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T^3 transcriptomics

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We will discuss a new method for analyzing transcription factors and other proteins involved in transcription using MALDI-TOF mass spectrometry. MALDI plates coated with polyvinylpyrrolidone do not bind protein but avidly bind DNA and DNA-protein complexes.The DNA sequence determines which proteins bind. When DNA, either oligonucleotides or longer DNAs such as core promoter sequences is mixed with nuclear extract and spotted on the plate, DNA-protein complexes are bound while other proteins can be simply washed away. The samples are then digested with trypsin and matrix is added on the plate and the plate is then ready for mass spectrometry analysis. Highest signal-to-noise is obtained with core promoters (in this case a 300 bp human telomerase (hTERT) promoter) than with 20-mer duplex oligonucleotides but usable data is obtained with either. Using overlapping oligonucleotides representing the entire hTERT promoter binding sites for TCF23 (an E-box binding protein), SP1 and AP2 were all localized to specific sequence regions. When the intact promoter is used, SP1 and AP2 are also found but instead of TCF23, USF2 is found. This suggests that USF2 binds to the E-boxes in the promoter context. Other components of the RNA polymerase 2 transcription complex such as TF2H were also characterized. The method allows as many as 384 samples to be analyzed on a single plate in a matter of three days and thus the method is capable of high-throughput analysis of DNA-binding proteins.

Biography

Harry W Jarrett received his PhD from the University North Carolina-Chapel Hill in 1976. After Postdoctoral fellowships at the Mayo Clinic, UCSD, and U.GA, he began an Assistant Professorship at IN U-Purdue U at Indianapolis. After promotion he joined the faculty at the U. TN Health Science Center where he was promoted to Professor and remained for 18 years. In 2006, he joined the faculty at U. TX San Antonio as the Lutcher Brown Distinguished Professor of Biochemistry where he remains. His research has involved the purification of transcription factors and the RNA pol 2 transcription complex. His discoveries have included coupling methods which led to the first DNA-silica HPLC columns and developed both oligonucleotide trapping and promoter trapping to purify transcription proteins for mass spectrometry characterization. Most recently, this research has involved coating MALDI plates to direct proteomic discovery to DNA-binding proteins for a new method called T3.

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