



A Laboratory Guide to In Vitro Studies of Protein-DNA Interactions (Biomethods)

Jost, Saluz

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A Safety Considerations Many techniques described here involve a number of hazards, such as high electrical current and voltage, radioactivity and highly toxic chemicals. It is absolutely essential that the instructions of equipment manufacturers be followed, and that particular attention be paid to the local and federal safety regulations. **B Introduction** The expression of prokaryotic and eukaryotic genes has been shown most often to be regulated at the level of mRNA synthesis. Thanks to the rapid development of methods for dissecting DNA sequences, cis-acting regulatory elements such as promoters and enhancers have been recognised. More recently, the widely expressed intuition that discrete sequences within these elements constitute binding sites for sequence-specific binding proteins has been confirmed, especially through the use of "footprinting" assays (for examples, Galas and Schmitz, 1978). This and similar assays have already resulted in the recognition, isolation and analysis of DNA-binding proteins for several genes. Excellent reviews exist of the structural studies on these transcription regulatory proteins and related DNA elements (for example, Glover, 1989 and Johnson and McKnight, 1989), to which the reader is referred for detailed information. To set the scene for applications of the techniques described in this volume, only the barest outline of previous studies is presented here. Protein-DNA interactions are dependent on very specific tertiary configurations of the binding protein which allow the closest contact with the DNA helix.

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