Next-generation sequencing

Comparison of hands-on time in next-generation sequencing workflows for the Ion Torrent Genexus Integrated Sequencer and the Illumina MiSeq System

Key points

- Preparing samples for sequencing on the Genexus Integrated Sequencer requires much less hands-on time than preparing samples for sequencing on the MiSeq System.
- The workflow for the Genexus sequencer is simpler, which makes training less onerous and reduces variability between users.
- With rapid turnaround from sample to report, the Genexus Integrated Sequencer provides next-generation sequencing results in as little as one day to inform decision making.

Introduction

Next-generation sequencing (NGS) has revolutionized genomics research and is now transforming clinical research and precision medicine. With NGS, large-scale gene panels can be sequenced rapidly and interrogated without bias. Unlike traditional Sanger sequencing, NGS is not limited to the identification of substitutions and small insertions or deletions [1]. NGS is highly effective for identifying and detecting various types of biomarkers. These biomarkers are found in specific subsets of relevant genes, so performing targeted sequencing to detect them is often more cost-effective than sequencing the whole genome.

Two of the biggest obstacles to widespread NGS implementation are its cost and the level of expertise required to prepare sequencing libraries and analyze and interpret the large volume of data generated [1]. Even laboratories that can afford NGS systems may find hiring and training the personnel to operate them cost-prohibitive. Learning an NGS workflow can take time, and novices often work more slowly than expert users. Variability between users, due to the complexity of many NGS workflows, often has a negative impact on reproducibility and the reliability of results. Workflow complexity also directly impacts turnaround time, and the average turnaround time for an NGS report is over two weeks. Reporting delays can have adverse impacts, particularly in clinical research settings [2].

For next-generation sequencing to reach its full potential, more laboratories must be able to get up and running with NGS quickly and easily. Novices and intermediate users should be able to learn workflows quickly and complete them efficiently. Reproducibility is imperative, so minimizing the number of user interventions (touchpoints) and hands-on time is generally recommended. Researchers at Thermo Fisher Scientific recently conducted an internal activity-based cost analysis to compare the labor costs and time required to prepare sequencing libraries and perform NGS on the Ion Torrent™ Genexus™ Integrated Sequencer and the Illumina™ MiSeq™ System using a two-pool targeted NGS panel.

Materials and methods

Four Thermo Fisher Scientific employees and an intern were each asked to prepare a total of 16 sequencing libraries for NGS. Two of the operators were novices with little or no NGS experience, two had five or more years of NGS experience, and one had ten months of NGS experience. None of the operators had prior experience with the Genexus Integrated Sequencer.

Each operator prepared two-pool Ion AmpliSeq™ sequencing libraries with eight samples for sequencing on the Genexus Integrated Sequencer. Each operator also used the PCR-based AmpliSeq™ for Illumina™ workflow to prepare libraries from the same eight samples for sequencing on the MiSeq System. This workflow is an amplicon-based enrichment protocol for targeted NGS on Illumina systems.

A Thermo Fisher Scientific auditor recorded hands-on time for every operator at each step of the workflows for the two sequencers (Figure 1). An hourly rate of $33 per full-time equivalent (FTE) based on technical laboratory positions posted on the websites of Glassdoor and Indeed was used to model and compare the labor costs associated with operating the Genexus Integrated Sequencer and the MiSeq System.

Results

The hands-on times needed to complete the workflows for the Genexus and MiSeq systems are shown for each operator in Table 1. The operators required 13 minutes of hands-on time on average (±1 min) to prepare eight nucleic acid samples for sequencing on the Genexus Integrated Sequencer. Completing the workflow for the MiSeq System with the AmpliSeq for Illumina library preparation protocol took 174 minutes on average with a standard deviation of 43 minutes.

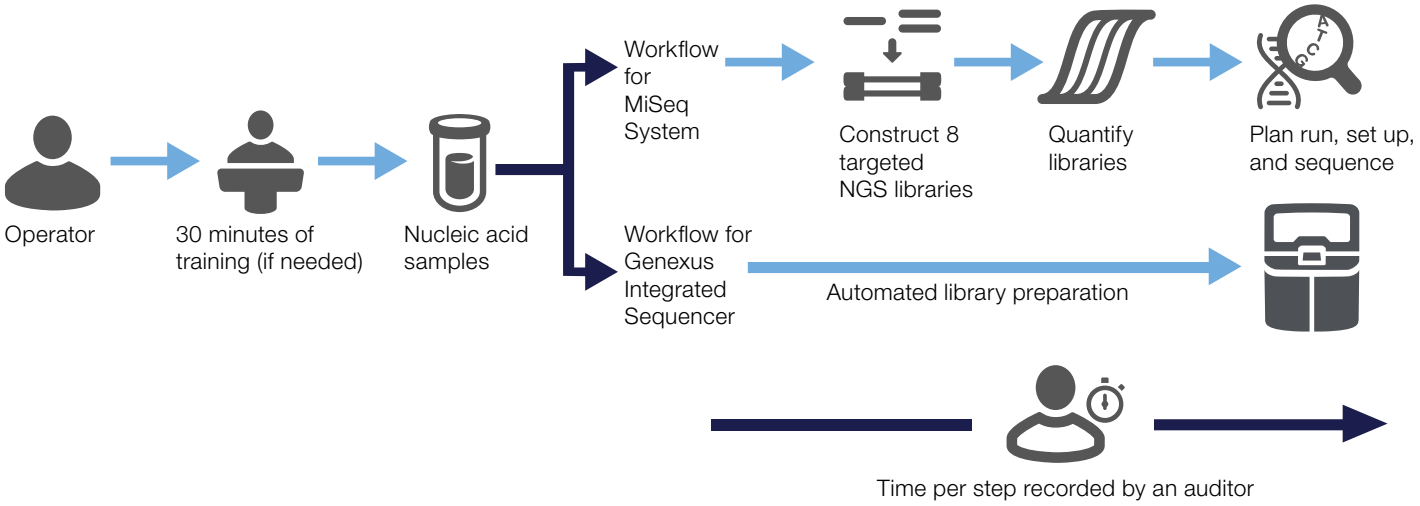

Figure 1. Comparison of the workflows for the Genexus and MiSeq systems. Both workflows require library preparation and sequencing run setup upstream of NGS. These steps are performed manually for the MiSeq System and are automated for the Genexus sequencer.

Table 1. Hands-on time (HOT) required to complete the workflows.*

Operator	Months of NGS experience	HOT	
		MiSeq System	Genexus sequencer
1**	1 (novice)	125 min	NA
2	0 (novice)	229 min	12 min
3	60 (expert)	154 min	12 min
4	10 (intermediate)	206 min	14 min
5	84 (expert)	154 min	14 min
	Average:	**174 min**	**13 min**

* Thermo Fisher Scientific internal data on file.

** Operator 1 was an intern who completed their internship before the study was completed.

Based on the average hands-on times (HOTs) needed to prepare NGS libraries and sequence eight samples using the two workflows, we calculated the total HOTs required to perform one to five NGS runs per week over the course of a year for a total of 416 to 2,080 samples. With an average HOT of 13 minutes, a single operator could theoretically prepare NGS libraries and sequence 2,080 nucleic acid samples in seven business days using the workflow for the Genexus system. It would take more than 94 business days to prepare libraries and sequence the same number of samples using the workflow for the MiSeq System (Figure 2). Switching to the Genexus sequencer could thus save laboratories about 87 business days, or nearly 700 operator hours, per year.

The time needed to complete the workflow for the MiSeq System varied widely, and much of it was spent preparing and quantifying sequencing libraries. Much less variability was observed between operators on the Genexus Integrated Sequencer, even though none of them had prior experience with it.

Cost analysis

Preparing and performing a run with eight nucleic acid samples on the Genexus Integrated Sequencer has an operational labor cost of $7.15 on average, whereas a run with eight samples on the MiSeq System has an operational labor cost of $95.70 on average. Our experimental results indicate that laboratories currently using the MiSeq System and workflow could significantly reduce their annual labor costs by switching to the Genexus Integrated Sequencer and workflow.

Our FTE labor expense model indicates the amount of money saved on labor will increase as the number of samples run on the Genexus system per year increases (Figure 3). According to the model, the annual cost of labor to perform one run per week with eight samples per run on the Genexus Integrated Sequencer would be $4,604 less than the cost of running the same number of samples on the MiSeq System. The annual labor cost savings would increase to $23,023 if an FTE using the Genexus system performed five runs per week with eight samples per run.

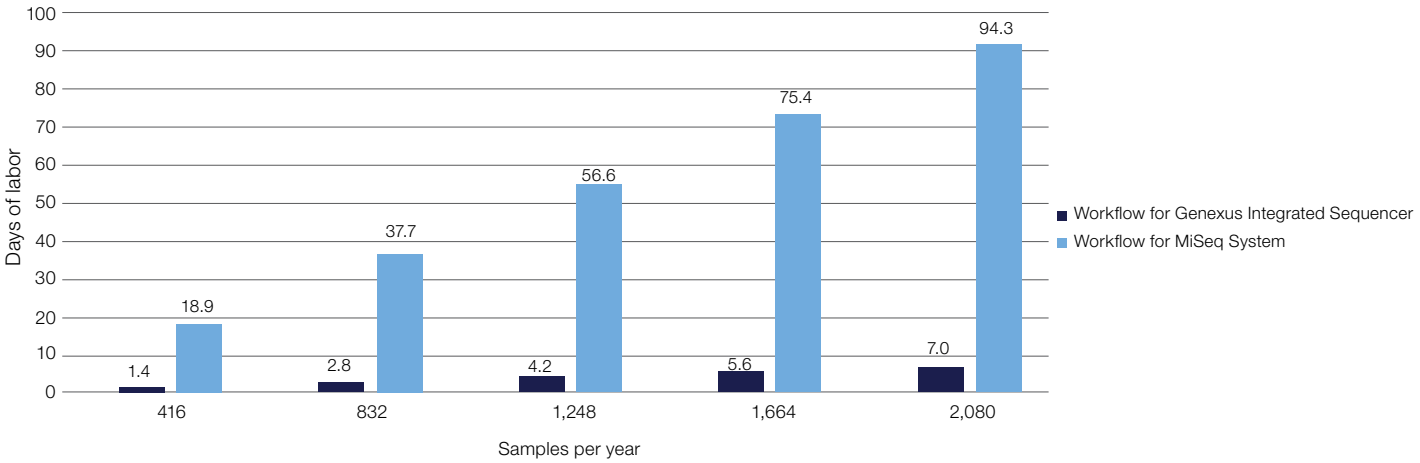

Figure 2. Days of labor (HOT) required for an operator to prepare and sequence nucleic acid samples using the workflows for the Genexus and MiSeq systems. Preparing and sequencing libraries for eight samples took 13 minutes on average using the Genexus sequencer. HOT for the MiSeq System was calculated assuming an FTE would take two hours and 54 minutes to prepare two-pool AmpliSeq libraries for eight samples per run and perform one to five NGS runs per week for a total of 416 to 2,080 samples.

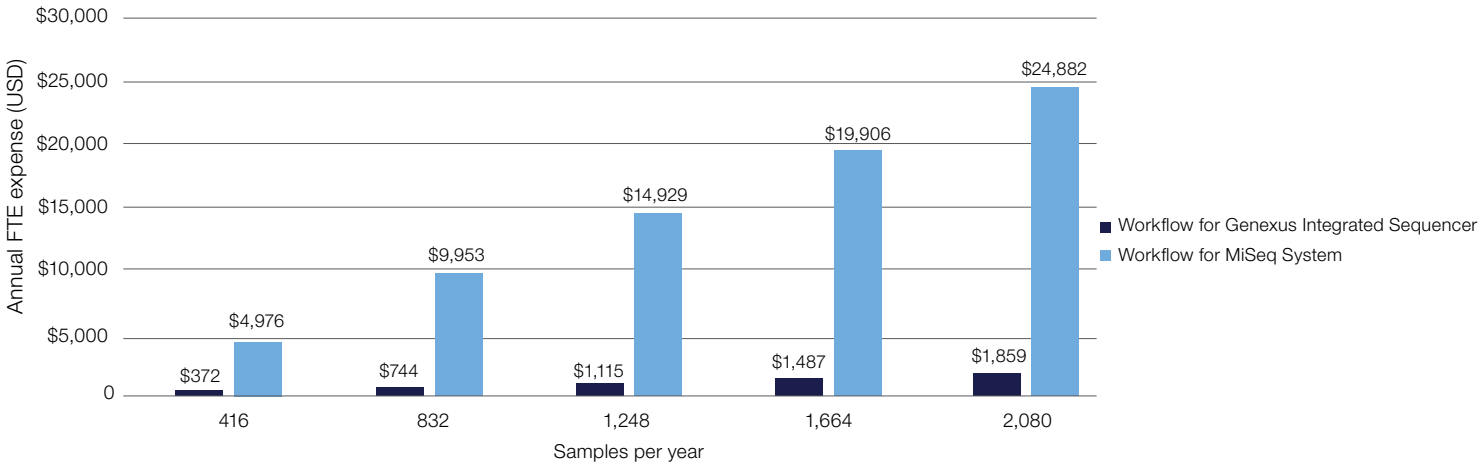

Figure 3. Estimated annual FTE labor costs associated with the workflows for the Genexus and MiSeq systems.

Conclusions

In this internal study, the workflow for the MiSeq System with manual library preparation took approximately 2.5 hours more hands-on time on average than the workflow for the Genexus sequencer (Thermo Fisher Scientific internal data on file). This means laboratories could get back an average of ~2.5 hours per NGS run per operator, or about 33% of an eight-hour day, by switching to the Genexus Integrated Sequencer. Users of the Genexus sequencer can be more productive than users of the MiSeq System and still have time to perform other laboratory tasks. NGS on the Genexus Integrated Sequencer is considerably easier and more user-friendly than NGS on the MiSeq System, even for novice operators, which can significantly reduce labor costs over time. The cost of labor for an average run with eight samples on the Genexus Integrated Sequencer was just over $7 in this study, while an equivalent run on the MiSeq System cost nearly $96.

Automated library preparation on the Genexus system takes far less hands-on time than manual library preparation using the AmpliSeq for Illumina protocol, and it involves fewer manual pipetting steps. It is thus easier to train inexperienced users to operate the Genexus sequencer. Since the workflow for the Genexus sequencer is simpler and requires less hands-on time than that of the MiSeq System, there is less variability between operators. This can improve data reproducibility and reduce the frequency of repeat testing, which translates to additional savings in terms of labor and the cost of reagents and consumables.

References

1. Behjati S, Tarpey PS (2013) What is next generation sequencing? *Arch Dis Child Educ Pract Ed* 96(6):236–238.
2. Sheffield BS, Beharry A, Diep J et al. (2022) Point of care molecular testing: community-based rapid next-generation sequencing to support cancer care. *Curr Oncol* 29(3):1326–1334.

Learn more at **thermofisher.com/genexus**