Abstract

Eukaryotic genomes are packaged into chromatin, a dynamic nucleoprotein complex in which nucleosomal units are connected by stretches of linker DNA. Its length is variable, quantized and highly controlled. Spacing strongly influences higher-order chromatin folding, accessibility, DNA integrity and potentially the recruitment of chromatin-associated factors. However, the precise biological reasons why eukaryotes set specific spacing patterns remain largely unknown. To study the impact of internucleosomal spacing on protein interactions in a controlled biochemical setting, a library of DNA constructs was generated based on the Widom 601 nucleosome positioning sequence, with defined linker lengths and compatible cloning sites. The constructs were designed to allow easy multiplication of repeats, enabling the assembly of longer arrays. DNA fragments were biotinylated for immobilization and reconstituted with recombinant histone octamers to yield variably spaced nucleosomes. As a proof of principle, dinucleosomal arrays were immobilized on streptavidin-coated magnetic beads and incubated with nuclear extracts in a pulldown experiment. Captured proteins were analyzed, revealing preliminary insight on protein binding patterns and linker-dependent differences. These experiments establish the feasibility of using this system for quantitative proteomic analysis to map spacing-dependent chromatin interactors. The modular design of the DNA library and labeling strategy makes the system applicable to a range of biochemical and structural methods, biolayer interferometry and cryo-EM. This system provides a versatile tool for dissecting the basis of chromatin-protein interactions with control over the spacing parameter.