PROTOCOL

Covaris™

TITLE: DNA Shearing with microTUBEs (<1.5kb fragments)

Summary of Operating Conditions:

Target Base Pair (Peak)	150	200	300	500	800	1000	1500
Duty Cycle	10%	10%	10%	5%	5%	5%	2%
Intensity	5	5	4	3	3	3	4
Cycles per Burst	200	200	200	200	200	200	200
Time (seconds)	430	180	120	90	50	40	15

Temperature (water bath)	6-8°C
Power mode	Frequency Sweeping
Degassing mode	Continuous
Volume	120µl
Buffer	Tris EDTA, pH 8.0
DNA mass	<5µg
Starting material	> 50kb
Water level (FILL/RUN)	S2 – level 12 E210 – level 6
AFA Intensifier	**Yes**

Methods are transferable between the S2 system and the automated E210 (batch) system.
Please see specific recommendation chapter regarding optimization of operating conditions

Supplies

		Part Number
Sample vessel	Snap-Cap microTUBE with AFA fiber and pre-split Teflon/silicone/Teflon septa	520045
	Crimp-Cap microTUBE with AFA fiber and pre-split Teflon/silicone/Teflon septa	520052
	96 microTUBE Plate (E210) See www.covarisinc.com/pdf/pn_010112.pdf	520051
Preparation station	Snap-Cap microTUBE loading and unloading station	500142
Holder for S2	microTUBE holder (single tube) NOTE: Snap- or Crimp-Cap	500114
Holders for E210	Snap-Cap microTUBE rack (24 tubes) 6mm Z	500111
	Crimp-Cap microTUBE rack (96 tubes) 7mm Z	500143
	Intensifier: IE-DNA (required for E210)	500141

Recommended settings are subject to change without notice.
See following link www.covarisinc.com/pdf/pn_400056.pdf for updates to this document.

Recommendations specific for microTUBEs

The Covaris AFA process is highly reproducible, however attention must be paid to the following treatment attributes to ensure best results:

Sample volume: At present, the volume range of the microTUBE for DNA shearing is 120 µl, however, when using less than 120 µl to reduce splashing, either reduce the Intensity AND/OR the Duty Cycle while simultaneously increasing the process time. This is especially a concern when running high energy processes (for example, 150 bp) with lower volumes as an air-space may form in the sample fluid; thus, partitioning the sample which may result in a broad peak.

Treatment: As the DNA fragmenting process is rate-limited, fragment size generation (defined by mean peak base-pair size) is affected by treatment duration and parameters:

1. **Duration:** Minor adjustments in treatment duration may be made to optimize results for various sample types and concentrations. Duration listed in this document is a recommended guideline. Actual results may vary depending on the amount and type of starting material, concentration, and/or viscosity. Covaris recommends setting up a time dose response experiment for determining appropriate treatment times. Larger length starting material (e.g., 100kb) and larger mass (above. 10 µg) may require a longer dose to ensure a homogeneous shearing result.
   A longer duration will produce smaller fragments.
2. **Parameters**: Control should be maintained over editing of Method files, so that acoustic parameters (intensity, duty cycle, cycles per burst) and well plate definitions, once proven effective, are preserved.

Water: The bath water is employed to couple acoustic energy to the sample vessel:

1. **Purity:** When applying acoustics in rate-limited applications, foreign materials such as algae and particulates may scatter the high frequency focused acoustic beam, resulting in a shift to larger mean fragment size. Bath water should be pure distilled or DI water, changed daily or cleansed by a Covaris Water Conditioning System.
2. **Degas Level**: Similarly, insufficient degas levels within the bath may result in poor acoustic coupling and thereby shift the mean fragment size. System degas pumps should be run in advance of and during AFA treatments, as detailed in instrument User Manuals.
3. **Temperature**: Warmer temperatures promote less forceful collapse of acoustic cavities within the sample fluid, causing a shift toward larger mean fragment size. Bath temperature (as reported by SonoLAB software) should therefore be closely controlled and matched run-to-run and day-to-day. Employ the temperature alert feature in SonoLAB to warn of a failure to maintain control of bath temperature**.**
4. **Level**: **Attention should be paid to maintaining a consistent water level, according to published protocols.** If using a Covaris Water Conditioning System, check levels daily to restore water lost to evaporation.

In summary, when employing the Covaris AFA, control and verification of treatment attributes and water quality will reduce variance and promote consistent, satisfactory shearing results.

Materials

1. Sample Vessel: Covaris Snap-Cap or Crimp-Cap microTUBEs
2. Buffer: Tris EDTA, pH 8.0.
3. Sample Concentration: 100 ng – 5µg DNA in 120µl
4. **E210** Rack and intensifier or **S2** holder

Operating Conditions

1. Fill the tank with fresh de-ionized water to proper fill line. The S2 or E210 tank should be equipped with a graduated water level label. If the tank lacks this label, please contact Covaris. During treatments, the microTUBE should be partially immersed in the water to ensure a good acoustic path from the AFA transducer.
    a. For S2 system equipped with a graduated fill line label, level = 12
    b. For E210 system equipped with a graduated fill line label, level = 6

2. Degas water for recommended time period. To maintain degassed water, keep the pump continuously on during operation and sample processing. Do not turn the pump off.
    a. For S2 system: at least 30 minutes
    b. For E210 system: at least 60 minutes

3. Set the chiller to the right temperature.
    a. When set at 3°C, the S2 and E210 temperature software display should settle near 7°C.
    b. Depending on environmental conditions (for example, high relative humidity) the chiller may have to be set a little lower (or higher) to maintain the bath temperature between 6 – 8°C to offset the thermal transfer loss between the chiller and the apparatus.

Method

1. Set up the Covaris S2 or E210 at the appropriate temperature following the operating conditions above.

2. Place unfilled Covaris microTUBE into loading station for the S2 system, or for the E210, place into the bottom portion of the 24 or the 96 tube rack.

3. Keeping the cap on the tube, using a tapered pipette tip, transfer 120 µl of DNA sample (in TE buffer) by inserting the pipette tip through the pre-split septa.
    - With the pipette tip approximately half way down the interior of the tube and alongside the interior wall, slowly discharge the fluid into the tube.
    - Be careful not to introduce a bubble into the bottom of the tube. This may happen if the sample is loaded too quickly.

CAUTION: the bottom of the tube is in the acoustic field, therefore, a bubble in the sample will deflect energy and induce variable results.

CAUTION: Do not remove the snap-cap prior to sample processing. The pre-split septa should self-seal after removal of the pipette; be careful not to pressurize the sample during loading

4. After the microTUBEs are carefully loaded in either the Covaris approved S2 or E210 holders, be sure to keep the tube in a vertical orientation; again, it is important NOT to have any bubbles at the bottom of the tubes. Inspect every tube by raising the holder and check for bubbles in the tubes. Briefly and gently centrifuge to remove any bubbles.

5. Take care not to bounce the rack or holder and carefully insert into the either the S2 or E210 instrument. For the E210 system, double check to make sure the holder is fully inserted into the instrument platform. If the holder is not fully engaged, this may result in variable doses to the samples.

6. Initiate and Run process according to desired base pair target peak range.

7. Following a process, remove holder or rack from apparatus. Check to see if any tubes had a bubble at the bottom; again, this bubble would introduce variable results.

8. Transfer processed sample to another vessel:
    1) S2 - Remove tube from S2 holder and place into prep station holder. While keeping the snap-cap on, insert a pipette tip through the pre-split septa and slowly remove fluid. Alternatively, the snap-cap may be removed with the tool supplied with the prep station
    2) E210 - It is possible to remove samples while the tubes are still in the rack through the top of the holder. Insert a pipette tip through the pre-split septa and slowly remove fluid.

Supplementary Data:

Please note that there are two sources of variation in both peak value and distribution: 1) the physical process of DNA fragmentation is random and 2) the analytical process to determine fragment size has inherent variances (for example, gel electrophoresis and electropherograms). Therefore, fragment distributions and peak values, even from technical replicates, may not appear identical.

If the sheared DNA sample will be column purified or concentrated prior to analysis on an agarose gel or Bioanalyzer, please remember to take out an aliquot for use as control prior to that step. Column purification and concentration of the sheared DNA will generate a biased fragment distribution profile due to the inherent greater loss of the smaller DNA fragments.

Concentration independent 150bp DNA shearing

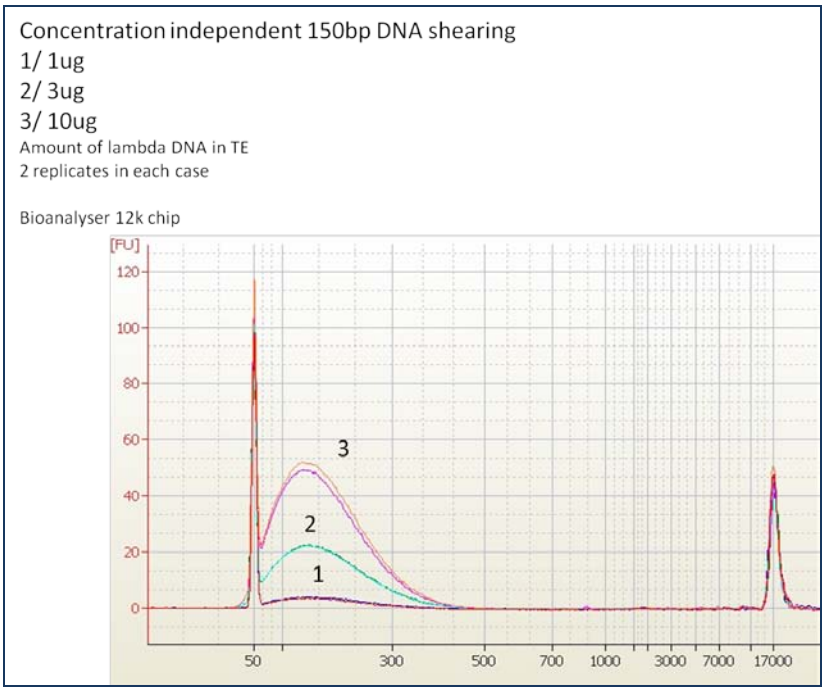


Figure 1 - Agilent 2100 Bioanalyzer electropherogram (12k chip) Different mass of lambda DNA (in 120 µl TE buffer) sheared in microTUBE operating conditions on page 1

Concentration independent 200bp DNA shearing

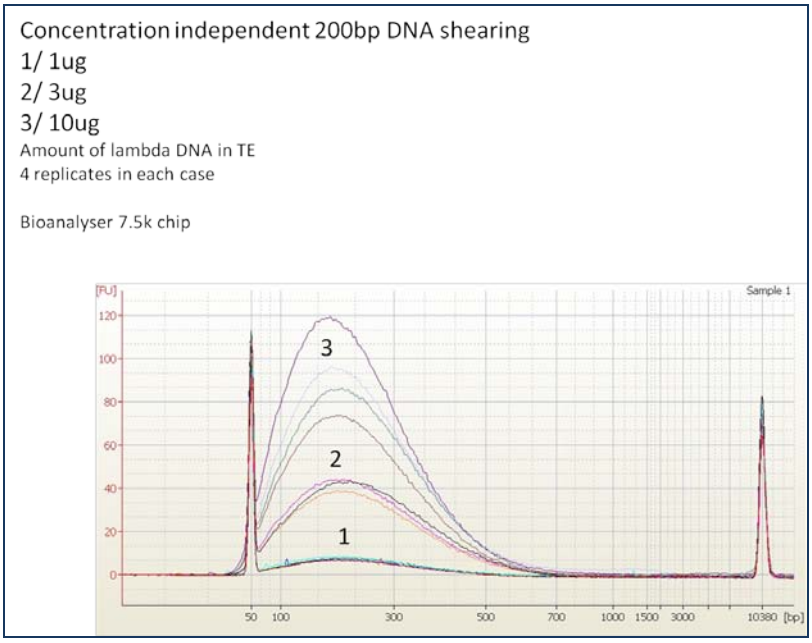


Figure 2 - Agilent 2100 Bioanalyzer electropherogram (12k chip) Different mass of lambda DNA (in 120 µl TE buffer) sheared in microTUBE operating conditions on page 1

Concentration independent 450bp DNA shearing

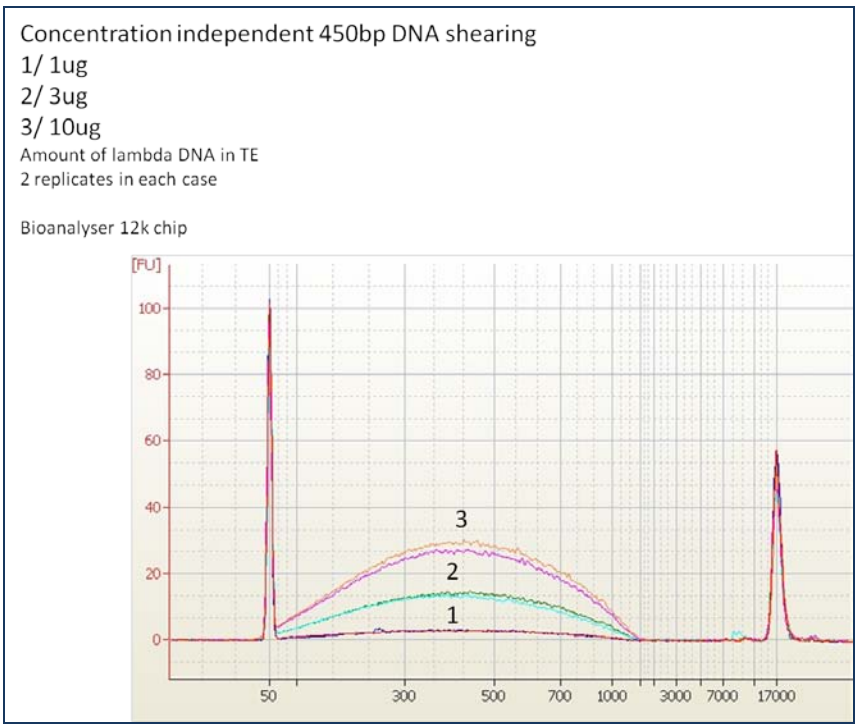


Figure 3 - Agilent 2100 Bioanalyzer electropherogram (12k chip) Different mass of lambda DNA (in 120 µl TE buffer) sheared in microTUBE operating conditions on page 1

Concentration independent 1500bp DNA shearing

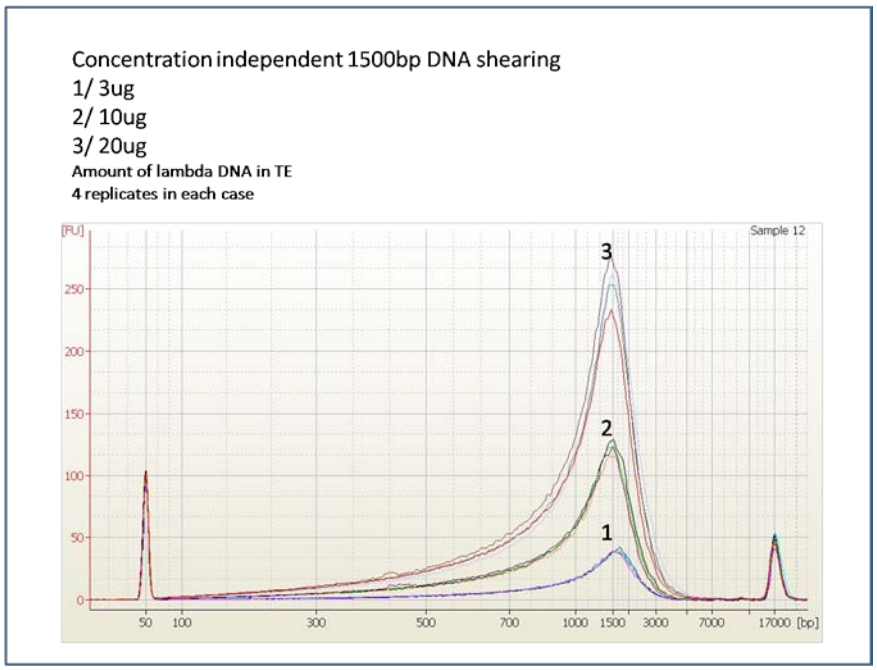
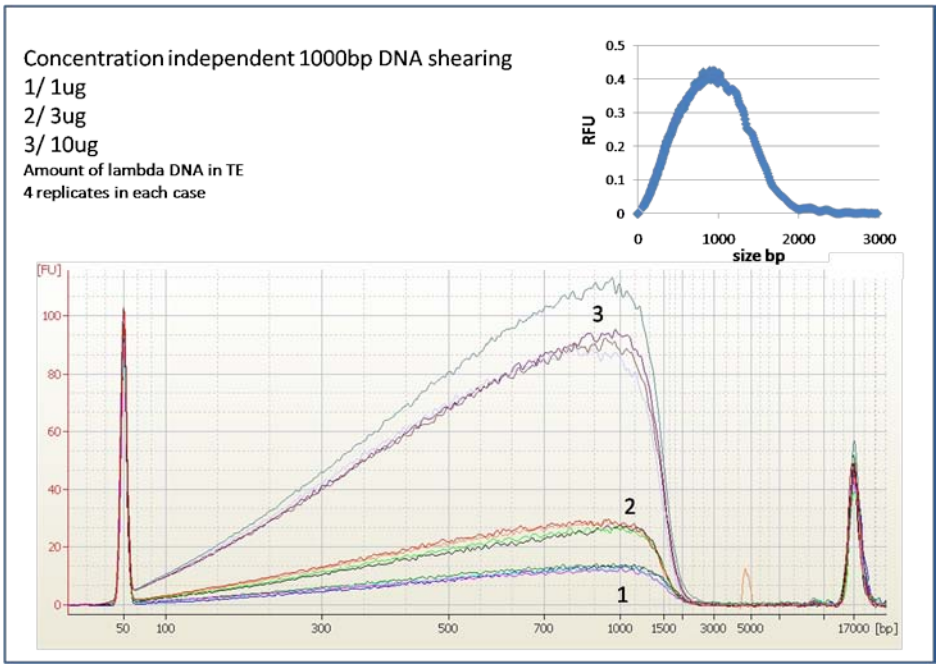


Figure 4 - Agilent 2100 Bioanalyzer electropherogram (12k chip) Different mass of lambda DNA (in 120 µl TE buffer) sheared in microTUBE operating conditions on page 1

Concentration independent 1000bp DNA shearing



NOTE: Top right graph is one of the electropherogram as plotted on a linear scale

Figure 5 - Agilent 2100 Bioanalyzer electropherogram (12k chip) Different mass of lambda DNA (in 120 µl TE buffer) sheared in microTUBE operating conditions on page 1.