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SUPPLEMENTARY INFORMATION

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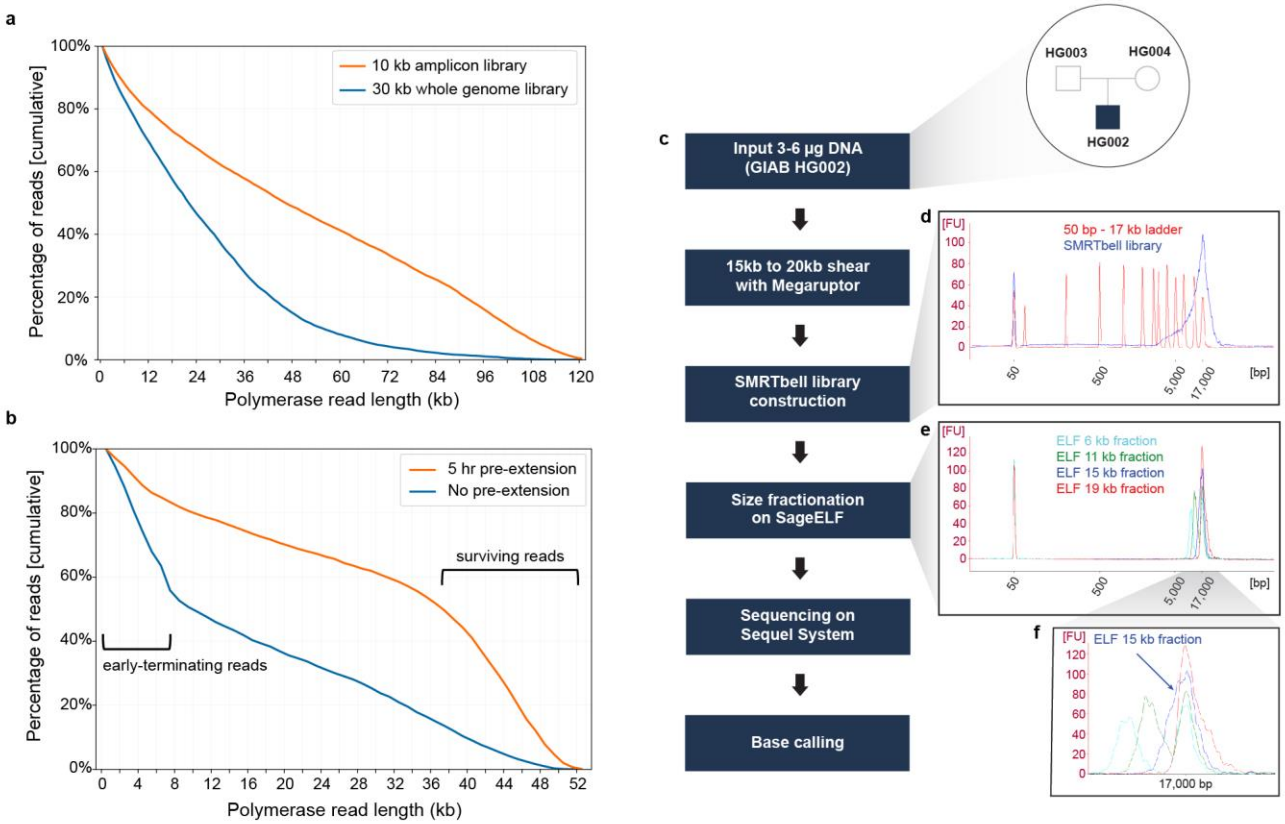
Accurate circular consensus long-read sequencing improves variant detection and assembly of a human genome

Aaron M. Wenger[1,14], Paul Peluso[1,14], William J. Rowell[1], Pi-Chuan Chang[2], Richard J. Hall[1], Gregory T. Concepcion[1], Jana Ebler[3,4,5], Arkarachai Fungtammasan[6], Alexey Kolesnikov[2], Nathan D. Olson[7], Armin Töpfer[1], Michael Alonge[8], Medhat Mahmoud[9], Yufeng Qian[1], Chen-Shan Chin[6], Adam M. Phillippy[10], Michael C. Schatz[8], Gene Myers[11], Mark A. DePristo[2], Jue Ruan[12], Tobias Marschall[3,4], Fritz J. Sedlazeck[9], Justin M. Zook[7], Heng Li[13], Sergey Koren[10], Andrew Carroll[2], David R. Rank[1*] and Michael W. Hunkapiller[1*]

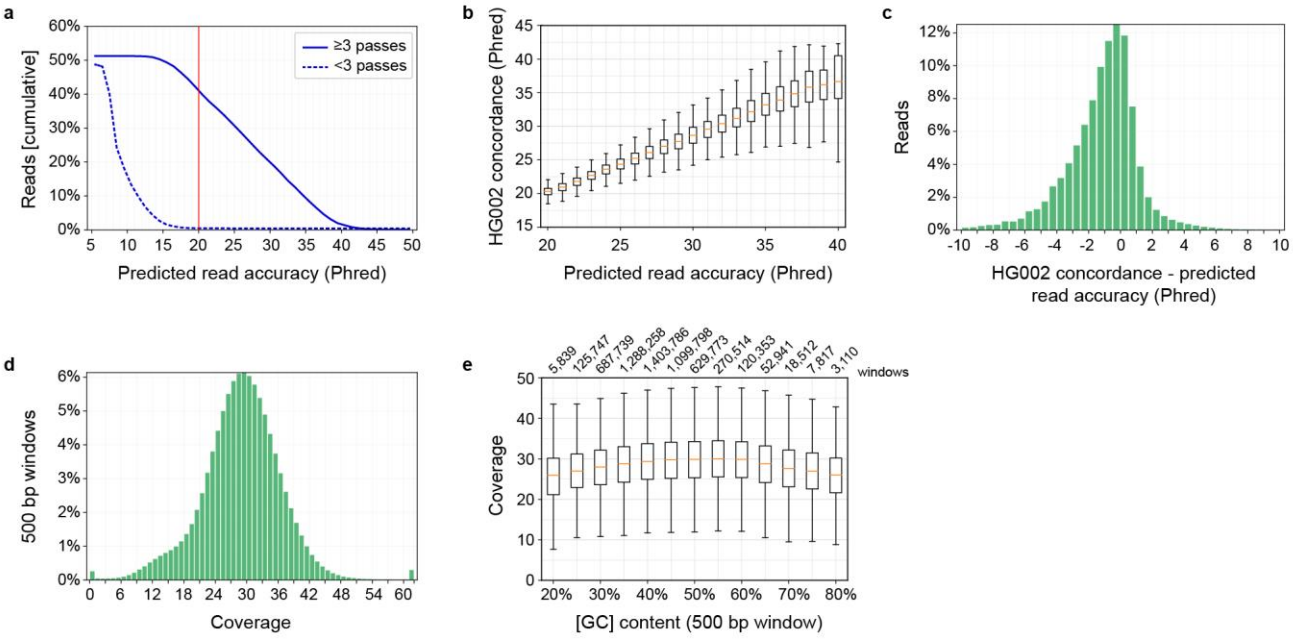
[1]Pacific Biosciences, Menlo Park, CA, USA. [2]Google Inc., Mountain View, CA, USA. [3]Center for Bioinformatics, Saarland University, Saarbrücken, Germany. [4]Max Planck Institute for Informatics, Saarbrücken, Germany. [5]Graduate School of Computer Science, Saarland University, Saarbrücken, Germany. [6]DNAnexus, Mountain View, CA, USA. [7]National Institute of Standards and Technology, Gaithersburg, MD, USA. [8]Department of Computer Science, Johns Hopkins University, Baltimore, MD, USA. [9]Human Genome Sequencing Center, Baylor College of Medicine, Houston, TX, USA. [10]Genome Informatics Section, Computational and Statistical Genomics Branch, National Human Genome Research Institute, Bethesda, MD, USA. [11]Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany. [12]Agricultural Genomics Institute, Chinese Academy of Agricultural Sciences, Shenzhen, China. [13]Dana-Farber Cancer Institute, Boston, MA, USA. [14]These authors contributed equally: Aaron M. Wenger, Paul Peluso. *e-mail: drank@pacb.com; mhunkapiller@pacb.com



Supplementary Figure 1

CCS protocol development.

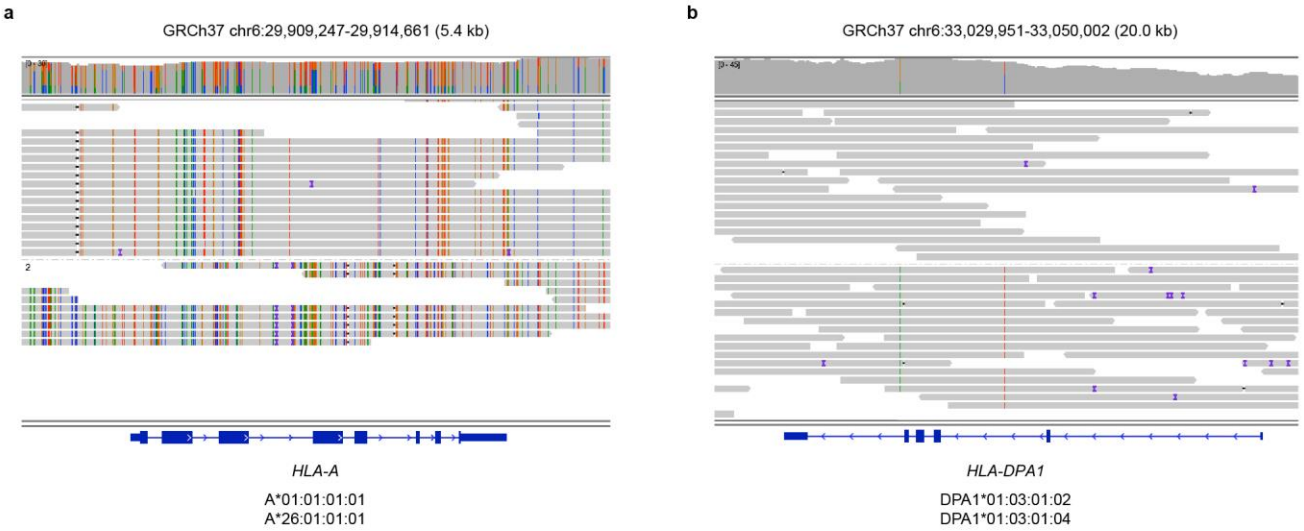
(a) Distribution of polymerase read lengths for a 10 kb *E.coli* amplicon library and 30 kb *E. coli* whole genome library sequenced for 10 hours with identical conditions. (b) Distribution of polymerase read lengths for an 8 kb fragment from a BsaAI-digested lambda library sequenced for 4 hours with (5 hour) and without (0 hour) pre-extension to reduce "early-terminating" reads and select surviving polymerase-template complexes. (c) Sample preparation and sequencing workflow. (d) BioAnalyzer trace for the SMRTbell library, sheared to target 15-20 kb fragments. "FU" is fluorescence units. (e) BioAnalyzer trace for ELF fractions of the SMRTbell library. (f) The fraction centered around 15 kb was used for sequencing.



Supplementary Figure 2

CCS read accuracy and coverage uniformity.

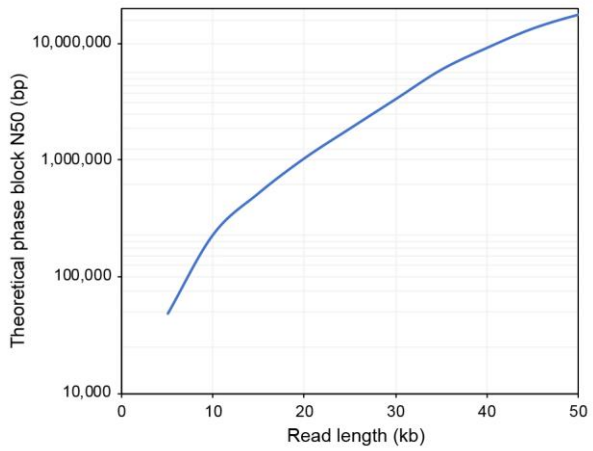
(a) Distribution of accuracy predicted by the CCS algorithm for reads with fewer than 3 passes and at least 3 passes, which we consider a minimum pass count for CCS. Approximately half of reads have 3 or more passes; among those nearly all achieve Q20 predicted accuracy. (b) Distributions of HG002 concordance, measured against the GIAB benchmark, at levels of predicted read accuracy (R^2 of median = 0.9980). Orange lines are medians; boxes extend from lower to upper quartiles; whiskers extend 1.5 interquartile distances; n=1,000 reads at each predicted accuracy. (c) Distribution of difference between concordance and predicted read accuracy shows that the prediction is well-calibrated to the empirical concordance. (d) Distribution of coverage in 500 bp windows at non-gap positions in GRCh37. (e) Coverage distributions at levels of [GC] content, measured in 500 bp windows. Orange lines are medians; boxes extend from lower to upper quartiles; whiskers extend 1.5 interquartile distances; n per distribution is listed above the plot.



Supplementary Figure 3

CCS read pileups at HLA genes.

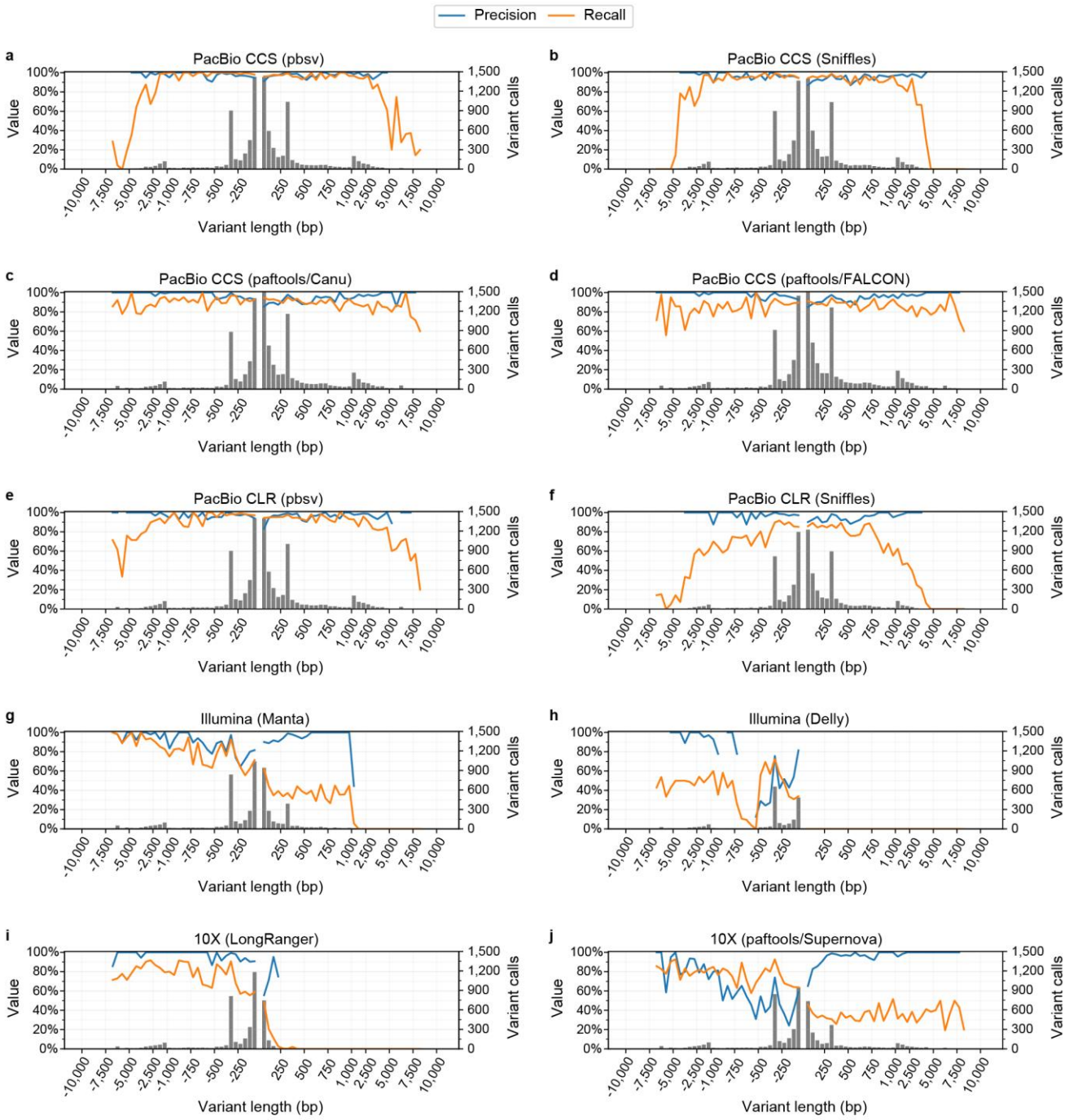
The 13.5 kb CCS reads provide phasing and full four-field resolution of HLA class I and II genes (Methods Mol. Biol. 1802, 135-153, 2018), including (a) *HLA-A* for which HG002 has alleles that differ in the first field, and (b) *HLA-DPA1* for which HG002 has alleles that differ only in the fourth field from two intronic single nucleotide polymorphisms across 20 kb.



Supplementary Figure 4

Theoretical phase block N50 in HG002 at different read lengths.

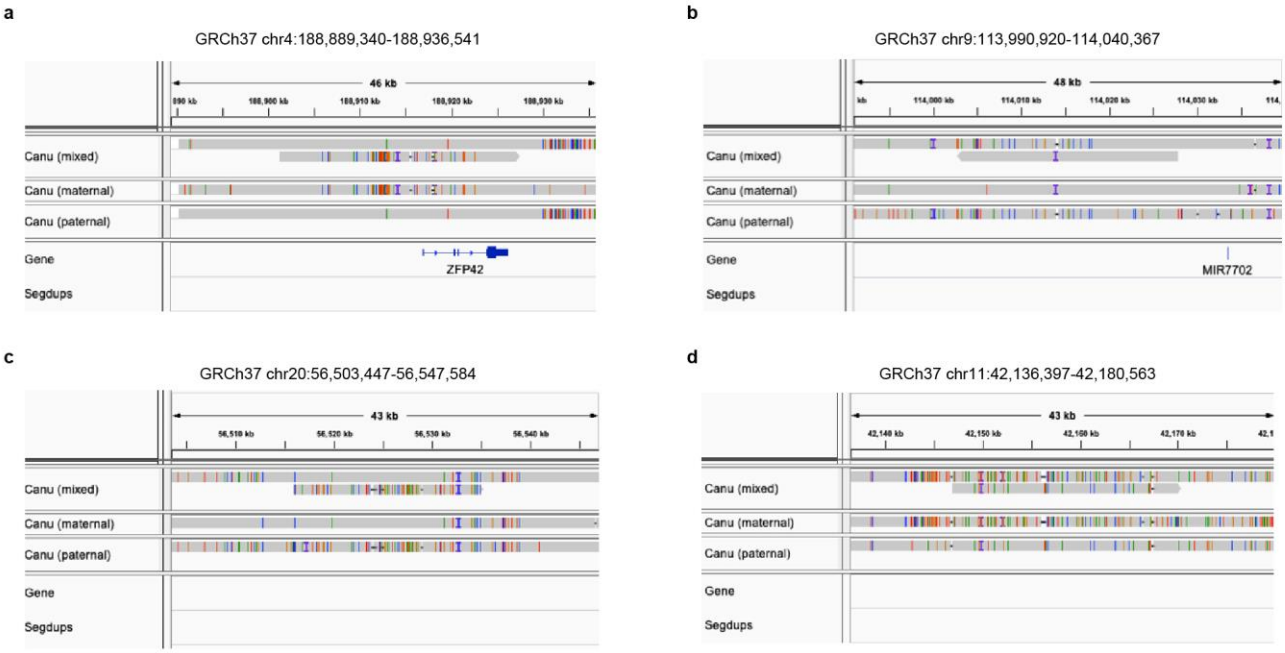
To model the phase blocks achievable with a given read length, cuts were introduced between heterozygous variants in the GIAB trio-phased HG002 variant callset that are separated by more than the read length, which effectively assumes that adjacent heterozygous variants separated by less than the read length can be phased.



Supplementary Figure 5

Structural variant calling performance.

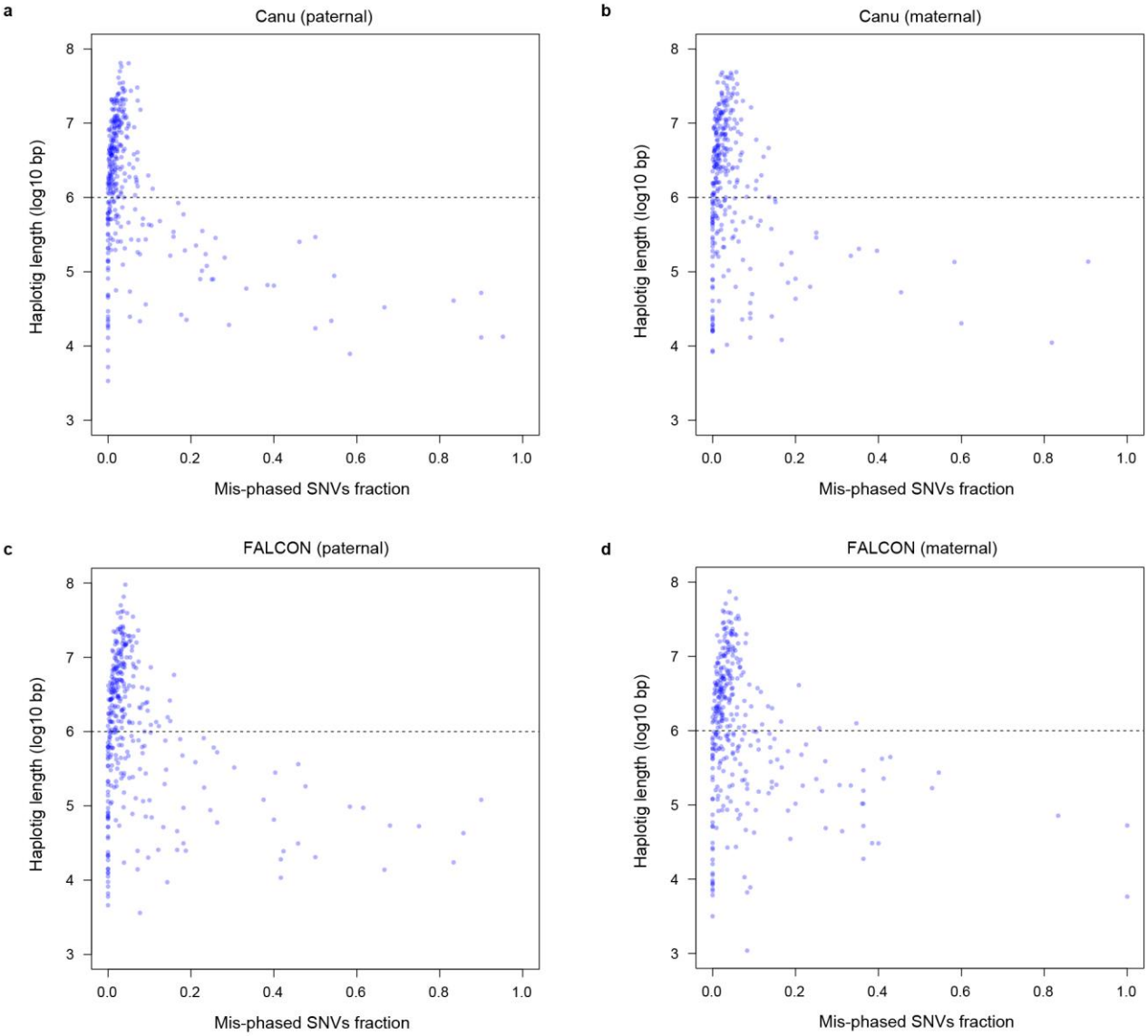
Precision, recall, and number of variant calls in the GIAB benchmark regions for the PacBio CCS mapping-based variant callers (a) pbsv and (b) Sniffles; the PacBio CCS assembly-based callers (c) paftools/Canu (polished) and (d) paftools/FALCON (unpolished); the PacBio CLR mapping-based callers (e) pbsv and (f) Sniffles; the Illumina short-read callers (g) Manta and (h) Delly; and the 10X Genomics callers (i) LongRanger and (j) paftools/Supernova. Negative length indicates a deletion; positive length indicates an insertion. The histogram bin size is 50 bp for variants shorter than 1 kb, and 500 bp for variants >1 kb. Precision and recall are measured with Truvari against the GIAB benchmark.



Supplementary Figure 6

Haplotype resolution in the Canu mixed assembly.

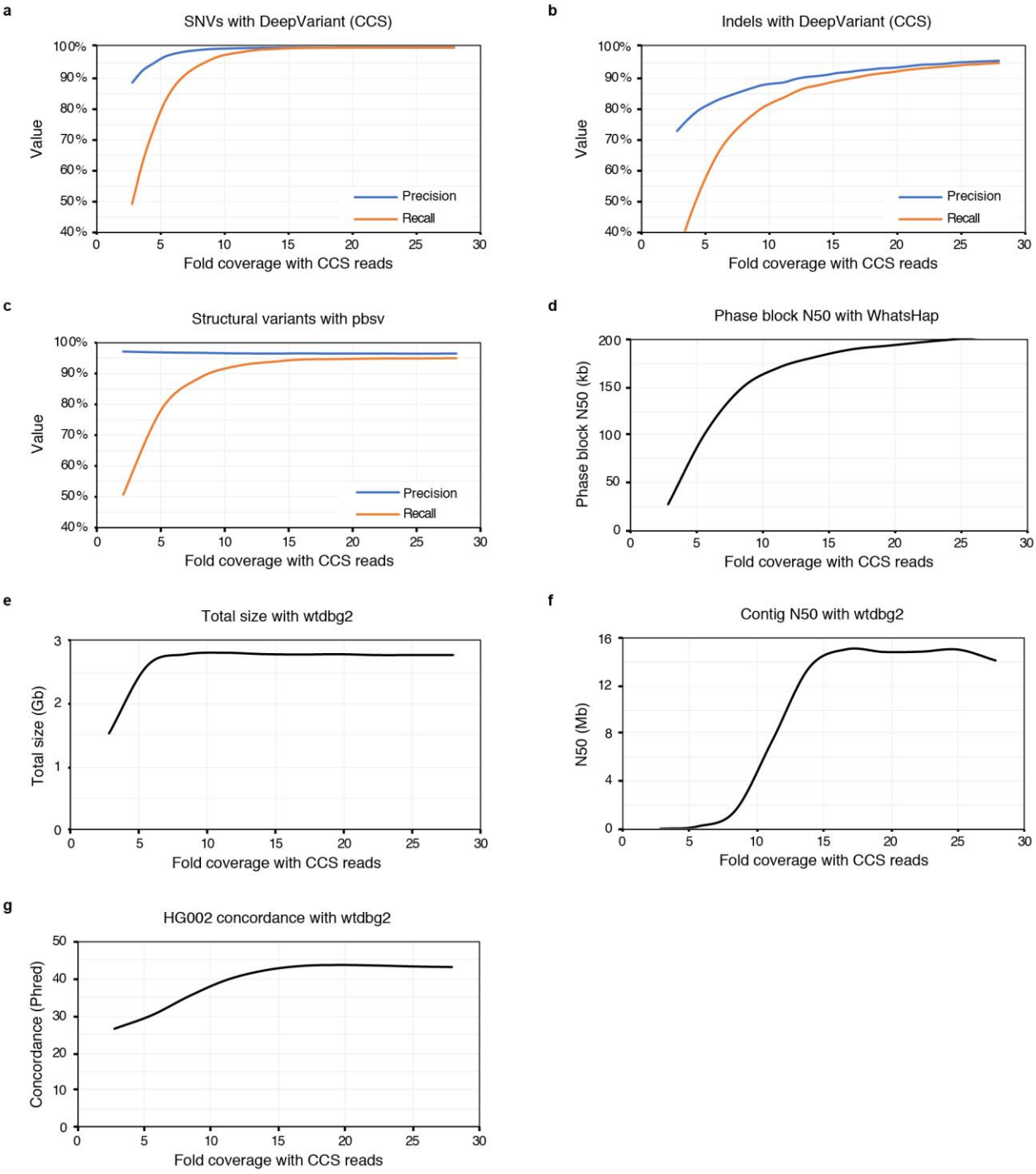
The Canu mixed assembly is larger than the haploid human genome size because it resolves some heterozygous loci into separate maternal and paternal haplotypes. (a) (b) Loci where the long primary contig matches the paternal haplotype and a smaller contig matches the maternal haplotype. (c) (d) Similar loci where the long primary contig matches the maternal haplotype and a smaller contig matches the paternal haplotype.



Supplementary Figure 7

Mis-phasing analysis of parental assemblies.

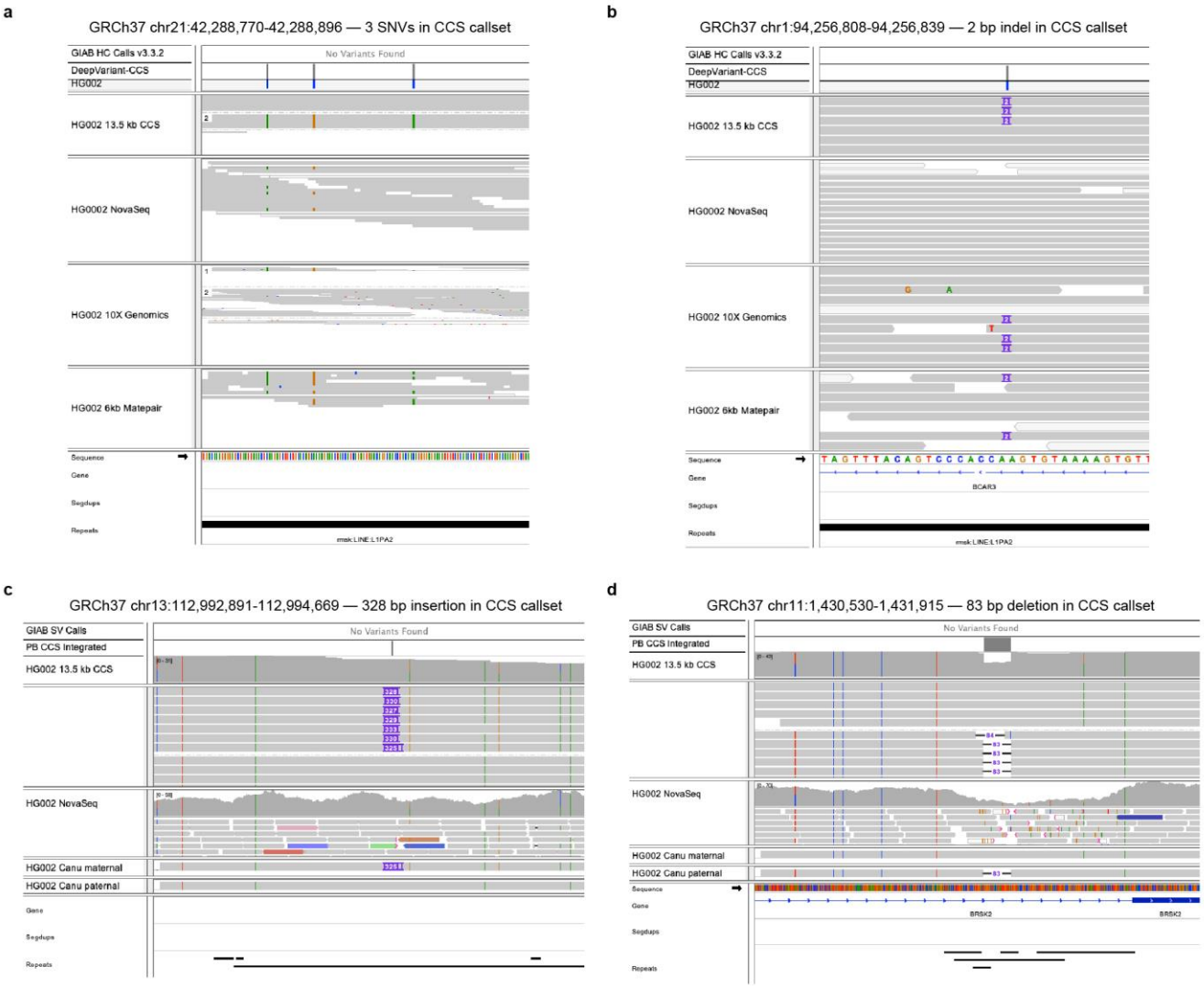
Parent-specific heterozygous SNVs were identified in the GIAB benchmark callset. The "Mis-phased SNVs fraction" is the fraction of parent-specific SNVs from the wrong parent (e.g. $[SNV_{pat}]/[SNV_{pat}+SNV_{mat}]$ in a maternal contig). No large contigs have a high mis-phased SNVs ratio, which suggests proper phasing of the (a) Canu paternal, (b) Canu maternal, (c) FALCON paternal, and (d) FALCON maternal assemblies.



Supplementary Figure 8

Coverage titration for variant calling, phasing, and assembly.

Precision and recall for (a) SNVs and (b) indels called with DeepVariant (CCS), subsampling in steps of 3%. (c) Precision and recall for structural variants called with pbsv, subsampling in steps of 10%. (d) Phase block N50 for phasing of the 28-fold DeepVariant (CCS) callset with WhatsHap, subsampling in steps of 10%. Phasing performance is similar with a callset produced at matched coverage (not shown). *De novo* assembly (e) completeness measured as total assembly size, (f) contiguity measured as contig N50, and (g) correctness measured as concordance to the HG002 GIAB benchmark for wtdbg2 assembly, subsampling reads in steps of 10%.



Supplementary Figure 9

Likely errors in the GIAB benchmark identified by CCS callsets.

Manual curation of discrepancies between the GIAB benchmark and CCS variant callsets identifies benchmark errors for all variant types that are correctable using the CCS variant callsets. Shown are four loci that the GIAB benchmark records as homozygous reference where CCS reads identify likely heterozygous variation: (a) Three SNVs supported by CCS reads and 6 kb matepair reads. (b) A 2 bp insertion supported by CCS reads, 10X Genomics reads, and 6 kb matepair reads. (c) A 328 bp insertion supported by CCS reads and assemblies. (d) An 83 bp deletion supported by CCS reads.

Supplementary Table 1

Selected insert size (kb)	Pre-extension time (hrs)	Collection time (hrs)	Average polymerase read length (kb)	Average CCS read length (kb)	Yield of CCS reads (Gb)	Average predicted CCS read accuracy (Phred)
10	4	20	64.9	9.6	2.3	25
* 15	12	24	100.3	13.5	2.3	27
18	12	30	115.0	17.4	2.1	25

Supplementary Table 1. CCS read yield and accuracy for different insert sizes. Run design and output for three size fractions of an HG002 library. The polymerase read is the full sequence of nucleotides detected for a circular SMRTbell template, which includes one or more passes of the template and intervening adapters. "*" Insert size selected for high-coverage sequencing.

Supplementary Table 2

	CCS reads			
Discordance	**%**	**Frequency (1/bp)**	**Concordance**	
Mismatch	3.4%	13,048	99.992%	(Q41)
Non-homopolymer indel	4.6%	9,669	99.990%	(Q39)
Non-homopolymer insertion	3.6%	12,359	99.991%	(Q41)
Non-homopolymer deletion	1.0%	44,425	99.998%	(Q46)
Homopolymer indel	92.0%	477	99.790%	(Q27)
Homopolymer insertion	42.3%	1,037	99.903%	(Q30)
Homopolymer deletion	49.7%	884	99.887%	(Q29)
Total	**100%**	**439**	**99.772%**	**(Q26)**

	NGS (2×151 bp NovaSeq) reads			
Discordance	**%**	**Frequency (1/bp)**	**Concordance**	
Mismatch	99.1%	761	99.869%	(Q29)
Non-homopolymer indel	0.3%	241,876	99.999%	(Q54)
Non-homopolymer insertion	0.1%	884,457	99.999%	(Q59)
Non-homopolymer deletion	0.2%	332,920	99.999%	(Q55)
Homopolymer indel	0.6%	124,943	99.999%	(Q51)
Homopolymer insertion	0.1%	523,665	99.999%	(Q57)
Homopolymer deletion	0.5%	164,096	99.999%	(Q52)
Total	**100%**	**748**	**99.866%**	**(Q29)**

	NGS (2×250 bp HiSeq 2500) reads			
Discordance	**%**	**Frequency (1/bp)**	**Concordance**	
Mismatch	99.0%	218	99.542%	(Q23)
Non-homopolymer indel	0.4%	53,619	99.998%	(Q47)
Non-homopolymer insertion	0.1%	216,562	99.999%	(Q53)
Non-homopolymer deletion	0.3%	71,264	99.999%	(Q49)
Homopolymer indel	0.6%	37,648	99.997%	(Q46)
Homopolymer insertion	0.2%	137,601	99.999%	(Q51)
Homopolymer deletion	0.4%	51,828	99.998%	(Q47)
Total	**100%**	**216**	**99.537%**	**(Q23)**

Supplementary Table 2. Discordances between read alignments and the HG002 GIAB benchmark for CCS and NGS reads. An indel is considered a homopolymer event if the inserted/deleted basepairs match either the preceding or following reference basepair. “Percentage” is over all discordances, by type. "Frequency" is the number of read basepairs between discordances. “Concordance” considers only discordances of the given type. The "Q" value is concordance in Phred scale.

Supplementary Table 3

		SNVs			Indels		
Platform	**Variant caller (training model)**	**Precision**	**Recall**	**F1 ^**	**Precision**	**Recall**	**F1**
Illumina (NovaSeq)	DeepVariant (Illumina model)	**99.925%**	**99.940%**	**99.933%**	**99.450%**	**99.233%**	**99.341%**
Illumina (NovaSeq)	GATK HaplotypeCaller (no filter)	99.824%	99.920%	99.872%	99.230%	98.898%	99.064%
PacBio (CCS)	DeepVariant (haplotype-sorted CCS model)	99.778%	99.937%	99.858%	96.860%	96.035%	96.446%
PacBio (CCS)	DeepVariant (CCS model)	99.807%	99.904%	99.855%	95.387%	94.501%	94.942%
PacBio (CCS)	DeepVariant (Illumina model)	99.533%	99.793%	99.663%	23.991%	81.692%	37.090%
PacBio (CCS)	GATK HaplotypeCaller (hard filter)	99.408%	99.531%	99.469%	77.137%	79.941%	78.514%

Supplementary Table 3. Performance of small variant calling with CCS reads on chromosome 20. DeepVariant models were not presented with chromosome 20 data before variant calling, so accuracy evaluations between GATK and DeepVariant are most comparable for chromosome 20. **Bold** indicates the highest value in each column. Underline indicates a value higher than the GATK HaplotypeCaller run on 30-fold Illumina NovaSeq reads. Coverage is 28-fold for PacBio CCS and 30-fold for Illumina NovaSeq. Callers are sorted ("^") based on F1 for SNVs.

Supplementary Table 4

GRCh37 chrom	Heterozygous variants	% phased	Phase blocks	Phase block N50 (bp)	Hamming error rate	Switch errors	Switch error rate
1	220,180	99.61%	1,585	225,534	1.53%	1,168	0.65%
2	212,809	99.62%	1,879	179,190	1.53%	373	0.21%
3	193,762	99.73%	1,312	259,761	1.63%	408	0.25%
4	199,451	99.70%	1,338	238,088	1.65%	547	0.33%
5	186,023	99.75%	1,115	277,697	1.06%	237	0.15%
6	177,458	99.71%	1,160	265,656	0.96%	303	0.20%
7	166,051	99.70%	1,048	246,748	2.23%	1,105	0.80%
8	153,941	99.71%	1,002	250,705	1.22%	322	0.25%
9	119,897	99.72%	778	207,951	1.30%	362	0.36%
10	141,433	99.72%	840	255,026	2.13%	344	0.29%
11	128,503	99.67%	948	203,073	1.24%	169	0.16%
12	135,470	99.72%	832	292,306	3.51%	229	0.20%
13	100,628	99.69%	638	244,289	2.19%	123	0.14%
14	93,645	99.68%	548	292,617	2.70%	520	0.66%
15	81,981	99.61%	609	188,168	0.71%	411	0.61%
16	87,697	99.71%	596	198,059	4.69%	455	0.63%
17	78,865	99.65%	569	209,363	3.06%	380	0.61%
18	74,575	99.68%	568	215,577	2.44%	95	0.15%
19	70,975	99.78%	345	283,264	2.17%	149	0.26%
20	61,413	99.65%	425	207,556	3.53%	165	0.33%
21	44,142	99.49%	257	178,353	4.29%	545	1.60%
22	38,604	99.71%	249	221,143	1.29%	87	0.28%
Autosomes	**2,779,801**	**99.64%**	**19,215**	**206,063**	**1.91%**	**8,497**	**0.37%**

Supplementary Table 4. WhatsHap phasing performance on DeepVariant (CCS) callset. WhatsHap provides highly complete phasing (99.64%) of heterozygous variants in the DeepVariant (CCS) callset that is concordant with the GIAB Trio/10X Genomics phasing benchmark set. Statistics are reported by WhatsHap with Hamming and switch error rates evaluated against the benchmark.

Supplementary Table 5

		All variants			Deletions			Insertions		
Platform	**Caller**	*Prec.*	*Recall*	*F1 ^*	*Prec.*	*Recall*	*F1*	*Prec.*	*Recall*	*F1*
PacBio (CCS)	Integrated	96.13%	**95.99%**	**96.06%**	**97.66%**	**96.88%**	**97.27%**	94.97%	**95.30%**	95.13%
PacBio (CCS)	pbsv	**96.26%**	94.93%	95.59%	96.71%	94.98%	95.84%	**95.95%**	94.89%	**95.42%**
PacBio (CLR)	pbsv	94.64%	94.48%	94.56%	96.70%	95.57%	96.13%	93.11%	93.64%	93.37%
PacBio (CCS)	Sniffles	94.28%	91.76%	93.01%	96.56%	92.19%	94.32%	92.59%	91.44%	92.01%
PacBio (CCS)	paftools/Canu †‡	93.16%	92.32%	92.74%	95.84%	92.76%	94.28%	91.48%	91.99%	91.73%
PacBio (CCS)	paftools/FALCON †	93.25%	89.14%	91.15%	95.99%	89.00%	92.36%	91.64%	89.25%	90.43%
PacBio (CLR)	Sniffles	95.66%	79.33%	86.73%	98.19%	80.07%	88.21%	93.80%	78.76%	85.62%
Illumina	Manta	85.34%	55.88%	67.53%	85.95%	76.90%	81.17%	92.12%	39.65%	55.44%
10X	paftools/Supernova	64.52%	52.74%	58.04%	55.37%	73.71%	63.24%	82.74%	36.57%	50.72%
10X	LongRanger	83.79%	39.83%	53.99%	94.66%	70.18%	80.60%	59.39%	16.41%	25.71%
Illumina	Delly	65.92%	19.90%	30.58%	65.92%	45.70%	53.98%	0.00%	0.00%	0.00%

Supplementary Table 5. Structural variant calling performance. Precision and recall are measured with Truvari against the GIAB benchmark. **Bold** indicates the highest value in each column; callers are sorted ("^") based on F1 for all variants. † union of maternal and paternal assemblies; ‡ polished with Arrow.

Supplementary Table 6

		CPU core hours		
Haplotype	**Assembler**	**Trio binning**	**Assembly**	**Arrow polishing**
Mixed	Canu	n/a	2,136	-
Mixed	FALCON	n/a	2,650	-
Mixed	wtdbg2	n/a	380	-
Maternal	Canu	350	751	71,226*
Maternal	FALCON	350	1,683	26,137
Maternal	wtdbg2	350	182	-
Paternal	Canu	350	841	70,069*
Paternal	FALCON	350	1,568	26,183
Paternal	wtdbg2	350	187	-

Supplementary Table 6. CPU core hours for *de novo* assembly and polishing. The CPU core hours required for trio binning, assembly, and polishing were recorded using the Unix `time` command. Assembly time includes read correction built into the assembler but excludes the total upfront CCS read generation (118,365 CPU core hours). The assemblers were run by different groups on different hardware, and thus times are not directly comparable. "*" Arrow polishing was run for one round on FALCON and two rounds on Canu; "n/a" = not applicable; "-" = not done

Supplementary Table 7

	% reads assigned to haplotype		
k-mer (bp)	Maternal	Paternal	Unassigned
21	35.3%	33.6%	31.1%
51	40.4%	38.1%	21.5%
91	40.5%	38.7%	20.8%

Supplementary Table 7. CCS read classification by trio binning. The percentage of CCS reads assigned to the maternal and paternal haplotype by k-mer size used in trio binning. CCS reads with an insufficient number of distinguishing k-mers are assigned to the "unassigned" haplotype, which includes reads from homozygous regions.

Supplementary Table 8

NCBI accession	Platform	Sample	Assembler + polish	Concordance ^	
GCA_004796285.1	PacBio (CCS)	HG002 (pat.)	Canu + Arrow	99.9983%	(Q47.7)
GCA_004796485.1	PacBio (CCS)	HG002 (mat.)	Canu + Arrow	99.9981%	(Q47.2)
-	PacBio (CCS)	HG002	wtdbg2	99.9965%	(Q44.6)
GCA_001542345.1	PacBio (CLR)	HG002	PBcR + Quiver	99.9900%	(Q40.0)
GCA_002077035.3	PacBio (CLR)	HG001	FALCON + Quiver	99.9893%	(Q39.7)
GCA_900232925.2	ONT + Illumina	HG001	Canu + Nanopolish×2, Pilon×2, Racon×2	99.8694%	(Q28.8)
-	ONT	HG001	Canu + Nanopolish×2	99.6566%	(Q24.6)

Supplementary Table 8. Reference concordance of assemblies from different platforms. Concordance is measured against the GIAB HG002 benchmark. The three CCS read assemblies have higher concordance than accessioned assemblies provided with PacBio continuous long reads (CLR) or Oxford Nanopore read (ONT). ONT HG001 assembly is from https://obj.umiacs.umd.edu/marbl_publications/triobinning/albacore_canu_nanopolish2.fasta or https://bit.ly/2HBOjyq. "pat.": paternal, "mat.": maternal.

Supplementary Table 9

		Segdups	
Haplotype	**Assembler**	**Spanned (Mb)**	**Not spanned (Mb)**
Mixed	Canu	63.6	111.8
Mixed	FALCON	46.1	129.3
Mixed	wtdbg2	26.4	149.0
Maternal	Canu	60.2	115.2
Maternal	FALCON	43.2	132.2
Maternal	wtdbg2	28.9	146.5
Paternal	Canu	60.0	115.4
Paternal	FALCON	41.7	133.7
Paternal	wtdbg2	27.2	148.2

Supplementary Table 9. Assembly of segmental duplications. A segmental duplication in GRCh38 is considered spanned by an assembly if a contig alignment extends fully through the segmental duplication and into at least 50 kb of unique sequence on each flank as reported by segDupPlots (https://github.com/mvollger/segDupPlots) (Nat. Methods 16, 88-94, 2019).

Supplementary Table 10

Discrepancy	Variant type	Repeat family (if ≥1 kb)	Homopolymer length (bp) (if ≥6 bp)	Correct call	Chr	Position	Variant
AM	INDEL		19	GIAB	2	9,591,845	CT/C
AM	INDEL			GIAB	2	232,051,483	GCA/GCATCATGGAGAATGGGACATCTC
AM	INDEL			GIAB	3	37,083,407	G/GA
AM	INDEL			CCS	4	11,468,804	CACACATATAT/C
AM	INDEL	L1PA2		CCS	5	42,740,225	CT/C
AM	INDEL		Nearby 17	GIAB	6	41,984,320	ACTAT/A
AM	INDEL		16	GIAB	8	73,675,279	TAAAA/T
AM	INDEL		13	GIAB	13	76,646,445	G/GA
AM	INDEL		16	GIAB	15	44,350,983	C/CA
AM	INDEL		13	GIAB	19	1,586,670	A/ATTT
AM	SNP	HERVH-int		CCS	2	5,143,996	G/A
AM	SNP			GIAB	2	230,174,543	A/G
AM	SNP	L1PA2		CCS	4	165,276,021	T/C
AM	SNP		8	GIAB	5	16,287,108	A/C
AM	SNP		8	GIAB	11	41,384,344	C/T
AM	SNP		20	GIAB	12	51,793,781	A/C
AM	SNP		9	GIAB	13	34,840,815	G/T
AM	SNP	L1PA3		CCS	13	48,291,499	A/C
AM	SNP		12	GIAB	13	71,512,745	A/T
AM	SNP			GIAB	21	25,668,597	G/A
FN	INDEL			GIAB	1	162,491,859	A/ATGTCTAG
FN	INDEL		12	GIAB	2	152,262,374	G/GTT
FN	INDEL		14	GIAB	2	236,062,930	G/GTT
FN	INDEL	L1PA2	9	GIAB	3	107,982,543	AT/A
FN	INDEL		18	GIAB	4	149,672,221	A/ATT
FN	INDEL			CCS	8	5,930,728	TACAC/T
FN	INDEL		6	GIAB	10	29,087,199	T/TCC
FN	INDEL			GIAB	15	26,120,981	C/CTTACACTGGGCTTTTTGTAAGGA
FN	INDEL			CCS	15	41,943,823	T/TCCTCTTCTCTCCTCTCC
FN	INDEL		15	GIAB	17	5,198,683	C/CA
FN	SNP		16	GIAB	5	55,201,041	A/G
FN	SNP			GIAB	6	8,353,625	C/T
FN	SNP			CCS	6	9,737,425	T/C
FN	SNP			GIAB	6	57,283,620	T/C
FN	SNP		13	GIAB	7	135,981,582	T/A
FN	SNP			CCS	7	157,385,671	A/G
FN	SNP			GIAB	9	117,917,190	A/C
FN	SNP		13	GIAB	9	129,471,234	T/A
FN	SNP		5	CCS	17	32,064,214	A/G
FN	SNP		25	GIAB	17	68,021,050	T/A
FP	INDEL	L1PA2		CCS	1	94,256,825	A/AAC
FP	INDEL	L1HS		CCS	2	153,864,971	AT/A
FP	INDEL	L1M2	13	GIAB	3	97,014,398	AT/A
FP	INDEL	L1HS	7	CCS	4	112,819,087	GA/G
FP	INDEL	L1PA2		CCS	4	165,026,074	A/AG
FP	INDEL		10	GIAB	6	64,897,720	A/AT
FP	INDEL		15	GIAB	7	38,338,238	C/CA
FP	INDEL			GIAB	8	132,575,025	C/CAAAAAAAAA
FP	INDEL	L1P1		CCS	11	23,338,682	C/CT
FP	INDEL		20	GIAB	11	61,993,476	CA/C
FP	SNP	L1HS		CCS	1	35,034,071	T/C
FP	SNP	L1HS		CCS	3	79,181,734	C/T
FP	SNP		Nearby 8	GIAB	4	55,520,593	G/A
FP	SNP	L1HS	7	CCS	4	94,532,444	T/G
FP	SNP	ALR/Alpha		CCS	8	46,873,565	C/T
FP	SNP		11	GIAB	9	6,900,971	C/T
FP	SNP	L1PA2		CCS	9	22,350,168	A/C
FP	SNP		13	GIAB	20	1,347,896	A/G
FP	SNP		12	GIAB	20	4,159,335	C/T
FP	SNP	L1PA2		CCS	21	42,288,851	C/A

Supplementary Table 10. Manual curation of small variant discrepancies between CCS callset and GIAB benchmark. For the "Discrepancy" column, "AM" means genotype difference, "FN" means false negative (in benchmark but not callset), and "FP" means false positive (in callset but not benchmark). "Repeat family" column is from the RepeatMasker track from the UCSC Genome Browser. "Correct call" column is "GIAB" when the benchmark was deemed correct by expert curators, and "CCS" when the CCS callset was deemed correct. Rows where the correct call is from the CCS callset are colored blue.

Supplementary Table 11

Discrepancy	Variant type	Length (bp)	Simple repeat length (bp) (if ≥100 bp)	Simple repeat period (bp)	Correct call	Chr	Position
FN	DEL	-32,196			GIAB	1	152,555,543
FN	DEL	-2,269			GIAB	2	159,958,799
FN	DEL	-49,058	172	71	-	4	34,779,881
FN	DEL	-127	466	127	GIAB	4	123,733,539
FN	DEL	-357	1,921	20	GIAB	13	30,131,788
FN	DEL	-108	589	54	GIAB	13	114,841,327
FN	DEL	-52			GIAB	16	85,800,468
FN	DEL	-565	1,403	561	-	19	4,884,873
FN	DEL	-55			GIAB	19	57,683,315
FN	DEL	-120	917	40	GIAB	20	62,510,913
FN	INS	62			GIAB	2	228,113,946
FN	INS	104	163	27	GIAB	3	66,992,107
FN	INS	52			GIAB	3	172,678,665
FN	INS	125	230	23	GIAB	5	105,107,607
FN	INS	727	651	65	-	6	40,459,830
FN	INS	172	815	4	-	9	135,394,538
FN	INS	6,179			GIAB	12	71,053,961
FN	INS	51	125	3	GIAB	13	29,161,602
FN	INS	3,268			GIAB	14	67,862,850
FN	INS	58	472	33	CCS	19	14,488,489
FP	DEL	-1,432			-	1	108,735,819
FP	DEL	-50			CCS	2	65939,406
FP	DEL	-80	1,274	16	CCS	6	167,162,349
FP	DEL	-80	332	40	CCS	7	129,149
FP	DEL	-65	632	168	-	10	134,253,963
FP	DEL	-83	333	83	CCS	11	1,431,223
FP	DEL	-74	1,674	74	CCS	12	6,038,958
FP	DEL	-128			-	13	107,435,844
FP	DEL	-63	1,227	22	GIAB	17	230,498
FP	DEL	-300	1,923	60	GIAB	18	77,569,248
FP	INS	103			CCS	4	141,283,453
FP	INS	52	588	26	CCS	4	190,329,327
FP	INS	202	893	18	-	8	146,172,196
FP	INS	176	1,184	24	-	10	132,840,681
FP	INS	783	1,184	24	-	10	132,841,387
FP	INS	328			CCS	13	112,993,782
FP	INS	60	312	4	CCS	16	85,867,748
FP	INS	54	527	18	-	17	10,662,861
FP	INS	84			CCS	18	53,029,667
FP	INS	267	641	37	CCS	X	67,035,046

Supplementary Table 11. Manual curation of structural variant discrepancies between CCS callset and GIAB benchmark. For the "Discrepancy" column, "FN" means false negative (in benchmark but not callset), and "FP" means false positive (in callset but not benchmark). "Simple repeat length" and "Simple repeat period" are from the simpleRepeat track from the UCSC Genome Browser. "Correct call" column is "GIAB" when the benchmark was deemed correct by expert curators, "CCS" when the CCS callset was deemed correct, and "-" when it is unclear which callset is correct (typically due to complex tandem repeats that permit multiple representations of the same variant). Rows where the correct call is from the CCS callset are colored blue.

Supplementary Table 12

Step	Instrument	Notes	Time
Library preparation			
Input DNA	-	3-6 µg	-
Shear DNA	Megaruptor	15-20 kb	2 hrs
Ligate hairpin adapters	Thermocycler	500:1 adapter:template	Overnight
Size select library	SageELF	6 fractions 8-18 kb	6 hrs
Adjust concentration	Ampure beads	-	2 hrs
Anneal primer	Thermocycler	20:1 primer:template	0.5 hrs
Bind polymerase	Thermocycler	10:1 polymerase:template	4 hrs
Sequencing			
Sequence to 15-fold coverage	Sequel System	20 SMRT Cells 1M × 24 hrs + 5 × 12 hrs pre-extension†	23 days
	-or-		
Sequence to 15-fold coverage	Sequel II System	2-3 SMRT Cells 8M* × 30 hrs + 12 hrs pre-extension†	3-5 days
Consensus read generation			
Generate CCS reads	Compute cluster	1,000 CPU cores in cluster	5 days

Supplementary Table 12. Basic workflow, resources, and time required to generate 15-fold CCS read coverage of a human genome. "*" The number of SMRT Cells 8M required for 15-fold coverage is based on SRX5527202 and SRX5633451. The Sequel II System was not available at the time of this study. "†" Pre-extension time delays the start of the only the first of 4 SMRT Cells per run.

Supplementary Note – Detailed Author Contributions

CCS Library Preparation and Sequencing: D.R.R., P.P., Y.Q.

Quality Evaluation of CCS Reads: A.M.W., G.M., R.J.H.

Increased Mappability of CCS Reads: R.J.H.

Small Variant Detection in CCS Reads: A.C., A.K., C-S.C., F.J.S., J.M.Z., M.A.D., N.D.O., P.C., W.J.R.

Phasing Small Variants: J.E., T.M., W.J.R.

Improving Small Variant Detection with Haplotype Phasing: A.C., A.K., M.A.D., P.C., W.J.R.

Structural Variant Detection in CCS Reads: A.M.W., A.T., F.J.S., H.L., M.C.S., M.A., M.M.

De Novo Assembly of CCS Reads: A.F., A.M.P., A.M.W., D.R.R., J.R., G.T.C., S.K.

Coverage Requirements for Variant Calling and De Novo Assembly: A.C., A.K., A.M.W., G.T.C., J.E., T.M., W.J.R.

Revising and Expanding Genome in a Bottle Benchmarks: A.M.W., J.M.Z., N.D.O.