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US/EG Bulletin 1736

PFGE Analysis of Chromosomal DNA from a Single Hair Root

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Introduction

Tumor diagnoses, DNA fingerprinting, and other genetic analysis often require DNA obtained in a non-invasive manner. For example, peripheral blood may be used as a source from solid tumors, but not for haematological malignancies. We first reported the isolation and pulsed-field electrophoresis of genomic DNA obtained from a single hair root.[1] Additional details are presented below.

Sample Preparation

One percent low melt agarose (SeaPlaque, FMC) was prepared in 1x PBS, 1 mM EDTA and added to a rectangular mold while molten. A single human hair was positioned in the middle of the block. After solidification, the block was placed in a NDS lysis solution consisting of 10 mM Tris, 0.5 mM EDTA, 1% sarcosyl, pH 9.5, 1 mg/ml Proteinase K, and 25 mM dithiothreitol. Blocks were incubated 24 hours at 50 °C, with a fresh change of lysis buffer after 24 hours. Blocks were rinsed three times in NDS and stored at 4 °C until required.

Enzyme digests were performed according to manufacturers directions. Prior to digestions, excess agarose was trimmed to within 2 mm of the hair.

Electrophoresis and Detection

The samples were resolved using the CHEF-DR® II pulsed-field electrophoresis system. The gel was 1.0% agarose (SeaPlaque) in 0.5x TBE buffer chilled at 14 °C. Electrophoresis was conducted at 200 volts for 24 hours with a 50-90 second switch time ramp.

Gels were first stained with ethidium bromide (Figure 1A). Southern blotting and hybridization were performed as previously described.[2,3] The autoradiograph of the gel with the hybridized, labeled DNA probe is shown in Figure 1B.

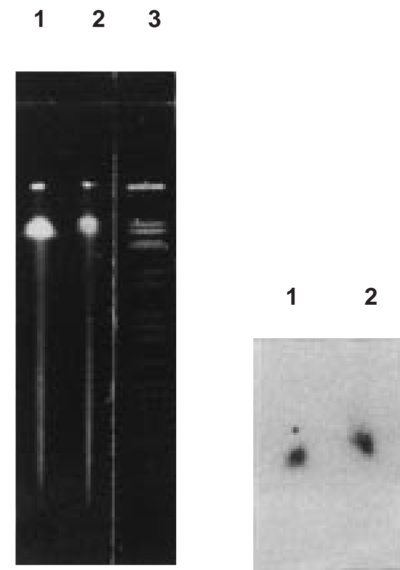

Fig.1. Detection of DNA obtained from a single human hair root. A) Ethidium bromide-stained CHEF gel. Lane 1: Peripheral blood DNA. Lane 2: hair root DNA. Lane 3: *S. cerevisiae* DNA marker (Bio-Rad). DNAs in lanes 1 and 2 were previously digested with restriction endonuclease *Mlu* I. **B)** Hybridization signal from blot probed with 5' genomic sequences encoding c-*fms*. Lane 1: peripheral blood DNA. Lane 2: hair root DNA.

Conclusion

We have shown that DNA may be prepared from a single hair root and detected after pulsed-field electrophoresis. Smaller-sized DNAs (*e.g.* < 20 kb) may be analyzed on conventional submarine gels using the same sample preparation method as the above analysis. Based on earlier analyses using 40 hair roots, we estimate that a single hair yielded 0.1-0.2 micrograms total genomic DNA. Conventional phenol/chloroform extraction procedures typically result in substantial loss of DNA. It is possible that other small biological structures, both plant and animal in origin, could be analyzed using the agarose block method described here.

Acknowledgements

This work was supported by the Leukemia Research Fund of the United Kingdom. The probes were kindly provided by Professor A. Jeffreys and Dr. N. Spurr.

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Bulletin 1736 US/EG REV A 95-0695f 1295 *SIG 020996* *Printed in USA*