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

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Overview

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

This poster highlights TGen-North's process 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

Manual Processing: Identifying Areas for Improvement

Why automate?

- Fast & 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

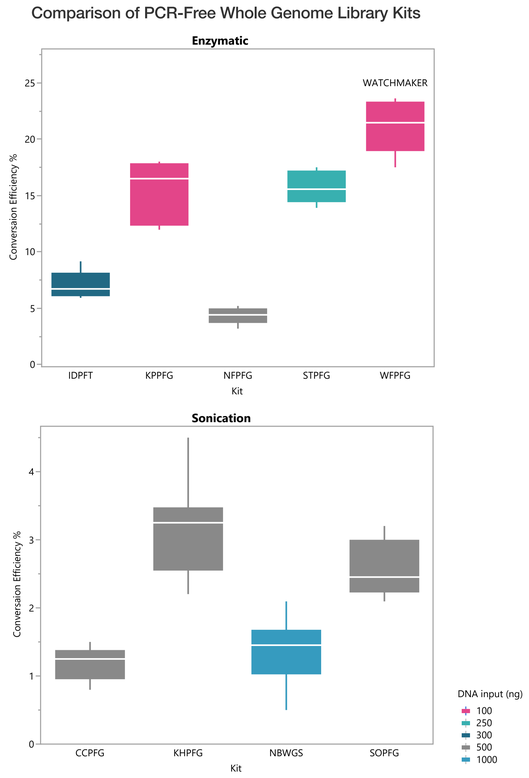
Automation platform evaluation criteria, why firefly?

- **Affordability:** Roughly half the cost of other systems
- **Compact footprint:** firefly utilizes z-height space allowing it to fit on a standard bench top
- **Usability:** Intuitive cloud-enabled software
- **Support:** Access to SPT Labtech's Application Scientists and Engineers to support in new method development
- **Versatility:** The system has 16 deck positions, a positive displacement non-contact dispenser and a dual core pipetting head, along with an optional integrated shaker and plate thermal module
- **Efficiency:** Significant decrease in required reagent dead volume with non-contact dispensing methods vs standard SBS reservoirs (~200 µL vs mLs respectively)

Whole genome library preparation evaluation criteria:

- Quality fragmentation enzyme
- Consistent results
- Avoid need for double-sided size selection, reducing product loss
- Ligation and conversion efficiency
- Input quantity range
- Ease of protocol
- Time requirements
- Support
- Minimal sequencing artifacts and bias

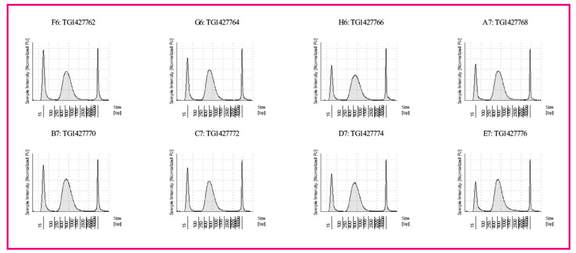
Kit Comparison

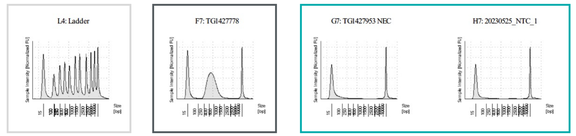


Conversion Efficiency Comparison

7 DNA library prep kits were compared using 8 independent DNA isolates from GM12978 cells extracted with 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.

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





D5000 TS assay for Watchmaker DNA Library Prep 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).

The conversion efficiency of libraries prepped using Watchmaker WGS with 4 cycles of PCR amplification and 100 ng of DNA was 70-80% (likely higher, post-ligation product was diluted 2X). Conversion efficiency was calculated using Tapestation data as follows: (((Library [ng/µL] x volume [µL]) / 1.65^#PCR cycles)) / Input DNA [ng]) x 100.

Watchmaker DNA Library Prep Kit with Fragmentation on firefly

Watchmaker DNA Library Prep Kit with Fragmentation — FRAG/AT | LIGATION | CLEANUP | PCR | CLEANUP — **130 min**

Protocol	Protocol name	firefly run time (min)*	Thermal cycler run time (min)**	96 well tip arrays required***	Dispense head syringes required	Reservoir type and dead volumes required for 96 samples****	Additional plates required
1 of 5	1.0 Frag/AT	5	35 - 55	0	1	5X Frag/AT Master Mix: 1 LDV, 75 µL	1 x DNA input plate
2 of 5	2.0 Adapter Ligation	5	20	1	2	Adapters: 1 LDV, 75 uL Ligation Master Mix: 1 Standard, 240 µL	n/a
3 of 5	3.0 Post-Ligation Purification	35	0	4	6	SPRI beads: 4 Standard, 240 µL 80% Ethanol: Standard, 240 µL 10 mM Tris-HCL: Standard, 240 µL	1 x Elution plate 1 x Waste plate
4 of 5	4.0 Library Amplification	5	15 - 60	1	1	2X Equinox Amplification Master Mix: Standard, 240 µL	1x PCR plate
5 of 5	5.0 Post-Amplification Cleanup	35	0	4	6	SPRI beads: 4 Standard, 240 µL 80% Ethanol: Standard, 240 µL 10 mM Tris-HCL: Standard, 240 uL	1 x Elution plate 1 x Waste plate

Time estimate and consumable estimates are based on 96 samples
**firefly run times vary with the number of samples processed*
*** thermal cycling time dependent on intended fragment size and PCR cycle count*
**** tip array or strip tips may be used. # per run can vary based on mix type (tip mix vs vortex) preferences*
***** Standard reservoir capacity = 10 mL with 240 µL dead volume, Low Dead Volume reservoir capacity = 1.5 mL with 75 µL dead volume. 200 and 35 µL recoverable respectively.*

Conclusions

- The transition from manual to automated library preparation can be streamlined by using efficient, cost-effective, user-friendly automation with support from applications teams for both the instrument and kits being used.
- firefly's dispense head significantly reduces reagent dead volumes compared to other liquid handlers where SBS reservoirs are required for reagent addition.
- The Watchmaker DNA Library Prep Kit with Fragmentation was seen to have in this experiment a conversion efficiency of >70-80% using 100 ng of DNA input with 4 cycles of amplification.

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