Comparison of calling pipelines for whole genome sequencing: an empirical study demonstrating the importance of mapping and alignment

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Abstract

Rapid advances in high-throughput DNA sequencing technologies have enabled the conduct of whole genome sequencing (WGS) studies, and several bioinformatics pipelines have become available. We conducted a WGS study involving >9000 subjects, sequenced on one Illumina NovaSeq 6000 with an average coverage of 35x using a PCR-free protocol and unique dual indices (UDI). As part of the quality control, we sequenced one genome in a bottle (GIAB) sample 70 times in different runs, and one GIAB trio in triplicate. In this study, we compared the performance of 6 pipelines, involving two mapping and alignment approaches (GATK utilizing BWA-MEM2 2.2.1, and DRAGEN 3.8.4) and three variant calling pipelines (GATK 4.2.4.1, DRAGEN 3.8.4 and DeepVariant 1.1.0). The truth set of the GIABs was used for comparison, and performance was assessed by computation time, F_1 score, precision and recall. In the mapping and alignment step, the DRAGEN pipeline was faster than the GATK with BWA-MEM2 pipeline. DRAGEN showed systematically higher F_1 score, precision, and recall values than GATK for single nucleotide variations (SNVs) and Indels in both

simple-to-map and complex-to-map regions. In the variant calling step, DRAGEN was fastest. In terms of accuracy, DRAGEN and DeepVariant performed similarly and both superior to GATK, with slight advantages for DRAGEN for Indels and for DeepVariant for SNVs. The DRAGEN pipeline showed the lowest Mendelian inheritance error fraction for the GIAB trios. Mapping and alignment played a key role in variant calling of WGS, with the DRAGEN substantially outperforming GATK.

Key words: DNA sequencing, High-Throughput Sequencing, Illumina NovaSeq 6000, Next Generation Sequencing

Introduction

Whole genome sequencing (WGS) of a human genome for less than 1000 USD has become reality[1], and cost might drop to even 100 USD with the Illumina NovaSeq system[2]. Large-scale WGS studies have already been initiated or even completed because of the greater cost efficiency, and the number of such studies is expected to increase, with several to be run in smaller labs[3-5]. The availability of fast, simple to use and accurate genotype calling pipelines is therefore of utmost importance.

GATK is the most frequently used pipeline[6], but other pipelines have outperformed it in terms of the F_1 statistic, precision and recall. Specifically, DeepVariant[7] won the first precisionFDA Truth Challenge for short-read sequencing in 2016 and had the highest accuracy of single nucleotide polymorphisms (SNPs). The winner of the second precisionFDA Truth Challenge for short-read sequencing in 2020 was Illumina's DRAGEN[8]. It outperformed other pipelines[6,9-12], in particular in difficult-to-call genomic

regions[13]. It is, however, unclear which pipeline should be used for secondary analysis of WGS data, especially in terms of speed and accuracy. The choice is further complicated by the fact that recent pipeline comparisons relied on synthetic-diploid and simulated data[14] or did not include the award-winning DeepVariant and/or DRAGEN pipelines[6,9,10,12,15]. Furthermore, other comparisons focused only on variant calling, the last step in the secondary analysis pipeline.

For our WGS project, in which we sequenced more than 9000 unrelated individuals using the Illumina sequencing technology, we had to select the most appropriate pipeline. Therefore, we compared DRAGEN against GATK with bwa-mem in the mapping and alignment steps (upstream pipeline), and DeepVariant and DRAGEN against GATK in the variant calling step (downstream pipeline, Figure 1). To assess the performance of the pipelines, we used samples from the genome in a bottle (GIAB) consortium as a reference truth set. We successfully sequenced one GIAB sample (NIST ID HG002) 70 times in different runs, i.e., in approximately one third of all runs, and one trio (NIST IDs HG002, HG003 and HG004) three times. These sequences allowed for the comparison of different pipelines against truth sets for single nucleotide variations (SNVs), insertions/deletions (Indels) in both simple- and difficult-to-map regions.

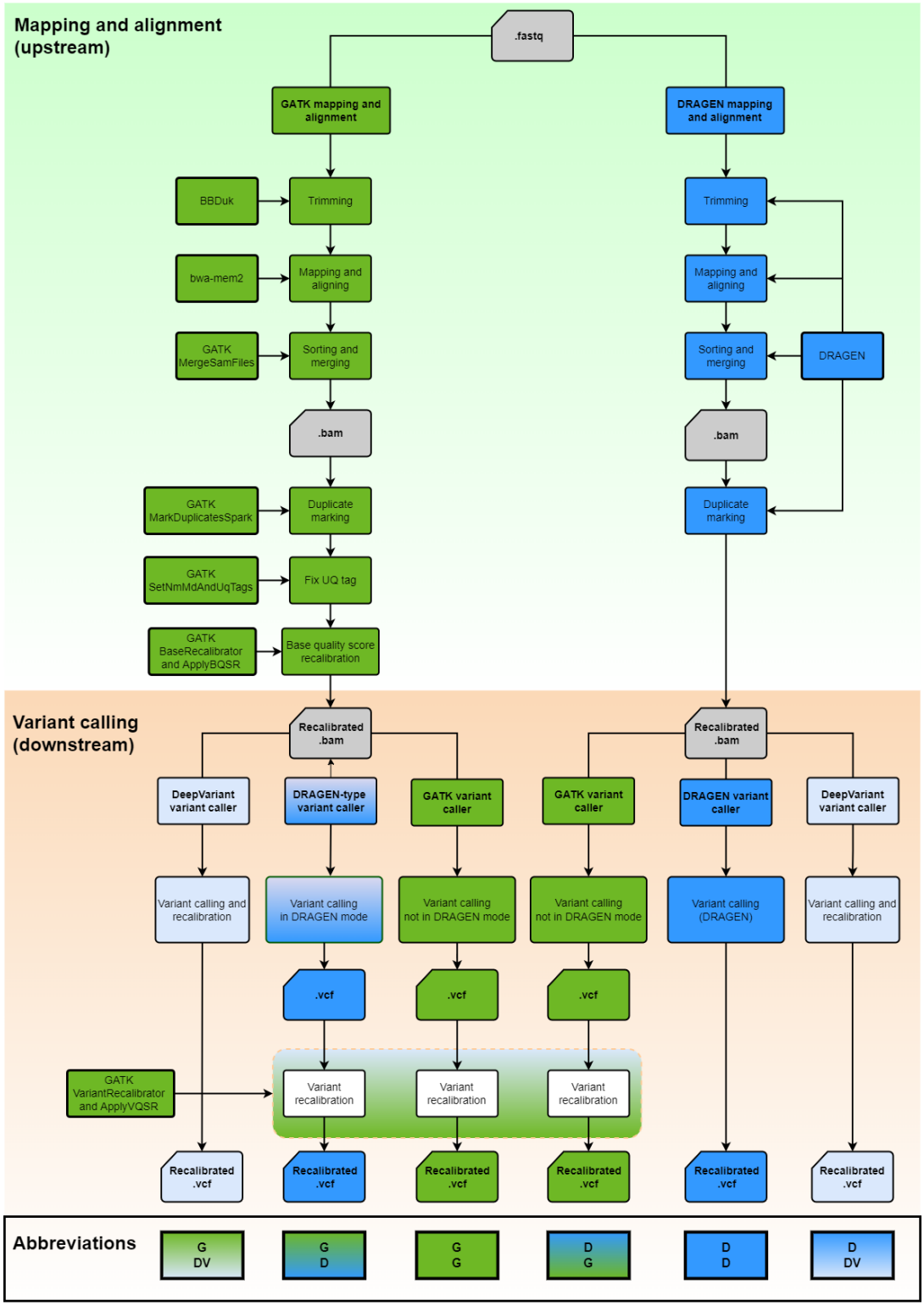

Figure 1. Flowchart of pipelines used in the benchmark analysis. The two upstream pipelines GATK and DRAGEN for mapping and alignment were used in conjunction with the four variant calling pipelines DRAGEN, DeepVariant, GATK Haplotypecaller in DRAGEN mode and GATK Haplotypecaller not in DRAGEN mode downstream.

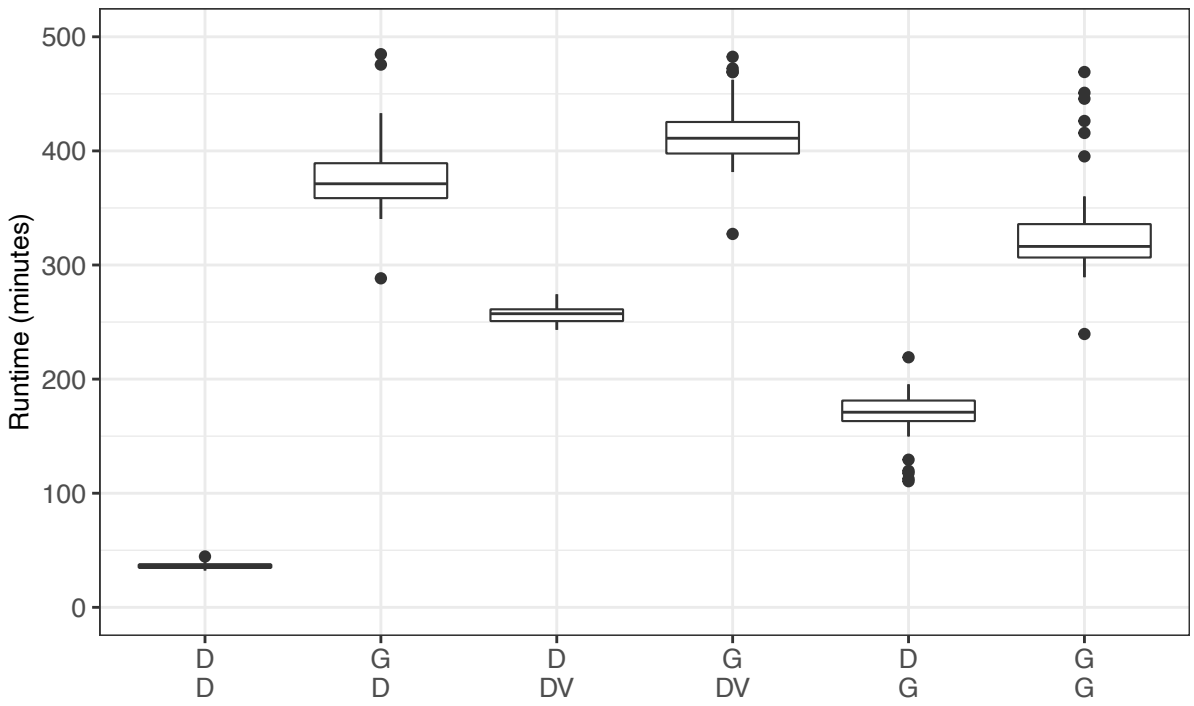

Figure 2. Runtime comparison of the 6 pipelines. Labels on the x-axis were defined in detail in Figure 1. D: DRAGEN; DV: DeepVariant; G: GATK.

Results

Run times. Total run times are displayed in Figure 2. The DRAGEN pipeline was fastest and required 36 ± 2 min (mean ± standard deviation) per sample; detailed comparisons are provided in Suppl. Table S1. The DRAGEN also showed the most homogeneous run time across all GIAB samples. The other pipelines were substantially slower (min average run time ≥ 180 min). For example, DeepVariant variant calling following DRAGEN mapping and alignment required 256 ± 7 min. The long run time of DeepVariant was most likely caused by the single threading of the software in several of the computing steps. Run time for the upper part of the pipeline, i.e., up to the bam file, was 182 ± 36 min for GATK and 18 ± 1 min for the DRAGEN (Suppl. Fig. S1). Run times

for variant calling were 18 ± 1 min for the DRAGEN and 231 ± 16 min for DeepVariant (Suppl. Fig. S1). The GATK Haplotypecaller in the DRAGEN mode took 189 ± 26 min to run, and 134 ± 20 min were required when GATK was run in the standard mode.

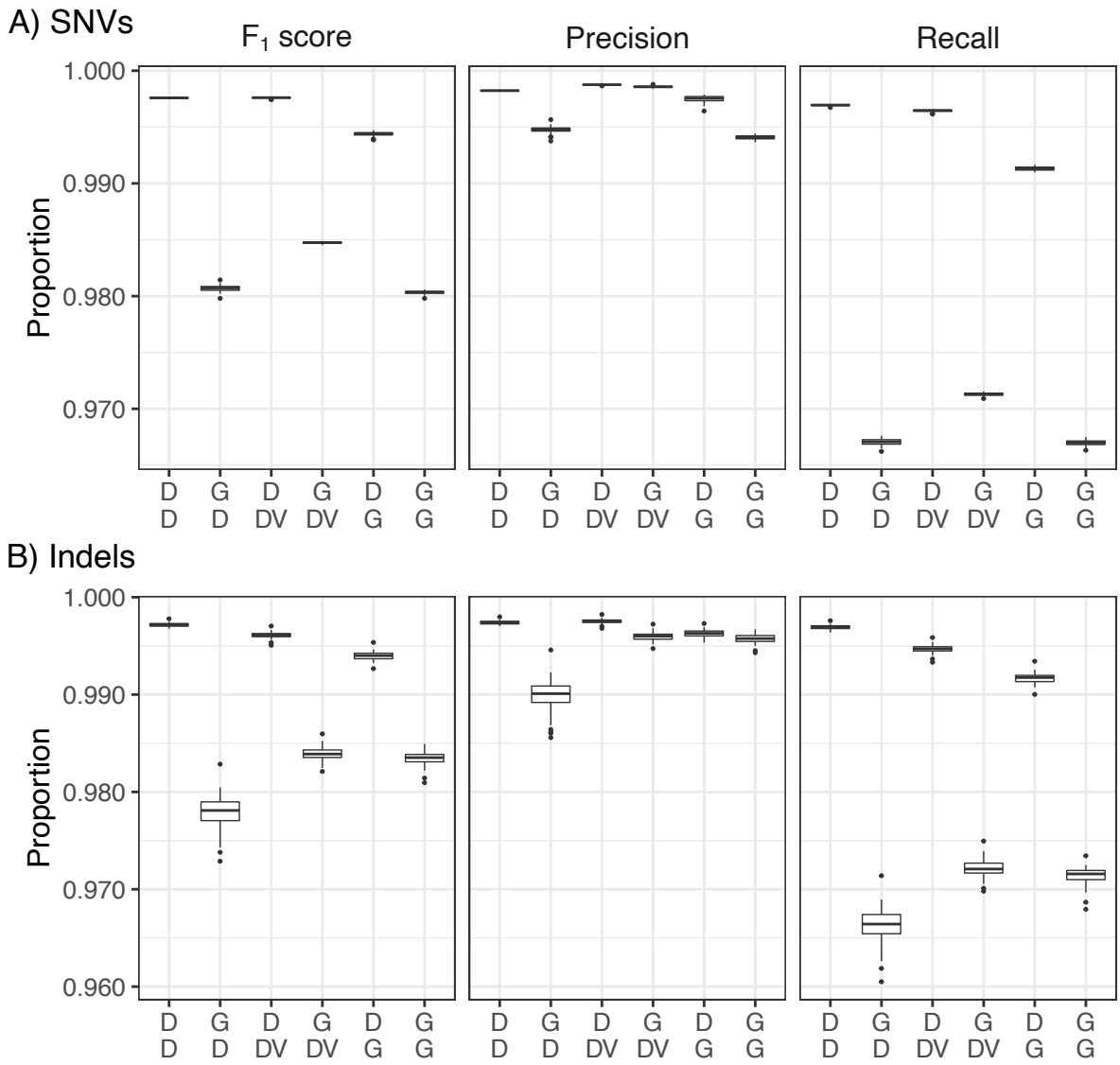

Figure 3. Percentages of F_1 score, precision and recall for single nucleotide variation (SNV) (upper three panels) and Indels (lower three panels) for the 6 pipeline combinations. Labels on the x-axis were defined in detail in Figure 1. D: DRAGEN; DV: DeepVariant; G: GATK.

Called variants and Ti/Tv ratio. Table 1 shows the number of polymorphic sites that failed and passed the filtering step for HG002. Between 4,680,047 (GATK-DV) and 5,066,532 (DRAGEN-DRAGEN) variants passed the filtering step. Both DeepVariant variant calling pipelines had the highest number of positions with failed variants. When DRAGEN was used in the alignment step, on average >100,000 more filter passing variants were detected as compared to using GATK for mapping and alignment. Furthermore, when DRAGEN was used for variant calling, approximately 200,000 additional variants passed filtering as compared with DeepVariant or GATK in the variant calling (Table 1).

The transition-to-transversion (Ti/Tv) ratio is an important measure to assess the quality of SNV calling. After stringent quality control, WGS studies are expected to have a Ti/Tv ratio around 2.0 – 2.2[16]. For our GIAB samples, Ti/Tv ratios varied between 1.960 (DRAGEN-DRAGEN and DRAGEN-GATK) and 1.998 (DRAGEN-DeepVariant) prior to stringent quality control (Table 1).

Table 1. Comparison of variant counts for the 6 pipelines with HG002 sequenced 70 times in different runs. Displayed are means and standard deviations (in parenthesis).

Mapping/alignment	**DRAGEN-**	**GATK-**	**DRAGEN-**	**GATK-**	**DRAGEN-**	**GATK-**
Variant calling	**DRAGEN**	**DRAGEN**	**DeepVariant**	**DeepVariant**	**GATK**	**GATK**
Failed filters	58,072 (1290)	78,883 (2164)	2,464,646 (195,004)	1,992,543 (116,083)	64,495 (1374)	47,017 (1075)
Passed filters	5,066,013 (3669)	4,840,437 (7688)	4,839,100 (2611)	4,679,657 (3452)	4,826,743 (5615)	4,772,371 (9821)
SNVs	4,080,672 (2417)	3,849,055 (3745)	3,914,177 (1177)	3,789,168 (1608)	3,889,807 (3161)	3,860,497 (5909)
Insertions	478,685 (996)	441,575 (1876)	444,619 (1122)	425,348 (1658)	447,413 (1662)	434,662 (2302)
Deletions	481,852 (603)	440,488 (1451)	455,649 (650)	441,116 (998)	450,115 (972)	439,814 (1735)
Indels	24,803 (198)	22,476 (454)	24,097 (162)	23,352 (194)	21,378 (334)	20,547 (439)
Ti/Tv	1.960 (0.00116)	1.997 (0.00194)	1.998 (0.00073)	1.992 (0.00074)	1.960 (0.00152)	1.971 (0.00217)

Target sensitivities of 99.95% for SNVs and 99.75% for Indels were used for pipelines involving the GATK Haplotypecaller. SNV: single nucleotide variation; Indels: insertions/deletions; Ti/Tv: transition/transversion.

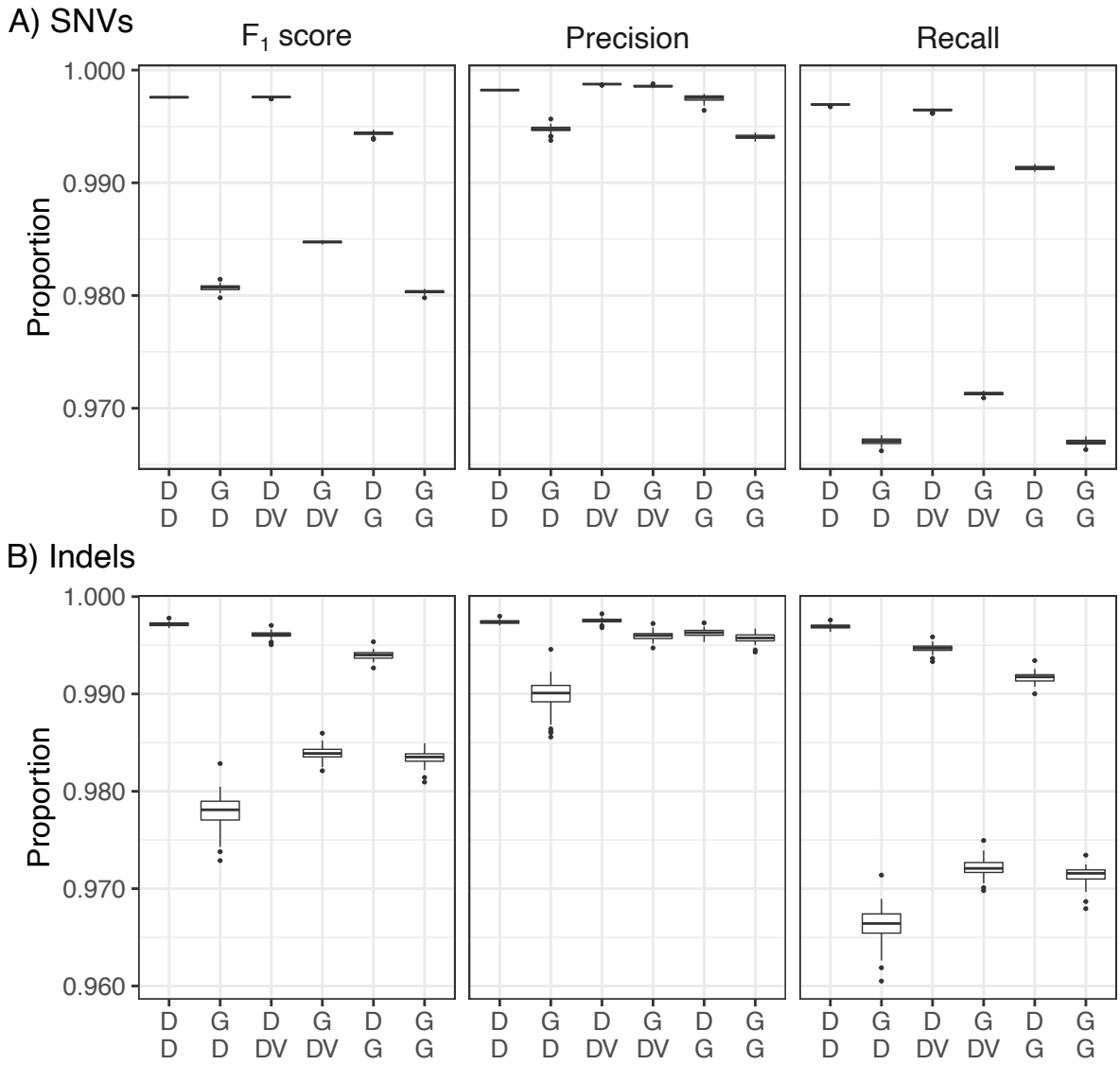

Figure 4. Percentages of F_1 score, precision and recall for single nucleotide variation (SNV) (upper three panels) and Indels (lower three panels) for the 6 pipeline combinations. Labels on the x-axis were defined in detail in Figure 1. D: DRAGEN; DV: DeepVariant; G: GATK.

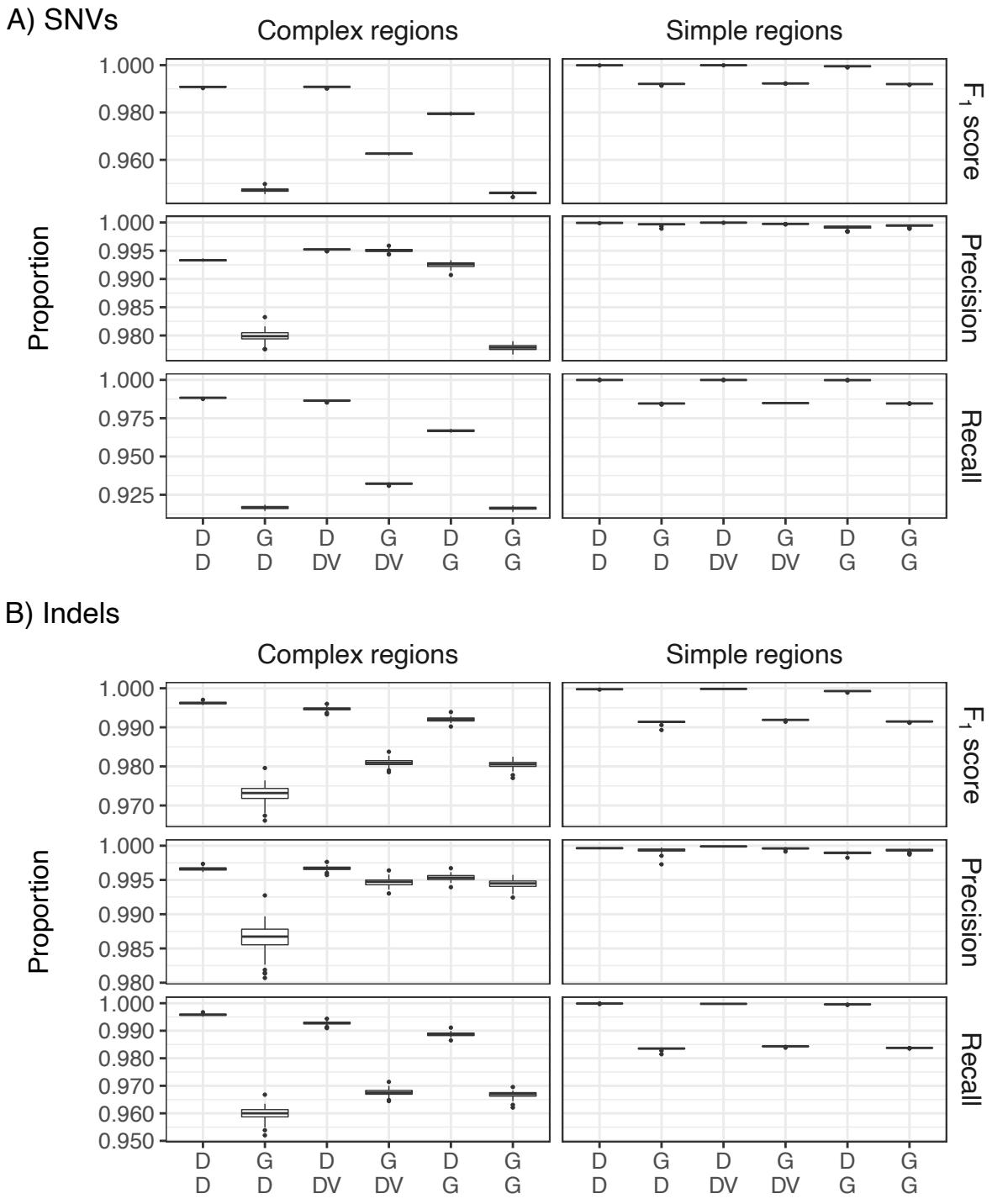

Figure 5. Percentages of F_1 score, precision and recall for complex-to-map (complex) and simple-to-map (simple) regions for single nucleotide variation (SNV) (upper 6 panels) and Indels (lower 6 panels) for the 6 pipeline combinations. Labels on the x-axis were defined in detail in Figure 1. D: DRAGEN; DV: DeepVariant; G: GATK.

Benchmarking for SNVs and Indels.

Figure 4 displays F_1 scores, precision and recall for SNVs (upper part A) and Indels (lower part B), respectively, for the 6 different pipelines after filtering. Both parts of the figure show substantial higher F_1 scores when DRAGEN was used upstream instead to GATK. DeepVariant and DRAGEN performed similarly in the variant calling step when mapping and alignment was done with the DRAGEN. F_1 scores and precision were slightly higher for SNVs with DeepVariant compared to DRAGEN, which has its basis in higher precision. In contrast, the recall for SNVs was lower for DeepVariant than for DRAGEN. In the case of Indels, the F_1 score of the DRAGEN was higher than for DeepVariant, when the DRAGEN was used upstream.

Benchmarking for SNVs and Indels in simple-to-map (simple) and difficult-to-map (complex) regions. Results were similar for difficult-to-map (complex) and simple-to-map (simple) regions (Figure 5). These regions have been defined for the truth set of the GIAB samples (for details, see Methods). For SNVs, F_1 scores were lower in complex regions, when GATK was used upstream compared to any DRAGEN pipeline upstream. These differences in the F_1 statistic were primarily caused by low recall values for GATK-based pipelines. However, even precision values were lower for SNVs in complex regions when GATK was used for mapping and alignment. In simple regions, precision was similar for all pipelines in case of SNVs, while recall was lower for GATK was used.

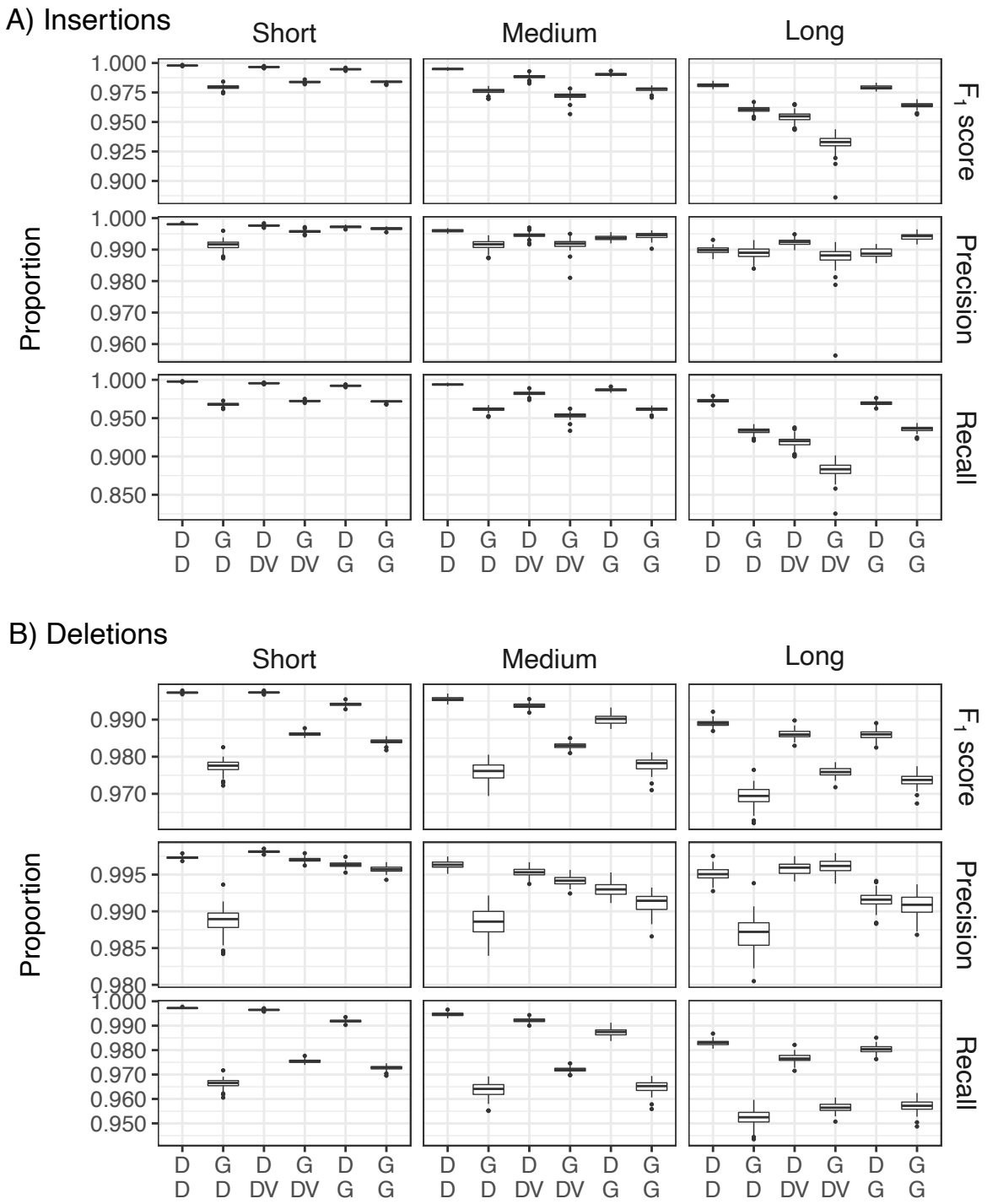

Figure 6. Percentages of F_1 score, precision and recall for deletions (upper part A) and insertions (lower part B) for short (1-5 bp), medium (6-15 bp) and long (> 15 bp) insertions / deletions for the 6 pipeline combinations. Labels on the x-axis were defined in detail in Figure 1. D: DRAGEN; DV: DeepVariant; G: GATK.

Consequently, F_1 scores were higher for all DRAGEN-based pipelines than for all GATK-based pipelines.

Benchmarking using precision, recall and F_1 scores for Indels of different size. Figure 6 shows F_1 scores, precision and recall for insertions (upper part) and deletions (lower part) of different sizes. Again, pipelines that used DRAGEN for mapping and alignment outperformed comparable GATK pipelines. Differences increased with the size of the insertions or deletions.

Mendelian inheritance error fractions. The GIAB trio consisting in HG002, HG003, and HG004 was sequenced three times to estimate Mendelian inheritance error fractions. Figure 7 shows that Mendelian inconsistencies were lowest for the DRAGEN pipeline. Error fractions were similar when GATK or DRAGEN were used upstream, followed by DeepVariant for variant calling (downstream). A substantial difference in error fractions was observed between GATK and DeepVariant in the variant calling step when GATK was used upstream.

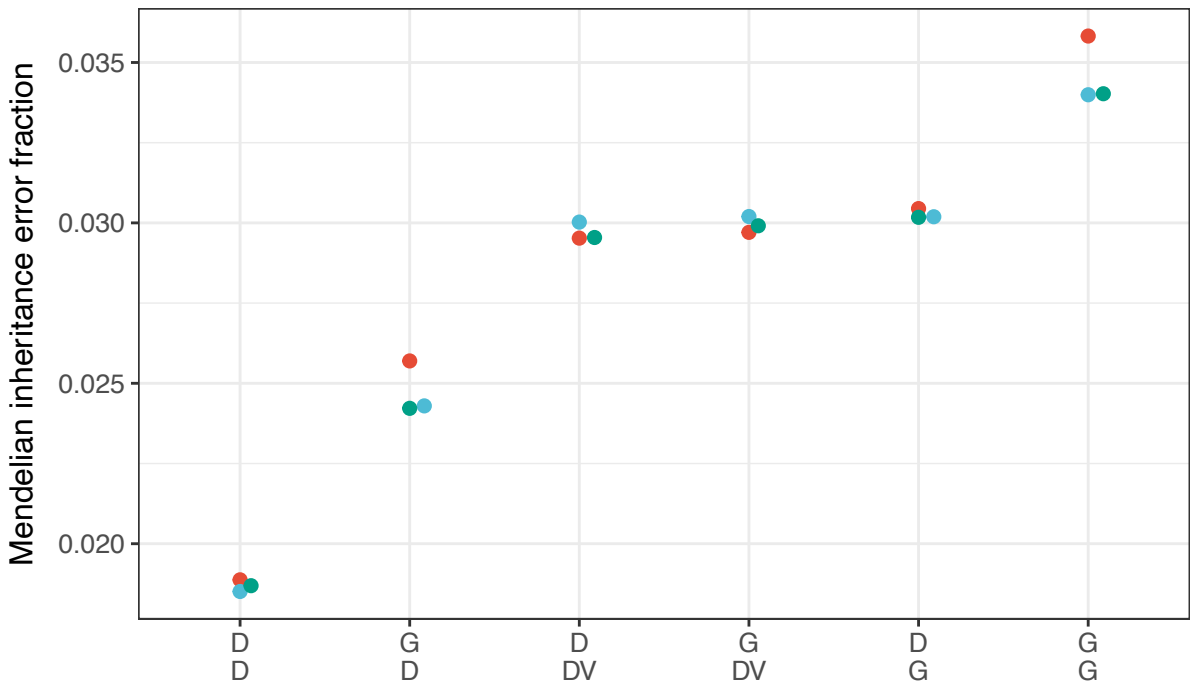

Figure 7. Mendelian inheritance error fraction for the GIAB trio, which was sequenced three times. The three different GIAB trio sequences are colored in red, blue, and green. Labels on the x-axis were defined in detail in Figure 1. D: DRAGEN; DV: DeepVariant; G: GATK.

Discussion

Our empirical comparison of 6 different pipeline combinations for mapping/alignment and variant calling of WGS data demonstrated that mapping and alignment with DRAGEN had higher F_1, precision and recall compared to GATK with BWA-MEM2. Specifically, the DRAGEN outperformed GATK in the in the upstream part of the pipeline for both SNVs and Indels, even when stratified by mapping complexity or by Indel size. Differences were most pronounced for the recall, termed sensitivity in statistics, i.e., the probability to detect a true variant. In line with previous precisionFDA Truth Challenges and other work[8,9], DeepVariant and DRAGEN performed well in the

calling of both SNV and Indels. DeepVariant was optimized for SNV calling[7], and it performed slightly better than the DRAGEN on SNVs. In contrast, the DRAGEN performed better than DeepVariant on Indels. We failed to confirm the findings of Hwang and colleagues[15] who demonstrated an excellent performance of BWA-MEM for mapping/alignment when combined with the GATK Haplotypecaller.

Computational efficiency was another aspect of interest in our comparative study. The highest speed was observed for the DRAGEN. Given that our processed GIAB samples had an average coverage of approximately 35×, the processing time per sample was 36 ± 2 min for both mapping/alignment and variant calling, which was substantially faster than GATK.

While "computational time" was considered "the most important advantage of the DRAGEN platform"[14], our pipeline comparison shows that accuracy in the mapping and alignment step might be at least equally important for downstream association or functional annotation analyses. The aspect of accuracy is not only demonstrated by the repeated sequencing of the GIAB sample HG002 and its comparison with the truth set data, but also by the Mendelian inheritance errors detected in the repeatedly sequenced GIAB trio. On the one hand, DeepVariant had fewer Mendel errors than GATK-based variant calling on parent-offspring trios, which is in line with the findings of Lin and colleagues[6]. However, when the DRAGEN was used for mapping and alignment, there was almost no difference between DeepVariant and GATK in the variant calling step. Results might differ when DeepTrio is used rather than DeepVariant

for variant calling in trios. DRAGEN mapping and alignment combined with DRAGEN variant calling outperformed the other 5 pipelines.

A limitation of our trio analysis might be that all subjects were treated individually. Inheritance-exploiting variant callers, such as DeepTrio[17] were not taken into consideration. However, large WGS studies generally focus on the association analysis of unrelated subjects. Another limitation might be that DeepVariant was trained by incorporating all GIAB samples but HG003 and all autosomal data but chromosome 20[18]. However, when we restricted analyses to data from HG003 (Suppl. Fig. S2) or chromosome 20 data of HG002 (Suppl. Fig. S3) only, the performance of DeepVariant differed only marginally from the performance involving all data. We therefore decided to present all data in this work.

Future studies might consider constructing a super learner in the variant calling step involving the two best-performing variant callers in this study, DeepVariant and DRAGEN.

Conclusions

The DRAGEN mapper and aligner had higher accuracy than the GATK with BWA-MEM2 mapper and aligner. DeepVariant and DRAGEN performed similarly for SNV and Indel variant calling, and both outperformed GATK variant calling pipelines. The DRAGEN pipeline showed the lowest percentage of Mendelian inheritance errors and had the shortest execution times. Because of accuracy and speed, we recommend the use of the DRAGEN for secondary analysis of WGS data.

Methods

Sample preparation. GIAB samples were sequenced as part of the QC for the WGS of more than 9000 samples. We ordered the GIAB samples from the Coriell Institute (NA24385, NIST ID HG002; NA24149, NIST-ID HG003 and NA24143, NIST-ID HG004). DNA concentration was measured by Qubit.

The library was constructed according to Illumina TruSeq DNA PCR Free Library Prep protocol HT (Illumina Inc., San Diego, CA, USA) for whole genome sequencing. Briefly, the protocol steps were: (1) fragmentation of 1 µg genomic DNA to 350 bp inserts by Covaris LE220-plus, (2) cleanup of fragmented DNA, (3) repair ends, (4) removal of large and small DNA fragments, (5) 3′-end adenylation and (6) adapter ligation. The resulting library was quantified and quality-assessed with the iSeq100 (Illumina). We performed sequencing with the NovaSeq 6000 platform (Illumina) using S4 flow cells with 300 cycles (2x 150 reads).

The GIAB samples were included in several runs within the whole genome sequencing project. Pools of 56 WGS samples were measured 2x on a S4 flow cell to reach a depth of coverage of 30x.

Variant calling pipelines. The raw sequencing files (base call file) were converted to fastq format and demultiplexed in a single step using Illumina's bcl2fastq program on a single DRAGEN version 2[19]. First steps in secondary analysis, including mapping and alignment, sorting, duplicate marking, and base quality recalibration were done using the GATK pipeline (version 4.2.4.1)[20] and the DRAGEN pipeline (version 3.8.4)[21] (Figure

1). The GATK best practices workflow was applied[20]. Read trimming for the GATK pipeline was performed with BBDuk (version 38.90) using the following parameters: ktrim=r k=23 mink=11 hdist=1 tpe tbo threads=8 trimpolygright=10[22]. For the DRAGEN pipeline, the integrated soft read trimer with default parameters was used. The human reference genome hs38DH was used for mapping, which contains the primary assembly of GRCh38 plus ALT contigs, additional decoy contigs and HLA genes[23]. BWA-MEM2 2.2.1 was employed for mapping and alignment[24], which is faster than BWA-MEM[24]. In the GATK base quality score recalibration step, the genome was split into 64 fractions for higher computational efficiency (one fraction per core). In the variant calling step, 25 fractions were used. Variant calling for SNVs and Indels was performed using the GATK HaplotypeCaller not in the DRAGEN mode, the GATK HaplotypeCaller in the DRAGEN mode[25], DeepVariant v1.1.0[7], and DRAGEN[21]. Alignment and variant calling algorithms were combined to 6 different pipelines (Figure 1): 1) GATK upstream plus DeepVariant downstream, 2) GATK upstream plus GATK HaplotypeCaller in DRAGEN mode downstream, 3) GATK upstream plus GATK HaplotypeCaller not in DRAGEN mode downstream, 4) DRAGEN upstream and downstream, 5) DRAGEN upstream plus DeepVariant downstream and 6) DRAGEN upstream plus GATK Haplotypecaller not in DRAGEN mode downstream. To filter the variants produced by GATK Haplotypecaller, variant quality score recalibration (VQSR) was applied with the parameters recommended by GATK: For indel recalibration, the Mills_and_1000G_gold_standard.indels.hg38 and

Axiom_Exome_Plus.genotypes.all_populations.poly.hg38 datasets were used for training. For SNV recalibration, the Hapmap_3.3.hg38, 1000G_omni2.5.hg38 and the 1000G_phase1.snps.high_confidence.hg38 datasets were used for training. The dbSNP_v154_GRch38.p12 was used as truth set in both cases. For DeepVariant, default filtering parameters were used. For DRAGEN, default filtering parameters were used, except the QUAL threshold was changed to <5.0 for both SNVs and indels. vcf files were used for analysis. For each trio, the gvcf files created from each pipeline were jointly called with GLNexus (version 1.4.1)[26], except for the DRAGEN mapping/alignment and variant calling pipeline. Specifically, DRAGEN treats non-PAR regions of chromosome X as haploid, while GATK and DeepVariant treat them as diploid. So far, DRAGEN gvcf files are not supported by GLNexus. DRAGEN gvcf files were therefore jointly called with GenomicsDBImport and GenotypeVcfs, both part of GATK. The Mendelian inheritance error was calculated with bcftools mendelian plugin (version 1.11)[27].

Computing environment and resources. Analyses were run on a local high-performance computing cluster at Cardio-CARE (Davos, CH). Each compute node is equipped with 2 AMD EPYC 7742 CPUs, 2 TB RAM, approximately 11 TB NVMe, and CentOS 8 operating system. For pipeline comparisons, each pipeline was given 64 cores as in Ref. 14. Two DRAGEN Servers v3 were used for the DRAGEN pipeline.

Statistics. Precision, recall and F_1 scores were estimated for SNVs and Indels using all regions and regions stratified by difficult-to-map and simple-to-map regions. All comparisons were made against the GIAB truth set[28,29], available from

ftp.ncbi.nlm.nih.gov/giab/ftp/release/AshkenazimTrio/. Mappability of Indels was stratified by Indel size (1-5 bp, 6-15 bp, > 15 bp). Ti/Tv ratios were calculated to indicate potential sequencing error. The target sensitivity was varied and fixed to 99.75% for Indels and 99.95% for SNVs. Figures for varying target sensitivities are shown in the Supplementary material. Results from the 70 GIAB sample sequencing repetitions are summarized in boxplots. Performance metrics were calculated using hap.py v0.3.14, available from github.com/Illumina/hap.py. The numbers of passed/failed variants, SNVs, insertions and deletions for each pipeline were calculated using RTG tools (version 3.12.1)[30]. The statistical analysis was performed with R (version 4.1.1). Statistical tests for significance between runtimes (Suppl. Tab. S1) and performance metrics (Suppl. Tab. S2) were performed with a paired two-sided t-test. The confidence level was chosen as 0.95. No adjustments were made for multiple testing.

Genome regions for stratification analyses. To compare the performance of the calling algorithms in simple-to-map, sometimes termed easy-to-map, and difficult-to-map regions of the human genome, we stratified the data in genome-specific regions by using complexandSVs_alldifficultregions and notin_complexandSVs_alldifficultregions[29], annotations available at github.com/genome-in-a-bottle/genome-stratifications.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Author contributions

A.T., A.Z. and R.B. designed this study. A.T. and R.B. performed the bioinformatic and statistical analysis. D.A.G. performed the first quality analysis of the genome data. M.Z. and H.M. supervised the laboratory work. S.B. and T.Z. developed the concept of the whole genome sequencing study. A.Z. and R.B. wrote the paper. All authors commented on the manuscript.

Additional Information

Supplementary Information. The online version contains supplementary material.

Competing Interests

A.T., A.Z., R.B. and S.B. are employees of Cardio-CARE, a 100% daughter of the Kühne Foundation.