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Technical Note

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Pipetting Best Practices for Covaris 96 microTUBE Plate and 8 microTUBE Strip in Automated Liquid Handlers

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Introduction

Next-Generation Sequencing (NGS) platforms require controlled generation of DNA fragments to produce high-quality sequencing data. Covaris leverages its proprietary and patented Adaptive Focused Acoustics® (AFA®) technology to convert focused high frequency acoustic energy into mechanical force to randomly fragment nucleic acids in an isothermal, non-contact environment. For higher throughput settings, Covaris labware comes in several multi-well formats, including the 96 microTUBE Plate and 8 microTUBE Strip that can be mounted in 12 column SBS format racks. Hardware and methods are described here which maximize the robustness of robot pipetting in Covaris consumables, particularly strategies for piercing seals and recovering the maximum amount of sample after mechanical DNA shearing.

Additionally, Covaris has developed the oneTUBE-10 product line compatible with the LE220-plus and ME220 Focused-ultrasonicators. The newly designed 96 oneTUBE-10 AFA Plate can be used as a typical SBS microplate and can be paired with the Automation Clamp for robust thin foil piercing.

Automation Clamp and Liquid Handling Robot Adapter

We have developed a clamp system to hold the Covaris microTUBE Plate or Rack on deck during all pipetting steps. This is a two part system which includes a universal clamp and robot-specific adapter. ***Figure 1*** shows the Covaris plate clamp system which is common to the pedestals for all liquid handling robots. The plate clamp system prevents SBS plates from lifting off the liquid handler deck when the tips are withdrawn from the seals. This clamp is compatible with all current Covaris plates and racks. Robot-specific mounting pedestals allow it to be compatible with seven common liquid handlers (***See Table 1***) with additional adapters available upon request. The pedestals used in the plate clamp system are designed to positively attach to the deck of each robot, so they do not lift off, and can be used as a traditional deck location when the top of the universal clamp is not attached.

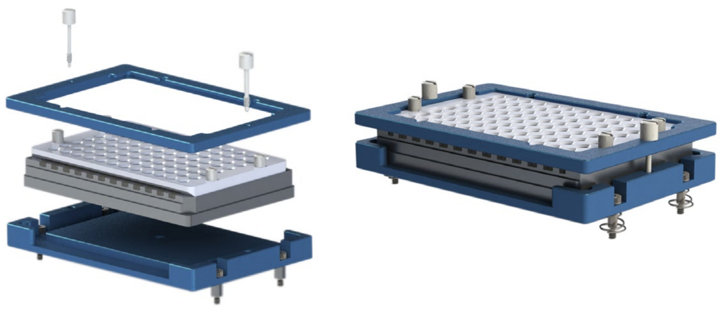


Figure 1. Left, an expanded view of the plate clamp system with rigid mounts. Right, the clamp spring mounts.

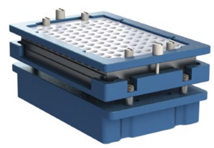
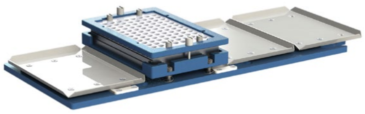
Liquid Handling Robot	Pedestal	Product Description
Universal Clamp (Required for all Liquid Handlers)		Automation Clamp Universal (PN 500600)
Agilent Bravo		Automation Adapter Agilent Bravo (PN 500598)
Beckman Coulter Biomek FX and NX		Automation Adapter Beckman Coulter Biomek FX/NX (PN 500599)
Hamilton Star and Starlet		Automation Adapter Hamilton Star/ Starlet (PN 500596)
Hamilton Nimbus		Automation Adapter Hamilton Nimbus (PN 500597)
Tecan Evo		Automation Adapter Tecan Evo (PN 500495)

Table 1. Pedestals for different liquid handlers.

Spring vs. Rigid Mounting of the Plate Clamp

The Covaris plate clamp system comes with two configurations: a rigid mount and a spring loaded mount (allowing about 5 mm of travel). Rigid mounting is easier to program since the plates are always at the same height. Pipetting and piercing seals on a spring-mounted plate requires more optimization, as the deflection of the plate will vary with type of pipette tip and the number of tips in use. Spring mounting allows for optimal sample recovery since the pipette tips can start below the level of the plate bottom, typically 0.5 mm, and be withdrawn during aspiration ensuring all tips touch the bottom of their respective wells. In general pipetting, this corrects for any lack of levelness or warping in the labware.

Liquid Handling Robot Setup

It is crucial to ensure a robot is properly trained on the deck layout and labware to ensure the instrument arrives at the desired height above the center bottom of each well. This requires setting X, Y, and Z coordinates for the deck locations and defining the labware correctly. Covaris microTUBE plate and strip definitions are available in product inserts, but direct measurement of source and destination plates with a micrometer may be required. It is also important to calibrate pipetting and ensure different liquid classes are optimized, so the robot is pipetting all reagents accurately.

Pipetting techniques for the following consumables are covered in this document:

1. 96 microTUBE Plate (PN 520078 and PN 520230)
2. 8 microTUBE Strip (PN 520053)
3. 8 microTUBE-130 AFA Fiber H Slit Strip V2 (PN 520239)
4. 8 microTUBE-15 AFA Beads H Slit Strip V2 (PN 520241)
5. 96 microTUBE-50 Plate (PN 520168 and PN 520232)
6. 8 microTUBE-50 AFA Fiber H Slit Strip V2 (PN 520240)

NOTE: Typical dead volume is around 3 to 5 µl. When looking to reduce this value, we have found that the most reliable method for sample transfer is multiple transfers to a destination plate with a centrifugation step. For example, up to five transfers with a quick spin of the plate after the fourth transfer (180 g, 1 minute) greatly reduces dead volume. With the spring mounted plate clamp, this method leaves <1 µl dead volume per well. A quick spin after shearing followed by successive transfers without intermediate spinning will typically leave 2 to 3 µl dead volume.

Pipetting Strategy: Piercing, Dispensing, and Aspirating

The goal when piercing seals is to ensure that the tips can get to the bottom of the wells and the pierced holes are large enough to prevent sealing around the tips. Various approaches are described in the robot specific methods below. When dispensing, tips should be near the top of the final liquid level such that they are submerged once dispensing is complete. This prevents droplets remaining on the tips and obviates the need to do a tip touch on the edge of the wells. After shearing, the sample needs to be aspirated out of the tube, typically leaving as little remaining as possible.

Summary of Best Practices for Pipetting from Covaris Labware

1. Designating one set of tips for piercing only and transferring with fresh tips is the most robust.
2. For offset pipetting, piercing one column at a time works well, although all robots tested could pierce all 96 at once (N.B., The Tecan was only tested with the 8 channel configuration).
3. Piercing with larger tips and pipetting with smaller tips, even if multiple liquid transfers are required, is robust.
4. Post shearing, re-piercing techniques should be the same as initial piercing of the new plate.

Agilent Bravo

Testing on the Bravo took place with the Agilent 250 µl clear filter tips (PN 19477-022). Alternatively, 180 µl sterile filter tips (PN 08585-102) could also be used. Since the 250 µl tips are slender and flexible, we do not recommend their use for multiple offset piercing. The same method was done for both the pre- and post-shearing pierce. Aspiration was performed using the spring plate starting from the bottom of the wells while withdrawing slowly during aspiration to progressively break any tip or well vacuums. With full tubes, an intermediate aspiration should be performed first before going to the bottom of the well. Three transfers were performed without a quick spin before the last transfer, leaving 2 to 3 µl remaining.

Tips for Piercing	250 µl Filter (PN 19477-022)
Tips for Aspiration and Dispensing	250 µl Filter (PN 19477-022)

Table 2. Pipetting details for Agilent Bravo.

Beckman Coulter Biomek FX & NX

A method was developed using the Biomek scripting language to pierce in a star pattern around the top of each well, resulting in a larger hole in the foil. This method is not critical with the 96 microTUBE AFA Plate Thin Foil and 8 microTUBE Strip, however it is very robust. Piercing is performed with Axygen P165 tips, whereas pipetting is done with Beckman P50s. When using the microTUBE-130 consumable, we recommend using multiple transfers with the smaller P50 tips rather than using larger tips.

Tips for Piercing	Axygen P165 (PN FXF-180-L-R-S)
Tips for Aspiration and Dispensing	Beckman P50 (PN A21586)

Table 3. Pipetting details for Beckman Coulter Biomek FX.

Hamilton Star, Starlet and Nimbus

Hamilton's Star, Starlet, and Nimbus share tips and software, though the deck layouts and pedestals are different. The Nimbus was tested using offset pipetting for the 8 microTUBE Strip, which we used to pick up as many columns of tips required for the number of samples being processed. On the Starlet, we have demonstrated an alternative method by piercing the 96 microTUBE Plate in one step with the P300s and successfully aspirating and dispensing with P50s.

Tips for Piercing	Hamilton P300 Clear Filter (PN 235832)
Tips for Aspiration and Dispensing	Hamilton P50 Clear Filter (PN 235831)

Table 4. Pipetting details for Hamilton Starlet and Nimbus.

Tecan Evo

An eight channel Evo was tested using 200 µl Tecan LiHa tips for piercing and pipetting, changing tips in between. Similar methods to the Bravo were used with deep piercing and multiple transfers. Alternatively, the Evo 1000 µl LiHa tips (PN 30057817) may be used for piercing and 50 µl LiHa tips (PN 30057813) may be used for transfers.

Tips for Piercing	Tecan LiHa 200 µl Filtered (PN 30057815)
Tips for Aspiration and Dispensing	Tecan LiHa 200 µl Filtered (PN 30057815)

Table 5. Pipetting details for Tecan Evo.

Conclusion

This document outlines solutions developed for Covaris consumables on the Agilent Bravo, the Beckman Coulter Biomek NX and FX, the Hamilton Star and Starlet, the Hamilton Nimbus, and the Tecan Evo. The pipetting techniques focus on the microTUBE-130 and microTUBE-15 consumables and can be edited for use with additional Covaris consumables, such as the microTUBE-50. Please note, pedestals for other liquid handlers not covered in this document are available upon request (contact Application Support for more information). Pipetting techniques described above may also be used with alternative adapters provided by liquid handler manufacturers.

Please contact Covaris at applicationsupport@covaris.com for support using the Automation Clamp and Adapter system.

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