ChimeraLM filters amplification artifacts for accurate structural variant calling in long-read single-cell sequencing

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Abstract

Single-cell genomics provides unprecedented insights into cellular heterogeneity, but Whole Genome Amplification (WGA)—required to obtain sufficient DNA—introduces chimeric artifacts that generate false-positive Structural Variations (SVs) and undermine biological interpretations. Current computational methods cannot reliably distinguish amplification-induced artifacts from genuine rearrangements. Here we present ChimeraLM, a genomic language model that learns sequence-level features to discriminate biological sequences from WGA artifacts. Validated on nanopore long-read data, ChimeraLM achieves 95% recall with 70% precision, reduces chimeric reads by ∼90%, and preserves 72–92% of true SVs. This improves SV validation rates 8.5–11.0 fold and eliminates artifactual inversion (INV) bias, restoring SV type distributions to bulk-like profiles. Attention visualization reveals that ChimeraLM focuses on chimeric junction regions, learning mechanistically interpretable features that generalize across sequencing platforms. By enabling reliable SV detection at single-cell resolution, ChimeraLM addresses a critical data quality barrier in cancer genomics, developmental biology, and somatic mosaicism studies. ChimeraLM is available at https://github.com/ylab-hi/ChimeraLM.

Keywords: Whole Genome Amplification, Single Cell, Genomic Language Model, Structural Variation

Main

Single-cell and ultra-low-input genomics have transformed our ability to resolve biological heterogeneity, enabling the discovery of rare cell states and the reconstruction of clonal evolution in cancer and development [1–3]. However, the limited DNA input (on the order of picograms per cell) makes comprehensive genome-wide profiling technically challenging [4, 5]. Whole Genome Amplification (WGA) therefore remains a prerequisite for high-coverage sequencing [6–8], yet it introduces systematic errors that compromise genomic fidelity, particularly for Structural Variation (SV) detection [9–11].

A prominent source of error is amplification-induced chimera formation, in which highly processive polymerases—such as phi29 used in Multiple Displacement Amplification (MDA)—switch templates and join discontinuous genomic loci into a single molecule [9–13]. In long-read sequencing, which is otherwise well suited for resolving complex SVs, chimeric reads can constitute a substantial fraction of WGA data [9], generating alignment patterns that resemble genuine translocations (TRAs) and inversions (inversions (INVs)) [10]. As a result, SV callers that rely on alignment-based signals (for example, split-read and supplementary alignments) and coverage-derived evidence frequently misinterpret amplification artifacts as true rearrangements, inflating false positives and distorting SV spectra [14–21].

Distinguishing biological rearrangements from amplification artifacts remains a major computational bottleneck. Existing quality-control approaches typically rely on handcrafted rules or alignment-derived features, such as read orientation signatures or local coverage anomalies [11, 13, 22]. These heuristics are often sensitive to platform- and protocol-specific variation and fail to capture sequence-intrinsic patterns near chimera junctions as well as long-range context within individual reads. This limitation has constrained the practical use of low-input long-read sequencing in applications where precision is essential, including somatic mosaicism profiling and validation of CRISPR off-target effects.

To address this challenge, we present ChimeraLM, an interpretable Genomic Language Model (GLM) for single-read identification and filtering of WGA-induced artifacts. Unlike traditional approaches that depend on alignment heuristics, ChimeraLM formulates artifact detection as a sequence modeling task, learning discriminative features directly from raw DNA sequences [23]. Leveraging advances in DNA foundation models [24–27], it captures latent motifs and structural dependencies that generalize across nanopore platforms. Across nanopore WGA datasets, ChimeraLM reduces chimeric reads by ∼90% while preserving 72–92% of bulk-supported SVs, improving SV validation rates by 8.5- to 11.0-fold and restoring bulk-like SV-type distributions. By enabling reliable SV discovery in long-read sequencing, ChimeraLM removes a critical data-quality barrier for single-cell genomics.

Results

Overview of ChimeraLM workflow and model architecture

Single-cell long-read genomics requires WGA to obtain sufficient DNA for sequencing (Fig. 1a). However, WGA introduces artifacts that generate chimeric reads composed of segments from distant genomic loci, which can confound SV detection. ChimeraLM integrates into this workflow as a post-alignment filtering module (Fig. 1a). It evaluates each chimeric read prior to variant calling, predicting whether the sequence reflects a genuine genomic rearrangement or a WGA-induced artifact. This binary classification enables the selective removal of false positives while preserving authentic biological signal.

To train and evaluate the model, we constructed a high-confidence labeled dataset using two WGA datasets generated from the same monoclonal PC3 lineage on independent Oxford Nanopore Technologies (ONT) platforms. The ONT PromethION WGA dataset was designated for model training, whereas the ONT MinION WGA dataset was reserved exclusively for the generalization test. For supervised labeling, chimeric reads from the PromethION WGA dataset were compared against unamplified bulk DNA sequenced on three long-read platforms (ONT PromethION, ONT MinION, and Pacific Biosciences (PacBio) (see Methods; Fig. 1b; Extended Data Fig. 1a)). WGA reads were labeled biological if their chimeric structures were supported by any bulk dataset, and artificial if they were absent from all bulk references.

This labeling procedure yielded two groups of WGA chimeric reads (Extended Data Fig. 1b). Most reads—12,670,396 in total—showed no supporting alignments in any bulk dataset and were classified as artificial. The remaining 293,180 reads had at least one matching breakpoint in bulk sequencing, providing evidence that they represent biological rearrangements rather than amplification artifacts. To construct the supervised training dataset, we retained all 293,180 biological reads and selected an equal number of artificial reads through random subsampling. We further expanded the biological class by adding 178,748 chimeric reads sampled directly from bulk sequencing data, which represent genuine structural rearrangements independent of WGA-induced artifacts. This augmentation ensures that the classifier is exposed to a broader spectrum of true biological chimeric structures. The final labeled dataset (765,108 reads) was split into training (70%), validation (20%), and test (10%) sets using stratified sampling.

Effective classification of WGA chimeric reads requires a model that can process long, variable-length DNA sequences while retaining single-nucleotide resolution (Fig. 1c). ChimeraLM addresses these challenges through a sequence encoder based on HyenaDNA [24], a genomic foundation model pre-trained on diverse DNA sequences. Input reads are tokenized at single-nucleotide resolution and processed by Hyena operators [28], which are designed to model long-range dependencies on long reads while still allowing full-length analysis without splitting the sequence. The encoder outputs a matrix of hidden states across the read. To obtain a fixed-length representation, an attention-pooling module (Fig. 1d) assigns learned, position-specific weights and computes a weighted sum over the sequence. The pooled vector is then processed by MLP

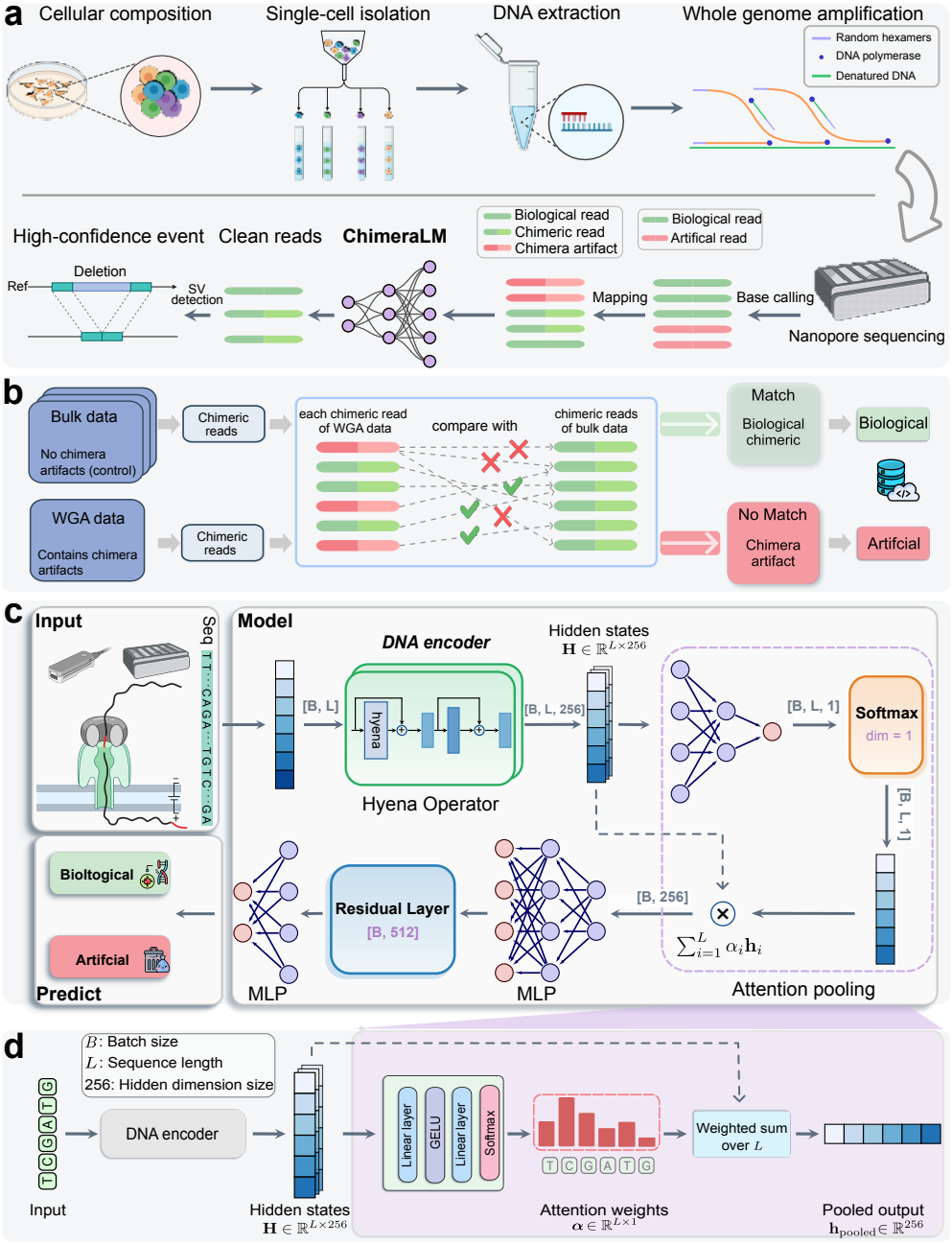


Fig. 1 ChimeraLM workflow and architecture for detecting WGA artifacts. (a) Single-cell genomic workflow and ChimeraLM integration. Single cells are isolated, followed by DNA extraction and WGA. During amplification, chimeric artifacts (red) are generated alongside biological reads (green). After base calling and mapping, ChimeraLM classifies chimeric reads as biological or artificial, enabling downstream SV detection on filtered data. **(b)** Ground truth label generation. Chimeric reads from WGA data are compared against chimeric reads from bulk sequencing of the same cell line. Reads matching bulk data are labeled biological (green); non-matching reads are labeled artificial (red). **(c)** ChimeraLM architecture. Input DNA sequences (batch size B, sequence length L) are tokenized at single-nucleotide resolution and encoded into hidden states $\mathbf{H} \in \mathbb{R}^{L\times 256}$ through DNA encoder (HyenaDNA [24]). Hyena operators capture long-range dependencies. Attention pooling aggregates position-specific features, and multilayer perceptron (MLP) layers with residual connections process pooled representations for binary classification. **(d)** Attention pooling mechanism. Attention weights $\boldsymbol{\alpha} \in \mathbb{R}^{L\times 1}$ are computed through linear layers with GELU activation and softmax normalization, assigning importance scores to each position. The weighted sum produces a fixed-dimensional representation $\mathbf{h}_{\text{pooled}} \in \mathbb{R}^{256}$. Created with BioRender.com.

blocks with residual connections, and a final softmax layer outputs the probability that each read is biological or artificial.

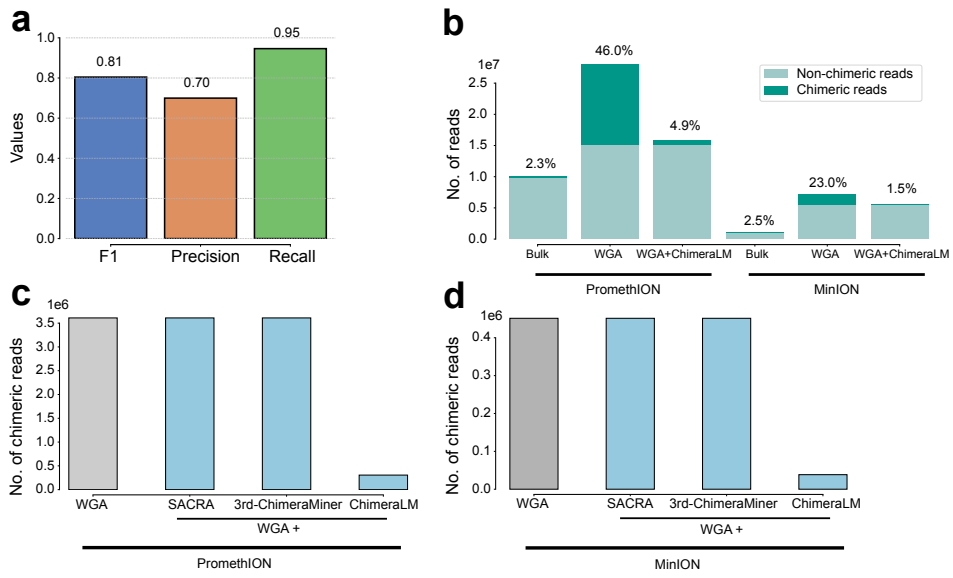


Fig. 2 ChimeraLM accurately identifies and removes WGA-induced chimeric artifacts. **(a)** Classification performance on held-out test data. ChimeraLM achieves recall of 0.95, precision of 0.70, and F1 score of 0.81. **(b)** Chimeric read reduction across sequencing platforms. Stacked bars show proportions of chimeric (dark teal) and non-chimeric (light teal) reads in bulk, WGA, and ChimeraLM-filtered samples. ChimeraLM reduces chimeric read frequencies from 46.0% to 4.9% (PromethION) and from 23.0% to 1.5% (MinION), approaching bulk levels (2.3% and 2.5%, respectively). **(c,d)** Benchmarking against existing methods on PromethION (c) and MinION (d). The bar represents the total count of chimeric reads. ChimeraLM achieves approximately 90% reduction in chimeric reads on both platforms; SACRA and 3rd-ChimeraMiner show no detectable reduction.

ChimeraLM achieves high accuracy and reduces artifacts to near-bulk levels across platforms

We first evaluated ChimeraLM on the held-out test set derived from the labeled dataset (Fig. 2a; see Methods). This test set comprises chimeric reads with known biological or artificial status based on the ground-truth labeling procedure described above. On this benchmark, ChimeraLM achieved an F1 score of 0.81, with a recall of 0.95 and a precision of 0.70. The high recall indicates that 95% of artificial chimeric reads were correctly identified and removed, which is critical for minimizing downstream false-positive SV calls, while the precision confirms that most flagged reads correspond to true artifacts rather than biological rearrangements.

We next asked whether ChimeraLM filtering could restore chimeric read rates in full PC3 WGA datasets to bulk baselines on both PromethION and MinION platforms (Fig. 2b). Bulk sequencing established low baseline chimeric read rates of 2.3%

(PromethION) and 2.5% (MinION). In contrast, WGA increased the chimeric fraction to 46.0% and 23.0%, respectively. After ChimeraLM filtering, chimeric content dropped to 4.9% on PromethION and 1.5% on MinION, corresponding to 10- to 15-fold reductions, while retaining 15.8 million and 5.6 million biological reads. These post-filtering rates approach bulk baselines, indicating effective removal of WGA-induced artifacts while preserving authentic biological signal.

We benchmarked ChimeraLM against SACRA [22] and 3rd-ChimeraMiner [13], two existing tools for detecting amplification-induced chimeras (Fig. 2c,d). ChimeraLM achieved approximately 90% reduction in chimeric reads on both platforms, whereas neither SACRA nor 3rd-ChimeraMiner produced a detectable reduction (0%).

The MinION results are particularly informative because this platform was never used during model training. ChimeraLM was trained exclusively on PromethION WGA data, yet achieved comparable chimeric read reduction on MinION. This cross-platform generalization indicates that ChimeraLM captures sequence-level features intrinsic to WGA-induced artifacts rather than platform-specific signatures, supporting its potential applicability to additional long-read and short-read sequencing technologies.

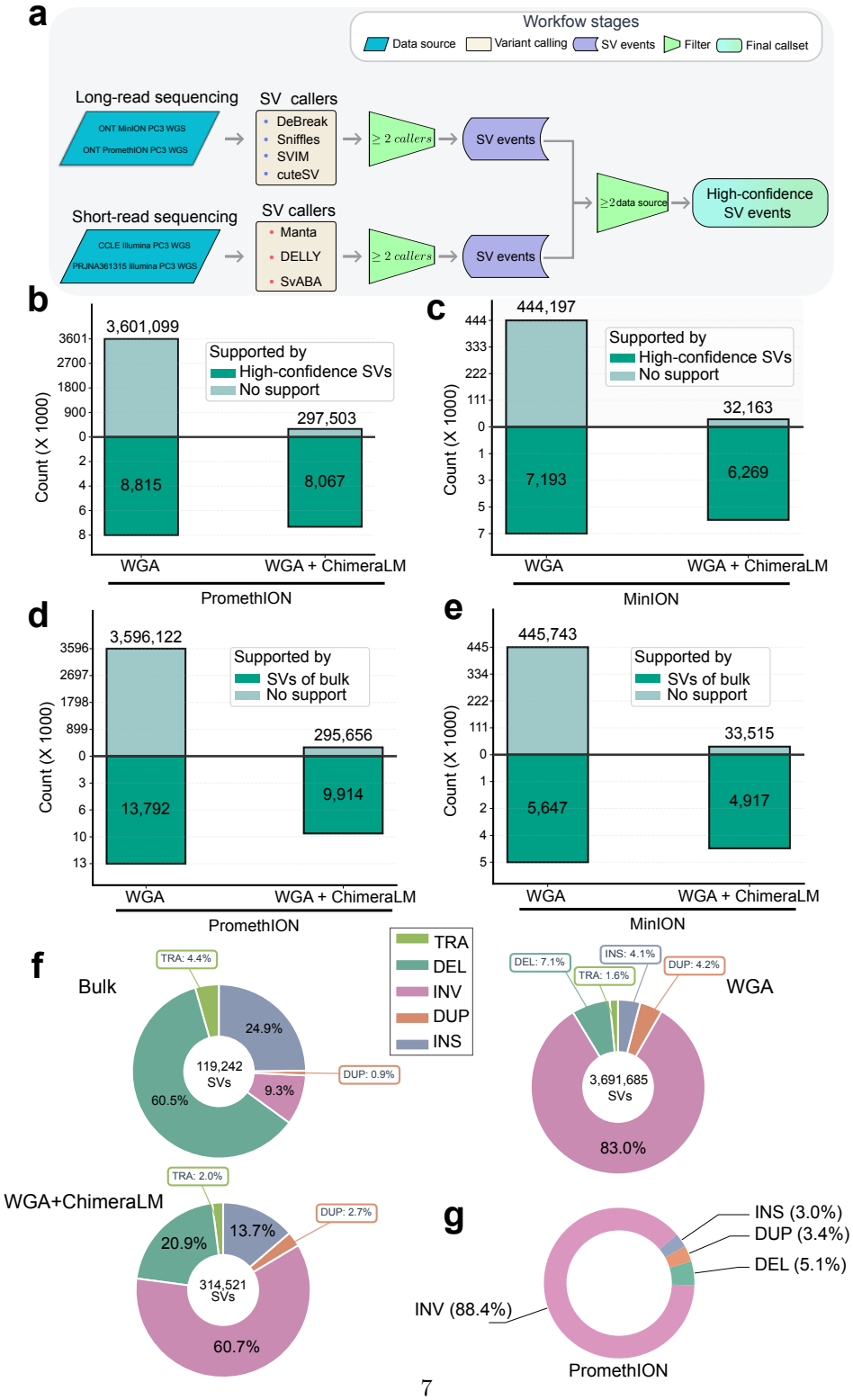


Fig. 3 ChimeraLM improves structural variant detection accuracy. (a) Construction of a high-confidence SV reference dataset from bulk PC3 sequencing. Four bulk datasets were integrated: ONT MinION Mk1C, ONT PromethION P2, the CCLE Illumina Whole Genome Sequencing (WGS) dataset, and the PRJNA361315 Illumina WGS dataset. SVs were called independently within each dataset using multiple callers, and events supported by ≥2 callers per dataset were retained. SVs were then compared across datasets, and events observed in ≥2 of the four bulk datasets were designated as gold-standard SVs. **(b,c)** SV validation against the gold-standard reference for PromethION (b) and MinION (c). Bars show SV calls supported by the gold standard (dark teal) or unsupported (light teal). **(d,e)** SV validation against platform-matched long-read bulk sequencing for PromethION (d) and MinION (e), capturing true long-read SVs that may not be represented in the multi-platform reference. Bars show SV calls supported by the platform-matched long-read bulk data (dark teal) or unsupported (light teal). **f** SV type distributions for PromethION across bulk, unfiltered WGA, and WGA+ChimeraLM. Unfiltered WGA shows an excess of INVs, which is reduced after ChimeraLM filtering. **g** Composition of artifact-supported SVs for PromethION. Donut charts summarize SV types among events supported exclusively by chimeric reads, representing artificial SVs preferentially removed by ChimeraLM.

ChimeraLM substantially reduces unsupported structural variant calls

Accurate SV detection from single cells is essential for understanding genomic diversity and disease mechanisms. However, WGA-induced chimeric artifacts can be misidentified as genuine SVs, leading to incorrect biological conclusions. To quantify ChimeraLM's impact on SV calling, we compared SV callsets generated from unfiltered WGA reads with those generated after ChimeraLM filtering (WGA + ChimeraLM). We evaluated both callsets against two complementary references (Fig. 3): (i) a stringent gold-standard SV set derived from bulk PC3 DNA by cross-dataset consensus (Fig. 3a), and (ii) platform-matched long-read bulk SV callsets used as a platform-specific reference for recall (Fig. 3d,e).

We first constructed a high-confidence gold-standard SV set from bulk PC3 DNA using four independent sequencing datasets: ONT PromethION, ONT MinION, and two Illumina whole-genome datasets (the CCLE PC3 WGS dataset and PRJNA361315 PC3 WGS dataset) (Fig. 3a; Extended Data Table 1). SVs were called separately within each dataset using multiple SV callers. Events supported by at least two callers within a dataset were retained, and only SVs observed in at least two of the four datasets were kept as gold-standard events.

Relative to this stringent gold standard, unfiltered WGA produced extensive unsupported SVs (Fig. 3b,c). On PromethION, WGA yielded 3,601,099 SV calls, of which only 8,815 (0.24%) overlapped gold-standard events. After ChimeraLM filtering, total calls dropped to 305,570 while retaining 8,067 gold-standard events (91.5% retention), increasing the validation rate to 2.64% (11-fold) (Fig. 3b). On MinION, calls decreased from 451,390 to 38,432, while gold-standard-supported events decreased from 7,193 to 6,269, corresponding to 87.2% retention. The validation rate increased from 1.59% to 16.3% (10-fold) (Fig. 3c).

Because the gold standard is intentionally stringent and may miss true SVs detectable only in long-read data, we next performed platform-matched validation using long-read bulk sequencing from the same platform (Fig. 3d,e). This analysis provides a platform-specific estimate of recall and reduces bias introduced by the strict gold-standard definition. ChimeraLM increased validation rates from 0.38% to 3.24% on PromethION (8.5-fold) and from 1.25% to 12.79% on MinION (10-fold), while retaining 71.9% and 87.1% of bulk-supported events, respectively. Together, these results show that ChimeraLM removes an order of magnitude of unsupported SV calls while preserving the majority of bulk-supported variants across platforms.

ChimeraLM restores bulk-like SV-type distributions

Amplification artifacts can distort the apparent spectrum of SVs. We therefore compared SV type distributions across bulk, unfiltered WGA, and ChimeraLM-filtered datasets on both nanopore platforms (Fig. 3f; Extend Data Fig. 2). Bulk sequencing showed a balanced mixture of deletions (DELs), duplications (DUPs), insertions (INSs), INVs, and TRAs. In contrast, unfiltered WGA callsets were dominated by INVs on both platforms. After ChimeraLM filtering, excessive INVs were markedly

reduced, and the overall SV type profile shifted toward the bulk distribution, while the relative proportions of other SV classes remained largely stable.

To identify which SV types were primarily driven by WGA-induced artifacts, we examined SV calls supported exclusively by reads classified as chimera artifacts (Fig. 3g; Extend Data Fig. 3). These artifact-supported events were overwhelmingly INVs, accounting for 88.4% on PromethION and 92.4% on MinION. The remaining calls included smaller fractions of DELs (5.1% and 3.8%), DUPs (3.4% and 2.4%), and INSs (3.0% and 1.4%), indicating that WGA-induced chimeras can generate false positives across multiple SV categories.

Together, these results show that WGA artifacts preferentially inflate INVs but are not limited to a single SV class. By selectively removing artifact-supported events and restoring SV type distributions toward bulk-like patterns, ChimeraLM improves the robustness and interpretability of single-cell SV analyses.

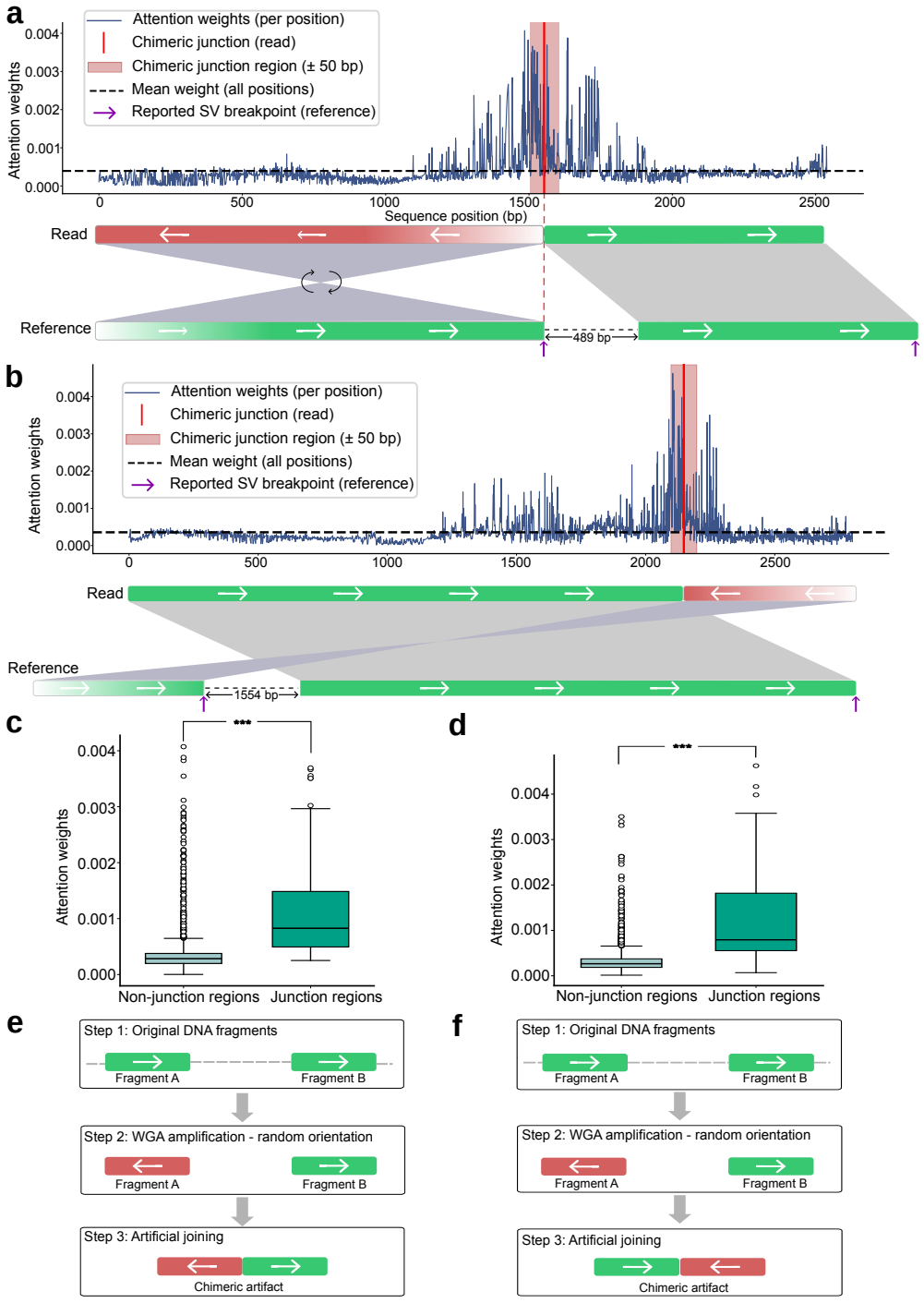


Fig. 4 ChimeraLM attention weights are enriched at chimeric junction regions. **(a,b)** Attention weight profiles for two representative chimeric reads exhibiting distinct junction configurations. Upper panels show per-position attention weights (blue) with the mean attention across the read indicated by a dashed line. Red vertical lines mark inferred chimeric junction positions, and pink shading denotes the junction-centered region (±50 bp). Lower panels display read-level alignments, highlighting orientation transitions at the junctions (green, forward orientation; red, reverse-complemented orientation). **(c,d)** Quantitative comparison of attention weights between junction and non-junction regions. Junction-centered windows show significantly elevated attention weights relative to non-junction regions ($P = 5.3 \times 10^{-14}$ and $P = 6.8 \times 10^{-15}$; Wilcoxon rank-sum test). **(e,f)** Schematic illustration of WGA-induced chimera formation. During amplification, DNA fragments originating from distant genomic loci can be amplified in either orientation, joining them into a single molecule with discordant orientations, producing INV-like alignment signatures. The two examples illustrate forward-to-reverse and reverse-to-forward orientation transitions.

Attention visualization reveals interpretable classification features

We examined whether ChimeraLM's attention weights provide an interpretable signal by focusing on mechanistically relevant regions of chimeric reads, particularly the junctions during WGA (Fig. 4). We inspected two representative chimeric reads exhibiting distinct junction configurations (Fig. 4a,b). In both cases, attention remained relatively flat across most positions but formed sharp, concentrated peaks at the inferred junction regions. These peaks aligned with the read-level breakpoint separating two genomic loci and coincided with an orientation transition between adjacent alignment segments.

To quantify this effect, we compared attention weights within junction-centered windows (±50 bp) against weights from non-junction regions (Fig. 4c,d). Junction windows showed significantly higher attention weights (Wilcoxon rank-sum test, $P = 5.3\times10^{-14}$ and $P = 6.8\times10^{-15}$), indicating that ChimeraLM preferentially emphasizes sequence context proximal to chimeric junctions.

This attention enrichment is consistent with the expected structure of WGA-induced chimeras (Fig. 4e,f): DNA fragments from distant loci can be amplified in either orientation and subsequently joined, generating junctions with discordant orientations. Together, these results suggest that ChimeraLM's attention peaks provide a mechanistically interpretable signal that concentrates classification evidence to junction-proximal sequence positions within individual reads.

Discussion

WGA enables genomic analysis from single cells but introduces chimeric artifacts that compromise SV detection. ChimeraLM addresses this challenge by classifying chimeric reads as biological or artificial from sequence information and filtering WGA-induced artifacts before variant calling, rather than attempting to correct artifact-driven calls post hoc. Across nanopore platforms, ChimeraLM yielded consistent improvements at both read and variant levels. It reduced chimeric reads by ∼90% while retaining 72–92% of bulk-supported SVs, and it lowered unsupported SV calls by 8.5–11.0 fold. Performance generalized from PromethION (used for training) to MinION without platform-specific retraining, indicating that ChimeraLM captures properties shared by WGA-induced artifacts rather than instrument-specific signatures. In contrast, SACRA and 3rd-ChimeraMiner failed to reduce chimeric content on our long-read WGA datasets, underscoring the limitations of heuristic strategies and indicating the need for models that learn discriminative features directly from sequence data.

The efficacy of ChimeraLM highlights the utility of deep learning in quality control tasks where conventional metrics (e.g., mapping quality, read depth) provide limited resolution [13, 22, 23]. By learning directly from sequence data, ChimeraLM discovers subtle compositional and structural features that differentiate authentic sequences from amplification artifacts. The model also offers interpretability through attention visualization: attention weights concentrate at junction regions where template switching joins discordant loci, validating the biological relevance of the learned features. These methodological advances have direct implications for single-cell genomics,

where high false-positive rates in WGA data have constrained robust characterization of chromosomal instability, clonal evolution, and SV burden [20, 29]. By improving the signal-to-noise ratio and clarifying SV-type spectra that are otherwise distorted by amplification artifacts, ChimeraLM enables more confident identification of genuine SVs, supporting studies of cancer evolution, developmental biology, and somatic mosaicism where single-cell resolution is essential.

Several limitations warrant consideration. First, the current model processes reads independently; integrating contextual features such as coverage or phasing information may further enhance accuracy. Second, regarding computational resources, while Central Processing Unit (CPU) inference is feasible, Graphics Processing Unit (GPU) acceleration is recommended for processing large-scale datasets. Finally, future work should extend validation to diverse cell types, sequencing platforms (e.g., PacBio HiFi), and alternative WGA protocols—including Multiple Annealing and Looping-based Amplification Cycles (MALBAC) [30], Linear Amplification via Transposon Insertion (LIANTI) [5], Primary Template-directed Amplification (PTA) [19], and droplet-based MDA (dMDA) [31]. Although the sequence-level approach implies effective transferability, such broad validation is essential to optimize performance across specific amplification chemistries.

Broadly, ChimeraLM illustrates the potential of GLMs for genomic data quality control. This framework could extend to other amplification-dependent technologies, such as cell-free DNA analysis, ancient DNA studies, and metagenomics from low-biomass samples. Furthermore, attention-based interpretability opens opportunities for studying template-switching dynamics, potentially guiding the development of improved amplification protocols. In summary, ChimeraLM provides a practical and interpretable framework for enhancing long-read single-cell genomic fidelity, ensuring that downstream biological insights are derived from genuine SVs rather than technical artifacts.

Methods

Cell culture, single-clone preparation, and nanopore sequencing

Cell culture and single-clone establishment

PC3 prostate cancer cells (ATCC® CRL-1435™) were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum and 1% penicillin–streptomycin at 37 °C with 5% CO_2. To minimize biological heterogeneity, a monoclonal population was established by serial dilution in 96-well plates, ensuring that each culture originated from a single cell. Mycoplasma contamination was routinely tested and confirmed negative prior to DNA extraction.

DNA extraction and whole-genome amplification

From the monoclonal population, two types of DNA samples were prepared: a bulk (non-amplified) control and ten single-cell MDA-amplified genomes. Bulk high-molecular-weight DNA was extracted using the Monarch® HMW DNA Extraction Kit for Cells & Blood (New England Biolabs). Individual cells were isolated using

1CellDish-60 mm (iBiochips) and amplified using the REPLI-g Advanced DNA Single Cell Kit (Qiagen) following the manufacturer's protocol. DNA concentration and fragment integrity were assessed with a Qubit 4 fluorometer and Agilent TapeStation (DNA 1000/5000 ScreenTape). Only samples meeting quality standards were used for library construction.

Nanopore library preparation and sequencing

Libraries were prepared using the ONT Ligation Sequencing Kit V14 (SQK-LSK114) and sequenced on MinION Mk1C or PromethION P2 Solo devices with R10.4.1 flow cells following the manufacturer's genomic DNA workflow. Because all single-cell samples originated from the same monoclonal lineage, differences between amplified and bulk datasets primarily reflect MDA-induced artifacts rather than biological variation.

Basecalling and read processing

POD5 files were basecalled using Dorado v0.5.0 with the high-accuracy model `dna_r10.4.1_e8.2_400bps_hac@v4.3.0` [32]. Reads with mean quality < 10 or length < 500 bp were removed. Adapters and concatemers were trimmed using Cutadapt v4.0 [33] in a two-pass, error-tolerant procedure. Filtered reads were aligned to the GRCh38.p13 reference genome using minimap2 v2.26 (`map-ont` preset) [34]. BAM files were sorted and indexed using SAMtools v1.16 [35]. Read-length and mapping statistics were computed using NanoPlot v1.46.1 [36]. All samples were processed using identical parameters.

Chimeric read identification

Chimeric reads were identified from BAM files using Supplementary Alignment (SA) tags. Reads were classified as chimeric if they (i) were mapped, (ii) contained an SA tag, (iii) were primary alignments (not secondary), and (iv) were not supplementary alignments themselves. This definition counts each chimeric read once using its primary alignment while excluding secondary/supplementary records, thereby avoiding double-counting and reducing ambiguity from low-confidence alignments. Reads lacking SA tags were classified as non-chimeric.

Training data construction

Data generation and sources

To construct the training dataset, we generated WGA and bulk sequencing data from PC3 cells. The WGA sample was amplified and sequenced on the PromethION P2 platform (ONT), while three independent bulk datasets were produced from non-amplified genomic DNA: bulk PromethION P2, bulk MinION Mk1c (ONT), and bulk PacBio. These bulk datasets represent authentic biological sequences free from amplification-induced artifacts. In contrast, WGA sequencing includes both genuine genomic reads and artificial chimeras introduced during the amplification process.

Ground truth annotation and class definition

Ground truth labels were established by systematically comparing chimeric reads from the WGA PromethION P2 dataset against those from the three bulk datasets. For each WGA chimeric read, all alignment segments—defined by their genomic start and end coordinates—were compared to the corresponding segments of bulk chimeric reads. A WGA read was labeled as biological if every segment matched at least one bulk chimeric read within a 1 kb positional tolerance, indicating that the structural configuration is also present in non-amplified DNA. Reads lacking any matching pattern across all bulk datasets were labeled as artificial chimeras, presumed to arise from the amplification process. Additional chimeric reads were randomly sampled from the bulk datasets and labeled as biological, as these reads originate from genuine genomic rearrangements such as true SVs. The final labeled dataset combined the annotated WGA PromethION P2 reads with the subsampled bulk chimeric reads and was subsequently partitioned into training, validation, and test sets as described below.

Dataset partitioning and cross-platform validation

The combined labeled dataset, derived from WGA PromethION P2 and bulk sequencing data, was divided into training (70%), validation (20%), and test (10%) sets using stratified random sampling. These subsets were used respectively for model training, hyperparameter tuning, and performance evaluation on data from the same sequencing platform.

To evaluate cross-platform generalization, the complete WGA MinION Mk1c dataset was reserved. This dataset, generated on a different nanopore platform, was never used during model training or internal testing. This two-level evaluation design allowed us to test whether ChimeraLM captures general sequence features of amplification-induced chimeras rather than platform-specific artifacts.

Model architecture

DNA encoder

ChimeraLM employs the pre-trained HyenaDNA model [24] as its DNA encoder. This model was pre-trained on large-scale genomic data and provides robust sequence representations. DNA sequences are tokenized at single-nucleotide resolution, with each base (A, C, G, T, N) mapped to a unique integer token (7, 8, 9, 10, 11, respectively). Special tokens include [CLS]=0, [PAD]=4, and others for sequence processing. Input sequences are truncated at 32,768 bp or padded to enable batch processing.

For a tokenized input sequence $\mathbf{x} \in \mathbb{Z}^L$, the HyenaDNA generates contextualized hidden representations:

$$\mathbf{H} = \text{HyenaDNA}(\mathbf{x}) \in \mathbb{R}^{L \times 256}$$

where $\mathbf{H} = (\mathbf{h}_1, \mathbf{h}_2, \ldots, \mathbf{h}_L)$ represents position-wise hidden states with dimension 256. The Hyena operators [28] efficiently capture both local sequence motifs and long-range dependencies essential for distinguishing biological sequences from chimeric artifacts.

Attention pooling

To aggregate variable-length sequence representations into fixed-size vectors, ChimeraLM implements attention-based pooling. For hidden states $\mathbf{H} \in \mathbb{R}^{L\times 256}$, attention weights are computed through a two-layer network:

$$\begin{aligned}\mathbf{e} &= \text{GELU}(\text{Linear}_{256\to 256}(\mathbf{H})) \in \mathbb{R}^{L\times 256}\\ \mathbf{s} &= \text{Linear}_{256\to 1}(\mathbf{e}) \in \mathbb{R}^{L\times 1}\\ \boldsymbol{\alpha} &= \text{softmax}(\mathbf{s}) \in \mathbb{R}^{L\times 1}\end{aligned}$$

The pooled representation is the weighted sum of hidden states:

$$\mathbf{h}_{\text{pooled}} = \sum_{i=1}^{L} \alpha_i \mathbf{h}_i \in \mathbb{R}^{256}$$

This mechanism assigns learned importance weights to each sequence position, enabling the model to focus on informative regions while accommodating natural variability in read lengths.

Classification head

The pooled representation is processed through a MLP with residual connections. The first layer expands dimensionality:

$$\mathbf{f}_1 = \text{Dropout}_{0.1}(\text{GELU}(\text{Linear}_{256\to 512}(\mathbf{h}_{\text{pooled}}))) \in \mathbb{R}^{512}$$

Subsequent residual blocks with input $\mathbf{f}_{\text{in}} \in \mathbb{R}^{512}$ compute:

$$\mathbf{f}_{\text{out}} = \text{Dropout}_{0.1}(\text{Linear}_{512\to 512}(\text{GELU}(\text{Linear}_{512\to 512}(\mathbf{f}_{\text{in}})))) + \mathbf{f}_{\text{in}}$$

where the skip connection enables stable gradient flow during training. The final layer produces binary classification logits:

$$\mathbf{z} = [z_0, z_1] = \text{Linear}_{512\to 2}(\mathbf{f}_{\text{final}}) \in \mathbb{R}^2$$

where z_0 and z_1 represent logits for biological and artificial chimeric classes, respectively. During inference, the predicted class is $\hat{y} = \text{argmax}_{i\in\{0,1\}}\, z_i$.

Model summary

The complete ChimeraLM pipeline processes DNA sequences through: (1) single-nucleotide tokenization, (2) HyenaDNA backbone encoding to generate contextualized representations, (3) attention pooling to aggregate position-specific features, (4) MLP layers with residual connections to learn classification features, and (5) binary classification output. The entire model is trained end-to-end using labeled data.

Model training and optimization

Training configuration

ChimeraLM was trained using PyTorch [37] and PyTorch Lightning [38] frameworks. Input sequences were tokenized using the tokenizer with maximum sequence length of 32,768 bp. Sequences longer than this threshold were truncated; shorter sequences were padded to enable batch processing. Training employed mixed-precision computation (bf16) to accelerate training while maintaining numerical stability.

Optimization procedure

We used the AdamW optimizer [39] with learning rate $\eta = 1 \times 10^{-4}$ and weight decay $\lambda = 0.01$. AdamW implements adaptive learning rates with decoupled weight decay, combining the benefits of Adam optimization with proper L2 regularization. A ReduceLROnPlateau scheduler dynamically adjusted the learning rate based on validation loss, reducing it by a factor of 0.1 when no improvement occurred for 10 consecutive epochs. Early stopping with patience of 10 epochs prevented overfitting by terminating training when validation performance plateaued. A fixed random seed (12345) ensured reproducibility across training runs.

The training objective used cross-entropy loss for binary classification. For a training example with class label $y \in \{0, 1\}$ and model logits $\mathbf{z} = [z_0, z_1]$, the loss is:

$$\mathcal{L}(\mathbf{z}, y) = -\log\left(\frac{\exp(z_y)}{\exp(z_0) + \exp(z_1)}\right) = -z_y + \log(\exp(z_0) + \exp(z_1))$$

where z_0 and z_1 represent logits for biological and artificial chimeric classes, respectively.

Training implementation

Training used batch size of 16 sequences with 30 parallel data loading workers. GPU acceleration was employed for efficient processing, with training typically requiring 55 hours. Model checkpointing saved the best-performing model based on validation metrics. Configuration management used Hydra [40] to enable reproducible experimentation.

Model evaluation

Performance was monitored using precision, recall, and F1 score on the validation set after each epoch:

$$\text{Precision} = \frac{\text{TP}}{\text{TP} + \text{FP}}, \quad \text{Recall} = \frac{\text{TP}}{\text{TP} + \text{FN}}$$
$$\text{F1} = \frac{2 \times \text{Precision} \times \text{Recall}}{\text{Precision} + \text{Recall}}$$

where TP (true positives) are chimeric reads correctly classified as artificial, TN (true negatives) are biological reads correctly classified as biological, FP (false positives)

are biological reads misclassified as artificial, and FN (false negatives) are artificial reads misclassified as biological. Final model selection was based on best validation performance as determined by early stopping.

Model inference and application

Inference pipeline

To apply ChimeraLM to new WGA sequencing data, the model takes a BAM file as input. Chimeric reads are identified using SA tags and filtered to exclude unmapped, secondary, or supplementary alignments. Each chimeric read sequence is tokenized using the tokenizer (maximum length 32,768 bp, with truncation or padding as needed). The trained model processes sequences in batches, generating two logits $[z_0, z_1]$ for each read corresponding to biological and artificial chimeric classes. Classification is determined by $\hat{y} = \text{argmax}(z_0, z_1)$. ChimeraLM outputs a filtered BAM file containing only reads classified as biological, which can be directly used for downstream analyses including SV calling.

Performance evaluation

Test set evaluation

Final model performance was evaluated on the held-out test set and the independent MinION Mk1c dataset. Metrics (precision, recall, and F1 score) were computed as described in the training section, where true positives represent chimeric reads correctly classified as artificial and true negatives represent biological reads correctly classified as biological.

SV calling

SVs were called using multiple tools to ensure comprehensive detection. For long-read data (ONT PromethION P2 and MinION Mk1c), we used Sniffles v2.5 [14, 15], DeBreak v1.2 [16], SVIM v2.0.0 [17], and cuteSV v2.1.1 [18]. For short-read data of the PC3 cell line, we used both the CCLE Illumina WGS dataset and the PRJNA361315 Illumina WGS dataset, processed with Manta v1.6.0 [41], DELLY v1.5.0 [42], and SvABA v1.1.0 [43]. All tools were executed with default recommended parameters.

Gold standard SV dataset construction

To evaluate the impact of ChimeraLM on SV detection accuracy, we generated a high-confidence gold-standard SV set from bulk PC3 sequencing data. All SV comparisons and breakpoint corrections were performed using OctopuSV v0.2.3 [44]. Four bulk datasets were integrated: ONT MinION Mk1c, ONT PromethION P2, the CCLE Illumina WGS dataset, and the PRJNA361315 Illumina WGS dataset. SVs were called independently within each dataset, and events supported by at least two SV callers were retained. The remaining calls were then compared across datasets, and SVs observed in at least two of the four datasets were designated as gold-standard events for benchmarking.

SV benchmarking analysis

To assess the impact of ChimeraLM on SV calling accuracy, we compared SV calls from unfiltered WGA data and ChimeraLM-filtered WGA data against two references: (1) the stringent multi-platform gold standard dataset, and (2) platform-matched long-read bulk sequencing data. Benchmarking was performed using Truvari v4.2.2 [45] with default parameters. SVs were considered supported if they matched reference variants within the defined breakpoint tolerance. Validation rates were calculated as the proportion of called SVs supported by the reference. This dual benchmarking strategy quantifies both improvements in detecting high-confidence multi-platform SVs and the retention of platform-specific true variants.

Benchmarking against existing methods

ChimeraLM was compared to two existing computational methods for detecting amplification-induced chimeric artifacts: SACRA [22] (GitHub commit 9a2607e) and 3rd-ChimeraMiner [13] (GitHub commit 04b5233). Both tools were applied to WGA data from PromethION P2 and MinION Mk1c platforms using default parameters as recommended in their documentation. Performance was evaluated by measuring the percentage reduction in chimeric reads relative to unprocessed WGA data. Chimeric reads were identified using WGA tag-based alignment criteria (reads with SA tags indicating split alignments), and reduction rates were calculated as the proportion of chimeric reads removed by each method.

Attention weight analysis

To investigate ChimeraLM's interpretability, we analyzed attention weights from the pooling mechanism for representative chimeric reads. Attention weights indicate the relative importance assigned to each sequence position during classification. For selected reads, we extracted per-position attention weights and visualized them alongside read alignments to identify whether the model focuses on mechanistically relevant regions.

Chimeric junction positions were identified from alignment data (defined by breakpoints in SA tags). A region of ± 50 bp surrounding each junction was designated as the junction region. Attention weights within junction region were compared to non-junction regions using the Wilcoxon rank-sum test [46], with statistical significance assessed at $p < 0.001$.

Data visualization

Figures were generated using Python with Matplotlib [47] and Seaborn [48].

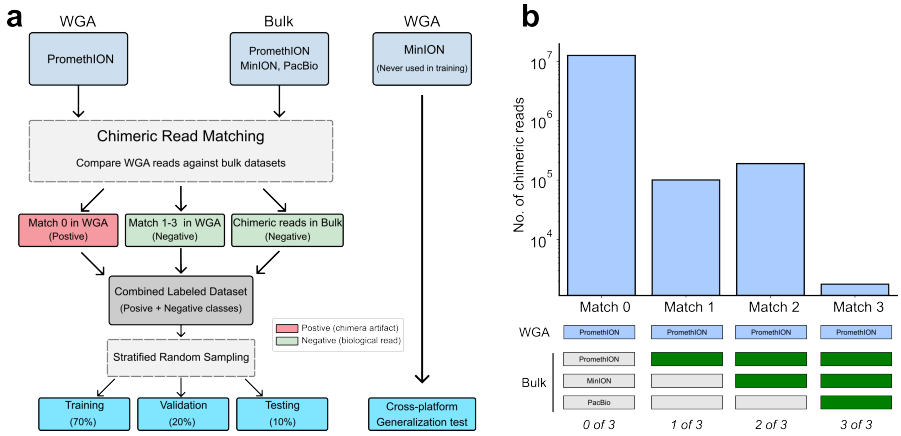
Computing resources

Computations were performed on a High Performance Computing (HPC) server with 64-core Intel Xeon Gold 6338 CPU, 256 GB RAM, and two NVIDIA A100 GPUs (80 GB memory each).

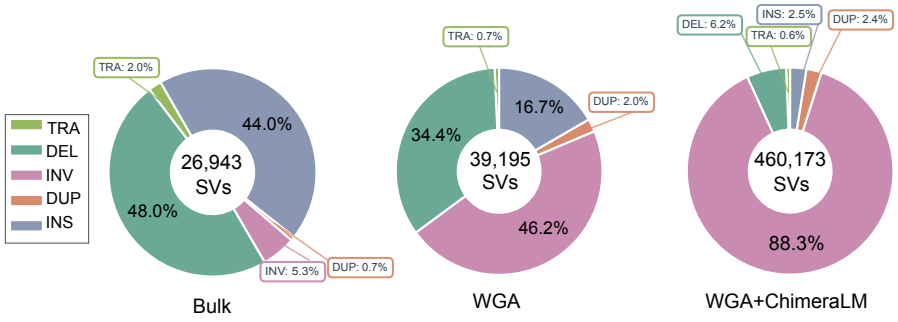
Supplementary information.

Extended Data Table 1 Sequencing and alignment statistics of PC3

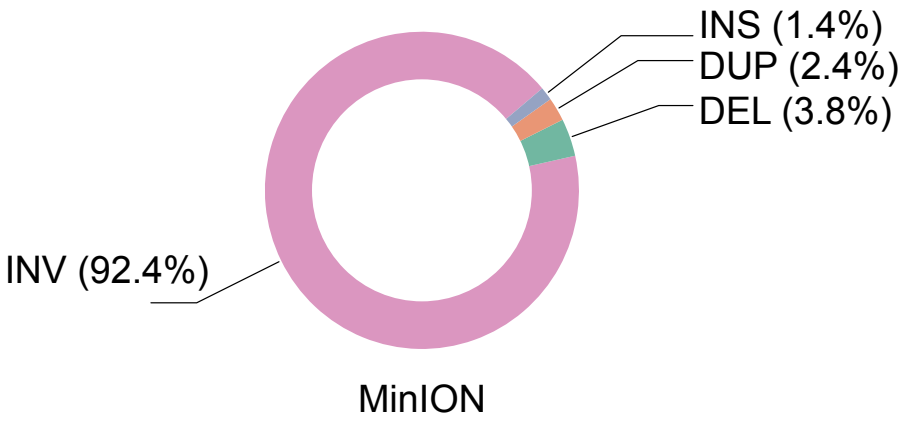
Sample	Platform	Reads ($\times 10^6$)	Total bases (Gb)	Total bases aligned (Gb)	Fraction aligned	Mean length (bp)	Mean quality (Q)	Average identity (%)
WGA	MinION	9.11	14.6	10.4	0.7	1,603	14.3	97.6
WGA	PromethION	44.69	128.2	69.2	0.5	2,869	14.5	96.1
Bulk	MinION	0.97	8.1	7.1	0.9	8,310	17.2	97.3
Bulk	PromethION	8.00	69.9	62.4	0.9	8,732	18.5	97.7



Extended Data Fig. 1 Training dataset construction and ground-truth labeling strategy. **(a)** Workflow for generating labeled training data. WGA PromethION data is compared against three independent bulk sequencing datasets (PromethION, MinION, and PacBio). Reads with no bulk matches (Match 0) are labeled artificial; reads matching one or more bulk datasets (Match 1–3) are labeled biological, along with chimeric reads sampled directly from bulk data. The labeled dataset is split into training (70%), validation (20%), and test (10%) sets. The WGA MinION dataset is reserved for independent cross-platform evaluation. **(b)** Distribution of chimeric read matches. Bar chart shows the number of WGA PromethION chimeric reads (log scale) by bulk dataset matches. Match 0 reads ($\sim 10^7$) lacking bulk validation are classified as artificial; Match 1–3 reads with bulk support are classified as biological. The substantial imbalance reflects high prevalence of WGA-induced artifacts.



Extended Data Fig. 2 SV type distributions for MinION across bulk, unfiltered WGA, and WGA+ChimeraLM. Unfiltered WGA shows an excess of INVs, which is reduced after ChimeraLM filtering.



Extended Data Fig. 3 Composition of artifact-supported SVs for MinION. Donut charts summarize SV types among events supported exclusively by chimeric reads, representing artificial SVs preferentially removed by ChimeraLM.

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Declarations

Author Contributions. YL, QG and RY designed the study. YL and QG performed the analysis. QG performed the experiments. YL and QG designed and implemented the model. YL built the command-line tool and documentation. YL, QG and RY wrote the manuscript. RY supervised this work.

Data Availability. The raw sequencing data generated in this study have been deposited in the NCBI Sequence Read Archive (SRA) under BioProject accession PRJNA1354861. The dataset includes Oxford Nanopore long-read whole-genome sequencing of PC3 prostate cancer cells and MDA-amplified single-cell derivatives. The individual SRA accessions are as follows: PC3 bulk (MinION Mk1C), SRR35904028; PC3 bulk (PromethION P2), SRR35904029; PC3 10-cell WGA (MinION Mk1C), SRR35904026; PC3 10-cell WGA (PromethION P2), SRR35904027. We can access the data at the following link: https://dataview.ncbi.nlm.nih.gov/object/PRJNA1354861?reviewer=viej6cv6mgbli3n7a9a5k1bsb3

Code Availability. ChimeraLM, implemented in Python, is open source and available on GitHub (https://github.com/ylab-hi/ChimeraLM) under the Apache License, Version 2.0. The package can be installed via PyPI (https://pypi.org/project/chimeralm) using pip, with wheel distributions provided for Windows, Linux, and macOS to ensure easy cross-platform installation. An interactive demo is available on Hugging Face (https://huggingface.co/spaces/yangliz5/ChimeraLM), allowing users to test DeepChopper's functionality without local installation. For large-scale analyses, we recommend using ChimeraLM on systems with GPU acceleration. Detailed system requirements and optimization guidelines are available in the repository's documentation (https://ylab-hi.github.io/ChimeraLM/).

Conflict of interest. RY has served as an advisor/consultant for Tempus AI, Inc. This relationship is unrelated to and did not influence the research presented in this study.

Acronyms

CPU Central Processing Unit 12

DEL deletion 8, 9
dMDA droplet-based MDA 12
DUP duplication 8, 9

GLM Genomic Language Model 2, 12
GPU Graphics Processing Unit 12, 16, 18, 21

HPC High Performance Computing 18

INS insertion 8, 9
INV inversion 1, 2, 7–10, 20

LIANTI Linear Amplification via Transposon Insertion 12

MALBAC Multiple Annealing and Looping-based Amplification Cycles 12
MDA Multiple Displacement Amplification 2
MLP multilayer perceptron 3, 4, 15

ONT Oxford Nanopore Technologies 3, 7, 8, 13, 17

PacBio Pacific Biosciences 3, 13
PTA Primary Template-directed Amplification 12

SA Supplementary Alignment 13, 17, 18
SV Structural Variation 1–5, 7–9, 11, 12, 14, 17, 18, 20

TRA translocation 2, 8

WGA Whole Genome Amplification 1–14, 17–20
WGS Whole Genome Sequencing 7, 8, 17

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