Volume 17 Number 2 1989 Nucleic Acids Research

Preparation of single stranded insert DNA free of vector sequences

Jacek Biernat, Ulf B.Göbel[1]* and Hubert Köster

Biosyntech Biochemische Synthesetechnik GmbH, Stresemannstrasse 268-280, D-2000 Hamburg 50 and [1]Institut für Medizinische Mikrobiologie und Hygiene, Klinikum der Albert Ludwigs Universität Freiburg, Hermann Herder Strasse 11, D-7800 Freiburg, FRG
Submitted December 8, 1988

There is an increasing demand for vector-free single stranded insert DNAs for the use as probes in either filter-, solution-, or in-situ hybridization experiments. This prompted us to modify the well established M13mp18/19 vectors (1) in a way that cloned single stranded inserts can be excised via inverted repeats forming a master restriction site upon annealing. The original M13mp18/19 polylinker region was removed by digestion with Hind III and Eco RI and replaced by a synthetic 96 bp polylinker (Fig. 1a). The original Eco RI and Hind III sites were inactivated by replacing G by A at both ends of the new polylinker (marked by stars; Fig 1a). The multiple cloning site of the new vectors SSEV18/19 thus contains all restriction sites present in M13mp18/19 as well as two inverted repeats (A & B) forming an Eco RI site in the stable stem structure of single stranded virus DNA (Fig. 1b). This "master restriction site" allows the excision of any cloned single stranded insert, which was shown by cloning of a 900 bp rDNA fragment, derived from the 23S rRNA gene of the mollicute *Mycoplasma hyorhinis* (2). After digestion of the recombinant ss virus DNA, the insert was separated on a denaturing polyacrylamide gel and electroeluted (3). The insert was then labeled by filling in with DNA Polymerase I in the presence of ^{32}P- or non-isotopically labeled dNTPs. By comparing these inserts as probes in a filter hybridisation experiment with stoichiometric amounts of the appropriate double stranded fragment labeled to identical specific activity, the single stranded probes showed an up to 30 fold higher hybridization efficiency in detecting homologous rRNA sequences in crude biological samples such as Mycoplasma-contaminated tissue cultures (4; unpublished results).

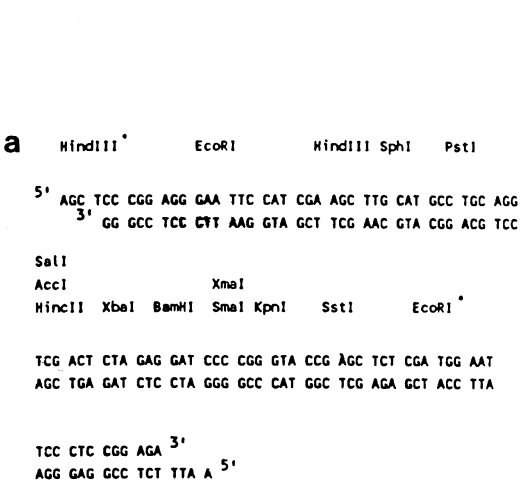

Fig 1a: SSEV Polylinker

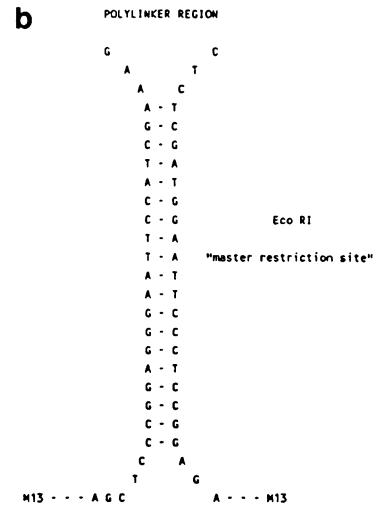

Fig. 1b: Stem loop structure in ss SSEV19 DNA

Acknowledgements: This work was funded by grant PTB038770 of the BMFT, FRG. We are thankfull to Dr. K.-E. Johansson, Uppsala Sweden, for kindly providing the tissue culture material.

*To whom correspondence should be addressed

References: (1) Yanisch-Perron, C. et al. (1985) Gene 33, 103-119. (2) Göbel,U.B. & Stanbridge,E.J. (1984) Science 226, 1211-1213. (3) Göbel, U.B. et al. (1987) J. Biochem. Biophys. Meth. 14, 245-260. (4) Biernat, J. et al. (1989) J. Biochem. Biophys. Meth., in the press.

