

TITLE: DNA miniTUBE - Red

Summary of Operating Conditions:

Target Base Pair (Peak) 5 kb (nominal)

Please see specific recommendation chapter regarding optimization of operating conditions

| | |
|------------------------|--|
| Temperature (bath) | 19-21°C |
| Duty Cycle | 20% |
| Intensity | 1 |
| Cycles per Burst | 1,000 |
| Total Treatment Time | 600 seconds |
| Power mode | Frequency Sweeping |
| Degassing mode | Continuous |
| Volume | 200µl |
| Buffer | Tris EDTA, pH 8.0 |
| DNA mass | 2- 20µg |
| Starting material | > 50kb |
| Water level (FILL/RUN) | S2 – level 16 E210 – level 13 |
| AFA Intensifier | No Intensifier (remove from E transducer) |

Supplies

| | | Part Number |
|---------------------|--|-------------|
| Sample vessel | miniTUBE - Red - polyethylene tube (Red) with Cap | 520066 |
| Preparation station | miniTUBE loading and unloading station | 500207 |
| Holder for S2 | miniTUBE holder (single tube) | 500206 |
| Holders for E210 | miniTUBE rack (24 tubes) | 500205 |

Recommended settings are subject to change without notice.

See following link www.covarisinc.com/pdf/pn_010301.pdf for updates to this document.

Recommendations specific for miniTUBEs Red

The Covaris AFA process is highly reproducible, however, in the extremely low power application of fragmenting DNA molecules to large mean fragment sizes, additional attention must be paid to the following treatment attributes to ensure best results:

DNA Sample size: Especially for the shearing into large fragments, it is very important to keep at least a 10 fold ratio as a minimum between the size of the starting material and the targeted mean of the fragment size distribution. For example, this means a starting material size should be > 50kb for the miniTUBE red to achieve a target fragment distribution with a mean of 5kb.

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 recommended guidelines. 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 (e.g., 10, 15, 20 minutes) for determining appropriate treatment times. Larger length starting material (e.g., 100kb) and larger mass (above. 20 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 low power 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:** The water level is a critical parameter in the red miniTUBE process; **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 for generation of large kilobase fragments, control and verification of treatment attributes and water quality will reduce variance and promote consistent, satisfactory shearing results.

Materials

1. Sample Vessel: Covaris miniTUBE - Red. The miniTUBE is a high density polyethylene tube (with a vented, separate polypropylene cap) with an AFA disc at the bottom.

**Warning: store the plugs in a cool, dry place before use.
Avoid contacting or touching the bottom of the MiniTUBE**

2. Buffer: Tris EDTA, pH 8.0.
3. Sample Concentration: 2-20µg DNA in 200 µl
4. Rack or holder: E210 miniTUBE rack (p/n: 500205) or S2 miniTUBE holder (p/n: 500206)

Operating Conditions

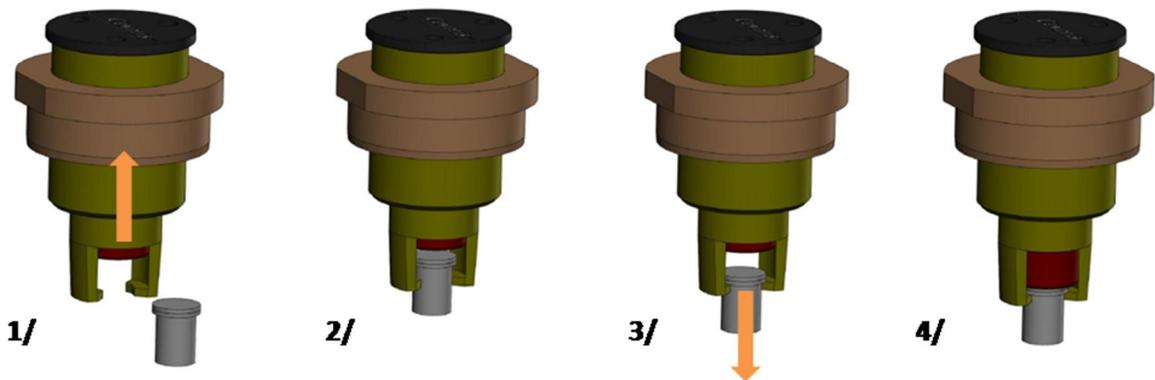
1. Fill the tank with fresh de-ionized water to proper fill line. The S2 or E210 tank should be equipped with a graduated water level label. If the tank lacks this label, please contact Covaris. During treatments, the miniTUBE should be slightly immersed in the water to ensure a good acoustic path from the AFA transducer.
 - a. For S2 system equipped with a graduated fill line label, level = 16
 - b. For E210 system equipped with a graduated fill line label, level = 13
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 18.5°C, the S2 and E210 temperature software display should settle near 20°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 19 – 21°C to offset the thermal transfer loss between the chiller and the apparatus.

Method for Using the S2

1. Set up the Covaris S2 at the appropriate temperature following the operating conditions above.
2. Slowly pipette 200 μ l of DNA solution into the miniTUBE. As the bottom is hydrophobic, the sample sometimes doesn't cover it completely. To break the surface tension, gently stir the sample with your pipette tip until the sample fully covers the bottom of the miniTUBE. Ensure that all of the solution is at the interior bottom of the tube and avoid droplets adhered to the interior sides of the tube. Note that this is a low power AFA process; the sample will not splash during the acoustic energy delivery.

CAUTION: keep the tube vertical at all times during the fragmentation process

3. Gently press the cap into place until it seats in the top of the tube. The caps provided in the miniTUBE kit are vented to relieve pressure within the tube.
4. Place the capped miniTUBE into the holder,



- 1) Depress the steel piston of the S2 holder.
 - 2) Gently insert the tube into the S2 holder from the side. The holder is not symmetrical – insert or remove the tube from the open side. NOTE: Do not contact the bottom of the tube.
 - 3) Pull tube to the bottom of the holder. Attempt to center the tube on the axis of the holder.
 - 4) Gently release the steel piston, taking care that the tube is centered and aligned to the axis of the holder.
5. Place the holder in the S2. Take care to slightly tilt the holder inserting it, in order to avoid trapping bubbles at the bottom of the miniTUBE. If a bubble is trapped at the bottom, remove the holder from the S2 and repeat this step.

CAUTION: Do not run treatments with a bubble trapped at the outside bottom of a miniTUBE

6. Process the samples according to the settings provided in the Summary of Operating Conditions on page 1.
7. At the completion of this treatment remove the miniTUBE from the holder. For maximum recovery of the sample, tilt the miniTUBE and slowly remove the sample with a pipette. Alternately, you may return the tube to the preparation station tilted approximately 30 degrees on its stand. Typically, you should recover more than 190 μ l. The sample should be transferred to another tube for long term storage.

Method for Using the E210



When operating an E210, please verify that any 500141 Intensifier has been removed from the transducer prior to running a method. The Intensifier may be removed by following the instruction in Covaris document 010111.

1. Set up the Covaris E210 at appropriate temperature following the operating conditions above.
2. Load up to 24 miniTUBEs in the E210 rack.
3. Slowly pipette 200 μ l of DNA solution into the miniTUBEs. As the bottom is hydrophobic, the sample sometimes doesn't cover it completely. To break the surface tension, gently stir the sample with your pipette tip until the sample fully covers the bottom of each miniTUBE. Ensure that all of the solution is at the interior bottom of the tube and avoid droplets adhered to the interior sides of the tube. Note that this is a low power AFA process; the sample will not splash during the acoustic energy delivery.
4. Gently press the caps into place until they seat in the top of the tube. The caps provided in the miniTUBE kit are vented to relieve pressure within the tube.
5. Press the rack cover into place until it rests on top of the caps. Tighten the center screw to secure the rack cover.

6. Set up your treatment in SonoLAB according to the settings provided in the Summary of Operating Conditions on page 1. Use plate definition 500205:

24 position MiniTube +15mm Z offset.wpl.

7. Before loading your rack in the holder, press the Check Alignment button in SonoLAB to bring the holder into position at the water surface. Then, place the rack into the holder, tipping the rack slightly as the tubes enter the water, so that any bubbles forming under the tubes can escape and are not trapped in the acoustic path. Observe the underside of the tubes - if a bubble is trapped at the bottom of a miniTUBE, remove the rack from the holder and repeat this step.

With a correct water level, you shouldn't see any air trap below the rack

Warning: Do not run treatments with a bubble trapped at the bottom of a miniTUBE

8. Run the treatment and process your samples.
9. At the completion of this treatment, slowly remove the rack from the holder keeping the samples in a vertical orientation. For maximum recovery of the sample, slightly tilt the rack and slowly remove the sample with a pipette. Alternately, you may return the tubes to the preparation station tilted approximately 30 degrees on its stand. Typically, you should recover more than 190 μ l. The sample should be transferred to another tube for long term storage.

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 Agilent 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.

Also please note that for the large fragment size, sizing by Bioanalyser and Agarose gel don't give exactly the same value.

Typical DNA Recovery

| Volume recovery (for 200 µl initial sample) | Concentration recovery (as measured with an Invitrogen Qubit fluorometer) | Overall recovery |
|---|---|------------------|
| > 95 % | > 95 % | > 90 % |

Sample to sample reproducibility

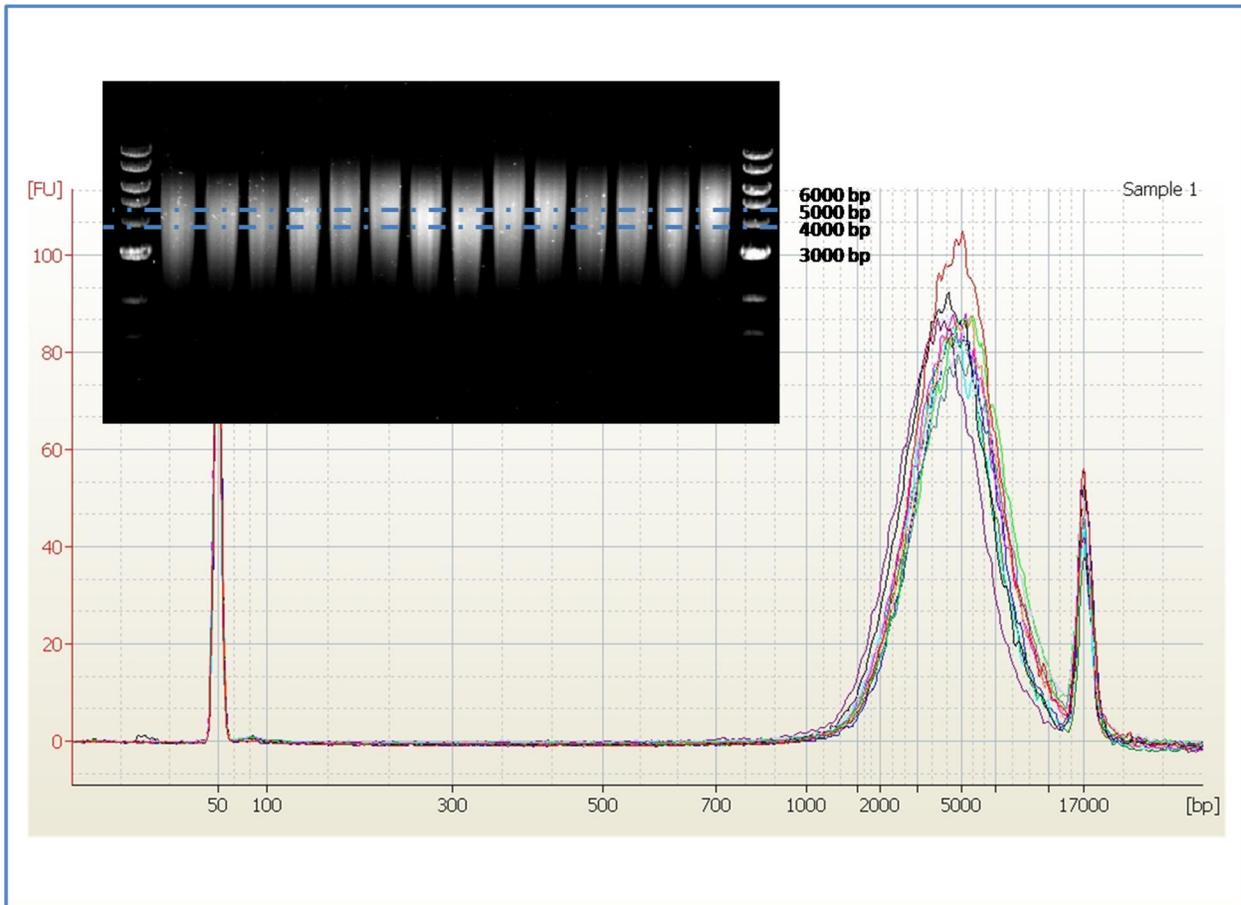


Figure 1 - Agilent 2100 Bioanalyzer electropherogram. 12 replicates using Covaris MiniTUBE - Red (10ug of E. Coli DNA in 200 µl TE)
 Inset: 0.7% Agarose gel. 14 replicates using Covaris MiniTUBE - Red

Concentration independent 5kb DNA shearing

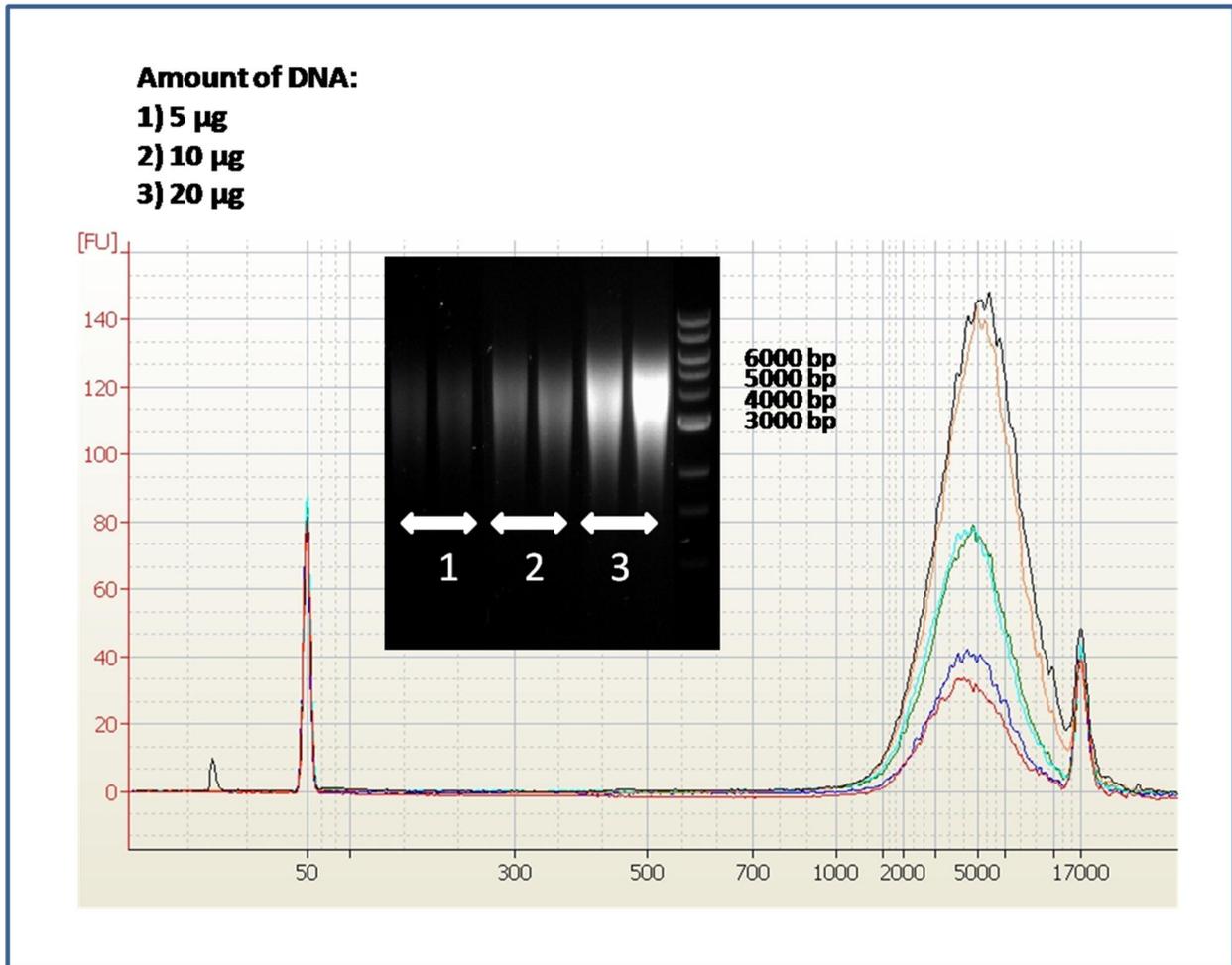


Figure 2 – Different mass of E. Coli DNA (in 200 µl TE buffer) sheared in miniTUBE red following the same operating conditions on page 1. 0.7% Agarose gel and Bioanalyzer 2100 electropherogram.