Lysines Acetylome Profiling of H3 and H4 Histones in TSA-Treated Stem Cells

P-01.4-32

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The DNA in eukaryotic cells is packed in chromatin with nucleosomes as basic unit. Nucleosomes are composed by octamer of four histones, and the global chromatin structure is altered by histone post-translational covalent modifications. Several types of histone modifications are well known such as acetylation, methylation, phosphorylation, and ubiquitination that play a role in the regulation of transcription activity. Histone modifications dysregulation as well as disruption of chromatin remodeling machinery play a fundamental role in many pathologies and cellular mechanisms. The analysis of histone modifications with standard mass mapping procedures is complicated by the highest occurrence of basic residues mainly in the regions interested by the modifications (methylation and acetylation). We developed a methodology based on limited proteolysis coupled to MALDI-MS to achieve a complete sequence coverage; then by LC-MS/MS and ion extract procedures, we got a relative quantification of the modification.
Once optimized the procedure on standard chicken core histones, we investigated the effects of TSA on H3 and H4 lysine acetylome in mice embryonic stem cells (ES14), treated with trichostatin A (TSA) by using the new, untargeted approach, consisting of trypsin-limited proteolysis experiments coupled with MALDI-MS and LC-MS/MS analyses. The proposed strategy was found in its simplicity to be extremely effective in achieving the identification and relative quantification of some of the most significant epigenetic modifications, such as lysines acetylation and methylation.