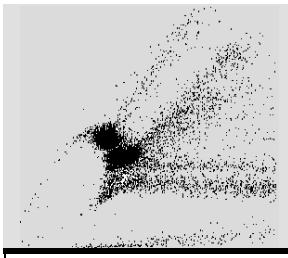


THE GOODELL LABORATORY

Author	Jonathan S. Berg	2/4/2009
Title	**Shearing DNA using the Bioruptor**	
Introduction	This protocol describes the use of the Bioruptor to shear DNA from cell lysates to prepare DNA fragments appropriate for chromatin immunoprecipitation. It assumes that the user has already prepared fixed cells according to the ChIP protocol. It can also be used with slight modifications to shear purified DNA.	
Materials	1. 1% SDS lysis buffer (use the stock buffer from any ChIP kit) 2. TE buffer 3. Bioruptor XL (in walk-in cold room) 4. Ice and water 5. Table-top centrifuge	
Protocol		*Notes*
1.	Resuspend fixed cells in 1% SDS lysis buffer to a final concentration of 1-5 x 10^6 cells/ml (or) Resuspend purified DNA in TE buffer at 100ng/ml	*One can use a lower concentration of cells/DNA but higher concentrations do not shear as well*
2.	Incubate lysates on ice for 10-15 minutes (ignore this step with purified DNA)	
3.	Prepare sonication tubes by aliquotting the sample into DNase/RNase free 1.5 ml or 0.65 ml tubes. • For 1.5 ml tubes use a volume of 200-300 ul • For 0.65 ml tubes use a volume or 100-200 ul	
4.	Load tubes into the holding ring so that they are "balanced" in the same way as a centrifuge. Add an extra tube containing just buffer if needed. Screw on the top of the ring so that it is snug, but not too tight.	*There are tube rings for:* *6 x 1.5 ml tubes and* *12 x 0.65 ml tubes* *(see Figure 1)*

5.	Prepare water bath by removing 100-200 ml of water and then adding wet ice so that there is a 1-2 cm layer of ice on top. Add back water until the level is at the blue line indicated on the water bath.	*Having the water level at the blue line is very important for even sonication*
6.	Place the lid on top of the water bath and put the tube holder into the opening such that the blue gear intercalates with the gear from the motor unit. Close the top of the enclosure.	
7.	Set the unit to the desired settings • Set output selector switch to High • Set time "on" to 0.5 min • Set time "off" to 0.5 min	*These settings are the typical ones for chromatin sonication using pulses of "30 seconds on, 30 seconds off" (see figure 2) but may need to be optimized*
8.	Put on your ear protection. It is also customary to tape a warning to the door of the cold room that "sonication is in progress".	*Once you turn on the sonicator (figure 3) it will begin the first "on" pulse*
9.	Set the timer to the desired number of minutes. • With 30 seconds "on", 30 seconds "off" each cycle will be ~1 min • Use 5-7 sonication cycles (each sonication cycle is 1-minute) • Remove the tube holder, replace the ice and repeat until the total sonication time is achieved	*The ice in the water bath will melt as sonication proceeds. By 7 cycles the ice is usually gone. By 10-15 cycles the water is lukewarm.So, after each 5-7 minute sonication cycle add more ice.*
10.	Remove tubes from tube holder and centrifuge in the 4 degree table top centrifuge at maximum for 20 minutes to pellet any insoluble material.	
11.	Process the sample according to the ChIP protocol you are using.	

A few notes on optimization:

DNA shearing should be tested and optimized when one is starting a ChIP project. In general, primary cells require more cycles to obtain the same shearing as cultured cells. For example, mouse splenocytes require ~35 cycles to obtain a good shear (200-500 bp), while 3T3 cells can be sheared adequately with only ~15 cycles. (For 35 cycles, 5X 7(1- minute) cycles and for 15 cycles 3X 5(1-minute) cycles)

In order to optimize your shearing, you need to start with an abundant source of cells and fix them according to the ChIP protocol you are using. These can then be aliquotted into tubes for empiric testing of conditions. The key variables seem to be cell concentration (no more than 1-5 x 10^6 cells/ml) and the number of sonication cycles (which really boils down to the total sonication time). The easiest experiment is to use a standard cell concentration, then shear samples of equal volume for different numbers of cycles (multiples of 5 or 7 are convenient). Other combinations (such as varying the sample volume, cell concentration, "on" and "off" times, etc.) are also possible. The sheared DNA is then reverse-crosslinked according to the ChIP protocol and run a portion of the shear on a 1% gel to evaluate the size range attained (figure 4). It usually requires at least 100,000-200,000 cell equivalents / tube in order to visualize the DNA on an ethidum-stained gel.

It is difficult to assess chromatin shearing in small numbers of cells because the DNA is generally not visible on an EtBr gel. To circumvent this problem, simulate the shearing of small numbers of cells (ex. 20,000) by preparing 12 x 0.65ml tubes with 20,000 cells per tube. Sonicate the tubes simultaneously at the desired conditions, then pool all of the samples together for reverse crosslinking and gel electrophoresesis. This yields an amount of DNA that can be analyzed on a gel and gives an "average" sense of the shearing attained in each tube.

Figures:

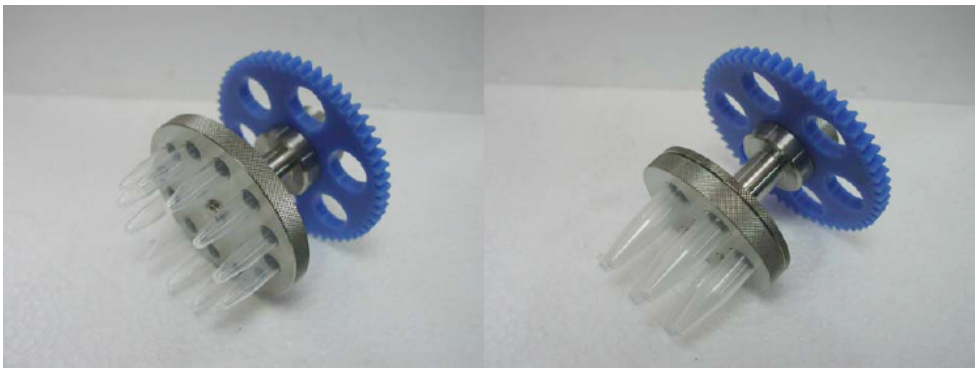
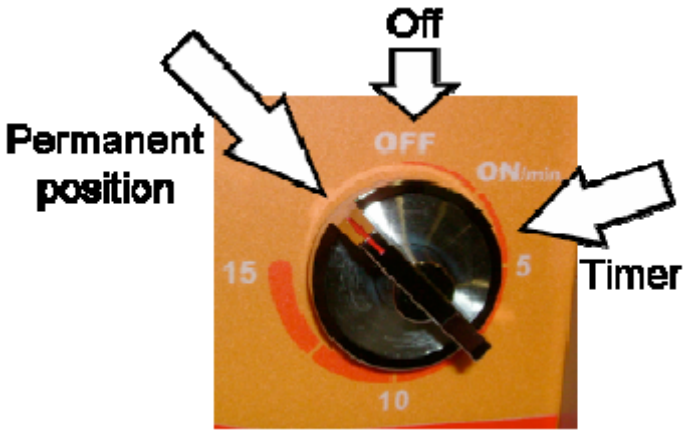


Figure 1: Tube holders for use in the Bioruptor
12 tube holder for 0.65 ml tubes (left) and 6 tube holder for 1.5 ml tubes (right). Note that the tubes are loaded so that the sample hangs down into the water bath.

Figure 2: The output selector switch (left) and interval control knob (right)
Low = 130 W
Medium = 160 W
High = 200 W
Time is indicated in minutes. The red line indicates the time spent "on" and the green line indicates the time spent "off".

Figure 3: The on/off switch and timer
The switch has three positions: Turn to the left for permanent "on", turn to the right for a timed sonication interval.



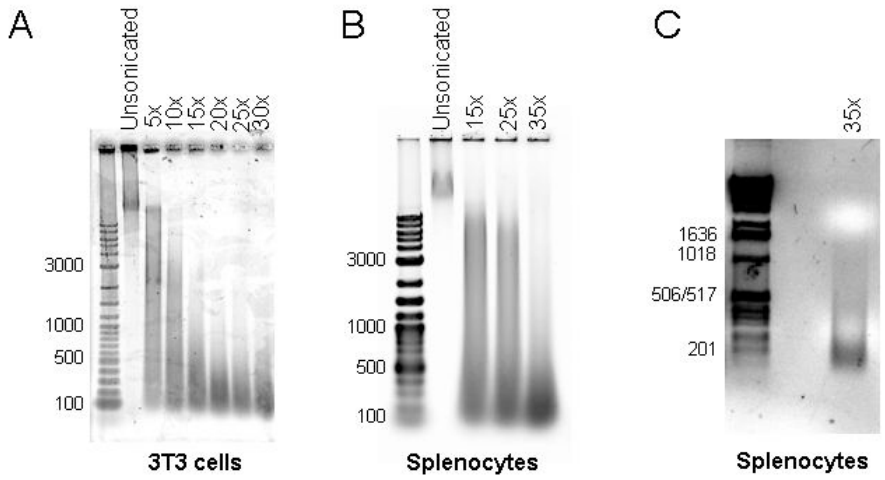


Figure 4: Examples of chromatin shearing
A) Equivalent samples of cultured 3T3 cells sonicated for increasing amounts of time. Maximal shearing is obtained at approximately 15-20 cycles of sonication at 30 seconds "on", 30 seconds "off".
B) Primary splenocytes sonicated for increasing amounts of time, demonstrating the need for longer sonication times with this cell type.
C) Shearing experiment using small numbers of cells (in this case, 12 tubes each containing 20,000 cells in 0.1 ml), then pooled and analyzed on the gel. This result confirms that dilute cell concentrations such as these (~0.2 x 10^6 cells/ml) give appropriate shearing.