

METHODS IN MOLECULAR BIOLOGY™

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DNA Electrophoresis

Methods and Protocols

Edited by

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 **Humana Press**

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Preface

Since the discovery of structure of DNA by Watson and Crick, electrophoresis has been established as the most commonly used approach to analyze size, shape, and structure of DNA molecules. DNA molecules vary greatly in size, from a few nucleotides one can easily synthesize in vitro to long chains of millions of base pairs such as eukaryotic chromosomes. DNA comes in a variety of forms: it can be single stranded or double stranded, linear or circular, or supercoiled or relaxed; it can be bound, bent, or modified by proteins; it forms sophisticated branched structures during replication and recombination. DNA electrophoresis is a powerful tool that allows separating DNA molecules according to their size and shape.

Beginning with a historic overview on DNA electrophoresis and its principles as an approach, each of the follow-up chapters is devoted to a single protocol and consists of a brief introduction, a list of materials and reagents, a step-by-step protocol, and a list of notes presenting the author's unique know-hows to help readers successfully navigate through the protocol. Chapter 2 is aimed at early career researchers, to introduce them to the basics of the most commonly used DNA electrophoresis in agarose gels to analyze samples of linear DNA such as plasmid digests and PCR products. Chapters 3–7 are devoted to 2-dimensional gel electrophoresis which allows resolving more complex DNA molecules: branched replication and recombination intermediates and supercoiled plasmids. Chapters 8–11 describe DNA electrophoresis under conditions in which DNA molecules are completely or partially denatured during the runs, allowing either analysis of ssDNA based on its mobility or comparison of sequences of dsDNA molecules derived from their mobility change due to partial in-gel melting. Chapters 12 and 13 are dedicated to pulse field gel electrophoresis employed to analyze very large DNA molecules such as full-length eukaryotic chromosomes or bacterial chromosomes cut into a relatively small number of fragments. Single-cell DNA electrophoresis (comet assay) used to analyze DNA damage and repair is described in Chapter 15. Chapter 16 adds another level of resolution to this approach by coupling the electrophoresis to fluorescence in situ hybridization which allows following the dynamics of damage repair at a specific locus in a genome. Finally, methods for studying protein–DNA interactions using electrophoreses are presented in Chapters 16–19.

DNA Electrophoresis: Methods and Protocols is written by expert scientists with hands-on experience. I would like to thank all of them for sharing their invaluable experience with the scientific community by contributing to this book.

Edinburgh, UK

Svetlana Makovets

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