

The Magic of DNA Fragmentation

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Introduction

The fragmentation of genomic DNA is an essential step during library preparation within NGS workflows, since it has a significant effect on the distribution of fragment sizes, the uniformity of coverage, and the performance of downstream analyses. Standard fragmentation strategies can be divided into enzymatic and mechanical fragmentation, with both having their advantages and disadvantages.

Pipette-Based DNA Shearing

Recent works demonstrate controlled pipette-based shearing on automated liquid-handling platforms enable high-throughput and reproducible DNA fragmentation. Achievable fragment size distributions are compatible with long-read sequencing platforms [K. Parliament et al. 2025; DOI: 10.1016/j.jbc.2025.109783]. Here, we evaluate an affordable alternative based on a semi-automated 96-well pipetting device (ePlateWizard™) that enables parallel, standardized liquid handling with variable volume control. The device comes equipped with different pipetting modes, comprising single pipetting, multi-step dispensing of defined volumes, recording and replay of user-defined pipetting steps as well as a programmable Macro-Mode.

Figure 1: Semi-automated 96-well pipetting device

Background

Mechanical fragmentation methods, including acoustic sonication and hydrodynamic or pipette-based shearing are largely sequence-agnostic and can provide a high level of reproducibility and fragment size control, which makes them the preferred choice for most PCR-free and long-read workflows. The drawbacks of mechanical fragmentation methods are the high initial capital investments and ongoing maintenance, and material costs compared to enzymatic fragmentation methods. Enzymatic methods are less costly compared to mechanical methods and easy and quick to use. However, they might introduce sequence-dependent biases and require additional steps to inactivate the enzymes. In addition, the control of the fragment size distribution is limited and not optimal for long-read workflows.



ePlateWizard™ DNA Shearing Profile

Step	Volume [μL]	Cycles	Speed
Aspirate	100	1x	5
Dispense	80	500x	5–15
Aspirate	80		
Dispense	100	1x	5

Setup

Genomic DNA, isolated from EDTA-blood or saliva, was diluted to a final concentration of 10 ng/μL. Utilizing the Macro-Mode of ePlateWizard™, we created a custom shearing profile, comprising of the steps listed in Table 1. The total volume was set to 120 μL. The mixing volume was set to 80 μL to ensure volume is retained in the wells and pipette tips preventing the aspiration of air.

Size Distribution is independent of pipetting Cycle Number

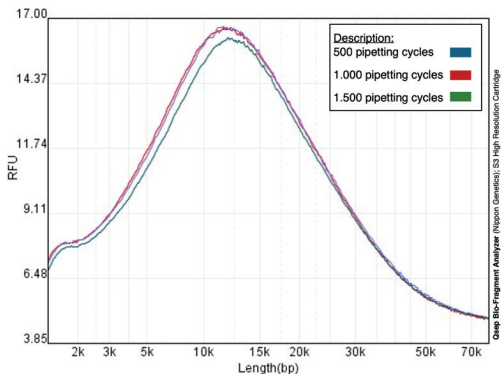


Figure 2: The size distribution does not change with more pipetting cycles. Shearing profile with mixing speed set to 15 and different number of pipetting cycles ranging from 500x (Blue), 1,000x (Red) and 1,500x (Green).

Size Distribution is Controlled by Mixing Speed

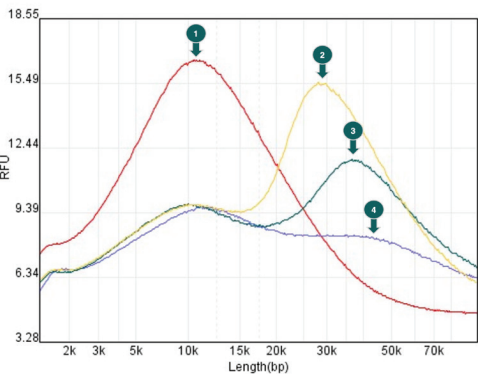


Figure 3: The size distribution is tightly linked to the mixing speed. The highest speed (15) produces fragments at around 11 kb (1), speed 8 fragments around 28 kb (2), speed 4 fragments around 35 kb, while speed 1 produces almost no fragments (4).

Discussion

Our approach preserves key advantages of mechanical fragmentation while substantially reducing capital and operational costs. We achieved consistent fragment sizes distributions compatible with downstream long-read sequencing applications. Utilizing the ePlateWizard™ platform for semi-automated pipette shearing represents a practical intermediate solution between enzymatic fragmentation and fully automated robotic platforms, especially for small to medium sized laboratories.



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