PROTOCOL

Covaris

Simultaneous Extraction and Purification of DNA and RNA from FFPE Tissue Samples with truXTRAC™

INTRODUCTION

Transcriptome analysis used in conjunction with Whole Genome Sequencing (WGS) is becoming a routine tool for assessing disease progression. Clinical samples are often stabilized in Formalin-Fixed, Paraffin-Embedded (FFPE) blocks, which were designed for histological analysis. The extreme formaldehyde fixation and harsh tissue dehydration of FFPE preserved tissue sections presents not only a technical challenge to reproducible RNA and DNA extraction for use in Next Generation Sequencing (NGS), but also a turn-around time challenge in clinical settings. We present a simple workflow to simultaneously extract and purify DNA and RNA from FFPE tissue samples utilizing Covaris Adaptive Focused Acoustics (AFA™) and truXTRAC™ FFPE DNA and RNA Kits.

SUPPLIED BY USERS

Covaris Instruments and Parts

Required parts					
truXTRAC Kits	truXTRAC FFPE RNA Kit (520161)				
	truXTRAC FFPE DNA Kit (520136)				
Focused-ultrasonicator	**LE220**	**E220 & E210**	**E220 evolution**	**S-Series**	**M220**
Rack/ Holder/ Insert	Rack-XT 24 Place microTUBE Screw-Cap (PN500388)	Rack 24 Place microTUBE Screw-Cap (PN 500308) and Intensifier (PN500141)	Rack E220e 4 Place microTUBE Screw Cap (PN500432)	Holder microTUBE Screw-Cap (PN500339)	Holder XTU PN500414 & Insert XTU PN500489 or Holder-XT PN500358 **(*)**
Accessories	Centrifuge and Heat Block microTUBE Adapter (PN500406)				
Optional parts					
Accessories	FFPE tissuePICK (PN520163)				
	FFPE sectionPICK (PN520149)				
	FFPE sectionWARMER (PN500403)				

(*) This holder has been discontinued

Other supplies:

- Microcentrifuge with 16,000 x g capability
- Dry heating block such as Eppendorf ThermoMixer or similar with either 1.5 or 2 mL heat block inserts. We recommend two heating blocks, preset at 56°C and 80°C respectively.
- Ethanol (>96%), MB Grade e.g., Thermo Scientific (PN BP2818-100).
- 1.5 mL nonstick nuclease free microfuge tubes e.g., Life Technologies (PN AM12450).
- Nuclease Free water, e.g., Life technologies (PN AM9932) or equivalent.

1. PREPARATION

See instruction of truXTRAC FFPE RNA Kit (PN 520161)

http://covarisinc.com/wp-content/uploads/pn_010268.pdf

2. PROTOCOL

A. TOTAL NUCLEIC ACID EXTRACTION FROM FFPE TISSUE

1. Open microTUBE Screw-Cap FFPE (from the truXTRAC RNA Kit), add 110 µL RNA Lysis Buffer into microTUBE and load FFPE tissue. 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 RNA Lysis Buffer addition to facilitate easier loading.
2. Process the samples using the settings provided in Table 2 below to dissociate the paraffin while simultaneously rehydrating the tissue.

NOTE: During the Adaptive Focused Acoustics (AFA) process, it is normal for the solution to turn milky white as the paraffin is emulsified.

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[(1)]	15%	450 Watts	200	300 sec	20 °C

Table 2 - Paraffin removal and tissue rehydration settings

3. Open Screw-Cap microTUBE, add 10 µl of PK solution (from the truXTRAC RNA Kit) to the sample and affix Screw-Cap back in place.
4. Process the sample using the settings provided in Table 3 below to properly mix Proteinase K with the sample.

System	Duty Factor	Peak Incident Power	Cycles per burst	Treatment Time	Temperature (Instrument)
S220 or E220	10%	175 Watts	200	10 sec	20 °C
S2 or E210	10%	5 (Intensity)	200	10 sec	20 °C
M220	20%	75 Watts	200	10 sec	20 °C
LE220[(1)]	15%	450 Watts	200	10 sec	20 °C

Table 3 – Proteinase K mixing settings

(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.

5. Digestion with Proteinase K at 56°C:
    a. Insert the required number of Heat Block microTUBE Adapters into a Heat Block.
    b. Load the microTUBEs into the adapters and incubate for 15 minutes at 56°C.

6. Using a wide bore pipet tip, transfer the sample to a clean 1.5 ml nuclease free microcentrifuge tube**.**

   NOTE: If any liquid is left in the microTUBE after the 1st transfer, use a regular sized pipet tip to transfer remaining sample

7. Centrifuge the microcentrifuge tube at 16,000 x g for 15 minutes.
8. Transfer the supernatant to a new nuclease free microcentrifuge tube taking care to leave the tissue pellet and residual wax behind for DNA solation (section C)

   NOTE: Small amounts of residual wax will not interfere with the RNA purification.

9. Store the microcentrifuge containing the tissue pellet on ice for the DNA isolation step.

B. RNA ISOLATION

1. Incubate the supernatant at 80**°**C for 15 minutes to reverse formaldehyde crosslinks.

   NOTE: If you are using the same heat block for both the 56°C & 80°C incubations, the tube should be kept at room temperature (20-25°) until the heat block reaches 80°C.

2. DNase I treatment (Optional): The sample can be treated with DNase I to remove residual
3. Prepare the DNase master mix as shown below.

Component	Volume Per Sample (in µL)
$MnCl_2$ Solution	**13**
DNase Buffer	**7**
DNase I	**10**
Total Volume per sample; 30µL	

NOTES – DNase I usage:

- **Prepare only the amount of DNase master mix required.**
- **Thaw and keep the DNase I enzyme on ice during use.**
- **The DNase I enzyme is sensitive to physical inactivation. Mix by gentle pipetting. Do not vortex.**
- **Prepare the DNase treatment mix immediately before use. The components of the DNase master mix should be stored separately and mixed fresh for each set of RNA extractions.**

4. Add 30 µL of freshly prepared DNase master mix to each sample and mix by pipetting gently and incubate for 15 minutes at room temperature (20–25°C.)

5. Proceed immediately to "Section 3– RNA Purification" of the truXTRAC FFPE RNA Kit User Manual.

C. DNA ISOLATION

1. Open microcentrifuge tube containing the tissue pellet and residual wax and load 100 µL Tissue SDS Buffer. Vortex to mix
2. Add 20 µL of Proteinase K solution (from truXTRAC FFPE DNA Kit) to the sample and vortex again.
3. Digestion with Proteinase K at 56°C
    a. Set heat block to 56°C.
    b. Insert microcentrifuge tube into a Heat Block once it has reached its 56°C set point.
    c. An incubation time of 1 hour at 56°C is sufficient for sections 10 µm or less in thickness;
4. Incubate the samples at 80**°**C for 1 hour to reverse formaldehyde crosslinks
    a. Set heat block to 80°C.
    b. Insert microcentrifuge tube into a Heat Block once it has reached its 80°C set point.

    NOTE: If you are using the same heat block for both the 56°C & 80°C incubations, the tube should be stored at room temperature until the heat block reaches 80°C.

5. Optional: The sample can be treated with RNase A to remove residual RNA before DNA purification. Add 5µl of RNase A solution and incubate for 5 minutes at room temperature.

    NOTE: If DNA is being extraction according to Option A or Option B of the truXTRAC FFPE DNA Kit protocol, then transfer the sample into a fresh microTUBE Screw-Cap FFPE (from the truXTRAC RNA Kit). If processing in accordance to Option C of the truXTRAC FFPE DNA Kit, then no transfer to a microTUBE Screw-Cap is required.

6. Proceed to "Section 3 – DNA Purification" of the truXTRAC FFPE DNA Kit User Manual