# Illumina® Nextera® Flex for Enrichment on the ep*Motion*® 5075t NGS Solution


## Abstract

Targeted enrichment workflows allow users to focus on informative DNA regions. Nevertheless, many enrichment workflows pose long and difficult procedures. Together with Illumina, Eppendorf has qualified an approach for a simpler, faster, and flexible workflow using the ep*Motion* liquid handling workstation. This workflow is compatible with classical enrichment oligo panels, used in e.g. Nextera Rapid Capture protocols, yet quicker being based on the new Nextera Flex chemistry. Here we demonstrate its implementation and performance of our solution. Overall with only a few minutes hands on time the automated workflow helps to further increase the productivity and efficiency of an NGS lab without compromising high quality results.


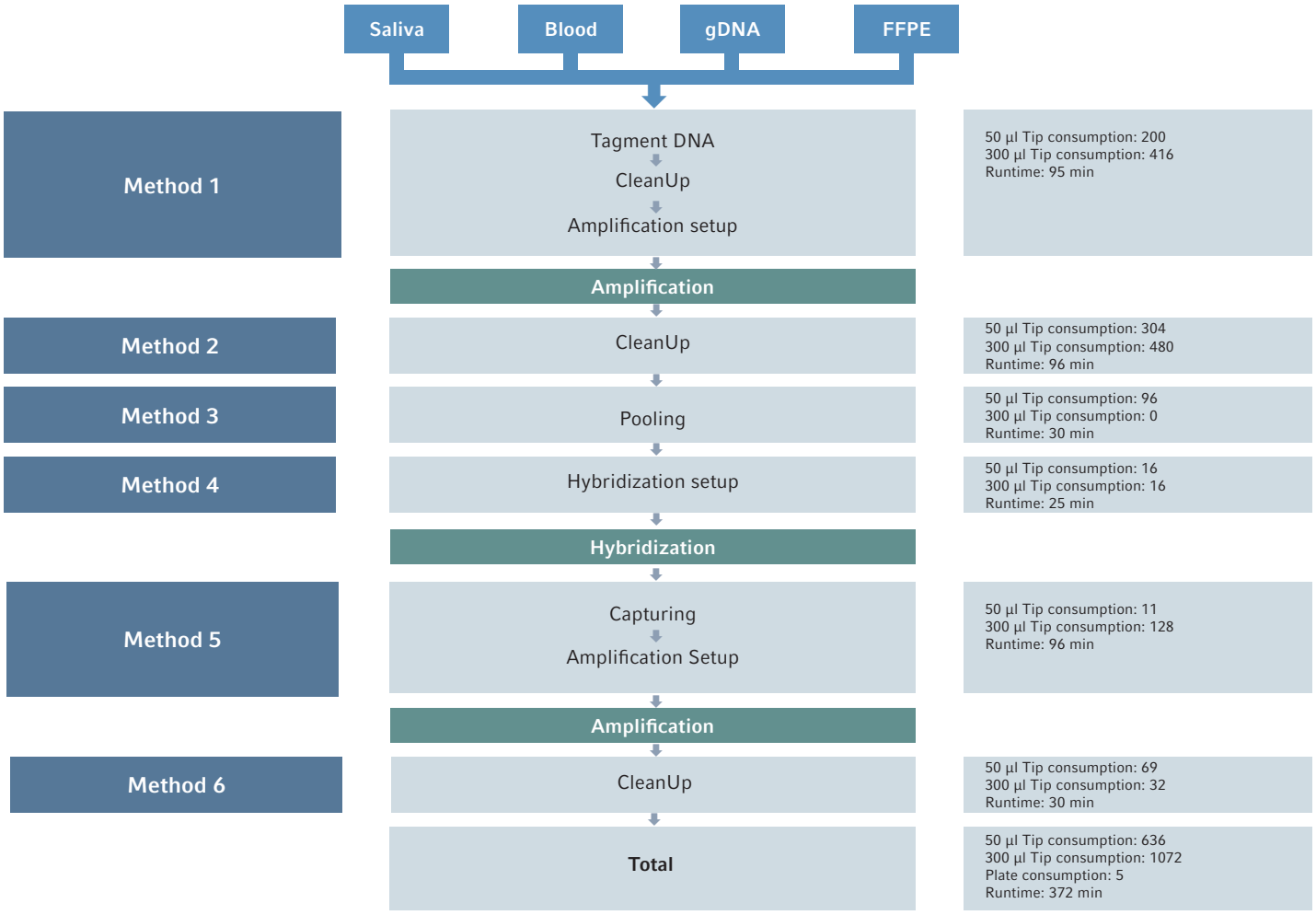


**Figure 1:** Demonstrated workflow for the Illumina Nextera Flex for Enrichment protocol on the ep*Motion*. The protocol accepts different inputs which are not part of this demonstrated workflow. The grey boxes show submethods processed on the ep*Motion*. Steps highlighted in green are performed off-deck using the Mastercycler® X50. In alignment with the protocol, workflows are compartmented into logical units. Detailed run times and for Methods 1-3: 96 samples; Methods 4-6: 8 pools are shown next to the methods, as well as consumption of epT.I.P.S.® Motion and Eppendorf twin.tec® PCR plates. The automated methods are capable of processing variable numbers of samples in approx. 10 hours considering PCR, hybridization and worktable setup, which are not included in this figure.

## Example Results for Illumina Nextera Flex for Enrichment

To demonstrate the performance of the automated solution we prepared libraries from 8 replicates of a gDNA input and the Illumina Nextera DNA Exome Panel. Fragment sizes and enrichment were reproducible among replicate experiments showing reproducible performance (figure 2 and table 2).

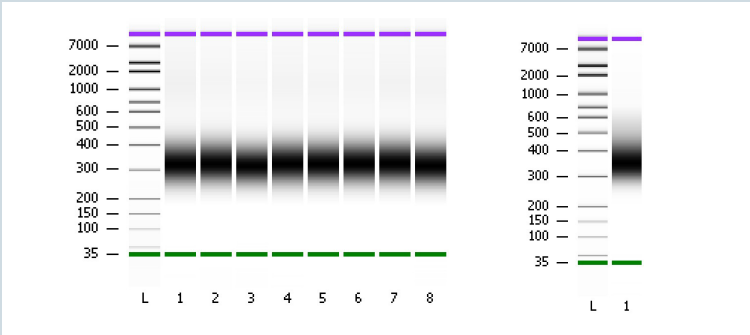


**Figure 2:** Example of 2100 BioAnalyzer® electropherograms showing the fragment distribution of libraries at the end of Method 2 (left) and post-enrichment as a pool at the end of Method 6 (right). Library preparation was automated with the ep*Motion* 5075t NGS.

**Table 1:** Sequencing results of 2 separate automated library preparation runs performed with the ep*Motion* 5075t NGS.

| | Sequencing Results Pool 1 | Sequencing Results Pool 2 |
|---|---|---|
| Total Aligned Reads | 18,899,580 | 18,693,439 |
| Fragment Length Median | 183 bp | 185 bp |
| Percent Aligned Reads | 94.3% | 95.62% |
| Read Enrichment | 74.96% | 76.37% |
| Padded Read Enrichment | 80.9% | 82.33% |
| Percent Padded Base Enrichment | 80.34% | 81.77% |
| Percent of Targeted Regions Covered End-to-End | 93.01% | 95.62% |
| Mean Region Coverage Depth (Fold) | 25.4x | 25.8x |
| Uniformity of Coverage (Pct > 0.2*mean) | 89.34% | 91.75% |

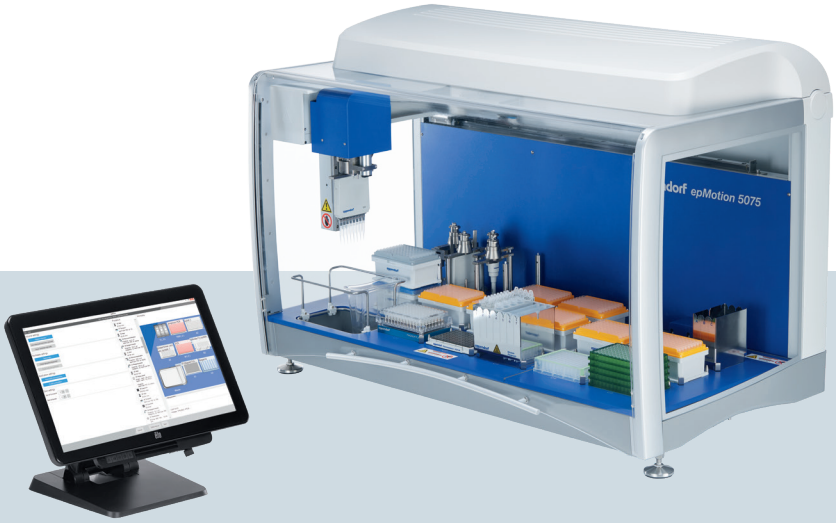


**Figure 3:** The Eppendorf ep*Motion* is a multi-purpose liquid handling workstation, suitable for many laboratory procedures. The ep*Motion* 5075t and other models in the family are ideal walk-away companions for labs that demand high efficiency, accuracy, and automated workflows. The on-deck thermal-mixing module and the incorporated thermal module provide incubation capability needed for NGS library preparation. Eight dispensing tools covering 0.2-1000 µL range are available in both single- and 8-channel formats to meet different throughput and volume requirements. For labware transports between e.g. magnets or temperature-controlled positions, a gripper is available.

**Ordering information**

| Description | Order no. international | Order no. North America |
|---|---|---|
| **ep*Motion*® 5075t NGS Solution** | 5075 000.962 | 5075000962 |
| **ep*Motion*® 5075tc NGS Solution** | 5075 787.963 | 5075000963 |
| **Thermoadapter PCR 96 (1x)** | 5075 787.008 | 960002199 |
| **Thermoadapter for Deep Well Plates, 96 wells/1,000 µL (1x)** | 5075 751.054 | 960002391 |
| **Mastercycler® X50s, silver block, 96-well or 0.1/0.2 mL tubes, with touchscreen interface** | 6311 000.010 | 6311000010 |

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