Automated Amplicon Library Preparation with MagicPrep™ NGS.

Application Note

AUTOMATED LIBRARY PREP THAT JUST WORKS

INTRODUCTION

Automated library prep for amplicon-based workflows
Routine manual preparation of amplicon-based libraries can be burdensome to personnel, requiring at least 5 hours of assay time and allots only 0.5 hours of uninterrupted walkaway time. MagicPrep™ NGS is an ideal solution for saving time by automating the generation of amplicon-based libraries.

MagicPrep NGS offers a fully automated workflow to generate high quality amplicon-based libraries. The workflow may be used with Revelo™ DNA-Seq Enz for MagicPrep NGS for large amplicon sizes (> 300 bp) or with Revelo™ DNA-Seq Mech for MagicPrep NGS for smaller amplicon sizes (< 300 bp). Running the instrument is a 1-step process allowing for 5.5 hours of uninterrupted walkaway time (**Figure 1**). This time savings of 9x over standard manual workflows provide lab scientists ample time to perform other tasks, including preparing the next set of samples.

CASE STUDY: MAGICPREP NGS WITH SARS-COV-2 AMPLICONS

Amplicon libraries generated from patient nasal swab
MagicPrep NGS was used to generate amplicon-based DNA libraries using primers from the QIAseq® SARS-CoV-2 Primer Panel with the goal of sequencing across the SARS-CoV-2 genome and subsequently identifying the SARS-CoV-2 strain for a given sample. Total RNA was extracted from a nasal swab sample from a SARS-CoV-2–positive patient and titrated down to Ct18, Ct21, and Ct24. Amplicons were generated from the titrated samples using the QIAseq SARS-CoV-2 Primer Panel RT-PCR protocol. PCR amplicons from the 3 samples were loaded with 2 different input amounts (25 ng and 100 ng) in duplicates for a total of 12 libraries. Once loaded into MagicPrep NGS, the Revelo DNA-Seq Enz for MagicPrep NGS kit was used in order to fragment the 400 bp amplicons down to 200 bp (**Figure 2**). The resulting libraries were sequenced on the MiniSeq System at 2x75 bp read length.

Library quality enabled SARS-CoV-2 strain detection with 1M read pairs
Sequencing data from each library were subsampled down to 1 million read pairs and aligned to the SARS-CoV-2 reference genome (MN908947.3). The alignment rate of the reads was over 99 % across all samples (**Figure 3**). The coverage pattern was, expectedly, uniform across most of the reference genome[1] (**Figure 4**). On average, the uniformity of coverage across all samples was approximately 89 % (**Figure 5**). The fidelity of the generated data allows for the identification of the SARS-CoV-2 strain across all samples (Omicron variant BA.1). The success of the SARS-CoV-2 amplicon library prep and sequencing analysis underscores the application of MagicPrep NGS for amplicon-based library preparation.

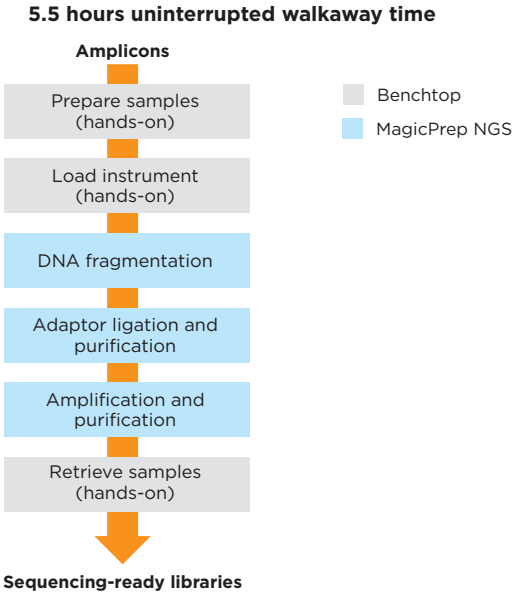

Figure 1: MagicPrep NGS automates the entire library prep workflow from DNA fragmentation through amplification, and purification to provide sequencing-ready libraries in 5.5 hours.

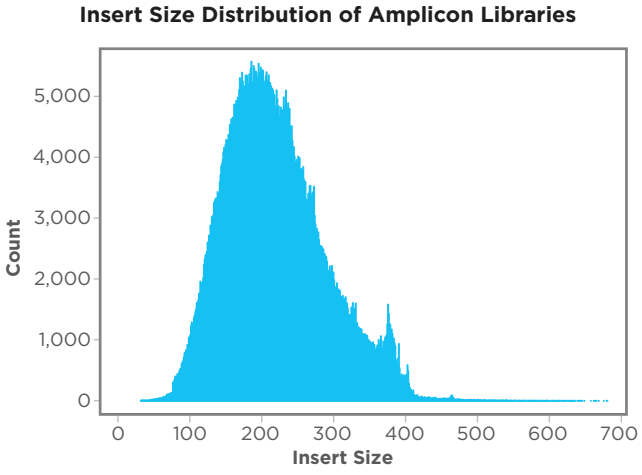

Figure 2: The insert size of the library after using the Revelo DNA-Seq Enz for MagicPrep NGS has a normal distribution averaging 200 bp.

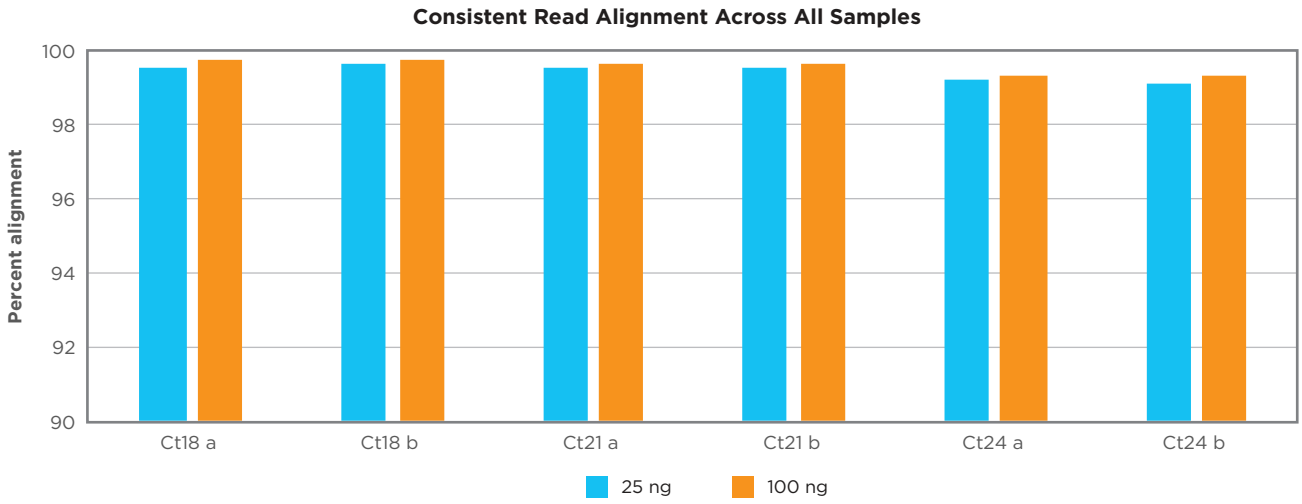

Figure 3: The alignment rate was over 99 % across all samples (a and b correspond to technical replicates) even at 25 ng input amounts, indicating minimal read artifact and contamination with the MagicPrep NGS workflow.

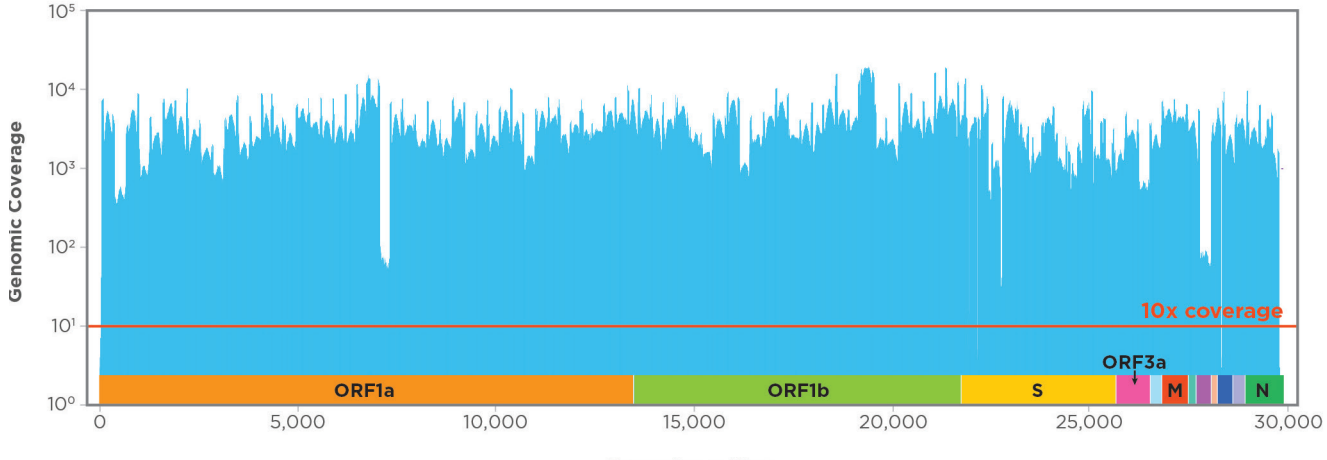

Figure 4: This is a representative coverage pattern as was observed across all samples (Ct18 sample shown). The level of coverage and uniformity was sufficient to determine that this sample is Omicron variant BA.1.

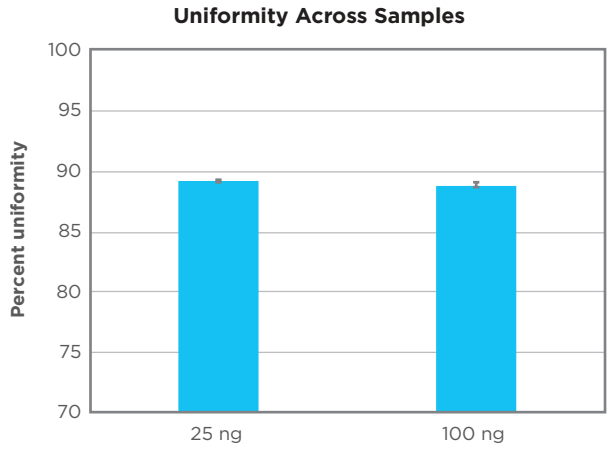

Figure 5: Coverage uniformity is consistent between the two input amounts for MagicPrep NGS, averaging ~89 %.

SUMMARY

The MagicPrep NGS platform is an automated solution for NGS library prep. The MagicPrep NGS alleviates the labor burden needed for manual amplicon-based library prep by providing 5.5 hours of uninterrupted walkaway time. The workflow can be used with shorter amplicons (< 300 bp) using Revelo DNA-Seq Mech for MagicPrep NGS and longer amplicons (> 300 bp) using Revelo DNA-Seq Enz for MagicPrep NGS.

This case study with SARS-CoV-2 amplicons highlights MagicPrep NGS' ability to generate amplicon-based libraries with high alignment rate and uniform coverage across all samples, allowing for the identification of the SARS-CoV-2 Omicron variant BA.1 for each sample.

REFERENCES

1) Lambisia AW, *et al.* Optimization of the SARS-CoV-2 ARTIC Network V4 Primers and Whole Genome Sequencing Protocol. *Front Med (Lausanne)*, 2022, **9**:836728. https://doi.org/10.3389/fmed.2022.836728

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