TECHNICAL NOTE

AUTOMATED DNA LIBRARY PREPARATION FOR PACBIO® LONG-READ WHOLE GENOME SEQUENCING WITH THE DREAMPREP® NGS COMPACT.

Minimal hands-on time solution that delivers up to 48 DNA libraries compatible with PacBio Revio™ and Sequel® IIe long-read sequencing systems.

TECAN

Introduction

Next-generation sequencing has transformed industry's comprehension, detection and treatment of infectious diseases, as well as those caused by genetic and epigenetic changes. It has also provided new insights into evolution and genome dynamics.

PacBio has developed long-read HiFi sequencing protocols to deliver highly accurate reads that span tens of thousands of bases, which is key for an insightful view of genomic structures. This allows users to analyze genomic variability across the population with unparalleled resolution, simultaneously detecting nucleotide polymorphisms, structural variants and epigenetic signatures.

This technical note demonstrates the use of the PacBio SMRTbell® prep kit 3.0 and DreamPrep NGS Compact platform to automate the generation of DNA sequencing libraries (Figure 1). It shows that running automated workflows with minimal user interaction delivers high quality and consistent libraries, allowing staff to undertake more meaningful work instead of performing long repetitive protocols.

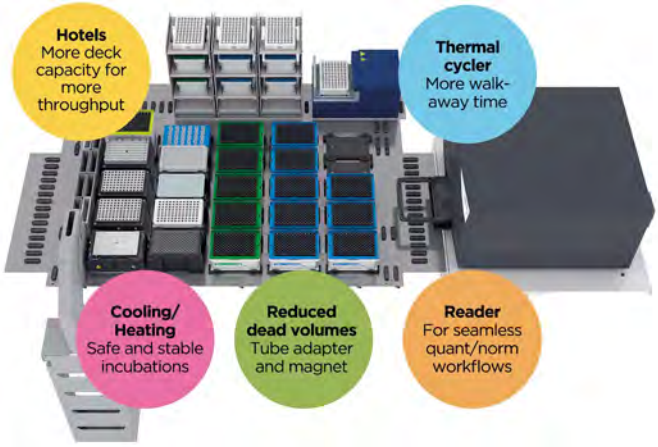

Figure 1: DreamPrep NGS Compact worktable layout. On-deck thermocycler and reader can be easily integrated for further functionality and increased walkaway times.

Materials

- **DreamPrep NGS Compact:** Based on the Fluent® workstation with two independent robotic arms, with integrated on-deck thermocycler (ODTC®, Inheco), Infinite® 200 PRO plate reader in F Nano+ configuration, three liquid-cooled thermal devices (CPLC, Inheco), shaker (BioShake 3000-T elm, QInstruments), 24-tube adapter (Alpaqua® POGO™, NimaGen) and magnet plate (Alpaqua MAGNUM FLX®, Nimagen).

- **Automation consumables and labware:**
  - SLAS tips (conductive), SLAS tip boxes and troughs (Tecan)
  - 96-Well Hard-Shell® Plates (Bio-Rad)
  - Axygen® 96-well Clear V-Bottom 500 µl Polypropylene Deep Well Plates (Corning)
  - 2 ml screw cap micro tubes (Sarstedt)
  - Arched Auto-Sealing Lids (Bio-Rad)

- **PacBio consumables:**
  - SMRTbell prep kit 3.0 (#102-82-700)
  - AMPure® PB beads size-selection kit (#102-182-500)
  - SMRTbell barcoded adapter plate 3.0 (#102-009-200)
  - Sequel II binding kit 3.2 and SMRTbell cleanup beads (#102-194-100) or Revio polymerase kit (#102-817-600)

- **Other consumables and reagents:**
  - Freshly prepared 80 % ethanol
  - Nuclease-free water
  - 4150 TapeStation System (#G2992AA, Agilent)
  - Genomic DNA ScreenTape Analysis (#5067-5365, Agilent)
  - Genomic DNA ScreenTape Analysis – Genomic DNA Reagents containing sample buffer and ladder (#5067-5366, Agilent)
  - g-TUBEs (#520079, Covaris)

- **Additional sample requirements:**
  - Revio sample input: 2 µg per 30x human genome or Revio SMRT® Cell
  - Sequel II or IIe sample input: 3 µg of sheared genomic DNA per 30x human genome, or 300 ng to 1 µg per SMRT Cell
  - Recommended DNA input size distribution: 90 % ≥7 kb (microbial) or 90 % ≥10 kb (non-microbial)

Method

The automated method is based on PacBio's SMRTbell prep kit 3.0 protocol for whole genome libraries, and can process up to 48 samples of mechanically-sheared gDNA. When running the protocol on the DreamPrep NGS Compact, interactive user prompts guide the user through the options and explain how to load the deck (Figure 2).

Figure 2: View of the touchscreen with TouchTools™ user prompts for definition of the run options.

The protocol includes three independent scripts: 1) an initial SMRTbell clean-up bead purification step, followed by 2) the library prep reactions and, finally, 3) the ABC workflow to anneal the polymerase for subsequent sequencing. QC steps should be performed after each part of the automated method according to PacBio's latest recommendations (DNA quantification and sizing).

When setting up the run, the user first inputs variables such as sample number (1-48) and any additional steps of interest, such as the use of barcoded adapters, post-nuclease AMPure bead size selection, or no size selection (Figure 2). Once these variables are chosen, the software will then automatically calculate the required labware and volumes for preparing master mixes and other reagents to run the desired SMRTbell protocol. The user is then instructed how to load labware onto the worktable, and guided through the preparation of master mixes and loading of reagents into troughs, as well as where to place the prepared master mixes in the POGO adapter to enable dead volume reduction. The final step of setting up the deck is loading the samples in a 450 µl microplate.

The recommended sheared DNA input should have a mean fragment size of 15-20 kb for optimal sequencing yields with human, animal or plant genomes. For microbial samples, mean sheared fragment sizes can be smaller – between 7 and 12 kb. The DNA input mass will depend on the sequencing system (1 µg for Sequel II/IIe systems or 2 µg for the Revio system). When pooling, as little as 300 ng per sample can be used if the total DNA input adds up to the required amount per SMRT Cell. All samples should be suspended in PacBio Low TE buffer after shearing and prior to library prep.

Once the library prep is complete, and a posterior QC check carried out, sample concentration should be adjusted using PacBio SMRT Link before performing sequencing primer annelaing and polymerase binding. After this, the libraries are ready to be pooled (if barcoded adaptors are used) and sequenced.

Qualification set-up

The qualification run with the AMPure PB size selection kit was performed with commercial human DNA (Promega) sheared with g-TUBEs and split into 48 preps. DNA quantities and sizes were measured using a TapeStation. Each prep used 2.5 µg of sheared DNA. Prior to sequencing, Femto Pulse (Agilent) and Qubit (Agilent) were used for final QC, and sequencing was performed with the Sequel IIe system.

Results and discussion

The automated workflow on the DreamPrep NGS Compact delivered an average library prep yield of 28.2 % across all 48 wells tested in the qualification run (Table 1). Furthermore, the libraries delivered high sequencing yields, with over 99 % of the reads containing a barcode (Table 2), and all 48 barcodes used were detected in the sequenced pool. Both the library prep and sequencing yields were comparable to those observed with manually prepared libraries. The HiFi read length distribution was consistent with the smaller size profile of the sheared input DNA and the use of bead-based size selection (Figure 3). Therefore, no degradation or performance trade-off was observed as a result of automating the SMRTbell prep kit 3.0 protocol. Increasing the average size of the sheared input DNA to 15-20 kb will increase the read length N50 and total HiFi base yield.

Statistic	Percent yield	Estimate SMRTbell library amount (ng)
Average	28.2	705
Median	27.5	688
Maximum	45.0	1,125
Minimum	18.2	455

Table 1: Automated library prep yields for a 48-sample qualification run, starting with approximately 2.5 µg of sheared DNA per sample.

Sequencing system	HiFi read length N50 (bp)	HiFi yield (QV20) (Gb)	Barcoded HiFi reads (%)
Sequel IIe	10,776	28.9	99.73

Table 2: Sequencing metrics obtained from a 48 sample pool of SMRTbell libraries prepared on the DreamPrep NGS Compact and sequenced on a Sequel IIe SMRT Cell. All 48 barcodes were detected.

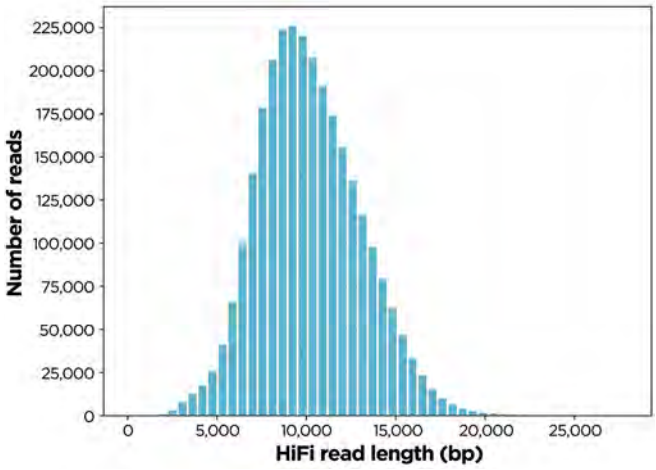

Figure 3: HiFi read length distribution of the 48 SMRTbell libraries prepared on the DreamPrep NGS Compact. DNA was sheared with g-TUBEs, resulting in a mean insert size of 10,000 bp.

Throughput and capacity

The SMRTbell prep kit 3.0 protocol for the DreamPrep NGS Compact can generate up to 48 libraries simultaneously. The full automated method can process 48 samples in 5 hours. In contrast, the manual process for library prep alone (without ABC) is estimated at 4 hours for 8 samples, with an additional 2 hours for every 8 additional samples added. As such, one DreamPrep NGS Compact run can dramatically increase sequencing throughput by preparing enough libraries for 6 fully loaded Revio runs (one library per SMRT Cell) in a single day.

Conclusions

Automation of the PacBio long read library prep protocol using SMRTbell prep kit 3.0 on the DreamPrep NGS Compact workstation represents a reliable solution with minimal user interaction. Final libraries are comparable to those produced manually, and are ready to be sequenced on Revio and/or Sequel IIe systems, depending on choice of sequencing binding kit. By combining automated solutions for library preparation with sequencing platforms that produce high quality and consistent datasets, it becomes feasible to support medium- to large-scale genomic projects in areas such as human genomics, microbiome or infectious diseases.

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