Preface

Maintenance of genome integrity is important for all aspects of cellular physiology and development. Alterations in our genome due to endogenous and exogenous factors threaten the integrity of our genome and therein have a direct consequence to human health. Evolution of DNA repair mechanisms has ensured that the majority of the DNA damage acquired is often quickly and efficiently reversed to restore genome stability. Based on the type of damage encountered, different repair proteins are mobilized to the site of damage for DNA repair. Over the past five decades, our understanding of these repair pathways has increased by convergent knowledge from chemists, biochemists, geneticists, molecular biologists, radiobiologists, and oncologists, among many others. At the heart of understanding the molecular details of these complex processes is the wealth of information that has been gained by the development of a wide range of experimental techniques.

This edition of *Methods in Molecular Biology* will outline techniques that will serve as a guide to assist researchers in their experimental design as they study the underlying mechanisms of these important DNA repair pathways. The chapters in this book have been divided into five general parts, each focused on specific aspects of repair biology.

In Part I, two timely reviews are provided on different aspects of DNA repair. Chapter 1 discusses the role of post-translational modifications on homology-directed repair (HDR) and recent advances in the treatment of HDR-defective cancers. The second review focuses on DNA damage caused by defective telomere replication, with a focus on telomere fragility in cells utilizing the recombination-based alternative lengthening of telomeres (ALT) pathway.

Part II, titled "Cellular Assays to Detect and Measure DNA Damage and Damage Response," describes methods in the detection and measurement of DNA breaks and proteins involved in the DNA damage response (DDR). Chapter 3 describes in detail the use of micro-irradiation to create DSBs followed by the use of live-cell imaging or immunofluorescence (IF) to quantify the dynamic association and dissociation of proteins involved in both the recognition and repair of DSBs. In Chapter 4, Pond and Ellis provide a detailed description of the use of pulse-field gel electrophoresis (PFGE) to detect and measure DSBs in genomic DNA. Chapter 5 then shifts focus to the detection of tri-nucleotide repeat (TNR) expansions and deletions caused by DNA base lesions. In this method, Lai et al. describe how to measure the distribution and location of these lesions and their affect TNR stability. Finally, Chapter 6 presents a qPCR method to measure cellular nucleotide pools, which, when not properly maintained, leads to DNA damage and defective DNA repair.

Part III, titled "Cellular and Cell Extract-Based Assays to Measure DNA Repair," encompasses methods designed to measure DNA repair efficiency and characterize factors involved in the repair process. Firstly, Chapter 7 focuses on a much-needed method to measure HDR following genome-editing (i.e., CRISPR, TALEN) through a fluorescence conversion assay, which allows for flow cytometry-based detection of converted cells followed by DNA sequencing to characterize the conversion tracts. Chapter 8 follows up with another reporter-based assay to directly measure base-excision repair (BER) in human cells. The next two chapters make use of *Xenopus* egg extracts, as a cell-free method to detect

DNA repair. Chapter 9 describes a newly developed assay, which creates site-specific ssDNA breaks on plasmid DNA. This plasmid can then be incubated with *Xenopus* egg extracts to measure ssDNA repair efficiency as well as protein recruitment and the DDR. Chapter 10 makes use of *Xenopus* extracts to perform chromatin-immunoprecipitation (ChIP) to measure dynamic protein associations and displacement in a synchronized, cell-free system, which has distinct advantages over cell-based assays. Chapter 11 then describes the use of cell viability assays, IF, and metaphase spread analysis to understand the functional importance of DNA helicases during DNA repair. The final two chapters of this part describe methods to screen for genetic complementation following DNA damage by the colony formation assay (Chapter 12) and the identification of small molecules that inhibit DNA repair proteins (Chapter 13).

Part IV, titled "In Vitro Biochemical and Biophysical Methods to Study DNA Repair," outlines different in vitro assays using purified recombinant proteins to study DNA repair enzyme mechanisms. Chapter 14 outlines the expression and purification of three proteins involved in DSB repair and use of a single-molecule DNA curtain assay to temporally and spatially assess the mechanisms of DNA resection end resection at DSBs. In Chapter 15, Sausen et al. describe two techniques to study the ATPase activity of recombinant helicase/ATPase enzymes. The first outlines a direct method for assaying ATPase activity using radiolabeled ATP and thin layer chromatography and the second uses a high-throughput 96-well plate assay that couples ATPase activity with nicotinamide adenine dinucleotide (NADH) oxidation for real-time monitoring. Chapter 16 details biochemical methods to assess the efficiency of repair enzymes during different DNA transactions, namely, DNA unwinding and annealing. Strand exchange mechanisms are frequently observed during homologous recombination and repair and Chapters 16 and 17 outline methodologies to study strand exchange reactions with purified recombination proteins. With a similar focus on the HDR pathway, Chapters 18 and 19 describe methodologies to assess branch migration and nucleoprotein filament formation, respectively.

The tail end of the book focuses on methods to study DNA damage and repair within the telomeric regions and/or arising during DNA replication. Part V is titled "Methods to Assess Telomeric and Replication-Induced DNA Damage and Repair." Fouquerel et al. in Chapter 20 present highly sensitive protocols, which allow for measurement of UV photoproducts that induce telomere damage in human cells and subsequent assessment of their removal and repair. Genome stability is directly impacted by the dynamics of replication fork movement during the duplication process. DNA fiber analysis allows for direct assessment of fork movement and replication dynamics. Assays describing DNA fiber methods to measure genome-wide and telomere replication are presented in Chapters 21 and 22, respectively. The presence of guanine-rich sequences at telomere ends promote the formation of G-quadraplex structures, which both protect the ends of the chromosomes as well as impede repair and replication. Chapter 23 outlines an in vivo method to detect these structures and their colocalization to telomeric regions using IF. The final technique (Chapter 24) describes the use of FISH to detect DNA damage on metaphase chromosomes, using telomeric FISH probes.

Many of the techniques outlined in this book can be easily adapted or modified by researchers interested in studying DNA transactions to assess their specific protein or repair pathway of interest. Our hope is that these methods will be useful for both those new and well-established in the field and help advance our understanding of DNA repair. We would like to specifically acknowledge and thank all the authors who contributed to this book with their expert descriptions and detailed outlines of various protocols, which will undoubtedly be an excellent resource to many in the field. We would also like to thank the series editor, John Walker, for assistance in the development and editing of this book.

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