

Covaris DNA Shearing Guide for Illumina TruSight Tumor 170 NGS Assay

INTRODUCTION

This document provides users with a complete set of protocols for all current Covaris Adaptive Focused Acoustics® (AFA®) instruments to use with the Illumina TruSight* Tumor 170 Kit. The following protocols have been developed to deliver the best outcome and compatibility for library preparation and subsequent sequencing applications.

Covaris offers a full suite of AFA instruments to suit different levels of throughput, ranging from a single sample up to 96 samples in one run. The precise engineering of Covaris instruments and the specific design of consumables and holders allow tunable, defined delivery of adaptive focused acoustic energy to mechanically shear DNA. Illumina provides DNA shearing settings for Covaris E220evolution and LE220 Focused-ultrasonicators (Table 1) for the 8 microTUBE Strip V1 consumable. This application note provides settings for other Covaris instruments for DNA shearing to 90 to 250 bp in 55 µl of sample volume with the microTUBE-50. The microTUBE-50 has been specifically developed and optimized for 55 µl processing volume, and is the recommended consumable for this application.

MATERIALS AND METHODS

- For DNA input requirements, please refer to the relevant Illumina TruSight Tumor 170 protocol
- Please refer to Tables 2 through 5 for microTUBE-50 compatibility with your Covaris instrument
- For detailed instructions, please refer to your instrument Quick Guide for DNA Shearing

- <http://covaris.com/resources/protocols>



NOTE: Please use 55 µl of sample volume with microTUBE-50 and use the settings provided below in Table 2 through 5.

Reference DNA Shearing Settings Published by Illumina for TruSight Tumor 170 Library Construction

| Instrument | E220evolution | LE220 |
|-------------------------|--|---|
| Consumable | 8 microTUBE Strip (520053) | |
| Holder | Rack E220evolution microTUBE Strip (500430) | Rack 12 place 8 microTUBE Strip (500191) |
| Bath Temperature (C) | 7 | 7 |
| Peak Incident Power (W) | 175 | 450 |
| Duty Factor (%) | 10 | 30 |
| Cycles Per Burst | 200 | 200 |
| Treatment Time (s) | 280 | 250 |
| Intensifier | Yes | N/A |

Table 1. Illumina shearing settings for Covaris E220evolution/LE220 instruments provided in the TruSight Tumor 170 Reference Guide for shearing to 90 to 250 bp with a peak at 125 bp (Document # 1000000024091 v01 April 2017).

| Instrument | S220 | M220 |
|-------------------------|---|---|
| Consumable | microTUBE-50 Screw-Cap (520166) | microTUBE-50 Screw-Cap (520166) |
| Holder | S-Series Holder microTUBE-50 Screw-Cap (500492) | M220 Holder XTU (500414) |
| Insert | N/A | M220 Holder XTU Insert microTUBE-50 µl (500488) |
| Bath Temperature (C) | 7 | 20 |
| Peak Incident Power (W) | 100 | 75 |
| Duty Factor (%) | 30 | 15 |
| Cycles Per Burst | 1000 | 1000 |
| Treatment Time (s) | 150 | 360 |

Table 2. Illumina published protocol for Covaris S220 and M220 instruments provided on the TruSight Tumor 170 Kit Support page (https://support.illumina.com/sequencing/sequencing_kits/trusight-tumor-170-kit/best_practices.html).



Note: Even if the Water Level check sensor is green in SonoLab, it is crucial to ensure the glass portion of the microTUBE-50 Screw-Cap is completely submerged before shearing on the M220.

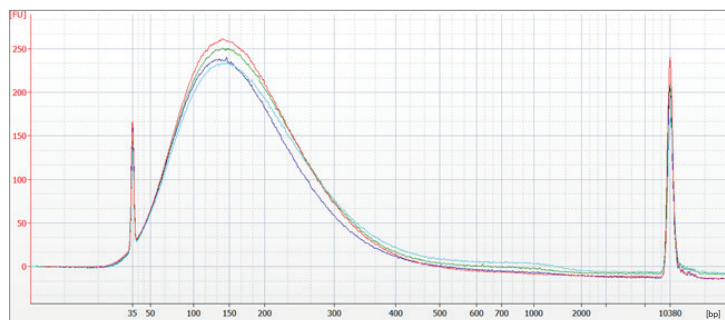


Figure 1. Observed DNA fragment size distribution profiles of 2 samples per instrument using Illumina settings listed in Table 1 on the LE220 and E220 measured by Agilent Bioanalyzer HS DNA Chip. Note, samples were human genomic DNA from Promega run at 10 ng/µl in low TE.

DNA Shearing Settings Developed by Covaris for TruSight Tumor 170 Library Construction

The following protocols were developed using purified genomic DNA as a model for FFPE DNA to ensure reproducible settings. Due to the variability of cross-linking in FFPE material from fixation, which can lead to variation in fragmentation, genomic DNA extracted from whole blood was chosen to match the settings developed by Illumina.

| Instrument | E220 | |
|-------------------------|---|---|
| Consumable | microTUBE-50 AFA Fiber Screw-Cap (520166) | 8 microTUBE-50 AFA Fiber Strip V2 (520174) 8 microTUBE-50 AFA Fiber H Slit Strip V2 (520240) |
| Holder | Rack 24 Place microTUBE Screw-Cap (500308) | Rack 12 Place 8 microTUBE Strip V2 (500444) |
| Plate Definition | "E220_500308 Rack 24 Place microTUBE-50 Screw-Cap +6.5 mm offset" | "E220_500444 Rack 12 Place 8 microTUBE-50 Strip V2 +10 mm offset" |
| Bath Temperature (C) | 7 | 7 |
| Water Level | 6 | -2 |
| Peak Incident Power (W) | 100 | 75 |
| Duty Factor (%) | 30 | 15 |
| Cycles Per Burst | 1000 | 500 |
| Treatment Time (s) | 160 | 500 |
| Intensifier | Yes | Yes |

Table 3. DNA shearing settings for the E220.

| Instrument | ME220 | |
|-------------------------|---|--|
| Consumable | microTUBE-50 AFA Fiber Screw-Cap (520166) | 8 microTUBE-50 AFA Fiber Strip V2 (520174) 8 microTUBE-50 AFA Fiber H Slit (520240) |
| Holder | Rack 4-place microTUBE Screw-Cap (500522) | Rack 8 microTUBE Strip V2 (500518) |
| Wave Guide | ME220 Waveguide 4 Place (500534) | ME220 Waveguide 8 Place (500526) |
| Bath Temperature (C) | 20 | 20 |
| Peak Incident Power (W) | 75 | 50 |
| Duty Factor (%) | 25 | 30 |
| Cycles Per Burst | 1000 | 1000 |
| Treatment Time (s) | 225 | 270 |

Table 4. DNA shearing settings for the ME220.

| Instrument | LE220 and LE220-plus | |
|-------------------------|---|--|
| Consumable | 8 microTUBE-50 AFA Fiber Strip V2 (520174) 8 microTUBE-50 AFA Fiber H Slit V2 (520240) | |
| Holder | Rack-XT 12 Place 8 microTUBE Strip V2 (500485) | |
| Plate Definition | "LE220_500485 Rack_XT 12 Place 8 microTUBE-50 Strip V2 -12 mm offset" | "LE220plus_500485 Rack-XT 12 Place 8 microTUBE-50 Strip V2 -12mm offset" |
| Dithering | 0.5 mm X-dither & 0.5 mm Y dither at 10 mm/sec | |
| Bath Temperature (C) | 7 | |
| Water Level | -2 (LE220-plus automatic adjustment) | |
| Peak Incident Power (W) | 450 | |
| Duty Factor (%) | 20 | |
| Cycles Per Burst | 1000 | |
| Treatment Time (s) | 360 | |

Table 5. DNA shearing settings for the L-series instruments.



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

RESULTS

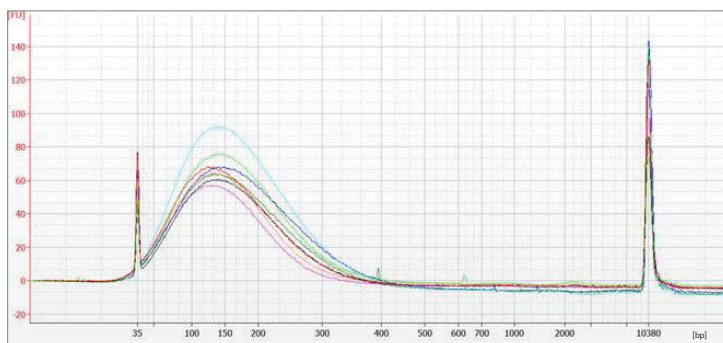


Figure 2. Summary of sheared DNA profiles measured by Agilent Bioanalyzer HS DNA Chip: Promega genomic DNA at 10 ng/μl in low TE was sheared following validated protocols. Representative profiles with average 125 bp size fragments were obtained using microTUBE-50 Screw-Cap (520166) with E220, ME220, ME220; using 8 microTUBE-50 AFA Fiber H Slit Strip V2 (520240) with E220, E220, ME220, and LE220 (replicates listed in electropherogram colors).

CONCLUSION

This guide provides flexible solutions for all current AFA-based NGS shearing platforms for users shearing to 90 to 250 bp in 55 μl of sample volume. The parameters developed have undergone rigorous testing to provide coefficient of variations (CV) of less than 10% in DNA sizing profile replicates, resulting in highly reproducible shearing suitable for Illumina TruSight Tumor 170 NGS protocols.

*TruSight is a registered trademark of Illumina.