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Maximizing Efficiency in NGS Workflows with NEBNext® UltraExpress® DNA Library Prep Kit using the epMotion® 5075 Automation System

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Introduction

Automating DNA library preparation is key to enhancing throughput, reproducibility, and efficiency in next-generation sequencing workflows. The NEBNext® UltraExpress® DNA Library Prep Kit (NEB E3325), enables the rapid construction of high-quality sequencing libraries from pre-fragmented DNA, making it an ideal choice for automation, such as the epMotion® 5075 system. This combination allows significant reductions in hands-on time, minimizes human error, and ensures consistent, high-quality results across samples. The epMotion 5075 enables seamless, walk-away processing of multiple samples. This makes it an optimal solution for laboratories aiming to scale up their sequencing operations without compromising performance. This library preparation kit and the epMotion device jointly provide a robust and efficient solution for modern genomics labs.

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

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User benefits

- **Walk-Away Automation:** Effortless preparation of up to 48 libraries from pre-fragmented DNA with minimal hands-on time
- **Rapid Turnaround:** Fast and streamlined protocol designed for automation, speeding up library preparation
- **Single-condition Workflow:** NEBNext® UltraExpress® DNA Library Prep kit workflow features a single-condition workflow across the input range regarding Adaptor dilution and PCR cycle number
- **Consistent NGS library quality:** Optimized reagents and streamlined workflow minimize variability between samples and runs
- **Ready-to-Use Workflow:** Pre-optimized reagents and optimized protocol designed for seamless automation and rapid setup
- **Simplified Training:** Standardized automated workflow makes it easy to train new users and maintain quality control
- **Scalable Throughput:** Automation solution keeps pace with increasing demands

Workflow

The automated workflow on the epMotion 5075t starts with 10–200 ng sheared (200 bp range) input DNA and includes end repair / dA tailing reaction setup, adaptor ligation, amplification setup, and cleanup steps.

Incubations, including End Prep, Adaptor Ligation, and PCR Enrichment, are performed off-deck on the Eppendorf Mastercycler® X50s, enabling a walk-away time savings of 142 minutes for 48 samples

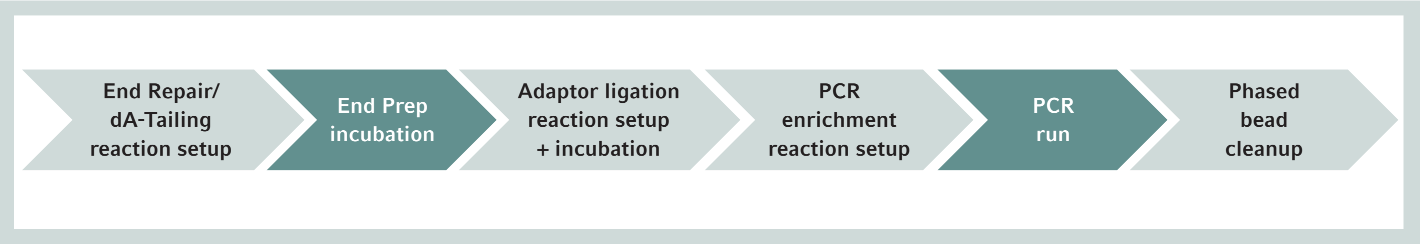

Figure 2: Schematic overview of the NGS library preparation using the NEBNext® UltraExpress® DNA Library Prep Kit and the Eppendorf epMotion (Light green background: step performed on the epMotion 5075t liquid handler; Dark green background: step performed off deck on the Eppendorf Mastercycler® X50s)

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 sheared gDNA input concentrations (10, 25, 200 ng) to assess performance of the automated process across 48 positions.

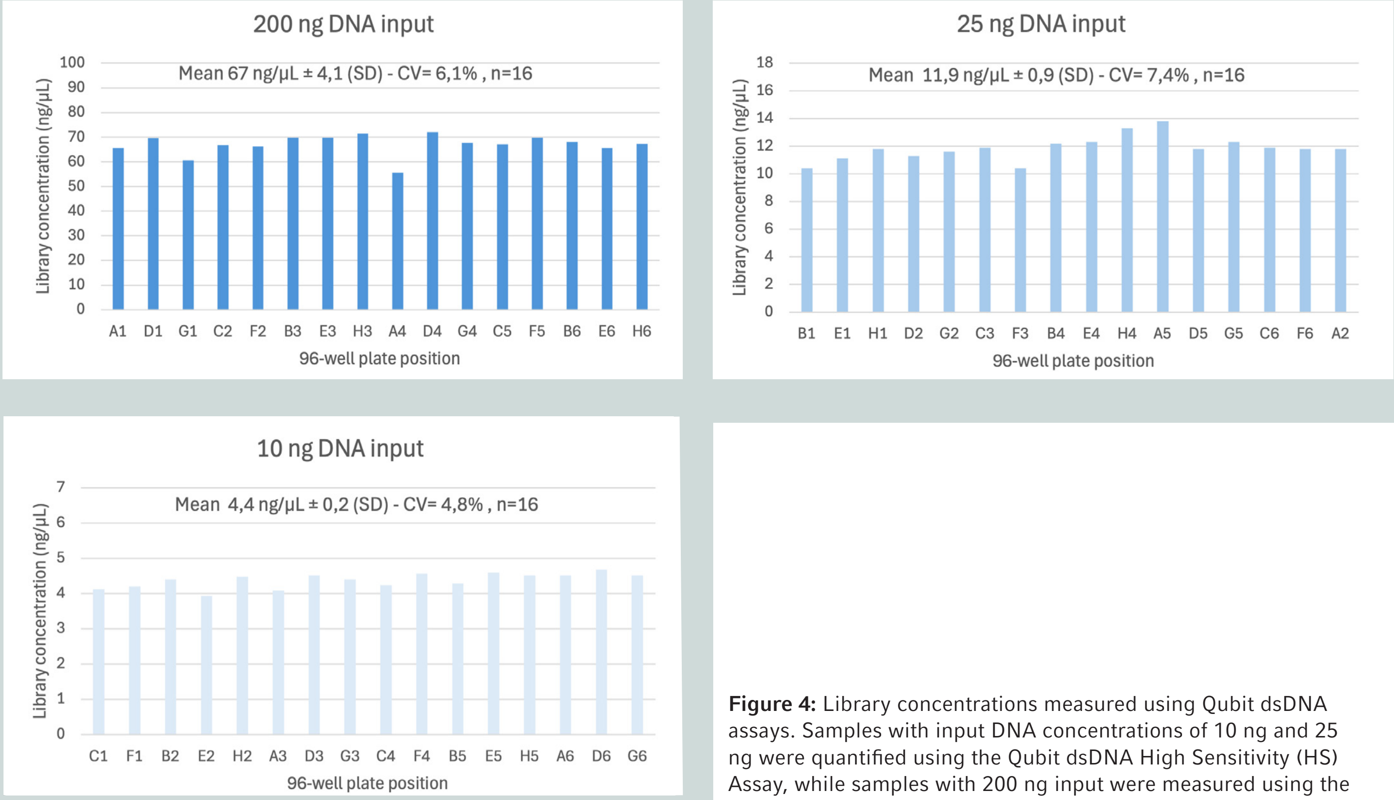

Figure 4: Library concentrations measured using Qubit dsDNA assays. Samples with input DNA concentrations of 10 ng and 25 ng were quantified using the Qubit dsDNA High Sensitivity (HS) Assay, while samples with 200 ng input were measured using the Qubit dsDNA Broad Range (BR) Assay.

Libraries were pooled and sequenced on an Illumina® NextSeq® 500/550 (2 x 75 cycles). Post-sequencing analysis showed uniformity in both GC coverage and insert size distribution among all prepared libraries. These results are consistent with those obtained through manual preparation.

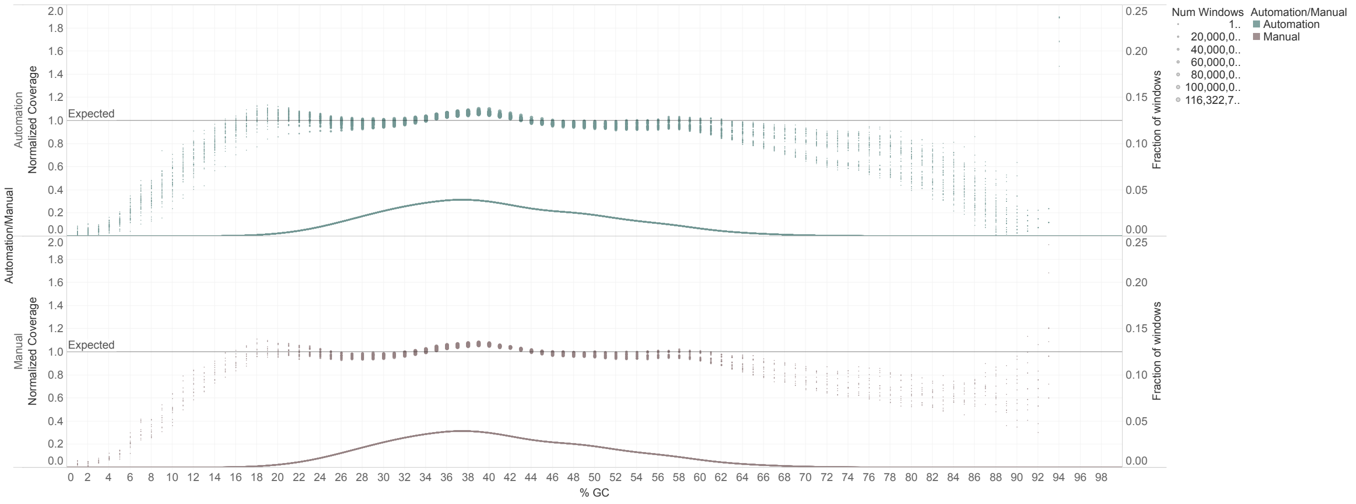

Figure 5: GC coverage of libraries prepared with the NEBNext® UltraExpress® DNA Library Prep Kit on the epMotion 5075t (green) and manually (brown) across three DNA input ranges (10, 25 and 200 ng).

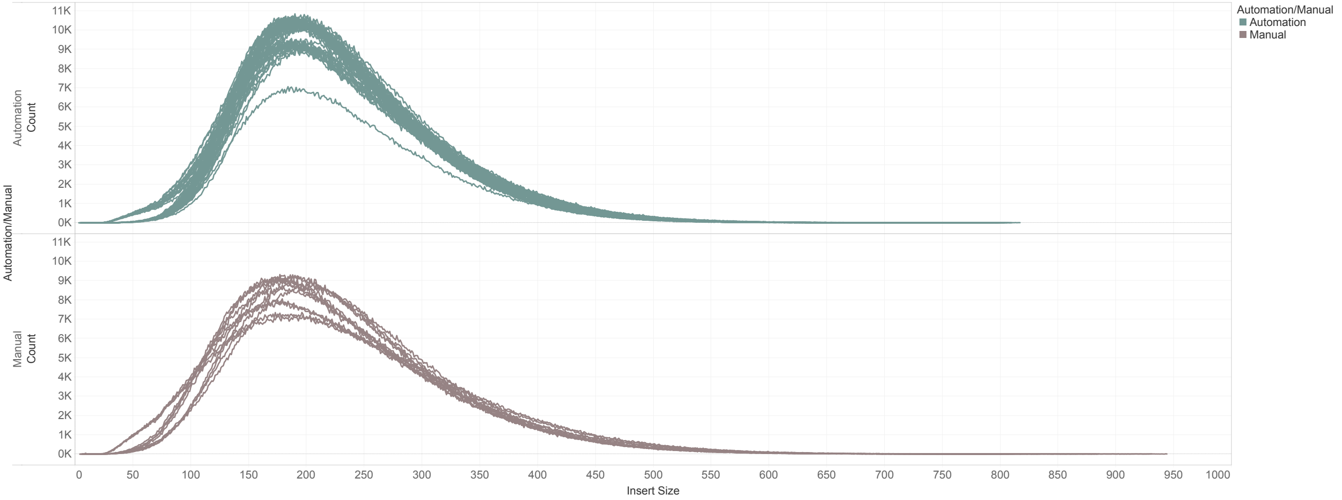

Figure 6: Insert size of libraries prepared with the NEBNext® UltraExpress® DNA Library Prep Kit on the epMotion 5075t (green) and manually (brown) across three DNA input ranges (10, 25 and 200 ng).

Conclusion

These results confirm that the automation of the NEBNext® UltraExpress® DNA Library Prep Kit delivers reproducibility- and high-quality performance with ready-to-implement protocols that bring efficiency and reliability to your sequencing workflows. The method described here is just one of many options available for the flexible epMotion system.

With its ability to support a wide range of applications – including additional workflow steps such as DNA purification – the epMotion 5075 offers laboratories a versatile solution for diverse automation needs.

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