

ChimeraLM: A genomic language model for detecting whole genome amplification artifacts in single-cell sequencing

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Abstract

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Main

Single-cell genomics has revolutionized our understanding of cellular heterogeneity and development by enabling the characterization of individual cells rather than bulk populations [1, 2]. This approach has proven instrumental in uncovering rare cell types, tracking developmental trajectories, and identifying somatic mutations that drive disease progression. However, the limited amount of DNA present in a single cell, typically only a few picograms, poses significant technical challenges for comprehensive genomic analysis [3, 4].

To overcome this fundamental limitation, [Whole Genome Amplification \(WGA\)](#) has become an essential preprocessing step in single-cell genomic studies [5, 6]. Various

WGA techniques, including Multiple Displacement Amplification (MDA), Multiple Annealing and Looping-based Amplification Cycles (MALBAC), and other emerging methods, can amplify the entire genome from a single cell by several orders of magnitude, generating sufficient DNA material for high-coverage sequencing [7–10]. This amplification enables researchers to achieve the depth and breadth of coverage necessary for reliable variant calling, copy number analysis, and structural variation detection.

Despite its critical role in single-cell genomics, WGA introduces systematic artifacts that can significantly impact downstream analyses [11, 12]. Among the most problematic are chimeric sequences—artificial DNA constructs formed when DNA fragments from different genomic loci are erroneously joined during the amplification process [10–12]. These chimeric artifacts can manifest as false-positive structural variations that do not exist in the original cell [11]. The presence of such artifacts poses a substantial challenge for accurate Structural Variation (SV) detection, potentially leading to misinterpretation of genomic rearrangements and their biological significance.

Current computational approaches for identifying WGA-induced artifacts rely primarily on coverage-based metrics and read-pair orientation patterns [12, 13]. However, these methods often fail to distinguish between genuine structural variations and amplification artifacts, particularly when chimeric sequences exhibit complex rearrangement patterns or occur in repetitive genomic regions [14, 15]. The lack of robust artifact detection methods has limited the reliability of structural variant analysis in single-cell studies and hindered the full realization of single-cell genomics’ potential.

To address these challenges, we developed ChimeraLM, a genomic language model specifically designed to detect chimeric artifacts introduced by whole genome amplification. By leveraging deep learning approaches to capture sequence patterns and contextual information in genomic reads [16–18], ChimeraLM can effectively distinguish between genuine biological sequences and WGA-induced chimeric artifacts. This approach represents a significant advancement in single-cell genomic analysis, offering improved accuracy in artifact detection and enabling more reliable structural variant analysis in single-cell studies. This methodology represents a significant advancement in single-cell genomic analysis, offering a principled approach to improve the reliability of structural variant detection and enable more precise characterization of genomic alterations in individual cells.

In this study, we present ChimeraLM, demonstrate its superior performance compared to existing methods, and illustrate its practical applications in genomic studies.

Results

ChimeraLM integrates seamlessly into single-cell genomic workflows

To systematically address WGA-induced chimeric artifacts, we developed ChimeraLM as an integrated component of single-cell genomic analysis pipelines (Figure 1a). Our approach leverages the standard single-cell workflow, beginning with cellular isolation through FACS or microfluidics-based sorting, followed by DNA extraction and whole

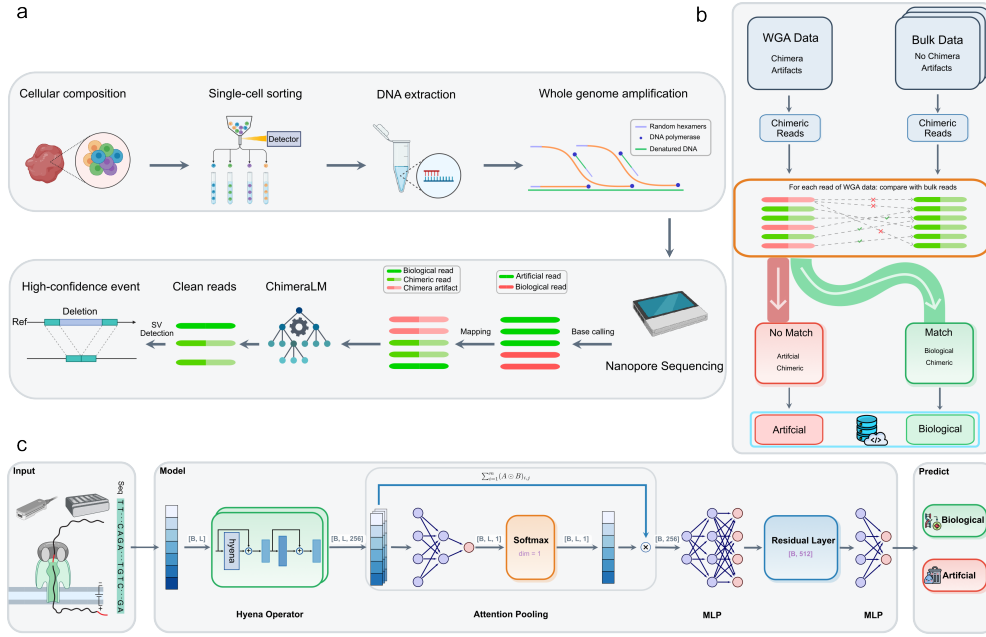


Fig. 1 ChimeraLM workflow and architecture for detecting whole genome amplification artifacts in single-cell sequencing. (a) Integration of ChimeraLM into single-cell genomic analysis workflows. Single cells are isolated from heterogeneous cellular populations through sorting technologies, followed by DNA extraction and WGA to generate sufficient material for sequencing. WGA introduces chimeric artifacts through random hexamers, DNA polymerase extension, and denatured DNA template switching. Sequencing reads from WGA-amplified samples contain both biological reads (green) and chimeric artifacts (red). ChimeraLM processes these reads to classify them as biological or artificial, enabling clean reads to proceed to structural variant analysis for high-confidence event detection such as deletions. (b) Dataset construction strategy for supervised learning. Training data is generated by comparing WGA sequencing reads against matched bulk sequencing data from the same biological sample. Bulk data contains only genuine biological sequences (no chimeric artifacts), while WGA data contains both biological reads and chimeric artifacts. Each WGA read is aligned against bulk data: reads that successfully match are labeled as "biological" (green pathway), while reads that fail to match are labeled as "artificial chimeric" (red pathway). This comparative approach provides reliable ground truth labels for training ChimeraLM in a supervised learning framework. (c) ChimeraLM neural network architecture. Input DNA sequences are tokenized and processed through a deep learning pipeline optimized for genomic sequence analysis. The architecture employs Hyena operators for efficient long-range dependency modeling, followed by attention pooling to aggregate variable-length sequence features. multilayer perceptron (MLP) components with residual connections process the pooled features to learn complex patterns distinguishing biological sequences from chimeric artifacts. The final output layer produces binary classification probabilities, predicting whether each input sequence represents a biological read or an artificial chimeric read.

genome amplification using established protocols. Amplified genomic material is then processed through long-read sequencing platforms such as Nanopore technology to generate comprehensive genomic coverage.

ChimeraLM operates at a critical juncture in the analysis pipeline, positioned between initial read processing and downstream analysis, for example, structural variants detection (Figure 1a). Following standard quality filtering and read cleaning

procedures, ChimeraLM evaluates each sequencing read to classify it as either biological or chimeric artifacts. This binary classification enables the selective retention of authentic genomic sequences while filtering out amplification artifacts before they can impact downstream analyses.

The filtered, high-quality biological reads are subsequently processed through conventional structural variant detection algorithms, enabling the identification of genuine genomic alterations such as deletions, duplications, and other rearrangements. By removing chimeric sequences upstream of variant calling, ChimeraLM ensures that detected structural variants represent true biological events rather than technical artifacts introduced during amplification (Figure 1a).

This workflow design allows ChimeraLM to integrate with existing single-cell genomic pipelines without requiring substantial modifications to established protocols. The method provides a versatile solution for improving the accuracy of single-cell genomic studies across diverse research applications.

Training dataset construction enables supervised learning of chimeric patterns

To train ChimeraLM for accurate chimeric artifact detection, we developed a novel dataset construction strategy that leverages paired WGA and bulk sequencing data from the same biological samples (Figure 1b). This approach exploits the fundamental difference between WGA and bulk sequencing: while WGA data contains both biological reads and chimeric artifacts introduced during amplification, bulk sequencing data from the same sample contains only genuine biological sequences.

Our ground truth labeling strategy compares each chimeric read of WGA data against the corresponding one of bulk sequencing dataset (Figure 1b). Chimeric reads that successfully matched to bulk data with high confidence are classified as “biological” indicating they represent authentic genomic sequences present in the original sample. Conversely, reads that fail to match bulk sequences are labeled as “artificial chimeric” artifacts, as they represent artificial constructs generated during the WGA process rather than genuine genomic content (Figure 1b).

This comparative approach generates a comprehensive labeled dataset where each chimeric read of WGA receives a binary classification based on its presence or absence in the matched bulk control. The resulting dataset captures the full spectrum of chimeric artifacts naturally occurring during WGA while providing reliable ground truth labels for model training (Figure 1b).

Following dataset construction, we partitioned the labeled reads into training, validation, and test sets to ensure robust model development and unbiased performance evaluation. The training set was used for model parameter optimization, the validation set for hyperparameter tuning and model selection, and the test set was reserved for final performance assessment. This rigorous data splitting strategy ensures that ChimeraLM’s reported performance metrics reflect its ability to generalize to previously unseen WGA data.

ChimeraLM architecture leverages modern genomic language modeling advances

ChimeraLM employs a sophisticated neural architecture specifically designed for genomic sequence analysis and chimeric artifact detection (Figure 1 c). The model enables single-base pair resolution and accepts DNA sequences as input, which are first tokenized and encoded into numerical representations suitable for deep learning processing. This encoding preserves the sequential nature of genomic information while enabling efficient computation on modern hardware.

The core of ChimeraLM’s architecture consists of Hyena operators, a recent advancement in sequence modeling that provides computational advantages over traditional transformer attention mechanisms while maintaining the ability to capture long-range dependencies in genomic sequences. Hyena operators enable ChimeraLM to process variable-length sequencing reads efficiently while learning complex patterns that distinguish biological sequences from chimeric artifacts. Moreover, the hyena operators are initialized with HyenaDNA [18], a pre-trained long-context genomic language model, which provides a strong foundation for learning genomic sequence features relevant to chimeric detection.

Following the Hyena operator layers, ChimeraLM incorporates an attention pooling mechanism that aggregates sequence-level features. This pooling strategy allows the model to handle reads of varying lengths while focusing computational attention on the most informative regions for chimeric detection. The attention weights learned during training provide interpretability regarding which sequence features contribute most strongly to classification decisions.

The aggregated features are then processed through multiple MLP components arranged in a residual architecture. This design enables gradient flow optimization during training while allowing the model to learn both low-level sequence motifs and high-level compositional patterns indicative of chimeric artifacts. The residual connections help prevent vanishing gradients and improve model convergence during training on large genomic datasets.

The final output layer produces a binary classification predicting whether each input sequence represents a biological read or an artificial chimeric artifact. This end-to-end architecture enables ChimeraLM to learn directly from raw sequence data without requiring manual feature engineering, allowing the model to discover complex patterns that may not be apparent through traditional bioinformatics approaches.

ChimeraLM achieves high performance in chimeric artifact detection

We evaluated ChimeraLM’s performance on held-out test data to assess its ability to accurately classify biological and chimeric reads (Figure 2a). ChimeraLM demonstrated robust performance across key classification metrics, achieving an F1 score of 0.805, which balances precision and recall for the binary classification task. The model exhibited high recall of 0.946, indicating that it successfully identified 94.6% of true chimeric artifacts in the test dataset, minimizing the risk of retaining false-positive structural variants in downstream analyses. The precision of 0.700 demonstrates that

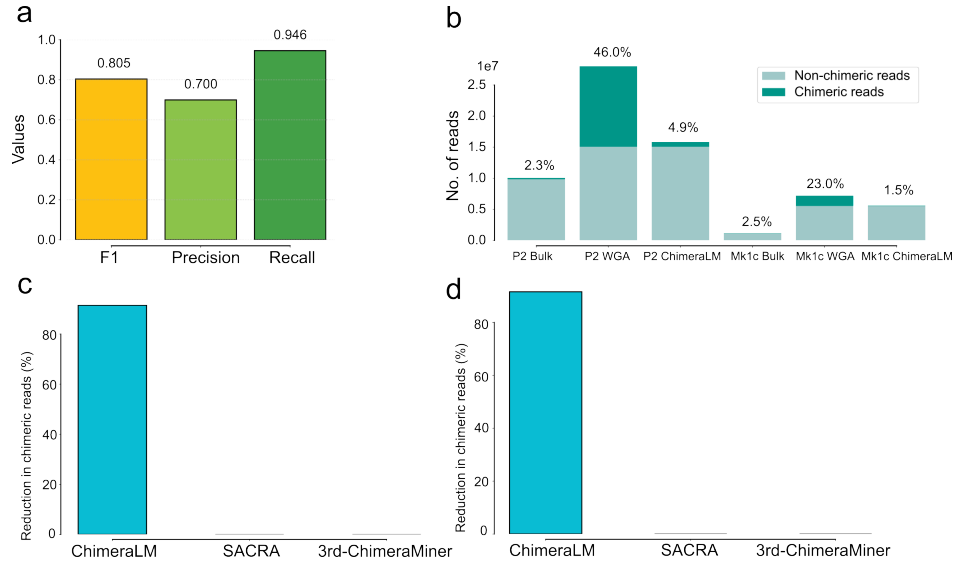


Fig. 2 ChimeraLM performance evaluation and benchmarking against existing methods. (a) ChimeraLM performance metrics on test dataset. Bar chart showing F1 score (0.805), precision (0.700), and recall (0.946) achieved by ChimeraLM on held-out test data for binary classification of biological versus chimera artifact. (b) Chimeric read reduction across sequencing platforms. Stacked bar charts comparing chimeric read proportions (dark teal) versus non-chimeric reads (light teal) across bulk sequencing, WGA, and ChimeraLM-processed samples for PC3 cell line data. Left side shows PromethION P2 platform data with chimeric rates of 2.3% (bulk), 46.0% (WGA), and 4.9% (ChimeraLM). Right side shows MinION Mk1c platform data with chimeric rates of 2.5% (bulk), 23.0% (WGA), and 1.5% (ChimeraLM). (c) Benchmarking on P2 platform data. Percentage reduction in chimeric reads achieved by ChimeraLM (90%) compared to existing computational methods SACRA and 3rd-ChimeraMiner (both 0% reduction). (d) Benchmarking on Mk1c platform data. Percentage reduction in chimeric reads achieved by ChimeraLM (90%) compared to SACRA and 3rd-ChimeraMiner (both 0% reduction) using MinION Mk1c sequencing data.

70.0% of reads classified as chimeric were indeed artifacts, representing an effective trade-off that prioritizes comprehensive artifact removal while maintaining reasonable specificity.

ChimeraLM reduces chimeric artifact burden across sequencing platforms

To evaluate ChimeraLM’s practical impact, we applied the trained model to PC3 cell line data generated on two Nanopore long-read sequencing platforms: PromethION P2 and MinION Mk1c (Figure 2b). The analysis revealed substantial differences in chimeric read proportions between bulk sequencing, WGA samples, and ChimeraLM-processed data.

For P2 platform data, bulk sequencing exhibited a low baseline chimeric rate of 2.3% among 10065403 total reads, consistent with the expected minimal artifact rate in non-amplified samples. In contrast, WGA amplification dramatically increased the chimeric burden to 46.0% of reads in a dataset containing 28027667 total sequences.

ChimeraLM processing effectively reduced this chimeric proportion to 4.9% while retaining 15833834 high-quality biological reads, representing a 10-fold reduction in artifact contamination compared to unprocessed WGA data.

Similar results were observed for Mk1c platform data, where bulk sequencing contained 2.5% chimeric reads among 1140363 total sequences. WGA amplification increased chimeric contamination to 23.0% of 7193945 total reads. ChimeraLM processing reduced the chimeric fraction to just 1.5% while preserving 5610252 biological reads, achieving nearly complete artifact removal that approached the quality of bulk sequencing data.

These results demonstrate ChimeraLM’s effectiveness across different Nanopore sequencing platforms and highlight the substantial chimeric artifact burden introduced by WGA, which varies between platforms but is consistently and dramatically reduced by ChimeraLM processing.

ChimeraLM outperforms existing chimera artifact detection tools

We benchmarked ChimeraLM against established computational methods for chimeric sequence detection, including SACRA [13] and 3rd-ChimeraMiner [12], using both P2 and Mk1c datasets (Figure 2c and Figure 2d). ChimeraLM achieved superior performance compared to existing approaches across both sequencing platforms.

For P2 data, ChimeraLM reduced chimeric read contamination by approximately 92%, demonstrating substantial improvement in data quality (Figure 2c). In contrast, both SACRA and 3rd-ChimeraMiner failed to achieve meaningful chimeric read reduction, showing 0% improvement over unprocessed WGA data. This stark difference highlights the limitations of existing rule-based and alignment-based approaches for detecting complex chimeric artifacts in long-read sequencing data.

Similar performance advantages were observed for Mk1c data, where ChimeraLM again achieved approximately 91% reduction in chimeric reads while SACRA and 3rd-ChimeraMiner provided no detectable improvement (Figure 2d). These results demonstrate that ChimeraLM’s deep learning approach captures complex sequence patterns that are not effectively identified by traditional computational methods.

The consistent superior performance across different sequencing platforms establishes ChimeraLM as a significant advance over existing tools, providing researchers with a reliable method for improving single-cell genomic data quality regardless of the specific long-read sequencing technology employed.

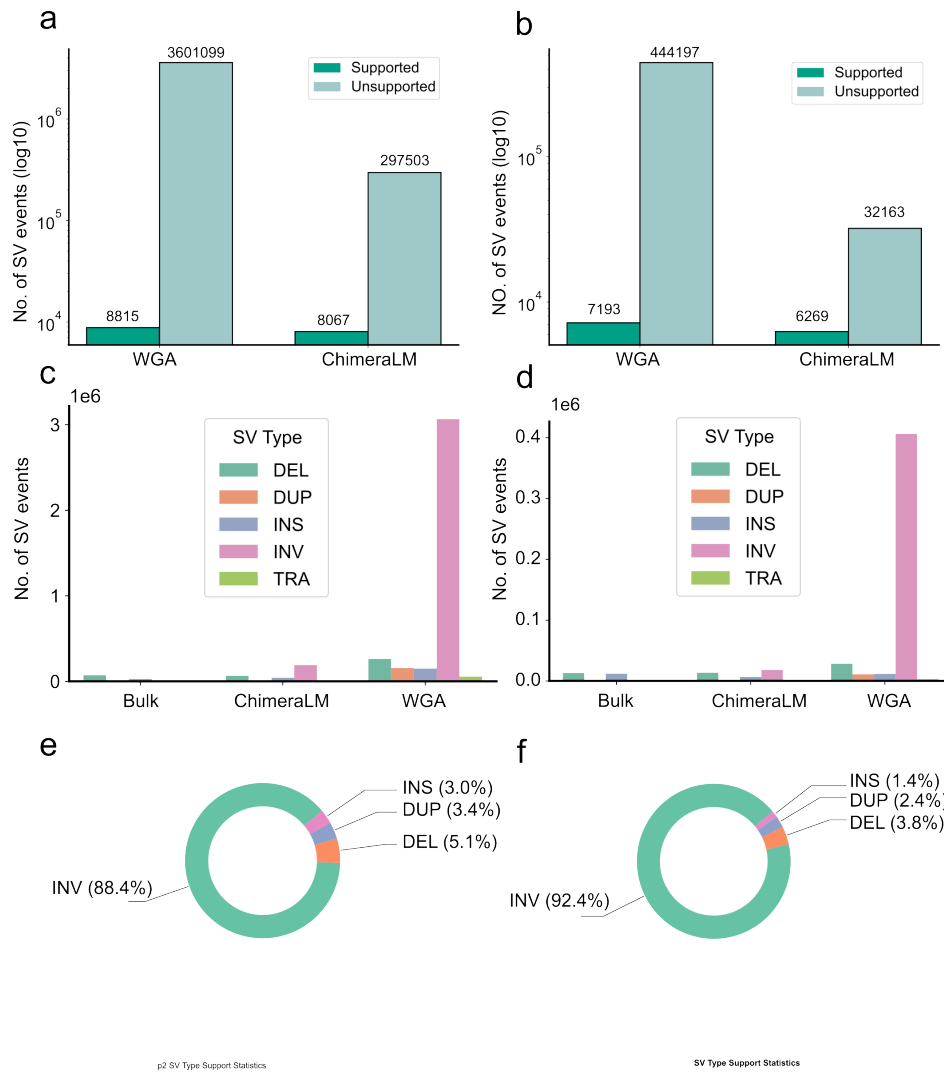


Fig. 3 Problem and Model

Methods

MDA sequencing

Train data construction

Model architecture

Model training

SV evaluation

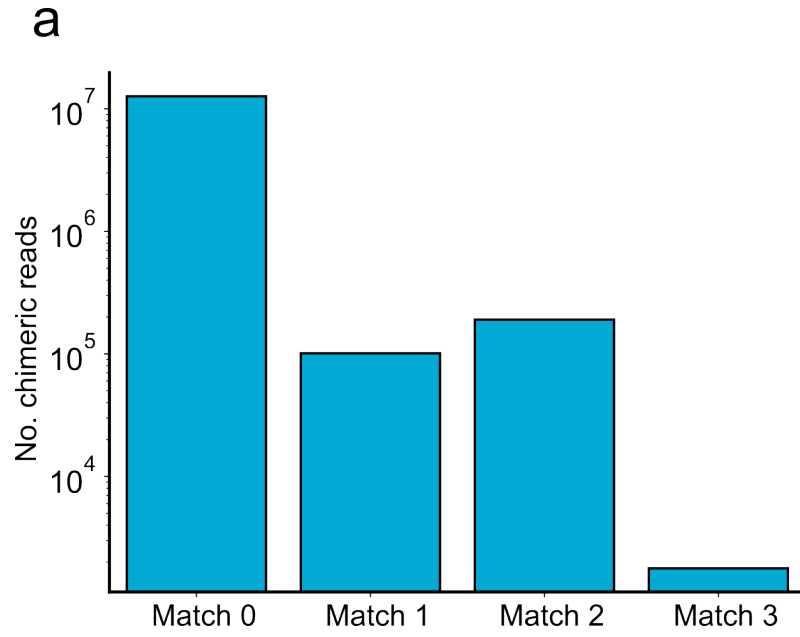


Fig. 4 Problem and Model

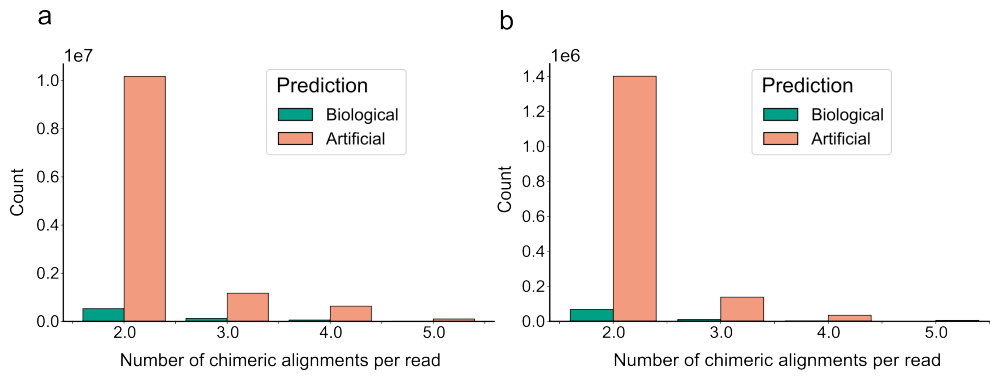


Fig. 5 Problem and Model

Supplementary information. This separation aligns with how many transcript assembly algorithms work:

1. First, chains of exons and splice junctions are identified from the data
2. Then, potential transcripts are derived by traversing the graph in different ways
3. Finally, relationships between different transcript graphs are established

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Acronyms

MALBAC Multiple Annealing and Looping-based Amplification Cycles [2](#)

MDA Multiple Displacement Amplification [2](#)

MLP multilayer perceptron [3](#), [5](#)

SV Structural Variation [2](#)

WGA Whole Genome Amplification [1–4](#), [6](#), [7](#)

Appendix A Section title of first appendix

An appendix contains supplementary information that is not an essential part of the text itself but which may be helpful in providing a more comprehensive understanding of the research problem or it is information that is too cumbersome to be included in the body of the paper.

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