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Heteroduplex formation and S1 digestion for mapping alternative splicing sites

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ABSTRACT. The identification of alternatively spliced transcripts has contributed to a better comprehension of developmental mechanisms, tissue-specific physiological processes and human diseases. Polymerase chain reaction amplification of alternatively spliced variants commonly leads to the formation of heteroduplexes as a result of base pairing involving exons common between the two variants. S1 nuclease cleaves single-stranded loops of heteroduplexes and also nicks the opposite DNA strand. In order to establish a strategy for mapping alternative splice-prone sites in the whole transcriptome, we developed a method combining the formation of heteroduplexes between 2 distinct splicing variants and S1 nuclease digestion. For 20 consensuses identified here using this methodology, 5 revealed a conserved splice site after inspection of the cDNA alignment against the human genome (exact splice sites). For 8 other consensuses, conserved splice sites were mapped at 2 to 30 bp from the border, called proximal splice sites; for the other 7 consensuses, conserved splice sites were mapped at 40 to 800 bp, called distal splice sites. These latter cases showed a nonspecific activity of S1 nuclease in digesting double-strand DNA. From the 20 consensuses identified here,

5 were selected for reverse transcription-polymerase chain reaction validation, confirming the splice sites. These data showed the potential of the strategy in mapping splice sites. However, the lack of specificity of the S1 nuclease enzyme is a significant obstacle that impedes the use of this strategy in large-scale studies.

Key words: Alternative splicing; S1 nuclease; Heteroduplex; Methodology

