APPLICATION NOTE

Automated NGS library preparation with Covaris® focused-ultrasonicator workflow solution.

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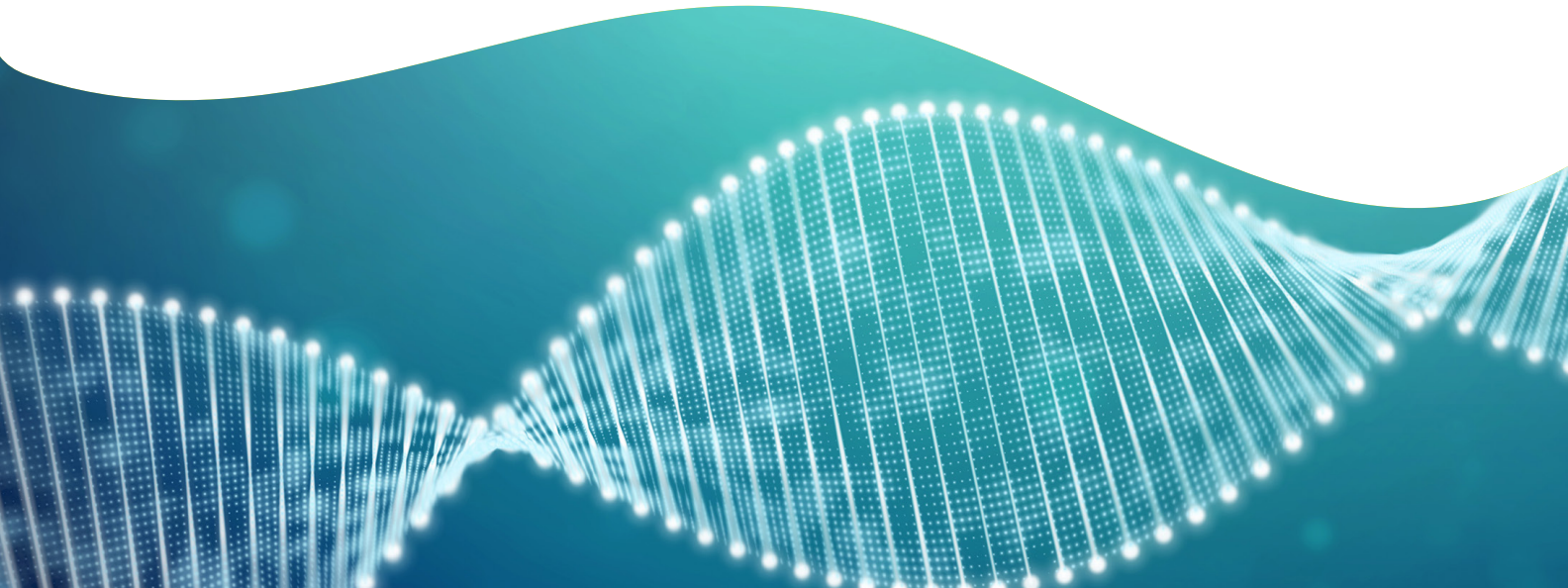
Introduction

Building on Revvity's established automation and applications expertise across the genomics workflow, we describe an automated workflow collaborating with Covaris®, Inc. to prepare next-generation sequencing (NGS) libraries with minimal process variability. The workflow described herein focuses on the upstream NGS library preparation for sample yield standardization including: mechanical DNA shearing, sample transfer, DNA quality assessment and subsequent automated library construction.

About Covaris® focused-ultrasonicators

Covaris® Focused-ultrasonicators are the gold standard for mechanical DNA shearing in NGS library preparation. It combines integration of high performance control electronics, medical-grade transducers, and custom engineered acoustical cuvettes. The technology uses controlled bursts of high power acoustic energy to process samples, resulting in highly tunable, randomly fragmented nucleic acids of similar size. Uniquely, all Covaris® Focused-ultrasonicators are calibrated to National Institute of Standards and Technology (NIST) traceable standards, ensuring highest quality and standardized results. These criteria enable robust and reproducible integration into high-throughput workflows and compatibility with liquid handlers to provide the most accurate and precise NGS data.

For research use only. Not for use in diagnostic procedures.



About Revvity solutions for NGS library prep

Revvity is eliminating the challenges associated with NGS analysis by providing labs with complete, single source solutions encompassing everything from sample to solution. With applications expertise spanning the NGS workflow, Revvity offers complete, automated solutions for NGS library prep to produce accurate, reproducible results.

Revvity's NGS offerings include nucleic acid extraction solutions, instrumentation, and assays to simplify nucleic acid and library analysis, workstations to automate library preparation, library preparation kits, and software for the analysis of complex sequencing data. Revvity's NGS workflow solutions are designed to solve the researcher's problems; from improving nucleic acid yields, to increasing throughput, to reducing bias, Revvity has the answer you need.

Revvity's NGS workflow solutions include:

- Automated Nucleic Acid Isolation
- Rapid Quantitation of Nucleic Acids
- Microfluidic Capillary Electrophoresis Analysis of Nucleic Acids and NGS Libraries
- Flexible NGS Library Prep Solutions
- Automated Library Preparation Solutions to Meet Your Unique Needs

Workflow overview

The DNA sample was sheared using a Covaris® Focused- ultrasonicator in a microTUBE™ consumable available in either 8 strip or 96 well SBS format. The microTUBE™ plates are sealed by either a slit-septa or aluminum foil (Figure 1), which typically leads to manual recovery of the process DNA with a multichannel pipet. To overcome this obstacle, we designed two automated solutions for the transfer of the fragmented DNA sample to the next step of the library preparation workflow. Post DNA shearing, the Covaris® microTUBE™ plate is placed by the user on either the JANUS® G3 or Sciclone® G3 NGS(x) Automated Workstation platform.

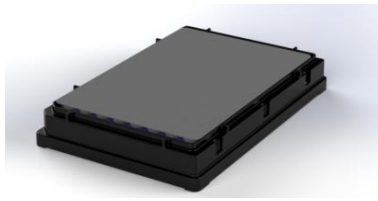


Figure 1: Covaris® 96 microTUBE™ AFA™ Fiber Plate Thin Foil

Depending on the expected plate throughput and the level of desired automation, we offer two options. For a fully-automated solution for high-throughput septum piercing, the microTUBE™ plate can be placed on a Cedrex® plate holder that has been integrated with both workstation platforms (Figure 2). Alternatively, for lower throughput a simple manually installable and removable plate holder (Figure 3) can be used. The plate holder was specifically designed to hold the microTUBE™ plate in-place during pipetting. The flexibility within our liquid handling software platforms enable sample processing of the total or cherry- picked wells from the Covaris® microTUBE™ plate using a simple method on our liquid handling platforms.

Figure 2: Integrating a Cedrex® plate holder provides a fully-automated solution for septum piercing. Compatible with either the JANUS G3 or Sciclone G3 NGS(x) Automated Workstations.

DNA samples were then run on the LabChip® GX Touch™ Nucleic Acid Analyzer to assess fragment size distribution of the genomic DNA.

Figure 3: Simple manually installable and removable plate holder for lower plate throughput workflows. Compatible with either the JANUS G3 or Sciclone G3 NGS(x) Automated Workstations.

Performances

To demonstrate the efficiency and versatility of this workflow, DNA samples were sheared using the Covaris® LE220-plus Focused-ultrasonicator in a 96 microTUBE™ AFA™ Fiber Plate. The plate was then removed from the instrument and placed under the manually installable plate adapter on the Sciclone G3 NGSx Workstation, as shown in Figure 4. The molecular weights were analyzed with the Revvity LabChip® GX Touch™ Nucleic Acid Analyzer using the High Sensitivity, DNA 3K NGS, or Genomic DNA Assay to evaluate sample integrity. Electropherograms in Figures 5 and 6 highlight the tunable shearing precision of the Covaris® Focused-ultrasonicator, with genomic DNA fragmented to specific sizes ranging from 150 to 550 bp. The overlay of 96 technical replicates in Figure 7 illustrates the high reproducibility of the DNA shearing.

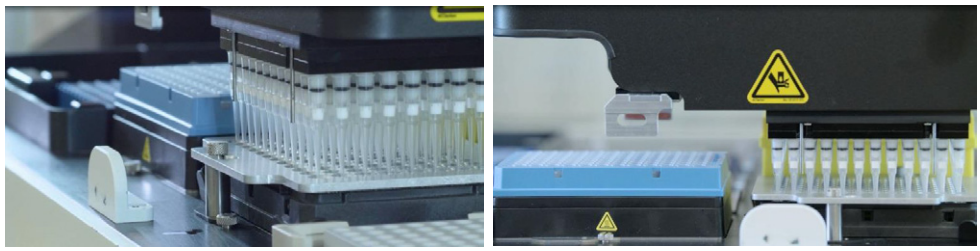


Figure 4: Sample removal post shearing by the Sciclone G3 NGS(x) Workstation pipetting through the manually installable plate adapter and piercing through the AFA™ fiber plate thin foil into the Covaris® 96 microTUBE™ plate.

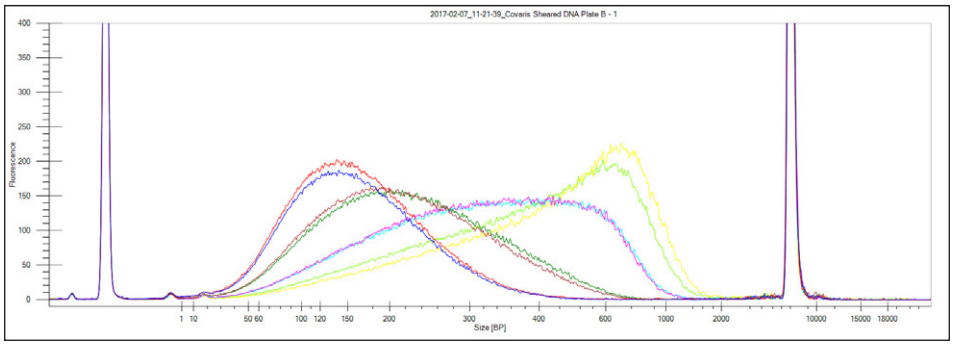


Figure 5: Representative electropherograms for isolated 150 bp, 200 bp, 350 bp and 550 bp DNA. Samples were run using the LabChip High Sensitivity Assay. Replicates were run to demonstrate reproducibility.

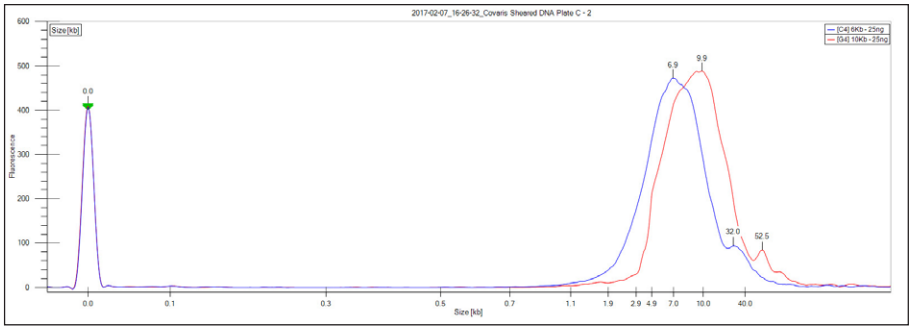


Figure 6: Representative electropherograms for isolated 6 kb and 10 kb DNA. Samples were run using the LabChip Genomic DNA Assay; concentrations were normalized to 25 ng/µL.

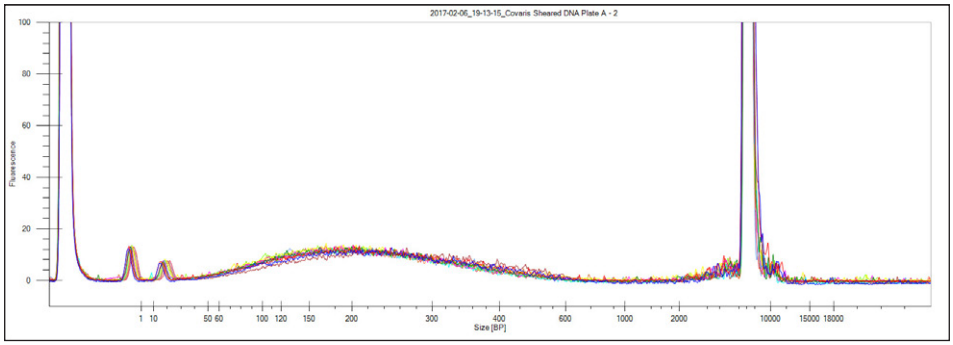


Figure 7: Representative electropherograms of eight 200 bp sheared DNA samples. Overlay illustrates accurate shearing and visualization. In total, 96 samples independent samples were run. Samples were diluted to 100 pg/µL to demonstrate the sensitivity (LOD) of the LabChip DNA NGS 3K Assay.

Automated FFPE sample processing

Revvity and Covaris® FFPE extraction and nucleic acid isolation

Active paraffin removal and tissue rehydration with Covaris® AFA™ technology. DNA is then isolated from solution with chemagic™ bead-based technology.

Covaris® focused-ultrasonicator for mechanical nucleic acid shearing

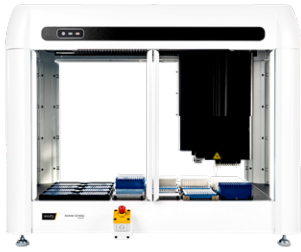
Covaris® employs AFA™ technology for controlled shearing of nucleic acids. Mechanical DNA shearing is isothermal; ensuring both unbiased fragmentation and high recovery of double-stranded DNA. The samples are processed in Covaris® microTUBE™ plates or strips that are sealed by slit-septa or aluminum foil.

Fast quantitative qnalysis of DNA/RNA samples with LabChip GX touch nucleic acid analyzer

The LabChip GX Touch Nucleic Acid Analyzer delivers electrophoresis separation for high-sensitivity DNA/RNA analysis, DNA smear analysis, and RNA and gDNA integrity analysis all on one platform, in as little as 30 seconds per sample. The system is available in both a 24-sample platform, saving time and reagent expense, and in a platform, accommodating up to 384 wells for high-throughput workflows.

Revvity and Covaris® automated sample processing of sheared DNA

Revvity has designed two automated solutions for the transfer the mechanically sheared DNA to the next step of the library preparation workflow with specially optimized adapter to hold the Covaris® microTUBE™ plate on the deck of JANUS G3 and Sciclone G3 liquid handling platforms. Depending on the expected plate throughput and the level of desired automation, we offer either a fully-automated or semi- automated option. This enables hands-free sheared DNA transfer onto the next steps within the library preparation workflow.



For more information, please visit www.revvity.com

For a short video on the simplicity of the semi-automated workflow using the Sciclone NGSx Workstation with the removable plate adapter, please visit www.covaris.com/automation

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