APPLICATION NOTE NO. 498

High-Molecular-Weight DNA Extraction Using PacBio® Nanobind® HT CBB Kit on epMotion® 5075t

[1]Antoine Huyghebaert, [1]Sandrine Hamels, [2]Rucha Datar, [3]Deborah Moine
[1]Eppendorf Application Technologies S.A.,[2] Eppendorf SE, [3]Pacific Biosciences Inc.

Introduction

High-quality DNA extraction is critical for achieving optimal sequencing performance in long-read sequencing applications. The PacBio Nanobind® HT CBB kit offers a reliable solution for extracting high-molecular-weight (HMW) DNA with minimal fragmentation and high purity. Automating this process on the Eppendorf epMotion liquid handling system enhances reproducibility, minimizes hands-on time, and reduces potential sources of human error.
This application brief details the successful implementation of the Nanobind HT CBB kit on the epMotion system, demonstrating high efficiency, yield, and quality of extracted DNA from cell culture.

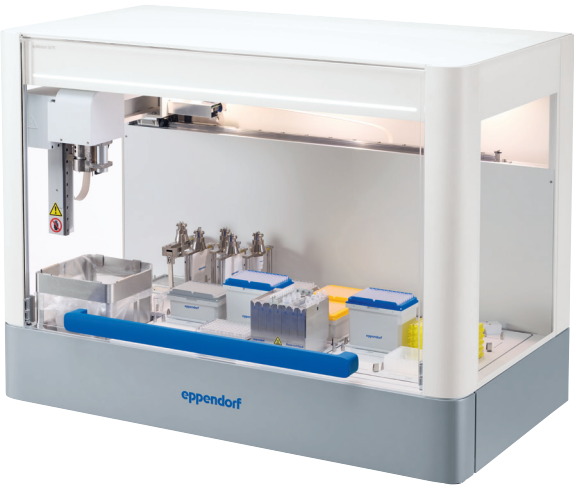


Figure 1: epMotion 5075t liquid handling system and PacBio® Nanobind® HT CBB kit (PN 102-762-700)

In cooperation with Pacific Biosciences Inc.

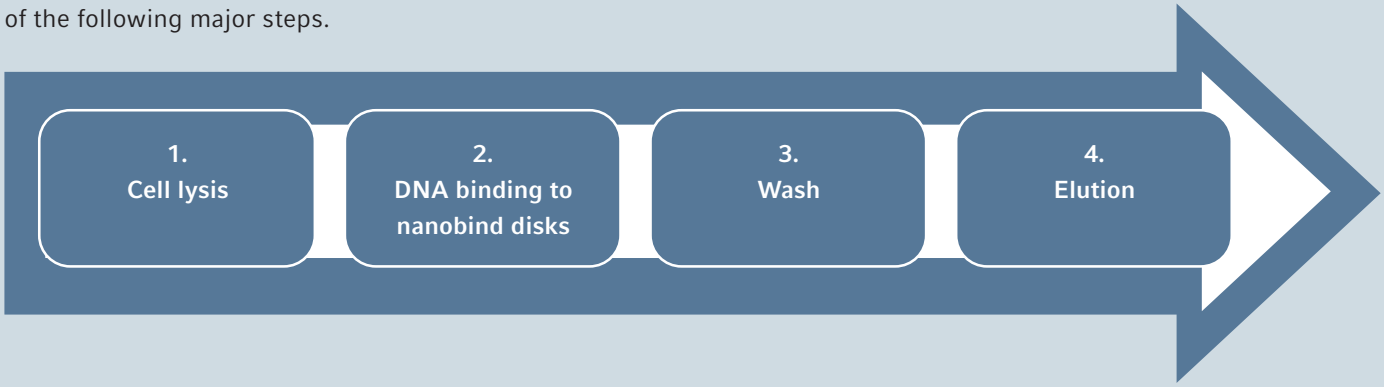
PacBio

User benefits

> High DNA Yield – Consistently extracts high molecular weight (HMW) DNA with excellent recovery rates.
> Superior DNA Integrity and purity – Preserves long and high-quality DNA fragments, essential for long-read sequencing applications, ideal for PacBio platforms.
> Suitable for Long-Read Sequencing – Produces high-quality DNA suitable for PacBio and other long-read sequencing platforms.
> Enhanced Consistency – Automated workflow, seamlessly integrated with the Eppendorf epMotion system, reduces variability between extractions, ensuring reproducible results and reduced manual effort.

Workflow

The automated DNA extraction workflow for processing 96 samples using epMotion 5075t consisted of the following major steps.



To evaluate this workflow, HeLa cells were used as input samples at 10^6 cells per well. The epMotion system was programmed to automate the cell lysis, binding, washing, and elution steps, ensuring consistent and high-quality DNA extraction with minimal manual intervention. In order to address the Nanobind magnetic discs effectively, a customized magnet from Ariumlab, Estonia (Levitation KF/D Magnet Plate, #70DM-L96-24) was used. The run time for the complete workflow using epMotion was 171 minutes for 96 samples processed in parallel (2 hours and 51 minutes).

Results

Yield and Quality Assessment

The automated workflow on the epMotion system consistently produced HMW DNA with high yield and purity. The following key metrics were observed:

> **DNA Yield:** Average yields by following automated workflow were comparable to that gDNA extracted manually (from 5-6 µg per sample), suitable for long-read sequencing applications.

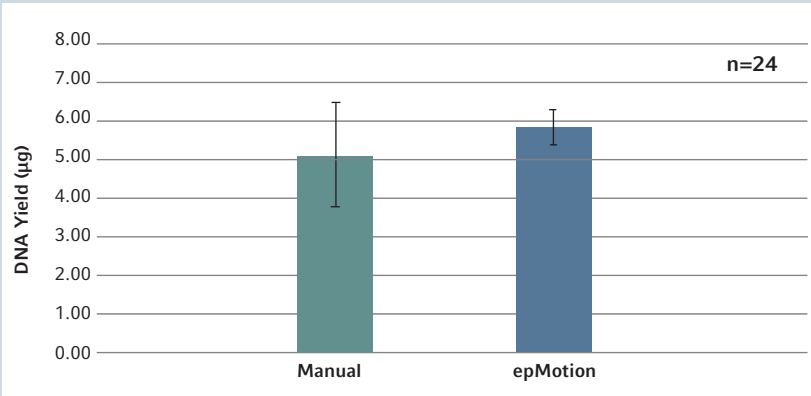


Figure 2: Automated workflow with epMotion produced yields comparable to manual gDNA extraction (5–6 µg per sample), supporting suitability for long-read sequencing applications.

> **Purity Ratios:**
Nanodrop measurements showed A260/A280 ratios of ~ 1.8 and A260/A230 ratios above 2.0, indicating minimal contamination.

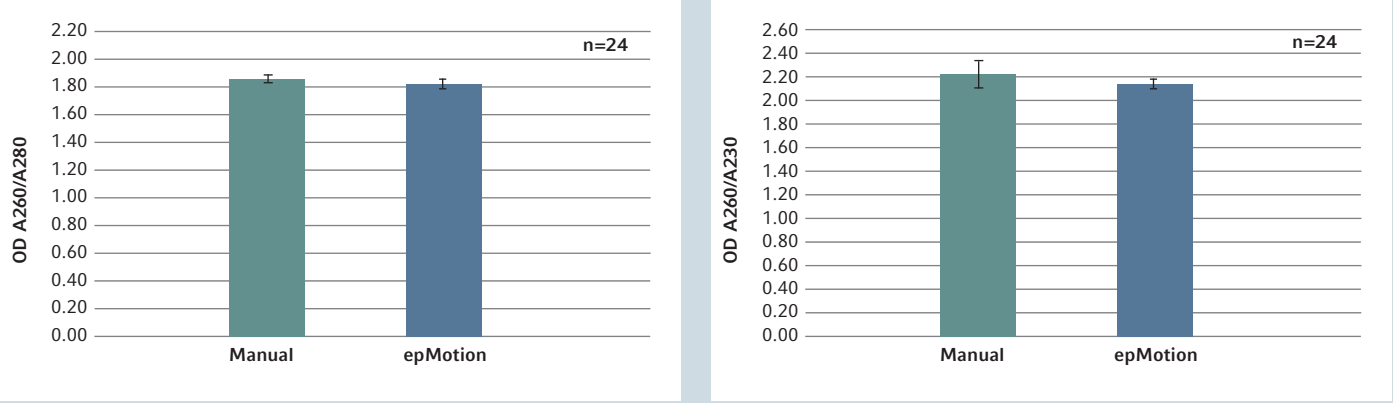
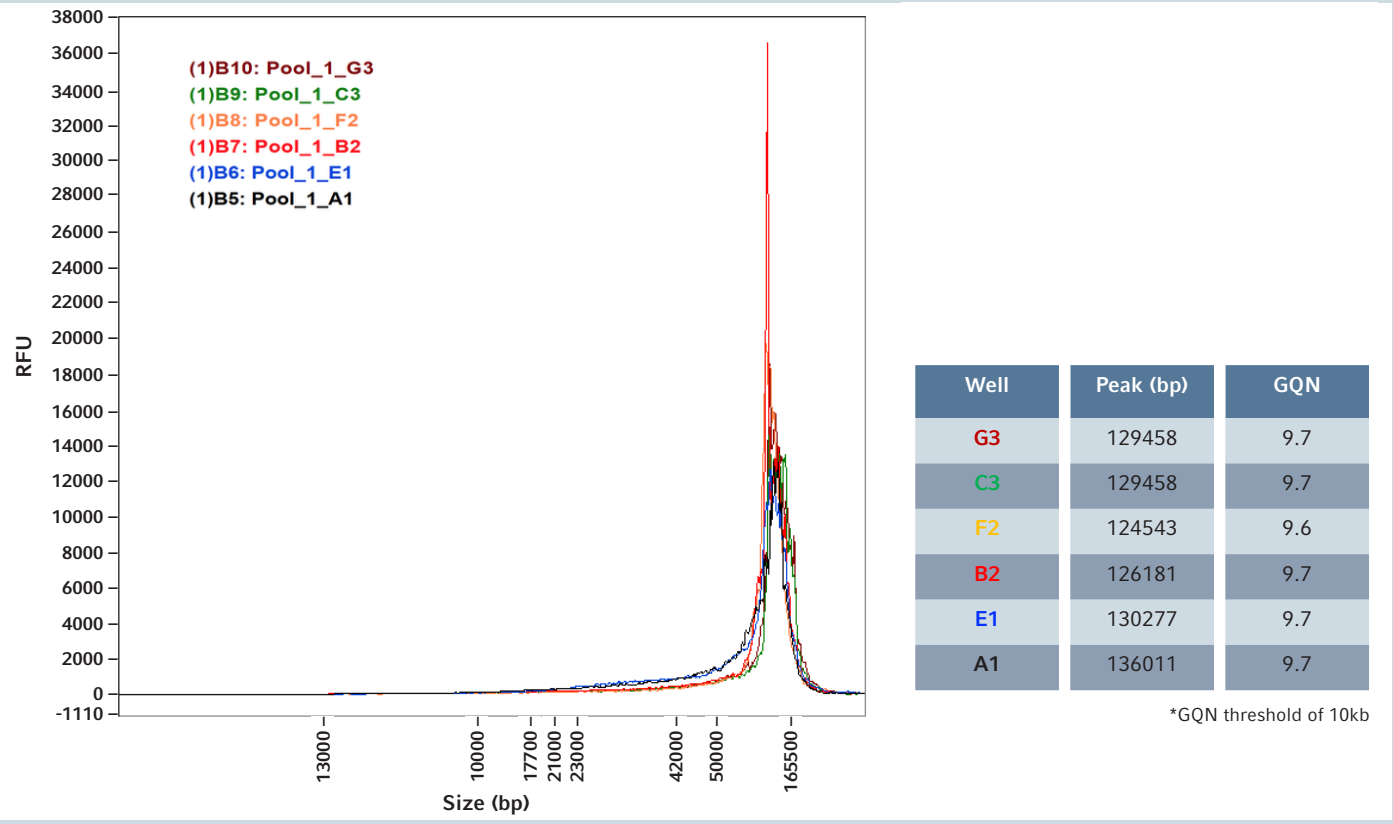


Figure 3: DNA extracted using epMotion showed A260/A280 ratios near 1.8 and A260/A230 ratios above 2.0 by Nanodrop analysis, demonstrating high DNA purity

DNA Quality:
The data from the Femto Pulse™ System (Agilent Technologies) confirmed the presence of HMW DNA with a GQN of 9.7 on an average, ideal for long-read sequencing.



Well	Peak (bp)	GQN
G3	129458	9.7
C3	129458	9.7
F2	124543	9.6
B2	126181	9.7
E1	130277	9.7
A1	136011	9.7

*GQN threshold of 10kb

Figure 4: DNA isolated with epMotion showed high molecular weight and an average GQN of 9.7 indicating suitability for long-read sequencing.

Reproducibility

Replicate extractions showed minimal variation in yield and purity metrics, highlighting the robustness and reproducibility of the automated workflow. The use of epMotion significantly reduced pipetting errors and hands-on time, improving overall laboratory efficiency.

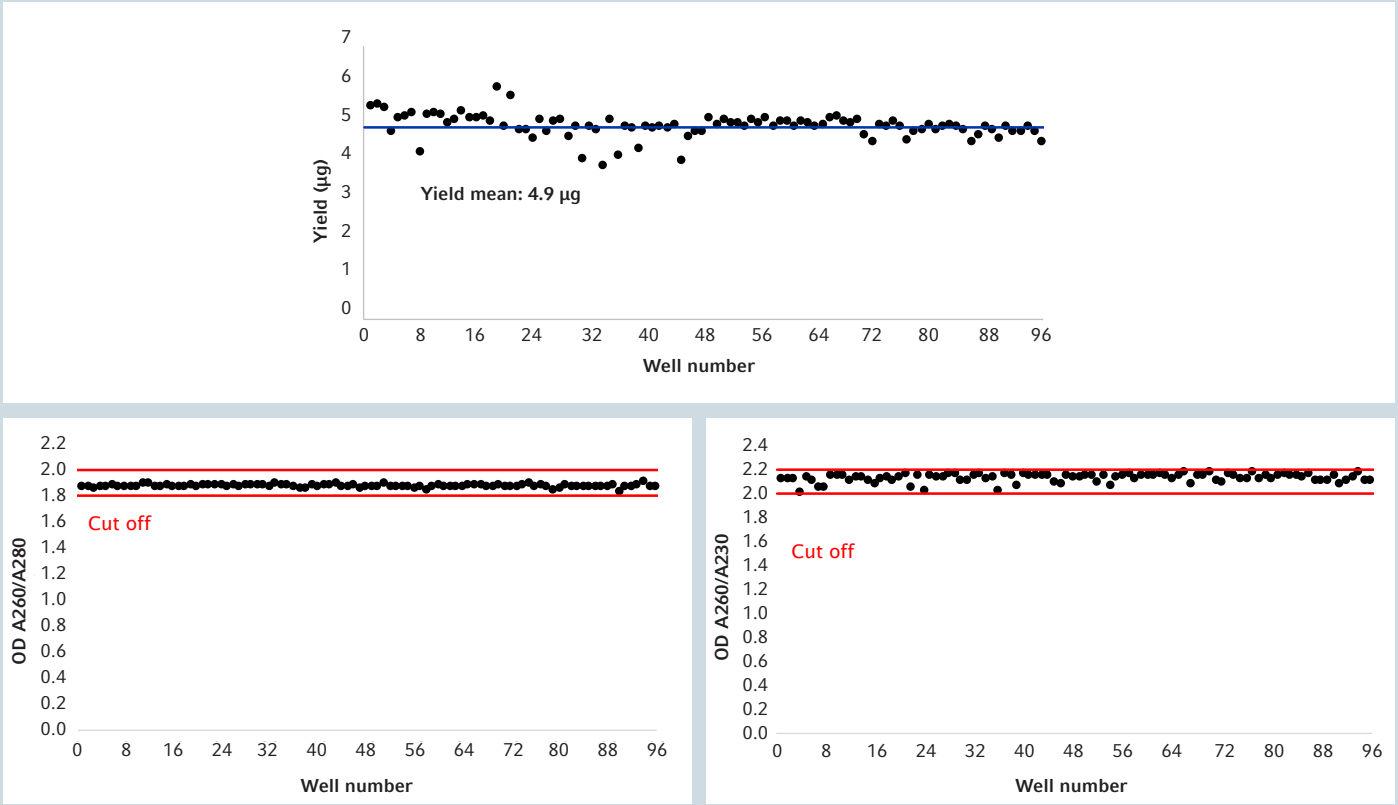


Figure 5: epMotion-enabled replicate extractions yielded consistent DNA quality and quantity, reducing pipetting errors and hands-on time for improved lab efficiency.

Summary

Overall, this data demonstrates the automated workflow for Nanobind HMW DNA extraction on the Eppendorf epMotion liquid handling system and showcases a high-quality, versatile, and robust solution for extracting DNA for PacBio® applications with high grade of reproducibility.

Your local distributor: www.eppendorf.com/contact
Eppendorf SE · 22339 Hamburg · Germany
eppendorf@eppendorf.com · www.eppendorf.com

www.eppendorf.com