truXTRAC™ FFPE DNA microTUBE Kit - Bead Purification (24)

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

Product PN 520210

Contents

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 is designed for the controlled and efficient extraction of DNA from Formalin Fixed, Paraffin Embedded (FFPE) tissue samples with Covaris Adaptive Focused Acoustics (AFA™) and subsequent magnetic bead based DNA purification.

AFA enables the active removal of paraffin from FFPE tissue samples in aqueous buffer, allowing simultaneous tissue rehydration. Compared to traditional passive, chemical-based methods of paraffin removal, this mechanical process is not as limited by the thickness of FFPE tissue sections. The AFA process enables the use of thicker sections, which can increase DNA yield and minimize the impact of increased DNA degradation at the exposed surfaces of a section. The truXTRAC process results in high yields of high-quality DNA well suited for analytical methods such as next-generation sequencing or qPCR.

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 can be highly variable. Factors such as fixation time, size and thickness of the sections, the ratio of tissue to paraffin, the type of tissue, and the age and storage conditions of the FFPE block are the main causes for this variability.

The quality of DNA isolated from FFPE samples can also be 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 applications such as PCR and 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 DNA 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
010339	A	04/16	As released
010339	B	06/16	Change storage temperature to 2-8 °C

KIT CONTENTS

Tissue SDS Buffer	3 ml
Proteinase K (PK Solution)	2x 275 µl
Bind Buffer	4.5 ml
Magnetic Beads	300 µl
Wash Buffer (Concentrate)	6.6 ml
Elution Buffer	7.5 ml
microTUBE-130 AFA Fiber Pre-Slit Screw-Cap	24

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

STORAGE

This kit should be stored at 2-8 °C.

SUPPLIED BY USERS

Covaris Instruments and Parts

Required parts					
Focused-ultrasonicator	**LE220**	**E220 & E210**	**E220 evolution**	**S-Series**	**M220**
Rack/ Holder	Rack-XT 24 Place microTUBE Screw-Cap PN500388	Rack 24 Place microTUBE Screw-Cap PN500308	Rack E220e 4 Place microTUBE Screw Cap PN500432	Holder microTUBE Screw-Cap PN500339	Holder XTU PN500414 & Insert XTU PN500489 **(*)**
Intensifier	NA	PN500141	PN500141	NA	NA
Accessories	Centrifuge and Heat Block microTUBE Adapter (PN500406)				
Optional parts					
Accessories	FFPE tissuePICK (PN520163) FFPE sectionPICK (PN520149) FFPE sectionWARMER (PN500403)				

(*) Holder PN500358, although discontinued, can also be used. This holder does not require an insert

Other supplies:

- Water bath, oven or dry block heater (e.g., Eppendorf ThermoMixer) for 1.5 or 2 mL tubes, capable of heating to 80°C.
- Magnetic Rack to hold 1.5 mL tubes (e.g., twelve-position Promega Magnetic Separation Stand PN Z5342) or alternatively 96 Magnetic Plate EX Model (Alpaqua, PN: A000380)
- RNase A (DNase free) at 10 mg/ml e.g., Thermo Scientific (PN EN0531)
- 80% ethanol made from nuclease-free water and ethanol (>96%), MB Grade e.g., Thermo Scientific (PN BP2818-100)
- 90% isopropanol made from nuclease-free water and isopropanol (100%), ultra pure, AmericanBio (PN AB07015)
- Isopropanol (100%), ultra pure, AmericanBio (PN AB07015)
- 1.5 mL nonstick nuclease free microfuge tubes e.g., Life Technologies (PN AM12450)

PROCEDURE WORKFLOW OVERVIEW

Three different options are possible with Covaris truXTRAC FFPE DNA Kit. The three options differ in the workflows for DNA extraction.

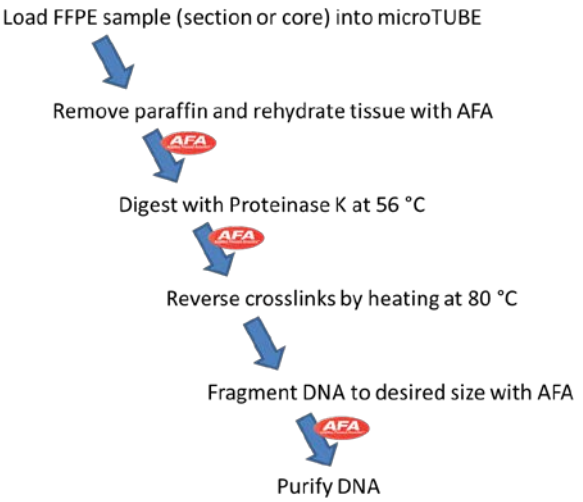
Option A: Shear DNA during extraction to a size suitable for next-generation sequencing library construction. Fragment size can be tuned between 200 and 400 bp.

Option B: Extract ~2kb DNA fragments. This protocol is recommended for most analytical applications, including PCR. Note that actual DNA fragment size will depend on the quality of the starting material.

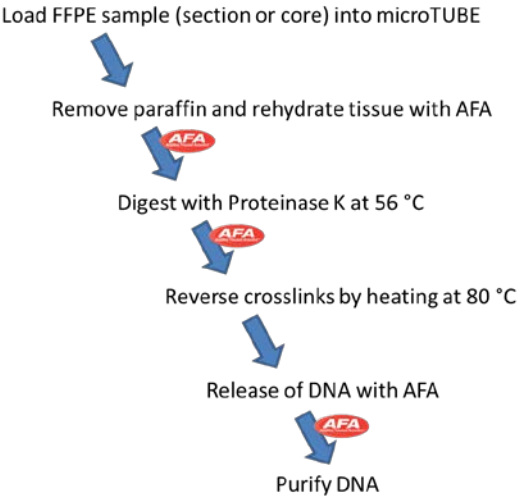
Option C: Extract large "genomic" DNA without any additional fragmentation. Actual DNA fragment size will depend on the quality of the starting material. For high-quality FFPE tissue blocks, we typically see an average fragment size of >= 8 kb.

Only Option C is described in the main part of the protocol, please refer to Appendix A for Option A and B.
Please refer to Appendix B for examples of final DNA fragment size distribution.

OPTION A – EXTRACT AND FRAGMENT DNA (FOR NGS) (APPENDIX A)

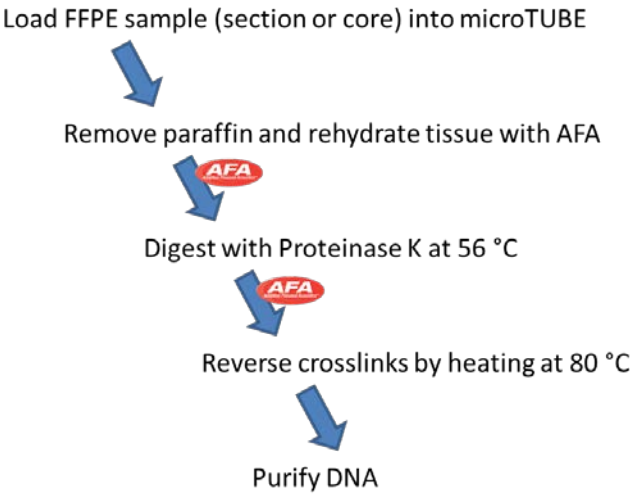


OPTION B - EXTRACT LARGE DNA FRAGMENTS (>2 KB)* WITH IMPROVED YIELD (APPENDIX A)



* 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.**

NOTE: 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.

	FFPE Sections Mounted on slide		FFPE Sections "scrolls" or "curls"		FFPE Cores
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 (Note A)		
Collection tool	tissuePICK (Note B)	sectionPICK (Note B)	NA		
Maximum number of samples Per Tube*	2x tissuePICK (200 mm^2 tissue for a 5 µm section)	2x sectionPICK	2*	1*	1*

* Numbers represent trimmed sections only

NOTES

A. If the FFPE sample is longer than about 10 mm, cut it in half before loading.

B. 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.

WARNING: The total mass of FFPE sample processed per extraction should be between 2 to 5 mg. Lower amounts may result in insufficient yield and higher amounts may cause spin columns to become partially or fully clogged.

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

Buffers

1. Add isopropanol to Wash Buffer: Add 4 ml of isopropanol (100%) to Wash Buffer concentrate and mark the label. After preparation, Wash Buffer can be stored for one year at room temperature. **Preheat wash buffer to 70°C**

2. Prepare fresh 80% ethanol using nuclease-free water (See Section 3)

3. Prepare fresh 90% isopropanol using nuclease-free water (See Section 3)

4. Preheat Elution Buffer to 70°C

5. Check Tissue SDS Buffer: A white precipitate may form during storage. Incubate the bottles at 50 – 60 °C before use to dissolve any precipitate.

Focused-ultrasonicator

- For S, E, or LE-Series Focused-ultrasonicators, set up the instrument as shown in table below. Wait for the water to reach temperature and to degas.

Focused-ultrasonicator setup

Instrument	Water level*	Chiller temp	Intensifier PN500141	Plate definition**	Holder or Rack
S-Series	**15**	**18°C**	**NA**	NA	PN500339
E220 & E210	**10**	**18°C**	**Yes**	500308 Rack 24 Place microTUBE Screw-Cap	PN500308
E220 evolution	**10**	**18°C**	**Yes**	500432 Rack E220e 4 Place microTUBE Screw Cap	PN500432
LE-Series	**10**	**18°C**	**NA**	500388 Rack-XT 24 Place microTUBE Screw-Cap	PN500388

***** Use RUN side of Fill/Run scale

**If you do not see a plate definition on your system, please contact Covaris technical support at TechSupport@covarisinc.com

- For the M220 Focused-ultrasonicators, put the Holder PN500414 and the Insert PN500489 (or the discontinued Holder PN500358 without insert) in place and fill the water bath until the water reaches the top of the holder. Allow system to reach temperature (20°C).

For detailed instructions on how to prepare your instrument please refer to the User Manual.

Heating Blocks, Water Baths, or Ovens

Preheat dry block heaters, water baths, or ovens to 56°C (or **T set$_1$** - see Appendix E) and 80°C (or **T set$_2$** - see Appendix E).

When using a dry block heater, microTUBEs need to be placed into microTUBE Adapters (PN500406) first.

It is important to confirm that the desired temperatures are actually reached. See Appendix E for instructions on how to calibrate your heating device.

Subsequent steps will require 70°C.

2 - DNA EXTRACTION FROM FFPE: TISSUE DE-PARAFFINIZATION, PROTEIN DIGESTION, AND DE-CROSSLINKING

Option C: Extract genomic DNA

Option C is designed for extraction of the largest possible DNA fragments from FFPE tissue. Note that actual DNA fragment size will depend of the quality of the starting material.

1. Using table 1 as a guide, generate a master mix of Processing Buffer by mixing Tissue SDS Buffer and PK Solution**.** Note that the volume includes 10% excess volume per sample to accommodate pipetting loss**.**

Table 1 – Processing Buffer master mix

Number of samples	Tissue SDS Buffer volume	PK Solution volume
8	**704** µl	**176** µl
16	**1408** µl	**352** µl
24	**2112** µl	**528** µl
x	**x * 88** µl	**x * 22** µl

2. Open Screw-Cap microTUBE, add 100 µl Processing Buffer master mix into each microTUBE and load FFPE tissue (section or core). Affix Screw-Cap back in place.

> **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.

3. Process the sample using the settings provided in Table 2 to dissociate the paraffin while simultaneously rehydrating the tissue. (Please see the example in Appendix C.) During the AFA process it is normal for the solution to turn milky white as the paraffin is emulsified.

> **NOTE***:* Processed samples can be kept at room temperature for 2 hours while remaining samples are processed.

Table 2 - Paraffin removal and tissue rehydration settings

System	Duty Factor	Peak Incident Power	Cycles per burst	Treatment Time	Temperature (Instrument)
S220 or E220	**10%**	**175 Watts**	**200**	**300 sec**	**20 °C**
S2 or E210	**10%**	**5 (Intensity)**	**200**	**300 sec**	**20 °C**
M220	**20%**	**75 Watts**	**200**	**300 sec**	**20 °C**
LE220	**30%**	**450 Watts** [(1)]	**200**	**300 sec**	**20 °C**

[(1)] As Covaris LE220 process multiple samples at a time, its PIP is distributed across microTUBEs, and power received by individual microTUBE stays within the 200 W limit.

4. **Proteinase K digestion at 56°C** (or **T set$_1$** - see Appendix E)**:** Incubate samples for 1 hour (sections <= 10 µm) or overnight (sections > 10 µm or cores) at 56°C for proteinase K digestion. If the digestion is incomplete after overnight incubation, add another 20 µl of PK solution, mix, and incubate for 1 more hour. **When using a dry block heater, microTUBEs need to be placed into microTUBE Adapters first.**

5. **Crosslink reversal at 80°C** (or **T set$_2$** - see Appendix E)**:** Incubate samples for 1 hour at 80**°**C to reverse formaldehyde crosslinks. **When using a dry block heater, microTUBEs need to be placed into microTUBE Adapters first.**

> **NOTE**: If you are using the same heating source for both the 56°C & 80°C incubations, the microTUBE should be stored at room temperature until the heating source reaches 80°C or **T set$_2$**

6. Proceed to Section 3 – DNA Purification

3 - DNA PURIFICATION

NOTE: Using table below as a guide, prepare required volume of Elution Buffer, Wash Buffer, 80% ethanol and 90% isopropanol.
Set heating element to 70°C and preheat Elution Buffer and Wash Buffer.
Note that the volume includes 10% excess volume per sample to accommodate pipetting loss.

	Elution Buffer	Wash Buffer	80% ethanol	90% isopropanol
	Preheated at 70°C		Room temperature	
8	352 µl	2.64 ml	13.2 ml	4.4 ml
16	704 µl	5.28 ml	26.4 ml	8.8 ml
24	1056 µl	7.92 ml	39.6 ml	13.2 ml
x	x * 44 µl	x * 330 µl	x * 1.65 ml	x * 550 µl

1. Transfer the sample to a clean 1.5 ml microcentrifuge tube.
2. **Optional**: The sample can be treated with RNase A to remove RNA before DNA purification. Add 5 µl of RNase A solution and incubate for 5 minutes at room temperature.
3. Add 140 µl **Bind Buffer** to the sample and mix by pipetting 10 times.
4. Vortex or shake the magnetic beads to resuspend before use. Add 8 µl **Magnetic Beads** to the sample.
5. Add 200 µl **isopropanol** (100%) to your sample and mix by pipetting 10 times with a volume of 350 µl.
6. Incubate at 56°C for 5 minutes.
7. Move tubes onto the magnetic stand and allow separating for 5 minutes or until solution is clear. Slowly aspirate and discard supernatant without disturbing the beads.
8. Remove tubes from magnet and add 300 µl of pre-warmed **Wash Buffer** (70 °C). Mix by pipetting 10 times and incubate for 1 minute.
9. Move tubes onto the magnet and let stand for 3 minutes or until solution is clear. Slowly aspirate and discard supernatant without disturbing the beads.
10. **1st EtOH wash**: Remove tubes from the magnet and add 750 µl **80% ethanol**. Mix by pipetting 10X and incubate for 1 minute.

NOTE: Magnetic bead clumping can occur at this stage with higher tissue content samples. If that occurs, please continue as the clumping will dissipate with the subsequent wash steps.

11. Move tubes onto the magnet and let stand for 3 minutes or until solution is clear. Slowly aspirate and discard supernatant without disturbing the beads.
12. **ISO wash**: Remove tubes from the magnet and add 500 µl **90% isopropanol**. Mix by pipetting 10 times and incubate for 1 minute.
13. Move tubes onto the magnet and let stand for 3 minutes or until solution is clear. Slowly aspirate and discard supernatant without disturbing the beads.
14. **2nd EtOH wash**: Remove tubes from the magnet and add 750 µl **80% ethanol**. Mix by pipetting 10 times and incubate for 1 minute.
15. Move tubes onto the magnet and let stand for 3 minutes or until solution is clear. Slowly aspirate and discard supernatant without disturbing the beads.
16. Allow the beads to air dry on the magnet for 8 minutes.

    Do not over dry the beads at this step as this may decrease yields.
17. **Elute DNA:** Remove tubes from the magnet and add 40 µL (up to 100 µl) of pre-warmed **Elution Buffer** (70 **°**C). Mix by pipetting 10 times and incubate for 3 minutes.
18. Move tubes onto the magnet and let stand for 2 minutes or until solution is clear. Slowly aspirate and pipet eluted DNA into a fresh tube.
19. ***(Optional)* Elute DNA – 2nd step**: Remove tubes from the magnet and add a second aliquot of 40 µl pre-warmed **Elution Buffer**. Mix by pipetting 10 times and incubate for 3 minutes. Move tubes onto the magnet and let stand for 2 minutes or until solution is clear. Slowly aspirate and pipet eluted DNA into a fresh tube.
20. **DNA is eluted in Elution Buffer** (5mM Tris HCl pH 8.5)**.**

APPENDIX A – ALTERNATIVE PROTOCOLS FOR DNA EXTRACTION FROM FFPE

Option A - Extract and fragment DNA (for NGS)

This protocol allows direct fragmentation of DNA to a size suitable for Next Generation Sequencing library construction during the extraction process.

1. Using table 1 below as a guide, generate a master mix of Processing Buffer by mixing Tissue SDS Buffer and Proteinase K**.** Note that the volume included 10% excess volume per sample to accommodate pipetting loss**.**

Table 1 – Processing Buffer master mix

Number of samples	Tissue SDS Buffer volume	Proteinase K volume
8	**704** µl	**176** µl
16	**1408** µl	**352** µl
24	**2112** µl	**528** µl
x	**x * 88** µl	**x * 22** µl

2. Open microTUBE Screw-Cap, add 100 µl Processing Buffer master mix into each microTUBE and load FFPE tissue (section or core). Affix Screw-Cap back in place.

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

3. Process the sample using the settings provided in Table 2 below to dissociate the paraffin while simultaneously rehydrating the tissue. (Please see the example in Appendix C.) During the AFA process it is normal for the solution to turn milky white as the paraffin is emulsified.

NOTE*:* Processed samples can be kept at room temperature for 2 hours while remaining samples are processed.

Table 2 - Paraffin removal and tissue rehydration settings

System	Duty Factor	Peak Incident Power	Cycles per burst	Treatment Time	Temperature (Instrument)
S220 or E220	**10%**	**175 Watts**	**200**	**300 sec**	**20 °C**
S2 or E210	**10%**	**5 (Intensity)**	**200**	**300 sec**	**20 °C**
M220	**20%**	**75 Watts**	**200**	**300 sec**	**20 °C**
LE220	**30%**	**450 Watts** (1)	**200**	**300 sec**	**20 °C**

(1) As Covaris LE220 process multiple samples at a time, its PIP is distributed across microTUBEs, and power received by individual microTUBE stays within the 200 W limit.

4. **Proteinase K digestion at 56°C** (or **T set$_1$** - see Appendix E)**:** Incubate samples for 1 hour (sections <= 10 µm) or overnight (sections > 10 µm or cores) at 56°C for proteinase K digestion. If the digestion is incomplete after overnight incubation, add another 20 µl of Proteinase K solution, mix, and incubate for 1 more hour. **When using a dry block heater, microTUBEs need to be placed into microTUBE Adapters first.**

5. **Crosslink reversal at 80°C** (or **T set$_2$** - see Appendix E)**:** Incubate samples for 1 hour at 80°C to reverse formaldehyde crosslinks. **When using a dry block heater, microTUBEs need to be placed into microTUBE Adapters first.**

> **NOTE**: If you are using the same heating source for both the 56°C & 80°C incubations, the microTUBE should be stored at room temperature until the heating source reaches 80°C or **T set$_2$**

6. Place tubes at room temperature for 5 minutes to cool down.

7. DNA fragments size can be tuned to the desired average fragment size by using the settings in Table 4 below.

> **NOTE**: If the target size is not achieved then the treatment time should be adjusted.

Table 3 - DNA Shearing settings

E- and S-Series Focused-ultrasonicator			
Targeted fragment size	**200 bp**	**300 bp**	**400 bp**
Treatment Time	**300 sec**	**110 sec**	**80 sec**
PIP (S220 and E220)	**175 W**	**175 W**	**175 W**
Intensity (S2 and E210)	**5**	**5**	**5**
Duty Factor	**10%**	**10%**	**10%**
Cycle per Burst	**200**	**200**	**200**
Temperature	**20 °C**	**20 °C**	**20 °C**

M220 Focused-ultrasonicator			
Targeted fragment size	**200 bp**	**300 bp**	**400 bp**
Treatment Time	**450 sec**	**200 sec**	**120 sec**
PIP	**75 W**	**75 W**	**75 W**
Duty Factor	**20%**	**20%**	**20%**
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	**300 sec**	**150 sec**	**80 sec**
PIP	**450 W** [(1)]	**450 W** [(1)]	**450 W** [(1)]
Duty Factor	**30%**	**30%**	**30%**
Cycle per Burst	**200**	**200**	**200**
Temperature	**20 °C**	**20 °C**	**20 °C**

8. Proceed to Section 3 – DNA Purification.

Option B - Extract large DNA fragments (>2 kb)* with improved yield

Option B enhances the release of DNA from the tissue while preserving a fragment size of >2kb. Note that actual DNA fragment size will depend of the quality of the starting material.

1. Using table 4 as a guide, generate a master mix of Processing Buffer by mixing Tissue SDS Buffer and Proteinase K**.** Note that the volume included 10% excess volume per sample to accommodate pipetting loss.

Table 4 – Processing Buffer master mix

Number of samples	Tissue SDS Buffer volume	Proteinase K volume
8	**704** µl	**176** µl
16	**1408** µl	**352** µl
24	**2112** µl	**528** µl
x	**x * 88** µl	**x * 22** µl

2. Open Screw-Cap microTUBE, add 100 µl Processing Buffer master mix into each microTUBE and load FFPE tissue (section or core). Affix Screw-Cap back in place.

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

3. Process the sample using the settings provided in Table 5 to dissociate the paraffin while simultaneously rehydrating the tissue. (Please see the example in Appendix C.) During the AFA process it is normal for the solution to turn milky white as the paraffin is emulsified.

NOTE*:* Processed samples can be kept at room temperature for 2 hours while remaining samples are processed.

Table 5 - Paraffin removal and tissue rehydration settings

System	Duty Factor	Peak Incident Power	Cycles per burst	Treatment Time	Temperature (Instrument)
S220 or E220	**10%**	**175 Watts**	**200**	**300 sec**	**20 °C**
S2 or E210	**10%**	**5 (Intensity)**	**200**	**300 sec**	**20 °C**
M220	**20%**	**75 Watts**	**200**	**300 sec**	**20 °C**
LE220	**30%**	**450 Watts** [(1)]	**200**	**300 sec**	**20 °C**

[(1)] As Covaris LE220 process multiple samples at a time, its PIP is distributed across microTUBEs, and power received by individual microTUBE stays within the 200 W limit.

4. **Proteinase K digestion at 56°C** (or **T set$_1$** - see Appendix E)**:** Incubate samples for 1 hour (sections <= 10 µm) or overnight (sections > 10 µm or cores) at 56°C for proteinase K digestion. If the digestion is incomplete after overnight incubation, add another 20 µl of Proteinase K solution, mix, and incubate for 1 more hour. **When using a dry block heater, microTUBEs need to be placed into microTUBE Adapters first.**

5. **Crosslink reversal at 80°C** (or **T set$_2$** - see Appendix E)**:** Incubate samples for 1 hour at 80°C to reverse formaldehyde crosslinks. **When using a dry block heater, microTUBEs need to be placed into microTUBE Adapters first.**

> **NOTE**: If you are using the same heating source for both the 56°C & 80°C incubations, the microTUBE should be stored at room temperature until the heating source reaches 80°C or **T set$_2$**

6. Place tubes at room temperature for 5 minutes to cool down.

7. Process the sample using the settings in Table 6 to release the DNA with AFA.

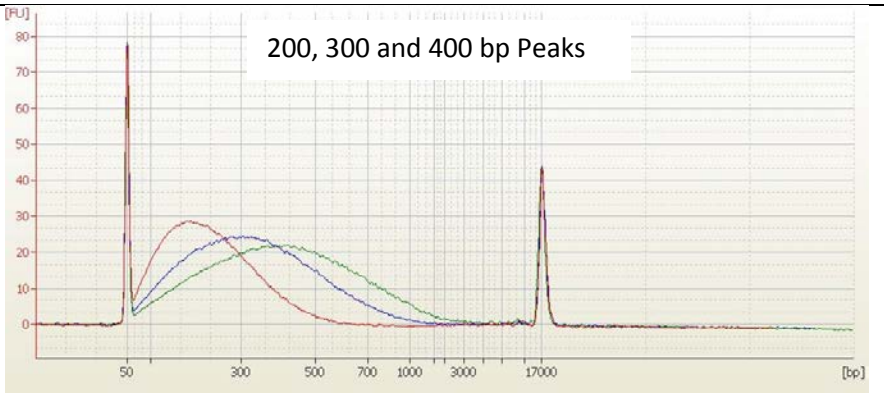
Table 6 – DNA release with AFA

System	Duty Factor	Peak Incident Power	Cycles per burst	Time	Temperature (Instrument)
S220 or E220	**10%**	**105 Watts**	**200**	**10 sec**	**20 °C**
S2 or E210	**10%**	**3 (Intensity)**	**200**	**10 sec**	**20 °C**
M220	**20%**	**75 Watts**	**200**	**10 sec**	**20 °C**
LE220	**30%**	**300 Watts** [(1)]	**200**	**10 Sec**	**20 °C**

8. Proceed to Section 3 – DNA Purification.

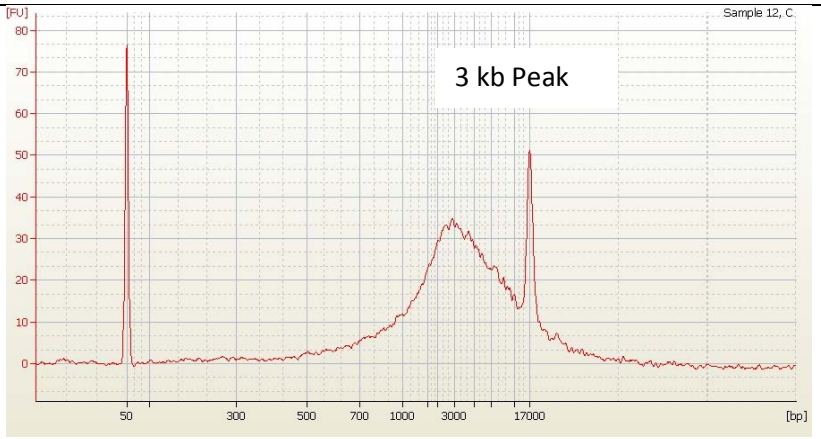
APPENDIX B – EXAMPLES OF DNA FRAGMENTS SIZE DISTRIBUTION

In these examples, DNA has been extracted from 10 µm sections off the same kidney tissue block with the Covaris FFPE kit following the 3 available options. The Bioanalyzer electropherograms below represent the fragment size distribution after purification. The size of the non-fragmented, genomic DNA (Option C) depends of the previous storage condition of the tissue block, including how it has been fixed and paraffin embedded.



Option A
Extract and fragment DNA (for NGS)

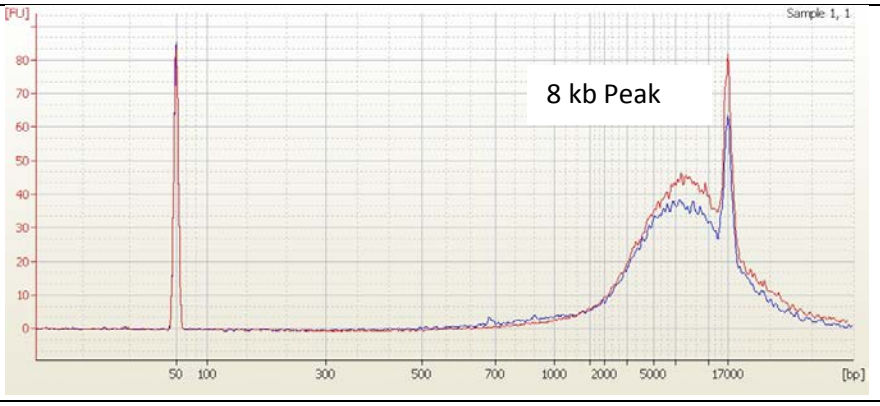
Subsequently to extraction, DNA is sheared to a size suitable for Next Gen Sequencing library construction. In this example, one sample has been sheared to 200 bp, one to 300 bp and one to 400 bp.



Option B
Extract large DNA fragments (>2 kb)*

AFA energy is used to release the DNA from the tissue. During this process the DNA is also sheared into fragments larger than 2 kb.

*Final size will depend of the quality of the starting tissue.



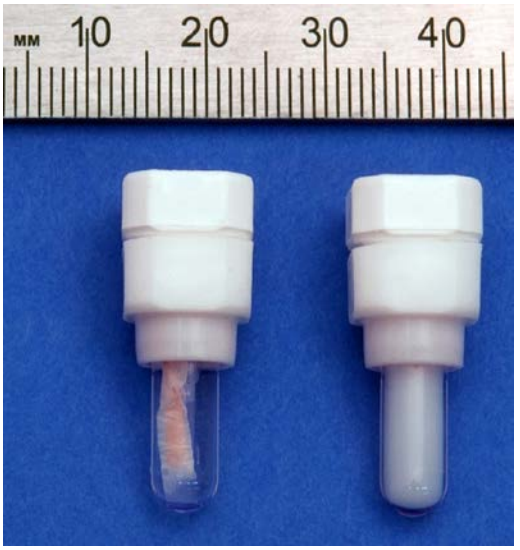
Option C
Extract genomic DNA*

DNA size will be the largest possible and will depend on the quality of the starting tissue.

*Final size will depend of the quality of the starting tissue

APPENDIX C – PARAFFIN EMULSIFICATION WITH AFA ENERGY

Paraffin is emulsified in 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 D – 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 2-8°C
	Incorrect amount of beads were added.	Resuspend beads well.	Vortex beads thoroughly before each use.
	Binding was incomplete.	Make sure the correct reagent volumes were used and mix well before the 56°C incubation.	
	Beads lost during pipetting steps.	Slowly draw up the supernatant into the pipet tip to not disturb the beads.	It is normal for beads to stick to the pipet tips during the elution step. This has no effect on yield.
Clumping of magnetic beads during wash steps	Some clumping of magnetic beads may occur with specific FFPE blocks.	Bead clumping will not affect the yield or purity of the DNA.	If bead clumping is severe enough to clog pipette tips reduce the amount input sample.
Sticking of magnetic beads to pipette tips during elution.	Some sticking of magnetic beads to pipette tips may occur with specific FFPE blocks	Magnetic beads sticking to pipette tips during elution will not affect the yield or purity of the DNA.	If magnetic bead sticking is severe enough to clog pipette tips reduce the amount input sample.
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 fragments 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 time course at step 7 and to increase the treatment time by 30 seconds steps.	Too much emulsified paraffin absorbs some of the acoustic energy and will adversely affect DNA Shearing efficiency.

APPENDIX E – HEATING SOURCE CALIBRATION PROCEDURE

1. If using a dry block heater, place the Covaris Heat Block microTUBE Adapters into the heating block.
2. Add water to one of the Heat Block microTUBE Adapters and insert a glass thermometer or place a glass thermometer into the water bath or the oven.
3. Set the heating source temperature to 56˚C.
4. Wait for the heating source to reach the set point.
5. Check temperature displayed by the thermometer (**Tth**).
6. If **Tth** is between 55˚C and 57˚C (setpoint +/- 1 ˚C), use 56˚C for **$Tset_1$**.
7. Otherwise, use the formula below to obtain **$T\ set_1$**:

$T\ set_1$ (˚C) = 120˚C- **Tth**

8. Repeat steps 3-7 with an initial set point of 80˚C to obtain **$T\ set_2$**:

$T\ set_2$ (˚C) = 160˚C- **Tth**

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_010339.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.