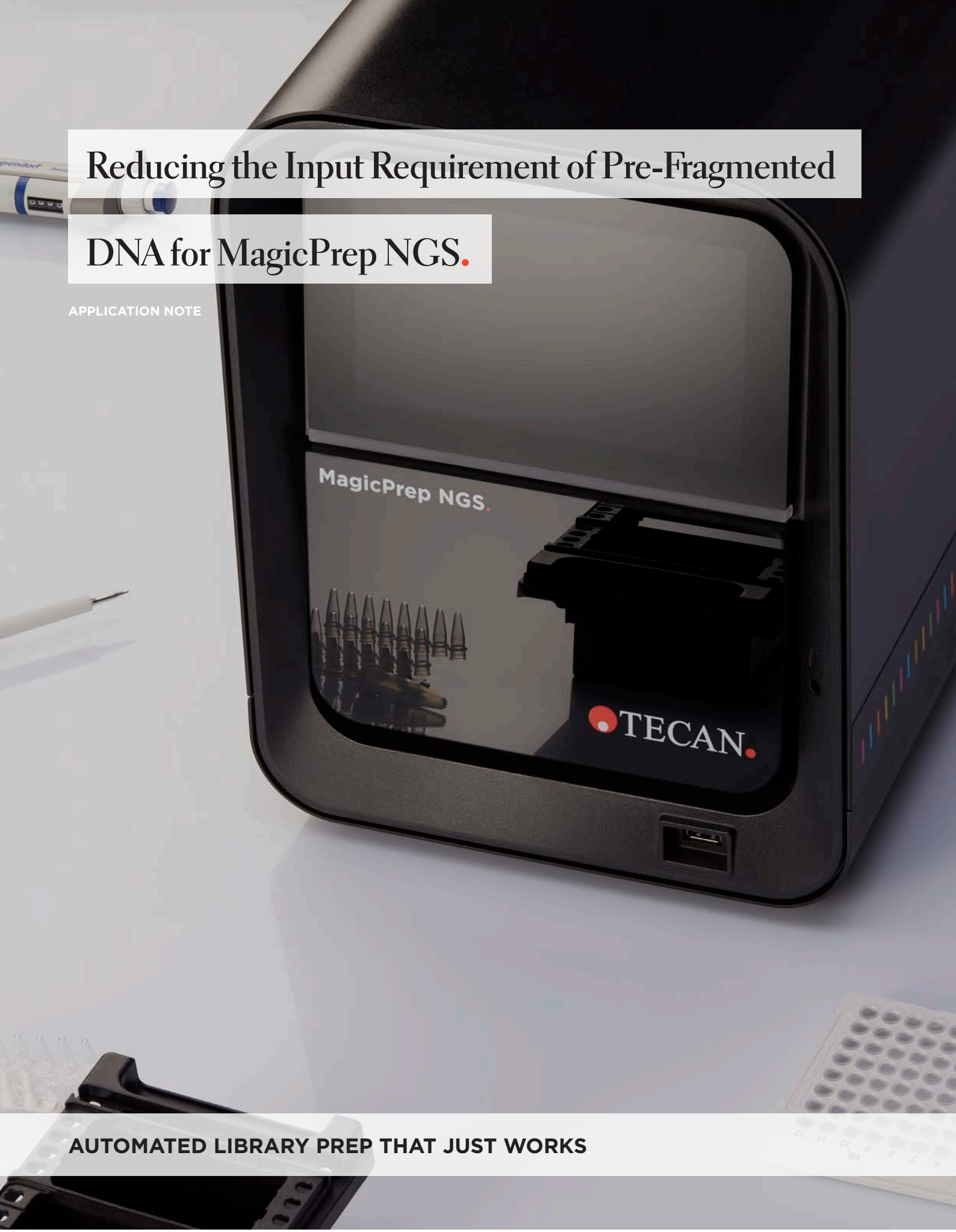
Reducing the Input Requirement of Pre-Fragmented DNA for MagicPrep NGS.

APPLICATION NOTE



AUTOMATED LIBRARY PREP THAT JUST WORKS

TECAN.

INTRODUCTION

Fully automated NGS library preparation
Tecan's MagicPrep™ NGS system is a unique solution for fully automated preparation of NGS libraries. The system combines hardware, software, reagents, and consumables to provide a complete solution that just works. After an initial 10-minute setup, the user can walk away and come back to finished NGS libraries (**Figure 1**). The instrument simultaneously produces 8 libraries, providing an automated solution for sequencing library preparation for mid- to low-throughput laboratories.

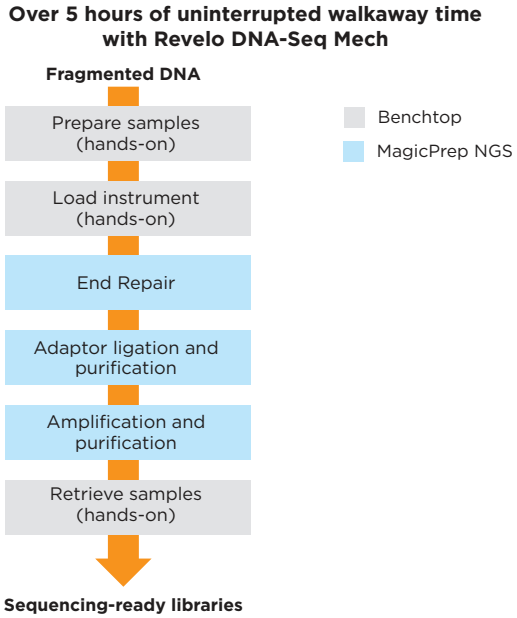


Figure 1: DNA-Seq Mech library preparation workflow on the MagicPrep NGS System. MagicPrep NGS performs the library preparation steps (in blue boxes) of end repair, ligation, and PCR amplification with optimized SPRI bead clean-ups. A DNA-Seq run can be started in about 10 minutes with no user interaction. Finished libraries can be stored in the instrument for up to 65 hours, allowing retrieval of libraries when you are ready.

CASE STUDY: USING LOW INPUT AMOUNTS ON MAGICPREP NGS

High-quality libraries from 1 ng of fragmented DNA
The specified minimum input for the Revelo DNA-Seq Mech workflow on the MagicPrep NGS system is 50 ng. There are, however, cases where the available material is limiting, such as cell-free DNA from plasma, or DNA from FFPE blocks. In this study, we tested changing the DNA-Seq Mech workflow to allow library preparation from lower inputs. We performed our tests with a mix of three bacteria corresponding to low, medium, and high GC content samples (*S. aureus* - 32%, *E. coli* - 50% GC, and *R. sphaeroides* - 68%). The three bacterial DNA mix was Covaris fragmented to a target of 300 bp using settings recommended by the manufacturer.

A total of 50 ng, 10 ng, or 1 ng of Covaris-sheared DNA was used as input. The recommended number of PCR cycles for 50 ng input is 8, and the number of PCR cycles was increased to 11 and 14 cycles for the 10 ng and 1 ng inputs, respectively. Tecan's DimerFree® technology obviates the need to titrate the adaptor concentration. All runs produced clean libraries with minimal primer-dimer or primer contamination regardless of input amount (**Figure 2**).

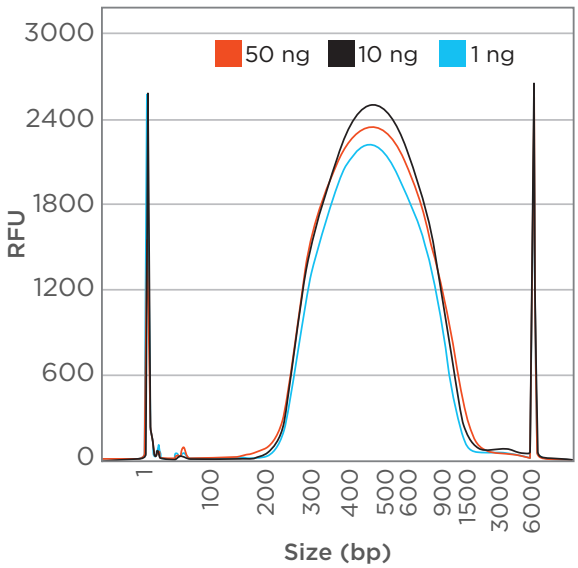


Figure 2: Example Fragment Analyzer traces of libraries generated with 50 ng, 10 ng, or 1 ng of Covaris-sheared bacterial DNA mix. Libraries show a single uniform peak centered around 450 bp with minimal primer-dimer.

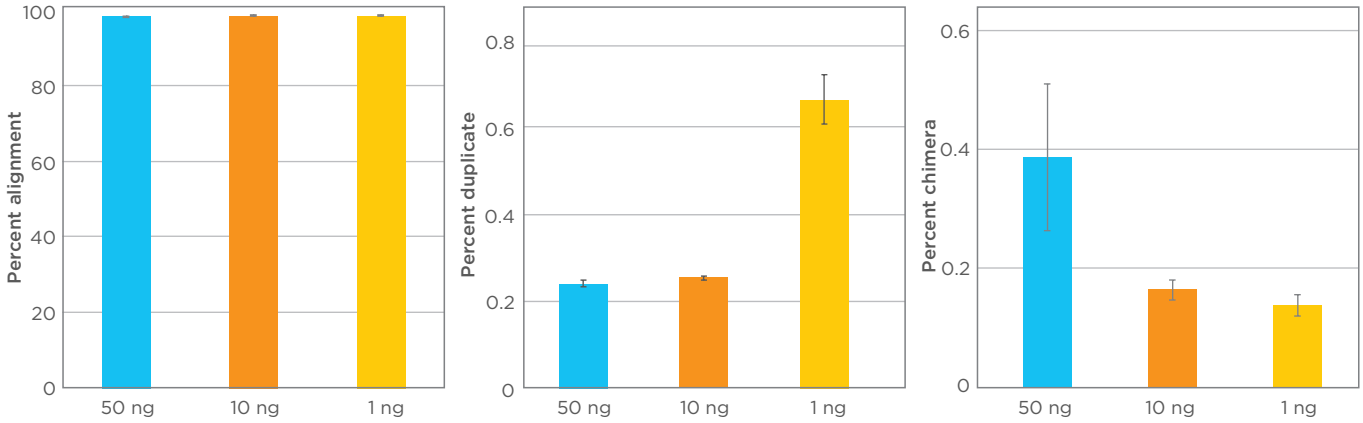


Figure 3: Alignment, duplication, and chimera rates for DNA-Seq Mech libraries from 50 ng, 10 ng, and 1 ng input. At all 3 input levels, alignment rates were above 98%, and duplication and chimera rates were below 1%, confirming that high quality libraries were generated. Percent alignment: Proportion of reads aligned to reference genomes. Percent duplicate: Proportion of reads that are marked as duplicate. Percent chimera: Proportion of read pairs that map >2 kb apart.

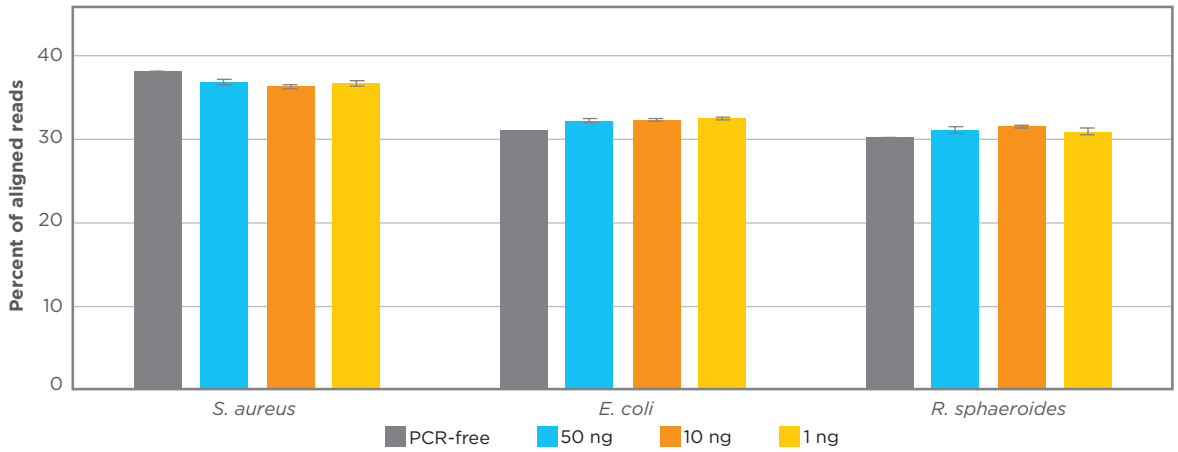


Figure 4: Read distribution for low input Revelo DNA-Seq Mech libraries are approximately equivalent to their PCR-free library. The additional PCR cycles used to amplify the low input samples did not skew their read distribution across the range of GC inputs tested.

Sequencing analysis of the libraries showed >98% alignment against all three bacterial genomes, with duplication and chimera rates below 1% (**Figure 3**). The low duplication rates confirm that adaptor ligation was efficient even with low DNA inputs, and that the increased number of PCR cycles amplified a diverse population of inserts, generating high-quality libraries.

The bacterial DNA mix was made into a PCR-free library and sequenced for reference. The read distribution of the three bacteria in the Revelo DNA-Seq Mech libraries at the different inputs was consistent with the distribution of the PCR-free library (**Figure 4**). Increasing the number of PCR cycles to account for the lower input introduced minimal bias to the sample's read distributions.

We also compared the coverage of the genomes (**Table 1**). 2.4 million read pairs were used for each library in this analysis. At all input levels, reads were evenly distributed across the reference genome of each bacteria with greater than 99% uniformity. This underscores the minimal bias in our DNA-Seq libraries even when the input amount is low.

We further examined the Revelo DNA-Seq Mech kit workflow using the ZymoBIOMICS™ Microbial Community DNA Standard (Zymo Research #D6306) sample, a known mixture of 10 microbes with genomes of varying GC content. This sample was measured independently, and is used to assess biases and errors in library preparation such as GC-bias from end repair or ligation. The distribution of reads was similar to the expected read distribution given by the manufacturer.

Bacteria	DNA Input	Average Coverage	Average Uniformity (%)
S. aureus	50 ng	41.92	100.00
	10 ng	41.94	100.00
	1 ng	41.97	100.00
E. coli	50 ng	20.32	99.50
	10 ng	20.34	99.52
	1 ng	20.29	99.52
R. sphaeroides	50 ng	21.85	99.83
	10 ng	21.92	99.91
	1 ng	21.93	99.72

Table 1: Revelo DNA-Seq Mech libraries have very similar coverage and uniformity across input levels. The lower input libraries showed minimal bias. The average coverage of *S. aureus* is higher due to its smaller genome size.

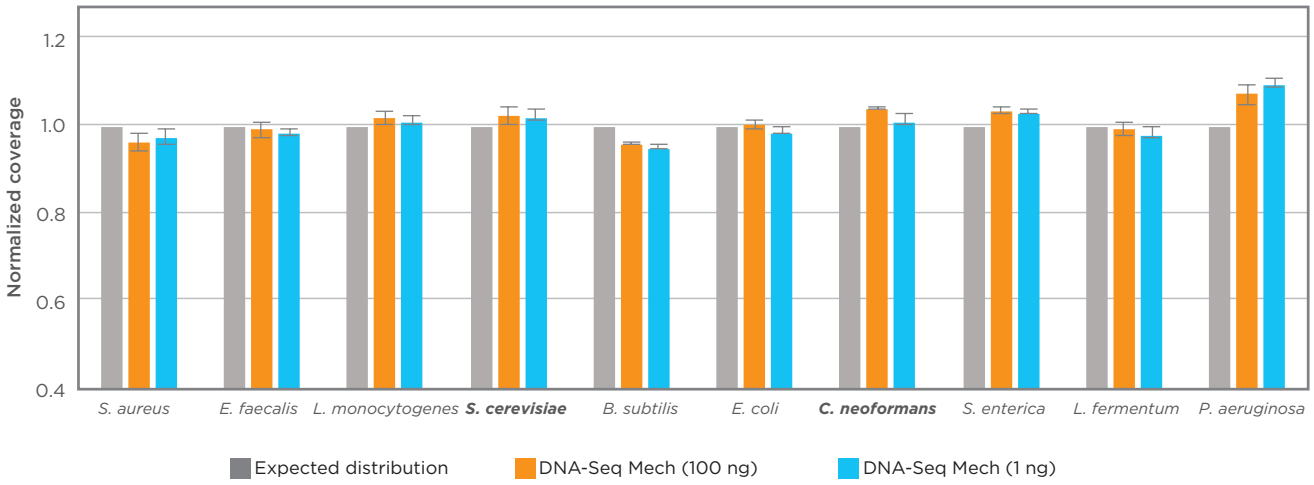


Figure 5: Coverage of the 10 microbial genome sample was normalized to their expected read distribution. The species in ***bold*** are expected to make up ~2% of the sample, while the others are expected at ~12%. The microbial genomes are arranged in ascending average GC content (*S. aureus* – 32.9%; *E. faecalis* – 37.5%; *L. monocytogenes* – 38.0%; *S. cerevisiae* – 38.3%; *B. subtilis* – 43.9%; *E. coli* – 46.7%; *C. neoformans* – 48.3%; *S. enterica* – 52.2%; *L. fermentum* – 52.4%; *P. aeruginosa* – 66.2%). Across the range of GC-content, the observed read distribution was within 5-10% of their expected distribution.

We divided our read distribution by the expected read distribution to quantify the deviation. Consistent with our three bacteria study, 9 out of 10 microbial genomes were represented within 5% of their expected distribution in the sequencing data (**Figure 5**), even for *S. cerevisiae* and *C. neoformans* that were expected at 2% of the community composition. The exception, *P. aeruginosa*, was within 10 % of its expected representation.

Low duplication rates in libraries constructed with 1 ng of fragmented human DNA

We also characterized 1 ng human DNA input on the Revelo DNA-Seq Mech for MagicPrep NGS workflow.

We used 50 ng and 1 ng of NA12878, human HapMap genomic DNA as input for the Revelo DNA-Seq Mech workflow. Both libraries had similar alignment and chimera rates (**Figure 6**). The duplicate rates remained below 1%, although higher for the 1 ng input than the 50 ng input library. This is expected due to the significantly lower input.

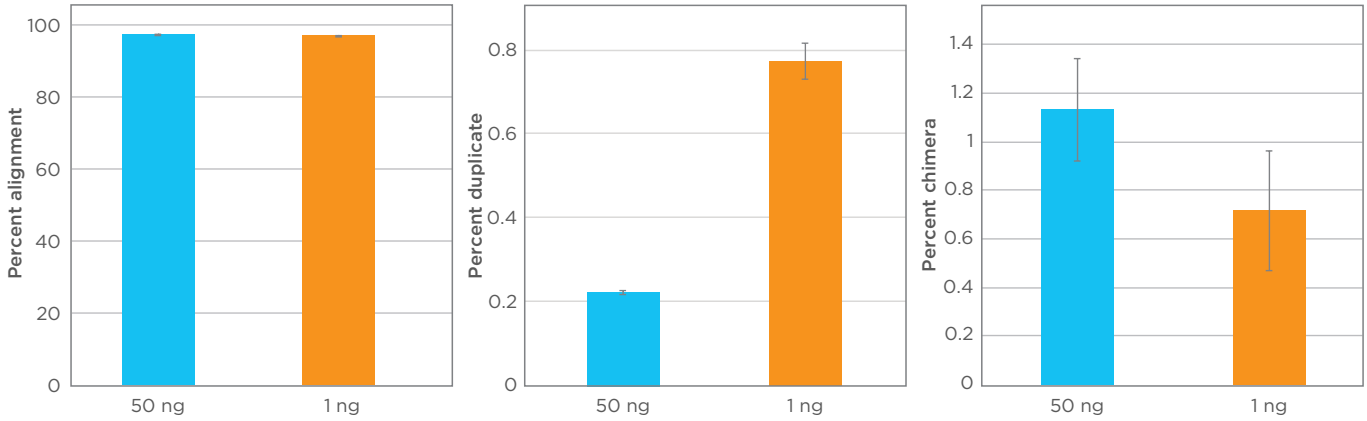


Figure 6: Alignment, duplication, and chimera rate for DNA-Seq Mech libraries made with 50 ng and 1 ng NA12878 input. Even at 1 ng input, alignment rate remains above 97%, duplication rate remains below 1%, and chimera rate was below 2% for the human samples compared to the library generated with 50 ng input.

SUMMARY

Here we demonstrate excellent performance from the MagicPrep NGS system using DNA input amounts well below the specifications of the Revelo DNA-Seq Mech kit. By simply increasing the number of PCR cycles via the touchscreen during run setup, users can obtain successful results from as little as 1 ng of pre-fragmented, high-quality DNA inputs. Results were consistent across genome inputs representing a range of GC content and complexity. The resulting libraries had high alignment rates (>97 %) with low duplication (< 1%) and chimera (< 2%) rates. With minimal effort and training, anyone can use the MagicPrep NGS system to reliably produce high-quality libraries from challenging low abundance and fragmented DNA samples.

Products Mentioned in this Application Note

Product Name	Part no.	No. of reactions
Revelo DNA-Seq Mech for MagicPrep NGS Kit A	30186627	32
Revelo DNA-Seq Mech for MagicPrep NGS Kit B	30186628	32
Revelo DNA-Seq Mech for MagicPrep NGS Kit C	30186629	32
MagicPrep NGS system	Contact your local sales representative.	

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