

# Automation of the Oxford Nanopore Ligation Sequencing Kit XL V14 on the HAMILTON NGS STARlet Generates High-Quality DNA Libraries for Nanopore Sequencing

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## Introduction

Oxford Nanopore Technologies offers nanopore sequencing of short to ultra-long fragments of native DNA or RNA with real-time data delivery, providing rich biological insights and streamlined workflows. Their highly flexible, scalable devices suits diverse experimental goals, from compact to high-throughput solutions. Automating nanopore sequencing library preparation based on the Oxford Nanopore Ligation Sequencing Kit XL V14 (SQK-LSK114-XL) on the NGS STARlet (Fig. 1) allows for the generation of sequencing libraries from various dsDNA input materials (e.g., genomic DNA or amplicons). With the automation of this kit, users can process up to 24 samples simultaneously with no need of user interaction.

- Standardized and reliable sample preparation for small to mid sample throughput and high-priority samples
- Complete walk-away solution with no user interaction
- Generates high-quality libraries with a broad range of input material

## Method Description

The Oxford Nanopore SQK-LSK114-XL V14 V1.0 method automates the Ligation Sequencing Kit XL V14 on the NGS STARlet. The workflow facilitates the preparation of sequencing libraries from dsDNA such as genomic DNA, complementary DNA, or amplicons in a straightforward manner (Fig. 2). The generated DNA libraries can then be sequenced on all Oxford Nanopore sequencing devices using the R10.4.1 flow cells.



Figure 1: The Hamilton NGS STARlet Assay-Ready Workstation.

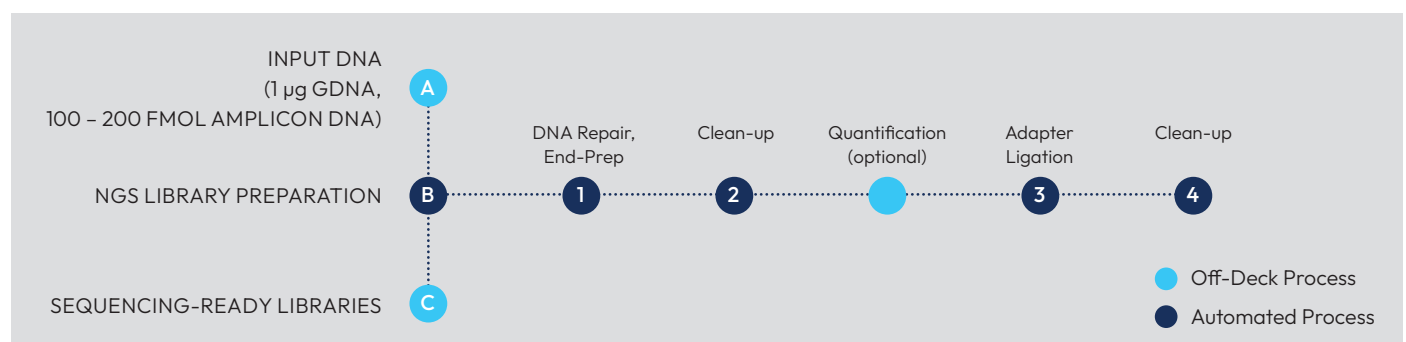


Figure 2: Graphical overview of the Oxford Nanopore SQK-LSK114-XL V14 V1.0 method. For the execution of this method, the DNA Repair and End-Prep reagents as well as T4 Ligase provided by the NEBNext® Companion Module v2 for Oxford Nanopore Technologies® Ligation Sequencing were used.

This method was performed with 1 µg genomic DNA (either Bacteriophage Lambda DNA (*E. coli dam*-) (NEB, #N3013S) or Human Genomic DNA (HG002, provided by Oxford Nanopore)) as input material. To enrich for DNA fragments >3kb, the Long Fragment Buffer was used for the clean-up after the Adapter Ligation step.

### System Description

The NGS STARlet is based on the Microlab STARlet platform and is equipped with 8 independent 1000 µL pipetting channels. The workspace is optimally tuned to high-quality generation of nucleic acid libraries for high priority samples or low-throughput sample numbers (Fig. 3). An On-Deck Thermal Cycler, two SBS cooling positions (CPACs), two Heater Shaker Modules (HHSs) and a magnet, together with carriers for tips, plates, reagents and samples create the optimal deck for nucleic acid library preparation of the NGS STARlet. The NGS STARlet enables fully-automated processing of up to 24 samples, depending on the kit used. This reduces the amount of manual work to a minimum. The samples and the required laboratory material (e.g., reagents, plates, tips) are identified by barcode verification. In addition, the user can define in-process protocols and a worklist for processing the samples. An easy-to-use Graphical User Interface ensures a smooth workflow.

- 1 On-Deck Thermal Cycler (ODTC)
- 2 Magnetic Stand
- 3 Heater Shaker Modules (HHSs)
- 4 High-Performance Cooling Modules (CPACs)

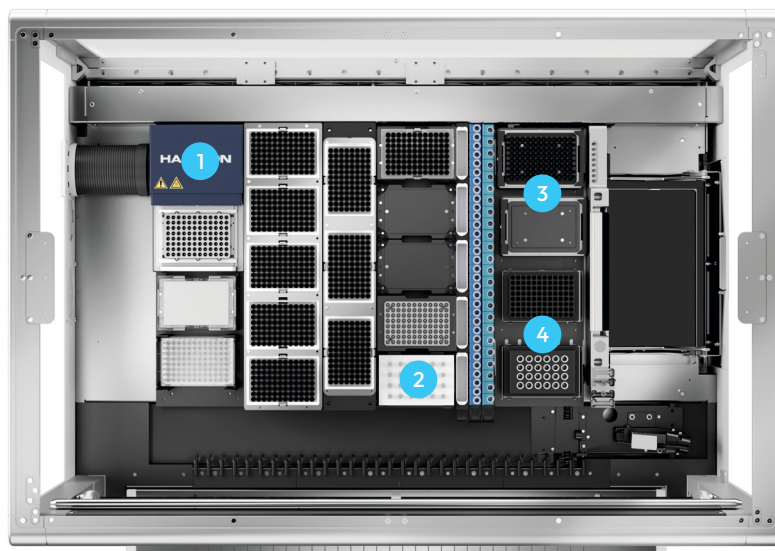


Figure 3: Deck Layout of the NGS STARlet.

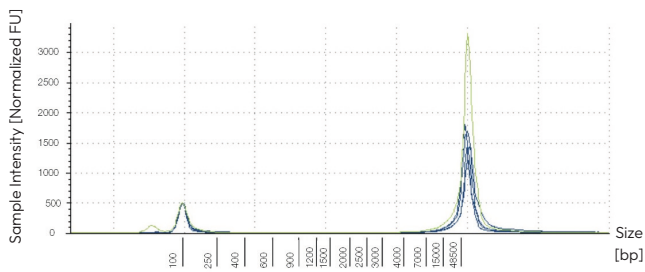
### Qualification Setup and Results

For biological verification of the Oxford Nanopore SQK-LSK114-XL V14 V1.0 method on the NGS STARlet, biological runs with either eight (four positive samples + four negative controls) or 24 samples (22 positive samples + two negative controls) were conducted. As input material,

1 µg of full length (48 kB) Bacteriophage Lambda DNA was used for the eight-sample run. For the 24-sample run, 1 µg of sheared (9kB) Human Genomic DNA served as input material.

DNA concentrations of the libraries obtained from the eight- and 24-sample biological verification run were determined using the Thermo Fisher Scientific Qubit 4 Fluorometer with the Quant-iT™ 1X dsDNA high-sensitivity Assay Kit (Thermo Fisher Scientific, #Q33232). The average sample yield was 344.3 ng (+/- 51.5 ng) for

Bacteriophage Lambda DNA samples and 452 ng (+/- 69.9 ng) for Human Genomic DNA samples. Subsequently, library size distribution of all four positive samples from the biological verification run with Bacteriophage Lambda DNA was determined with the Agilent TapeStation 4150 using the Genomic DNA ScreenTape (Agilent, #5067-5365) and Genomic DNA Reagents (Agilent, #5067-5366). The average library size was matching input DNA sizes at 48,000 bp for the eight sample run (Fig. 4).

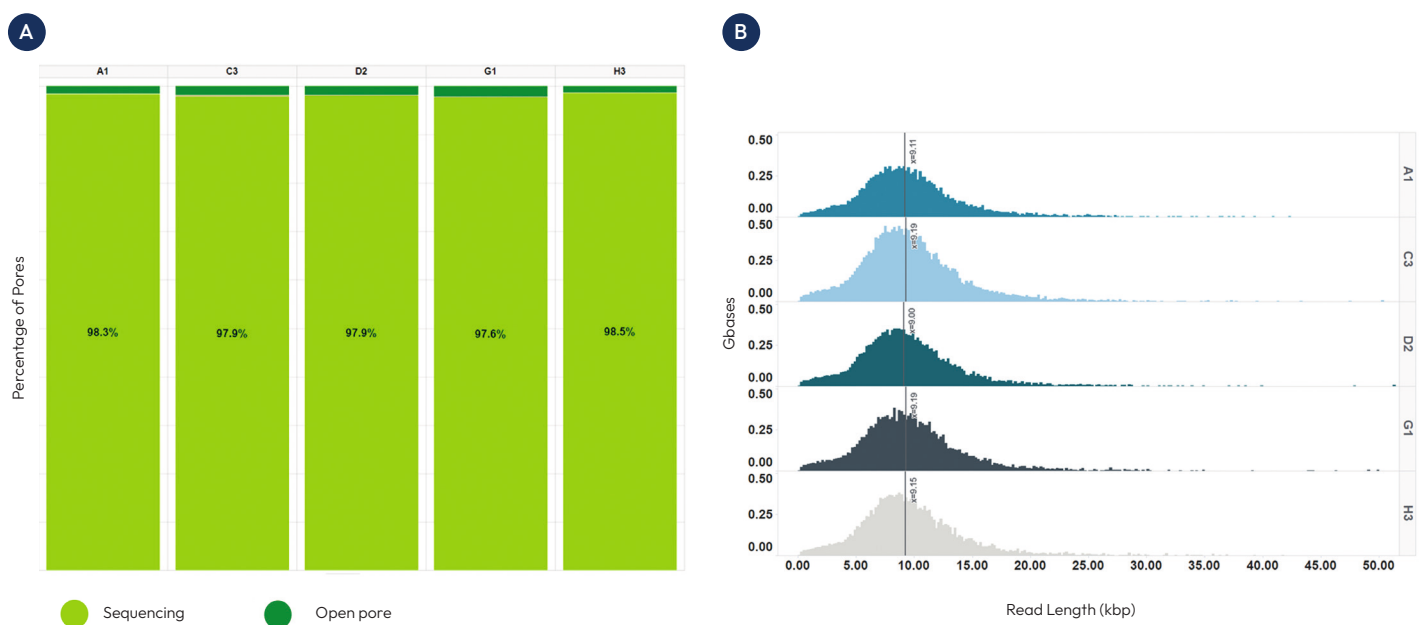


**Figure 4:** Size distribution of the library DNA generated from 1 µg Bacteriophage Lamda DNA as input material with the Oxford Nanopore SQK-LSK114-XL V14 V1.0 method; blue: generated DNA libraries; green: input DNA.

The DNA libraries obtained from both biological verification runs were sequenced using a MinION R10.4.1 Flow Cell on a GridION device and analyzed at Oxford Nanopore. Pore occupancy and read length (N50) were used for sequencing analysis. The libraries generated from Bacteriophage Lamda DNA revealed a modal peak at 48 kB with a pore occupancy of 92.2% ( $\pm$  2.4%); (Table 1). Human Genomic DNA libraries demonstrated an N50 read length of 9.12 kb ( $\pm$  0.05 kb) and a pore occupancy of 99.1% ( $\pm$  0.2%); (Table 1, Figure 5). All libraries passed the benchmark criteria.

Metric	8 Samples (Bacteriophage Lamda DNA)	24 Samples (Human Genomic DNA)
Pore Occupancy [% $\pm$ SD]	92.2 $\pm$ 2.4	99.1 $\pm$ 0.2
Read Length [kB $\pm$ SD]	Peak at 48 kB	9.12 $\pm$ 0.05

**Table 1:** Key sequencing metrics obtained from libraries generated on the NGS STARlet either within an 8 sample run using Bacteriophage Lamda DNA or within a 24 sample run using Human Genomic DNA.



**Figure 5:** Pore Occupancy (A) and Read Length distribution (B) of 5 exemplary sequenced libraries generated on the NGS STARlet from Human Genomic DNA.

## Summary

Samples processed on the NGS STARlet using the Oxford Nanopore Ligation Sequencing Kit XL V14 were found to deliver the expected library yields and size profiles as well as downstream sequencing performance for both Bacteriophage Lambda and Human Genomic DNA input samples. Automation of this kit on

the NGS STARlet allows to increase the number of libraries generated for medium throughput needs to up to 24 samples in under 3 hours on a relatively small footprint without additional resources or compromising quality.

## Requirements

System Requirements	Part Number
NGS STARlet Product Line + ODTc option	806799
Ambion Magnet Adapter	10107866

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
0.5 mL Screw Cap Micro Tubes	72.730.006	Sarstedt
2 mL Screw Cap Micro Tubes	72.694.006	Sarstedt
5 mL Screw Cap Micro Tubes	62.611	Sarstedt

Reagent Requirements	Part Number	Provider
Ligation Sequencing Kit XL V14	SQK-LSK114-XL	Oxford Nanopore Technologies
NEBNext Companion Module V2 for Oxford Nanopore Technologies Ligation Sequencing	E7672S or E7672L	New England Biolabs
Qubit dsDNA HS Assay Kit	Q33232	Thermo Fisher Scientific
Agencourt AMPure XP Beads	A63880 or A63881 or A63882	Beckmann Coulter

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