Development of Heterogeneous and Homogeneous Platforms for Rapid Analysis of DNA-Protein Interactions
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Abstract
Two plate-based assay platforms have been developed for rapid analysis of DNA-protein interactions. The first is a heterogeneous, ELISA-based platform in which a biotinylated dsDNA molecule is fixed to a streptavidin-coated plate. Cell-gate purified, protein or nuclear extract is incubated in the wells. The DNA-protein complex is then detected with target specific antibodies conjugated to subtilisin. This protocol results, based on a series of experiments, in the determination of DNA and protein concentration and in the analysis of consensus protein binding sequences and for analysis of each component protein complex assembly. The second is a homogeneous platform that utilizes fluorescence polarization to detect the binding of labeled DNA and target protein. The assay is highly sensitive, detecting single base pair variations within the binding sequence and target protein. Data from several model systems demonstrate that the assay is highly sensitive with easily measurable and repeatable signal strengths within the binding region. The platform is adaptable to 96 and 384-well formats, making it an ideal tool for research and high throughput drug screening applications.

Introduction
Assays that identify and analyze DNA-protein interactions are useful tools for studying cell functions such as gene expression, DNA repair and RNA packaging. These assays can also be used to determine the effects of drugs, antibodies and other factors that influence protein-DNA interactions. Standard methods for studying these interactions include the use of mobility shift assays. However, these assays can be time and labor intensive and often require the use of radioactive isotopes. The plate-based assay formats described here are versatile, sensitive, non-radioactive, and fast.

Results
Overview of Assay Platforms
A) Biotinylated DNA
B) Unlabeled competitor
C) Biotinylated DNA
D) Unlabeled competitor

Figure 1. Oligonucleotide Design for Four Model Systems. Cleotides were synthesized (Sigma-Genosys, Woodlands, TX) for the 1) human-antitrypsin promoter, and 4) human E-selectin promoter were cloned into Director-Ready pFLAG-CMV2 and transfected into cos-7 cells. Expressed FLAG-creb-1 was captured from lysate onto anti-FLAG-M2 agarose beads.

Figure 2. Fluorescein-c-Jun-mutant (negative control)
A) Fluorescein-c-Jun
B) Fluorescein-c-Jun-Mutant (negative control)
C) Fluorescein-c-Fos-Mut
D) Fluorescein-c-Fos

Conclusions
The heterogeneous, ELISA-based assay platform provides rapid analysis of DNA-protein interactions. This platform is non-radioactive, fast, and sensitive, and can be completed in as little as 3 hours. The homogeneous assay platform is suitable for the analysis of multiple binding domains and proteins in a high-throughput fashion. The platform has been demonstrated using a series of protein-DNA interaction model systems and was shown to be sensitive to single base pair mutations within the binding region of both protein and DNA.

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References

Related Sigma Products

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