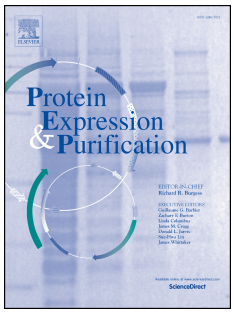
Accepted Manuscript



A dual affinity-tag strategy for the expression and purification of human linker histone H1.4 in *Escherichia coli*

Daniel P. Ryan, David J. Tremethick

PII: S1046-5928(15)30132-7

DOI: 10.1016/j.pep.2015.12.017

Reference: YPREP 4857

To appear in: *Protein Expression and Purification*

Received Date: 4 December 2015

Accepted Date: 24 December 2015

Please cite this article as: D.P. Ryan, D.J. Tremethick, A dual affinity-tag strategy for the expression and purification of human linker histone H1.4 in *Escherichia coli*, *Protein Expression and Purification* (2016), doi: 10.1016/j.pep.2015.12.017.

A dual affinity-tag strategy for the expression and purification of human linker histone H1.4 in *Escherichia coli*

Daniel P. Ryan[a*] and David J. Tremethick[a]

[*]Indicates corresponding author

Author affiliations:
(a) Department of Genome Sciences, The John Curtin School of Medical Research, Building 131, Garran Road, The Australian National University, Canberra, ACT, 2601

Corresponding Author:
Daniel P. Ryan, daniel.ryan@anu.edu.au, Ph: +61 2 6125 2549, Fax: +61 2 6125 2499

Abstract

Linker histones are an abundant and critical component of the eukaryotic chromatin landscape. They play key roles in regulating the higher order structure of chromatin and many genetic processes. Higher eukaryotes possess a number of different linker histone subtypes and new data are consistently emerging that indicate these subtypes are functionally distinct. We were interested in studying one of the most abundant human linker histone subtypes, H1.4. We have produced recombinant full-length H1.4 in *Escherichia coli*. An N-terminal Glutathione-S-Transferase tag was used to promote soluble expression and was combined with a C-terminal hexahistidine tag to facilitate a simple non-denaturing two-step affinity chromatography procedure that results in highly pure full-length H1.4. The purified H1.4 was shown to be functional via *in vitro* chromatin assembly experiments and remains active after extended storage at −80 ℃.

Keywords

Linker histones; chromatin; nucleosomes; affinity purification; recombinant protein; rhamnose

Highlights

- Overexpression of human linker histone H1.4 in *Escherichia coli* using a rhamnose-inducible vector
- A simple purification strategy for the full-length protein using tandem GSH- and Ni^{2+}-affinity columns
- Purified recombinant H1.4 readily incorporates into *in vitro*-assembled chromatin
- Recombinant H1.4 is stable and remains active after long periods of storage at −80 ℃

Abbreviations

Glutathione-S-Transferase (GST); Glutathione (GSH); hexahistidine (6xHis); Human rhinovirus 3C (HRV-3C); immobilised metal affinity chromatography (IMAC); Fast protein liquid chromatography (FPLC); polyacrylamide gel electrophoresis (PAGE); sodium dodecyl sulphate PAGE (SDS-PAGE); amino acid (aa); Tris-Borate-EDTA (TBE); base pair (bp); Molecular weight (MW); molecular weight cut-off (MWCO); kanamycin (kan); chloramphenicol (cml)