Quick Guide:

DNA Shearing with ME220 Focused-ultrasonicator

This Quick Guide provides DNA Shearing protocols for the Covaris microTUBE and miniTUBE consumables using a Covaris ME220 Focused-ultrasonicator instrument.

Revision History

Part Number	Revision	Date	Description of change
010349	H	2/2019	Update Quick Guide format. 8 microTUBE-50 AFA Fiber Strip 350bp shearing protocol with SonoLab version 8.0.2 or higher
010349	I	3/2019	Updated shearing protocol with SonoLab version 8.0.2 or higher
010349	J	4/2019	Formatting changes, updated rack definitions, addition of Appendix C

Values mentioned in this Quick Guide are nominal values. The tolerances are as follow:

- Temperature ± 5°C
- Sample volume
  - microTUBE-15: from 15 to 20 µL, ± 1 µL
  - microTUBE-50: 55 µL, ± 2.5 µL
  - microTUBE-130: 130 µL, ± 5 µL
  - microTUBE-500: 320 µL, ± 10 µL
  - miniTUBE: 200 µL, ± 10 µL
- Water Level ± 0.5

Sample preparation guidelines

- **DNA input:** microTUBE-130 and microTUBE-50 up to 5 µg purified DNA; microTUBE-15 up to 1 µg; microTUBE-500 minimum 320 ng and up to 5 µg.
- **Buffers:** TE **-** Tris-EDTA, pH 8.0.
- **DNA quality:** Genomic DNA (> 10 kb). For lower quality DNA, Covaris recommends setting up a time dose response experiment for determining appropriate treatment times.
- **WARNING**: DO NOT use the microTUBE or miniTUBE for long term sample storage. Samples should be transferred after processing.

Instrument setup

- Refer to the instrument manual for complete setup.
- DNA Shearing vessels have specific racks and waveguides associated with them.

Instrument settings

- Recommended settings are subject to change without notice.
- **DNA fragment representation will vary with analytical systems. Please carry out a time course experiment based on settings provided in this document to reach desired fragment size distribution (Appendix C).**

See http://www.covarisinc.com/wp-content/uploads/pn_010349.pdf for updates to this document.

Table of Contents

microTUBE-50 with SonoLab 8.0.2 or higher

Vessel	8 microTUBE-50 AFA Fiber Strip V2	8 microTUBE-50 AFA Fiber H Slit Strip V2
		
Part Number (PN)	**520174**	**520240**
Sample Volume	55 µL	
Rack	Rack 8 microTUBE Strip V2 PN 500518	
Rack Definition	8 microTUBE-50 Strip V2 PN 520174.2	8 microTUBE-50 H Slit Strip V2 PN 520240.2
Waveguide	PN 500526	
Temperature (°C)	12	
Analytical System	Agilent High Sensitivity DNA Kit cat# 5067-4626	
Base Pair Mode (bp)	**150**	**350**
Repeat/Iterations (#)	23	7
Repeat Process Treatment Duration (sec)	10	10
Peak Incident Power (W)	50	50
Duty Factor (%)	30	20
Cycles per Burst (#)	1000	1000
Total Treatment Time per sample (s)	230	70

See Appendix B for screenshots of protocols entered into SonoLab 8.0.2 or higher.

microTUBE-130 with SonoLab 8.0.2 or higher

Vessel	8 microTUBE-130 AFA Fiber Strip V2	8 microTUBE-130 AFA Fiber H Slit Strip V2	microTUBE-130 AFA Fiber Screw-Cap	
				
Part Number (PN)	**520217**	**520239**	**520216**	
Sample Volume	130 µL			
Rack	Rack 8 microTUBE Strip V2 PN 500518		Rack 4-place microTUBE Screw-Cap PN 500522	
Rack Definition	8 microTUBE-130 Strip V2 PN 520217.2	8 microTUBE-130 H Slit Strip V2 PN 520239.2	4 microTUBE-130 Screw-Cap PN 520216.2	
Waveguide	PN 500526		PN 500534	
Temperature (°C)	12			
Analytical System	Agilent High Sensitivity DNA Kit cat# 5067-4626			
Base Pair Mode (bp)	**150**	**350**	**150**	**350**
Repeat/Iterations (#)	30	6	25	6
Repeat Process Treatment Duration (sec)	10	10	10	10
Peak Incident Power (W)	75	70	75	70
Duty Factor (%)	25	20	25	20
Cycles per Burst (#)	50	1000	50	50
Total Treatment Time per sample (s)	300	60	250	60

See Appendix B for screenshots of protocols entered into SonoLab 8.0.2 or higher.

microTUBE-15

Vessel	8 microTUBE-15 AFA Beads Strip V2		8 microTUBE-15 AFA Beads H Slit		microTUBE-15 AFA Beads Screw-Cap			
								
Part Number (PN)	**520159**		**520241**		**520145**			
Sample Volume	15 µL							
Rack	Rack 8 microTUBE Strip V2 PN 500518				Rack 4-place microTUBE Screw-Cap PN 500522			
Rack Definition	8 microTUBE-15 Strip V2 PN 520159		8 microTUBE-15 H Slit Strip V2 PN 520241		4 microTUBE-15 Screw-Cap PN 520145			
Waveguide	PN 500534				PN 500526			
Temperature (°C)	20							
Analytical System	Agilent DNA 12000 Kit cat# 5067-1509							
Base Pair Mode (bp)	150	200	350	550	150	200	350	550
Duration (s)	140	70	40	55	140	70	40	45
Peak Power (W)	50	50	30	18	50	50	30	15
Duty Factor (%)	30	30	20	10	30	30	20	20
Cycles per Burst (#)	50	50	50	200	50	50	50	200

To ensure reproducible DNA shearing, it is required to centrifuge microTUBE-15 before processing. See Appendix A for instructions.

microTUBE-50 with SonoLab 8.0.1* or lower

Vessel	8 microTUBE-50 AFA Fiber Strip V2		8 microTUBE-50 AFA Fiber H Slit Strip V2		microTUBE-50 AFA Fiber Screw-Cap			
								
Part Number (PN)	**520174**		**520240**		**520166**			
Sample Volume	55 µL							
Rack	Rack 8 microTUBE Strip V2 PN 500518				Rack 4-place microTUBE Screw-Cap PN 500522			
Rack Definition	8 microTUBE-50 Strip V2 PN 520174		8 microTUBE-50 H Slit Strip V2 PN 520240		4 microTUBE-50 Screw-Cap PN 520166			
Waveguide	PN 500534				PN 500526			
Temperature (°C)	20							
Analytical System	Agilent DNA 12000 Kit cat# 5067-1509							
Base Pair Mode (bp)	**150**	**200**	**350**	**550**	**150**	**200**	**350**	**550**
Duration (s)	214	125	45	40	180	90	72	52
Peak Power (W)	50	50	50	50	75	75	50	25
Duty Factor (%)	30	30	20	10	25	25	10	10
Cycles per Burst (#)	1000	1000	1000	1000	1000	1000	1000	1000

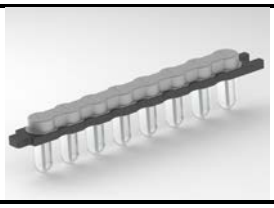
***For SonoLab version 8.0.2 or higher, refer to the prior table (page 3). To upgrade SonoLab, visit our website: https://covaris.com/resources/registered-users-login/m-series/**

microTUBE-130 with SonoLab 8.0.1* or lower

Vessel	8 microTUBE-130 AFA Fiber Strip V2		8 microTUBE-130 AFA Fiber H Slit Strip V2		microTUBE-130 AFA Fiber Screw-Cap			
								
Part Number (PN)	**520217**		**520239**		**520216**			
Sample Volume	130 µL							
Rack	Rack 8 microTUBE Strip V2 PN 500518				Rack 4-place microTUBE Screw-Cap PN 500522			
Rack Definition	8 microTUBE-130 Strip V2 PN 520217		8 microTUBE-130 H Slit Strip V2 PN 520239		4 microTUBE-130 Screw-Cap 520216			
Waveguide	PN 500526				PN 500534			
Temperature (°C)	20							
Analytical System	Agilent DNA 12000 Kit cat# 5067-1509							
Base Pair Mode (bp)	**150**	**200**	**350**	**550**	**150**	**200**	**350**	**550**
Duration (s)	225	130	42	65	225	140	45	62
Peak Power (W)	75	70	70	40	75	70	70	40
Duty Factor (%)	25	20	20	10	25	20	20	10
Cycles per Burst (#)	1000	1000	1000	1000	1000	1000	1000	1000

***For SonoLab version 8.0.2 or higher, refer to the prior table (page 4). To upgrade SonoLab, visit our website: https://covaris.com/resources/registered-users-login/m-series/**

microTUBE-130 with SonoLab 8.0.1 or lower

Vessel	microTUBE AFA Fiber Pre-Slit Snap-Cap				microTUBE AFA Fiber Crimp-Cap				8 microTUBE Strip V1			
												
Part Number (PN)	**520045**				**520052**				**520053**			
Sample Volume	130 µL											
Rack	Snap-Cap/Crimp-Cap/8 microTUBE Strip V1 Rack PN 500514											
Rack Definition	8 microTUBE-130 Snap-Cap PN 520045				8 microTUBE-130 Crimp-Cap PN 520052				8 microTUBE-130 Strip V1 PN 520053			
Waveguide	PN 500526											
Temperature (°C)	12											
Analytical System	Agilent DNA 12000 Kit cat# 5067-1509											
Base Pair Mode (bp)	**150**	**200**	**350**	**550**	**150**	**200**	**350**	**550**	**150**	**200**	**350**	**550**
Duration (s)	225	130	42	65	240	140	45	65	225	130	38	55
Peak Power (W)	75	70	70	40	75	70	70	40	75	70	70	40
Duty Factor (%)	25	20	20	10	25	20	20	10	25	20	20	10
Cycles per Burst (#)	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000

microTUBE-500

Vessel	microTUBE-500 AFA Fiber Screw-Cap
	
Part Number (PN)	**520185**
Sample Volume	320 µL
Rack	Rack 4-place microTUBE-500 PN 500525
Rack Definition	4 microTUBE-500 Screw-Cap PN 520185
Waveguide	PN 500534
Temperature (°C)	20
Analytical System	Agilent High Sensitivity DNA Kit cat# 5067-4626
Base Pair Mode (bp)	**500 - 600**
Duration (s)	65
Peak Power (W)	75
Duty Factor (%)	20
Cycles per Burst (#)	1000

To fragment DNA to sizes larger than 5 kb, Covaris offers the g-TUBE: a single-use device that shears genomic DNA into selected fragments sizes ranging from 6 kb to 20 kb. The only equipment needed is a compatible bench-top centrifuge.

miniTUBE

Vessel	miniTUBE Clear	miniTUBE Blue	miniTUBE Red
			
Part Number (PN)	**520064**	**520065**	**520066**
Sample Volume	200 µL		
Rack	Rack 4 Place miniTUBE PN 500521		
Rack Definition	4 miniTUBE		
Waveguide	PN 500534		
Temperature (°C)	9	20	20
Analytical System	Agilent DNA 12000 Kit cat# 5067-1509		
Base Pair Mode (bp)	**2,000**	**3,000**	**5,000**
miniTUBE	**Clear**	**Blue**	**Red**
Duration (s)	900	900	900
Peak Power (W)	8	8	10
Duty Factor (%)	20	20	25
Cycles per Burst (#)	1000	1000	1000

Additional Accessories

	Product Description	Part Number
Preparation Stations	microTUBE Prep Station Snap & Screw Cap	500330
	microTUBE-500 Screw-Cap Prep Station	500510
	ME220 Rack Loading Station	500523
Centrifuge and Heat Block microTUBE Adapter	Fits microTUBE Screw-Caps into bench top microcentrifuges	500406
Centrifuge 8 microTUBE Strip V2 Adapter	Fits the 8 microTUBE Strip into a Thermo Scientific™ mySPIN™ 12 mini centrifuge	500541
g-TUBE	g-TUBEs (10) and prep station	520079

Technical Assistance

- By telephone (+1 781 932 3959) during the hours of 9:00am to 5:00pm, Monday through Friday, United States Eastern Standard Time (EST) or Greenwich Mean Time (GMT) minus 05:00 hours
- By e-mail at ApplicationSupport@covaris.com

Appendix A – microTUBE-15 centrifugation before DNA shearing

1. Sample loading and centrifugation

microTUBE-15 AFA Beads Screw-Cap

Load and centrifuge microTUBE-15 Screw-Cap as described before placing the tubes in the rack.

Carefully load sample through the septa making contact with the glass wall of the microTUBE

Load microTUBE-15 into the centrifuge using microTUBE Adapter (PN 500406)

Balance centrifuge. Spin at 3000x *g* (RCF) for 30 seconds

If some of the sample splashes onto the wall of the microTUBE while removing from centrifuge or placing into rack, repeat centrifuge step. All liquid should be at the bottom of the microTUBE-15 before starting the AFA treatment.

8 microTUBE-15 AFA Beads Strip V2

The 8 microTUBE-15 AFA Beads Strip V2 will fit into the Covaris Centrifuge 8 microTUBE Strip V2 Adapter (PN 500541) for the Thermo Scientific™ mySPIN™ 12 mini centrifuge. Place the strip in the adapter and spin for a minimum of 1 minute.

2. Sample processing

Use settings provided on page 5.

3. Sample recovery

Repeat the centrifuge step before recovering sample from microTUBE-15.

Place microTUBE-15 in Preparation Station and unscrew the cap

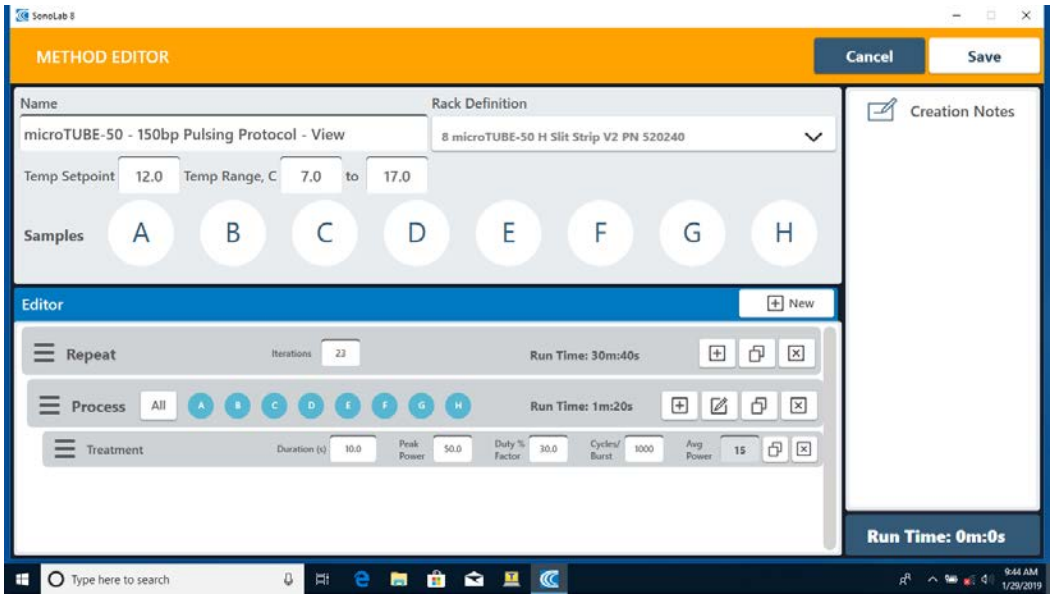
Retrieve the sample with a narrow bore 20 µL pipet tip. It may be necessary to push the beads aside for full recovery

Appendix B – Pulsing methods for microTUBE-50 in SonoLab version 8.0.1 or higher

Refer to the ME220 User Manual for detailed instructions on method creation.
https://covaris.com/wp-content/uploads/pn_010325.pdf

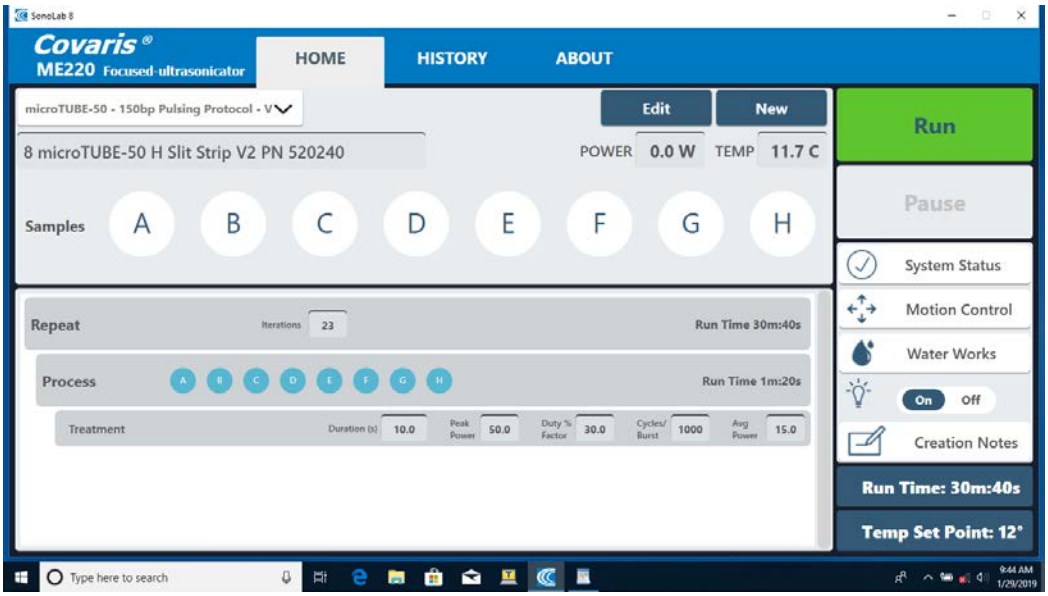
1. Method Editor

As an example, below is a screenshot of the 150 bp protocol for the microTUBE-50 in the Method Editor with all samples selected. **NOTE:** Repeat ("Iterations") is programmed before the Process.



2. Shearing Protocol

Screenshot of a complete shearing protocol with all samples selected for treatment.



Appendix C – Performing a Time Course to Create a Pulsing Method using SonoLab 8.0.1 or higher

Refer to the ME220 User Manual for detailed instructions on pulsing method creation.
https://covaris.com/wp-content/uploads/pn_010325.pdf

1. Time Course

A time course is necessary to optimize shearing protocols based on user needs. Covaris recommends using the maximum number of samples (N = 4 for screw-cap consumables or N = 8 for strip or snap-cap consumables) for each time point in the time course. The time course shall be performed by starting with the nearest recommended protocol (containing "Z" iterations) and increasing or decreasing the total number of iterations used to adjust total treatment time.

2. Time Course Set-Up

a) Fill Covaris consumable with lambda or genomic DNA in desired buffer at a concentration of 10 ng/µL or higher
   - Recommended buffer: Tris-EDTA

b) Input protocol settings into SonoLab with a value of Z – 1 iterations or Z – 2 iterations to begin:
   - I.e. Input method with 2 less iterations than stated for methods shearing to below 200 bp
   - I.e. Input method with 1 less iteration than stated for methods shearing to above 200 bp

c) Insert tubes into the ME220 and run protocol

d) Once the treatment is complete, remove samples from the instrument

e) Transfer a 5 µL aliquot of each sheared sample to a Lo-Bind vessel for sizing analysis

f) Retain used Covaris consumable and immediately replace the 5 µL missing volume with buffer for a full volume after each method is complete
   - i.e. microTUBE-50 would initially be filled to 55 µL for shearing. 5 µL would be removed post-shearing for a total volume of 50 µL. Buffer is added for a total volume of 55 µL for next time point.

g) Once Covaris consumable volume has been restored, adjust method iterations:
   - Input method with 2 iterations TOTAL for methods shearing to below 200 bp
   - Input method with 1 iteration TOTAL for methods shearing to above 200 bp

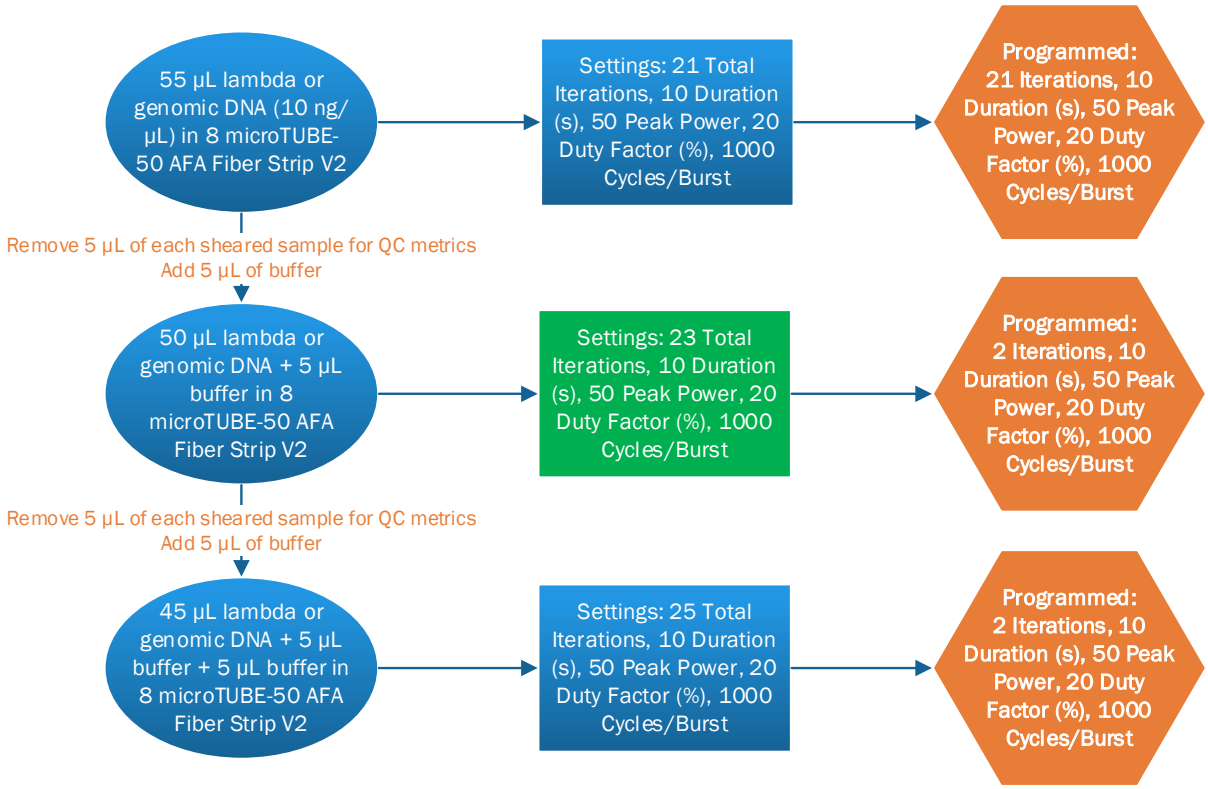
h) Repeat steps c – g.

i) If extra time points are necessary, a new consumable (Strip) or consumables (Snap-Caps, etc.) must be used (see flowchart below for details).

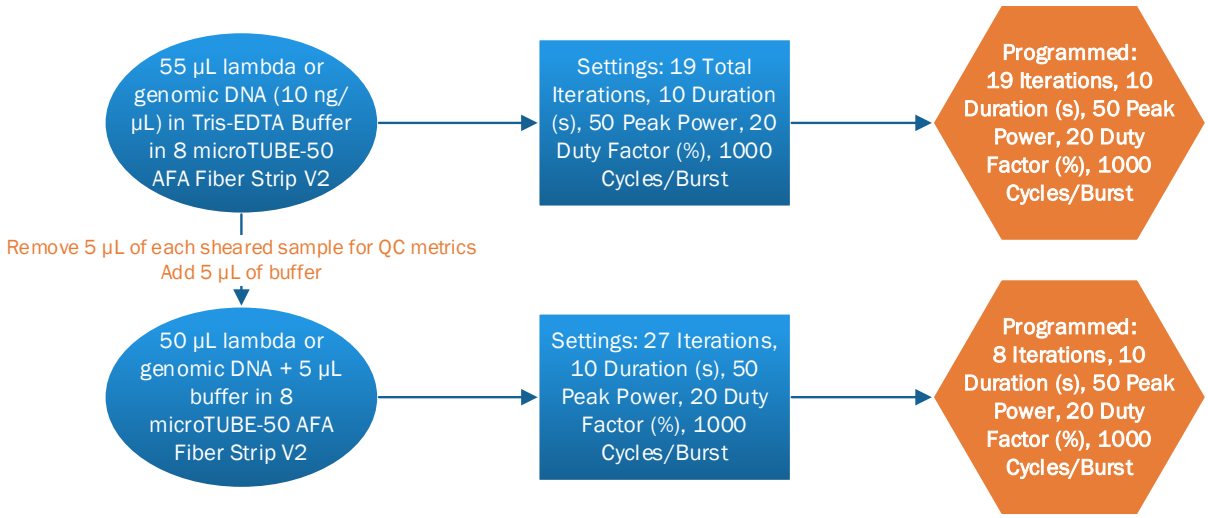
j) Retain **total** iterations or duration (s) used to obtain desired fragment sizes for programming into SonoLab and future use.

NOTE: Do not alter settings when running a time course. Only iterations should be altered.

8 microTUBE-50 AFA Fiber Strip 150bp Protocol DNA Shearing Time Course Example

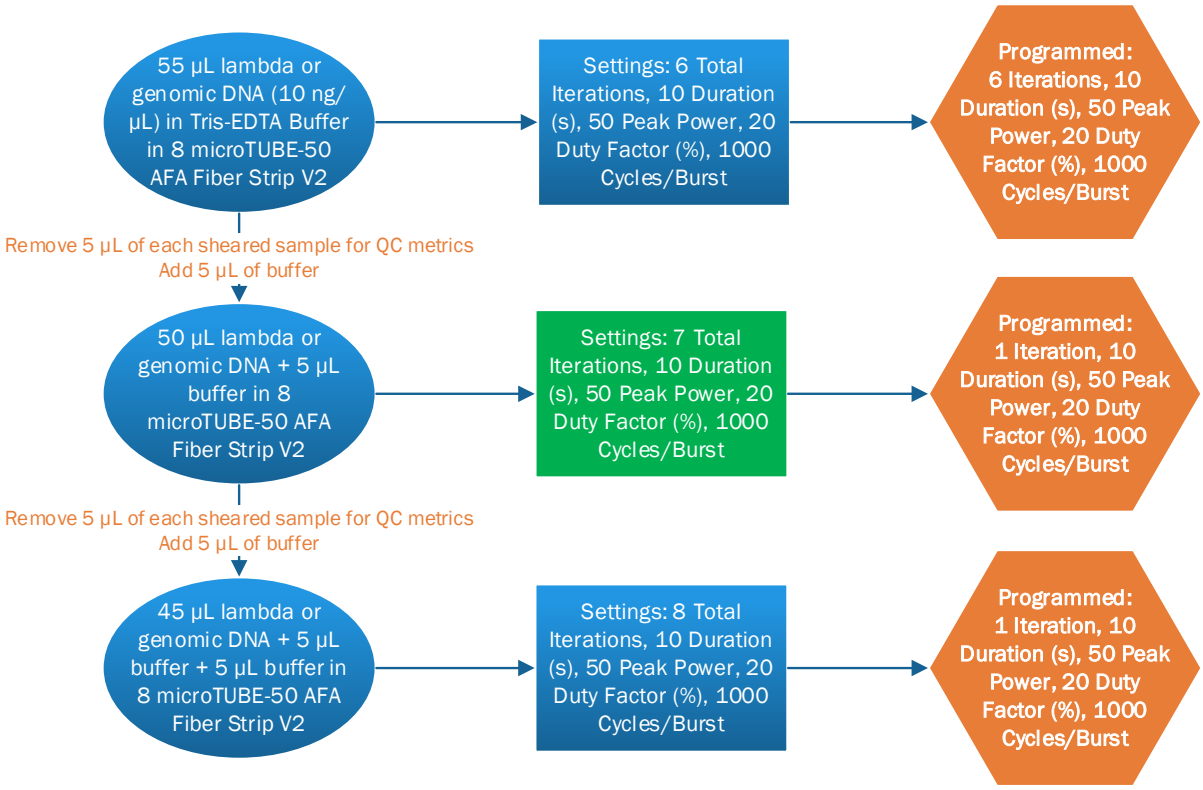


Note: new consumable(s) need to be used for additional time points

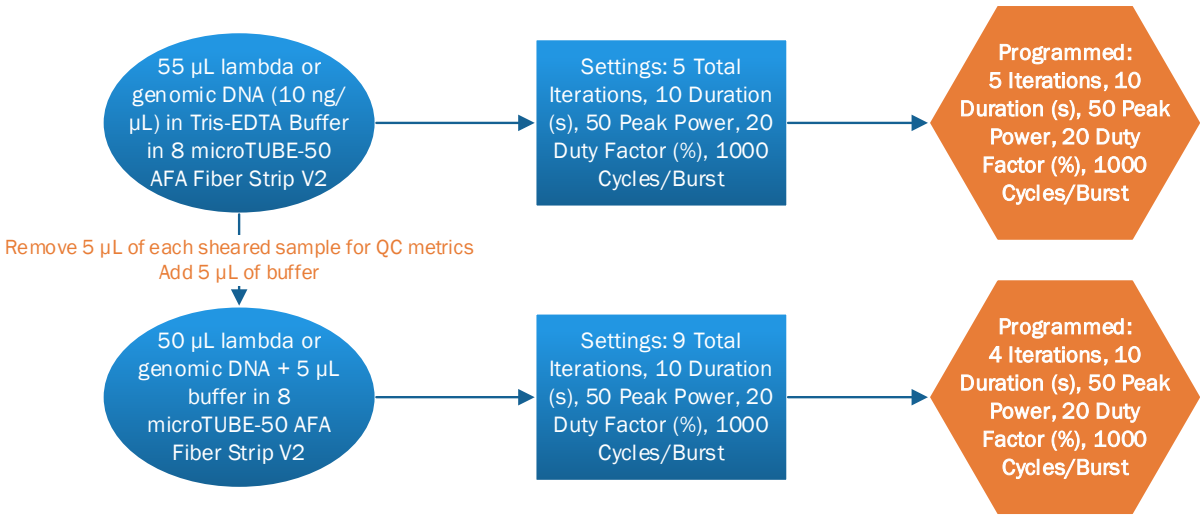


Workflow for targets lower than 200 bp. The **green** box indicates the Quick Guide protocol settings. The column of **orange hexagons** indicate treatment settings that should be programmed into the ME220 during the time course. The column of **blue rectangles** indicate settings to be programmed when the target base pair size has been achieved. The **orange text** indicates user intervention.

8 microTUBE-50 AFA Fiber Strip 350bp Protocol DNA Shearing Time Course Example



Note: new consumable(s) need to be used for additional time points



Workflow for targets lower than 200 bp. The **green** box indicates the Quick Guide protocol settings. The column of **orange hexagons** indicate treatment settings that should be programmed into the ME220 during the time course. The column of **blue rectangles** indicate settings to be programmed when the target base pair size has been achieved. The **orange text** indicates user intervention.