Covaris®

a PerkinElmer Company

truCOVER® Kits

WGS PCR-free Library Prep Kit (PN 520361KIT)

Library Amplification Kit (PN 520366KIT)

Table of Contents

Revision History

Part Number	Revision	Date	Description of Change
010673	A	10/2024	Initial Release
010673	B	07/2025	Amplification Kit was added
010673	C	03/2026	Trademark, Adapter Ligation step edits, & addition of "KIT" to PNs

Intended Use

The ***truCOVER WGS PCR-free Library Prep Kit*** is intended for generating DNA libraries compatible with next generation sequencing platforms. ***The truCOVER Library Amplification Kit*** is intended for post-library preparation amplification of the generated DNA libraries.

Product Description

The truCOVER WGS PCR-free Library Prep Kit and truCOVER Library Amplification Kit and associated workflows were developed to enhance the efficiency and quality of library preparation for next-generation sequencing (NGS) applications, particularly for whole genome sequencing (WGS). These kits combine the proven accuracy and reproducibility of mechanical DNA fragmentation using Adaptive Focused Acoustics® (AFA®) with the subsequent conversion of fragmented DNA into NGS-ready libraries. Please note that the truCOVER WGS Library prep workflow is a fine-tuned process, combining optimized mechanical fragmentation, library conversion, and post-library clean-up. Thus, mechanical shearing parameters are paired with an optimal downstream process, especially post-library size selection. Changes to this protocol may result in reduced performance.

truCOVER offers a streamlined workflow that integrates AFA-based shearing, library preparation, and magnetic bead clean-up with a single vessel, thus minimizing sample loss and maximizing convenience and process simplicity. A post-library preparation amplification step can be added for low DNA input.

The workflow begins with fragmentation of the input DNA via AFA-based shearing in the 8 AFA-TUBE® TPX Strip or plate. End-repair, 3' A-tail addition, and adapter ligation are directly performed in the same vessel without the need for DNA concentration adjustments. The final magnetic bead-based, clean-up step has been optimized to virtually eliminate the known bias towards small fragment amplification on Illumina® flow cells. When following this workflow, median insert sizes obtained by sequencing are generally greater than 360 bp. This optimized clean-up step also virtually eliminates fragment size biases if post-library prep amplification is desired.

Due to a highly optimized workflow, DNA can be pooled based on mass (fluorescent measurement) rather than qPCR-based quantification. The PCR-free truCOVER workflow for DNA extracted from various sources allows for inputs as low as 50–500 ng. If adding the library amplification, the inputs can be as low as 0.1 ng (100 pg). truCOVER reagents can also be used for library construction from DNA extracted from FFPE samples. For these samples, the initial shearing settings and final magnetic bead clean-up are modified to ensure maximum library conversion efficiency.

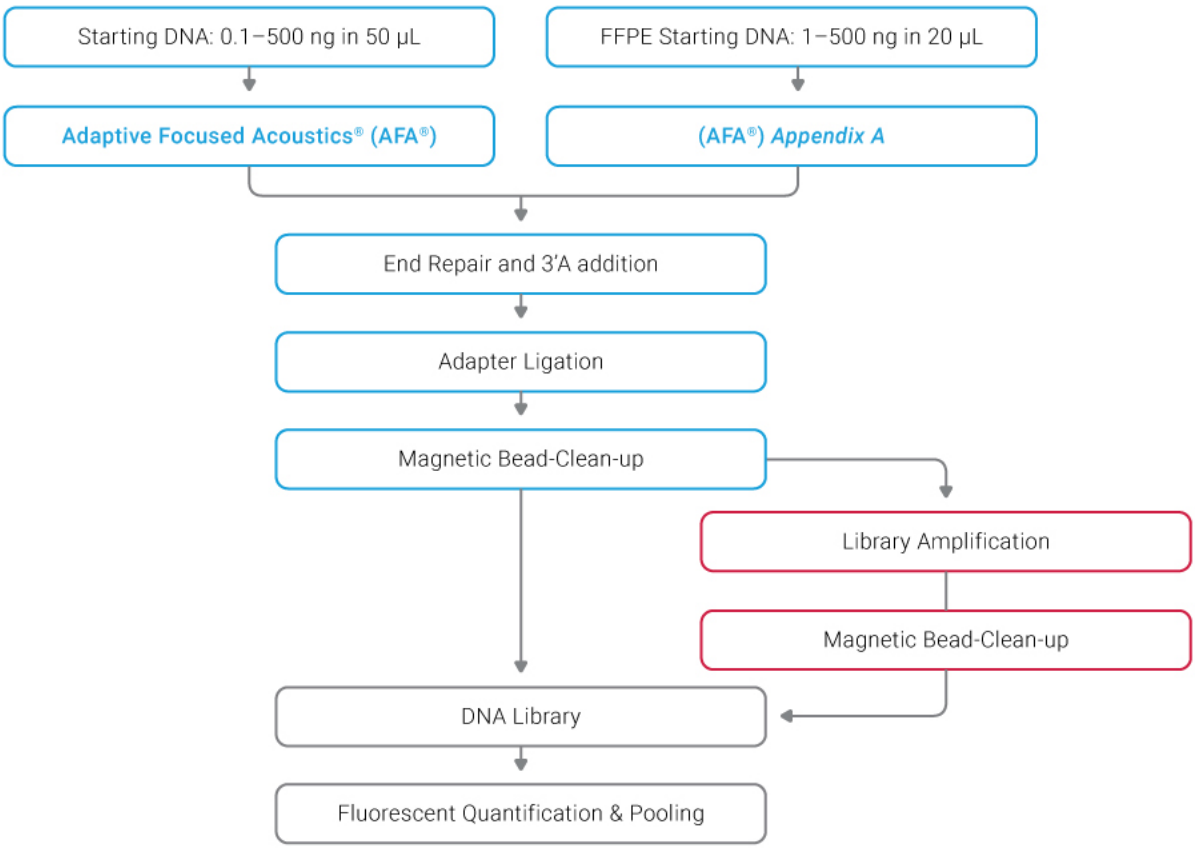
Important Considerations

Please note that the truCOVER DNA WGS PCR-Free Library Prep kit is not compatible with DNA eluted and dissolved in elution buffers supplied with the following DNA isolation kits:

- MagMAX™ Ultra 2.0 Kits (ThermoFisher Scientific®)
- Chemagic Nucleic Acid Isolation Kits (Revvity, Inc.)

The DNA should be dissolved in buffers free of additives such as detergents, salts and preservatives. Supported elution buffers are 10 mM Tris (pH 7.5–8.5)/0.1 mM EDTA (low-TE) or 5–10 mM Tris (pH 7.5–8.5). Plain, non-buffered water is not recommended. Please contact Covaris Application Support (applicationsupport@covaris.com) if you have any questions and for recommended buffer exchange protocols.

Workflow Steps



Sample Input Requirements and Considerations

The truCOVER PCR-free Library Prep workflow is designed for DNA inputs ranging from 50–500 ng for a PCR-free workflow. For lower input concentrations between 0.1 and 50 ng, additional use of the truCOVER Library Amplification kit (PN 520366KIT) is recommended.

> **NOTE:** Due to the inherent variation in quality of DNA isolated from FFPE tissues, it is recommended to use an amended protocol outlined in ***Appendix A***. This protocol starts with shearing conditions that result in shortened fragment sizes (200 bp), which will increase the conversion rates. If using the non-modified protocol that shears to a target fragment-size of 500 bp, it is strongly recommended to add library amplification (6–10 cycles) to obtain sufficient library DNA for NGS analysis.

The input DNA should be suspended in low EDTA TE. If detergent is present, the DNA should be diluted into low EDTA TE because detergent will interfere with the shearing process.

Adapters

The truCOVER WGS Library Prep workflow is compatible with full-length adapters with a 3' T overhang. Adapters containing sample indexes are added at molar excess to facilitate efficient ligation but DNA to adapter ratios are dependent on the initial DNA input

Full-length indexed adapters in a 96-well plate format can be obtained from different vendors. For multiplexing pooled libraries, it is important to choose unique dual-indexed adapters (UDI) with or without unique molecular identifiers (UMI) for each library.

Kit Contents and Storage

The reagents for both the truCOVER library Prep kit (PN 520361KIT) and the truCOVER DNA Library Prep Amplification Kit (PN 520366KIT) are shipped on cold packs. Each package contains 2 boxes with different storage conditions. See Safety Data Sheets (SDS) for more information.

> **IMPORTANT:** Upon delivery, store the Bead Purification Reagent and DNA Elution Buffer at 2–8°C (do not freeze). Store the remaining reagents at -15 to -25°C.

Reagents in truCOVER WGS PCR-free Library Prep Kit (PN 520361KIT)

Reagent	Volume	Box Number	Storage Temperature
Enzyme Mix Buffer (green cap)	840 µL	1	-15 to -25°C
Enzyme Mix (blue cap)	360 µL	1	-15 to -25°C
Ligase Buffer	3 mL	1	-15 to -25°C
Ligase (white cap)	600 µL	1	-15 to -25°C
Bead Purification Reagent	17.5 mL	2	2–8°C
DNA Elution Buffer	9 mL	2	2–8°C

Reagents in truCOVER DNA Library Amplification Kit (PN 520366KIT)

Reagent	Volume	Box Number	Storage Temperature
Amplification Master Mix	3 mL	1	-15 to -25°C
P5/P7 Primer Mix	600 µL	1	-15 to -25°C
Bead Purification Reagent	6 mL	2	2–8°C
Elution Buffer	3 mL	2	2–8°C

Laboratory Chemicals and Consumables Supplied by User

- 8 AFA-TUBE TPX Strip (Covaris, PN 520292)
- 8 AFA-TUBE TPX Strip Caps (Covaris, PN 500639)
  - Three (3) Strip Caps for EACH 8 AFA-TUBE TPX Strip are needed
- 8-strip 0.2 mL PCR tubes (e.g Eppendorf®, PN 951010022) or
- 96-well PCR plates (e.g Eppendorf, PN 0030129512)
- Strip caps or plate seals
- Low EDTA TE (10 mM Tris-HCl pH 8.0, 0.1 mM EDTA) (e.g., ThermoFisher, PN J75793.AP)
- Full length indexed adapters for Illumina sequencing (e.g., IDT PN 10005903, NEB PN E7395, E7874, E7876, E7878)
- Molecular biology grade Ethanol, 100% (e.g., Sigma®, PN E7023)
- PCR grade water (e.g., Millipore®,Omnipure WFI grade water PN 7732-18-5)

Equipment Required (Choose 1 Focused-ultrasonicator)

- R230 Focused-ultrasonicator (Covaris, PN 500620)
- ML230 Focused-ultrasonicator (Covaris, PN 500656)
- LE220-plus Focused-ultrasonicator (Covaris, PN 500659)
- LE220R-plus Focused-ultrasonicator (Covaris, PN 500578)
- LE220Rsc Focused-ultrasonicator (Covaris, PN 500652)
- Thermocycler (e.g., Eppendorf Mastercycler® X50 or equivalent)
- Magnet stand for 0.2 mL PCR tubes (e.g., Permagen®, PN MSRLV08 or equivalent) or magnet plate for 96-well plates
- Qubit Fluorometer (ThermoFisher, PN Q33238 or plate-reader equivalent)

Additional Notes

- Ensure that all buffers are fully thawed before use. Mix the contents by inverting or flicking, followed by a quick spin.
- When creating a master mix, vortex the tube for at least 3 seconds to ensure complete mixing. Centrifuge briefly before opening the tube.
- Keep all reagents on ice, except for the Bead Purification Reagent and the DNA Elution Buffer.

IMPORTANT: Equilibrate the Bead Purification Reagent to room temperature for at least 1 hour. Ensure that the beads are dispersed evenly by vortexing the bottle for at least 30 seconds just before using the Bead Purification Reagent for clean-up. Prepare fresh 80% Ethanol for the bead-based clean-up process. 800 µL of 80% Ethanol are required per sample.

AFA-enabled Shearing of DNA

1. Create the appropriate program for your Covaris Focused-ultrasonicator in SonoLab (***see table below***). The settings will shear DNA to fragments with an optimal size for WGS sequencing. This section also contains information about different Covaris consumables that can be used for shearing.

Instrument	R230		ML230	L-Series
SonoLab	10.0.1		10.1	8.5, 10.2 or higher
Consumable PN	8 AFA-TUBE TPX Strip (PN 520292)	96 AFA-TUBE TPX Plate (PN 520291)	8 AFA-TUBE TPX Strip (PN 520292)	8 AFA-TUBE TPX Strip (PN 520292)
Rack PN	Rack R230 AFA-TUBE TPX Plate & Strip (PN 500668)	Rack R230 AFA-TUBE TPX Plate & Strip (PN 500668)	ML230 Rack 8 AFA-TUBE TPX Strip (PN 500660)	Rack 8 AFA-TUBE TPX Strip (PN 500685)
Tube Holder	PSU Rack R230 8 AFA-TUBE TPX Strip (PN 500723)	N/A	N/A	N/A
Plate Definition	R230_500723 PSU Holder 8 AFA-TUBE TPX Strip +0.5 offset	R230_520291 96 AFA-TUBE TPX Plate +0.5 offset	ML230_500660 Rack 8 AFA-TUBE TPX Strip +12.7 mm offset	LE220plus_500685 8 AFA-TUBE TPX Strip +1.8 offset
Volume	50 µL		50 µL	50 µL
Peak Incident Power	250 W		160 W	200 W
Duty Factor	20%		16%	26%
Cycles Per Burst	50		50	50
Temperature	10°C		12°C	10°C
Dithering	3 mm Y at 20 mm/sec		3 mm Y at 20 mm/sec	1 mm Y at 20 mm/sec
Repeat Process Duration	10 sec		10 sec	10 sec
Number of Iterations	5		7	5
Delay Time	10 sec		10 sec	10 sec
Total Treatment Time	50 sec		70 sec	50 sec

2. Dilute the starting DNA with Low EDTA TE, pH 8.0 to the desired concentration. The volume in each AFA vessel should be 50 µL and the amount of DNA between 50–500 ng (1–10 ng/µL). Before starting the shearing process, seal the 8 AFA-TUBE TPX Strip with the 8 AFA-TUBE TPX Strip Caps.
3. Briefly centrifuge the 8 AFA-TUBE TPX Strip to bring the volume to the bottom of the tubes.
4. Place the 8 AFA-TUBE TPX Strip into the appropriate rack and start the AFA treatment to shear the DNA.

End Repair and A-Tailing

1. Create the "End Repair and A-Tailing" program in a thermocycler:

Step	Temperature	Time
Lid Temperature	75°C	n/a
End Repair	20°C	30 min
A-Tailing	65°C	30 min
Hold	4°C	Indefinitely

2. Prepare the End Repair and A-tailing Master Mix by combining Enzyme Mix Buffer and Enzyme Mix in a microtube. When preparing the Master Mix for multiple samples, add 10% extra volume.

Component	Volume for 1 sample
Enzyme Mix Buffer (green cap)	7 µL
Enzyme Mix (blue cap)	3 µL

When preparing the Master Mix for multiple samples, add 10% extra volume.

3. Mix the End Repair-A-tailing Master Mix by vortexing, followed by a brief centrifugation.
4. After the DNA is sheared in the Focused-ultrasonicator, remove the 8 AFA-TUBE TPX Strips from the appropriate rack. Briefly centrifuge to bring the volume to the bottom of the tubes.
5. Open the 8 AFA-TUBE TPX Strip and add 10 µL of the End Repair and A-tailing Master Mix to the 50 µL sheared DNA.
6. Mix thoroughly by pipetting up and down 10 times. Close the tubes with the 8 AFA-TUBE Strip Cap, place the 8 AFA-TUBE TPX Strip into the thermocycler and start the program "End Repair and A-tailing".

Adapter Ligation

1. After the End Repair and A-tailing program is finished and the sample temperature has reached 4°C, proceed with the adapter ligation step.
2. Prepare the "Adapter Ligation" program in a thermocycler or set the temperature on a calibrated heatblock:

Step	Temperature	Time
Lid Temperature	Off	n/a
Ligation	20°C	15 min

3. Prepare a Ligation Master Mix by combining Ligase Buffer and Ligase in a microtube. The Ligase Buffer is viscous and should be mixed thoroughly by pipetting up and down 10 times before use.

Component	Volume for 1 sample
Ligase Buffer	25 µL
Ligase (white cap)	5 µL

When preparing the Master Mix for multiple samples, add 10% extra volume.

4. Mix the Ligation Master Mix by vortexing for 4 seconds. Briefly centrifuge the tube to bring the volume to the bottom of the tube. Store on ice until use.
5. Briefly centrifuge the full-length indexed adapter plate.
6. Use the table below to determine the amount of adapter needed. If necessary, dilute the adapters to the appropriate concentration using the adapter diluent provided by the manufacturer.

DNA Input (ng)	Adapter Concentration (µM)
51–500	15
25–50	7.5
10–24	3
2–9	1.5
< 2	0.3

7. Add 5 µL of the appropriate adapter dilution to the end repaired DNA in the 8 AFA-TUBE TPX Strip.

> **NOTE:** Make sure to keep track of the IDs used per sample to allow library pooling and multiplexing.

8. Add 30 µL of the Ligation Master Mix to the sample in the 8 AFA-TUBE TPX Strip. The final volume is now 95 µL.
9. Mix thoroughly by pipetting up and down 10 times. Close the tubes with the 8 AFA-TUBE Strip Cap, place the 8 AFA-TUBE TPX Strip into the thermocycler, and start the program "Adapter Ligation."

Library Clean-up and Fragment Size Selection

The dual magnetic bead-based clean-up using the Bead Purification Reagent in combination with the fragmentation target length is optimized for superior size selection. This process effectively removes adapter dimers and shorter DNA fragments, which can contribute to small insert size sequence bias on Illumina platforms.

1. Add 5 µL of Elution Buffer to each end-repaired and adapter-ligated sample for a final volume of 100 µL.
2. Add 60 µL of the Bead Purification Reagent. Mix by pipetting up and down at least 20 times (IMPORTANT!) and incubate the tubes at room temperature for 5 minutes.
3. Place the 8 AFA-TUBE TPX Strip on the magnet rack for 3 minutes or until the magnetic beads are separated from the supernatant.
4. Carefully remove the supernatant without disturbing the bead pellet. Do not remove the 8 AFA-TUBE TPX Strip from the magnetic rack.
5. Add 200 µL 80% Ethanol to each tube and incubate for 30 seconds. Do not mix the beads.
6. Remove the supernatant.
7. Repeat the wash steps 5 & 6.
8. After removing the supernatant, carefully remove any residual liquid using a 20 µL pipette
9. With the 8 AFA-TUBE TPX Strip on the magnet rack and the tubes open, air-dry the beads for 3–5 minutes.
10. Remove the 8 AFA-TUBE TPX Strip from the magnet rack.
11. Add 52 µL of Elution Buffer to each tube and re-suspend the beads by pipetting up and down at least 20 times. Ensure all beads are in suspension.
12. Incubate the Elution Buffer with the beads for 2 minutes at room temperature.
13. Place the 8 AFA-TUBE TPX Strip back on the magnet rack for 3 minutes or until the magnetic beads are separated from the supernatant.
14. Transfer 50 µL of the supernatant into a clean PCR tube 8-Strip. Be sure not to carry over any beads. Discard the 8 AFA-TUBE TPX Strip with the beads.
15. Add 37.5 µL of the Bead Purification Reagent to the supernatant in the PCR tube 8-strip. Mix by pipetting up and down at least 20 times (IMPORTANT!) and incubate the tubes at room temperature for 5 minutes.
16. Place the PCR tube 8-strip on the magnet rack for 3 minutes or until the magnetic beads are separated from the supernatant.
17. Carefully remove supernatant without disturbing the bead pellet. Do not remove the PCR tube 8-strip from magnet rack.
18. Add 200 µL 80% Ethanol to each tube and incubate for 30 seconds. Do not mix the beads.
19. Remove the supernatant.
20. Repeat the wash steps 17 & 18.
21. After removing the supernatant, carefully remove the residual liquid using a 20 µL pipette tip.
22. With the PCR tube 8-Strip on the magnet rack and the tubes open, air-dry the beads for 3–5 minutes.
23. Remove the PCR tube 8-Strip from the magnet rack.
24. Add 22 µL of Elution Buffer to each tube and re-suspend the beads by pipetting up and down at least 20 times. Ensure all beads are in suspension.
25. Incubate the Elution Buffer with the beads for 2 minutes at room temperature.
26. Place the PCR tube 8-Strip back on the magnet rack for 2 minutes or until the magnetic beads are separated from the supernatant.
27. Transfer 20 µL of the supernatant into a clean PCR tube 8-Strip or into 0.5 mL Eppendorf LoBind® tubes.

The libraries are now ready for library QC, pooling and sequencing. For libraries with low concentrations (0.05–2.5 ng/µL), amplification with the truCOVER DNA Library Amplification Kit (PN 520366KIT) is recommended. The finished libraries should be stored at -20°C.

PCR Amplification of Low-Input Libraries

1. Create the "Amplification" program in a thermocycler:

Step	Temperature	Time	Number of Cycles
Initial Denaturation	98˚C	45 sec	1
Denaturation	98˚C	15 sec	Variable (see Table)
Annealing	60˚C	30 sec	
Extension	72˚C	30 sec	
Final Extension	72˚C	60 sec	1
Hold	4˚C	Hold	1

2. Use the table below to determine the recommended number of PCR cycles.

> **NOTE:** To avoid amplification bias and minimize sequence duplications, it is advisable to keep the number of PCR cycles as low as possible. The amplification can be optimized for different input library concentrations. The table below shows recommended amplification cycles that result in a final library amount that is sufficient for pooling and sequencing.

Recommended number of PCR cycles

Library Input DNA	Recommended PCR cycles
50 ng	4
10 ng	6
5 ng	8
1 ng	10
0.5 ng	12
0.1 ng	12

3. Prepare the Amplification Mix by combining the Amplification Master Mix and the P5/P7 Primer Mix in a microtube.

Component	Volume for 1 sample
Amplification Master Mix	25 µL
P5/P7 Primer Mix	5 µL

When preparing the Master Mix for multiple samples, add 10% extra volume.

4. Mix the Amplification Mix by vortexing, followed by a brief centrifugation.
5. Add 30 µL of the Amplification Mix to the 20 µL prepared library.
6. Mix thoroughly by pipetting up and down 10 times. Cap the 8-strip 0.2 mL PCR tubes, place into the thermocycler and start the program "Amplification".

Post Amplification Clean-up

The magnetic bead-based clean-up using the Bead Purification Reagent is optimized for complete removal of primers while retaining the library.

1. Add 50 µL of the Bead Purification Reagent. Mix by pipetting up and down at least 20 times (IMPORTANT!) and incubate the tubes at room temperature for 5 minutes.
2. Place the 8-strip 0.2 mL PCR tubes on the magnet rack for 3 minutes or until the magnetic beads are separated from the supernatant.
3. Carefully remove the supernatant without disturbing the bead pellet. Do not remove the 8-strip 0.2 mL PCR tubes from the magnetic rack.
4. Add 200 µL 80% Ethanol to each tube and incubate for 30 seconds. Do not mix the beads.
5. Remove the supernatant.
6. Repeat the wash steps 3 & 4.
7. After removing the supernatant, carefully remove any residual liquid using a 20 µL pipette tip.
8. With the 8-strip 0.2 mL PCR tubes on the magnet rack and the tubes open, air-dry the beads for 3–5 minutes.
9. Remove the 8-strip 0.2 mL PCR tubes from the magnet rack.
10. Add 22 µL of Elution Buffer to each tube and re-suspend the beads by pipetting up and down at least 20 times. Ensure all beads are in suspension.
11. Incubate the Elution Buffer with the beads for 2 minutes at room temperature.
12. Place the 8-strip 0.2 mL PCR tubes back on the magnet rack for 3 minutes or until the magnetic beads are separated from the supernatant.
13. Transfer 20 µL of the supernatant into a clean 8-strip 0.2 mL PCR tubes. Be sure not to carry over any beads. Discard the 8-strip 0.2 mL PCR tubes with the beads.

Library QC

Quantitation

The recommended method to determine the library DNA concentration is by using fluorescent dyes, such as a Qubit or Pico Green™ based assay. Measuring the UV absorbance is not recommended for quantitation as the ligated adapters are partially single stranded which can significantly skew results.

For most sample types such as DNA from blood and saliva, the purified libraries can be pooled for sequencing based on Qubit or plate reader/PicoGreen measurements.

> **NOTE:** When pooling libraries by mass (Qubit), do not combine amplified and non-amplified libraries. Amplified and non-amplified libraries may cluster differently on a flow cell.

qPCR (optional)

Libraries can also be quantified using qPCR. Several commercial qPCR-based library quantification kits are available that use primers complementary to the P5 and P7 Illumina adapters.

Fragment Sizes (optional)

The library fragment size distribution can be determined using capillary electrophoresis, such as the TapeStation, Fragment Analyzer or BioAnalyzer (all Agilent). For non-amplified libraries, please note that the migration of the libraries during electrophoresis is affected by the partially single stranded Illumina adapters which can result in incorrect determination of the fragment sizes. The TapeStation (Agilent) is not recommended for QC of PCR-free Illumina-compatible libraries because the adapters cause artifacts.

Appendix A: Protocol Modifications for DNA Extracted from FFPE Samples

DNA extracted from FFPE samples is often degraded and chemically damaged, which can inhibit amplification of larger fragments. Taking this into account, a modified protocol is suggested. If desired, the quality of the extracted DNA can be determined either by electrophoretic methods (i.e. TapeStation) or by qPCR (i.e. KAPA NGS FFPE DNA QC Kit, Roche PN 0921719300) before starting the library preparation. Optimized settings for shearing of DNA from FFPE in 8 AFA-TUBE TPX Strips are shown in the table below.

The sample volume for shearing should be 20 µL with a DNA input between 50 and 500 ng (2.5–25 ng/µL). The DNA should be diluted in low EDTA TE.

Instrument	R230		ML230	L-Series
SonoLab	10.0.1		10.1	8.5, 10.2 or higher
Consumable PN	8 AFA-TUBE TPX Strip (PN 520292)	96 AFA-TUBE TPX Plate (PN 520291)	8 AFA-TUBE TPX Strip (PN 520292)	8 AFA-TUBE TPX Strip (PN 520292)
Rack PN	Rack R230 AFA-TUBE TPX Plate & Strip (PN 500668)	Rack R230 AFA-TUBE TPX Plate & Strip (PN 500668)	ML230 Rack 8 AFA-TUBE TPX Strip (PN 500660)	Rack 8 AFA-TUBE TPX Strip (PN 500685)
Tube Holder	PSU Rack R230 8 AFA-TUBE TPX Strip (PN 500723)	N/A	N/A	N/A
Plate Definition	R230_500723 PSU Holder 8 AFA-TUBE TPX Strip +0.5 offset	R230_520291 96 AFA-TUBE TPX Plate +0.5 offset	ML230_500660 Rack 8 AFA-TUBE TPX Strip +12.7 mm offset	LE220plus_500685 8 AFA-TUBE TPX Strip +1.8 offset
Volume	20 µL		20 µL	20 µL
Peak Incident Power	280 W		210 W	260 W
Duty Factor	25%		25%	26%
Cycles Per Burst	50		50	50
Temperature	10°C		10°C	10°C
Dithering	3 mm Y at 20 mm/sec		3 mm Y at 20 mm/sec	1 mm Y at 20 mm/sec
Repeat Process Duration	10 sec		10 sec	10 sec
Number of Iterations	11		14	11
Delay Time	10 sec		10 sec	10 sec
Total Treatment Time	110 sec		140 sec	110 sec

After shearing, add 30 µL of Low EDTA TE to each sample for a final volume of 50 µL before commencing with End Repair and A-Tailing and Adapter Ligation following the general protocol above.

Library Clean-up and Fragment Size Selection for DNA from FFPE

The dual magnetic bead-based clean-up using the Bead Purification Reagent is optimized for complete removal of adapter dimers.

> **IMPORTANT:** This clean-up protocol differs from the one described for non-FFPE DNA above!

1. Add 5 µL of Elution Buffer to each end-repaired and adapter ligated sample for a final volume of 100 µL.
2. Add 90 µL of the Bead Purification Reagent. Mix by pipetting up and down at least 20 times (IMPORTANT!) and incubate the tubes at room temperature for 5 minutes.
3. Place the 8 AFA-TUBE TPX Strip on the magnet rack for 3 minutes or until the magnetic beads are separated from the supernatant.
4. Carefully remove the supernatant without disturbing the bead pellet. Do not remove the 8 AFA-TUBE TPX Strip from the magnetic rack.
5. Add 200 µL 80% Ethanol to each tube and incubate for 30 seconds. Do not mix the beads.
6. Remove the supernatant.
7. Repeat the wash steps 5 & 6.
8. After removing the supernatant, carefully remove any residual liquid using a 20 µL pipette tip.
9. With the 8 AFA-TUBE TPX Strip on the magnet rack and the tubes open, air-dry the beads for 3–5 minutes.
10. Remove the 8 AFA-TUBE TPX Strip from the magnet rack.
11. Add 52 µL of Elution Buffer to each tube and re-suspend the beads by pipetting up and down at least 20 times. The beads must be all in suspension.
12. Incubate the Elution Buffer with the beads for 2 minutes at room temperature.
13. Place the 8 AFA-TUBE TPX Strip back on the magnet rack for 3 minutes or until the magnetic beads are separated from the supernatant.
14. Transfer 50 µL of the supernatant into a clean PCR tube 8-Strip. Be sure not to carry over any beads. Discard the 8 AFA-TUBE TPX Strip with the beads.
15. Add 55 µL of the Bead Purification Reagent to the supernatant in the PCR tube 8-Strip. Mix by pipetting up and down at least 20 times (IMPORTANT!) and incubate the tubes at room temperature for 5 minutes.
16. Place the PCR tube 8-Strip on the magnet rack for 3 minutes or until the magnetic beads are separated from the supernatant.
17. Carefully remove the supernatant without disturbing the bead pellet. Do not remove the PCR tube 8-Strip from the magnetic rack.
18. Add 200 µL 80% Ethanol to each tube and incubate for 30 seconds. Do not mix the beads.
19. Remove the supernatant.
20. Repeat the wash steps 17 & 18.
21. After removing the supernatant, carefully remove the residual liquid using a 20 µL pipette tip.
22. With the PCR tube 8-Strip on the magnet rack and the tubes open, air-dry the beads for 3–5 minutes.
23. Remove the PCR tube 8-Strip from the magnet rack.
24. Add 22 µL of Elution Buffer to each tube and re-suspend the beads by pipetting up and down at least 20 times. The beads must be all in suspension.
25. Incubate the Elution Buffer with the beads for 2 minutes at room temperature.
26. Place the PCR tube 8-Strip back on the magnet rack for 2 minutes or until the magnetic beads are separated from the supernatant.
27. Transfer 20 µL of the supernatant into a clean PCR tube 8-Strip.

The libraries are now ready for library QC, pooling and sequencing. For libraries with low concentrations (0.05–2.5 ng/µL), amplification with the truCOVER DNA Library Amplification Kit (PN 520366KIT) is recommended. Follow the instructions starting on ***Page 11***: PCR Amplification of low input libraries. The finished libraries should be stored at -20°C.

> **NOTE:** For DNA from FFPE, library quantitation using a qPCR-based method is recommended to determine the optimal concentrations for pooling.

Support and Technical Assistance

Tech Support: Ongoing assistance with the operation or application of the equipment and/or troubleshooting is provided via:

- **Telephone:**
  - **US & APAC:** +1 781.932.3959, during the hours of 8:30 a.m. to 5:00 p.m. EST, Monday through Friday
  - **EU:** +44 (0) 845 872 0100, during the hours of 9:00 a.m. to 5:00 p.m. GMT, Monday through Friday
- **E-mail:**
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