Preface

Agrobacterium tumefaciens is a soil bacterium that for more than a century has been known as a pathogen causing the plant crown gall disease. Unlike many other pathogens, *Agrobacterium* has the ability to deliver DNA to plant cells and permanently alter the plant genome. The discovery of this unique feature 30 years ago has provided plant scientists with a powerful tool to genetically transform plants for both basic research purposes and for agricultural development.

Compared to physical transformation methods such as particle bombardment or electroporation, *Agrobacterium*-mediated DNA delivery has a number of advantages. One of the features is its propensity to generate a single or a low copy number of integrated transgenes with defined ends. Integration of a single transgene copy into the plant genome is less likely to trigger "gene silencing" often associated with multiple gene insertions.

When the first edition of *Agrobacterium Protocols* was published in 1995, only a handful of plants could be routinely transformed using *Agrobacterium*. *Agrobacterium*-mediated transformation is now commonly used to introduce DNA into many plant species, including monocotyledon crop species that were previously considered non-hosts for *Agrobacterium*. Most remarkable are recent developments indicating that *Agrobacterium* can also be used to deliver DNA to non-plant species including bacteria, fungi, and even mammalian cells.

While the list of organisms that can be infected by *Agrobacterium* has increased significantly over the past decade, the success in transformation also relies on culture responsiveness of the target cells/tissues subsequent to the co-cultivation with *Agrobacterium*. Essentially, the dynamic interactions between the two living organisms are critical for development of transformation methods.

The second edition of *Agrobacterium Protocols* contains 80 chapters (two volumes) divided into 14 parts. Part I in Volume 1 (*Agrobacterium* Handling) provides six chapters describing basic techniques in *Agrobacterium* manipulation and strategies for vector construction, major components of plant transformation that are often neglected by many plant biologists. Part II in Volume 1 (Model Plants) consists of seven chapters describing various ways to introduce DNA into three major model plant species, *Arabidopsis thaliana*, *Medicago truncatula*, and *Nicotiana*. Although most plant laboratories transform these model plants on a routine basis, protocols from leading experts may further enhance their capabilities. Parts III through VI in Volume 1 and Parts I through

VII in Volume 2 collect 61 chapters covering protocols for 59 plant species. The plants are grouped according to their practical utilization rather than their botanical classification. The significant expansion of this section reflects the remarkable advancements in plant transformation technology during the past decade. Part VIII in Volume 2 (Non-plants) contains six chapters with protocols for introducing DNA into non-plant species such as bacteria, fungi, algae, and mammalian cells. The description of this unique capacity of *Agrobacterium* is a new addition to this edition.

Agrobacterium Protocols provides a bench-top manual for tested protocols involving *Agrobacterium*-mediated transformation. All chapters are written in the same format as that used in the Methods in Molecular Biology series. Each chapter is contributed by authors who are leaders or veterans in the respective areas. The Abstract and Introduction sections provide outlines of protocols, the rationale for selection of particular target tissues, and overall transformation efficiency. The Materials section lists the host materials, *Agrobacterium* strains and vectors, stock solutions, media, and other supplies necessary for carrying out these transformation experiments. The Methods section is the core of each chapter. It provides a detailed step-by-step description of the entire transformation procedure from the preparation of starting materials to the harvest of transgenic plants. To ensure the reproducibility of each protocol, the Notes section supplies additional information on possible pitfalls in the protocol and alternative materials or methods for generating transgenic plants.

Typically, most laboratories only work on one or a few plant species. Of course, each laboratory or individual researcher has his/her own favorite variation or modification of any given plant transformation protocol. The protocols presented in this edition represent the most efficient methods used in the laboratories of these contributors. They are by no means the only methods for successful transformation of your plant of interest. The broad range of target tissue selection and in vitro culture procedures indicate the complexity in plant transformation. It is the intention of this book to facilitate the transfer of this rapidly developing technology to all researchers for use in both fundamental and applied biology. I take this opportunity to thank all my colleagues whose time and effort made this edition possible. Special thanks go to my family for their unconditional love and support during the process of editing this book.

Kan Wang

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