Automated Library Preparation using Watchmaker DNA Library Prep Kit with Fragmentation on firefly®

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Abstract

With the rapid growth of Next Generation Sequencing (NGS), having a robust automation-friendly library preparation solution is a minimum requirement for the handling of precious samples across many applications.

Automating the library prep process has the benefits of not only increasing throughput, but also minimizing performance variability and can even help to eliminate errors in sample-tracking. However, the transition from manual workflows to automation can often be daunting and time-consuming. This application note describes one lab's process, TGen North, to evaluate several library prep solutions and automation systems with the goal of transitioning from their current manual library prep to an automated workflow using SPT Labtech's firefly® along with the Watchmaker DNA Library Prep Kit with Fragmentation.

Introduction

TGen North is the home of TGen's Pathogen and Microbiome Division, located in Flagstaff, Arizona. The HIVE Laboratory at TGen North is involved in surveillance programs for several pathogens enabling the state of Arizona to help inform the public, as well as provide aid to underserved communities.

They use whole genome sequencing to track emerging outbreaks and enable informed public health responses and are responsible for high throughput sample processing including NGS. This group supports large projects at TGen from specimen accession, extraction and QC through sequencing and data analysis. With the critical need for accurate pathogen surveillance, manual library preparation processes have posed challenges including monotonous workflows with an increased risk of potential human errors and inconsistencies between lab personnel. Seeking improved efficiency and data quality, this application note explores the transition to automated library preparation workflows. The transition to automation was ultimately enabled by first identifying areas for improvement in the overall workflow, then determining evaluation criteria for both sample prep solutions and automation platforms to be considered, next executing comparison experiments to identify the most robust sample prep solution and last working with the selected automation vendor, SPT Labtech, to automate the library prep workflow of choice, the Watchmaker DNA Library Prep Kit with Fragmentation. The following sections will discuss this shift towards automation and how it holds the potential to revolutionize NGS workflows, ensuring reliable and reproducible results for more efficient and accurate analyses.

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Manual Processing – Identifying Areas for Improvement

Automating library preparation can minimize several of the error prone steps and decrease time required by scientists in the laboratory enabling them to focus on more complex tasks.

Time Consuming and Error Prone Steps

Normalization

- Sample array
- Normalization of samples with variable dilution requirements

Library Construction

- Bead purification and size selection
- Liquid handling variability across scientists
- Sample transfers and tracking

Library Pooling

- Missed samples, pipetting errors
- Uneven sequencing results

Why automate?

- Fast and reliable
- Prevent cross-contamination
- Reduce human errors in indexing, pooling and sample tracking
- Protocol version tracking
- Consistency in library yields
- Reliable sequencing results, reducing sequencing costs

Several automated liquid handling systems from a range of vendors were considered. Ultimately, TGen North decided on the firefly for the following reasons:

- **Cost:** affordable, roughly half the cost of other systems
- **Lab footprint:** firefly utilizes z-height space allowing it to fit on a standard bench top
- **Intuitive software:** user-friendly, cloud-enabled
- **Support:** SPT Labtech's field application scientists and field service engineers are responsive and helpful in developing new protocols
- **Flexibility:** the system has 16 deck positions, a positive displacement non-contact dispenser, dual core capable of picking up 384, 96 and strip tips, along with optional integrated shaker and plate thermal module
- **Efficiency:** significantly less reagent dead volume required for automating library prep workflows due to integrated positive displacement non-contact dispenser

Determining the appropriate DNA Library Prep Kit to scale

7 DNA library prep kits were compared extracted with the same 8 independent DNA isolates from GM12878 cells using the Chemagic Cell Pellet protocol from Revvity, eliminating process variability. In addition to the positive controls processed, negative extraction controls (NEC) and non-template controls (NTC) were processed for each kit. Input quantities used were those recommended by each kit.

Evaluation Criteria for Whole-Genome Library Preparation

Quality fragmentation enzyme

- Consistent results
- Avoid need for double-sided selection
  - Product loss
- Ligation and conversion efficiency
- Input quantity range
- Ease of protocol
- Time requirements
- Support
  - Technical, sales, automation
- Minimal sequencing artifacts and bias
  - i.e. GC, start-bias

Figure 1: Conversion efficiency comparison.

Calculation assumes no sample loss through process. (((qPCR Molarity [pM] / 1x10^9) x (size [bp] x 660 x volume [uL]) / (input DNA [ng])) x 100

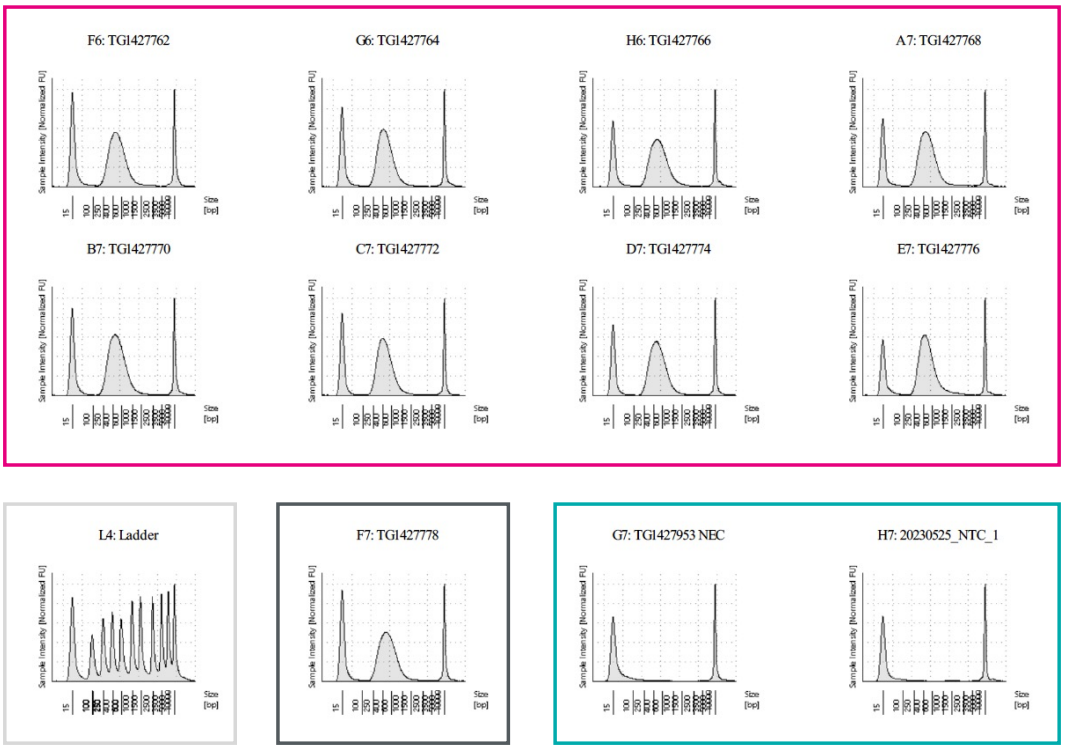

Figure 2: D5000 TS final library traces for Watchmaker DNA Library Kit with Fragmentation.

No additional size selection required. Even sizing and yield across samples (pink), Clean negative controls (teal), Positive control (dark grey), Ladder (light grey). A conversion efficiency for Watchmaker WGS with 4 cycles of PCR amplification and 100ng of DNA was 70-80% (likely higher, post ligation product is diluted 2x). Conversion efficiency was calculated using Tapestation data as follows: (((Library [ng/uL] x volume [uL]) / (1.65^#PCR cycles)) / Input DNA [ng]) x100

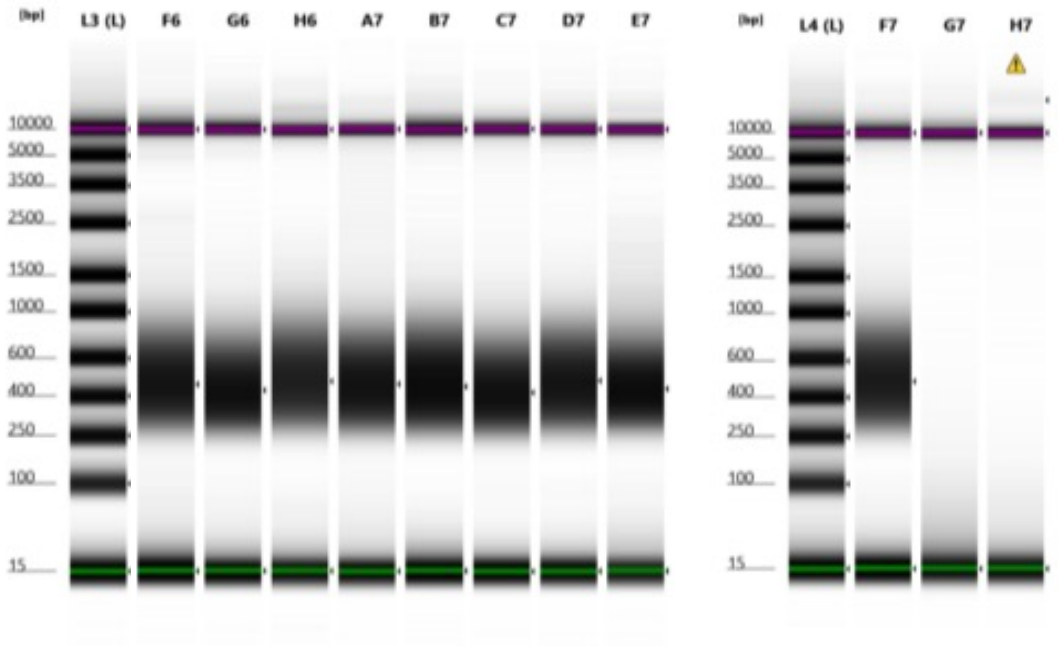

Figure 3: Virtual Gel Image of Watchmaker final libraries from 8 independent DNA isolates (F6-E7), ladder (L4), positive control (F7), negative controls (G7-H7).

The Watchmaker DNA Library Prep Kit with Fragmentation includes a robust enzymatic fragmentation module that allows for both tunable and reproducible results (Figure 2), even across inputs, without requiring the use of additional size selection steps. Out of the 7 kits tested, the Watchmaker DNA Library Prep Kit with Fragmentation offered the highest conversion rates (Figure 1) with a streamlined automation-friendly workflow (Figure 4) that's compatible with PCR-free options. The kit includes adequate overage for automation and Watchmaker has a knowledgeable scientific support team willing to help as needed.

Figure 4: Watchmaker DNA Library Prep Kit with Fragmentation workflow overview.

Conclusion

In conclusion, the transition to automated library preparation using the Watchmaker DNA Library Prep Kit with Fragmentation on the firefly system has significantly improved TGen North's pathogen surveillance workflows. The Watchmaker kit's robust enzymatic fragmentation module, high conversion rates, and streamlined automation-friendly workflow have been instrumental in achieving enhanced efficiency and data quality. The selected firefly automation system offers cost-effectiveness, space efficiency, user-friendly software, and outstanding support from SPT Labtech's scientific team. With increased flexibility and reduced reagent dead volume, the automated library preparation has empowered TGen North to handle precious samples more effectively, ensuring timely pathogen surveillance and freeing up resources for more complex tasks in the laboratory.

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