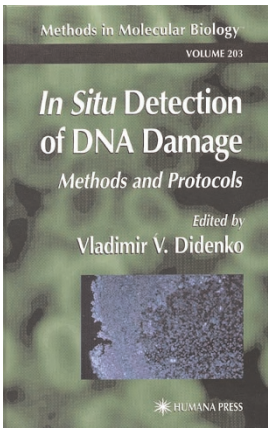
Slings and arrows



***In situ* detection of DNA damage**
edited by Vladimir V. Didenko
Human Press • 2002
Hardback £/$89.50

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Our genomes are subject to the slings and arrows of outrageous fortune. In each of us, cosmic radiation is inflicting a DNA double-stranded break (DSB) in 10^5 cells every second. To add insult to injury, we are self-destructive. In 1955, Isaac Asimov published a proposal that life only became possible as ^{40}K decayed (Asimov, I. "The Radioactivity of the Human Body". *J. Chem. Edu.*, February 1955). ^{40}K has a half-life of 1.26×10^9 years, with the result that 8.4% of the amount in the big bang is still around, and enough of it is in us to cause a DNA DSB in 2×10^4 cells every second. Even 'getting some sun' damages our DNA. In addition, DNA can be broken or otherwise damaged by what we eat, and our metabolism makes mistakes that result in DNA damage. In contrast, programmed DNA breaks occur in recombination during development of the immune system and germ cell development — processes essential for our existence. Programmed cell death also causes extensive DNA fragmentation. DNA continuity is important for replication and transcription, and essential for normal mitosis. Fortunately for us, our cells contain a host of systems to restore, repair and rejoin DNA. It is defects in these systems that typically result in sensitivity to radiation and a propensity towards cancer.

Many techniques are used in the study of DNA damage. Volume 203 of *Methods in Molecular Biology* is devoted to methods and protocols for *in situ* detection of DNA damage. Although the title suggests a broad coverage, the main biological application discussed is apoptosis (programmed cell death) and most of the techniques discussed are limited by their sensitivity to analysing the extensive DNA fragmentation that occurs during apoptosis. The first three parts of the book contain chapters on end-labelling DNA fragments with terminal transferase, DNA polymerase I and ligase, respectively. Each part begins with one or two chapters that provide a useful overview of techniques and a discussion of their applicability, specificity and limitations. Part I concentrates on the terminal uridine nucleotide end labelling (TUNEL) and *in situ* end labeling (ISEL) assays. Techniques starting with cells and tissue sections and finishing with flow cytometry, and light, confocal, and electron microscopy are presented in detail.

The fourth part contains chapters on the comet assay, in which single cells are subjected to gel electrophoresis and the size and shape of the resulting comet-shaped DNA distribution are analysed by a variety of detection methods. The last two parts contain chapters covering the detection of modified DNA bases and some other methods for detecting DNA damage.

There is a great deal of useful information in this manual, but in spite of its title and claim to be state-of-the-art, the book does not include all known techniques in the area. Indeed, it would have benefited greatly from an overview of all known *in situ* methods for detecting DNA damage to help researchers decide what is the best method for their application. In particular, there is no mention of the use of antibodies to proteins such as γ-H2AX and other DNA DSB repair proteins to detect foci that form at DNA DSB sites, techniques that are used widely. These foci detection methods cover non-apoptotic applications, such as V(D)J recombination and exposure to ionizing radiation, applications in which cells contain DNA DSBs in small numbers.

Researchers also need to be aware of other limitations of this book. Readers are confronted with a format that often obfuscates rather than clarifies in the name of stylistic uniformity. Most chapters include multiple related techniques that are dismembered and sorted into 'Materials, Methods and Notes'. Thus, researchers must endure constant cross-referencing to get a complete depiction of any technique. Simply subdividing each chapter primarily by technique would have eliminated this difficulty.

To make this process of matching relevant material across irrelevant paragraphs more challenging, the cross-referencing is sometimes compromised by damaged links. For example, in the methods section of each chapter, researchers are sometimes referred to various parts of the materials sections only to come up empty-handed. Sometimes the misreferenced item is located in a neighbouring materials list. In at least one case, it took a whole body search of the chapter to find the desired item buried in one of the notes. I wonder if some of the authors may have experienced similar frustrations in trying to present their methods in this format.

Unfortunately, the index of the book is also found wanting. Researchers cannot rely on it to find where, or even if, a topic of interest is discussed in the book. For example, references to DNA DSBs during V(D)J recombination and a PCR method of detecting DNA DSBs were found by browsing, but are absent from the index. The index also assumes a high degree of knowledge about the field. Mentions of techniques by their acronyms and nicknames in the text are indexed as discrete items. Mentions of SCGE are indexed only under "single cell gel electrophoresis", and mentions of comet assay, another name for SCGE, are indexed separately as if the two were unrelated. A similar situation exists for AP and apurinic/apyrimidinic sites.

Clearly, this book would work better as a CD-ROM so that researchers could use a search program to bypass the poor indexing and cross-referencing. Most researchers lack the time for patient browsing and deserve a clear presentation of methods and protocols — at least clearer than the one offered by this book. While this book may be useful for researchers interested in apoptosis, those interested in DNA DSB detection techniques in areas other than apoptosis must still consult the original research literature. □

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