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The unique alteration of electrophoretic mobility of fragile-X-expanded fragments in the presence of ethidium bromide

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The DNA intercalative dye ethidium bromide is commonly added directly to agarose gels and buffer prior to the electrophoresis of restricted DNA to allow visualization of migrated DNA after electrophoresis. Our laboratory routinely used this technique but we recently experienced a significant problem with the electrophoretic migration of specific DNA fragments in the presence of ethidium bromide.

Our laboratory employs a simple Southern blot assay for the detection of the CGG triplet repeat expansion associated with the fragile X syndrome (FRAXA). The restriction enzyme *Pst*I is used to cut isolated patient genomic DNA which is run out on a 1% agarose gel in TAE buffer (40 mM Tris-acetate, 1 mM EDTA, pH 8.0), supplemented with 5 μg/ml ethidium bromide. Electrophoresed DNA is next depurinated with 0.75 N HCl for 15 min, denatured with 1 N NaOH for 30 min and finally transferred by vacuum to a nylon membrane. Fragments are detected using a 440 bp *Bam*HI/*Xho*I subclone of PE5.1 (Ref. 1) which hybridizes downstream of the CGG expansion site and slightly upstream of the second *Pst*I cutting site. This yields restriction fragments containing the expansion region of 1–1.1 kb in normal individuals, greater than 1.7 kb in phenotypically affected individuals and 1.1–1.7 kb among 'phenotypically normal' carriers. A typical autoradiogram obtained before any problems arose is shown (Fig. 1a).

Our difficulties arose when the distributor of our agarose (ONCOR, Inc.) switched their manufacturer and sent us a different agarose. The electrophoretic mobility of fragile-X-positive samples through the new agarose (Infinity LE, cat#4330) demonstrated a drastic reduction in migration distance as well as a decrease in band resolution (smearing of the bands; see Fig. 1b). Normal samples, on the other hand, showed little or no change in electrophoretic mobility or band resolution. Furthermore, the difference was only apparent on the autoradiograms; the ethidium bromide gels appeared normal with good separation of the restriction fragments indicating complete digestion of the DNA. Moreover, the larger the expanded fragment the greater the decrease in electrophoretic migration and the poorer the resolution of the RFLPs. ONCOR stated that the old agarose (cat#S4300-1) was SeaKem LE (cat#50001) purchased in bulk from FMC, Inc. and further aliquoted under their label with no further modifications. We tested SeaKem LE at this point and achieved results similar to those obtained with Infinity LE. Because the problems clearly appeared to be with the agarose, numerous other types and lots of FMC agarose were tested. All agaroses tested showed the same inhibition of migration and decrease in resolution as initially experienced with the Infinity LE.

Further investigation led to the modification of our standard protocol. Ethidium bromide, normally added to a final concentration of 5 μg/ml, was excluded from a gel made from an older lot of SeaKem LE agarose and run in tandem with the same agarose supplemented with ethidium

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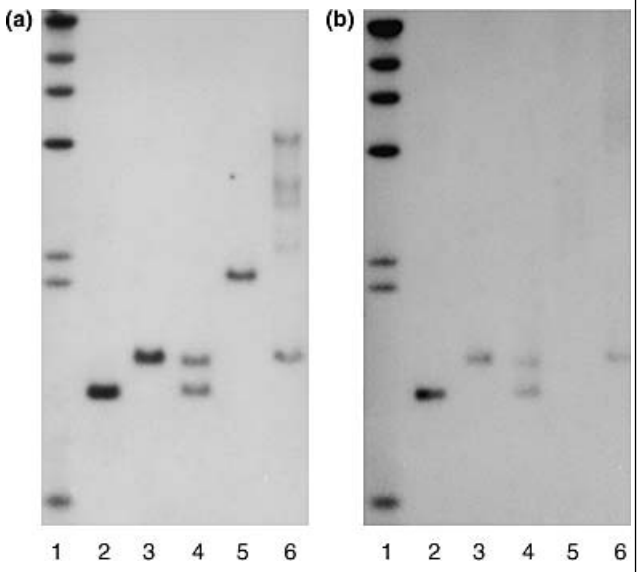

FIGURE 1. (a) Autoradiogram from ONCOR S4300 agarose gel supplemented with ethidium bromide. Lane 1, *Hin*dIII cut λ phage end-labeled with [α^{32}P]dCTP; lane 2, normal male; lane 3, fragile X premutation male carrier; lane 4, fragile X premutaion female carrier; lane 5, fragile X full mutation male carrier (single allele); lane 6, fragile X full mutation male carrier (4 major alleles present demonstrating somatic instability of full mutation). (b) Autoradiogram from Infinity LE agarose gel supplemented with ethidium bromide. All individuals and lane positions are identical to those in (a).

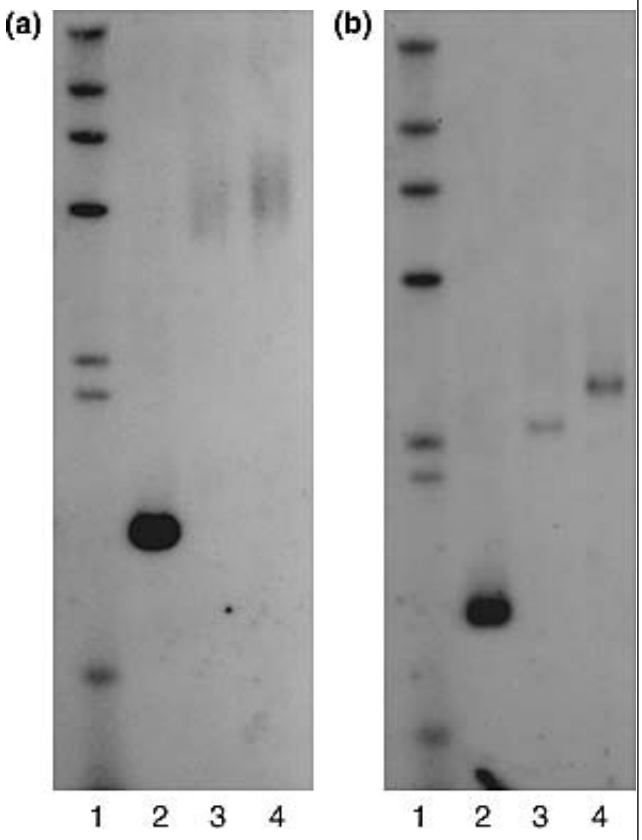

FIGURE 2. (a) Autoradiogram from SeaKem LE agarose gel supplemented with ethidium bromide. Lane 1, *Hin*dIII cut λ phage end-labeled with [α^{32}P]dCTP; lane 2, normal male; lanes 3 and 4, fragile X full mutation male carriers. (b) Autoradiogram from SeaKem LE agarose gel without ethidium bromide. All individuals and lane positions are identical to those in (a).

bromide. Unexpectedly, the unsupplemented run demonstrated a well-defined fragile-X-positive band on analysis of the autoradiogram compared with the smear evident on the supplemented agarose (Fig. 2). Further testing of the other problematic agaroses also yielded good results when ethidium bromide was not added before electrophoresis. The only difference between the ethidium-bromide-supplemented ONCOR S4300 agarose and all the other unsupplemented agaroses appears to be the expected reduction in overall migration of approximately 15% (Ref. 2).

Two phenomena are apparent from these experiments. First is the specific electrophoretic migrational inhibition of p(CGG)$_n$-expanded fragments in the presence of ethidium bromide. Second is the apparently unique 'non-interaction' between the S4300 agarose and the ethidium-bromide-intercalated p(CGG)$_n$-expanded fragments. FMC, Inc. ran several tests on a sample of the S4300 agarose including electroendomosis (a functional measure of the number of sulfate and pyruvate residues present on the agarose polysaccharide) and gelling temperature. The results showed the 'signature of SeaKem LE' (Doug Robinson, director of technical services. pers. commun.). Interestingly, Chastain *et al.* (Ref. 3) demonstrated that DNA fragments containing stretches of CTG or CGG triplet repeats migrated at a faster rate than expected through unstained polyacrylamide and agarose gels. Their results show, as do ours, the unique interaction between DNA fragments containing triplet repeats and their electrophoretic medium. The exact interaction between p(CGG)$_n$-expanded fragments and ethidium bromide and their electrophoretic mobility through the agarose matrix remains unexplained. Major conformational changes in these triplet-repeat-containing fragments could easily be implicated as the cause, but further studies are required. The purpose of this article, however, is not to elucidate this phenomenon thoroughly but to make researchers aware of this unexpected interaction between agarose and ethidium-bromide-intercalated DNA. We recommend that agarose gels are stained with ethidium bromide after electrophoresis, especially if the assay involves

detection of regions rich in CGG repeats. The amount of time saved by running ethidium bromide in a gel during electrophoresis is minimal (20–30 min) when compared to the problems that can arise from the misinterpretation of results.

References

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