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# Preface

The aim of *Transgenic Plants: Methods and Protocols* is to provide a source of information to guide the reader through a wide range of frequently used, broadly applicable, and easily reproducible techniques involved in the generation of transgenic plants. Its step-by-step approach covers a series of methods for genetically transforming plant cells and tissues, and for recovering whole transgenic plants from them. The volume then moves on to the use of selectable and reporter markers, positive selection, marker elimination after recovery of transgenic plants, and the analysis of transgene integration, expression, and localization in the plant genome. Although contributors usually refer to model plants in most chapters, the protocols described herein should be widely applicable to many plant species. The last two sections are devoted to methods of risk assessment and to exploring the current and future applications of transgenic technology in agriculture and its social implications in a case study.

*Transgenic Plants: Methods and Protocols* is divided into six major sections plus an introduction, comprising 27 chapters. Part I, the Introduction, is a review of the past, present, and perspectives of the transgenic plants, from the discovery of *Agrobacterium tumefaciens* as a feasible transformation vector, to its use as a tool to study gene expression and function, and the current and possible future applications of this technology in agriculture, industry, and medicine. Part II covers the most commonly used transformation systems, including *Agrobacterium tumefaciens*, *Agrobacterium rhizogenes*, particle bombardment, electroporation, floral dip, and chloroplast transformation. Part III covers regeneration of whole transgenic plants by both organogenesis and somatic embryogenesis from different explant cells/tissues and from such diverse plant species as tomato, cassava, conifers, and citrus. Part IV covers the use of selectable and reporter markers, exemplified by the utilization of the *nptII* and *bar* genes for wheat transformation, and by  $\beta$ -glucuronidase (GUS) and green fluorescent protein (GFP) detection and quantification, respectively. Positive selection (for maize transformation) is also described as an alternative to the use of antibiotic and herbicide resistance genes as selectable markers. Also covered in this section is the controlled excision and removal of marker genes from both nuclei and plastids once transgenic shoots have been efficiently generated. Part V treats the study of transgene copy number and organization by quantitative real-time polymerase chain reaction (PCR), and the analysis of transgene expression by Northern and dot-blot hybridizations using nonradioactive probing methods, by reverse transcription (RT)-PCR, and by

RNA *in situ* hybridization. Also described is the use of matrix attachment regions (MARs) flanking the transgenes to obtain predictable and stable expression of the transgenic traits. Fluorescence *in situ* hybridization (FISH) is described as a method to map transgenes physically in specific plant chromosome regions. This section also covers the use of thermal asymmetric interlaced (TAIL)-PCR to amplify (and precisely determine by sequencing) genomic sequences flanking transgene insertions. Part VI covers risk assessment methods for studying *Agrobacterium* persistence in plant tissues and to investigate the possibility of transgene dispersal through pollen. Part VII provides an overview of the current and next generations of transgenic crops based not only in the most recent scientific literature, but also in patent applications. Social implications of the transgenic crops are exemplified by the development and impact of the virus-resistant transgenic papayas in Hawaii, Jamaica, and Venezuela.

*Transgenic Plants: Methods and Protocols* has been planned, written, and edited with the intention of being useful for those beginners and experienced scientists looking for a laboratory manual covering all aspects of plant genetic transformation. I greatly hope you will find it helpful.

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