

NGS DreamPrep™ – automated DNA

library preparation and quantification on

the Fluent® Automation Workstation.

Application Note



A TURNKEY WORKFLOW FOR DNA LIBRARY PREPARATION, QUANTIFICATION,
NORMALIZATION AND POOLING IN LESS THAN FOUR HOURS



INTRODUCTION

Celero DNA-Seq kits with NuQuant® provide an innovative and streamlined DNA library preparation workflow for generating quantified libraries that are ready for sequencing on Illumina instruments (Figure 1). The Celero PCR Workflow with Enzymatic Fragmentation DNA-Seq library preparation kit features a fast, easy-to-use, addition-only protocol, and allows researchers to determine library molarity without using time consuming methods, such as qPCR, that are subject to sample-to-sample variability. Celero DNA libraries can be prepared, quantified, normalized and pooled in less than four hours on the Fluent Automation Workstation, compared to more than seven hours for library preparation and qPCR quantification with other kits. Tecan's touchscreen interface – TouchTools™ – simplifies run set-up and makes deck preparation more intuitive, reducing the need for operator training.

The NGS DreamPrep is a turnkey solution designed and optimized for completely automated DNA library preparation and quantification. It combines Celero

DNA-Seq kits with a Fluent workstation and integrated Infinite® plate reader, allowing users to transform up to 96 DNA samples into readily normalized libraries. The instrument has an eight-channel Flexible Channel Arm™, a Multiple Channel Arm™ with a 96-channel head and Robotic Gripper Arm™, as well as integrated temperature-controlled devices, an ODTC® 96 thermal cycler (INHECO) and an Infinite plate reader, reducing manual steps and processing times.

This application note describes a fully automated protocol for generating quantified DNA libraries using the NGS DreamPrep and a Celero PCR Workflow with Enzymatic Fragmentation DNA-Seq library preparation kit. It enables highly reproducible DNA library preparation from a wide range of inputs, with minimal user intervention, using the integrated plate reader to generate quantified and normalized DNA libraries with no sample loss (Figure 2). Unlike other automated solutions, this setup eliminates the need of additional manual qPCR or capillary electrophoresis steps to quantify libraries, saving significant time, resources and valuable library material.

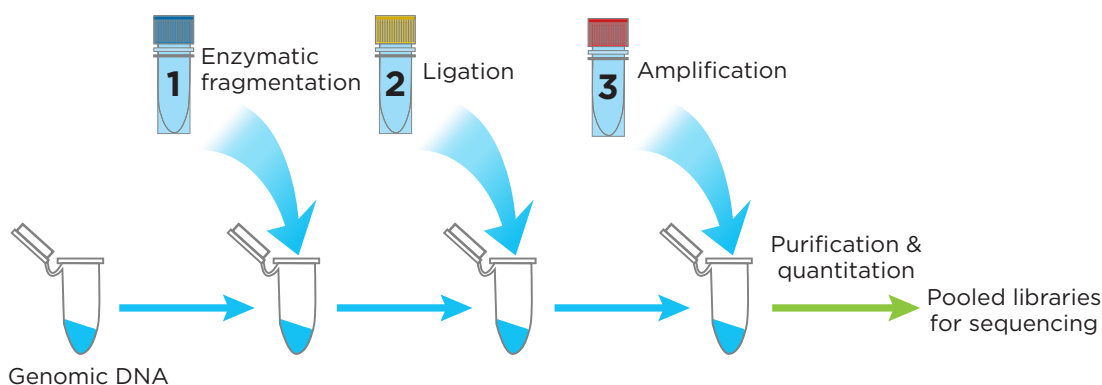


Figure 1: The Celero PCR Workflow with Enzymatic Fragmentation DNA-Seq Kit offers a simple, addition-only workflow for generating quantified libraries ready for sequencing. A separate kit is available for mechanical fragmentation.

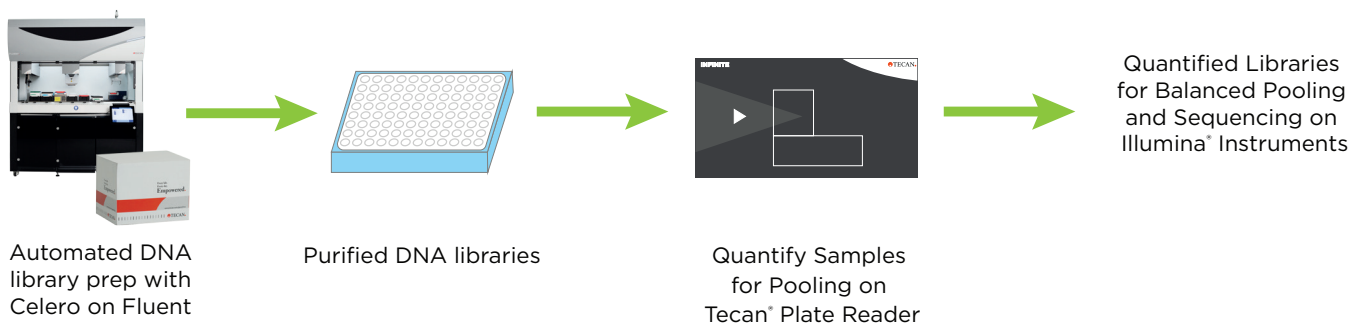


Figure 2: After final purification of DNA libraries in a 96-well plate with the Fluent, the fluorescence of the libraries and standards is measured on the Infinite plate reader. The molarity determined for each library can then be used for balanced pooling and subsequent sequencing on Illumina instruments.



MATERIALS AND METHODS

Materials

- 10 or 75 ng of human genomic DNA NA12878 (Coriell Institute)
- 10 ng of a 3-bacterial DNA blend containing *Staphylococcus aureus* (33% GC), *Escherichia coli* (51% GC), and *Rhodobacter sphaeroides* (69% GC) at equal mass.
- Celero PCR Workflow with Enzymatic Fragmentation DNA-Seq library preparation kit (9363A-A01)

Instruments

- Fluent automation workstation integrated with integrated Infinite plate reader and ODT 96 thermal cycler.

Experimental design and protocol

The Celero enzymatic fragmentation kit was used to prepare 12 DNA libraries from 10 ng replicate aliquots of a bacterial blend and 12 libraries from 10 ng human genomic DNA, arranged in a checkerboard pattern. Separately, 96 individual libraries were prepared from 75 ng of human genomic DNA in two separate runs, to demonstrate run-to-run consistency in library yield. All samples were sheared by enzymatic fragmentation for 20 min to yield 300 bp inserts. Libraries were amplified by PCR for nine cycles (for 10 ng input) or six cycles (for 75 ng input), with one post-amplification bead purification at 0.7x bead-to-sample ratio. Samples were arranged in alternating wells to assess possible edge effects. Finished libraries in their original plate were quantified using the NuQuant method integrated with the Infinite plate reader. The molarity of each library was determined by fluorescence measurement and compared to a standard curve. The fluorescence was measured directly in the PCR plate (excitation 627, emission 690), and comparing it to a standard curve. Libraries were then pooled based on the molarity and sequenced on the MiSeq (Illumina).

RESULTS AND ANALYSIS

Library size

Library QC

Electrophoretic analysis of finished individual libraries was conducted on the HS NGS Fragment Kit (Agilent Technologies). Figure 3 illustrates consistent size distribution from four representative samples of 10 ng 3-bacterial DNA blend, 10 ng NA12878, and 75 ng NA12878 genomic DNA. The average library size from 24 samples (12 bacterial DNA blend at 10 ng, 12 human genomic DNA at 10 ng) was 467 bp with a standard

deviation of 15.8, corresponding to insert size between 275 bp and 303 bp (Table 1). Automation of Celero kits on the Fluent achieved consistent size distribution, with no adaptor dimers, across sample types and inputs.

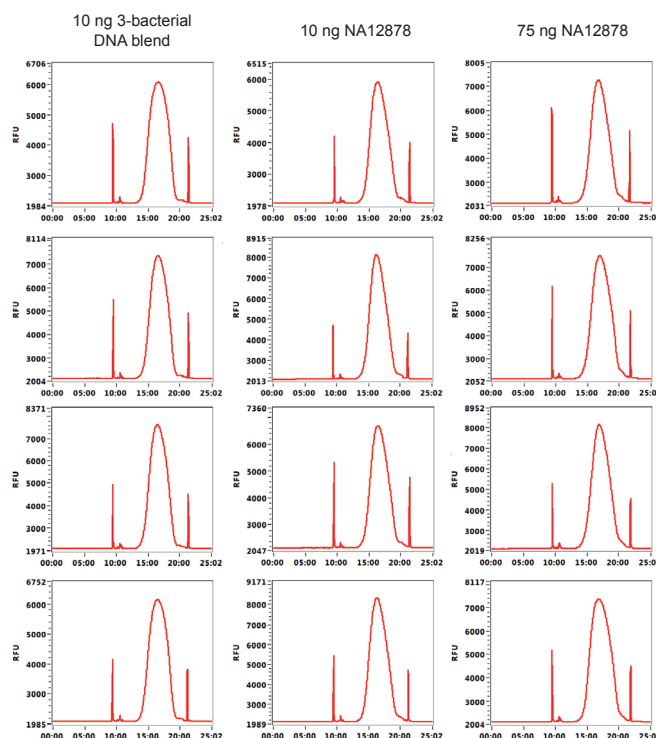


Figure 3: Library size distribution from bacterial (10 ng input) and human genomic DNA (10 and 75 ng input).

Sample	# of replicates	Avg insert size	Avg % aligned reads
3-bacterial DNA blend	12	294 bp (2.27 % CV)	98.8 (0.09 % CV)
Human Genomic DNA (NA12878)	12	280 bp (1.42 % CV)	96.5 (0.09 % CV)

Table 1: Sequencing results from 10 ng of bacterial blend and human genomic DNA.

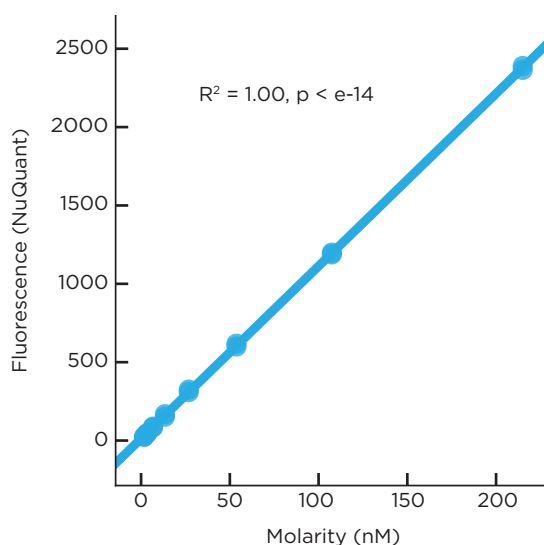


Figure 4: Standard curve generated using NuQuant standards. Library molarity used for pooling is determined by standard curve calculation.

The NuQuant library quantification was used to measure the molarity of the purified DNA libraries. Figure 4 shows the standard curve for the NuQuant standards. Figure 5 shows the library yield from 10 ng of both the bacterial and the human genomic DNA (Figure 5A, n=12) and from 75 ng starting input of human genomic DNA (Figure 5B, n=96), as well as library reproducibility.

Sequencing results

The sequencing results from 10 ng input of bacterial and human genomic DNA are shown in Table 1. The equimolar parsing of total reads from 10 ng and 75 ng input quantified by NuQuant had a %CV of 12.8 % and 11.0 % respectively. The automated library preparation provided the desired insert size of ~300 bp with high alignment rates for both bacterial and genomic DNA. The sequencing results were consistent with manual Celero library preparation (results not shown). Figure 6 shows the sequencing efficiency of automated DNA preparation for high and low GC organisms, showing no significant GC bias and a very uniform coverage.

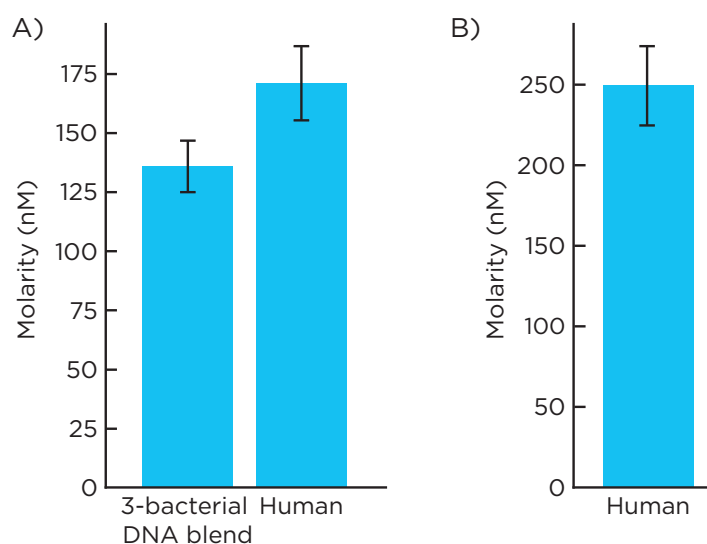


Figure 5: A) Library yield from 10 ng input of bacterial blend and human genomic DNA with nine PCR cycles. B) Library yield from 75 ng input of human genomic DNA with six PCR cycles. Final library volume for both inputs was 20 μ l.

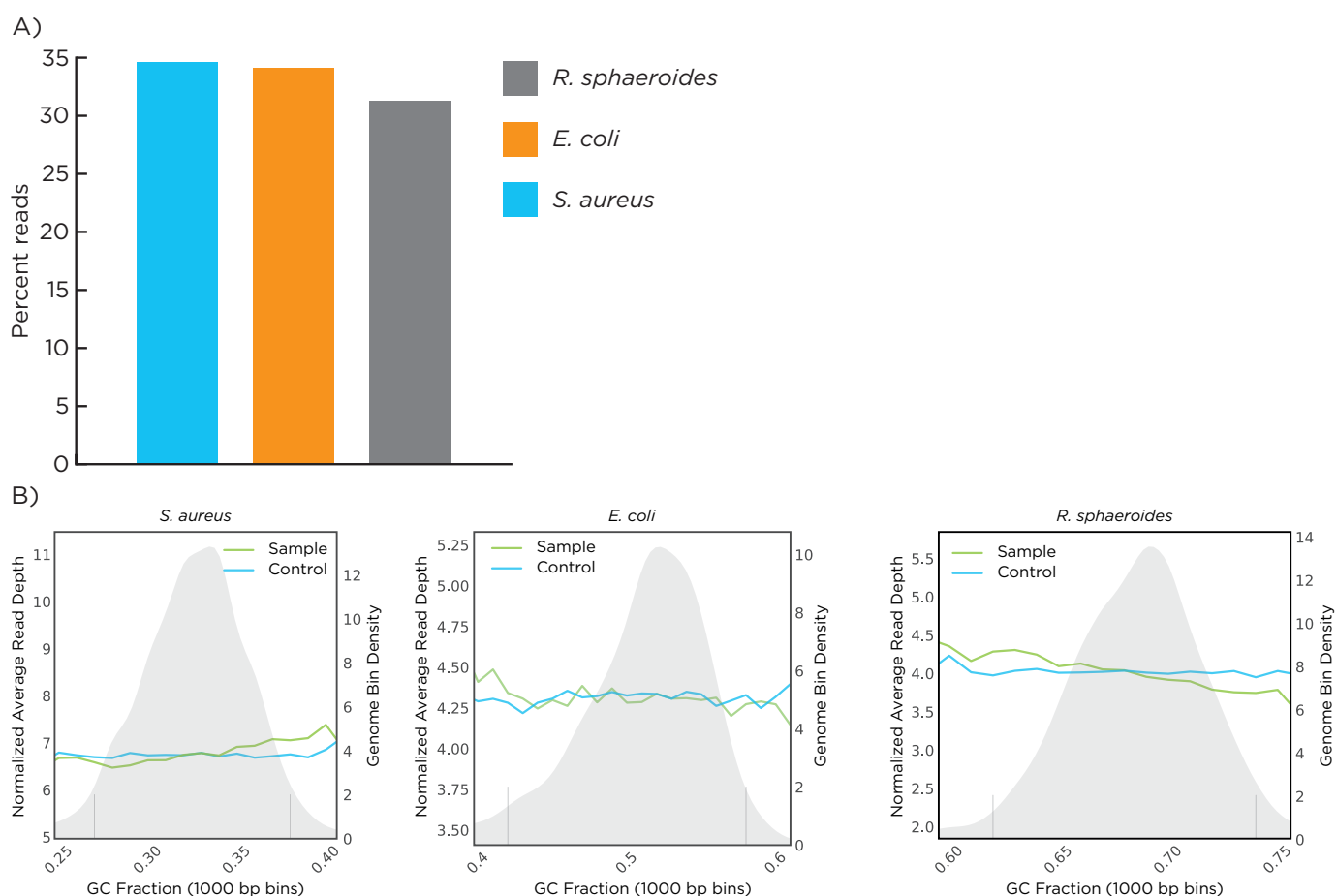


Figure 6: A) Sequencing efficiency of DNA libraries generated by automated prep from high and low GC organisms.

B) Uniformity of coverage of a representative library from bacterial DNA blend with varying GC content and comparison to control.



SUMMARY

The results presented in this application note highlight the benefits of automating DNA library preparation, quantification, normalization and pooling using Tecan's NGS reagent and automation solutions. The protocol can be completed in less than four hours, generating quantified DNA libraries for balanced pooling and sequencing with no manual intervention.

The sequencing results show the robustness of pooling using NuQuant with CVs of less than 13 % for 24 samples at 10 ng input, and 96 samples at 75 ng input. The results also show a high alignment rate for bacterial and human genomic DNA. The sequencing of blended bacterial DNA from three organisms with varying GC content showed uniform coverage.

About the authors



Mike Benway has been automating laboratory workflows for over two decades, and NGS Sample preparation exclusively since 2010. During that time he has served in dozens of sequencing centers and NGS research labs throughout the US and Europe. He has taken his degrees in Molecular Biology and Computer Science from The University of Massachusetts at Boston. He has come to Tecan Genomics from TTP Labtech, and is now helping to automate the entire Tecan NGS sample prep reagent portfolio.



Joe Don Heath has been a scientific leader and customer advocate at Tecan Genomics since 2003, in which time he has had several responsibilities, including leadership roles in technical service, training, collaborations, and marketing. He presently is responsible for technical support, bioinformatics, and automation. JD earned his PhD in biochemistry and molecular biology from the University of Texas Health Science Center in Houston and completed a postdoctoral fellowship in plant-microbe interactions at the University of Washington in Seattle.

The NGS DreamPrep method needs to be validated in your lab based on your workflow and according to your desired use and laboratory protocols. Tecan makes no claims regarding the performance of the example methods. For Research Use Only. Not for use in diagnostic procedures.

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