Covaris®

truChIP® Chromatin Shearing Kit

Adaptive Focused Acoustics® (AFA®)-based Chromatin Shearing for ChIP-based Applications

PN 520154 (with Formaldehyde) and PN 520127 (without Formaldehyde)

Patents Granted and Pending

Part Number: 010179 Rev N | November 2019

Table of Contents

General Information

Intended Use

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

Introduction

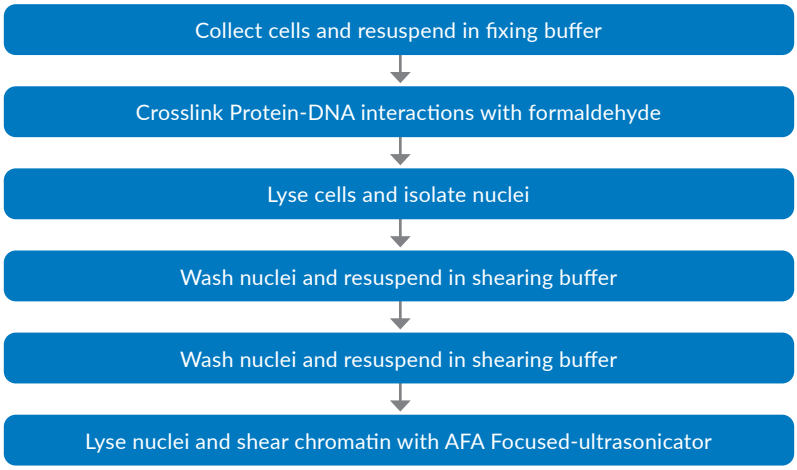
The truChIP Chromatin Shearing Kit is optimized for the efficient and reproducible shearing of chromatin from adherent and suspension mammalian cells using Covaris AFA Focused-ultrasonicators. Focused-ultrasonicators provide a non-contact and isothermal method of shearing chromatin without compromising the structural integrity of the target epitopes of interest. This kit can be used to prepare samples for ChIP-qPCR, ChIP-ChiP, and ChIP-Seq applications.

truChIP is compatible with a variety of cell types including primary, stem cells, and sorted cells. The Covaris shearing buffer contains SDS, which may not be compatible with immunoprecipitations (IP) using antibodies against some commonly used protein tags. Therefore, Covaris recommends contacting the antibody manufacturer prior to using this kit. To start, Covaris recommends users to perform a one-time fixation and shearing time course study to empirically determine the optimal treatment conditions.

Revision History

Part Number	Revision	Date	Description of Change
010179	I	03/17	Update template and publish ME220 settings
010179	J	7/17	Remove specific content description of Buffer D3
010179	K	7/17	Correct procedure overview
010179	L	11/18	Correct step 10 on the nuclei prep protocol and add IP Dilution Buffer
010179	M	6/19	Adding water level clarification
010179	N	10/19	Change Protease inhibitor cocktail, change LE220 settings, add Buffer for reverse X-link, change input cell numbers for M-Series Instruments, adjust fixation times for TFs, added LE220-plus settings, added IP part and description, added NEXSON settings as Appendix C

Procedure Overview



Sample Input Requirements

The truChIP Chromatin Shearing Kit is compatible with a range of inputs from less than 1 million ($1x10^6$) and up to 200 million ($2x10^8$) cells. The Low Cell protocol has been optimized for chromatin shearing of 1 to 3 million cells using the microTUBE-130 with AFA Fiber; the High Cell protocol uses the milliTUBE–1 mL with AFA Fiber to process up to 30 million cells; and the batch protocol is optimized for preparing up to 200 million cells cultured as a suspension for chromatin shearing in 6 separate milliTUBE–1 mL with AFA Fiber.

Single Sample	Low Cell	High Cell	Batch
Input cell number	1 to 3 million* (1 to 3 x 10^6) cells	5 to 30 million* (0.5 to 3 x 10^7) cells	50 to 200 Million* (0.5 to 2 x 10^8) cells
Number of samples sheared per kit	50	15	2
AFA tube	microTUBE–130	milliTUBE–1 mL with AFA Fiber	6 × milliTUBE–1 mL with AFA Fiber
Shearing volume	130 µl	1 mL	6 x 1 mL

Note: Please note that for M-Series Instruments we suggest the following upper limits for input cell numbers: Low Cell: 1.5M, High Cell: 15M, Batch: 90M

Kit Contents

Buffer A	7.5 mL	10X Fixing Buffer
Buffer B	5 mL	5X Lysis Buffer
Buffer C	2.5 mL	10X Wash Buffer
Buffer D3	6 mL	10X SDS Shearing Buffer
Buffer E	6 mL	1X Quenching Buffer
Buffer F	Protease Inhibitor Cocktail Tablet	50X Protease Inhibitor Cocktail (PIC) – See Note
Buffer G	1.0 ml	Reverse Crosslinking Buffer
Dilution Buffer	20 mL	2X IP Dilution Buffer
Formaldehyde	5 x 1 mL ampules (PN 520154 only)	16% Methanol-free Formaldehyde

Note: To prepare the 50X Protease Inhibitor Cocktail add 1 ml of 18 megohm (mΩ) water to the Protease Inhibitor Cocktail Tablet in vial and vortex the tube until the powder is fully dissolved. The buffer is stable for 1 to 2 weeks at 2 to 8 °C and at least 12 weeks at -15 to 25 °C. We suggest to aliquot the buffer in working aliquots for storage at -15 to 25 °C.

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

Storage

The kit is shipped at room temperature and should be stored at 2 to 8 °C upon arrival. Prior to use, kit reagent Buffers D3 and E may have to be warmed to 55 °C to dissolve precipitate and then cooled to room temperature before use.

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

Supplied by User

- Molecular Biology Grade Water – Thermo Scientific (Cat. No. SH3053802), Mo Bio (Cat. No. 17012-200), or equivalent
- Phosphate Buffered Salt Solution (PBS) – Mo Bio (Cat. No. 17330-500), Thermo Scientific (Cat. No. SH30256.FS), or equivalent
- RNase A (DNase free) Thermo Scientific (Cat. No. EN0531) or equivalent
- Proteinase K (RNase and DNase free) Thermo Scientific (Cat. No. 17916), NEB (Cat. No. P8102S), or equivalent
- Covaris Focused-ultrasonicator (M220, ME220, S220, E220*evolution*, E220, or LE220-plus)
- Refrigerated centrifuge having 15,000 x g capability
- Rocker – Nutator® or equivalent
- AFA Tubes, Holders, and Racks

Low Cell Protocol Consumables, Holder and Racks Required

Part Number	Description	M220 Holder & Insert	ME220 Holder & Insert	S-Series Holder	E220*evolution* Rack	E220 Rack	LE220-plus Rack
520045	microTUBE AFA Fiber Pre-Slit Snap-Cap	500414 & 500489	500514 & 500526	500114	500433	500111	N/A
520052	microTUBE AFA Fiber Crimp-Cap	N/A	500514 & 500526	500114	500433	500282	500282
520216	microTUBE-130 AFA Fiber Screw-Cap	500414 & 500489	500522 & 500534	500339	N/A	N/A	N/A
520053	8 microTUBE Strip V1	N/A	500514 & 500526	N/A	500430	500191	500191
520217	8 microTUBE-130 AFA Fiber Strip V2	N/A	500518 & 500526	N/A	N/A	N/A	N/A
520078	96 microTUBE Plate	N/A	N/A	N/A	N/A	No Rack Required	500329

High Cell Protocol Consumables, Holder, and Racks Required

Part Number	Description	M220 Holder & Insert	ME220 Holder & Insert	S-Series Holder	E220*evolution* Rack	E220 Rack	LE220-plus Rack
520130	milliTUBE–1 mL with AFA Fiber	500414 & 500422	500520 & 500534	500371	500431	500368	500368

Protocol

A. Cell Preparation and Crosslinking

The truChIP protocol uses a two-step lysis method to ensure reproducible and efficient shearing of both suspension and adherent cells. Follow the Cell Preparation and Crosslinking method (***A.1 – Suspension and A.2 – Adherent***) for your cell culture type.

Note: ChIP assays are sensitive to crosslinking and shearing conditions. Therefore, Covaris recommends users to include multiple fixation and shearing time points to empirically determine the optimal treatment conditions.

A.1 Suspension Cells

1. Prepare solutions for the appropriate number of samples being processed before starting. Volumes in the table below are for a single sample.

Buffer	Low Cell	High Cell	Batch
Cold 1X PBS	**Final Volume: 2.0 mL per sample** - Store on ice	**Final Volume: 4 mL per sample** - Store on ice	**Final Volume: 40 mL per batch** - Store on ice
1X Fixing Buffer A	**Final Volume: 0.5 mL per sample** - Mix 50 µl of Fixing Buffer A with 0.450 mL of molecular biology grade water	**Final Volume: 2 mL per sample** - Mix 200 µl of Fixing Buffer A with 1.8 mL of molecular biology grade water	**Final Volume: 20 mL per batch** - Mix 2.0 mL of Fixing Buffer A with 18 mL of molecular biology grade water
Fresh 11.1% Formaldehyde	**Final Volume: 1 mL per 1 to 20 samples** - Mix 690 µl of 16% Fresh Formaldehyde with 310 µl of molecular biology grade water	**Final Volume: 1 mL per 1 to 5 samples** - Mix 690 µl of 16% Fresh Formaldehyde with 310 µl of molecular biology grade water	**Final Volume: 2 mL per batch** - Mix 1.38 mL of 16% Fresh Formaldehyde with 0.62 mL of molecular biology grade water
Quenching Buffer E	Place in a 55 °C water bath to dissolve crystals, then place at ambient		

Important Notes
- The use of fresh methanol-free formaldehyde is required to achieve reproducible results
- The methanol-free formaldehyde ampule is for one-time use only–storage for later use is not recommended

2. Collect cells by centrifugation at 200 x g for 5 minutes at room temperature. Remove media and wash cells once with cold 1X PBS and collect cells again by centrifugation.

   Note: Some cells do not pellet well at 200 x g. If a "spongy" pellet is not visible, increase speed at 100 x g intervals until a pellet is visible.

Reagent	Low Cell	High Cell	Batch
Cold 1X PBS	400 µl	1.5 mL	20 mL
Input cell number	1 to 3 x 10^6 Cells	1 to 3 x 10^7 Cells	0.5 to 2 x 10^8 Cells
Centrifuge Tube	2.0 mL	2.0 mL	50 mL

3. Re-suspend cells in room temperature Fixing Buffer A.

Reagent	Low Cell	High Cell	Batch
Fixing Buffer A	400 µl	1.5 mL	20 mL

4. Crosslink cells by adding freshly prepared 11.1% formaldehyde solution to a final concentration of 1% and set timer.

   Note: The use of fresh methanol-free formaldehyde is required to achieve reproducible results.

Reagent	Low Cell	High Cell	Batch
Fresh 11.1% Formaldehyde	40 µl	150 µl	2.0 mL

5. Place cells on a shaking platform at room temperature for the recommended time.

   Note: We recommend including two fixation times. Typically, ***2.5 and 5 minutes*** for stem, blood-derived and primary cells, and ***5 and 10 minutes*** for all other cell types. The fixation time will also depend on the respective target for IP and must be determined empirically. For transcription factors a fixation time of 10 minutes is recommended.

6. Quench the crosslinking reaction by adding the appropriate volume of Quenching Buffer E to the fixed cells. Keep cells on rocker at room temperature for an additional 5 minutes.

Reagent	Low Cell	High Cell	Batch
Quenching Buffer E	23 µl	87 µl	1.2 mL

7. Collect cells by centrifuging at 500 x g for 5 minutes at room temperature.

8. Aspirate the supernatant and wash twice with cold 1X PBS.

Reagent	Low Cell	High Cell	Batch
Cold 1X PBS	300 µl	1.0 mL	5.0 mL

9. Collect cells by centrifugation at 200 x g for 5 minutes, 4 °C.

10. Proceed to nuclei preparation and chromatin shearing steps.

    Note: You may flash-freeze the fixed cells in liquid nitrogen at this point and store at -80 °C for short periods of time (*e.g.*, 2 to 3 days). Longer-term storage is not recommended.

A.2 Adherent Cells

1. Grow the proper number of cells to conduct a single ChIP assay or the initial time course until they are 80 to 90% confluent.

   Note: Cell densities provided above are estimates provided as a general guideline. Accurate cell densities for your cell lines should be determined.

	35 mm Plate	60 mm Plate	100 mm Plate	150 mm Plate
Cell Density	~0.8 x 10^6	~2.0 x 10^6	~5.5 x 10^6	~15 x 10^6
Protocol	Low Cell	Low Cell	High Cell	High Cell
Number of Plates	1 to 3	1 to 2	2 to 5	1 to 2

2. Prepare solutions for the appropriate number of samples being processed fresh before starting. Volumes in the table below are for a single sample.

Buffer	35 and 60 mm Dish	100 and 150 mm Plate
Cold 1X PBS	**Final Volume: 14 mL per Plate** - Store on ice	**Final Volume: 25 mL per Plate** - Store on ice
1X Fixing Buffer A	**Final Volume: 2 mL per Plate** - Mix 200 µl of Fixing Buffer A with 1.8 mL of molecular biology grade water	**Final Volume: 2 mL per sample** - Mix 500 µl of Fixing Buffer A with 4.5 mL of molecular biology grade water
Fresh 11.1% Formaldehyde	**Final Volume: 1 mL per 1 to 20 samples** - Mix 208 µl of 16% Fresh Formaldehyde with 92 µl of molecular biology grade water	**Final Volume: 1 mL per 1 to 5 samples** - Mix 0.69 mL of 16% Fresh Formaldehyde with 0.31 mL of molecular biology grade water
Quenching Buffer E	Place in a 55 °C water bath to dissolve crystals, then place at ambient	

Important Notes
- The use of fresh methanol-free formaldehyde is required to achieve reproducible results
- The methanol-free formaldehyde ampoule is for one-time use only–storage for later use is not recommended

3. Remove media and wash each plate one time with cold 1X PBS.

Reagent	35 and 60 mm Plate	100 and 150 mm Plate
Cold 1X PBS	2 mL	5 mL

4. Remove PBS and add room temperature Fixing Buffer A to each dish.

Reagent	35 and 60 mm Plate	100 and 150 mm Plate
Fixing Buffer A	2 mL	5 mL

5. Crosslink cells by adding freshly prepared 11.1% formaldehyde solution to a final concentration of 1% and start timing the crosslinking reaction.

   Note: The use of fresh methanol-free formaldehyde is required to achieve reproducible results.

Reagent	35 and 60 mm Plate	100 and 150 mm Plate
Fresh 11.1% Formaldehyde	200 µl	500 µl

6. Place cells on a shaking platform at room temperature for the recommended time.

   Note: We recommend including two fixation times. Typically, ***2.5 and 5 minutes*** for stem, blood-derived and primary cells, and ***5 and 10 minutes*** for all other cell types. The fixation time will also depend on the respective target for IP and must be determined empirically. For transcription factors a fixation time of 10 minutes is recommended.

7. Quench the crosslinking reaction by adding the appropriate volume of Quenching Buffer E to fixed cells. Keep on a shaking platform at room temperature for an additional 5 minutes.

Reagent	35 and 60 mm Plate	100 and 150 mm Plate
Quenching Buffer E	120 µl	300 µl

8. Completely aspirate the solution from the plate.

9. Add cold 1X PBS to each dish and scrape cells from the plate into a proper vessel.

Reagent	35 and 60 mm Plate	100 and 150 mm Plate
Cold 1X PBS	450 µl	5 mL
Centrifuge Tube	2.0 mL tube	15 mL conical

10. Wash the plate with an additional volume of cold 1X PBS to collect any remaining cells.

Reagent	35 and 60 mm Plate	100 and 150 mm Plate
Cold 1X PBS	450 µl	5 mL
Centrifuge Tube	2.0 mL tube	15 mL conical

11. Collect cells at 200 x g for 5 minutes, 4 °C.

    Note: Some cells do not pellet well at 200 x g. If a "spongy" pellet is not visible, increase speed at 100 x g intervals until a pellet is visible.

12. Wash cells twice by resuspending in cold 1X PBS, and collecting by centrifugation at 200 x g, 4 °C.

Reagent	35 and 60 mm Plate	100 and 150 mm Plate
Cold 1X PBS	450 µl	5 mL

13. Carefully and completely aspirate the supernatant from the tube(s), and place on ice. Proceed to nuclei preparation and chromatin shearing steps.

    Note: You may flash-freeze the fixed cells in liquid nitrogen at this point and store at -80 °C for short periods of time (e.g., 2 to 3 days). Longer-term storage is not recommended.

B. Nuclei Preparation

1. Prepare the proper number of suspension or adherent cells according to ***Step 1 on page 7***. Place the required number of AFA tubes on ice to pre-chill while preparing samples to shear.

   Note: Prepare the correct volume of fresh solutions for the nuclei preparation and chromatin shearing prior to beginning. Substituting any of the reagents or changing any steps will adversely affect shearing efficiency and reproducibility.

   Note: Volumes in the table below are for a single sample.

	Low Cell	High Cell	Batch
Number of fixed cells	1 to 3 x 10^{6}*	1 to 3 x 10^{7}*	0.5 to 2 x 10^{8}*
AFA Tube	microTUBE-130	milliTUBE-1 mL	6 × milliTUBE-1 mL

   ***Note:** Please note that for M-series Instruments we suggest the following upper limits for input cell numbers:
   Low Cell: 1.5M, High Cell: 15M, Batch: 90M

Buffer	Low Cell	High Cell	Batch
1X Lysis Buffer B	**Final Volume: 0.5 mL per sample** - Mix 100 µl 5X Lysis Buffer B with 390 µl of molecular biology grade water - Add 10 µl of 50X Buffer F - Store on ice	**Final Volume: 1.0 mL per sample** - Mix 200 µl 5X Lysis Buffer B with 0.79 mL of molecular biology grade water - Add 20 µl of 50X Buffer F - Store on ice	**Final Volume: 10 mL per sample** - Mix 2 mL 5X Lysis Buffer B with 7.9 mL of molecular biology grade water - Add 200 µl of 50X Buffer F - Store on ice
1X Wash Buffer	**Final Volume: 0.5 mL per sample** - Mix 50 µl 10X Wash Buffer C with 440 µl of molecular biology grade water - Add 10 µl of 50X Buffer F - Store on ice	**Final Volume: 1.0 mL per sample** - Mix 100 µl 10X Wash Buffer C with 0.89 mL of molecular biology grade water - Add 20 µl of 50X Buffer F - Store on ice	**Final Volume: 10 mL per sample** - Mix 1.0 mL 10X Wash Buffer C with 8.9 mL of molecular biology grade water - Add 200 µl of 50X Buffer F - Store on ice
1X Shearing Buffer D3 (1mM EDTA, 10mM Tris-HCl pH 7.6, 0.1% SDS)	**Final Volume: 1 mL per sample** - Mix 100 µl 10X Shearing Buffer D3 with 0.89 mL of molecular biology grade water - Add 20 µl of 50X Buffer F - Store on ice	**Final Volume: 3 mL per sample** - Mix 300 µl 10X Shearing Buffer D3 with 2.67 mL of molecular biology grade water - Add 60 µl of 50 X Buffer F - Store on ice	**Final Volume: 10 mL per sample** - Mix 1.0 mL 10X Shearing Buffer D3 with 8.9 mL of molecular biology grade water - Add 200 µl of 50 X Buffer F - Store on ice

2. Add Lysis Buffer B containing 1× protease inhibitors to cross-linked cells to lyse plasma membrane, gently resuspend by aspirating/dispensing 4 times. If cells were frozen after formaldehyde fixation, thaw cells on ice first.

Reagent	Low Cell	High Cell	Batch
Lysis Buffer B	300 µl	1 mL	10 mL

3. If processing using Low Cell or High Cell volumes, then transfer to 1.5 mL microcentrifuge tube. If processing for batch, then transfer to a 15 mL conical tube.

4. Incubate for 10 minutes on a rocker at 4 °C.

   Note: For very hard to lyse cells the nuclear extraction incubation step can be substituted with AFA lysis per the NEXSON protocol[8]. The AFA-mediated lysis settings are provided in Appendix D.

5. Collect intact nuclei by centrifugation at 1,700 x g for 5 minutes, 4 °C. Decant the supernatant without disturbing the nuclei pellet.

6. Gently resuspend pellet in Wash Buffer C containing protease inhibitor (Buffer F) and incubate on a rocker for 10 minutes, 4 °C.

   Note: The purpose of this wash is to significantly dilute the salts remaining from the Wash Buffer. Shearing in the presence of high salt concentrations may result in the reversal of formaldehyde cross-links during the process.

Reagent	Low Cell	High Cell	Batch
Wash Buffer C	300 µl	1 mL	10 mL

7. Collect nuclei by centrifugation at 1,700 x g for 5 minutes, 4 °C. Carefully remove and discard the wash solution, taking care not to disturb the nuclei pellet.

8. Gently rinse the sides of the tube with Shearing Buffer D3 containing Protease inhibitor (Buffer F). Slowly dispense the buffer down the entire circumference of the upper-inside of the tube, taking care not to disturb the nuclei pellet.

Reagent	Low Cell	High Cell	Batch
Shearing Buffer D3	300 µl	1 mL	1.5 mL

9. Collect nuclei by centrifugation at 1,700 x g for 5 minutes, 4 °C. Decant the supernatant without disturbing the nuclei pellet.

10. Repeat steps 8 and 9 an additional time. Carefully remove and discard the supernatant, taking care not to disturb the nuclei pellet.

C. Chromatin Shearing

1. Resuspend nuclei pellet in the Shearing Buffer D3 and transfer to appropriate AFA Tube(s).

Reagent	Low Cell	High Cell	Batch
Shearing Buffer D3	130 µl	1 mL	6 mL
AFA Tube	1 x microTUBE	1 x milliTUBE	6 x milliTUBE

2. Shear chromatin with an AFA Focused-ultrasonicator with appropriate rack or holder; settings are provided in ***Appendix A***.

   Note: Optimization of shearing time should be conducted whenever experimental parameters (e.g., cell type, cell number, or sample volumes) are changed.

3. If processing samples for Low Cell Chromatin Shearing Optimization in microTUBEs, please aliquot 130 µl of the nuclei preparation into 6 microTUBEs for carrying out the shearing time course of 2, 4, 6, 8, 10, and 12 minutes.

   If processing samples for High Cell Chromatin Shearing Optimization, please aliquot 1 mL of the nuclei into one milliTUBE–1 mL with AFA Fiber for carrying out the shearing time course. of 2, 4, 8, 12, 15, and 20 minutes.

4. If processing samples using the milliTUBE–1 mL with AFA Fiber, you may process all time points of the time course study in the same tube according to the figure below. After each time point interval, remove 35 µl of the sample and place in a pre-chilled microcentrifuge tube labeled with the total processing time – store on ice. Replace the removed volume with 35 µl of Covaris Shearing Buffer D3 before running the next time point.

Programmed Interval Processing Time (min)	2	2	4	4	3	5
Total Processing Time (min)	2	4	8	12	15	20

5. Place the milliTUBE in the holder/rack and process on the ultrasonicator for the next programmed interval processing time. 25 µl will be used for DNA shearing size range analysis, and 10 µl will be used for epitope integrity analysis using western. Replace the removed volume with 35 µl of Covaris Shearing Buffer D3 before running the next time point.

   Note: Carefully remove any traces of foam from the solution and the cap prior to placing sample back in the ultrasonicator for the next shearing time point when carrying out the time course experiment in the milliTUBE-1mL with AFA fiber.

6. After shearing, transfer samples into a pre-chilled microcentrifuge tube and place on ice until all tubes are processed. If batch processing using a high-throughput ultrasonicator (e.g. E220), samples can be maintained in the instrument's water bath at 4 °C before and after processing.

   Note: Do not proceed to IP before analyzing the sheared chromatin to make sure it is at the correct size and distribution for your downstream analysis.
   Note: To check the efficiency of your shearing, reserve 25 µl of the sheared chromatin.
   Note: Sheared chromatin can be stored at 4 °C for up to 2 days.
   Note: Freezing sheared chromatin is not recommended. Freeze/thaw cycles reduce IP efficiency and reproducibility.

D. Chromatin Shearing Efficiency Analysis

1. Take a 25 µl aliquot of the sheared sample and transfer to 0.6 mL microcentrifuge tube.
2. Add 3 µl of Buffer G.
3. Add 1 µl of RNase A (10 mg/mL) and incubate at 37 °C for 30 min.
4. Add 1 µl of Proteinase K (10 mg/mL) and reverse crosslink by heating at 65 °C overnight in a PCR cycler with a heated lid.
5. Purify DNA using either a commercial column-based kit (*e.g.*, Qiagen QIAquick PCR Purification Kit, Cat. No. 28104)
6. Elute from column or resuspend pellet with 50 µl of elution buffer (10 mM Tris-HCl, pH 8.5).
7. If analyzing on an agarose gel, add 1 volume of loading dye to 5 volumes of purified DNA.

   Note: The use of loading dye without Bromophenol Blue is recommended. Bromophenol.

8. Load 300 to 600 ng of purified DNA per lane of a 1.2% agarose TAE gel, and run at 30 Volts for 2.5 to 4 hours for optimal resolution.

   Note: We strongly advise the use of a Bioanalyzer DNA 12000 chip for chromatin shearing smear analysis.

9. Analyze 1 µl of purified DNA on an Agilent 2100 BioAnalyzer to provide an accurate representation of the shearing size range and distribution.

Supplemental Material

Appendix A. AFA Focused-ultrasonicator Operating Condition

Low Cell Chromatin Shearing Protocol						
Instrument	M220	ME220	S220	E220*evolution*	E220	LE220/LE220-plus
Target Size (bp)	200 to 700	200 to 700	200 to 700	200 to 700	200 to 700	200 to 700
PIP	75	75	105	105	105	300
Duty Factor (%)	5	5	2	2	2	15
CPB	200	1000	200	200	200	200
Treatment Time (minutes)	2 to 20	2 to 20	2 to 12	2 to 12	2 to 12	2 to 12
Setpoint Temperature (C)[1]	7	9	6	6	6	6
Min/Max Temperature (C)	4/10	6/12	3/9	3/9	3/9	3/9
Max Cell Number (Million)	3M	3M	3M	3M	3M	3M
AFA Intensifier Required[2]	N/A	N/A	Integrated	Yes	Yes	N/A
Water Level (run)[3]	Full	9	12	6	6	6
Sample Volume (µl)[4]	130	130	130	130	130	130

Important Notes

1. If using the S220, E220*evolution*, E220, or LE220, set the temperature on the external chiller 3 °C below the setpoint temperature for the run. The min/max is set in SonoLab. 2. If intensifier is required, please ensure PN 500141 is used. 3. Water level should always be 1 mm below the neck of the microTUBE–130 cap.
4. Always fill the microTUBE-130 with 130 µl of sample.

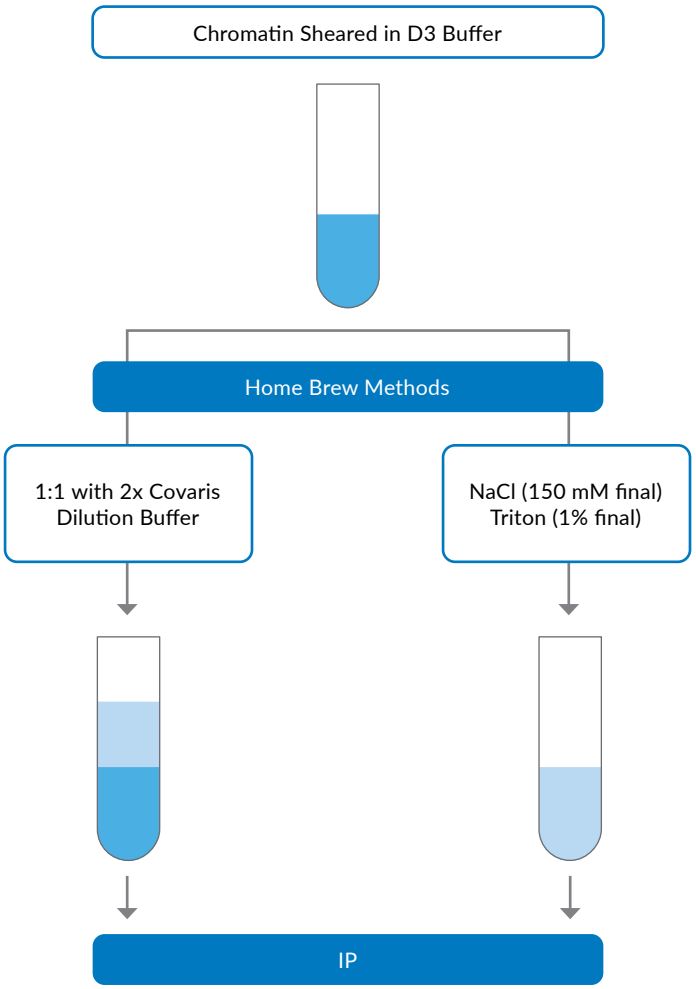
High Cell Chromatin Shearing Protocol						
Instrument	M220	ME220	S220	E220*evolution*	E220	LE220/LE220-plus
Target Size (bp)	200 to 700	200 to 700	200 to 700	200 to 700	200 to 700	200 to 700
PIP	75	75	140	140	140	420
Duty Factor (%)	10	15	5	5	5	30
CPB	200	1000	200	200	200	200
Treatment Time (minutes)	2 to 20	2 to 20	2 to 20	2 to 20	2 to 20	5 to 30
Setpoint Temperature (C)[1]	7	9	6	6	6	6
Min/Max Temperature (C)	4/10	6/12	3/9	3/9	3/9	3/9
Max Cell Number (Million)	30M	30M	30M	30M	30M	30M
AFA Intensifier Required	N/A	N/A	N/A	No	No	N/A
Water Level (run)[2]	Full	9	8	5	0	-4
Sample Volume (ml) [3]	1	1	1	1	1	1

Important Notes

1. If using the S220, E220*evolution*, E220, or LE220, set the temperature on the external chiller 3 °C below the setpoint temperature for the run. The min/max is set in SonoLab. 2. Water level should always be 1 mm below the neck of the milliTUBE–1 mL with AFA Fiber cap. 3. Always fill the milliTUBE–1 mL with AFA Fiber with 1.0 mL of sample.

Appendix B. Preparing Sheared Chromatin for IP

1. Before proceeding to IP, dilute the chromatin 1:1 with the Covaris 2X IP Dilution Buffer, and vortex for 3 seconds.
2. Centrifuge sample after the addition of the IP Dilution buffer at 10,000 x g for 5 minutes at 4 °C to prepare the supernatant for IP.



Sheared chromatin must be diluted prior to IP.

This is required to equilibrate the sheared chromatin in a buffer that is compatible with antibody binding. Therefore, dilute the chromatin sheared in the Covaris chromatin shearing buffer D3 1:1 with the 2X Dilution Buffer. Alternatively, if the chromatin sample should not be diluted further, add NaCl to a final concentration of 150 mM and Triton to a final concentration of 1%. If you do not adjust these conditions prior to IP the enrichment of your target will not occur, or it will be suboptimal.

Appendix C. NEXSON[8]

The efficiency of nuclei isolation can be enhanced by supporting cellular lysis with AFA. This has especially proven useful for difficult to lyse cells.

1. Transfer the fixed cells resuspended in 1ml of Buffer B into 1 ml milliTUBEs
2. Process the samples for AFA-mediated lysis according to the settings in the table below

AFA-mediated Cell Lysis Operating Conditions for up to 12 Million cells.

Up to 12 million cells in milliTUBE 1 ml AFA Fiber (PN 520130)						
Instrument	M220	ME220	S220	E220*evolution*	E220	LE220/LE220-plus
PIP	75	75	75	75	75	N/A
Duty Factor (%)	5	5	2	2	2	N/A
CPB	200	1000	200	200	200	N/A
Treatment Time (minutes)	30 sec to 5 min	30 sec to 5 min	30 sec to 5 min	30 sec to 5 min	30 sec to 5 min	30 sec to 5 min
Setpoint Temperature (C)[1]	7	9	6	6	6	6
Min/Max Temperature (C)	4/10	6/12	3/9	3/9	3/9	3/9
Max Cell Number (Million)	12M	30M	30M	30M	30M	30M
AFA Intensifier Required	N/A	N/A	N/A	No	No	N/A
Water Level (run)[2]	Full	9	8	5	0	-4
Sample Volume (ml) [3]	1	1	1	1	1	1

Important Notes

1. Treatment time must be determined for the cell type of interest and nuclei lysis should be determined by microscopy as described in the original publication[8].
2. If using the S220, E220*evolution*, E220, or LE220, set the temperature on the external chiller 3 °C below the setpoint temperature for the run. The min/max is set in SonoLab.
3. Water level should always be 1 mm below the neck of the milliTUBE–1 mL with AFA Fiber cap.
4. Always fill the milliTUBE–1 mL with AFA Fiber with 1.0 mL of sample.

3. Continue with the nuclei preparation protocol beginning with step 5 (Section B, page 10).

Appendix D. Additional Notes

1. The treatment settings listed in this document are recommended guidelines. Actual results may vary depending on the cell type and mass.
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 daily 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.

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