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

Technical Manual No. 042

INSTRUCTIONS FOR USE OF PRODUCTS A1360, A1380, A3600 AND A3610.
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I. Description

The pGEM®-T and pGEM®-T Easy Vector Systems[a,b] are convenient systems for the cloning of PCR 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 region 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 multiple cloning region. These restriction sites allow for the release of the insert by digestion with a single restriction enzyme. The pGEM®-T Easy Vector multiple cloning region is flanked by recognition sites for the restriction enzymes *Eco*R I, *BstZ* I and *Not* I, thus providing three single-enzyme digestions for release of the insert, while the pGEM®-T Vector cloning region is flanked by recognition sites for the enzyme *BstZ* 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 1, Panels A and B, for the pGEM®-T and pGEM®-T Easy Vectors (nonrecombinant), respectively.

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

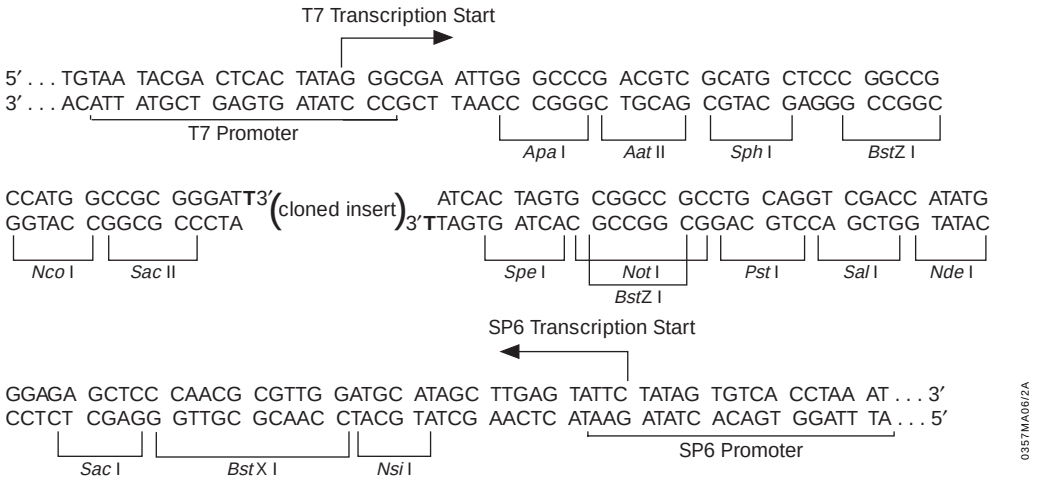
Table 1. Comparison of PCR Product Properties for Some Thermostable DNA Polymerases.

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

II. Vector Maps

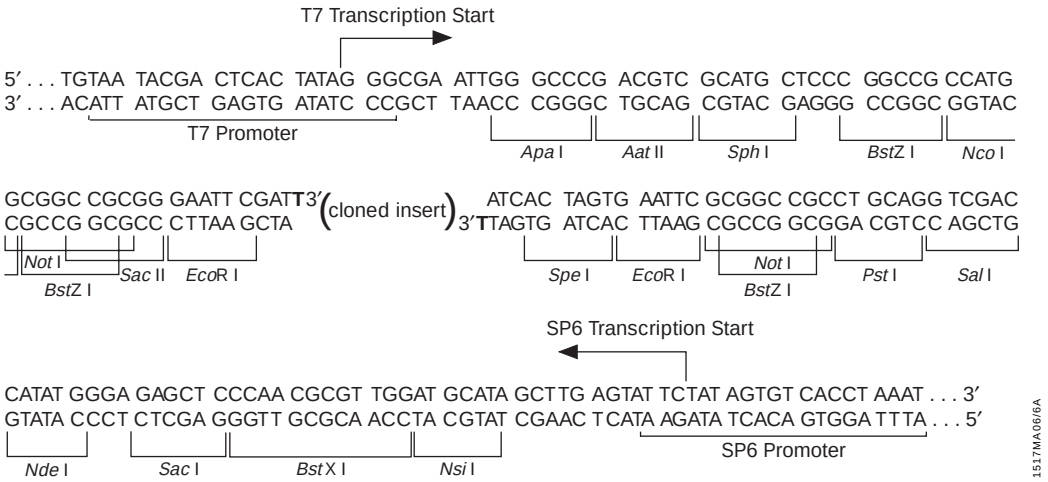
A. pGEM®-T Vector and pGEM®-T Easy Vector Multiple Cloning Sequences

pGEM®-T Vector



Note: A single digest with *BstZ* I (Cat.# R6881) will release inserts cloned into the pGEM®-T Vector.

pGEM®-T Easy Vector



Note: A single digest with *BstZ* I (Cat.# R6881), *Eco*R I (Cat.# R6011) or *Not* I (Cat.# R6431) will release inserts cloned into the pGEM®-T Easy Vector.

Figure 1. The promoter and multiple cloning sequence of the pGEM®-T and pGEM®-T Easy 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.

B. pGEM®-T Vector Map and Sequence Reference Points

Note: Inserts can be sequenced using the following primers: SP6 Promoter Primer (Cat.# Q5011), T7 Promoter Primer (Cat.# Q5021), pUC/M13 Forward Primer (Cat.# Q5601), pUC/M13 Reverse Primer (Cat.# Q5421).

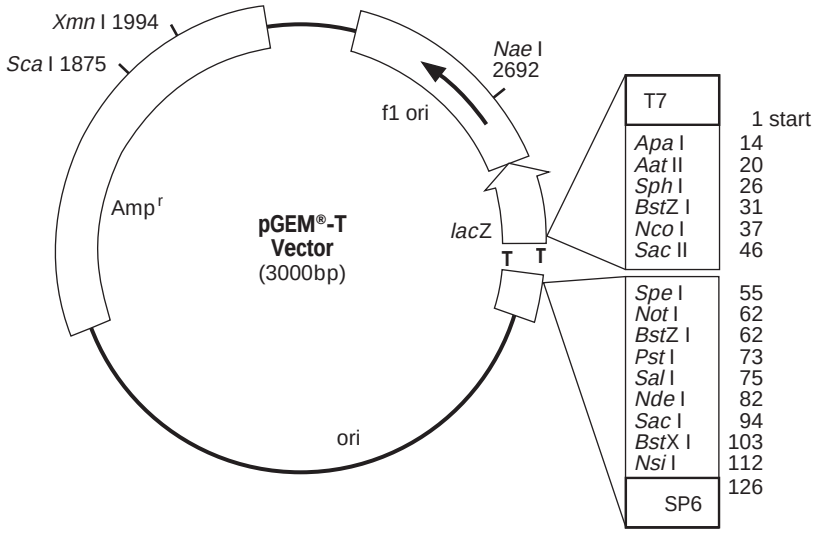


Figure 2. pGEM®-T Vector circle map and sequence reference points.

Note: A single digest with *Bst*Z I (Cat.# R6881) will release inserts cloned into the pGEM®-T Vector. Double digests can also be used to release inserts.

pGEM®-T Vector sequence reference points:

T7 RNA polymerase transcription initiation site	1
multiple cloning region	10–113
SP6 RNA polymerase promoter (–17 to +3)	124–143
SP6 RNA polymerase transcription initiation site	126
pUC/M13 Reverse Sequencing Primer binding site	161–177
*lac*Z start codon	165
lac operator	185–201
β-lactamase coding region	1322–2182
phage f1 region	2365–2820
lac operon sequences	2821–2981, 151–380
pUC/M13 Forward Sequencing Primer binding site	2941–2957
T7 RNA polymerase promoter (–17 to +3)	2984–3

C. pGEM®-T Easy Vector Map and Sequence Reference Points

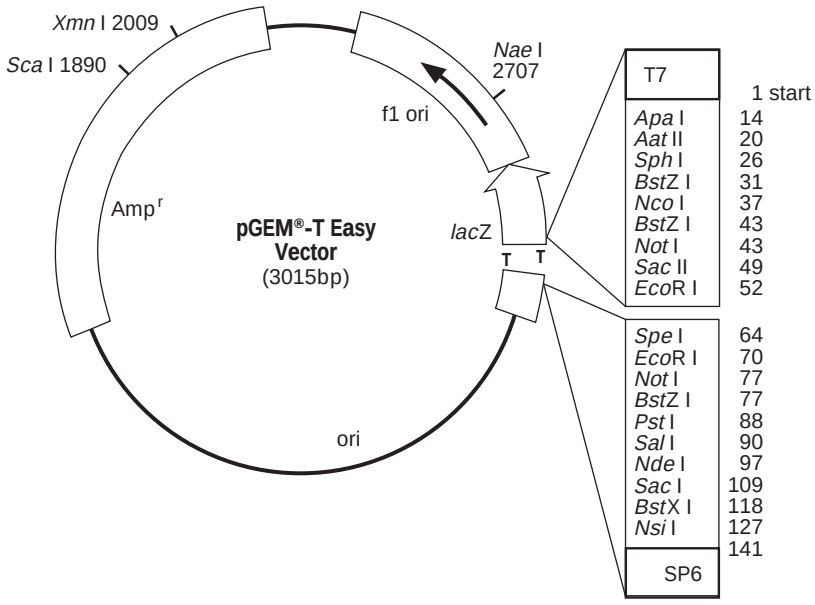


Figure 3. pGEM®-T Easy Vector circle map and sequence reference points.

pGEM®-T Easy Vector sequence reference points:

T7 RNA polymerase transcription initiation site	1
multiple cloning region	10–128
SP6 RNA polymerase promoter (–17 to +3)	139–158
SP6 RNA polymerase transcription initiation site	141
pUC/M13 Reverse Sequencing Primer binding site	176–197
*lac*Z start codon	180
lac operator	200–216
β-lactamase coding region	1337–2197
phage f1 region	2380–2835
lac operon sequences	2836–2996, 166–395
pUC/M13 Forward Sequencing Primer binding site	2949–2972
T7 RNA polymerase promoter (–17 to +3)	2999–3

Note: Inserts can be sequenced using the following primers: SP6 Promoter Primer (Cat.# Q5011), T7 Promoter Primer (Cat.# Q5021), pUC/M13 Forward Primer (Cat.# Q5601), pUC/M13 Reverse Primer (Cat.# Q5421).

Note: A single digest with *BstZ* I (Cat.# R6881) will release inserts cloned into the pGEM®-T Vector. Double digests can also be used to release inserts.

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

- Cloning PCR products.
- Construction of unidirectional nested deletions with the Erase-a-Base® System.
- Production of ssDNA.
- Blue/white screening for recombinants.
- In vitro transcription from dual opposed promoters. (For protocol information, please request Promega's *Riboprobe® in vitro Transcription Systems*(c) *Technical Manual* #TM016.)

III. Product Components

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

For Laboratory Use. 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

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

For Laboratory Use. 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 × 200µl)
- 1 Protocol

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

For Laboratory Use. 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

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

For Laboratory Use. 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 × 200µl)
- 1 Protocol

Storage Conditions: For Cat.# A3610, A1380, store the Competent Cells at –70°C. All other components can be stored at –20°C or –70°C.

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

1. Briefly centrifuge the pGEM®-T or pGEM®-T Easy Vector and Control Insert DNA tubes to collect contents at the bottom of the tubes.
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 VI.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 deoxythymidines 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. Protocol for Transformations Using the pGEM®-T and pGEM®-T Easy Vector Ligation Reactions

Use high-efficiency competent cells (≥1 × 10^8cfu/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 × 10^8cfu/µg DNA (or higher) in order to obtain a reasonable number of colonies (see Section VI.E).

We recommend using JM109 High Efficiency Competent Cells (Cat.# L2001); these cells 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

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, containing both the *pro*AB genes, which complement proline auxotrophy in a host with a (*pro*AB) deletion, and *lac*I^{q}ZΔM15, required in blue/white color screening. 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 XI.A). For best results, do not use plates that are more than 1 month 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^{q}ZΔM15] (5).

Materials to Be Supplied by the User
(Solution compositions are provided in Section XII.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 VI.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 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 VI.E).

In Step 3, avoid excessive pipetting as the competent cells are extremely fragile.

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.
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 LB/ampicillin/IPTG/X-Gal 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 × *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 × 10^8cfu/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 color development. White colonies generally contain inserts; however, inserts may also be present in blue colonies. Please see Section VI.D for more information.

Notes:

1. In our experience, the use of larger (17 × 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. General Considerations

A. PCR Product Purity

An aliquot of the PCR reaction should be analyzed on an agarose gel before use in the ligation reaction. The PCR product to be ligated can be gel-purified or purified directly from the PCR amplification reaction using the Wizard® SV Gel and PCR Clean-Up System(d) (Cat.# A9281). 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 and purify the DNA with Wizard® SV Gel and PCR Clean-Up System. Even if distinct bands of expected size are observed, primer-dimers should be removed by gel purification or by using the Wizard® SV Gel and PCR Clean-Up System to purify the bands of interest directly from the reaction mix. Use of crude PCR product may 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-Ended 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-ended fragments during PCR amplification. Nevertheless, PCR fragments generated using these polymerases can be modified using the A-tailing procedure (Figure 4) and ligated into the pGEM®-T and pGEM®-T Easy Vectors (6). Using this method, only one insert will be ligated into the vector as opposed to multiple insertions that can occur with blunt-ended cloning. In addition, with T-vector cloning there is no need to dephosphorylate the vector, and there is a low background of religated vector.

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Using this procedure with optimized insert:vector ratios, 55–95% recombinants were obtained when *Pfu* and *Tli* DNA Polymerases were used to generate the insert DNA (Table 2). It is critical that the PCR fragments are purified using the Wizard® SV Gel and PCR Clean-Up System (Cat.# A9281) or by direct isolation from a gel by other means. In the absence of purification, the proofreading activity of the *Pfu*, *Pwo* and *Tli* DNA Polymerases will degrade the PCR fragments or remove the 3´-terminal deoxyadenosine added during tailing or the 3´-terminal deoxythymidine from the vector during the A-tailing reaction or ligation.

To optimize cloning efficiency, the amount of DNA in the A-tailing reaction and the ligation volumes must be adjusted depending on the molar yield of the purified PCR product. When molar concentrations are high due to small fragment size and/or good amplification, small volumes of the PCR fragment are needed for the A-tailing and ligation reactions. However, when molar concentration is low due to large fragment size and/or poor amplification, large volumes of the PCR fragment are needed for the A-tailing and ligation reactions. We have successfully used 1–7µl of the purified PCR fragment in the A-tailing reaction using *Taq* DNA Polymerase(e) to optimize the insert:vector ratio. See Section IV.C for further discussion of optimizing the insert:vector ratio. Recombinants were identified by blue/white screening, and 70–100% of the recombinants were shown to have the correct size insert by PCR amplification of DNA. Few recombinants were observed in the control reactions 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 and that, during the A-tailing, *Taq* DNA Polymerase added a 3´-terminal deoxyadenosine to a significant proportion of the PCR fragment.

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

	% Recombinants[1]			
	1-Hour Ligation at 24°C (Standard)		16-Hour Ligation at 4°C (Alternative)	
Polymerase	**542bp**	**1.8kb**	**542bp**	**1.8kb**
Pfu DNA Polymerase	65–84%[2]	31–55%[3]	81–95%[2]	50–75%[3]
Tli DNA Polymerase	68–77%[4]	37–65%[5]	85–93%[4]	60–81%[5]

PCR fragments generated by *Pfu* and *Tli* DNA Polymerase were A-tailed and ligated into pGEM®-T Easy Vector for 1 hour at 24°C or for 16 hours at 4°C. Two microliters of ligation mix was transformed into JM109 Competent Cells and plated on LB/amp/IPTG/X-gal plates.

[1]% Recombinants = % white and/or pale blue colonies. PCR fragments were purified with the Wizard® PCR Preps DNA Purification System(f) prior to A-tailing.

[2]Insert:vector ratios tested: 5:1, 3:1, 1:1. Volume of PCR amplification product used in A-tailing: 1–2µl.

[3]Insert:vector ratios tested: 3:1, 2:1, 1:1. Volume of PCR amplification product used in A-tailing: 3–7µl.

[4]Insert:vector ratios tested: 3:1, 2:1, 1:1. Volume of PCR amplification product used in A-tailing: 1–2µl.

[5]Insert:vector ratios tested: 2:1, 1:1. Volume of PCR amplification product used in A-tailing: 4–7µl.

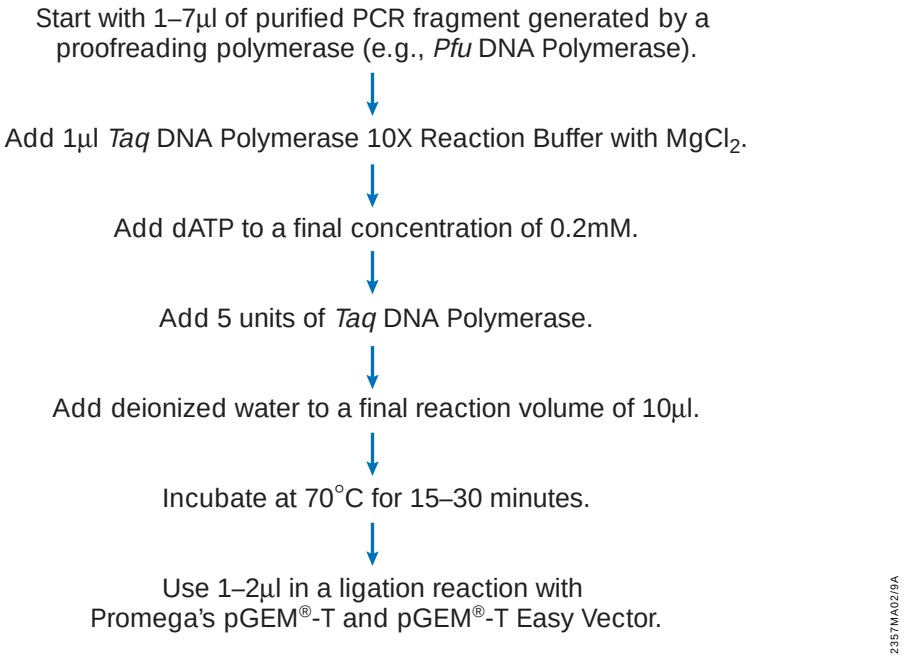

Figure 4. An A-tailing procedure for blunt-ended PCR fragments purified with Wizard® SV Gel and PCR Clean-Up System (Cat.# A9281) and used in T-vector cloning.

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 used successfully. 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 (7). The pGEM®-T and pGEM®-T Easy Vectors are approximately 3kb and are supplied at 50ng/µl. To calculate the appropriate amount of PCR product (insert) to include in the ligation reaction, use the following equation.

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

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?

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

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), and do not contain in-frame stop codons. There have been reports of DNA fragments of up to 2kb that have been cloned in-frame and have 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 pGEM®-*luc* Vector[b,g] DNA (Cat.# E1541). 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) 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, 10–40% of which are blue, when competent cells that have a transformation efficiency of 1×10^8cfu/µg DNA are transformed. Greater than 60% of the colonies should be white. The Control Insert DNA is specifically designed to produce white colonies; however, other insert DNA may not yield white colonies (see Section VI.D). Background blue colonies from the positive control ligation reaction 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 1×10^8cfu/µg DNA. (Under these conditions, cells that have an efficiency of 1×10^7cfu/µg DNA would yield 1–3 blue colonies and cells with a transformation efficiency of 1×10^9cfu/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 VI.D).

Transformation Control

Check the transformation efficiency of the competent cells by transforming them with an uncut plasmid (not pGEM®-T or pGEM®-T Easy since these vectors are linearized) and calculating cfu/µg DNA. If the transformation efficiency is lower than 1×10^8cfu/µg DNA, prepare fresh cells. (Competent cells are available from Promega. See Section XII.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 have a transformation efficiency of at least 1×10^8cfu/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?

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

VII. 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* (8). 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* SV Minipreps DNA Purification System[h,i] (Cat.# A1330).

VIII. 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 (e.g., R408 Helper Phage, Cat.# P2291). 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 the *Protocols and Applications Guide* (Cat.# P1610). For further information, please contact your local Promega Branch Office or Distributor. In the U.S., contact Technical Services at 1-800-356-9526.

IX. 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 VI.E). Use high efficiency competent cells (≥1 × 10^8cfu/µg DNA). Test the efficiency by transforming the cells with an uncut plasmid that allows for antibiotic selection, such as the pGEM®-5Zf(+) Vector. If the guidelines in Section V.A are followed, cells at 1 × 10^8cfu/µg DNA typically yield 100 colonies. Therefore, you would not see any colonies from cells that are <1 × 10^7cfu/µg DNA (Section VI.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.
		To test the activity of the ligase and buffer set up a ligation with ~20ng 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.

IX. Troubleshooting (continued)

Symptom	Possible Cause	Comments
Less than 10% white colonies with Control Insert DNA (continued)	T-overhangs have been removed allowing blunt-ended ligation of vector and giving rise to more blue than white colonies	Avoid introduction of nucleases that may degrade the T-overhangs. Use only T4 DNA Ligase provided with the system, which has been tested for minimal exonuclease activity.
High colony number, but low percentage of white colonies with Control Insert DNA	Competent cells may have a high transformation efficiency (≥1 × 10^9cfu/µg) but there is a ligation problem	Approximately 1,000 colonies can be obtained in the positive control ligation using cells that are 10^9cfu/µg DNA with 70–90% white colonies. If ligation is suboptimal or fails, the total number of colonies will be high (up to 300 cells at 1 × 10^9cfu/µg), but the amount of white colonies will be low or zero. See comments under "Ligation reaction has failed" (above).
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-ended ligation of vector and giving rise to more blue than white colonies	Avoid introduction of nucleases that may degrade the T-overhangs. Use only 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 of 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 positive 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 (9,10).

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**

IX. Troubleshooting (continued)

Symptom	Possible Cause	Comments
Low number of 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 a glass plate between the gel and UV source to 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 VI.C).
	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 VI.C).
	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. More blue colonies may be seen with the ligation than on the background control plates. The PCR fragment should be gel-purified.
	Multiple PCR products are generated and cloned into the 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.

IX. 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 1 month. Test ampicillin activity by streaking plates, with and without ampicillin, using 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 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 (9,10). Clean up the sample and proceed with the protocol.
	Insert:vector ratio is not optimal	Check the integrity and quality of your PCR fragment by gel analy--sis. Optimize the insert:vector ratio (see Section VI.C).
	Multiple PCR products are generated and cloned into the pGEM®-T or pGEM®-T Easy Vector	Gel purify the PCR fragment of interest.

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**

X. References

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XI. Appendix A: Vector Sequences and Restriction Sites

A. pGEM®-T Vector Sequence

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 at: www.promega.com/vectors/.

```
  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
551  GTTGCTGGCG TTTTTCCATA GGCTCCGCCC CCCTGACGAG CATCACAAAA
601  ATCGACGCTC AAGTCAGAGG TGGCGAAACC CGACAGGACT ATAAAGATAC
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 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  AAGAACAGTA 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 ACGTTGTTGC 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 CTGATGCGGT GTGAAATACC GCACAGATGC GTAAGGAGAA
2351  AATACCGCAT CAGGAAATTG TAAGCGTTAA TATTTTGTTA AAATTCGCGT
2401  TAAATTTTTG TTAAATCAGC TCATTTTTTA ACCAATAGGC CGAAATCGGC
2451  AAAATCCCTT ATAAATCAAA AGAATAGACC GAGATAGGGT TGAGTGTTGT
2501  TCCAGTTTGG AACAAGAGTC CACTATTAAA GAACGTGGAC TCCAACGTCA
2551  AAGGGCGAAA AACCGTCTAT CAGGGCGATG GCCCACTACG TGAACCATCA
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2601  CCCTAATCAA GTTTTTTGGG GTCGAGGTGC CGTAAAGCAC TAAATCGGAA
2651  CCCTAAAGGG AGCCCCCGAT TTAGAGCTTG ACGGGGAAAG CCGGCGAACG
2701  TGGCGAGAAA GGAAGGGAAG AAAGCGAAAG GAGCGGGCGC TAGGGCGCTG
2751  GCAAGTGTAG CGGTCACGCT GCGCGTAACC ACCACACCCG CCGCGCTTAA
2801  TGCGCCGCTA CAGGGCGCGT CCATTCGCCA TTCAGGCTGC GCAACTGTTG
2851  GGAAGGGCGA TCGGTGCGGG CCTCTTCGCT ATTACGCCAG CTGGCGAAAG
2901  GGGGATGTGC TGCAAGGCGA TTAAGTTGGG TAACGCCAGG GTTTTCCCAG
2951  TCACGACGTT GTAAAACGAC GGCCAGTGAA TTGTAATACG ACTCACTATA
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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 3. 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, 2626
***Ban* II**	3	14, 94, 2664
***Bbu* I**	1	26
***Bgl* I**	3	39, 1515, 2833
Bsa I	1	1456
*Bsa*A I	1	2589
*Bsa*H I	2	17, 1932
*Bsa*J I	5	37, 43, 241, 662, 2936
*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, 2937
***Bst*X I**	1	103
***Bst*Z I**	2	31, 62
*Cfr*10 I	2	1475, 2690
***Dde* I**	4	777, 1186, 1352, 1892
***Dra* I**	3	1261, 1280, 1972
Dra III	1	2589
Drd I	2	610, 2544
Dsa I	2	37, 43
Eag I	2	31, 62
Ear I	3	386, 2190, 2878
***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, 2840
***Hae* II**	4	380, 750, 2740, 2748
Hga I	4	613, 1191, 1921, 2806
***Hin*c II**	1	77
*Hin*d II	1	77
***Hsp*92 I**	2	17, 1932
Mae I	5	56, 997, 1250, 1585, 2740
***Mlu* I**	1	99

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

Table 3. Restriction Enzymes That Cut the pGEM®-T Vector Between 1 and 5 Times (continued).

Enzyme	# of Sites	Location	Enzyme	# of Sites	Location
***Nae* I**	1	2692	***Sac* II**	1	46
***Nci* I**	4	30, 882, 1578, 1929	***Sal* I**	1	75
			***Sca* I**	1	1875
***Nco* I**	1	37	***Sfi* I**	1	39
***Nde* I**	1	82	***Sin* I**	2	1533, 1755
***Ngo*M IV**	1	2690	***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**	2	2199, 2381
*Ppu*10 I	1	108	***Sty* I**	1	37
***Pst* I**	1	73	***Taq* I**	4	76, 602, 2046, 2622
***Pvu* I**	2	1765, 2861			
***Pvu* II**	2	326, 2890	*Tfi* I	2	337, 477
***Rsa* I**	1	1875	***Vsp* I**	3	273, 332, 1567
***Sac* I**	1	94	***Xmn* I**	1	1994

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

Table 4. Restriction Enzymes That Do Not Cut the pGEM®-T Vector.

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

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

Table 5. Restriction Enzymes That Cut the pGEM®-T Vector 6 or More Times.

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

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

C. pGEM®-T Easy Vector Sequence

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 at: www.promega.com/vectors/.

```
   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 ACTAGAAGAA 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 GTTGCCATTG
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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 GCCACCTGAT GCGGTGTGAA ATACCGCACA
2351  GATGCGTAAG GAGAAAATAC CGCATCAGGA AATTGTAAGC GTTAATATTT
2401  TGTTAAAATT CGCGTTAAAT TTTTGTTAAA TCAGCTCATT TTTTAACCAA
2451  TAGGCCGAAA TCGGCAAAAT CCCTTATAAA TCAAAAGAAT AGACCGAGAT
2501  AGGGTTGAGT GTTGTTCCAG TTTGGAACAA GAGTCCACTA TTAAAGAACG
2551  TGGACTCCAA CGTCAAAGGG CGAAAAACCG TCTATCAGGG CGATGGCCCA
2601  CTACGTGAAC CATCACCCTA ATCAAGTTTT TTGGGGTCGA GGTGCCGTAA
2651  AGCACTAAAT CGGAACCCTA AAGGGAGCCC CCGATTTAGA GCTTGACGGG
2701  GAAAGCCGGC GAACGTGGCG AGAAAGGAAG GGAAGAAAGC GAAAGGAGCG
2751  GGCGCTAGGG CGCTGGCAAG TGTAGCGGTC ACGCTGCGCG TAACCACCAC
2801  ACCCGCCGCG CTTAATGCGC CGCTACAGGG CGCGTCCATT CGCCATTCAG
2851  GCTGCGCAAC TGTTGGGAAG GGCGATCGGT GCGGGCCTCT TCGCTATTAC
2901  GCCAGCTGGC GAAAGGGGGA TGTGCTGCAA GGCGATTAAG TTGGGTAACG
2951  CCAGGGTTTT CCCAGTCACG ACGTTGTAAA ACGACGGCCA GTGAATTGTA
3001  ATACGACTCA CTATA
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Promega

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

Enzyme	# of Sites	Location	Enzyme	# of Sites	Location
***Aat* II**	1	20	***Fok* I**	5	134, 1376, 1557, 1844, 2931
***Acc* I**	1	91			
***Acy* I**	2	17, 1947	*Fsp* I	2	1632, 2855
Afl III	2	114, 517	***Hae* II**	4	395, 765, 2755, 2763
***Alw*26 I**	2	1471, 2247			
***Alw*44 I**	2	831, 2077	*Hga* I	4	628, 1206, 1936, 2821
*Alw*N I	1	933			
***Apa* I**	1	14	***Hin*c II**	1	92
*Asp*H I	4	109, 835, 1996, 2081	*Hin*d II	1	92
			***Hsp*92 I**	2	17, 1947
***Ava* II**	2	1548, 1770	*Mae* I	5	65, 1012, 1265, 1600, 2755
***Ban* I**	3	261, 1358, 2641			
***Ban* II**	3	14, 109, 2679	***Mlu* I**	1	114
***Bbu* I**	1	26	***Nae* I**	1	2707
***Bgl* I**	4	39, 42, 1530, 2848	***Nci* I**	4	30, 897, 1593, 1944
Bsa I	1	1471			
*Bsa*A I	1	2604	***Nco* I**	1	37
*Bsa*H I	2	17, 1947	***Nde* I**	1	97
*Bsa*J I	5	37, 46, 256, 677, 2951	***Ngo*M IV**	1	2705
			***Not* I**	2	43, 77
*Bsp*120 I	1	10	***Nsi* I**	1	127
*Bsp*H I	2	1237, 2245	*Nsp* I	2	26, 521
*Bsp*M I	1	77	*Ppu*10 I	1	123
*Bss*S I	2	690, 2074	***Pst* I**	1	88
***Bst*O I**	5	257, 545, 666, 679, 2952	***Pvu* I**	2	1780, 2876
			***Pvu* II**	2	341, 2905
***Bst*X I**	1	118	***Rsa* I**	1	1890
***Bst*Z I**	3	31, 43, 77	***Sac* I**	1	109
*Cfr*10 I	2	1490, 2705	***Sac* II**	1	49
***Dde* I**	4	792, 1201, 1367, 1907	***Sal* I**	1	90
			***Sca* I**	1	1890
***Dra* I**	3	1276, 1295, 1987	***Sin* I**	2	1548, 1770
Dra III	1	2604	***Spe* I**	1	64
Drd I	2	625, 2559	***Sph* I**	1	26
Dsa I	2	37, 46	*Sse*8387 I	1	88
Eag I	3	31, 43, 77	***Ssp* I**	2	2214, 2396
Ear I	3	401, 2205, 2893	***Sty* I**	1	37
***Ecl*HK I**	1	1410	***Taq* I**	5	56, 91, 617, 2061, 2637
***Eco*52 I**	3	31, 43, 77			
***Eco*ICR I**	1	107	*Tfi* I	2	352, 492
EcoR I	2	52, 70	***Vsp* I**	3	288, 347, 1582
***Eco*R V**	1	60 (see above)	***Xmn* I**	1	2009

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

Table 7. Restriction Enzymes That Do Not Cut the pGEM®-T Easy Vector.

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

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

Table 8. Restriction Enzymes That Cut the pGEM®-T Easy Vector 6 or More Times.

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

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

XII. 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 1 month or at room temperature for up to 1 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.

A. Composition of Buffers and Solutions (continued)

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
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. 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

PCR Cloning Systems

Product	Size	Cat.#
pTARGET™ Mammalian Expression Vector System[a,l]	20 reactions	A1410

Direct mammalian expression from a T-Vector.

Product	Size	Cat.#
PinPoint™ Xa-1 T-Vector System I[a,b,m]	20 reactions	V2610
PinPoint™ Xa-1 T-Vector System II with Competent Cells[a,b,m]	20 reactions	V2850

Direct bacterial expression from a T-vector.

Thermostable Polymerases

Product	Size	Cat.#
PCR Master Mix[n,o]	100 reactions	M7501
	1,000 reactions	M7502

Premixed 2X solution containing *Taq* DNA Polymerase, dNTPs, reaction buffer and magnesium. Simply add template and primers. For Laboratory Use.

Product	Size	Cat.#
GoTaq™ DNA Polymerase*(e,o)	100u	M3001
	500u	M3005
Taq DNA Polymerase*(e)	100u	M1661
	500u	M1665
Pfu DNA Polymerase(e)	100u	M7741
(not available in the U.S.)	500u	M7745
Tli DNA Polymerase*(e)	50u	M7101

*For Laboratory Use.

RT-PCR Systems

Product	Size	Cat.#
Access RT-PCR System(n)	20 reactions	A1260
	100 reactions	A1250
	500 reactions	A1280
AccessQuick™ RT-PCR System(n,p)	20 reactions	A1701
	100 reactions	A1702
	500 reactions	A1703
Improm-II™ Reverse Transcription System	100 reactions	A3800

For Laboratory Use.

PCR Purification Systems

Product	Size	Cat.#
Wizard® SV Gel and PCR Clean-Up System(d)	50 preps	A9281
	250 preps	A9282

For Laboratory Use.

Product	Size	Cat.#
Wizard® SV 96 PCR Clean-Up System	1 × 96 preps	A9340
	4 × 96 preps	A9341
	8 × 96 preps	A9342
Wizard® MagneSil™ PCR Clean-Up System(q)	4 × 96 preps	A1930
	8 × 96 preps	A1931
	100 × 96 preps	A1935

For Laboratory Use.

dNTPs

Product	Size	Cat.#
PCR Nucleotide Mix (10mM each)	200µl	C1141
	1,000µl	C1145
dATP, dCTP, dGTP, dTTP, each at 100mM	10µmol of each	U1330
dATP, dCTP, dGTP, dTTP, each at 100mM	40µmol of each	U1240
dATP, dCTP, dGTP, dTTP, each at 100mM	200µmol of each	U1410

For Laboratory Use.

Sequencing Primers

Product	Size	Cat.#
SP6 Promoter Primer	2µg	Q5011
T7 Promoter Primer	2µg	Q5021
pUC/M13 Primer, Forward (24mer)	2µg	Q5601
pUC/M13 Primer, Reverse (22mer)	2µg	Q5421

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