Preface

The integrity of the genome is a fundamental determinant of cellular identity, cellular fitness, and interactions between a cell and its environment. The study of genome integrity is now a mature field, but one marked by continuous innovations in techniques, technology, and systems, both in vitro and in vivo. We present 42 methods and protocols to analyze diverse aspects of genome instability.

Beginning in the realm of mutagenesis and repair, we present classic genetic assays to detect chromosome loss, mutation, and genome rearrangements, whole genome approaches to mapping base modifications and repair events, and a method for analyzing engineered base lesions in the genomic context. Methods to quantify and analyze the properties of DNA double-strand breaks include traditional and single-molecule approaches to measure double-strand break resection, and modern methods to map double-strand breaks at high resolution and high sensitivity. Given the importance of DNA replication errors as a source of genome instability, we include methods to profile replication, to probe replication and replication proteins strand specifically, to analyze replication intermediates at high resolution, and to specifically perturb DNA synthesis at specific sites. The increasing interest in the role of ribonucleotides and RNA–DNA hybrids in genome instability is reflected in methods to detect and map ribonucleotides and RNA-DNA hybrids. Techniques to study genome instability at specialized regions, in particular the telomeres and triplet nucleotide repeats, are presented and include molecular biological, genetic, and imaging-based methods. The application of imaging techniques to study genome instability has become common in the field. We present fluorescence microscopic techniques to detect and analyze genome instability, including single-molecule and single-cell analysis, as well as high-resolution methods to probe DNA structural properties. Finally, the contributions of genomic and proteomic approaches to identifying and defining genome instability pathways and networks are reflected in procedures for measuring cell fitness, protein interactions, gene and protein expression, protein-DNA interactions, and protein modifications, on a genome/proteome scale.

Together, the methods and protocols here form a comprehensive resource for the discovery and analysis of the proteins and pathways that are critical for stable maintenance of the genome.

Milano, Italy — *Marco Muzi-Falconi*
Toronto, Canada — *Grant W. Brown*

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