truXTRAC® DNA Kit for truCOLLECT™-plus - Magnetic Bead (10)

Adaptive Focused Acoustics® (AFA) -based
DNA extraction from truCOLLECT-plus
using magnetic bead-based purification

PN 520260

INTENDED USE

The truXTRAC® DNA Kit for truCOLLECT™-plus - Magnetic Bead is intended for use in life science applications, such as molecular biology. This kit is designed to be used in conjunction with the truCOLLECT™-plus Stabilization and Transport kit (PN 520254) to extract and purify DNA from dry stabilized blood specimens from 80 µl of EDTA-stabilized whole blood.

This Research Use Only product is not intended for the diagnosis, prevention, or treatment of a disease.

INTRODUCTION

DNA extraction from blood is the method of choice for researchers, however, the logistics of collection, stabilization, and long-term storage, as well as DNA extraction from dried blood specimens remains difficult to adapt for use in downstream NGS-based analysis, due to inherently low DNA yields. DNA recovery from such dry-stabilized blood samples is performed using Covaris Adaptive Focused Acoustics (AFA®), which enables rapid rehydration and detachment of blood cells from the truCOLLECT-plus swabs. Furthermore, optimized DNA extraction buffers in combination with AFA ensures the efficient isolation and subsequent magnetic bead-based purification of high quality, molecular biology grade DNA. The yield of genomic DNA is dependent on white blood cell count and is generally in the range of 5 to 15 ng per microliter of blood.

If you require any assistance with this product please refer to Troubleshooting (Appendix B) in this protocol, check the FAQs found on our website, or contact Covaris Application Support at ApplicationSupport@covaris.com.

REVISION HISTORY

Part Number	Revision	Date	Description of change
010463	01 DRAFT	7/9/18	Kit Release of truXTRAC DNA Kit for truCOLLECT-plus – Magnetic Beads (E220)
010463	02	8/18	Adding M220, ME220, LE220

KIT CONTENTS

- AFA Conditioning Buffer 5 ml
- Buffer M1 5 ml
- Proteinase K (PK Solution) 1.25 ml
- Magnetic Bead Suspension 0.25 ml
- Buffer BB2 4.5 ml
- Buffer WB2* 4.5 ml
- Buffer WB5* 4.5 ml
- Buffer BE 3 ml
- microTUBE-500 AFA tube 10 tubes

***BUFFER CONCENTRATES. SEE PAGE 6, SECTION 1 FOR PREPARATION.**

SDS INFORMATION IS AVAILABLE AT http://covaris.com/resources/safety-data-sheets/

STORAGE

Upon arrival, store the Proteinase K solution and Magnetic Bead Suspension at 2 to 8C.

Store all other kit components at room temperature.

LABORATORY EQUIPMENT, CHEMICALS, AND CONSUMABLES TO BE SUPPLIED BY USER

Required Laboratory Equipment and Accessories

- truCOLLECT-plus dry-stabilized sample (Covaris, PN 520254)
- Magnet Stand for 2 ml tubes (e.g., Thermo Fisher Scientific, DynaMag™-2 Magnet, PN 12321D)
- Dry block heater with block to accommodate 2 ml tubes or temperature-controlled water bath able to accurately heat between 54-58C (56C ± 2C)

Required Chemicals

- 100% Isopropanol, ultra-pure (e.g., AmericanBio, PN AB07015)
- 200 proof Ethanol (e.g., AmericanBio, PN AB00515)

Required Consumables

- 2.0 ml nuclease-free microfuge tubes (e.g., Eppendorf Safe-Lock Tubes, PN 022363352)

Covaris Focused-ultrasonicator Accessories and Plate Definition

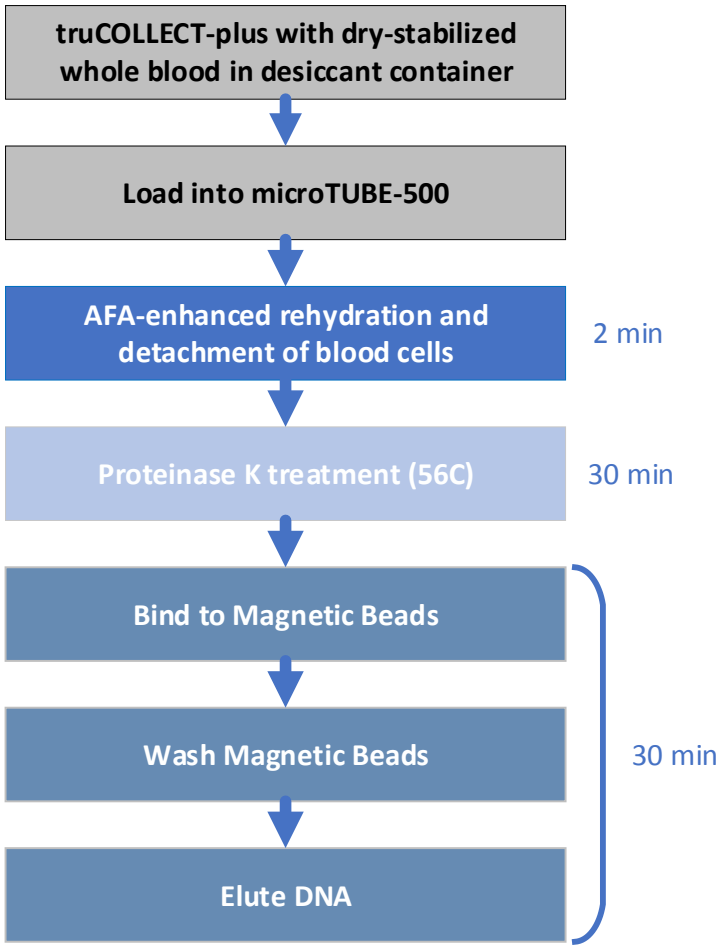
The table below contains the parts and plate definitions necessary to run the protocol.

Use the parts and plate definitions specific to your Covaris Focused-ultrasonicator.

Instrument	M220	ME220	E220	LE220
Holder/Rack Description (PN)	Holder XTU (500414)	Rack 4 Position microTUBE-500 (500525)	Rack 24 microTUBE-500 Screw-Cap (500452)	Rack 24 microTUBE-500 Screw-Cap (500452)
Plate definition file name	NA	<microTUBE-500 Screw-Cap>	<500452-24 microTUBE-500 Screw-Cap +6mm offset z enabled>	<500452-24 microTUBE-500 Screw-Cap +6mm offset z enabled>
Required Accessories (PN)	Insert XTU (500471)	ME220 Waveguide 4 Place (500534)	NA (Intensifier not required)	NA

truCOLLECT-plus Extraction And Purification Workflow

Using the Adaptive Focused Acoustics (AFA) process, dry-stabilized whole blood samples are rehydrated and detached from the swabs. Samples are treated with Proteinase K for 30 minutes at 56C. The genomic DNA is released and purified using magnetic beads.



1 – Preparation of Reagents

Follow these instructions before starting the DNA isolation process.

- **80% ethanol:** Prepare 80% ethanol by mixing 4 parts 100% ethanol with 1 part nuclease free water. One sample requires 1 ml of 80% ethanol. To prepare the total amount of 80% ethanol needed, multiply the number of samples to be processed by 1.1 ml.

- **Buffer WB2:** Before the 1st use of the kit, add 3 ml 100% Isopropanol to the Buffer WB2, close bottle tightly and mix by inverting the bottle 5 times. Mark the bottle to indicate that the Isopropanol was added.

- **Buffer WB5:** Before the 1st use of the kit, add 3 ml 100% Isopropanol to the Buffer WB5, close bottle tightly and mix by inverting the bottle 5 times. Mark the bottle to indicate that the Isopropanol was added.

2 – Preparation of Heat Block

1. Preheat dry block heater to 56C ± 2C. It is critical that this temperature is accurate to successfully execute the protocol.

2. Test the temperature of your heat block:
    a. Place a microcentrifuge tube (1.5 or 2 ml) filled with water into the heat block.
    b. Immerse a thermometer into the tube.
    c. Wait until the temperature has reached the plateau.
    d. Adjust the Set-temperature accordingly until the temperature inside the microcentrifuge tube has reached 56C ± 2C.

4 – FOCUSED-ULTRASONICATOR SETUP

For detailed instructions on how to prepare and use your instrument, please refer to the respective Covaris User Manual. If you do not see a Plate Definition on your system, please contact Covaris Technical Support (techsupport@covaris.com)

Refer to page 4 for Plate Definitions and required Focused-ultrasonicator accessories

1. **Create "truCOLLECT-plus" program in SonoLab™**
   Use the settings provided in the table below, specific to your Covaris instrument type, to create a program called "truCOLLECT-plus" using the Covaris SonoLab method editor. The truCOLLECT-plus protocols require the set up of dithering parameters. Save the program for later use. Refer to Appendix A for protocol creation.

Instrument	M220	ME220	E220	LE220
Peak Incident Power (PIP) (Watt)	50	75	75	350
Duty Factor (%)	25	25	25	25
Cycles Per Burst (CPB)	1000	1000	1000	1000
Treatment time (seconds)	120	120	120	120
Bath temperature (°C)	20	20	20	20
Water Level (run)	Fill to sample	Automatic	5	5
Dithering (See Appendix A)	NA	X Dither: 2.0mm Z Dither: 2.0mm Dither Speed: 10mm/sec	X Dither: 1.0mm Y Dither: 1.0mm X-Y Dither Speed: 10mm/sec Z Dither: 3.0mm Z Dither Speed: 10mm/sec	X Dither: 1.0mm X-Y Dither Speed: 10mm/sec Z Dither: 3.0mm Z Dither Speed: 10mm/sec
Plate definition	NA	<microTUBE-500 Screw-Cap>	<500452-24 microTUBE-500 Screw-Cap +6mm offset z enabled>	<500452-24 microTUBE-500 Screw-Cap +6mm offset z enabled>

5 – DNA PURIFICATION

1. Set up the dry-heat block as explained in Section-3 and verify the block temperature to be 56C. The heat block set to 56C is required for Proteinase K incubation (Step 13) and DNA elution after purification via magnetic beads (Step 43).

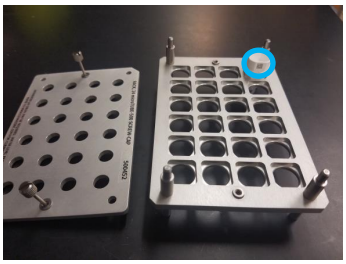
2. Add 400 µl of **AFA Conditioning Buffer** to a microTUBE-500 AFA processing tube. Prepare the appropriate number of microTUBE-500 tubes for the number of samples to be processed.

3. Open the truCOLLECT-plus desiccant storage container and remove the truCOLLECT-plus cap/swab.

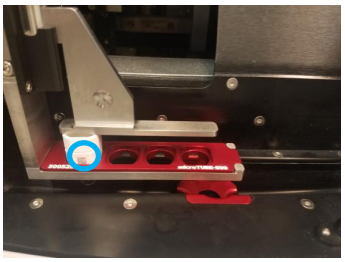
4. Carefully insert the swab into a microTUBE. Avoid spilling Conditioning Buffer. Seal the microTUBE-500 by turning the cap until it stops.

5. Place the microTUBE-500s in the appropriate rack for your Focused-ultrasonicator. The rack must be positioned how it will fit into the instrument. The barcodes on the truCOLLECT cap/swab must be positioned facing you. See Appendix B.

E220 and LE220



ME220



6. Load the rack into the Focused-ultrasonicator for processing.

7. Process the sample using the "***truCOLLECT-plus***" program on your Covaris Focused-ultrasonicator.

8. Remove the microTUBE-500 rack from the Focused-ultrasonicator and remove the microTUBE-500s from the rack.

9. Carefully unscrew and remove the cap/swab. Slowly remove 350 µl of the lysate and transfer to a 2 ml microcentrifuge tube. (See Appendix C - Troubleshooting for any issues.)

> CAUTION: Some swabs may break at the top of the handle due to the AFA-energetics. This will not impact DNA yields. Use caution when removing the cap/swabs.

10. Add 390 µl of Buffer M1.

11. Add 80 µl of Proteinase K.

12. Cap the tube and vortex for 5 seconds at maximum speed.

13. Place 2 ml tube into heat block pre-heated to 56C and incubate for 30 minutes at 56C.

14. During the incubation, prepare the IPA/Magnetic Bead Mix according to Table 1 and mix thoroughly.

Table 1 – IPA/Magnetic Bead Mix

Reagent	Volume for one sample*	Volume for N samples*
Isopropanol (IPA)	**860** µl	**860** µl x N
Magnetic Bead Suspension	**16.5** µl	**16.5** µl x N

* calculation includes 10% excess in final volume

15. Remove the tube from the 56C heat block.

16. Add 350 µl of BB2 to the 2 ml tube.

17. Cap the tube and vortex for 5 seconds at maximum speed.

CAUTION: Thoroughly vortex the Magnetic Bead Suspension and IPA/Magnetic Bead Mix before using to ensure a homogenous suspension. Beads will settle when left standing.

18. Add 800 µl of the IPA/Magnetic Bead Mix.

19. Cap the tube and invert the solution 2-3 times. Then vortex for 15 seconds, or until homogenous.

20. Incubate the tube on the benchtop at room temperature for 5 minutes to ensure complete binding of the DNA.

21. Vortex for 15 seconds or until solution is homogenous.

22. Place the tube on the magnetic stand and incubate for 5 minutes until the beads have been pulled to the magnet.

23. With the tube still on the magnet, carefully remove and discard the supernatant using a 1000 µl pipette. Avoid touching or disturbing the bead pellet.

24. Add 500 µl of prepared Buffer WB2.

25. Cap the tube and vortex for 10 seconds. Confirm that all beads are resuspended. If the beads are still sticking to the wall, continue vortexing until all beads are resuspended.

26. Place the tube on the magnetic stand and incubate for 2 minutes until the beads have been pulled to the magnet.

27. Remove and discard the supernatant without disturbing the bead pellet using a 1000 µl pipette.

28. Add 500 µl of prepared Buffer WB5.

29. Cap the tube and vortex for 10 seconds. Confirm that all beads are resuspended. If the beads are still sticking to the wall, continue vortexing until all beads are resuspended.

30. Place the tube on the magnetic stand and incubate for 2 minutes until the beads have been pulled to the magnet.

31. Remove and discard the supernatant without disturbing the bead pellet using a 1000 µl pipette.

32. Add 500 µl 80% ethanol.

33. Cap the tube and vortex for 10 seconds. Confirm that all beads are resuspended. If the beads are still sticking to the wall, continue vortexing until all beads are resuspended.

34. Place the tube on the magnetic stand and incubate for 2 minutes until the beads have been pulled to the magnet.

35. Remove and discard the supernatant without disturbing the bead pellet.

36. Add 500 µl 80% ethanol.

37. Cap the tube and vortex for 10 seconds. Confirm that all beads are in suspension. If beads are still sticking to the wall continue vortexing until all are suspended.

38. Place the tube on the magnetic stand and incubate for 2 minutes until the beads have been pulled to the magnet.

39. Remove and discard as much of the supernatant as possible using a 1000 µl pipette. Use a 20 µl pipette to remove the remaining liquid from the bottom of the tube.

40. Leave the tube open on the magnetic stand and let the beads dry for 5 minutes at room temperature.

CAUTION: Make sure that the ethanol has evaporated before continuing with elution. Residual ethanol can inhibit the elution and impact downstream applications such as PCR.

41. Remove the tube from the magnetic stand and add 50 µl to 100 µl of Buffer BE (5 mM TrisCl pH 8.5) into the tube. A 50 µl elution volume will result in a higher DNA concentration.

42. Resuspend the beads by pipetting up and down 20 times. Ensure that all the beads are resuspended in the buffer and none are still sticking to the wall of the tube.

43. Cap the tube and incubate the microcentrifuge tube in the heat block set to 56C for 5 minutes.

44. Remove the tube from the heat block, place it on the magnetic stand, and incubate for 2 minutes.

45. Transfer the eluate into a clean elution tube without transferring beads.

    Isolated DNA should be kept at 2-8C for short term storage (1 to 2 days) and -20C for long term storage.

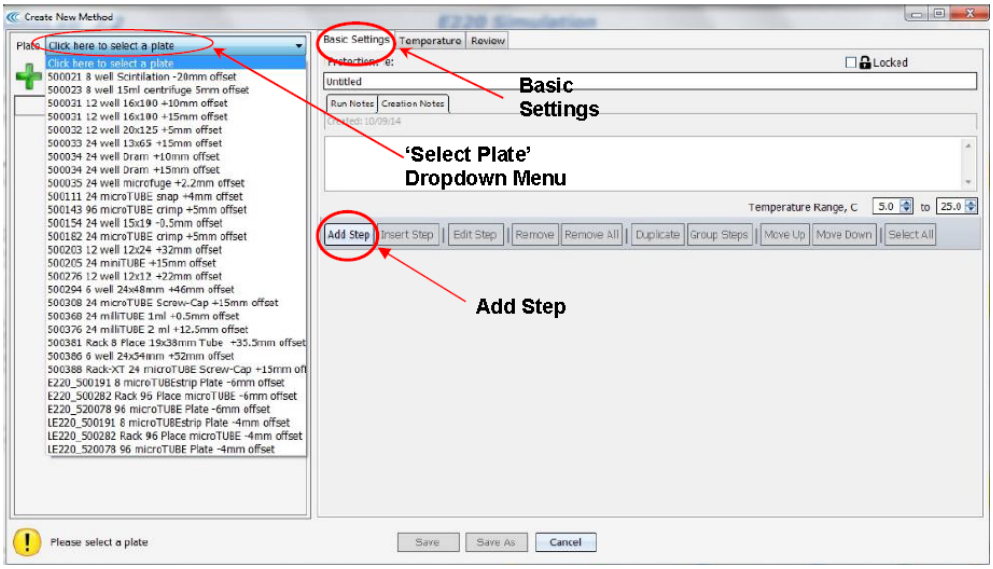
APPENDIX A – PROTOCOL CREATION IN SONOLAB

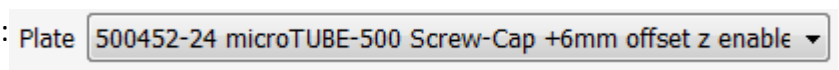
E220 and LE220 with SonoLab 7.3

1. Refer to your instrument user manual.
   a. https://covaris.com/wp-content/uploads/010277-E-and-LE-series-Manual_Rev-F.pdf
2. Refer to the protocols.

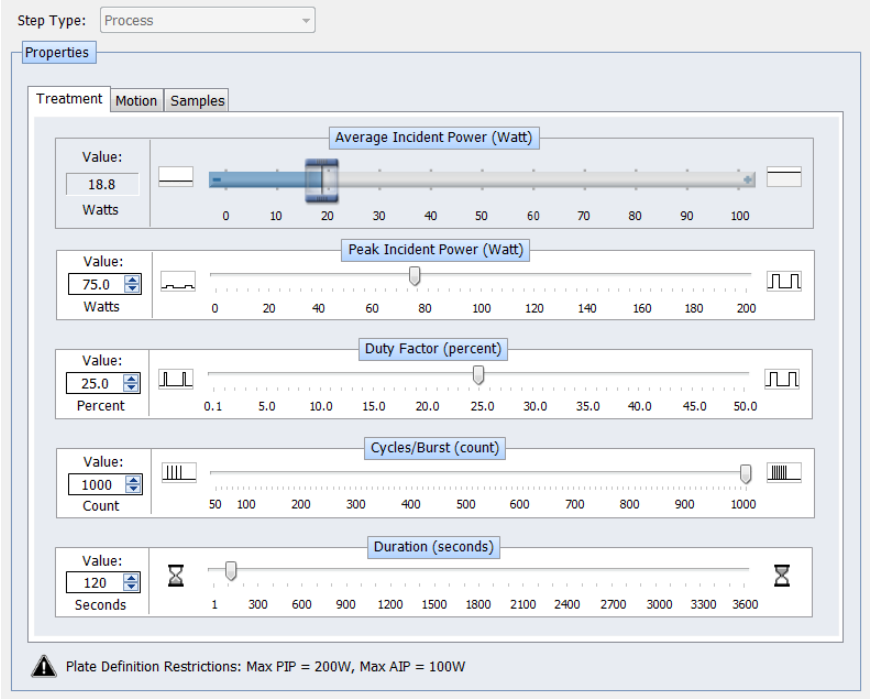
Instrument	E220	LE220
Peak Incident Power (PIP) (Watt)	75	350
Duty Factor (%)	25	25
Cycles Per Burst (CPB)	1000	1000
Treatment time (seconds)	120	120
Bath temperature (°C)	20	20
Water Level (run)	5	5
Dithering	X Dither: 1.0mm Y Dither: 1.0mm X-Y Dither Speed: 10mm/sec Z Dither: 3.0mm Z Dither Speed: 10mm/sec	X Dither: 1.0mm X-Y Dither Speed: 10mm/sec Z Dither: 3.0mm Z Dither Speed: 10mm/sec
Plate definition	<500452-24 microTUBE-500 Screw-Cap +6mm offset z enabled>	<500452-24 microTUBE-500 Screw-Cap +6mm offset z enabled>

3. On the Run screen, click the “New…” button in the Method window.
4. In the Basic Settings tab, first select the 500452 plate with z dither enabled.

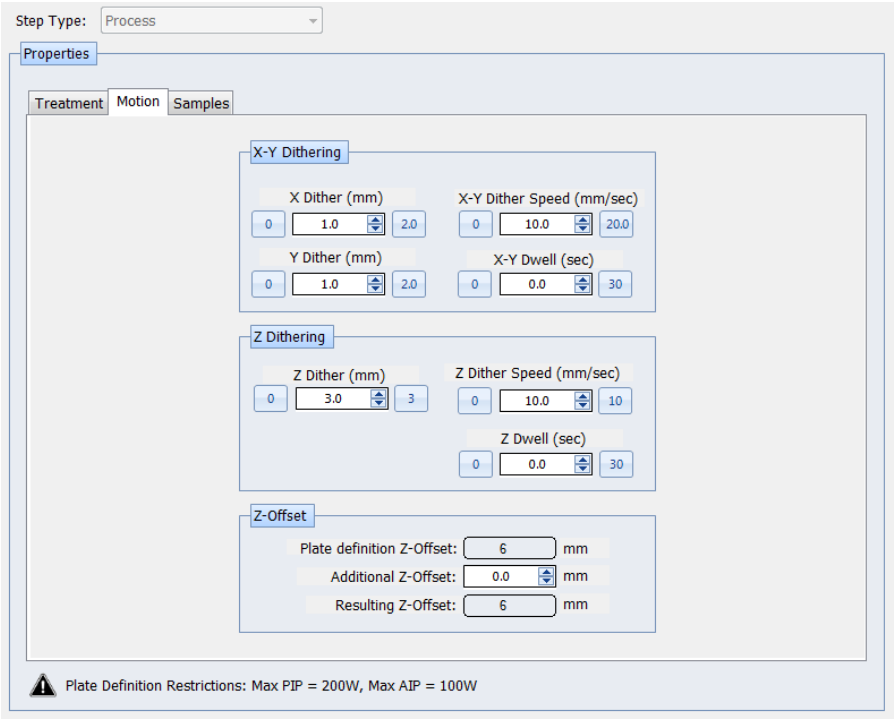


a. Plate: 

5. Rename the “Untitled” protocol to “truCOLLECT-plus.”
6. Click the “Add Step” button. In the Treatment tab, input the settings for the truCOLLECT-plus. (The image below is for the E220.)



7. In the Motion tab, input the motion settings to enable x, y, and z dithering. (The image below is for the E220.)



8. Save the protocol.

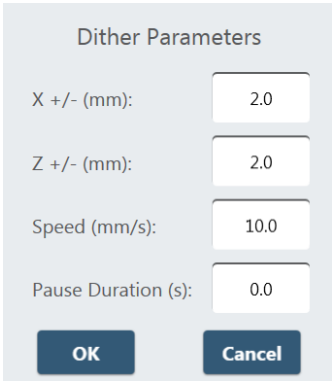
ME220 with SonoLab 8

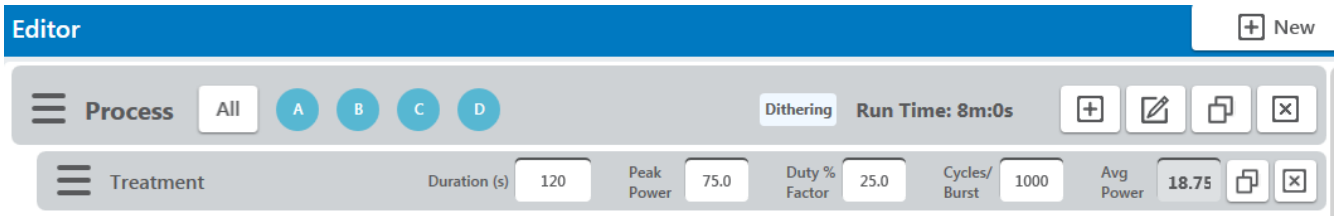
1. Refer to the ME220 User Manual https://covaris.com/wp-content/uploads/pn_010325.pdf
2. Select <microTUBE-500 Screw-Cap> from the drop down menu.
3. Click “Edit.”
4. In the Editor box, select the number of samples to run in the Process bar.
5. Change the AFA settings in the Treatment bar.

Instrument	ME220
Peak Incident Power (PIP) (Watt)	75
Duty Factor (%)	25
Cycles Per Burst (CPB)	1000
Treatment time (seconds)	120
Bath temperature (°C)	20
Water Level (run)	Automatic
Dithering	X Dither: 2.0mm Z Dither: 2.0mm Dither Speed: 10mm/sec
Plate definition	<microTUBE-500 Screw-Cap>

6. Click the “Edit Process Settings” button.

7. Input the X- and Z-dithering values and click OK.



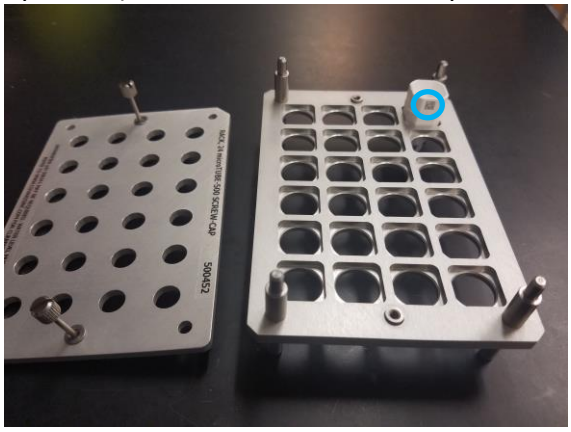


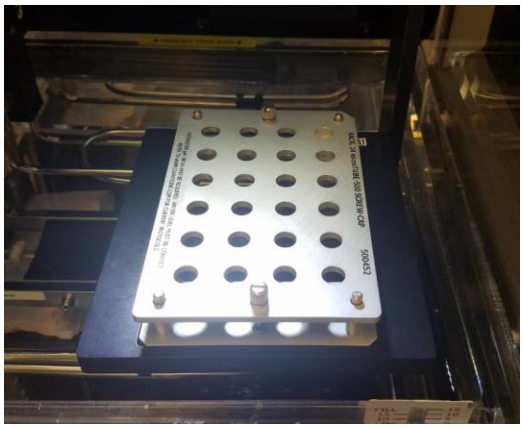
8. Rename the protocol as “truCOLLECT-plus.”
9. Click “Save.”

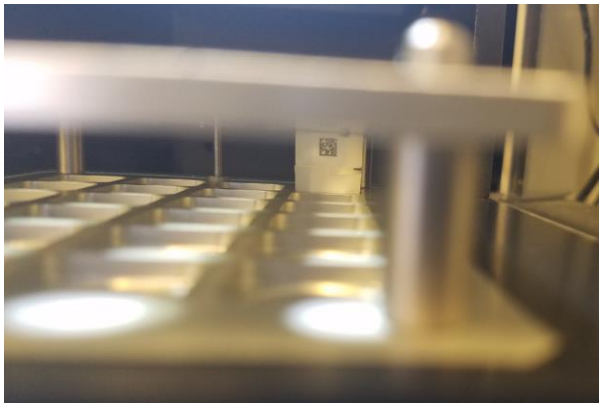
APPENDIX B – LOADING TRUCOLLECT-PLUS MICROTUBES IN THE FOCUSED-ULTRASONICATOR

E220 and LE220

1. The barcodes of the truCOLLECTs should be facing the bottom the rack 500452 (toward the operator) so the swabs are lined up in the same direction. Refer to the pictures below.

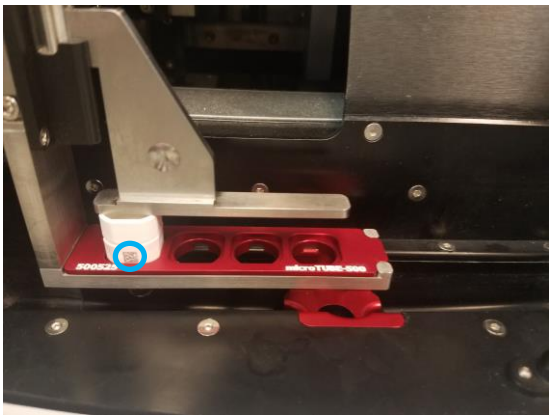






ME220

1. The barcodes of the truCOLLECTs should be facing the operator. Refer to the picture below.



APPENDIX C – TROUBLESHOOTING GUIDE

Issue	Cause	Solution	Comments / Suggestions
Low yield of DNA	Loss of magnetic beads during purification steps	Remove supernatant of bind and wash steps slowly and carefully. If beads appear in the pipette tip, eject the liquid back into the tube, wait for 1 minute, and try aspirating the supernatant again.	The viscosity of buffers BB2, WB2, and WB5 can make supernatant removal difficult.
	Too much liquid in the cap	Pipette cap after vortexing.	Beads can get stuck in the cap of the 2 ml tube.
Low A260/230	Residual salt	After vortexing the beads during the wash WB2 and WB5, quickly centrifuge samples to remove residual buffer from the sides of the tube.	
Swab handle breaks	AFA-energetics	Carefully remove the cap after AFA treatment. The swab may stay in the tube while removing the 350 µl for purification.	Sample loss will be negligible with remaining liquid.
Low concentration of DNA	Elution volume too high	Use lower elution volume in Step 41.	Only 50 µl and 100 µl have been tested.
Low sample volume recovery after AFA	Liquid absorbed by the swabs	Pipette liquid off of the swab.	Due to the viscosity and absorbance capacity of the swab, the liquid can stick to the swab while removing it from the AFA tube.

ADDITIONAL NOTES

1. See following link: http://covaris.com/resources/protocols/ for updates to this document.
2. Patents pending