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The pGEM®-T and pGEM®-T Easy Vector Systems

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The use of T vectors can greatly facilitate the cloning of PCR products. In this update, we describe a new version of the pGEM®-T Vector available in the pGEM®-T Easy Vector Systems. The pGEM®-T Easy Vector is produced by modifying the multiple cloning site (MCS) of the original pGEM®-T Vector so that a single restriction digestion with either* Not *I or* Eco*R I may be performed to remove the insert DNA. We also describe ways to optimize the results obtained using the pGEM®-T and pGEM®-T Easy 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.*

Introduction

Cloning of PCR products generated by many thermostable DNA polymerases presents unique challenges to the researcher. Many of the enzymes used for PCR (such as *Taq* DNA Polymerase**) add single deoxyadenosine residues to the 3´-ends of PCR products (1,2). So-called "T" vectors, including Promega's pGEM®-T Vectors, are manufactured with 3´-T overhangs complementary to these A-overhangs. Promega's pGEM®-T and pGEM®-T Easy Vectors are prepared by cutting either the pGEM® -5Zf(+) or the pGEM®-T Easy Vector with *Eco*R V and adding a 3´-terminal thymidine to both ends. The pGEM®-T Easy Vector is further modified by the addition of 15 nucleotides to create *Not* I and *Eco*R I restriction sites flanking the cloning site.

Features of the pGEM®-T and pGEM®-T Easy Vectors

The high copy number pGEM®-T and pGEM®-T Easy Vectors contain T7 and SP6 RNA Polymerase promoters flanking a multiple cloning region within the alpha-peptide coding region of the enzyme beta-galactosidase (Figure 1). Insertional inactivation of the alpha-peptide allows recombinant clones to be directly identified by blue/white color screening on indicator plates. The multiple cloning region (Figure 2) includes restriction sites conveniently arranged for use with Promega's Erase-a-Base® System (Cat.# E5750) for generating nested sets of deletions. The pGEM®-T and pGEM®-T Easy Vectors also contain the origin of replication of the filamentous phage f1 for the preparation of single-stranded DNA (ssDNA). The ssDNA molecule exported corresponds to the bottom strand shown in Figure 2 for the pGEM®-T Easy Vector.

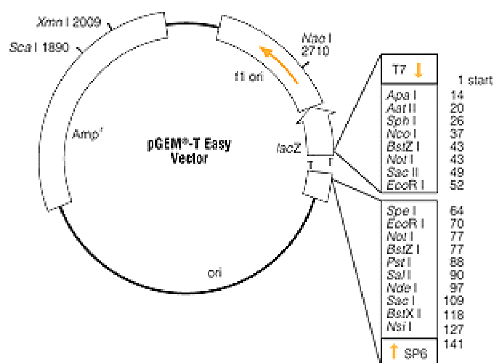

Figure 1. pGEM®-T Easy Vector circle map.

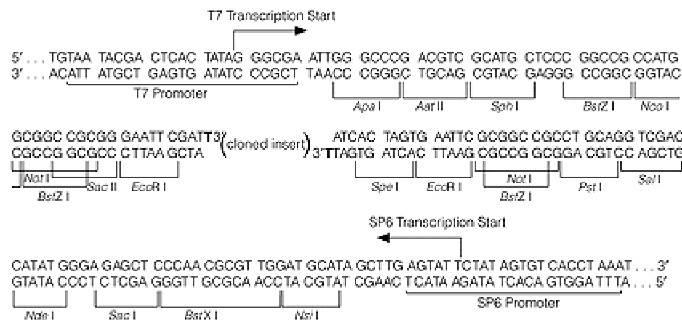

Figure 2. The promoter and multiple cloning site of the pGEM®-T Easy Vector. The top strand of the sequence shown corresponds to the RNA synthesized by T7 RNA Polymerase. The bottom strand corresponds to the RNA synthesized by SP6 RNA Polymerase.

Specialized applications

Specialized applications of the pGEM®-T and pGEM®-T Easy Vector Systems include: cloning PCR products, construction of unidirectional nested deletions using the Erase-a-Base® System, ssDNA production, blue/white screening for recombinants and transcription *in vitro* from dual opposed promoters. The T7 Promoter Primer (Cat.# Q5021) or the pUC/M13 Forward Primer (Cat.# Q5391) may be used to sequence ssDNA produced by the pGEM®-T Easy Vector. For an *in vitro* transcription protocol, please request Promega's *Riboprobe®* in vitro *Transcription Systems Technical Manual,* #TM016.

Restriction digests

The pGEM®-T and pGEM®-T Easy Vectors offer a number of options for removal of the insert DNA of interest. With the new pGEM®-T Easy Vector Systems, either of the popular restriction enzymes *Not* I or *Eco*R I may be used in a single digest. *Bst*Z I may be used in a single digest with both the original pGEM®-T Vector and the pGEM®-T Easy Vector. Alternatively, *Pst* I may be combined with either *Sph* I, *Nco* I or *Sac* I in a double digest in Promega's Buffer H using either vector. The choice of method to be used will depend upon the sequence of the insert to be cloned and the availability of the restriction enzymes.

Optimization of ligation

Efficiency of ligation of the insert into the vector is affected by a number of variables. We recommend ligating at 4°C overnight. Low temperature ligations favor the desired annealing of the A-overhang of the insert with the T-overhang of the vector. When higher temperatures (i.e., room temperature) are used, a high background of blue colonies resulting from optimization of blunt-end ligations of non-T-tailed vector may result. It is also critical to use only the T4 DNA Ligase provided with the system. Other preparations may contain exonuclease contaminants which result in degradation of the T-overhangs. Finally, an overnight ligation (rather than three hours) will ensure that the ligation reaction goes to completion. To determine if the ligation conditions used are optimal, we suggest performing a ligation reaction with the positive control DNA. If the ligation temperature used is too high, the number of white colonies in the positive control ligation will be less than 60%.

Choice of thermostable polymerases for use with pGEM®-T Vectors

The pGEM®-T and pGEM®-T Easy Vectors are designed for use with enzymes which generate A-overhangs. These enzymes do not possess 3´ --> 5´ exonuclease proofreading activity. In contrast, enzymes such as *Tli* DNA Polymerase** do possess proofreading activity. Consequently, the majority of the PCR products (amplicons) produced by these enzymes are blunt-ended. The use of proofreading enzymes is increasing due to their higher fidelity and ability to generate long (up to 10kb) fragments when used in combination with nonproofreading enzymes (3,4). One can readily adapt PCR products generated using proofreading enzymes for use with the pGEM®-T and pGEM®-T Easy Vector Systems. To A-tail the products of *Tli* DNA Polymerase and other proofreading enzymes, dATP and *Taq* DNA Polymerase can be added to the final cycles of the PCR reaction (5,6). A list of some of the more common thermostable DNA polymerases and their characteristics is provided in Table 1 (7).

Table 1. Characteristics of Thermostable DNA Polymerases.

DNA Polymerase**	Promega Catalog Number(s)	5′→3′ exonuclease activity	3′→5′ exonuclease activity ["Proofreading"]	Resulting DNA ends[1]
Taq[2]	**M1861 M1661**	**Yes**	**No**	**3′-A**
Tfl	**M1941**	**Yes**	**No**	**3′-A**
Tbr	–	Yes	No	3′-A
Tth	**M2101**	**Yes**	**No**	**3′-A**
Stoffel Fragment	–	No	No	3′-A
$Vent_R$® (exo-)	–	No	No	70% Blunt 30% Single base overhangs
Tli	**M7101**	**No**	**Yes**	**>95% Blunt**
Deep $Vent_R$®	–	No	Yes	>95% Blunt
Pfu	–	No	Yes	Blunt
Pwo	–	No	Yes	NA
Ul*Tma*™	–	No	Yes	Blunt

Enzymes in boldface type are available from Promega

NA: not available

[1] Sequence content may influence the polymerase's addition of a 3′-A overhangs

[2] Also includes AmpliTaq®

[3] After primer extension, a 15 minute incubation at 72°C is required to add 3′-A overhangs to DNA products

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Summary

The pGEM®-T and pGEM®-T Easy Vector Systems are convenient systems for the cloning of PCR products. The vectors are prepared by cutting Promega's pGEM®-5Zf(+) and pGEM®-T Easy Vectors with *Eco*R V and adding a 3´-terminal thymidine to each end. This provides a convenient cloning site for PCR products generated by certain thermostable DNA polymerases which add a single deoxyadenosine, in a template-independent fashion, to the 3´-ends of amplified fragments. In the pGEM®-T Easy Vector, the multiple cloning site is flanked by recognition sites for the restriction enzymes *Eco*R I, *Bst*Z I and *Not* I, providing three single-enzyme digestions for release of the insert.

References

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3. Barnes, W.M. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 2216.
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Ordering Information

Product	Size	Cat.#
pGEM®-T Easy Vector System I	20 reactions	A1360
pGEM®-T Easy Vector System II	20 reactions	A1380
pGEM®-T Vector System I	20 reactions	A3600
pGEM®-T Vector System II	20 reactions	A3610

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