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BOOK REVIEW

p53 Protocols

(Deb, S., and Deb, S. P. (eds.) in Series "Methods in Molecular Biology", Vol. 234 (Series Editor John Walker), Humana Press, 2003, 300 p., $89.50)

p53 is the most significant tumor suppressor protein discovered to date. Epidemiological studies indicate that well over half of human malignancies display genetic alterations at the p53 locus. In 25 years since the discovery of p53, significant advances have been made in elucidating its biological role. It is now well-established that p53 functions as a sequence-specific transcription factor that activates various gene expression in response to genotoxic stress leading to the onset of either cell cycle arrest, apoptosis, premature senescence, or a host of other cellular effects. However, significant gaps persist in our knowledge of the multifaceted role that p53 plays in the cell.

The book is comprised of eighteen chapters and covers some of the most widely used techniques in p53 research.

Chapter 1 describes in detail the generation of adenovirus expressing p53, including techniques for cloning of p53 cDNA into the adenoviral vectors, generation of adenovirus in HEK293 cells, characterization of p53 adenovirus and, finally, large scale preparation of adenoviral stocks. Adenovirus-mediated delivery of wild-type p53 into tumor cells has been shown to sensitize them to apoptosis following exposure to genotoxic stress.

Chapter 2 deals with the purification of recombinant p53 protein from Sf9 insect cells. This chapter also describes the use of chromatographically purified recombinant p53 in the DNA binding assay known as electromobility shift assay (EMSA). Recombinant p53 can also be utilized in a number of other critical experiments *in vitro*, such as protein–protein interaction studies and degradation assays involving purified components. The methods outlined in this chapter include the infection of cells for virus production, the virus plaque assay, calculation of viral titers, the optimization of protein expression in Sf9 cells, anion-exchange chromatography, followed by p53 concentration using ultrafiltration.

Chapter 3 provides an overview of the necessary steps and protocols involved in producing a mutant mouse as well as p53 vectors for embryonal stem cells.

Chapters 4 and 5 describe, respectively, fluorescent differential display (FDD) and Affymetrix GeneChip assay (otherwise known as gene microarray) to identify novel p53 target genes.

In chapter 6, the method of quantitative analysis of the reverse transcriptase (RT-PCR) of one of genes (which is p53-regulated) is described.

Chapters 7 and 8 describe several methods to study p53-mediated transactivation and transrepression. Detailed protocols are provided for the transfection of cells with various plasmids and p53-responsive luciferase reporters, preparation of cell lysates for luciferase activity assays, and chromatin immunoprecipitation (ChIP) and McKay's assay.

In chapter 9 several classical methods to detect p53 protein–protein interactions are described. Guidance on the choice of p53 antibodies to be used in co-immunoprecipitation experiments is also provided.

Chapter 10 is devoted to the description of methods used to study the interaction of p53 with adenoviruses including co-immunoprecipitation, luciferase reporter assay, and immunofluorescence.

In chapter 11 *in vivo* foot-printing assay, which is used to assess p53 binding to nuclear chromatin, is described.

Chapter 12 is devoted to the discussion of phospho-specific antibodies that could be raised against a particular phosphorylated residue on p53.

Chapter 13 provides an overview of the subcellular fractionation method used to reveal p53 in mitochondria.

Chapter 14 also describes subcellular fractionation procedure in monitoring nuclear and cytoplasmic degradation of p53.

Chapters 15 and 16 describe methods that allow the detection of p53 in frozen and paraffin-embedded human tumor specimens. Techniques described include extraction of DNA from frozen or paraffin-embedded tissues, PCR amplification of p53 coding sequences, purification and sequence analysis of PCR products, and immunocytochemical methods.

In chapters 17 and 18, methods to study p53-mediated apoptosis and cell cycle arrest are presented. These approaches include fluorescent-activated cell sorter (FACS) analysis and bromodeoxyuridine incorporation assay, both of which are discussed in substantial detail.

Each chapter begins with an introduction describing the theoretical basis of a particular method and its applications in p53 research. A materials section includes a list of required commercially available reagents, as well as

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provides instructions on how to prepare buffer solutions that will be used during an experiment. A detailed methods section that follows is presented in a "step-by-step" format, which allows easy use right at the bench. Most chapters are well illustrated with diagrams, tables, and figures depicting representative results of a particular experiment.

Publication of "p53 Protocols" as part of the "Methods in Molecular Biology" series from the Humana Press should aid both experienced researchers and entry-level scientists in their efforts to elucidate molecular and biochemical properties of p53 and other tumor suppressor proteins.

Inclusion of all the methods used in studying p53 in a single publication is nearly impossible. However, "p53 Protocols" offers a good compilation of useful techniques that will facilitate p53 exploration by anyone intrigued by its biology. The book will be also valuable to biochemists, molecular biologists, and investigators in cell carcinogenesis and proteomics.

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