Insertion of rare cutting sites nearby genes allows their rapid physical mapping: localization of the *E.coli map* locus

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High (1) and low (2, 3) resolution restriction maps can assist rapid physical mapping of genes on the *E. coli* chromosome. An efficient manual method (4) already allows alignment of restriction map data of cloned genes on high resolution genomic restriction maps. Here, we describe a physical method for rapidly mapping genes on low resolution genomic restriction maps. This method was used to determine the location of the methionine aminopeptidase (*map*) gene (5) in *E. coli*.

The cloned *map* (5) gene was modified (a) by placing the chloramphenicol acetyltransferase (*cat*) gene directly downstream to *map* (*map - cat*) and (b) additionally by inserting a linker (GATCGATCGATGCGGCCGCCATGGCC) containing M.*Cla* I/ *Dpn* I(6), *Not* I, and *Sfi* I sites between the two genes (*map*-linker-cat). DNA fragments containing modifications (a) or (b) were transformed into *E. coli* strain H205 (*recB*$^-$, *recC*$^-$, *sbcB*$^-$). Chloramphenicol resistant transformants arise when these fragments replace the chromosomal *map* locus by homologous recombination (7). The location of the integrated fragments was determined by PFG analysis of *Not* I digested chromosomal DNA. Examination of an ethidium bromide strained gel revealed that *Not* I fragment D (275 kb) had increased slightly in size in cells containing *mat-cat*, while in cells containing *mat*-linker-*cat* (see fig.) fragment D was cut into two smaller pieces (d: 110 and d': 165 kb). The latter construct marks the location of the *map* gene with rare cutting sites. Precise localization requires that the orientation of the new *Not* I fragments be determined by hybridization with a gene from one end of the parental *Not* I fragment. The *lps* gene contains both *Not* I and *Sfi* I sites (2, 3) and maps to the proximal end of fragment D at 1 min. Since the *lps* gene hybridizes to the new 165 kb *Not* I fragment the *map* gene is located at about 4 min. Hybridization with the *map* gene confirmed the chromosomal *map* locus is on parental *Not* I fragment D and show that it is on the 110 kb derivative fragment. Since the linkers are positioned 3' to the gene, the *map* gene is transcribed counterclockwise on the chromosome. These results were confirmed by similar experiments using *Sfi* I cleavage and by DNA sequence analysis of the region flanking the *map* locus which reveals homology with the *rpsB* gene located at 4 min. The approach allows physical mapping of essential genes. The use of vectors that contain rare cutting sites will expedite these experiments. This work was supported by a grant from DOE (DG-FG-02-87ER-GD825).

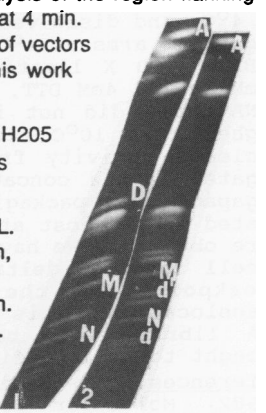

Fig. *E. coli* chromosomal DNA cut with *Not* I. Genotypes are lane 1. H205 (*map*+) and lane 2. H205 (*mat*-linker-*cat*). Some *Not* I fragments (A, D, N, M, d and d' (modified D)) are indicated.

References: 1. Kohara, Y. *et al.* 1987. Cell **50**, 495. 2. Smith, C. L. *et al.* 1987. Science **236**, 4481. 3. Condemine, G. and C. L. Smith, 1988, In. The Bacterial Chromosome, ed. M. Riley and K. Drlica, J. Wiley and Son, New York, in press. 4. Aasland, R. and C. L. Smith. 1988. Nuc. Acids. Res., in press. 5. Ben-Bassat, A. *et al.*, 1987. J. Bact. **169**, 751. 6. McClelland, M. *et al.*, 1984. Proc. Nat. Acad. Sci. **81**, 983. 7. Winans, S. C. *et al.* 1985. J. Bact. **161**, 1219.