Size and composition of haplotype reference panels impact the accuracy of imputation from low-pass sequencing in cattle

Lloret-Villas A.[1], **Pausch H.**[1], **and Leonard A.S.**[1]

[1]Animal Genomics, ETH Zürich, Universitätstrasse 2, 8092 Zurich, Switzerland

Motivation: Low-pass sequencing followed by sequence variant genotype imputation is an alternative to the routine microarray-based genotyping in cattle. However, the impact of haplotype reference panel composition and its interplay with the coverage of low-pass whole-genome sequencing data has not been sufficiently explored in typical livestock settings where only a small number of reference samples are available.

Results: Benchmarking against a microarray truth set confirms that DeepVariant is a suitable variant caller to establish bovine haplotype reference panels. We build haplotype reference panels of varying sizes and composition with DeepVariant and use GLIMPSE to impute genotypes for cattle that had between 0.01- and 4-fold genome sequence data. Same-breed haplotype reference panels perform better than equally-sized multibreed haplotype reference panels for all target sample coverages and allele frequencies. F1 scores greater than 0.9 can be achieved with 0.25-fold sequencing coverage when large breed-specific haplotype reference panels (n = 150) are used. In absence of such large same-breed haplotype panels, sequence variant genotyping accuracy can be increased either by adding non-related samples or by increasing the coverage of the low-pass sequencing data. Sequence variant genotyping from low pass sequencing is substantially less accurate when the reference panel lacks individuals from the target breed.

Availability: Sequencing data are publicly available as indicated in the Supplementary Files. Scripts and workflows are available on Github: https://github.com/AnimalGenomicsETH/Low_pass_imputation.

Correspondence: ***avillas@ethz.ch***

Introduction

More than a million cattle are genotyped every year with microarray technology for the purpose of genomic prediction (1). Access to whole genome sequence variants can improve the accuracy of genomic predictions and facilitates the monitoring of trait-associated alleles (2). However, costs are still too high to sequence all individuals from a population to a sufficient coverage to call variants.

Low-coverage whole-genome sequencing (lcWGS) followed by genotype imputation has emerged as an alternative with comparable costs to genotyping microarrays but with substantially higher marker density (tens of millions versus tens of thousands) for obtaining genotypes for a target population (3–6). Sequencing coverage as low as 0.1-fold can be used to infer sequence variant genotypes that are as accurate as those obtained from genotyping microarrays, especially for rare variants, while sequencing coverage greater than 1-fold can have much higher accuracy (5). Reference panels that are representative for the target populations are a prerequisite for the accurate imputation of genotypes from lcWGS (7–9). The 1000 Genomes Project (1KGP) and the Haplotype Reference Consortium (HRC) established such reference panels for several human ancestry populations (10, 11) and made them available through dedicated imputation servers (12). A bovine imputation reference panel established by the 1000 Bull Genomes project is frequently used to infer sequence variant genotypes for large cohorts of genotyped taurine cattle, thus enabling powerful genome-wide analyses at the nucleotide level (13). Sequenced reference panels are available for other animal species (14, 15). However, these haplotype panels lack diversity as they were established mainly with data from mainstream breeds and thus are depleted for individuals from local or rare populations.

An exhaustive set of variants and accurate genotypes are crucial to compile informative haplotype reference panels. The Genome Analysis Toolkit (GATK) has been frequently applied to discover and genotype sequence variants in large reference populations of many livestock species (3, 14). DeepVariant has recently emerged as an alternative machine learning-based variant caller (16). Several studies suggest that DeepVariant has superior genotyping accuracy over GATK (17–20). However, DeepVariant had rarely been applied to call variants in species other than humans (21, 22).

In this study, we benchmark sequence variant genotyping of DeepVariant and GATK in a livestock population. We then build haplotype reference panels of varying sizes and composition with DeepVariant, and use GLIMPSE to impute sequence variant genotypes for cattle that had been sequenced at between 0.01- and 4-fold genome coverage. We show that within-breed haplotype reference panels outperform multibreed reference panels across all tested scenarios, provided that enough sequenced samples are available.

Materials and methods

Data availability and code reproducibility.

Short paired-end whole-genome sequencing reads of 501 cattle from nine breeds were used: 327 Brown Swiss (BSW), 50 Fleckvieh, 13 Hereford, 57 Holstein, 2 Nordic Red, 14 Rätisches Grauvieh, 10 Simmental, 25 Tyrolean Grauvieh and 3 Wagyu cattle. Accession numbers for the raw data are available in the Supplementary file 1. Computational workflows were implemented using Snakemake (23) (version 7.5.0 or newer). The R software environment (version 4.0.2) and ggplot2 package (24) (version 3.3.2) were used to create figures and perform statistical analyses. Scripts and workflows are available online.

Alignment, mapping quality and depth of coverage.

Raw short sequencing reads were filtered with fastp (25) (version 0.23.1), and MultiQC (26) (version 1.11) was applied to collect the quality metrics across samples. Reads were split per read groups with gdc-fastq-splitter (27) (version 1.0.) and subsequently aligned with bwa-mem2 (28) using the -M and -R flags to a manually curated version of the current bovine Hereford-based reference genome (ARS-UCD1.2) (29) that included a Y chromosome as described in (30). Samblaster (31) (version 0.1.26), Sambamba (32), samtools (33, 34) (version 1.12), and Picard tools (35) (version 2.25.7) were used to deduplicate and sort the BAM files. We calculated average coverage with mosdepth (36) (version 0.3.2) considering all aligned reads that had MQ $\geq$ 10.

Comparison of variant callers.

Testing set. 50 BSW cattle with coverages ranging from 4 to 63-fold were selected as testing set for a comparison between GATK and DeepVariant.

GATK. We used the BaseRecalibrator module of GATK (37, 38) (version 4.2.2.0) to adjust the base quality scores of the deduplicated bam files using 115,815,224 unique positions from the Bovine dbSNP version 150 as known variants. Multi-sample variant calling was performed with the GATK HaplotypeCaller, GenomicsDBImport and GenotypeGVCFs modules according to the best practice guidelines (39, 40). We applied the VariantFiltration module for site-level filtration with thresholds indicated in (30) to retain high-quality SNP and INDELs.

DeepVariant + GLnexus. DeepVariant (16) (version 1.2) was run on the deduplicated bam files using the "WGS" Illumina-trained model, producing gVCF output per sample. The gVCF files were then merged and filtered using GLnexus (41) (version 1.4.1) with the "DeepVariantWGS" configuration but with the "revise_genotypes" flag set to false.

VCF imputation and statistics. We used Beagle 4.1 (42) (27Jan18.7e1) to improve genotype calls and impute sporadically missing genotypes from genotype likelihoods (gl mode). INDELs were left-normalised using bcftools (34) (version 1.12 or 1.15) norm. Variant and genotype counts, and Ti:Tv ratios were calculated with bcftools stats and bcftools query. VCF files were indexed with tabix (43, 44).

Variant annotation. Functional consequences of SNPs were predicted based on the Ensembl (release 104) annotation of the bovine reference assembly using the Variant Effect Predictor tool (VEP) (45) (version 106) with default parameter settings.

Variant accuracy evaluation. We intersected the truth and query VCF files using bcftools isec with both the "-c none" (exact) and "-c all" (position) flags, and retained biallelic SNPs with bcftools view to compare the genotypes for 33 cattle that had both microarray- and sequence-derived genotypes (Supplementary File 1). Intersection overlaps were counted and then plotted with UpSetR (46, 47). Since the microarray data contains fewer sites than WGS, we intersected the truth (microarray) and query (WGS variants) sets to retain only positions where the truth genotypes were not homozygous for the reference allele. We calculated recall, precision, and F1 scores for the truth and query sets using hap.py (48) (version 0.3.9) on a per-sample basis. Agreement between the imputed variant alleles/genotypes and raw sequencing reads was assessed with Merfin's k-mer-based filtering method (49) (commit fc4f89a). A k-mer database was prepared using Meryl (commit 51fad4b) with a k-mer size of 21 and minimum k-mer occurrence of 2 in the short sequencing reads.

We assessed mendelian consistency in filtered but not-imputed data from parent-offspring pairs and trios (Supplementary File 2) using the bcftools +mendelian plugin (34). We calculated discrepancy rate as the number of inconsistent sites divided by the total number of non-missing sites. For duos (dam-offspring or sire-offspring) only homozygous sites were considered.

Imputation of low-pass sequencing data.

Haplotype panel generation. The BSW reference panels contained 150, 75 and 30 samples that were randomly selected from 303 BSW samples. The non-BSW panels contained 150, 75 and 30 samples that were randomly selected from 174 non-BSW samples. The multibreed panels were randomly selected from a combination of the above, and they contained 150 samples of which 50%, 25%, and 10% were BSW samples and the remaining were non-BSW. Three random replicates for each panel were created. Sequence variant genotypes were called for each panel with DeepVariant and sporadically missing genotypes were imputed with Beagle 4.1 (42) (27Jan18.7e1) as described above.

Truth sequencing set, truth variants and subsampling. Variants were called with DeepVariant and GLnexus as described previously for 24 BSW samples with coverage above 20-fold to generate a truth set for assessing imputation accuracy. The raw sequencing reads of the 24 BSW samples were

then downsampled with seqtk (50) to mimic 4x, 2x, 1x, 0.5x, 0.25x, 0.1x, and 0.01x coverage, and subsequently aligned to ARS-UCD12 as described previously.

Genotype likelihoods for the variants that are present in the haplotype reference panel were estimated from the subsampled read alignments with bcftools mpileup and bcftools call. These were then imputed using the different haplotype panels using GLIMPSE (51) (version 1.1.1). We used 2 Mb windows and 200 Kb buffer sizes during the chunk step followed by phasing and ligation to produce the final imputed variant calls.

Comparison of true and imputed variants. The accuracy of the imputed sequence variant genotypes was assessed with hap.py as described above. The minor allele frequency (MAF) of the imputed sequence variants was calculated with PLINK (52) (version 1.9). The estimated imputation quality was retrieved from the INFO flag from the VCF files produced by GLIMPSE with bcftools query. Pearson squared correlation between expected and actual dosages (r^2) was calculated with the bcftools stats.

Results

Variant calling with GATK and DeepVariant.

We compared sequence variant discovery and genotyping between GATK and DeepVariant in 50 Brown Swiss (BSW) cattle that had between 4 and 63-fold sequencing depth (19.26 ± 11.09) along the autosomes. GATK and DeepVariant identified 18,654,649 and 18,748,114 variants, respectively, of which 7.79% and 8.38% were filtered out due to low quality (Table 1). There were 16,147,567 filtered variants identified by both callers, but 1,053,716 and 1,292,671 variants were private to GATK and DeepVariant, respectively (Figure 1A). Overall, DeepVariant had more private SNPs than GATK, but GATK had more private INDELs than DeepVariant (Supplementary Table 1). 416,642 variants had the same coordinates but different alternative alleles. These discrepant sites were primarily INDELs (83%, as opposed to the 12% of INDELs in all shared variants). Multiallelic sites accounted for 3.44% and 3.31% of the variants (0.33% and 0.28% of the SNPs, and 23.22% and 23.94% of the INDELs) that passed the quality filters of GATK and DeepVariant, respectively. Multiallelic sites were enriched among the variants private to either GATK or DeepVariant (Supplementary Table 2).

The biallelic variants called by GATK had a higher percentage of homozygous reference (HOMREF) and heterozygous (HET) genotypes whereas the biallelic variants called by DeepVariant had a higher percentage of homozygous alternative (HOMALT) genotypes (Figure 1B, Supplementary Figure 1A). Missing genotypes were very rare (<0.01%) for GATK-called biallelic variants but accounted for 2.72% of the DeepVariant-called genotypes (Supplementary Figure 1B). Beagle phasing and imputation increased the number of HET genotypes for both GATK - mainly transitioning from HOMREF - and DeepVariant - mainly due to the refinement of missing genotypes (Supplementary Figure 1C).

Functional consequences on the protein sequence were predicted for all biallelic variants. DeepVariant identified more SNPs that were predicted to have a high impact on protein function than GATK in the raw (+24%) and filtered (+9%) sets (Table 1 & Supplementary Table 3). Around one fourth of the high impact SNPs detected by DeepVariant (24%) were not detected by GATK. GATK identified more INDELs that were predicted to have a high impact on protein function than DeepVariant in the raw (+62%) and filtered (+78%) sets. More than half of the high impact INDELs detected by GATK (52%) were not detected by DeepVariant.

We investigated the ratio of transitions to transversions (Ti:Tv) to assess variant quality. The Ti:Tv ratio was 2.16 and 2.24 for raw SNPs identified by GATK and DeepVariant, respectively (Table 1). While the Ti:Tv ratio was higher (2.20) for the GATK variants that met the quality filters, variant filtration had no impact on the Ti:Tv ratio for SNPs called by DeepVariant. The Ti:Tv ratio of the filtered out SNPs was substantially lower for GATK (1.66) than DeepVariant (2.19). SNPs private to GATK had lower Ti:Tv ratios than the SNPs private to DeepVariant (Figure 1A). Substantial differences in the Ti:Tv ratio (0.81 points) existed between overlapping and GATK-private SNPs but were less (0.18 points) between overlapping and DeepVariant-private SNPs.

Variant calling accuracy.

Thirty-three sequenced cattle also had between 17,575 and 490,174 SNPs genotyped with microarrays. The filtered biallelic SNPs called with GATK and DeepVariant (query sets) were compared to those genotyped with the microarrays (truth chip set). The vast majority (98.82%) of the SNPs present in the truth chip set was called by both tools (Figure 1C). The overlap of SNPs present in the truth chip set was slightly higher for DeepVariant than GATK. 1.06% (n = 5,309) of the SNPs present in the truth chip set were not called by any of the software as biallelic SNPs (exact match). However, 3,497 of these SNPs were either multiallelic SNPs or INDELs (position match) in DeepVariant/GATK while the other 1,812 were truly missing. Most of the SNPs private to the chip set (5,265 exact match / 1,767 position match) were also missing in the raw calls from the variant callers. DeepVariant filtered out more variants present in the truth chip set than GATK.

The analysis of variant effect predictions for the filtered variants revealed that most low/moderate/high impact variants were called by both GATK and DeepVariant (99.9%, 99.8%, and 95.4%, respectively). However, DeepVariant additionally called 5/2/4 variants predicted as low/moderate/high impact respectively, while GATK only called 0/1/1 (Figure 1C). Some of the low/moderate/high impact biallelic SNPs private to GATK and DeepVariant (5/11 private to DeepVariant and 1/2 private to DeepVariant) were called either as multiallelic

Table 1. Summary of the variants called by GATK and DeepVariant (DV). Multiallelic sites are presented in parentheses. Ti:Tv ratios are restricted to biallelic SNPs. Functional consequences are predicted for biallelic SNPs / biallelic INDELs.

Variant caller	Sets	Variants	SNPs	INDELs	Ti:Tv ratio	High impact predicted SNPs / INDELs
GATK	Raw	18,654,649 (831,391)	16,135,130 (58,049)	2,617,546 (773,342)	2.16	2,680 / 4,493
GATK	Filtered out	1,453,366 (239,008)	1,271,522 (8,577)	279,871 (230,431)	1.66	428 / 500
GATK	Filtered	17,201,283 (592,383)	14,863,608 (49,472)	2,337,675 (542,911)	2.20	2,252 / 3,993
DV	Raw	18,748,114 (702,173)	16,554,438 (54,438)	2,401,933 (647,735)	2.24	3,530 / 2,778
DV	Filtered out	1,571,454 (270,963)	1,174,815 (11,834)	393,927 (259,108)	2.19	1,061 / 612
DV	Filtered	17,440,238 (577,997)	15,361,785 (42,899)	2,240,627 (535,098)	2.24	2,474 / 2,240

SNPs or as INDELs by the other caller (Supplementary Table 4). Only half (1/2) of GATK's private variants annotated with low/moderate/high have minor allele frequencies (MAF) $>$ 0.05, while most (9/11) of DeepVariant's do, suggesting that GATK misses more variants that might have larger impact in populations.

Genotyping accuracy of variant calls.

GATK and DeepVariant called 492,265 and 493,145 variants from the truth chip set, respectively. GATK missed and miscalled more truth variants than DeepVariant: 8.13% and 10.13%, respectively. Around 90.6% of the discrepancies between the sequence variant genotypes and the truth chip set in both variant callers were due to missing genotypes. Of those, GATK missed proportionally more HOMALT than DeepVariant and DeepVariant missed proportionally more HET variants. For the remaining $\sim$9.4% of mismatching genotypes (miscalled), also GATK miscalled proportionally more HOM variants and DeepVariant significantly miscalled proportionally more HET variants (Supplementary Figure 2). After imputation, however, the proportion of HET positions miscalled was higher in the GATK set and the proportion of HOMREF positions miscalled as HET was significantly higher in the DeepVariant set.

Recall, precision and F1 score of the filtered query sets were calculated to assess the genotyping accuracy for both variant callers. DeepVariant had strictly better F1 scores than GATK for the filtered data (mean of 0.9719 versus 0.9694, Figure 2A-B). The difference was small but significant (Wilcoxon signed-rank test, p=$2.3x10^{-10}$). As expected, lower coverage ($<$20x) samples benefited from imputation, improving their F1 scores to be comparable to high coverage samples. Imputation improved GATK genotypes more than DeepVariant genotypes at lower coverages, potentially due to better calibration of genotype likelihoods, but DeepVariant was still strictly better above 7x coverage. Overall, DeepVariant still had a significantly higher mean F1 score for the imputed data (0.9912 versus 0.9907, Wilcoxon signed-rank test p=$4.2x10^{-05}$, Figure 2C).

We further examined variant genotyping accuracy through Merfin (49). This approach assumes that variant calls producing k-mers not observed in the sample's short sequencing reads are likely to be wrong. HET genotypes of both GATK and DeepVariant had less support from the sequencing reads, as they are harder to genotype correctly than HOM genotypes. For both HET and HOMALT, more of DeepVariant's than GATK's variants were supported (Figure 3A). The difference between the tools was statistically significant for both genotypes (two-sided paired Wilcoxon test, p_{HET}=$3.6x10^{-19}$, p_{HOMALT}=$1.8x10^{-19}$).

In addition, we compared mendelian concordance rate among sequenced duos and trios across the two variant callers. There were only two family relationships in the previously examined 50 samples, and so we evaluated the concordance on a separate set of 206 samples (Supplementary File 2) forming 7 trios (both parents available) and 142 duos (one parent available). DeepVariant had less genotypes conflicting with mendelian inheritance compared to GATK (2.3% versus 3.8%, Figure 3B, one-sided paired Wilcoxon signed-rank test p=$1.3x10^{-24}$). This was due to DeepVariant calling both more genotypes that were compatible as well as fewer that were incompatible with parent-offspring relationship.

Generation of a sequencing validation set for lcWGS imputation.

We benchmarked the accuracy of low-pass sequence variant imputation in a target population consisting of 24 BSW samples with mean autosomal coverage of 28.12 ± 9.07-fold. DeepVariant identified 15,948,663 variants (87.77% SNPs and 12.23% INDELs) in this 24-samples cohort of which we considered 13,854,932 biallelic SNPs as truth set.

The sequencing reads of these 24 samples were randomly downsampled to mimic mid (4x and 2x), low (1x, 0.5x, 0.25x, and 0.1x), and ultralow (0.01x) sequencing coverage. We then aligned the reads to the reference sequence and produced genotype likelihoods from the pileup files. Subsequently, genotypes were imputed with GLIMPSE considering nine haplotype reference panels, and compared to the truth set to determine the accuracy of imputation.

The nine haplotype reference panels varied in size and com-

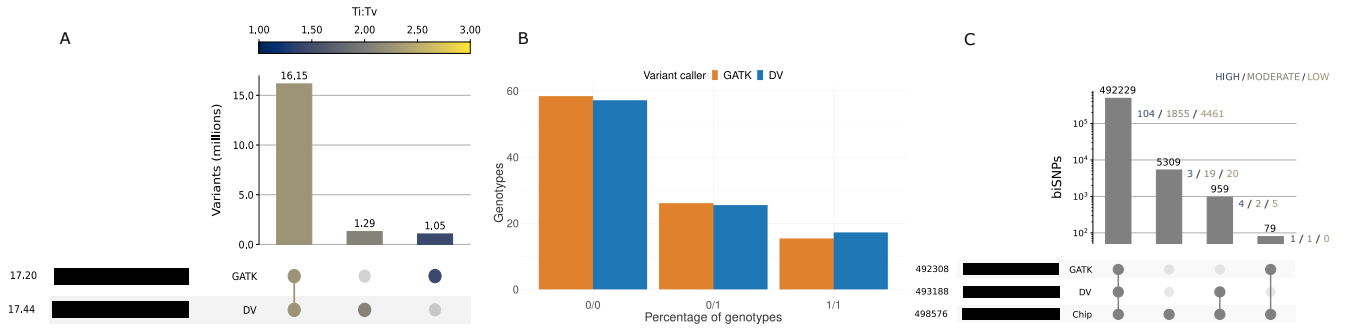

Fig. 1. Variant call comparison between DeepVariant (DV) and GATK. a) Intersection of variants called with each variant caller (or both) and the Ti:Tv ratio of the biallelic SNPs of each set. b) Percentage of imputed genotypes called by each variant caller. c) Intersection of variant calls with truth genotyping arrays, where only positions intersecting truth are retained. Low, moderate, and high predicted impact variants from the intersection sets are indicated.

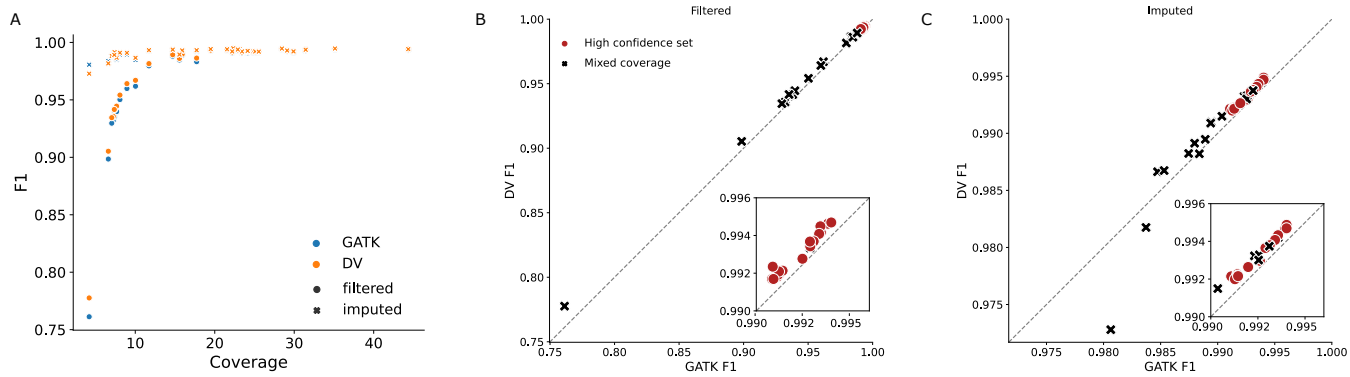

Fig. 2. Comparison of the F1 values obtained with hap.py from GATK and DeepVariant (DV) variant calls against the truth chip set for 33 samples. a) Imputation improves genotype accuracy below 20x coverage but has minor impact above that. b) DV has a higher F1 score for every sample than GATK for post-filter variants. The high confidence set indicates the 17 microarray genotyped samples out of the 24 samples used later as a truth set for GLIMPSE imputation. c) Similar to (b) but for post-imputation variants.

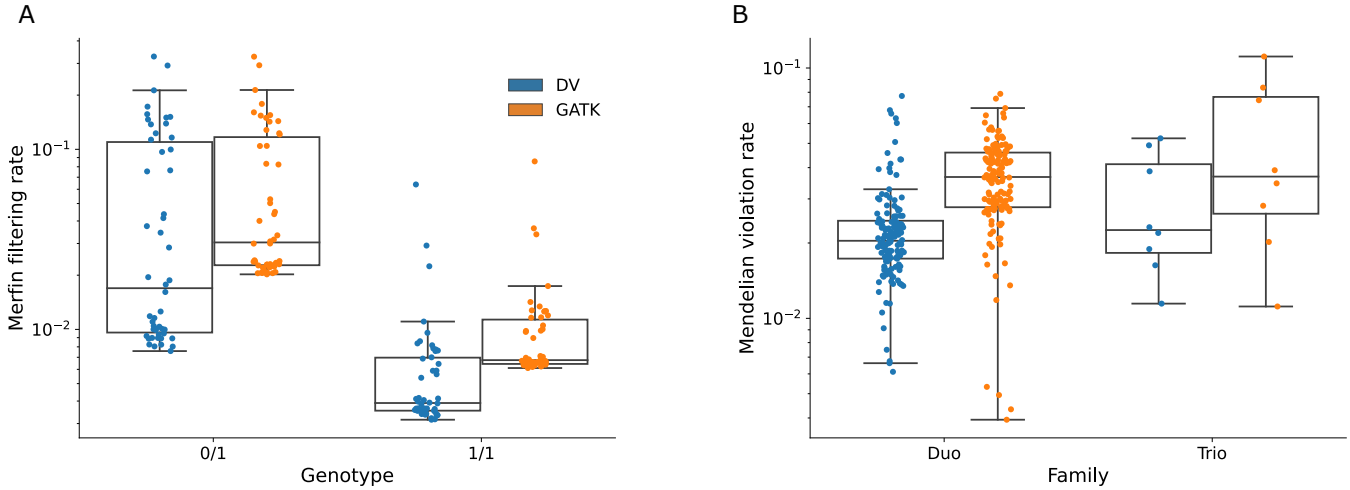

Fig. 3. Genotyping accuracy of variant calls validated with sequencing reads and mendelian relationships. a) Filtering rate of heterozygous (0/1) and homozygous alternate (1/1) variant calls post-imputation for GATK and DV. Higher filtering rate indicates the genotype/allele is not consistent with k-mers from the same-sample sequencing reads. b) Mendelian violation rate for 206 separate samples, with either 2 family members (Duo) or all 3 (Trio). Mendelian violations are defined as genotypes in the offspring that could not have been inherited from the parents. In the case of duos, only homozygous variants can be assessed.

position. Five haplotype reference panels contained 150 cattle (full panels) of which either 0%, 10%, 25%, 50% or 100% were from the BSW breed (*i.e.*, the breed of the target samples). The other four panels contained either 75 or 30 cattle (reduced panels) that were either from the BSW breed or from breeds other than BSW. DeepVariant identified between 17,035,514 and 28,755,400 sequence variants in the nine haplotype reference panels (Table 2). The full BSW panel contained around 5,167,875 less biallelic SNPs than the full non-BSW panel. The 50% multibreed panel had the highest number of variants shared with the truth set and the lowest number of variants present in the truth set but missing in the reference panel, closely followed by the BSW panel. The reduced non-BSW panel (30 samples) had the lowest number of shared variants and the highest number of variants that were present in the truth but missing in the reference.

Assessment of lcWGS imputation with the different haplotype panels.

Increasing the number of reference haplotypes enabled higher F1, recall and precision scores in all tested scenarios (Figure 4A & Supplementary Table 5). Imputation accuracy also improved with increasing lcWGS coverage, with the largest change between 0.01x and 1x coverage. Accuracy continued to improve with diminishing returns between 1x and 4x coverage. The difference in accuracy between panels also reduced as coverage increased.

The largest BSW haplotype reference panel (n = 150) performed better than any of the multibreed panels at all sequencing coverages. Multibreed panels outperformed BSW panels with a larger number of BSW samples, especially at low coverage. For instance, a large multibreed panel containing 10% BSW samples (n = 15) produced higher F1 scores than a smaller breed-specific panel containing two times more BSW samples (n = 30). Similarly, a large multibreed panel containing 25% BSW samples (n = 37) provided higher F1 scores than a smaller breed-specific panel containing two times more BSW samples (n = 75) for lcWGS below 1-fold coverage. Accuracies were similar between large multibreed panels and smaller breed-specific panels when the coverage of the lcWGS was higher than 1-fold. All results were validated by three different conformations of the haplotype reference panels (replicas). Standard errors accounting for all the replicas did not overlap for any of the haplotype panels (Supplementary Figure 3A).

The imputation accuracy estimated by GLIMPSE (INFO score) was higher for all BSW panels than for the multibreed panels across all coverages (Figure 4B). A higher proportion of variants were imputed with an INFO greater than 0.6 in the BSW than in non-BSW or multibreed panels (Supplementary Figure 3B). Therefore, panels for which the average INFO was higher had also a major proportion of variants with high imputation quality, potentially selected for downstream analyses. Differences between BSW panels and the rest were higher than the differences between multibreed and non-BSW. The average values of F1 and the average INFO scores were closer for the variants imputed with BSW panels (Figure 4C). The differences between both metrics decreased as the coverage of the lcWGS increased (Supplementary Figure 3B-C).

The variants were then stratified by MAF, and the squared correlation of genotype dosages (r^2) was calculated (Figure 4D). The correlations increased along with the MAF similarly for all the panels. The highest correlations were for BSW panel (150 samples) and multibreed panels (50% and 25%). The values increased substantially between 0-0.1 MAF and continued slowly incrementing until 0.5 for all panels.

Discussion

Higher F1 scores against a microarray truth set, improved k-mer based variant filtering, and less mendelian errors suggest that DeepVariant is a superior variant caller to GATK for bovine short read sequencing. These results extend evidence of DeepVariant's greater accuracy established in multiple human studies (17–20). Ti:Tv ratios in the expected range of 2-2.2 suggest that variant calls private to DeepVariant contain genuine variants, whereas a lower Ti:Tv ratio in variants private to GATK is indicative for an excess of false positives. DeepVariant revealed more SNPs with impactful annotations, likely providing additional putative trait-associated candidates for downstream analyses. While our benchmark analysis focused on variant genotyping accuracy, different compute requirements between GATK and DeepVariant (18, 20) also require consideration particularly when implementing variant calling for large reference panels.

To the best of our knowledge, our study is the first to establish bovine haplotype reference panels with DeepVariant. A within-breed panel consisting of 75 samples enabled us to genotype more than 13 million sequence variants in animals sequenced at 0.5-fold sequencing coverage with F1 scores greater than 0.9. Larger haplotype reference panels (n = 150) from the same breed as the lcWGS data outperform multibreed panels across all low coverage spectrum (from 0.1- to 1-fold) and MAFs, including rare variants. The development of such panels is a feasible alternative option to using much bigger multibreed panels, such as the 1000 Bull Genomes project imputation reference panel (13) and may be particularly useful to implement lcWGS for breeds that are under-represented in current bovine reference panels.

In absence of an adequately sized breed-specific panel (*e.g.*, below 30 animals), F1 scores of 0.9 can also be accomplished either by increasing the coverage of the lcWGS or by adding distantly related samples from other breeds to the haplotype panels as even animals from seemingly unrelated breeds may share short common haplotypes. Both options will provide accurate sequence variant genotypes at affordable costs for samples from rare breeds, where large breed-specific haplotype reference panels can't be easily established. For instance, F1 scores > 0.92 are observed at

Table 2. General overview of the haplotype reference panels: number of samples, coverage and number of variants called. Shared and private variants are considered through exact matching (position and alleles). Values are the mean of 3 replicas per haplotype panel.

Panel	Samples	Coverage	Variants	Biallelic SNPs	SNPs shared truth-query sets	Truth SNPs missing in haplotype panel	SNPs private to haplotype panel
BSW	150	9.40	22,493,568	19,682,362	13,537,126	317,806	6,145,236
BSW	75	9.65	19,883,488	17,345,201	13,373,462	481,470	3,971,739
BSW	30	9.42	17,035,514	14,839,600	12,810,541	1,044,391	2,029,059
Multibreed (50%)	150	10.48	27,710,504	24,325,185	13,568,744	286,188	10,756,441
Multibreed (25%)	150	10.86	28,755,400	25,266,484	13,531,721	323,211	11,734,763
Multibreed (10%)	150	11.44	28,608,506	25,126,433	13,427,451	427,481	11,698,982
Non-BSW	150	11.78	28,303,738	24,850,237	13,075,827	779,105	11,774,410
Non-BSW	75	11.78	25,059,239	21,968,792	12,868,909	986,023	9,099,883
Non-BSW	30	11.45	21,011,311	18,402,870	12,283,284	1,571,648	6,119,586

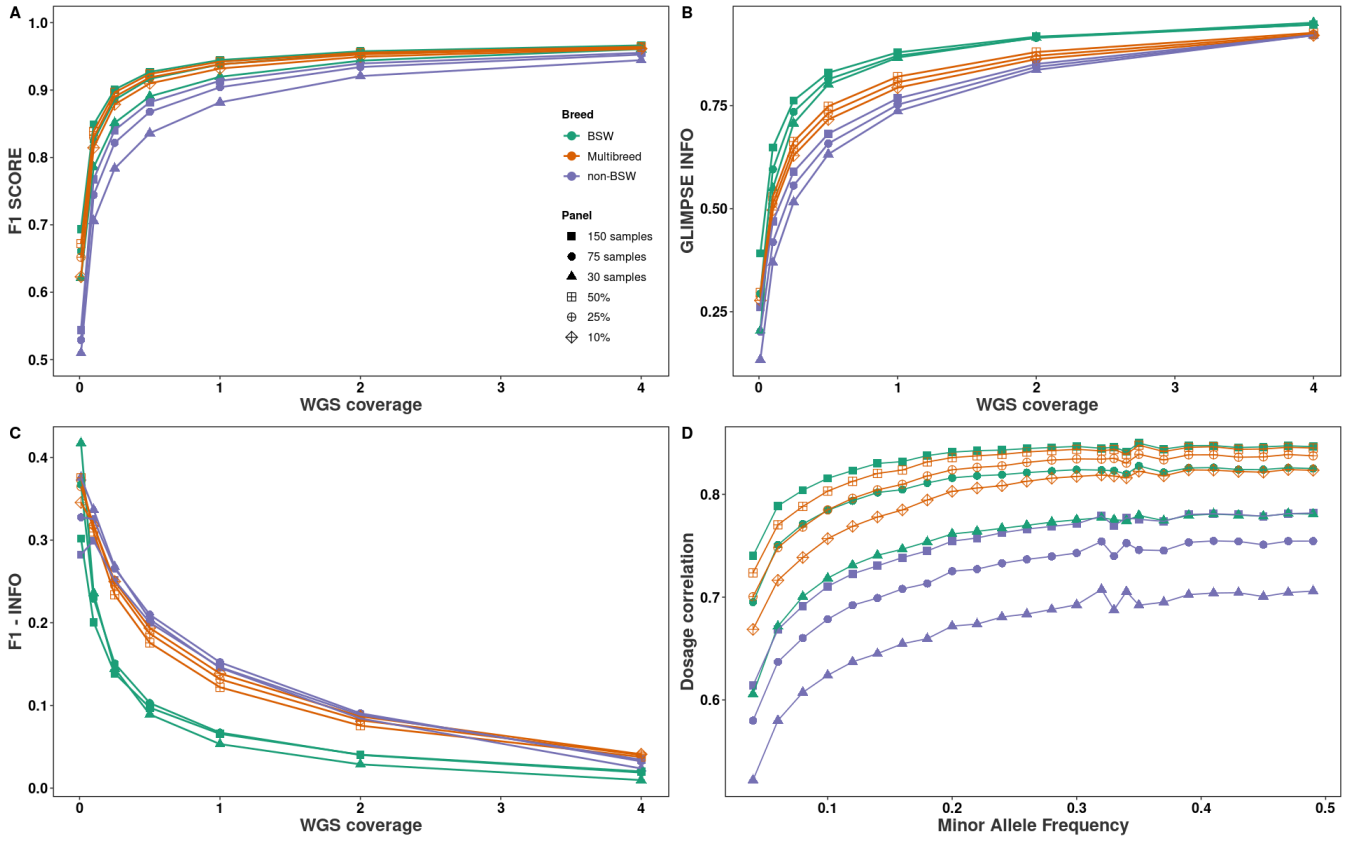

Fig. 4. Genotyping accuracy from low-pass whole-genome sequencing. a) F1 score between truth and imputed variants. b) GLIMPSE INFO score achieved with different sequencing coverages and haplotype panels. c) Differences (subtraction) between F1 and GLIMPSE INFO average scores for different sequencing coverages and haplotype panels. d) Squared dosage correlation (r^2) between imputed data and truth set, stratified by MAF for lcWGS at 0.5x. Panels are indicated with colours and number/percentages of BSW samples in different point shapes. Points indicate the average of the results for all variants in three different replicates.

2-fold sequencing coverage for all tested haplotype panels with small differences among them. This is likely caused because higher coverages provide more information for imputation from the own sequencing reads, while lower coverages rely on the information from haplotypes in the panels. We also achieved F1 scores of 0.9 with large multibreed panels containing only 10% same-breed samples (n = 15). However, reference panels that contain only few samples from the target breed are in general less informative as evidenced by the lack of around 100K truth SNPs that were present in same-size breed-specific panels. Additionally, a threshold of non-related haplotypes from where only marginal gains to imputation accuracy are observed have been described (15, 53, 54). Overall results are compatible with similar studies with haplotype panels of both bigger and smaller sample sizes (15, 54, 55).

Although imputation accuracy (F1) and GLIMPSE's predicted imputation accuracy (INFO score) are respectively averaged over each sample and each variant, we note that F1 (truth) is strictly higher than INFO (estimation). The differences appear to be more pronounced for reference haplotype panels that are of different breed to the target sample and at lower coverages (*i.e.*, less than 0.25-fold coverage, where accuracies estimated by GLIMPSE's are inaccurate (6)). While, for example, multibreed panels are near equally accurate to the 150 sample BSW panel, the INFO scores are noticeably lower. Similarly, the INFO score drops more rapidly for lower coverages, suggesting that a fixed threshold may be unnecessarily conservative given the slower decay in F1. The GLIMPSE INFO score is also positively correlated with variant MAF, and thus filtering based on INFO predominantly removes low-frequency variants. While INFO and other imputation accuracy scores are still useful, additional care should be taken in determining a constant filtering threshold as more and different panels become available for use.

ACKNOWLEDGEMENTS & FUNDING

The authors acknowledge the Functional Genomics Center Zürich for generating DNA sequencing data.

This work was supported by a grant from the Swiss National Science Foundation (310030_185229).

Conflict of interests: none declared.

Bibliography

1. Michel Georges, Carole Charlier, and Ben Hayes. Harnessing genomic information for livestock improvement. *Nature Reviews Genetics*, 20(3):135–156, March 2019. ISSN 1471-0064. doi: 10.1038/s41576-018-0082-2.
2. Paul M. VanRaden, Melvin E. Tooker, Jeffrey R. O'Connell, John B. Cole, and Derek M. Bickhart. Selecting sequence variants to improve genomic predictions for dairy cattle. *Genetics Selection Evolution*, 49(1):32, March 2017. ISSN 1297-9686. doi: 10.1186/s12711-017-0307-4.
3. Warren M. Snelling, Jesse L. Hoff, Jeremiah H. Li, Larry A. Kuehn, Brittney N. Keel, Amanda K. Lindholm-Perry, and Joseph K. Pickrell. Assessment of Imputation from Low-Pass Sequencing to Predict Merit of Beef Steers. *Genes*, 11(11):E1312, November 2020. ISSN 2073-4425. doi: 10.3390/genes11111312.
4. Roger Ros-Freixedes, Andrew Whalen, Gregor Gorjanc, Alan J. Mileham, and John M. Hickey. Evaluation of sequencing strategies for whole-genome imputation with hybrid peeling. *Genetics, selection, evolution: GSE*, 52(1):18, April 2020. ISSN 1297-9686. doi: 10.1186/s12711-020-00537-7.
5. Robert W. Davies, Marek Kucka, Dingwen Su, Sinan Shi, Maeve Flanagan, Christopher M. Cunniff, Yingguang Frank Chan, and Simon Myers. Rapid genotype imputation from sequence with reference panels. *Nature Genetics*, 53(7):1104–1111, July 2021. ISSN 1546-1718. doi: 10.1038/s41588-021-00877-0.
6. Jun Teng, Changheng Zhao, Dan Wang, Zhi Chen, Hui Tang, Jianbin Li, Cheng Mei, Zhangping Yang, Chao Ning, and Qin Zhang. Assessment of the performance of different imputation methods for low-coverage sequencing in Holstein cattle. *Journal of Dairy Science*, 105 (4):3355–3366, April 2022. ISSN 0022-0302. doi: 10.3168/jds.2021-21360.
7. Bogdan Pasaniuc, Nadin Rohland, Paul J. McLaren, Kiran Garimella, Noah Zaitlen, Heng Li, Namrata Gupta, Benjamin M. Neale, Mark J. Daly, Pamela Sklar, Patrick F. Sullivan, Sarah Bergen, Jennifer L. Moran, Christina M. Hultman, Paul Lichtenstein, Patrik Magnusson, Shaun M. Purcell, David W. Haas, Liming Liang, Shamil Sunyaev, Nick Patterson, Paul I. W. de Bakker, David Reich, and Alkes L. Price. Extremely low-coverage sequencing and imputation increases power for genome-wide association studies. *Nature Genetics*, 44(6): 631–635, May 2012. ISSN 1546-1718. doi: 10.1038/ng.2283.
8. Roger Ros-Freixedes, Andrew Whalen, Ching-Yi Chen, Gregor Gorjanc, William O. Herring, Alan J. Mileham, and John M. Hickey. Accuracy of whole-genome sequence imputation using hybrid peeling in large pedigreed livestock populations. *Genetics, selection, evolution: GSE*, 52(1):17, April 2020. ISSN 1297-9686. doi: 10.1186/s12711-020-00536-8.
9. Runyang Nicolas Lou, Arne Jacobs, Aryn P. Wilder, and Nina Overgaard Therkildsen. A beginner's guide to low-coverage whole genome sequencing for population genomics. *Molecular Ecology*, 30(23):5966–5993, 2021. ISSN 1365-294X. doi: 10.1111/mec.16077.
10. 1000 Genomes Project Consortium, Adam Auton, Lisa D. Brooks, Richard M. Durbin, Erik P. Garrison, Hyun Min Kang, Jan O. Korbel, Jonathan L. Marchini, Shane McCarthy, Gil A. McVean, and Gonçalo R. Abecasis. A global reference for human genetic variation. *Nature*, 526(7571):68–74, October 2015. ISSN 1476-4687. doi: 10.1038/nature15393.
11. Shane McCarthy, Sayantan Das, Warren Kretzschmar, Olivier Delaneau, Andrew R. Wood, Alexander Teumer, Hyun Min Kang, Christian Fuchsberger, Petr Danecek, Kevin Sharp, Yang Luo, Carlo Sidore, Alan Kwong, Nicholas Timpson, Seppo Koskinen, Scott Vrieze, Laura J. Scott, He Zhang, Anubha Mahajan, Jan Veldink, Ulrike Peters, Carlos Pato, Cornelia M. van Duijn, Christopher E. Gillies, Ilaria Gandin, Massimo Mezzavilla, Arthur Gilly, Massimiliano Cocca, Michela Traglia, Andrea Angius, Jeffrey C. Barrett, Dorrett Boomsma, Kari Branham, Gerome Breen, Chad M. Brummett, Fabio Busonero, Harry Campbell, Andrew Chan, Sai Chen, Emily Chew, Francis S. Collins, Laura J. Corbin, George Davey Smith, George Dedoussis, Marcus Dorr, Aliki-Eleni Farmaki, Luigi Ferrucci, Lukas Forer, Ross M. Fraser, Stacey Gabriel, Shawn Levy, Leif Groop, Tabitha Harrison, Andrew Hattersley, Oddgeir L. Holmen, Kristian Hveem, Matthias Kretzler, James C. Lee, Matt McGue, Thomas Meitinger, David Melzer, Josine L. Min, Karen L. Mohlke, John B. Vincent, Matthias Nauck, Deborah Nickerson, Aarno Palotie, Michele Pato, Nicola Pirastu, Melvin McInnis, J. Brent Richards, Cinzia Sala, Veikko Salomaa, David Schlessinger, Sebastian Schoenherr, P. Eline Slagboom, Kerrin Small, Timothy Spector, Dwight Stambolian, Marcus Tuke, Jaakko Tuomilehto, Leonard H. Van den Berg, Wouter Van Rheenen, Uwe Volker, Cisca Wijmenga, Daniela Toniolo, Eleftheria Zeggini, Paolo Gasparini, Matthew G. Sampson, James F. Wilson, Timothy Frayling, Paul I. W. de Bakker, Morris A. Swertz, Steven McCarroll, Charles Kooperberg, Annelot Dekker, David Altshuler, Cristen Willer, William Iacono, Samuli Ripatti, Nicole Soranzo, Klaudia Walter, Anand Swaroop, Francesco Cucca, Carl A. Anderson, Richard M. Myers, Michael Boehnke, Mark I. McCarthy, Richard Durbin, and Haplotype Reference Consortium. A reference panel of 64,976 haplotypes for genotype imputation. *Nature Genetics*, 48(10):1279–1283, October 2016. ISSN 1546-1718. doi: 10.1038/ng.3643.
12. Sayantan Das, Lukas Forer, Sebastian Schönherr, Carlo Sidore, Adam E. Locke, Alan Kwong, Scott I. Vrieze, Emily Y. Chew, Shawn Levy, Matt McGue, David Schlessinger, Dwight Stambolian, Po-Ru Loh, William G. Iacono, Anand Swaroop, Laura J. Scott, Francesco Cucca, Florian Kronenberg, Michael Boehnke, Gonçalo R. Abecasis, and Christian Fuchsberger. Next-generation genotype imputation service and methods. *Nature Genetics*, 48(10):1284–1287, October 2016. ISSN 1546-1718. doi: 10.1038/ng.3656.
13. Hans D. Daetwyler, Aurélien Capitan, Hubert Pausch, Paul Stothard, Rianne van Binsbergen, Rasmus F. Brøndum, Xiaoping Liao, Anis Djari, Sabrina C. Rodriguez, Cécile Grohs, Diane Esquerré, Olivier Bouchez, Marie-Noëlle Rossignol, Christophe Klopp, Dominique Rocha, Sébastien Fritz, André Eggen, Phil J. Bowman, David Coote, Amanda J. Chamberlain, Charlotte Anderson, Curt P. VanTassell, Ina Hulsegge, Mike E. Goddard, Bernt Guldbrandtsen, Mogens S. Lund, Roel F. Veerkamp, Didier A. Boichard, Ruedi Fries, and Ben J. Hayes. Whole-genome sequencing of 234 bulls facilitates mapping of monogenic and complex traits in cattle. *Nature Genetics*, 46(8):858–865, August 2014. ISSN 1546-1718. doi: 10.1038/ng.3034.
14. Wenqian Yang, Yanbo Yang, Cecheng Zhao, Kun Yang, Dongyang Wang, Jiajun Yang, Xiaohui Niu, and Jing Gong. Animal-ImputeDB: A comprehensive database with multiple animal reference panels for genotype imputation. *Nucleic Acids Research*, 48(D1):D659–D667, January 2020. ISSN 1362-4962. doi: 10.1093/nar/gkz854.
15. Zhen Wang, Zhenyang Zhang, Zitao Chen, Jiabao Sun, Caiyun Cao, Fen Wu, Zhong Xu, Wei Zhao, Hao Sun, Longyu Guo, Zhe Zhang, Qishan Wang, and Yuchun Pan. PHARP: A pig haplotype reference panel for genotype imputation. *Scientific Reports*, 12(1):12645, July 2022. ISSN 2045-2322. doi: 10.1038/s41598-022-15851-x.
16. Ryan Poplin, Pi-Chuan Chang, David Alexander, Scott Schwartz, Thomas Colthurst, Alexander Ku, Dan Newburger, Jojo Dijamco, Nam Nguyen, Pegah T. Afshar, Sam S. Gross, Lizzie Dorfman, Cory Y. McLean, and Mark A. DePristo. A universal SNP and small-indel variant caller using deep neural networks. *Nature Biotechnology*, 36(10):983–987, November 2018. ISSN 1546-1696. doi: 10.1038/nbt.4235.
17. Taedong Yun, Helen Li, Pi-Chuan Chang, Michael F. Lin, Andrew Carroll, and Cory Y. McLean. Accurate, scalable cohort variant calls using DeepVariant and GLnexus. *Bioinformatics (Oxford, England)*, page btaa1081, January 2021. ISSN 1367-4811. doi: 10.1093/bioinformatics/btaa1081.
18. Yi-Lin Lin, Pi-Chuan Chang, Ching Hsu, Miao-Zi Hung, Yin-Hsiu Chien, Wuh-Liang Hwu, FeiPei Lai, and Ni-Chung Lee. Comparison of GATK and DeepVariant by trio sequencing. *Scientific Reports*, 12:1809, February 2022. ISSN 2045-2322. doi: 10.1038/s41598-022-05833-4.
19. Jared O'Connell, Taedong Yun, Meghan Moreno, Helen Li, Nadia Litterman, Alexey Kolesnikov, Elizabeth Noblin, Pi-Chuan Chang, Anjali Shastri, Elizabeth H. Dorfman, Suyash Shringarpure, Adam Auton, Andrew Carroll, and Cory Y. McLean. A population-specific reference panel for improved genotype imputation in African Americans. *Com-*

munications Biology, 4:1269, November 2021. ISSN 2399-3642. doi: 10.1038/s42003-021-02777-9.

20. Raphael O. Betschart, Alexandre Thiéry, Domingo Aguilera-Garcia, Martin Zoche, Holger Moch, Raphael Twerenbold, Tanja Zeller, Stefan Blankenberg, and Andreas Ziegler. Comparison of calling pipelines for whole genome sequencing: An empirical study demonstrating the importance of mapping and alignment. *Scientific Reports*, 12(1):21502, December 2022. ISSN 2045-2322. doi: 10.1038/s41598-022-26181-3.
21. Justin M. Zook, Jennifer McDaniel, Nathan D. Olson, Justin Wagner, Hemang Parikh, Haynes Heaton, Sean A. Irvine, Len Trigg, Rebecca Truty, Cory Y. McLean, Francisco M. De La Vega, Chunlin Xiao, Stephen Sherry, and Marc Salit. An open resource for accurately benchmarking small variant and reference calls. *Nature Biotechnology*, 37(5):561–566, May 2019. ISSN 1546-1696. doi: 10.1038/s41587-019-0074-6.
22. Blog DeepVariant. Improved non-human variant calling using species-specific deepvariant models, 2018.
23. Felix Mölder, Kim Philipp Jablonski, Brice Letcher, Michael B. Hall, Christopher H. Tomkins-Tinch, Vanessa Sochat, Jan Forster, Soohyun Lee, Sven O. Twardziok, Alexander Kanitz, Andreas Wilm, Manuel Holtgrewe, Sven Rahmann, Sven Nahnsen, and Johannes Köster. Sustainable data analysis with Snakemake. *F1000Research*, 10:33, 2021. ISSN 2046-1402. doi: 10.12688/f1000research.29032.2.
24. Hadley Wickham. *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York, 2016. ISBN 978-3-319-24277-4.
25. Shifu Chen, Yanqing Zhou, Yaru Chen, and Jia Gu. Fastp: An ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics (Oxford, England)*, 34(17):i884–i890, September 2018. ISSN 1367-4811. doi: 10.1093/bioinformatics/bty560.
26. Philip Ewels, Måns Magnusson, Sverker Lundin, and Max Käller. MultiQC: Summarize analysis results for multiple tools and samples in a single report. *Bioinformatics (Oxford, England)*, 32(19):3047–3048, October 2016. ISSN 1367-4811. doi: 10.1093/bioinformatics/btw354.
27. Kyle Hernandez. Cli for splitting a fastq that has multiple readgroups, 2022.
28. Vasimuddin Md, Sanchit Misra, Heng Li, and Srinivas Aluru. Efficient Architecture-Aware Acceleration of BWA-MEM for Multicore Systems. *arXiv:1907.12931 [cs, q-bio]*, July 2019.
29. Benjamin D. Rosen, Derek M. Bickhart, Robert D. Schnabel, Sergey Koren, Christine G. Elsik, Elizabeth Tseng, Troy N. Rowan, Wai Y. Low, Aleksey Zimin, Christine Couldrey, Richard Hall, Wenli Li, Arang Rhie, Jay Ghurye, Stephanie D. McKay, Françoise Thibaud-Nissen, Jinna Hoffman, Brenda M. Murdoch, Warren M. Snelling, Tara G. McDaneld, John A. Hammond, John C. Schwartz, Wilson Nandolo, Darren E. Hagen, Christian Dreischer, Sebastian J. Schultheiss, Steven G. Schroeder, Adam M. Phillippy, John B. Cole, Curtis P. Van Tassell, George Liu, Timothy P. L. Smith, and Juan F. Medrano. De novo assembly of the cattle reference genome with single-molecule sequencing. *GigaScience*, 9(3):giaa021, March 2020. ISSN 2047-217X. doi: 10.1093/gigascience/giaa021.
30. Audald Lloret-Villas, Meenu Bhati, Naveen Kumar Kadri, Ruedi Fries, and Hubert Pausch. Investigating the impact of reference assembly choice on genomic analyses in a cattle breed. *BMC Genomics*, 22(1):363, May 2021. ISSN 1471-2164. doi: 10.1186/s12864-021-07554-w.
31. Gregory G. Faust and Ira M. Hall. SAMBLASTER: Fast duplicate marking and structural variant read extraction. *Bioinformatics (Oxford, England)*, 30(17):2503–2505, September 2014. ISSN 1367-4811. doi: 10.1093/bioinformatics/btu314.
32. Artem Tarasov, Albert J. Vilella, Edwin Cuppen, Isaac J. Nijman, and Pjotr Prins. Sambamba: Fast processing of NGS alignment formats. *Bioinformatics (Oxford, England)*, 31 (12):2032–2034, June 2015. ISSN 1367-4811. doi: 10.1093/bioinformatics/btv098.
33. Heng Li, Bob Handsaker, Alec Wysoker, Tim Fennell, Jue Ruan, Nils Homer, Gabor Marth, Goncalo Abecasis, Richard Durbin, and 1000 Genome Project Data Processing Subgroup. The Sequence Alignment/Map format and SAMtools. *Bioinformatics (Oxford, England)*, 25 (16):2078–2079, August 2009. ISSN 1367-4811. doi: 10.1093/bioinformatics/btp352.
34. Petr Danecek, James K Bonfield, Jennifer Liddle, John Marshall, Valeriu Ohan, Martin O Pollard, Andrew Whitwham, Thomas Keane, Shane A McCarthy, Robert M Davies, and Heng Li. Twelve years of SAMtools and BCFtools. *GigaScience*, 10(2):giab008, February 2021. ISSN 2047-217X. doi: 10.1093/gigascience/giab008.
35. Picard. Picard toolkit, 2022.
36. Brent S. Pedersen and Aaron R. Quinlan. Mosdepth: Quick coverage calculation for genomes and exomes. *Bioinformatics (Oxford, England)*, 34(5):867–868, March 2018. ISSN 1367-4811. doi: 10.1093/bioinformatics/btx699.
37. Aaron McKenna, Matthew Hanna, Eric Banks, Andrey Sivachenko, Kristian Cibulskis, Andrew Kernytsky, Kiran Garimella, David Altshuler, Stacey Gabriel, Mark Daly, and Mark A. DePristo. The Genome Analysis Toolkit: A MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Research*, 20(9):1297–1303, September 2010. ISSN 1549-5469. doi: 10.1101/gr.107524.110.
38. Mark A. DePristo, Eric Banks, Ryan Poplin, Kiran V. Garimella, Jared R. Maguire, Christopher Hartl, Anthony A. Philippakis, Guillermo del Angel, Manuel A. Rivas, Matt Hanna, Aaron McKenna, Tim J. Fennell, Andrew M. Kernytsky, Andrey Y. Sivachenko, Kristian Cibulskis, Stacey B. Gabriel, David Altshuler, and Mark J. Daly. A framework for variation discovery and genotyping using next-generation DNA sequencing data. *Nature Genetics*, 43(5): 491–498, May 2011. ISSN 1546-1718. doi: 10.1038/ng.806.
39. Geraldine A. van der Auwera, Mauricio O. Carneiro, Christopher Hartl, Ryan Poplin, Guillermo Del Angel, Ami Levy-Moonshine, Tadeusz Jordan, Khalid Shakir, David Roazen, Joel Thibault, Eric Banks, Kiran V. Garimella, David Altshuler, Stacey Gabriel, and Mark A. DePristo. From FastQ data to high confidence variant calls: The Genome Analysis Toolkit best practices pipeline. *Current Protocols in Bioinformatics*, 43:11.10.1–11.10.33, 2013. ISSN 1934-340X. doi: 10.1002/0471250953.bi1110s43.
40. GATK. Gatk blog, 2022.
41. Michael F. Lin, Ohad Rodeh, John Penn, Xiaodong Bai, Jeffrey G. Reid, Olga Krasheninina, and William J. Salerno. GLnexus: Joint variant calling for large cohort sequencing, June 2018.
42. Brian L. Browning and Sharon R. Browning. Genotype Imputation with Millions of Reference Samples. *American Journal of Human Genetics*, 98(1):116–126, January 2016. ISSN 1537-6605. doi: 10.1016/j.ajhg.2015.11.020.
43. Heng Li. Tabix: Fast retrieval of sequence features from generic TAB-delimited files. *Bioinformatics*, 27(5):718–719, March 2011. ISSN 1367-4803. doi: 10.1093/bioinformatics/btq671.
44. James K. Bonfield, John Marshall, Petr Danecek, Heng Li, Valeriu Ohan, Andrew Whitwham, Thomas Keane, and Robert M. Davies. HTSlib: C library for reading/writing high-throughput sequencing data. *GigaScience*, 10(2):giab007, February 2021. ISSN 2047-217X. doi: 10.1093/gigascience/giab007.
45. William McLaren, Laurent Gil, Sarah E. Hunt, Harpreet Singh Riat, Graham R. S. Ritchie, Anja Thormann, Paul Flicek, and Fiona Cunningham. The Ensembl Variant Effect Predictor. *Genome Biology*, 17(1):122, June 2016. ISSN 1474-760X. doi: 10.1186/s13059-016-0974-4.
46. Alexander Lex, Nils Gehlenborg, Hendrik Strobelt, Romain Vuillemot, and Hanspeter Pfister. UpSet: Visualization of Intersecting Sets. *IEEE Transactions on Visualization and Computer Graphics*, 20(12):1983–1992, December 2014. ISSN 1941-0506. doi: 10.1109/TVCG.2014.2346248.
47. Jake R Conway, Alexander Lex, and Nils Gehlenborg. UpSetR: An R package for the visualization of intersecting sets and their properties. *Bioinformatics*, 33(18):2938–2940, September 2017. ISSN 1367-4803. doi: 10.1093/bioinformatics/btx364.
48. hap.py. hap.py github, 2022.
49. Giulio Formenti, Arang Rhie, Brian P. Walenz, Françoise Thibaud-Nissen, Kishwar Shafin, Sergey Koren, Eugene W. Myers, Erich D. Jarvis, and Adam M. Phillippy. Merfin: Improved variant filtering, assembly evaluation and polishing via k-mer validation. *Nature Methods*, pages 1–9, March 2022. ISSN 1548-7105. doi: 10.1038/s41592-022-01445-y.
50. seqtk. seqtk github, 2022.
51. Simone Rubinacci, Diogo M. Ribeiro, Robin J. Hofmeister, and Olivier Delaneau. Efficient phasing and imputation of low-coverage sequencing data using large reference panels. *Nature Genetics*, 53(1):120–126, January 2021. ISSN 1546-1718. doi: 10.1038/s41588-020-00756-0.
52. Christopher C. Chang, Carson C. Chow, Laurent Cam Tellier, Shashaank Vattikuti, Shaun M. Purcell, and James J. Lee. Second-generation PLINK: Rising to the challenge of larger and richer datasets. *GigaScience*, 4:7, 2015. ISSN 2047-217X. doi: 10.1186/s13742-015-0047-8.
53. Sanne van den Berg, Jérémie Vandenplas, Fred A. van Eeuwijk, Aniek C. Bouwman, Marcos S. Lopes, and Roel F. Veerkamp. Imputation to whole-genome sequence using multiple pig populations and its use in genome-wide association studies. *Genetics, Selection, Evolution : GSE*, 51:2, January 2019. ISSN 0999-193X. doi: 10.1186/s12711-019-0445-y.
54. Muhammad Yasir Nawaz, Priscila Arrigucci Bernardes, Rodrigo Pelicioni Savegnago, Dajeong Lim, Seung Hwan Lee, and Cedric Gondro. Evaluation of Whole-Genome Sequence Imputation Strategies in Korean Hanwoo Cattle. *Animals*, 12(17):2265, January 2022. ISSN 2076-2615. doi: 10.3390/ani12172265.
55. Aine C. O'Brien, Michelle M. Judge, Sean Fair, and Donagh P. Berry. High imputation accuracy from informative low-to-medium density single nucleotide polymorphism genotypes is achievable in sheep. *Journal of Animal Science*, 97(4):1550–1567, April 2019. ISSN 1525-3163. doi: 10.1093/jas/skz043.