RESEARCH PAPER

Cost-Effective Manual DNA Fragmentation Method for Nanopore PromethION Human Whole Genome Next Generation Sequencing

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ABSTRACT

Background

Human whole genome sequencing (WGS) is a powerful tool for understanding complex genetic disorders. One of the critical steps in next-generation sequencing (NGS) is library preparation, which significantly influences the quality and quantity of the sequencing data. DNA quality plays a critical role in ensuring the accuracy and efficiency of Next-Generation Sequencing (NGS). Human whole genome sequencing (WGS) using Oxford Nanopore Technologies (ONT) platforms requires careful optimization of library preparation steps to ensure high sequencing yield and efficient pore utilization. DNA fragmentation is a critical determinant of read length distribution and sequencing performance, yet commonly used mechanical fragmentation systems remain costly and inaccessible to many laboratories. This paper reviews the factors influencing DNA quality, the methods of assessment, and the implications of DNA quality on NGS outcomes. The analysis includes common issues like DNA degradation, contamination, and the importance of quantification. This study evaluates a cost-effective manual DNA fragmentation method using syringes with different needle gauges (30G, 32G, and 34G) and varying pass-throughs (20, 50, 75, and 100 times) to optimize the fragment size for Nanopore PromethION sequencing. Fourteen blood samples were divided into five groups, including a control group and four treatment groups with different needle sizes and pass-through counts. Sequencing performance was assessed using normalized metrics, including N50 read length and yield per active pore.

Results

Manual syringe fragmentation effectively reduced DNA fragment size in a controlled manner. The combination of a 34G needle with 75 pass-throughs consistently produced fragment sizes of approximately 7–8 kb, which was associated with improved pore efficiency and higher sequencing output after normalization to initial active pore counts.

Conclusions

Manual DNA fragmentation using a fine-gauge syringe represents a reproducible and economical alternative to mechanical shearing methods for Nanopore WGS. This approach is particularly suitable for laboratories with limited resources, without compromising sequencing performance.

Keywords: PromethION, Syringe, DNA fragmentation, library preparation

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INTRODUCTION

Whole genome sequencing (WGS) has become an essential tool for investigating genetic variation

underlying complex diseases and for advancing precision medicine. Long-read sequencing technologies, particularly those developed by Oxford Nanopore Technologies (ONT), offer advantages over short-read platforms by enabling the detection of structural variants, repetitive regions, and epigenetic modifications. Human whole-genome sequencing is a comprehensive method for analyzing the entire human genome to understand the biological basis of complex disorders resulting from the interplay of multiple genetic and environmental influences, such as diabetes, heart disease, cancer, and many other illnesses.

Nanopore technology refers to nano-scale holes embedded in a thin membrane structure to detect the potential change when charged biological molecules smaller than Nanopore pass through the hole. Therefore, Nanopore technology has the potential to sense and analyze single-molecule amino acid, DNA, RNA, etc. Nucleic acid is an important genetic material for most of the living body, and accurate sequencing of the nucleic acids is important for biomedical research, which would be useful for diagnosing human diseases and providing personalized medicine. Since the last century, gene sequencing technology has developed dramatically. (Lin, Hui, and Mao 2021)

Rapid advances in Nanopore technologies for sequencing single long DNA and RNA molecules have led to substantial improvements in accuracy, read length, and throughput. These breakthroughs have required extensive development of experimental and bioinformatics methods to fully exploit Nanopore long reads for investigations of genomes, transcriptomes, epigenomes, and epi-transcriptomes. Nanopore sequencing is being applied to genome assembly, full-length transcript detection, base modification detection, and more specialized areas, such as rapid clinical diagnosis and outbreak surveillance. Many opportunities remain to improve data quality and analytical approaches by developing new Nanopores, base-calling methods, and experimental protocols tailored to specific applications. (Wang et al. 2021)

The PromethION reaction system is carried out in a flow cell, in which two ionic solution-filled compartments are separated by membranes containing 12,000 Nanopores. The process of Nanopore gene sequencing can be divided into three parts: library preparation, sequencing, and base calling. (Technologies 2020)

Despite these advantages, the success of Nanopore sequencing depends heavily on upstream library preparation, particularly DNA fragmentation. Excessively long DNA fragments may block nanopores and reduce pore lifespan, whereas overly fragmented DNA may compromise long-range genomic information. Commercial mechanical fragmentation systems such as Megaruptor and Covaris g-TUBEs provide precise control over fragment size but are often cost-prohibitive for laboratories in low- and middle-income settings.

Library preparation is a determining factor in getting the desired amount of data, especially in human whole-genome sequencing. Two crucial elements in library preparation contribute to the success of human whole-genome sequencing: library concentration and fragment size. While library concentration can be adjusted readily during library preparation, adjusting library fragment size is less so.

Since Nanopore core technology utilizes Nanopores embedded in a thin membrane structure to detect the potential change when charged biological molecules smaller than the Nanopore pass through the hole, it is crucial to obtain a library fragment size that is short enough to easily pass through the pores, as longer molecules may clog the pores and reduce the potential and lifetime of the pores. The more pores available for sequencing, the more data can be obtained.

Next-Generation Sequencing (NGS) has revolutionized genomics, enabling high-throughput DNA sequencing with unprecedented speed and accuracy. However, the quality of DNA is crucial in determining the success of NGS. Poor DNA quality can lead to sequencing errors, poor coverage, or even sequencing failure. (Satam et al. 2023) Manual DNA shearing using syringe needles has been reported as a simple and inexpensive alternative; however, systematic evaluation of needle gauge and pass-through number in the context of PromethION-based human WGS remains limited. This study aims to optimize a manual DNA fragmentation protocol by assessing its impact on fragment size distribution, pore efficiency, and sequencing yield, with particular emphasis on identifying a practical balance between read length and sequencing performance.

This study introduces a systematic and experimentally validated manual DNA fragmentation protocol designed specifically for Nanopore PromethION human whole genome sequencing. Unlike conventional fragmentation approaches that rely on expensive mechanical systems, this research evaluates

the combined effects of needle gauge size and pass-through frequency on fragment size distribution and sequencing efficiency. The novelty of this work lies in identifying an optimized low-cost fragmentation condition—using a 34G syringe needle with 75 pass-throughs—that achieves consistent fragment sizes and improved pore utilization efficiency. This approach provides a practical and scalable alternative for laboratories operating under resource constraints while maintaining reliable sequencing performance.

The novelty of this study lies in the development and optimization of a cost-effective manual DNA fragmentation protocol for Nanopore PromethION sequencing. By systematically evaluating needle gauge size and pass-through frequency, this research establishes an optimized fragmentation condition that improves sequencing efficiency while reducing reliance on expensive mechanical equipment.

This study contributes methodological novelty by introducing a reproducible manual DNA fragmentation workflow that integrates controlled mechanical shearing parameters with normalized sequencing performance metrics. The proposed protocol provides an experimentally validated alternative to conventional mechanical fragmentation systems, enabling reliable library preparation for long-read sequencing in laboratories with limited resources.

METHODS AND MATERIALS

Ethical Approval and Sample Collection

This study was approved by an institutional ethics committee (approval number: KET-972/UN2.F1/ETIK/PPM. 00.02/2022). Written informed consent was obtained from all participants prior to blood collection. Peripheral blood samples were collected from fourteen adult volunteers using EDTA-coated tubes.

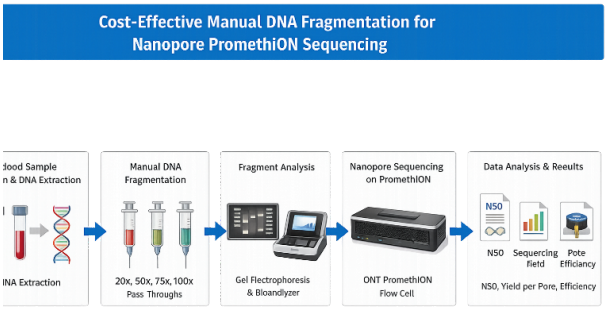

Figure 1. Workflow Diagram

Blood collection & DNA extraction

Blood samples were collected from the peripheral vein of 14 subjects with EDTA-coated tubes. DNA was then extracted using a Favorgen Blood Genomic DNA Extraction Kit (Favorgen Biotech Corp, Taiwan) according to the manufacturer's standard protocol. The quality of the extracted DNA was then measured using a Qubit Fluorometer (Thermo Fisher Scientific, USA). DNA was extracted from human blood samples using the Favorgen DNA extraction kit, which employs a spin-column-based method for efficient genomic DNA isolation. First, 200 µL of whole blood was added to a 1.5 mL microcentrifuge tube, followed by the addition of 200 µL of Favorgen lysis buffer and 20 µL of Proteinase K solution. The mixture was then incubated at 56°C for 10 minutes to ensure thorough cell lysis and protein denaturation.(Thermo Fisher Scientific 2018)

After the lysis step, 200 µL of ethanol (95-100%) was added to the lysate and mixed thoroughly to facilitate DNA binding. The entire mixture was transferred to a Favorgen spin column, and the column was centrifuged at 12,000 × g for 1 minute. This step enabled the genomic DNA to bind to the silica membrane of the column. The flow-through was discarded, and the column was washed twice with 500 µL of wash buffer, each wash followed by centrifugation at 12,000 x g for 1 minute to remove residual contaminants.

For DNA elution, the spin column was transferred to a clean microcentrifuge tube. Then, 200 µL of elution buffer, preheated to 70°C, was applied directly onto the membrane. After a 5-minute incubation, the column was centrifuged at 12,000 × g for 1 minute to elute the purified genomic DNA. The extracted DNA was subsequently stored at -20°C until further use.(Zeolite Products 2021)

After DNA extraction, the concentration of genomic DNA was measured using the Qubit Fluorometer (ThermoFisher Scientific). The Qubit system provides a highly sensitive and specific method for quantifying double-stranded DNA by using fluorescent dyes that selectively bind to DNA. This method is particularly advantageous over spectrophotometric techniques because it is less affected by contaminants such as RNA, proteins, and free nucleotides.

To begin, the Qubit dsDNA HS (High Sensitivity) assay kit was used following the manufacturer's protocol. A working solution was prepared by diluting the Qubit

reagent in Qubit buffer at a 1:200 ratio. DNA standards (provided with the kit) and sample dilutions were set up in appropriate microcentrifuge tubes. For each measurement, 2 µL of the extracted DNA was added to the prepared working solution and incubated at room temperature for 2 minutes to allow the dye to bind to the DNA.

The samples were then loaded into the Qubit Fluorometer, which quantified the DNA concentration by measuring the fluorescence intensity of the dye-DNA complex. The results were displayed in ng/µL, and the average DNA concentration across multiple extractions was between 60 and 80 ng/µL, aligning with the expected yield for high-quality genomic DNA. The Qubit system ensured accurate quantification, particularly for low-concentration samples, and provided more reliable results compared to spectrophotometric methods.(Versmessen et al. 2024)

DNA fragmentation method

We examine the effect of DNA fragmentation using a 1 cc syringe paired with 30G, 32G, and 34G needles, with varying numbers of pass-throughs (20, 50, 75, and 100 times). To begin, high-molecular-weight genomic DNA (5-10 µg) is dissolved in TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0). The DNA solution (approximately 200 µL) is loaded into a 1 mL syringe equipped with a 34G needle. Careful loading is necessary to avoid introducing bubbles, which can affect the shearing efficiency.

The DNA is then sheared by repeatedly passing it through the needle. Each cycle involves gently pushing the DNA solution through the needle into a clean microcentrifuge tube, then pulling it back into the syringe. This cycle is repeated between 20 to 100 times, depending on the desired fragment size. Fewer passes (e.g., 10) yield larger DNA fragments, while more passes (15-20) create smaller, more uniform fragments.(Cook et al. 2018)

Human whole genome next-generation sequencing (NGS)

After DNA fragmentation, library preparation was performed using the Nanopore Ligation Sequencing Kit V14 (SQK-LSK114). Human whole-genome next-generation sequencing was performed using the Nanopore P2Solo platform with a PromethION flow cell (FLO-PRO114M). Basecalling was done using MinKNOW version 24.02.6. Next-Generation Sequencing (NGS) technology enables rapid sequencing of entire genomes, providing high-throughput and long-read capabilities that are especially useful for de novo genome assembly, structural variant detection, and epigenetic studies. One of the most advanced platforms for NGS is Oxford Nanopore Technologies (ONT), which provides long-read sequencing using the MinION or PromethION devices. The Nanopore LSK114 kit is a popular library preparation kit that facilitates the preparation of DNA samples for sequencing.

High-molecular-weight (HMW) genomic DNA is required for optimal long-read sequencing using Nanopore technology. For this protocol, genomic DNA was extracted from human blood samples, and DNA concentrations were measured using a Qubit 4 Fluorometer. An ideal DNA concentration for Nanopore sequencing is 50-100 ng/µL, with a minimum input of 1-2 µg of DNA. For this experiment, 1.5 µg/60 µL DNA was used for all samples.

The LSK114 kit from Oxford Nanopore Technologies provides a streamlined process for preparing DNA libraries, enabling direct sequencing of native DNA molecules without amplification. The workflow begins with DNA repair and end-prep. The genomic DNA (1-2 µg) is incubated with the NEBNext FFPE DNA repair mix and NEBNext Ultra II End-Repair/dA-Tailing Module to ensure that damaged ends are repaired and overhangs are prepared for adapter ligation.(Oxford Nanopore Technologies 2022)

Following the DNA repair step, adapters from the LSK114 kit are ligated to the DNA fragments using T4 DNA ligase. These adapters enable the DNA to be recognized and processed by the Nanopore during sequencing. The ligation reaction is incubated at room temperature for 20 minutes, and then the adapter-ligated DNA is purified using AMPure XP beads to remove any unligated fragments or contaminants. The resulting library is eluted in a small volume of elution buffer (typically 25 µL) for loading onto the Nanopore device.

The prepared DNA library is then loaded into a flow cell installed on a MinION or PromethION device. Before loading, the flow cell quality is checked to ensure that enough active pores are available for sequencing. Approximately 75-100 µL of the library, mixed with sequencing buffer and loading beads (provided in the LSK114 kit), is carefully applied to the flow cell.

The sequencing process begins immediately, with the Nanopore device reading the DNA sequence as molecules pass through Nanopores embedded in the flow

cell membrane. As DNA passes through the Nanopores, changes in ionic current are recorded, and these signals are converted into nucleotide sequences in real time.(Jain et al. 2016) The long-read nature of Nanopore sequencing allows for reads exceeding 100 kb in length, depending on the input DNA quality.

Data Normalization and Analysis

To account for variability between flow cell batches, sequencing output was normalized to the number of active pores reported at the start of each run. Yield per pore was calculated by dividing the total bases generated by the initial active pore count. Fragment size distribution was assessed using the N50 read length.

Ethics approval and consent to participate

Ethical approval for this research was granted by the Ethics Committee of the Faculty of Medicine, University of Indonesia – Cipto Mangunkusumo Hospital, with number KET-972/UN2.F1/ETIK/PPM. 00.02/2022, and informed consent was obtained from all individual participants included in the study

RESULTS AND DISCUSSIONS

In this research, we evaluate the use of a syringe and needle to shear, creating smaller fragments that easily pass through the Nanopores, thereby increasing the efficiency and durability of the pores. The results were obtained from 14 blood samples, and extraction and whole-genome sequencing were performed. The samples were divided into 5 groups, namely the control group without treatment (S04, S05), the 20x manual syringe needle and wash treatment group (S08, S09), the 50x manual syringe needle treatment group (S29, S30, S11, S12), the manual syringe treatment group needle 75x (S13, S14, S15, S16), manual syringe needle treatment group 100x (S27, S28). Each sample is shown in Table 1 below.

Table 1. Treatment, DNA Quality, Initial Pores, Estimated Bases, and N50

Sample	DNA Quality (ng/µL)	Needle Size	Pass-through	Wash	Initial Pore	Estimated bases (72 hrs)	N50 (kb)
S04	102.0	-	-	No	7200	101.1 Gb	11.41
S05	192.0	-	-	No	6200	44.81 Gb	21.95
S08	102.0	30 G	20 x	Yes	6836	88.37 Gb	12.63
S09	106.0	32 G	20 x	Yes	7626	95.88 Gb	11.89
S29	108.0	32 G	50 x	No	5366	86.01 Gb	11.25
S30	61.0	32 G	50 x	No	5798	77.72 Gb	8.64
S11	99.4	32 G	50 x	No	5111	79.53 Gb	10.1
S12	65.0	32 G	50 x	No	4451	67.57 Gb	10.04
S13	63.2	32 G	75 x	No	6414	114.2 Gb	8.92
S14	61.8	32 G	75 x	No	7342	129.3 Gb	8.43
S15	80.8	34 G	75 x	No	7787	185.8 Gb	7.81
S16	66.0	34 G	75 x	No	6668	114.3 Gb	8.77
S27	88.2	32 G	100 x	No	6221	107.7 Gb	8.19
S28	89.8	34 G	100 x	No	5863	83.94 Gb	9.32

Manual syringe fragmentation produced a clear reduction in DNA fragment size with increasing numbers of pass-throughs and smaller needle gauges. Untreated samples showed N50 values ranging from 11.41 to 21.95 kb, whereas fragmented samples demonstrated progressively lower N50 values.

The most consistent fragment size distribution was observed using a 34G needle with 75 pass-throughs, yielding N50 values of approximately 7–8 kb. After normalization to the initial active pore counts, this group demonstrated higher sequencing yield per pore than in untreated samples and under other fragmentation conditions. Although initial active pore counts varied between flow cells due to manufacturing batch differences, normalization enabled fair comparison of sequencing performance across experimental conditions.

The initial number of pores of the flowcell varied between 5366 to 7787, this was due to the difference in production batches of each flowcell.

The impact of DNA quality on NGS output is substantial. Poor-quality DNA can lead to low sequencing yields, high error rates, and biased data. Studies have shown that optimizing DNA quality leads to improved read length and coverage.(Cheng, Fei, and Xiao 2023)

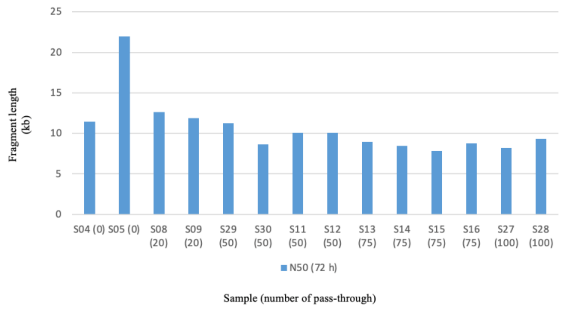

Figure 2. Fragment sizes in kbp (kilobases) from the sample with various pass-throughs.

DNA shearing is essential in preparing high-molecular-weight genomic DNA for applications such as Next-Generation Sequencing (NGS), where precise fragmentation is required. Manual DNA shearing with a fine-gauge needle, such as a 34-gauge (34G) needle, offers a cost-effective alternative to mechanical shearing devices, including acoustic, hydrodynamic, and nebulization shearing. Enzyme-based methods include transposons, restriction enzymes, and nicking enzymes. (Apone, Dimalanta, and Stewart 2017) This method involves physically passing the DNA solution through the needle multiple times to break the strands into smaller fragments.

Fragment size is described by the N50 metric, where N is a measure of the closeness of a series of sequences and is often used to assess genome assemblies. N50 is related to the median and average length of a set of sequences. The value represents the shortest read length in a group of the longest sequences that together represent (at least) 50% of the nucleotides in the sequence. The N50 value can be interpreted as the weighted midpoint of the read-length distribution for a sequencing run. However, the N50 value must be interpreted in the context of the total number of reads in the sequencing process. A larger N50 value indicates a larger fragment size in DNA sequencing. When using Nanopore PromethION, DNA fragments go in and out of the pores, and the changes in the electric signal will be called into bases. The number of available pores determines how many bases can be called. The larger the fragment size, the greater the likelihood of blockages in the pores, rendering them unusable and reducing the amount of data obtained. The N50 values for the samples without treatment were 11.41 kb and 21.95 kb. In contrast with the samples without treatment, samples treated with 20x pass-throughs demonstrate fragment sizes between 11-12kb. In the 50x pass-through samples, the fragments ranged from 8-10kb. The 75x and 100x pass-throughs resulted in fragments between 7-8kb and 8-9kb, respectively. This manual shearing method is advantageous for labs without access to more advanced mechanical shearing tools and provides a simple, reproducible approach to DNA fragmentation, although the size distribution may vary slightly due to manual handling.

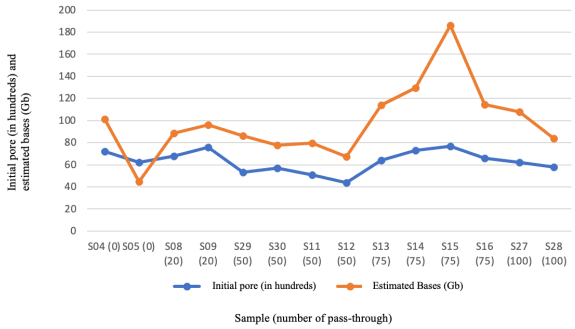

Figure 3. Pore and Bases called after 72 hours of run for samples with various pass-throughs

In Figure 3, it can be seen that there is a difference in the number of initial pores in each flow cell and the bases produced after 72 hours of running. For samples sequenced directly without syringe fragmentation, the results varied widely, with 101.1 Gb and 44.81 Gb generated. The treated samples, however, showed better results, with all samples generating more than 67 Gb of bases called, with samples treated with 75x pass-throughs averaging more than 100 Gb (114.2-185.8 Gb) bases called. The smaller fragment sizes might increase the durability and effectiveness of the pores for sequencing DNA and, therefore, generate more reads, whereas larger fragments either permanently block the pores or reduce the number of times the pores can be used to sequence DNA.

Another factor that may affect the amount of data generated is the amount of DNA loaded into the flow cell. Accurate quantification of DNA is essential for successful library preparation. In this experiment, we

used 1500 ng/60 µL for all samples to eliminate bias due to differences in DNA concentration. Insufficient or excessive amounts of DNA can affect the efficiency of enzymatic reactions used in NGS workflows, and methods like fluorometric quantification are more reliable than spectrophotometry.(Bruijns et al. 2022)

The sizes of the needles were also evaluated to see the effect of different gauges in the process of DNA shearing. The result showed that a 34G needle provides the most precise mechanical shearing of DNA, obtaining between 7.81 kb and 8.92 kb fragments. The size of the needle is crucial for minimizing the risk of generating excessively fragmented DNA or introducing artifacts, as it ensures that DNA molecules are broken into uniform fragments, which is critical for obtaining high-quality sequencing data.

This study also aims to determine the optimal number of pass-throughs to achieve the best fragment size. By performing a 75X pass-through, we optimized the fragmentation process, achieving a balance between adequate shearing and maintaining uniform DNA fragment size. This approach was found to produce DNA with an average fragment size of 7-8 kb, which is ideal for human whole-genome sequencing on the PromethION platform.

In this study, the results showed that using a combination of a 34G needle and a 75X pass-through yielded the most consistent, high-quality DNA fragments suitable for human whole-genome sequencing on the PromethION platform. The choice of needle size and the number of pass-throughs significantly impact the quality of DNA fragments. Using the wrong needle size or fewer pass-throughs can result in suboptimal fragment sizes, potentially leading to poor sequencing performance and reduced data quality, highlighting the importance of fine-tuning the shearing parameters to match the requirements of specific sequencing technologies.

Moreover, the manual syringe method offers several advantages over automated shearing devices. It is cost-effective and provides greater control over the shearing process, enabling researchers to adjust parameters to meet their specific needs. Despite the labor-intensive nature of manual shearing, the precision achieved with a 34G needle and 75X pass-throughs ensures reproducibility and reliability, making it a viable alternative to more expensive mechanical shearing methods. In laboratory research and clinical settings, the choice of equipment and materials is often influenced by both cost and functionality. Manual syringes with 34G needles, priced between $0.10 and $0.50 per unit, offer a cost-effective solution for precise sampling of small volumes. Their low cost makes them an attractive option for budget-conscious laboratories, especially for applications requiring high accuracy in small quantities. However, their suitability diminishes if the samples require further processing or if the sample volume is large, which could lead to increased costs over time due to repeated use.

In contrast, the Megaruptor, a high-efficiency device for cell and tissue disruption, is a significant investment, with costs ranging from $20,000 to $30,000. Despite the high upfront cost, Megaruptor's ability to process large sample volumes consistently can make it a more economical choice in the long run. The efficiency and speed of sample processing with Megaruptor may offset the initial cost by reducing labor and time required for sample preparation, ultimately lowering the cost per sample.

Covaris g-TUBEs, used for sample fragmentation with a bench-top centrifuge, are priced between $2 and $5 each. These tubes provide high precision and uniformity in sample homogenization, which is crucial for analyses demanding consistent results. However, the cost of Covaris g-TUBEs can add up significantly if used frequently or in large quantities. The overall cost-effectiveness of Covaris g-TUBEs depends on sample volume processed and frequency of use, as recurring costs can impact laboratory budgets for processing large volumes of samples.

Ultimately, while manual syringes offer a low-cost option for small-volume applications, Megaruptor and Covaris g-TUBEs are better suited for high-efficiency and high-volume scenarios. The decision on which tool to use should take into account both the initial purchase cost and the operational expenses, as well as the specific needs and scale of the laboratory's research activities.

DNA fragmentation is a critical determinant of Nanopore sequencing efficiency, particularly in whole-genome sequencing workflows, where pore availability and durability directly influence sequencing yield. In many low- and middle-income settings, access to advanced mechanical DNA fragmentation instruments remains limited due to high procurement and maintenance costs. Consequently, there is a practical need for alternative fragmentation strategies that are both technically reliable and economically feasible.

In this study, manual syringe-based DNA fragmentation demonstrated consistent, reproducible control over fragment size, with smaller needle gauges and higher pass-through numbers yielding shorter read-length distributions. The combination of a 34G needle with 75 pass-throughs achieved an optimal balance between effective fragmentation and preservation of sufficient read length for downstream genomic analyses. Normalization of sequencing output to initial active pore counts showed that this condition improved pore utilization efficiency and overall sequencing yield compared with untreated high-molecular-weight DNA.

While a reduction in N50 read length represents a trade-off for applications requiring highly contiguous de novo genome assemblies, this limitation does not substantially affect many clinically and translationally relevant applications. In regional diagnostic and research settings, Nanopore sequencing is frequently used for variant detection, gene-level analysis, pathogen surveillance, and targeted genomic investigations, rather than for reference-grade genome assembly. For these use cases, improved pore efficiency and higher data output may be more advantageous than maximizing ultra-long read length.

From a cost and accessibility perspective, the manual syringe method offers a significant advantage. Syringe needles are inexpensive, widely available, and require no specialized infrastructure, making them particularly suitable for laboratories operating under constrained budgets. This approach can facilitate broader adoption of long-read sequencing technologies in many low- and middle-income countries, supporting local capacity building in genomic research, molecular diagnostics, and precision medicine initiatives.

Table 2. Previous Research

No	Author / Year	Focus of Study	Method / Approach	Key Findings (State of the Art)	Research Gap	Novelty of the Present Study
1	Jain et al., 2016	Introduction of Nanopore sequencing	Long-read sequencing platform	Nanopore sequencing enables real-time genome sequencing with long-read capability	Limited optimization of upstream library preparation processes	Development of an optimized manual DNA fragmentation workflow tailored for Nanopore PromethION sequencing
2	Wang et al., 2021	Advances in Nanopore sequencing and bioinformatics	Review of sequencing technologies and applications	Improvements in sequencing accuracy, throughput, and read length	Insufficient focus on laboratory-level protocol optimization for sequencing preparation	Experimental validation of practical fragmentation parameters affecting sequencing performance
3	Satam et al., 2023	Next-Generation Sequencing (NGS) technology development	Technology trend analysis	DNA quality strongly influences sequencing accuracy and coverage	Lack of low-cost and accessible DNA preparation methods	Demonstration of a cost-effective manual fragmentation method suitable for resource-limited laboratories
4	Apone et al.,	Enzymatic DNA fragment	Enzyme-based DNA	Enzymatic fragmen	High dependency on	Introduction of a mechani

No	Author / Year	Focus of Study	Method / Approach	Key Findings (State of the Art)	Research Gap	Novelty of the Present Study
	2017	ation methods	shearing techniques	tation provides controlled DNA fragment size	specialized reagents and equipment	cal manual fragmentation alternative without enzymatic dependency
5	Cook et al., 2018	Comparison of DNA fragmentation and extraction methods	Experimental evaluation of DNA fragment yield	DNA fragment size significantly affects sequencing performance	Limited systematic evaluation of manual mechanical fragmentation parameters	Systematic comparison of needle gauge size and pass-through frequency in manual DNA fragmentation
6	Cheng et al., 2023	Improving sequencing accuracy in NGS workflows	Optimization of sequencing quality parameters	Optimized DNA quality improves sequencing output and data reliability	Lack of direct linkage between the fragmentation method and pore efficiency	Introduction of normalized sequencing yield per active pore as a performance metric
7	Present Study	Manual DNA fragmentation for	Experimental laboratory	Manual fragmentation using a	Absence of standardized	Establishment of a reproduc
	(2024)	Nanopore PromethION WGS	optimization using syringe needles	**34G needle with 75 pass-throughs** produces optimal fragment size (7–8 kb) and improved sequencing yield	low-cost fragmentation protocols for long-read sequencing platforms	ible, economical, and scalable DNA fragmentation protocol for resource-limited genomic laboratories

Overall, the findings highlight that carefully optimized manual DNA fragmentation can serve as a practical and scalable solution for Nanopore-based WGS in resource-limited environments, without compromising sequencing performance metrics essential for most clinical and research applications.

Table 3. State of the Art, Research Gap, and Novelty

Aspect	State of the Art	Research Gap	Novelty
DNA Fragmentation Technology	Mechanical and enzymatic fragmentation methods provide precise DNA fragment control	High cost and limited accessibility of mechanical fragmentation systems	Development of a low-cost manual fragmentation method using syringe needles
Library Preparation Optimization	DNA quality and fragment size strongly influence	Limited systematic evaluation of manual	Optimization of needle gauge size and pass-through

Aspect	State of the Art	Research Gap	Novelty
	sequencing performance	fragmentation parameters	frequency for improved sequencing output
Sequencing Efficiency	Long-read sequencing technologies improve genomic analysis	Lack of standardized fragmentation protocols for Nanopore PromethION sequencing	Identification of optimal fragmentation condition (34G needle + 75 pass-throughs)
Laboratory Accessibility	Advanced sequencing technologies are widely used in developed laboratories	Resource-limited laboratories face infrastructure and cost constraints	Demonstration of an affordable and scalable sequencing preparation workflow
Performance Metrics	Sequencing performance is typically evaluated using read length and coverage	Limited use of normalized efficiency metrics in fragmentation studies	Introduction of normalized yield per active pore as a robust evaluation indicator

CONCLUSION

Manual DNA fragmentation using a 34G syringe needle with 75 pass-throughs provides an effective, low-cost alternative for preparing human genomic DNA for Nanopore PromethION WGS. By improving pore efficiency and sequencing yield without reliance on expensive equipment, this method offers a practical solution for expanding access to long-read sequencing technologies in diverse laboratory settings.

Competing interests

The authors declare that there is no conflict of interest. The funders had no role in the design, data collection, analysis, manuscript writing, or decision to publish

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Authors contribution

The author's contributions to this study are as follows: Tiar Masykuroh Pratamawati was responsible for conceptualization, methodology, investigation, project administration, supervision, funding acquisition, writing of the original draft, and review and editing of the manuscript. Idrus Alwi and Asmarinah contributed to the writing through critical review and editing. Donny Nauphar carried out the investigation, formal analysis, and data curation. Vincentius S.W. Budhyanto provided essential resources for the study.

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REFERENCES

- Apone, L., Dimalanta, E., & Stewart, F. Improving Enzymatic DNA Fragmentation for Next Generation Sequencing Library Construction. *New England BioLabs* 1–4 (2017).
- Bruijns, B., Hoekema, T., Oomens, L., Tiggelaar, R. & Gardeniers, H. Performance of Spectrophotometric and Fluorometric DNA Quantification Methods. *Analytica* 3, 371–384 (2022).
- Cheng, C., Fei, Z. & Xiao, P. Methods to improve the accuracy of next-generation sequencing. *Front Bioeng Biotechnol* 11, 1–13 (2023).
- Cook, L. *et al.* Does size matter? Comparison of extraction yields for different-sized DNA fragments by seven different routine and four new circulating cell-free extraction methods. *J Clin Microbiol* 56, 1–13 (2018).
- Jain, M., Olsen, H. E., Paten, B. & Akeson, M. The Oxford Nanopore MinION: delivery of

nanopore sequencing to the genomics community. *Genome Biol* 17, 1–11 (2016).

- Lin, B., Hui, J. & Mao, H. Nanopore Technology and Its Applications in Gene Sequencing. (2021).
- Oxford Nanopore Technologies. Ligation sequencing DNA V14 Library Preparation. 14, 1–37 (2022).
- Satam, H. *et al.* Next-Generation Sequencing Technology: Current Trends and Advancements. *Biology (Basel)* 12, (2023).
- Technologies, O. N. Introduction to Real Time Analysis. https://www.youtube.com/watch?%0Av=8oNEjt5Ov_Q (2020).
- Thermo Fisher Scientific. Qubit ™Fluorometer User Guide. *Software* (2018).
- Versmessen, N. *et al.* Comparison of DeNovix, NanoDrop and Qubit for DNA quantification and impurity detection of bacterial DNA extracts. *PLoS One* 19, 1–14 (2024).
- Wang, Y., Zhao, Y., Bollas, A., Wang, Y. & Au, K. F. Nanopore sequencing technology, bioinformatics and applications. 39, (2021).
- Zeolite Products. Material Safety Data Sheet Induex-25 MATERIAL SAFETY DATA SHEET. *Material Safety Data Sheet* 1–5 (2021).