
Preface

The development of cellular sciences has now entered the stage which requires the evaluation of DNA damage at both the single-cell and the whole organism levels. New approaches are developed to satisfy this need, and the older established techniques were adapted to the task. Advances in organic chemistry, fluorescent microscopy, and materials science have created a whole new range of techniques and probe for imaging DNA damage in molecular and cell biology.

The volume presents all major assays used in molecular and cell biology for the labeling of DNA damage *in situ*, *ex vivo*, and *in vivo*. It brings together recently introduced techniques, as well as those established earlier, which detect and quantify DNA damage at the scales ranging from subcellular to the level of a whole, living organism.

Historically, many techniques which detect DNA damage were originally introduced to solve a utilitarian task of labeling apoptotic cells. These include methods designed to detect specific single- and double-stranded DNA breaks in tissue sections using terminal transferase (TUNEL assay), T4 DNA ligase (ISL assay), T7 DNA polymerase, and many others. These techniques' association with specific cellular process provides an additional bonus of their utilization. Therefore, their application for apoptosis detection is described in the volume. In such cases, detailed analysis from the DNA damage detection's point of view is also provided.

The book does not contain a description of MRI-based or other similar approaches, which use high-end medical diagnostic instrumentation. These belong to a different field due to their specialized nature.

Proper decision-making on what technique to choose requires clear understanding of the terms "*in vivo*," "*ex vivo*," and "*in situ*" in their application to the detection methods.

In vivo means "within the living" or "inside the living body." In application to detection techniques, it refers to measurements done in cells and tissues which remain in a whole, living organism.

Ex vivo translates as "out of the living" or "outside the body." In application of detection techniques, it refers to measurements done in cells or other materials taken from a living organism and performed outside the body. *Ex vivo* measurements are made under conditions impossible in the living organism. The term is used in many different contexts with different emphases. In a more narrow definition, not used here, *ex vivo* indicates a procedure in which cells are taken from a living organism for a treatment, and then put back into the body. Another related term – *in vitro* (i.e., "within a glass," "in a test tube"), is opposite *in vivo*, and indicates a work done in permanent cell cultures, when cells are grown and always remain in the artificial environment.

In situ means "on site" or "in place." The term is not in opposition to either *in vivo* or *ex vivo*. It just denotes the processes detected in their place of origin. In molecular and cell biology, this usually refers to undisrupted mounted cells or tissue sections. In that meaning, "*in situ*" is used as part of the terms "*in situ* PCR," "*in situ* transcription," "*in situ* hybridization," "*in situ* end labeling," and "*in situ* ligation." Sometimes, the "*in*

situ” term is applied at the subcellular level to cells disrupted in the process of analysis, for example, the detection of specific sequences in chromosomes in fluorescent *in situ* hybridization (FISH). Historically, the term was used primarily in methods dealing with nucleic acids. *In situ* methods are advantageous in the analysis of heterogeneous cellular populations. Their attractive features include single-cell detection level, potential to colocalize DNA damage and cellular proteins, the ability to use cellular morphology to verify cellular phenomena, and small sample size. The opposite of *in situ* assays are biochemical techniques, which detect their targets in bulk samples without reference to specific individual cells.

The book is divided into three parts. The first part deals with fixed tissue sections. It contains a complete set of enzymatic approaches to study DNA breaks and apoptosis. The second part describes the detection of DNA damage and apoptosis in cultured cells. It includes instrumental approaches, such as those which use flow- and image cytometry or electrophoresis of agarose-trapped cells. All of the techniques presented in these two parts are the *in situ* approaches. The third part describes methods developed either for *in vivo* detection, directly in a living organism, or for samples taken from the body, *ex vivo* – in blood, urine, and sperm. These are, somewhat, closer to diagnostic procedures. The presented assays often permit monitoring levels of DNA damage and can provide conclusions at the scale of the whole organism. They are either *in situ* assessments or biochemical bulk measurements.

The volume is self-sufficient and easy to understand the source of information needed to reproduce its protocols and interpret their results. Each chapter presents a single protocol or a group of techniques which are related either by their mechanisms or by the molecular targets they detect. This serves to better understand the concepts underlying the methods and the types of DNA damage they label. Such structure also makes it easier to select an approach which better suits the specific needs of a particular study.

The book is equally useful for newcomers to the field and for experienced researchers. A novice molecular biology scientist will use the volume as a guide for selecting and mastering the technique most suitable for his specific needs. To help with this task, the chapters provide detailed, simple-to-follow protocols and describe possible technical pitfalls and limitations.

The rapid growth of specialization makes it increasingly difficult even for the most experienced scientists to follow the never-ending stream of technical innovations which could be beneficial for their research. For the more experienced, the volume serves as a source of novel, recently developed methods and a resource of the new technical possibilities provided by familiar approaches.

It can also help scientists from other more distant fields, such as clinical scientists and nanobiotechnology specialists, who would like to familiarize themselves with research possibilities on DNA damage detection and explore the benefits of this unique technical arsenal.

Researchers in many fields, including molecular and cell biology, experimental and clinical pathology, toxicology, radiobiology, oncology, embryology, experimental pharmacology, drug design, and environmental science, benefit from the book.

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