TECHNICAL NOTE

NGS Automation

Improving the Efficiency of Metagenomic Analysis of Stool Samples

illumina®

A Valued PerkinElmer Collaborator

INTRODUCTION

Understanding the abundance, distribution, collective gene expression, and function of microbiota provides intricate insights into an individual at a specific time. Gut bacteria play an important role in human health as changes in the microbiome can be potentially harmful, often linking to chronic conditions such as inflammatory bowel disease, obesity, cancer, and autism. NGS technologies deliver the detail necessary to characterize microbial communities, providing information about the genetic profile, population structure, and role of microorganisms within a given sample.

Here PerkinElmer and Illumina® describe a streamlined, automated workflow from DNA extraction to sequence-ready libraries, which results in high quality data for species identification, metagenomic profiling, and de novo genome assembly.

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For the Better

WORKFLOW OVERVIEW

Automation of an NGS-based metagenomics workflow offers significant advantages over manual sample preparation. Increased throughput and scalability, reduction in human touch-points and human error, enhanced consistency and reproducibility, and increased speed all contribute to reliable data production that is amenable to varying throughputs for metagenomics labs.

Figure 1. The automated Illumina® Nextera® DNA Flex library preparation workflow for metagenomics—Illumina® and PerkinElmer collaborated to create a fully automated NGS library preparation workflow for high-throughput metagenomics. The times indicated above are for the processing of 96 samples.

Optimized DNA Isolation from Stool Samples

Correctly representing bacterial complexity and genetic diversity is a challenging problem, complicated further because it is difficult to isolate DNA from stool samples. The matrix contains inhibitors which can interfere with downstream applications, leading to incorrect microbial profiles and misrepresentation of the microbiota. Efficient washing procedures are required in the DNA extraction process to eliminate these inhibitors. The DNA in stool samples is also frequently heavily fragmented, resulting in DNA that is not suitable for many downstream applications. Therefore, for improved NGS results and identification of rare microbial species, it is critical that the extraction protocol does not further fragment the DNA.

PerkinElmer chemagen™ technology addresses the challenges associated with the isolation of DNA from stool samples while offering high recovery of clean DNA suitable for downstream applications. Shaking and centrifugation steps that typically result in nucleic acid denaturation and fragmentation are avoided with chemagen™ technology. This automated magnetic separation procedure uses chemagen™ M-PVA magnetic beads to isolate and purify nucleic acids from human sample material. The beads have a high affinity for nucleic acids and low inhibitor binding, resulting in ultra-pure DNA or RNA. chemagen™ technology features magnetizable rotating rods, combining the transfer and suspension of magnetic beads to extract DNA, preventing further fragmentation.

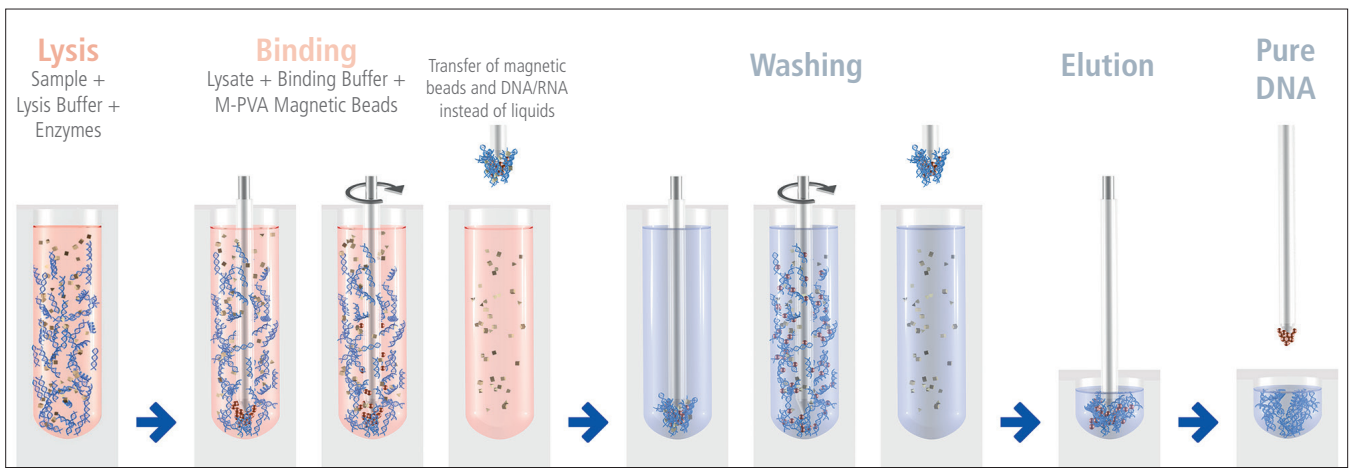

Figure 2. chemagen™ technology uses automated magnetizable rotating rods, combining the transfer and suspension of magnetic beads, to isolate ultrapure, high molecular weight DNA.

SAMPLE COLLECTION & DNA ISOLATION

Stool samples were collected multiple times from 4 donors: 2 adults and 2 children below 2 years of age. Before DNA isolation, collected stool samples were stored at 4°C for 20 hours. DNA was isolated from the samples using the chemagic™ 360 instrument and the chemagic™ DNA stool kit.

DNA Yield

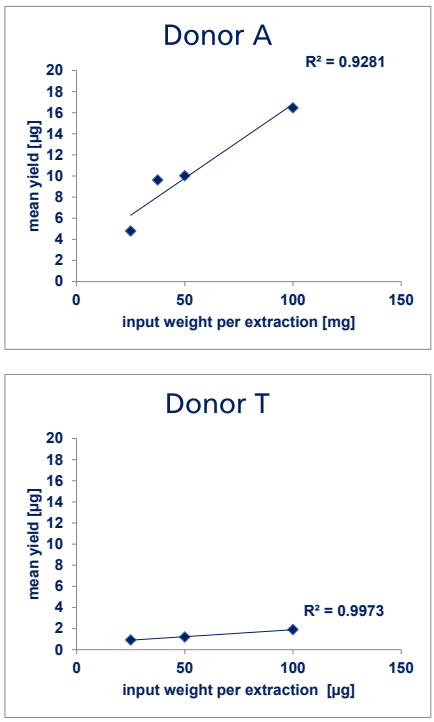

Figure 3. DNA yield dependent upon sample input amount (Qubit® fluorometer)—A strong linear correlation exists between stool input amount and the amount of double-stranded DNA isolated. DNA yield between donors can vary drastically, which makes it necessary for the extraction system to be able to handle extremes on both ends of the spectrum.

DNA Quality Assessment

LabChip® GX Touch™ nucleic acid analyzer microfluidics technology delivers rapid capillary electrophoresis analysis for DNA sample quality control. Eluates were analyzed on the LabChip GX Touch nucleic acid analyzer using the genomic DNA chip and reagents. The genomic quality score (gQC) refers to the degradation degree of the sample; 5 stands for intact gDNA and 0 for degraded DNA. In the gQS, the size distribution of the sample is included; the peak shifts to the left with increasing degradation, and the gQS is reduced.

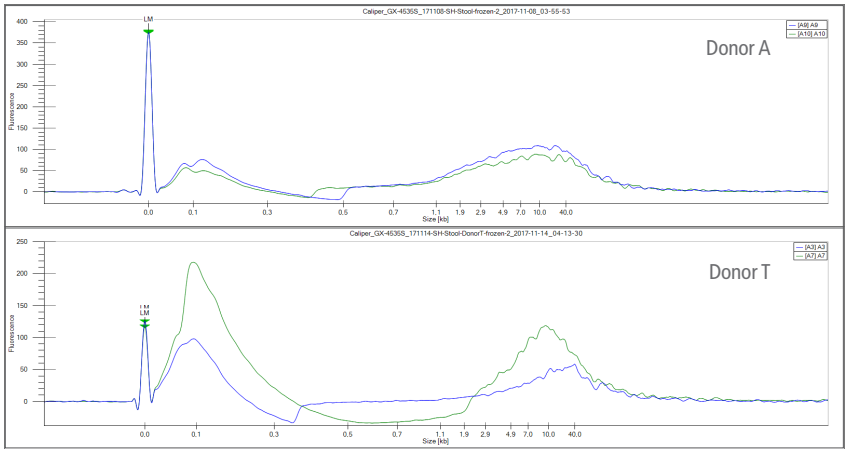

Figure 4. DNA gQC was determined using the LabChip® GX Touch nucleic acid analyzer and the genomic DNA chip and reagents. Donor A and Donor T show relatively high quality scores (gQS: 3,7/ 3,9 and 4,2/ 4,7).

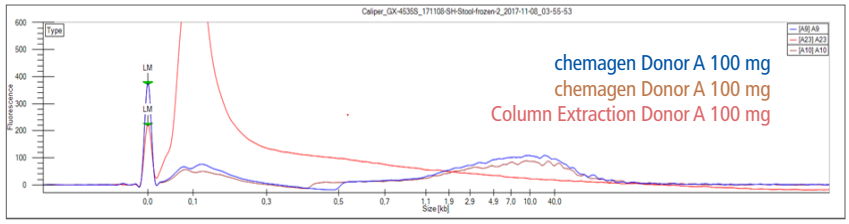

Figure 5. Size distribution of DNA extracted from Donor A (100 mg) using the chemagic™ technology compared to a column-based extraction method. Figure 5 shows that column-based manual DNA extraction methods can fragment DNA. This additional fragmentation is not seen when using the chemagen™ technology.

PERFORMANCE IN QPCR

DNA eluates obtained from different sample input weights from Donor T were tested in a qPCR targeting the human beta2 microglobulin (gB2M) gene.

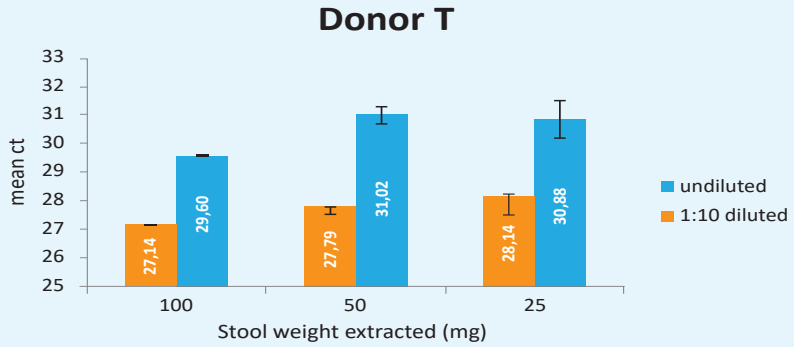

Figure 6. Mean ct values of eluates from donor T in quantitative PCR for a human target. Eluates were tested undiluted and tenfold diluted. The ct-differences for diluted eluates versus undiluted eluates are close to the perfect value of 3,3 indicating the absence of any inhibitors left in the eluates.

LIBRARY PREPARATION

Post nucleic acid extraction, the Illumina® Nextera™ DNA Flex library prep kit was used for library construction. The Illumina® Nextera™ DNA Flex library prep kit employs on-bead tagmentation that allows for the use of a broad range of input material (1 – 500 ng) from both large and small genomes, delivering consistent insert sizes, minimizing bias and opportunities for error, making it highly suitable for the metagenomics studies. Additionally, the Illumina® Nextera™ DNA Flex library prep kit innovates sample normalization at inputs greater than 100 ng, eliminating the need to quantify and normalize individual libraries generated within a single experiment. The Illumina® Nextera™ DNA Flex kit offers manual users a host of benefits, including decreased time and greater economies of scale, and to the automation user a promise of even better precision for higher sample throughput.

AUTOMATION

The PerkinElmer Sciclone® G3 NGSx liquid handling station is an automated benchtop solution optimized for high throughput library preparation. Automated library preparation enjoys the benefit of precision pipetting and therefore significant reductions in error, as well as flexible tip and waste management solutions for even the most complex NGS workflows. Protocols can be run from 8 to 96 samples, where the number of consumables required to complete the run is constant regardless of sample number. For manual library preparations, the Illumina® Nextera™ DNA Flex workflow already decreases laboratory time to a mere 3 hours; however, automating this workflow reduces library preparation to as little as 2.5 hours for 8 samples and 3.5 hours for 96 samples, while reducing hands-on time to 45 minutes to an hour.

Illumina® Nextera™ DNA Flex libraries were prepared on the PerkinElmer Sciclone G3 NGSx workstation with variable DNA input volumes (5-30 µL). The DNA input amount was between 100-600 ng for a single library and within the optimal range of 1-500 ng required for Illumina® Nextera™ DNA Flex libraries. To ensure the automated script fully aligns with the Illumina® Nextera™ DNA Flex manual protocol, a few sets of libraries, from the same DNA isolates used for automation, were prepared manually as internal controls. Between 2 independent runs, 90 Illumina® Nextera™ DNA Flex libraries were prepared on a Sciclone G3 NGS workstation, and a sub-set of 42 libraries from the same DNA isolates was prepared manually as an internal control.

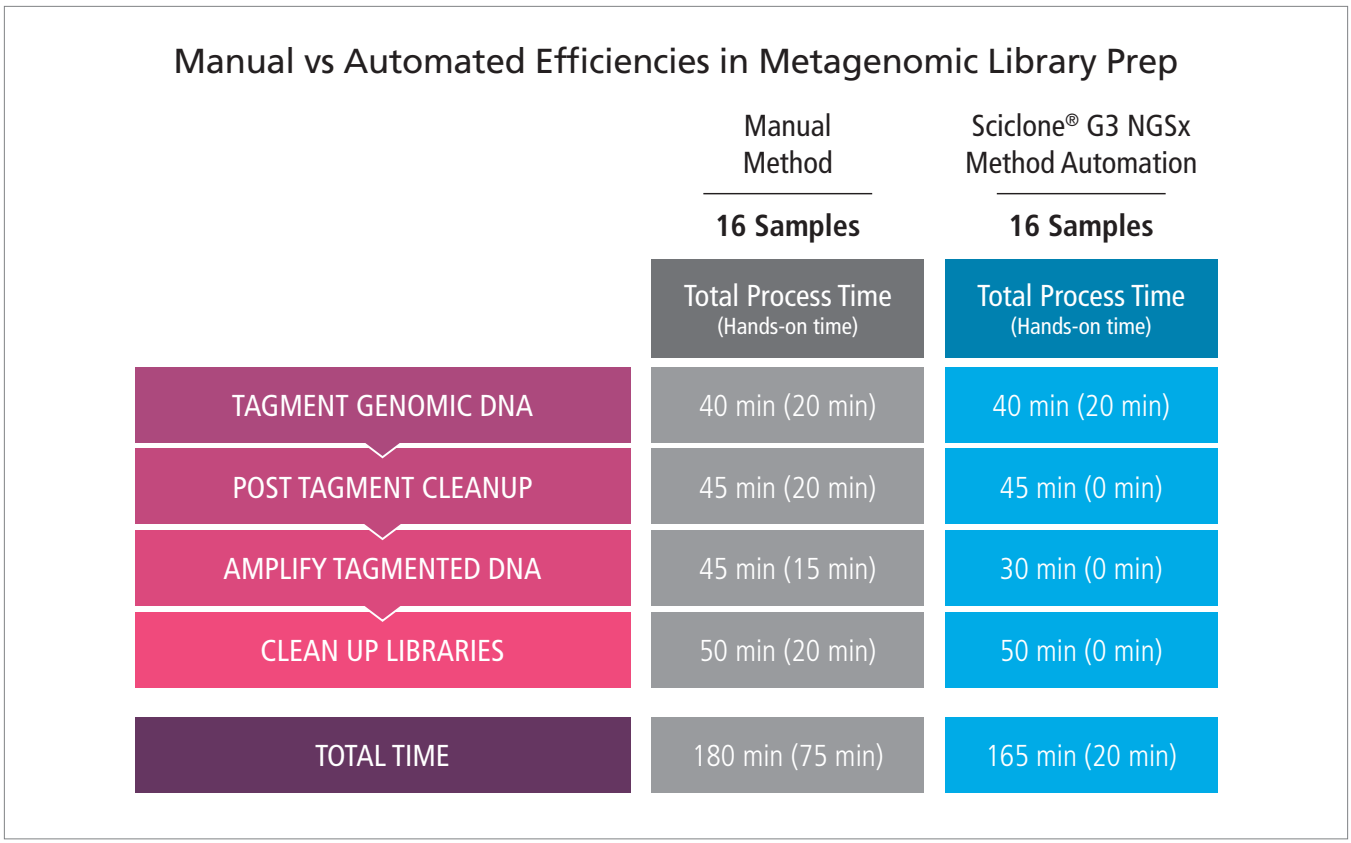

Figure 7. The automated Illumina® Nextera™ DNA Flex library preparation workflow program on the Sciclone® G3 NGS workstation reduces hands on time and total sample prep time for construction of metagenomic libraries.

The Illumina® Nextera™ DNA Flex Library Prep workflow program on the Sciclone G3 NGSx workstation is intuitively designed to quickly set up, start, and track a run. The Application Selection Menu offers the ability to easily run the entire protocol or split the workflow into modules.

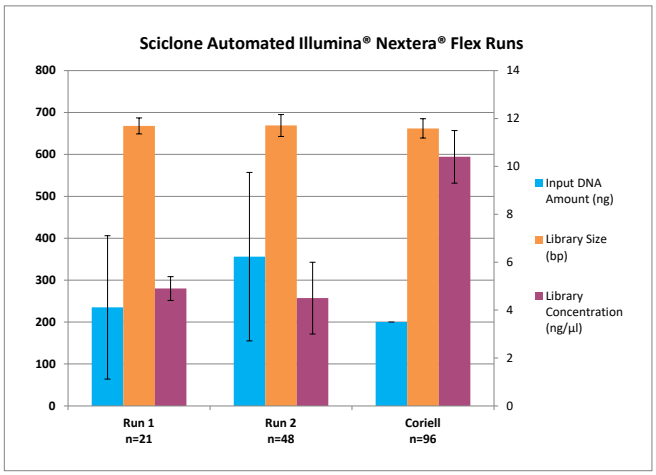

Figure 8. Input DNA amounts (ng), library size (bp), and library concentration (ng/µL) of automated Illumina® Nextera™ DNA Flex libraries prepared from stool metagenomic DNA and Coriell NA12878. Metagenomic libraries generated on the Sciclone® G3 NGS workstation from stool produced repeatable data that is similar to libraries generated from NA12878 gDNA.

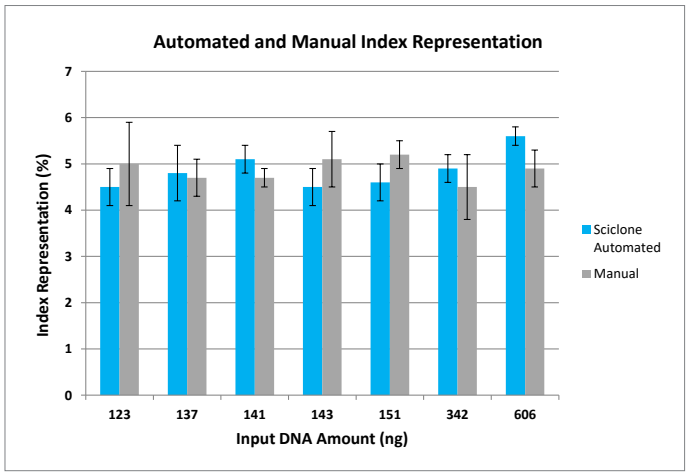

Figure 9. Automated Run 1 and Manual Run Index representation (%) of Illumina® Nextera™ DNA Flex libraries prepared from various amounts of stool metagenomic DNA and from an Illumina® HiSeqX® sequencing run (n=3). The automated and manual libraries were prepared from the same Donor DNA and split into each workflow. The input amounts were dictated by concentration of DNA isolates and maximum library input volume (30 µL). Index representation was similar across multiplexed sequencing libraries prepared either manually or automated and was not impacted by amount of input DNA.

LIBRARY PREPARATION QC

Electrophoretic analysis assays are a core component of quality control (QC) for next generation sequencing libraries, providing assessment of both the size and quantity of DNA fragments in a sample. Both capabilities serve to ensure that library samples for sequencing are properly prepared. The LabChip® GX Touch™ nucleic acid analyzer automates nucleic acid sizing and quantitation of both fragments and smears. These assays, including NGS 3K and gDNA assay, provide unparalleled genomic and NGS library data quality with minimal input requirements.

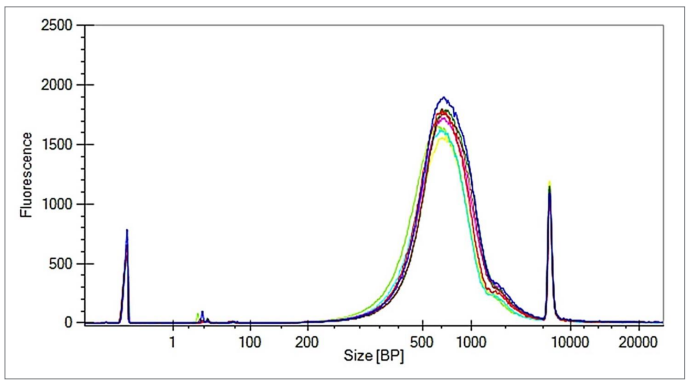

Figure 10. Quality Control overlay of Illumina® Nextera™ DNA Flex sequencing libraries from Automated Run 2 on the Sciclone® G3 NGSx workstation, and analyzed on the LabChip® GX Touch™ nucleic acid analyzer using the DNA NGS 3K Assay. The average insert sizes of 600 base pairs are fully aligned with the quality and sizing expected from manual library preparations.

RESULTS

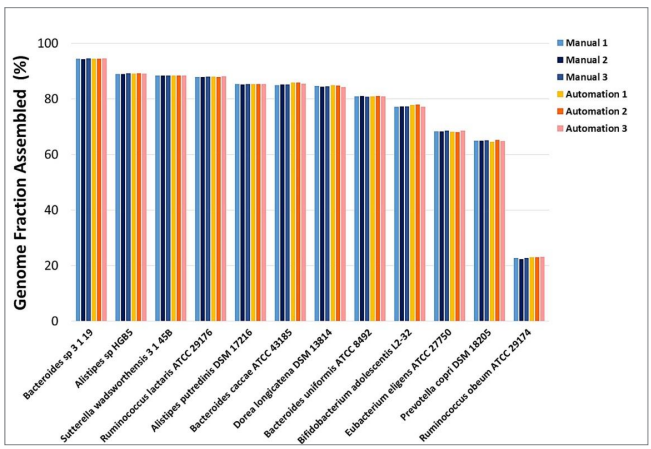

Figure 11. MetaQUAST genome assembly analysis for a sub-set of the top abundant bacteria species detected in Illumina® Nextera™ DNA Flex libraries by Genius Metagenomics.

Manual and automated libraries were prepared in triplicate from DNA extracted from a fecal sample originating from a single donor. For all organisms analyzed, the automated and manually prepared libraries generated comparable, high-quality genome assembly results.

The percent of genome assembled strongly depends on alignment between available reference genome and genome of particular species present in the tested stool samples. Reference genomes were obtained from NCBI database. Preferentially, whole genomes were used, and in the instances where whole genomes could not be found, mostly complete genomes consisting of contigs and scaffolds were used instead.

SUMMARY

The Illumina® Nextera™ DNA Flex library preparation kit offers the most flexible protocol in the Illumina® portfolio. The kit supports a broad range of input amounts and versatile applications. Its ability to generate normalized libraries eliminates the burden of accurate quantification for both input DNA and final library preparations.

The automated Illumina® Nextera™ DNA Flex protocol, combined with chemagic™ DNA extraction, is a convenient solution for researchers whose microbiome projects require a high level of automation. Stool storage conditions which limit DNA degradation (Omega Bio-tek® tubes, (AC7055), 4ºC storage), DNA isolation protocol optimized for stool material, and the consistent, highly-uniform libraries delivered by the Illumina® Nextera™ DNA Flex kit allow thorough testing of complex human gut bacterial communities.

LEARN MORE

To learn more about the Illumina® Nextera™ DNA Flex library prep kit, visit the **Nextera DNA Flex Library Prep page**.

For more on microbial genome sequencing with the Illumina® Nextera™ DNA Flex library prep kit, read the **Microbial WGS with Nextera DNA Flex Application Note**.

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