

truXTRAC™ FFPE DNA 8 microTUBE Strip Kit for chemagen Technology (96)

Adaptive Focused Acoustics™ (AFA) -based DNA extraction and purification from Formalin-Fixed, Paraffin-Embedded (FFPE) Tissue

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INTENDED USE

The truXTRAC FFPE DNA Kit is intended for use in molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

INTRODUCTION

The truXTRAC FFPE DNA Kit for chemagen Technology is designed for the efficient extraction of DNA from Formalin Fixed, Paraffin Embedded (FFPE) tissue samples with Covaris Adaptive Focused Acoustics (AFA™) and subsequent purification with PerkinElmer chemagen Technology. The process results in high yields of high-quality DNA well suited for analytical methods such as next-generation sequencing or qPCR.

AFA enables effective removal of paraffin from FFPE tissue samples in aqueous buffer, with simultaneous tissue rehydration. Compared to chemical-based methods of paraffin removal, this mechanical process is not as limited by the thickness of FFPE tissue sections. The ability to use thicker sections can increase DNA yield and minimize the impact of increased DNA degradation at the exposed surfaces of a section.

This protocol is optimized for sections up to 25 µm in thickness and cores up to 1.2 mm in diameter.

Important Notes on FFPE Samples:

The yield of DNA from FFPE tissue blocks is highly variable. Factors such as fixation time, size and thickness of the sections, the ratio of tissue to wax, the type of tissue, and the age of the FFPE block are the main causes for this variability.

The quality of DNA isolated from FFPE samples depends on many of the same factors and is thus also highly variable. During the fixation process, DNA is cross-linked to proteins and other nucleic acid molecules to varying degrees. Incomplete reversal of this crosslinking may cause the isolated DNA to perform less well in downstream enzymatic applications such as qPCR. In addition, the size of DNA fragments isolated from FFPE samples is generally smaller than that of DNA isolated from fresh or frozen tissues. This is particularly evident in older FFPE sample blocks or sample blocks stored at elevated temperatures.

Note for first time users:

Given the highly variable yield of DNA from FFPE tissue blocks, we recommend using FFPE blocks that have been well characterized for yield and quality for initial testing of the truXTRAC FFPE kit. Ideally, samples should be extracted immediately after sectioning.

Please contact Covaris at Application Support (ApplicationSupport@covarisinc.com) if you have any questions.

REVISION HISTORY

Part Number	Revision	Date	Description of change
010295	A	July 2015	Initial release

KIT CONTENTS

Tissue SDS Buffer	10 ml
PK Solution	2x 1.25 ml
8 microTUBE-130 AFA Fiber Uncapped Strip V2	12

SDS information available at <http://covarisinc.com/resources/safety-data-sheets/>

STORAGE

This kit should be stored at room temperature (18 – 25 °C).

SUPPLIED BY USERS

Covaris Instruments and Parts

Required parts			
Focused-ultrasonicator	LE220	E220 & E210	E220 evolution
Rack/ Holder/ Insert	Rack-XT 12 Place 8 microTUBE Strip V2 (PN500485)	Rack 12 Place 8 microTUBE Strip V2 (PN500444)	Rack E220e 8 microTUBE Strip V2 (PN500437)
Accessories	Heat Block Rack 12 Place 8 microTUBE Strip V2* (PN500481) (x2)		Heat Block Rack E220e 8 microTUBE Strip V2* (PN500493) (x2)
	*Referred to as Covaris Heat Block in text. Cap Press Tool 8 microTUBE Strip V2 (PN 500469)		
Chemagen Kit	Prepito truXTRAC DNA FFPE Kit (CMG-2037)		
Optional parts			
Accessories	FFPE tissuePICK (PN 520163)		
	FFPE sectionPICK (PN 520149)		
	FFPE sectionWARMER (PN 500403)		

PerkinElmer chemagic Instruments and Parts

- chemagic Prepito-D or equivalent
- Prepito truXTRAC DNA FFPE Kit (CMG-2037)

Other supplies:

- Dry block heater (eg, from VWR) with at least two-blocks capacity. We recommend two dry block heaters, preset at **T set₁** and **T set₂** respectively. See Appendix D and E for nominal dimensions of the Covaris Heat Block and calibration procedure.
- RNase A (DNase free) at 10 mg/ml e.g., Thermo Scientific (PN EN0531)

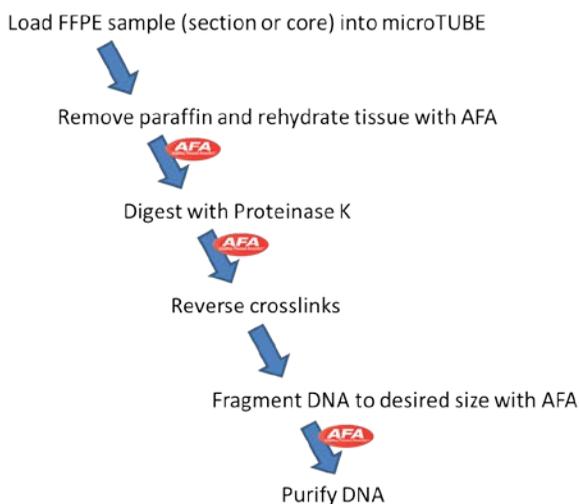
PROCEDURE WORKFLOW OVERVIEW

Three different protocols are supported for the Covaris truXTRAC FFPE DNA Kit. The three options have different DNA extraction workflows. The DNA purification workflow is identical for all three options.

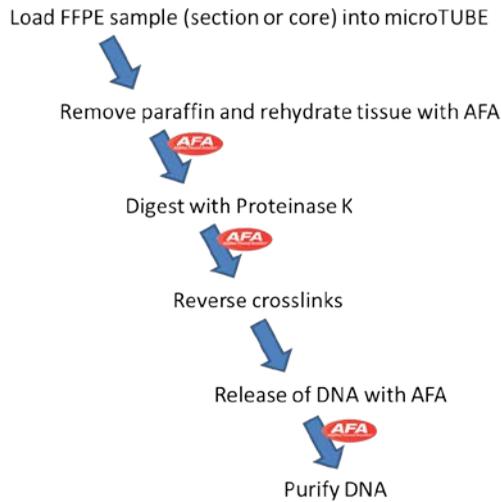
- Option A:** Shear DNA during extraction to a size suitable for next-generation sequencing library construction. Fragment size can be tuned between 200 and 400 bp.
- Option B:** Extract ~2kb DNA fragments. This protocol is recommended for most analytical applications, including PCR. Note that actual DNA fragment size will depend of the quality of the starting material.
- Option C:** Extract large “genomic” DNA without any additional fragmentation. Actual DNA fragment size will depend on the quality of the starting material. For high-quality FFPE tissue blocks, we typically see an average fragment size of ≥ 8 kb.

Please refer to Appendix A for examples of final DNA fragment size distribution.

OPTION A – EXTRACT AND FRAGMENT DNA (FOR NGS)

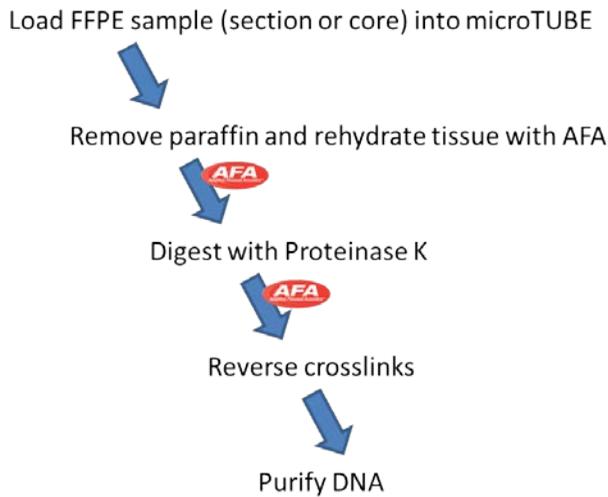


OPTION B - EXTRACT LARGE DNA FRAGMENTS (>2 KB)* WITH IMPROVED YIELD



* Actual DNA fragment size will depend of the quality of the starting tissue block

OPTION C - EXTRACT “GENOMIC” DNA*



* Actual DNA fragment size will depend of the quality of the starting tissue block

1 - PREPARATION

FFPE Tissue Sample

1. Sample Input requirements

The truXTRAC process is highly efficient at removing paraffin even from relatively thick FFPE sections while simultaneously rehydrating the tissue. Use of thicker sections is often desirable, both for increased yield and since DNA or RNA in the exposed surfaces of a section tends to degrade quickly. **We recommend using sections between 15 and 25 µm thick, or cores of 1.2 mm.**

IMPORTANT: Excess paraffin will adversely affect the yield and quality of DNA and RNA extracted from FFPE. We strongly advise trimming off any excess of paraffin before sectioning a FFPE tissue block, or after the section has been cut from the FFPE block. A ratio of 80% tissue to 20% paraffin or higher is ideal.

The total mass of FFPE sample processed per extraction should be between 2 to 5 mg. Lower amounts may result in insufficient yield.

	FFPE Sections		FFPE Sections		FFPE Cores
	Mounted on slide		"scrolls" or "curls"		
Size (thickness or diameter)	4 to 10 µm	7 to 10 µm	7 to 15 µm	16 to 25 µm	< 1.2 mm diameter
Size (length)	NA		<10 mm (if longer, cut in half before loading)		
Collection tool	tissuePICK	sectionPICK	NA		
Maximum number of TRIMMED samples Per Tube	2x tissuePICK (200 mm ² tissue for a 5 µm section)	2x sectionPICK	2	1	1

NOTE: For optimal tissuePICK and sectionPICK performances, tissue section should be mounted on uncoated slides. The tissuePICK and sectionPICK should always be used in conjunction with a sectionWARMER.

2. Tissue Fixation Requirements

The yield and quality of DNA extracted from FFPE tissue blocks is highly dependent on tissue collection and paraffin embedding procedures. For good yields of high quality DNA:

- Use a maximum fixation time of 24 hours.
- Use Formalin solution, neutral buffered, 4%.
- Fix sample tissue sample as quickly as possible after collection.

Buffer

1. **Check Tissue SDS Buffer:** A white precipitate may form during storage. Incubate the bottle at 50 – 70 °C before use to dissolve any precipitate.

Instruments

NOTE: For detailed instructions on how to prepare your particular instrument, please refer to your instrument’s User Manual.

1. For E and LE-Series Focused-ultrasonicators, fill the water bath, set the chiller temperature as described in Table 1, and allow the system temperature to equilibrate and the water bath to degas.
 - For E210 or E220, load the plate definition “E220_500444 Rack 12 Place 8 microTUBE Strip V2 -1.5mm offset” and check that the intensifier is in place.
 - For E220 evolution load the plate definition “500437 Rack E220e 8 microTUBE Strip V2 - 6mm offset” and check that the intensifier is in place.
 - For LE-Series: Load the plate definition “LE220_500485 Rack-XT 12 Place 8 microTUBE Strip V2 -1.5mm offset.”

NOTE: If you do not see the correct plate definition on your system, please contact Covaris technical support at TechSupport@covarisinc.com.

Table 1 - Focused-ultrasonicator setup

Instrument	Water level (Fill/RUN scale)	Chiller temperature
E-Series & L-Series	6	18°C

Prepare Heating Block

Dry block heaters should be preset at **T set₁** and **T set₂**. See Appendix D and E for instructions on how to calibrate and install the Covaris Heat Blocks.

PerkinElmer chemagic Prepito[®]-D

Please refer to the chemagic Prepito-D instrument user manual (revision 2022-0020)

2 - DNA EXTRACTION FROM FFPE TISSUE

1. Using Table 2 below as a guide, generate the Processing Buffer master mix by mixing Tissue Lysis Buffer and Proteinase K.

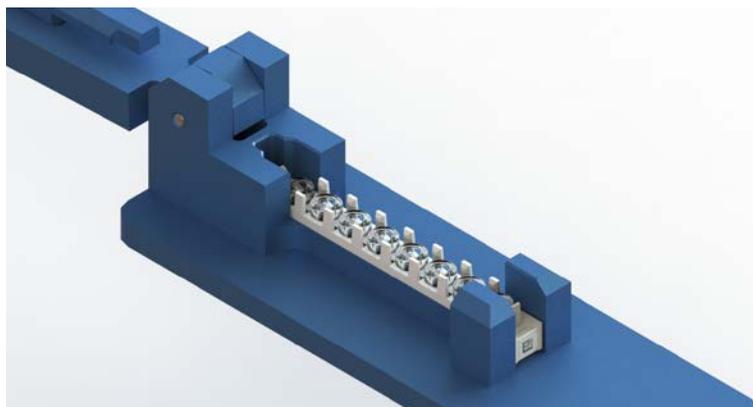
Table 2 – Processing Buffer master mix

Number of samples	Tissue SDS Buffer volume	Proteinase K volume
8	704 μ l	176 μ l
16	1408 μ l	352 μ l
x	x * 88 μ l	x * 22 μ l

2. Carefully peel off the blue tape from the 8 microTUBE 130 Strip and remove the Cap Strip. Keep 8 microTUBE-130 Strip upright while handling, to avoid losing the tube insert!



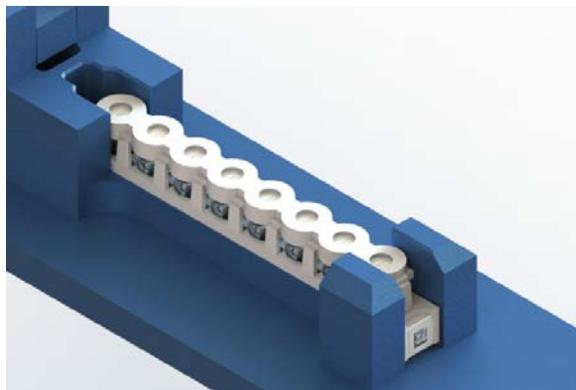
3. Load the 8 microTUBE-130 Strip (without Cap Strip) into the Cap Press Tool as shown.



4. Add 100 μ l Processing Buffer master mix into each microTUBE and load FFPE tissue (section or core).

NOTE: if the FFPE tissue samples are loose or broken, the samples may be added to the microTUBE prior to Processing Buffer addition to facilitate easier loading.

5. Place the Cap Strip on top of the 8 microTUBE-130 Strip, with the white side facing up.



6. Close the Cap Press Tool and apply pressure until the lever bottoms out, snapping the Cap Strip into place. Lift the Cap Press Tool and make sure that the Cap Strip is level.



7. Load 8 microTUBE-130 Strip into Rack.



8. Repeat steps 2 to 7 until all samples have been loaded.
9. Close the Rack using the screws affixed to the cover.



10. Process the sample using the settings provided in Table 3 below to dissociate the paraffin while simultaneously rehydrating the tissue. (Please see the example in Appendix B.) During the AFA process, it is normal for the solution to turn milky white as the paraffin is emulsified.

NOTE: Processed samples are stable for up to 8 hours while remaining samples are treated.

Table 3 - Paraffin removal and tissue rehydration settings

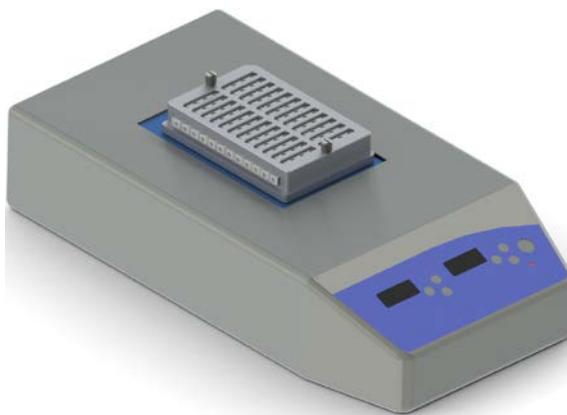
System	Duty Factor	Peak Incident Power	Cycles per burst	Treatment Time	Temperature (Instrument)
E220	10%	175 Watts	200	300 sec	20 °C
E210	10%	5 (Intensity)	200	300 sec	20 °C
LE220	20%	450 Watts⁽¹⁾	200	300 sec	20 °C

⁽¹⁾ Since PIP is distributed across multiple microTUBEs in an LE220, the power received by individual microTUBE stays within the 200 W limit.

11. Protein digestion at **T set₁**

IMPORTANT: Use a Covaris Heat Block inside the dry block heater (instead of a standard heat block for microfuge tubes).

Calibrate the dry block heater before use (see Appendix E for instructions). Failure to calibrate the dry block heater may lead to incomplete tissue digestion and/or incomplete crosslink reversal.



- a. Insert a Covaris Heat Block into a dry block heater set at temperature **T set₁**.
- b. Once **T set₁** has been reached, load the rack containing the samples into the Covaris Heat Block.
- c. An incubation time of 1 hour at **T set₁** is sufficient for sections 10 µm or less in thickness; 12-hour (i.e. overnight) incubation should be used for larger samples, such as 25 µm sections and cores. If the digestion is incomplete after 12 hours, add an additional 20 µl of Proteinase K solution, mix, and incubate for 1 more hour.

12. Incubate the samples at **T set₂** for 1 hour to reverse formaldehyde crosslinks.

- a. Insert a Covaris Heat Block into a dry block heater set at temperature **T set₂**. (If using the same dry block heater for both the **T set₁** & **T set₂** incubations, the rack containing the samples should be stored at room temperature until the Covaris Heat Block reaches **T set₂**.)

Once **T set₂** has been reached, load the rack containing the samples into the Covaris Heat Block.

13. For **Option C “Extract Genomic DNA”** Proceed to Section 3 – **DNA Purification**

14. For **Option B, “Extract large DNA Fragments (>2 kb) with Improved yield”**, process the samples using the settings in Table 4 below to release the DNA with AFA and proceed to Section 3 – DNA Purification with Prepito.

Table 4 – DNA release with AFA

System	Duty Factor	Peak Incident Power	Cycles per burst	Time	Temperature (Instrument)
E220	10%	105 Watts	200	10 sec	20 °C
E210	10%	3 (Intensity)	200	10 sec	20 °C
LE220	30%	300 Watts ⁽¹⁾	200	10 Sec	20 °C

15. For **Option A, “Extract and Fragment DNA (for NGS)”**, process the samples using the appropriate settings in Table 5 below for the desired DNA fragment size, then proceed to Section 3 – DNA Purification with Prepito

NOTE: If the target size is not achieved using the settings shown in Table 5, the treatment time should be adjusted.

Table 5 - DNA Shearing settings

E- Series Focused-ultrasonicator			
Targeted fragment size	200 bp	300 bp	400 bp
Treatment Time	300 sec	110 sec	80 sec
PIP (E220)	175 W	175 W	175 W
Intensity (E210)	5	5	5
Duty Factor	10%	10%	10%
Cycle per Burst	200	200	200
Temperature	20 °C	20 °C	20 °C

LE220 Focused-ultrasonicator			
Targeted fragment size	200 bp	300 bp	400 bp
Treatment Time	450 sec	225 sec	120 sec
PIP	450 W	450 W	450 W
Duty Factor	20%	20%	20%
Cycle per Burst	200	200	200
Temperature	20 °C	20 °C	20 °C

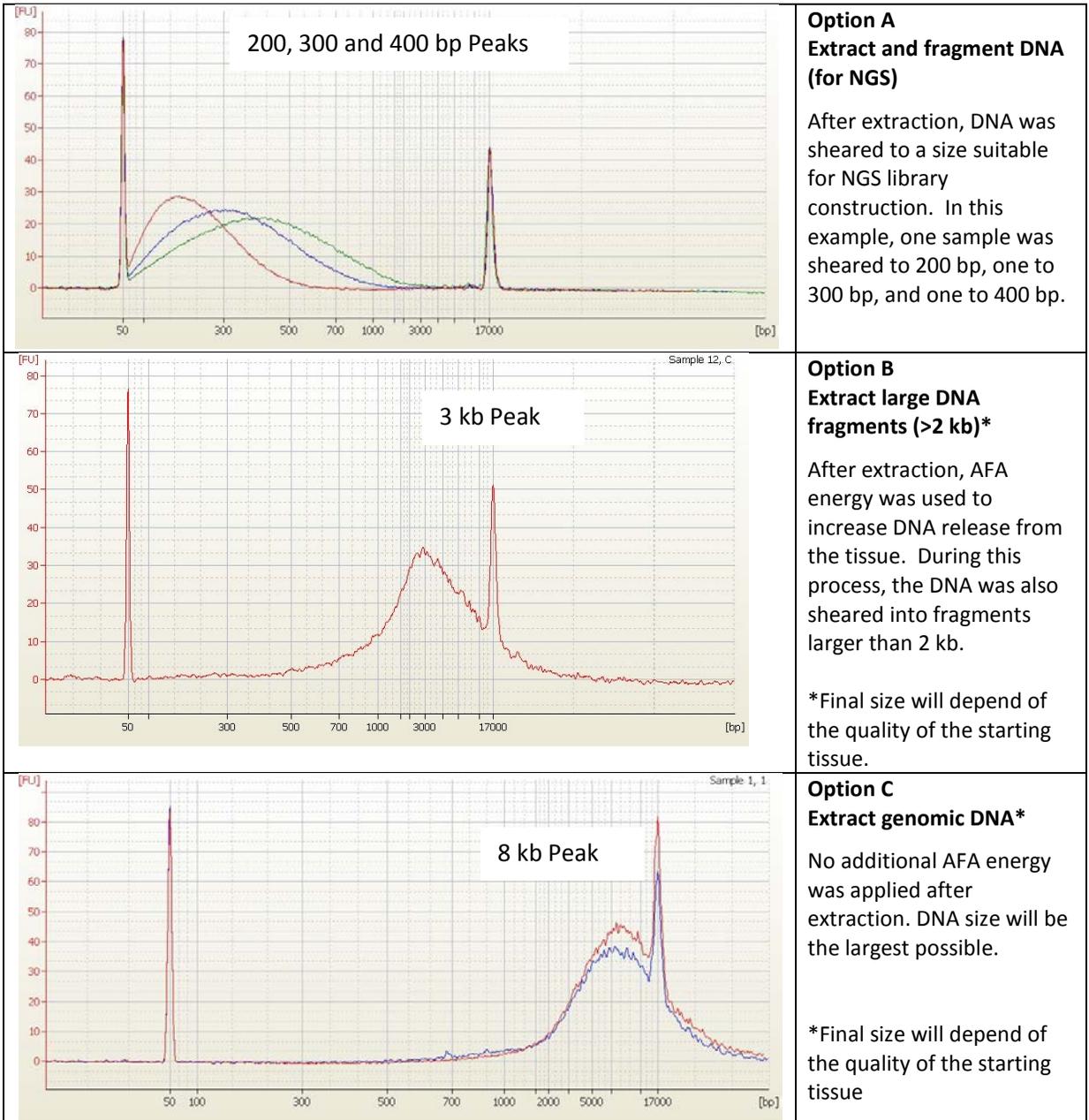
3 - DNA PURIFICATION WITH CHEMAGEN TECHNOLOGY

This step requires the Prepito truXTRAC DNA FFPE Kit (CMG-2037) and associated script.

1. Place the rack with the samples on a Covaris Heat Block set at **T set₁** to prevent paraffin from solidifying. (If amounts of emulsified paraffin are small, it may be possible to keep the samples at room temperature.)
2. Transfer the FFPE lysates to the first row of a 96 well Deep Well Plate (DWP - included in the Prepito truXTRAC DNA FFPE Kit). Sample should be retrieved by pipetting through the septum. For easy pipetting of sample in a single step, we recommend using pipette tips with ribs (e.g., Hamilton 250 µl Ribbed Tip or Agilent Bravo 250 µl Tip). Otherwise, two pipetting steps with 50 µl tips may be necessary.
3. **Optional:** The sample can be treated with RNase A to remove RNA before DNA purification. Add 5µl of RNase A solution to the FFPE lysate and incubate for 5 minutes at room temperature.
4. Follow Prepito truXTRAC DNA FFPE Kit instructions to purify the DNA.

APPENDIX A – EXAMPLES OF DNA FRAGMENTS SIZE DISTRIBUTION

Multiple 10 µm sections were cut off the same FFPE kidney tissue block, and DNA was extracted with the Covaris FFPE kit using Option A, B, or C. Extracted DNA was purified and analyzed on a Bioanalyzer. The size of the non-fragmented, genomic DNA (Option C) depends on how the tissue block was generated and stored as well as the age of the tissue block.



APPENDIX B – EXAMPLE OF PARAFFIN EMULSIFICATION WITH AFA ENERGY

Paraffin was emulsified in a microTUBE Screw-Cap using a Covaris S220 Focused-ultrasonicator. Sample before (left side) and after (right side) processing. Sample was a 10 μm kidney tissue section.

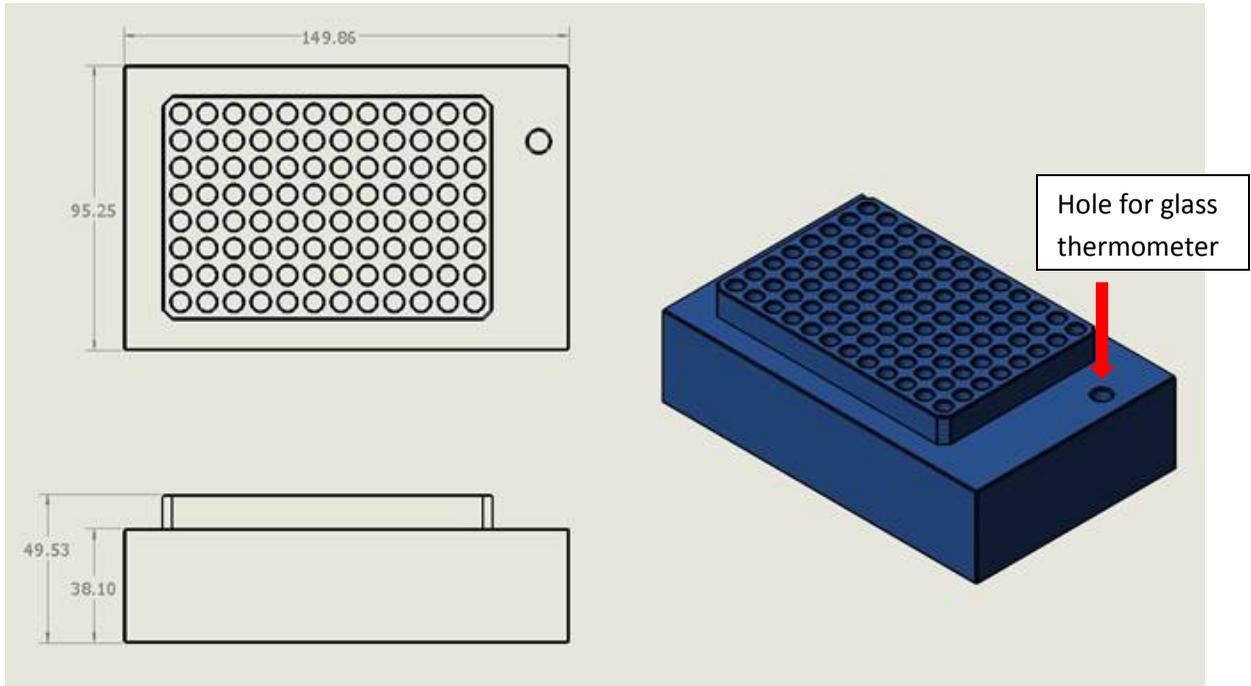


APPENDIX C – TROUBLESHOOTING GUIDE

Issue	Cause	Solution	Comments / Suggestions
Low yield of DNA	Low tissue to wax ratio in FFPE section.	Repeat the procedure using additional sections until desired yield is achieved.	In your initial use of the truXTRAC FFPE kit, use FFPE blocks that have been well characterized for yield and quality.
	Proteinase K stored above recommended temperature or expired.	Repeat the procedure using fresh Proteinase K.	Always store proteinase K solution at Room Temperature or 4°C.
DNA does not perform well in downstream applications such as qPCR	DNA in FFPE sample blocks is severely cross-linked or degraded.	Design amplicons to be as small as possible (<100 bp).	DNA isolated using Covaris AFA technology is of the highest possible quality. Some FFPE sample blocks may be too degraded or cross-linked for some applications.
DNA fragment size too large when following Option A	Too much emulsified paraffin in the sample	Trim any excess paraffin from tissue blocks before proceeding with protocol. If it isn't possible to completely trim the paraffin from the FFPE block, we recommend running a treatment time course by increasing the treatment time by 30 seconds steps.	Too much emulsified paraffin absorbs some of the acoustic energy and will adversely affect DNA shearing efficiency.
Low yield for NGS library construction or by qPCR	Incomplete crosslink reversal	Check that dry block heater was calibrated following procedure in Appendix E	Incomplete crosslink reversal will prevent DNA amplification

APPENDIX D – COVARIS HEAT BLOCK NOMINAL DIMENSIONS

Heat Block Rack 12 Place 8 microTUBE Strip V2 (PN 500481) (referred to as Covaris Heat Block in text)
(in millimeters)



APPENDIX E – DRY BLOCK HEATER CALIBRATION PROCEDURE

1. Place the Covaris Heat Block into the dry block heater.
2. Add water to the separate hole in the Covaris Heat Block and insert a glass thermometer.
3. Set the dry block heater temperature to 60°C.
4. Wait for the dry block heater to reach the set point.
5. Check temperature displayed by the thermometer (**T_{th}**).
6. If **T_{th}** is between 59°C and 61°C (setpoint +/- 1 °C), use 60°C for **T_{set1}**.
7. Otherwise, use the formula below to obtain **T_{set1}**:

$$T_{set_1} (^{\circ}C) = 120^{\circ}C - T_{th}$$

8. Repeat steps 3-7 with an initial set point of 90°C to obtain **T_{set2}**:

$$T_{set_2} (^{\circ}C) = 180^{\circ}C - T_{th}$$

Additional Notes

1. Covered by US Patent 9,080,167
2. Other patents pending
3. Best Practices for determining the yield and purity of isolated DNA:
 - To determine DNA yield with the highest level of accuracy, a fluorometric assay such as Qubit™ (Life Technologies) should be used.
 - In addition, spectrophotometric analysis of DNA for A260/280 and A260/230 ratios will determine if protein or peptide/salt contamination is present in the sample.
4. Tissue Blocks were obtained from: Theresa Kokkat, PhD and Diane McGarvey, Cooperative Human Tissue Network (CHTN), Eastern Division, University of Pennsylvania, USA
5. See following link: http://covarisinc.com/wp-content/uploads/pn_010295.pdf for updates to this document.
6. The treatment settings listed in this document are recommended guidelines. Actual results may vary depending on the tissue type, mass, and previous handling of FFPE samples.