PROTOCOL

Covaris

truChIP® FFPE Chromatin Shearing Kit

Adaptive Focused Acoustics™ (AFA)-based chromatin shearing for ChIP-based applications from FFPE tissues

Contents

INTENDED USE

The truChIP™ FFPE Chromatin Shearing kit is intended for use in research applications (RUO). This product is not intended for the diagnosis, prevention, or treatment of disease.

INTRODUCTION

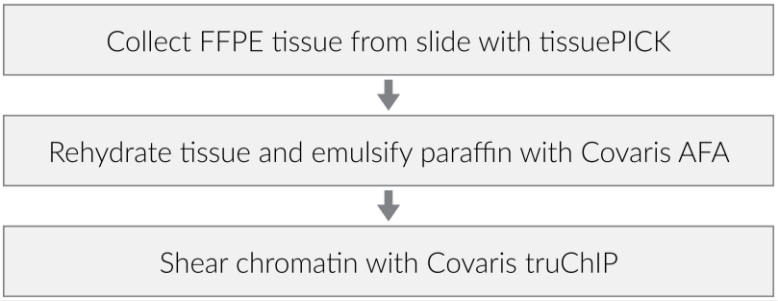
The truChIP FFPE Chromatin Shearing Kit utilizes an active-based parrafin emulsification process prior to shearing chromatin using Covaris AFA Focused-ultrasonicators. This protocol provides a fast, non-contact, isothermal, and reprodubilce shearing method for histone and transcription factor ChIP assays. FFPE samples have not been routninely used in chromatin immnopreciptiation because of limited amounts of material, and reduced antigen availability due to the harsh fixation process. This truChIP FFPE Chromaitn Shearing Kit can be used as an alternative to cumbersome methods that require multiple organic solvents. This protocol enables investigators to rapidly process archived FFPE tissue to study the epigenetic landscape in normal and tumor samples.

To start, Covaris recommends users to perform a one-time titration of Proteinase K and a shearing time course study to empirically determine the optimal treatment conditions for your sample.

REVISION HISTORY

Part Number	Revision	Date	Description of change
010464	A	07/18	As released

PROCEDURE OVERVIEW



SAMPLE INPUT REQUIREMENTS

The truChIP FFPE Chromatin Shearing Kit performs optimally with at least two tissue picks from 10 µm slides. The deparaffinization step uses AFA technology to emulsify the parrafin in the microTUBE-130 Pre-slit Screw Cap followed by chromatin shearing in microTUBE-130 AFA Fiber Snap Cap.

This truChIP FFPE kit efficiently isolates high-quality soluble chromatin that can be immunoprecipitated (IP) for downstream application, such as ChIP-Seq. The kit contains enough reagents and consumables to process up to 25 samples.

KIT CONTENTS

Buffer	**Volume (mL)**
Tissue SDS Buffer (1x)	3
Proteinase K Solution (10µg/µl)	0.125
Chromatin Shearing Buffer (1x)	2 mL
Protease Inhibitor Cocktail (100x)	0.2
Tubes	**Amount**
microTUBE AFA Fiber Screw Cap FFPE	25
microTUBE AFA Fiber Snap Cap	25

Safety Data Sheets: http://covaris.com/resources/safety-data-sheets

STORAGE

The kit is shipped cold and should be stored at 2-8C.

Note: Mix solutions well before use to ensure solutions are completely solubilized.

SUPPLIED BY USER

- Microcentrifuge (5,000 x g capability)
- Heat Block with adapter 1.7 or 2 ml tubes (Eppendorf, PN5383000027; PN5362000036; 5362000035) and ThermoTop (PN5308000003); VWR Advanced Mini Dry Block Heater with Heated Lid (PN10153-348) in combination with MiniBlock 2ml tubes (PN10153-366) is recommended
- microTUBE-130 Centrifuge and Heat Block Adapter (PN: 500406)

Consumables

- 1.5 ml nuclease-free microfuge tubes (e.g., Eppendorf Safe-Lock Tubes, PN 022363212)

Optional Supplies for Sample Collection from Slides

- FFPE tissuePICK (PN: 520163)
- tissuePICK Forceps (5) (PN: 520164)
- FFPE sectionPICK (PN: 520149)
- FFPE sectionWARMER (PN: 500403)

FOCUSED-ULTRASONICATOR SETUP

M220

Required Accessories: Holder XTU (PN: 500414), Insert XTU (PN: 500489)

System Setup

Holder	Holder XTU (PN500414)
Insert	Insert XTU (PN500489)

Position Holder XTU and Insert into place and fill the water bath until the water level reaches the top of the holder. After setting up the system, wait until the water bath has reached the set temperature.

PROTOCOL

A. Paraffin Emulsification

1. Set up the dry-heat block and set to to 40C. Insert the required number of microTUBE-130 centrifuge and heat block adapters into the heat block.

2. Capture two ROIs from FFPE tissue slides using the sectionPICK or tissuePICK. These methods are specifically designed to collect FFPE tissue from non-stained sections mounted on slides. For further guidance, please view the video below. http://covaris.com/products/ffpe-extraction/tissuepick-instructions-2/

Material	tissuePICK	sectionPICK
Capture ROIs from FFPE tissue slides with the appropriate method	PN: 520163	PN: 520149

3. Release the tissue into the **microTUBE Screw-Cap FFPE** (blue cap).
4. Add 100 µl of **Tissue SDS Buffer** and 0.8 µl of **Proteinase K** (final concentration 80ng/µl)
5. Load the microTUBE into the microTUBE-130 Centrifuge and Heat Block adapter.
6. Incubate at 40C for 10 minutes with mixing after 5 minutes of incubation.

Note: Depending of tissue type and quantity, we recommend testing different quantities of tissue and Proteinase K concentration. When starting with 2 tissue picks we recommend to perform an initial Proteinase K titration using 40, 80, 120, 160 and 200 ng/µl.

Please contact applicationsupport@covaris.com for further support.

7. Process the sample on a Covaris M220 using the following settings:
   - Time 5 min
   - Duty Factor 20%
   - Peak incident 75W
   - 200 cycles per burst
   - 20C

Note: Please contact Covaris at applicationsupport@covaris.com if using another instrument than the Covaris M220

8. Add 1 µl of Protease Inhibitor Cocktail (PIC). From here proceed at 4C.

B. Chromatin Shearing

1. Using the table below, prepare a sufficient volume of 1× Shearing Buffer D3. A 15% excess volume is recommended when preparing this buffer.

Number of Samples	1X Shearing Buffer	100X PIC
1	46 µl	0.5 µl
6	276 µl	3 µl
12	552 µl	6 µl
25	1150 µl	12.5 µl
X	X 46 µl	X µl

* Calculations include 15% excess

1. Transfer 90 µl of the paraffin emulsified sample generated in A. 5. into a microTUBE Snap Cap.

2. Adjust the final volume in the microTUBE Snap Cap to 130µl with Chromatin Shearing Buffer D3 supplemented with PIC (100x).

3. Process the sample on a Covaris M220 using the following settings:

   - Time course: 10, 15, 20 and 30 min (once you have determined the optimal processing time for your tissue of choice directly process with this one timepoint only)
   - Duty Factor 15%
   - Peak incident 75W
   - 200 cycles per burst
   - 7C

Note: Please contact Covaris at applicationsupport@covaris.com if using another instrument than Covaris M220

4. Place microTUBE in the microTUBEadapter into a microcentrifuge (fixed angle rotor) and centrifuge at 5000 x g for 5 minutes at 4C and retain the supernatant.
5. Place the sample on ice and determine chromatin concentration using Qubit fluorometric quantification.
6. Transfer an appropriate amount of the sample to a clean tube and proceed with IP.

Note: For histone and CTCF ChIPs from FFPE tissue, we achieved satisfactory results starting with ~500ng input chromatin. For most IP workflows, the chromatin sheared in D3 has to be diluted before proceeding to IP step. Therefore:

(a) Prepare a 2x IP Dilution buffer (10mM tris-HCl pH 8.1, 300mM NaCl, 2% Triton) with which you dilute your chromatin 1:1 before proceeding to IP.

(b) In case your starting chromatin concentration is low, sipmly add NaCl to a final concentration of 150mM and Triton to a final concentration of 1% before proceeding to IP.

To check the efficiency of your shearing, reserve 25 µl of the sheared chromatin and see **Appendix A** for detailed instructions.

Storing sheared chromatin is not recommended.

Freezing sheared chromatin is not recommended. Freeze/thaw cycles reduce IP efficiency and reproducibility.

SUPPLEMENTAL MATERIAL

Appendix A: Chromatin Shearing Efficiency Analysis Protocol

1. Take a 25 µl aliquot of the sheared sample and transfer to 0.6 mL microcentrifuge tube.
2. Add 1 µl of RNase A (10 mg/mL) and incubate at 37C for 30 min.
3. Add 1 µl of Proteinase K (10 mg/mL) and incubate at 56C for 2 hours in a PCR cycler with a heated lid.
4. Purify DNA using a commercial column based kit (*e.g.,* Qiagen QIAquick PCR Purification Kit, Cat. No. 28104)
5. Elute from column, or resuspend pellet with 50 µl of elution buffer (10 mM Tris-HCl, pH 8.5).
6. Load 1µl of purified DNA onto the DNA 12000 chip and run on the Agilent 2100 BioAnalyzer.

Appendix B: Additional Notes

1. The treatment settings listed in this document are recommended guidelines. Actual results may vary depending on the tissue type, mass, amount of paraffin and duration of formalin fixation.
2. The Covaris process uses high intensity focused ultrasonic (HIFU) energy and as such is influenced by objects in the acoustic path from the transducer surface to the fluid sample. For example, particles and bubbles in the water bath may scatter the acoustic energy from the sample. Replace the bath water on a daily basis and ensure that appropriate time has been allowed for degassing and water bath temperature to stabilize prior to use of the instrument.
3. Bubbles in the sample fluid in the tube may diminish the acoustic dose effectiveness. Be sure to fill the tubes slowly with the recommended volumes and avoid the use of additional detergents that may induce foaming.