Supplementary Material for “Accurate, scalable cohort variant calls using DeepVariant and GLnexus”

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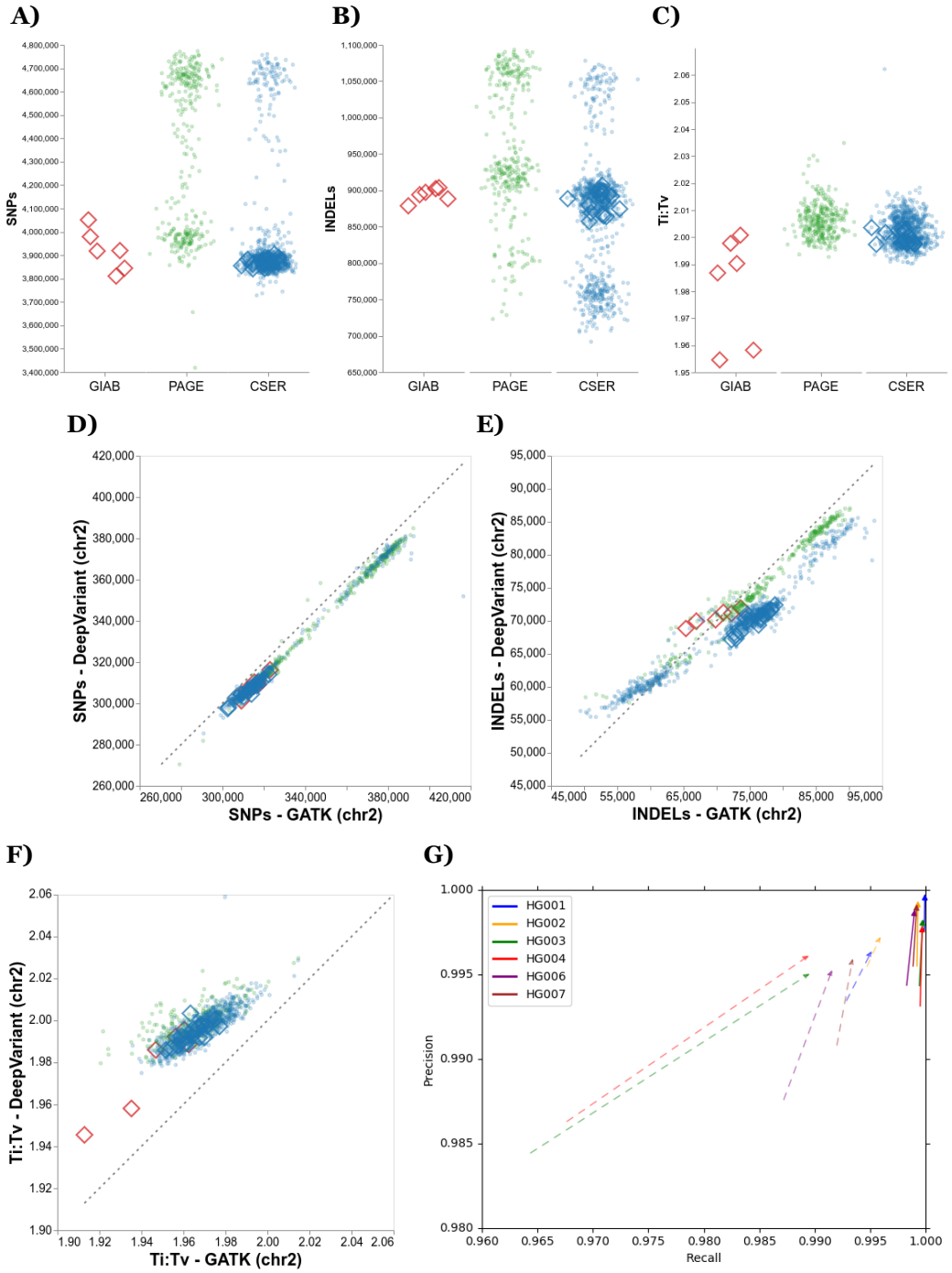
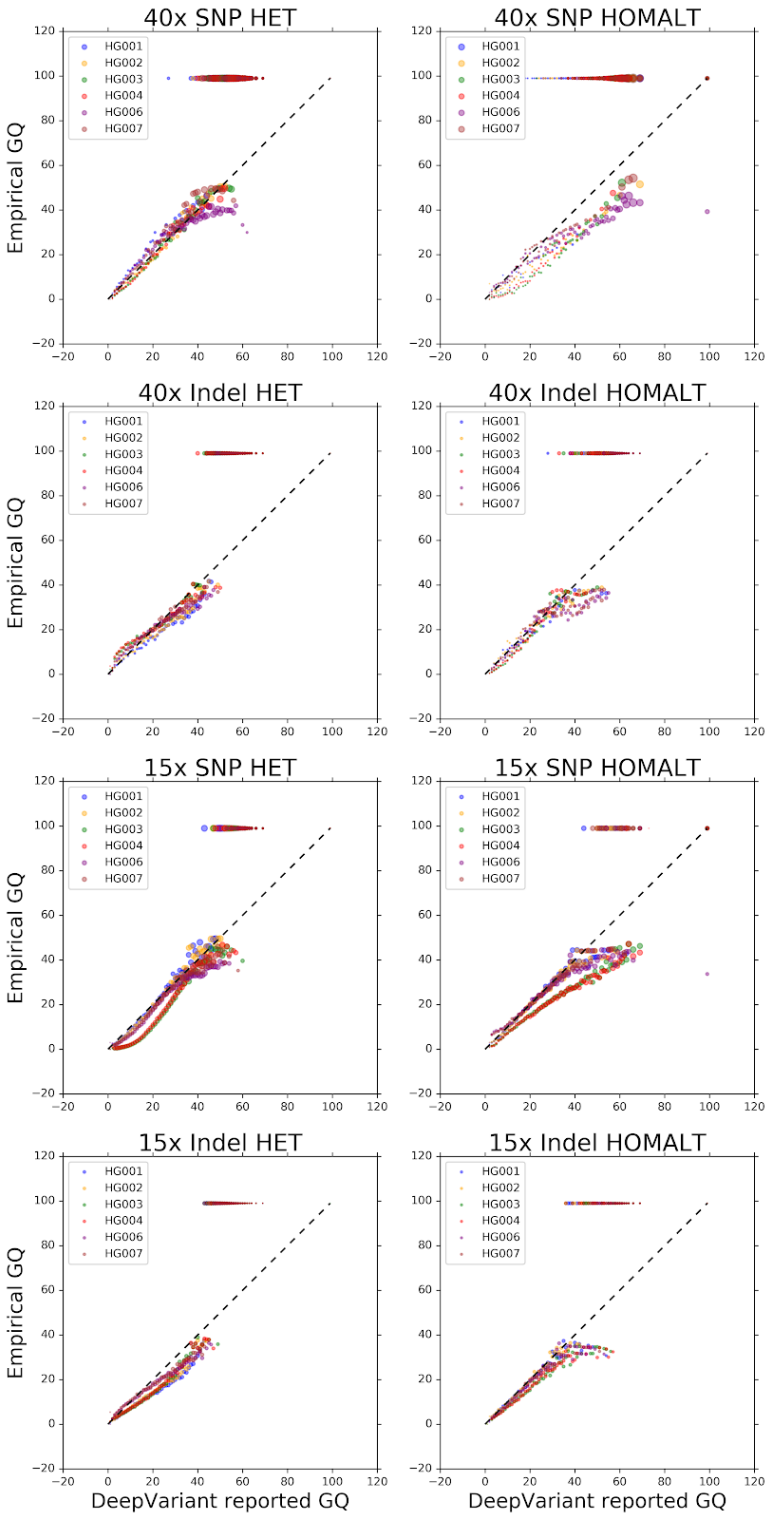
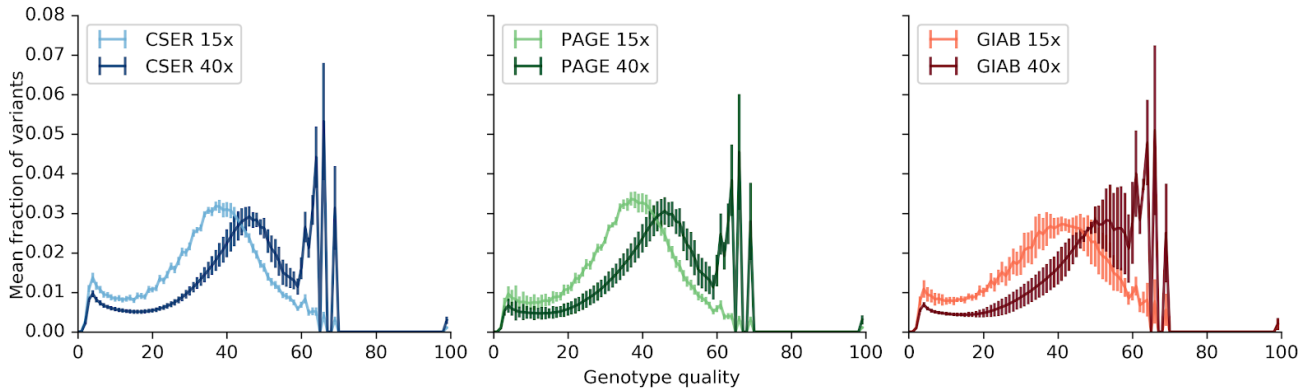
Supplementary Figures



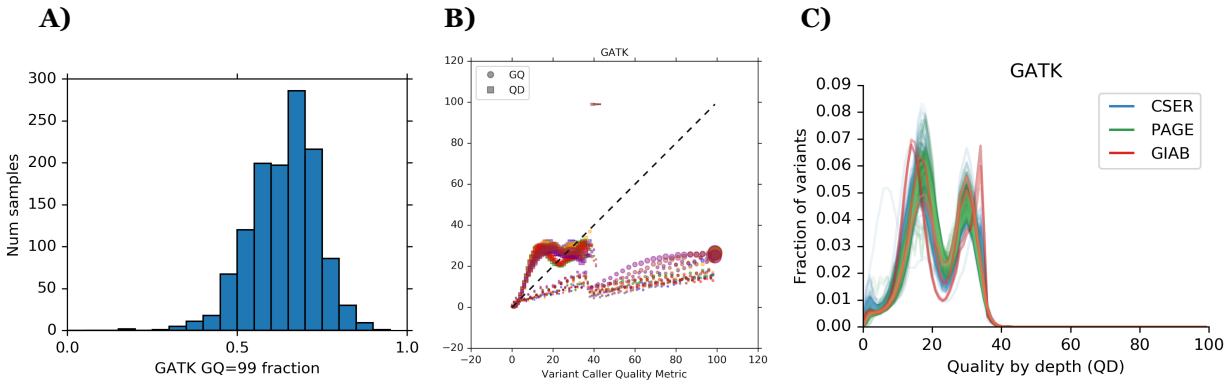
Supplementary Figure 1. Single-sample call statistics for GIAB, PAGE, and CSER datasets. **A, B, C)** The number of SNPs (A), indels (B), and Ti:Tv ratio (C) reported in each individual genome-wide using DeepVariant. Diamond markers indicate samples used for evaluation (GIAB samples for benchmark call accuracy and CSER samples for Mendelian violation rate). **D, E, F)** Comparison of DeepVariant and GATK4 HaplotypeCaller single-sample calls for number of SNPs (D), indels (E), and Ti:Tv ratio (F) computed on chromosome 2. **G)** Comparison of GATK4 HaplotypeCaller (line starts) and DeepVariant (arrowheads) recall and precision scores for SNPs (solid lines) and indels (dashed lines) computed in the GIAB samples on chromosome 2.



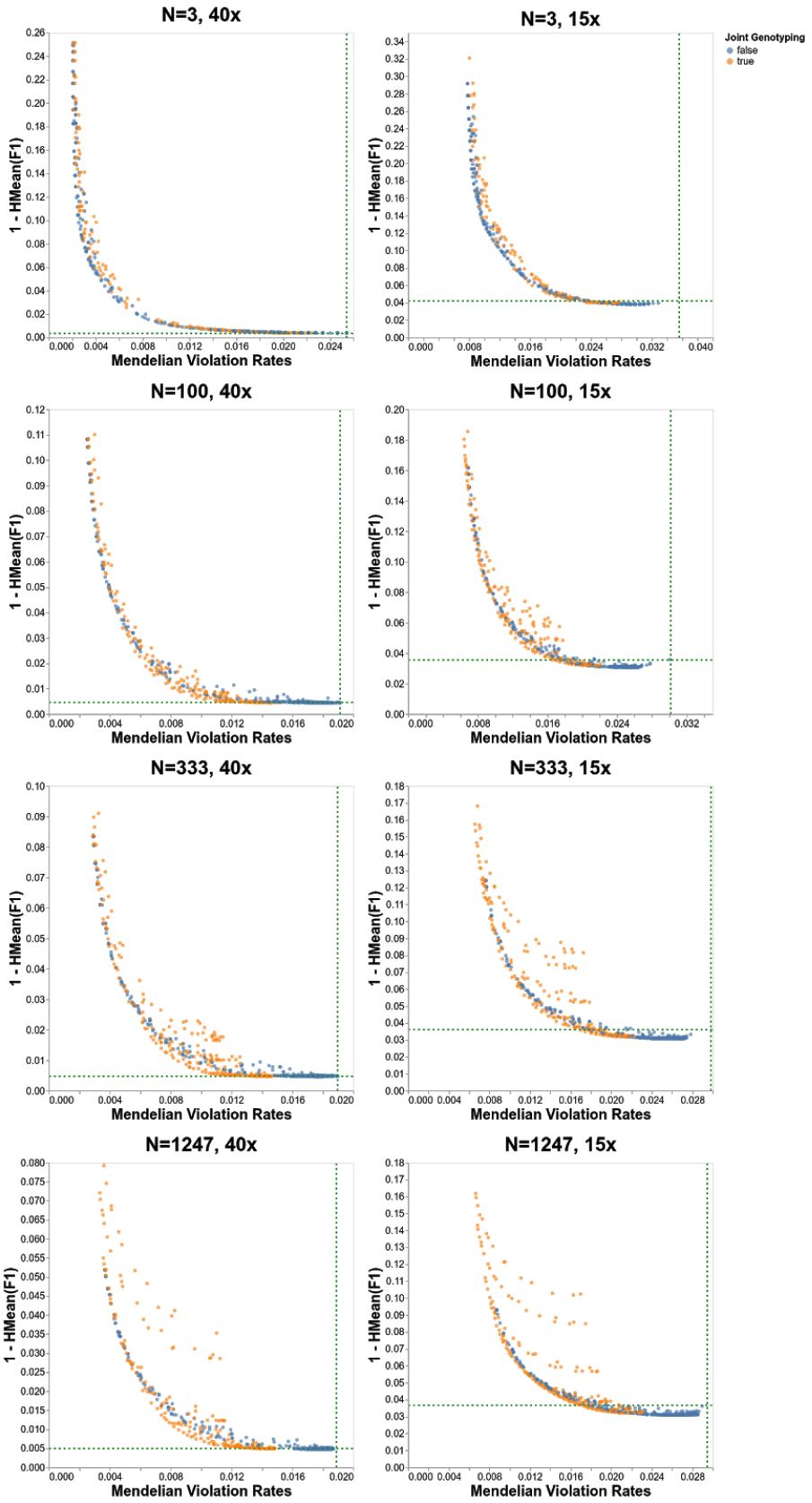
Supplementary Figure 2. DeepVariant 0.8 genotype quality (GQ) score calibration stratified by variant type. Similar to Figure 1, for both ~40x and 15x coverage reads and computed separately per variant type (SNP, indel) and zygosity (heterozygous reference/alternate, homozygous alternate).



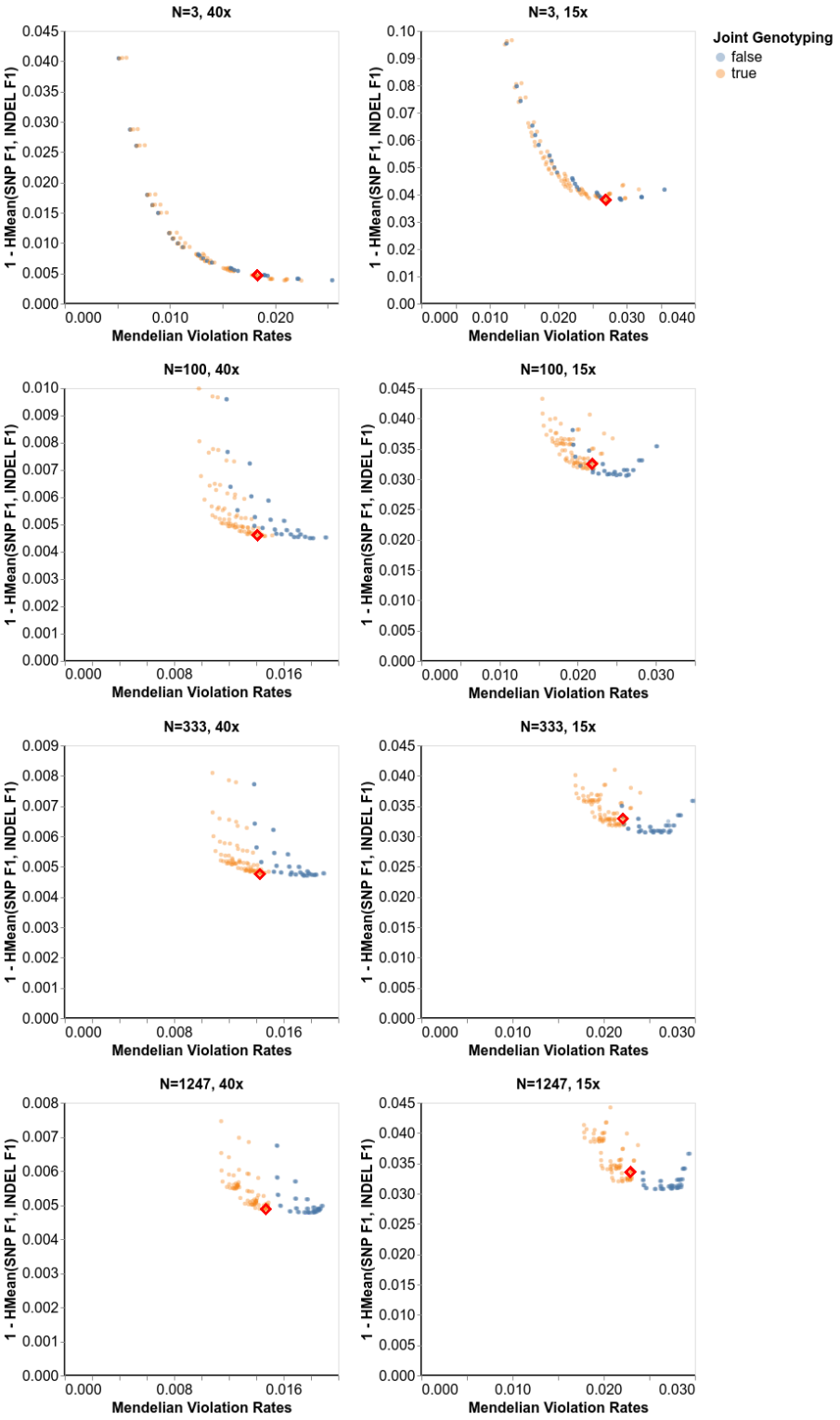
Supplementary Figure 3. Sample genotype quality distributions for DeepVariant v0.8.0 calls as a function of sequence coverage. For each of the three development datasets, average fractions of variants at each estimated genotype quality are plotted at both 15x and 40x sequence coverage. Error bars represent sample standard deviations.



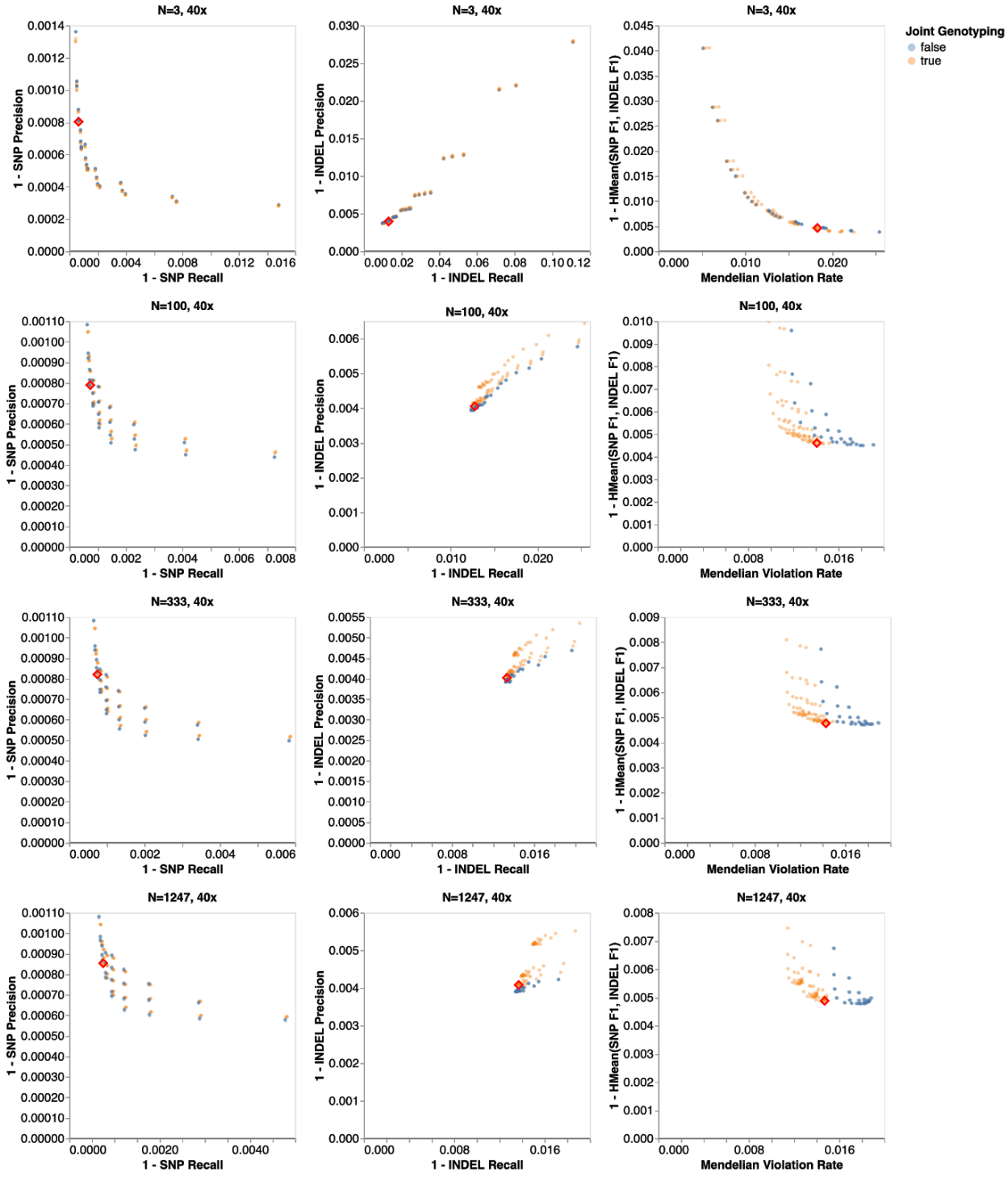
Supplementary Figure 4. Genotype quality distribution properties of GATK PASS variants. **A)** The fraction of variant calls with GQ=99 from GATK4 HaplotypeCaller across the 1,248 samples. On average, 63.81% of variants have GQ=99. **B)** Variant calibration for GATK4 HaplotypeCaller. Both reported GQ (circles, also shown in **Figure 1B**) and reported QD (variant quality normalized by read depth; squares) are plotted against empirical GQ. Colors correspond to GIAB samples as in **Figure 1**. **C)** Distributions of reported QD ("Qual by Depth") for GATK4 HaplotypeCaller in all 1,248 samples computed on chromosome 2 only. Distributions of both QD and GQ (Figure 1D) for GIAB samples genome-wide show qualitatively similar results (data not shown).



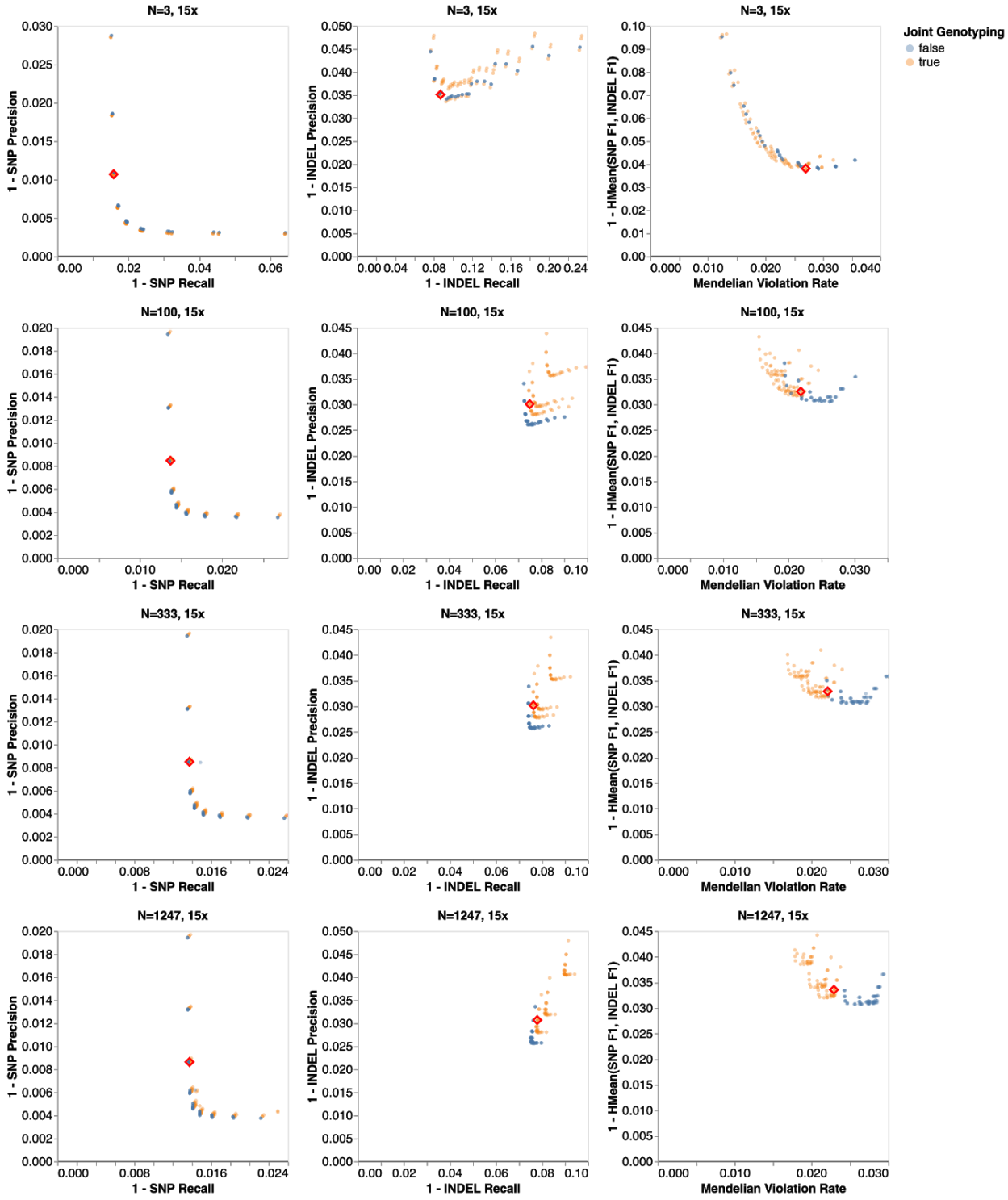
Supplementary Figure 5. Pareto-optimal search for all WGS cohorts. See also **Figure 2**.



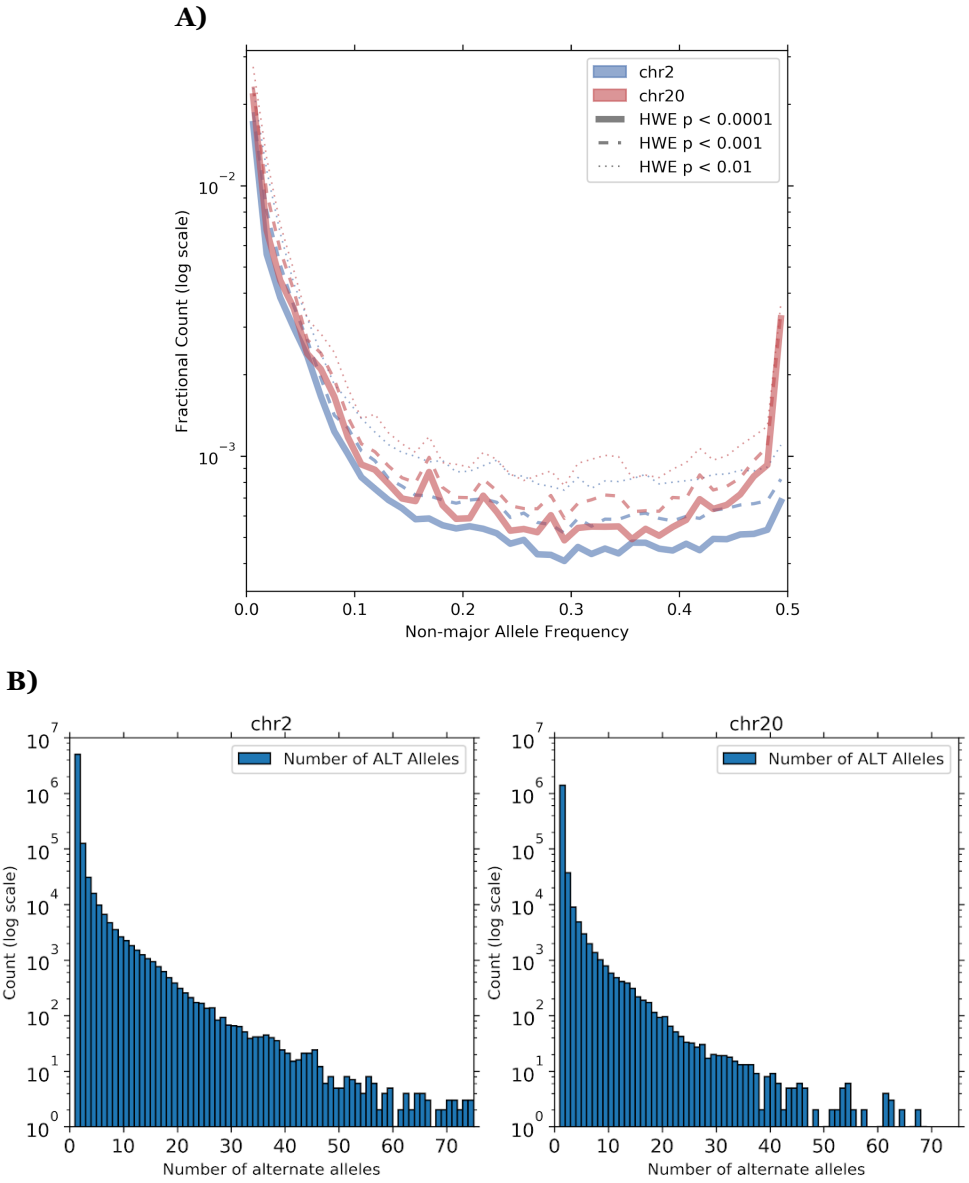
Supplementary Figure 6. Grid search for GLnexus parameters. Each data point represents a unique parameter combination. The x-axis shows the rates of Mendelian violations and the y-axis shows one minus the harmonic mean of SNP F1 and indel F1 using GIAB benchmark calls (lower numbers are better). The red highlighted points are the optimized parameter set used for DV-GLN-OPT. See also **Supplementary Figures 7** and **8**.



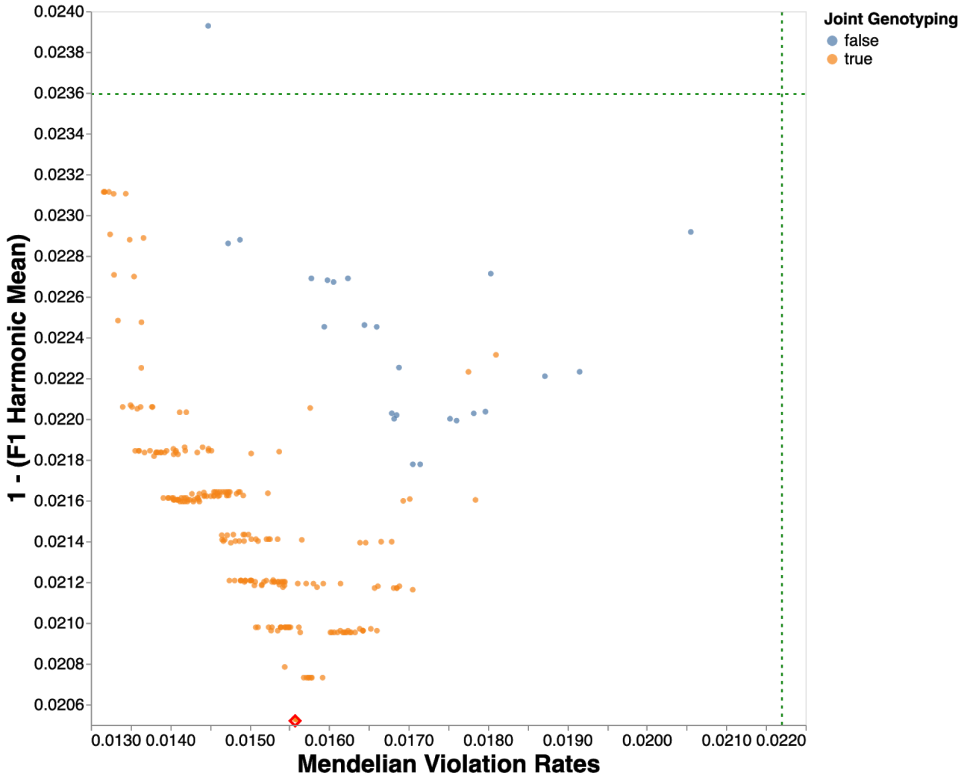
Supplementary Figure 7. Optimized parameter performance for all 40x sequence coverage cohorts, compared to other parameter sets explored by grid search. The red highlighted points are the optimized parameter set.



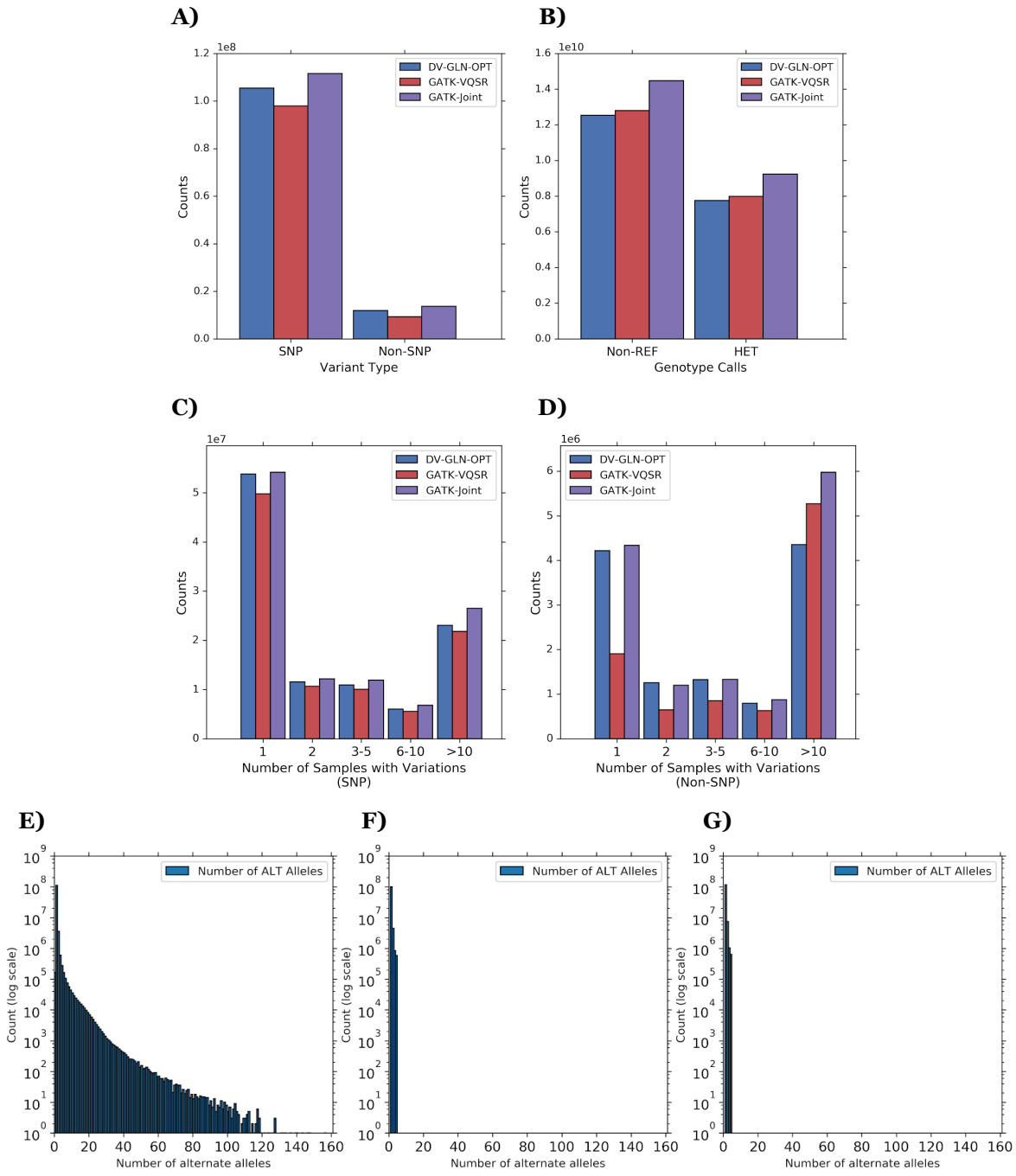
Supplementary Figure 8. Optimized parameter performance for all 15x sequence coverage cohorts, compared to other parameter sets explored by grid search. The red highlighted points are the optimized parameter set.



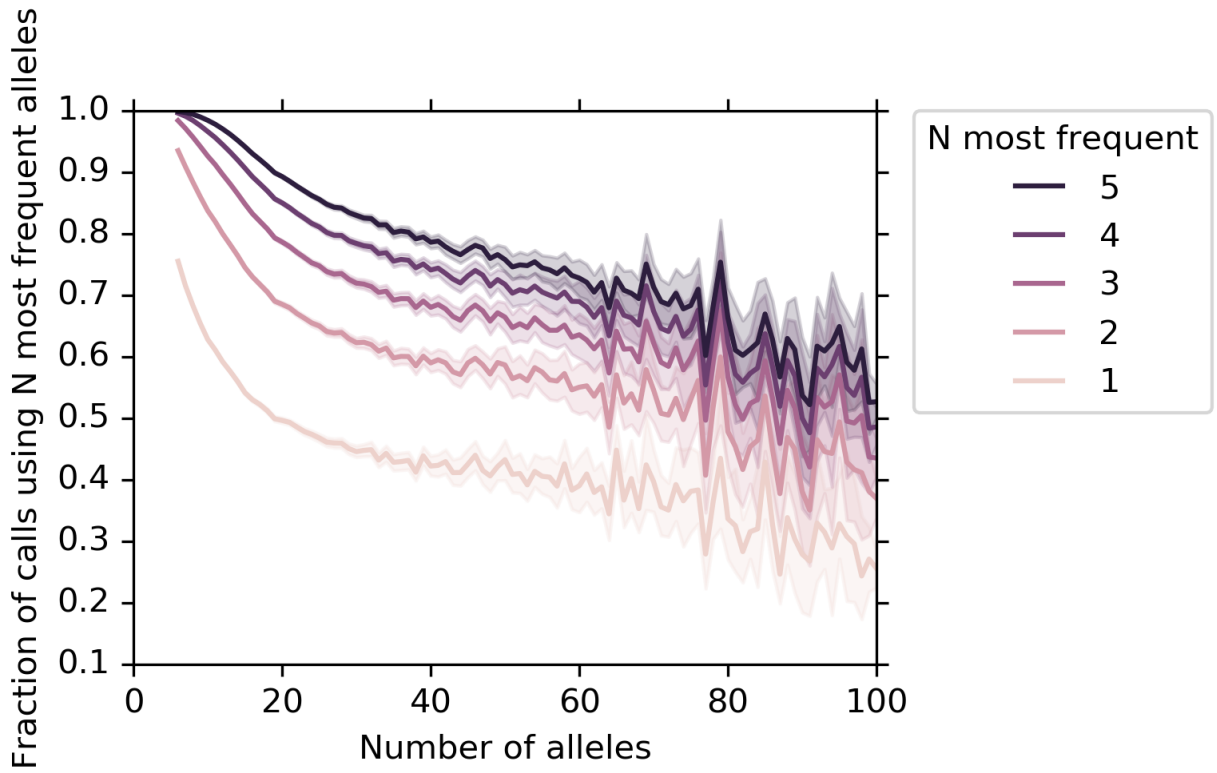
Supplementary Figure 9. Similarity of calls in chr2 and chr20 of N=1,247 cohort with 40x coverage generated by the optimized DeepVariant+GLnexus pipeline. A) Fractional counts of variants with low HWE p-values, binned by non-major allele frequency in chromosome 2 and chromosome 20. **B)** Histogram of the number of alternate alleles in variants in chr2 and chr20. Note: chr2 is ~4x larger than chr20.



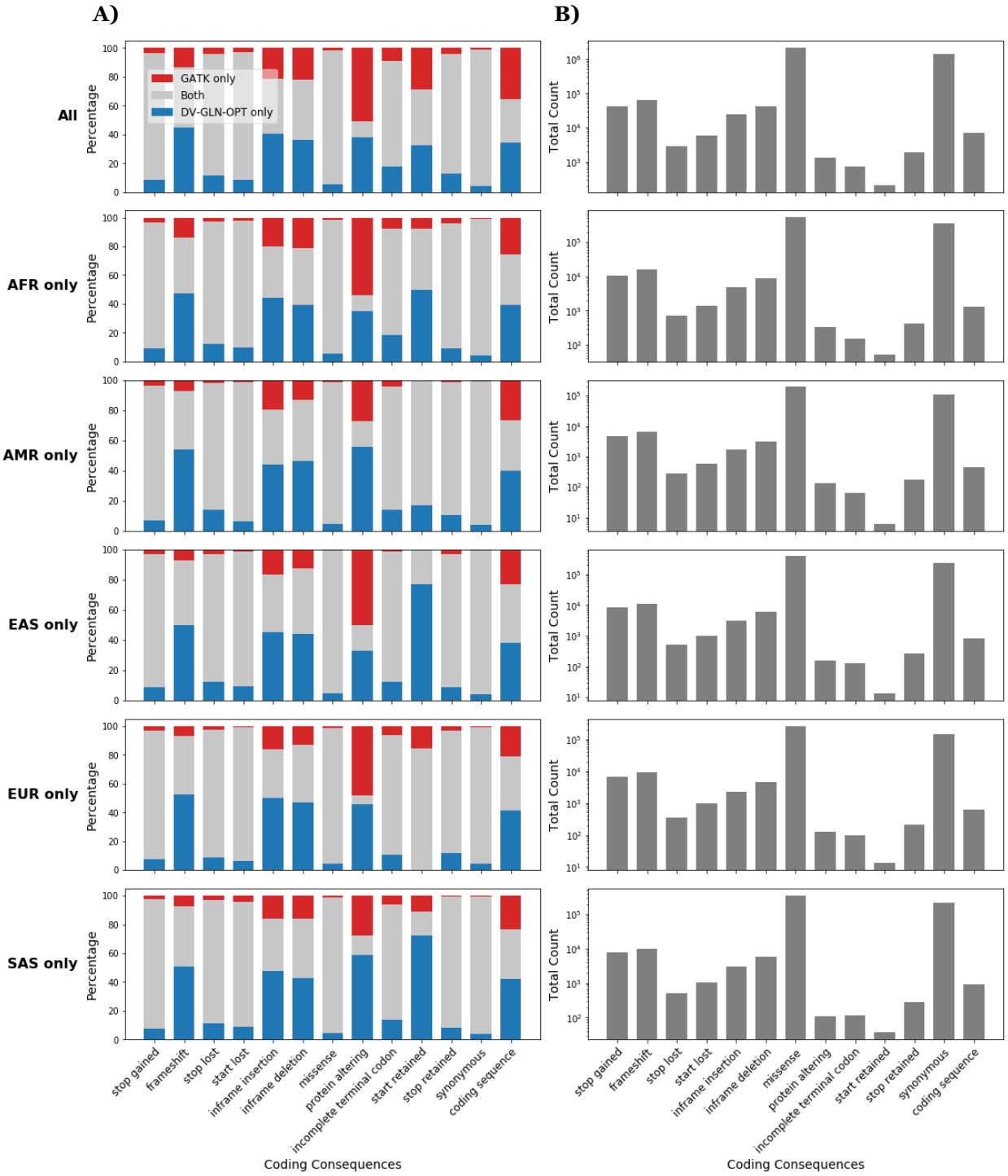
Supplementary Figure 10. Pareto optimal search for exomes. The red diamond indicates the exome-optimized DeepVariant+GLnexus pipeline.



Supplementary Figure 11. Comparison of 1KGP cohort callset properties.
A) The number of variants generated by each method per variant type. **B)** The number of all genotype calls excluding homozygous reference calls (Non-REF), and the number of heterozygous genotype calls in each method. **C, D)** Histogram of number of samples with variations in SNPs (C) and non-SNPs (D). Per each cohort variant (i.e. a row in a cohort VCF) of each type, we count the number of samples with a non-reference genotype (i.e. the leftmost bins are singletons). **E, F, G)** Histogram of the count of the number of alternate alleles from DV-GLN-OPT (E), GATK-VQSR (F), and GATK-Joint (G). Note that GATK limits the number of alternate alleles to 6 by default (gatk.broadinstitute.org/hc/en-us/articles/360036734631-GenotypeGVCFs).

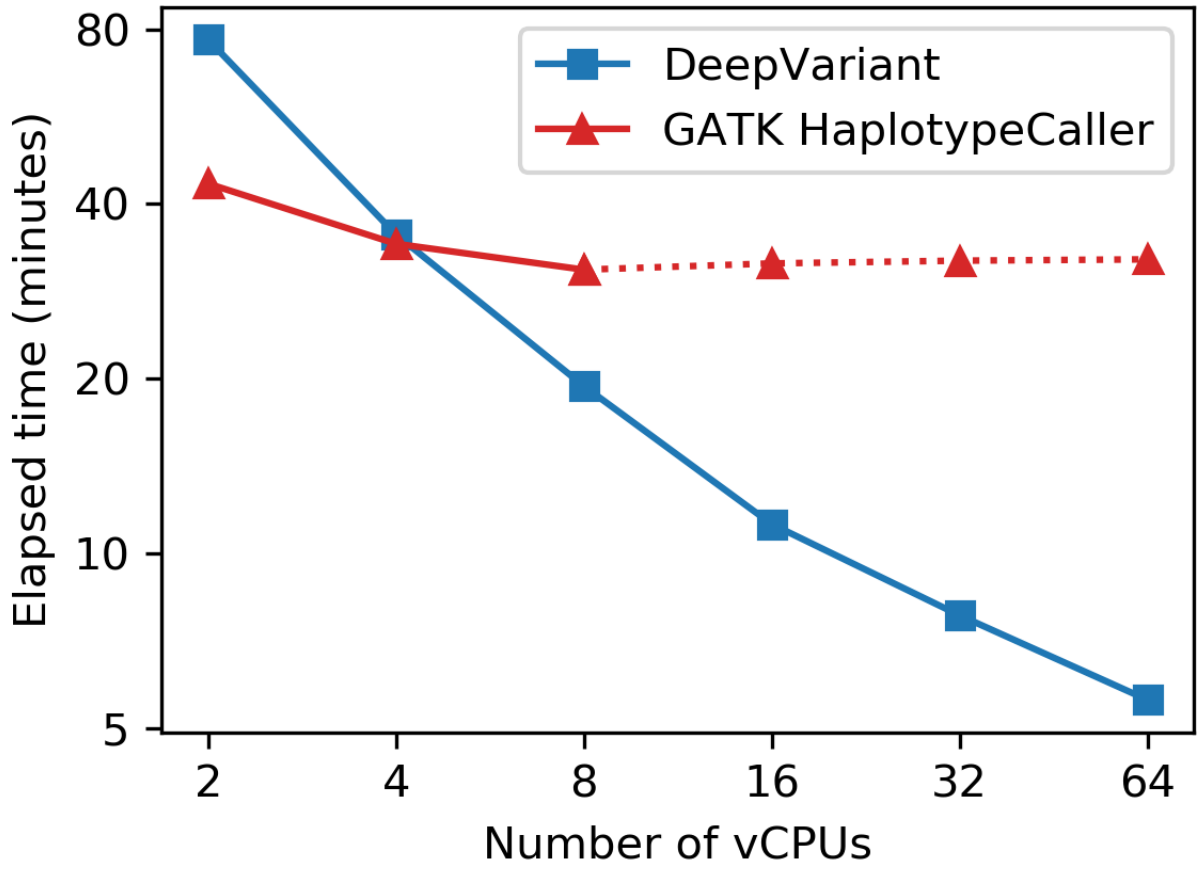


Supplementary Figure 12. Distribution of allele usage in DeepVariant multiallelic sites. All variants in the 1KGP cohort with at least six total alleles were analyzed to see the fraction of calls using the most frequently-called alleles. The "100" allele bin contains all variants (n=97) with 100 or more alleles (the maximal variant contained 162 alleles). Bands represent 95% confidence intervals.



Supplementary Figure 13: Functional annotations of variants in 1KGP populations.

A) Coding consequences of variants discovered by DV-GLN-OPT and GATK (GATK-VQSR) in 1KGP, and variants exclusive to a superpopulation in 1KGP. A variant is considered exclusive to a superpopulation if an alternate allele is called in at least one member of that superpopulation and *no* alternate allele is called in any member of other superpopulations. AFR: African; AMR: American; EAS: East Asian; EUR: European; SAS: South Asian. Variants discovered by either of the two systems are partitioned into (1) variants called by both systems (light gray), (2) variants called by DV-GLN-OPT but not by GATK (blue), (3) *vice versa* (red). The proportion of each of the three partitions within each coding consequence category is shown in percentages. **B)** The total number of variants (displayed in log scale) discovered by either DV-GLN-OPT or GATK. Note the scale changes on the y-axis.



Supplementary Figure 14. Log-scaled elapsed real times to generate chr22 gVCF of one sample (NA12878) using a varying number of vCPUs. Identical to **Figure 7B**, but using log scales for both axes.

Supplementary Tables

Supplementary Table 1: Calibration Scores of DeepVariant and GATK calls.
For both Brier scores and Spiegelhalter's Z statistics, lower numbers (**bold**) indicate better calibration. The mean Brier score across all samples is 0.00091816 for DeepVariant and 0.00537084 for GATK. The mean Spiegelhalter's z statistic is 0.5249 for DeepVariant and 1141.0275 for GATK. For more discussion of the Brier score and Spiegelhalter's Z, see (Schmid and Griffith, 2014) for example.

Sample	Caller	Brier Score	Spiegelhalter's Z
HG001	DV	**0.00052899**	**10.6434**
	GATK	0.00424776	1,629.6215
HG002	DV	**0.00064753**	**15.0890**
	GATK	0.00445521	1,504.1458
HG003	DV	**0.00121769**	**2.1718**
	GATK	0.00551098	726.1125
HG004	DV	**0.00111538**	**-3.6398**
	GATK	0.00540835	756.5146
HG006	DV	**0.00113718**	**-7.4291**
	GATK	0.00659380	1,009.0203
HG007	DV	**0.00086219**	**-13.6857**
	GATK	0.00600893	1,220.7504
All (average)	DV	**0.00091816**	**0.5249**
	GATK	0.00537084	1,141.0275

Supplementary Table 2: Cohort evaluation experimental setup.

A) Cohort subset definitions.

Source	Subset Name	Size	Description
GIAB	*GIAB3*	3	HG002, HG003, HG004 (son, father, mother) trio.
	GIAB5	5	HG001, HG003, HG004, HG006, HG007 (mutually non-descendant samples).
	GIAB_WES	2	HG001, HG002 exomes sequences.
CSER	*CSER15*	15	Randomly selected 5 WGS trios, excluding outliers. See **Supplementary Table 2**.
	CSER	929	All available WGS CSER samples.
	CSER15_WES	15	Randomly selected 5 WES trios, excluding outliers. See **Supplementary Table 2**.
	CSER_WES	344	All available WES CSER samples.
PAGE	*PAGE80*	80	Randomly selected 80 PAGE samples, excluding outliers. See **Supplementary Table 7**.
	PAGE	313	All PAGE samples.

B) Custom cohorts for cohort evaluation. Each WGS cohort has two versions for 40x and 15x coverage.

Cohort Name	Size	Definition	Single-sample benchmark samples	Evaluation trios (for Mendelian violation)
GIAB3	3	GIAB3	GIAB3	GIAB3
GIAB5_CSER15_PAGE80	100	GIAB5 + CSER15 + PAGE80	GIAB5	CSER15
GIAB5_CSER15_PAGE	333	GIAB5 + CSER15 + PAGE	GIAB5	CSER15
GIAB5_CSER_PAGE	1,247	GIAB5 + CSER + PAGE	GIAB5	CSER15
GIAB_CSER_WES	346	GIAB_WES + CSER_WES	GIAB_WES	CSER15_WES

C) Cohort evaluation metrics.

Cohort Metric Name	**Definition**
Mendelian Violation	Arithmetic mean of Mendelian violation rates on evaluation trios.
SNP Precision	Arithmetic mean of SNP precisions of all single-sample benchmark samples.
SNP Recall	Arithmetic mean of SNP recalls of all single-sample benchmark samples.
Indel Precision	Arithmetic mean of indel precisions of all single-sample benchmark samples.
Indel Recall	Arithmetic mean of indel recalls of all single-sample benchmark samples.

Supplementary Table 3: CSER15 and CSER15_WES trio sample names.

	Child	**Father**	**Mother**
CSER15	SRR4370493	SRR4370494	SRR4370495
	SRR6706862	SRR6707105	SRR6707106
	SRR6706955	SRR6706956	SRR6706957
	SRR6707156	SRR6707157	SRR6707158
	SRR6707268	SRR6707269	SRR6707270
CSER15_WES	SRR3406206	SRR3406207	SRR3406279
	SRR3406280	SRR3406209	SRR3406430
	SRR3406315	SRR3406316	SRR3406317
	SRR3406410	SRR3406404	SRR3406373
	SRR3406427	SRR3406428	SRR3406429

Supplementary Table 4: GLnexus configurable parameters.

Name	Type	Default Value	Tuned	Description
min_AQ1	Numeric	0	Y	The minimum allele quality in phred scale to be used for all alleles. Alleles lower than this quality will be pruned. Increasing this will increase specificity and decrease sensitivity.
min_AQ2	Numeric	0	Y	The minimum allele quality in phred scale to be used for alleles that have multiple observations. min_AQ1 ≥ min_AQ2.
min_GQ	Numeric	0	Y	The minimum genotype quality in phred scale to be used for copy number estimates for the constituent alleles.
min_allele_copy_number	Numeric	1		The minimum number of observations an allele needs to have in order to be kept.
revise_genotypes	Boolean	false	Y	If true, joint calling is enabled - use genotype likelihoods and allele frequencies to revise low quality genotype calls.
min_assumed_allele_frequency	Numeric	0.0001		Allele frequency lower than this value will be fixed to be this minimum value so rare alleles are less likely to be lost in a large cohort.
required_dp	Numeric	0		The minimum depth required for any allele call.

Supplementary Table 5. Comparison of variant calling-merging methods.
A) GATK-Joint, GATK-VQSR, DV-GLN-NOMOD, and DV-GLN-OPT pipelines were compared by GIAB sample concordance and trio sample Mendelian violation rates for all 40x sequence coverage cohorts. All evaluation metrics were computed on chromosome 20. The F1 score is the harmonic mean of precision and recall. Bold numbers are the best values across three methods, or the values that are within 0.001 difference from the best value. The parameters and resources used for GATK-VQSR can be found in **Supplementary Note 3**. GATK-VQSR is skipped for the trio cohort due to the insufficient size. The trio cohort also includes a cohort generated from DeepVariant single-sample calls merged using GATK GenotypeGVCFs ("DV-GATK (Joint)" method in light gray numbers), but this setup was skipped for the other cohorts due to substantially lower indel recall & precision compared to other methods in the trio experiment. Prec, precision; MV, Mendelian violation rate; Std, standard deviation. **B)** Similar to A), for the 15x sequence coverage cohorts. **C)** Similar to A) and B), for a single 346-individual exome cohort. A separate parameter set "OPT-WES" for DV+GLnexus, optimized specifically for exomes, is used.

A) 40x coverage

Size	Method	SNP F1	SNP Recall	SNP Recall Std	SNP Prec	SNP Prec Std	Indel F1	Indel Recall	Indel Recall Std	Indel Prec	Indel Prec Std	MVR	MVR Std
3	GATK (Joint)	0.99788	**0.99945**	0.00013	0.99631	0.00033	0.97950	0.97285	0.01233	0.98624	0.00551	6.61%	.
	DV-GATK (Joint)	0.97547	0.98700	0.00787	0.96420	0.02269	0.92497	0.90430	0.04933	0.94660	0.03019	**1.66%**	.
	DV-GLN (NOMOD)	**0.99936**	**0.99937**	0.00019	**0.99936**	0.00025	**0.99036**	**0.98556**	0.00685	**0.99521**	0.00131	4.72%	.
	DV-GLN (OPT)	**0.99932**	**0.99903**	0.00011	**0.99962**	0.00013	0.98753	0.98057	0.00988	**0.99459**	0.00193	3.32%	.
100	GATK (Joint)	0.99657	**0.99916**	0.00057	0.99399	0.00205	0.97858	0.97727	0.01019	0.97989	0.00502	5.97%	0.26%
	GATK (VQSR)	0.98638	0.97429	0.00145	0.99877	0.00071	0.97175	0.96146	0.01064	0.98226	0.00614	4.15%	0.30%
	DV-GLN (NOMOD)	**0.99935**	**0.99929**	0.00021	**0.99941**	0.00024	**0.98959**	**0.98429**	0.00727	**0.99495**	0.00065	2.46%	0.21%
	DV-GLN (OPT)	**0.99934**	**0.99917**	0.00023	**0.99951**	0.00015	**0.98926**	**0.98387**	0.00698	**0.99472**	0.00031	**1.61%**	0.19%
333	GATK (Joint)	0.99634	**0.99915**	0.00056	0.99355	0.00234	0.97745	0.97633	0.01060	0.97858	0.00487	6.37%	0.27%
	GATK (VQSR)	0.98886	0.97935	0.00135	0.99855	0.00074	0.97156	0.96195	0.01126	0.98137	0.00625	4.42%	0.30%
	DV-GLN (NOMOD)	**0.99932**	**0.99922**	0.00024	**0.99942**	0.00025	**0.98907**	**0.98334**	0.00790	**0.99487**	0.00069	2.44%	0.20%
	DV-GLN (OPT)	**0.99931**	**0.99910**	0.00027	**0.99951**	0.00017	**0.98903**	**0.98332**	0.00741	**0.99480**	0.00039	**1.63%**	0.19%
1247	GATK (Joint)	0.99609	**0.99912**	0.00057	0.99309	0.00264	0.97666	0.97523	0.01057	0.97811	0.00540	7.03%	0.29%
	GATK (VQSR)	0.98771	0.97709	0.00526	**0.99857**	0.00072	0.97081	0.96101	0.01148	0.98082	0.00636	5.00%	0.33%
	DV-GLN (NOMOD)	**0.99931**	**0.99922**	0.00025	**0.99941**	0.00025	**0.98868**	**0.98245**	0.00855	**0.99500**	0.00065	2.43%	0.20%
	DV-GLN (OPT)	**0.99930**	**0.99913**	0.00028	**0.99948**	0.00017	**0.98860**	**0.98261**	0.00806	**0.99466**	0.00047	**1.69%**	0.20%

B) 15x coverage

Size	Method	SNP F1	SNP Recall	SNP Recall Std	SNP Prec	SNP Prec Std	Indel F1	Indel Recall	Indel Recall Std	Indel Prec	Indel Prec Std	MVR	MVR Std
3	GATK (Joint)	0.98176	0.97426	0.00274	**0.98937**	0.00284	0.88575	0.84140	0.03604	0.93503	0.01991	8.64%	.
	DV-GATK (Joint)	0.96691	0.94780	0.02786	0.98680	0.00564	0.86053	0.80400	0.05586	0.92560	0.02171	**2.39%**	.
	DV-GLN (NOMOD)	0.97596	**0.98108**	0.00198	0.97090	0.01670	**0.92567**	**0.90176**	0.01695	0.95090	0.01376	5.20%	.
	DV-GLN (OPT)	**0.98448**	**0.98043**	0.00230	**0.98857**	0.00590	**0.92479**	0.89072	0.02129	**0.96158**	0.00667	3.81%	.
100	GATK (Joint)	0.98498	0.98079	0.00584	0.98920	0.00205	0.91734	0.88600	0.04947	0.95099	0.01625	8.25%	0.31%
	GATK (VQSR)	0.97231	0.95344	0.00759	**0.99194**	0.00179	0.91038	0.87252	0.04989	0.95168	0.01729	6.93%	0.50%
	DV-GLN (NOMOD)	0.98260	**0.98479**	0.00461	0.98042	0.01743	0.93578	**0.91087**	0.02878	0.96210	0.01692	3.46%	0.25%
	DV-GLN (OPT)	**0.98789**	**0.98453**	0.00417	**0.99128**	0.00562	**0.93720**	0.90884	0.02721	**0.96738**	0.00972	**2.33%**	0.16%
333	GATK (Joint)	0.98480	0.98079	0.00578	0.98885	0.00245	0.91739	0.88560	0.04938	0.95156	0.01514	8.66%	0.29%
	GATK (VQSR)	0.97298	0.95401	0.00722	**0.99271**	0.00183	0.91152	0.87378	0.05004	0.95266	0.01643	7.18%	0.47%
	DV-GLN (NOMOD)	0.98254	**0.98466**	0.00467	0.98043	0.01743	0.93494	**0.90900**	0.02968	0.96241	0.01679	3.43%	0.25%
	DV-GLN (OPT)	**0.98780**	**0.98444**	0.00419	0.99120	0.00565	**0.93655**	0.90755	0.02804	**0.96746**	0.00979	**2.37%**	0.15%
1247	GATK (Joint)	0.98447	0.98082	0.00575	0.98815	0.00284	0.91630	0.88610	0.04937	0.94863	0.01606	9.37%	0.25%
	GATK (VQSR)	0.97513	0.95795	0.00971	**0.99293**	0.00155	0.91099	0.87506	0.04970	0.95001	0.01698	8.06%	0.47%
	DV-GLN (NOMOD)	0.98248	**0.98454**	0.00474	0.98043	0.01743	0.93324	**0.90532**	0.03144	0.96294	0.01651	3.38%	0.25%
	DV-GLN (OPT)	**0.98773**	**0.98440**	0.00416	0.99108	0.00572	**0.93506**	**0.90492**	0.02936	**0.96727**	0.00945	**2.46%**	0.16%

C) Exome

Size	Method	SNP F1	SNP Recall	SNP Recall Std	SNP Prec	SNP Prec Std	INDEL F1	INDEL Recall	INDEL Recall Std	INDEL Prec	INDEL Prec Std	MVR	MVR Std
346	GATK (Joint)	0.98918	**0.99409**	0.00482	0.98433	0.00940	0.81343	0.91792	0.03306	0.73030	0.11674	2.69%	0.29%
	GATK (VQSR)	0.99122	0.99318	0.00472	0.98928	0.00825	0.81945	0.90645	0.03309	0.74769	0.11412	2.16%	0.21%
	DV-GLN (NOMOD)	**0.99591**	**0.99464**	0.00383	**0.99718**	0.00058	0.95813	0.93523	0.00595	0.98218	0.00571	2.15%	0.36%
	DV-GLN (OPT-WES)	**0.99600**	**0.99468**	0.00380	**0.99732**	0.00050	**0.96356**	**0.94096**	0.00839	**0.98727**	0.00408	**1.56%**	0.20%

Supplementary Table 6. Imputation accuracy of GIAB benchmark callsets. The imputed variant calls of HG002 and HG005 are scored using the GIAB benchmark variants v3.3.2 (GRCh38) and hap.py v0.3.9. Two evaluation regions are used: "*full conf. region*" is the intersection of the HG002 and HG005 benchmark regions, agnostic to either reference panel, and "*shared conf. region in both panels*" is the subset of full conf. region that also intersects both the DV-GLN-OPT panel and GATK panel regions.

Eval. region	Sample	Ref. panel method	Type	F1	Recall	Precision	TP	FN	FP	FP.gt
Full conf. region	HG002	DV-GLN-OPT	INDEL	**0.90307**	**0.88392**	**0.92308**	325366	42729	27115	14189
			SNP	**0.94555**	**0.92818**	**0.96359**	2596802	200944	98143	39149
		GATK	INDEL	0.89921	0.87839	0.92106	323329	44766	27721	14140
			SNP	0.94219	0.92176	0.96354	2578852	218894	97606	38968
	HG005	DV-GLN-OPT	INDEL	**0.89325**	**0.88232**	**0.90446**	319121	42564	33714	15193
			SNP	**0.93832**	**0.93058**	0.94618	2566405	191442	146023	41306
		GATK	INDEL	0.88989	0.87706	0.90310	317221	44468	34052	15051
			SNP	0.93511	0.92425	**0.94624**	2548940	208921	144832	41045
Shared conf. region in both panels	HG002	DV-GLN-OPT	INDEL	**0.90621**	0.88959	**0.92345**	323048	40094	26778	14018
			SNP	**0.95033**	**0.93721**	**0.96382**	2579381	172822	96838	38671
		GATK	INDEL	0.90549	**0.88971**	0.92183	323092	40050	27399	14126
			SNP	0.95016	0.93696	0.96373	2578713	173490	97058	38965
	HG005	DV-GLN-OPT	INDEL	**0.89633**	0.88827	**0.90455**	316799	39850	33430	15031
			SNP	**0.94287**	**0.93947**	0.94630	2549478	164268	144708	40856
		GATK	INDEL	0.89593	**0.88863**	0.90336	316929	39720	33907	15032
			SNP	0.94276	0.93917	**0.94638**	2548675	165071	144442	41046

Supplementary Table 7. DeepVariant and GATK HaplotypeCaller benchmark.
Summary statistics of elapsed real time, user CPU time, and system CPU time spent on running DeepVariant and GATK HaplotypeCaller across 2,504 1KGP samples, chromosome 22 only, using 8-vCPU virtual machines.

	DeepVariant (seconds)			**GATK HaplotypeCaller** (seconds)		
	Real	**User CPU**	**System CPU**	**Real**	**User CPU**	**System CPU**
Mean	1,201.26	7,733.31	146.56	1,989.20	5,346.24	9.63
St. dev.	75.44	470.21	10.20	203.66	511.87	1.48
Min	1,044.64	6,795.50	125.96	1,617.60	4,442.35	7.59
25%	1,144.85	7,386.25	138.91	1,849.41	4,986.84	8.51
50%	1,178.51	7,594.58	143.52	1,953.21	5,223.30	9.09
75%	1,263.78	8,113.92	154.78	2,082.78	5,636.03	10.66
Max	1,484.26	9,279.03	191.90	3,601.13	9,196.31	18.43

Supplementary Table 8. PAGE80 sample names.

SRR2993850	SRR2994215	SRR2994285	SRR2994293	SRR2994301
SRR2994861	SRR2995075	SRR2995970	SRR2996055	SRR2996085
SRR2996123	SRR2996131	SRR2996243	SRR2996321	SRR2996337
SRR2996373	SRR3003654	SRR3003716	SRR3003902	SRR3004018
SRR3004154	SRR3004266	SRR3010823	SRR3010896	SRR3010944
SRR3011061	SRR3011110	SRR3011469	SRR3011551	SRR3011961
SRR3012267	SRR3012323	SRR3012447	SRR3012511	SRR3012726
SRR3012734	SRR3012758	SRR3012834	SRR3012951	SRR3012975
SRR3013049	SRR3013065	SRR3013089	SRR3013153	SRR3013161
SRR3013177	SRR3013201	SRR3013202	SRR3013242	SRR3013338
SRR3013370	SRR3013378	SRR3013430	SRR3013508	SRR3013524
SRR3013587	SRR3013603	SRR3013793	SRR3013843	SRR3013881
SRR3014027	SRR3014035	SRR3014051	SRR3014088	SRR3014096
SRR3014120	SRR3014152	SRR3014168	SRR3014200	SRR3014306
SRR3014314	SRR3014338	SRR3014370	SRR3014378	SRR3014418
SRR3014442	SRR3014520	SRR3014536	SRR3014653	SRR3014824

Supplementary Notes

Supplementary Note 1: "DV-GLN-OPT" optimized GLnexus WGS configuration

This configuration is available as “DeepVariantWGS” in GLnexus v1.2.2: https://github.com/dnanexus-rnd/GLnexus/blob/v1.2.2/src/cli_utils.cc#L808-L852.

```
# Custom configuration for joint calling DeepVariant whole genome sequencing gVCFs.
unifier_config:
    min_AQ1: 10
    min_AQ2: 10
    min_GQ: 0
    monoallelic_sites_for_lost_alleles: true
genotyper_config:
    required_dp: 0
    revise_genotypes: true
    more_PL: true
    trim_uncalled_alleles: true
    liftover_fields:
        - orig_names: [MIN_DP, DP]
          name: DP
          description: '##FORMAT=<ID=DP,Number=1,Type=Integer,Description="Approximate read
depth (reads with MQ=255 or with bad mates are filtered)">'
          type: int
          combi_method: min
          number: basic
          count: 1
          ignore_non_variants: true
        - orig_names: [AD]
          name: AD
          description: '##FORMAT=<ID=AD,Number=R,Type=Integer,Description="Allelic depths
for the ref and alt alleles in the order listed">'
          type: int
          number: alleles
          combi_method: min
          default_type: zero
          count: 0
        - orig_names: [GQ]
          name: GQ
          description: '##FORMAT=<ID=GQ,Number=1,Type=Integer,Description="Genotype
Quality">'
          type: int
```

```
          number: basic
          combi_method: min
          count: 1
          ignore_non_variants: true
        - orig_names: [PL]
          name: PL
          description: '##FORMAT=<ID=PL,Number=G,Type=Integer,Description="Phred-scaled
genotype Likelihoods">'
          type: int
          number: genotype
          combi_method: missing
          count: 0
          ignore_non_variants: true
```

Supplementary Note 2: Data preparation details

Reference genome

Throughout this study we used the human GRCh38 reference genome that contains the "no alt" analysis set and human decoy sequences from hs38d1 (GCA_000786075.2) (ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/001/405/GCA_000001405.15_GRCh38/seqs_for_alignment_pipelines.ucsc_ids/GCA_000001405.15_GRCh38_no_alt_plus_hs38d1_analysis_set.fna.gz ; ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/001/405/GCA_000001405.15_GRCh38/seqs_for_alignment_pipelines.ucsc_ids/README_analysis_sets.txt).

Genome in a Bottle

We used sequencing reads and benchmark callsets of the seven individuals currently in GIAB. HG001 (a female of Utah/European ancestry) and HG002 (Ashkenazi Jewish son) samples were sequenced by Illumina HiSeq 2500 in Rapid Mode (v1) with 2x148bp read length and ~50x coverage, and were aligned to GRCh38 by BWA-MEM version 0.7.17 (Li, 2013). The Ashkenazi Jewish parent samples, HG003 and HG004, were sequenced with Illumina HiSeq 2500 in Rapid mode (v2) with 2x250 paired-end reads and ~40-50x coverage, and were aligned to GRCh38 using Novoalign version 3.02.07 (www.novocraft.com/products/novoalign). HG005, the Chinese son sample, was used only for imputation evaluation based on the benchmark callset. The Chinese parent samples HG006 and HG007 were sequenced by Illumina HiSeq 2500 in Rapid mode (v1) with 2x148 paired end reads to 100x coverage, mapped to GRCh38 with BWA-MEM, and downsampled to ~40x coverage to match the coverage of other samples using samtools v1.6 (Li *et al.*, 2009).

Clinical Sequencing Evidence-Generating Research

We downloaded SRA files for 929 WGS samples (among 931 WGS samples, SRR6706856 and SRR4370311 repeatedly failed to download) and 346 WES samples from the CSER project from dbGaP (project ID: 20844). All samples were sequenced with the Illumina HiSeq X platform. We generated FASTQ files using the "fastq-dump" command in the NCBI SRA Toolkit (github.com/ncbi/sra-tools). Finally, all FASTQ files were mapped to GRCh38 with BWA-MEM version 0.7.17 and duplicate reads were marked by samblaster version 0.1.24 (Faust and Hall, 2014).

We identified all mother-father-child trio samples in the CSER dataset using the "Library_Name" field in the associated SRA Run Table. The values of the field are formatted as "A-[Family ID]-[Family relation]". Of 249 disjoint trios we identified, we randomly selected five trios (15 total individuals, IDs in **Supplementary Table 2**) among all non-outlier trios to use for Mendelian violation rate estimation during callset evaluation. To define non-outlier samples, we examined six variant summary statistics for each sample: the number of records, the number of SNPs, the number of indels, the Ti:Tv ratio, the mean SNP quality, and the mean indel quality. Non-outliers are defined as the samples for which all six statistics are within one standard deviation of the mean (i.e. the magnitude of the Z-score is at most one).

Population Architecture Using Genomics and Epidemiology

We processed the PAGE samples in the same way as those from CSER. We downloaded 313 sample SRA files from dbGaP (project ID: 17123), which were also generated by Illumina HiSeq X platform, converted them to FASTQ, mapped them to GRCh38, and marked duplicates as described above. We also generated a subset we call *PAGE80* by randomly selecting 80 non-outlier samples among the 313 samples (**Supplementary Table 7**). The same six summary statistics were used to select non-outliers, except that we used a maximum Z-score magnitude of 1.25 (instead of one) to include more samples.

Using the 6 GIAB samples, 929 CSER samples, and 313 PAGE samples above, we created custom cohorts of size 3, 100, 333, and 1247 (**Supplementary Table 1**) for which both GIAB concordance and Mendelian violation rate could be evaluated. We used the GIAB benchmark variant version 3.3.2 for GRCh38 to evaluate concordance. Finally, we created 15x autosomal coverage BAMs from all BAM files from GIAB, CSER, and PAGE datasets by downsampling full BAMs with samtools v1.6 using the "samtools view -s" command.

1000 Genomes Project

The 2,504-sample cohort callset we release is based on the recent deep sequencing of the 1KGP phase3 samples by New York Genome Center. The input reads were sequenced at 30x coverage using the new Illumina NovaSeq 6000 system with 2x150bp reads, and then aligned to GRCh38 using BWA-MEM v0.7.15 (Li, 2013). More details about their pipeline can be found on EBI 1000 Genomes FTP (ftp://ftp.1000genomes.ebi.ac.uk/vol1/ftp/data_collections/1000G_2504_high_coverage/20190405_NYGC_b38_pipeline_description.pdf). We used samtools v1.6 to convert the original CRAM files to BAM format for downstream tasks.

1KGP imputation reference panel creation

We generated the 1KGP reference panels from DV-GLN-OPT and GATK-VQSR callsets by applying identical minimal transformations to them and phasing them with Eagle (Loh *et al.*, 2016). We followed a standard pipeline for generating a reference panel recommended in Eagle's website (data.broadinstitute.org/alkesgroup/Eagle/#x1-300005.3). Starting from each cohort callset, we removed singleton variants and kept only variants with either "PASS" or "." (empty) filter. We also converted multi-allelic variants to multiple biallelic variants and removed duplicate variants, as required by Eagle, using bcftools v1.9 (samtools.github.io/bcftools). Finally, we ran Eagle v2.4.1 with the hg38 genetic map file released with the software, without supplying any additional reference panel. A script for running all the above steps can be found in **Supplementary Note 5**.

Supplementary Note 3: GATK command details

Here we give details of GATK Best Practices v4.1.2.0 commands we used for generating the custom GIAB, CSER, and PAGE cohorts, following the official GATK documentation. Please note that the 1KGP GATK cohort was generated independently by NYGC (for more details see: http://ftp.1000genomes.ebi.ac.uk/vol1/ftp/data_collections/1000G_2504_high_coverage/20190405_NYGC_b38_pipeline_description.pdf).

HaplotypeCaller (for each sample and chromosome):

```
gatk --java-options -Xmx10g HaplotypeCaller
    -R GRCh38_reference_genome.fa
    -I sample_input.bam
    -O sample_output.g.vcf.gz
    -ERC GVCF
    -L chr20
    --native-pair-hmm-threads 4
```

GenomicsDBImport and GenotypeGVCFs (for each chromosome):

```
# "cohort.sample_map" is a tab-separated text file with two columns.
# The first column contains the sample names and the second column has
# the corresponding paths to the gVCF files generated by HaplotypeCaller.

gatk --java-options -Xmx200g GenomicsDBImport
    --genomicsdb-workspace-path gdi
    --batch-size 50
    --sample-name-map cohort.sample_map
    --reader-threads 5
    -L chr20

gatk --java-options -Xmx200g GenotypeGVCFs
    -R GRCh38_reference_genome.fa
    -V gendb://gdi
    -O cohort.vcf.gz
```

For running VQSR, we followed the settings used for the deep coverage 1KGP phase 3 release from NYGC (http://ftp.1000genomes.ebi.ac.uk/vol1/ftp/data_collections/1000G_2504_high_coverage/20190405_NYGC_b38_pipeline_description.pdf), but using GATK v4.1.2.0 instead of GATK v3.5 for the custom GIAB, CSER, and PAGE cohorts. Note that some parameters in GATK v3.5 have different names in GATK4. All resource files can be found in Broad Institute's public resource directory on Google Cloud Storage (gs://genomics-public-data/resources/broad/hg38/v0).

```
RES_HAPMAP="hapmap_3.3.hg38.vcf.gz"
RES_1KG_OMNI="1000G_omni2.5.hg38.vcf.gz"
RES_1KG_P1_SNP="1000G_phase1.snps.high_confidence.hg38.vcf.gz"
```

```
RES_MILLS_INDEL="Mills_and_1000G_gold_standard.indels.hg38.vcf.gz"
RES_DBSNP="Homo_sapiens_assembly38.dbsnp138.vcf"

gatk --java-options -Xmx8g VariantRecalibrator
    -R "${ref_genome_fasta}"
    -V "${input_vcf}"
    -O "${output_snp_basepath}.recal"
    --tranches-file "${output_snp_basepath}.tranches"
    --rscript-file "${output_snp_basepath}.plots.R"
    -mode SNP
    "--resource:hapmap,known=false,training=true,truth=true,prior=15.0" "${RES_HAPMAP}"
    "--resource:omni,known=false,training=true,truth=true,prior=12.0" "${RES_1KG_OMNI}"
    "--resource:1000G,known=false,training=true,truth=false,prior=10.0" "${RES_1KG_P1_SNP}"
    "--resource:dbsnp,known=true,training=false,truth=false,prior=2.0" "${RES_DBSNP}"
    -an QD
    -an MQ
    -an FS
    -an MQRankSum
    -an ReadPosRankSum
    -an SOR
    -an DP
    -tranche 100.0
    -tranche 99.8
    -tranche 99.6
    -tranche 99.4
    -tranche 99.2
    -tranche 99.0
    -tranche 95.0
    -tranche 90.0
    --max-attempts 3

gatk --java-options -Xmx8g VariantRecalibrator
    -R "${ref_genome_fasta}"
    -V "${input_vcf}"
    -O "${output_indel_basepath}.recal"
    --tranches-file "${output_indel_basepath}.tranches"
    --rscript-file "${output_indel_basepath}.plots.R"
    -mode INDEL
    "--resource:mills,known=true,training=true,truth=true,prior=12.0" "${RES_MILLS_INDEL}"
    "--resource:dbsnp,known=true,training=false,truth=false,prior=2.0" "${RES_DBSNP}"
    -an QD
    -an FS
    -an ReadPosRankSum
    -an MQRankSum
    -an SOR
    -an DP
    -tranche 100.0
```

```
    -tranche 99.0
    -tranche 95.0
    -tranche 92.0
    -tranche 90.0
    --max-gaussians 4
    --max-attempts 3

gatk --java-options -Xmx8g ApplyVQSR
    -R "${ref_genome_fasta}"
    -V "${input_vcf}"
    -O "${vqsr_snp_vcf}"
    -mode SNP
    --truth-sensitivity-filter-level 99.80
    --recal-file "${output_snp_basepath}.recal"
    --tranches-file "${output_snp_basepath}.tranches"

gatk --java-options -Xmx8g ApplyVQSR
    -R "${ref_genome_fasta}"
    -V "${vqsr_snp_vcf}"
    -O "${vqsr_final_vcf}"
    -mode INDEL
    --truth-sensitivity-filter-level 99.0
    --recal-file "${output_indel_basepath}.recal"
    --tranches-file "${output_indel_basepath}.tranches"
```

Supplementary Note 4: Parameter optimization details

We used Google Vizier (Golovin *et al.*, 2017), a Google-internal service for performing black-box optimization, for optimizing the configurable parameters of GLnexus (**Supplementary Table 3**).

The first iteration of parameter search used the *Pareto-optimal* search algorithm, where we set two optimization objectives: maximizing GIAB benchmark call concordance and minimizing the rate of Mendelian violations. GIAB benchmark call concordance was defined as the harmonic mean of the SNP F1 score (which in turn is defined as the harmonic mean of SNP recall and precision values) and the indel F1 score. The precision/recall of the two types of variants was defined as the arithmetic mean of the precision/recall values for the GIAB benchmark samples (three samples for the cohorts of size three, and five samples for all other cohorts). The purpose of the first iteration of parameter search was to explore the general trends and reduce the search space volume. To this end, we explored a wide range of possible parameter values: "min_AQ2" could be any integer from 0 to 50 (inclusive), "min_AQ1" could be any integer from "min_AQ2" to 80 where the difference between "min_AQ1" and "min_AQ2" was at most 30, "min_GQ" was a multiple of 10 from 0 to 50 (because GLnexus quantizes this value as a multiple of ten), and "revise_genotypes" could be True or False.

After manually investigating points on the Pareto optimal frontier of the above search we substantially reduced the search space as follows: "min_AQ2" between 0 and 20, "min_AQ1" defined by ($0 \le$ min_AQ1 - min_AQ2 ≤ 20), "min_GQ" in {0, 10, 20}, and "revise_genotypes" could be True or False. In this reduced search space, we performed exhaustive grid search where the size of the grid for "min_AQ1" and "min_AQ2" was 5 (so the values are multiples of 5). This resulted in 150 (=5×5×3×2) total configurations. We merged all eight cohorts with all possible configurations in this space, resulting in 1,200 total experiments.

Formally, we have the following five evaluation metrics, each of which is a function of the read coverage, cohort size, and configuration parameters:

$$\begin{aligned} \mathit{ErrorMetrics} = \{\ & \mathit{MendelianViolationRate},\ 1 - \mathit{SNPPrecision},\ 1 - \mathit{SNPRecall}, \\ & 1 - \mathit{IndelPrecision},\ 1 - \mathit{IndelRecall}\ \} \end{aligned}$$

The value of every metric is between 0 to 1, where 0 is the most desirable. Note that 1-precision is also called the *false discovery rate* and 1-recall is called the *false negative rate*.

Let P be the set of all parameter tuples within our search space, namely,

$$\begin{aligned} P = \{\ & (\mathit{minAQ1},\ \mathit{minAQ2},\ \mathit{minGQ},\ \mathit{reviseGenotypes}) \mid \\ & 0 \le \mathit{minAQ2} \le 20, \\ & 0 \le \mathit{minAQ1} - \mathit{minAQ2} \le 20, \\ & \mathit{minGQ} \in \{0,\ 10,\ 20\}, \\ & \mathit{reviseGenotypes} \in \{\mathit{True},\ \mathit{False}\}\ \} \end{aligned}$$

For each parameter set p in P, we define the objective function $L(p)$ by this formula:

$$L(p) = \sum_{\mathit{coverage} \in \{15,\ 40\}} \sum_{\mathit{size} \in \{3,\ 100,\ 313,\ 1247\}} \sum_{m \in \mathit{ErrorMetrics}} \frac{m(\mathit{coverage}, \mathit{size}, p) - m(\mathit{coverage}, \mathit{size}, p_0)}{m(\mathit{coverage}, \mathit{size}, p_0)}$$

where p_o is the GLnexus parameter with no modification of input calls, namely p_o=(0, 0, 0, *False*). Finally we search for the parameter p^* that minimizes the objective.

$$p^* = argmin_{p \in P} L(p)$$

This implies that we try to maximize the *rate of error reduction* per metric over the "no modification" parameter setting, and sum them with equal weights.

The optimized DeepVariant+GLnexus callsets use this p^* configuration (see **Supplementary Note 1** for the parameter values).

Supplementary Note 5: Reference panel creation

This is a Bash script for creating a reference panel from a 1KGP cohort VCF (DV-GLN-OPT or GATK-VQSR), closely following a standard pipeline in Eagle's website (https://data.broadinstitute.org/alkesgroup/Eagle/#x1-300005.3)

```
# Required tools: bcftools, tabix, Eagle.

# Input cohort VCF (from DeepVariant-GLnexus or GATK)
cohort_vcf="cohort-chr22.vcf.gz"

# 1KGP Reference genome:
ftp://ftp.1000genomes.ebi.ac.uk/vol1/ftp/technical/reference/GRCh38_reference_genome/GRCh38_full_analysis_set_plus_decoy_hla.fa

ref_genome="GRCh38_full_analysis_set_plus_decoy_hla.fa"

# Genetic map file from Eagle repo:
https://data.broadinstitute.org/alkesgroup/Eagle/downloads/tables/genetic_map_hg38_withX.txt.gz

genetic_map_file="genetic_map_hg38_withX.txt.gz"

# Intermediate/output file names
cohort_processed_vcf="cohort-chr22-processed.bcf"
eagle_output_prefix="cohort-chr22-reference-panel"

# Filter singletons, apply variant filter, and convert to bcf.
bcftools view --no-version \
    -c 2 \
    -f ".,PASS" \
    "${cohort_vcf}" | \
  bcftools norm --no-version -Ou -m -any | \
  bcftools norm --no-version -Ob -o "${cohort_processed_vcf}" \
    -d none -f "${ref_genome}" && \
  bcftools index -f "${cohort_processed_vcf}"

# Run Eagle for phasing
eagle \
  --geneticMapFile=${genetic_map_file}" \
  --vcf="${cohort_processed_vcf}"
  --outPrefix="${eagle_output_prefix}" \
  --vcfOutFormat=z \
  --numThreads="$(nproc)"

# Index the output
tabix "${eagle_output_prefix}.vcf.gz"
```

Supplementary Note 6: Genotype imputation

This is a Bash script for imputing phased (pseudo-)microarray variants with Beagle 5.0 using a reference panel.

```
# Required tools: Beagle 5.0, tabix

# Input files
reference_panel="cohort-chr22-reference-panel.vcf.gz"
input_vcf="HG002.pseudo-microarray.phased.chr22.vcf.gz"

# Output file name
output_prefix="HG002.imputed-pseudo-microarray.phased.chr22.vcf.gz"

chrom="chr22"

# Run Beagle for imputation
java -Xss2048k -Xmx50G -jar beagle.jar \
  "ref=${reference_panel}" \
  "gt=${input_vcf}" \
  "out=${output_prefix}" \
  "chrom=${chrom}"

tabix "${output_prefix}.vcf.gz"
```

Supplementary Note 7: Software versions

DeepVariant (Google Brain): **v0.8.0** and **custom model** (included in 1000 Genomes data release) for NovaSeq reads.
GLnexus (DNAnexus): This study was run using **v1.2.0-pre.0**. The optimized parameters from this study are now included in GLnexus **v1.2.2** as two presets: `DeepVariantWGS` and `DeepVariantWES`.
GATK (Broad Institute): **v4.1.2.0**, except for the GATK 1KGP VCFs released by the New York Genome Center which used GATK **v3.5**.
Hap.py (Illumina): **v0.3.9**.
Eagle: **v2.4.1**.
Beagle: **v5.0**.
bcftools: **v1.9**.

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