Application Note

HAMILTON

Automating the **KAPA EvoPlus** Kit from Roche on the Hamilton **NGS STAR V** Generates High-Quality DNA Libraries for Whole Genome Sequencing

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Introduction

Library preparation is a key requirement for Next-Generation Sequencing (NGS) applications and is among the most critical segments of the sequencing workflow. The full automation of the KAPA EvoPlus workflow on the Hamilton NGS STAR V 2.0 MPH96 (further referred to as NGS STAR V; Fig. 1) allows efficient NGS library preparation of 96 samples for Whole Genome Sequencing in a single run.

The appropriate placement of reagents, plates and tips is guaranteed, using automated barcode verification. The user can also define in-process controls and upload a worklist with the combination of indexes and samples. The automated error handling and the easy-to-use VENUS framework ensure a smooth setup of the workflow, which can also be started and stopped at specific steps within the process.

Method Description

The KAPA EvoPlus DNA Library Prep Kit 2.0 MPH96 method (further referred to as KAPA EvoPlus method) automates the KAPA EvoPlus Kit protocol (Version 1.0_11/21) on the NGS STAR V. The workflow facilitates conversion of input DNA material into high-quality NGS libraries to conduct Whole Genome Sequencing on Illumina platforms. With this method, either 10 – 50 ng, 75 – 250 ng or 500 ng of genomic DNA can be processed using slightly different workflows (Fig. 2). The method was developed using the KAPA EvoPlus Kit in a 96 well plate format (#09420428001).

- Hamilton's NGS STAR V offers several temperature-controlled positions to also meet the needs of highly-demanding NGS library protocols
- Speeds up library preparation and maximizes walk-away time
- Optimal process reliability via safety light and standardized workflows

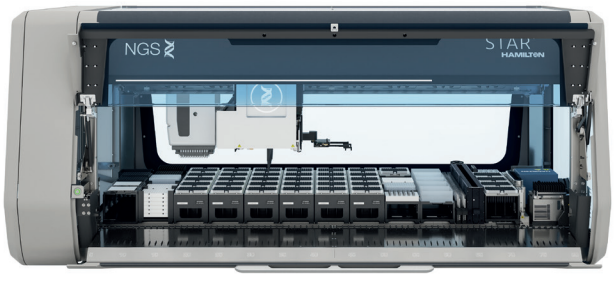


Figure 1: The Hamilton NGS STAR V Assay Ready Workstation

Roche

Visual Workflow

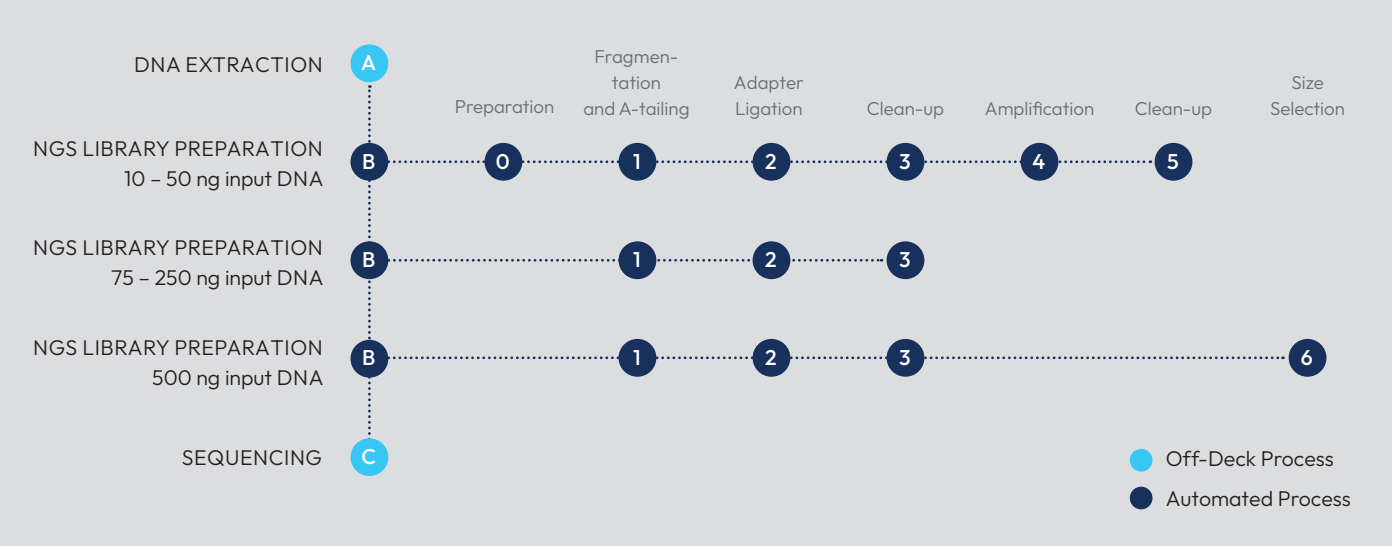
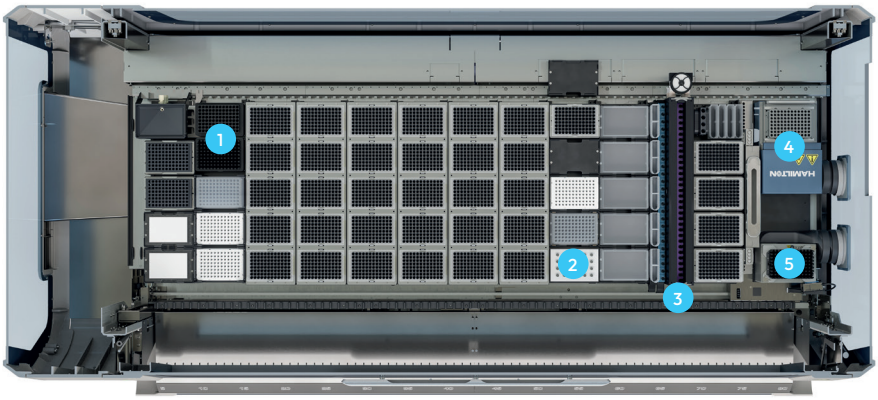


Figure 2: Graphical Overview of the KAPA EvoPlus Method

System Description

The NGS STAR V workspace (Fig. 3) is optimally equipped to generate high-quality NGS libraries, also for highly demanding protocols. The Cooling Carrier Module, the On-Deck Thermal Cycler (ODTC), and two SBS cooling positions (CPACs) as well as a Thermoshake, ensure optimal temperature-controlled sample handling.

A magnetic stand and tip carriers, together with carriers for samples and reagents complete the ideal deck for NGS library preparation of the NGS STAR V.



1. High Performance Cooling Modules (CPACs)
2. Magnetic Stand
3. Linear Cooling Carrier
4. On-Deck Thermal Cycler (ODTC)
5. Heating-Shaking Module

Figure 3: Deck Layout of the NGS STAR V

Yield and Quality

The performance of the KAPA EvoPlus method on the NGS STAR V was evaluated by preparing NGS libraries, using the KAPA Unique Dual-Index Adapter Kit (Roche, #08861919702). A maximum number of 96 samples per run with either 10 ng or 500 ng Human Genomic DNA (Roche, #11691112001) as input material was tested for technical and biological performance on the NGS STAR V.

KAPA EvoPlus method using 10 ng input DNA

For biological verification of the KAPA EvoPlus method using 10 ng Human Genomic DNA as input material, NGS library preparation was performed. The workflow included the Amplification step using six PCR cycles but excluded the Size Selection step. A fragmentation time of 10 min was chosen. Final library concentrations of the samples from either eight-, 48- or 96-sample biological verification runs have been determined using the QuantIT 1X dsDNA HS Assay Kit (ThermoFisher Scientific, #Q33266). The average sample yield was 77.2 ± 4.6 ng for the eight-sample run, 84.8 ± 19.7 ng for the 48-sample run and 105.0 ± 13.8 ng for the 96-sample run.

Subsequently, library size distribution of 15 samples randomly selected from the 96-sample biological verification run was determined with the Agilent TapeStation 4150 using the D5000 ScreenTape (Agilent, # 5067-5592) with the D5000 Reagents Kit (Agilent, #5067-5593) (Fig. 4). The average library size was 449 ± 12 bp.

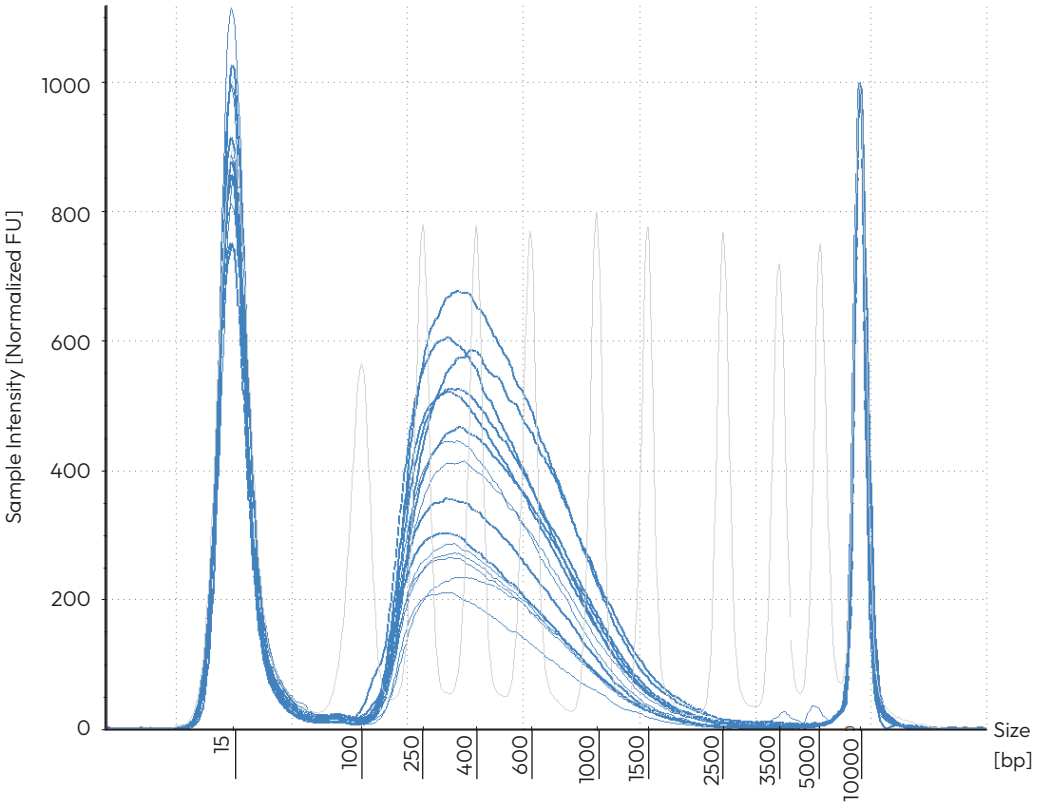


Figure 4: Size distribution of the NGS library DNA generated from 10 ng input DNA with the KAPA EvoPlus method; from 15 samples out of the 96-sample biological verification run, library size distribution was determined. Therefore, the generated library DNA was loaded on the TapeStation4150 and analyzed using the D5000 ScreenTape with the D5000 Reagents Kit

KAPA EvoPlus method using 500 ng input DNA

For biological verification of the KAPA EvoPlus method using 500 ng Human Genomic DNA as input material, NGS library preparation was performed. The workflow included the Size Selection step but excluded the Amplification step (PCR-free). A fragmentation time of 10 min was chosen. Final library concentrations of the samples from either eight-, 48- or 96-sample biological verification runs have been determined using the QuantIT 1X dsDNA HS Assay Kit. The average sample yield was 60.1 ± 7.0 ng for the eight-sample run, 68.6 ± 8.9 ng for the 48-sample run and 79.8 ± 9.1 ng for the 96 sample run.

Subsequently, library size distribution of 10 samples randomly selected from the 96-sample biological verification run was determined with the Agilent TapeStation 4150 using the D5000 ScreenTape with the D5000 Reagents Kit (Fig. 5). To enable visualization of PCR-free libraries on a TapeStation, an amplification with four PCR cycles had to be performed beforehand, followed by a 1x/1x bead clean-up with KAPA HyperPure beads. The average library size detected with the TapeStation was 517 ± 40 bp.

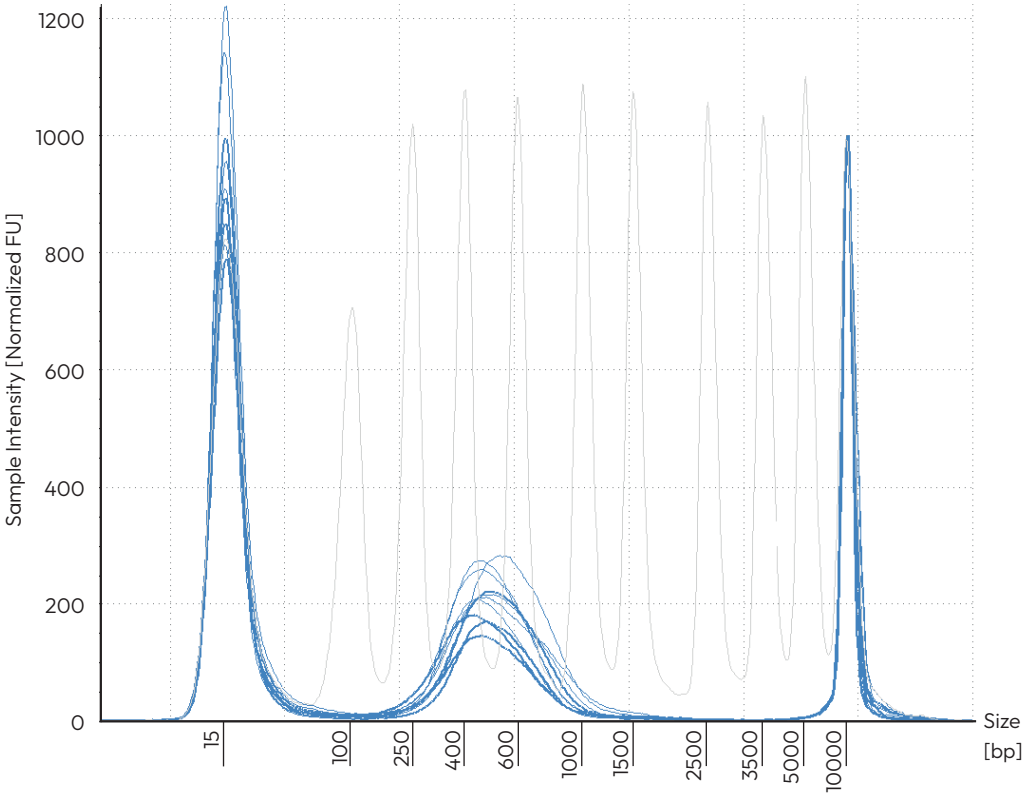


Figure 5: Size distribution of the NGS library DNA generated with the KAPA EvoPlus method; from 10 samples out of the 96-sample biological verification run, library size distribution was determined. To enable visualization of PCR-free libraries on a TapeStation, PCR amplification with four cycles of libraries was performed, followed by a 1x/1x bead clean-up. For TapeStation analysis, the generated DNA libraries were loaded on the TapeStation4150 and analyzed using the D5000 ScreenTape with the D5000 Reagents Kit

To determine the sequencing metrics, eight randomly selected libraries out of the 96-sample biological verification runs with either 10 ng (samples 1 - 4) or 500 ng (samples 5 - 8) input DNA were sequenced at the Functional Genomics Center Zürich (FGCZ) on an Illumina NovaSeq 6000 sequencer (2x150 bp, SP flowcell) (Fig. 6). Sequencing data was analyzed with the Sushi data analysis framework (Hatakeyama et al., BMC Bioinformatics: 17, 2016), using Bowtie 2 (v2.4.2) to align the sequencing reads to the human reference genome (GRCh38.p13) and estimate mapping rates. Additional sequencing quality metrics were collected with Picard (Picard toolkit, Broad Institute, v2.27.5).

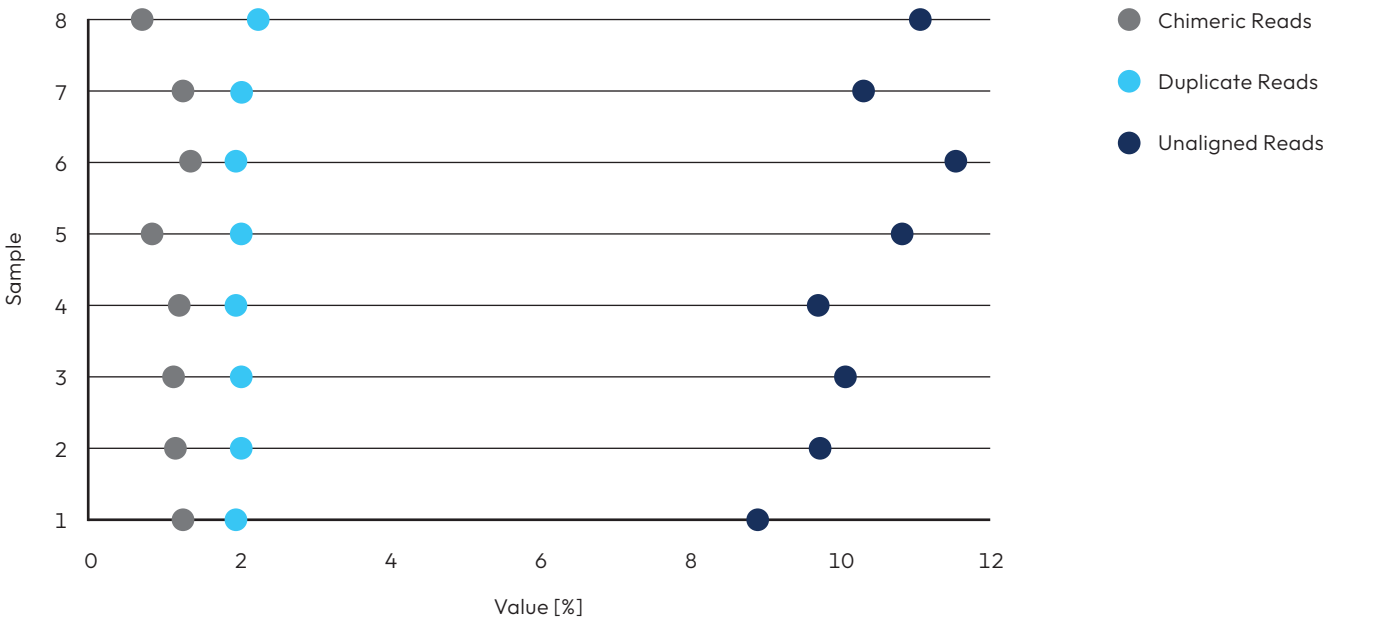


Figure 6: Mapping quality metrics of the DNA libraries generated with KAPA EvoPlus method; from eight randomly selected samples generated from the 96-sample biological verification runs with either 10 ng or 500 ng input material, generated DNA libraries were sequenced with the Illumina NovaSeq 6000 sequencer at the Functional Genomics Center Zürich (FGCZ). Samples 1-4: 10 ng input DNA; samples 5-8: 500 ng input DNA

All four NGS libraries generated from 10 ng input DNA displayed a high mapping rate of 98.04 ± 0.04%, a low percentage of chimeric reads 1.12 ± 0.06% and an average duplication rate of 9.6 ± 0.5%.

Similarly, the four NGS libraries generated from 500 ng input material depicted a high mapping rate of 97.95 ± 0.11%, a low percentage of chimeric reads 0.96 ± 0.34%, and an average duplication rate of 10.9 ± 0.5%

Throughput and Capacity

The KAPA EvoPlus method was automated for the generation of NGS libraries for up to 96 samples in a single run. Biological verification runs have been conducted with eight, 48 or 96 samples, using either 10 ng or 500 ng input material. Processing times for different sample numbers and input DNA amounts are listed in Table 1.

DNA Input Material	Sample Number		
	8	48	96
10 ng	02:53	03:11	03:18
500 ng	02:45	03:04	03:10

Table 1: Processing times [hh:mm] for biological verification runs with different sample numbers and input DNA amounts

Others

System Requirements	Part Number / Provider	Provider
NGS STAR V 2.0 MPH96 Base incl. VENUS four + Deck Components	827008	Hamilton Bonaduz AG
2nd Adapter PCR Plate	10087667	Hamilton Bonaduz AG
Huber Mini Chiller Olé 280 for Cooling Carrier	3006.0105.98	Huber
Huber Tubing Adapter NW8	6086	Huber

Labware Requirements	Part Number	Provider
50 µL CO-RE Filter Tips	235948	Hamilton Bonaduz AG
300 µL CO-RE Filter Tips	235903	Hamilton Bonaduz AG
1000 µL CO-RE Filter Tips	235905	Hamilton Bonaduz AG
PCR ComfortLid	814300	Hamilton Bonaduz AG
PCR FramePlate 96-well	814302	Hamilton Bonaduz AG
20 mL Reagent Reservoirs	96424-02	Hamilton Bonaduz AG
60 mL PP Reagent Trough with Lid	56694-01	Hamilton Bonaduz AG
2 mL Screw Cap Micro Tubes	72.694.406	Sarstedt

The Hamilton Genomics Squad

NGS STARlet

- Traceability of samples and reagents
- Minimal hands-on time for almost any NGS library preparation workflow
- Automated library preparation for high priority samples or smaller sample throughput

NGS STAR

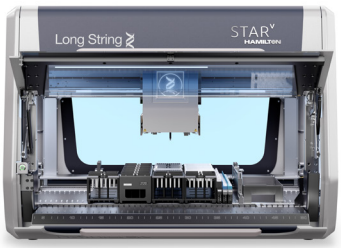
- Tried and true performance for many years
- Proven design, including qualified methods from many kit providers
- Upgradable to higher throughput needs

NIMBUS Presto

- More than 15 biologically-verified nucleid acid extraction protocols from leading kit vendors
- NOW available: PacBio Nanobind HMW DNA extraction application
- Configurable loading area for 96 samples

Long String STAR V

- Automated walk-away UHMW DNA extraction and labeling
- Optimal magnetic disk handling via Hamilton MagRod technology
- Scalable preparation of UHMW DNA for Bionano OGM for increased number of samples



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