
Preface

Cytochromes P450 (CYPs) comprise a large superfamily of proteins that are of central importance in the detoxification or activation of a tremendous number of natural and synthetic hydrophobic xenobiotics, including many therapeutic drugs, chemical carcinogens, and environmental pollutants. Some CYPs catalyze the metabolism of endogenous compounds, particularly the ones involved in signaling. CYPs, therefore, are important in mediating interactions between an organism and its chemical environment and in the regulation of physiological processes. Many CYPs are inducible by the compounds they metabolize. In addition, genetic polymorphisms of *CYP* genes affect expression or activity of the enzymes, which can result in adverse drug reactions or genetic diseases. Consequently, CYPs are among the most extensively studied groups of proteins, being investigated by researchers in fields as diverse as biochemistry, molecular biology, pharmacology, toxicology, environmental biology, and genetics. The wide range of techniques that have been applied to the CYPs reflects the diverse backgrounds of the many researchers active in this field.

Previous editions of *Cytochrome P450 Protocols* contained collections of key “core” techniques, most of which are still relevant. The emphasis, however, was on methods for the investigation of individual CYPs and substrates, mostly in an *in vitro* context. The current edition focuses on high-throughput methods for the simultaneous analysis of multiple CYPs, substrates, or ligands. Although the emphasis is on CYPs of mammalian origin, it reflects an increasing interest in CYPs of bacterial species. However, most of the methods described are suitable for the investigation of CYPs from any source. Also included are chapters on CYP reductase (the redox partner of CYPs) and the flavin-containing monooxygenases (FMOs), another family of proteins that are important in the metabolism of foreign chemicals, and that share several substrates in common with the CYPs.

The chapters of this edition of *Cytochrome P450 Protocols*, although not formally divided into sections, are grouped loosely according to topic. Included are high-throughput methods for identification of substrates, ligands, and inhibitors of CYPs; metabolomic and lipidomic approaches for identification of endogenous substrates of CYPs (“de-orphanizing” CYP substrates); reconstitution systems for the incorporation of modified and novel metalloporphyrins into CYPs *in vivo* or for developing nanoparticle bioreactors for biophysical and mechanistic studies of CYPs and drug-metabolite profiling; high-throughput assays for measuring the activity of CYPs and for identification of their substrates and adducts; methods for the generation and quantification of novel CYPs and for identification of their potential substrates; techniques for phenotyping, genotyping, and identification of transcriptional regulatory sequences; a high-throughput method for the generation of libraries of redox-self-sufficient CYP biocatalysts; a guide to CYP allele nomenclature; and methods for the isolation of mouse primary hepatocytes, for the differentiation of a hepatoma cell line into cells with hepatocyte-like metabolic properties, and for transfection of such cells with DNA and siRNA constructs to investigate the function and regulation of expression of CYPs.

Each chapter is written by researchers who have been involved in the development and application of the particular technique. Protocols are presented in a step-by-step manner,

with extensive cross-references to notes that highlight critical steps, potential problems, and alternative methods. We hope that this format will enable researchers who have no previous knowledge of the technique to understand the basis of the method and to perform it successfully.

We are extremely grateful to all the authors who contributed so generously to this volume and to John Walker, the series editor of *Methods in Molecular Biology*, for his advice and patience.

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