One-STrEP Application Note

One-STrEP-tag for protein:protein-interaction analysis

Groth et al., 2007 and Jasencakova et al., 2010

Protein:protein-interactions (PPI) govern almost all important processes in living organisms. Thus, their rapid and accurate determination and investigation is a major challenge in life sciences. The extremely efficient and fast One-STrEP system is suitable for the isolation of functional protein complexes and subsequent mass spectrometry analysis leads to the identification of protein complex components (Junttila et al., 2005) as it is shown in the following studies.

Groth et al., 2007, analysed DNA replication in eukaryotes. In this context they isolated and characterized *in vivo* a complex in which the human histone chaperone Asf1 and MCM2-7, the putative replicase helicase, are connected through a histone H3-H4 bridge. One-STrEP-tagged Asf1

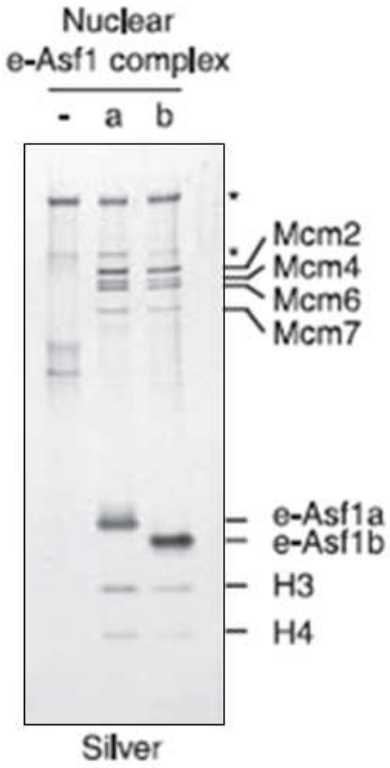

Figure 1: Purification analysis of a human Asf1-(H3-H4)-MCM2-7 complex by silver staining. Control extracts without e-Asf1 (-) was included to identify unspecific proteins (asterisks).

*From **`Regulation of Replication Fork Progression Through Histone Supply and Demand´.** A. Groth, A. Corpet, A. J. L. Cook, D. Roche, J. Bartek, J. Lukas, and G. Almouzni. Science 21 Dec. 2007: 318 (5858), 1928-1931. Reprinted with permission from AAAS.*

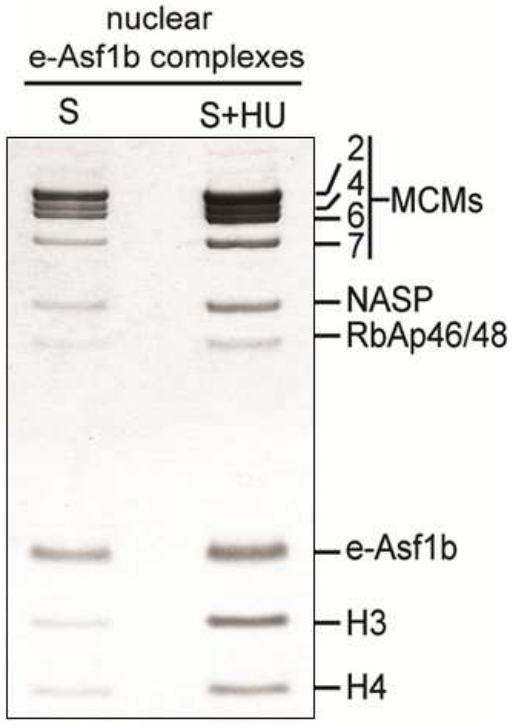

Figure 2: Coomassie staining of e-Asf1b complexes. Cells were harvested in mid-S phase (S) or after 1.5 h hydroxyurea treatment (S+HU) for complex purification. Mass spectrometry identified the indicated proteins. (*Kindly provided by Zuzana Jasencakova, Biotech Research and Innovation Centre, University of Copenhagen*)

(e-Asf1) was expressed in asynchronous HeLa S3 cells and the nuclear extract was used for the purification of the e-Asf1 complex via *Strep*-Tactin® Superflow®. Mass spectrometry and Western blotting revealed the presence of Mcm2, 4, 6, and 7 in the nuclear e-Asf1 (a and b) complexes, together with histone H3 and H4 (Figure 1). In a second study Jasencakova et al., 2010, isolated e-Asf1 complexes from HeLa S3 cells synchronized in mid-S phase before and after treatment with the replication inhibitor hydroxyurea. In contrast to Groth et al., 2007 they used larger amounts of starting material and modified the washing conditions during the purification process. Applying this approach, they got more interactors and were not only able to identify MCM2-7, H3 and H4 as e-Asf1 complex components but also the additional interacting chaperones NASP and RbAp46/48 in the nuclear extract (Figure 2). Analysis by size-exclusion chromatography revealed that chromatin-associated Asf1 (a and b) are part of two separate nuclear complexes, a larger complex with MCM6 and a smaller one containing NASP.

Due to its high efficiency and specificity the One-STrEP system is a reliable tool to isolate protein complexes in a simple and short one-step purification procedure. In combination with mass spectrometry it allows the identification of new protein interaction partners. Supplemental analysis could provide the facility to identify different complexes of one and the same bait protein.

"We chose the One-STrEP-tag for the following reasons:

The One-STrEP system provides a reliable, efficient and **rapid one-step purification** method useful for **isolation of protein complexes** from stable human cell lines. It is compatible with high stringency washing to obtain complexes of **high purity** and it is reasonably **low cost** compared to other systems."

Anja Groth, Biotech Research and Innovation Centre, University of Copenhagen

References:

1. Groth A, Corpet A, Cook AJL, Roche D, Bartek J, Lukas J, Almouzni G, 2007: Science 318: 1928-1931. Regulation of replication fork progression through histone supply and demand. Abstract
2. Jasencakova Z, Scharf AND, Ask K, Corpet A, Imhof A, Almouzni G, Groth A, 2010: Mol Cell 37:736-743. Replication stress interferes with histone recycling and predeposition marking of new histones.
3. Junttila MR, Saarinen S, Schmidt T, Kast J, Westermarck J, 2005: Proteomics 5: 1199-1203. Single-step Strep-tag® purification for the isolation and identification of protein complexes from mammalian cells.