BECKMAN COULTER
Life Sciences

Automating NGS workflows: A comparison between a traditional liquid handler and Biomek NGeniuS Next Generation Library Prep System

Introduction

Technological advancements in Next Generation Sequencing (NGS) have enabled researchers to collect information about the function and structure of genomes and transcriptomes. Deep sequencing of the gene-coding and non-coding regions of the genome over a large number of samples is a common practice in many research laboratories. Researchers can reliably detect genetic basis of various biological conditions. Thanks to the ultra-deep coverage and throughput offered by NGS (Ma et al., 2019).

However, there are several barriers to robust detection of genetic conditions, one of which is sample throughput (Hess et al., 2020). Current sequencing technologies require dedicated sample preparation to create the sequencing library loaded onto the instrument. Depending on the required throughput and type of sequencing, the library preparation can be cumbersome when done manually. Automating NGS library preparation enables the use of complex protocols in high-throughput scenarios. In addition, automation provides high reproducibility, reduced error rates and decreased contamination due to minimal human interactions (Fleischer and Thurow, 2018; Pawliszyn and Wilson, 2002; Vonderschmitt, 1991). Despite these advantages, not all automated workflows are the same. Therefore, we compare a traditional liquid handler with the Beckman Coulter Biomek NGeniuS Next Generation Library Prep System, a purpose-built liquid handler for NGS library preparation. For the comparison, we used automated Roche KAPA HyperPrep protocol, at a throughput of 24 samples (Figure 1, Table 1).

Figure 1. Workflow for Roche KAPA HyperPrep protocol (Roche.com). Fragmentation is done off-deck.

Workflow comparison: Traditional liquid handler vs. Biomek NGeniuS Next Generation Library Prep System

Automated method features	Biomek NGeniuS system	Traditional liquid handler
Method setup	Complimentary library of data-demonstrated methods, no programming required	User must write or purchase the data-demonstrated method
	Method can be setup off-site	Method setup done at the instrument
	Synchronize up to 50 instruments	No synchronization
	Different levels of user authorizations	Different levels of user authorizations
	Printable batch-specific Work-Aid	None
Fragmentation	off-line	off-line
Sample aliquoting	Samples in multi-purpose 24-well Reaction Vessel (RV)	24 samples in 96 well plate
Deck setup validation	Dynamic DeckOptix	Manual
Normalization	Automated	Manual calculation & programming (24 manual pipetting steps)
End repair and A-tailing	On-deck, incubation in on-deck thermal cycler	Off-deck incubation, optional on-deck integration
Ligation	On-deck, incubation in on-deck thermal cycler	Off-deck incubation, optional on-deck integration
PCR	On-deck	Off-deck, optional on-deck integration
Cleanup	Automated, using on-deck magnetic station	Automated using magnet plates
Monitoring system status	Onsite: 360° Multicolor Light Bar on top, Offsite: Biomek NGeniuS Portal Software	On site: through software

Table 1. Automation of Roche KAPA HyperPrep protocol, Biomek NGeniuS system vs traditional liquid handler.

Setting up the method

Unlike traditional systems, the Biomek NGeniuS system comes with a complimentary collection of data-demonstrated NGS library construction methods. These include DNA Sequencing (Whole genome and Targeted re-sequencing) and RNA Sequencing (mRNA, Total RNA, Targeted RNA) methods. The application library is designed to evolve with changing customer needs. From the Biomek NGeniuS application library, the user gets to select the desired method to run and enter kit-associated method parameters (Figure 2). The software lets the user select the reagent vendor's recommended safe stop points. The user can synchronize up to 50 Biomek NGeniuS systems simultaneously on one network, providing the flexibility to expand the lab. The lab manager also has the ability to set up different user authorizations to provide specific levels of access to various users. The process of setting up a run on the Biomek NGeniuS Next Generation Library Prep System does not require any programming skills, and can be done from anywhere using a Google Chrome or Microsoft Edge-enabled computer. In comparison, traditional liquid handlers require users to program the method using the instrument software, often on-site.

Once the method options are selected, Biomek NGeniuS software automatically calculates reagent volumes and needed to complete the run. Then the user can generate a batch-specific work aid that lists the reagents needed for the batch. This means the user knows exactly what's needed for the run, even before entering the lab.

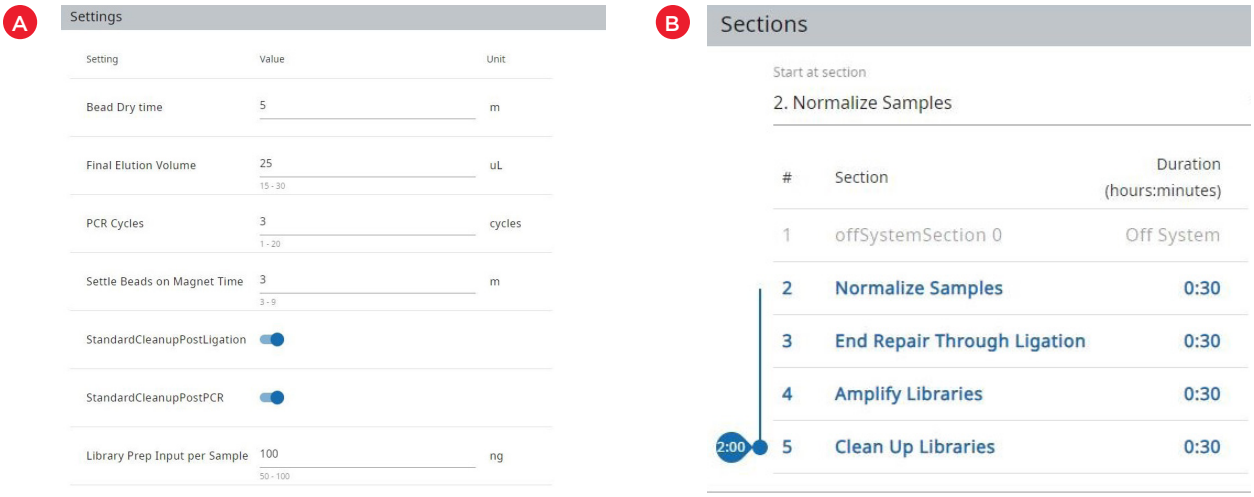

Figure 2. Biomek NGeniuS portal software (a) Method configuration screen showing the vendor recommended method parameter options (b) Method configuration screen showing vendor recommended safe stop points.

Roche KAPA HyperPrep protocol: Fragmentation

Roche KAPA HyperPrep protocol prepares sequencing-ready DNA libraries. The protocol starts with mechanical fragmentation of DNA samples to achieve fragments of 180 – 220 bp (Figure 1). Both liquid handler protocols perform off-deck fragmentation using a Covaris instrument (Covaris, Woburn, MA).

Roche KAPA HyperPrep protocol: Sample aliquoting

Both instrument protocols require manual aliquoting of sheared DNA into automation-friendly labware. Even if the user is running 24 samples, traditional liquid handlers require a 96-well sample plate for sample input. In contrast, Biomek NGeniuS system uses a 24-well Reaction Vessel (RV) as the input plate (Figure 3). The RV serves as a sample input plate with up to 100 million unique barcodes for positive ID of samples. A typical NGS method is labware heavy, requiring at least 3 different plate types (e.g., sample input plate containing nucleic acid samples, PCR plate for thermal cycling and a deep-well plate for cleanup). The Biomek NGeniuS Reaction Vessel replaces all three of these plate types, reducing the need to manage multiple consumable inventories, saving time and money.

Figure 3. Biomek NGeniuS multi-purpose Reaction Vessel (RV) with 600 µL well volume with lid.

Roche KAPA HyperPrep protocol: Reagent aliquoting

Automation on a traditional liquid handler requires all required reagents to be manually aliquoted into automation-friendly tubes (e.g., for small-volume reagents such as enzymes) or into reservoirs (e.g., for large-volume reagents such as ethanol). It is also important to make sure that all tubes are of one type. Because the kit reagents come in different tube types, the user must manually aliquot reagents from their original tubes to automaton-friendly tubes. In addition, temperature-sensitive reagents must be placed on a cold Peltier with a cold block. As these hardware components are not included in the instrument configuration, the user must purchase them separately, at an additional cost.

In comparison, the Biomek NGeniuS Next Generation Library Prep System protocol eliminates these unnecessary pipetting steps by having the user place original reagent tubes on the reagent input carousel (Figure 4). The carousel is compatible with many of the industry tubes, thus reducing the need to transfer reagents to plates. With its advanced optical character recognition technology, the system can automatically identify which reagents are loaded on the carousel and which required tubes are missing (with or without barcodes). It minimizes waste from under-utilization of plated reagents and gives the flexibility to run any batch size from 4 - 24 samples. The Biomek NGeniuS 8 channel LLS (Liquid Level Sensing) pipetting pod detects insufficient reagents prior to the run. Temperature-sensitive reagents are placed in the Biomek NGeniuS cold storage area (2-65 °C). This does not require additional hardware purchases.

Figure 4. Biomek NGeniuS reagent input carousel can take many of the industry tubes including 0.5 mL, 5 mL.

Roche KAPA HyperPrep protocol: Deck setup validation

Automation of KAPA HyperPrep protocol on the traditional liquid handler requires the user to carefully scan the deck to make sure all reagents are placed in the correct deck location, and check labware types and conditions (e.g., capped tubes), before starting the automated run. In contrast, the Biomek NGeniuS Dynamic DeckOptix system scans the deck to identify common setup errors (Figure 5). Using sophisticated optical analytics and a 24-inch head-up display, Dynamic DeckOptix provides real-time feedback on labware placement to virtually eliminate loading errors. The software can identify labware conditions such as capped reagent tubes, incorrect sample input RV, misplaced foil plate covers, missing thermal cycling pads. This system also allows users to use partial tip boxes in the method. Using optical analytics, the system automatically counts the number of tips remaining in the box and determines if there are enough to complete the desired workflow.

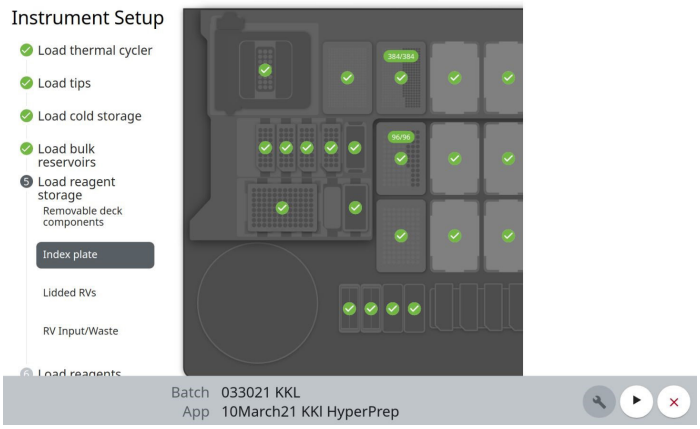

Figure 5. Biomek NGeniuS Dynamic DeckOptix software scans the deck for common setup errors.

Roche KAPA HyperPrep protocol: Normalization

Normalization in the NGS workflows refers to the process of equalizing the concentration of nucleic acids. The traditional liquid handler protocol requires that the user carries out normalization manually, by calculating the required volumes and reprogramming the liquid handler. In contrast, Biomek NGeniuS system protocol has an automated normalization step to further reduce hands-on time.

Roche KAPA HyperPrep protocol: End repair and A-tailing

Both liquid handlers automate the end-repair and A-tailing reaction setup with tip-mixing. The traditional liquid handler requires integration of a thermal cycler to carry out the incubation reaction. In the absence of the integration, the user must take the plate out, perform the reaction in a separate thermal cycler and bring the plate back to the liquid handler for the next step. This requires user-intervention and significant hands-on time. With the Biomek NGeniuS system, the End Repair and A-Tailing incubation is performed in the built-in thermocycler and labware transport system (Gripper; Figure 6). This increases walk-away time and minimizes manual interactions with the liquid handler.

Figure 6. Biomek NGeniuS deck with thermal cycler.

Roche KAPA HyperPrep protocol: Ligation

Both protocols automate the adapter ligation reaction setup. The traditional liquid handler requires a thermocycler integration for on-deck ligation incubation. In comparison, the Biomek NGeniuS system has a built-in thermal cycler and labware transport system to automate incubation and thermal cycling, without the need to have additional hardware and software integrations (Figure 6).

Roche KAPA HyperPrep protocol: PCR

As in the case of End repair/A-tailing and ligation incubations, the traditional liquid handler requires an integrated thermal cycler for on-deck PCR. In contrast, Biomek NGeniuS system has a built-in thermal cycler, enabling users to walk away from the automated run, once the run is started (Figure 6). The Biomek NGeniuS system has an integrated gripper for labware transport, to minimize interactions with the system. Without the on-deck thermal cycler, the traditional liquid handler requires six user interactions with the system, while the Biomek NGeniuS Next Generation Library Prep System requires none once the deck is set up.

Whereas the traditional liquid handler method required a fully skirted PCR plate for thermal cycling, the Biomek NGeniuS workflow does not require a specific labware type for PCR. The multi-purpose RV is made with heat-resistant plastic and therefore can be used for thermal cycling (Figure 3).

Roche KAPA HyperPrep protocol: Cleanup

Both instrument protocols carry out the magnetic bead-based cleanup step with tip-mixing. The traditional liquid handler requires a separate magnetic plate for bead separation. In contrast, the Biomek NGeniuS Next Generation Library Prep System has an integrated magnetic station on the deck, eliminating the need to purchase additional labware (Figure 7). Due to the high volumes associated with cleanup, the traditional automation protocol requires a deep-well plate for cleanups. On the other hand, the Biomek NGeniuS RV handles up to 600 µL and is therefore suitable for cleanup steps.

Figure 7. Biomek NGeniuS deck with magnet station (A) and reagent storage zones (B)

Monitoring system status

Traditional liquid handlers require the user to check the instrument to monitor system status. Biomek NGeniuS Portal Software lets the user monitor the system status from any Google Chrome/Microsoft edge-enabled computer (Figure 8). In addition, the Biomek NGeniuS Multi-color 360° Light Bar, shows the system status through color changes.

Figure 8. Biomek NGeniuS 360° Multicolor Light Bar on top.

Summary

The benefits of switching from manual to automated NGS library preparation are many. Automated more efficient, less prone to human errors (such as during pipetting), less susceptible to contamination, and more reliable and consistent. However, not all liquid handlers are equipped with similar hardware and software features to provide all the benefits of automation. Therefore, we compared a traditional liquid handler with the Beckman Coulter Biomek NGeniuS Next Generation Library Prep System. Compared to the traditional liquid handler, the Biomek NGeniuS system has hardware and software capabilities that enable users to fully reap the benefits of NGS library preparation automation (Table 1).

References

1. Fleischer, H & Thurow, K. (2018) Automation Solutions for Analytical Measurements: Concepts, and Applications, Wiley-VCH, Weinheim, Germany
2. Hess, J. F. et al. (2020) Library preparation for next generation sequencing: A review of automation strategies, Biotechnology Advances, 41. doi.org/10.1016/j.biotechadv.2020.107537
3. Ma, X. et al. (2019) Analysis of error profiles in deep next-generation sequencing data. Genome Biol 20, 50. doi.org/10.1186/s13059-019-1659-6
4. Pawliszyn, J & Wilson, C.L. (Eds.), Sampling and Sample Preparation for Field and Laboratory: Fundamentals and New Directions in Sample Preparation (1st ed.), Elsevier, Amsterdam (2002)
5. Ma, X. et al. (2019) Analysis of error profiles in deep next-generation sequencing data. Genome Biol 20, 50. doi.org/10.1186/s13059-019-1659-6
6. Roche.com Retrieved from **https://sequencing.roche.com/content/dam/rochesequence/worldwide/resources/brochure-kapa-hyperprep-kits-SEQ100003.pdf**

For Beckman Coulter's worldwide office locations and phone numbers, please visit Contact Us at **beckman.com**

21.04.1301 GEN