A simple and rapid amplification procedure for cDNA cloned in dephosphorylated plasmid

Mitsuoki Morimyo and Kazuhide Mita¹
Division of Genetics and ¹Division of Biology, National Institute of Radiological Sciences, 9-1, Anagawa-4-chome, Inageku, Chiba 263, Japan

Received February 8, 1993; Accepted March 5, 1993

The preparation of cDNA libraries usually involves multiple steps, such as isolation of polyA⁺ mRNA, synthesis of cDNA, cloning of cDNA into plasmid, and amplification of cloned plasmid. The last step, the amplification of insert DNA fragment after cloned in dephosphorylated plasmid vector, is done in either of two ways. The recombinant DNA can be extracted after amplification in host cells, or the amplification of insert DNA can be done by the PCR using primers complementary to both ends of the insertion site of the vector. However, the ligation of dephosphorylated nicks or nick translation beyond the primer sites is essential in the latter case prior to the PCR (see step 1 in Figure 1 (B)). Taq DNA polymerase, by virtue of its 5' to 3' exonuclease activity (Ref 1), may be utilized for nick translation instead of E.coli DNA polymerase 1. This would simplify the amplification of insert cDNA by doing nick translation and PCR steps successively, controlling each reaction condition.

We examined the conditions for nick translation by Taq DNA polymerase. The reaction mixture, 20 µl containing 25 µCi ³²P-dNTPs (600 Ci/mmol), 0.5 µg RF-M13mp18 DNA, 0.02 ng pancreatic DNase I, and 2.5 units of Taq DNA polymerase was incubated at 37, 50 and 65°C and the incorporation of ³²P-dNTPs into acid-insoluble fraction was measured. Radioactivities incorporated were 167 k, 323 k, and 286 k DPM, respectively, compared with 82 k DPM by a standard nick translation reaction at 12°C using DNA polymerase I and DNase I. These results indicated that the nick translation reaction by Taq DNA polymerase worked normally at all temperatures examined, although the incorporation was reduced at 65°C.

Then we amplified insert DNA by nick translation reaction

---

Figure 1. (A) Comparison of the PCR products with (lane 3) and without (lane 2) nick translation step. The conditions of PCR and nick translation steps are presented in the text. The length of insert DNA fragment is 561 bp. The amplified DNA is 684 bp because of carrying M4 and RV primers at both ends. Lanes 1 and 4 are a mixture of λ-HindIII and φX174-HaeII digest. (B) Schematic procedure for nick translation (step 1) and PCR (step 2). Hatched boxes and thick lines represent insert DNA and vector DNA, respectively. Primers M4 and RV are marked by short arrows.
at 37°C for 10 min followed by PCR with M4 and RV primers by Taq DNA polymerase (step 2 in Figure 1 (B)).

We observed the amplification of the 0.6 kb insert DNA when the two steps were done as described below (lane 3 in Figure 1 (A)), whereas the reaction without the nick translation step failed to amplify (lane 2 in Figure 1 (A)).

Here, we report a very fast and simple procedure for amplification of cloned insert DNA by controlling temperature and time.

A detailed description of the method is presented below:

1. Digest RF-M13mp18 DNA with restriction enzyme Smal.
2. Dephosphorylate plasmid DNA by incubation twice with alkaline phosphatase.
3. Inactivate alkaline phosphatase by treating 3 times with phenol.
4. Separate dephosphorylated plasmid DNA by agarose gel electrophoresis.
5. Ligate insert DNA with dephosphorylated plasmid DNA.
6. Prepare a 100 μl reaction mixture containing:
   1 μl 10⁻³ diluted ligation product (ca. 2 ng vector DNA),
   79 μl water, 10 μl 10× buffer for Taq DNA polymerase,
   8 μl 2.5 mM dNTP mixture, 1 μl 0.5 μg primer 1 (M13 primer M4, Takara Shuzo Co.),
   1 μl 0.5 μg primer 2 (M13 primer RV, Takara Shuzo Co.), and 0.5 μl Taq DNA polymerase (5 units/μl).
7. Incubate for 10 min at 37°C for nick translation reaction.
8. Warm immediately at 95°C.
9. PCR; 30 cycles at (30 sec at 94°C, 30 sec at 55°C and 1 min at 72°C).

The simple and rapid procedure described here is very useful to amplify cDNA species without bias since a uniform amplification of cDNA species would be expected by PCR using primers located on vector DNA.

ACKNOWLEDGMENTS

This work was partially supported by International Core System for Basic Research, Science and Technology Agency of Japan and by a grant from the Ministry of Education, Science and Culture of Japan.

REFERENCE