Volume 14 Number 22 1986 Nucleic Acids Research

Restriction mapping by preferential ligation of adjacent digestion fragments

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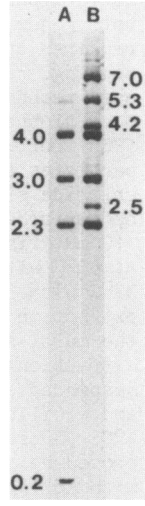
Submitted 6 October 1986

I present a simple, rapid method to define the order of sites recognized by a single restriction enzyme in a circular or linear DNA molecule. The specific subset of type 2 restriction endonucleases that can be used generates protruding single stranded 3' or 5' termini of undefined sequence (eg. BglI, BstXI, SfiI). For an infrequent cutter, it is unlikely that these sequences are identical at different loci in a single DNA molecule. This probability is, for example, the product of the number of sites (n) and 1/64 for a 3 base pair overhang. Therefore, the rapid religation of fragments produced by a complete digestion results primarily in the formation of bimolecular products composed of fragments occurring at neighboring positions in the uncut DNA molecule.



Since the reaction is terminated at an early stage, both reactants and ligation products are present. For a circular or a linear molecule with cohesive termini, n religated fragments are expected. The map for such a linear molecule will be circularly permuted. Linear molecules with incompatible termini result in a linear map with n-1 religation products. The nearest neighbor relationships of fragments in the complete digestion are deduced from the sizes of the religated products (which are the sum of the lengths of the constituent fragments) on agarose gels. The method depends on the accurate determination of these fragment lengths.

product	components
2.5	2.3, 0.2
4.2	4.0, 0.2
5.3	2.3, 3.0
7.0	4.0, 3.0

The reaction should be carried out under conditions which limit the formation of products containing more than 2 ligated constituents. The duration of the reaction and the T4 DNA ligase concentration should be optimized. We religate at a DNA concentration of 30 ug/ml for 5' to 10' at 17° C while varying the enzyme concentration between 0.2 and 20 Weiss units/ul. The figure shows the religation of BglI fragments generated from a digestion of recombinant SV40 viral DNA fused to pBR322 at their respective BamH1 sites. The complete BglI digest of this construct contains fragments of 0.2, 2.3, 3.0, and 4.0 kb (lane A). In addition to these bands, ligation products of 2.5, 7.0, 5.3 and 4.2 kb are observed (lane B). A unique ordering of sites can be deduced in this case (see accompanying table and map). Sometimes, the assignment of components of a ligation product appears to be equivocal because other pairwise combinations could generate a fragment of the same length. By initially constructing the map with unambiguous assignments, incorrect bimolecular fragment combinations are usually eliminated.