

PCR-aided DNaseI footprinting of single copy gene sequences in permeabilized cells

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Ligation mediated PCR (LMPCR) has been used for in vivo dimethylsulfate (DMS) footprinting (1, 2) and genomic sequencing (3). Genomic sequencing by LMPCR uses a linker-ligation step to introduce a common oligonucleotide sequence to DNA ends. This enables all linker-containing molecules to be PCR amplified by use of a linker-specific primer along with a gene-specific primer. LMPCR amplification gives the sensitivity and specificity necessary to analyze single copy genes in relatively small amounts of DNA. Successful ligation of the linker rerquires a 5′ phosphate group on the genomic DNA fragments, a feature provided by DNaseI cleavage. We report here that DNaseI footprinting can be done for single copy genes on permeabilized cells. Y162−11C is a Chinese hamster human hybrid cell line containing an active human X chromosome (4).

Permeabilized cells were prepared by treating cell monolayers (4×10^6 cells) with 0.5 mg/ml lysolecithin for 1 min at 37°C as described by Zhang and Gralla (5). These cells were treated with DNaseI (25 to 100 μg/ml) at room temperature for 10 min (5) and DNA then isolated (3). Controls were obtained by DNaseI digestion (2 μg of DNA with 0.1 to 0.2 μg/ml DNaseI for 2 min at room temperature) of purified DNA from Y162−11C cells. DNA fragments containing human phosphoglycerate kinase-1 (*pgk-1*) gene sequences were specifically amplified by LMPCR (3). Direct LMPCR amplification of DNaseI generated fragments resulted in a low amplification efficiency and high background. Results were considerably improved when the 3′ OH groups of genomic DNA fragments were first blocked by addition of a dideoxynucleotide. After DNaseI treatment, DNA (10 μg in 50 μl) was denatured and incubated with 5 units of Sequenase 2.0 (USB), 5 μM ddNTPs in 40 mM Tris-HCl, pH 7.7, 25 mM NaCl, 6.8 mM $MgCl_2$ for 20 min at 45°C. The DNA was then denatured again and incubated with 30 units terminal transferase (BRL) at 37°C for 30 min in the same reaction mixture supplemented to 200 mM potassium cacodylate, pH 7.0, 1 mM 2-mercaptoethanol. After phenol/chloroform extraction and selective ethanol precipitation, the samples were processed for LMPCR as described (3). Figure 1 shows in vivo DNaseI digestion patterns compared to the digestion of naked genomic DNA. The region shown includes the transcription start site and upstream sequences of *pgk*-1 (6). When compared to naked DNA, permeabilized cells show several distinct DNaseI hypersensitive bands (Figure 1, closed symbols) and protected bands (Figure 1, open symbols). The nucleotides protected in vivo from DNaseI attack are located within two adjacent SP1 consensus sequences (nt. −40 to −65) previously shown to be protected in vivo from DMS modification (2). Hyperreactive sites may indicate regions of higher order nucleoprotein structure. The purpose of this report is to communicate that it is feasible to use enzymes and LMPCR-aided genomic sequencing for the in vivo study of single-copy genes.

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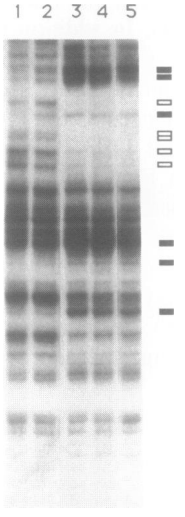

Figure 1. In vivo DNaseI footprinting by LMPCR. Primers specific for the *pgk-1* promoter region (5′-CGTCCAGCTTGTCCAGC, and 5′-TCCAGCGTCAGC-TTGTTAGAAAGCG, nt. + 134 to +99) were used for LMPCR (3). Lanes 1 and 2 show the DNaseI digestion pattern of 2 μg of purified genomic DNA from Y162−11C cells. Lane 1, 0.1 μg/ml DNaseI; lane 2, 0.2 μg/ml DNAseI. Lanes 3 to 5: in vivo DNaseI footprinting of permeabilized cells. Lane 3, 25 μg/ml DNaseI; lane 4, 50 μg/ml DNAseI; lane 5, 100 μg/ml DNaseI (2.5 μg DNA per lane). Open symbols, protected nucleotides; closed symbols, hyperreactive nucleotides.

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