

A Proteomics Approach to Reversing the Chromatin Immunoprecipitation Methodology.

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Abstract

The focus of the Wisconsin Center of Excellence in Genomics Science (CEGS) is to develop a general technology capable of identifying proteins bound to any specific region of the genome. In contrast to ChIP-Chip (ChIP-Seq), the technology being developed by this CEGS requires neither an antibody nor any prior knowledge about proteins binding to the region of interest for isolation of the desired region. Instead, this technology uses sequence-specific capture to isolate a locus with its constitutively bound proteins prior to mass-spectrometric (MS) analysis.

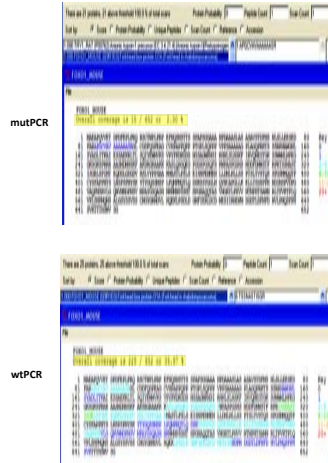
Using two model systems (the IGFBP1 promoter region in the mouse, and the UAS_{Gal} system in yeast), we explored the ability of tandem mass spectrometry to identify specific proteins bound to a fragment of DNA that was captured on solid support using complementary oligonucleotides. The solid support used included both, gold-coated microscope slides and streptavidin-coated beads, with the goal of capturing a DNA fragment in a sequence-specific manner.

For the IGFBP1 promoter region, we synthesized a 180 bp PCR product that contained the binding sites for the transcription factor FoxO1. As a negative control, we synthesized a similar 180 bp PCR product that contained two point mutations within the binding site of FoxO1, thus disrupting the binding ability of the protein to the DNA. FoxO1 was bound to the DNA sequence in solution, the DNA-protein complex was captured, and the bound proteins were identified and quantified using mass spectrometry.

In our analysis of the UAS_{Gal} yeast system, we used a similar approach to identify Gal4p and other yeast proteins binding to a PCR amplicon spanning the Gal4 binding sites in this interval. Protein binding and capture were in buffer as well as in the presence of yeast whole cell lysate.

In addition to demonstrating the feasibility of this approach using the two different systems, we also assessed the limit of detection for this analysis to determine the amount of protein needed for successful mass spectral identification. Here, we compared the sensitivity of two separate LC-MS/MS systems.

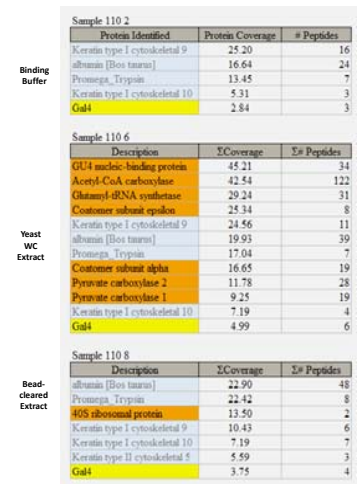
Analysis of FoxO1 bound to the IGFBP1 region



FoxO1 protein captured using mutant PCR vs. wild-type PCR Experiment run on LTQ XL (Thermo)

Comparison of protein coverage (%) of mutPCR vs. wtPCR.

Analysis of Captured Gal4p



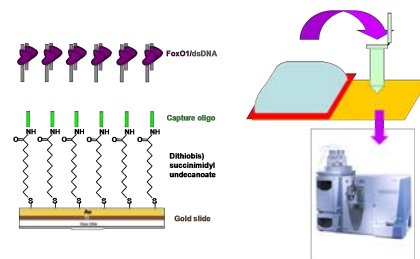
Mass spectrometry analysis of eluted DNA:Gal4p complexes under three different conditions using the LTQ Velos-Orbitrap discovery method. Notice other DNA-bound proteins identified using this method (orange).

Sequence-Specific DNA Capture

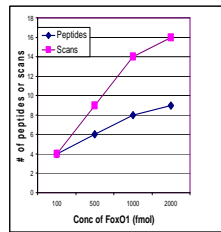
Capture on streptavidin beads
Amino-terminated capture oligonucleotides complementary to the capture sequence of interest were attached to streptavidin-coated beads. Amino-terminated oligonucleotides were used as a negative control. Hybridization was tested with complementary Fam-labeled oligonucleotides.



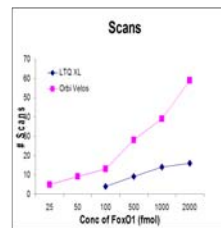
Capture on gold-coated slides
Amino-terminated capture oligonucleotides were attached to gold surfaces using linker chemistry. PCR products with FoxO1 bound to the sequence of interest were treated with Exonuclease to generate complementary single-stranded ends. The resulting products were hybridized to the surface, washed, and the captured DNA-protein complex was digested on-chip with trypsin for MS analysis.



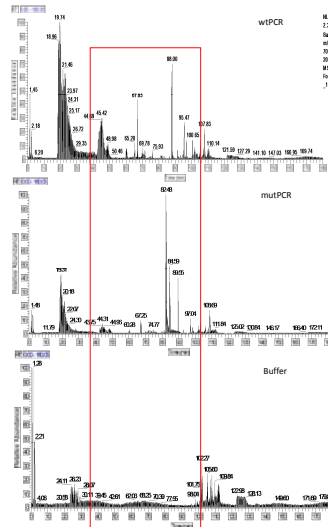
Sensitivity of FoxO1 Detection



MS analysis of varying FoxO1 concentrations using an LTQ XL mass spectrometer



Comparison of scan counts for FoxO1 analysis using an LTQ XL vs. LTQ Velos-Orbitrap mass spectrometer



Base Peak Chromatogram comparison for FoxO1 peptides run on the LTQ XL. Area highlighted in Red shows differences in eluted peptides.

Conclusions

- Our novel technology allows the mass spectral detection of proteins using our model systems (mouse IGFBP1 promoter region and yeast GAL_{UAS} region).
- Using the LTQ Orbitrap Velos, we are able to identify less than 100 fmol of captured and eluted protein from a solid surface.
- DNA-protein complexes can be captured in a sequence-specific manner using beads or gold surfaces as solid support.
- Both proteins (FoxO1 and Gal4p) can be successfully detected, even in the presence of proteins from whole cell lysate.

Acknowledgements

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