

Covaris DNA shearing guide for the Agilent SureSelect Target Enrichment System[©]

INTRODUCTION

This document provides users with a complete set of protocols that encompass all Covaris Adaptive Focused Acoustics™ (AFA) instruments for DNA shearing to 150 to 200 bp with 50 to 55 µl of sample volume, to be readily compatible with the Agilent SureSelect Target Enrichment System (XT or XT2). The goal is to provide the most precise, pre-validated protocols to ensure the best outcome and compatibility for library preparation and subsequent sequencing applications.

Covaris offers a full lineup of AFA instruments to suit different levels of throughput, ranging from processing 1 sample up to a 96-well format. Precise engineering of these instruments has been the key to achieve tunable, defined delivery of the adaptive focused acoustic energy to mechanically shear DNA. Various pairings of shearing consumables and holders are integrated components of our technology that enables this control. Agilent provides DNA shearing settings for Covaris S220 and E220 Focused-ultrasonicators (Table 1). This application note was developed to provide settings for other Covaris instruments and for use with the microTUBE-50, the latest Covaris consumable optimized for 55 µl processing volume.

MATERIALS & METHODS

1. For DNA input requirements, please refer to the relevant Agilent SureSelect Target Enrichment System protocol.
2. Please refer to the table below for microTUBE-50 compatibility with your Covaris instrument.

! **NOTE:** Please use 55 µl of sample volume with microTUBE-50 and use the settings provided below.

Table 1 provides the settings developed by Agilent for DNA shearing with Covaris for the Agilent SureSelect Target Enrichment System.

Tables 2 to 5 provide the settings developed by Covaris to match these Agilent parameters using the microTUBE-50, encompassing all Covaris instruments.

TABLE 1. Agilent shearing settings for Covaris S220/E220 instruments provided in the SureSelect Target Enrichment System protocols for shearing to 150 – 200 bp (PN G7530-90000 revB5 & PN G9630-90000 revE2)

Instrument	S220/E220
Consumable	microTUBE AFA Fiber Pre-Slit Snap-Cap (520045)
Holder	S220 Holder microTUBE (500114)
Bath Temperature (°C)	4 - 8
Peak Incident Power (W)	175
Duty Factor (%)	10
Cycles Per Burst	200
Treatment Time (s)	360

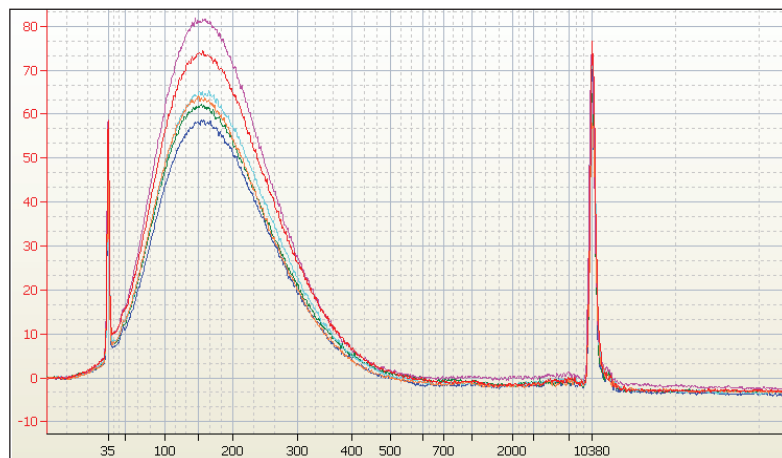


FIGURE 1. Observed DNA fragment size distribution profiles of 6 replicates using Agilent settings listed in Table 1, measured by Agilent Bioanalyzer HS DNA Chip.

TABLE 2. DNA shearing settings for the S220

Instrument	S220
Consumable	microTUBE-50 AFA Fiber Screw-Cap (520166)
Holder	S-Series Holder microTUBE-50 Screw-Cap (500492)
Bath Temperature (°C)	7
Peak Incident Power (W)	100
Duty Factor (%)	30
Cycles Per Burst	1000
Treatment Time (s)	130


SureSelect compatible Covaris consumables	Part number	M220	ME220	S220	E220 evolution	E220	LE220	LE220-plus
microTUBE-50 AFA Fiber Screw-Cap	520166	✓	✓	✓	✓	✓		
8 microTUBE-50 AFA Fiber Strip V2 8 microTUBE-50 AFA Fiber H Slit Strip V2	520174 520240		✓		✓	✓	✓	✓
96 microTUBE-50 AFA Fiber Plate 96 microTUBE-50 AFA Fiber Plate Thin Foil	520168 520232					✓	✓	✓

TABLE 3. DNA shearing settings for M-series instruments

Instrument	M220	ME220	
Consumables	microTUBE-50 AFA Fiber Screw-Cap (520166)	microTUBE-50 AFA Fiber Screw-Cap (520166)	8 microTUBE-50 AFA Fiber Strip V2 / H Slit (520174/520240)
Holder/Rack	M220 Holder XTU (500414)	ME220 Rack 4-place microTUBE Screw-Cap (500522)	ME220 Rack 8 microTUBE Strip V2 (500518)
Insert/Waveguide	M220 Holder XTU Insert microTUBE 50 µl (500488)	ME220 Waveguide 4 Place (500534)	ME220 Waveguide 8 Place (500526)
Water Level	N/A	5.5	5.5
Bath Temperature (°C)	20	20	20
Peak Incident Power (W)	75	75	50
Duty Factor (%)	20	25	30
Cycles Per Burst	200	1000	1000
Treatment Time (s)	205	180	214

TABLE 4. DNA shearing settings for E-series instruments

Instrument	E220 and E220 evolution			
Consumables		microTUBE-50 AFA Fiber Screw-Cap (520166)	8 microTUBE-50 AFA Fiber Strip V2 / H slit (520174/520240)	96 microTUBE-50 AFA Fiber Plate / Thin Foil (520168/520232)
E220	Plate Definition	"E220_500308 Rack 24 place microTUBE-50 screw-cap+6.5mm offset"	"E220_500444 Rack 12 Place 9 microTUBE-50 Strip V2-10 mm offset"	"E220_520168 96 microTUBE-50 plate -10.5 mm offset"
	Rack	Rack 24 Place microTUBE Screw-Cap (500308)	Rack 12 Place 8 microTUBE Strip V2 (500444)	No Rack Needed
E220evolution	Plate Definition	"500432 E220e 4 microTUBE-50 Screw Cap -8.32mm offset"	"500437 E220e 8 microTUBE-50 Strip V2 -10mm offset"	N/A
	Rack	Rack E220e 4 Place microTUBE Screw Cap (PN 500432)	Rack E220e 8 microTUBE Strip V2 (PN 500437)	N/A
Y-dithering	N/A	N/A	N/A	0.5mm Y-dither at 10mm/s
Bath Temperature (°C)	7	7	7	7
Water Level	6	-2	-2	0
Peak Incident Power (W)	100	100	100	100
Duty Factor (%)	30	30	30	30
Cycles Per Burst	1000	1000	1000	1000
Treatment Time (s)	130	180	180	145

 **NOTE:** Please make sure that the intensifier (500141) is in place while using the E220 instrument.


 The Y-dithering function is required for shearing with 96 microTUBE-50 AFA Fiber Plates. This function is only available on SonoLab version 7.3 and up. Please refer to the DNA Shearing Quick Guide for detailed instructions.

TABLE 5. DNA shearing settings for L-series instruments

Instrument	LE220 and LE220-plus	
Consumables	8 microTUBE-50 AFA Fiber Strip V2 / H Slit (520174/520240)	96 microTUBE-50 AFA Fiber Plate / Thin Foil (520168/520232)
Plate Definition	"LE220_500485 Rack-XT 12 Place 8 microTUBE-50 Strip V2 -12mm offset"	"LE220_520168 96 microTUBE-50 Plate -12mm offset"
Rack	Rack-XT 12 Place 8 microTUBE Strip V2 (500485)	No Rack Needed
X and/or Y-dithering	0.5mm X-dither & 0.5mm Y-dither at 10mm/sec	0.5mm X-dither & 0.5mm Y-dither at 10mm/sec
Bath Temperature (°C)	7	7
Water Level	-2	-2
Peak Incident Power (W)	450	450
Duty Factor (%)	20	20
Cycles Per Burst	1000	1000
Treatment Time (s)	360	360

! The X-dithering and Y-dithering functions are both required for shearing with the 8 microTUBE-50 AFA Fiber Strips V2 and 96 microTUBE-50 AFA Fiber Plates. These functions are only available on SonoLab version 7.3 and up. Please refer to the DNA Shearing Quick Guide for detailed instructions.

RESULTS

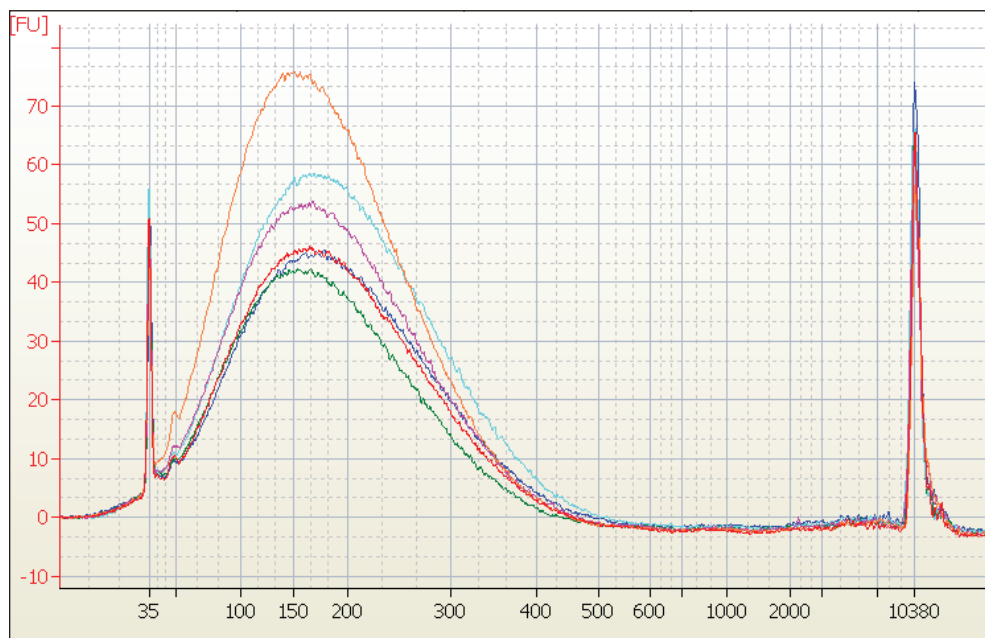


FIGURE 2. Summary of sheared DNA profiles measured by Agilent Bioanalyzer HS DNA Chip: genomic DNA was sheared following validated protocols. Representative profiles with average 150-200 bp size fragments were obtained using microTUBE-50 Screw-Cap (520166) with S220, M220, ME220; using 8 microTUBE-50 AFA Fiber Strip V2 (520174) with ME220 and E220; using 96 microTUBE-50 AFA Fiber Plate (520168) with LE220.

CONCLUSION

As shown with robust DNA shearing size profiles for a range of flexible throughput requirements, this guide provides users with a turn-key solution for all current AFA-based NGS shearing platforms. The parameters developed have undergone rigorous testing to provide coefficient of variations (CV) of less than 5% in DNA sizing profile replicates, resulting in highly reproducible shearing suitable for Agilent SureSelect Target Enrichment System protocols.