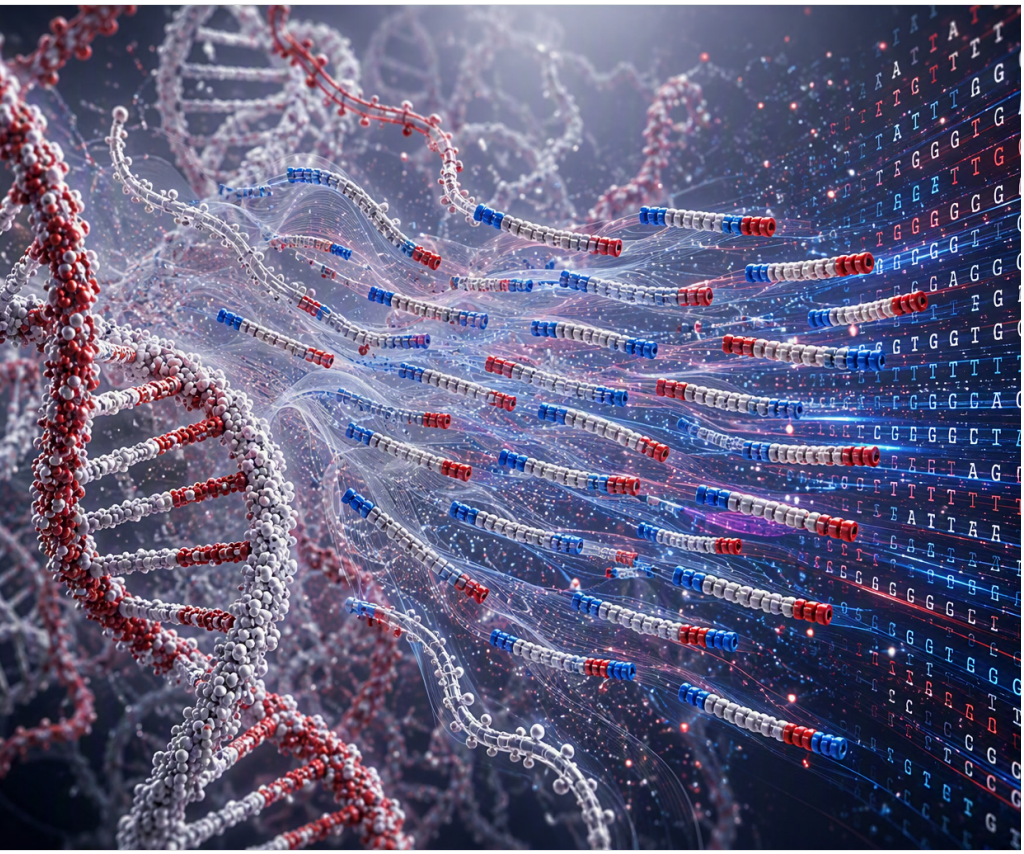

Challenge
Manual NGS library preparation is time-consuming, variable, and difficult to scale for DNA and RNA workflows.

Solution
Automated NEBNext DNA/RNA kits on liquid handlers with Biometra TRobot II for robust and reproducible library preparation.

Intended audience
Genomics laboratories in academia, CROs, and pharmaceutical R&D.

Automated NGS library preparation using the Biometra TRobot II

Introduction

Laboratories performing genomic workflows that involve any type of Next Generation Sequencing (NGS) methods are under constant pressure to process more samples, reduce hands-on time, and maintain consistent library quality across projects and operators. Automated library preparation addresses these needs by transferring repetitive pipetting, timed incubations, and protocol-critical reaction handling from manual workflows to integrated platforms.[1] Reducing manual intervention lowers the risk of pipetting errors, sample mix-ups, and process-to-process variability, while improving throughput and standardization.

A key success factor in automated library preparation is the performance of the integrated thermal cycler. Many NGS workflows depend on precise and reproducible temperature control during multiple sensitive enzymatic reaction steps such as fragmentation, end repair, dA-tailing, adapter ligation, reverse transcription, and PCR enrichment. The Biometra TRobot II was developed specifically for integration into automated workflows and supports reliable 24/7 operation, making it well suited for high-throughput NGS approaches. The Biometra TRobot II combines a compact footprint, robotic access from three sides, and a plate lifting function that supports seamless unloading of plates. These features help minimize integration complexity on liquid handling platforms while improving robustness in automated operation. Furthermore, the instrument provides outstanding heating and cooling rates and excellent temperature homogeneity across the sample block, both of which are critical for reproducible enzymatic reactions and consistent amplification.

This application note focuses on three representative NEB workflows that cover major automated library preparation use cases. The NEBNext Ultra II DNA Library Prep Kit for Illumina is designed for high-quality DNA libraries from low or challenging inputs and supports streamlined, automation-compatible processing. The NEBNext Ultra II FS DNA Library Prep Kit for Illumina further simplifies automation by combining fragmentation, end repair, and dA-tailing in

a single tube, reducing transfer steps and sample loss while supporting variable insert sizes via incubation time control. The NEBNext Ultra II Directional RNA Library Prep Kit for Illumina extends the concept to RNA-seq with a strand-specific workflow, broad input range, and automation-compatible design.

Together, these kit options illustrate how an integrated liquid handling system plus a high-performance automated thermal cycler can create a flexible platform for DNA and RNA library preparation. Such a setup can support improved reproducibility, increased throughput, reduced hands-on time, and more reliable process transfer from development into routine operation.

Materials and methods

Sample preparation

This automated workflow demonstrates how three NEBNext library preparation kits can be implemented on a liquid handling platform with an integrated Biometra TRobot II thermal cycler. The approach enables standardized processing for DNA and RNA applications while leveraging the thermal cycler's automation-oriented design, robust unattended operation, and high-performance temperature control.

By combining automated reagent dispensing, plate-based magnetic bead cleanups, and precise incubation/PCR handling on the Biometra TRobot II, laboratories can create a scalable workflow architecture that supports both development and routine operation. This is particularly relevant where laboratories want to consolidate multiple library preparation formats on one automation backbone without compromising thermal performance.

NEBNext Ultra II workflows are streamlined, automation-compatible solutions designed for low input amounts, reduced hands-on time, and scalable implementation on liquid handling instruments. Three kits were chosen:

A) NEBNext UltraExpress™ FS DNA Library Prep Kit (FS UE DNA)

This kit includes DNA fragmentation, end repair, and dA-tailing in the same well without intermediate cleanup, helping reduce sample handling complexity.

B) NEBNext UltraExpress™ DNA Library Prep Kit (UE DNA)

This kit requires pre-fragmented DNA and is suitable for low-input DNA, FFPE-compatible DNA workflows, and general DNA library preparation.

C) NEBNext UltraExpress™ RNA Library Prep Kit (UE mRNA)

This kit includes strand-specific RNA-seq after poly(A) enrichment or rRNA depletion. This workflow is suitable for low-input RNA, including low-quality RNA such as FFPE-derived material.

All liquid handling steps were conducted on a Biomek i7 robot integrating the Biometra TRobot II on the deck. Thermal cycling programs were selected according to the respective kit protocols and sample input amounts (Table 1-3).

Samples and reagents

- NEBNext UltraExpress™ FS DNA Library Prep Kit (E3340S/L)
- NEBNext UltraExpress™ DNA Library Prep Kit (E3325S/L)
- NEBNext UltraExpress™ RNA Library Prep Kit (E3330S/L)

Sample preparation

- Genomic DNA, Roche
- Total human RNA

Instrument settings

- Biometra TRobot II 96 SG automated thermal cycler, Analytik Jena (846-2-070-902)
- Biomek i7 automated workstation, Beckman Coulter
- Qubit, fluorometric quantification, Thermo Fisher Scientific
- 5200 Fragment Analyzer, capillary electrophoresis, Agilent

Method parameters

Table 1: Thermal cycler program for FS UE DNA kit

Step	Cycle	Profile	Temperature	Duration	Ramp rates
Fragmentation[I] End prep[I]	1	Incubation	37 °C	20 min	max
	1	Incubation	65 °C	15 min	max
	1	Hold	4 °C	Inf	max
Adapter ligation	1	Incubation adapter[II]	20 °C[II]	15 min	max
	1	Hold	4 °C	Inf	max
	1	Incubation USER enzyme[III]	37 °C[III]	5 min	max
	1	Hold	4 °C	Inf	max
PCR enrichment	1	Initial denaturation	98 °C	30 sec	max
	7	Denaturation	98 °C	10 sec	max
	7	Extension	65 °C	75 sec	max
	1	Final extension	65 °C	5 min	max
	1	Hold	4 °C	Inf	max

I - Heated lid ≥ 75 °C, II - Heated lid off, III - Heated lid ≥ 47 °C

Table 2: Thermal cycler program for UE DNA kit

Step	Cycle	Profile	Temperature	Duration	Ramp rates
End prep[I]	1	Incubation	20 °C	15 min	max
	1	Incubation	65 °C	15 min	max
	1	Hold	4 °C	Inf	max
Adapter ligation	1	Incubation Adapter[II]	20 °C[II]	15 min	max
	1	Hold	4 °C	Inf	max
	1	Incubation USER enzyme[III]	37 °C[III]	5 min	max
	1	Hold	4 °C	Inf	max
PCR enrichment	1	Initial denaturation	98 °C	30 sec	max
	8	Denaturation	98 °C	10 sec	max
	8	Extension	65 °C	75 sec	max
	1	Final extension	65 °C	5 min	max
	1	Hold	4 °C	Inf	max

I - Heated lid ≥ 75 °C, II - Heated lid off, III - Heated lid ≥ 47 °C

Table 3: Thermal cycler program for UE mRNA kit (with NEBNext Poly(A) mRNA Magnetic Isolation Module)

Step	Cycle	Profile	Temperature	Duration	Ramp rates
mRNA isolation[I]	1	Denaturation[I]	65 °C[I]	5 min	max
	1	Hold	4 °C	Inf	max
mRNA isolation[II]	1	Elution[II]	80 °C[II]	2 min	max
	1	Hold	25 °C	Inf	max
mRNA elution	1	Denaturation[III]	94 °C[III]	15 min	max
	1	Hold	4 °C	Inf	max
First strand cDNA synthesis	1	cDNA synthesis[IV]	25 °C[IV]	10 min	max
	1	cDNA synthesis	42 °C	10 min	max
	1	cDNA synthesis	70 °C	5 min	max
	1	Hold	4 °C	Inf	max
Second strand cDNA synthesis	1	cDNA synthesis[V]	16 °C[V]	30 min	max
	1	Hold	4 °C	Inf	max
End prep[VI]	1	Incubation	20 °C	15 min	max
	1	Incubation	65 °C	15 min	max
	1	Hold	4 °C	Inf	max
Adapter ligation	1	Incubation Adapter[VII]	20 °C[VII]	15 min	max
	1	Hold	4 °C	Inf	max
	1	Incubation USER enzyme[VIII]	37 °C[VIII]	5 min	max
	1	Hold	4 °C	Inf	max
PCR enrichment	1	Initial denaturation	98 °C	30 sec	max
	14	Denaturation	98 °C	10 sec	max
	14	Extension	65 °C	75 sec	max
	1	Final extension	65 °C	5 min	max
	1	Hold	4 °C	Inf	max

I - Heated lid ≥ 75 °C, II - Heated lid ≥ 90 °C, III - Heated lid 105 °C, IV - Heated lid ≥ 80 °C, V - Heated lid ≤ 40 °C or off, VI - Heated lid ≥ 75 °C, VII - Heated lid ≤ 40 °C or off, VIII - Heated lid ≥ 45 °C

Results and discussion

Library quantification

Automated library preparation workflows using the three NEBNext kits generated libraries across the range of tested input amounts with generally increasing yields as the input increased. For the FS UE DNA, library concentrations increased from approximately 4 ng/µL at 10 ng input DNA to 24 ng/µL at 200 ng input DNA (Figure 1). The UE DNA produced substantially higher yields, reaching approximately 31, 62, 89, and 95 ng/µL from 25, 50, 100, and 200 ng DNA inputs, respectively. The UE mRNA generated reproducible libraries across all tested mRNA inputs, with concentrations ranging from approximately 3.5 to 6.5 ng/µL. Error bars indicated low to moderate variation between replicates, demonstrating robust automated processing and consistent thermal control throughout the workflow. Overall, the liquid handling system and Biometra TRobot II thermal cycler enabled reliable library generation for DNA and RNA applications, with library yields scaling according to input material and kit chemistry.

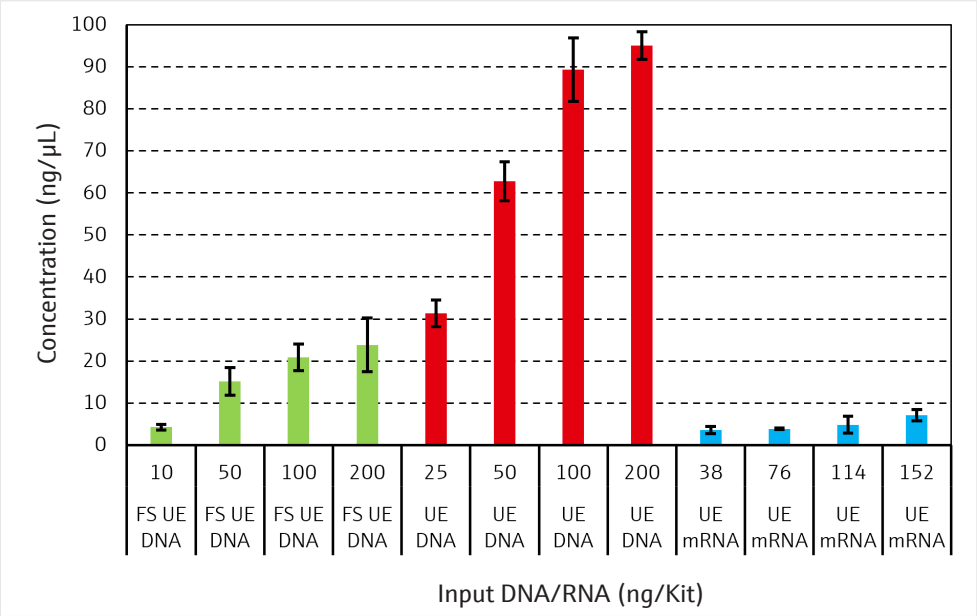

Figure 1: Quantification of the final libraries after preparation using fluorescence detection. Four different amounts of initial DNA or RNA were tested for each of the three used library preparation Kits. Every bar represents the mean of at least four replicates. Error bars show the standard deviation.

Library size distribution and quality control

Fragment analysis was performed following automated library preparation to verify library integrity and assess fragment size distribution. All analyzed libraries (A1–H1; Figure 2) exhibited highly comparable electropherogram profiles, with a broad and uniform fragment distribution predominantly ranging from approximately 250 to 1,000 bp and peak intensities centered around 350–500 bp. The absence of significant low-molecular-weight peaks indicated effective removal of adapter dimers and other small reaction by-products. Furthermore, the consistent size profiles observed across all samples demonstrate excellent reproducibility of the automated workflow and confirm that library construction, cleanup, and thermal processing were performed reliably. These results indicate that the integrated liquid handling platform and Biometra TRobot II thermal cycler generated high-quality NGS libraries suitable for downstream sequencing applications.

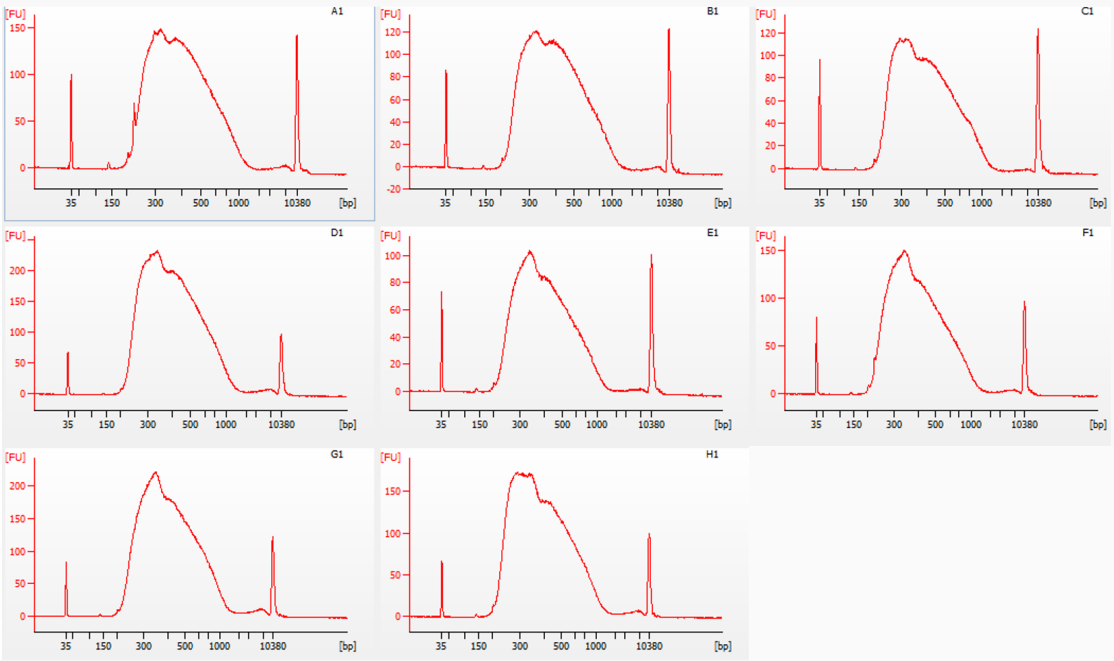

Figure 2: Size validation of eight replicated final libraries using capillary electrophoresis. Exemplarily results are shown for UE mRNA. Samples A1-E1, B1-F1, C1-G1, and D1–H1 correspond to initial RNA inputs of 38, 76, 114, and 152 ng, respectively. Electropherograms of each panel show the fluorescent signals against the size of an individual library ranging from 250 to 1,000 bp including a small and large marker.

Summary

Automated NGS library preparation was successfully implemented on a liquid handling platform equipped with the integrated Biometra TRobot II thermal cycler for three representative NEBNext workflows covering DNA, fragmented DNA, and mRNA library construction. Across all tested input amounts, the automated process generated reproducible library yields and consistent fragment size distributions, demonstrating reliable workflow execution and robust assay performance.

A key contributor to this performance was the Biometra TRobot II, which provided precise thermal control for critical incubation and amplification steps throughout the library preparation process. Its excellent temperature homogeneity, rapid heating and cooling rates, and reliable unattended operation ensured consistent reaction conditions across all samples. Furthermore, the automation-oriented design, including compact footprint, robotic accessibility, and seamless integration into liquid handling workflows, enabled efficient hands-off processing while minimizing the risk of user-induced variability.

The combination of automated liquid handling and high-performance thermal cycling resulted in a scalable and standardized NGS library preparation solution capable of supporting routine laboratory operation. The results obtained in this study demonstrate that the Biometra TRobot II is a valuable component for automated NGS workflows, delivering the reproducibility, robustness, and throughput required for modern sequencing laboratories.

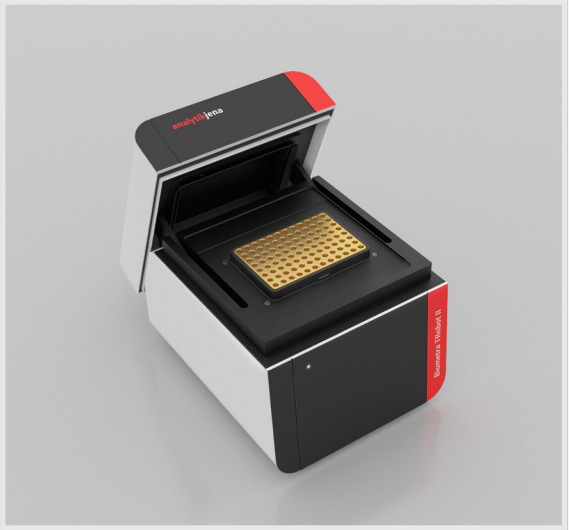

Figure 3: Biometra TRobot II

Recommended device configuration

Table 4: Overview of devices, accessories, and consumables

Article	Article number	Description
Biometra TRobot II 96 SG, 230 V*	846-2-070-902	Automated thermal cycler with a 96 well silver block for highest demands in research and routine.

* available as 100 V, 115 V and 230 V version

Acknowledgements

We gratefully acknowledge Ferris Jung & Genomics Core Facility of EMBL Heidelberg for providing the data used in this application note. Visit www.embl.org/groups/genomics

GeneCore

References

[1] Hess, J. F. et al. Library preparation for next generation sequencing: A review of automation strategies. Biotechnol. Adv. 41, 107537 (2020).

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Version 1.0 · Author: Markus Krüger, Ferris Jung
en · 08/2026