

## MS-based protein profiling and bottom-up approaches for the characterization of histone post-translational modifications: Application to the study of cellular senescence

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The repeating unit structure of chromatin is the nucleosome; it contains ~147 base pairs of DNA wrapped around a histone octamer, which comprises pairs of the core histones H2A, H2B, H3 and H4 and/or their variants. Canonical histones and histone variants are subjected to extensive post-translational modifications (PTMs) including mainly acetylation, mono-, di- and trimethylation of lysine residues. These PTMs are known to play key roles in nearly all cellular processes that involve DNA.

In this study, we report a fast, efficient, highly reproducible, and easily automated method involving ultra-high performance liquid chromatography (U-HPLC) coupled to a high resolution/high mass accuracy LTQ-Orbitrap mass spectrometer to profile core histone modifications/variants from WI-38 primary human fibroblasts. The whole analysis was performed on intact unfractionated histones within 19 min, which is ~3 fold faster than previously published procedures. High mass accuracy measurements combined with top-down tandem mass spectrometry (MS) experiments enable accurate histone identification. Experimental and biological variations were thoroughly assessed. With a sample preparation reduced to the minimum, characterization of the most abundant histones can be achieved in a single experiment. Semi-quantitative information can be obtained with respect to the relative abundances of the detected isoforms through a label-free approach. Isoform identities and relative distributions were further confirmed by bottom-up MS after thorough optimization and validation. Noteworthy, this approach comprises a chemical derivatization step (i.e. propionylation) prior to trypsin digestion to generate reproducible and MS-friendly Arg-C-type peptides. These MS-based approaches were shown to be complementary, bottom-up MS is perfectly suited to obtain site-specific information while intact protein MS approach is more relevant to decipher combinatorial patterns. Overall, our U-HPLC-MS approach for histone profiling offers a sensitive and reproducible tool that will be of great value for exploring PTMs and variants, and can readily be applied to clinical or pharmaceutical studies.

This MS work-flow has been applied to diverse biological issues. First of all, quantitative evidence of distinct PTM profiles between histone H3 variants from WI-38 primary human fibroblasts was obtained. Histones H3.1 and H3.2 were shown to carry comparable PTM relative abundances, while H3.3 appeared to be enriched in marks associated with transcriptional activation. More importantly, we assessed the impact of oncogene-induced cellular senescence on histone PTMs, and a senescent-specific modification was identified and will be presented.