

Automated KAPA HyperPlus DNA Library Preparation for Illumina® Sequencing on the Eppendorf epMotion® 5075t

Nathan Quon¹, Cheng Liu², Ph.D., Eleanor Cowley¹, M.S., Rachel Kasinskas¹, Ph.D. and Dan Stover¹, M.S.

¹Roche Sequencing & Life Science | Kapa Biosystems, Wilmington, USA; ²Eppendorf AG, Hamburg, Germany

Abstract

The rapid growth and declining costs of Next Generation Sequencing (NGS) have increased the demand for high throughput sequencing capacity. Automation of library preparation offers customers efficient and effective tools for meeting the increased throughput demands of the NGS

world. Eppendorf has partnered with Kapa Biosystems, an industry leader in life science reagents, to develop an automated method for the KAPA HyperPlus Library Preparation Kit on the Eppendorf epMotion 5075t automated liquid handling system, as described in this application note.

Introduction

The KAPA HyperPlus Kit enables rapid construction of DNA libraries for Illumina sequencing. It is compatible with a wide range of sample types and input amounts (1 ng – 1 µg), making it one of the most versatile kits on the market. The novel one-tube chemistry streamlines the DNA fragmentation and library construction processes, yielding libraries of similar or better quality than those produced with KAPA HyperPrep Kits and Covaris®-sheared DNA. This highly optimized protocol, including engineered enzymes, optimally formulated buffers, and minimal cleanup steps, results in efficient conversion of input DNA to adapter-ligated library, enabling deep and uniform sequence coverage.

The method developed on the Eppendorf epMotion 5075t offers an automated solution for preparation of up to 48 samples, with the capability of scaling up to 96 samples

per run. Modular programming gives the user flexibility to run a size selection step if desired, before or after PCR amplification. The system's user-friendly interface also guides the user through the run setup, including placement of the labware and required reagent volumes. An optical sensor verifies that all labware is correctly placed before the run starts. Moreover, epMotion's walk-away potential for generating PCR-free libraries is maximized by an on-deck ThermoMixer that provides homogeneous reaction mixtures, a thermal module that enables on-deck incubation steps, and a gripper that transports plates to various deck positions. Workflows with PCR require the use of an off-deck thermocycler.



Materials and Methods



Figure 1: Eppendorf epMotion 5075t automated liquid handling system.

Experimental Design

Sixteen libraries were prepared from varying input amounts of *Escherichia coli* (*E.coli*) genomic DNA to ensure the automated method performed within the KAPA HyperPlus Kit specifications. All incubations were performed on-deck, with the exception of the library amplification. Molecular biology grade mineral oil, an inert overlay, was used to prevent evaporation during the end repair and A-tailing incubation. Four replicates each of 1 ng, 10 ng, 50 ng and 200 ng inputs were fragmented at 37 °C for 35 minutes. End repair and A-tailing was performed at 65 °C for 30 minutes. The adapter concentrations were matched to the input amounts according to the kit specifications, as shown below in Table 1. The ligation reaction was performed at 20 °C for 15 minutes, followed by a 0.8× post-ligation cleanup and 0.6× – 0.8× size

selection. The number of library amplification cycles was adjusted for each input amount to achieve final library yields between 100 ng – 1 µg. The method was completed with a final 1× post-amplification cleanup.

Quality control (QC) samples at several stages in the workflow were collected during the validation of the automated method. QC samples were recovered after the post-ligation cleanup, size selection, and the post-amplification cleanup as shown in Figure 2. For each QC sample, 2 µL of sample was stored in 18 µL of 10 mM Tris-HCl (pH 8.0 – 8.5) to prevent degradation. Samples were quantified using the KAPA Library Quantification Kit (LQK), which measures library fragments containing the sites necessary for flow cell hybridization and Illumina sequencing. This quantification is a cost-effective alternative to sequencing that can indicate viability of the prepared libraries prior to downstream sequencing.

Post-ligation and post-size selection QC samples were diluted 1:10,000 and post-amplification QC samples diluted 1:100,000 to ensure that all data points fell within the standard curve of the KAPA LQK assay. All qPCR samples and standards were run in triplicate (outlier data points were excluded from the analyses) on the Eppendorf MasterCycler® thermal cycling device. Final library size distributions were determined by running 1:5 dilutions of the post-amplification libraries on the Agilent® 2100 BioAnalyzer® with the High Sensitivity DNA Assay.

Table 1: Experimental summary of epMotion 5075t validation run.

Sixteen libraries were prepared: four replicates each from 1 ng, 10 ng, 50 ng, and 200 ng input amounts with appropriate adapter concentrations and PCR cycles to generate between 100 ng – 1 µg of final library.

DNA Input	Adapter Stock Concentration	Number of PCR Cycles
1 ng	0.3 µM	14
10 ng	3 µM	10
50 ng	15 µM	6
200 ng	15 µM	4

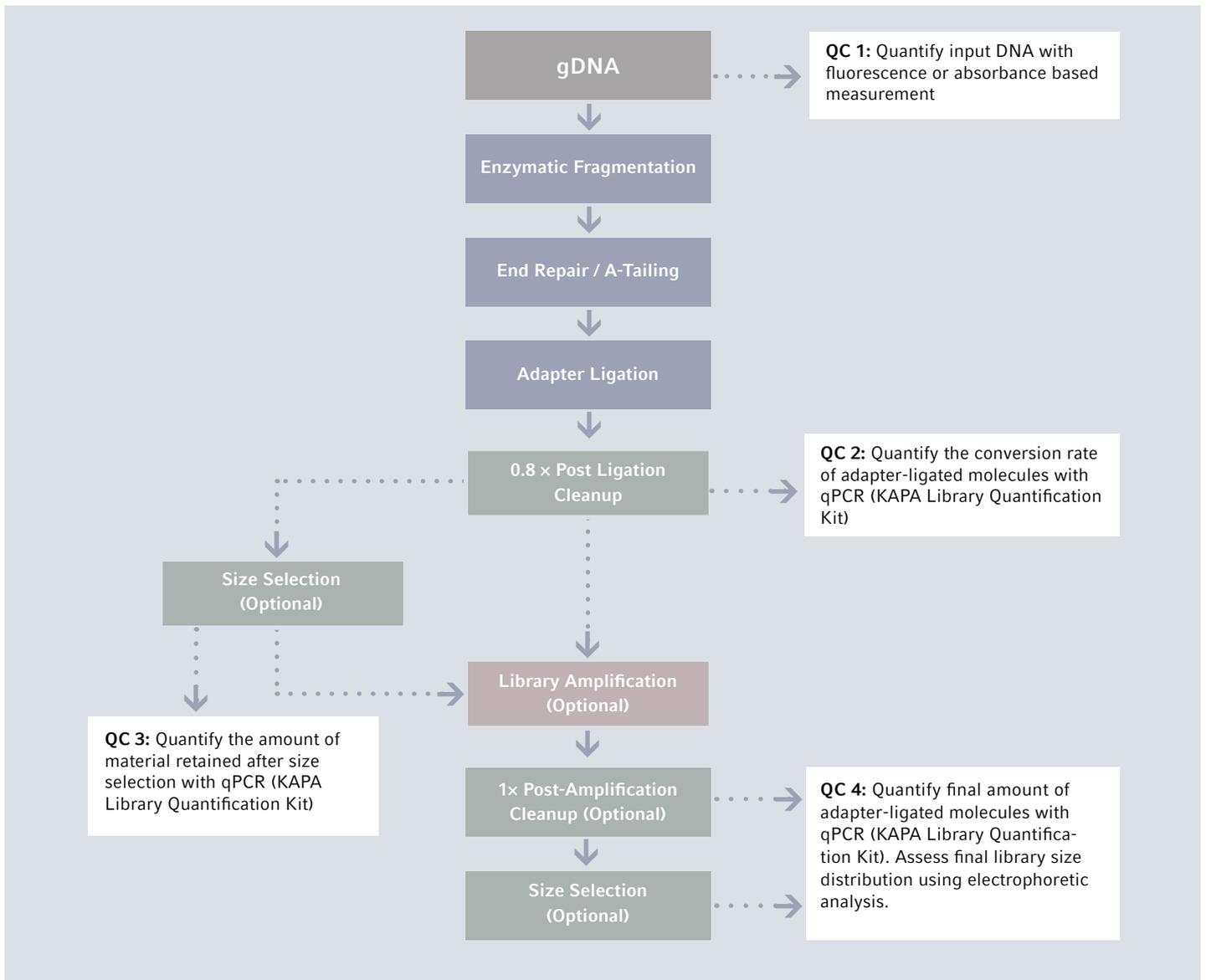


Figure 2: KAPA HyperPlus workflow shown with optional QC checkpoints. The approximate total instrument time to run the 16 samples was 4 hours, including on-deck incubations. Size Selection, Library Amplification, and Post-Amplification Cleanup are optional steps that can be omitted, which would reduce the run time to ~2.5 hours.

Results and Discussion

The post-ligation qPCR results were used to calculate the percentage of starting material that was successfully adapter-ligated, or the conversion rate as shown in Figure 3. Higher conversion rates are generally achieved with higher input amounts into library preparation, which results in libraries with greater complexity. With lower input amounts, a larger proportion of material may be lost to DNA adsorption to plastic surfaces, which can contribute to decreased conversion rates. For inputs above 100 ng, conversion rates typically

range between 50 – 100%. For inputs between 10 ng – 100 ng, conversion rates range between 10 – 50%. For inputs 1 – 10 ng, conversion rates range between 5 – 20%. Across all replicates, each input exceeded the expected conversion rates. The high conversion rates indicate that the Eppendorf LoBind® quality consumables (PCR plates, tubes) may improve sample recovery yields throughout the library construction process.

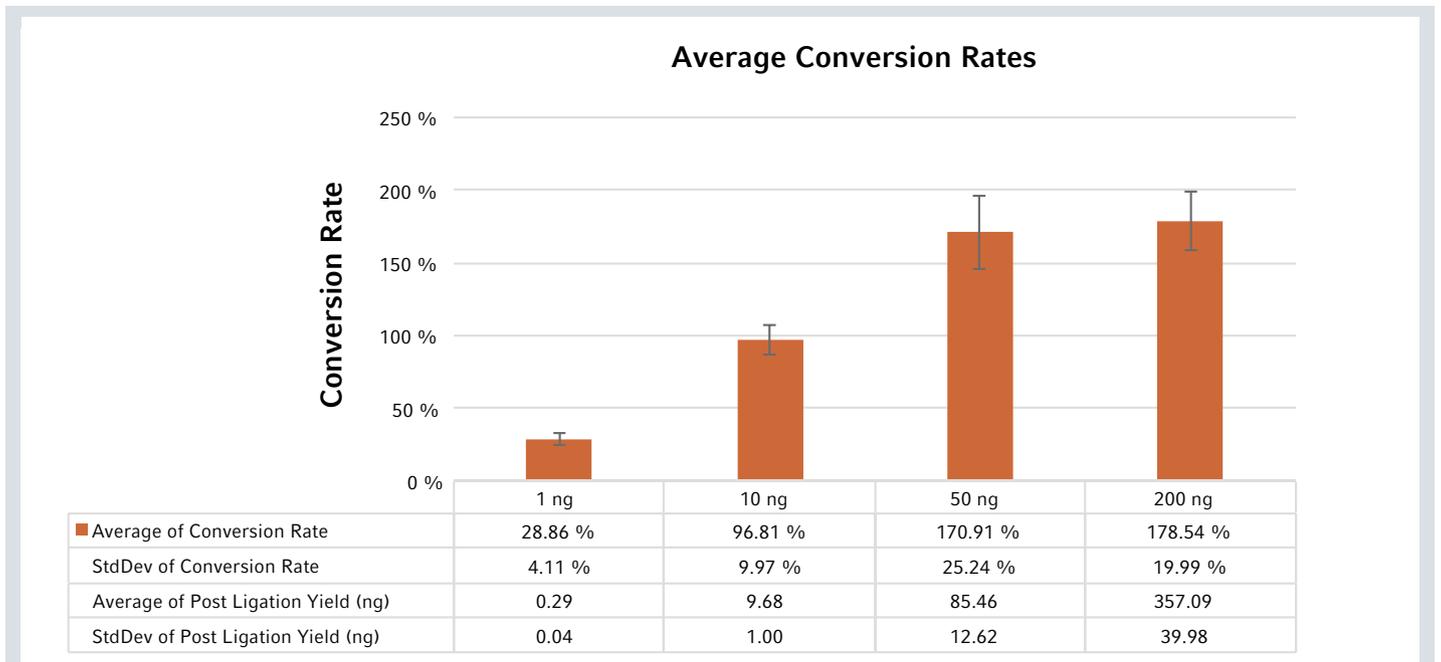


Figure 3: Post-Ligation Yields and Conversion Rates. All input amounts resulted in higher conversion rates than typically observed in manual experiments. 1 ng inputs had an average conversion rate of 28.86% compared to 5 – 20% typical conversion rates. 10 ng inputs and 50 ng inputs had average conversion rates of 96.81% and 170.91%, respectively, compared to 10 – 50% typical conversion rates. 200 ng inputs had an average conversion rate of 178.54% compared to 50 – 100% typical conversion rates. Conversion rates above 100% can be explained by highly efficient adapter ligation with little to no sample loss during the post-ligation cleanup. The addition of the ~140 bp adapters to the DNA fragments increases the overall molecular weight of the samples. If most of the DNA fragments were adapter-ligated and carried through the post-ligation cleanup, then the net yield can be higher than the initial input concentration.

The post-size selection qPCR results were used to calculate the percentage of material retained from the post-ligation cleanup as shown in Figure 4. Typically, 5 – 20% of

material is retained from size selection. Across all inputs, the size selection retention fell within the expected range.



Figure 4: Post-Size Selection Yields and Percentage Retained from Post-Ligation material. Following the 0.6x – 0.8x size selection, all input amounts resulted in 10 – 17% retention from the post-ligation cleanup, which falls within the expected range of 5 – 20%

The post-amplification qPCR results were used to calculate the amplification efficiency as shown in Figure 5. Library amplification efficiency is typically $\geq 80\%$, but can vary depending on the quality of sample and number of PCR cycles. With the chosen PCR cycling parameters, both the 1 ng and 10 ng input samples had an average amplification efficiency greater

than 80%. The 50 ng and 100 ng input samples had an average amplification efficiency of approximately 74%, still being within the normal range. The number of PCR cycles should be optimized according to the requirements for downstream processes.

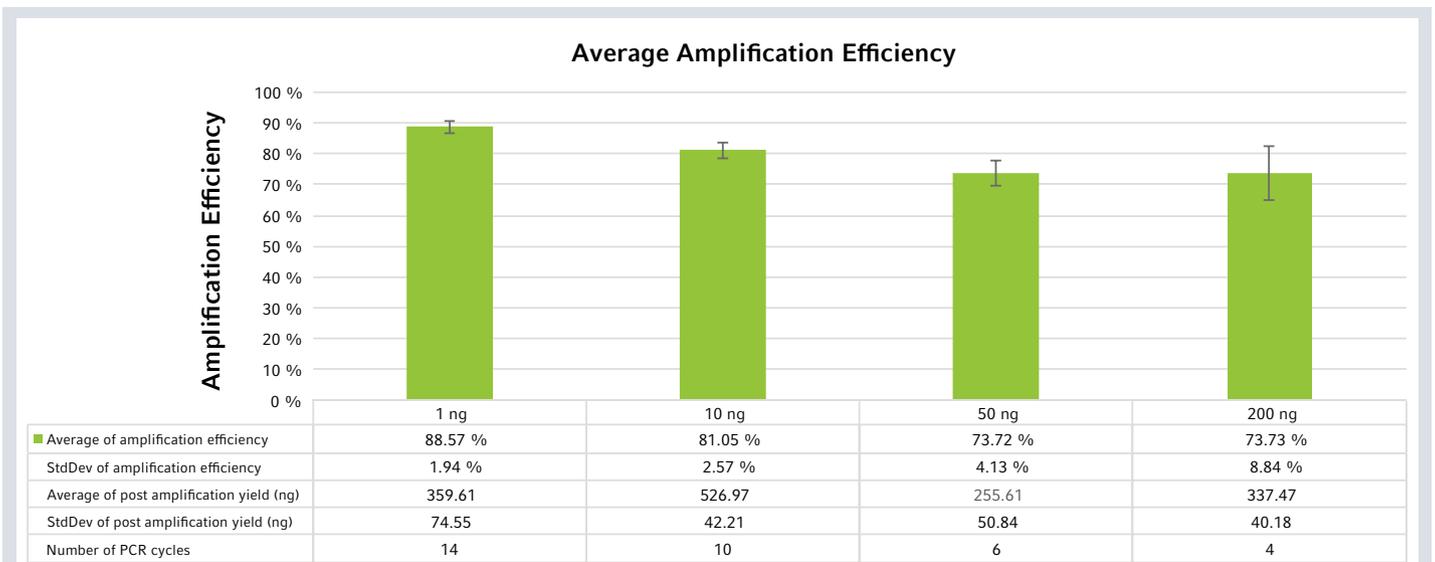


Figure 5: Post-Amplification Yields and Amplification Efficiency. The number of PCR cycles varied across input amounts to achieve final library yields between 100 ng – 1 μ g. Across all inputs, the final library yields fell within the expected range.

The Agilent 2100 BioAnalyzer was used to determine the final library size distributions shown in Figure 6. The 0.6x – 0.8x size selection was expected to retain fragments between 250 – 450 bp. Across all inputs, the average final library sizes

were between 305 – 330 bp, with narrow and reproducible size distributions. The absence of adapter-dimer (~140 bp) and large fragments (> 450 bp) shows the size selection process to be highly effective.

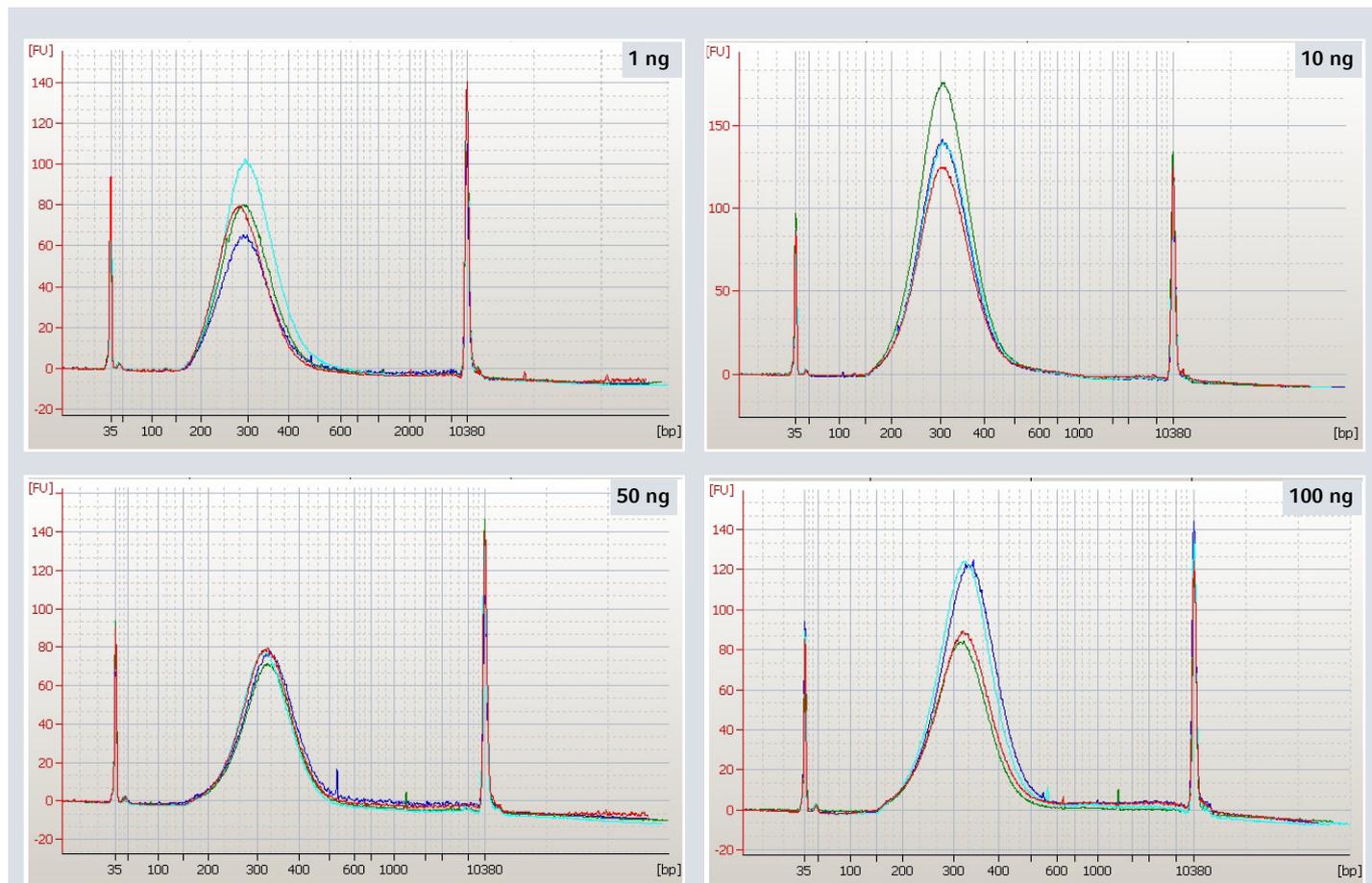


Figure 6: Electrophoretic Size Analysis of Final Libraries across all inputs. Size analysis of all 16 replicates was performed. The average size of the 1 ng (top left) and 10 ng inputs (top right) were 305 bp +/- 8 bp and 317 bp +/- 1 bp, respectively. The average size of the 50 ng (bottom left) and 200 ng inputs (bottom right) were 330 bp +/- 5 bp and 330 bp +/- 7 bp, respectively. Within all replicates, the average size varied by less than 8%, which shows that size selection was highly robust.

Conclusion

NGS library construction is a critical step in sample preparation for sequencing on Illumina platforms. The increased demand for high-quality NGS libraries in high-throughput laboratories has necessitated the development of robust, automated methods for library preparation. Kapa Biosystems, an industry leader in NGS library preparation, has partnered with Eppendorf to automate the KAPA HyperPlus Kit on the Eppendorf epMotion 5075t.

The experimental data shows that the automated method performs within the KAPA HyperPlus Kit specifications at a wide range of input amounts from 1 ng – 200 ng. The highly flexible and modular automated solution for NGS library preparation will allow laboratories to easily scale up experiments without sacrificing quality in the process.

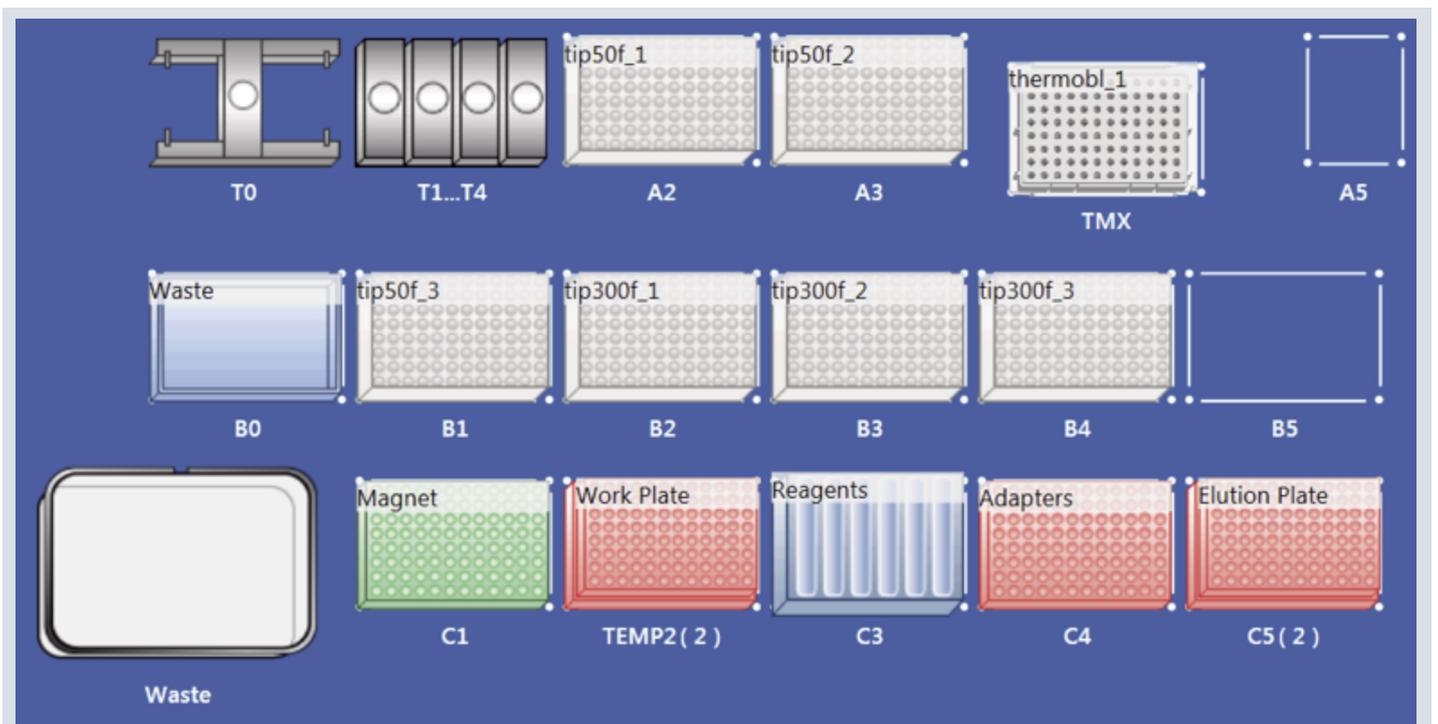


Figure 7: Eppendorf epMotion 5075t Deck Layout. This figure shows the fully walk-away setup for fragmentation, end repair, A-tailing, adapter ligation and bead-based purification steps before the library amplification.

Ordering information

Description	Order no. international
epMotion® 5075t	5075 000.302
Thermal module on position C2	5075 757.001
TS 50 Dispensing Tool	5280 000.010
TM50-8 Dispensing Tool	5280 000.215
TM300-8 Dispensing Tool	5280 000.231
Gripper	5282 000.018
Thermoblock PCR 96 OC	5075 751.666
Thermoadapter PCR 96 (2x)	5075 787.008
Reservoir rack	5075 754.002
Reservoir Rack Module TC, 4 × 0.5/1.5/2.0 mL	5075 799.081
Reservoir Rack Module TC, 4 × 5.0 mL	5075 799.340
epT.I.P.S.® Motion, 50 µL, filtered	0030 014.413
epT.I.P.S.® Motion, 300 µL, filtered	0030 014.456
epMotion® Reservoir, 30 mL	0030 126.505
epMotion® Reservoir, 400 mL	5075 751.364
Eppendorf twin.tec® PCR Plate 96, semi-skirted	0030 128.575
Eppendorf twin.tec® PCR Plate 96, skirted	0030 128.648
Eppendorf Safe-Lock Tubes, 1.5 mL	0030 120.086
Eppendorf Safe-Lock Tubes, 5.0 mL	0030 108.310
Alpaqua® MAGNUM FLX® Enhanced Universal Magnet Plate	A000400
KAPA HyperPlus Kits (96 reactions)	07962428001

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