To the Editor:

In their recent letter, Novak et al. report the identification of two novel CBFB-MYH11 fusion transcripts detected with heminested reverse transcription-polymerase chain reaction (RT-PCR) in a patient with inversion of chromosome 16 and acute nonlymphocytic leukemia. In both transcripts an internal deletion of MYH11 cDNA sequences from position 1017 to 2095 is present (Fig 1). Novak et al. conclude that the absence of these MYH11 sequences are the result of alternative splicing at position 1017 and 2095.

We have recently resolved the intron-exon structure of 9 contiguous MYH11 exons (the first identified exon begins at cDNA position 1201 and the ninth ends at position 2632; B.A. van der Reijden, unpublished results, August 1995). MYH11 cDNA position 2095 is located within a 213-bp exon (cDNA positions 1921-2133; data not shown). Surprisingly, there is no splice acceptor site (requiring the sequence AG) upstream of cDNA position 2095 (Fig 2). Because
Fig 1. Sequences of the two CBFB-MYH11 fusion transcripts reported by Novak et al.’ CBFB is fused at nucleotide (nt) 495 to MYH11 at nt 994 or to unknown sequences (the junction is indicated with a vertical arrow and the unknown sequences are indicated with a question mark). Both products miss the MYH11 region between nt 1017 and 2095. The MYH11 junction 1017-2095 is located immediately upstream of primer M1.

Novak et al.'s suggest that splicing occurs at position 2095, we were puzzled by the fact that no splice acceptor site (essential for splicing) is present. To investigate whether the reported fusion transcripts may have been amplified due to aspecific annealing of primer M1 (used in their PCR), we compared its sequence with MYH11 sequences directly downstream of position 1017. Interestingly, two stretches of 6 bp in the sequence of primer M1 are identical to the MYH11 sequences (Fig 3). Such an homology may, especially in heminested PCRs, lead to an aspecific annealing and consequently lead to the amplification of artifact PCR products. Moreover, the fact that the MYH11 splice junction is detected directly upstream of primer M1 (Fig 1) suggests that this primer has aspecifically annealed in this PCR.

The absence of a splice acceptor site at MYH11 cDNA position 2095 and the homology between primer M1 and the sequence directly downstream of MYH11 cDNA position 1017 suggests that the detected products are the result of reproducible PCR artifacts and hence do not represent novel CBFB-MYH11 transcripts. We propose that the amplification of possible alternatively spliced fragments should be reproduced using additional primers and that the genomic sequence of the relevant gene(s) should be analyzed for cryptic splice sites. This work must be performed before it can be concluded that any newly identified cDNA is the result of alternative splicing.2

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van der Reijden et al raise an important question, because the primer M1 is widely used for the amplification of CBFβ-MYH11 fusion transcripts. We have performed RT-PCR after optimizing the PCR conditions with a sample from a type A patient and found conditions similar to those proposed by others. The sequences described were only found in 1 of 17 patients tested so far, arguing against technical problems. Nevertheless, the notion that no ideal splice site (AC) is present at position 2095 is puzzling, although the sequence AC (in position 2093/94) has been shown to represent a weak acceptor splice site in rare instances. To exclude unspecific annealing of primer M1 to sequences downstream of nucleotide 1017, we are currently constructing a new assay with primers 3’ of M1. We agree that problems with this primer may have general implications for the standardization of the CBFβ-MYH11 assay.

Apart from this technical question, we still note that two different transcripts unrelated to the primer sequence (5’ of MYH11 position 994) were simultaneously isolated in our patient. We are in the process of identifying the origin of one of them, which has not been found in the Genbank.

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Reticuloendothelial origin and metastasis of Kaposi's sarcoma: historical update on the literature [letter; comment]

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