Genome analysis

**Accurate haplotype-resolved assembly reveals the origin of structural variants for human trios**

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**Abstract**

**Motivation:** Achieving a near complete understanding of how the genome of an individual affects the phenotypes of that individual requires deciphering the order of variations along homologous chromosomes in species with diploid genomes. However, true diploid assembly of long-range haplotypes remains challenging.

**Results:** To address this, we have developed Haplotype-resolved Assembly for Synthetic long reads using a Trio-binning strategy, or HAST, which uses parental information to classify reads into maternal or paternal. Once sorted, these reads are used to independently de novo assemble the parent-specific haplotypes. We applied HAST to cobar-coded second-generation sequencing data from an Asian individual, resulting in a haplotype assembly covering 94.7% of the reference genome with a scaffold N50 longer than 11 Mb. The high haplotyping precision (≈99.7%) and recall (≈95.9%) represents a substantial improvement over the commonly used tool for assembling cobar-coded reads (Supernova), and is comparable to a trio-binning-based third generation long-read-based assembly method (TrioCanu) but with a significantly higher single-base accuracy [up to 99.99997% (Q65)]. This makes HAST a superior tool for accurate haplotyping and future haplotype-based studies.

**Availability and implementation:** The code of the analysis is available at https://github.com/BGI-Qingdao/HAST

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**Supplementary information:** Supplementary data are available at Bioinformatics online.

1 **Introduction**

Determining the combination of allelic variants along each homologous chromosome, haplotyping, provides more information than nonhaplotype-resolved genetic variations for diploid or polyploid species. Population-variation-based haplotype inference has been used for many years (Hill, 1974; O’Connell et al., 2016), but the direct determination of an individual’s haplotype has been challenging. More recently, whole genome sequence data have been applied to reconstructing haplotypes. By aligning the sequencing data to the reference genome, reads harboring two or more heterozygous variants can be used to capture allelic variations (alignment-based haplotyping) (Snyder et al., 2015). Despite the high efficiency of this approach, especially when using long reads (Edge et al., 2017) or cobar-coded (Peters et al., 2014) long fragment reads (Peters et al., 2012) (LFR), alignment-based haplotyping relies on the reference genome and variation calling. This makes it difficult to phase haplotypes that include structural variations (SVs) or sequences not found in the reference genome. One solution to this problem would be to de novo assemble the complete sequence of each haplotype (Myers, 2005). For example, during graph-based genome assembly, handling the variation-induced ‘bubble’ structures can facilitate the
construction of haplotypes (Chin et al., 2016; Weisenfeld et al., 2017). Although assembly-based haplotyping performs better in regions with SV (Zhang et al., 2020), the overall efficiency and precision underperforms that of alignment-based haplotyping.

To improve upon this, trio-binning-based strategies have been developed (Eberle et al., 2017; Marchini et al., 2006). These strategies use heterozygous variants not shared between the two parents to separate the child’s sequencing data into maternal and paternal groups. With the read data classified, genome assembly is more efficient and results in improved haplotype contiguity and precision (Koren et al., 2018; Low et al., 2020). However, it is difficult for haplotype-specific k-mers to capture heterozygous alleles in all three individuals in the trio without local phasing information (Garg et al., 2018).

Recently, cobarcoding-based strategies that retain long DNA information along with cost-effective, highly accurate short reads, such as MGE’s single-tube long fragment read (Wang et al., 2019) (stLFR) and 10x Genomics’ linked-read (Zheng et al., 2016) technologies, have been successfully used in alignment-based haplotyping (Wang et al., 2019), SV detection (Bishara et al., 2015; Zhou et al., 2019) and de novo genome assembly (Kuleshov et al., 2016).

In this study, we describe Haplotyper-resolved Assembly for Synthetic long reads using a Trio-binning strategy (HAST, https://github.com/BGI-Qingdao/HAST), which is the first trio-binning-based haplootyping tool for cobarcoded reads. The principle of this pipeline is to reconstruct two haplotype-resolved assemblies with high precision and continuity based on the combination of global haplotyping information from trios and local phasing information from barcodes. HAST first identifies haplotype-specific k-mers using sequencing data from the parents, then employs these markers to partition the offspring cobarcoded sequencing data into haplotypes, and finally assembles each haplotype independently. Using HAST to assemble haplotypes of an Asian individual, we generated results comparable to trio-binning-assemble-based haplotyping using long third generation reads (TrioCanau) (Koren et al., 2018).

2 Materials and methods

2.1 Sample preparation and sequencing of the HJ trios

We sequenced a Han Chinese volunteer (Research ethics ID: XHEC-C-2019-086, HJ) using the MGIEasy stLFR Library Prep Kit on the MGISeq-2000 platform (DNBSEQ-G400, RRID: SCR_017980) and generated a total of 632 Gb of read data. We applied SOAPfilter (Luo et al., 2012) (version 2.2) to remove possible adapter contamination, low quality reads, duplicated reads and PE reads with a short insert size (<300 bp). This reduced the amount of data to 355 Gb. Both of HJ’s parents were sequenced to ~30× coverage using massively parallel sequencing (MPS) short reads (Rogers and Venter, 2005). From this data, haplotype k-mers were extracted. In addition, to construct haplotypes for comparison with HAST results, PacBio SMRT libraries were sequenced on a Sequel instrument (PacBio Sequel System, RRID: SCR_017989). In total, we generated 138 Gb PacBio high-fidelity circular consensus sequence (CCS) long reads.

2.2 Generation of parental unique markers

The classification of parent-specific k-mers is similar to the method described in TrioCanau (Koren et al., 2018). Due to its high speed and low requirement of memory, Jellyfish (Marcais and Kingsford, 2011) (version 0.6.1; Jellyfish, RRID: SCR_005491) was chosen to generate, count and output distinct k-mers in the parental genomes. The haplotype-specific k-mers were identified by mixing 1 copy of parental k-mers with 2 copies of maternal and then counting the total frequencies. The k-mers that occurred exactly once in the mixture were identified to belong to the paternal group, while those that occurred twice were maternal-specific.

The majority of unshared k-mers originated from the sequencing errors and as such were present in low copy numbers. In addition, there were a number of k-mers with excessively high coverage due to repetitive regions of the genome. To reduce the computational load from these useful k-mers, we limited the parental k-mer library size based on the coverage distribution. For the plot of k-mer frequency f against coverage c, the low coverage range C_{low} was determined based on the first lowest point of the profile C_1 and the highest point of the main peak C_2 (Supplementary Fig. S1). The profile can be fitted by a mixture model of negative binomial model terms with a long tail in the high-coverage region (Vurture et al., 2017). We simplified the variances in the tail region and defined the high-coverage range C_{high} as twice of C_{low}. It can also be calculated by C_1 and C_2:

$$ C_{high} = 3 \times C_2 - 2 \times C_1 $$

Only those k-mers within the thresholds were exported to identify long fragments in the next step. Note that the numbers of unshared k-mers for two haplotypes are not necessarily equal.

The performance of the classification also substantially depends on the k value. Theoretically, a read has a greater possibility of matching heterozygous markers by using a smaller k-mer size. However, too small of a k value can cause random k-mer collisions, especially for large genomes. According to the formula of k-mer collision, for a rate r given a random distribution of k-mers in the genome G (Fotanov et al., 2004),

$$ 1/r = 1 + (4^{k} / G) $$

a k of 19 provides a collision rate of 1% for the human genome, while k = 21 and 31 reduce r below 0.1% and 1e-8, respectively.

2.3 Partition of stLFR long fragments

The concept of read binning prior to assembly has been used in long reads (Koren et al., 2018) and metagenomics (Wu and Ye, 2011). Ideally, cobarcoded reads can be categorized if they possess individual k-mers from one haplotype and none from others. Assuming that heterozygous sites and sequencing errors are randomly distributed in the genome and there are no overlaps between long DNA fragments or between reads sharing the same barcode, the expected number of unshared k-mers, p in one long DNA fragment can be derived as

$$ p = 2 \cdot b \cdot k \cdot N \cdot (l - k + 1)(1 - e)^k $$

where b is the genome heterozygosity, N is the number of read pairs in one long DNA fragment, l is the read length and e is the sequencing error rate. The read clustering based on barcodes overcomes the limit of short-read length, especially for genomes with low heterozygosity. The high base-calling accuracy also improves the sensitivity and precision of k-mer mapping. Given b = 0.1%, N = 20, l = 100, k = 21, e = 0.1% for a typical stLFR library of a human sample, the expected number of unshared k-mers is larger than 6 δ for each barcode.

In the case of trios, long DNA fragments are assigned to the paternal group if they only own paternal-specific k-mers and vice versa. If both types of parental k-mers match different portions of the same DNA fragment, then the proper partitioning will be determined by which parental genome has a higher probability to be mapped by the fragment. The probability is first normalized to the parent-specific k-mer libraries with different sizes, and then multiplied by a correction factor of sex chromosome size variance. The factor attempts to neutralize the inherent discrepancy of parents due to the constitution of sex chromosomes (Bachtrog and Charlesworth, 2001). The remaining DNA fragments with equal k-mer counts are discarded as errors, while those with no k-mers are regarded as homozygous. Note that each DNA fragment is identified by its barcode, which corresponds to all fragments in the same cobarcoding compartment (Wang et al., 2019). To accelerate millions of k-mer queries in the partitioning, we binarized the k-mer characters, hashed the k-mer database and parallelized the classifying procedure. Note that HAST (version 1.0.6; HAST, RRID: SCR_018247) also accepts 10× Genomics reads, PacBio and ONT long reads, and shows significant improvements in speed and memory efficiency.
relative to the trio-binning scripts in the published TrioCanu (Koren et al., 2018) (Supplementary Table S19). We notice that the latest version of Canu (Koren et al., 2017) has also optimized its thread and memory consumption.

2.4 HAST assembly pipeline
HAST utilizes parental variation information to facilitate a completely haplotype-resolved diploid assembly in the following three steps (Fig. 1): (a) generation of parental unshared k-mers from MPS reads and sequencing the child with cobarcode reads, (b) determination of the parent of origin of each of the child’s long fragments (the set of cobarcode reads sharing the same barcode and representing a single long DNA fragment) based on the parental unshared k-mer sets, and finally (c) haplotype-resolved assemblies of both parental-inherited chromosomes.

The global haplotyping information from the trio straightforwardly partitioned reads with haplotype-specific k-mers. Meanwhile, reads without global markers could also be grouped if they share the same barcode with adjacent alleles that are trio-resolved due to the long-range information. All DNA fragments in paternal or maternal groups along with homozygous fragments were transformed into $10^5$ Genomics data format, and passed to Supernova (Weisenfeld et al., 2017) (version 2.1.1; Supernova assembler, RRID: SCR_016756) to assemble. The assembly graph was simplified by barcode information to resolve repeat-induced junctions.

Note that the direct Supernova output for both groups still retains phasing errors. For errors due to inaccurate base calling, we recovered the bubble structures in the assembly graph, and used the haplotype-specific k-mers again to determine which arm truly represented the allelic variants. For phase variants which are heterozygous in all three individuals in the trio, their corresponding reads were identified as homozygous and used for both assemblies if they have no adjacent marked alleles sharing the same barcode. We also distinguished two arms of each bubble to determine if they belong to heterozygous variants in all three individuals, and kept both allelic sequences in the final output.

2.5 Haplotyped-resolved assembly for CCS data
We used the TrioCanu module implemented in Canu (version 2.0; Canu, RRID: SCR_015880) to classify the PacBio CCS long reads with an average read length of 10.2 kb. The identified ratio was 34.3% and 34.8% for paternal and maternal, respectively (Supplementary Table S5). To compare with HAST, the haplotypes were individually assembled by Canu using corresponding haplotype-specific long reads and unassigned long reads without any markers.

2.6 Validation of haplotyping effects
The hg19 assembly (Church et al., 2011) was used as the reference for Homo sapiens. The QUAST (Gurevich et al., 2013) (version 5.0.2; QUAST, RRID: SCR_001228) was used with default parameters to report the assembly statistics including total length, scaffold N50, contig N50, as well as scaffold NGA50, contig NGA50, genome fraction, misassemblies and local misassemblies based on the mapping relation with the reference. Note that QUAST splits scaffolds as contigs when there is a continuous stretch of N’s of length ≥10. In addition, mercury (Rhiie et al., 2020) (version 1.0) was applied for the evaluation of haplotyping precision and recall according to the reliable specific k-mers from the child’s and parental reads. In total, we generated 18.6 Mb paternal hap-mers and 16.3 Mb maternal hap-mers to evaluate. To quantify the effect of HAST on the downstream bioinformatics analysis, we ran BUSCO (Simao et al., 2015) (version 3.0.2; BUSCO, RRID: SCR_015008) analysis for all the human assemblies against the vertebrata_odb9.

3 Implementation
3.1 Assignment of long fragments into haplotype groups
The effectiveness of grouping the raw sequencing data into their specific haplotypes is important for an assembly-based haplotyping method. The MPS datasets of ~30× coverage per parent were used to generate unique markers (unshared k-mers) to partition the child’s cobarcode reads to separate haplotypes. The accurate selection of markers depends on the sequencing error rate and influences the accuracy of partitioning. In this study, we first applied 21-mers to partitioning those unassigned reads, and then used 19- and 31-mers to enhance the efficiency. Based on the k-mer distribution, 21-mers that occurred <9 times (sequencing errors) or >58 (high-frequency duplications) were removed from the dataset (Supplementary Fig. S1) resulting in ~40 M unique markers for maternal and ~60 M for paternal (Supplementary Table S1). Due to the relatively short-read length (100 bp), only ~1.4% of reads had at least one unshared k-mer (Supplementary Table S2). Nonetheless, stLFR barcodes enabled the clustering of short reads and extended the haplotype information to the entire long fragment. There were total of 155 196 643 barcodes for two stLFR libraries in the child’s clean reads, of which 23.8% (correspondingly 58.3% read pairs) were uniquely assigned into haplotypes.

To investigate the grouping effectiveness, we calculated the assigned ratio for four groups: paternal, ambiguous, maternal and homozygous. We observed that all the ratios showed a clear dependence of read-pair number sharing the same barcode. The ratio of cobarcode read groups with no haplotype-specific k-mers were exponentially decreasing from 93% to 0% with the increasing number of read pairs per barcode, while the assigned barcode numbers were evenly growing (Fig. 2a). This suggested that the distinguishable
3.2 Collision rates of cobarcoded read groups

The haplotyping precision of cobarcoded reads is dependent on how often an stLFR barcode is associated with long DNA fragments from disparate parts of the genome (the stLFR collision rate). To better understand the stLFR collision effect, all the cobarcoded reads were mapped against the human reference genome (hg19). For the child’s stLFR library the overall collision rate was 1.47, similar to what as previously been reported [Wang et al., 2019]. The barcode assignment was used to further differentiate the collision rate. The group of paternal-only, maternal-only, shared and homozygous had a collision rate of 1.58, 1.54, 1.19 and 2.12, respectively (Supplementary Table S5).

The distribution of long fragment counts per barcode shows that the majority of paternal-only, maternal-only and homozygous barcodes have only one long fragment, while the shared barcode group exhibits a peak at two (Fig. 2b). The shared barcodes can also be classified based on the relative marker ratio to tolerate mis-mapped k-mers by possible sequencing errors. Although this data tends to introduce defective long-range information, the overall amount is insignificant thanks to the high base-calling accuracy and low LFR collision rate of short-read-based cobarcoding techniques. Moreover, this spurious information can be avoided during the assembly process by considering the fact that the stLFR collision rate randomly occurs among the long fragments and thus two fragments from disparate parts of the genome are practically never found repeated on another barcode.

3.3 Complete reconstruction of individual haplotypes

Using HAST, we individually assembled the classified stLFR data from an Asian male (HJ) to reconstruct haplotypes, and compared these to direct pseudo-haplotyping assembly that did not use trio-binning information. The HAST generated assembly was entirely in phase and the origin of the next sequence was unambiguous, while the pseudo-haplotype assembly arbitrarily combined one arm of each ‘bubble’ structure in the graph resulting in many incorrectly linked haplotypes. Although it was complicated to maintain the long phasing information due to the relatively sparse heterozygous variant sites in humans (~0.1%), HAST was able to cluster most of the fragments based on the long-range cobarcoding information and successfully assemble haploid chromosomes. For the genomes with higher heterozygosity, this complexity would decrease.

HAST generated a 6.0Gb assembly of HJ with a scaffold N50 of 18.3 and 11.4 Mb for the maternal and paternal portions, respectively (Supplementary Table S7). The longer scaffold length of the maternal assembly is likely due to the presence of large X chromosome. The contig N50 is about 60 kb. The alignment to the human reference genome resulted in 94.95% and 94.40% coverages with a scaffold NGA50 ≥ 1 Mb. This suggests the assembly is highly accurate and complete. In contrast, the pseudo-haplotype assembly without trio binning produced a comparable scaffold N50 (16.7 Mb) but a shorter contig N50 (38 kb). The base-level accuracy (quality value, QV) and the reappearance capacity of reliable k-mers from input reads (completeness) were estimated for each assembly. The HAST assembly showed a slightly higher average QV value (Q63 vs Q61) with similar completeness (~97%) to the pseudo-haplotype assembly (Table 1).

The contiguity metrics were reported by QUAST. The assembly quality and haplotyping were evaluated by Merqury, including k-mer-based QV, assembly completeness and haplotyping precision and recall. The QV was calculated using the following equation: $QV = \frac{\log_{10}E}{\log_{10}C}$, where $E$ refers to the single-base-level assembly errors. The haplotyping estimates were given by the reliable haplotype-specific k-mers. BUSCO v3 was run with vertebrata_odb9 database, where Comp., Complete; Dup., Duplicated; Frag., Fragmented and Mis., Missing.

We also examined HAST effect using other two pilot human genomes HG001/NA12878 and HG002/NA24385. Compared with HJ, the diploid assemblies showed average scaffold N50s of 4.3 and 5.7 Mb due to relatively shorter DNA fragment length but larger average contig N50s of 123 and 93 kb for two datasets, respectively (Supplementary Table S11). The single-base accuracy reached Q66 and Q62 with a similar completeness (~99%) (Supplementary Table S9).

3.4 Assembly haplotype validation

As a sanity check, we applied Merqury (Rhiie et al., 2020), a reference-free phasing assessment tool based on the k-mer analysis, to haplotype evaluation. We chose this method because the direct alignment to the human reference does not provide phasing information and currently there are no other assemblies available for HJ or HJ’s parents. By counting the number of expected parent-specific k-mers present in the child’s diploid assembly, it demonstrated that each haplotype recovered >95% of the parental heterozygous sites (Table 1). This was significantly higher than the pseudo-haplotypes without trio binning, which recovered only 64.91% and 54.05% of the paternal and maternal alleles, respectively (Table 1).

The haplotyping error is related to the presence of unexpected haplotype-specific k-mers in the assembly. The haplotype precisions of the HAST paternal and maternal assemblies were 99.42% and 99.94%, respectively. In contrast, it was only 64.09% or 44.05% for Supernova assembled pseudo-haplotypes. The Haplotype recall and precision rates achieved 95.73% and 99.89% for HG001 and 91.81% and 99.81% for HG002 (Supplementary Table S9 and Fig. S2). Moreover, the HAST haplotypes contained fewer fragmented or missing BUSCO genes compared to the pseudo-haplotype and achieved 94.8% complete genes. This suggests that there are more allelic variations improperly assembled in the pseudo-haplotype.

In the k-mer multiplicity copy-number plots (Fig. 3a), the first small 1-copy peak (red) represents k-mers unique to each parental haplotype, the second large 2-copy peak (green) corresponds to sequences shared between both haplotypes or haplotype-specific duplications and higher copies (blue, purple, orange) are repetitive regions. Those k-mers that only occur in the sequence reads (grey) possibly came from sequencing errors or indicated missing genomic

Fig. 2. Statistics for the clustering of cobarcoded reads. (a) The assigned ratio of barcodes for different numbers of read pairs per barcode. The assigned ratio is plotted on the x-axis and consists of four groups: paternal or maternal (contain more maternal or paternal-specific k-mers than the other), ambiguous (have equal numbers of both types of haplotype-specific k-mers) or homozygous (have no haplotype-specific k-mers). Barcodes with a smaller number of reads (<10 read pairs) are likely to be from shorter DNA fragments and result in mostly homozygous assignments. As the number of cobarcoded read pairs per barcode increases (likely due to longer DNA fragments being captured on the stLFR beads), the majority of these barcodes are assigned to a haplotype-specific group. The ambiguous barcodes are likely to be caused by multiple DNA fragments being captured on a single stLFR bead, but they are relatively infrequent in our data. (b) The stLFR collision rate for four groups: paternal-only or maternal-only (only contain at least one paternal or maternal-specific k-mer), shared (have both types of haplotype-specific k-mers) or homozygous (have no haplotype-specific k-mers). The x-axis represents the number of DNA fragments per barcode, and y-axis represents the ratio of barcodes for each group to the total barcode number. Not surprisingly, the ambiguous barcodes have a peak at 2, indicating a higher possibility of collision barcodes had more read pairs and the unassigned barcodes contained DNA fragments that were of a shorter overall length and lacked heterozygous variants. When we filtered out the short fragments (i.e. <20 read pairs per barcode), there were total of 31.0% and 32.7% of barcodes (42.0% and 44.1% of read pairs) classified as inherited from the child’s father and mother, respectively. This demonstrates the feasibility and high efficiency of our trio-binning strategy using stLFR data.
regions that could not be assembled. HAST and Supernova had comparable assembly completeness and quality. The >Q60 single-base-level QV was evident in the k-mer analysis as a relatively small bar at the zero multiplicity which corresponded to k-mers found in the assembly but absent from the reads. We also analyzed the k-mer spectra of each haplotype individually. In the HAST assembly, the paternal (blue) and maternal (red) haplotypes generated approximately identical numbers of k-mers in the heterozygous peak (Fig. 3b). This was also true for the Supernova assembly, where the arbitrary combination of 'heterozygous sites' resulted in equal numbers of k-mers across all scaffolds (round). The accurate reconstruction of haplotypes is not limited to stLFR format data. We also applied HAST to a 10× Genomics format dataset for HG001. HAST partitioned 10× Genomics barcodes and read pairs into haplotype-specific and homozygous groups for assembling. However, there were up to 95.09% read pairs (correspondingly 34.93% barcodes) have both types of parental unique markers (Supplementary Table S12). This is because each barcode corresponds to more DNA long fragments relative to stLFR cobar-coded reads, and the higher LFR collision ratio makes the clustering more complex and error-prone. Two assemblies showed scaffold N50s of 6.84 and 13.7 Mb, contig N50s of 97 and 123 kb with single-base accuracy of Q46 and Q48 for the paternal and maternal haplotypes, respectively (Supplementary Table S13). Compared to the stLFR data, the 10× Genomics linked reads also obtained a similar haplotyping precision rate of 96.85% and a recall rate of 93.66% on average, beyond those of Supernova pseudo-haplotypes (Supplementary Table S13 and Fig. S3). However, the relatively higher LFR collision rate leads to more switch errors, reducing the contiguity of phase blocks (Supplementary Table S14). A phase block refers to the region in the sequence where at least two paternal or maternal-specific k-mers occur. Two consecutive conflicting haplotype-specific k-mers within a certain range are marked as a switch error and split the sequence into two phase blocks. We allowed at most 100 switches within 20 kb range to calculate the phase block.

### 3.5 Investigation of phased SVs

As an additional validation, we generated ~46× coverage PacBio CCS long reads from the same diploid sample and examined the phased SVs by HAST and Supernova assemblies. Currently there are no reference assemblies for the parents so the relatively long and accurate CCS reads were ideal to inspect the haplotypes and the associated phased SVs. The CCS reads were partitioned using TrioCanu (Koren et al., 2018) and individually mapped against each haplotype or pseudo-haployte. We investigated regions corresponding to all 52 high-confidence SVs that were discovered by various long read
assemblies were aligned with the reference hg19 to obtain proper coordinates. Individually mapped to the corresponding assemblies with minimap2 (Li, 2018). All 10,137,719 bp. The CCS reads were first partitioned by TrioCanu, and then individually mapped to the corresponding assemblies with minimap2 (Li, 2018). In total, almost all the haplotype-resolution contigs were aligned along either the X-axis or Y-axis in the blob plot, which demonstrated the excellent haplotyping precision and efficiency resulting from Trio binning (Fig. 3c). In addition, we also investigated the phased SVs recovered by TrioCanu assemblies and compared with those by HAST. Totally 93.8% of 52 phased SVs were detected by TrioCanu, among which the haplotyping consistency with HAST is 92.9% and 100.0% for paternal- and maternal-specific groups, respectively (Supplementary Table S16). In general, the reconstruction of two haplotypes by TrioCanu was equivalent to that of HAST and superior to Supernova.

The average k-mer-level accuracy in the CCS assemblies was Q46 (Table 1) substantially lower than stLFR assemblies by HAST or Supernova (>Q60). The base-calling accuracy has been improved by the consensus procedure during the CCS read generation. The residual errors in the input reads introduced a remarkable number of mis-assembled k-mers in the final result (Fig. 3a and b), with an error bar higher than the stLFR assemblies. Nevertheless, the haplotype-resolved CCS assembly produced a 60-fold longer contig N50 compared to that of HAST (Table 1), but with a 4-fold shorter phase block N50 (Table 2). It indicates the absence of long-range scaffolding information as CCS reads are typically around 10 kb but stLFR long fragment length can reach almost 300 kb.

### 3.7 Assembled MHC/KIR regions with accurate phasing
To better understand the structural accuracy of phased assemblies, we investigated the highly repetitive and polymorphic regions of biological importance such as Major Histocompatibility Complex (MHC) and Killer-cell Immunoglobulin-like Receptor (KIR). The MHC region contains human leukocyte antigen (HLA) genes, important to cancer and autoimmunity studies (Brandt et al., 2015). A single long scaffold of HAST maternal haplotype covered the entire MHC region, while the paternal haplotype assembled two scaffolds to cover (>99%) (Fig. 5). There were obvious distinctions between two haplotype-specific assemblies. Previous studies showed that HLA type phasing was consistent with a trio structure (Chin et al., 2020). We observed that 23 and 22 out of 24 HLA class I and II genes (Horton et al., 2004) were recovered by HAST maternal and paternal haplotypes, respectively (Supplementary Table S20).
Accurate haplotype-resolved assembly

Fig. 5. Alignments of two haplotypes to the GRCh38 MHC region. The haplotype-resolved scaffolds or contigs were mapped to the reference using minimap2 (Li, 2018). The scaffolds or contigs were locally anchored to the reference using a greedy strategy. The edit distance between 10 kb bins and reference was individually computed using a modified O(ND) dynamics programming alignment algorithm (Myers, 1986) (penalty score: 1 for an indel, 1 for a substitution, 0 for an N). The white strips corresponding to the regions with >30% N’s were rescaled based on the aligned positions of adjacent regions, and their differences were not computed.

By comparison, Supernova assemblies were more fragmented in this region covering ~86% bases, and erroneously showed overall homozygous structures. As a result, Supernova failed to assemble 4 HLA genes for each pseudo-haplotype, including HLA-DRB1, HLA-DQB1, MICA and MICB. TrioCanu also correctly reconstructed most of the MHC region (>99%) for two haplotypes with several contigs, and only paternal haplotype missed 1 HLA genes.

Similarly, all three methods successfully assembled the KIR region with one scaffold or contig for each haplotype (Supplementary Fig. S5). However, only Supernova failed to represent the structural heterogeneity between two haplotypes.

4 Results

In this study, we provided a direct way to individually assemble the haplotypes of an individual using parental sequencing data. Although the DNA fragment length and read coverage of each fragment vary for different cobarcode datasets, HAST can cluster reads sharing the same barcodes and retain the long-range phased sequence information. The application of trio binning by HAST simplifies the assembly problem and achieves significant improvements in the contiguity, precision and recall of haplotyping. The assembly of sex chromosomes could be further enhanced by the identification of male-specific k-mers (Wang et al., 2020). This concept could also be applied to complex polyplid genomes if parents or related species are available, which is a potentially important application for animal and plant genetics and breeding programs.

The long-range Hi-C conformation data and long reads can also provide accurate phasing information ranging from single-base level to chromosome level (Garg et al., 2020). Following the HAST assembly, two sets of haplotype-resolved scaffolds were possible to be improved in collaboration with other sequencing platforms. With additional haplotype-resolved Hi-C data, the chromosome-level genomes were obtained with scaffold N50s of 145.0 and 153.2 Mb (Supplementary Table S17). Additionally, the PacBio CCS reads could extend contigs with a >10-fold increase in contig N50 (Xu et al., 2020) (Supplementary Table S18). The improved contiguity, haplotype precision and recall of the final assembly will provide access to the reference-level genomes for different individuals with haplotype-specific SVs, which is essential for the studies of genomic diseases and evolutionary relationships.

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The data that support the findings of this study have been deposited into CNGb Nucleotide Sequence Archive (CNSA) (Guo et al., 2020) of China National GeneBank (CNGbDB) (Chen Fengzhen, 2020) with accession number CNP0001199.

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Conflict of Interest:

Some of the authors are employees of BGI Group. The authors otherwise declare that they have no competing interests.

Data availability

The sequencing data for the sample HJ (including stLR and PacBio CCS) has been deposited in the CNGb under accession number CNP0000091. The stLR data for HG001/NA12878 and HG002/NA24385 is available in the CNGb under accession number CP00000066 (PRJEB27414). We downloaded the 10X Genomics linked reads of HG001/NA12878 from GIAB (http://ftp.ncbi.nlm.nih.gov/giab/ftp/data/NA12878/10xgenomics_Chromium_Genome_LongRanger2.0_06202016NA12878.fasta.gz). All the evaluated assemblies generated by us can be obtained in the CNGb under accession number CNP0001199. All codes, scripts and manuals to build haplotype-specific k-mer sets, and classify stLR reads are available at https://github.com/BIGI-Qingdao/HAST.

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