|  |  |
| --- | --- |
| Step 2/6  Chromatin shearing by MNase digestion  Time  1h  Vol.  20 -> 60µl/Sample  Vol.  60 -> 210µl/Sample | 2 Chromatin shearing and nuclear membrane solubilization  Digest chromatin with 10U/µl MNase for 5min on ice:  1) Thaw cells (nucleus) on ice.  2) Make the volume to 10 µl by quick spin the nucleus (on a table rotator for 5-10s) and discard the extra volume.  3) Dilute stock MNase to 10 U/µl with MNase Storage Buffer (10x dilution: add 0.5µl MNase to 9.5µl MNase Storage Buffer to get 10µl 10U/µl MNase).  4) Prepare MNase Master mix as table I. Add MNase just before use.  5) Add 40µl MNase Master Mix to each sample, mix well by pipetting or gentle vertex.  6) Transfer the sample from ice to 25°C water bath for exactly 6 min then transfer back on ice. Add 5.5 µl MNase Stop solution (100mM EDTA), mix well by gentle vertex.  7) Add 5.5µl Nuclear Break Buffer (1% Triton, 1% DOC solution) into the tube, mix well by gentle vertex. These are the sheared chromatin ready to be ChIPed.  8) Add 40µl of Protease Inhibitor mix per ml to ChIP Buffer. This is the Complete ChIP Buffer to be used next.  9) Add 150µl Complete ChIP Buffer to each sheared chromatin to make 210µl per 2000 cells.  For 3000 cells, add 255µl of Complete ChIP Buffer to 60µl sheared chromatin to make 315µl total volume.  For 4000 cells: Add 360 µl of Complete ChIP Buffer to the 60µl sheared chromatin to make 420µl total volume.  You will have 100µl sheared chromatin for IP and 10µl for Input. See table II for a detailed volume added along the process.  Note if pre-clear is desired, deduct the volume of beads needed. See step3 pre-clearing for detail.  This step diluted Triton and DOC to ~0.1% before the addition of antibodies. |