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## **MASS SPECTROMETRIC ANALYSIS OF DNA-BOUND PROTEINS FROM A COMPLEX MATRIX**

The focus of the Wisconsin CEGS is to develop a general technology capable of identifying proteins bound to any specific region of the genome. In contrast to Chromatin Immunoprecipitation methods (ChIP-Chip or ChIP-Seq), the technology being developed by this CEGS requires neither an antibody nor any prior knowledge of proteins binding to the region of interest for isolation of the desired region. Rather, it uses sequence specific capture to isolate locus with its constitutively bound proteins prior to mass-spectrometric (MS) analysis. We tested our approach, which we refer to as GENECAPP, for Global ExoNuclease-based Enrichment of Chromatin-Associated Proteins for Proteomics, using two model systems (the IGFBP1 promoter region in the mouse, and the Gal/<sub>UAS</sub> system in yeast). For the IGFBP1 region, a synthesized 180 bp PCR product containing binding sites of the transcription factor FoxO1 was used. As a negative control, a second 180 bp PCR product with two point mutations that occur within the binding site of FoxO1 was synthesized, thus disrupting the binding ability of the protein to the DNA. Using an LTQ Orbitrap Velos, our results indicate that the wild-type DNA sequence with bound FoxO1 had 12 peptides with 26 scans while the mutant-type DNA sequence with bound FoxO1 which had 1 peptide with 1 scan. This relates to a total protein coverage of 35% with the wild-type DNA sequence vs. only 2% with the mutant-type DNA sequence. We have also been able to detect as little as 100 fmol of FoxO1 protein that was captured and eluted from solid support surfaces.

In our analysis of the Gal/<sub>UAS</sub> yeast system, we utilized the same approach to identify Gal4 protein and other yeast proteins binding to a PCR amplicon spanning the Gal4 binding sites. Experiments were performed in aqueous buffer as well as in a complex matrix, such as yeast lysate. In all experiments, Gal4 protein was successfully identified along with other yeast proteins that are bound to the PCR amplicon.

Overall, these results confirm that DNA binding proteins can be successfully identified with our approach using solid support surfaces, oligonucleotides, and LC-MS/MS analysis. Our methodology also showcases the power and capability of high resolution mass spectrometers to identify DNA-bound proteins from a complex matrix, such as cell lysate.