Automation of the sparQ DNA Library Prep Kit on the PerkinElmer® Sciclone G3

Keywords: NGS, library preparation, sparQ DNA Library Prep Kit, automation, PerkinElmer Sciclone G3, sparQ PureMag Beads

ABSTRACT

Automation of next generation sequencing library preparation increases throughput of samples, decreasing costs and widening utility. In this application note, we detail the conditions used to transfer the sparQ DNA Library Prep Kit on to the PerkinElmer Sciclone G3 instrument for successful, automated library preparation.

INTRODUCTION

Increased throughput and reduced costs of next generation sequencing (NGS) have vastly increased its research and clinical applications. In particular the ability to sequence multiple bacterial genomes in a few hours makes NGS ideal for microbiology translational research applications. Whole genome sequencing can be used to identify strains and determine strain characteristics informing epidemiology and treatment options. NGS library preparation can consist of a series of highly repetitive steps and so is amendable for automation. Only after these steps have provided consistent and reliable results can they be automated to provide robust libraries for sequencing.

The sparQ DNA Library Preparation Kit is a fast and flexible method for preparing NGS libraries for sequencing on Illumina® instruments (Figure 1). The streamlined workflow can be completed in less than 2.5 h including library amplification or under 2 h with an amplification-free protocol. The first step is a one-tube DNA Polishing reaction which combines end-repair and dA-tailing. This is followed by adapter ligation in the same tube, then clean-up or size selection using high recovery DNA-binding magnetic beads, such as sparQ PureMag Beads.

There is an optional library amplification step for low-input workflows to make this single kit applicable to a whole range of applications (Figure 1).

This simple workflow maximizes purity and quality resulting in high yield NGS libraries. The kit is compatible with a wide DNA input range, from 1 ng to 1 µg, and has been validated with mechanically sheared DNA, FFPE DNA and chromatin immunoprecipitated DNA. High efficiency ligation, validated with various Y-adapter kits/chemistries, maximizes sensitivity and yields ensuring sequenceable libraries from even the lowest inputs. The amplification step uses sparQ HiFi Master Mix, a high efficiency, high fidelity PCR Master Mix that ensures uniform amplification over a wide range of GC-content, eliminating PCR-induced gaps in coverage.

The high quality, high repeatability library preparation achieved with the sparQ DNA Library Preparation Kit makes it ideal for high-throughput NGS workflows. In this application note, we detail the automation of a sparQ DNA Library Preparation Kit PCR-free workflow on a PerkinElmer Sciclone G3 instrument for microbiology applications.

Figure 1 Overview of the workflow for preparation of NGS libraries using the sparQ DNA Library Prep Kit.

METHODOLOGY

DNA extraction

Genomic DNA was isolated from single-colony bacterial cultures using QIAamp spin procedures (Qiagen). Extracted DNA was qualified by measuring the $A_{260}/_{280}$ ratio on a NanoDrop® Spectrophotometer (Thermo Scientific) and quantified by Qubit® dsDNA assay (Thermo Scientific).

Fragmentation

High throughput DNA shearing was carried out using Covaris LE2200 Focused-ultrasonicator in a 96-well Covaris plate (Covaris Inc.) (Figure 2). The Covaris instrument uses adaptive focused acoustic energy to fragment nucleic acids, providing unbiased fragmentation and high sample yields. The samples were transferred between the Sample Plate and Covaris plate using the Sciclone G3 Workstation. The fragmentation protocol was optimized to give an average library size of 670 bp (post-ligation) suitable for 250 bp paired-end reads on the Illumina MiSeq™ instrument (Illumina Inc.). The workflow allows for a wide input range of extracted microbial gDNA; here 2 – 5 µg input to DNA shearing, in a volume of 130 µl. Sheared DNA was stored at 4°C for immediate use or –20°C for use within 3 days.

The preparation of sequencing libraries from sheared DNA was fully automated on the Sciclone G3 Workstation using the sparQ DNA Library Prep Kit with sparQ PureMag Beads. The deck setup is illustrated in Figure 3 and the optimized workflow is detailed below. Samples were loaded into the Sample Plate (Figure 3, position D3).

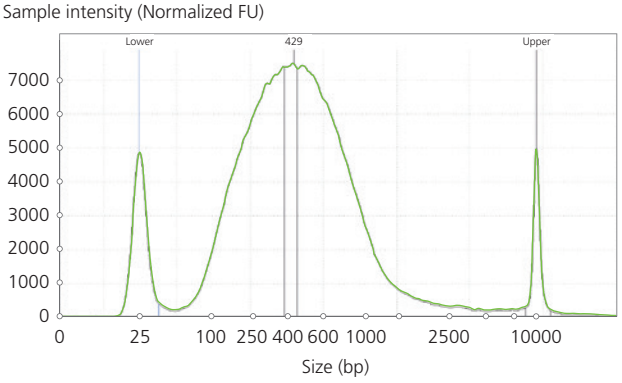

Figure 2 Example Bioanalyzer trace of bacterial DNA after Covaris shearing. Peaks at 15 and 10,000 bp show lower and upper markers.

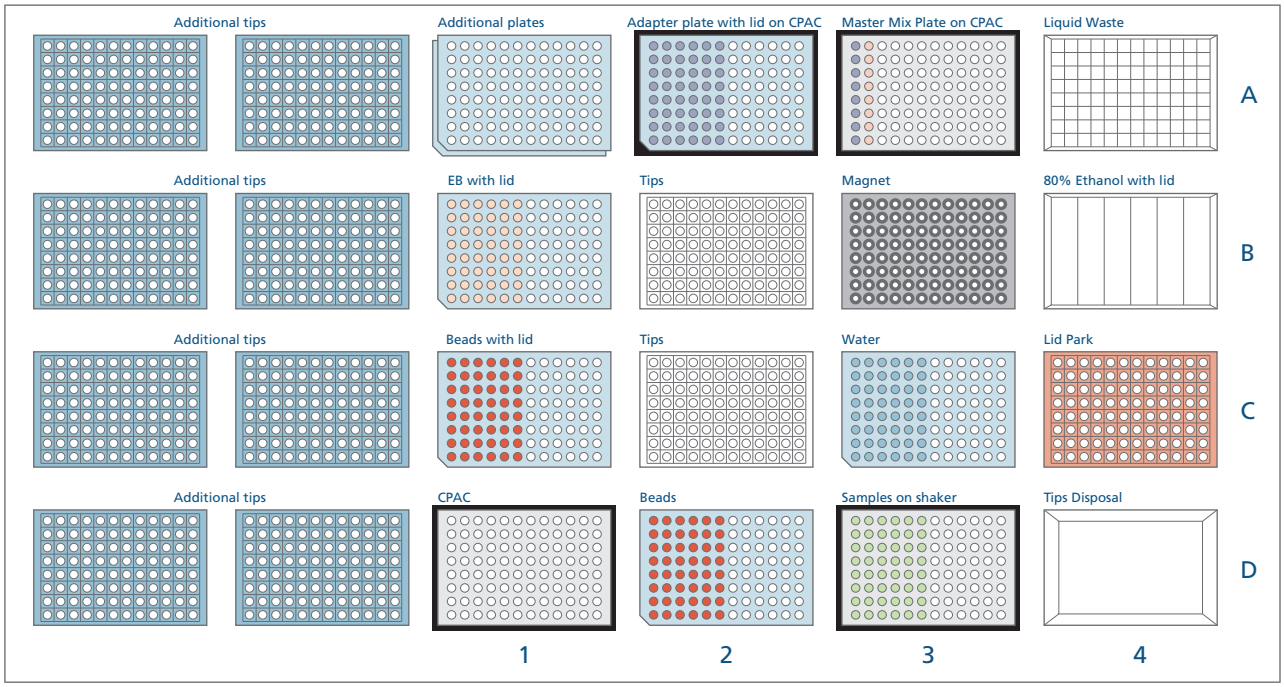

Figure 3 Labeled diagram of PerkinElmer Sciclone G3 deck layout for initial cleanup and library preparation using sparQ DNA Library Prep Kit, with labeled positions.

Initial cleanup

Following DNA shearing, initial cleanup was carried out to remove any over-fragmented DNA and unwanted buffer components. The initial cleanup protocol took around 1 hour. A ratio of 1X sparQ PureMag Beads to sheared DNA sample was used for stringent DNA cleanup as DNA input amount was high and loss of sample was not a concern. For maximum DNA recovery, a 1.8X ratio of sparQ PureMag Beads to sample can be used. The DNA was eluted in 80 µl Tris-HCl pH 8.0 and 30 µl carried forward for library preparation.

DNA Polishing

The sparQ DNA Library Preparation Kit was used for the remainder of the library preparation process which took around 2 hours. First, the DNA polishing reagent was used for single-step 5' phosphorylation and 3' dA-tailing of DNA fragments. The combination of these two steps into one reaction reduces pipetting and sample transfer, decreasing protocol time and increasing yield retention. The DNA polishing reagents were prepared as a master mix with overage (Equation 1-2, Table 1) and loaded into the Master Mix plate (Figure 3 position A3).

DNA Polishing	1 Sample (µl)	96 Samples (µl)
DNA Polishing Buffer	5	539.7
DNA Polishing Enzyme Mix	10	1079.4
Nuclease-free water	5	539.7
Total	20	2158.8

Table 1 Example table showing volumes required for preparation of DNA Polishing Master Mix for 96 samples.

$$\text{Vol Pol MM per row (µl)} = (50\ \text{µl} - \text{DNA sample vol (µl)} + 1) * \text{No. of columns used on plate} + 5$$

Equation 1 Calculation for the volume of DNA Polishing Master Mix (Pol MM) required, per row, to be loaded into the Master Mix Plate.

$$\text{Total vol (µl)} = \frac{\text{Vol for one sample (µl)}}{\text{Total vol Pol MM one sample (µl)} * \text{Total vol Pol MM for all rows} * 1.05}$$

Equation 2 Calculation for the volume of each component of the DNA Polishing Master Mix (Pol MM) required to prepare the Master Mix.

Adapter ligation

The quality of library preparation depends heavily on the efficiency and sensitivity of the enzymes involved in DNA polishing and adapter ligation steps. The sparQ enzymes have been engineered for optimal sensitivity and efficiency, supporting the construction of adapter-ligated libraries from a broad range of input DNA and so were ideal for this workflow as DNA input was highly varied. The adapters used were IDT for Illumina TruSeq™ DNA UD Indexes (Illumina) which were diluted 2-fold (1.5 µl adapter and 1.5 µl nuclease-free water) before use, resulting in a concentration of adapters of 112.5 nM in the ligation reaction. Optimization of adapter concentration in the ligation reaction for individual applications is recommended. For workflows with high input DNA (>500 ng), adapter concentration of up to 750 nM may result in increased library yield. An alternative option for unique dual indexed adapters for the workflow is sparQ UDI Adapters (#95211-096, Quantabio).

The ligation reaction was prepared as a master mix consisting of DNA Rapid Ligation Buffer (5X), DNA Ligase and water with overage (Equation 3, Table 2) and loaded into the Master Mix plate (Figure 3, position A3). The ligation master mix was distributed to the Ligation Plate. Adapters and fragmentation reactions were then transferred to the Ligation Plate in a single pipetting step (Figure 3, position D3).

Ligation	1 Sample (µl)	96 Samples (µl)
DNA Rapid Ligation Buffer (5X)	20	2168.4
DNA ligase	10	1084.2
Nuclease-free water	18.5	2005.8
Total	48.5	5258.4

Table 2 Example table showing volumes required for preparation of Ligation Master Mix for 96 samples.

A <7 columns used : Vol Ligation MM per row (µl) = (51 * No. of columns used on plate) + 7

B ≥7 columns used : Vol Ligation MM per row (µl) = (51 * 6) + 7

Equation 3 Calculation for the volume of Ligation Master Mix (Ligation MM) required, per row, to be loaded into the Master Mix Plate for A <7 columns used or B ≥7 columns used.

$$\text{Total vol (µl)} = \frac{\text{Vol for one sample (µl)}}{\text{Total vol Ligation MM one sample (µl)} * \text{Total vol Ligation MM for all rows} * 1.05}$$

Equation 4 Calculation for the volume of each component of the Ligation Master Mix (Ligation MM) required to prepare the Master Mix.

Post-ligation cleanup

Following ligation, a final cleanup was carried out to remove enzymes, excess adapters or adapter dimers. This was achieved with a 0.8X sparQ PureMag Beads to sample ratio, removing any adapter dimers while retaining a high yield of the library (Figure 4). The library was eluted in 12.5 µl Tris-HCl pH 8.0 or nuclease-free water and 10 µl collected for downstream analysis. The library was analyzed immediately or stored at –20°C for up to 2 weeks.

This workflow is PCR-free; the high efficiency ligation reaction and high recovery of DNA from bead cleanup steps results in sufficient library yield for sequencing without PCR amplification. For applications requiring amplification, the sparQ DNA Library Prep Kit and the sparQ DNA Frag and Library Prep Kit include the sparQ HiFi Master Mix and Illumina p5 and p7 primers. The sparQ HiFi Master Mix is formulated to increase library yields while maintaining library complexity. Efficient amplification across challenging AT- and GC-rich regions ensures even coverage of the target region. Automation of library amplification using sparQ HiFi Master Mix has also been demonstrated on the Sciclone G3 instrument and an instrument script is available from PerkinElmer.

Libraries were quantified using qPCR with Illumina p5 and p7 primers by absolute quantification against a standard curve. Libraries were normalized and samples were pooled for sequencing on MiSeq (up to 48 samples per run with MiSeq Reagent Kit V2, or up to 96 samples per run with MiSeq Reagent Kit V3) and run for 500 cycles (Illumina Inc.). The sequencing run met Illumina sequencing performance metrics for yield and percent quality scores, confirming a successful run. The sequencing data was analyzed through an automated pipeline to determine key bacterial features including serotype and antibiotic resistance determinants.

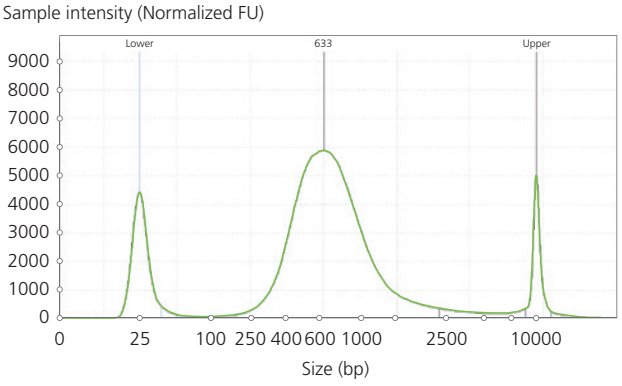

Figure 4 **Example Bioanalyzer trace of final library.** Library was prepared from 1200 ng DNA using the protocol and conditions described, in a manual workflow, yielding on average 1.6 µg adapter-ligated DNA library. Peaks at 15 and 10,000 bp show lower and upper markers.

CONCLUSION

Automation of NGS workflows can massively increase throughput and reduce the need for specialist expertise. This further drives the reduced cost of sequencing, expanding its utility in healthcare and surveillance. We have described automation of the sparQ DNA Library Prep Kit on the PerkinElmer Sciclone G3 instrument and a script for this protocol is available from PerkinElmer. This workflow was focused on microbial applications, but the method presented is widely applicable, opening the way for automation of the sparQ DNA Library Prep Kit for many NGS applications.

MK-AN-0011 REV 01 sparQ DNA Library Prep Kit PerkinElmer Sciclone G3