

Simplifying NGS library preparation with

MagicPrep™ NGS.

Application Note



AUTOMATED LIBRARY PREP THAT JUST WORKS



INTRODUCTION

Next generation sequencing (NGS) is a standard tool in academic and clinical research. Since the original Illumina NGS platforms were introduced, there have been significant improvements in throughput, turnaround time and cost of NGS. The process for NGS library construction, however, has not significantly changed. While there have been some innovations, this process still follows the basic step of first and second strand cDNA synthesis (for RNA), end repair, adaptor ligation and PCR amplification. The preparation of libraries can be:

- Time consuming
- Error prone
- Costly

Laboratory automation is one solution to reduce the burden of manual NGS library preparation. Laboratory automation, however, has typically been geared to high-throughput labs and requires considerable expertise to utilize efficiently. Consequently, automation has not been accessible or affordable for lower or medium throughput labs. The MagicPrep NGS system is Tecan's solution to address these issues and provide a simple solution for low-throughput automated library preparation for Illumina sequencing platforms (**Figure 1**).

The MagicPrep NGS system

The MagicPrep NGS system combines hardware, software and scripts, reagents, and consumables to provide a unique, fully-walkaway solution for simple automated NGS library preparation that just works. Specifically designed for library preparation



Figure 1: The MagicPrep NGS system. The MagicPrep NGS system is a fully integrated system without the need for external computers. The MagicPrep NGS instrument comes preloaded with all scripts, allowing a user to start a run right out of the box. The MagicPrep NGS (58 cm x 31 cm x 25 cm (DxHxW)) is designed to minimize use of the laboratory bench while maximizing the lab space.

of 8 samples at a time, MagicPrep NGS utilizes novel dispensing technology along with standard technology for robust library construction.

In addition to the instrument and scripts, the cartridge-based reagents and consumables simplify the run setup and reduce the possibility of errors. The MagicPrep NGS kit consists of beads, a reagent cartridge with pre-aliquoted reagents and sample deck consumables containing the buffers and tips used for library construction (**Figure 2A**). The MagicPrep NGS kit provides a complete solution, enabling a highly



Figure 2: Components of a MagicPrep NGS kit. The kits supplied for the MagicPrep NGS system provide everything necessary for a run. **A)** Each MagicPrep NGS kit comes with magnetic beads (not shown), reagent cartridges and sample deck consumables. These consumables provide all the reagents, adaptors, buffers, bead, and tips needed for library prep. **B)** Sample deck consumables are inserted into the sample deck frame to assemble the final sample deck. After adding the samples and beads, the assembled sample deck is ready to be inserted into the MagicPrep NGS system to start a run.



simplified workflow that is not possible with other systems.

Rather than a complicated liquid handler, the MagicPrep NGS system is a laboratory device that allows anyone to start a run in 10 minutes and with about 30 minutes of training. Briefly, the MagicPrep NGS run setup consists of 3 steps:

Step 1. Combine your samples together with the provided beads.

Step 2. Set up the sample deck by transferring the consumables to the sample deck frame (**Figure 2B**). All of the sample deck consumables are keyed to minimize errors during the assembly process. Add the sample and bead mix to the sample deck.

Step 3. Insert the reagent cartridge and sample deck into the MagicPrep NGS system and follow the on-screen instructions to start the run.

Once the run has been started, there are no additional user interaction steps allowing extended walkaway time. The MagicPrep NGS system allows anyone to generate high quality libraries with minimal training and regardless of laboratory experience. The MagicPrep NGS system is available with workflows for DNA-Seq starting with either intact DNA or pre-fragmented DNA and mRNA-Seq starting with total RNA.

Reliability and reproducibility

In addition to simplicity, reliability of library prep is a major requirement for automated library prep systems. Previous low-throughput automation solutions used challenging technologies such as electrowetting and suffered from poor reliability. The MagicPrep NGS system uses a combination of a novel reagent dispensing technology, proven automation technology and robust Tecan reagents to provide a reliable solution for library preparation.

The MagicPrep NGS system demonstrates a mean success rate of >99%* for generating libraries from control samples. A successful library is characterized as having sufficient yield for sequencing and the expected library distribution when starting with a defined input amount and number of PCR cycles. The mean success rate is defined as the number of successful libraries generated across all available workflows. Analysis of 100 DNA-Seq and mRNA-Seq MagicPrep NGS runs (800 samples) using 10 different instruments, a mean success rate of over 99%* (**Figure 3**) was demonstrated. The runs were performed with a range of input amounts and different

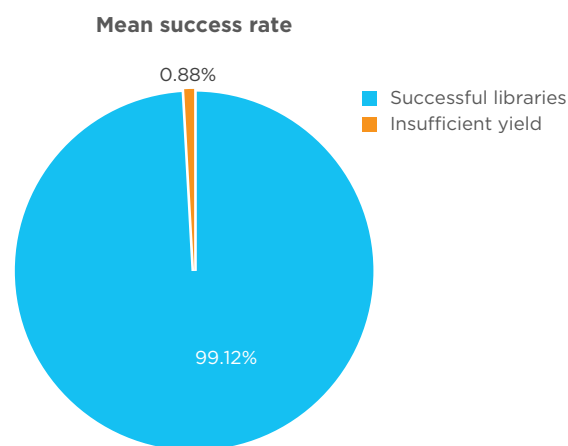


Figure 3: Mean success rate for library preparation. A total of 100 runs, using a mix of both DNA-Seq or mRNA-Seq kits, were performed on 10 different MagicPrep NGS instruments. Over 99% of the samples generated high quality libraries demonstrating the reliability of the MagicPrep NGS system.

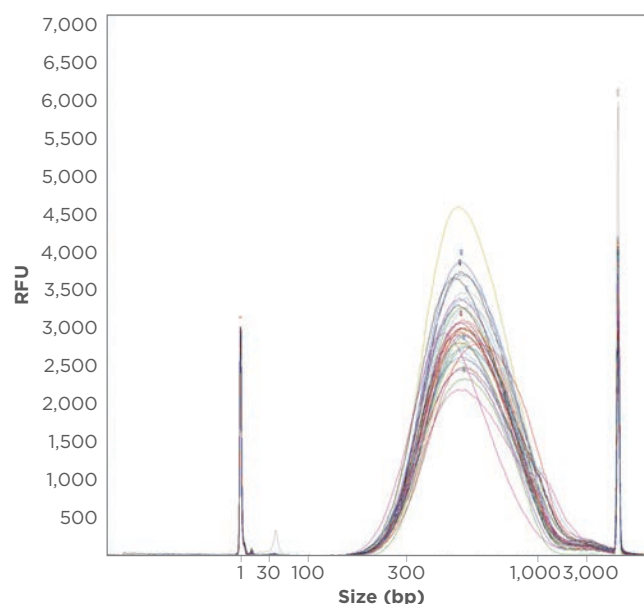


Figure 4: Consistent library size distribution. Fragment analyzer traces are shown from 6 runs (48 samples) using 100 ng of HapMap DNA or the Zymo bacterial control using the Revelo DNA-Seq Enz for MagicPrep NGS kit. All the samples produced libraries with similar library distributions.

control sample types. Analysis of library distributions (**Figure 4**) shows consistent results indicating the construction of a successful library.

The MagicPrep NGS system processes 8 samples during each run. For larger projects, multiple runs on the same instrument or across multiple MagicPrep NGS instruments may be required. Analysis of libraries across runs on the same instrument or multiple instruments show consistent library construction (**Figure 5**).

*Success rate is based on mean internal data. Sample quality and variability may have an impact on the success rate. For more information, visit www.tecan.com/magicprep-ngs/reliability.



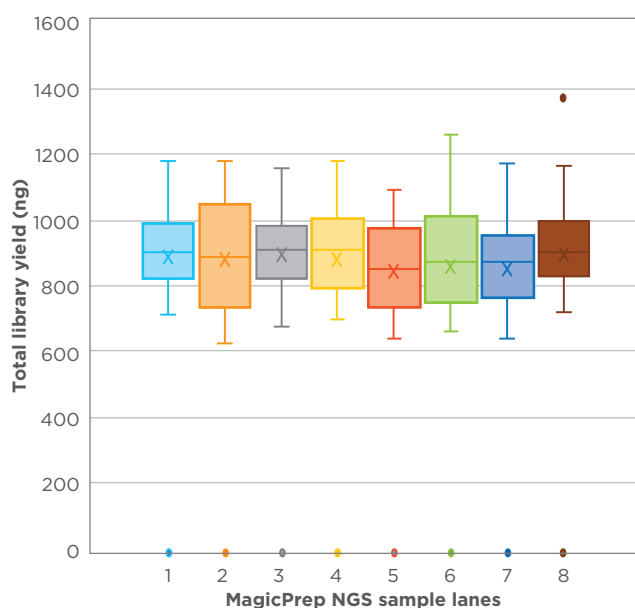


Figure 5: Consistency between runs. The plot represents the average library yield for each sample lane across 34 runs (272 samples) performed on 10 different instruments. The runs were performed with the DNA-seq kits starting with either 50 or 100 ng of HapMap control DNA or bacterial genomic DNA. The data shows that the MagicPrep NGS system provides consistent results for run performed on the same instrument and across multiple instruments.

Data quality

Tecan library preparation reagents for Illumina sequencing platforms have proven performance that has been validated by thousands of peer-reviewed publications. Additionally, Tecan reagents contain proprietary technology that allow compatibility across a wide range of sample types and inputs. For example, Tecan's DimerFree technology virtually eliminates the formation of adaptor dimers, reducing the need for additional bead purification steps to remove these contaminants while the enzymatic DNA fragmentation provides consistent DNA fragmentation with minimal bias regardless of input or %GC content. These proprietary technologies and reliable reagents enable the simplicity and reliability of the MagicPrep NGS system. To demonstrate the data quality, libraries were constructed on the MagicPrep NGS system and sequenced.

The Revelo mRNA-Seq for MagicPrep NGS kit includes all the reagents and consumables needed to convert total RNA to mRNA-Seq libraries. Starting with inputs of 10 – 1000 ng of total RNA, the MagicPrep NGS system, performs all the necessary steps for library prep, including the poly(A) selection and RNA fragmentation (**Figure 6**).

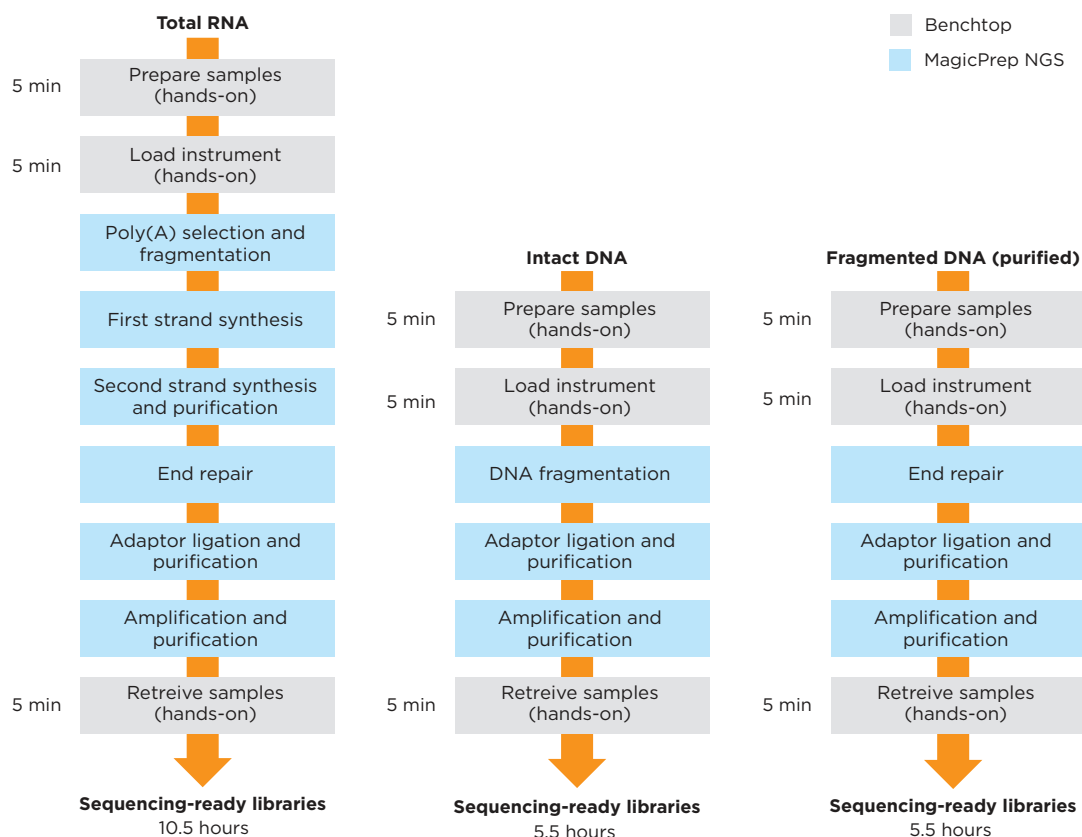


Figure 6: NGS library prep workflow on the MagicPrep NGS system. Steps performed on the MagicPrep NGS instrument are colored light blue.



Libraries were generated using 10 ng, 100 ng and 1000 ng of K562 total RNA and sequenced on an Illumina miniSeq. Analysis of the sequencing results showed that the data is highly consistent despite a 100-fold difference in input (**Figure 7**).

The MagicPrep NGS system was also tested with Universal Human Reference (UHR) total RNA and compared to manual library construction using a competitor kit. The libraries from MagicPrep NGS showed similar general sequencing metrics to the manual libraries (**Figure 8**). Overall, these results suggest that the MagicPrep NGS system produces high quality libraries, across a broad input range and multiple RNA samples types with data, similar to manually constructed libraries.

In addition to mRNA-Seq, the MagicPrep NGS system also provides a solution for DNA-Seq applications. The Revelo DNA-Seq Enz for MagicPrep NGS and the Revelo DNA-Seq Mech for MagicPrep NGS kits provide solutions for DNA-Seq starting with either intact or pre-fragmented DNA, respectively (**Figure 6**). These workflows were tested using both human and bacterial genomic DNA to demonstrate the flexibility of these workflows for a range of sample types.

Testing both workflows with a bacterial control (**Figure 9**) showed that libraries constructed on the MagicPrep NGS system provide high quality data with

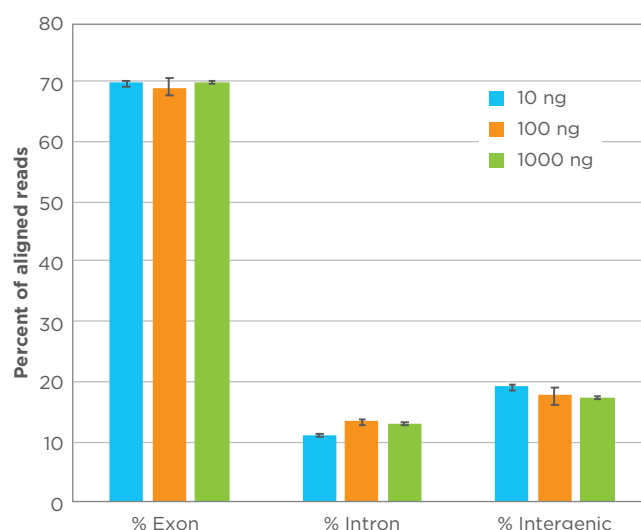


Figure 7: Consistent data across a broad input range. mRNA-Seq libraries were constructed on the MagicPrep NGS system starting with 10, 100 or 1000 ng of K562 total RNA and sequenced on an Illumina miniSeq. All three total RNA input amounts showed a similar percent of exon, intron and intergenic reads in the sequencing data demonstrating that the MagicPrep NGS system provides similar data across a broad input range.

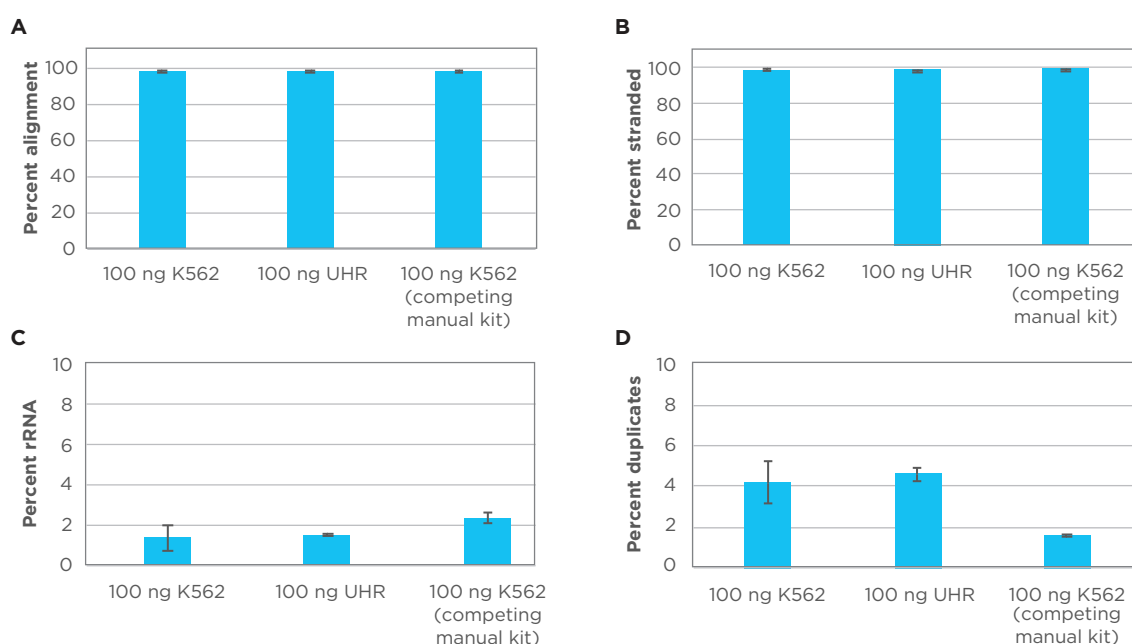


Figure 8: MagicPrep NGS provides similar data to manual workflows. mRNA-Seq libraries were constructed on the MagicPrep NGS system and with a competitor's manual kit using 100 ng of K562 or UHR total RNA. **A)** Analysis of the sequencing data showed that MagicPrep NGS and manually prepared libraries with a competitor kit had similar alignment rates. **B)** The library analysis also shows that all libraries have a similar strandedness. **C)** Both the MagicPrep NGS and the competitor's manual kit perform a poly(A) selection to remove unwanted rRNA reads. Analysis of the sequencing results showed that both workflows efficiently removed rRNA transcripts, although the MagicPrep NGS libraries had slightly lower rRNA reads. **D)** Both the MagicPrep NGS and manual libraries prepared with a competitor kit showed less than 5% duplicate reads. The MagicPrep NGS libraries had a slightly higher percent of duplicate reads compared to the manual workflow. Overall, the sequencing data and library quality are similar between libraries generated with the MagicPrep NGS system and a competitor manual kit.



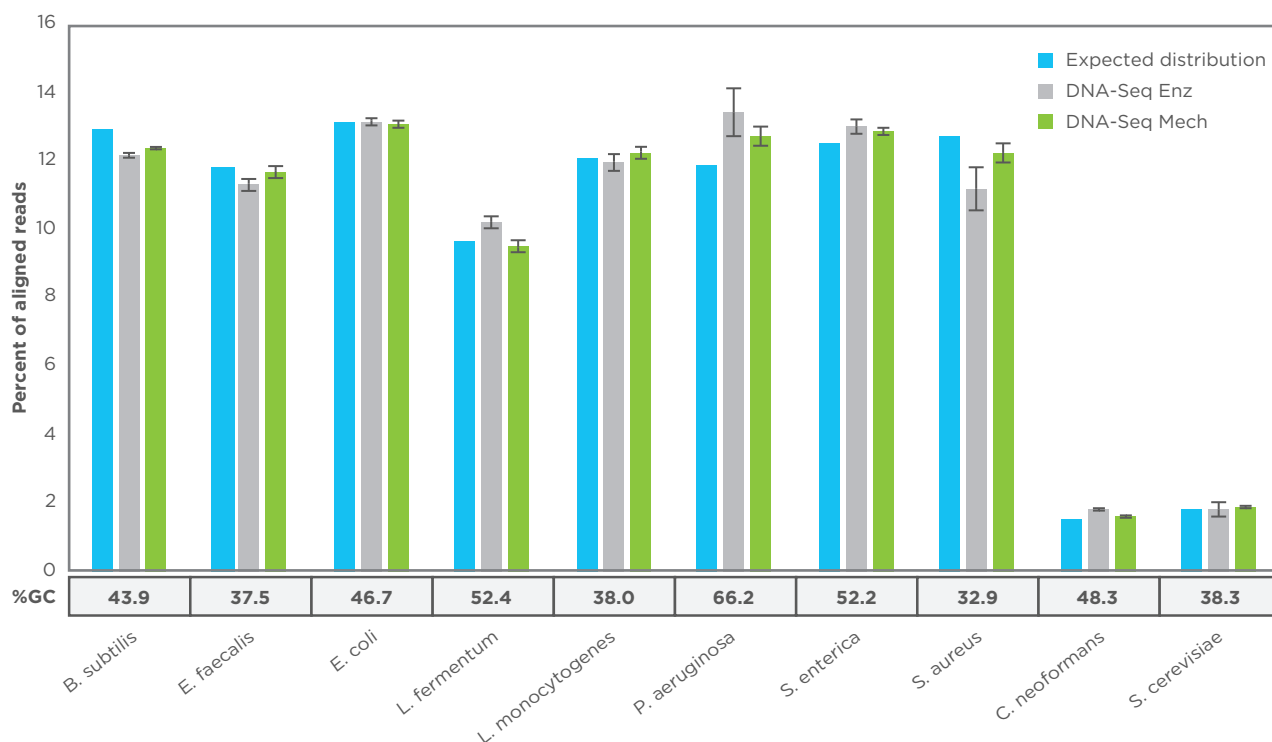


Figure 9: MagicPrep NGS libraries have minimal GC bias. Libraries were prepared using the MagicPrep NGS system starting with 100 ng of the Zymo bacterial control with either enzymatically or Covaris fragmented DNA. The sequencing data showed that the genome distribution in the sequencing data, is very similar to the expected results with minimum variation. This indicates that the MagicPrep NGS minimizes bias during library preparation across a broad range of GC content.

minimal bias due to GC content. The minimal bias seen in the Revelo DNA-Seq Enz for MagicPrep NGS kits also indicates that the enzymatic DNA fragmentation step is consistent across genomes with different GC content.

(NA12878). The resulting data was compared to manually constructed libraries using a competitor kit (**Figure 10**). The sequencing data was comparable between the MagicPrep NGS libraries and the manual libraries (data not shown).

The Revelo DNA-Seq Enz for MagicPrep NGS kit was also tested with different inputs of a HapMap DNA

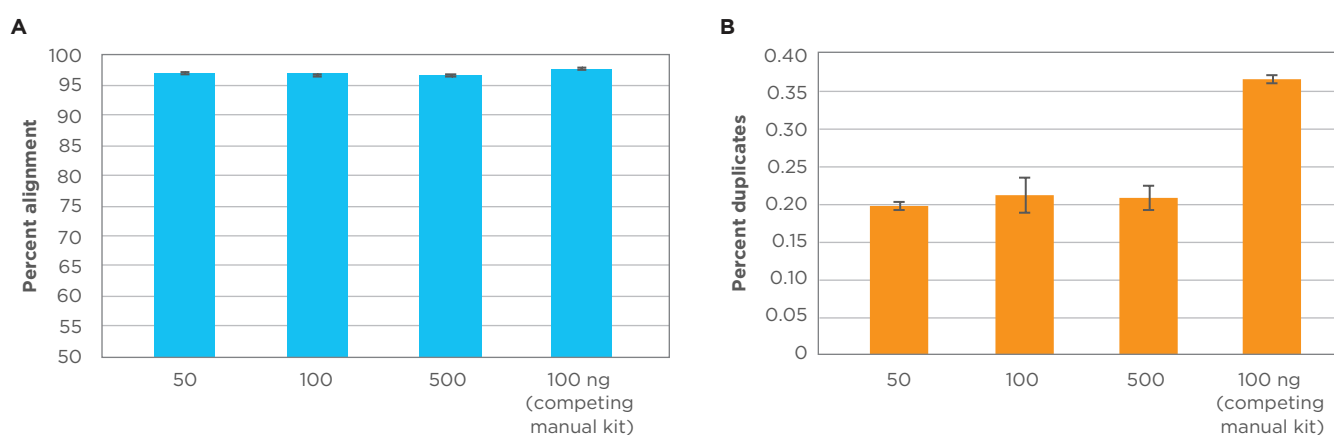


Figure 10: MagicPrep NGS produces consistent results across a broad input range. DNA-Seq libraries were constructed, starting with varying inputs of intact HapMap control DNA, using either the MagicPrep NGS system or a competitor's manual kit. **A)** Analysis of the sequencing data showed that both the MagicPrep NGS and libraries manually prepared with the competitor kit produced high quality sequencing data with a high alignment rate. For the MagicPrep NGS libraries, this was consistent across the input range tested. **B)** The percent of duplicate reads is consistently less than 0.5% across all inputs of the MagicPrep NGS constructed libraries. Libraries generated with the competitor kit showed a higher percent of duplicate reads in the sequencing data. The libraries generated with the MagicPrep NGS system provide similar, high quality data compared to a competitor's manual kit.



SUMMARY

Tecan's MagicPrep NGS system is a novel solution, combining automation, software, scripts, reagents, and consumables into a seamless workflow to generate libraries for Illumina sequencing platforms. Utilizing cartridge-based reagents and pre-validated scripts, new users can learn to use the MagicPrep NGS system in 30 minutes and setup a run in 10 minutes. With the MagicPrep NGS system, all you need to supply are the samples so you can start a run in the morning, after lunch or just before going home. The MagicPrep NGS system is a new breakthrough in fully-walkaway, library preparation automation that is designed for everyday use by anyone. No installation, no scripting, no master mixes...Just Magic!

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