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Characterization of the Saturation Level of Nucleosome Arrays and Plasmids by MNase Digestion

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Studying processes involving DNA such as DNA replication and transcription is best done in the context of chromatin because the structure of DNA influences the efficiency of such processes. Chromatin structure is derived from DNA wrapping around histone octamers with the histone octamer density termed saturation level. For in vitro studies on DNA replication and transcription, it is important to maintain a constant saturation level so that saturation level can be removed as a variable. Our goal is to characterize the saturation level of plasmids using the rate of MNase digestion. MNase digestions were first performed on reconstituted nucleosome arrays with known saturation levels. Nucleosome array saturation levels were determined through restriction sites between each nucleosome array. The array is digested at the restriction sites into individual nucleosomes and unoccupied nucleosome sites show as a low band on a polyacrylamide gel. A gel image analysis tool is used to characterize MNase digestion pattern of these arrays and relate it to the saturation level of array. We have shown that lower octamer:DNA ratios of reconstituted arrays digested faster and have correlated their digestion patterns to saturation level. This correlation can be used to maintain a constant saturation level on reconstituted plasmids. Additionally, MNase digestion patterns were used to show the histone H3 and H4 tail effects on chromatin structure through MNase accessibility.