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Cloning of all *Eco*RI fragments from phage λ in *E. coli*

CLONING of DNA fragments is a new powerful technique in molecular genetics and a number of interesting experiments involving both prokaryotic and eukaryotic genomes have already been reported (see ref. 1 for review). We present here results concerning the cloning of all *Eco*RI (ref. 2) fragments from phage λ DNA in *Escherichia coli* using as a vector plasmid pSC101 (ref. 3). To achieve this, we had to solve the problem of propagating the λ fragment containing the immunity region since the phage strain used had a temperature-sensitive mutation in that region.

DNA from the lysogenising strain cI857 of phage λ and the supercoiled form of plasmid pSC101, were digested at 37 °C with restriction enzyme *Eco*RI (prepared according to a modification of the method of Yoshimori[4]) in 100 mM Tris, *p*H 7.5, and 10 mM $MgCl_2$ (ref.5). After digestion was complete, as judged by electrophoresis of the DNAs, *Eco*RI was inactivated by heating the incubation mixtures at 65 °C for 5 min. The two digests were then mixed together, slowly chilled to 0 °C to allow reannealing of the "sticky ends" formed by *Eco*RI to occur and incubated for 16 h at 0 °C and 1 h at 14 °C in 50 mM Tris, *p*H 7.5, 10 mM $MgCl_2$, 1 mM EDTA, 0.035 mM ATP, 10 mM dithiothreitol[6] and polynucleotide ligate from T4-infected *E.coli* (from Miles or from Dr F. Rougeon). The ligated mixture was used to transform strain HB 101 (ref. 7) of *E.coli*. The transfectants were plated on L agar[8] containing 15 (or 10, ref. 9) $\mu g\ ml^{-1}$ tetracycline, grown overnight at 37 °C and further purified on plates containing tetracycline. Selection of recombinant clones was carried out either by a group screening using gel electrophoresis, or by the hybridisation technique of Grunstein and Hogness[10].

In an experiment, in which 85 clones were screened in groups of 7–8, at least 10 were found to contain λ fragments 3, 4 or 5 (the numbering of the six *Eco*RI λ fragments is according to decreasing molecular weight[11]); these range in molecular weight from 3.5 to 3×10^6. One additional clone contained two λ fragments, 3 and 5, which are non-adjacent in the genome and account together for 6.5×10^6 of DNA. Still another clone contained fragments 1 and 6; these fragments are bound together through the natural cohesive ends of λ DNA; they contain the whole left arm of the genome and have a molecular weight of 16×10^6; obviously, these fragments cannot be inserted separately since each one of them lacks an *Eco*RI sticky end in the linear form of λ DNA. The easier integration of low molecular weight compared to high molecular weight fragments is expected; our failure to find recombinants harbouring λ fragment 2, which has a molecular weight of 4×10^6, was, therefore, at first sight, surprising.

An experiment in which λ fragment 2 was eluted from Agarose[12], ligated with pSC101 and used for transfection, did not yield any recombinant plasmid, in spite of the fact that 600 colonies were examined by the hybridisation technique: in contrast, a parallel experiment with fragments 3 and 4 was successful; this suggests that our negative results with fragment 2 were not due to the fact that fragments eluted from Agarose cannot be ligated.

Since fragment 2 comprises the λ immunity region, which is the locus of the temperature-sensitive mutation that induces the lysogen at 37 °C, an experiment was carried out in which Agarose-eluted fragment 2 was ligated to pSC101 and transfectants were grown overnight at the permissive temperature of 30 °C. In this case, 4 out of 600 transfectants were found to be positive by

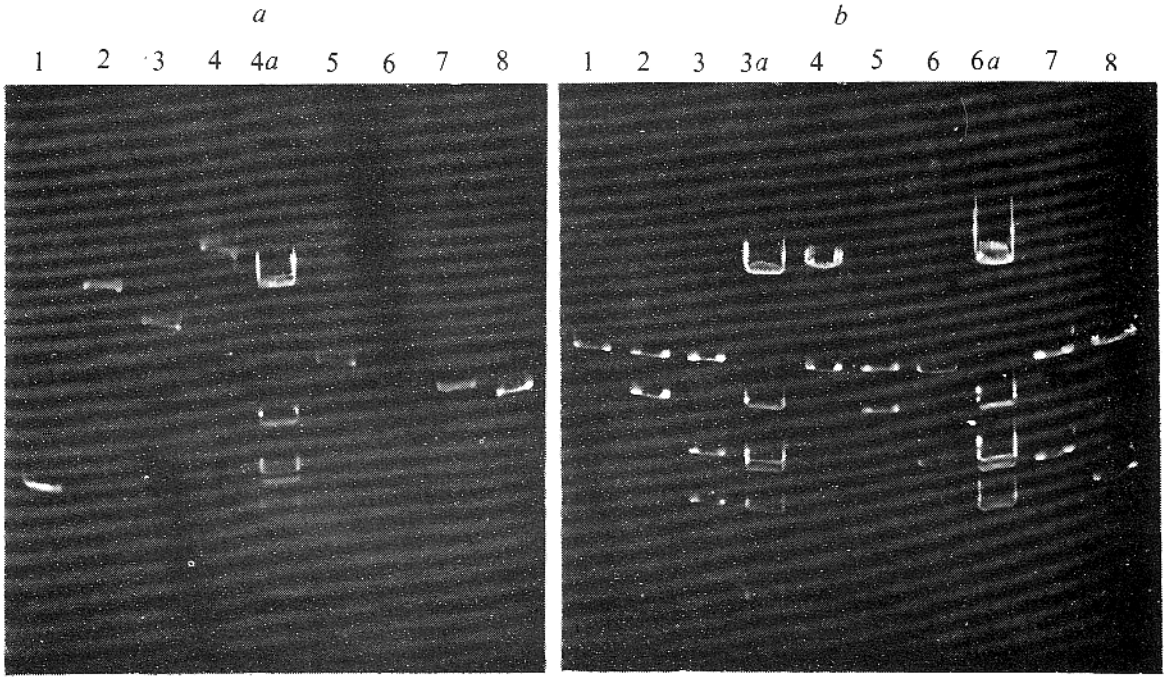

Fig. 1. Electrophoresis of recombinant pSC101-λ fragment plasmids before (*a*) and after (*b*) *Eco*RI digestion. *a*, Slots contain: 1, supercoiled circular pSC101; 2, pSC101 + fragment 2 (two copies); 3, pSC101 + fragments 3+5; 4, pSC101 + fragments 1+6; 4*a*, *Eco*RI fragments of λDNA; 5, pSC101 + fragment 2; 6, pSC101 + fragment 3; 7, pSC101 + fragment 4; 8, pSC101 + fragment 5. *b*, Slots 1–8 contain the same plasmids as in *a* after digestion with *Eco*R1; slots 3*a* and 6*a* contain *Eco*RI digests of λDNA; in slot 3*a* fragments were heated for 5 min at 70 °C to show the separation of fragments 1 and 6, which gives a single band in non-heated *Eco*RI fragments (slot 6*a*).

the hybridisation test. The plasmids prepared from three colonies contained a single copy of fragment 2, whereas the fourth clone contained two copies of this fragment.

It may be interesting to remark that recombinant plasmids containing λ fragments may themselves be useful in that they represent new vectors carrying restriction sites brought in by the λ fragments which may not exist in the parent plasmids.

Figure 1 presents the gel electrophoresis results obtained with the recombinant plasmids described above, before and after *Eco*RI digestion.

These experiments were carried out in low-risk containment conditions, as defined by the Asilomar guidelines. All the clones described in the present paper are available on request.

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