Quantification of lysine acetylation

(Cotter Lab, 2006)

A method to determine the percent acetylation at any lysine residue in histones (or other acetylated proteins) by quantitatively acetylating a protein preparation with deuterated acetic anhydride. Any non-acetylated sites will be converted to deuteroacetylated sites, where the mass of the acetyl group in these molecules will be 3 Da higher than those acetylated in-vivo.

1. Deuterated (d_4) acetic acid, deuterated (d_6) acetic anhydride, ammonium bicarbonate (AmBic), trifluoroacetic acid (TFA), and a Trypsin Profile IGD Kit for in-gel digests are from Sigma (St. Louis, MO).

2. Purify histones from yeast cells (Poveda et al., 2004) and fractionate in SDS-15% polyacrylamide gels.

3. Excise Coomassie-stained histone H3 bands from the gel, cut into 1mm pieces, wash with 50% methanol and dry in a SpeedVac. Destain the gel pieces following the protocol included in the IGD kit.

4. Incubate at room temperature for 5 hours in a solution of deuterated acetic acid containing 15% deuterated acetic anhydride.

5. Remove the acetylation solution and cover the gel pieces with 1M AmBic solution (as much as needed to bring the final pH to approximately 8) and leave at room temperature for 20 minutes. Remove the AmBic solution and dry the gel pieces in a Speed-vac for 15 minutes.

6. Carry out trypsin digestion and peptide extraction overnight following the IGD kit protocol. (Note: assuming complete lysine acetylation of histone with acetic anhydride, trypsin will cleave only at arginine residues).

7. Obtain mass spectrum directly from the tryptic digest or following fractionation of peptides by reversed-phase HPLC.

8. The relative abundance of the acetylated and d3-acetylated (naturally unacetylated) species is determined quantitatively based on the surface areas under the monoisotopic peaks of both species (Yao et al., 2001; Yao et al., 2004; and Celic et al. 2005 Supplemental Figure S4).

References Cited

Poveda, A., Pamblanco, M., Tafrov, S., Tordera, V., Sternglanz, R. and Sendra, R. (2004) Hif1 is a component of a yeast histone acetyltransferase B, a complex mainly localized in the nucleus, J. Biol. Chem. 279, 16033-16043

Yao, X., Freas, A., Ramirez, J., Demirev, P.A., and Fenselau, C. (2001). Proteolytic ^{18}O labeling for comparative proteomics: model studies with two serotypes of adenovirus. Anal. Chem. *73*, 2836-2842.

Yao, X., Freas, A., Ramirez, J., Demirev, P.A., and Fenselau, C. (2004). Proteolytic ^{18}O labeling for comparative proteomics: model studies with two serotypes of adenovirus - Correction. Anal. Chem. *76*, 2675.

Celic, I., Masumoto, H., Griffith, W.P., Meluh, P., Cotter, R.J., Boeke, J.D. and Verreault, A., (2006) The sirtuins Hst3 and Hst4p preserve genome integrity by controlling histone H3 lysine 56 deacetylation, Current Biology 16, 1280-1289.