Histone extraction from cell pellets

1. Thaw the sample at 37°C water bath (if it was frozen at -80°C) and then transfer the sample to 15 mL tube. Centrifuge at 300g for 5 minutes at room temperature (RT) and discard the supernatant.
2. Suspend the pellet with 6mL ice-cold PBS and centrifuge at 300g for 5 minutes at 4°C. PBS can be supplemented with 5 mM sodium Butyrate to retain levels of histone acetylation.
3. Repeat step 2.
4. Re-suspend cells in ice-cold TEB (Triton Extraction Buffer: PBS containing 0.5% Triton X100 (v/v). 2mM phenylmethylsulfonyl fluoride (PMSF), 0.02% (V/V) NaN_3 and protein inhibitor cocktail) at a cell density of $5X10^6$ cells ml^{-1}.
5. Lyse the sample on rotator for 30 minutes at 4°C.
6. Centrifuge at 2000rpm for 10 minutes at 4°C. Remove and discard the supernatant.
7. Wash the cells in half of the volume of TEB and centrifuge at 2000rpm for 10 minutes at 4°C. Remove and discard the supernatant.
8. Entirely discard supernatant and re-suspend the pellet (nuclei) in 0.4 N H_2SO_4 (0.4 volume of TEB used in step 4). Note: Nuclei have to be re-suspended very well, with no obvious clumps left in solution, vortex the solution is necessary.
9. Incubate on rotator for 4 hours or overnight at 4°C.
10. Centrifuge the sample at 2000rpm for 10 minutes at 4°C, transfer the supernatant containing histones into a fresh 1.5 mL tube.
11. Add TCA drop by drop to the histone solution and invert the tube several times to mix the solution (final concentration of TCA is 33%). Incubate on ice for 30 minutes.
12. Pellet histones by spinning in cooled tabletop centrifuge at16000g for 10minutes at 4°C. Carefully remove the supernatant.
13. Wash histone pellet with ice-cold acetone without disturbing and spin at 16000g for 5 minutes at 4°C. Carefully remove the supernatant.
14. Repeat step 13. Air-dry histone pellet for 10 minutes at RT.