

Series Editor: *John M. Walker***PLANT VIROLOGY PROTOCOLS***From Virus Isolation to Transgenic Resistance*

Edited by

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and

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Plant Virology Protocols: From Virus Isolation to Transgenic Resistance offers for the first time a comprehensive collection of state-of-the-art techniques for generating transgenic plants that are resistant to plant viruses via the cloning and expression of the coat protein gene. Its unfailingly reproducible methods, perfected by hands-on masters, cover the entire process from virus isolation, RNA extraction, and cloning coat protein genes, to the introduction of the coat protein gene into the plant genome and the testing of transgenic plants for resistance. Methods for testing for transformation by PCR and Southern blotting, the detection of RNA transcripts by Northern blotting, and the production of protein by Western analysis are provided, as are methods for challenging the transgenic plants produced and for detecting and measuring the levels of virus. The authoritative contributors also discuss the history and mechanisms of coat protein-mediated protection and the key ethical issues surrounding transgenic technology.

Unprecedented in its comprehensiveness and its many time-proven methods, *Plant Virology Protocols: From Virus Isolation to Transgenic Resistance* is certain to become the authoritative standard reference for plant scientists who want to tap the enormous benefits this technology has to offer.

FEATURES

- Comprehensive collection of plant virus purification protocols
- State-of-the-art protocols for cloning coat protein genes
- Easily reproducible methods for plant transformation and primary analysis
- Up-to-date review of plant resistance and current opinions about its mechanisms
- Techniques equally applicable to other viral genes and nucleotide sequences
- Methods for generating and identifying cDNA clones representing the coat protein gene

CONTENTS

PART I. INTRODUCTION. Introduction to Plant Virology. Introduction to Classical Crossprotection. History of Coat Protein-Mediated Protection. **PART II. VIRUS ISOLATION, RNA EXTRACTION, AND LOCATION OF COAT PROTEIN GENES.** Geminivirus Isolation and DNA Extraction. Caulimovirus Isolation and DNA Extraction. Reovirus Isolation and RNA Extraction. Procedures for Plant Rhabdovirus Purification, Polyribosome Isolation, and Replicase Extraction. Hordeivirus Isolation and RNA Extraction. Furovirus Isolation and RNA Extraction. Tobravirus Isolation and RNA Extraction. Tobamovirus Isolation and RNA Extraction. Potexvirus Isolation and RNA Extraction. Carlavirus Isolation and RNA Extraction. Potyvirus Isolation and RNA Extraction. Trichovirus Isolation and RNA Extraction. Ilarvirus Isolation and RNA Extraction. Bromovirus Isolation and RNA Extraction. Cucumovirus Isolation and RNA Extraction. Nepovirus Isolation and RNA Extraction. Comovirus Isolation and RNA Extraction. Carmovirus Isolation and RNA Extraction. Tymovirus Isolation and Genomic RNA Extraction. Tombusvirus Isolation and RNA Extraction. Luteovirus Isolation and RNA Extraction. **PART III. CLONING OF COAT PROTEIN GENES.** RNA Analysis: Size and 3' End Group Determination. RNA Fractionation by Density Gradient Centrifugation. cDNA Library Construction for Lambda ZAP®-Based Vectors. PCR Cloning of Coat Protein Genes. Antibody Production. Expression Library Screening. In Vitro Transcription and Translation. Analysis of Coat Expression Cassettes in Protoplasts. DNA Sequencing. Computer Analysis of Amino Acid Sequences: *The Case of Plant Virus Capsid Proteins*. **PART IV. PLANT TRANSFORMATION AND PRIMARY ANALYSIS.**

Preparation of Coat Protein-Containing Binary Vectors for Use in *Agrobacterium*-Mediated Transformation. Potato Transformation. Transformation of Tomato. Tobacco Transformation. Genetic Transformation of Wheat. Production of Transgenic Rice. Molecular Analysis of Transgenic Rice. PCR Analysis of Transgenic Tobacco Plants. Southern Analysis of Transgenic Tobacco Plants. Detection and Quantification of Transcript RNA in Transgenic Plants Using Digoxigenin-Labeled cDNA Probes. Western Analysis of Transgenic Plants. **PART V. EVALUATION OF RESISTANCE.** Assaying Levels of Plant Virus by ELISA. Detection of Plant RNA Viruses by Nonisotopic Dot-Blot Hybridization. Detection and Quantification of Plant Viruses by PCR. Assaying Levels of Virus with Local Lesion Hosts. Field Testing Resistance of Transgenic Plants. Agronomic Performance of Transgenic Plants. **PART VI. MECHANISMS OF RESISTANCE.** Mechanisms of Resistance: *Expression of Coat Protein*. Mechanisms of RNA-Mediated Resistance to Plant Viruses. Detection of Risks Associated with Coat Protein Transgenics. Potential Benefits of the Transgenic Control of Plant Viruses in the United Kingdom. Index.

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