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Cloning *E.coli* genes by oligonucleotide hybridization

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Until now, the cloning of specific genes from E.coli or close relatives still largely depends either on the complementation of defined chromosomal mutations (1) or on the overproduction of the desired enzymatic activity (2). Traditional hybridization procedures (3) of plasmid or phage libraries are generally avoided due to the undesirably high background caused by the host chromosomal copy. Recently, it has been shown (4) that the use of bacteriophage M13 as a cloning vector, combined with a very simple blotting method allowing to transfer only phage single-stranded DNAs, should obviate this problem. However, the analysis of the clones obtained by this procedure, in that case containing Chlamydomonas reinhardtii DNA hybridizing at low stringency with an E.coli rpoC probe, was not reported. Here we show that, using this method, classical oligonucleotide hybridization can be used with high selectivity and efficiency to rapidly clone E.coli genes. As an example, we report the cloning of a 3.5 Kbp PstI fragment carrying the whole gene coding for the E.coli methionine aminopeptidase (map) which overlaps the fragment already described (5; Fig.2) and contains 2.3 Kbp of the 5' region.

A 30-mer oligonucleotide corresponding to codons 1-10 of map was synthesized, purified and used as a probe in the following experiments. Since the absence of PstI site in map was known (5), the size of the PstI fragment from the E.coliK12 C600 chromosome carrying the gene was first estimated by Southern blot hybridization. A single chromosomal fragment of about 3.5 Kbp was detected with the probe. Such a preliminary estimation allowed us to construct a size-restricted genomic library and therefore to screen only a few hundreds recombinant clones. A total PstI digest of C600 DNA (30µg) was electrophoresed on a 0.8% agarose gel; fragments from ≈3 to ≈4.5 Kbp were electroeluted from gel slices and ligated to the PstI-cut replicative form of M13mp19. This phage was used because of its enhanced stability when harbouring large inserts in the E.coli strain JM105 (6), as compared with previous M13 vectors and host strains.

A total of 6 transformation plates containing about 100 white and 200 to 300 blue plaques were used directly in the screening. Such plates usually allow an easy identification of a given individual plaque. Plaques were transferred as described (4) onto nitrocellulose filters. Duplicate filters are not necessary, given the high signal/noise ratio of these experiments. Briefly, circular filters are carefully placed onto the surface of plates, previously chilled during 1 h at 4°C, and removed after 5 min, without tearing the top-agar overlay. Filters are washed 4 times during 15 min in 10xSSC buffer under gentle agitation. After a brief rinse in TE buffer, filters are then placed onto Whatman 3MM paper, air-dried and baked in a vacuum oven at 80°C during 1 h. Standard hybridization conditions (7,8) were then followed to identify plaques specifically hybridizing with the 30-mer probe. Up to 5 filters are placed in a boilable bag containing 10 ml of prehybridization solution (5 x SSC; 10 x Denhardt; 2% SDS) and the sealed bag is incubated 1 h at 65°C. Following this, the prehybridization solution is removed and the filters are air-dried, then placed in a new bag containing 10 ml of probe solution (0.1 µg of 5'-labeled oligonucleotide in 5 x SSC; 10 x Denhardt; 40% formamide). It is not necessary at this step to separate the labeled probe from unincorporated label. Hybridization is carried out overnight at 50°C. Filters are then washed three times at 45°C in 2 x SSC, 0.02% SDS. After autoradiography for ≈ 2 h without screen, three independant clones on a

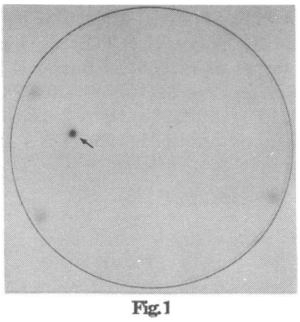

Fig.1

total of about 600 white plaques were detected by a very dark spot over an almost undetectable background (Fig.1) and were further purified by a single streaking on a JM105 lawn. These clones were found identical by restriction mapping and contain the same 3.5 Kbp fragment. Sequence analysis showed that they carry the whole map gene, as expected, and overlap 1244 bp of the previously cloned fragment (5; Fig.2). We found no evidence suggesting that the recombinant phages, which are 50% larger than wild-type M13mp19 phages, were unstable. This suggests that this type of phage can be a suitable vector, in JM105, for such a cloning experiment.

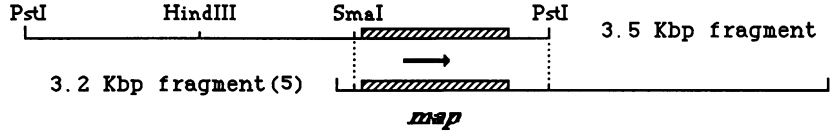

Fig.2

In conclusion, we have demonstrated that oligonucleotide hybridization provides a very efficient way to clone homologous genes in E.coli. The whole described procedure, from the synthesis of the probe up to the sequence analysis of the recombinant phages can be carried out in three weeks. Besides its use in finding overlapping sequences to a given fragment, the main application of this method should be to clone genes from E.coli or related organisms, for which a partial aminoacid sequence of the corresponding product is known, especially when no characterized mutation can be used in a complementation test. In this case, this method is indeed by far more simple than the screening of an overproduced enzymatic activity, particularly since peptide sequencing techniques are getting very sensitive. Modifications from the procedure described above should include the use of a partially degenerated probe, as in cloning experiments of genes/cDNAs from higher organisms, and the construction of a genomic library from partially digested DNA. Hybridization conditions should be adapted, of course, to the used probe. Given the very important signal/noise ratio found in the above experiment, these adaptations should not appreciably modify the efficiency of the described technique.

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