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A one tube reaction for the synthesis of blunt-ended double-stranded cDNA

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In 1983, we described a simple protocol to synthesize cDNA by a combination of reverse transcriptase, RNAseH and DNA polymerase I to generate large plasmid libraries (1). This protocol has now been modified to a simple one tube reaction with bluntended cDNA as the final product that can be cloned into bacteriophage lambda or plasmids. Preparation of the cDNA takes about 4 hours; a library in lambda phage can be obtained in 2 to 3 days. First strand cDNA is synthesized in 40 μl of 50 mM Tris-HCl pH 8.3, 10 mM $MgCl_2$, 10 mM DTT, 100 mM NaCl, 4mM NaPyrophosphate, 0.625 mM dNTPs, 15-20 uCi α-^{32}P-dCTP, 100 μg/ml oligodT_{12-18}, 125 μg/ml polyA+ RNA, 2500 u/ml AMV reverse transcriptase at 43°C for 40 minutes. Cool on ice and remove 1 μl for analysis of the first strand products. To the remaining 39 μl, add 125 μl of 40 mM Tris-HCl pH 7.5, 10 mM $MgCl_2$, 20 mM $(NH_4)_2SO_4$, 200 mM KCl, 100 μg/ml BSA, followed by water, 4.6 units of RNAse H and 115 units of DNA pol I. Final volume is 250 μl. Incubate at 12°C for 60 minutes and at 22°C for 60 minutes. Heat to 70°C for 10 minutes. Cool on ice. Add 2 units of T4 DNA polymerase per μg of starting mRNA. Incubate at 37°C for 10 minutes. Add EDTA to 20 mM and SDS to 1% and extract with phenol twice. Precipitate with ethanol and NH_4OAc twice as described (1) and redissolve in 20 μl water. Determine incorporation of radioactivity by TCA precipitation. Total incorporation minus incorporation into the first strand equals incorporation into the second strand. Run denaturing gels on the singlestranded and doublestranded cDNA to determine its size. The size distribution should be the same for both products. For cloning in lambda, linkers can be added by standard procedures. Alternatively, the cDNA can be tailed for cloning into tailed plasmid vectors. Before cloning, the cDNAs can be sized on Sepharose 4B or Sephacryl S500. We have found that Sephacryl will give a better resolution in the size range above 2 kb. To concentrate the cDNA after the column fractionation, use sec-butanol followed by ethanol precipitation in the presence of tRNA carrier (Boehringer Mannheim cat No 109541). We have found that up to 20 μg tRNA in a final preparation of as little as 20 ng cDNA will not impair efficiencies of ligation and cloning into lambda phage.

Reference: 1) Gubler, U. and Hoffman, B.J. (1983): Gene 25, 263-269.