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A simplified, orientation-specific cDNA cloning strategy

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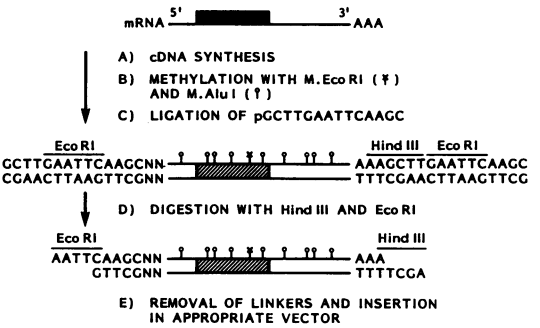
A cDNA cloning strategy has been devised, that creates different nuclease recognition sites at both ends.



PROTOCOL. a) cDNA synthesis by Reverse Transcriptase is initiated with oligo(dT) according to Gubler and Hofmann (1). b) The double stranded cDNA is methylated, and (c) a synthetic oligonucleotide pGCTTGAATTCAAGC is added. This generates a HindIII recognition sequence (AAGCTT) when ligated to the oligo(dA) track at the 3' terminal end of the cDNA molecule and an EcoRI site at the 5' terminus. In our protocol, the cDNA should be methylated with M.EcoRI and M.HindIII (2). Since M.HindIII was not commercially available, M.AluI (New England Biolabs) was tested and shown to provide complete protection of lambda DNA against HindIII digestion. d) The ligation mixture is digested with HindIII and subsequently with EcoRI, in order to prevent inefficient cleavage of the terminal HindIII site. e) After removal of excess linker molecules (3) the cDNA is inserted in a specialized vector.

DISCUSSION. We have used this procedure to construct a human leukocyte cDNA library. Sequence analysis of randomly picked clones showed a correct fusion of the linker to the cDNA molecule, resulting in 3' terminal HindIII sites. Furthermore, cDNA inserts were found with internal HindIII sites, indicating successful inhibition of cDNA digestion by HindIII following methylation by M.AluI. A 5' terminal HindIII site could theoretically be generated due to the presence of a TT dinucleotide. This chance was estimated from human mRNA sequences present in GenBank. Of 21 randomly chosen mRNA's (total of 43,884 bases) the average occurrence of TT was found to be 5.25% (range 1.4-8.7%), indicating that 1 out of 19 cDNA molecules cannot be cloned by this technique. Reduction of transformation efficiencies due to specific restriction of cytosine methylated DNA (4), may be prevented by cloning in Mcr negative strains (New England Biolabs). The presented orientation-specific cDNA cloning procedure for the construction of expression libraries is simple, widely applicable and may substitute the Okayama-Berg protocol (5).

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