BECKMAN COULTER
Life Sciences

Reducing Errors associated with NGS library preparation

Abstract

Technological advancements in nucleic acid sequencing have decreased the cost of sequencing and improved the sequencing depth. However, library preparation is a bottleneck that can introduce human errors to the process. Given that each genomic region is sequenced at a 10x to 60x coverage, the errors introduced at the library preparation step magnify, eventually resulting in significant data errors. Compared to manual prep, automation reduces the errors associated with NGS sample preparation by decreasing sample-to-sample variation, but not all automated liquid handlers have the same hardware and software capabilities to reduce human errors. In this application note, we compare Beckman Coulter Next Generation Library Prep System and a traditional low-throughput liquid handler. Compared to the traditional liquid handler, Biomek NGeniuS system has a variety of hardware and software features purpose-built to significantly reduce NGS library prep errors.

Introduction

The introduction of high-throughput sequencing, also known as Next Generation Sequencing (NGS), has revolutionized the field of biology by enabling researchers to gain a deeper understanding of a wide variety of organisms. With the advent of highly parallel sequencing in the past decade, the cost of sequencing and the time required to obtain sequencing information has reduced dramatically, making NGS an accessible option for many molecular biology laboratories. As a result, the number and range of sequencing applications continue to increase exponentially. NGS library preparation methods have also evolved, along with sequencing technologies. Despite the progression, NGS library preparation remains time consuming and error prone when done manually (Ma et al., 2019).

The purpose of library preparation is to convert the nucleic acid target into a form that is compatible with the sequencing system to be used. In DNA sequencing, DNA is first fragmented through physical, enzymatic or chemical cleaving approaches, the fragments are size selected and converted into a library by ligating sequencing adapters containing specific sequences to interact with the sequencing platform. Unique barcodes are also added to facilitate sample indexing, which is important for high-throughput preparations. A final size selection step, typically a bead-based cleanup is performed to refine the library size and remove library preparation artifacts such as adapter dimers. The specifics of each step vary based on the library preparation kit and workflow. Depending on the application, the NGS library prep process can take a couple of hours to a couple of days (Head et al., 2014).

As sample throughput increases, manual library preparation becomes burdensome, and automating library preparation on a liquid handler becomes crucial to keep up with the workload. Apart from being more efficient, automated library preparation is less prone to human errors and therefore can be more reliable and consistent than manual processing. However, liquid handlers have varied built-in capabilities to reduce the library preparation errors. In this application note we review the common sources of library preparation errors and compare features of two liquid handlers (Beckman Coulter Biomek NGeniuS Next Generation Library Prep System and a traditional low- throughput liquid handler) focusing on reducing NGS library preparation errors.

Methods

We compared the automation of the Roche Kapa HyperPrep protocol on Biomek NGeniuS system with another low-throughput liquid handler on the market. Technical details of the automated method, including the number of manual interactions, the number of pipetting steps and additional hardware and software features, were compared for a sample size of 24.

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

Results

Table 1 shows the comparison of the Roche Kapa HyperPrep protocol automated on Biomek NGeniuS Next Generation Library Prep System and a low-throughput liquid handler. The automation on Biomek NGeniuS system allows more walkaway/less hands-on time with few manual steps. Unlike the low throughput liquid handler, the Biomek NGenuS's Dynamic DeckOptix system scans the deck to identify common setup errors.

Automated method features	Biomek NGeniuS Next Generation Library Prep System	Traditional liquid handler
Sample size	24	24
Reagent aliquoting	Original tubes placed on reagent input carousel. Automated reagent aliquoting	All reagents aliquoted manually (24 manual pipetting steps)
Deck setup validation	Dynamic DeckOptix	Manual
Normalization	Automated	Manual calculation & programming (24 manual pipetting steps)
End repair and A-tailing	Automated	Automated
Ligation	Automated	Automated
Cleanup	Automated	Automated
PCR	On-deck	Off-deck, optional on-deck integration
Temperature control	Integrated Reagent storage zones (4)	Optional Peltier integration
Input flagging	Yes	No
Number of interactions after starting the run	None	6 (with no on-deck thermal cycler)

Table 1. Comparison of automated Roche Kapa HyperPrep protocol on Biomek NGeniuS system vs. on a traditional liquid handler.

Discussion

Recent developments in highly parallel sequencing technologies have decreased the cost of sequencing and enabled in-depth sequencing coverage across many samples. This enables researchers to study complex biological phenomenon. However, there are many challenges associated with reaping the full benefits of NGS. These challenges include bioinformatics limitations to process large amounts of data, sequencing errors and NGS library preparation errors (Robasky, Lewis & Church, 2014; Ma et al, 2019). Because of the time it takes to sequence and analyze large data sets, it could be months until the researcher realizes that there was a library prep error. This means loss of time and money, in addition to the loss of precious and sometimes irreplaceable samples.

Ideally, the prepared libraries should reflect the natural complexity of the genetic material, without introducing technical errors during the process. The biases or the batch effects created during library prep can introduce noise, making it difficult to tease out biological variation. One way of minimizing such NGS errors is to minimize operator error. For instance, pipetting technique is known to vary across operators, resulting in inaccurate dispense volumes, a variability that can affect the NGS library prep (Artel.com). Automation can minimize the human error by making the sample processing consistent and less biased.

However, automated liquid handlers vary in their ability to provide a hands-free solution, as some require multiple manual pipetting steps and processing steps. In this application note we compared the features of two liquid handlers, Beckman Coulter Biomek NGeniuS Next Generation Library Prep System and a traditional liquid handler, to differentiate the hardware and software features that reduce human error. We found that Biomek NGeniuS System requires less hands-on time and includes features to identify potential human error (Table 1). Below we describe NGS sample prep errors and discuss the features of Biomek NGeniuS system that help reduce these errors.

- **Deck setup errors**

  User errors such as mislabeling and using the wrong reagents contribute to NGS library preparation errors (Robasky, Lewis & Church, 2014). If certain reagents are not added or added in the wrong order, the library preparation fails. For instance, before the addition of adaptors, A-tailing reagents should be added because A-tailing of the 3′ ends of DNA facilitates ligation of sequencing adapters. Such human errors can be significantly reduced by the Biomek NGeniuS's Reagent Identification System, and its carousel, which is compatible with many industry vials and tubes, thus reducing the need to transfer reagents to intermediate plates or tubes. Its optical character recognition technology identifies which reagents are loaded on the carousel and which required vials are missing, with or without barcodes. The demonstrated methods include the programmed sequence of processing of the kit components, with kit vendor recommended safe stop points. In the case of actions needed off the system, the Biomek NGeniuS system includes a work Aid specifically designed for the run/batch being executed.

- **Labware placement**

  On an automated liquid handler, placing the wrong labware in the wrong location can lead to instrument crashing, method termination and ultimately loss of precious samples, expensive reagents and loss of valuable time. Biomek NGeniuS software calculates the number of consumables needed on the deck, based on the number of samples. The Dynamic DeckOptix System on the Biomek NGeniuS system uses the on-deck camera to scan the deck in real time, providing feedback on labware placement to virtually eliminate loading errors. Many traditional liquid handlers lack this level of deck verification, and therefore rely on the user to assess the deck (Table 1). Manual checks increase deck loading time and are not as effective in preventing human error.

- **Insufficient reagents**

  Having insufficient reagents in reagent vials can create unexpected shortage of reagents. The 8-channel pipettor on Biomek NGeniuS system has a Liquid Level Sensing (LLS) capability, detecting reagent levels and alerting users if the reagent volume is insufficient. This check point before sample processing helps users identify the insufficient reagents and make necessary adjustments before the run starts, helping to avoid downstream sample processing issues.

- **Manual pipetting**

  Manual pipetting can introduce technical variability and can lead to erroneous libraries. For instance, working too quickly—and with insufficient training—have been identified as sources of pipetting errors (Artel.com). Such variation in pipetting technique can either over-deliver or under-deliver the required volume, ultimately causing library preparation errors. For example, too much adapter favors formation of adapter dimers that can be difficult to separate and magnify in the subsequent PCR amplification. Insufficient adapters could result in some fragments not having adapters at all (Head et al, 2014). Manual aliquoting of reagents and mastermixes in traditional liquid handlers can introduce pipetting errors and batch-to-batch variation in pipetting. The Biomek NGeniuS reagent input carousel significantly reduces manual reagent aliquoting. Many of the reagents are loaded onto the carousel in their original tubes, thereby reducing the propensity for errors. The reagents are aliquoted by the Biomek NGeniuS system to prepare mastermixes on-deck. In comparison, the traditional low throughput liquid handler requires all reagents to be aliquoted manually (Table 1).

- **Manual data entry errors**

  Multiplex deep sequencing is a powerful approach to sequencing a large number of samples at one time. Instead of sequencing one at a time, libraries are tagged with barcodes and then pooled to sequence in one run using high-throughput sequencing machines. However, if not added properly, barcode and adapter errors are a significant source of NGS sequencing errors. Manual entry of barcodes and pooling combinations can easily introduce errors (Robasky, Lewis & Church, 2014).

Biomek NGeniuS software lets users upload the sample information in .csv file format. After the file is uploaded, the software checks for errors (E.g. Indices, concentration range). This again ensures that sample parameters match vendor recommendations, helping to reduce library preparation issues. For instance, if the user inputs duplicate indices, the system flags the .csv file, encouraging the user to review the input.

- **Sub-optimal sample processing conditions**

  Consistent processing of NGS samples is important to reduce technical variability (the variability due to sample preparation conditions and measurements). To reduce the technical variability, it is important to process each sample in the same manner (e.g. same processing conditions and timings). Technical variability is increased due to the lag between adding reagents to the first sample versus the last. Compared to manual processing, automated library preparation can reduce such variability by parallel processing of samples. However, sample processing conditions can still affect the success of library preparation. For instance, sample evaporation or degradation can change input concentrations (Akbari et al, 2005). Low DNA template input in PCR can generate false mutations, affecting the results of sequencing. Temperature-sensitive reagents and temperature- sensitive reactions must be carried out at optimal conditions to obtain the desired results. Integrated temperature-controlled reagent storage zones provide the right storage temperature for reagents and ensures that input reagent temperature is the same during dispense (from 2°C to 65°C). In addition, these zones can be used as storage zones for unused stock solutions. The on-deck thermal cycler minimizes evaporation on the longest hybridization capture protocols. It is also used to hold finished libraries at 4°C until the user is ready to retrieve them—all contributing to optimizing sample processing conditions.

Conclusion

In this application note we compared Biomek NGeniuS Next Generation Library Prep System with a traditional liquid handler to identify the hardware and software features that help to reduce library prep errors. We reviewed the common sources of library prep errors and discussed how automation can minimize them. The specific features of the Biomek NGeniuS system, including Dynamic DeckOptix system, reagent identification system, temperature management system, Multi-channel liquid level sensing and sample input tracking, minimize human errors, which helps to save time and money.

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