

Express protocol for generating G+A sequencing ladders

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The precise position in a DNA sequence is determined in a wide spectrum of assays, by electrophoretic separation and comparison with size marker sequencing ladders analysed in parallel. Such assays include localisation of transcription initiation sites and a variety of DNA–protein interaction studies. For many purposes, a single lane of a Maxam–Gilbert G+A ladder then provides adequate information as size markers.

We here report a procedure for generating G+A ladders which greatly reduces the time and which is considerably simpler to perform compared to the canonical Maxam–Gilbert sequencing reaction (1). The procedure is based on partial acidic hydrolyses of DNA, in the presence of diphenylamine and proceeds via depurination/5′,3′-elimination (2).

In a typical experiment, the reaction mixture was prepared by mixing 10 µl of a solution of 3% diphenylamine in formic acid with 5 µl of DNA-solution, containing 0.1–5 µg of DNA (the amount of DNA can be increased to 10–20 µg with a proportional increase of all other components). The tube was allowed to stand at room temperature for 3–5 min and 100 µl of 0.3 M sodium acetate (pH 5.5) was then added. The 'milky' mixture obtained was extracted three to four times with water-saturated ether. The tube was left for 5 min at room temperature or 2–3 min at 37°C, to discard traces of ether and the DNA was subsequently precipitated by addition of 2.5 vol of ethanol. Carrier DNA can be added if desirable. DNA was pelleted for 15 min at 15 000*g* and resuspended in loading buffer. The entire procedure takes ~30 min.

In Figure 1 we have used this method in *in vivo* DNaseI footprinting experiments and detection of the DNAs, including the ladder by electroblotting and indirect end-labelling by hybridisation. The G+A ladder can also be detected via labelling of the DNA fragment prior to hydrolysis or via linear amplification with *Taq* polymerase and a kinased primer (data not shown).

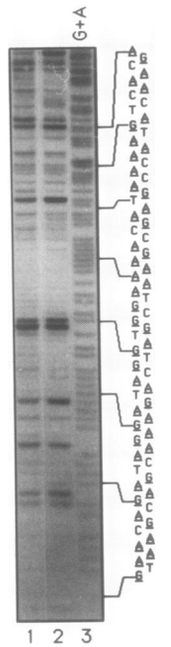

Figure 1. Genomic footprinting (3) of tandem repeats in the Balbiani ring 2.1 gene of *Chironomus tentans* (4). 1. *Chironomus tentans* DNA digested with DNase I. 2. DNA isolated from tissue culture cell nuclei digested with DNase I. 3. G+A ladder of the coding strand of the Balbiani ring 2.1 gene tandem repeat obtained according to the protocol presented here. After electrophoretic separation, the DNA fragments were electroblotted onto a nylon membrane and visualised by hybridisation with a ^{32}P-labelled probe corresponding to a 53 bp fragment of the tandem repeat of the gene.

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