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Wang, Shuai; Wang, Mengyao; Chen, Lingxi; Pan, Guangze; Wang, Yanfei; Li, Shuai Cheng

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SpecHLA enables full-resolution HLA typing from sequencing data

Graphical abstract

Highlights
- SpecHLA accurately reconstructs diploid sequences of HLA genes
- SpecHLA accepts diverse sequencing data protocols
- SpecHLA can leverage pedigree data and genotype frequency to refine typing
- SpecHLA accurately detects HLA loss-of-heterozygosity events

Authors
Shuai Wang, Mengyao Wang, Lingxi Chen, Guangze Pan, Yanfei Wang, Shuai Cheng Li

Correspondence
shuaicli@cityu.edu.hk

In brief
Wang et al. present SpecHLA, a software package leveraging reads binning and local assembly to achieve accurate full-resolution HLA typing and loss-of-heterozygosity detection. SpecHLA accepts various sequencing data protocols and can incorporate pedigree data and genotype frequency for better performance.
SpecHLA enables full-resolution HLA typing from sequencing data

Shuai Wang,1,2 Mengyao Wang,1,2 Lingxi Chen,1 Guangze Pan,1 Yanfei Wang,1 and Shuai Cheng Li1,3,*
1City University of Hong Kong, Department of Computer Science, Kowloon, Hong Kong
2These authors contributed equally
3Lead contact
*Correspondence: shuaicli@cityu.edu.hk
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SUMMARY
Reconstructing diploid sequences of human leukocyte antigen (HLA) genes, i.e., full-resolution HLA typing, from sequencing data is challenging. The high homogeneity across HLA genes and the high heterogeneity within HLA alleles complicate the identification of genomic source loci for sequencing reads. Here, we present SpecHLA, which utilizes fine-tuned reads binning and local assembly to achieve accurate full-resolution HLA typing. SpecHLA accepts sequencing data from paired-end, 10\(^3\)-linked-reads, high-throughput chromosome conformation capture (Hi-C), Pacific Biosciences (PacBio), and Oxford Nanopore Technology (ONT). It can also incorporate pedigree data and genotype frequency to refine typing. In 32 Human Genome Structural Variation Consortium, Phase 2 (HGSVC2) samples, SpecHLA achieved 98.6% accuracy for G-group-resolution HLA typing, inferring entire HLA alleles with an average of three mismatches fewer, ten gaps fewer, and 590 bp less edit distance than HISAT-genotype per allele. Additionally, SpecHLA exhibited a 2-field typing accuracy of 98.6% in 875 real samples. Finally, SpecHLA detected HLA loss of heterozygosity with 99.7% specificity and 96.8% sensitivity in simulated samples of cancer cell lines.

INTRODUCTION
Human leukocyte antigen (HLA) genes encode the major histocompatibility complex (MHC) molecules essential in adaptive immune system regulation.\(^1\) HLA typing assists in organ transplantation and preimplantation genetic diagnosis\(^2\) and yields insights into the molecular mechanism of autoimmune disorders,\(^3\) infectious diseases,\(^4\) cancer immunotherapy,\(^5\) etc. Many HLA typing methods identify the most compatible pair of alleles for each gene that match the sequencing data from the database.\(^6\) For instance, OptiType\(^7\) maximizes the number of mapped reads on the inferred alleles by employing integer linear programming. PolySolver\(^8\) selects alleles via a Bayesian model by integrating base qualities of aligned reads, observed insert sizes, and ethnicity-dependent allele frequency. HLA-VBSeq\(^9\) optimizes both read alignments to alleles and relative quantities of reads based on variational Bayesian inference. HLA-HD\(^10\) maps reads to exons and introns of alleles in the database and utilizes weighted read counts to choose suitable allele pairs. HLA\(^*\)PRG\(^11\) transforms the allele database to a population reference graph and applies the read-to-graph alignment algorithm to infer the most likely allele pair. The method arcasHLA\(^12\) aligns reads to the de Bruijn graph constructed from the reference transcriptome and then identifies the allele pair using the k-mer structure. HLA\(^*\)LA\(^13\) projects the linear read alignment on the reference graphs, allowing allele inference from short- or long-read data. However, these methods have several limitations: (1) they are imprecise if the target alleles are incomplete or absent from the
HLA-A and 590 bp less edit distance than HISAT-genotype compared entire HLA alleles with three mismatches fewer, ten gaps fewer, 10
tocols, SpecHLA accepts the sequencing data of paired-end (PE),
tocols. Unlike most methods that are limited to a single data pro-
make it necessary to support HLA typing for multiple data pro-
HLA genes possess a high similarity,7 making it complicated to
effective typing using both actual and simulated samples (Figure 1G). We validated
allele data-
capture (Hi-C).20 Pacific Biosciences (PacBio), and Oxford
Nanopore Technology (ONT).21 Additionally, it has been recog-
nize that utilizing pedigree relations22 and genotype fre-
that splits aligned reads into k-mers and resolves assembly am-
generate full-length HLA alleles.
Assembly and alignment are indispensable for full-resolution
HLA typing. The assembly-based approach is to assemble reads into haplotype-resolved sequences, and the alignment-based
loci from other homologous loci. For instance, reads originating from
the HLA-A-like pseudogene HLA-Y hamper typing HLA-A.10
Alleles of different HLA genes possess a high similarity,7 making it complicated to
identify the genomic sources of reads unambiguously. Hence,
typing an HLA locus would be interfered with by reads derived from
other homologous loci. For instance, reads originating
the HLA-A-like pseudogene HLA-Y hamper typing HLA-A.10
Although a previous method attempted to discard the reads potentially generated from other loci to type the target
locus, the method cannot reconstruct HLA allele sequences and
is not open source.16 Second, HLA alleles of the same gene are
exceptionally polymorphic; consequently, the reads might be
highly different from the reference allele. Due to the limitation of
scoring systems of alignment algorithms, homologous reads are
possibly mapped to alternative coordinates. Moreover, the
highly variable reads render assembly highly fragmented and
prone to error.17
Here, we present a software package, SpecHLA, for accurate
full-resolution HLA typing on HLA-A, -B, -C, -DPA1, -DPB1,
-DQA1, -DQB1, and -DRB1 genes. The package adopts fine-
tuned reads binning and local assembly for precise read align-
ment and reconstruct complete diploid sequences of HLA loci
through variant phasing. SpecHLA determines the reads
belonging to an HLA locus by aligning the reads to known HLA
alleles to solve the first challenge. Furthermore, SpecHLA as-
sembles reads mapped to each highly divergent region into con-
tigs independently and realigns the reads to the reference allele
through the assembled contigs to overcome the second challenge.
SpecHLA achieved 97.6%, 98.4%, and 99.1% 2-field typing accuracies in 230 whole-genome sequencing (WGS)
samples, 183 whole-exome sequencing (WES) samples, and
462 RNA sequencing (RNA-seq) samples from the 1000 Genomes Project, respectively. In the 32 WGS samples from the
Human Genome Structural Variation Consortium, Phase 2
(HGSVC2) Project, SpecHLA achieved 98.6% HLA typing ac-
curacy at G group resolution. Also, on average, it reconstructed
entire HLA alleles with three mismatches fewer, ten gaps fewer,
and 590 bp less edit distance than HISAT-genotype compared
with ground-truth per allele.
Furthermore, the development of sequencing technologies
makes it necessary to support HLA typing for multiple data pro-
tocols. Unlike most methods that are limited to a single data proto-
col, SpecHLA accepts the sequencing data of paired-end (PE),
10x Genomics,19 high-throughput chromosome conformation
scan.
reference alleles and the default IMGT representative reference. For most genes, the 2-field HLA typing results showed no significant differences when using different reference alleles, except for the HLA-DRB1 gene (see Figure S1C). Notably, when using the randomly selected allele DRB1*09:01:02:02 as the reference, the accuracy of HLA-DRB1 significantly decreased to 65.3%. Conversely, employing the default reference allele resulted in relatively high accuracy across all genes, suggesting that...
### Table A

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### Figure D

- **MHCI**: 2-field typing accuracy vs. sequencing depth.
- **MHCII**: 2-field typing accuracy vs. sequencing depth.
- **Sequencing depth**: 10x to 40x.

### Figure F

- **Recall**: Hit rate vs. sequencing depth.
- **Sequence Precision**: Hit rate vs. sequencing depth.

### Figure M

- **HLA-A**: NA12891, NA12878, NA12892, NA19239, NA19240, NA19238
- **Zoom Bar**: Exons and cDNA sequences.
- **DNA**: Genetic variation sites.
SpecHLA exhibits greater robustness when utilizing the default reference allele. Second, in terms of computational resource consumption, SpecHLA exhibited relatively higher CPU time requirements but demanded less memory compared with other tools (Figures S1D and S1E). Third, we evaluated the effect of alignment tools on the read-binning performance in 2,000 simulated samples. When using Novoalign, the read-binning step exhibited an average precision of 99.8% and a recall of 89.4%. Conversely, employing Bowtie2 resulted in an average precision of 99.6% and a recall of 49.9% (Figures S1F and S1G). The result demonstrated that Novoalign outperforms Bowtie2 in terms of read-binning accuracy.

**SpecHLA is accurate in 2-field HLA typing**

First, SpecHLA performed accurately in 2-field HLA typing on actual data. We evaluated SpecHLA with 230 WGS, 183 WES, and 462 RNA-seq samples containing validated HLA typing results for the five genes, HLA-A, -B, -C, -DQB1, and -DRB1 from the 1000 Genomes Project (Figures 2A–2C). SpecHLA achieved an average typing accuracy of 98.6% in the 875 samples, while HISAT-genotype, HLA-HD, HLA*LA, HLA-VBseq, Kourami, OptiType, Polysolver, and arcashHLA exhibited accuracies of 94.7% (875 samples); 93.1% (875 samples); 97.1% (413 samples); 66.3% (413 samples); 90.7% (413 samples); 97.8% (875 samples); 88.9% (183 samples); and 98.5% (462 samples), respectively. SpecHLA demonstrated the highest average accuracy across a range of experiments involving different genes and sequencing protocols on actual data. Second, the typing accuracy of SpecHLA was robust with varying sequencing depths. The 230 WGS samples have sequencing depths ranging from 10x to 49x in the MHC region. We observed that the 2-field typing accuracy of SpecHLA raised from 98.1% to 99.7%, with the depth cutoff increasing from 10x to 22x (Figures S2A and S2B). Moreover, we subsampled the 87 IHWG cell line WES samples for various sequencing depths (10x, 15x, 20x, 30x, 35x, and 40x). The average MHC class I gene typing accuracies of SpecHLA, HISAT-genotype, HLA-HD, HLA*LA, HLA-VBseq, Kourami, OptiType, Polysolver, and arcashHLA were 94.3%, 98.6%, 94.3%, and 395 bp, respectively, while HISAT-genotype achieved 93.1%, 89%, 89%, 84%, 95.7%, and 85.5%, respectively. Also, SpecHLA, HISAT-genotype, HLA-HD, HLA*LA, HLA-VBseq, and Kourami exhibited average typing accuracies on class II genes of 94.4%, 89.3%, 87%, 84.7%, 83.2%, and 92%, respectively.

**SpecHLA accurately reconstructs HLA sequences**

First, SpecHLA reconstructed entire HLA alleles of higher quality than HISAT-genotype on actual and simulated data. We measured the sequence quality according to five criteria: mismatch rate, gap rate, sequence precision, sequence recall, and edit distance, as they covered completeness and precision. SpecHLA outperformed HISAT-genotype to reconstruct sequences in 32 WGS samples from the HGSVC2 Project (Figures 2F–2J). SpecHLA achieved average mismatch rate, gap rate, sequence precision, sequence recall, and edit distance of 2.99e−4, 1.75e−4, 98.8%, 94.3%, and 395 bp, respectively, while HISAT-genotype achieved 1.02e−3, 2.37e−3, 98.6%, 85.5%, and 985 bp, respectively. SpecHLA inferred entire HLA alleles with an average of three mismatches fewer, ten gaps fewer, and 590 bp less edit distance than HISAT-genotype per allele. SpecHLA reconstructed 71.1% more sequences than HISAT-genotype in all the loci (100% vs. 28.9%), highlighting SpecHLA’s robustness (Figure 2K). Moreover, we compared the two methods for sequence reconstruction in the 2,000 simulated samples (Figures S4A–S4E). SpecHLA and HISAT-genotype had average mismatch rates of 1.96e−3 vs. 1.45e−2, gap rates of 5.14e−4 vs. 1.01e−2, sequence precisions of 93.3% vs. 87%, sequence recalls of 97.7% vs. 83%, and edit distances of 658 vs. 1,315 bp. SpecHLA was more accurate than HISAT-genotype across different sequencing settings.

### Figure 2. Validation of SpecHLA for 2-field and full-resolution HLA typing

(A–C) The 2-field typing accuracy in 230 WGS (A), 183 WES (B), and 462 RNA-seq (C) samples from the 1000 Genomes Project.
(D and E) The 2-field HLA typing accuracy of MHC class I (D) and MHC class II (E) genes with different sequencing depths in the IHWG cell line subsampling dataset.
(F–K) The accuracy of inferring full-length HLA alleles with 32 HGSVC2 samples, measured by mismatch rate (F), gap rate (G), sequence precision (H), sequence recall (I), edit distance (J), and the number of samples with reconstructed sequences (K).
(L) The accuracy of G-group-resolution HLA typing on 32 HGSVC2 samples.
(M) The illustration of HLA-A genotypes phased by SpecHLA in two trios; each dot symbol represents a variant.
See also Figures S2–S4.
also applied SpecHLA and HISAT-genotype to recover novel alleles in the 50 simulated samples, and they achieved average mismatch rates of 1.74e−6 vs. 3.48e−4 and gap rates of 9.24e−6 vs. 2.59e−3 (Figures S2C–S2H). SpecHLA recovered simulated novel alleles more precisely than HISAT-genotype, suggesting that SpecHLA could reconstruct the HLA alleles absent from the database.

Also, SpecHLA inferred HLA exon sequences accurately in actual and simulated samples. In the HGSCV2 dataset, the G-group-resolution typing accuracies of SpecHLA, Kourami, HISAT-genotype, HLA-VBSeq, HLA*LA, and HLA-HD were 98.6% (505/512), 98.7% (379/384), 96.3% (493/512), 86.5% (443/512), 97.1% (497/512), and 94.7% (485/512), on average, respectively (Figure 2L). In the 2,000 simulated samples, SpecHLA yielded more accurate exon sequences of MHC class I genes than Kourami for all the datasets (Figures S4F and S4G), with average mismatch rates of 9.32e−4 vs. 6.05e−3 and gap rates of 3.30e−5 vs. 2.44e−3. SpecHLA was slightly less robust for MHC class II genes than Kourami in low-depth and short-read samples. With sequencing depth greater than 20x and reads longer than 90 bp, SpecHLA exhibited higher accuracies than Kourami, and they achieved average mismatch rates of 6.25e−4 vs. 2.04e−3 and gap rates of 7.35e−5 vs. 1.02e−3. Only exons 2 and 3 for MHC class I and only exon 2 for MHC class II genes were considered in the comparison.

Furthermore, SpecHLA reconstructed sequences highly trio consistent in two family trios. We calculated the difference between the child’s inherited and the parent’s original sequences. The difference was measured by mismatch rate and gap rate. On average, the mismatch and gap rates were 7.69e−4 and 2.63e−4, respectively (Figure S2L). We visualized the phased exonic variants of the trios using Oviz-Bio. The phased genotypes were consistent with the trio structure (Figure 2M). Also, the 4-field typing results of SpecHLA were trio consistent for 93.8% (30/32) of the alleles in the children. These results consistently implied that SpecHLA is accurate in full-resolution HLA typing.

**SpecHLA works with different data types**

SpecHLA can integrate short and long reads for HLA typing. Seven individuals of HGSCV2 have both PE and PacBio data, and with these data, we performed SpecHLA using PE data and PE plus PacBio data separately. The mean mismatch rates without and with PacBio data were 1.95e−3 vs. 1.37e−3, and gap rates were 3.16e−4 vs. 2.98e−4 (Figures 3A and 3B). The result indicated that SpecHLA could efficiently integrate short and long reads to yield better results. SpecHLA can also accept long reads independently for HLA typing. Apart from the seven HGSCV2 PacBio samples, we collected a PacBio and an ONT sample of the individual NA12878, resulting in a total of nine long-read samples. In the nine long-read samples, SpecHLA and HLA*LA achieved G-group-resolution typing accuracies of 95.1% and 94.4%, respectively (Figure S2J).

In addition, simulated data showed that SpecHLA could combine PE data with PacBio, ONT, 10x, and Hi-C data. We generated 50 individuals using simulated novel alleles and produced sequencing reads with different protocols. We performed SpecHLA with different data permutations of the 50 individuals. The average mismatch rates were 5.84e−4, 5.46e−6, 8.92e−6, 2.61e−6, 2.61e−6, 2.03e−5, and 1.97e−4 using PE, PacBio, ONT, +PacBio, +ONT, +10x, and +Hi-C data, respectively (Figure 3C). The plus symbol (+) indicates the integration of PE and the present data. The highest mismatch rate was observed when utilizing only PE data. The lowest mismatch rate was achieved by integrating PE data with either PacBio or ONT data. Incorporating Hi-C data was not as efficient as long-read and 10x data.

SpecHLA could refine HLA typing with pedigree relations in actual and simulated family trios. We executed SpecHLA without and with pedigree relations in two family trios from the 1000 Genomes Project. The mean mismatch rates for children without and with pedigree relations were 1.10e−3 and 7.89e−4, respectively, while the corresponding gap rates were 3.39e−4 and 3.21e−4 (Figures 3D and 3E). We also simulated 50 family trios with PE reads; the read length, depth, and error rate were 75 bp, 30x, and 1%, respectively. In simulated trios, SpecHLA exhibited average mismatch rates of 3.59e−3 and 2.10e−3 for children without and with pedigree relations, respectively, and the corresponding gap rates were 9.06e−4 and 7.73e−4 (Figures S2K and S2L). The results indicated that SpecHLA reduced variant phasing ambiguities using pedigree relations.

SpecHLA demonstrated the ability to incorporate genotype frequency for HLA typing in allelic imbalance samples. We ran SpecHLA separately using only reads (“read”), using the integration of reads and genotype frequency (“hybrid”), and using only genotype frequency (“geno”). First, to investigate whether SpecHLA can incorporate genotype frequency, we performed SpecHLA on 200 allelic imbalance samples, which were LOH samples simulated from cancer cell lines. When solely using genotype frequency, SpecHLA achieved a 2-field typing accuracy of 90% (Figure 3F). Next, we examined the sequence reconstruction performance of SpecHLA after using genotype frequency. We ran SpecHLA in a simulated dataset comprising 80 allelic imbalance samples. Each gene contained one haplotype with a depth of 20x and the other haplotype with a depth of 80x. On average, in the dataset, the mismatch rates for “read,” “hybrid,” and “geno” were 3.49e−4, 3.98e−4, and 2.79e−4, respectively (Figure 3G). Notably, using only genotype frequency achieved the lowest mismatch rate. Furthermore, we tested SpecHLA’s genotype frequency incorporation ability with different allelic depths. SpecHLA was applied to a dataset of 100 simulated novel-allele samples with varying allelic depths (Figure 3H). In this dataset, the average mismatch rates for “read,” “hybrid,” and “geno” were 9.93e−4, 4.34e−4, and 6.40e−4, respectively. The combined utilization of sequencing reads and genotype frequency achieved the best performance. These results showed that SpecHLA can efficiently utilize genotype frequency for HLA typing.

**SpecHLA precisely infers HLA LOH events**

SpecHLA identified HLA LOH events more accurately than DASH and LOHHLA in simulated data from cancer cell lines. We simulated 300 samples by combining HLA exonic data from cancer cell lines and matched normal cells, in which 200 samples possessed LOH events. For MHC class I genes, the LOH detection specificities of SpecHLA (99.7%) and LOHHLA...
(100.0%) were much higher than that of DASH (44.3%) (Figure 4). SpecHLA exhibited a higher sensitivity for LOH detection than LOHHLA across a range of tumor purities from 0.2 to 0.9, and the average sensitivities of SpecHLA and LOHHLA with tumor purity ranging from 0.1 to 1 were 96.8% and 92.8%, respectively. SpecHLA was more accurate than DASH and LOHHLA with MHC class I genes in the 300 samples. Additionally, SpecHLA exhibited a high LOH detection accuracy with a specificity of 97.5% and a sensitivity of 92.0% for MHC class II genes (Figure 4), but DASH and LOHHLA cannot handle such genes. Furthermore, through additional simulated data, we demonstrated the robustness of SpecHLA in handling allelic imbalance samples (Figure S2M) and different tumor purity and ploidy conditions (Figure S2N).

**DISCUSSION**

HLA genes persist in high sequence identity with the genes in the same class. For instance, *HLA-A* has high identities with *HLA-B*, *HLA-C*, and the pseudogene *HLA-Y*. Read accuracies from different HLA genes are mixed during sequencing; some of them are prone to align to the faulty gene, introducing errors in HLA typing. SpecHLA identifies the exact HLA locus for sequencing reads based on two assumptions: (1) HLA alleles belonging to the same HLA gene have a higher sequence identity than those from different genes, and (2) *de novo* alleles have high sequence identities with the known ones. SpecHLA permits adjusting read-assignment parameters to retain more novel small variants for *de novo* allele reconstruction. The curated HLA database grows rapidly. SpecHLA has the advantage of adapting the iterative updates of the database. Based on the reads-binning strategy, we can use continuously added alleles in the database to assign reads more precisely. On the contrary, the database-matching methods optimized for a specific collection of alleles thus cannot efficiently remodel the ever-expanding list of alleles. Furthermore, they may even suffer from increased HLA alleles due to the increment of searching complexity.
Previous research has highlighted the incompleteness of current HLA allele databases,\textsuperscript{14} which poses a challenge for accurate HLA designation. Even if the reconstructed sequence is correct, the absence of the allele in the database can lead to erroneous designation results. Conversely, directly analyzing the HLA sequences is not affected by the incompleteness of the database. Furthermore, clustering the HLA sequences into groups using self-defined distance measurements has the potential to offer novel biological insights. As a result, we anticipate that conducting HLA typing at full resolution will become a prominent trend in future research endeavors.

MHC class II molecules present processed antigens to CD4(+) T lymphocytes, which is crucial for antigen-specific immune response.\textsuperscript{32} Notably, LOH events can occur in MHC class II genes, as observed in a previous study on a patient with acute myeloid leukemia.\textsuperscript{33} Tumor-specific MHC class II expression may be a clinically actionable biomarker of response to immune checkpoint inhibition, while LOH of MHC class II genes may reduce response to immunotherapies.\textsuperscript{34} Many HLA-related tools merely focus on MHC class I genes. SpecHLA can perform full-resolution HLA typing and detect LOH events in MHC class II genes, which provides the opportunity to study MHC class II genes further.

Limitations of the study
To achieve accurate reconstruction of HLA sequences, our method, SpecHLA, requires a relatively high read alignment depth, typically 5x or greater. Any sequence regions with insufficient read depth are automatically masked by SpecHLA. Moreover, low-depth data can negatively impact the accuracy of SpecHLA's long indel detection, leading to lower completeness and increased contamination in the reconstructed HLA sequences.

STAR★METHODS
Detailed methods are provided in the online version of this paper and include the following:

- **KEY RESOURCES TABLE**
- **RESOURCE AVAILABILITY**
  - Lead contact
  - Materials availability
  - Data and code availability
- **METHOD DETAILS**
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  - Incorporating genotype frequency to phase in allelic imbalance samples
  - Benchmark datasets
  - Evaluation methods
- **QUANTIFICATION AND STATISTICAL ANALYSIS**

SUPPLEMENTAL INFORMATION
Supplemental information can be found online at https://doi.org/10.1016/j.crmeth.2023.100589.

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AUTHOR CONTRIBUTIONS
S.C.L. designed and supervised the study. S.W. and M.W. developed and evaluated the software. L.C. was involved in software implementation. G.P.
contributes to pedigree integration. M.W. and Y.W. were engaged in figure design. S.W. and M.W. wrote the manuscript, and all authors contributed to manuscript revisions.

DECLARATION OF INTERESTS

The authors declare no competing interests.

DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

During the preparation of this work, the authors used ChatGPT to refine English sentences. After using this tool, the authors reviewed and edited the content as necessary and take full responsibility for the publication’s content.

References


**STAR METHODS**

**KEY RESOURCES TABLE**

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<td>DASH HLA LOH cancer cell lines</td>
<td>Pyke et al.</td>
<td><a href="https://www.nature.com/articles/s41467-022-29203-w">https://www.nature.com/articles/s41467-022-29203-w</a></td>
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<td>LOH results of DASH data as ground truth</td>
<td>Pyke et al.</td>
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**Software and algorithms**

| SpecHLA v1.0.1 | This paper | [https://doi.org/10.5281/zenodo.8232273](https://doi.org/10.5281/zenodo.8232273) |
| BWA-0.7.17-r1188 | Li | [https://github.com/lh3/bwa](https://github.com/lh3/bwa) |
| Novoalign V4.02.01 | NOVOCRAFT | [https://www.novocraft.com/products/novoalign/](https://www.novocraft.com/products/novoalign/) |
| Bowtie2 v2.3.4.1 | Langmead and Salzberg | [https://github.com/BenLangmead/bowtie2](https://github.com/BenLangmead/bowtie2) |
| ScanIndel v1.3 | Yang et al. | [https://github.com/cauyrd/ScanIndel](https://github.com/cauyrd/ScanIndel) |
| Fermikit-0.13 | Li | [https://github.com/lh3/fermikit](https://github.com/lh3/fermikit) |
| SpecHap v1.0.1 | Yu et al. | [https://github.com/deepomicslab/SpecHap](https://github.com/deepomicslab/SpecHap) |
| arcasHLA v0.5.0 | Orenbuch et al. | [https://github.com/RabadanLab/arcasHLA](https://github.com/RabadanLab/arcasHLA) |
| HISAT-genotype v1.3.2 | Kim et al. | [https://github.com/DaehwanKimLab/hisat-genotype](https://github.com/DaehwanKimLab/hisat-genotype) |
| HLA-HD v1.4.0 | Kawaguchi et al. | [https://www.genome.med.kyoto-u.ac.jp/HLA-HD/](https://www.genome.med.kyoto-u.ac.jp/HLA-HD/) |
| HLA*LA v1.0.2 | Dilthey et al. | [https://github.com/DiltheyLab/HLA-LA](https://github.com/DiltheyLab/HLA-LA) |
| HLA-VBseq v2 | Nariai et al. | [http://nagasakilab.csml.org/hla/](http://nagasakilab.csml.org/hla/) |
| Kourami v0.9.6 | Lee and Kingsford | [https://github.com/Kingsford-Group/kourami](https://github.com/Kingsford-Group/kourami) |
| OptiType v1.3.1 | Szolek et al. | [https://github.com/FRED-2/OptiType](https://github.com/FRED-2/OptiType) |
| PolySolver v1.0.0 | Shukla et al. | [https://github.com/jason-weirather/hla-polysolver](https://github.com/jason-weirather/hla-polysolver) |
| DASH training.xgboost_model.2021_05_10.p | Pyke et al. | [https://github.com/Personalis-DASH/DASH](https://github.com/Personalis-DASH/DASH) |
| LOHHLA v1.1.6 | McGranahan et al. | [https://github.com/mskcc/lohHLA](https://github.com/mskcc/lohHLA) |
| ABSOLUTE v1.2 | Carter et al. | [https://bioinformaticshome.com/tools/cnv.descriptions/ABSOLUTE.html#gsc.tab=0](https://bioinformaticshome.com/tools/cnv.descriptions/ABSOLUTE.html#gsc.tab=0) |
| pbsv Version 2.6.2 | PacificBiosciences | [https://github.com/PacificBiosciences/pbbioconda](https://github.com/PacificBiosciences/pbbioconda) |
| pbmm2-1.4.0 | PacificBiosciences | [https://github.com/PacificBiosciences/pbbioconda](https://github.com/PacificBiosciences/pbbioconda) |
| LRSIM v1.0 | Luo et al. | [https://github.com/aquaskyline/LRSIM](https://github.com/aquaskyline/LRSIM) |
| LongRanger v2.2.2 | 10x Genomics | [https://support.10xgenomics.com/genome-exome/software/downloads/latest](https://support.10xgenomics.com/genome-exome/software/downloads/latest) |

(Continued on next page)
RESOURCE AVAILABILITY

Lead contact
Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Shuai Cheng Li (shuaicli@cityu.edu.hk).

Materials availability
This study did not generate new unique reagents.

Data and code availability
- This paper analyzes existing, publicly available data. The accession numbers for the datasets are listed in the key resources table.
- The software package SpecHLA is publicly available at https://github.com/deepomicslab/SpecHLA. Detailed benchmark experiment results have also been deposited in this repository. Additionally, the original source code has been deposited at Zenodo and is publicly available as of the date of publication. DOIs are listed in the key resources table.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

METHOD DETAILS

Algorithm of SpecHLA

Binning reads
SpecHLA employs a read-binning procedure to reduce the ambiguity of HLA read alignment. We conduct read binning by assigning reads to HLA loci based on their sequence identity to existing HLA alleles. A lite version of IMGT/HLA database29 (Release 3.37.0) is constructed with 6,172 alleles of 39 HLA genes and pseudogenes (Table S1). Given a sample, we first collect the reads mapped to the HLA region with a method similar to Kourami6 and align them to the lite IMGT/HLA database using Novoalign v.4.2.1 (http://www.novocraft.com/products/novoalign/). In the absence of the Novoalign license, Bowtie2 v2.3.4.137 is offered as an alternative. Each read is then assigned to its best-matched allele, that is, the allele with the least number of mismatches with the read. Then a PE read is assigned to an HLA locus to which the best-matched allele belongs if it satisfies the four additional criteria: (i) Both its two ends are aligned to the same best-matched allele. (ii) The number of mismatches between each end and the best-matched allele is no more than \( q \) (\( q \) is 2 by default). (iii) The sequence identity between the read and the alleles of any other HLA loci is at least \( v \) higher than the sequence identity between the read and the best-matched allele. By default, \( v \) is 0.1. (iv) The read is aligned to the best-matched allele without soft-clipping. The accuracy of read binning with different \( q \) values is tabulated in Figure S1H-M.

In 50 simulated samples, we validated SpecHLA’s ability to accurately type alleles that are not included in the lite IMGT/HLA database, achieving a 2-field typing accuracy of 93.38%.

Mapping reads to the reference
SpecHLA further utilizes local assembly to align the binned reads to the reference more accurately. Alignment algorithms rely on scoring systems. If the reads from alleles heavily diverge from the reference, homologous reads might be aligned to alternative coordinates on the reference. First, we construct the IMGT representative reference by concatenating the first recorded allele at each HLA locus from the IMGT/HLA database (Table S1). Given a sample, we first collect the reads mapped to the HLA region with a method similar to Kourami5 and align them to the lite IMGT/HLA database using Novoalign v.4.2.1 (http://www.novocraft.com/products/novoalign/). In the absence of the Novoalign license, Bowtie2 v2.3.4.137 is offered as an alternative. Each read is then assigned to its best-matched allele, that is, the allele with the least number of mismatches with the read. Then a PE read is assigned to an HLA locus to which the best-matched allele belongs if it satisfies the four additional criteria: (i) Both its two ends are aligned to the same best-matched allele. (ii) The number of mismatches between each end and the best-matched allele is no more than \( \theta \) (\( \theta \) is 2 by default). (iii) The sequence identity between the read and the best-matched allele is at least \( \nu \) higher than the sequence identity between the read and the alleles of any other HLA loci. By default, \( \nu \) is 0.1. (iv) The read is aligned to the best-matched allele without soft-clipping. The accuracy of read binning with different \( \theta \) values is tabulated in Figure S1H-M.

In 50 simulated samples, we validated SpecHLA’s ability to accurately type alleles that are not included in the lite IMGT/HLA database, achieving a 2-field typing accuracy of 93.38%.

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<td>Jia et al.31</td>
<td><a href="https://bio.oviz.org/">https://bio.oviz.org/</a></td>
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</table>

RESOURCES OR REAGENT SOURCE IDENTIFIER

- Minimap 2.17-r941 : Li42 https://github.com/lh3/minimap2
- fastp 0.20.0 : Chen et al.43 https://github/OpenGene/fastp
- PBSIM v1.0.3 : Ono et al.44 https://doi/10.1093/bioinformatics/bts649
- NanoSim v3.1.0 : Yang et al.46 https://github.com/bgsc/NanoSim
- sim3C v0.2 : DeMaere and Darling46 https://github.com/cerebis/sim3C
- DWGSIM v0.1.13 : Github https://github.com/nh13/DWGSIM
- Longshot v0.4.1 : Edge and Bansal47 https://github.com/pedge/longshot
- bcftools-Version: 1.9 : Danecek and McCarthy48 http://www.htslib.org/doc/1.0/bcftools.html
- Oviz-bio : Jia et al.31 https://bio.oviz.org/

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- fastp 0.20.0 : Chen et al.43 https://github/OpenGene/fastp
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- Longshot v0.4.1 : Edge and Bansal47 https://github.com/pedge/longshot
- bcftools-Version: 1.9 : Danecek and McCarthy48 http://www.htslib.org/doc/1.0/bcftools.html
- Oviz-bio : Jia et al.31 https://bio.oviz.org/
them into contigs with Fermikit v.0.13. We may have one or more assembled contigs for each highly divergent region. After that, each extracted read is then mapped to the assembled contig with the highest alignment score by BWA MEM. We also map the contigs to the IMGT reference using Blastn v.2.3.0. Last, we re-build the alignment between the extracted reads and the IMGT reference by projecting the reads onto the IMGT reference according to aligned loci between the reads and the contig, and between the contig and the IMGT reference.

**Calling variants**

With the reads aligned to the IMGT reference, we identify the variants. We categorized the variants into two types, small variants and long indels. Small variants consist of single nucleotide variants (SNVs) and short indels. Long indels are insertions and deletions longer than 150 bp. We detect both small variants and long indels from the reads alignment. Freebayes v.1.2.0 is used to call small variants, and ScanIndel v.1.3 is adopted to detect long indels. To handle PacBio reads, we also allow adopting pbsv v.2.6.2 to call long indels based on the alignment file processed by pbmm2 v.1.4.0 (https://github.com/PacificBiosciences/pbbioconda). At the variant locus with more than two alleles, if the sum of the frequency of the two highest-frequency alleles is larger than 0.7, we retain the two highest-frequency alleles of the variant. Otherwise, we discard the variant.

**Phasing small variants guided by spectral graph theory**

SpecHLA phases small variants based on spectral graph theory using SpecHap v.1.0. We model the variant phasing problem as a graph-bipartition problem. With $n$ heterozygous variants, we construct an undirected graph $G$ of 2$n$ vertices. Assume each locus has two variants. Each vertex represents an allele of the variant locus (0 or 1). The edge among two vertices of different loci indicates the two variants are from the same haplotype; the edge weight is the logarithmic likelihood of the corresponding haplotype.

Given conflicting haplotype $H_1$, and $H_2$ as the index of the read in the set $R$. Then, we assume the edge weight of conflicting haplotype $H_1$ and $H_2$ as

$$E_{H_1} = \max \left\{ \log \frac{p(H_1)}{p(H_2)} : 0 \right\},$$

$$E_{H_2} = \max \left\{ \log \frac{p(H_2)}{p(H_1)} : 0 \right\}.$$

In this way, we deduce the edge weight between every two variant loci. After the graph construction, the 2$n$ vertices of the graph $G$ can be partitioned into two subgroups by the sign (+/-) of the Fiedler vector. Therefore, we obtain the haplotypes equivalent to vertex subgroups. The locus with the absolute value of the Fiedler vector lower than 1e-5 will not be phased. We illustrate an example of phasing variants based on the Fiedler vector in Figure S1A.

**Incorporating genotype frequency to phase in allelic imbalance samples**

The alleles from different variant loci on the same haplotype should share similar frequencies. Hence, genotype frequency can guide variant phasing in allelic imbalance samples. SpecHLA incorporates genotype frequencies in edge weight computation. Assume $\beta(h_j)$ as the frequency of allele $h_j$ at variant locus $j$. At two variant loci, given a self-complement haplotype pair $H = (h, \bar{h})$, we define the function $f$ as

$$f(H) = \max \left\{ \prod_j \beta(h_j) \cdot \prod_j \beta(h_j) \right\}.$$  

Given conflicting haplotype $H_1$ and $H_2$, we calculate the edge weight from both sequencing reads and genotype frequency as

$$G_{H_1} = (1 - \omega)E_{H_1} + \omega \max \left\{ \log \frac{f(H_1)}{f(H_2)} : 0 \right\}.$$
where \( w \) is hyper-parameter, \( 0 \leq w \leq 1 \), \( w = 0 \) by default. The genotype frequency is incorporated into variant linkage graph construction for variant phasing. Evaluation of SpecHLA with different values of \( w \) can be seen in Figures S2O–S2Q.

**Adopting pedigree relations to phase**

SpecHLA has the capability to enhance phasing results through the utilization of pedigree relations when such relations are accessible. We refine the phasing outcome by establishing a connection between the child’s unlinked blocks guided by the parents’ phased blocks. Moreover, at each phased block of the child, we employ parental locus linkage as a reference, enabling the correction of the child’s haplotype in instances where conflicts arise.

Specifically, let \( S \) represent the child’s phased blocks, \( F \) denote the father’s phased blocks, and \( M \) denote the mother’s phased blocks. SpecHLA identifies connections between distinct phased blocks of the child based on parental blocks. Let \( S_i \) and \( S_j \) represent two unconnected phased blocks of the child. If a parental block \( \phi \in \{F, U, M\} \) encompasses both blocks, we consider a link to exist between \( S_i \) and \( S_j \). Two types of links may exist: (i) direct link from \( S_i \) to \( S_j \), and (ii) link from \( S_i \) to the flipped version of \( S_j \). If the parental blocks support different link types, SpecHLA assigns a score \( \sigma \) to each link type and determines the final link type with a higher \( \sigma \) value. The calculation of \( \sigma \) involves examining the variant sites in the overlap region of \( \phi \), for \( S_i \) and \( S_j \) separately. Consider one haplotype of a parental block \( \phi \). If the alleles from the child and parents are the same at a site, \( \sigma \) is incremented by one; otherwise, it is decremented by one. This process is repeated for the other haplotype of \( \phi \) (i.e., the complement haplotype), and the maximum value is selected as the final \( \sigma \) score.

SpecHLA can also correct falsely phased variants of children using parental phased blocks. If a variant within the child-phased block conflicts with both the paternal and maternal haplotypes, SpecHLA identifies this site as wrongly phased and flips it. For instance, consider a child’s phased block with two variant sites: the first haplotype is \([0,1]\), and the other haplotype is \([1,0]\). If the variant sites in the overlap region of \( \phi \) with \( S_i \) and \( S_j \) separately. Consider one haplotype of a parental block \( \phi \). If the alleles from the child and parents are the same at a site, \( \sigma \) is incremented by one; otherwise, it is decremented by one. This process is repeated for the other haplotype of \( \phi \) (i.e., the complement haplotype), and the maximum value is selected as the final \( \sigma \) score.

SpecHLA uses known HLA alleles from the IMGT/HLA database to phase unlinked blocks based on spectral graph theory. The procedure is based on the assumption that a haplotype with higher similarity to known HLA alleles is more likely to be correct. To construct the linkage graph, we denote the self-complement haplotype pair of each phased block as two vertices. The edge indicates that the haplotypes should be linked. We merge the haplotypes from two blocks and align the merged haplotype \( h \) to known HLA alleles. For each mapped allele, we calculate an alignment score \( \gamma \) according to the mapping identity \( I \) and the mapped length \( L \) by \( \gamma = IL \). \( \delta(h) \) represents the highest \( \gamma \) value among the mapped alleles of \( h \). At two blocks, for a self-complement merged haplotype pair \( H = (h, \bar{h}) \), we have

\[
\delta(H) = \delta(h) + \delta(\bar{h}).
\]  

(Equation 8)

The edge weight of conflicting haplotype \( H_1 \) and \( H_2 \) is defined as \( \delta(H_1) \) and \( \delta(H_2) \), respectively. Afterward, unlinked blocks are phased by partitioning the vertices with the Fiedler vector.

**Phasing long indel loci**

We link the long indel to the small variant phased haplotypes. The long insertion sequences and the IMGT representative reference are combined to generate a modified reference. The modified reference is split into segments based on the breakpoints of long indels. In particular, if a deletion overlaps with another, it would be divided into two segments at the overlap boundary. The segments can be classified into three categories: insertion, deletion, and regular. We align reads to the modified reference using BWA MEM. The copy number of long indels is estimated based on the reads alignment. We compute the average depth of all regular segments (\( \mu \)) and each insertion segment (\( \sigma \)). The copy number of the insertion segment is deduced as two if \( \sigma / \mu > 0.85 \) and one otherwise. Phasing of small variants takes place within insertion segments characterized by a copy number of two. For each deletion segment, the unmapped ratio (representing the proportion of zero-depth regions) is assessed. A copy number of zero is inferred for deletion segments if the unmapped ratio exceeds 0.2; otherwise, it is determined as one.

The heterozygous long indels are phased with the supporting reads. This involves the collection of reads that independently support each haplotype phased for small variants. Additionally, the determination of which allele is supported by the reads at each long indel locus allows for a linkage to a haplotype featuring a greater number of shared supporting reads. The diploid sequences are generated with phased small variants and long indels. Finally, we slide a window of 20 bp along the gene; the window with a mean depth less than \( \zeta \) is masked with ‘N’ (\( \zeta \) is five by default).

**Sequence inference of the HLA-DRB1 duplication**

The region spanning 3,900 to 4,400 bp within the HLA-DRB1 locus harbors a long duplication in the IMGT representative reference. In response to this challenge, a strategic approach was implemented: homologous sequences of this region were extracted from the entire ensemble of HLA-DRB1 alleles contained within the IMGT/HLA database. Through subsequent multiple sequence alignments, a discernable set of unique sequences emerged from this process. Among these, a judicious selection was made of the eight most indicative sequences, which collectively constituted a targeted regional reference database. For a given sample, we extract reads
mapped to this region and methodically align them to this reference database. The two sequences commanding the highest rankings in terms of coverage and depth are then chosen for inclusion. These selected sequences are seamlessly integrated into the phased haplotypes. Within each haplotype, the sequence possessing the greatest aligned read count is identified. The net outcome of these procedures culminates in the comprehensive reconstruction of full-length diploid sequences of the HLA-DRB1 locus.

**Reporting HLA official designations**

SpecHLA assigns each reconstructed sequence an official designation corresponding to the best-matched allele sourced from the IMGT/HLA database. To achieve this assignment, a comprehensive comparison is conducted between the reconstructed sequence and all alleles documented in the database, leveraging Blastn. During this process, a sequence is annotated with the official designation of the allele exhibiting the highest identity to this sequence. In instances where multiple alleles share the same highest identity, the allele endowed with the highest ethnicity-dependent prior frequency is prioritized as the best-matched allele. Notably, all alleles achieving the highest identity are duly reported in the results. Furthermore, we undertake a direct alignment of the inferred HLA sequences with the exon database to extract G group resolution annotations.

**Detecting LOH events in cancer samples**

To detect LOH events in cancer samples, we first compute haplotype frequencies (\(\alpha_1\) and \(\alpha_2\)) with the genotype frequency of phased small variants. At each variant locus \(j\), there are two alleles 0 and 1, and the frequency of allele 1 is denoted as \(\beta_j\). Values can be observed from sequencing data and deduced from the haplotype. The observed allele frequency \(\hat{\beta}_j\) is obtained from the VCF file at each locus \(j\). Moreover, we deduce the expected allele frequency \(\hat{\beta}_j\) with phased genotype \(g_j = (g_{1,j}, g_{2,j})\), where \(g_{1,j}, g_{2,j} \in \{0, 1\}\). Alleles are on the first haplotype, and \(g_{2,j}\) are on the other haplotype. We have

\[
\hat{\beta}_j = g_{1,j} \times \alpha_1 + g_{2,j} \times \alpha_2.
\] (Equation 9)

The observed and expected \(\beta\) values should be the same in the ideal setting. To infer haplotype frequencies, we minimize the difference between observed and expected \(\beta\) values at all loci. Assume the number of small variant loci is \(n\), and our optimization objective is

\[
\text{argmin} \sum_{j=1}^{n} (\beta_j - \hat{\beta}_j)^2.
\] (Equation 10)

Based on the least squares method, the haplotype frequencies can be estimated by

\[
\alpha_1 = \frac{\sum_{j=1}^{n} [g_{1,j} \times \hat{\beta}_j + (1 - g_{1,j}) \times (1 - \hat{\beta}_j)]}{n},
\] (Equation 11)

\[
\alpha_2 = \frac{\sum_{j=1}^{n} [g_{2,j} \times \hat{\beta}_j + (1 - g_{2,j}) \times (1 - \hat{\beta}_j)]}{n}.
\] (Equation 12)

Based on the inferred haplotype frequencies, we then calculate the copy number of two alleles \((\epsilon_1, \epsilon_2)\) at each HLA locus. Calculating the copy number of HLA alleles should consider tumor purity because the cancer tissue is contaminated by normal cells. With tumor purity \(\rho\) and tumor ploidy \(\psi\) predicted by ABSOLUTE v1.2,\(^{40}\) we have the following equations:

\[
\epsilon_1 + \epsilon_2 = \psi,
\] (Equation 13)

\[
\frac{\epsilon_1}{\epsilon_2} = \frac{\epsilon_1 \times \rho + 1 - \rho}{\epsilon_2 \times \rho + 1 - \rho}.
\] (Equation 14)

The copy number of the two alleles is then

\[
\epsilon_1 = \frac{\alpha_1 \times \rho \times \psi - \alpha_1 \times \rho + \alpha_2 \times \rho + \alpha_1 - \alpha_2}{\rho}.
\] (Equation 15)
The minor allele with a copy number less than 0.5 \((\min(\epsilon_1, \epsilon_2) < 0.5)\) is identified as the lost allele.

**Long-read based HLA typing**

Long reads are binned to the HLA loci using a method similar to short-read binning. Given the long-read data, SpecHLA aligns the reads to the HLA allele database using Minimap2 \(^{42}\) with the parameter \(-p 0.1 -N 100000\) allowing multiple alignments. We then calculate the identity for each read alignment. If a read is mapped to the alleles of only a single HLA locus, we assign the read to the locus. If the read is mapped to several loci, we assign the read to a locus that (i) has the highest sequence identity and (ii) the identity difference between the locus and the second-highest-identity locus is greater than the value 0 (default to 0.001).

We then map the binned reads to the IMGT representative reference of each locus using Minimap2 \(^{42}\) with the default parameter. Subsequently, we adopt Longshot v0.4.1 \(^{47}\) to detect and phase SNVs with the default setting. Since Longshot is unable to handle indels, the indels are ignored. We generate the diploid sequence of each gene using the consensus function of bcftools. \(^{48}\) Finally, the diploid sequences are mapped to the IMGT/HLA database to obtain the official designations.

**Benchmark datasets**

**1000 Genomes Project data**

We downloaded 183 WES samples, 230 WGS samples, and 462 RNA-seq samples from the 1000 Genomes Project to evaluate SpecHLA. All the samples contain validated 2-field HLA typing results for HLA-A, -B, -C, and -DRB1; and 71 WGS, 127 WES, and 356 RNA-seq samples possess validated results for HLA-DQB1. The sequencing reads from these samples underwent refinement using the fastp tool v.0.20.0. \(^{43}\) Furthermore, to validate SpecHLA’s sequence reconstruction and pedigree relations integration abilities, we downloaded two family trios from the 1000 Genomes Project (NA12878-NA12891-NA121892 and NA19238-NA19239-NA19240).

**IHKG cell line WES data**

The IHKG cell line WES data were obtained from the NCBI BioProject with the accession number BioProject: PRJEB6763, and 89 of these samples have validated 2-field HLA typing results. \(^{30}\) The mean sequencing depth of the MHC region is 59x in this dataset. To evaluate the robustness of SpecHLA, the data were subsampled at various sequencing depths, specifically at 10x, 15x, 20x, 30x, 35x, and 40x. This subsampling approach allows for the assessment of SpecHLA’s performance under different sequencing depth conditions.

**HGSVC2 project data**

To assess SpecHLA in full-resolution HLA typing, we collected 32 WGS samples with PE data from the HGSVC2 project. Previous research has generated haplotype-resolved assemblies for these samples by combining the long-read technology and single-cell sequencing. \(^{18}\) The haplotype-resolved assemblies were applied as ground truth. Further, seven out of the 32 samples possess matched PacBio HiFi data. We also collected the PacBio and ONT data for the sample NA12878. These samples were used to evaluate SpecHLA’s ability in incorporating long reads.

To obtain the HLA allele sequences from the haplotype-resolved assemblies of HGSVC2 samples, we aligned each assembly onto the IMGT/HLA database using Minimap2. \(^{42}\) As 20 samples have validated 2-field HLA types, we removed the alleles that did not fit the validated HLA types for these samples. Next, we identified the allele that best matched the assembly. Specifically, we collected the matched length and identity of each allele. If the allele with the longest matched length also had the highest identity, we selected it as the best-matched allele. However, if the allele with the longest matched length \(A_m\) differed from the allele with the highest identity \(A_i\), we used the following criteria to choose the best-matched allele: (i) if the identity score of \(A_m\) is less than 99.9%, we select \(A_i\); (ii) if the matched length difference ratio of \(A_m\) and \(A_i\) is smaller than the identity difference ratio, we choose \(A_i\); (iii) If the identity difference ratio between \(A_m\) and \(A_i\) is less than 0.5%, we select \(A_m\). We only consider the subsequent criteria if the previous ones are not satisfied. Finally, we extracted the assembly sequence that mapped to the best-matched allele as the ground truth for sequence inference assessment.

**Synthetic cancer data**

We simulated cancer data by mixing exonic reads of cancer cell lines and matched normal cells to evaluate SpecHLA’s LOH detection ability. We downloaded the WES data of three cancer cell lines (CRL-2314, CRL-5915, and CRL-5922) and matched normal cells. \(^{27}\) We sub-sampled the cancer cell line data and the matched normal data and combined them to achieve different tumor purity (from 0.1 to 1, step by 0.1). Ten replicates were generated for each tumor purity. Together, it led to 300 samples; among them, there are 200 samples with LOH events, as only CRL-2314 and CRL-5922 have HLA LOH events.

**Synthetic data of different protocols**

To validate SpecHLA, we simulated PE data with different sequencing parameters and generated 50 replicates for each parameter combination (sequencing depth: 10x, 20x, 30x, 50x, and 100x; read length: 75bp, 90bp, 100bp, and 150bp; sequencing error rate: 0% and 1%). For each sample, we included 31 homologous genes and pseudogenes such as HLA-H and HLA-DRB3 (see Table S1). Two alleles were randomly selected from the IMGT/HLA database for each gene. Then DWGSIM was utilized to simulate PE...
sequencing reads (https://github.com/nh13/DWGSIM). To assess SpecHLA in typing rare alleles, we simulated a dataset comprising HLA alleles that are not included in the lite IMGT/HLA database (50 replicates; sequencing depth: 100x; read length: 150bp; sequencing error rate: 0%). For the purpose of contrasting the performance of SpecHLA and HISAT-genotype in deducing new alleles, we simulated a set of 50 samples. These samples were created by introducing ten random SNVs to the IMGT representative reference, thus generating novel alleles for detection.

To evaluate the effectiveness of SpecHLA in incorporating genotype frequency, we conducted tests using three distinct datasets. Initially, we utilized the 200 LOH samples simulated from cancer cell lines, and subsequently simulated two additional datasets. In the first dataset, we simulated an allelic imbalance dataset with 10 replicates for each combination of parameters, including read lengths of 75bp, 90bp, 100bp, and 150bp, as well as sequencing error rates of 0% and 1%. This resulted in a total of 80 samples. Each gene within these samples consisted of one allele with a depth of 20x and another allele with a depth of 80x. The alleles were randomly selected from the database. For the second dataset, we introduced random SNVs to the IMGT reference allele at a rate of 0.2%, generating novel alleles. These novel alleles were then employed to simulate samples with varying allelic depths, specifically: 20x-80x, 30x-70x, 40x-60x, 48x-52x, and 50x-50x. This simulation was performed 20 times, using a fixed read length of 75bp and a sequencing error rate of 0%. Consequently, a total of 100 samples were generated.

To test SpecHLA in adopting different sequencing protocols, we generated the sequencing reads of 10x, Hi-C, PacBio, and ONT. By introducing random SNVs at a rate of 0.1% to the IMGT representative reference, we effectively created new alleles, which in turn served as the basis for simulating a cohort of 50 individuals. Given the relatively sparse variant density, the challenge arose in acquiring ample phasing evidence for PE reads. Diverse read-generation protocols were then applied to each individual. We simulated the data of 10x, Hi-C, PacBio, and ONT using LRSIM, sim3C, PBSIM, and NanoSim, respectively (detailed parameters provided in Table S2). Minimap2 was applied for aligning PacBio and ONT reads. We used BWA MEM with parameter -S to align Hi-C data and employed LongRanger (https://support.10xgenomics.com/genome-exome/software/downloads/latest) for aligning 10x linked reads.

**Evaluation methods**

**Assessing 2-field resolution HLA typing accuracy**

To guarantee an equitable comparison of HLA typing, we standardize the obtained typing results from different methodologies to the latest IMGT alleles, employing the most up-to-date file available (https://github.com/ANHIG/IMGTHLA/blob/Latest/Allelelist_history.txt). As the ground truth and inferred HLA types may contain ambiguities, we compare the reference HLA type tuple \( R \) with the inferred HLA type tuple \( I \) using a compatible function \( F \), where \( F(R, I) \) is 1 only if the intersection between \( R \) and \( I \) is not empty. For an HLA locus, suppose the reference HLA type tuples as \( R_1 \) and \( R_2 \), and the inferred HLA type tuples as \( I_1 \) and \( I_2 \). The number of correctly inferred alleles is calculated by

\[
\max(F(R_1, I_1) + F(R_2, I_2), F(R_1, I_2) + F(R_2, I_1)).
\]

The HLA type truth of the 1000 Genomes Project is at 2-field resolution; we thus compare the benchmark methods at 2-field resolution. All of the methods can provide HLA typing results with at least 2-field resolution, with the exception of Kourami, which only generates results at the G group resolution. To solve the problem, we collect all alleles corresponding to the G group resolution HLA type of Kourami to evaluate its accuracy at 2-field resolution.

**Metrics for assessing reconstructed HLA sequences**

To assess the reconstructed sequence, we align it to the true sequence using Blastn. Since Blastn is a local alignment method, the alignment fragments might overlap. We remove the redundant alignment fragments. The number of mismatches \( M \) and the total number of gaps \( G \) on the non-redundant alignment fragments are counted. Assume the total length of the non-redundant alignment fragments as \( A \). We measure the mismatches and gaps in the alignment using mismatch rate and gap rate, respectively, which are calculated by

\[
mismatch rate = \frac{M}{A},
\]

\[
gap rate = \frac{G}{A}.
\]

Moreover, we define sequence recall and sequence precision to measure the inferred sequence’s completeness and contamination. With the length of the true sequence \( E \) and the inferred sequence \( I \), sequence recall and sequence precision are deduced as

\[
sequence recall = \frac{A}{E},
\]

\[
sequence precision = \frac{A}{L}.
\]

In addition, we employ edit distance to comprehensively measure the difference between the true and inferred sequences, which is obtained by the Python module edlib. We calculate the mean value of the diploid sequences in a sample for each metric.
Assessing HLA sequence reconstruction

We compared the full-resolution HLA typing utility of SpecHLA and HISAT-genotype in the HGSVC2 dataset. The extracted HLA allele sequences of HGSVC2 samples were used as ground truth to assess the sequence reconstruction accuracy. We counted the number of samples with reconstructed sequences for each HLA locus separately. In addition, we evaluated the exon reconstruction accuracy of SpecHLA in the HGSVC2 dataset. The alleles in the same G group have identical exons encoding the peptide binding domains (exon 2 and 3 for MHC class I and exon 2 only for MHC class II alleles). We compared the HLA typing accuracy of SpecHLA, Kourami, HISAT-genotype, HLA-VBSeq, HLA*LA, and HLA-HD at G group resolution. The methods were executed with default settings. The typing results of all methods were converted to G group resolution using the same G grouping file (date: 2023-01-12). The ground truth G group resolution HLA types were inferred by mapping the phased assemblies of HGSVC2 samples onto the HLA exons database using Minimap2.42

In assessing trio-consistency of two family trios, the exclusion of HISAT-genotype was necessary due to its inability to successfully recover alleles for all trio members of a given HLA locus, rendering an assessment of trio consistency unfeasible. To gauge sequence accuracy, we opted to select the most closely inferred sequence from the parental pool for each child’s sequence. A comparative analysis of these two sequences was performed, employing metrics such as mismatch rate and gap rate. Notably, a lower mismatch rate and gap rate indicate a heightened level of trio consistency.

Additionally, the two trios were employed to assess SpecHLA’s prowess in integrating pedigree relations for phasing. This evaluation involved separate runs of SpecHLA on the trios, both with and without pedigree relations. A subsequent comparison of sequence accuracy between these runs was undertaken. During the SpecHLA runs, the guidance of block linking from the database was intentionally omitted (-b 0). Moreover, to exemplify SpecHLA’s ability to effectively integrate short and long reads, SpecHLA was also carried out with -b 0.

Computational resource comparison

We conducted a comparative analysis of the computational resource utilization across various HLA typing tools on a LINUX cluster featuring 8-core processors, a 143 MB cache, and a total of 16 TB global shared memory. We employed a random simulation involving a sample with PE150 reads, a sequencing depth of 50x, and a 1% sequencing error rate. Each tool was executed five times, and we documented both the CPU time and the peak RAM usage for each individual run of all the tools.

Benchmark tool parameter setting

We compared SpecHLA with ten state-of-the-art and widely used tools: Polysolver, OptiType, HLA-VBseq, Kourami, HISAT-genotype, HLA-HD, HLA*LA, arcasHLA, DASH, and LOHHLA. The default parameters were employed for running these tools (details provided in Table S3). Running LOHHLA to detect LOH was based on the HLA typing results of OptiType, as the authors of LOHHLA suggested. In the simulated datasets, the alleles were chosen from the database randomly, so we ran SpecHLA without considering the HLA population frequency using nonuse. We employed our read extraction procedure to obtain HLA-related reads for all the tools to save computational resources. The extracted reads were mapped to the HG19 reference as input for Polysolver and HLA*LA.

QUANTIFICATION AND STATISTICAL ANALYSIS

All procedures involving statistical analysis were described in the Method details section. The number of samples used in each experiment was described in both the figure legend and Results sections.