TWIST BIOSCIENCE

TECH NOTE

Covaris DNA Fragmentation

FOR TARGET ENRICHMENT WITH TWIST CUSTOM AND HUMAN EXOME PANELS

Twist Bioscience's Target Enrichment System for next-generation sequencing (NGS) of human genomic DNA (gDNA) optimizes probe balance and design to provide industry-leading performance. A critical step that precedes target enrichment is gDNA fragmentation, and many researchers depend on mechanical shearing using Covaris' Adaptive Focused Acoustics (AFA) technology for this initial library preparation step. We provide here instructions for gDNA fragmentation with AFA technology and subsequent library preparation. This method has been optimized to yield high-quality gDNA fragments for use in target enrichment and NGS analysis.

RECOMMENDED MATERIALS

Table 1. Consumables and equipment.

COMPONENT	VENDOR	PART NUMBER
CONSUMABLES		
TE buffer with low EDTA (10 mM Tris-HCl, pH 8.0)	Thermo Fisher	#12090015
oneTUBE-10 AFA vessel, choice of: · 8 oneTUBE-10 AFA Strip (12) with caps · 96 oneTUBE-10 AFA Plate with foil seals	Covaris	#520225, #500613 #520249, #520073
KAPA HyperPrep Kit	Roche	#KK8504
Twist Adapter Set (or equivalent TruSeq DNA barcode adapters compatible with TA ligation)	Twist Bioscience	#100577 (96 samples) #100255 (16 samples)
Qubit dsDNA BR Kit	Invitrogen	#Q32853
Agilent 7500 DNA Kit	Agilent Technologies	#5067-1506
Agilent High Sensitivity DNA Kit (optional)	Agilent Technologies	#5067-4626
EQUIPMENT		
LE220-plus Focused-ultrasonicator with Rack 96 oneTUBE-10 AFA Plate or LE220-plus Focused-ultrasonicator with Rack 8 oneTUBE-10 AFA Strip ME220 Focused-ultrasonicator with ME220 Rack 8 oneTUBE-10 AFA Strip and Waveguide	Covaris	#500569, #500588, or #500608 #500506, #500609, and #500534
Pipets and tips	Rainin	–
Benchtop mini-centrifuge for 0.2 ml tube	Spectrafuge	#C1301
Thermal cycler (96 well) with heated lid	Thermo Fisher	#4452300
Qubit 3.0 fluorometer	Thermo Fisher	#Q33216
2100 Bioanalyzer	Agilent Technologies	#G2939BA

Table 2. Instrument settings.

	LE220-PLUS FOCUSED-ULTRASONICATOR		ME220 FOCUSED-ULTRASONICATOR
	96 oneTUBE-10 AFA Plate (#520249)	8 oneTUBE-10 AFA Strip (#520225)	8 oneTUBE-10 AFA Strip (#520225)
Software	SonoLab 8.4 or higher recommended		SonoLab 8.0.1 or higher required
Rack	Rack 96 one-TUBE AFA Plate (#500588)	Rack 8 one-TUBE AFA Strip (#500608)	ME220 Rack 8 one-TUBE AFA Strip (#500609)
Plate Definition	LE220plus_520249 96 oneTUBE Plate -2.2mm offset	LE220plus_500608 8 oneTUBE Strip -2.2mm offset	8 oneTUBE-10 Strip PN 520225
Waveguide	N/A	N/A	PN 500534
Dithering	1mm Y-dither at 20mm/s	1mm Y-dither at 20mm/s	N/A
Volume (µl)	15	15	15
Time (sec)	150	150	150 (10 pulses, 15 sec/pulse)
Peak Incident Power (W)	200	200	20
Duty Factor (%)	25	25	25
Cycles per Burst (CpB)	50	50	50
Temperature (°C)	20	20	20

STEP 1: FRAGMENTATION OF gDNA

NOTES:

- Use a Covaris LE220-plus or ME220 Focused-ultrasonicator with compatible oneTUBE-10 AFA vessels. Set the instrument for gDNA fragmentation to a size distribution mode of 200 bp (see **Table 2** for recommended settings). If using another Covaris machine and AFA vessel, target a library mode of 200 bp by adjusting sample and reaction volumes as needed.
- 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 oneTUBE-10 vessel used for fragmentation.
- For support using your Covaris Focused-ultrasonicator, please contact applicationsupport@covaris.com

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

1.2 In a Covaris oneTUBE-10 AFA vessel (strip or plate), dilute 100 ng of each gDNA sample in TE buffer with low EDTA (pH 8.0) to a final volume of 15 µl (final concentration 6.67 ng/µl).

For example, with a 25 ng/µl gDNA sample, dilute 4 µl gDNA into 11 µl TE buffer with low EDTA (pH 8.0).

1.3 Seal the oneTUBE-10 vessel with either a strip-cap or foil seal. Collect the liquid at the bottom of the vessel by centrifuging for up to 60 sec at 2,000 RCF.

1.4 Ensure there are no air bubbles at the bottom of the oneTUBE-10 vessels, then place the vessel containing the samples into the proper rack and load onto the Covaris Focused-ultrasonicator.

1.5 Process the samples using the settings for your instrument (Covaris LE220-plus or ME220 Focused-ultrasonicator, **Table 2**).

1.6 OPTIONAL: Analyze the size distribution of the fragmented material, for example with an Agilent High Sensitivity DNA Kit.

For optimum performance with the Twist Target Enrichment System, the mode of the fragment size distribution should be ~200 bp (**Figure 1**). Adjust the AFA program time in increments of 30 seconds to reach this mode of distribution (increase the time to yield smaller fragments, or decrease it for larger fragments).

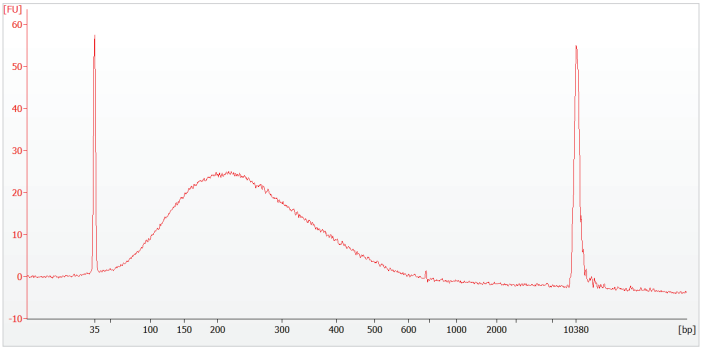

Figure 1. DNA fragment size distribution of a gDNA fragment library immediately after Covaris AFA shearing, as analyzed using an Agilent High Sensitivity DNA Assay.

STEP 2: LIBRARY PREPARATION

For best results with the Twist Target Enrichment System, use a Twist Adapter Set during ligation. Alternatively, use any other indexed adapters that are Illumina TruSeq compatible, capable of participating in TA-ligation, and compatible with Twist Universal Blockers (#100767 or #100578).

2.1 Process the 15-µl fragmented DNA sample with the KAPA HyperPrep Kit for end repair, A-tailing, ligation, amplification, and purification of the indexed library.

NOTE: If the total mass of the fragmented gDNA sample is 100 ng and volume is <50 µl, perform the steps in the KAPA HyperPrep Kit in the Covaris oneTUBE-10 vessel until transfer to a new vessel is required. This is useful for adapting this workflow for use with automated systems.

2.2 Quantify each indexed library, for example with the Invitrogen Qubit dsDNA BR Kit, according to the manufacturer's instructions. Final concentrations should be ≥80 ng/µl.

2.3 Evaluate the fragment size distribution using an Agilent 7500 DNA Assay. Average fragment length should be 375–425 bp using a range setting of 150–1,000 bp (**Figure 2**).

2.4 Pool the amplified indexed libraries, or store them at –20°C.

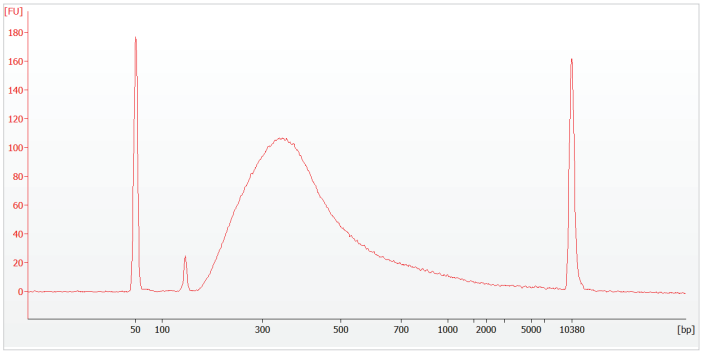

Figure 2. DNA fragment size distribution of a gDNA fragment library, as analyzed using an Agilent 7500 DNA Assay.

PERFORMANCE IN DOWNSTREAM TARGET ENRICHMENT AND NGS

A variety of methods are available for gDNA fragmentation, but Covaris AFA technology is the industry standard for mechanical shearing with high sensitivity and unbiased results. The shearing and library preparation protocol provided here has been optimized to exploit the benefits of the Covaris system to generate gDNA libraries that provide excellent results upon target enrichment with Twist's Human Core Exome Kit and NGS (**Table 3**).

Table 3. Performance of gDNA libraries prepared using the Covaris AFA system with oneTUBE-10 consumables and instructions provided here. Fragments (187.5 ng) were subjected to capture in a multiplexed reaction with the Twist Core Exome Panel in 16-hour hybridization reactions. Samples were sequenced on a NextSeq system (Illumina) with a NextSeq 500/550 High Output v2 kit (150 cycles) and downsampled to 150x of targeted bases for evaluation. Picard metric tools with default values were utilized for sequence analysis.

METRIC	PICARD VARIABLE	VALUE
Fold-80 base penalty	FOLD_80_BASE_PENALTY	1.31
Percent duplication rate	PCT_EXC_DUPE	3.1%
30x depth of coverage	PCT_TARGET_BASES_30X	93.3%
Percent bases on target	1 - (PCT_OFF_BAIT)	83.4%

DOC-001034 REV 2.0