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

Technical Manual No. 042

**INSTRUCTIONS FOR USE OF PRODUCTS A1360, A1380, A3600 and A3610.**
***PLEASE DISCARD PREVIOUS VERSIONS.***
**All Technical Literature is Available on the Internet at www.promega.com**




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Part# TM042

## I. Introduction

The pGEM®(a,b)-T and pGEM®-T Easy Vector Systems are convenient systems for the cloning of PCR(c) products. The vectors are prepared by cutting Promega's pGEM®-5Zf(+)(b) and pGEM®-T Easy Vectors with *Eco*R V and adding a 3´ terminal thymidine to both ends. These single 3´-T overhangs at the insertion site greatly improve the efficiency of ligation of a PCR product into the plasmids by preventing recircularization of the vector and providing a compatible overhang for PCR products generated by certain thermostable polymerases (1,2). As summarized in Table 1, these polymerases often add a single deoxyadenosine, in a template-independent fashion, to the 3´-ends of the amplified fragments (3,4).

The high copy number pGEM®-T and pGEM®-T Easy Vectors contain T7 and SP6 RNA Polymerase promoters flanking a multiple cloning site (MCS) within the α-peptide coding region of the enzyme β-galactosidase. Insertional inactivation of the α-peptide allows recombinant clones to be directly identified by color screening on indicator plates. The multiple cloning region of the two vectors includes restriction sites conveniently arranged for use with Promega's Erase-a-Base® System (Cat.# E5750) for generating nested sets of deletions.

Both the pGEM®-T and pGEM®-T Easy Vector contain multiple restriction sites within the MCS. These restriction sites allow for the release of the insert by digestion with a single restriction enzyme. The pGEM®-T Easy Vector MCS is flanked by recognition sites for the restriction enzymes *Eco*R I, *Bst*Z I and *Not* I, thus providing three single-enzyme digestions for release of the insert, while the pGEM®-T Vector cloning site is flanked by recognition sites for the enzyme *Bst*Z I. Alternatively, a double-digestion may be used to release the insert from either vector.

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; see Section VII). The ssDNA molecule exported corresponds to the bottom strand shown in Figure 1A and 1B for the pGEM®-T and pGEM®-T Easy Vectors (nonrecombinant), respectively.

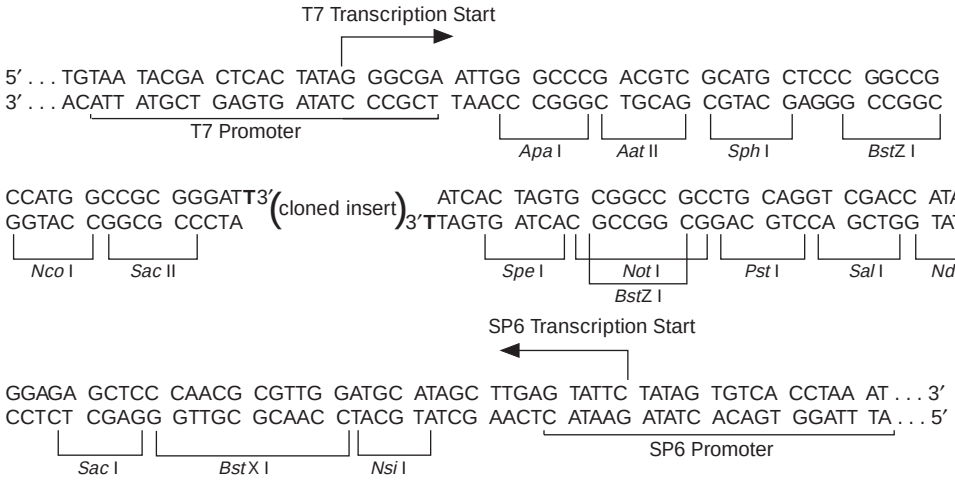
**The pGEM®-T and pGEM®-T Easy Vector Systems now include a 2X Rapid Ligation Buffer for ligation of PCR products. Reactions using this buffer may be incubated for 1 hour at room temperature. The incubation period may be extended to increase the number of colonies after transformation. Generally, an overnight incubation at 4°C will produce the maximum number of transformants.**

**Table 1. Comparison of PCR Product Properties for Some Thermostable DNA Polymerases.**

| Characteristic | *Taq*I AmpliTaq® | *Tfl* | *Tth* | Vent$_R$®/ (*Tli*) | Deep Vent$_R$® | *Pfu* | *Pwo* |
|---|---|---|---|---|---|---|---|
| Resulting DNA ends | 3´ A | 3´ A | 3´ A | >95% Blunt | >95% Blunt | Blunt | N.A. |
| 5´→3´ exonuclease activity | Yes | Yes | Yes | No | No | No | No |
| 3´→5´ exonuclease activity | No | No | No | Yes | Yes | Yes | Yes |

N.A.: not available

**Panel A**




**Panel B**

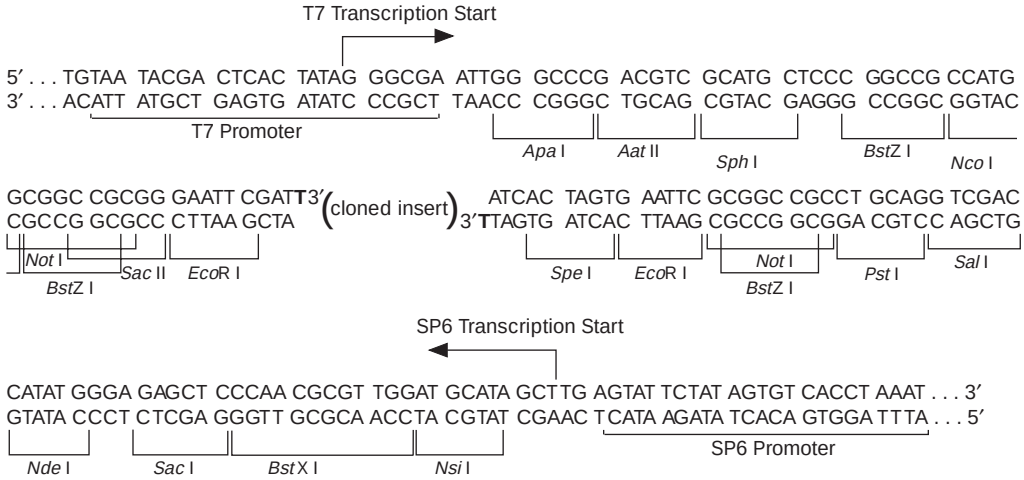



**Figure 1. The promoter and multiple cloning sequence of the pGEM®-T (Panel A) and pGEM®-T Easy (Panel B) Vectors.** The top strand of the sequence shown corresponds to the RNA synthesized by T7 RNA Polymerase. The bottom strand corresponds to the RNA synthesized by SP6 RNA Polymerase.

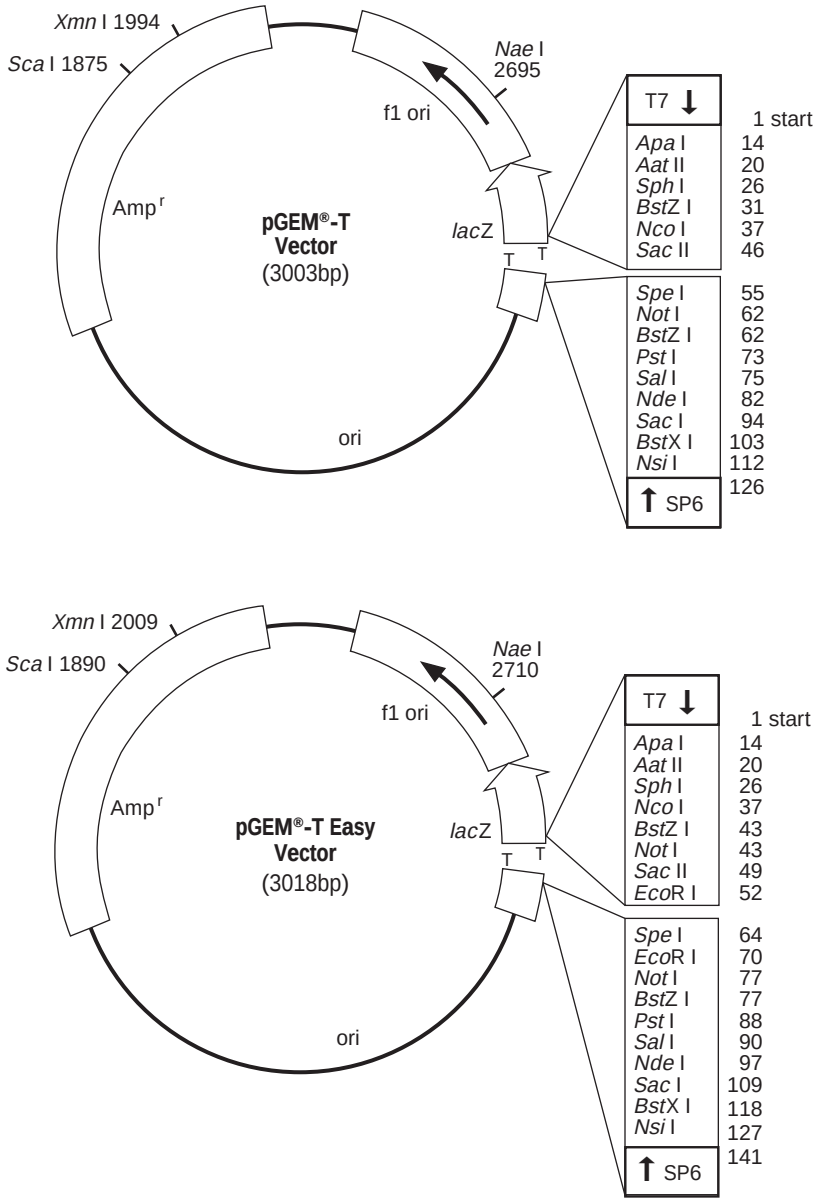



**Figure 2. pGEM®-T and pGEM®-T Easy Vector circle maps.**

**Figure 2 Circle Map Notes:**

**pGEM®-T Vector Sequence reference points:**

| | |
|---|---|
| T7 RNA Polymerase transcription initiation site | 1 |
| SP6 RNA Polymerase transcription initiation site | 126 |
| T7 RNA Polymerase promoter | 2987-6 |
| SP6 RNA Polymerase promoter | 121-143 |
| multiple cloning site | 10-113 |
| *lac*Z start codon | 165 |
| *lac* operon sequences | 2824-2984, 151-380 |
| *lac* operator | 185-201 |
| β-lactamase coding region | 1322-2182 |
| phage f1 region | 2368-2823 |
| binding site of pUC/M13 Forward Sequencing Primer | 2944-2960 |
| binding site of pUC/M13 Reverse Sequencing Primer | 161-177 |

**pGEM®-T Easy Vector Sequence reference points:**

| | |
|---|---|
| T7 RNA Polymerase transcription initiation site | 1 |
| SP6 RNA Polymerase transcription initiation site | 141 |
| T7 RNA Polymerase promoter | 3002-6 |
| SP6 RNA Polymerase promoter | 136-158 |
| multiple cloning site | 10-128 |
| *lac*Z start codon | 180 |
| *lac* operon sequences | 2839-2999, 166-395 |
| *lac* operator | 100-216 |
| β-lactamase coding region | 1337-2197 |
| phage f1 region | 2383-2838 |
| binding site of pUC/M13 Forward Sequencing Primer | 2959-2975 |
| binding site of pUC/M13 Reverse Sequencing Primer | 176-192 |

**Specialized applications of the pGEM®-T and pGEM®-T Easy Vectors:**

1. cloning PCR products
2. used with the Erase-a-Base® System for construction of unidirectional nested deletions
3. ssDNA production
4. blue/white screening for recombinants
5. transcription *in vitro* from dual opposed promoters (For protocol information, please request Promega's *Riboprobe*® in vitro *Transcription Systems*[d] *Technical Manual* #TM016.)

   Use the T7 Promoter Primer or the pUC/M13 Forward Primer to sequence ssDNA produced by the pGEM®-T and pGEM®-T Easy Vectors.

## II. Product Components

| Product | Size | Cat.# |
|---|---|---|
| pGEM®-T Vector System I | 20 reactions | A3600 |

Includes:

- 1.2µg pGEM®-T Vector (50ng/µl)
- 12µl Control Insert DNA (4ng/µl)
- 100u T4 DNA Ligase
- 200µl 2X Rapid Ligation Buffer, T4 DNA Ligase
- 1 Protocol

**Storage Conditions:** Store all components at –20°C or –70°C. pGEM®-T Vector System I is guaranteed for at least 1 year from date of purchase when stored and handled properly.

| Product | Size | Cat.# |
|---|---|---|
| pGEM®-T Vector System II | 20 reactions | A3610 |

Includes:

- 1.2µg pGEM®-T Vector (50ng/µl)
- 12µl Control Insert DNA (4ng/µl)
- 100u T4 DNA Ligase
- 200µl 2X Rapid Ligation Buffer, T4 DNA Ligase
- 1.2ml JM109 Competent Cells, High Efficiency (6 x 200µl)
- 1 Protocol

**Storage Conditions: Store the Competent Cells at –70°C.** All other components can be stored at –20°C or –70°C. Components of pGEM®-T Vector System II are guaranteed for at least 1 year from date of purchase when stored and handled properly, except for JM109 Competent Cells, which are guaranteed until the expiration date that is printed on the tube label.

| Product | Size | Cat.# |
|---|---|---|
| pGEM®-T Easy Vector System I | 20 reactions | A1360 |

Includes:

- 1.2µg pGEM®-T Easy Vector (50ng/µl)
- 12µl Control Insert DNA (4ng/µl)
- 100u T4 DNA Ligase
- 200µl 2X Rapid Ligation Buffer, T4 DNA Ligase
- 1 Protocol

**Storage Conditions:** Store all components at –20°C or –70°C. pGEM®-T Easy Vector System I is guaranteed for at least 1 year from date of purchase when stored and handled properly.

| Product | Size | Cat.# |
|---|---|---|
| pGEM®-T Easy Vector System II | 20 reactions | A1380 |

Includes:

- 1.2µg pGEM®-T Easy Vector (50ng/µl)
- 12µl Control Insert DNA (4ng/µl)
- 100u T4 DNA Ligase
- 200µl 2X Rapid Ligation Buffer, T4 DNA Ligase
- 1.2ml JM109 Competent Cells, High Efficiency (6 x 200µl)
- 1 Protocol

**Storage Conditions: Store the Competent Cells at –70°C.** All other components can be stored at –20°C or –70°C. Components of pGEM®-T Easy Vector System II are guaranteed for at least 1 year from date of purchase when stored and handled properly, except for JM109 Competent Cells, which are guaranteed until the expiration date that is printed on the tube label.

## III. General Considerations

### A. PCR Product Purity

An aliquot of the PCR reaction should be analyzed on an agarose gel before using it in the ligation reaction. The PCR product to be ligated can be gel-purified or directly purified using the Wizard® PCR Preps DNA Purification System (Cat.# A7170) or used directly from the reaction. Exposure to shortwave ultraviolet light should be minimized in order to avoid the formation of pyrimidine dimers. If smearing of the PCR product or inappropriate banding is observed on the gel, excise the bands to be cloned from a low-melt agarose gel and purify the DNA with Wizard® PCR Preps or Agar*ACE*® Agarose-Digesting Enzyme (Cat.# M1741). Even if distinct bands of expected size are observed, primer-dimers should be removed. Use Wizard® PCR Preps to purify the bands of interest directly from the reaction mix. Use of crude PCR product may also produce successful ligations in some cases; however, the number of white colonies containing the relevant insert may be reduced due to preferential incorporation of primer-dimers or other extraneous reaction products. Therefore, it may be necessary to screen numerous colonies in order to identify clones that contain the PCR product of interest.

### B. Blunt End PCR Products

Thermostable DNA Polymerases with proofreading activity, such as *Pfu* DNA Polymerase(e) (Cat.# M7741), *Pwo* DNA polymerase and *Tli* DNA Polymerase(e) (Cat.# M7101) generate blunt-end fragments during PCR amplification. Nevertheless, PCR fragments generated using these polymerases can be modified and ligated to the pGEM®-T and pGEM®-T Easy Vectors (5). Greater than 80% recombinants were obtained (Table 2) when the fragments were purified using the Wizard® PCR Preps DNA Purification System (Cat.# A7170) and A-tailed with *Taq* DNA Polymerase.

The standard A-tailing protocol outlined in Figure 3 requires purification of the PCR product. When working with a large number of different amplified fragments this tailing procedure may prove cumbersome. To determine whether the protocol can be abbreviated in any manner, we have performed several A-tailing reactions using the alternative procedure (Figure 3). In this shortened protocol, the PCR product is not purified and *Taq* DNA Polymerase and dATP are added directly to the 50µl PCR mix after the amplification profile is complete.

Using this alternative procedure, 20-30% recombinant colonies were obtained when *Pfu* DNA polymerase was used in the amplification reaction (Table 2). Recombinants were identified by blue/white screening and were confirmed by restriction digestion analysis. Few positives were observed in the control reaction, in which the PCR fragment was not tailed. These control results confirm that the majority of the pGEM®-T Easy Vector used contains 3´-terminal deoxythymidine. Although the efficiency of this shortened protocol is reduced compared to the standard tailing procedure, the low background exhibited by the pGEM®-T Vectors means that this procedure is a useful way to obtain clones when processing large numbers of samples.

**The alternative tailing protocol outlined in Figure 3 cannot be used with all thermostable DNA polymerases.** The DNA polymerase used in the amplification is a critical factor. The results in Table 2 show that no recombinants were obtained using the alternative tailing procedure when *Tli* DNA Polymerase was used in the amplification reaction. This may be due to the greater thermostablity of *Tli* DNA Polymerase compared with *Pfu* DNA Polymerase (6). However, greater than 90% recombinant colonies were obtained using the standard tailing procedure in conjunction with a *Tli* DNA Polymerase-generated amplification fragment.

**Table 2. Comparison of A-Tailing Procedures Used With Different DNA Polymerases.**

| | A-Tailing Procedure | |
|---|---|---|
| **Polymerase** | **Standard** | **Alternative** |
| *Pfu* DNA Polymerase | 85-90% | 20-30% |
| *Tli* DNA Polymerase | 80-90% | 0% |

Table 2 lists percent recombinants obtained following PCR amplification with *Pfu* DNA Polymerase and *Tli* DNA Polymerase in combination with either a standard or alternative A-tailing procedure.

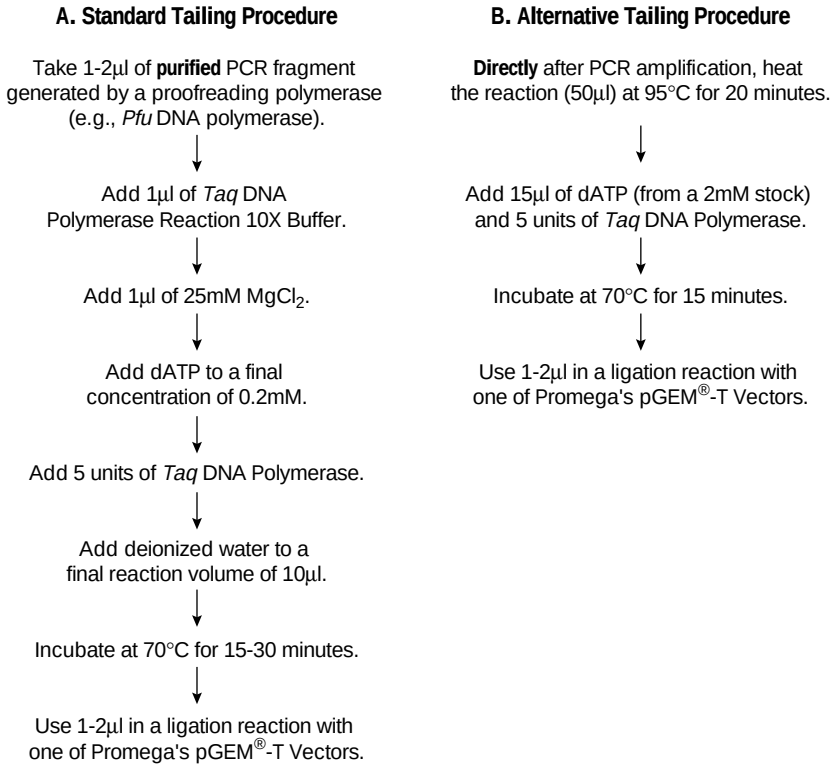



**Figure 3. A-Tailing procedures. Panel A:** Standard tailing procedure for blunt-end PCR fragments purified with the Wizard® PCR Preps DNA Purification System (Cat.# A7170). **Panel B:** Alternative tailing procedure for blunt-end PCR fragments.

## C. Optimizing Insert:Vector Molar Ratios

The pGEM®-T and pGEM®-T Easy Vector Systems have been optimized using a 1:1 molar ratio of the Control Insert DNA to the Vectors. However, ratios of 8:1 to 1:8 have been successfully used. If initial experiments with your PCR product are suboptimal, ratio optimization may be necessary. Ratios from 3:1 to 1:3 provide good initial parameters. The concentration of PCR product should be estimated by comparison to DNA mass standards on a gel or by using a fluorescent assay (5). The pGEM®-T and pGEM®-T Easy Vectors are approximately 3kb and are supplied at 50ng/ml. To calculate the appropriate amount of PCR product (insert) to include in the ligation reaction, use the following equation.

$$\frac{\text{ng of vector x kb size of insert}}{\text{kb size of vector}} \times \text{insert:vector molar ratio} = \text{ng of insert}$$

Sufficient pGEM®-T or pGEM®-T Easy Vector is provided to vary insert:vector ratios as recommended and to perform control reactions.

**Example of insert:vector ratio calculation:**

How much 0.5kb PCR product should be added to a ligation in which 50ng of 3.0kb vector will be used if a 3:1 insert:vector molar ratio is desired?

$$\frac{\text{50ng vector x 0.5kb insert}}{\text{3.0kb vector}} \times \frac{3}{1} = \text{25ng insert}$$

**Note:** Using the same parameters for a 1:1 insert:vector molar ratio, 8.3ng of a 0.5kb insert would be required.

## D. Screening Transformants for Inserts

Successful cloning of an insert in the pGEM®-T and pGEM®-T Easy Vectors interrupts the coding sequence of β-galactosidase; recombinant clones can usually be identified by color screening on indicator plates. However, the characteristics of PCR products cloned into the pGEM®-T and pGEM®-T Easy Vectors can significantly affect the ratio of blue:white colonies obtained following transformation of competent cells. Clones that contain PCR products, in most cases, produce white colonies, but blue colonies can result from PCR fragments that are cloned in-frame with the *lac*Z gene. Such fragments are usually a multiple of 3 base pairs long (including the 3´-A overhangs), which do not contain in-frame stop codons. It has been reported in the literature that DNA fragments up to 2kb have been cloned in-frame and produced blue colonies.

Even if your PCR product is not a multiple of 3 bases long, the amplification process can introduce mutations (e.g., deletions or point mutations) that may result in blue colonies when competent cells are transformed with the fragment inserted into the pGEM®-T or pGEM®-T Easy Vectors.

The Control Insert DNA supplied with the pGEM®-T and pGEM®-T Easy Systems is a 542bp fragment from Promega's pGEM®-*luc*(f) DNA. This sequence has been mutated to contain multiple stop codons in all six reading frames, which ensures a low background of blue colonies for the control reaction. Results obtained with the Control Insert DNA may not be representative of those achieved with your PCR product.

## E. Experimental Controls

Promega strongly recommends performing the controls detailed below. These are necessary to accurately assess the performance of the pGEM®-T and pGEM®-T Easy Vector Systems.

### Positive Control

Set up a ligation reaction with the Control Insert DNA as described in the protocol (Section IV.A) and use it for transformations as described in Section V. This control will allow you to determine whether the ligation is proceeding efficiently. Typically, approximately 100 colonies should be observed, 10-30% of which are blue, when competent cells that have a transformation efficiency of 1 x $10^8$ cfu/µg DNA are transformed. Greater than 60% of the colonies should be white, and therefore recombinant since the Control Insert DNA is designed to reduce the number of background blue colonies (discussed in Section III.D). Background blue colonies arise from non-T-tailed or undigested pGEM®-T or pGEM®-T Easy Vector. These blue colonies are a useful internal transformation control; if no colonies are obtained, the transformation has failed. If blue colonies are obtained, but no whites, the result suggests that the ligation reaction failed. If <50% white colonies are seen in this positive control reaction, then the ligation conditions were probably suboptimal.

The concentration of the Control Insert DNA is such that 2µl (4ng/µl) can be used in a 10µl ligation reaction to achieve a 1:1 molar ratio with 50ng of the pGEM®-T or pGEM®-T Easy Vectors.

### Background Control

Set up a ligation reaction with 50ng of pGEM®-T or pGEM®-T Easy Vector and no insert as described in the protocol (Section IV.A) and use it for transformations as described in Section V. This ligation will allow determination of the number of background blue colonies resulting from non-T-tailed or undigested pGEM®-T or pGEM®-T Easy Vector alone. If the recommendations in Section V are followed closely, 10-30 blue colonies will, typically, be observed if the transformation efficiency of the competent cells is 1 x $10^8$ cfu/µg DNA. (Under these conditions, cells that are 1 x $10^7$ cfu/µg DNA would yield 1-3 blue colonies and cells with a transformation efficiency of 1 x $10^9$ cfu/µg DNA would yield 100-300 blue colonies). Compare the number of blue colonies obtained with this background control to the number of blue colonies obtained in the standard reaction using the PCR product. If ligation of the PCR product yields dramatically more blue colonies than the background control reaction, then recombinants are probably among these blue colonies (see Section III.D).

### Transformation Control

Check the transformation efficiency of the competent cells by transforming them with an uncut plasmid and calculating cfu/µg DNA. If the transformation efficiency is lower than 1 x $10^8$ cfu/µg DNA, prepare fresh cells. (Competent cells are available from Promega. See Section X.B.) If you are not using JM109 High Efficiency Competent Cells (provided with pGEM®-T and pGEM®-T Easy Vector Systems II; Cat.# A3610 and A1380, respectively), be sure the cells are compatible with blue/white screening and standard ampicillin selection, and are at least 1 x $10^8$ cfu/µg DNA.

**Example of Transformation Efficiency Calculation:**

After 100µl competent cells are transformed with 0.1ng uncut plasmid DNA, the transformation reaction is added to 900µl of SOC medium (0.1ng DNA/ml). From that volume, a 1:10 dilution with SOC medium (0.01ng DNA/ml) is made and 100µl plated on two plates (0.001ng DNA/100µl). If 200 colonies are obtained (average of two plates), what is the transformation efficiency?

$$\frac{200\text{cfu}}{0.001\text{ng}} = 2 \times 10^5 \text{ cfu/ng} = 2 \times 10^8 \text{ cfu/µg DNA}$$

## IV. Ligations Using the pGEM®-T and pGEM®-T Easy Vectors and the 2X Rapid Ligation Buffer

This protocol uses the **new 2X Rapid Ligation Buffer**. Use the appropriate volume when setting up the ligation reaction.

### A. Protocol

1. Briefly centrifuge the pGEM®-T or pGEM®-T Easy Vector and Control Insert DNA tubes to collect contents at the bottom of the tube.
2. Set up ligation reactions as described below. Note: Use 0.5ml tubes known to have low DNA-binding capacity (e.g., VWR Cat.# 20170-310).
3. Vortex the 2X Rapid Ligation Buffer vigorously before each use.

| | Standard Reaction | Positive Control | Background Control |
|---|---|---|---|
| 2X Rapid Ligation Buffer, T4 DNA Ligase | 5µl | 5µl | 5µl |
| pGEM®-T or pGEM®-T Easy Vector (50ng) | 1µl | 1µl | 1µl |
| PCR product | Xµl* | – | – |
| Control Insert DNA | – | 2µl | – |
| T4 DNA Ligase (3 Weiss units/µl) | 1µl | 1µl | 1µl |
| deionized water to a final volume of | 10µl | 10µl | 10µl |

*Molar ratio of PCR product:vector may require optimization (see Section III.C).

4. Mix the reactions by pipetting. Incubate the reactions 1 hour at room temperature.

   Alternatively, if the maximum number of transformants is required, incubate the reactions overnight at 4°C.

**Notes:**

1. Use only Promega T4 DNA Ligase supplied with this system in performing pGEM®-T and pGEM®-T Easy Vector ligations. Other commercial preparations of T4 DNA ligase may contain exonuclease activities that may remove the terminal thymidines from the vector.
2. 2X Rapid Ligation Buffer contains ATP, which degrades during temperature fluctuations. Avoid multiple freeze-thaw cycles and exposure to frequent temperature changes by making single-use aliquots of the buffer.
3. It is important to vortex the 2X Rapid Ligation Buffer before each use.
4. Longer incubation times will increase the number of transformants. Generally, incubation overnight at 4°C will produce the maximum number of transformants.

## V. Transformations Using the pGEM®-T and pGEM®-T Easy Vector Ligation Reactions

Use high efficiency competent cells (1 x $10^8$ cfu/µg DNA) for transformations. The ligation of fragments with a single-base overhang can be inefficient, so it is essential to use cells with a transformation efficiency of 1 x $10^8$ cfu/µg DNA (or higher) in order to obtain a reasonable number of colonies (see Section III.E).

We recommend using JM109 High Efficiency Competent Cells (Cat.# L2001); these are provided with the pGEM®-T and pGEM®-T Easy Vector Systems II. Other host strains may be used, but they should be compatible with blue/white color screening and standard ampicillin selection. JM109 cells should be maintained on M9 minimal medium plates supplemented with thiamine hydrochloride prior to the preparation of competent cells. This selects for the presence of the F´ episome, which carries both the *pro*AB genes, which complement proline auxotrophy in a host with a (*pro*AB) deletion, and *lac*$I^qZ\Delta M15$, which is required in the blue/white color screening process. If you are using competent cells other than JM109 High Efficiency Competent Cells purchased from Promega, it is important that the appropriate transformation protocol be followed. Selection for transformants should be on LB/ampicillin/IPTG/X-Gal plates (see Section X.A). For best results, do not use plates more than 30 days old.

The genotype of JM109 is *rec*A1, *end*A1, *gyr*A96, *thi*, *hsd*R17 ($r_{K-}$,$m_{K+}$), *rel*A1, *sup*E44, Δ(*lac-pro*AB), [F´, *tra*D36, *pro*AB, *lac*$I^qZ\Delta M15$] (8).

### A. Protocol

**Reagents to Be Supplied by the User**
(Solution compositions are provided in Section XI.A.)

- LB plates with ampicillin/IPTG/X-Gal
- SOC medium

1. Prepare 2 LB/ampicillin/IPTG/X-Gal plates for each ligation reaction, plus two plates for determining transformation efficiency (see Section III.E). Equilibrate the plates to room temperature prior to plating (Step 10).
2. Centrifuge the tubes containing the ligation reactions to collect contents at the bottom of the tube. Add 2µl of each ligation reaction to (a) sterile 1.5ml microcentrifuge tube(s) on ice (see Note 1). Set up another tube on ice with 0.1ng uncut plasmid for determination of the transformation efficiency of the competent cells (see Section III.E).
3. Remove tube(s) of frozen JM109 High Efficiency Competent Cells from –70°C storage and place in an ice bath until just thawed (about 5 minutes). Mix the cells by **gently** flicking the tube.
4. **Carefully** transfer 50µl of cells into each tube prepared in Step 2 (100µl cells for determination of transformation efficiency).
5. **Gently** flick the tubes to mix and place them on ice for 20 minutes.
6. Heat-shock the cells for 45-50 seconds in a water bath at exactly 42°C (DO NOT SHAKE).
7. Immediately return the tubes to ice for 2 minutes.

(!) Avoid excessive pipetting as the cells are very fragile.

8. Add 950µl room temperature SOC medium to the tubes containing cells transformed with ligation reactions and 900µl to the tube containing cells transformed with uncut plasmid (LB broth may be substituted, but colony number may be lower).

9. Incubate for 1.5 hours at 37°C with shaking (~150rpm).

10. Plate 100µl of each transformation culture onto duplicate antibiotic plates. For the transformation control, a 1:10 dilution with SOC medium is recommended for plating. If a higher number of colonies is desired, the cells may be pelleted by centrifugation at 1,000 x *g* for 10 minutes, resuspended in 200µl of SOC medium, and 100µl plated on each of 2 plates.

11. Incubate the plates overnight (16–24 hours) at 37°C. In our experience, approximately 100 colonies per plate are routinely seen when using competent cells that are 1 x $10^8$ cfu/µg DNA, if 100µl is plated. Longer incubations or storage of plates at 4°C (after 37°C overnight incubation) may be used to facilitate blue/white screening. White colonies generally contain inserts; however, inserts may also be present in blue colonies. Please see Section III.D for more information.

**Notes:**

1. In our experience, the use of larger (17 x 100mm) polypropylene tubes (e.g., Falcon Cat.# 2059) has been observed to increase transformation efficiency. Tubes from some manufacturers bind DNA, thereby decreasing the colony number, and should be avoided.

2. Colonies containing β-galactosidase activity may grow poorly relative to cells lacking this activity. After overnight growth, the blue colonies may be smaller than the white colonies which are approximately one millimeter in diameter.

## VI. Isolation of Recombinant Plasmid DNA

A standard plasmid miniprep procedure, which takes 30-60 minutes to perform, is described in Promega's Protocols and Applications Guide (9). The miniprep process can be both laborious and time-consuming, particularly when large numbers of minipreps are required. A convenient and reliable method is the Wizard® *Plus* Minipreps DNA Purification System(g).

The Wizard® line of DNA purification products offers five alternatives for plasmid DNA preparation which are distinguished by the scale of the isolation desired (see Table 3). Wizard® *Plus* Miniprep DNA isolations can be completed in 15 minutes and the DNA is ready for other molecular biology applications without prior ethanol precipitations. For best results, a vacuum manifold, such as Promega's Vac-Man® (Cat.# A7231) or Vac-Man® Jr. (Cat.# A7660) Laboratory Vacuum Manifold, should be used to process the minipreps. If a vacuum source is not available, the minipreps may be processed individually using a disposable 3ml Luer-Lok® syringe and a microcentrifuge. A vacuum source and manifold is required for the Wizard® *Plus* Midipreps, Maxipreps, Megapreps and Series 9600™ DNA Purification Systems(h). Refer to Section X.B for ordering information.

**Table 3. Wizard® *Plus* Plasmid DNA Purification Systems Selection Guide**

| Wizard® *Plus* DNA Purification System | Number of Isolations | Culture Volume | Typical DNA Yields* |
|---|---|---|---|
| Minipreps | 50-250 | 1-3ml | 3-10µg |
| Midipreps | 25 | 10-100ml | 10-200µg |
| Maxipreps | 10 | 100-500ml | 300-1,000µg |
| Megapreps | 5 | 500-1,000ml | 700-3,000µg |
| Series 9600™ | 8-192 | 1-5ml | 5-20µg |

*Typical DNA yields are based on results obtained with high copy number plasmids.

## VII. Generation of Single-Stranded DNA from the pGEM®-T and pGEM®-T Easy Vectors

For induction of ssDNA production, bacterial cells containing either the pGEM®-T or pGEM®-T Easy Vector are infected with an appropriate helper phage. The plasmid then enters the f1 replication mode, and the resulting ssDNA is exported as an encapsulated virus-like particle. The ssDNA is purified from the supernatant by simple precipitation and extraction procedures, which are described in detail in Technical Bulletin #TB187, *pGEM® Vector Cloning and Single-Stranded DNA Generation*. For further information, please contact your local Promega Branch Office or Distributor. In the U.S., contact Technical Services at 1-800-356-9526.

## VIII. Troubleshooting

| Symptom | Possible Cause | Comments |
|---|---|---|
| No colonies | A problem has occurred with the transformation reaction or the cells have lost competence | Background undigested vector and religated non-T-tailed vector should yield 10–30 blue colonies, independent of the presence of insert DNA. Check the background control (Section III.E). |
| | | Use high efficiency competent cells (1 x $10^8$ cfu/µg DNA). Test the efficiency of the cells by transforming them with an uncut plasmid that allows for antibiotic selection, such as the pGEM®-5Zf(+) Vector. If the recommendations in Section V.A are followed, cells that are 1 x $10^8$ cfu/µg DNA typically yield 100 colonies. Therefore, you would not see any colonies from cells that are 1 x $10^6$ cfu/µg DNA (Section III.E). |
| Less than 10% white colonies with Control Insert DNA | **Improper dilution of the 2X Rapid Ligation Buffer** | The T4 DNA ligase buffer is provided at a concentration of 2X. **Use 5µl in a 10µl reaction.** |
| | Ligation reaction has failed | Ligase buffer may have low activity. The 2X Rapid Ligation Buffer contains ATP, which degrades during temperature fluctuations. Avoid multiple freeze-thaw cycles by making single-use aliquots of the buffer. Use a fresh vial of buffer. |
| | | Ligase activity may be low. To test the activity of the ligase and buffer, set up a ligation with approximately 200ng of DNA markers (e.g., Lambda DNA/*Hin*d III Markers, Cat.# G1711). Compare ligated and nonligated DNA on a gel and check that the fragments have been religated into high molecular weight material. |
| | T-overhangs have been removed allowing blunt-end ligation of vector and giving rise to more blue colonies than white colonies | Avoid introduction of nucleases that may degrade the T-overhangs. Use only the T4 DNA Ligase provided with the system, which has been tested for minimal exonuclease activity. |

For questions not addressed here, please contact your local Promega branch office or distributor (contact information available at: **www.promega.com**.

E-mail: **techserv@promega.com**

**VIII. Troubleshooting (continued)**

| Symptom | Possible Cause | Comments |
|---|---|---|
| High colony number, but low percentage of white with Control Insert DNA | The competent cells have a high transformation efficiency (>1 x $10^9$ cfu/µg DNA), and there is a ligation problem | Approximately 1,000 colonies can be obtained with cells that are 1 x $10^9$ cfu/µg DNA with 70-90% of those being white. If the ligation is suboptimal or fails, the total number of colonies will be high, but the amount of white colonies will be low or zero. See comments under "Ligation reaction has failed" (above). Also, optimize vector:insert ratio. |
| Less than 60% white colonies with Control Insert DNA | **Improper dilution of the 2X Rapid Ligation Buffer** | The T4 DNA ligase buffer is provided at a concentration of 2X. **Use 5µl in a 10µl reaction.** |
| | T-overhangs have been removed allowing blunt-end ligation of vector and giving rise to more blue colonies than white colonies | Avoid introduction of nucleases that may degrade the T-overhangs. Use only the T4 DNA Ligase provided with the system, which has been tested for minimal exonuclease activity. |
| | Ligation temperature is too high | Higher temperatures (>28°C) give rise to increased background and fewer recombinants. |
| Low number or no white colonies with PCR product | **Improper dilution of the 2X Rapid Ligation Buffer** | The T4 DNA ligase buffer is provided at a concentration of 2X. **Use 5µl in a 10µl reaction.** |
| | Ligation incubation is not long enough | Optimal results are seen with an overnight ligation. |
| | Failed ligation due to an inhibitory component in the PCR product | Mix some of the PCR product with the control ligation to see if it is inhibiting the reaction. If an inhibitor is suspected, repurify the PCR fragment. |
| | PCR product is not ligating because there are no 3´-A overhangs | As summarized in Table 1, not all thermostable DNA polymerases create a 3´-A overhang (3,4). Blunt-ended fragments may be subsequently A-tailed by treatment with an appropriate polymerase and dATP (10,11). |

## VIII. Troubleshooting (continued)

| Symptom | Possible Cause | Comments |
|---|---|---|
| Low number or no white colonies with PCR product (continued) | PCR product cannot be ligated due to pyrimidine dimers formed from UV overexposure | This is a common problem with gel purified DNA. There is no way to fix this; the DNA must be remade. Exposure to shortwave UV should be limited as much as possible. Use of a glass plate between the gel and UV source will decrease UV overexposure. If possible, only visualize the PCR product using a longwave UV source. |
| | The PCR fragment is inserted, but it is not disrupting the *lac*Z gene | If there are a higher number of blue colonies resulting from the PCR fragment ligation than with the background control, some of these blue colonies may contain insert. Screen blue and pale blue colonies (see Section III.D). |
| | Insert:vector ratio is not optimal | Check the integrity and quantity of your PCR fragment by gel analysis. Optimize the insert:vector ratio (see Section III.C). |
| | Ligation temperature is too high | Higher temperatures (>28°C) give rise to increased background and fewer recombinants. |
| | There may be primer-dimers present in PCR fragment preparation | Primer-dimers will ligate into the pGEM®-T or pGEM®-T Easy Vector, but may not be seen after restriction digestion and gel analysis because of their small size. The vector will appear to contain no insert. The PCR fragment should be gel purified. |
| | Multiple PCR products are generated and cloned into pGEM®-T or pGEM®-T Easy Vector | Gel purify the PCR fragment of interest. |
| | DNA has rearranged | Check a number of clones to see whether the rearrangement is random. If so, the clone of interest should be present and can be identified by screening several clones. If the same rearrangement is found in all of the clones, use a repair deficient bacterial strain to protect the insert (e.g., SURE® cells), which may reduce recombination events. |

For questions not addressed here, please contact your local Promega branch office or distributor (contact information available at: **www.promega.com**.

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**VIII. Troubleshooting (continued)**

| Symptom | Possible Cause | Comments |
|---|---|---|
| PCR product ligation reaction produces white colonies only (no blue colonies are present) | Ampicillin is inactive, allowing ampicillin-sensitive cells to grow | Check that ampicillin plates are made properly and used within 2 weeks. Test ampicillin activity by streaking plates, with and without ampicillin, with an ampicillin-sensitive clone. |
| | The bacterial strain (e.g., JM109) has lost its F´ episome | Check the background control. If these colonies are not blue, the cells may have lost the F´ episome (assuming *lac*I$^q$ZΔM15 is located on the F´ in the transformed strain and appropriate plates were used). Be sure that the cells are prepared properly for use with this system (see Section V). |
| | Plates are incompatible with blue/white screening | Check the background control. If these colonies are not blue, check that the plates have ampicillin/IPTG/X-Gal, and that they are fresh. If there is any question about the quality of the plates, repeat plating with fresh plates. |
| Not enough clones contain the PCR product of interest | Insufficient A-tailing of the PCR fragment | After purification of the PCR fragment, set up an A-tailing reaction (10,11). Clean up the sample and proceed with the protocol. |
| | Insert:vector ratio is not optimal | Check the integrity and quantity of your PCR fragment by gel analysis. Optimize the insert:vector ratio (see Section III.C). |

## IX. References


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## X. Appendix A: Vector Sequences and Restriction Sites

### A. Sequence of the pGEM®-T Vector

The sequence supplied below is that of the circular pGEM®-5Zf(+) Vector from which the pGEM®-T Vector is derived. The pGEM®-T Vector has been linearized with *Eco*R V at base 51 of this sequence (indicated by an asterisk) and a T added to both 3´-ends. The added T is not included in this sequence. The sequence shown corresponds to RNA synthesized by T7 RNA Polymerase and is complementary to RNA synthesized by SP6 RNA Polymerase. The strand shown is complementary to the ssDNA produced by this vector. Vector sequences are also available on the Internet at www.promega.com.

```
  1  GGGCGAATTG GGCCCGACGT CGCATGCTCC CGGCCGCCAT GGCCGCGGGA
 51  T*ATCACTAGT GCGGCCGCCT GCAGGTCGAC CATATGGGAG AGCTCCCAAC
101  GCGTTGGATG CATAGCTTGA GTATTCTATA GTGTCACCTA AATAGCTTGG
151  CGTAATCATG GTCATAGCTG TTTCCTGTGT GAAATTGTTA TCCGCTCACA
201  ATTCCACACA ACATACGAGC CGGAAGCATA AAGTGTAAAG CCTGGGGTGC
251  CTAATGAGTG AGCTAACTCA CATTAATTGC GTTGCGCTCA CTGCCCGCTT
301  TCCAGTCGGG AAACCTGTCG TGCCAGCTGC ATTAATGAAT CGGCCAACGC
351  GCGGGGAGAG GCGGTTTGCG TATTGGGCGC TCTTCCGCTT CCTCGCTCAC
401  TGACTCGCTG CGCTCGGTCG TTCGGCTGCG GCGAGCGGTA TCAGCTCACT
451  CAAAGGCGGT AATACGGTTA TCCACAGAAT CAGGGGATAA CGCAGGAAAG
501  AACATGTGAG CAAAAGGCCA GCAAAAGGCC AGGAACCGTA AAAAGGCCGC
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 551  GTTGCTGGCG TTTTTCCATA GGCTCCGCCC CCCTGACGAG CATCACAAAA
 601  ATCGACGCTC AAGTCAGAGG TGGCGAAACC CGACAGGACT ATAAAGATAC
 651  CAGGCGTTTC CCCCTGGAAG CTCCCTCGTG CGCTCTCCTG TTCCGACCCT
 701  GCCGCTTACC GGATACCTGT CCGCCTTTCT CCCTTCGGGA AGCGTGGCGC
 751  TTTCTCATAG CTCACGCTGT AGGTATCTCA GTTCGGTGTA GGTCGTTCGC
 801  TCCAAGCTGG GCTGTGTGCA CGAACCCCCC GTTCAGCCCG ACCGCTGCGC
 851  CTTATCCGGT AACTATCGTC TTGAGTCCAA CCCGGTAAGA CACGACTTAT
 901  CGCCACTGGC AGCAGCCACT GGTAACAGGA TTAGCAGAGC GAGGTATGTA
 951  GGCGGTGCTA CAGAGTTCTT GAAGTGGTGG CCTAACTACG GCTACACTAG
1001  AAGGACAGTA TTTGGTATCT GCGCTCTGCT GAAGCCAGTT ACCTTCGGAA
1051  AAAGAGTTGG TAGCTCTTGA TCCGGCAAAC AAACCACCGC TGGTAGCGGT
1101  GGTTTTTTTG TTTGCAAGCA GCAGATTACG CGCAGAAAAA AAGGATCTCA
1151  AGAAGATCCT TTGATCTTTT CTACGGGGTC TGACGCTCAG TGGAACGAAA
1201  ACTCACGTTA AGGGATTTTG GTCATGAGAT TATCAAAAAG GATCTTCACC
1251  TAGATCCTTT TAAATTAAAA ATGAAGTTTT AAATCAATCT AAAGTATATA
1301  TGAGTAAACT TGGTCTGACA GTTACCAATG CTTAATCAGT GAGGCACCTA
1351  TCTCAGCGAT CTGTCTATTT CGTTCATCCA TAGTTGCCTG ACTCCCCGTC
1401  GTGTAGATAA CTACGATACG GGAGGGCTTA CCATCTGGCC CCAGTGCTGC
1451  AATGATACCG CGAGACCCAC GCTCACCGGC TCCAGATTTA TCAGCAATAA
1501  ACCAGCCAGC CGGAAGGGCC GAGCGCAGAA GTGGTCCTGC AACTTTATCC
1551  GCCTCCATCC AGTCTATTAA TTGTTGCCGG GAAGCTAGAG TAAGTAGTTC
1601  GCCAGTTAAT AGTTTGCGCA ACGTTGTTGG CATTGCTACA GGCATCGTGG
1651  TGTCACGCTC GTCGTTTGGT ATGGCTTCAT TCAGCTCCGG TTCCCAACGA
1701  TCAAGGCGAG TTACATGATC CCCCATGTTG TGCAAAAAAG CGGTTAGCTC
1751  CTTCGGTCCT CCGATCGTTG TCAGAAGTAA GTTGGCCGCA GTGTTATCAC
1801  TCATGGTTAT GGCAGCACTG CATAATTCTC TTACTGTCAT GCCATCCGTA
1851  AGATGCTTTT CTGTGACTGG TGAGTACTCA ACCAAGTCAT TCTGAGAATA
1901  GTGTATGCGG CGACCGAGTT GCTCTTGCCC GGCGTCAATA CGGGATAATA
1951  CCGCGCCACA TAGCAGAACT TTAAAAGTGC TCATCATTGG AAAACGTTCT
2001  TCGGGGCGAA AACTCTCAAG GATCTTACCG CTGTTGAGAT CCAGTTCGAT
2051  GTAACCCACT CGTGCACCCA ACTGATCTTC AGCATCTTTT ACTTTCACCA
2101  GCGTTTCTGG GTGAGCAAAA ACAGGAAGGC AAAATGCCGC AAAAAAGGGA
2151  ATAAGGGCGA CACGGAAATG TTGAATACTC ATACTCTTCC TTTTTCAATA
2201  TTATTGAAGC ATTTATCAGG GTTATTGTCT CATGAGCGGA TACATATTTG
2251  AATGTATTTA GAAAAATAAA CAAATAGGGG TTCCGCGCAC ATTTCCCCGA
2301  AAAGTGCCAC CTGTATGCGG TGTGAAATAC CGCACAGATG CGTAAGGAGA
2351  AAATACCGCA TCAGGCGAAA TTGTAAACGT TAATATTTTG TTAAAATTCG
2401  CGTTAAATAT TTGTTAAATC AGCTCATTTT TTAACCAATA GGCCGAAATC
2451  GGCAAAATCC CTTATAAATC AAAAGAATAG ACCGAGATAG GGTTGAGTGT
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2501  TGTTCCAGTT TGGAACAAGA GTCCACTATT AAAGAACGTG GACTCCAACG
2551  TCAAAGGGCG AAAAACCGTC TATCAGGGCG ATGGCCCACT ACGTGAACCA
2601  TCACCCAAAT CAAGTTTTTT GCGGTCGAGG TGCCGTAAAG CTCTAAATCG
2651  GAACCCTAAA GGGAGCCCCC GATTTAGAGC TTGACGGGGA AAGCCGGCGA
2701  ACGTGGCGAG AAAGGAAGGG AAGAAAGCGA AAGGAGCGGG CGCTAGGGCG
2751  CTGGCAAGTG TAGCGGTCAC GCTGCGCGTA ACCACCACAC CCGCCGCGCT
2801  TAATGCGCCG CTACAGGGCG CGTCCATTCG CCATTCAGGC TGCGCAACTG
2851  TTGGGAAGGG CGATCGGTGC GGGCCTCTTC GCTATTACGC CAGCTGGCGA
2901  AAGGGGGATG TGCTGCAAGG CGATTAAGTT GGGTAACGCC AGGGTTTTCC
2951  CAGTCACGAC GTTGTAAAAC GACGGCCAGT GAATTGTAAT ACGACTCACT
3001  ATA
```

### B. pGEM®-T Vector Restriction Sites

The following restriction enzyme tables are based on those of the circular pGEM®-5Zf(+) Vector from which the pGEM®-T Vector is derived. The pGEM®-T Vector has been linearized at base 51 with *Eco*R V and a T added to both 3´-ends. This site will not be recovered upon ligation of the vector and insert. The tables were constructed using DNASTAR® sequence analysis software. Please note that we have not verified this information by restriction digestion with each enzyme listed. The location given specifies the 3´-end of the cut DNA (the base to the left of the cut site). Please contact your local Promega Branch Office or Distributor if you identify a discrepancy. In the U.S., contact Technical Services at 1-800-356-9526.

**Table 4. Restriction Enzymes That Cut the pGEM®-T Vector Between 1 and 5 Times.**

| Enzyme | # of Sites | Location |
|---|---|---|
| ***Aat* II** | 1 | 20 |
| ***Acc* I** | 1 | 76 |
| ***Acy* I** | 2 | 17, 1932 |
| *Afl* III | 2 | 99, 502 |
| ***Alw*26 I** | 2 | 1456, 2232 |
| ***Alw*44 I** | 2 | 816, 2062 |
| *Alw*N I | 1 | 918 |
| ***Apa* I** | 1 | 14 |
| *Asp*H I | 4 | 94, 820, 1981, 2066 |
| ***Ava* II** | 2 | 1533, 1755 |
| ***Ban* I** | 3 | 246, 1343, 2629 |
| ***Ban* II** | 3 | 14, 94, 2667 |
| ***Bbu* I** | 1 | 26 |
| ***Bgl* I** | 3 | 39, 1515, 2836 |
| *Bsa* I | 1 | 1456 |
| *Bsa*A I | 1 | 2592 |
| *Bsa*H I | 2 | 17, 1932 |
| *Bsa*J I | 5 | 37, 43, 241, 662, 2939 |
| *Bsp*120 I | 1 | 10 |
| *Bsp*H I | 2 | 1222, 2230 |
| *Bsp*M I | 1 | 62 |
| *Bss*S I | 2 | 675, 2059 |
| ***Bst*O I** | 5 | 242, 530, 651, 664, 2940 |
| ***Bst*X I** | 1 | 103 |
| ***Bst*Z I** | 2 | 31, 62 |
| *Cfr*10 I | 2 | 1475, 2693 |
| ***Dde* I** | 4 | 777, 1186, 1352, 1892 |
| ***Dra* I** | 3 | 1261, 1280, 1972 |
| *Dra* III | 1 | 2592 |
| *Drd* I | 2 | 610, 2547 |
| *Dsa* I | 2 | 37, 43 |
| *Eag* I | 2 | 31, 62 |
| *Ear* I | 3 | 386, 2190, 2881 |
| ***Ecl*HK I** | 1 | 1395 |
| ***Eco*52 I** | 2 | 31, 62 |
| ***Eco*ICR I** | 1 | 92 |
| ***Eco*R V** | 1 | 51 (see above) |
| ***Fok* I** | 5 | 119, 1361, 1542, 1829, 2919 |
| *Fsp* I | 2 | 1617, 2843 |
| ***Hae* II** | 4 | 380, 750, 2743, 2751 |
| *Hga* I | 4 | 613, 1191, 1921, 2809 |
| ***Hinc* II** | 1 | 77 |
| *Hin*d II | 1 | 77 |
| ***Hsp*92 I** | 2 | 17, 1932 |
| *Ksp* I | 1 | 46 |
| *Mae* I | 5 | 56, 997, 1250, 1585, 2743 |

| Enzyme | # of Sites | Location | Enzyme | #of Sites | Location |
|---|---|---|---|---|---|
| ***Mlu* I** | 1 | 99 | ***Sac* II** | 1 | 46 |
| ***Nae* I** | 1 | 2695 | ***Sal* I** | 1 | 75 |
| ***Nci* I** | 4 | 30, 882, 1578, 1929 | ***Sca* I** | 1 | 1875 |
| ***Nco* I** | 1 | 37 | ***Sfi* I** | 1 | 39 |
| ***Nde* I** | 1 | 82 | ***Sin* I** | 2 | 1533, 1755 |
| ***Ngo*M I** | 1 | 2693 | ***Spe* I** | 1 | 55 |
| ***Not* I** | 1 | 62 | ***Sph* I** | 1 | 26 |
| ***Nsi* I** | 1 | 112 | *Sse*8387 I | 1 | 73 |
| *Nsp* I | 2 | 26, 506 | ***Ssp* I** | 3 | 2199, 2384, 2408 |
| *Ppu*10 I | 1 | 108 | ***Sty* I** | 1 | 37 |
| ***Pst* I** | 1 | 73 | ***Taq* I** | 4 | 76, 602, 2046, 2625 |
| ***Pvu* I** | 2 | 1765, 2864 | *Tfi* I | 2 | 337, 477 |
| ***Pvu* II** | 2 | 326, 2893 | ***Vsp* I** | 3 | 273, 332, 1567 |
| ***Rsa* I** | 1 | 1875 | ***Xmn* I** | 1 | 1994 |
| ***Sac* I** | 1 | 94 | | | |

**Note:** The enzymes listed in boldface type are available from Promega.

**Table 5. Restriction Enzymes That Do Not Cut the pGEM®-T Vector.**

| | | | | | |
|---|---|---|---|---|---|
| ***Acc* III** | *Bbs* I | *Bst*1107 I | ***Eco*R I** | *Pae*R7 I | ***Sma* I** |
| ***Acc*65 I** | ***Bcl* I** | ***Bst*98 I** | *Ehe* I | *Pfl*M I | ***Sna*B I** |
| ***Acc*B7 I** | ***Bgl* II** | ***Bst*E II** | *Fse* I | *Pin*A I | *Spl* I |
| *Afl* II | *Blp* I | ***Bsu*36 I** | ***Hin*d III** | *Pme* I | *Srf* I |
| ***Age* I** | *Bpu*1102 I | ***Cla* I** | ***Hpa* I** | *Pml* I | ***Stu* I** |
| *Asc* I | *Bsa*B I | ***Csp* I** | **I-*Ppo* I** | *Ppu*M I | *Swa* I |
| ***Ava* I** | ***Bsa*M I** | ***Csp*45 I** | *Kas* I | *Psh*A I | ***Tth*111 I** |
| *Avr* II | *Bsi*W I | *Dra* II | ***Kpn* I** | *Psp*5 II | ***Xba* I** |
| ***Bal* I** | *Bsm* I | ***Eco*47 III** | ***Nar* I** | *Psp*A I | *Xcm* I |
| ***Bam*H I** | ***Bsr*Br I** | *Eco*N I | ***Nhe* I** | *Rsr* II | ***Xho* I** |
| *Bbe* I | *Bsr*G I | *Eco*72 I | ***Nru* I** | ***Sgf* I**(i) | ***Xma* I** |
| *Bbr*P I | ***Bss*H II** | *Eco*81 I | *Pac* I | *Sgr*A I | |

**Note:** The enzymes listed in boldface type are available from Promega.

**Table 6. Restriction Enzymes That Cut the pGEM®-T Vector 6 or More Times.**

| | | | | | |
|---|---|---|---|---|---|
| *Aci* I | ***Bst*71 I** | ***Hae* III** | *Mae* II | ***Msp*A1 I** | *Sfa*N I |
| ***Alu* I** | *Bst*U I | ***Hha* I** | *Mae* III | *Nla* III | ***Tru*9 I** |
| *Bbv* I | ***Cfo* I** | ***Hin*f I** | ***Mbo* I** | *Nla* IV | ***Xho* II** |
| ***Bsa*O I** | ***Dpn* I** | *Hin*P I | ***Mbo* II** | *Ple* I | |
| ***Bsp*1286 I** | *Dpn* II | ***Hpa* II** | *Mnl* I | ***Sau*3A I** | |
| *Bsr* I | *Eae* I | *Hph* I | *Mse* I | ***Sau*96 I** | |
| ***Bsr*S I** | *Fnu*4H I | ***Hsp*92 II** | ***Msp* I** | *Scr*F I | |

**Note:** The enzymes listed in boldface type are available from Promega.

## C. Sequence of the pGEM®-T Easy Vector

The pGEM®-T Easy Vector has been linearized with *Eco*R V at base 60 of this sequence (indicated by an asterisk) and a T added to both 3´-ends. The added T is not included in this sequence. The sequence shown corresponds to RNA synthesized by T7 RNA Polymerase and is complementary to RNA synthesized by SP6 RNA Polymerase. The strand shown is complementary to the ssDNA produced by this vector. Vector sequences are also available on the Internet at www.promega.com.

```
   1  GGGCGAATTG GGCCCGACGT CGCATGCTCC CGGCCGCCAT GGCGGCCGCG
  51  GGAATTCGAT*ATCACTAGTG AATTCGCGGC CGCCTGCAGG TCGACCATAT
 101  GGGAGAGCTC CCAACGCGTT GGATGCATAG CTTGAGTATT CTATAGTGTC
 151  ACCTAAATAG CTTGGCGTAA TCATGGTCAT AGCTGTTTCC TGTGTGAAAT
 201  TGTTATCCGC TCACAATTCC ACACAACATA CGAGCCGGAA GCATAAAGTG
 251  TAAAGCCTGG GGTGCCTAAT GAGTGAGCTA ACTCACATTA ATTGCGTTGC
 301  GCTCACTGCC CGCTTTCCAG TCGGGAAACC TGTCGTGCCA GCTGCATTAA
 351  TGAATCGGCC AACGCGCGGG GAGAGGCGGT TTGCGTATTG GGCGCTCTTC
 401  CGCTTCCTCG CTCACTGACT CGCTGCGCTC GGTCGTTCGG CTGCGGCGAG
 451  CGGTATCAGC TCACTCAAAG GCGGTAATAC GGTTATCCAC AGAATCAGGG
 501  GATAACGCAG GAAAGAACAT GTGAGCAAAA GGCCAGCAAA AGGCCAGGAA
 551  CCGTAAAAAG GCCGCGTTGC TGGCGTTTTT CCATAGGCTC CGCCCCCCTG
 601  ACGAGCATCA CAAAAATCGA CGCTCAAGTC AGAGGTGGCG AAACCCGACA
 651  GGACTATAAA GATACCAGGC GTTTCCCCCT GGAAGCTCCC TCGTGCGCTC
 701  TCCTGTTCCG ACCCTGCCGC TTACCGGATA CCTGTCCGCC TTTCTCCCTT
 751  CGGGAAGCGT GGCGCTTTCT CATAGCTCAC GCTGTAGGTA TCTCAGTTCG
 801  GTGTAGGTCG TTCGCTCCAA GCTGGGCTGT GTGCACGAAC CCCCCGTTCA
 851  GCCCGACCGC TGCGCCTTAT CCGGTAACTA TCGTCTTGAG TCCAACCCGG
 901  TAAGACACGA CTTATCGCCA CTGGCAGCAG CCACTGGTAA CAGGATTAGC
 951  AGAGCGAGGT ATGTAGGCGG TGCTACAGAG TTCTTGAAGT GGTGGCCTAA
1001  CTACGGCTAC ACTAGAAGGA CAGTATTTGG TATCTGCGCT CTGCTGAAGC
1051  CAGTTACCTT CGGAAAAAGA GTTGGTAGCT CTTGATCCGG CAAACAAACC
1101  ACCGCTGGTA GCGGTGGTTT TTTTGTTTGC AAGCAGCAGA TTACGCGCAG
1151  AAAAAAAGGA TCTCAAGAAG ATCCTTTGAT CTTTTCTACG GGGTCTGACG
1201  CTCAGTGGAA CGAAAACTCA CGTTAAGGGA TTTTGGTCAT GAGATTATCA
1251  AAAAGGATCT TCACCTAGAT CCTTTTAAAT TAAAAATGAA GTTTTAAATC
1301  AATCTAAAGT ATATATGAGT AAACTTGGTC TGACAGTTAC CAATGCTTAA
1351  TCAGTGAGGC ACCTATCTCA GCGATCTGTC TATTTCGTTC ATCCATAGTT
1401  GCCTGACTCC CCGTCGTGTA GATAACTACG ATACGGGAGG GCTTACCATC
1451  TGGCCCCAGT GCTGCAATGA TACCGCGAGA CCCACGCTCA CCGGCTCCAG
1501  ATTTATCAGC AATAAACCAG CCAGCCGGAA GGGCCGAGCG CAGAAGTGGT
1551  CCTGCAACTT TATCCGCCTC CATCCAGTCT ATTAATTGTT GCCGGGAAGC
1601  TAGAGTAAGT AGTTCGCCAG TTAATAGTTT GCGCAACGTT GTTGGCATTG
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1651  CTACAGGCAT CGTGGTGTCA CGCTCGTCGT TTGGTATGGC TTCATTCAGC
1701  TCCGGTTCCC AACGATCAAG GCGAGTTACA TGATCCCCCA TGTTGTGCAA
1751  AAAAGCGGTT AGCTCCTTCG GTCCTCCGAT CGTTGTCAGA AGTAAGTTGG
1801  CCGCAGTGTT ATCACTCATG GTTATGGCAG CACTGCATAA TTCTCTTACT
1851  GTCATGCCAT CCGTAAGATG CTTTTCTGTG ACTGGTGAGT ACTCAACCAA
1901  GTCATTCTGA GAATAGTGTA TGCGGCGACC GAGTTGCTCT TGCCCGGCGT
1951  CAATACGGGA TAATACCGCG CCACATAGCA GAACTTTAAA AGTGCTCATC
2001  ATTGGAAAAC GTTCTTCGGG GCGAAAACTC TCAAGGATCT TACCGCTGTT
2051  GAGATCCAGT TCGATGTAAC CCACTCGTGC ACCCAACTGA TCTTCAGCAT
2101  CTTTTACTTT CACCAGCGTT TCTGGGTGAG CAAAAACAGG AAGGCAAAAT
2151  GCCGCAAAAA AGGGAATAAG GGCGACACGG AAATGTTGAA TACTCATACT
2201  CTTCCTTTTT CAATATTATT GAAGCATTTA TCAGGGTTAT TGTCTCATGA
2251  GCGGATACAT ATTTGAATGT ATTTAGAAAA ATAAACAAAT AGGGGTTCCG
2301  CGCACATTTC CCCGAAAAGT GCCACCTGTA TGCGGTGTGA AATACCGCAC
2351  AGATGCGTAA GGAGAAAATA CCGCATCAGG CGAAATTGTA AACGTTAATA
2401  TTTTGTTAAA ATTCGCGTTA AATATTTGTT AAATCAGCTC ATTTTTTAAC
2451  CAATAGGCCG AAATCGGCAA AATCCCTTAT AAATCAAAAG AATAGACCGA
2501  GATAGGGTTG AGTGTTGTTC CAGTTTGGAA CAAGAGTCCA CTATTAAAGA
2551  ACGTGGACTC CAACGTCAAA GGGCGAAAAA CCGTCTATCA GGGCGATGGC
2601  CCACTACGTG AACCATCACC CAAATCAAGT TTTTTGCGGT CGAGGTGCCG
2651  TAAAGCTCTA AATCGGAACC CTAAAGGGAG CCCCCGATTT AGAGCTTGAC
2701  GGGGAAAGCC GGCGAACGTG GCGAGAAAGG AAGGGAAGAA AGCGAAAGGA
2751  GCGGGCGCTA GGGCGCTGGC AAGTGTAGCG GTCACGCTGC GCGTAACCAC
2801  CACACCCGCC GCGCTTAATG CGCCGCTACA GGGCGCGTCC ATTCGCCATT
2851  CAGGCTGCGC AACTGTTGGG AAGGGCGATC GGTGCGGGCC TCTTCGCTAT
2901  TACGCCAGCT GGCGAAAGGG GGATGTGCTG CAAGGCGATT AAGTTGGGTA
2951  ACGCCAGGGT TTTCCCAGTC ACGACGTTGT AAAACGACGG CCAGTGAATT
3001  GTAATACGAC TCACTATA
```

## D. pGEM®-T Easy Vector Restriction Sites

The pGEM®-T Easy Vector has been linearized at base 60 with *Eco*R V and a T added to both 3´-ends. This site will not be recovered upon ligation of the vector and insert. The tables were constructed using DNASTAR® sequence analysis software. Please note that we have not verified this information by restriction digestion with each enzyme listed. The location given specifies the 3´-end of the cut DNA (the base to the left of the cut site). Please contact your local Promega Branch Office or Distributor if you identify a discrepancy. In the U.S., contact Technical Services at 1-800-356-9526.

**Table 7. Restriction Enzymes That Cut the pGEM®-T Easy Vector Between 1 and 5 Times**

| Enzyme | # of Sites | Location |
|---|---|---|
| ***Aat* II** | 1 | 20 |
| ***Acc* I** | 1 | 91 |
| ***Acy* I** | 2 | 17, 1947 |
| *Afl* III | 2 | 114, 517 |
| ***Alw*26 I** | 2 | 1471, 2247 |
| ***Alw*44 I** | 2 | 831, 2077 |
| *Alw*N I | 1 | 933 |
| ***Apa* I** | 1 | 14 |
| *Asp*H I | 4 | 109, 835, 1996, 2081 |
| ***Ava* II** | 2 | 1548, 1770 |
| ***Ban* I** | 3 | 261, 1358, 2644 |
| ***Ban* II** | 3 | 14, 109, 2682 |
| ***Bbu* I** | 1 | 26 |
| ***Bgl* I** | 4 | 39, 42, 1530, 2851 |
| *Bsa* I | 1 | 1471 |
| *Bsa*A I | 1 | 2607 |
| *Bsa*H I | 2 | 17, 1947 |
| *Bsa*J I | 5 | 37, 46, 256, 677, 2954 |
| *Bsp*120 I | 1 | 10 |
| *Bsp*H I | 2 | 1237, 2245 |
| *Bsp*M I | 1 | 77 |
| *Bss*S I | 2 | 690, 2074 |
| ***Bst*O I** | 5 | 257, 545, 666, 679, 2955 |
| ***Bst*X I** | 1 | 118 |
| ***Bst*Z I** | 3 | 31, 43, 77 |
| *Cfr*10 I | 2 | 1490, 2708 |
| ***Dde* I** | 4 | 792, 1201, 1367, 1907 |
| ***Dra* I** | 3 | 1276, 1295, 1987 |
| *Dra* III | 1 | 2607 |
| *Drd* I | 2 | 625, 2562 |
| *Dsa* I | 2 | 37, 46 |
| *Eag* I | 3 | 31, 43, 77 |
| *Ear* I | 3 | 401, 2205, 2896 |
| ***Ecl*HK I** | 1 | 1410 |
| ***Eco*52 I** | 3 | 31, 43, 77 |
| ***Eco*ICR I** | 1 | 107 |
| ***Eco*R I** | 2 | 52, 70 |
| ***Eco*R V** | 1 | 60 (see above) |

| Enzyme | # of Sites | Location |
|---|---|---|
| ***Fok* I** | 5 | 134, 1376, 1557, 1844, 2934 |
| *Fsp* I | 2 | 1632, 2858 |
| ***Hae* II** | 4 | 395, 765, 2758, 2766 |
| *Hga* I | 4 | 628, 1206, 1936, 2824 |
| ***Hin*c II** | 1 | 92 |
| *Hin*d II | 1 | 92 |
| ***Hsp*92 I** | 2 | 17, 1947 |
| *Ksp* I | 1 | 49 |
| *Mae* I | 5 | 65, 1012, 1265, 1600, 2758 |
| ***Mlu* I** | 1 | 114 |
| ***Nae* I** | 1 | 2710 |
| ***Nci* I** | 4 | 30, 897, 1593, 1944 |
| ***Nco* I** | 1 | 37 |
| ***Nde* I** | 1 | 97 |
| ***Ngo*M I** | 1 | 2708 |
| ***Not* I** | 2 | 43, 77 |
| ***Nsi* I** | 1 | 127 |
| *Nsp* I | 2 | 26, 521 |
| *Ppu*10 I | 1 | 123 |
| ***Pst* I** | 1 | 88 |
| ***Pvu* I** | 2 | 1780, 2879 |
| ***Pvu* II** | 2 | 341, 2908 |
| ***Rsa* I** | 1 | 1890 |
| ***Sac* I** | 1 | 109 |
| ***Sac* II** | 1 | 49 |
| ***Sal* I** | 1 | 90 |
| ***Sca* I** | 1 | 1890 |
| ***Sin* I** | 2 | 1548, 1770 |
| ***Spe* I** | 1 | 64 |
| ***Sph* I** | 1 | 26 |
| *Sse*8387 I | 1 | 88 |
| ***Ssp* I** | 3 | 2214, 2399, 2423 |
| ***Sty* I** | 1 | 37 |
| ***Taq* I** | 5 | 56, 91, 617, 2061, 2640 |
| *Tfi* I | 2 | 352, 492 |
| ***Vsp* I** | 3 | 288, 347, 1582 |
| ***Xmn* I** | 1 | 2009 |

**Table 8. Restriction Enzymes That Do Not Cut the pGEM®-T Easy Vector.**

| | | | | | |
|---|---|---|---|---|---|
| ***Acc* III** | *Bbs* I | *Bst*1107 I | *Ehe* I | *Pfl*M I | ***Sma* I** |
| ***Acc*65 I** | ***Bcl* I** | ***Bst*98 I** | *Fse* I | *Pin*A I | ***Sna*B I** |
| ***Acc*B7 I** | ***Bgl* II** | ***Bst*E II** | ***Hin*d III** | *Pme* I | *Spl* I |
| *Afl* II | *Blp* I | ***Bsu*36 I** | ***Hpa* I** | *Pml* I | *Srf* I |
| ***Age* I** | *Bpu*1102 I | ***Cla* I** | **I-*Ppo* I** | *Ppu*M I | ***Stu* I** |
| *Asc* I | *Bsa*B I | ***Csp* I** | *Kas* I | *Psh*A I | *Swa* I |
| ***Ava* I** | ***Bsa*M I** | ***Csp*45 I** | ***Kpn* I** | *Psp*5 II | ***Tth*111 I** |
| *Avr* II | *Bsi*W I | *Dra* II | ***Nar* I** | *Psp*A I | ***Xba* I** |
| ***Bal* I** | *Bsm* I | ***Eco*47 III** | ***Nhe* I** | *Rsr* II | *Xcm* I |
| ***Bam*H I** | ***Bsr*Br I** | *Eco*N I | ***Nru* I** | ***Sfi* I** | ***Xho* I** |
| *Bbe* I | *Bsr*G I | *Eco*72 I | *Pac* I | ***Sgf* I**(i) | ***Xma* I** |
| *Bbr*P I | ***Bss*H II** | *Eco*81 I | *Pae*R7 I | *Sgr*A I | |

**Note:** The enzymes listed in boldface type are available from Promega.

**Table 9. Restriction Enzymes That Cut the pGEM®-T Easy Vector 6 or More Times.**

| | | | | | |
|---|---|---|---|---|---|
| *Aci* I | ***Bst*71 I** | ***Hae* III** | *Mae* II | ***Msp*A1 I** | *Sfa*N I |
| ***Alu* I** | *Bst*U I | ***Hha* I** | *Mae* III | *Nla* III | ***Tru*9 I** |
| *Bbv* I | ***Cfo* I** | ***Hin*f I** | ***Mbo* I** | *Nla* IV | ***Xho* II** |
| ***Bsa*O I** | ***Dpn* I** | *Hin*P I | ***Mbo* II** | *Ple* I | |
| ***Bsp*1286 I** | *Dpn* II | ***Hpa* II** | *Mnl* I | ***Sau*3A I** | |
| *Bsr* I | *Eae* I | *Hph* I | *Mse* I | ***Sau*96 I** | |
| ***Bsr*S I** | *Fnu*4H I | ***Hsp*92 II** | ***Msp* I** | *Scr*F I | |

**Note:** The enzymes listed in boldface type are available from Promega.

## XI. Appendix B: Reference Information

### A. Composition of Buffers and Solutions

**IPTG stock solution (0.1M)**

1.2g IPTG (Cat.# V3951)

Add water to 50ml final volume. Filter-sterilize and store at 4°C.

**X-Gal (2ml)**

100mg 5-bromo-4-chloro-3-indolyl-β-D-galactoside (Cat.#V3941)

Dissolve in 2ml N,N´-dimethyl-formamide. Cover with aluminum foil and store at –20°C.

**LB medium (per liter)**

10g Bacto®-Tryptone
5g Bacto®-Yeast Extract
5g NaCl

Adjust pH to 7.0 with NaOH.

**LB plates with ampicillin**

Add 15g agar to 1 liter of LB medium. Autoclave. Allow the medium to cool to 50°C before adding ampicillin to a final concentration of 100µg/ml. Pour 30-35ml of medium into 85mm petri dishes. Let the agar harden. Store at 4°C for up to one month or at room temperature for up to one week.

**LB plates with ampicillin/IPTG/X-Gal**

Make the LB plates with ampicillin as above; then supplement with 0.5mM IPTG and 80µg/ml X-Gal and pour the plates. Alternatively, 100µl of 100mM IPTG and 20µl of 50mg/ml X-Gal may be spread over the surface of an LB-ampicillin plate and allowed to absorb for 30 minutes at 37°C prior to use.

**SOC medium (100ml)**

2.0g Bacto®-tryptone
0.5g Bacto®-yeast extract
1ml 1M NaCl
0.25ml 1M KCl
1ml 2M $Mg^{2+}$ stock, filter-sterilized (as prepared below)
1ml 2M glucose, filter-sterilized

Add Bacto®-tryptone, Bacto®-yeast extract, NaCl and KCl to 97ml distilled water. Stir to dissolve. Autoclave and cool to room temperature. Add 2M $Mg^{2+}$ stock and 2M glucose, each to a final concentration of 20mM. Bring to 100ml with sterile, distilled water. Filter the complete medium through a 0.2µm filter unit. The final pH should be 7.0.

**2M $Mg^{2+}$ stock**

20.33g $MgCl_2 \cdot 6H_2O$
24.65g $MgSO_4 \cdot 7H_2O$

Add distilled water to 100ml. Filter sterilize.

**2X Rapid Ligation Buffer, T4 DNA Ligase (provided)**

60mM Tris-HCl (pH 7.8)
20mM $MgCl_2$
20mM DTT
2mM ATP
10% polyethylene glycol (MW8000, ACS Grade)

Store in single-use aliquots at –20°C. Avoid multiple freeze/thaw cycles.

**TYP broth (per liter)**

16g Bacto®-Tryptone
16g Bacto®-Yeast Extract
5g NaCl
2.5g $K_2HPO_4$

## B. Related Products

### Thermostable Enzymes

| Product | Size | Cat.# |
|---|---|---|
| PCR Core System I | 200 reactions | M7660 |
| PCR Core System II | 200 reactions | M7665 |
| *Taq* DNA Polymerase | 100u | M1661 |
| | 500u | M1665 |
| *Pfu* DNA Polymerase | 100u | M7741 |
| | 500u | M7745 |
| *Tli* DNA Polymerase | 50u | M7101 |

### Wizard® *Plus* SV Minipreps DNA Purification Systems(j)

*Components of the Wizard® *Plus* SV Minipreps DNA Purification System should not be exchanged for or replaced with components from any Wizard® *Plus* DNA Purification System. Components for the Wizard® *Plus* Systems and the Wizard® *Plus* SV Systems are not interchangeable.

| Product | Cat.# |
|---|---|
| Wizard® *Plus* SV Minipreps DNA Purification System* | A1330 |
| Wizard® *Plus* SV Minipreps DNA Purification System* | A1340 |

Each system contains sufficient reagents for 50 isolations from 1-10ml of culture. Cat.# A1340 includes 20 Miniprep Vacuum Adapters.

| Product | Cat.# |
|---|---|
| Wizard® *Plus* SV Minipreps DNA Purification System* | A1460 |
| Wizard® *Plus* SV Minipreps DNA Purification System* | A1470 |

Each system contains sufficient reagents for 250 isolations from 1-10ml of culture. Cat.# A1470 includes 20 Miniprep Vacuum Adapters.

### Wizard® DNA Purification Systems

| Product | Size | Cat.# |
|---|---|---|
| Wizard® *Plus* Minipreps DNA Purification System | 50 preps | A7100 |
| Wizard® *Plus* Minipreps DNA Purification System | 100 preps | A7500 |
| Wizard® *Plus* Minipreps DNA Purification System | 250 preps | A7510 |
| Wizard® *Plus* Midipreps DNA Purification System | 25 preps | A7640 |
| Wizard® *Plus* Maxipreps DNA Purification System | 10 preps | A7270 |
| Wizard® *Plus* Megapreps DNA Purification System | 5 preps | A7300 |
| Wizard® *Plus* Series 9600™ DNA Purification System | 2 x 96 preps | A7000 |

Each system contains sufficient reagents for isolating DNA as described in Table 3.

| Product | Cat.# |
|---|---|
| Wizard® PCR Preps DNA Purification System | A7170 |

Each system contains sufficient reagents to purify 50 DNA samples, either with or without prior gel extraction.

| Product | Capacity | Cat.# |
|---|---|---|
| Vac-Man® Laboratory Vacuum Manifold | 20 samples | A7231 |
| Vac-Man® Jr. Laboratory Vacuum Manifold | 2 samples | A7660 |

### Competent Cells

| Product | Efficiency | Size | Cat.# |
|---|---|---|---|
| JM109 Competent Cells, High Efficiency | $>10^8$ cfu/µg | 1ml (5 x 200µl) | L2001 |

Each order of competent cells is provided with control DNA.

## Reagents and dNTPs

| Product | Size | Cat.# |
|---|---|---|
| X-Gal | 100mg | V3941 |
| IPTG, Dioxane-Free | 1g | V3955 |
| | 5g | V3951 |
| | 50g | V3953 |
| dATP, 100mM | 40µmoles | U1201 |
| dCTP, 100mM | 40µmoles | U1221 |
| dGTP, 100mM | 40µmoles | U1211 |
| dTTP, 100mM | 40µmoles | U1231 |
| dATP, dCTP, dGTP, dTTP, each at 100mM | 40µmoles of each | U1240 |
| dATP, dCTP, dGTP, dTTP, each at 100mM | 10µmoles of each | U1330 |
| PCR Nucleotide Mix, 40mM | 200µl | C1141 |
| | 1,000µl | C1145 |
| Lambda DNA/*Hin*d III Markers | 100µg | G1711 |
| 100bp DNA Ladder | 250µl | G2101 |

(a)Licensed under one or both of U.S. Pat. No. 5,487,993 and European Pat. No. 0 550 693.

(b)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.

(c)The PCR process is covered by patents issued and applicable in certain countries. Promega does not encourage or support the unauthorized or unlicensed use of the PCR process.

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(f)The method of recombinant expression of *Coleoptera* luciferase is covered by U.S. Pat. No. 5,583,024 assigned to The Regents of the University of California.

(g)Patent Pending.

(h)U.S. Pat. Nos. 5,658,548 and other patents.

(i)U.S. Pat. No. 5,391,487 has been issued to Promega Corporation for Restriction Endonuclease Sgf I.

(j)Patent Pending.

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Product claims are subject to change. Please contact Promega Technical Services or access the Promega online catalog for the most up-to-date information on Promega products.

**Ligations Using the 2X Rapid Ligation Buffer** (Section IV.A)

1. Briefly centrifuge the pGEM®-T or pGEM®-T Easy Vector and Control Insert DNA tubes to collect contents at the bottom of the tube.
2. Set up ligation reactions as described below. **Note:** Use 0.5ml tubes known to have low DNA-binding capacity (e.g., VWR Cat.# 20170-310).
3. Vortex the 2X Rapid Ligation Buffer vigorously before each use.

| | Standard Reaction | Positive Control | Background Control |
|---|---|---|---|
| 2X Rapid Ligation Buffer, T4 DNA Ligase | 5µl | 5µl | 5µl |
| pGEM®-T or pGEM®-T Easy Vector (50ng) | 1µl | 1µl | 1µl |
| PCR product | Xµl | – | – |
| Control Insert DNA | – | 2µl | – |
| T4 DNA Ligase (3 Weiss units/µl) | 1µl | 1µl | 1µl |
| deionized water to a final volume of | 10µl | 10µl | 10µl |

4. Mix the reactions by pipetting. Incubate the reactions 1 hour at room temperature.

   Alternatively, if the maximum number of transformants is required, incubate the reactions overnight at 4°C.

! This protocol uses the **new 2X Rapid Ligation Buffer**. Use the appropriate volume when setting up the ligation reaction.