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Enhancing Library Prep Efficiency with NEBNext® UltraExpress® FS DNA Library Prep Kit on the epMotion® 5075 Platform

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Introduction

Efficient and reliable DNA library preparation is crucial for sequencing workflows, particularly when handling large sample volumes. The NEBNext® UltraExpress® FS DNA Library Prep Kit (NEB E3340) simplifies this process by enabling streamlined library construction directly from intact DNA enabled by integrated enzymatic fragmentation and library prep protocol. When combined with the epMotion® 5075 automation system, the workflow reduces operator intervention and ensures consistent performance across samples. This combination saves valuable time and supports the generation of high-quality libraries with minimal effort for laboratories seeking to improve productivity and standardize processes.

Figure 1: The Eppendorf epMotion 5075 liquid handling system and NEBNext® UltraExpress® FS DNA Library Prep Kit

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User benefits

> **Integrated Workflow:** Enzymatic Fragmentation, End Prep, and Adaptor Ligation in a single streamlined automated protocol
> **Single-condition Workflow:** NEBNext® UltraExpress® FS DNA Library Prep kit workflow features a single-condition workflow (Adaptor dilution and PCR cycle number) across the input range
> **Walk-Away Automation:** Hands-free preparation of up to 48 libraries with minimal supervision
> **Time-Saving Efficiency:** Drastically reduce hands-on time and accelerate turnaround without compromising quality
> **Reproducible Results:** Consistent library yields and performance across runs and operators
> **Improve sample throughput:** to meet growing sequencing demands without adding staff or lab time.

Workflow

The automated workflow on the epMotion 5075t starts with 10–200 ng intact DNA and includes enzymatic fragmentation, end repair/dA tailing reaction setup, adaptor ligation, amplification setup, and cleanup steps.

Incubations, including Fragmentation/End Prep and PCR enrichment, are performed off-deck on the Eppendorf Mastercycler® X50s. The automated workflow enables a walk-away time savings of 150 minutes when processing up to 48 samples simultaneously.

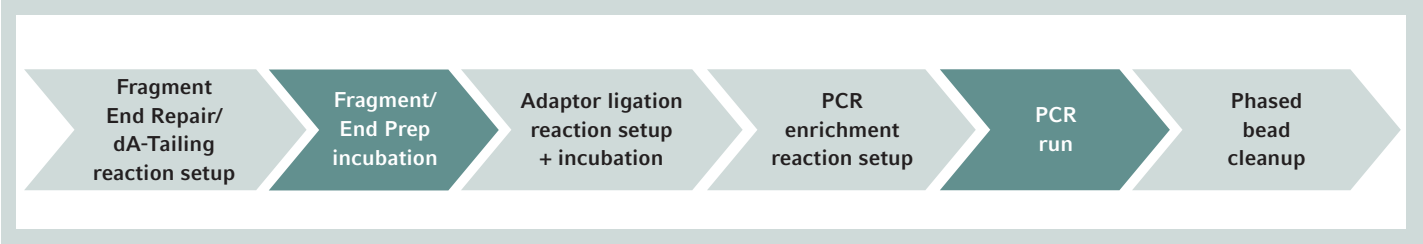


Figure 2: Schematic overview of the NGS library preparation using the NEBNext® UltraExpress® FS DNA Library Prep Kit and the Eppendorf epMotion (Light green background: step performed on the epMotion 5075t liquid handler; Dark green background: step performed off deck on the Eppendorf Mastercycler® X50s)

Result and Discussion

Libraries were prepared either via automation using the epMotion or manually from 10, 25, or 200 ng of Human Genomic DNA (Promega®, G3041) using the same adaptor amount and 8 PCR cycles. The input material used was sheared using the Covaris system.

The libraries generated on the epMotion 5075t with the NEBNext® UltraExpress® DNA Library Prep Kit demonstrated excellent reproducibility, exhibiting less than 10% coefficient of variation (CV). Library concentrations were consistent across all replicates for each input amount and adequate for sequencing across the full input DNA range (10 – 200 ng) specified by the kit.

	1	2	3	4	5	6
A	200ng	25 ng	10 ng	200ng	25 ng	10 ng
B	25 ng	10 ng	200ng	25 ng	10 ng	200ng
C	10 ng	200ng	25 ng	10 ng	200ng	25 ng
D	200ng	25 ng	10 ng	200ng	25 ng	10 ng
E	25 ng	10 ng	200ng	25 ng	10 ng	200ng
F	10 ng	200ng	25 ng	10 ng	200ng	25 ng
G	200ng	25 ng	10 ng	200ng	25 ng	10 ng
H	25 ng	10 ng	200ng	25 ng	10 ng	200ng

Figure 3: DNA input plate layout showing 48 samples distributed across three enzymatically fragmented gDNA input concentrations (10, 25, 200 ng) to assess performance of the automated process across 48 positions.

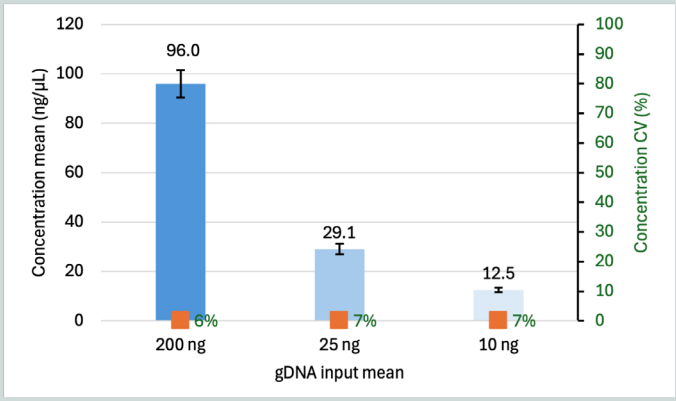
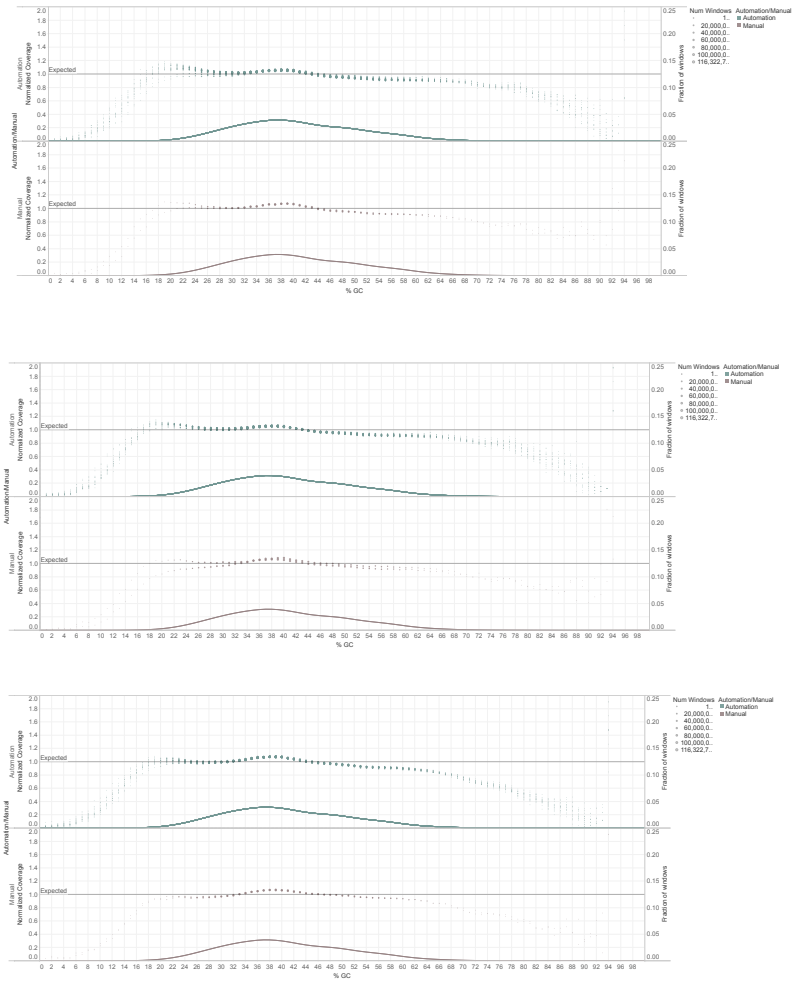


Figure 4: Library concentrations measured using Qubit dsDNA assays. Samples with input DNA concentrations of 10 ng and 25 ng were quantified using the Qubit dsDNA High Sensitivity (HS) Assay, while samples with 200 ng input were measured using the Qubit dsDNA Broad Range (BR) Assay.

Libraries were pooled and sequenced on an Illumina NextSeq® 500/550 (2 x 75 cycles). Sequencing results further confirmed consistent GC content and fragment size distribution across all libraries.

(A)



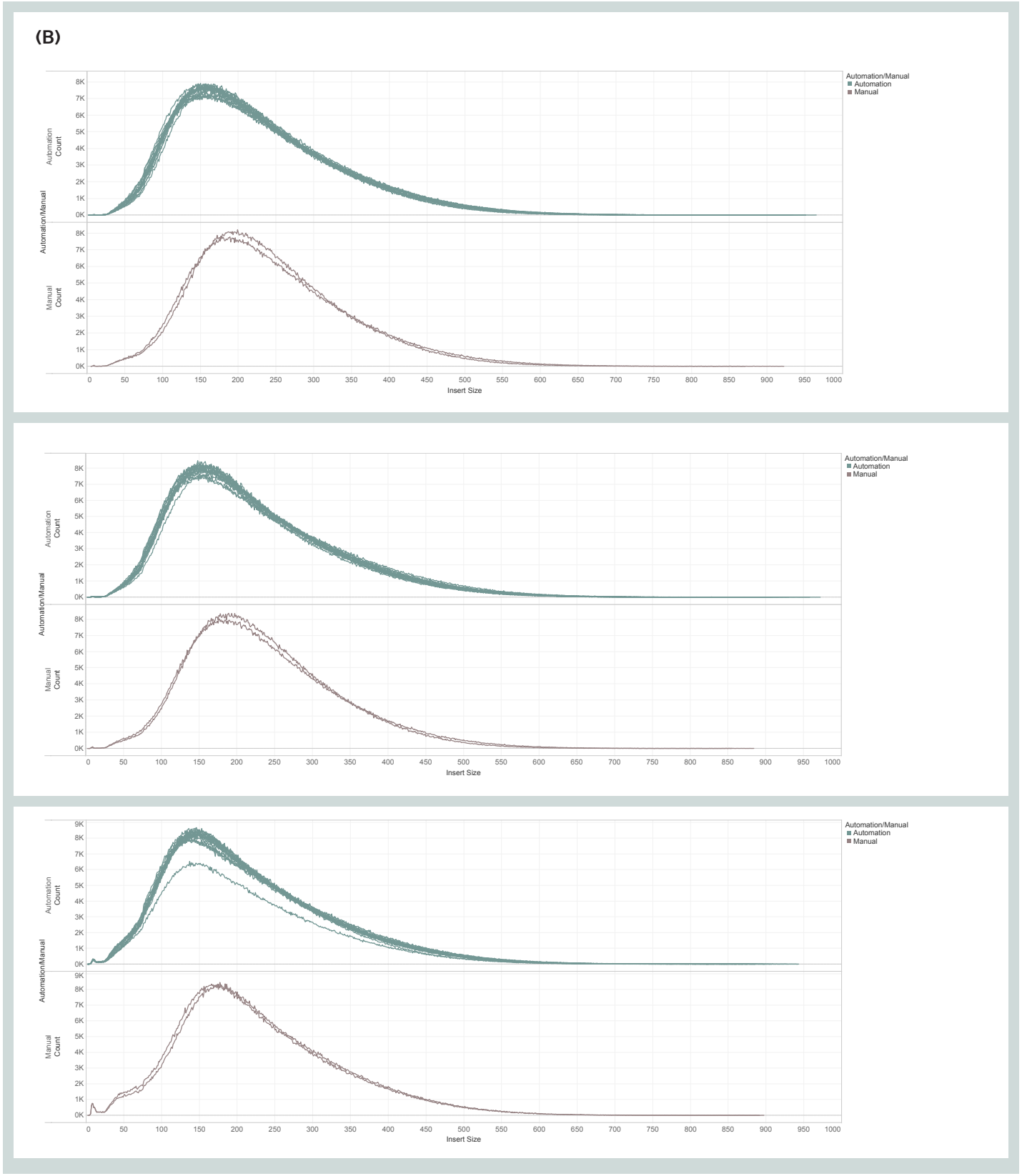


Figure 5: GC coverage (A) and Insert size (B) of libraries prepared with the NEBNext® UltraExpress® FS DNA Library Prep Kit on the epMotion 5075t (green) and manually (brown) across 3 DNA input ranges (10, 25 and 200 ng).

Conclusion

The automation of the NEBNext® UltraExpress® FS DNA Library Prep Kit provides robust and reproducible results, with optimized methods that are ready for immediate integration into your high-throughput sequencing workflows. Leveraging the flexibility of the epMotion 5075 system, users can go beyond this protocol.

The instrument supports a wide range of applications, with additional validated methods readily available to maximize lab productivity and adaptability.

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