Magnetic Bead-based Clean-up Using AFA-energetics™

Abstract

DNA shearing is the first step in NGS library prep workflow and AFA-based DNA shearing from Covaris is the industry gold standard. In order to support downstream NGS library prep applications, Covaris protocols support contact-free micromixing during magnetic bead-based clean-up steps. Here, we show how Adaptive Focused Acoustics® (AFA®) technology can replace conventional pipetting to mix a sample, specifically during a SPRI* clean-up step, in the new, automatable 96 oneTUBE-10 AFA plate.

Introduction

AFA-enabled Micromixing is an Alternative to Conventional Pipette-Mixing

Covaris technology is based on patented AFA-energetics, which delivers a highly controlled dose of acoustic energy to the sample. When precisely tuned, this stream of acoustic energy enables DNA shearing precisely and reliably to desired fragment sizes, in addition to gentle contact-free sample mixing.

To support an automation-friendly workflow, Covaris has developed the oneTUBE-10 product line, compatible with the LE220-plus and ME220 Focused-ultrasonicators. For DNA shearing, the oneTUBE-10 is validated for volumes between 10 and 50 µL and comes in 2 formats, the 96 oneTUBE-10 AFA Plate and the 8 oneTUBE-10 AFA Strip. This consumable line is constructed using a unique polymer for low impedance and better transmission of acoustic energy, which eliminates the need for an AFA fiber. The newly designed 96 oneTUBE-10 AFA Plate can be used as a typical SBS microplate and can be paired with an automation clamp for robust thin foil piercing. The oneTUBE format allows the full integration of an NGS library prep to be performed in the same vessel.

As depicted in ***Figure 1*** and ***Table 1***, decoupling mixing from pipetting using controlled AFA energy to resuspend beads provides cost-savings benefits in time and consumable needs to automated bead clean-up protocols.

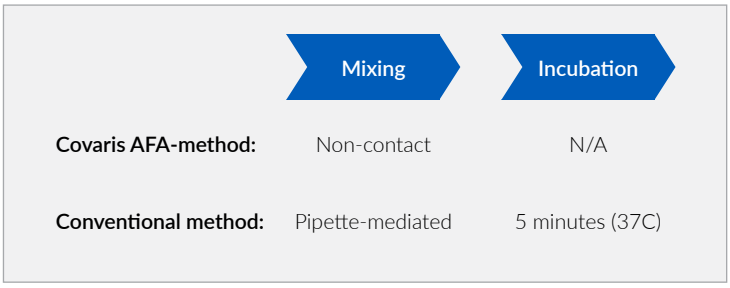

Figure 1. Introducing Covaris AFA into the binding and elution steps of SPRI workflows, removes conventional pipetting-based mixing and the subsequent incubation for AFA-based, non-contact micromixing.

	AFA-micromixing	Pipette-mixing
Reaction volume during bead binding/elution	10 to 50 µl	10 to 150 µl
Number of Pipette Tip boxes required per SPRI prep (8-channel head)	4	6
Elapsed Time per bead-binding step and bead-elution step (8 oneTUBE AFA Strip in ME220)	5 seconds (bind); 2 seconds (elute)	320 seconds (bind); 320 seconds (elute)
Elapsed Time per bead-binding step and bead-elution step (96 oneTUBE AFA Plate in LE220 plus)	300 seconds (bind); 120 seconds (elute)	320 seconds (bind); 320 seconds (elute)
Contact-free mixing	Y	N
Pipette-tip, capacity-independent mixing	Y	N

Table 1. Comparison of AFA-and conventional pipette-based mixing during the SPRI clean-up workflow. The data are for the liquid handler with an 8-channel pipette head.

Magnetic Bead-based Nucleic Acid Isolation

Bind-and-release of nucleic acids to solid-phase matrices is an essential tool in molecular biology. It is applied during isolation & purification of DNA and RNA from crude biological sample extracts, for desalting, buffer exchange, sample concentration, and separation by molecular weight/fragment length (Ali et al. 2017).

Solid-phase reversible immobilization (SPRI) (DeAngelis et al. 1995), using carboxylated silica-coated magnetic beads, is preferred over column- or filter-based immobilization for high-throughput Next Generation Sequencing (NGS) library preparation. The use of magnetic beads does not require filtration or centrifugation and is compatible with a wide range of volumes and nucleic acid concentrations. These features make SPRI beads easily adaptable into automation workflows on liquid handling platforms. Most NGS library prep protocols incorporate at least two and sometimes up to four magnetic bead clean-up steps (e.g., TruSeq DNA PCR-free, Illumina; Accel-NGS 2S, Swift Biosciences). A generic SPRI workflow used in NGS library prep is shown in ***Figure 2***.

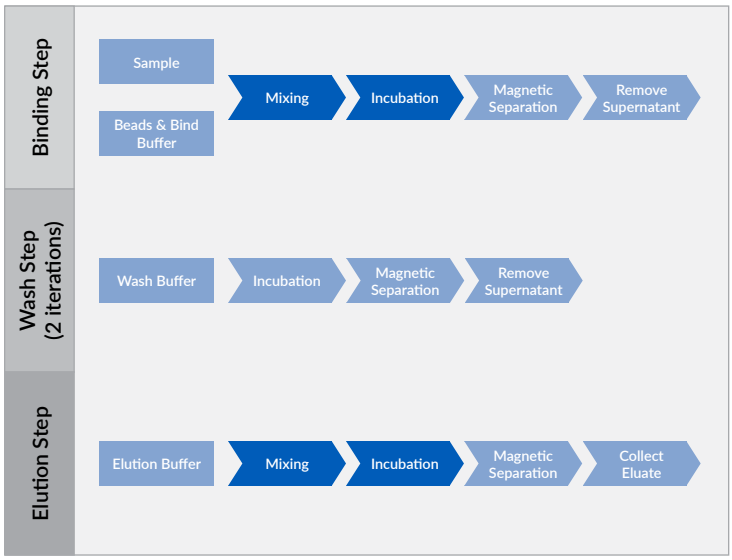

Figure 2. A magnetic bead-based nucleic acid clean-up workflow and its division into the bind step, wash step and elution step. Two critical events are the mixing and incubation steps (shown in black) essential for homogeneous distribution of magnetic beads and nucleic acids to ensure quantitative binding and recovery.

Materials and Methods

Required Materials

Covaris

- microTUBE-130 (PN 520045)
- 96 oneTUBE-10 AFA Plate (PN 520249)
- M220 Focused-ultrasonicator (PN 500295)
- LE220-plus Focused-ultrasonicator (PN 500569)

Other

- AMPure XP beads (Beckman Coulter, NC9933872)
- Lynx LM1200 Automated Platform with a 96-channel VVP head (Dynamic Devices)
- 80% ethanol
- 1x low-TE buffer (pH 8.0)

Methods

DNA was sheared to 300 bp (mode peak size) in a Covaris microTUBE-130 on a Covaris M220 and batched for all experiments. Magnetic bead binding, wash, and elution was done using 1.2x AMPure XP beads. The total binding volume was between 20 and 50 µl. Washing was done by adding 200 µl of 80% ethanol to magnet-immobilized beads and subsequently removing the supernatant. Elution of DNA from magnetic beads was achieved by adding between 10 and 50 µl 1x low-TE buffer (pH 8.0). All liquid handling/pipetting steps were performed in a 96 oneTUBE-10 AFA Plate on a Lynx LM1200 Automated Platform with a 96-channel VVP head (Dynamic Devices). AFA-induced Micromixing (AFA-Micromixing) was performed on a Covaris LE220-plus.

Results

Binding and elution steps using AFA-Micromixing where validated in separate experiments. For the bead binding evaluation, only the mixing step was performed using AFA-Micromixing; all steps other steps, including the mixing during elution, was done by pipetting. Conversely, for evaluating the bead elution step, only the mixing during elution was done with AFA-Micromixing.

AFA-Micromixing can be used instead of conventional pipette-mixing during bead binding and bead elution steps. There is no significant difference between yields when comparing AFA to pipette mixing. This holds true across a wide range of input DNA, i.e. 100 ng, 500 ng and 1 µg (***Figure 3***), as well as across different sample volumes (***Figure 4***).

As expected from these prior results, when AFA-micromixing is applied during bead binding and bead elution, the DNA yields are indistinguishable from pipette-mixing (***Figure 5***).

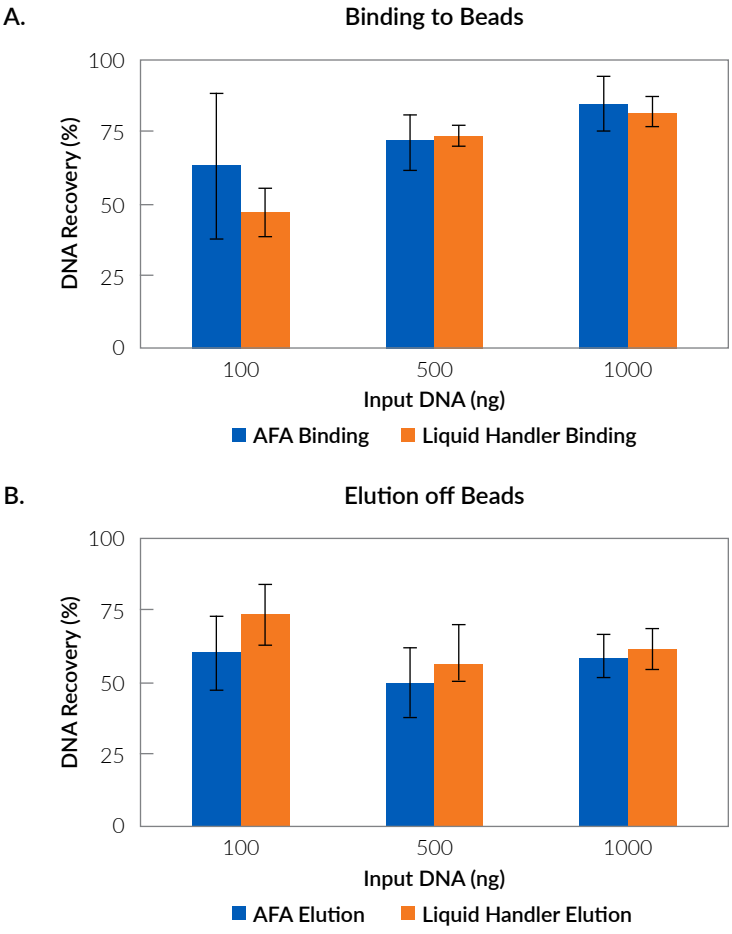

Figure 3. DNA yields across three different DNA input ranges obtained after (a) AFA-Micromixing during bead binding compared to conventional pipette-mixing, and after (b) AFA-Micromixing during bead elution.

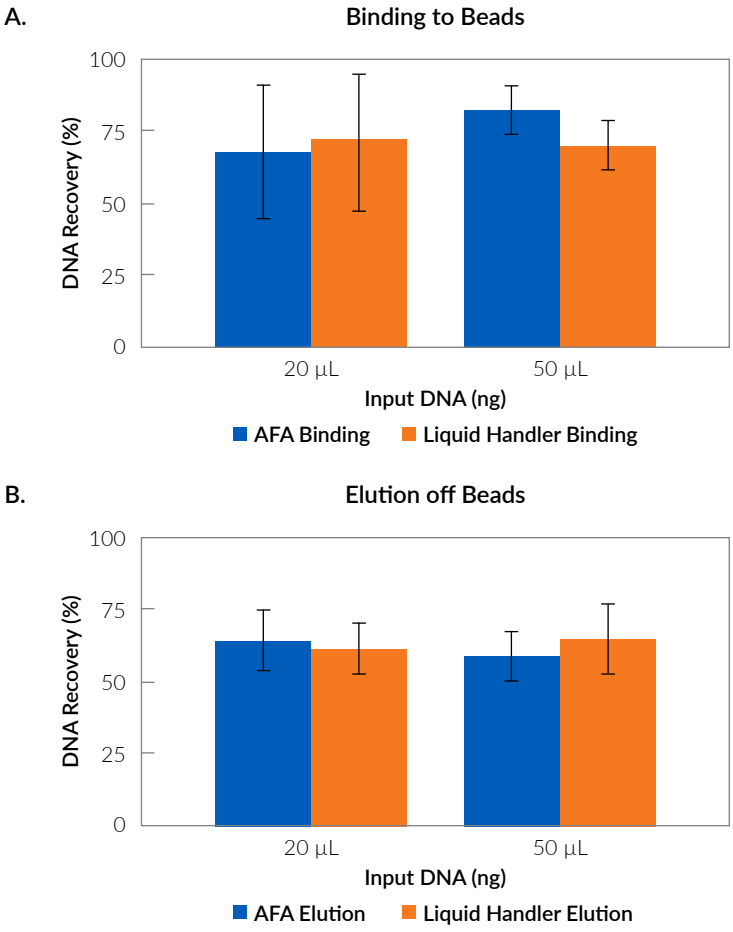

Figure 4. DNA yields across two different binding and elution volumes obtained after (a) AFA-Micromixing during bead binding compared to conventional pipette-mixing, and after (b) AFA-Micromixing during bead elution.

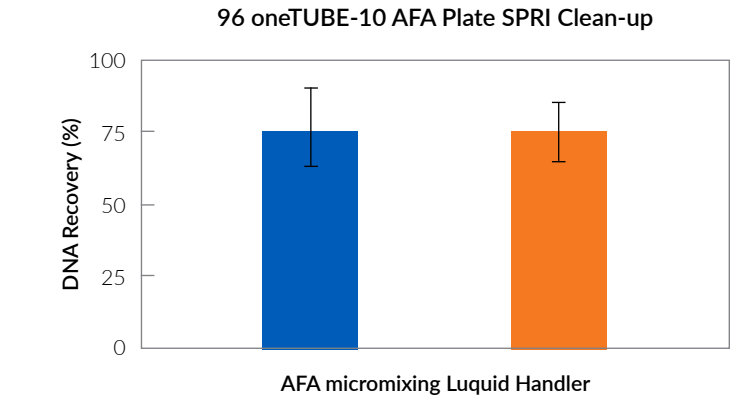

Figure 5. DNA yields in % to input after SPRI clean-up performed in a 96 oneTUBE-10 AFA plate (N=96) applying AFA-Micromixing as compared to conventional pipette-mixing.

DNA fragment distribution profiles as analyzed before and after bead binding and elution, and the two compared methods are indistinguishable, proving that AFA-Micromixing does not introduce biases during binding or elution (***Figure 6***).

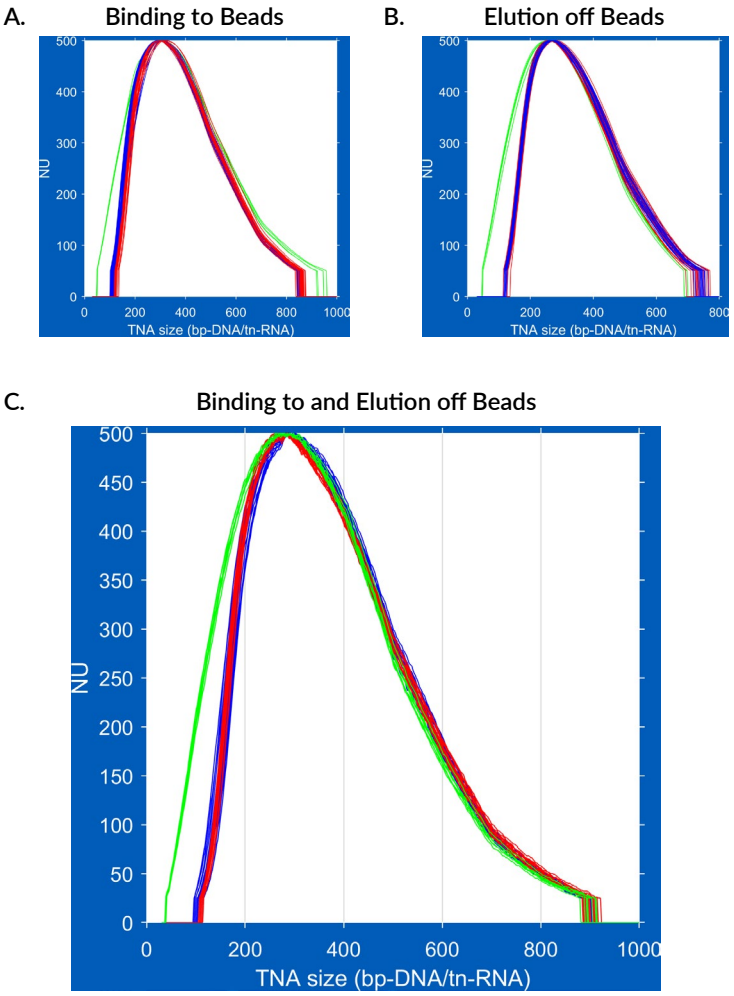

Figure 6. An electropherogram of 300 bp sheared DNA (green traces) is overlayed onto traces obtained from the same fragments after bead binding and bead elution. A and B: Fragment traces obtained by testing either binding or elution to or off beads (blue trace: AFA-Micromixing; red trace: pipette-mixing). C: Fragment traces obtained after binding and elution using AFA-Micromxing (blue) or pipette-mixing (red). All electropherograms are normalized to peak (mode) using Fragment Analyzer (AATI) output data.

Combined with the 96 oneTUBE-10 AFA plate and the LE220-plus or LE220R Focused-ultrasonicators, AFA-Micromixing will integrate seamlessly into higher-throughput sample clean-up steps during NGS library prep. The binding process for a whole 96- well plate currently requires 300 seconds, almost identical to pipette-mixing (320 seconds) with an 8-channel head. Elution, however, can be done in 120 seconds as compared to 320 seconds using a pipettor. The LE220-plus was modified to allow a continuous AFA sweeping mode that allows for a fast, very homogeneous AFA-treatment of the entire plate. This process is highly reproducible as indicated by the DNA yield shown in a heat map across plate wells (***Figure 7***).

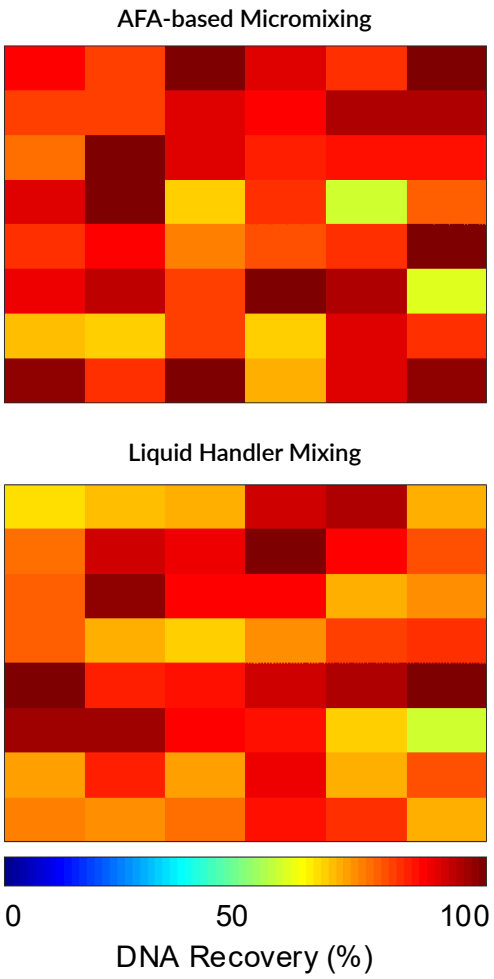

Figure 7. A heat map representing DNA yields in a 96 oneTUBE AFA plate obtained after AFA-Micromixing (N=48) as compared to pipette-induced mixing (N=48).

Conclusions and Outlook

- The 96 oneTUBE AFA Plate and 8 oneTUBE Strip open the possibilities to perform DNA shearing and subsequent downstream processing in the same vessel.
- The LE220-plus and LE220R Focused-ultrasonicators are automation-compatible units that can be used to integrate AFA into the library prep workflow.
- Covaris has developed protocols to perform non-contact micromixing during the binding and elution steps in the SPRI clean-up step. Please, inquire about details (applicationsupport@covaris.com).
- Non-contact, AFA-Micromixing is an alternative to conventional pipette-mixing, which offers consumable savings, reduction of cross-contamination risk, and freeing pipette head scheduling time.

Literature Cited

1. Ali N, Rampazzo RCP, Costa ADT, Krieger MA. Current Nucleic Acid Extraction Methods and Their Implications to Point-of-Care Diagnostics. Biomed Res Int. 2017;2017:9306564.
2. Deangelis MM, Wang DG, Hawkins TL. Solid-phase reversible immobilization for the isolation of PCR products. Nucleic Acids Res. 1995;23(22):4742-3.

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