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Letter to the Editor

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To the editor: We read with interest the article by Lapointe *et al*,[1] who reported a novel fusion of variant *TMPRSS2* isoform with *ERG*, resulting in a variant *TMPRSS2 (isoforms 2)-ERG* fusion in prostate cancer. This finding has important implications for accurate detection of *TMPRSS2-ERG* fusion transcript, since 10% of specimens in their series expressed only the novel variant isoforms *TMPRSS2-ERG* fusion, which could be missed using the published RT-PCR assays for the known *TMPRSS2* transcript.[2–6] This is a particularly important issue for the development of PCR-based diagnostic tests. For tissue-based FISH assays, the two *TMPRSS2* isoforms cannot be distinguished using the standard *ERG* break-apart assay.[2,4,6]

There appears to be some possibility of confusion regarding the ERG break-apart assays presented in their paper. The FISH images presented in Figure 3a are described as negative for the *TMPRSS2-ERG* fusion. Because *TMPRSS2* and *ERG* are located so close together on 21q, all non-rearranged alleles should be immediately adjacent to each other (or even overlapping). In this figure, it appears more consistent with a break-apart signal pattern, suggesting gene fusion. This may be due to overlapping nuclei, making it unclear which signals belong to which nucleus, and whether all signals have been captured, as they may not all be on the same plane. Figure 3b demonstrates very well the *TMPRRS2-ERG* rearrangement through deletion. There is no significant visible difference between the relative signal positions of the non-rearranged allele and the allele with the rearrangement. As the signal distance is not informative, the rearrangement only becomes obvious because of the missing green signal between the red and blue signals. The signal patterns in Figure 3c are equally confusing. As in Figure 3b, the rearrangement is most clearly shown by the absence of the green signals (indicating fusion through deletion). However, the signals for the non-rearranged allele in the upper-right nucleus seem to be too distant to be from the same nucleus. In Figure 3b and the other nuclei in Figure 3c, the non-rearranged aqua and red signals are immediately adjacent to each other. Also, there appears to be an additional non-rearranged allele in the large nucleus in the bottom center. We believe that these inconsistencies are due to overlapping nuclei as shown in Figure 3a.

We believe it is important to point out the ambiguity of the images to avoid possible confusion for the reader and in interpreting future *TMPRSS2-ERG* FISH assays.

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References

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In reply: In response to the letter by Drs Perner and Rubin, the apparent confusion appears to arise from a misunderstanding of the genome map location and/or the colors of the fluorescence *in situ* hybridization (FISH) probes used. As indicated in the legend to Figure 3 of the article,[1] the green/blue pseudocolored probes map, respectively, to the 5′ and 3′ portions of *ERG*, whereas the red

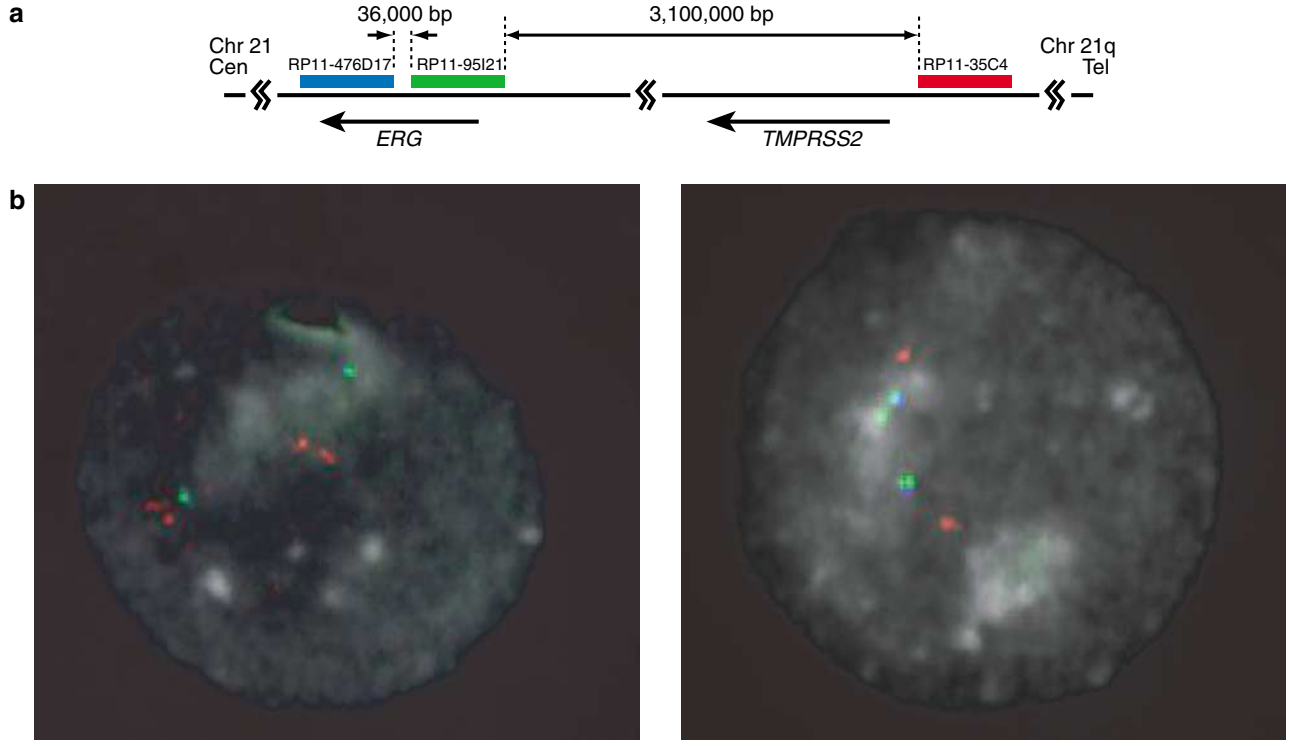

Figure 1 Three-color FISH assay of *TMPRSS2–ERG* rearrangement. (**a**) Schematic depiction of pseudocolored FISH probes in relation to *TMPRSS2* and *ERG* loci. (**b**) Three-color FISH analysis of representative normal human lymphocytes. Note that the red (*TMPRSS2*) signal can be observed at a substantial distance from the green/blue (*ERG*) signals; the latter two, by their proximity to each other, can appear aqua-colored.

pseudocolored probe maps telomeric to *TMPRSS2*. These relationships are shown schematically in Figure 1a. In Figure 3 of the article, the green/blue signals do not appear split apart, indicating, as reported, that the specimen is indeed negative for *ERG* rearrangement. The distance observed between the red (*TMPRSS2*) and green/blue (*ERG*) signals, which map to DNA sequences approximately 3 million base pairs apart on chromosome 21, varies based on the condensation state of the chromatin in the interphase nuclei and on the orientation of the FISH signals with respect to the photographed plane. It is not unusual to observe substantial spacing between the red and green/blue signals in normal nuclei (see Figure 1b). Thus, we believe that the interpretations of the FISH studies are accurate as reported.

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