

Automation of the KAPA RNA HyperPrep with RiboErase (HMR) Protocol from Roche on NGS STAR V

Genomics

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Figure 1: The Hamilton NGS STAR V Assay Ready Workstation.

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 workflow on the Hamilton NGS STAR V 2.0 MPH96 (further referred to as NGS STAR V, Figure 1) significantly reduces hands-on time and allows for the processing of up to 96 samples in a single run. The appropriate placement of reagents, plates and tips is guaranteed through the use of 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 framework ensures a smooth setup of the workflow, which can also be started and stopped at specific steps within the process.

- Hamilton's NGS STAR V offers several temperature-controlled positions, in order to meet the needs of NGS library protocol steps.
- Maximal walk-away time for NGS library preparation.
- Optimal process reliability via safety light and standardized workflows.

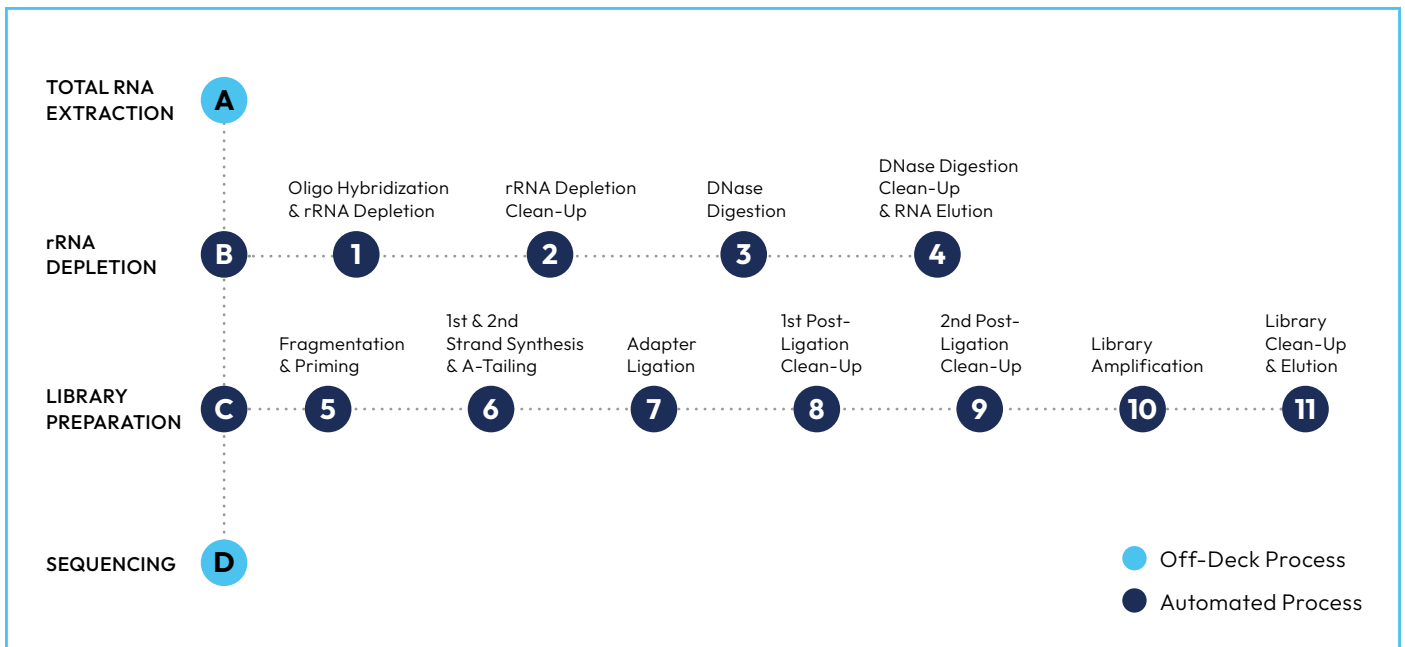


Figure 2: Graphical Overview of the KAPA RNA HyperPrep with RiboErase (HMR) Workflow.

Method Description

The “KAPA RNA HyperPrep with RiboErase (HMR)” workflow automates the KAPA RNA HyperPrep with RiboErase (HMR) protocol from Roche on the NGS STAR V. The workflow can be used to construct stranded ribosomal rRNA-depleted RNA libraries, from 25–1000 ng total RNA input material, suitable for sequencing on Illumina sequencers to conduct high-throughput whole transcriptome sequencing.

Each of these two workflows (B or C) can be performed individually on the NGS STAR V, if required. Running time: for rRNA Depletion, approximately 4 hours and for Library Preparation workflow, approximately 6.5 hours (96-sample run for both). The total run time is approximately 10.5 hours.

Application Software

NGS STAR V Framework (based on VENUS software) ensures that programming new methods is even more user-friendly. This means that even complex NGS methods can be programmed quickly and be tailored to the needs of the user.

Qualification Setup

The performance of the Roche KAPA RNA HyperPrep with RiboErase (HMR) protocol on the Hamilton NGS STAR V was evaluated by preparing libraries, using the KAPA Unique Dual-Indexed Adapter Kit (Roche, #KK8727).

Eight samples with either 25 ng or 1000 ng input RNA (Universal Human Reference RNA, Agilent #740000), as well as 96 samples with 500 ng input RNA were processed. Library amplification was performed with an On-Deck Thermal Cycler (ODTC) using 14 (25 ng input RNA) or 7 (500 ng and 1000 ng input RNA) PCR cycles, respectively. The elution volume was 22 µL.

Final library concentrations were determined using the Qubit 1X dsDNA HS Assay Kit (Thermo Fisher Scientific, #Q33231). Subsequently, library size distribution of the generated library DNA was measured with the Agilent TapeStation 4150 using the D5000 ScreenTape (Agilent, #5067-5592) with the D5000 Reagents (Agilent, #5067-5593).

Results

The average concentration of the final libraries was 3.8 ± 0.3 ng/ μ L for 25 ng of total input RNA, 5.8 ± 1.4 ng/ μ L for 500 ng total input RNA, and 3.5 ± 0.2 ng/ μ L for 1000 ng of total input RNA. Libraries were normalized to 10 nM in the pooling process prior to sequencing. The average library size was assessed by TapeStation and was measured at 331.5 ± 9.1 bp, 344.5 ± 17.9 bp, and 323.7 ± 29.0 bp on average for 25 ng, 500 ng, and 1000 ng input samples, respectively (Figure 3).

Summary

Step into the future with Hamilton's automation technology on the NGS STAR V for NGS library preparation.

By automating the KAPA RNA HyperPrep with RiboErase (HMR) protocol on NGS STAR V, Hamilton provides state of the art automated library preparation for RNA sequencing.

The assay delivers reliable results with superb mapping rates and minimal residual ribosomal RNA content. Also, the separation of rRNA Depletion and Library Preparation steps into two individual workflows adds the flexibility required to choose both starting and stopping points for the user, based on experimental needs.

When using the KAPA RNA HyperPrep with RiboErase (HMR) protocol on NGS STAR V, nothing can stop you from achieving superior outcomes for measuring gene expression profiles.

KAPA mRNA HyperPrep Kit is Research Use Only. Not for use in diagnostic procedures. KAPA is a trademark of Roche.

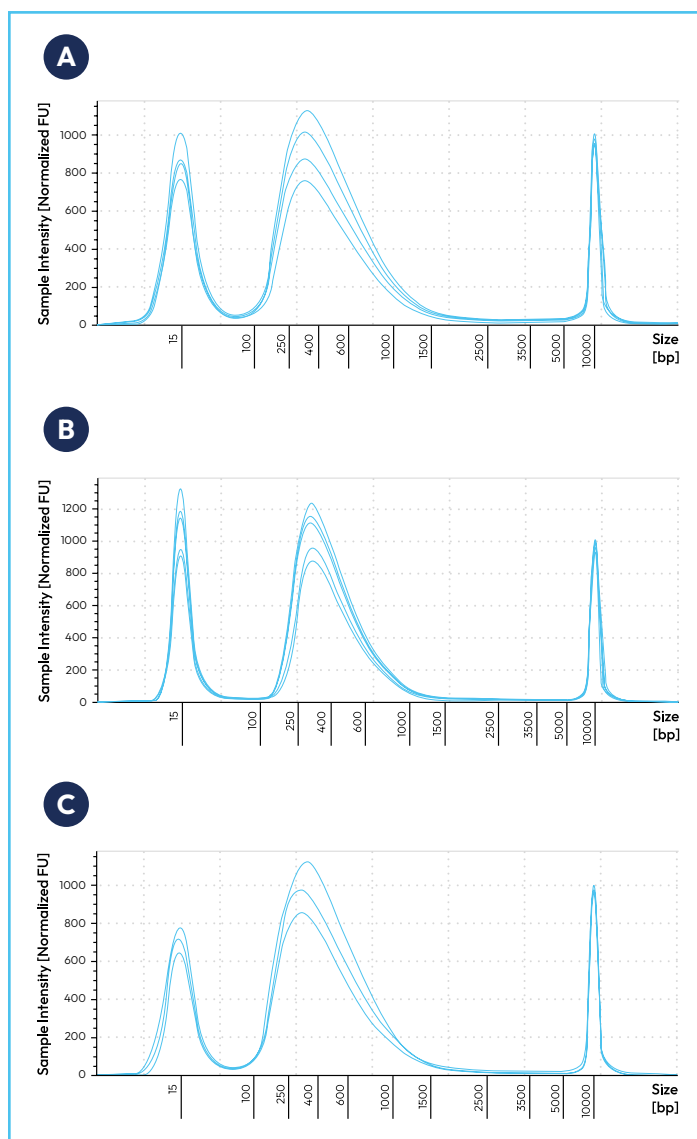


Figure 3: Size distribution of the NGS library DNA generated from (A) 25 ng, (B) 500 ng, or (C) 1000 ng input RNA using the KAPA RNA HyperPrep with RiboErase (HMR) method.

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