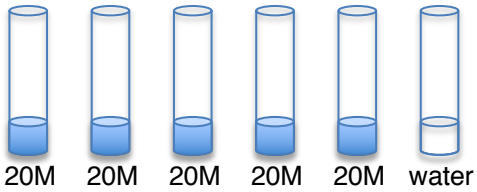
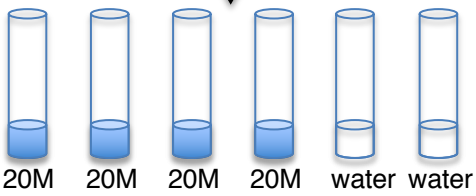
3 cycles
30 sec on, 30 sec off



Diagenode Biorupter
4°C water bath
10 ml tubes

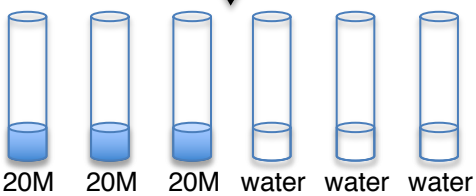
Remove one 20M sample and place on ice. Briefly (~1 sec) vortex remaining samples.

2 additional cycles
(= total of 5 cycles)
30 sec on, 30 sec off



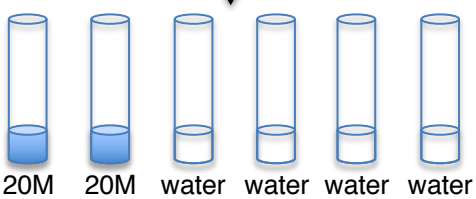
Remove one 20M sample and place on ice. Briefly (~1 sec) vortex remaining samples.

2 additional cycles
(= total of 7 cycles)
30 sec on, 30 sec off



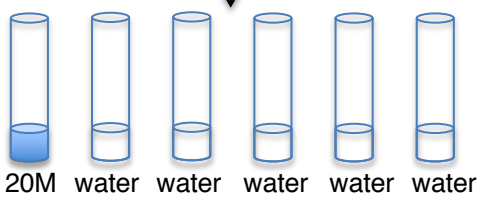
Remove one 20M sample and place on ice. Briefly (~1 sec) vortex remaining samples.

3 additional cycles
(= total of 10 cycles)
30 sec on, 30 sec off

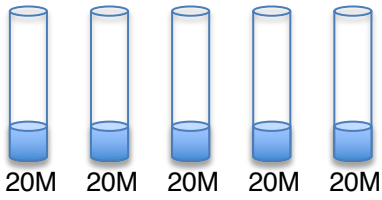


Remove one 20M sample and place on ice. Briefly (~1 sec) vortex remaining samples.

5 additional cycles
(= total of 15 cycles)
30 sec on, 30 sec off



Remove last 20M sample and place on ice



Sheared chromatin on ice

Either preclear chromatin o/n
or
Freeze at -20°C for short term storage
or
Freeze at -80°C for long term storage

Remove 50 µL of each condition for testing*

Bring volume up to 200 µL with ChIP Elution Buffer
Add: 16 µl of 5M NaCl
1 µl of 1 mg/ml of RNase A
Incubate o/n at 65°C

ChIP Elution Buffer
100 mM $NaHCO_3$
1% SDS

3µL of 20 mg/mL proteinase K to each tube and incubate for 2 hours at 45°C.

Purify samples using a Qiagen PCR purification kit
Elute samples in 12 µl of Qiagen EB solution according to kit protocol.

Qiagen EB solution
(from Qiagen kit)

Run samples on Agilent Bioanalyzer.
Region 100-500 bp and determine average length.
Proceed with ChIP-seq and determine which size range may be best for your factor of interest.

Once you have determined an acceptable range, you do not need to repeat titration, but you should always check your samples to make sure they are in the predetermined acceptable range.

*You can use less than 50 µl, however, we always increase the volume to 200 µl with ChIP elution buffer and process samples in the same way that ChIP samples are processed for reverse cross-linking.

Figure S1: Optimization of chromatin shearing. This protocol, while specific for the Diagenode Biorupter Plus, can be used as a guide to determine the range of chromatin needed for successful ChIP-seq experiments for various factors of interest.