Improvement in automated NGS sample preparation workflows: combining the Agilent Bravo Liquid Handling Platform with Covaris Focused-ultrasonicators

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Introduction

While most NGS automation workflows have focused on Library Preparation and Target Enrichment protocols, there have been only a few commercial solutions presented around the initial DNA sample transfer from the Covaris microTUBE consumables. Here we introduce an automated workflow for extracting sheared DNA samples from a 96 microTUBE Plate and 8 microTUBE Strip 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. Automation is a mandatory requirement for any high-throughput project that reduces variability, improves workflow efficiency and increases walk-away time. Utilizing the Agilent Bravo Liquid Handling Platform for automating extraction of sheared DNA from the Covaris 96 microTUBE Plate and 8 microTUBE Strip offers a reliable and reproducible solution for high-throughput studies.

Agilent/Covaris Workflow Overview

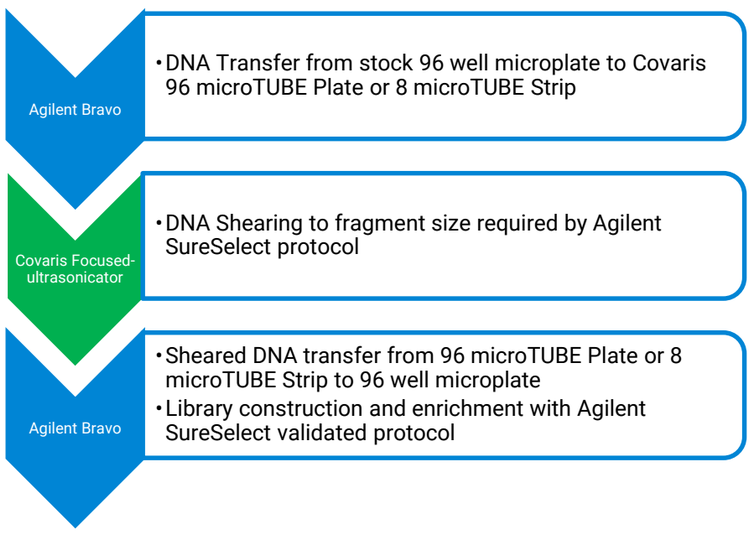


Figure 1 – Overview of the automated Covaris sample preparation workflow performed using the Agilent Bravo Liquid Handling Platform. All samples transfer steps are automated on Agilent Bravo Liquid Handling Platform, thus reducing variability, while improving workflow efficiency and bringing end to end sample tracking.

Covaris AFA Technology

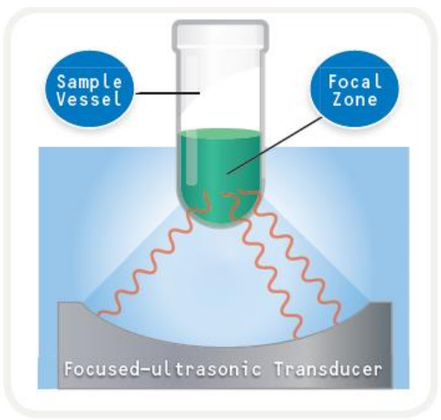


Figure 2 – Illustration of Covaris Adaptive Focused Acoustics™ (AFA) technology for the controlled mechanical shearing of the phosphodiester backbone of nucleic acids. AFA hydrodynamic shear force-based fragmentation is purely mechanical and designed to be isothermal, ensuring both unbiased fragmentation and high recovery of double-stranded and single–stranded DNA.

Bravo NGS and NGS Workstations

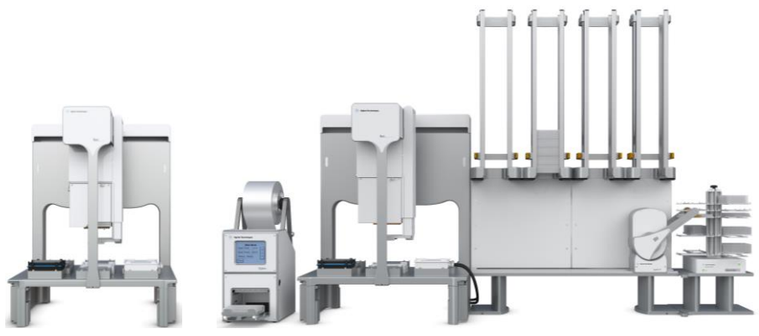


Figure 3 – NGS Automation Solutions from Agilent compatible with Covaris Plate Adaptor. A) Bravo NGS (Option A, NGS A). B) NGS Workstation (Option B, NGS B).

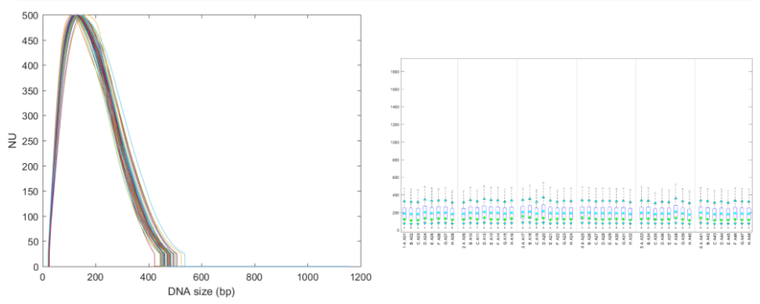
Covaris Consumables for Agilent Bravo

Figure 4 – Covaris consumables developed for compatibility with Agilent Bravo Liquid Handling Platform. A) 8 microTUBE-50 AFA Fiber Strip V2 H Slit (PN 520240). B) 8 microTUBE-50 strip into rack defined for SBS microplate standards. C) 96 microTUBE Plate Thin Foil (PN 520232) is a single use pre-pierced, pre-assembled, ready to use plate

Agilent Covaris Plate Adaptor

Figure 5 – Covaris Plate Adaptor with compatible consumables for the Agilent Bravo NGS and NGS Workstation. Plate adaptor assembly is comprised of three separate components: two base pieces secured by screws on CPAC base and one top piece that is height-adjustable and secured by four side screws. Adaptor is installed at Bravo Deck position 4. Configuration is adaptable for Covaris 96 microTUBE plates (A) and 8 microTUBE Strips (B).

Covaris DNA Shearing Metrics



	Average	StDev	%Cv
Mean	192.5	7.9	4.1
Median	179.83	7.89	4.39

Figure 6 – DNA Shearing with Covaris for Agilent SureSelect XT2 NGS Library Preparation. 200 ng human genomic DNA (Promega PN G3041) was sheared on a Covaris LE220 with the following settings: 450W PIP, 20% Duty Factor, 1000 cpb and 160 seconds per row in a 96 microTUBE-50 AFA Fiber Plate Thin Foil (PN 520232). 48 DNA replicates were run the first 6 rows of the plate. A) Overlay of 48 replicates after amplitude normalization performed on Fragment Analyzer. B) Box plot of the 48 replicates, organized by row and position. C) Summary of DNA shearing metrics for the 48 DNA sample replicates.

Full Microplate Puncture and Transfer

Figure 7 – Image of Covaris 96 microTUBE Plate being punctured by 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.

Simplified VWorks User Experience

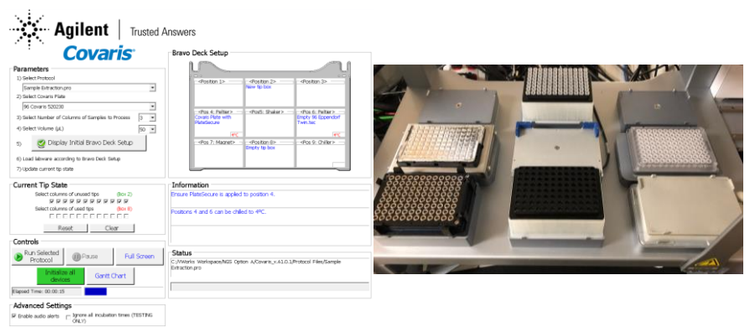


Figure 8 – Simplified Agilent VWorks Automation Control Software GUI for loading and extracting samples from Covaris consumables. The form is tunable so a user can process 1, 2, 3, 4, 6 or 12 columns of samples with minimal user intervention. Protocol is designed to work with Bravo NGS and NGS Workstation.

Bravo Head Pipetting Performance

	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

Figure 9 – Volumetric assessment of accuracy with sample transfer from Covaris 96 microTUBE Plate to 96-well microplate using Artel MVS Platform. 96 microTUBE plate was pre-loaded with 120 uL of prepared Artel calibration standard. 110 uL volume of solution was transferred into a 96-well MVS Verification Plate and measured with the Artel MVS. Mean volume transferred was 111.40 uL, relative inaccuracy was 1.2% and CV was 0.83% for all channels.

Performance: SureSelect^{XT} Low Input

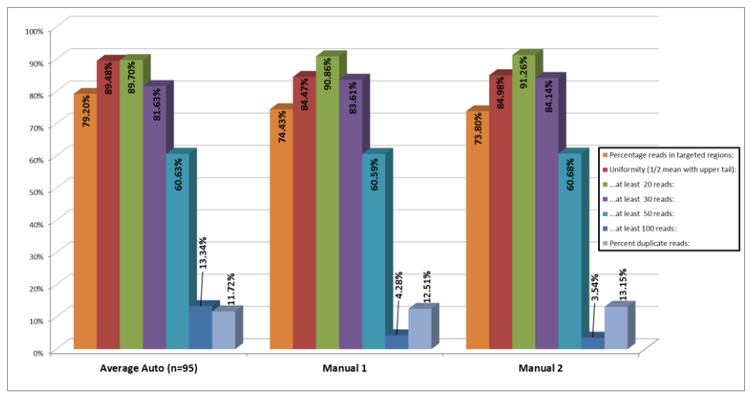


Figure 10 – Consistent, high specificity and coverage depth after SureSelect XT Low Input capture using 10ng HapMap DNA samples with a 0.2Mb design on Illumina MiSeq. Specificity of capture (>70% on target) and sequence coverage (>80% at 20X) is very reproducible across all indexed samples normalized to 100x bait size. The automated samples were extracted from the Covaris shear plate using the developed Covaris plate holder and protocol then taken through the soon-to-be released SureSelect^{XT} Low Input protocol for the NGS Workstation. One sample dropped out due to user error, unrelated to Bravo processing. The two manual samples shown were processed at Agilent La Jolla R&D site.

Conclusions

The Agilent Covaris Plate Adaptor

- Provides an adjustable and highly consistent automated solution for processing genomic DNA using Covaris AFA technology
- Adaptable to Covaris 8 microTUBE strips and 96 microTUBE plates
- Operated with user-friendly software for seamless processing
- Directly compatible with Agilent Bravo NGS and NGS Workstation platforms

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