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Improved control of partial DNA restriction enzyme digest in agarose using limiting concentrations of Mg^{++}

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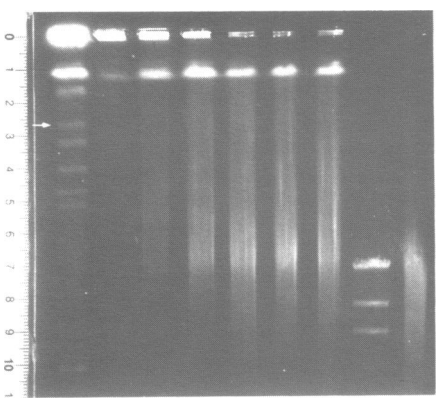
Partial digests of DNA for cloning experiments are usually performed using either the quantity of restriction enzyme or incubation time, or both, as controlling factors. However, we have found that more precise and reproducible partial digests are obtained, when a limiting concentration of the required cofactor Mg^{++} is used. This method is especially useful for partial digests of DNA contained in agarose. In agarose, diffusion time of the much smaller Mg^{++} ion is shorter than that of a restriction enzyme. The result is a more homogenous digestion of the DNA throughout the agarose block. Since the concentration of Mg^{++} in this procedure is usually only 10% or less of that normally used, the digestion is easily interrupted by excess EDTA.

1) Equilibrate at 4°C agarose blocks (0.5% SeaPlaque,FMC) each of 100 µl volume containing 5-20 µg of DNA, in 10 vol. of an appropriate restriction buffer lacking Mg^{++}.
2) Replace the buffer with 100 µl of fresh Mg^{++} free buffer per block, supplemented with 5 units of restriction enzyme per µg of DNA. Incubate at 4°C for 30 min.
3) Transfer the samples to the proper incubation temperature and add Mg^{++} from a $MgCl_2$ stock solution, to final concentrations varying from 0.01 mM to 1.0 mM. Incubate for one hour. As a complete digest control, adjust one sample to 10 mM $MgCl_2$ with 10 units/µg of restriction enzyme.
4) Stop the reaction by aspirating the incubation buffer and replacing it with 2 ml. of cold TE (10mM,10mM).



The figure shows a series of partial EcoRI digests size separated by CHEF. Lanes 1 to 9:
1) YAC 6B5 chromosomes,
2) Incubation with enzyme, but without Mg^{++}.
3) 0.01 mM Mg^{++},
4) 0.03 mM Mg^{++},
5) 0.10 mM Mg^{++},
6) 0.30 mM Mg^{++},
7) 1.00 mM Mg^{++},
8) λ-phage HindIII marker. (only three top bands)
9) Total digest

We have successfully applied this partial digestion technique with EcoRI to obtain large human DNA fragments that have been used to construct artificial yeast chromosomes (YAC) of several hundred kb in length. The arrow indicates one such novel chromosome of 490 kb.

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