Preface

It was discovered back in 1977 that for most genes the genetic code is dispersed over the gene. Before the messenger RNA can be translated into protein noncoding intron fragments have to be removed from RNA transcripts during a process called splicing. This complex process is coordinated by the splicing machinery, which consists of hundreds of proteins, and sequence motifs in introns and exons are important for recognition by splicing factors and proper processing of pre-mRNA into mRNA. Some exons are not always included in the mRNA depending, e.g., on the developmental state of an organism or the type of tissue (alternative splicing). Soon after the discovery of splicing, it became apparent that genetic mutations affecting splicing motifs or introducing "false" splicing motifs can disrupt splicing and underlie many genetic diseases. In addition, the disruption of alternative splicing can give rise to or exacerbate genetic and acquired disease processes.

Due to their larger size, introns are generally not included in standard diagnostic protocols. Nevertheless, for multiple diseases it has been shown that deep intronic mutations can activate false splice sites, leading to the aberrant inclusion of a piece of intron into the mRNA. Furthermore, previously silent mutations (or substitutions) within an exon were often thought to be polymorphic. Now, it is recognized that these mutations can also cause an exon to be no longer recognized by the splicing machinery, leading to exon skipping.

Even though the splicing process has not been elucidated completely, it is possible to intentionally manipulate it. This can be achieved by preventing binding of splicing factors to their respective motifs, e.g., using chemical compounds, modified pieces of RNA or DNA (antisense oligonucleotides) or through expression of a small nuclear ribonucleoprotein in which the natural antisense part is replaced with an antisense sequence targeting the splicing motif. These tools all induce "skipping" of the targeted exon and can be used to prevent the inclusion of an aberrant exon, to modify levels of alternatively spliced exons or to decrease protein expression levels by skipping an exon.

I felt that a Methods book on exon skipping was timely for two reasons: (1) Now that "next generation" sequencing techniques allow a more detailed analysis of exons and introns in multiple genes at the same time, many alterations will be identified in the near future for which the impact on splicing is uncertain. Methodology on how to assess this will be crucial to discriminate "real polymorphisms" from mutations that affect splicing. (2) Antisense-mediated exon skipping is currently tested in phase 3 clinical trials for Duchenne muscular dystrophy and the encouraging results in this field have incited many groups to apply the antisense-mediated exon skipping approach to their own favorite gene(s). Methodology on how to go about this should facilitate obtaining proof of concept for new exon skipping applications and prevent duplication of errors that have been made already by others (after all as Einstein pointed out: "An expert is someone who has made all possible mistakes in a limited field.").