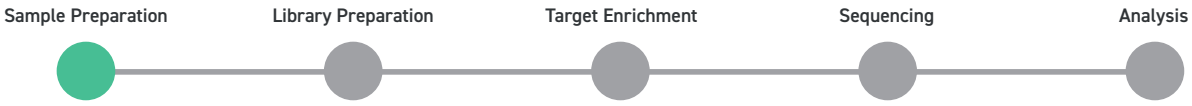
# Twist Sample Preparation Protocol: Covaris FFPE DNA Extraction

## For use with the Twist Library Preparation Protocol– Mechanical Fragmentation

An optimal NGS workflow starts with an optimal Sample Preparation step, followed by the Library Prep. Sample preparation is a critical step, and often challenging because of the quality of the samples. This is the case with FFPE tissue samples, where the starting material could be degraded. Adaptive Focused Acoustics (AFA) technology, developed by Covaris for improving the Sample prep step, drastically improves the yield and the quality of extracted nucleic acids. Covaris is the gold standard technology for NGS fragmentation thanks to its unparalleled results.

The protocol has been developed using state of the art technologies from Covaris and Twist for improving Sample prep and Library Prep. Twist Bioscience's Target Enrichment System for next-generation sequencing (NGS) of genomic DNA (gDNA) optimizes probe balance and design to provide industry-leading performance.

Instructions for gDNA extraction from FFPE samples and fragmentation with AFA technology, will be detailed in this protocol. This method has been optimized to yield high-quality gDNA fragments for use with the Twist Library Preparation Kit, Mechanical Fragmentation.




**Twist NGS workflow.** The complete NGS workflow takes you from sample preparation to NGS sequencing and data analysis. A component of this workflow, this Sample Preparation Protocol works in conjunction with the other component protocols.

*This product is for **research use only**.*

## RECOMMENDED MATERIALS

The following materials or their equivalents are required to generate fragmented gDNA samples from FFPE samples using Covaris AFA technology.

| COMPONENT | SUGGESTED SUPPLIER | PART NUMBER |
|---|---|---|
| **REAGENTS AND CONSUMABLES** | | |
| TE buffer with low EDTA (10 mM Tris-HCl, pH 8.0) | Thermo Fisher Scientific | 12090015 |
| truXTRAC FFPE total NA Plus Kit – Magnetic Bead or truXTRAC FFPE total NA Plus Kit – Column | Covaris | 520255 (Magnetic bead)<br>520252 (Column) |
| (Optional) KAPA hgDNA Quantification and QC Kit | Kapa Biosystems or Roche | Kapa KK4960<br>Roche 07960590001 |
| AFA-TUBE, choice of:<br>8 AFA-TUBE TPX Strip (12) with caps<br>96 AFA-TUBE TPX Plate (1) with thin foil seals | Covaris | 520275, 500639<br>520272, 520235 |
| (Optional) Agilent High Sensitivity Kit | Agilent Technologies | 5067-4626 |
| Twist Library Preparation Kit, Mechanical Fragmentation (for subsequent library preparation) | Twist Bioscience | 101280: 16 rxn<br>101281: 96 rxn |
| **EQUIPMENT** | | |
| LE220-plus Focused-ultrasonicator | Covaris | 500569 |
| Rack 96 AFA-TUBE TPX or Rack 8 AFA-TUBE TPX AFA Strip | Covaris | 500637 (Plate)<br>500608 (Strip) |
| Pipette and tips | Rainin | – |
| Benchtop mini-centrifuge for 0.2 ml tubes | Spectrafuge | C1301 |

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# GENERAL NOTES AND PRECAUTIONS

Wear appropriate protective equipment (lab coat, gloves, and protective glasses or goggles) at all times when performing this protocol.

For best results, read this document before performing the protocol and follow the provided instructions. Twist cannot guarantee the performance of this protocol if modifications are made.

For technical support, contact NGSsupport@twistbioscience.com.

## STEP 1 EXTRACT gDNA FROM FFPE SAMPLES

**1.1**

Use the Covaris truXTRAC FFPE total NA Plus Magnetic Bead or Column Kit and instructions to extract the gDNA from FFPE samples. Find the latest protocol on the Covaris website www.covaris.com or contact Covaris Applications Support at applicationsupport@covaris.com.

**1.2**

OPTIONAL: Use a KAPA hgDNA Quantification and QC Kit to determine the quality of the extracted gDNA. If the Q305/Q41 ratio for an FFPE extracted sample is <0.20, the amount of sequencing depth required to limit the impact to certain critical sequencing metrics may increase when compared directly to samples from high-quality sources.

## STEP 2 FRAGMENT gDNA USING AFA TECHNOLOGY

**Notes:**

- Use Covaris AFA equipment with compatible AFA-TUBE TPX vessels. Set the instrument for gDNA fragmentation to target a size distribution with a mode of 200–250 bp (see Step 2.5 for recommended settings). If using another Covaris machine and AFA vessel, target a library size mode in a range of 200–250 bp by adjusting sample and reaction volumes as required.
- Covaris AFA shearing is concentration-independent when machine settings are held constant.
- This method can be adapted for use with automated systems, as the downstream library preparation steps can be performed in the same AFA-TUBE TPX vessel used for fragmentation.

**2.1**

Determine the gDNA concentration of the samples, for example with a Qubit dsDNA Broad Range Quantitation Assay.

**2.2**

In a Covaris AFA-TUBE TPX vessel (strip or plate), dilute 100 ng each gDNA sample in TE buffer with low EDTA (pH 8.0) to a final volume of 10 µl (final concentration 10 ng/µl).

EXAMPLE: With a 25 ng/µl gDNA sample, dilute 4 µl gDNA into 6 µl TE buffer with low EDTA (pH 8.0).

**2.3**

Seal the AFA-TUBE TPX vessel with either a strip-cap (for 8 AFA-TUBE TPX strip) or foil seal (96 AFA-TUBE TPX Plate) and centrifuge for 10 sec at 2,000 RCF.

**2.4**

Ensure there are no air bubbles at the bottom of the AFA-TUBE TPX vessels, then place the vessel containing the samples into the Covaris AFA instrument.

**2.5**

Process the samples using the following settings (for use with a Covaris AFA LE220-plus instrument with AFA-TUBE TPX consumables).

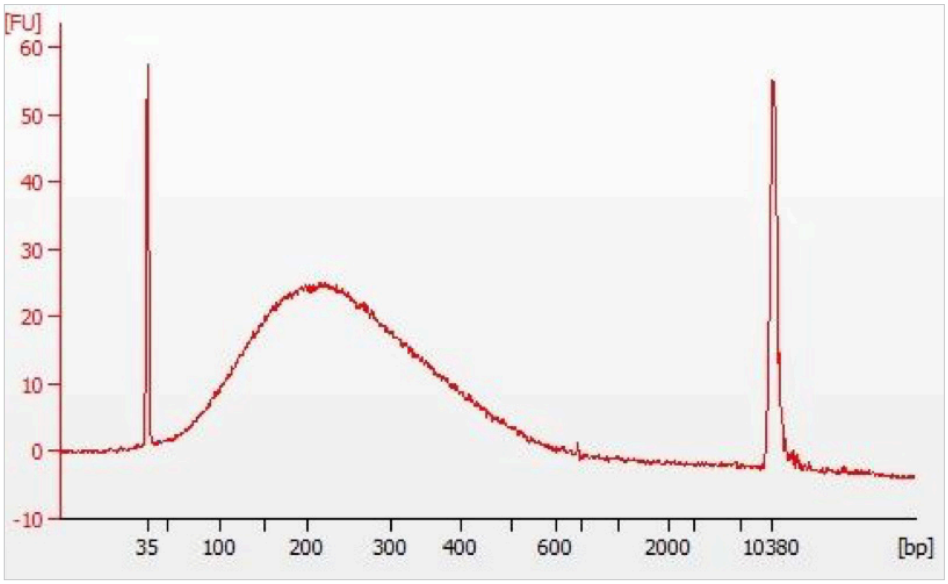
For the most current settings refer to the Covaris website www.covaris.com or contact Covaris Applications Support at applicationsupport@covaris.com.

## INSTRUMENT SETTINGS FOR gDNA FRAGMENTATION USING AN LE220-PLUS FOCUSED-ULTRASONICATOR

| | LE220-PLUS FOCUSED-ULTRASONICATOR | |
|---|---|---|
| | 96 AFA-TUBE TPX Plate (520272) | 8 AFA-TUBE TPX Strip (520275) |
| Software | SonoLab 8.4 or higher recommended | |
| Rack | Rack 96 AFA-TUBE TPX Plate (500637) | LE220-plus Rack 8 AFA-TUBE TPX Strip (500608) |
| Plate Definition | LE220plus_520637<br>96 AFA-TUBE TPX Plate -2.2 offset | LE220plus_500608<br>8 AFA-TUBE TPX Strip -2.2 offset |
| Waveguide | — | |
| Volume (µl) | 10 | |
| Dithering (distance) | 1 mm Y-dither | |
| Dithering (speed) | 20 mm/sec | |
| Time (sec) | 140 sec (250 bp target mode) to 220 sec (200 bp target mode) | |
| Peak Incident Power (W) | 200 | |
| Duty Factor (%) | 25 | |
| Cycles per Burst (CpB) | 50 | |
| Pulsing (on, sec) | 10 | |
| Pulsing (off, sec) | 20 | |
| Temperature (°C) | 10 | |

**2.6**

OPTIONAL: For optimum performance, analyze the size distribution of the fragmented gDNA with an Agilent High Sensitivity DNA Kit before proceeding with library preparation using the Twist Library Preparation Kit, Mechanical Fragmentation. Ensure the mode of the fragment size distribution is 200–250 bp. Adjust the AFA program time in increments of 10 seconds until the mode reaches this range of distribution (increase the time to yield smaller fragments or decrease it for larger fragments).




*DNA fragment size distribution of a gDNA fragment library immediately after mechanical shearing, as analyzed using an Agilent High Sensitivity DNA Assay.*

STOPPING POINT: If not proceeding immediately to the Twist Library Preparation Protocol – Mechanical Fragmentation, store the DNA samples at –20°C.

**END WORK FLOW**