A new vector and RNase H method for the subtractive hybridization

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Subtractive hybridization is an attractive method to clone cDNAs but requires a large amount of mRNA. In addition, non-hybridized cDNA is not always completely separated from hybridized cDNAs by a hydroxyapatite column. To overcome these limitations, we explored a new vector (T7 λgt10-1) and procedure (RNase H method) for preparing subtracted cRNA probes of a given cDNA library. A synthetic 57mer fragment, 5'-AATTGTAATACGACTCACT ATAGGGTCTAGACCCCGAATTCGCGGCCGCAGCGGCCA-3', was inserted into the *Eco* RI site of λgt10 to make a vector that has the T7 promoter containing *Sfi* I, *Not* I, *Eco* RI and *Xba* I sites (Fig.1). cDNA inserts were synthesized according to Gubler-Hoffman (1) using $GGCGGCCGC(T)_{12}$ as a primer. After ligation with *Eco RI* linker, cDNAs were digested with *Eco* RI and *Not* I, and inserted into *Eco* RI-*Not* I site of T7 λgt10-1. From this cDNA library, we could produce transcripts equivalent to mRNA by T7 RNA polymerase without any plasmid sequence. To separate non-hybridized RNA from the hybrid, we introduced the RNase H digestion method. A model experiment is described below. We have prepared two RNA probes by transcribing human IgCγ and $V_H$par gene plasmids (templates A and B respectively) with T7 polymerase(2). A mixture of probes A and B (50ng each) was hybridized with 5 μg of denatured template DNA B at 65 °C for 20 hrs in 200μl of 0.4 M NaCl-1mM EDTA-40mM PIPES(pH6.7). Hybridized RNA was digested with 65 units of RNase H(Takara) in 300μl of 40mM Tris-Cl(pH7.5)-4mM MgCl2-1mM DTT at 30°C for 40 min. After removal of digested oligonucleotides by ethanol precipitation, probes and a template were denatured and the same process was repeated once again. The template DNA in the subtracted probe was digested with RNase-free DNase(Promega). This subtracted probe and the original mixture of probes for A and B were hybridized with various amounts of template DNAs on nitrocellulose filters. The subtracted probe hybridized to template B 1/ 100 as strongly as the original mixture probe whereas both probes hybridized to template A with the same intensity (Fig.2). The result indicates that the subtracted RNA probes prepared as above were enriched at least 100 folds.

Fig.1

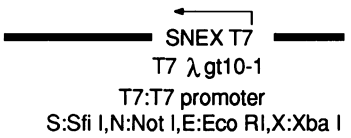
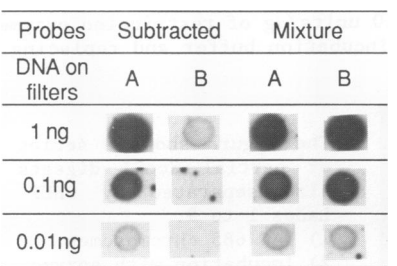


Fig.2



Reference
(1) Gubler, U. and Hoffman, B.J. (1983) Gene:**25**, 263.
(2) Melton, D.A. *et al.* (1984) Nucleic Acids Res.:**12**, 7035.

