Analysis of DNA curvature using circular permutation of 5' end labelled fragments

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In studies of DNA curvature, DNA molecules of the same size in which the position of the bend is changed are obtained by circular permutation through cloning a tandem dimer (1) and this has been facilitated by construction of special plasmids (2, 3). A rapid procedure for such studies consists of cyclization or concatemerization of 5' end labeled fragments with regions of interest (alone or precloned in a reporter fragment) followed by digestion with single site enzymes (Fig. 1).

To cyclize, a few ng are ligated overnight at 15°C using DNA concentrations of 50—100 ng/ml (for a fragment of about 250—450 bp, respectively) and 50 U/ml T4 DNA ligase in 50 mM Tris—HCl, pH 7.7, 10 mM MgCl2, 0.2 mM ATP, 10 mM DTT and 50 µg/ml BSA. During subsequent phenol—chloroform extraction, carrier DNA (usually pBR322) is added. Equal aliquots of the dissolved pellet that may contain less than 1 ng of labeled fragment but should contain no less than about 100 ng pBR322 are digested each with one of unique site enzymes. In studies of intrinsic curvature, phenol extraction of digests is not needed before loading on gels. When used in protein-induced curvature, the experiment is fully quantitative because of the constant specific radioactivity in different permutation contexts.

Concatemerization (15—25 µg/ml DNA during ligation that may include added unlabeled fragment) is used for studies (on intrinsic curvature only) of fragments that are too short to cyclize or as a first step to permute an unlabeled blunt ended fragment, to a context with 5' protruding ends that can be then labeled. Subsequent digestion with unique cutters should produce 5 products: two half-molecules (in very low amounts due to high DNA concentrations), one of the two possible palindromic dimers of each half-molecule, and the permuted fragment (only these are shown in Figure 1A). Ambiguities may arise with cleavage sites in the center or at one-third from one end of the original fragment.

Figure 2 shows that cyclization of small amounts of DNA in separate tubes produced reasonable recovery leaving little linear fragment, and that favoring the formation of one or the other topoisomer product did not seem to affect the outcome of subsequent digestion. Other hints: Ends labeled with ATP (3000 Ci/mmol) to the highest practical level (unnecessary for this method) produced circles usable for no less than 8 days. A 2 bp GC overlap was the shortest tested and found to suffice. Carrier DNA need not contain the cleavage site (Figure 1A, lane 1’) and seems to be replaceable by salmon sperm DNA; poly(dl)·poly(dC) or poly(dl-dC)·poly(dl-dC) produced partial digestion.

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REFERENCES

Figure 1. A 26 bp DNA (5' GATCTAATAAAACATAAAAATAATAG 3' and its complement to produce 5' GATC ends) with homology of an early vaccinia virus promoter was cloned in the BgIII site of a 150 bp (reporter) MluI fragment (4) as a single (A) or divergent double (B) insert, which was then concatemerized or cyclized, respectively, before digestion with enzymes identified in A (sites at vertical interruptions above the lines with MluI junctions below; arrows localize inserts). BglII digestion in B is partial due to cruciform exit. Apparent sizes (on 8% gels) were 180 to 193 in A and 210 to 222 in B (marker lanes show pBR322 Mspl fragment).

Figure 2. DNA recovery during cyclization and cleavage. Aliquots (1.3 ng) of a labeled 406 bp XbaI fragment were either loaded (lane 1) on a 4% gel (top on the left) or ligated in two pairs of tubes in 15 µl volumes in presence of 0.15 µg/ml ethidium bromide (lanes 2 and 4) or its absence (lanes 3 and 5). Lanes 4 and 5 are cyclization products. Lanes 2 and 3 show the linear (L) products after digestion at the unique HinfI site. The major product of ligation has a linking difference of +1 compared to topoisomer 0, defined as the one closest to relaxed state, which becomes the major product by including ethidium bromide during ligation.