

Bauer Core Standard Protocol		
Title: Using the Covaris S220 to shear DNA in a microTube or microTube-15		
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Contact: claire@cgr.harvard.edu	Comment: DNA shearing protocol. See separate general protocol for other shearing applications	

1. Purpose

To shear DNA samples using the Covaris S220.

2. Materials

2.1. DNA sample(s) in TE Buffer

2.2. Microtube of desired volume

2.2.1 MicroTube (6 x 16 mm) part # 520045 (provided by core)

2.2.2. MicroTube-15 part # 520145

3. Instrumentation:

3.1. Covaris S220

3.2. Microtube holder (provided by core)

3.2.1. For MicroTube use holder # 500114

3.2.2. For MicroTube-15 use holder # 500427

3.3. Microcentrifuge (minifuge may be used for MicroTubes, but NOT MicroTube-15)

3.3.1. Microcentrifuge adapter #500406

4. Reagent preparation

4.1. DNA Sample Quantity: up to 5µg (1µg for MicroTube-15)

4.2. DNA Sample Volume: MicroTube: 130µl or 50µl, MicroTube-15: 15µl

5. Procedure

5.1. Start Up: Allow 30 minutes for start up procedure.

5.1.1. Turn on the chiller beneath the bench (power switch on back).

5.1.1.1. Set to 2.5 °C to give water at ~7°C in the Covaris tank.

5.1.1.2. To adjust, push the knob, turn to desired temp, and push again.

5.1.2. Fill the Covaris tank with DI H₂O to ~ level 12 (level 15 for MicroTube-15).

5.1.3. Turn on the Covaris instrument (power switch on front).

5.1.4. Open the SonoLab software.

5.1.5. Allow the water bath to degas and come to temperature.

5.2. Create a new method or select an existing method

5.2.1. Methods for standard sizes/volumes are saved in the “Bauer Core” folder.

5.2.2. To create a method, use settings from a library prep protocol or the tables below:

For 130ul

Target BP	150	200	300	400	500	800	1000	1500
Duty Factor	10%	10%	10%	10%	5%	5%	5%	2%
Peak Incident Power	175	175	140	140	105	105	105	140
Cycles Per Burst	200	200	200	200	200	200	200	200
Duration	430	180	80	55	80	50	40	15

For 50ul

Target BP	150	200	300	400	500	1000	1500
Duty Factor	10%	10%	10%	5%	5%	2%	1%
Peak Incident Power	175	175	175	175	175	175	175
Cycles Per Burst	200	200	200	200	200	200	200
Duration	280	120	50	55	35	45	20

For 15ul

Target BP	150	200	250	350	550
Duty Factor	20%	20%	20%	20%	20%
Peak Incident Power	18	18	18	18	18
Cycles Per Burst	50	50	50	50	50
Duration	300	120	80	45	22

5.3. Preparing the Sample Tube

5.3.1. Pipette 15µl (MicroTube-15), 50µl or 130µl (MicroTube) through the septum.

5.3.1.1. MicroTube: Check for air pockets and spin down if present.

5.3.1.2. MicroTube-15: Spin @ 3000xg for 30s using adapter 500406

5.3.2. Place the tube into the holder so that it sits straight.

5.3.3. Load the holder into the instrument.

5.4. Running a Saved Method.

5.4.1. Select a method (standard methods are in the “Bauer Core” folder).

5.4.2. Wait for the instrument status to be ready and then hit Run.

5.5. Cleanup

5.5.1. Remove the sample tube.

5.5.1.1. MicroTube: If necessary, spin tube briefly to collect sample at the bottom

5.5.1.2. MicroTube-15; Spin @ 3000xg for 30s using adapter 500406

5.5.1.3. Remove cap to pipette out sample.

5.5.2. Turn off the degas pump

5.5.3. Empty the water tank.

5.5.4. Run the degas pump to purge residual water from the lines.

The pump will automatically stop after 10 seconds.

5.5.5. Empty residual water from the tank and allow to air dry.