

UV Nicking of DNA in Agarose Gels for Enhanced Transfer and Detection of Megabase Size DNAs

Introduction

The transfer efficiency of large (>100 kb) DNAs from agarose gels to membrane is poor unless the DNAs are nicked prior to transfer. Although this has traditionally been accomplished with HCl depurination, UV irradiation (254 nm) provides a faster and more consistent alternative to HCl nicking. The GS Gene Linker® UV chamber contains preprogrammed routines for many UV crosslinking and other controlled dosage applications, including UV nicking of pulsed field gels prior to transfer to membrane. The chamber's unique sensor measures only the effective wavelengths of UV light, insuring reproducible results every time.

Results

High molecular weight DNA from a human HeLa cell line was cleaved with various restriction enzymes, resolved on the CHEF Mapper™ XA pulsed field electrophoresis system using the Auto Algorithm mode (50-750 kb), and irradiated with 60 mJoules of energy in the GS Gene Linker UV chamber after staining with ethidium bromide. The DNA was alkaline transferred to Zeta-Probe® GT membrane, and probed with a human T cell receptor variable region (single copy gene) probe. As illustrated in Figure 1, bands ranging in size from 100 kb to greater than 700 kb were detected by the probe in the DNA lanes.

UV Nicking Protocol

Note: This protocol must be followed rigorously to obtain optimal results.

1. Following electrophoresis, stain the gel with 1.0 µg/ml ethidium bromide (EtBr) for exactly 30 minutes with constant agitation. Use a fresh dilution of EtBr for each gel. Do not destain the gel.
2. Place the gel on a glass tray for transportation to the GS Gene Linker chamber, place the tray inside the GS Gene Linker chamber, and close the door.
3. Irradiate the gel with 60 mJoules of energy (nic program). The gel can now be photographed, but further exposure to UV light must be minimized (<10 seconds). The gel can be destained prior to photography if desired.

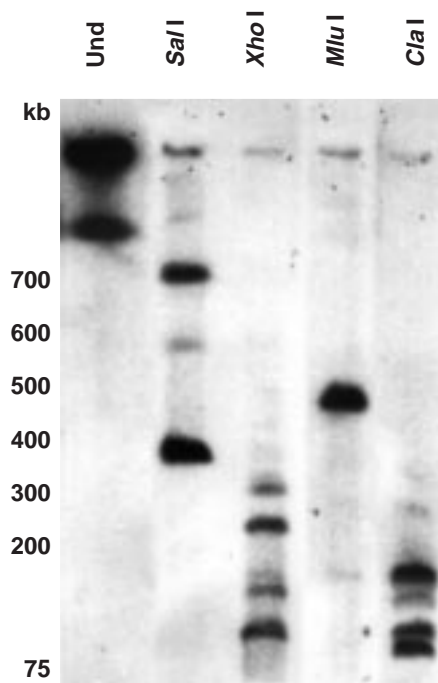


Fig. 1. Southern genomic hybridization analysis of restriction digests of human HeLa cell line DNA probed with a human T cell receptor variable region probe. The DNA was resolved on the CHEF Mapper system, nicked in the GS Gene Linker chamber, and transferred to Zeta-Probe membrane. Courtesy of Dr. E. Lai, University of North Carolina.

4. Soak the gel in 0.4 N NaOH, 1.5 M NaCl for 15 minutes. Transfer the DNA for at least 16 hours to Zeta-Probe GT membrane according to standard procedures, using 0.4 N NaOH, 1.5 M NaCl as the transfer solvent.

This protocol is for use with the GS Gene Linker UV chamber, with gels approximately 6 mm thick. If thicker gels are used, the staining period should be prolonged. DNA that is not stained with EtBr will not be nicked by UV irradiation, and will not transfer efficiently.

Ordering Information

Catalog Number	Product Description	Price	Catalog Number	Product Description	Price
165-5031	GS Gene Linker UV Chamber, 120 VAC/60 Hz, includes five 254 nm bulbs, applications card, and instruction manual	\$1,295.00	162-0190	Zeta-Probe GT Membrane, sheets, 9 x 12 cm, 15	\$90.00
165-5032	GS Gene Linker UV Chamber, 220 VAC/50 Hz, includes five 254 nm bulbs, applications card, and instruction manual	1,350.00	162-0191	Zeta-Probe GT Membrane, 10 x 15 cm, 15	110.00
165-5033	GS Gene Linker UV Chamber, 240 VAC/50 Hz, includes five 254 nm bulbs, applications card, and instruction manual	1,350.00	162-0192	Zeta-Probe GT Membrane, sheets, 15 x 15 cm, 15	130.00
165-5034	GS Gene Linker UV Chamber, 100 VAC/50 Hz, includes five 254 nm bulbs, applications card, and instruction manual	1,350.00	162-0193	Zeta-Probe GT Membrane, sheets, 15 x 20 cm, 15	175.00
165-5035	GS Gene Linker UV Chamber Replacement Bulbs, 254 nm, 5	95.00	162-0194	Zeta-Probe GT Membrane, sheets, 20 x 20 cm, 15	235.00
			162-0195	Zeta-Probe GT Membrane, sheets, 20 x 25 cm, 3	70.00
			162-0196	Zeta-Probe GT Membrane, roll, 30 cm x 3.3 m, 1	225.00
			162-0197	Zeta-Probe GT Membrane, roll, 20 cm x 3.3 m, 1	175.00
			162-0198	Zeta-Probe GT Membrane, roll, 30 cm x 30 m, 1	1,490.00

For information on the GS Gene Linker UV chamber, request bulletin 1667. For more information on the CHEF Mapper XA pulsed field electrophoresis system, request bulletin 1604. For more information on Zeta-Probe GT membrane, request bulletins 1110 and 1645.

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