Automation of Oxford Nanopore General Ligation LSK114 on the Agilent Bravo NGS Workstation

Authors: Katherine Roper[1], Harry T. Child[2], Gabrielle R. Joslin[2], Lucy Wierzbicki[2], Richard K. Tennant[2]

[1] *Agilent Technologies LDA UK Limited,*
[2] *University of Exeter,*

SLAS Europe

Poster # 1065-B

Agilent Trusted Answers

Introduction

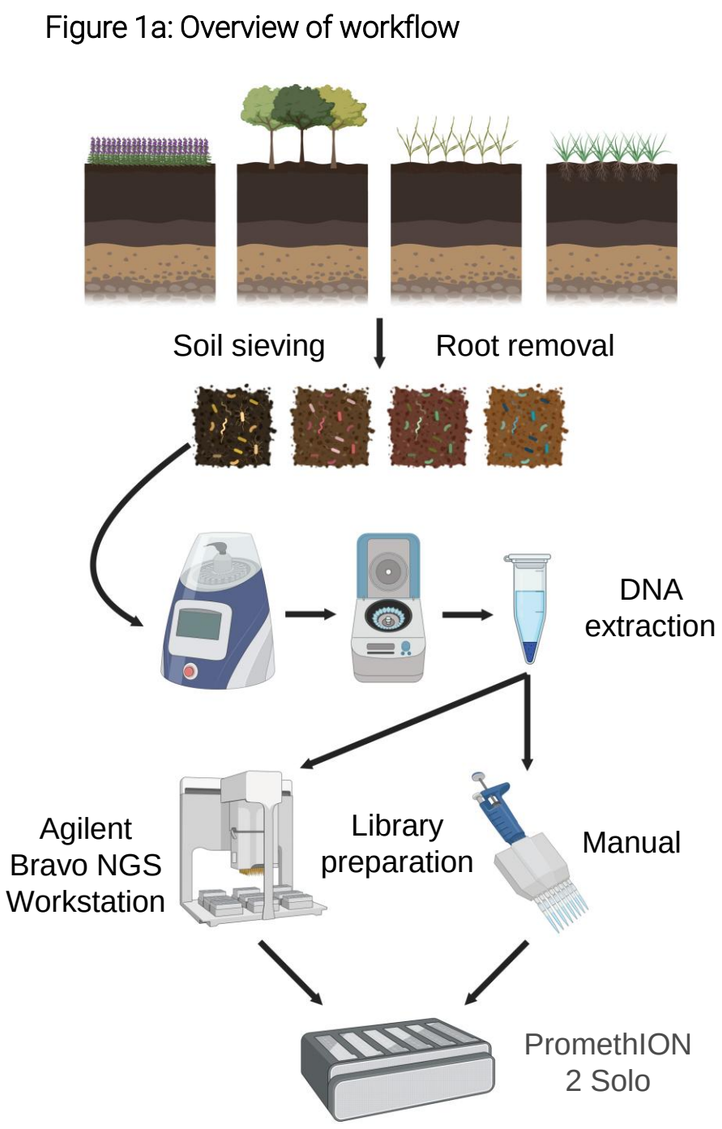
Long-read sequencing (LRS) is becoming increasingly popular in next-generation sequencing (NGS), however long-read workflows are difficult to scale up when performed manually. Automating LRS library preparation minimizes the potential for human error, improves consistency when working with multiple samples, increases walkaway time and facilitates multiplexing of samples.

We automated the Oxford Nanopore Technologies (ONT) LSK114 General Ligation library preparation workflow on the Agilent Bravo NGS workstation. 24 soil-derived DNA samples were processed on the Bravo NGS Workstation and sequenced with an ONT PromethION flow cell. This data set demonstrates the suitability of Agilent NGS automation for preparation of LRS libraries for metagenomic analysis.

Experimental

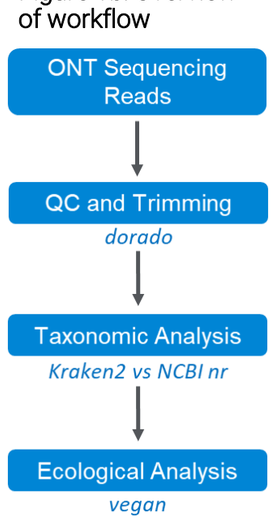
ONT sequencing libraries were prepared using 24 DNA samples extracted from soils from arable, pasture, heath and woodland locations. DNA was manually extracted using the DNeasy PowerSoil Pro Kit (Qiagen, UK), with 4 to 8 extractions per soil type. The DNA input for library preparations was normalized to 1 µg. Libraries were prepared on the Bravo NGS Workstation using the Ligation Sequencing Kit (SQK-LSK114; ONT, UK) and PCR Barcoding Expansion 96 (EXP-PBC096; ONT, UK). 20ng of DNA was input into PCR barcoding reactions. Normalized pools of barcoded libraries were sequenced using an R10.4.1 PromethION flow cell.

Figure 1a: Overview of workflow



Reads were base called and demultiplexed using guppy v7.1.4 and adapters were trimmed using dorado v0.6.0. Taxonomic classification was performed using Kraken2 v2.1.2 against the NCBI nr database, with a confidence score threshold of 0.05. Count tables were compiled using MEGAN Ultimate Edition v6.25.6 and filtered to remove taxa occurring at an abundance of <0.1% across all samples. Ecological statistics were calculated using the vegan v2.6-4 R package.

Figure 1b: Overview of workflow



Experimental

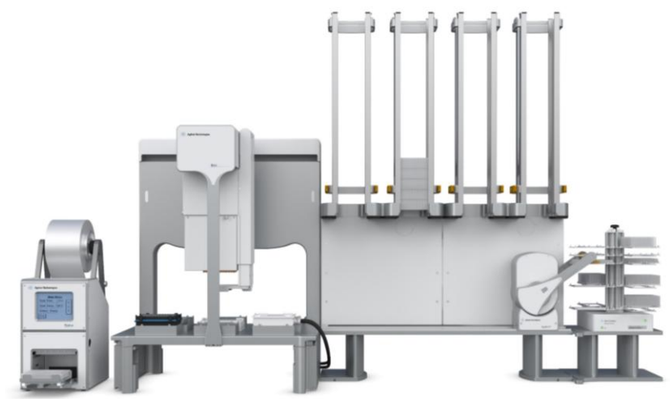


Image 1: Agilent Bravo NGS Workstation

This process was automated in VWorks automation control software for the Bravo NGS workstation (Image 1). The VWorks module includes two runsets that combine the end repair or adapter ligation setup with an Ampure bead cleanup. The incubations for these steps are carried out on-deck, allowing full walkaway automation during each runset (Table 1).

Adapted runsets can be used for the post-library pooling round of end repair and adapter ligation.

Table 1: Protocol run times

Workflow Step	Walk Away Time
End Repair & Cleanup	53 minutes
Ligation & Cleanup	63 minutes
Barcoding PCR	8 minutes
Barcoding Cleanup	33 minutes

The module includes separate protocols for the barcoding PCR setup and barcoding PCR Ampure cleanup, with the PCR step carried out off-deck on a thermocycler. Additionally, the module features a sample pooling protocol, which takes sample volumes and locations from a CSV file to automate library pooling.

Each protocol has a unique deck layout displayed in the user interface (Image 2). This form allows users to select the sample numbers and PCR plate type.

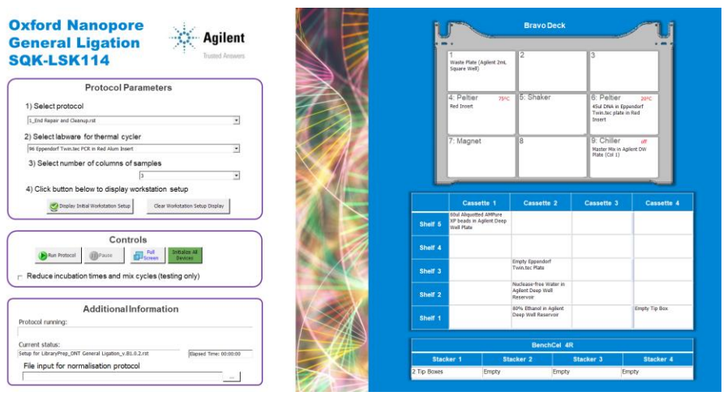
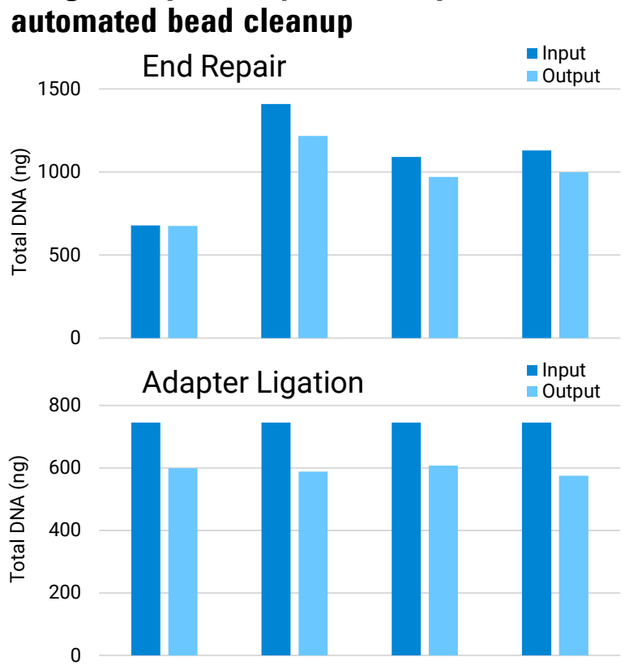


Image 2: Agilent Bravo NGS Workstation user interface

Results and Discussion

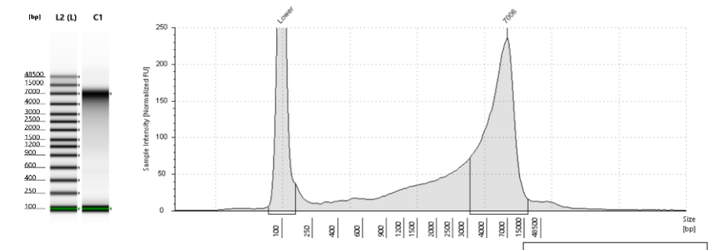
When automating LRS library preparation workflows, many technical challenges are similar to those in short-read workflows. An additional requirement is to minimize the risk of DNA shearing during sample pipetting, while maintaining high yields during bead cleanup steps. Using pooled DNA from sampling sites to optimize cleanups, homogenous mixing of beads with DNA and elution buffers was found to be critical for retention of DNA. With mix cycles set to 60, the average recovery rate was 90% for standalone runs of the end repair cleanup, and 79% for the post-adapter-ligation/PCR cleanup, which uses a lower DNA-to-bead ratio (Image 3).

Image 3: Input and yield DNA quantities for automated bead cleanup



Results and Discussion

Image 4a: TapeStation electropherogram of the 24 pooled libraries. 4b: Histogram plot showing distribution of lengths of reads for all libraries



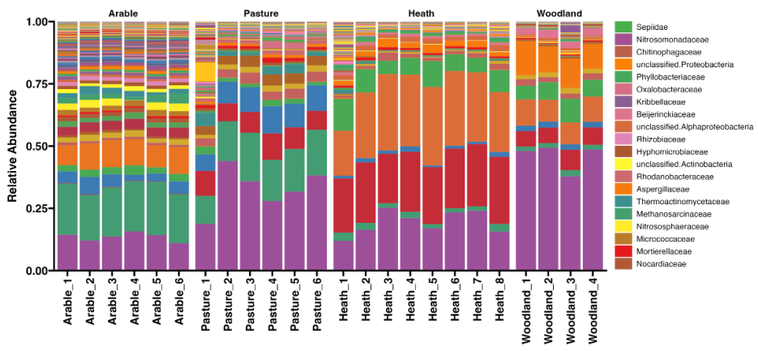
Sample Source	Heath	Pasture	Woodland	Arable
Sample Numbers	8	6	4	6
N50	3887.3	2984.0	3575.5	3347.5
Q20(%)	79.5	79.3	79.5	79.8
Q30(%)	62.8	62.5	62.7	63.2
GC(%)	57.1	53.8	56.9	57.0
Classified %	15.7	23.2	17.4	23.6
Classified Reads	151980	321539	217344	178383

Table 2: Mean sequencing metrics of the 24 libraries comparing DNA sampling sites

The pooled libraries were run on a Genomic DNA ScreenTape using an Agilent TapeStation system. DNA had a maximum peak size of just over 7kb with some smaller fragments present (Image 4). All 24 DNA generated libraries irrespective of the original source of the DNA. The mean Q20 and Q30 scores were very similar, whilst the libraries from the Pasture had a slightly lower N50 compared to those from other sample sites (Table 2)

Ecological analyses were performed on the results of taxonomic classification at a Family level. Variation in microbial community structure was investigated through calculation of Bray-Curtis distances with rarefaction. Soil type explained the vast majority of variation in community composition between samples, with consistent taxonomic makeup across sampling site types (Image 5).

Image 5: Relative abundance of microbial families across the four soil types



Classification detected the presence of rare taxa in the sequenced libraries, with more than 34 families identified. Detection of rare microorganisms in complex samples is an important objective of many metagenomic studies, due to their importance to ecosystem functions and community dynamics [1, 2]. Being able to detect these taxa is a potential benefit of automated library preparation.

Conclusions

- Automated library preparation with LSK114 on the Bravo NGS Workstation produces good quality sequencing data, allowing classification of microbial families.
- With automated library preparation, up to 96 samples can be processed simultaneously, significantly improving throughput compared to a manual approach.
- The automation protocol is easy to implement and increases walkaway time for users.
- Considering the benefits of reduced hands-on time, reproducibility and reliability, automated library preparation using the Agilent Bravo should be considered suitable for long-read metagenomics.

Agilent Bravo, TapeStation, Fragment Analyzer & Femto Pulse have been added to the ONT Compatible Products Program.

References

1. Shade A, Jones SE, Caporaso JG, Handelsman J, Knight R, Fierer N, et al. Conditionally Rare Taxa Disproportionately Contribute to Temporal Changes in Microbial Diversity. mBio. 2014 Jul 15;5(4):10.1128/mbio.01371-14.
2. Xiong C, He JZ, Singh BK, Zhu YG, Wang JT, Li PP, et al. Rare taxa maintain the stability of crop mycobiomes and ecosystem functions. Environ Microbiol. 2021;23(4):1907–24.

PR7001-4239