Preface

The first edition of this book, published in 1999 and called *DNA Repair Protocols: Eukaryotic Systems*, brought together laboratory-based methods for studying DNA damage and repair in diverse eukaryotes: namely, two kinds of yeast, a nematode, a fruit fly, a toad, three different plants, and human and murine cells. This second edition of *DNA Repair Protocols* covers mammalian cells only and hence its new subtitle, *Mammalian Systems*. There are two reasons for this fresh emphasis, both of them pragmatic: to cater to the interests of what is now a largely mammalocentric DNA repair field, and to expedite editing and production of this volume.

Although *DNA Repair Protocols: Mammalian Systems* is a smaller book than its predecessor, it actually contains a greater variety of methods. Fourteen of the book's thirty-two chapters are entirely new and areas of redundancy present in the first edition have been eliminated here (for example, now just two chapters describe assays for nucleotide excision repair [NER], rather than seven). All eighteen returning chapters have been revised, many of them extensively. In order to maintain a coherent arrangement of topics, the four-part partitioning seen in the first edition was dispensed with and chapters concerned with ionizing radiation damage and DNA strand breakage and repair were relocated to near the front of the book. Finally, an abstract now heads each chapter.

I have aimed to make *DNA Repair Protocols: Mammalian Systems* a well-rounded book, intended to address a broad range of questions about practical mammalian DNA repair, including arcana such as "what is radioresistant DNA synthesis and how is it measured?" (see Chapter 5, by Jaspers and Zdzienicka). The final selection of topics was influenced by both the contents of the first edition (naturally) and the willingness of its authors to contribute again; by my desire to correct deficiencies in the first edition (e.g., it lacks a chapter on DNA helicase assays); and by what new methods have come into use since 1998, when the first edition went into production. Below I summarize and put into context some of what's new, as a way of illustrating the diversity and scope of *DNA Repair Protocols: Mammalian Systems*. (My apologies to authors of chapters not mentioned; no slight is intended.)

Cytogenetic analysis, a topic that had scant coverage in the first edition, is emphasized in Chapters 3 and 4. In Chapter 3, Au and Salama detail a cytogenetic challenge assay in which blood lymphocytes obtained from individuals of interest (e.g., smokers, chemical workers, chemotherapy patients) are irradiated—that is, challenged—with ionizing or UV radiation and then scored for chromosomal anomalies. Abnormally high levels of induced chromosomal aberrations may be indicative of a constitutional repair deficiency, as the authors and their colleagues recently described for certain variant alleles of two DNA repair genes, *XPD* and *XRCC1*.

The cellular response to ionizing radiation is complex. For example, the irradiated cell may be killed outright or it may repair whatever damage occurs and continue to grow and divide no worse for wear. A third outcome, only recently appreciated, goes something like this: the cell survives irradiation apparently unharmed and proliferates, but then its descendents go on to display "genomic instabilities" of various kinds. This intriguing transgenerational phenomenon (or phenomena) and cytological methods for studying it are described by Nagar, Corcoran, and Morgan in Chapter 4.

In Chapter 7, Huang and Darzynkiewicz present a new cytometric approach for detecting DNA double-strand breaks (DSBs). Their method is based on work by W. M. Bonner and colleagues who discovered that a phosphorylated form of histone H2AX, called γH2AX, accumulates in quantity at chromosomal DSBs. Specific antibodies against γH2AX are now available commercially, making immunofluorescence detection of γH2AX a convenient way to detect DSBs induced by clastogenic agents (with attendant caveats noted in the chapter).

Two very different methods for quantifying levels of DNA damage (Chapters 14 and 21) both make use of PicoGreen, an ultrasensitive nucleic acid stain that shows especially strong enhancement of fluorescence when bound to double-stranded DNA (dsDNA). In Chapter 14, Santos, Meyer, Mandavilli and Van Houten describe a quantitative PCR (QPCR) method that can be used to measure DNA damage in specific regions of either the mitochondrial or nuclear genome. The crux of this method is that DNA lesions able to impede movement of the polymerase during PCR will cause less product to be synthesized compared to control reactions using a lesion-free template; in both cases the amount of dsDNA product is quantified with PicoGreen. The QPCR technique combines aspects of Bohr's pioneering Southern blot method, described by Anson, Mason, and Bohr in Chapter 13 (both approaches quantify underrepresentation of a specific DNA fragment and use Poisson probabilities to calculate levels of damage), and Pfeifer's ligation-mediated PCR in Chapter 15 (the stopping of polymerases by lesions in DNA). (Note that Hays and Hoffman in the first edition described a QPCR approach for measuring activities of photolyases, although that method uses UV-damaged plasmids as template and radiolabel to quantify PCR product levels.)

In Chapter 21, Schröder, Batel, Schwertner, Boreiko, and Müller detail their sensitive microtiter plate assay ("Fast Micromethod") for estimating levels of single-strand breaks (nicks) in DNA. The single-strand breaks are identified indirectly by measuring the reduction in amount of dsDNA—quantified by

PicoGreen—in mutagen-treated and untreated samples following alkaline denaturation. Nicked duplex DNA denatures more readily and hence loses fluorescence more rapidly than does intact DNA. The Fast Micromethod shares some methodological features with the alkaline version of the comet assay described by Speit and Hartmann in Chapter 20 (e.g., denaturation of DNA under alkaline conditions). In addition, it can be adapted for high throughput screening and allows testing of both cells and tissues, including frozen material.

Chapter 22, contributed by Shibutani, Kim, and Suzuki, describes a vastly improved ^{32}P-postlabeling protocol, which is a very sensitive method for detecting the presence of "adducted" bases in DNA. ^{32}P-postlabeling protocols have traditionally used thin layer chromatography to separate modified deoxynucleotides from their normal counterparts. The new technique, developed in Shibutani's laboratory, uses polyacrylamide gel electrophoresis, which allows multiple samples to be run out and compared on a single gel. Other strengths of the method are its improved handling and high sensitivity.

In Chapter 24, Wang and Hays describe a new and efficient protocol for preparing mismatch repair (MMR) plasmid substrates. Their method makes use of nicking endonucleases that introduce a single-strand nick at specific sequences in duplex DNA. One nicking endonuclease is used to create a pair of nicks separated by tens of nucleotides on one strand of a plasmid specially engineered for this purpose. The oligonucleotide defined by the nicks is melted out by heating, the resulting gapped plasmid is purified by BND-cellulose chromatography, and the desired mismatch-containing synthetic oligomer is annealed into the gap. Following ligation and purification, the mismatch-containing plasmid substrate is treated with a different nicking endonuclease to generate *the nick* that is essential for initiation of MMR excision.

In the first edition, Matsumoto described a base excision repair (BER) assay using *Xenopus* oocyte extracts. Here, in Chapter 26, he details an updated protocol for studying BER in mammalian cells that includes several important technical refinements. For example, in the synthetic BER substrate that he described in the first edition, the abasic site was positioned opposite an adenine. In his new construct the abasic site is opposite a cytosine, thus mimicking depurination of guanine, which in vivo occurs much more frequently than loss of thymine. Two further improvements to his assay are the use of SYBR Green (rather than radiolabel) to detect repair products and a simplified method for preparing whole-cell extracts.

Missing from the first edition was a chapter on DNA helicase assays. This significant lacuna has now been filled by Brosh and Sharma's excellent contribution, Chapter 28. New authors Scovassi and Prosperi have written a considerably expanded chapter (Chapter 31) on proliferating cell nuclear antigen (PCNA), in keeping with that molecule's central role in DNA repair. Those two chapters, together with one by Mello, Moggs, and Almouzni addressing the role of chromatin in repair (Chapter 32), round out a strong collection of BER- and NER-related chapters.

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