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Histone extraction protocol from the two model diatoms *Phaeodactylum tricornutum* and *Thalassiosira pseudonana*

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ABSTRACT

Post-translational modifications of histones affect many biological processes by influencing higher order chromatin structure that affects gene and genome regulation. It is therefore important to develop methods for extracting histones while maintaining their native post-translational modifications. While histone extraction protocols have been developed in multicellular and single celled organisms such as yeast and Arabidopsis, they are inefficient in diatoms that have a silica cell wall that is likely to hinder histone extraction. We report in this work a rapid and reliable method for extraction of large amounts of high quality histones from the two model diatoms *Phaeodactylum tricornutum* and *Thalassiosira pseudonana*. The protocol is an important enabling step permitting downstream applications such as western blotting and mass spectrometry.

1. Introduction

Post-translational modifications (PTMs) on the N-terminal tails of histones play important roles in many biological processes, e.g., by regulating chromatin dynamics and transcription. Combinatorial PTMs of histones give rise to the histone code which defines active or repressed chromatin states which in turn regulate gene expression. Chromatin, the site where all these modifications take place and are regulated, is composed of nucleosomes in which 146 bp DNA is wrapped around an octamer composed of two copies of each of the four core histones, H2A, H2B, H3 and H4. Nucleosomes are linked to each other with the linker histone H1 which is important for nucleosome stability and higher order chromatin structures.

The protruding tails of histones are subject to several covalent modifications that include methylation, acetylation, phosphorylation, ubiquitination, sumoylation, and adenosine-diphosphate ribosylation. The availability of fast, reliable and cheap methods for histone extraction that preserve native PTMs of histones is fundamental for understanding and deciphering the dynamics and complexity of the histone code. Protocols for histone extraction have been developed for many organisms ranging from yeast to human. However, very few protocols are known for eukaryotic photosynthetic single celled algae, with the exception of *Chlamydomonas reinhardtii* (Morris et al., 1990). We report in the present work a simple and rapid protocol for the isolation and purification of histones in two model eukaryotic diatoms *Phaeodactylum tricornutum* and *Thalassiosira pseudonana*.

Diatoms (Bacillariophyceae) are believed to be the most important group of eukaryotic phytoplankton (Bowler et al., 2010). They account for more than 20% of global carbon fixation and 40% of marine primary productivity. Thus, they have a crucial role in the food web and the biological pump that draws down atmospheric carbon dioxide to the ocean interior. With more than 100,000 species estimated, they are likely to be one of the most diverse groups of photosynthetic organisms on Earth (Round et al., 1992). Besides their biogeochemical importance, diatoms are also attracting interest for the nanotechnology, pharmaceutical and biofuel industries (Dismukes et al., 2008; Kroger and Poulsen, 2008).

P. tricornutum is a marine diatom whose genome has been fully sequenced (Bowler et al., 2008). It is widely used as a model for pennate diatoms because of its small genome (27 Mb), short life cycle, ease of transformation and growth in laboratory conditions (De Riso et al., 2009), availability of molecular tools for functional genomic characterization (Siaut et al., 2007; Maheswari et al., 2010), and genetic resources such as 12 ecotypes collected worldwide (De Martino et al., 2007), Bowler, unpublished). *P. tricornutum* is also an emerging model for epigenetic studies in single celled eukaryotes because it has all the components of the epigenetic machinery such as DNA methylation (Veluchamy et al., 2013), histones that can be modified (Lin et al., 2012), Veluchamy et al., in preparation), and small RNA-mediated regulation (De Riso et al., 2009; Huang et al., 2011), Angela Falciatore, personal communication). Likewise, *T. pseudonana* is a model diatom for centric diatoms, and

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for which a fully sequenced genome and molecular tools are also available (Armbrust et al., 2004; Poulsen and Kroger, 2005; Nicole Poulsen and Kröger, 2006.

Histones, their variants and modifications play an important role in transcriptional regulation but also in higher order chromatin structure. Characterization of native histone PTMs by mass spectrometry is a useful technology to survey PTMs prior to the mapping of modifications of interest in the genome by chromatin immunoprecipitation followed by high throughput sequencing (ChIP-seq). A prerequisite for this is to obtain native purified histones. The majority of protocols published so far use an acid or high salt (NaCl) extraction, which seems to work for most species (Shechter et al., 2007; Jufvas et al., 2011). However, both types of extraction failed in *P. tricornutum* (data not shown). We therefore adapted a protocol previously described for histone extraction from *Chlamydomonas reinhardtii*, where it was also reported that acid and salt extraction did not succeed in extracting histones (Morris et al., 1990). Our work describes a protocol for extracting core histones of good quality and pure enough to be used for western blot and mass spectrometry without the need to be purified over a reversed phase HPLC column.

2. Results and discussion

The protocol comprises a step of nuclei extraction before salt extraction of histones, acid precipitation followed by precipitation of histones with trichloroacetic acid (TCA) and several washes for purification of histones, which are then air dried and resuspended for gel visualization and mass spectrometry (Fig. 1). Cultures of *P. tricornutum* Bohlin clone Pt1 8.6 (CCMP2561) and *Thalassiosira pseudonana* (CCMP1335) were grown in artificial seawater at 19 °C under cool white fluorescent lights at approximately 75 μmol m^{-2} s^{-1} in 12 h light:12 h dark conditions and maintained in exponential phase in semi-continuous batch cultures. Cells were harvested at approximately 1.4×10^6 cells per ml by centrifugation at 4000 rpm for 20 min. Cell pellets can be stored at −80 °C for several months. Nuclei were then extracted as previously described (Lin et al., 2012). Pellets of nuclei can be stored at −20 °C for several weeks.

Histones were extracted from isolated nuclei with extraction buffer containing 1 M CaCl2, 20 mM Tris HCl pH 7.4 and 1 mini tablet of proteinase inhibitors cocktail (Complete protease cocktail Inhibitor (Roche cat. No. 11873 580 001)). High salt extraction disrupts DNA–histone interactions and precipitates contaminants such as nucleic acids and non-histone proteins while core histones remain soluble in the supernatant and thus can be enriched and purified. Samples were kept on ice for 10 min. HCl was then added to 0.3 N and samples were centrifuged at 4 °C at 10000 rpm for 5 min to precipitate the acid-insoluble fraction. TCA was added to 20% to the supernatant, which contains the acid soluble proteins. To precipitate histones, TCA was added drop wise to the sample kept on ice. Samples were kept on ice for a further 10 min and then centrifuged for 30 min at 13000 rpm at 4 °C to precipitate histones. We added in our protocol an extra centrifugation step

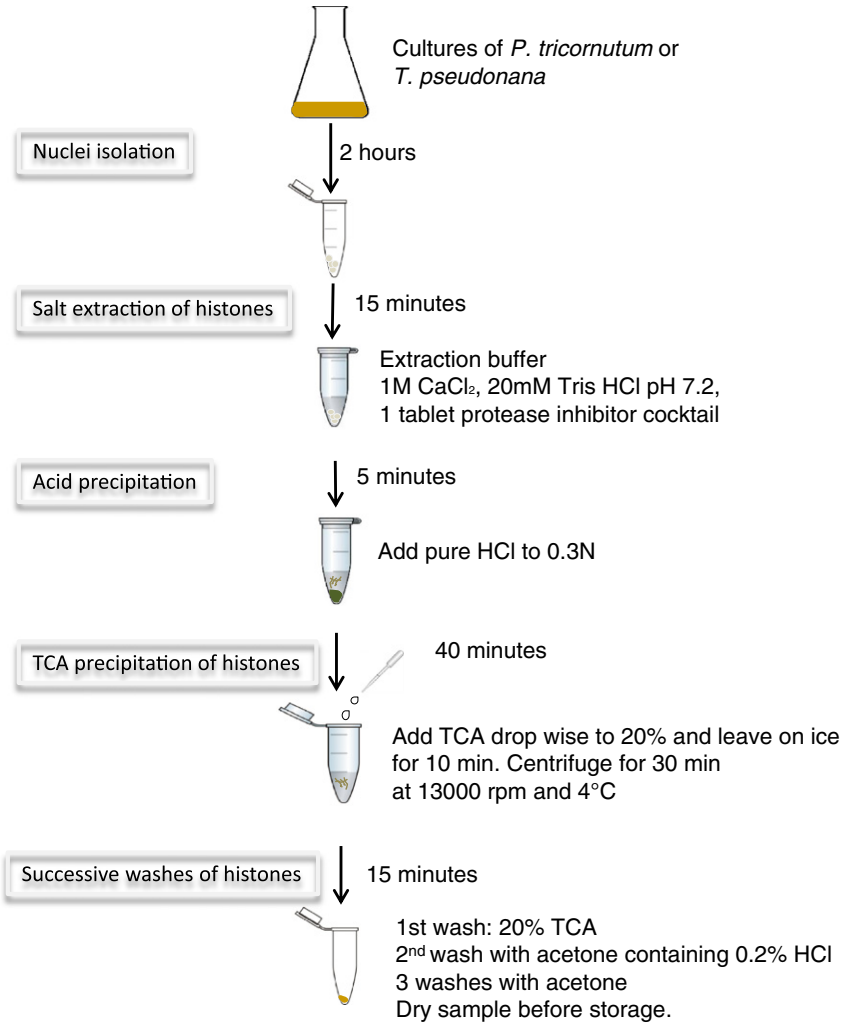

Fig. 1. Outline of the different steps of the histone extraction protocol. After centrifugation of diatom cultures, pellets can be stored at −80 °C for several months. Isolated nuclei can be stored at −20 °C for several weeks. Likewise, once histones are isolated, they can be stored at −20 °C for several weeks.