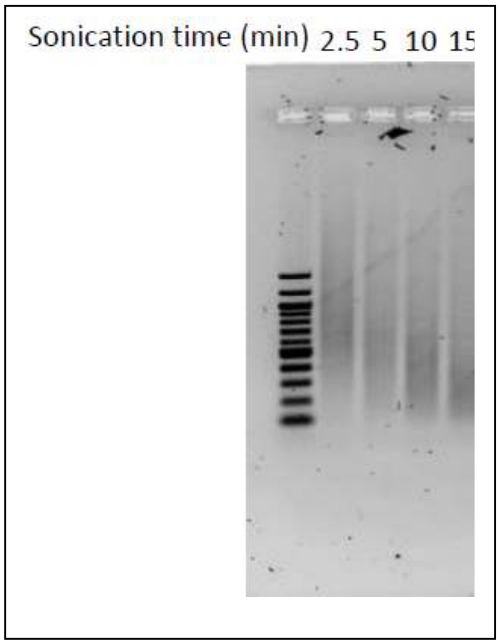
Yeast and Mammalian Chromatin Prep

Example protocols and results are based on customer feedback.

Yeast Protocol:

Cell Type: Budding yeast; 5 -20 ml worth of culture at OD around 1.0 per sample
Sample Lysis Solution: 50 mM Tris, pH 7.5, 300 mM NaCl, 1 mM EDTA, 0.5% Nonidet P-40, 10% Glycerol
Total Sample volume: 500ul
Fixation Time: 1% Formaldehyde, 5-15 min
Sonicator Amplitude Setting: 50%
Sonication Pulse Rate: 30 seconds On, 30 seconds Off
Total Sonication On Time: 5-30 min.
Sample Process Temperature: 4°C



Mammalian Cell Protocol:

Cell Type: Mammalian cells (mouse and human); 1-5 million cells per sample
Sample Lysis Solution: RIPA buffer
Total Sample volume: 500ul
Fixation Time: 1% Formaldehyde, 5-15 min
Sonicator Amplitude Setting: 50%
Sonication Pulse Rate: 30 seconds On, 30 seconds Off
Total Sonication On Time: 5-30 min.
Sample Process Temperature: 4°C

Customer Notes:

- In general, we use 5 min for ChIPping histones and histone modifications.
- For cultured human fibroblasts and MSCs, 15-20 min sonication is enough.
- You will need longer time for mouse or human tissues, and MEFs.
- Crosslinking time should also be empirically determined.
- The shortest effective time is the best because crosslinking can cause aggregation of soluble proteins and make chromatin harder to solubilize.
- It is not necessary to keep it in the cold room. We keep it in room temp on a bench and use the chiller.
- To reduce the time needed to chiller the water at the start of a cycle, we keep several bottles of water chilled in a cold room and pour into the water bath prior to use.
- We wrap the tubing to and from chiller in absorbent pads for insulation. It helps the chiller to cool the water more efficiently and prevents condensation from dripping all over the bench.
- Samples processed using 1.5ml polystyrene tubes (Evergreen).
- The Q800R has a more consistent and efficient sonication performance when compared to Bioruptor.