Application Note

Automation of the **PacBio Revio SPRQ HiFi prep kit 96** on the Hamilton **NGS STAR MOA** System – Next Level long-read sequencing library preparation for whole genome sequencing

Authors: Chantal Merz[1], Dominik Laubscher[1], Kaitlyn Scott[3], William Hutchens[2], Aurelie Souppe[3], Gregory Young[3], Camille Conner[3], Sha Liao[2], Birgit Ottenwälder[1]

1 Hamilton Bonaduz AG, Via Crusch 8, 7402 Bonaduz, Switzerland
2 Hamilton Company, 7970 Energy Way, Reno, NV 89502, USA
3 PacBio, 1305 O'Brien Dr., Menlo Park, CA 94025, USA

Introduction

Whole Genome Sequencing (WGS) is indispensable in modern research. The high-resolution data provided by long-read sequencing technology offers a comprehensive view of a genome, enabling the detection of single nucleotide variants, insertions/deletions, copy number changes, and large structural variants, even in genomic dark regions or structurally complex areas. However, WGS workflows can be both time-consuming and labor-intensive, potentially diverting valuable time from operators that could be more effectively spent on planning experiments or analyzing data. With the power of automation for long-read sequencing, new possibilities arise to advance scientific discoveries at elevated capacity.

With the NGS STAR MOA (Fig. 1), operators can benefit from unmatched potential for long-read sequencing by streamlined sample preparation and pooling optimized for Revio® sequencing.

- Flexible sample input from 8 to 96 samples
- Minimal hands-on time for sample and reagent preparation
- Robust and consistent high-quality results
- Solve traditional bottlenecks for long-read sequencing
- Method tested and validated by PacBio®

System Description

The NGS STAR MOA (Fig. 1) is designed for high-quality NGS libraries in high-throughput workflows.

With its 96 Multi-Probe Head (MPH 96), the NGS STAR MOA is equipped to generate high-quality NGS libraries for high-throughput sample numbers. It comes with five Heater Shaker modules (HHS), an active cooling position (CPAC) and the option for an On-Deck Thermal Cycler (ODTC) for optimal temperature control of samples and reagents. The deck features a Magnetic Stand as well as plenty of deck space reserved for tips. Special Carriers can be used for loading samples and reagents. The Autoloader with a barcode reading function ensures tracking of the loading process.

The NGS STAR MOA enables the fully automated processing of up to 96 samples, depending on the kit used. This reduces the amount of manual work to a minimum. Automated error handling and the easy-to-use software framework ensure a smooth setup of the workflow, which can also be started and stopped at specific steps within the process.

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

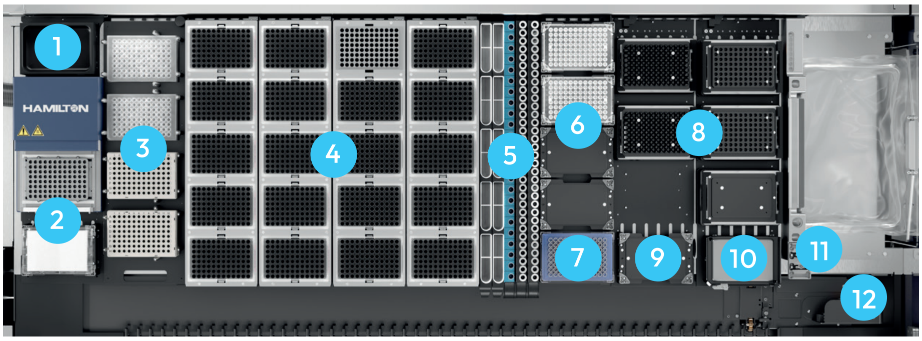

Figure 2: Deck Layout of an NGS STAR MOA.

1. Gravity Liquid Waste for Multi-Probe Head (MPH)
2. (Optional) On-Deck Thermal Cycler (ODTC) with lid parking position
3. Plate Stacker
4. Tip Carriers with MPH Tip Support Adapter
5. Reagent Carriers
6. Plate Carrier
7. Alpaqua Magnum FLX Magnetic Plate
8. Hamilton Heater Shakers with 96-well PCR Plate Adapters and Flat Bottom
9. Inheco CPAC* with 96-well PCR Plate Adapter
10. Inheco CPAC with 2mL Tube Adapter
11. CO-RE Gripper Paddles
12. Autoloader with Barcode Reader

* Must be purchased separately

Method Description

The PacBio HiFi prep and ABC methods automate the workflow (Fig. 3) for constructing polymerase-bound Whole Genome Sequencing (WGS) libraries from genomic DNA using the HiFi prep kit 96 and the Revio polymerase kit 96 for both SPRQ™ and V1 chemistry. All reagents are designed for a minimum of 24 and maximum of 96 samples per automated run. Running fewer than 24 samples at a time is possible but may result in not having enough reagents to support a total of 96 samples from each kit . PacBio Nanobind® DNA extraction kits are recommended to ensure sufficient amounts and quality of high molecular weight DNA.

To support low DNA input amounts for SPRQ sequencing on Revio, we have included low mass (0.5-1.25 µg) options for both SRE and shearing in addition to the V1 high mass (2-4 µg) protocol. Both options are optimized and provide comparable recoveries, size selection performance, and maintain size distribution between 15–20 kb.

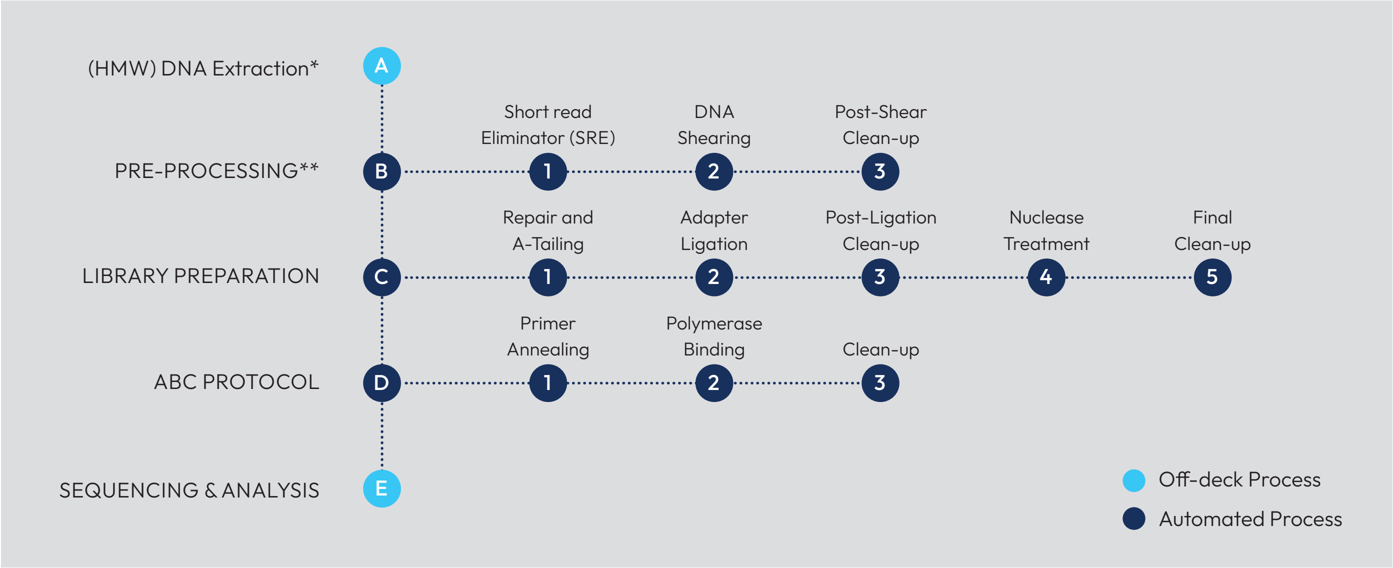

Figure 3: Workflow of the Revio SPRQ HiFi prep kit 96 automated on the NGS STAR MOA;
* (High Molecular Weight) DNA Extraction automated on NIMBUS Presto, ** Low or high mass settings available.

Kit Description

The HiFi prep kit 96 can be used in applications for WGS of human, animal, or plant samples.

It is a mid-to-high throughput bundle with improved cost per sample ratio, designed for optimized workflow efficiency and automation compatibility. SPRQ sequencing chemistry further enables lower genome costs by increasing HiFi yields, while requiring four times less input DNA and facilitating improved on-instrument DNA methylation detection.

This kit supports up to four 24-sample runs or a single 96-sample run using the NGS STAR MOA automated method.

Throughput and Capacity

The method can fully process 24-96 samples in just 13 hours with minimal hands-on time (Table 1).

Table 1: Processing times on the NGS STAR MOA for the Revio SPRQ HiFi prep kit 96 workflow.

Workflow Step	Automation Time for 96 Samples	Day
SRE	3.5 hours	1
Shearing	10 min	
Library Preparation	6.5 hours	2
Anneal, bind, clean-up	2.5 hours	
Average total time	13 hours	

Results

Biological runs with 96 samples were conducted using HG002 DNA extracted from frozen cells (QC conducted on Agilent Femto Pulse with GQN at 10 kb = 9.3). 515 ng input DNA per replicate (normalized to 20.6 ng/µl) was used and replicates were barcoded with SMRTbell® adapter index plate 96A (102-009-200).

Table 2 shows the gDNA recovery rate for the protocol steps SRE/shearing, library preparation, and polymerase binding (ABC; annealing, binding, clean-up) (steps B-D, Fig. 2). The observed values are in correspondence with the expected range.

Table 2: gDNA recovery rates during the different HiFi prep kit 96 method protocol steps and HiFi read numbers and lengths after sequencing.
* Sample in H12 was lost when removing the adhesive seal after the Repair and A-tailing step.

Equal Volume Pooling

n = 96 reps	SRE step recovery	Recovery (gDNA to library)*	Recovery (gDNA to ABC)	HiFi reads per rep (n = 95)	HiFi read length per rep (bp, n = 95)
Average	80%	26%	25%	82,172	16,074
St Dev	3%	4%	3%	15,006	520
% CV	3%	16%	11%	18%	3%

On average, the concentrations of each library replicate were enough to load 1.8 Revio SMRT® Cells at 250 pM at an 18 kb average insert size, requiring 72 ng of polymerase-bound SMRTbell library.

95 replicates were pooled in equal volume and sequenced on a single Revio SMRT Cell using the new SPRQ chemistry.

72 ng of polymerase-bound SMRTbell library was loaded at an on-plate concentration of 250 pM and sequenced on a PacBio Revio system. Sequencing metrics are reported in Table 3.

Figure 4 shows the read length distribution of sequenced libraries. The mean peak length is around 16 kb, which lies within the expected range of 15-20 kb.

Table 3: Sequencing metrics of the pooled 95 library replicates.

Value	Metric
7.9 M	HiFi reads
126 Gb	HiFi reads yield
18,274 bp	HiFi read length (N50)
Q34	HiFi read quality (median)
91.72%	Base Quality ≥ Q30 (%)

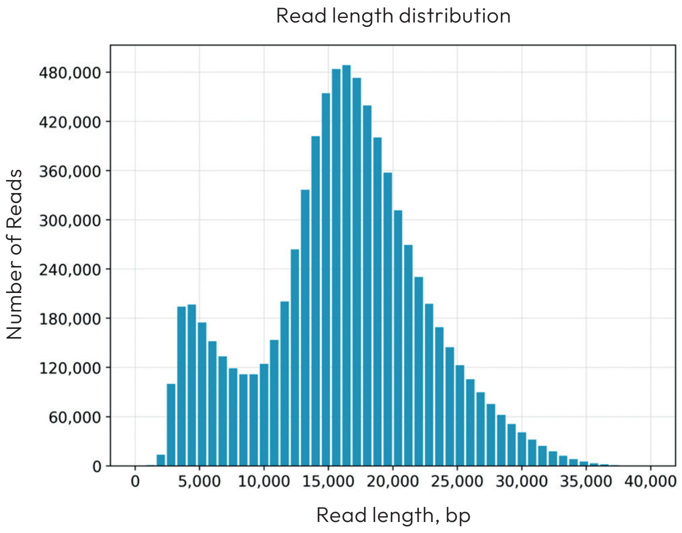

Figure 4: Read length distribution of sequenced libraries created by the HiFi prep kit 96 method on the NGS STAR MOA.

Technology

Using the 96 Multi-Probe Head (MPH) allows for the simultaneous processing of a high sample number throughput of up to 96 samples, significantly reducing processing times and reducing any column-based effects. In this method, the MPH is also used to perform on-deck mechanical shearing, providing even more walk-away time to the user, and represents a fast and highly cost-efficient alternative to conventional shearing processes. Additionally, the method utilizes capacitive Liquid Level Detection (cLLD) technology to increase robustness and consistency.

Table 4: Hamilton NGS STAR MOA: Methods and Compatible PacBio Kits.

NGS STAR MOA			
Method name	**Includes**	**PacBio kits**	
HiFi Prep	• SRE • DNA shearing • SMRTbell library preparation protocol	HiFi prep kit 96	Revio SPRQ HiFi prep kit 96
Revio SPRQ ABC	ABC protocol (polymerase binding)	Revio SPRQ polymerase kit 96	

Summary

The PacBio Revio SPRQ HiFi prep kit 96 workflow was successfully automated and verified to produce high-quality SMRTbell libraries on the NGS STAR MOA. The automation workflow seamlessly integrates three processing steps (SRE and shearing, Library preparation, polymerase binding) to streamline the creation of sequencing-ready SMRTbell libraries. This approach offers significant advantages in terms of library quality and operational efficiency. This solution addresses previous low-throughput, time- and cost-intensive size selection and fragmentation steps by automated processing.

Thereby, automating this method on the NGS STAR MOA significantly enhances throughput while maintaining cost-effectiveness. This automation provides valuable hands-off time for users, enabling them to focus on making meaningful scientific contributions rather than engaging in manual laboratory tasks. Finally, the presented automation workflow is set up to empower breakthrough discoveries at elevated scale.

System Requirements	Provider	Part Number
NGS STAR MOA (ODTC optional)	Hamilton Bonaduz AG	806800
2nd CPAC + Slot Module	Hamilton Bonaduz AG	282432 + 282437

Labware Requirements	Provider	Part Number
Hard Shell 96 PCR Plate*	Bio-Rad	HSP9601
Abgene 96 Well 0.8mL Polypropylene Deepwell Storage Plate**	ThermoFisher Scientific	AB0859
50 µL CO-RE II Tips (Filtered, Conductive)	Hamilton	235948
300 µL CO-RE II Tips (Filtered, Conductive)	Hamilton	235903
1000 µL CO-RE II Tips (Filtered, Conductive)	Hamilton	235905
60mL Reagent Reservoir Self-Standing	Hamilton	194051
Heat Sealing Foil	ThermoFisher Scientific	AB-0757 W
MicroAmp Clear Adhesive Film	ThermoFisher Scientific	00146104
2mL Sarstedt Tubes	Sarstedt Inc	72.694.306
300mL Reservoir***	Agilent	201244-100
2mL Amber Tubes	ThermoFisher Scientific	03-390-28

Reagent Requirements	Provider	Part Number
Revio® HiFi prep kit 96	Pac Bio	103-382-200 (Revio® HiFi prep kit 96) or 103-522-600 (Revio® SPRQ™ HiFi prep kit 96)
Femto Pulse gDNA 165kb Analysis Kit	Agilent	FP-1002-0275
Qubit 1x dsDNA HS (High Sensitivity) Assay Kit	ThermoFisher Scientific	Q33231
Quant-iT 1X dsDNA HS assay kit	ThermoFisher Scientific	Q33232

Equipment Requirements	Provider	Part Number
Vortex Mixer	Any major lab supplier	
Microcentrifuge	Any major lab supplier	
ALPS 50 V-Manual Heat Sealer	ThermoFisher Scientific	AB-1443A
Plate Centrifuge with 2250 g force capability	Any major lab supplier	
Femto Pulse System	Agilent	M5330AA
Qubit 4 or Qubit Flex Fluorometer	ThermoFisher Scientific	Q33238 (Qubit 4), Q33327 (Qubit Flex)
Varioskan LUX multimode microplate reader	ThermoFisher Scientific	VL0L00D0

* Alternative consumable: PCR FramePlate 96-well, PN 844302, Hamilton Bonaduz AG

** Alternative consumable: Deep Well Plate PP 0.8ML, Barcode, PN 10161066, Hamilton Bonaduz AG

*** Alternative consumable: 300 ml SBS Reagent Trough, PN 56669-01, Hamilton Bonaduz AG

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hamiltoncompany.com/contact
infoservice@hamiltonrobotics.com

United States
+1-775-858-3000
United Kingdom, Ireland
+44 121 272 92 80
Brazil
+55 11 95914 5000
China
+86 21 6164 6567

Denmark, Norway, Sweden, Finland
+46 8410 27 373
France
+33 184 008 420
Germany, Austria, Switzerland
+49 89 248 804 804

Benelux
+31 40 209 178 0
Italy
+39 039 930 06 06
Japan
+81 3 6435 6850
Spain, Portugal
+34 930 186 262