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2ND EDITION

Edited by

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Preface

The technique of *in situ* hybridization, in its various forms, has been used routinely in many laboratories for a number of years. However, *in situ* hybridization has retained a reputation as one of the more difficult molecular biological techniques.

This may be caused, in part, by the hybrid nature of the technique, which often requires a mixture of molecular biological and histological skills. The two techniques are usually taught and acquired in different streams of biological science. The step-by-step and detailed protocols provided in this book, by researchers active in the field, should make it possible for both the molecular biologist with little experience of histology and the histologist with little experience of molecular biology to use the technique successfully in their laboratories.

In Situ Hybridization Protocols aims to provide useful protocols for two groups of researchers who use the technique: those who use *in situ* hybridization to localize genes to particular chromosomes and those researchers who use DNA or RNA probes to localize expression of mRNA in tissue. This new edition also encompasses a number of techniques not covered in the previous edition, such as *in situ* hybridization of whole-mount embryo specimens, *in situ* hybridization at the electron microscopic level, and *in situ* detection of DNA fragmentation in apoptosis. The final two chapters of the book provide examples of application of *in situ* hybridization techniques to research and clinical problems.

In Situ Hybridization Protocols provides ample information for novices planning to set up the *in situ* hybridization technique and use it in their laboratories for the first time, as well as giving updates of recent developments, in step-by-step protocols for those laboratories where *in situ* hybridization techniques are already used. In particular, the use of nonisotopic methods for both forms of *in situ* hybridization has advanced rapidly since the well-received previous edition.

In my own laboratory we have used *in situ* hybridization on tissue sections for a number of years; when we look back on results gained even a few years ago, there has clearly been continual improvement in the technique. I trust that those who use this new edition will find it a valuable aid in setting

up the technique or improving the sensitivity and scope of applications of *in situ* hybridization in their own laboratories.

Lastly, I would like to acknowledge the contribution made by Andy Choo, who edited the first edition of the book, and thus provided a basis for the second edition, my colleagues Teresa Bisucci and Tim Hewitson for their participation in planning and writing our chapters, and all of the authors who have contributed their protocols.

Ian A. Darby

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Color Plates

Color Plates 1–5 appear as an insert following p. 176.

PLATE 1 Fig. 4. Results of FISH experiments. (*See* full caption on pp. 10–11.)

PLATE 2 Fig. 2. Double labeling for monoctyes/macrophages and TUNEL in a rat model of experimental renal infection. (*See* full caption on p. 159.)

Fig. 3. End-labeling of fragmented DNA in a rat tubular epithelial cell. (*See* full caption on p.159.)

PLATE 3 Fig. 1. Low- and high-power views of *in situ* hybridization. (*See* full caption on p. 172.)

PLATE 4 Fig. 2. Low-power view of *in situ* hybridization for TNFα. (*See* full caption on p. 173.)

PLATE 5 Fig. 3. Results after ISH and CARD signal amplification on human cell preparations, and routinely fixed, paraffin-embedded tissue sections. (*See* full caption on p. 200.)