ABSTRACT

TITLE:	Creating Nucleosomes In Vitro: Large-Scale Expression of Histone H3
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ABSTRACT: To explore molecular interactions of histones in a nucleosome, an intact nucleosome must be recreated in vitro. To do so each histone—H1, H2, H3 and H4—must be overexpressed and purified, refolded and then bound to a piece of DNA in a test tube. This experiment focused on the large-scale expression of histone H3 in E. coli followed by separation of the H3 histone away from other proteins. A plasmid encoding a Xenopus (frog) H3 gene was transformed into competent BDP cells followed by induction with IPTG. Cells were then lysed and proteins were separated from other cellular material by inclusion body prep. Remaining proteins were separated by cation exchange and molecular weight filtration. Gel data (using molecular weight markers) suggests that H3 was over-expressed; however, after cation and size separation, the final gel shows little protein present in any sample including the initial pellet. These results indicate possible degradation due to the presence of proteases.