Volume 15 Number 4 1987 Nucleic Acids Research

Construction of the full coding information for murine J-chain protein from cDNA and its homologous genomic clone

Rachel Gollop and Arieh Zaritsky

Department of Biology, Ben Gurion University of the Negev, PO Box 653, Beer Sheva 84105, Israel

Submitted December 31 1986,

The cDNA for murine J-chain (MJ-cDNA) (1) contains a short complementary inversion at its 5'-end (2). Replacing this segment by the appropriate genomic fragment was achieved through the HinfI restriction site immediately downstream the inversion, where no intron exists (2). The nearby single KpnI recognition site circumvented the complication caused by 10 additional HinfI sites on the pBR322 vector carrying the genomic clone (pPE_S). The 5'-end PstI-HinfI fragment of pPE_S and the appropriate HinfI fragment of MJ-cDNA were introduced into the PstI site of pUC9 (3) after removing a 15 bp PstI-SmaI fragment, sequentially and in the correct orientation because the nucleotide sequences of the two HinfI sites flanking this fragment (H_1 and H_2) are not identical (Figure). The remaining HinfI sticky end was filled-in, and the blunt end thus formed (H_2^*) ligated to the SmaI open end.

The resultant 585 bp fragment (Jcf) contains most coding information for J-chain and 122 bp upstream, but lacks the coding information for the five C-terminal amino acids. To obtain the complete coding information for J-chain (Jci), the BamHI-EcoRI large fragment from Jcf-containing pUC9 [EcoRI overlapping the original SmaI site of the polylinker (3)] was ligated to the BamHI-EcoRI fragment from MJ-cDNA (1).

(1) Cann, G.M., Zaritsky, A. & Koshland M.E. (1982) Proc. natl. Acad. Sci. USA 79, 6656-6660.
(2) Matsuuchi, L., Cann, G.M. & Koshland, M.E. (1986) Proc. natl. Acad. Sci. USA 83, 456-460.
(3) Vieira, J. & Messing, J. (1982) Gene 19, 259-268.

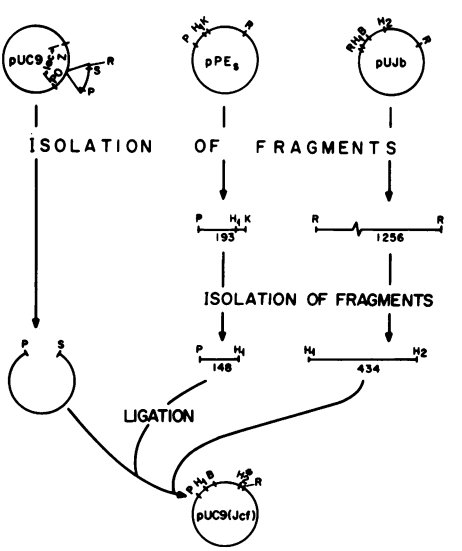

FIGURE LEGEND
Recognition sites for the following enzymes drawn to scale: B, BamHI; K, KpnI; P, PstI; E, EcoRI; S, SmaI; H_1 and H_2, two different sites for HinfI; H_2^*, the filled-in H_2. lacPOZ, promoter, operator and structural genes for beta-galactosidase, including a polylinker (3).

Thanks are due to M.E. Koshland et al. (2) for pPE_S, to N. Gollop for discussions, to D.M. Chipman for comments, and to the Fund for the Encouragement of Research, Histadrut-the General Federation of Labor in Israel (to R.G.).