

# Automated NGS Sample Preparation Workflows

Combining the Agilent Bravo Liquid Handling Platform with Covaris Focused-Ultrasonicators for Complete Automation of SureSelect Workflows

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## Abstract

Automation is a mandatory requirement for any high-throughput project that reduces variability, improves workflow efficiency, and increases walk-away time. While most NGS automation workflows have primarily been centered on library preparation and target enrichment protocols, there have been only a few commercial solutions offering a comprehensive workflow, from mechanical DNA shearing with a Covaris Focused-ultrasonicator to completed libraries. This White Paper introduces an automated workflow for transferring DNA samples to/from a Covaris 96 microTUBE Plate, 8 microTUBE Strip, or 96 oneTUBE-10 AFA Plate using the Agilent Bravo NGS liquid handling platform. We show the successful transfer of samples from each microTUBE product, and demonstrate that sample and data qualities are comparable between manual and automated handling.

**Covaris®**

## Agilent Covaris Plate Adaptor

To enable this fully automated workflow, Agilent and Covaris worked together to develop the Agilent Covaris Plate Adaptor (p/n G5498#068) (Figure 2). This adaptor provides an adjustable and highly consistent automated solution for processing DNA using Covaris AFA technology.

The Adaptor is compatible with all Covaris consumables designed for automation (Figure 3): 8 microTUBE Strips, 96 microTUBE Plates, and 96 oneTUBE-10 AFA Plates.

## Agilent/Covaris workflow overview

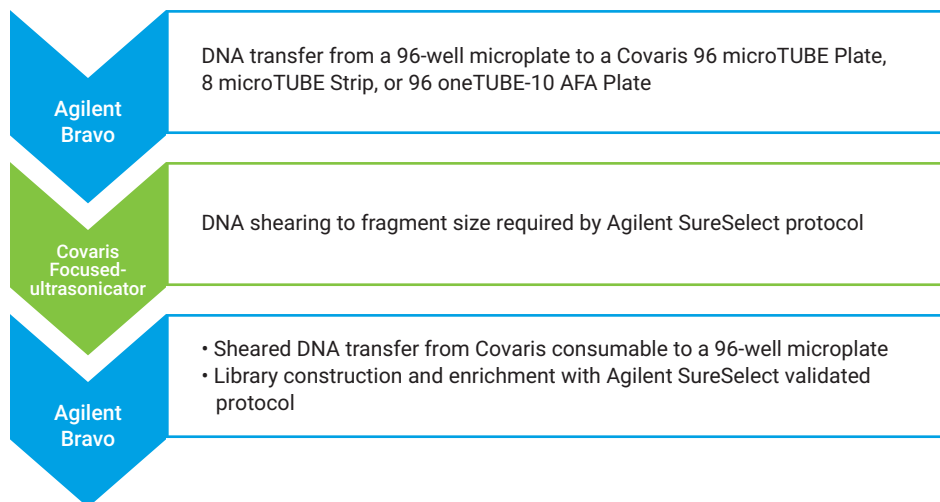
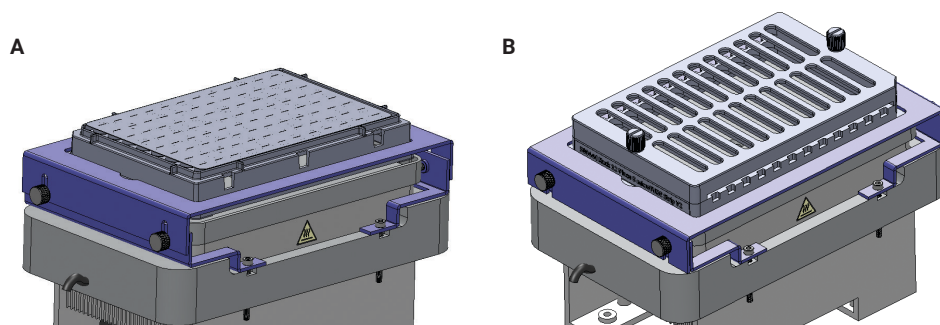
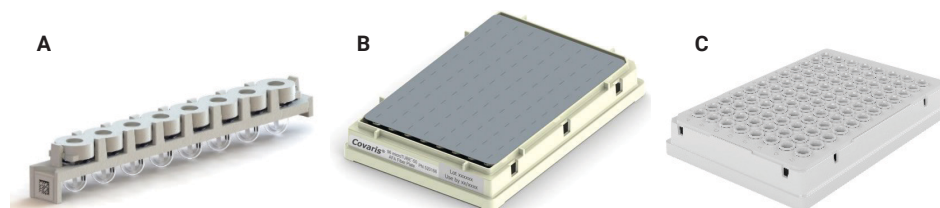


Figure 1. Workflow overview.



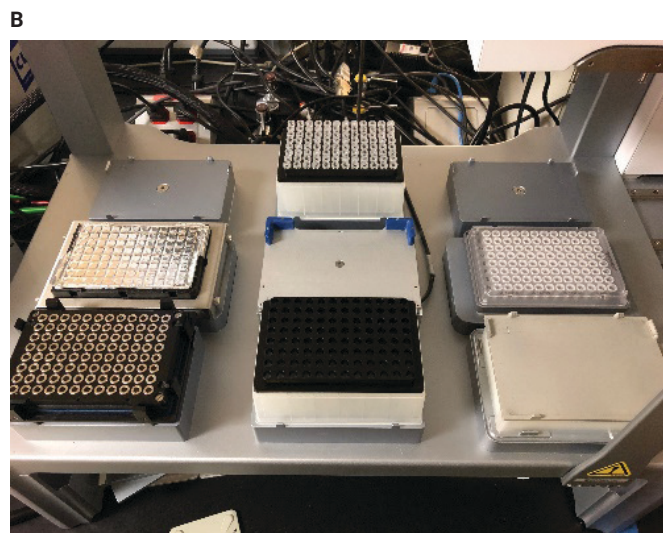
**Figure 2.** Covaris Plate Adaptor with compatible consumables for the Agilent Bravo NGS and NGS Workstation. The Plate Adaptor assembly is comprised of three separate components: two base pieces secured by screws on a CPAC base, and one top piece that is height-adjustable and secured by four side screws. The Adaptor is installed at Bravo Deck position 4. The configuration is adaptable for Covaris 96 microTUBE Plates (A) and 8 microTUBE Strips (B).



**Figure 3.** Examples of Covaris consumables compatible with the Agilent Bravo Liquid Handling Platform. A) 8 microTUBE-50 AFA Fiber Strip V2 H Slit (p/n 520240). B) 96 microTUBE Plate Thin Foil (p/n 520232). C) 96 oneTUBE AFA plate (p/n 520249).

The Plate Adaptor is easily integrated with the Agilent Bravo NGS and NGS Workstation platforms, and all steps are programmed with user-friendly software for seamless processing (Figure 4).

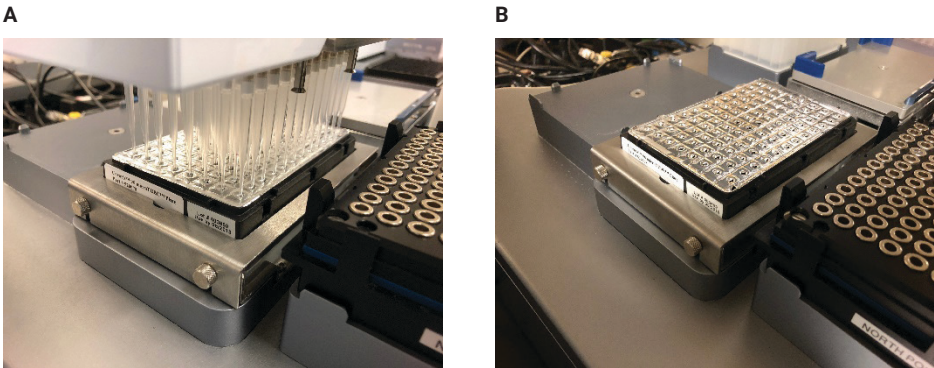
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**Figure 4.** Simplified Agilent VWorks Automation Control Software GUI for loading and extracting samples from Covaris consumables. A) VWorks GUI: The form is tunable so a user can process 1–12 columns of samples with minimal user intervention. The protocol is designed to work with Bravo NGS and NGS Workstation. B) Image of deck layout on Bravo.

# Precise and robust sample transfer from a Covaris consumable to microplate

The Plate Adaptor holds down and enables robust piercing and pipetting of the Covaris consumable on deck (Figure 5). Volume transfer is accurate and highly reproducible (Table 1).



**Figure 5.** Image of Covaris 96 microTUBE Plate being punctured by an Agilent Bravo 96LT Pipette Head for sample transfer. A) Puncture of all 96 wells of a Covaris 96 microTUBE plate. B) Post puncture view of a Covaris 96 microTUBE plate.

**Table 1.** Volumetric assessment of accuracy with sample transfer from a Covaris 96 microTUBE Plate to a 96-well microplate using Artel MVS Platform. The 96 microTUBE Plate was preloaded with 120 µL of prepared Artel calibration standard. Then, 110 µL of solution was transferred into a 96-well MVS Verification Plate and measured with the Artel MVS. The mean volume transferred was 111.40 µL, relative inaccuracy was 1.2 %, and CV was 0.83 % for all channels.

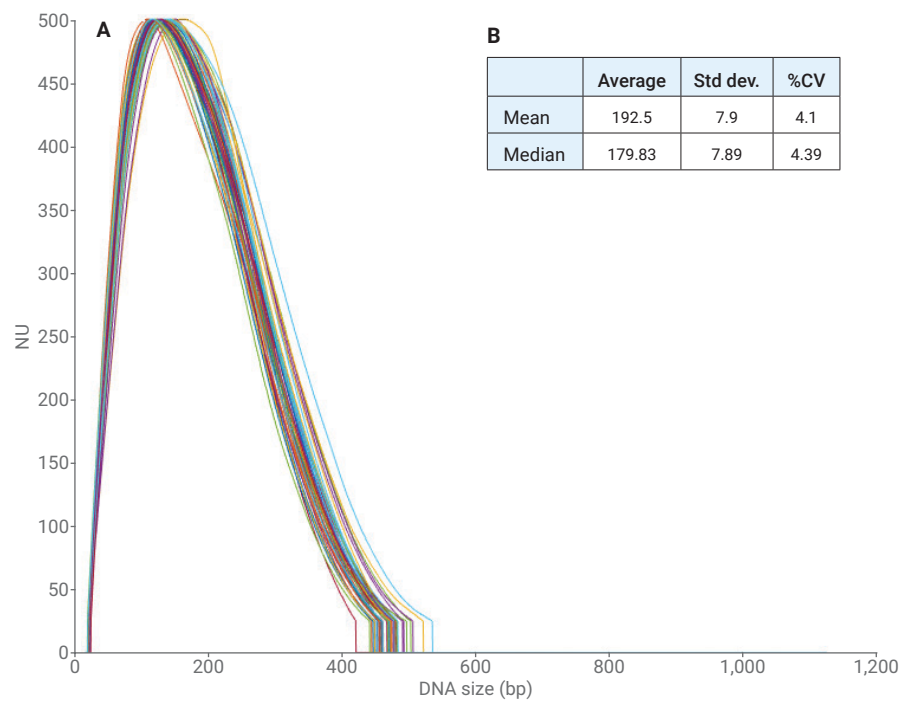
|   | 1     | 2     | 3     | 4     | 5     | 6     | 7     | 8     | 9     | 10    | 11    | 12    |
|---|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| A | 111.5 | 111.3 | 111.8 | 111.5 | 112.3 | 111.6 | 111.5 | 111.5 | 111.1 | 110.9 | 111.6 | 112.3 |
| B | 111.4 | 111.4 | 111.4 | 111.4 | 111.2 | 111.6 | 111.8 | 111.7 | 111   | 111.3 | 111.9 | 111.9 |
| C | 111.8 | 112.1 | 111   | 111.7 | 114.7 | 111.9 | 111.8 | 111.9 | 111.8 | 112.1 | 111.4 | 111.5 |
| D | 111.7 | 112.4 | 111.9 | 111.7 | 111.8 | 111.2 | 111.6 | 111.6 | 111.7 | 111.4 | 111.1 | 111   |
| E | 111.1 | 111.3 | 111.6 | 111.6 | 111.5 | 111.8 | 111.9 | 112.1 | 110.2 | 112   | 111.6 | 111.7 |
| F | 111.4 | 112   | 111.1 | 111.1 | 111.1 | 111.6 | 111.8 | 111.3 | 111.7 | 111.7 | 112.8 | 111.6 |
| G | 110.1 | 110.9 | 111.8 | 111.3 | 108.1 | 112   | 111.7 | 112.2 | 111.9 | 112   | 111.9 | 111.4 |
| H | 111.4 | 111.7 | 111.9 | 110.5 | 111.9 | 112.1 | 110.7 | 111.9 | 111.5 | 111.7 | 112   | 111.4 |

Highly reproducible  
mechanical DNA shearing

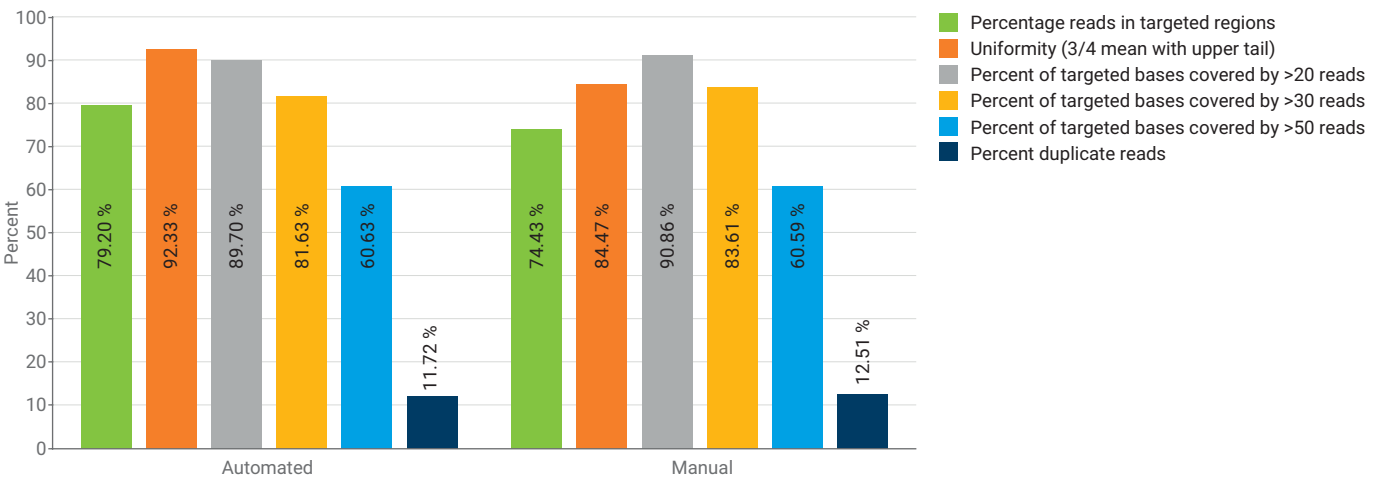
After loading DNA into the Covaris 96 microTUBE Plate, 48 technical replicates were mechanically sheared with a Covaris Focused-ultrasonicator, then transferred back to a 96 microwell plate using the Adaptor on the Bravo Liquid Handling Platform (Figure 6).

Sequencing metrics  
equivalent to manual  
workflow

Ninety-five technical replicates were run using the fully automated SureSelect<sup>XT</sup> Low Input workflow, and benchmarked to a manual protocol, showing highly reproducible and consistent sequencing results, as expected (Figure 7).



**Figure 6.** DNA Shearing with Covaris for Agilent SureSelect<sup>XT2</sup> NGS Library Preparation. Two hundred nanograms of human genomic DNA (Promega p/n G3041) was sheared on a Covaris LE220 with the following settings: 450W PIP, 20 % Duty Factor, 1,000 CpB, and 160 seconds per row in a 96 microTUBE-50 AFA Fiber Plate Thin Foil (p/n 520232). Forty-eight DNA replicates were run in the first six columns of the plate. A) Overlay of the DNA fragment distributions for the 48 replicates. B) Summary of DNA shearing metrics for the 48 DNA sample replicates.



**Figure 7.** Consistent high specificity and coverage depth after SureSelect<sup>XT</sup> Low Input capture using 10 ng HapMap DNA samples with a 0.2 Mb design on Illumina MiSeq. Specificity of capture (>70 % on target) and sequence coverage (>80 % at 20x) is very reproducible across all indexed samples normalized to 100x bait size. The automated samples were extracted from the Covaris microTUBE plate using the Adaptor on the Bravo Liquid Handling Platform, then taken through the SureSelect<sup>XT</sup> Low Input protocol for the NGS Workstation. One sample dropped out due to user error unrelated to Bravo processing. The manual samples were processed at the Agilent La Jolla R&D site.

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