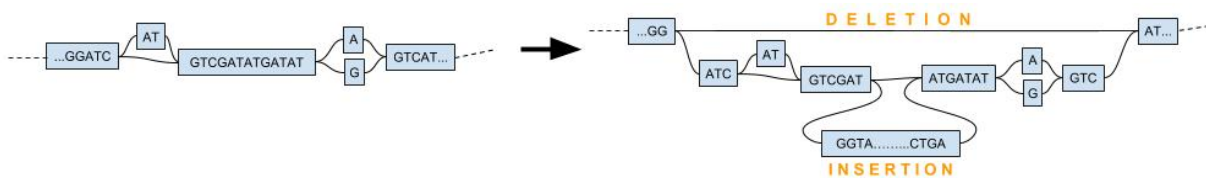


Figure 1: Large deletions and insertions in variation graphs

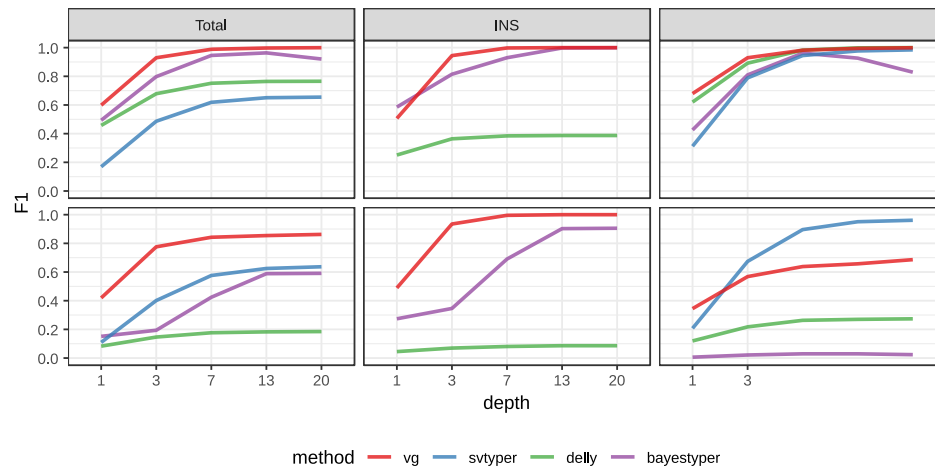


Figure 2: **Simulation experiment.** For each experiment (method, depth and input VCF with/without errors), the deciles of the call qualities were used as threshold and the maximum F1 is reported on the y-axis.

## HGSVC

Chaisson et al.[17] provide a high-quality SV catalog of three samples, obtained using a consensus from different sequencing, phasing and variant calling technologies.

### (Whole-genome) Simulation

The phasing information in the HGSVC VCF was used to extract two haplotypes for sample HG00514, and 30X paired-end reads were simulated using vg sim. The reads were used to call VCFs then compared back to the original HGSVC calls (Figure 3 and Table 1).

When restricting the comparisons to regions not identified as tandem repeats or segmental duplications in the Genome Browser (Table 2).

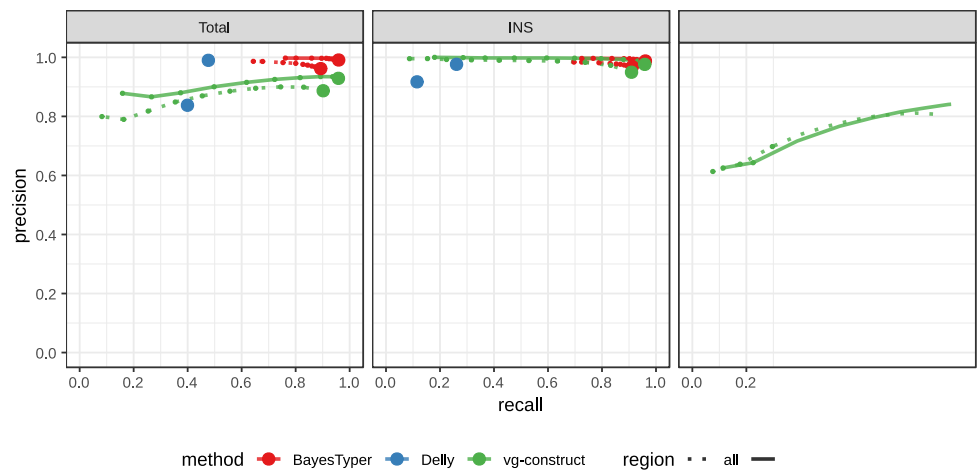


Figure 3: HGSVC simulated reads.

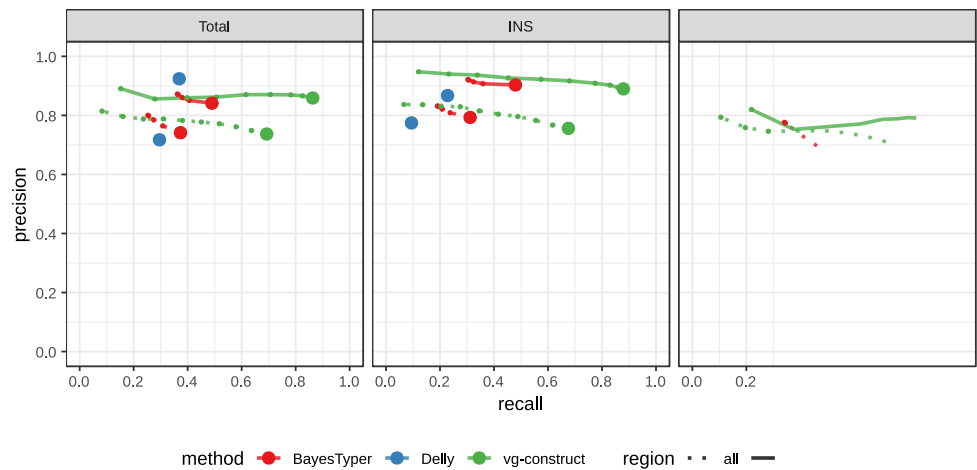


Figure 4: HGSVC real reads.

(Whole-genome) Real reads

Figure 4. Tables 3 and 4 for results over the genome or when restricting the comparisons to regions not identified as tandem repeats or segmental duplications in the Genome Browser.

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*) [18]. 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 [19], AsmVar [20] and paf tools [21]. 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 occurring 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 [22]. While the *construct graph* is still mainly linear and highly dependent on the reference genome, the cactus graph is completely unbiased in that regard.

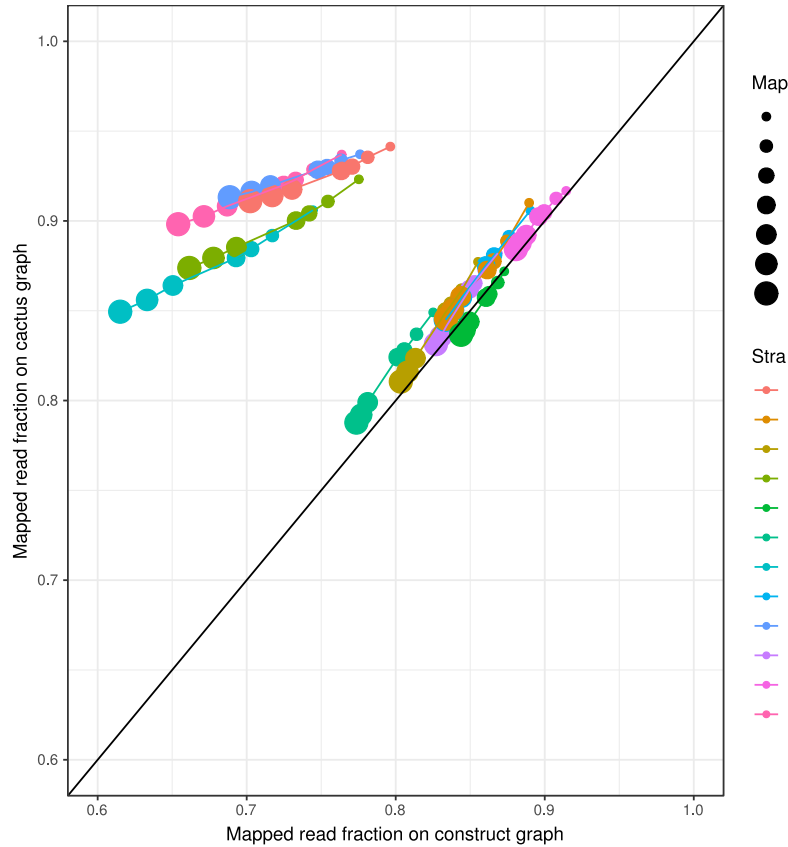


Figure 5: **Mapping quality comparison.** The fraction of reads mapped (with different mapping quality thresholds) to the cactus graph (y-axis) and the construct graph (x-axis) are compared

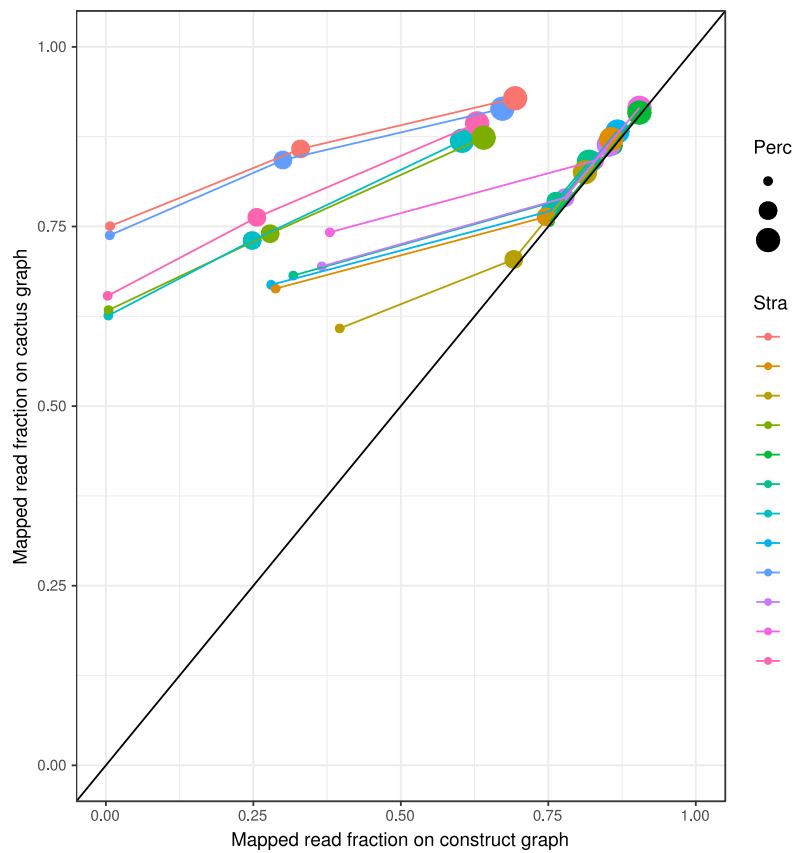


Figure 6: **Mapping identity comparison.** The fraction of reads mapped (with different percent identity thresholds) to the cactus graph (y-axis) and the construct graph (x-axis) are compared

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. When mapping short Illumina reads from the 12 strains to both graphs, we indeed observed a higher fraction of reads mapped to the *cactus graph* than to the *construct graph* (see Fig. 5). 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 gap is largest for strains in the *S. paradoxus* clade and smaller for reads from strains in the *S. cerevisiae* clade.

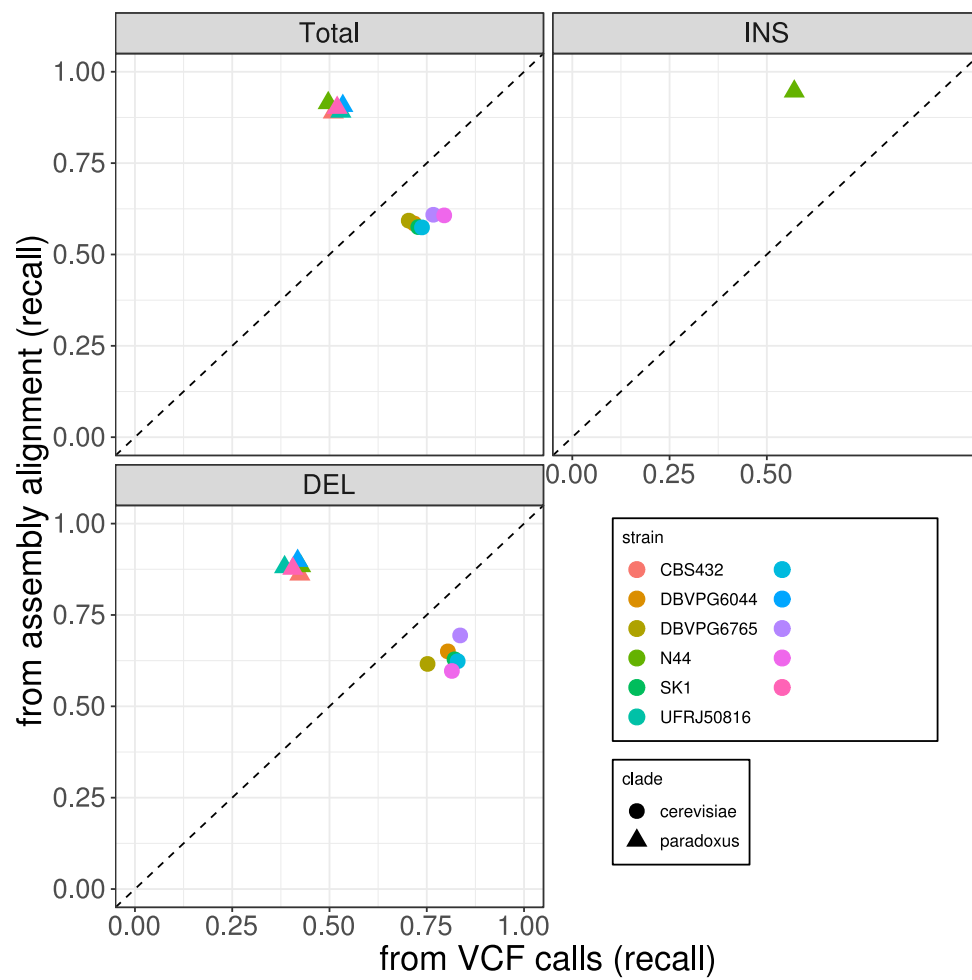


Figure 7: **SV genotyping comparison.** SV genotype recall from the *cactus graph* (y-axis) and *construct graph* (x-axis) are compared. Colors and shapes represent the 12 strains and two clades, respectively



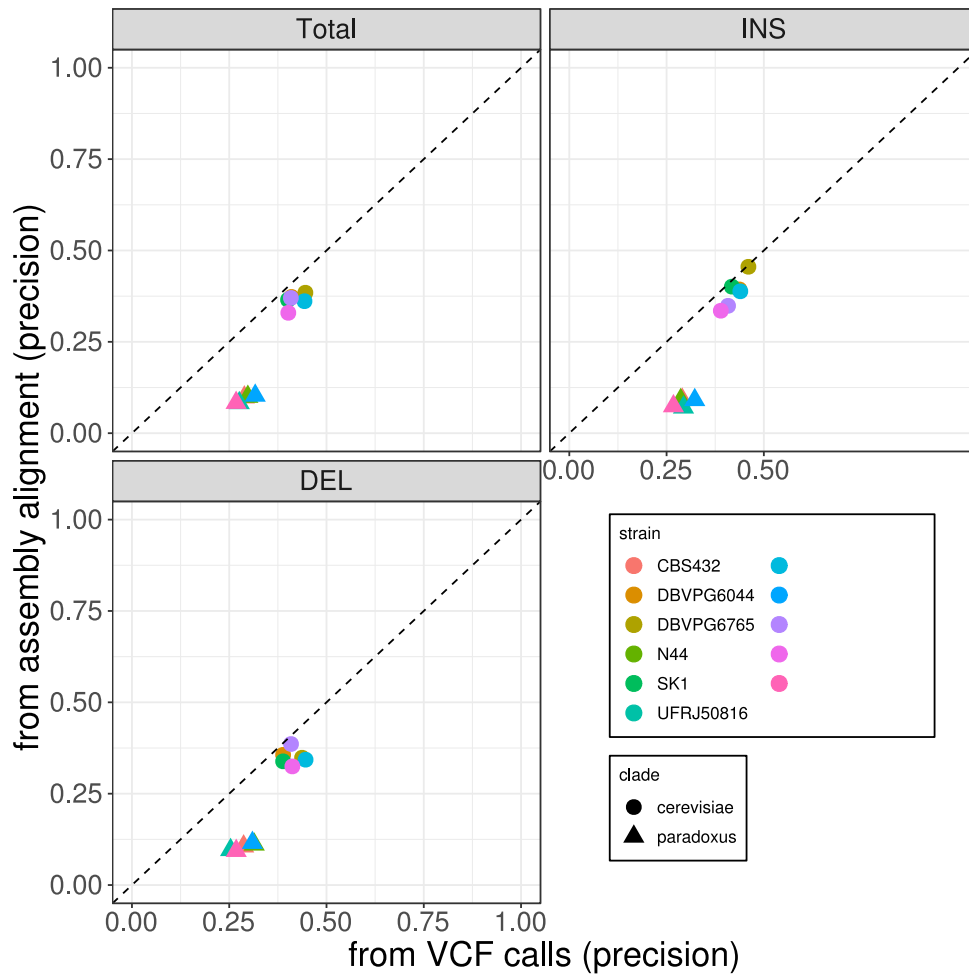


Figure 8: **SV genotyping comparison.** SV genotype precision from the *cactus graph* (y-axis) and *construct graph* (x-axis) are compared. Colors and shapes represent the 12 strains and two clades, respectively

Next, we compared the SV genotype performance of both graphs. To facilitate a fair evaluation of genotype performance, we combined all SVs that were detected by at least two of the three SV callers (Assemblytics, AsmVar and paftools) into a truth set. This truth set is a subset of the SV set used for construction of the *construct graph* which is important because only variants already present in the graph can be genotyped.

Figure 7 and 8 shows the results of our analysis. Depending on the clade, the *cactus graph* reaches either a substantially higher SV genotyping recall than the *construct graph* (*S. paradoxus*) or a substantially lower recall (*S. cerevisiae*).

**Methods**

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**Discussion**

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**Supplementary Material**

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Table 2: HGSVC experiment using simulated reads and restricting the comparisons to non-repeat regions.

Graph	type	TP	TP.baseline	FP	FN	precision	recall	F1
HGSVC-Construct	Total	5901	5822	452	253	0.928	0.9584	0.943
	INS	4026	3952	98	172	0.9758	0.9583	0.967
	DEL	1875	1870	354	81	0.8408	0.9585	0.8958
HGSVC-1KG-Construct	Total	5880	5785	486	290	0.9225	0.9523	0.9372
	INS	4024	3922	123	202	0.9696	0.951	0.9602
	DEL	1856	1863	363	88	0.8369	0.9549	0.892
HGSVC-Bayestyper	Total	3805	3863	8	2212	0.9979	0.6359	0.7768
	INS	2401	2430	6	1694	0.9975	0.5892	0.7408
	DEL	1404	1433	2	518	0.9986	0.7345	0.846
SVPOP-Construct	Total	3565	3856	390	2219	0.9081	0.6347	0.7472
	INS	3091	3246	239	878	0.9314	0.7871	0.8532
	DEL	474	610	151	1341	0.8016	0.3127	0.4499
SVPOP-1KG-Construct	Total	3574	3817	562	2258	0.8717	0.6283	0.7303
	INS	3066	3180	253	944	0.9263	0.7711	0.8416
	DEL	508	637	309	1314	0.6734	0.3265	0.4398

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Table 3: HGSVC experiment using real reads.

Graph	type	TP	TP.baseline	FP	FN	precision	recall	F1
HGSVC-Construct	Total	18436	18500	6575	8206	0.7378	0.6927	0.7145
	INS	10984	10600	3542	5085	0.7495	0.6758	0.7107
	DEL	7452	7900	3033	3121	0.7226	0.7168	0.7197
HGSVC-1KG-Construct	Total	17802	17946	6221	8760	0.7426	0.672	0.7055
	INS	10647	10262	3304	5423	0.7564	0.6543	0.7017
	DEL	7155	7684	2917	3337	0.7248	0.6972	0.7107
HGSVC-Bayestyper	Total	4342	4840	1048	21866	0.822	0.1812	0.2969
	INS	1786	1883	309	13802	0.859	0.1201	0.2107
	DEL	2556	2957	739	8064	0.8001	0.2683	0.4018
SVPOP-Construct	Total	9091	9931	10235	16775	0.4925	0.3719	0.4238
	INS	6972	7420	6706	8265	0.5253	0.4731	0.4978
	DEL	2119	2511	3529	8510	0.4157	0.2278	0.2943

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Table 4: HGSVC experiment using real reads and restricting the comparisons to non-repeat regions.

<b>Graph</b>	<b>type</b>	<b>TP</b>	<b>TP.baseline</b>	<b>FP</b>	<b>FN</b>	<b>precision</b>	<b>recall</b>	<b>F1</b>
HGSVC-Construct	Total	5197	5244	854	831	0.86	0.8632	0.8616
	INS	3708	3626	459	498	0.8876	0.8792	0.8834
	DEL	1489	1618	395	333	0.8038	0.8293	0.8164
HGSVC-1KG-Construct	Total	5103	5155	865	920	0.8563	0.8486	0.8524
	INS	3642	3555	464	569	0.8845	0.862	0.8731
	DEL	1461	1600	401	351	0.7996	0.8201	0.8097
HGSVC-Bayestyper	Total	1560	1731	274	4344	0.8633	0.2849	0.4284
	INS	883	901	69	3223	0.9289	0.2185	0.3538
	DEL	677	830	205	1121	0.8019	0.4254	0.5559
SVPOP-Construct	Total	3251	3480	941	2595	0.7872	0.5728	0.6631
	INS	2859	3009	780	1115	0.7941	0.7296	0.7605
	DEL	392	471	161	1480	0.7453	0.2414	0.3647

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