

pGEM®-T Vector: Cloning of Modified Blunt-ended DNA Fragments

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We describe a modification to standard methods for cloning blunt-ended DNA fragments, which allows efficient cloning of these DNA fragments into Promega's pGEM®-T Vector.

Introduction

The cloning of blunt-ended DNA fragments into plasmid vectors is a common laboratory practice in molecular biology. These DNA fragments are typically generated by one of two methods: direct digestion with a restriction enzyme that produces a blunt end (e.g., *Sma* I, *Stu* I or *Pvu* II) or by using the controlled exonuclease activity of either Klenow or T4 DNA Polymerase (1).

In order to efficiently clone a blunt-ended fragment into a suitable vector, several requirements must be met. The vector should be dephosphorylated using either Calf Intestinal Alkaline Phosphatase or bacterial alkaline phosphatase, in order to minimize high backgrounds resulting from religated vector. The dephosphorylated vector must then be purified prior to ligation. Additionally, it may be necessary to modify the standard ligation buffer to include polyethylene glycol (which promotes macromolecular crowding) and low concentrations of ATP. Finally, high concentrations of T4 DNA Ligase and insert are recommended (1).

We have modified a typical blunt-end cloning procedure to eliminate some of the requirements described above. The methods described here take advantage of the fact that *Taq* DNA Polymerase preferentially adds a single 3' A-deoxynucleotide to double-stranded DNA fragments by a non-template-dependent extension reaction (2). After a brief incubation of the blunt-ended DNA fragment with *Taq* DNA Polymerase and dATP, the resulting A-tailed fragment can be cloned using Promega's pGEM®*-T Vector Systems.

*U.S. Pat. No. 4,766,072 has been issued to Promega Corporation for transcription vectors having two different bacteriophage RNA polymerase promoter sequences separated by a series of unique restriction sites into which foreign DNA can be inserted.

DNA fragments generated by restriction digestion

The protocol summarized in [Figure 1](#) can be used for DNA fragments produced by restriction digestion with enzymes that generate blunt ends. The resulting 3'-tailed fragment can be ligated directly into the pGEM®-T Vector without further purification using standard ligation conditions (3). For optimal transformation results, we recommend using no more than 1-2µl of the tailing reaction in the ligation mixture. The molar ratio of insert to vector should fall within an 8:1 to 1:8 range, however, a 1:1 molar ratio reflects optimal conditions with the inserts tested in these experiments. Using these conditions, 80-90% recombinants are typically obtained.

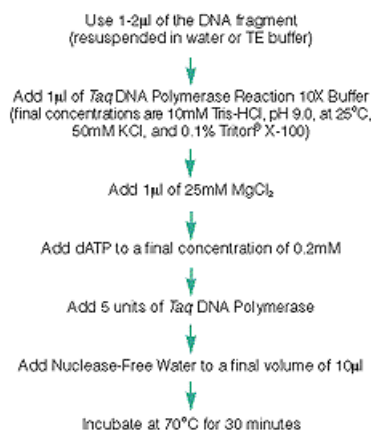


Figure 1. Tailing of blunt-ended DNA fragments with *Taq* DNA Polymerase.

We tested the results of this cloning method using a DNA fragment generated by restriction digestion with *Stu* I. The fragment was gel-purified using the Wizard(TM) PCR Preps DNA Purification System (Cat.# A7170). Following the protocol described in [Figure 1](#), we

obtained greater than 90% recombinants at 2-3 x 10⁵cfu/μg. Restriction digests of DNA from selected recombinants verified the presence of the insert fragment (Figure 2).

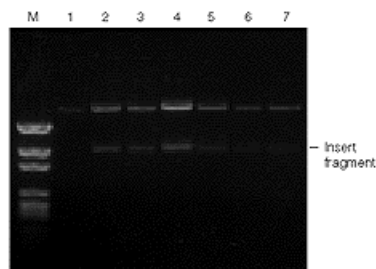


Figure 2. Analysis of recombinants obtained from cloning modified blunt-ended DNA. One hundred twenty-five nanograms of a *Stu* I restriction digestion fragment (1,519bp) was tailed using *Taq* DNA Polymerase in the presence of dATP as described in Figure 1. Twenty-five nanograms of the tailed fragment was ligated into 50ng of Promega's pGEM®-T Vector (1:1 molar ratio) and transformed into competent JM109 cells as described (3). The transformation produced 36 white (recombinant) and 3 blue colonies. Six recombinants were selected and plasmid DNA was isolated using the Wizard (TM) Minipreps DNA Purification System (Cat.# A7100) (7). The isolated DNA was digested with *Not* I and *Apa* I to release the insert from the pGEM®-T Vector and the DNA was analyzed on a 1% agarose gel followed by staining with ethidium bromide. Lane M, pGEM® DNA Markers (Cat.# G1741); Lane 1, pGEM®-T Vector; Lanes 2-7, *Not* I - *Apa* I digests of recombinants.

DNA fragments generated by thermostable polymerases

Blunt-ended DNA fragments can also be generated by some thermostable DNA polymerases (e.g., *Pfu* and *Tli* DNA polymerases) that have extensive 3'→5' exonuclease activity (4,5). A substantial proportion of the DNA fragments generated by these polymerases possesses blunt ends. The resulting fragments may be cloned by conventional methods or they may be modified by a second incubation with *Taq* DNA Polymerase in the presence of dATP (Figure 1) and then cloned into Promega's pGEM®-T Vector. We recommend that the amplification reaction products be purified prior to modification to eliminate nonspecific reaction products. Promega's Wizard(TM) PCR Preps DNA Purification System provides a convenient method for purifying amplification reaction products (6).

Following this procedure, we typically obtained 80-90% positive transformants. Control ligation reactions using non-tailed amplified DNA (i.e., blunt-ended DNA) resulted in minimal positive colonies (1-5cfu/μg; data not shown).

Summary

We describe an effective method for tailing blunt-ended DNA fragments with *Taq* DNA Polymerase, which facilitates cloning the fragments into Promega's pGEM®-T Vector. This method eliminates some requirements of conventional blunt-end cloning techniques including dephosphorylation of the vector and preparation of modified ligation buffers.

References

1. Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
2. Clark, J.M. (1988) *Nucl. Acids Res.* **19**, 1156.
3. *pGEM®-T Vector Technical Bulletin #TB150*, Promega Corporation.
4. Lundberg, K.S. *et al.* (1991) *Gene* **108**, 1.
5. Kong, H. *et al.* (1993) *J. Biol. Chem.* **268**, 1965.
6. *Wizard(TM) Minipreps DNA Purification System Technical Bulletin #TB118*, Promega Corporation.
7. *Wizard(TM) PCR Preps DNA Purification System Technical Bulletin #TB117*, Promega Corporation.

Ordering Information

Product	Size	Cat. #
pGEM®-T Vector System I	20 reactions	A3600
pGEM®-T Vector System II	20 reactions	A3610
dATP	40μmoles	U1201

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