Technical Manual

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

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

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

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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 the pGEM®-5Zf(+) and pGEM®-T Easy Vectors, respectively, with EcoRV 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 the 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. The pGEM®-T Easy Vector multiple cloning region is flanked by recognition sites for the restriction enzymes EcoRI, BstZI and NotI, thus providing three single-enzyme digestions for release of the insert. The pGEM®-T Vector cloning region is flanked by recognition sites for the enzyme BstZI. 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 VIII). The ssDNA molecule exported corresponds to the bottom strand shown in Figure 1.

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 Thermostable DNA Polymerases.

	Thermostable DNA Polymerase						
Characteristic	*Taq*/ 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

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

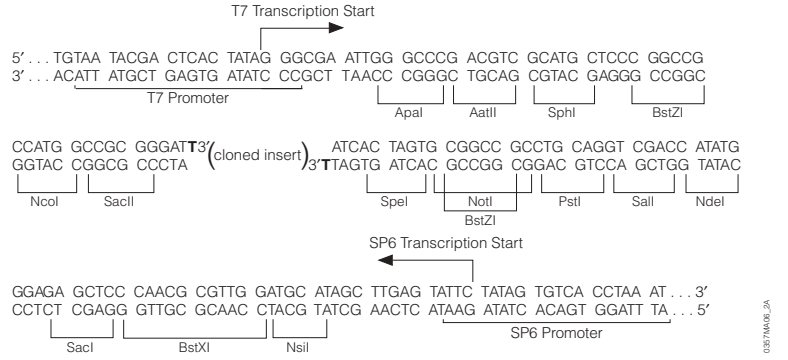
- Cloning PCR products.
- Construction of unidirectional nested deletions with the Erase-a-Base® System.
- Production of ssDNA.
- Blue/white screening for recombinants.
- In vitro transcription from dual-opposed promoters. (For protocol information, please request the *Riboprobe® in vitro Transcription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

For peer-reviewed articles that cite use of the pGEM®-T Vectors, visit: **www.promega.com/citations**

II. pGEM®-T and pGEM®-T Easy Vector Multiple Cloning Sequences and Maps

II.A. Multiple Cloning Sequences

pGEM®-T Vector



pGEM®-T Easy Vector

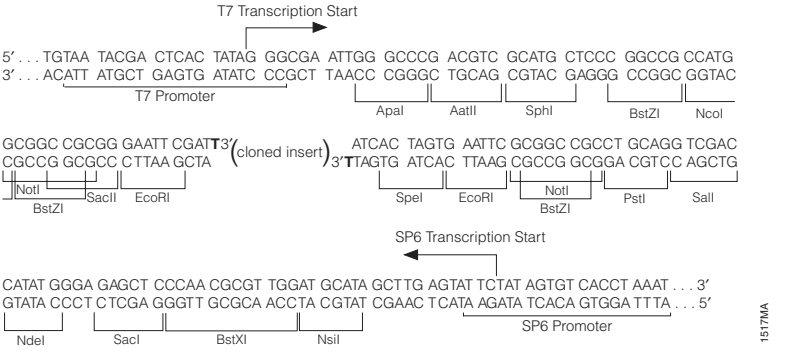


Figure 1. The promoter and multiple cloning sequence of the pGEM®-T and pGEM®-T Easy Vectors. The top strand of the sequence shown corresponds to the RNA synthesized by T7 RNA polymerase. The bottom strand corresponds to the RNA synthesized by SP6 RNA polymerase.

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

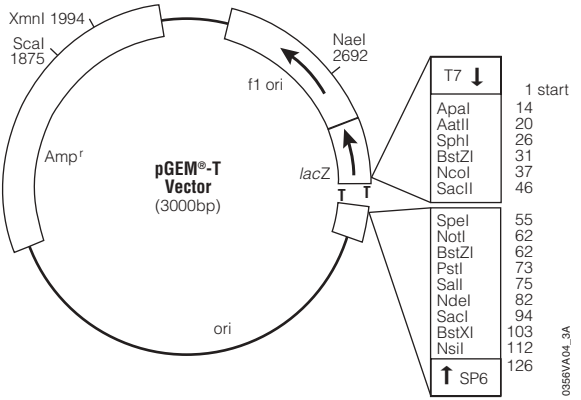


Figure 2. pGEM®-T Vector circle map and sequence reference points.

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

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

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

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

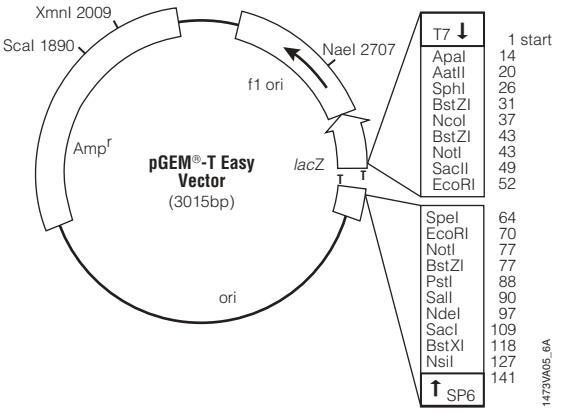


Figure 3. pGEM®-T Easy Vector circle map and sequence reference points.

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 SP6 Promoter Primer (Cat.# Q5011), T7 Promoter Primer (Cat.# Q5021), pUC/M13 Forward Primer (Cat.# Q5601), or pUC/M13 Reverse Primer (Cat.# Q5421).

Note: A single digest with BstZI (Cat.# R6881), EcoRI (Cat.# R6011) or NotI (Cat.# R6431) will release inserts cloned into the pGEM®-T Easy Vector. Double digests can also be used to release inserts.

III. Product Components and Storage Conditions

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.

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).

   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).

3. 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 to perform 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. 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 for blue/white 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.C). 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 (rK–,mK+), *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 XI.C.)

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

1. Prepare two 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).
3. Remove tube(s) of frozen JM109 High Efficiency Competent Cells from storage and place in an ice bath until just thawed (about 5 minutes). Mix the cells by **gently** flicking the tube.

Note: Avoid excessive pipetting, as the competent cells are extremely fragile.

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 two plates.
11. Incubate the plates overnight (16–24 hours) at 37°C. In our experience, if 100µl is plated approximately 100 colonies per plate are routinely seen when using competent cells that are 1×10^8cfu/µg DNA. 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

VI.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 using the Wizard® SV Gel and PCR Clean-Up System (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 the expected size are observed, primer-dimers should be removed by gel purification or by using the Wizard® SV Gel and PCR Clean-Up System. Use of crude PCR product may produce successful ligation 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.

VI.B. Blunt-Ended PCR Products

Thermostable DNA polymerases with proofreading activity, such as *Pfu* DNA Polymerase (Cat.# M7741), *Pwo* DNA polymerase and *Tli* DNA Polymerase (Cat.# M7101) generate blunt-ended fragments during PCR amplification. Nevertheless, PCR fragments generated using these polymerases can be modified using the A-tailing procedure outlined in 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.

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 purified PCR fragment in A-tailing reactions to optimize the insert:vector ratio. (See Section VI.C for further discussion of optimizing the insert:vector ratio.) Recombinants were identified by blue/white screening, and 70–100% were shown to have the correct size insert by PCR. Few recombinants were observed in control reactions in which the PCR fragment was not tailed. These control results confirm that the majority of the pGEM®-T Easy Vector used contained 3´-terminal deoxythymidine and that, during the A-tailing, *Taq* DNA Polymerase added a 3´-terminal deoxyadenosine to a significant proportion of the PCR fragments.

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 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.

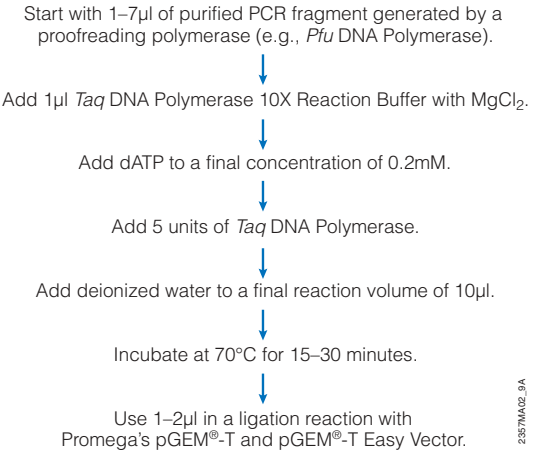


Figure 4. An A-tailing procedure for blunt-ended PCR fragments purified with the Wizard® SV Gel and PCR Clean-Up System (Cat.# A9281) and used in T-vector cloning.

VI.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?

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

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

VI.D. Screening Transformants for Inserts

Successful cloning of an insert into 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 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.

VI.E. Experimental Controls

Promega strongly recommends performing the controls described 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 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 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 ligation reaction may have failed. If <50% white colonies are seen in the 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 Section IV and use it for transformations as described in Section V. This control allows 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, 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 XI.D.) 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

Standard plasmid miniprep procedures may be used to isolate the recombinant plasmid DNA. The DNA Purification Chapter of the Promega P*rotocols and Applications Guide* provides an overview of plasmid DNA purification methods (8). A convenient and reliable method is the Wizard® *Plus* SV Minipreps DNA Purification System (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 (9). 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

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**

Symptom	Causes and 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.
	If the total number of colonies is high, but there are few/no white colonies, competent cells may be high efficiency (≥1 × 10^9cfu/µg) but there may be a ligation problem. Approximately 1,000 colonies can be obtained from 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 below on ligation failure.

IX. Troubleshooting (continued)

Symptoms	Causes and Comments
Less than 10% white colonies with Control Insert DNA (continued)	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.
	T-overhangs have been removed, allowing blunt-ended ligation of vector and giving rise to more blue than white colonies. Avoid introduction of nucleases, which may degrade the T-overhangs. Use only the T4 DNA Ligase provided with the system, which has been tested for minimal exonuclease activity.
Less than 60% white colonies with Control Insert DNA	Improper dilution of the Rapid Ligation Buffer. The Rapid Ligation Buffer is provided at a 2X concentration. 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, which 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 containing PCR product	Improper dilution of the Rapid Ligation Buffer. The Rapid Ligation Buffer is provided at a 2X concentration. 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 determine whether an inhibitor is present. If an inhibitor is indicated, repurify the PCR fragment.

IX. Troubleshooting (continued)

Symptoms	Causes and Comments
Low number or no white colonies containing PCR product (continued)	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 (10–12).
	PCR product cannot be ligated due to pyrimidine dimers formed from UV over-exposure. 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.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 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 may have been generated and cloned into the pGEM®-T or pGEM®-T Easy Vector. Gel-purify the PCR fragment of interest.

IX. Troubleshooting (continued)

Symptom	Causes and Comments
Low number or no white colonies with PCR product (continued)	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 of a repair-deficient bacterial strain (e.g., SURE® cells) may reduce recombination events.
PCR product ligation reaction produces white colonies only (no blue colonies)	Ampicillin is inactive, allowing ampicillin-sensitive cells to grow. Check that ampicillin plates are made properly and used within one 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 *lacI*^{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 (10–12). 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 analysis. 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.

X. References

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XI. Appendix

XI.A. pGEM®-T Vector Restriction Enzyme Sites

The pGEM®-T Vector is derived from the circular pGEM®-5Zf(+) Vector (GenBank® Accession No. X65308). The pGEM®-5Zf(+) Vector sequence is available on the Internet at: **www.promega.com/vectors/**

The following restriction enzyme tables are based on those of the circular pGEM®-5Zf(+) Vector. The pGEM®-T Vector has been created by linearizing the pGEM®-5Zf(+) Vector with EcoRV at base 51 and adding a T to both 3´-ends. This site will not be recovered upon ligation of the vector and insert. The tables below 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 1–5 Times.

Enzyme	# of Sites	Location
AatII	1	20
AccI	1	76
AcyI	2	17, 1932
AflIII	2	99, 502
Alw26I	2	1456, 2232
Alw44I	2	816, 2062
AlwNI	1	918
ApaI	1	14
AspHI	4	94, 820, 1981, 2066
AvaII	2	1533, 1755
BanI	3	246, 1343, 2626
BanII	3	14, 94, 2664
BbuI	1	26
BglI	3	39, 1515, 2833
BsaI	1	1456
BsaAI	1	2589
BsaHI	2	17, 1932
BsaJI	5	37, 43, 241, 662, 2936
Bsp120I	1	10
BspHI	2	1222, 2230
BspMI	1	62
BssSI	2	675, 2059
BstOI	5	242, 530, 651, 664, 2937
BstXI	1	103
BstZI	2	31, 62
Cfr10I	2	1475, 2690
DdeI	4	777, 1186, 1352, 1892
DraI	3	1261, 1280, 1972
DraIII	1	2589
DrdI	2	610, 2544
DsaI	2	37, 43
EagI	2	31, 62
EarI	3	386, 2190, 2878
EclHKI	1	1395
Eco52I	2	31, 62
EcoICRI	1	92
EcoRV	1	51*

Enzyme	# of Sites	Location
FokI	5	119, 1361, 1542, 1829, 2919
FspI	2	1617, 2840
HaeII	4	380, 750, 2740, 2748
HgaI	4	613, 1191, 1921, 2806
HincII	1	77
HindII	1	77
Hsp92I	2	17, 1932
MaeI	5	56, 997, 1250, 1585, 2740
MluI	1	99
NaeI	1	2692
NciI	4	30, 882, 1578, 1929
NcoI	1	37
NdeI	1	82
NgoMIV	1	2690
NotI	1	62
NsiI	1	112
NspI	2	26, 506
Ppu10I	1	108
PstI	1	73
PvuI	2	1765, 2861
PvuII	2	326, 2890
RsaI	1	1875
SacI	1	94
SacII	1	46
SalI	1	75
ScaI	1	1875
SfiI	1	39
SinI	2	1533, 1755
SpeI	1	55
SphI	1	26
Sse8387I	1	73
SspI	2	2199, 2381
StyI	1	37
TaqI	4	76, 602, 2046, 2622
TfiI	2	337, 477
VspI	3	273, 332, 1567
XmnI	1	1994

*The pGEM®-T Vector has been created by linearizing the pGEM®-5Zf(+) Vector with EcoRV at base 51 and adding a T to both 3´-ends. This site will not be recovered upon ligation of the vector and insert.

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

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

AccB7I	BbsI	**BstEII**	FseI	PinAI	SplI
AccIII	**BclI**	**Bsu36I**	**HindIII**	PmeI	SrfI
Acc65I	**BglII**	**ClaI**	**HpaI**	PmlI	**StuI**
AflII	BlpI	**CspI**	**I-PpoI**	PpuMI	SwaI
AgeI	Bpu1102I	**Csp45I**	KasI	PshAI	**Tth111I**
AscI	BsaBI	DraII	**KpnI**	Psp5II	**XbaI**
AvaI	**BsaMI**	**Eco47III**	**NarI**	PspAI	XcmI
AvrII	BsmI	Eco72I	**NheI**	RsrII	**XhoI**
BalI	BsrGI	Eco81I	**NruI**	**SgfI**	**XmaI**
BamHI	**BssHII**	EcoNI	PacI	SgrAI	
BbeI	Bst1107I	**EcoRI**	PaeR7I	**SmaI**	
BbrPI	**Bst98I**	EheI	PflMI	**SnaBI**	

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

AciI	Bst71I	**HaeIII**	MaeIII	**NdeII**	SfaNI
AluI	BstUI	**HhaI**	**MboI**	NlaIII	**Tru9I**
BbvI	**CfoI**	**HinfI**	**MboII**	NlaIV	**XhoII**
BsaOI	**DpnI**	**HpaII**	MnlI	PleI	
Bsp1286I	DpnII	HphI	MseI	**Sau3AI**	
BsrI	EaeI	**Hsp92II**	**MspI**	Sau96I	
BsrSI	Fnu4HI	MaeII	**MspA1I**	ScrFI	

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

XI.B. pGEM®-T Easy Vector Restriction Enzyme Sites

The sequence of the pGEM®-T Easy Vector is available on the Internet at: **www.promega.com/vectors/**

The pGEM®-T Easy Vector has been linearized at base 60 with EcoRV and a T added to both 3´-ends. This site will not be recovered upon ligation of the vector and insert. The tables below 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 1–5 Times.

Enzyme	# of Sites	Location	Enzyme	# of Sites	Location
AatII	1	20	**FokI**	5	134, 1376, 1557, 1844, 2931
AccI	1	91			
AcyI	2	17, 1947	FspI	2	1632, 2855
AflIII	2	114, 517	**HaeII**	4	395, 765, 2755, 2763
Alw26I	2	1471, 2247			
Alw44I	2	831, 2077	HgaI	4	628, 1206, 1936, 2821
AlwNI	1	933			
ApaI	1	14	**HincII**	1	92
AspHI	4	109, 835, 1996, 2081	HindII	1	92
			Hsp92I	2	17, 1947
AvaII	2	1548, 1770	MaeI	5	65, 1012, 1265, 1600, 2755
BanI	3	261, 1358, 2641			
BanII	3	14, 109, 2679	**MluI**	1	114
BbuI	1	26	**NaeI**	1	2707
BglI	4	39, 42, 1530, 2848	**NciI**	4	30, 897, 1593, 1944
BsaI	1	1471			
BsaAI	1	2604	**NcoI**	1	37
BsaHI	2	17, 1947	**NdeI**	1	97
BsaJI	5	37, 46, 256, 677, 2951	**NgoMIV**	1	2705
			NotI	2	43, 77
Bsp120I	1	10	**NsiI**	1	127
BspHI	2	1237, 2245	NspI	2	26, 521
BspMI	1	77	Ppu10I	1	123
BssSI	2	690, 2074	**PstI**	1	88
BstOI	5	257, 545, 666, 679, 2952	**PvuI**	2	1780, 2876
			PvuII	2	341, 2905
BstXI	1	118	**RsaI**	1	1890
BstZI	3	31, 43, 77	**SacI**	1	109
Cfr10I	2	1490, 2705	**SacII**	1	49
DdeI	4	792, 1201, 1367, 1907	**SalI**	1	90
			ScaI	1	1890
DraI	3	1276, 1295, 1987	**SinI**	2	1548, 1770
DraIII	1	2604	**SpeI**	1	64
DrdI	2	625, 2559	**SphI**	1	26
DsaI	2	37, 46	Sse8387I	1	88
EagI	3	31, 43, 77	**SspI**	2	2214, 2396
EarI	3	401, 2205, 2893	**StyI**	1	37
EclHKI	1	1410	**TaqI**	5	56, 91, 617, 2061, 2637
Eco52I	3	31, 43, 77			
EcoICRI	1	107	TfiI	2	352, 492
EcoRI	2	52, 70	**VspI**	3	288, 347, 1582
EcoRV	1	60*	**XmnI**	1	2009

*The pGEM®-T Easy Vector has been linearized at base 60 with EcoRV and a T added to both 3´-ends. This site will not be recovered upon ligation of the vector and insert.

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

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

AccB7I	BbsI	**BstEII**	**HindIII**	PmeI	SplI
AccIII	**BclI**	**Bsu36I**	**HpaI**	PmlI	SrfI
Acc65I	**BglII**	**ClaI**	**I-PpoI**	PpuMI	**StuI**
AflII	BlpI	**CspI**	KasI	PshAI	SwaI
AgeI	Bpu1102I	**Csp45I**	**KpnI**	Psp5II	**Tth111I**
AscI	BsaBI	DraII	**NarI**	PspAI	**XbaI**
AvaI	**BsaMI**	**Eco47III**	**NheI**	RsrII	XcmI
AvrII	BsmI	Eco72I	**NruI**	**SfiI**	**XhoI**
BalI	BsrGI	Eco81I	PacI	**SgfI**	**XmaI**
BamHI	**BssHII**	EcoNI	PaeR7I	SgrAI	
BbeI	Bst1107I	EheI	PflMI	**SmaI**	
BbrPI	**Bst98I**	FseI	PinAI	**SnaBI**	

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

AciI	Bst71I	**HaeIII**	MaeIII	**NdeII**	SfaNI
AluI	BstUI	**HhaI**	**MboI**	NlaIII	**Tru9I**
BbvI	**CfoI**	**HinfI**	**MboII**	NlaIV	**XhoII**
BsaOI	**DpnI**	**HpaII**	MnlI	PleI	
Bsp1286I	DpnII	HphI	MseI	**Sau3AI**	
BsrI	EaeI	**Hsp92II**	**MspI**	Sau96I	
BsrSI	Fnu4HI	MaeII	**MspA1I**	ScrFI	

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

XI.C. Composition of Buffers and Solutions

IPTG stock solution (0.1M)

1.2g IPTG

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

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.

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 $MgCl_2 \bullet 6H_2O$
24.65g $MgSO_4 \bullet 7H_2O$

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

XI.D. Related Products

PCR Cloning Systems

Product	Size	Cat.#
pTARGET™ Mammalian Expression Vector System	20 reactions	A1410

Direct mammalian expression from a T-Vector.

Amplification Products

Please request our Amplification Products Brochure #BR139 or visit our Web site at **www.promega.com/applications/pcr/** to see a complete listing of our amplification products.

Thermostable DNA Polymerases

Product	Size	Cat.#
GoTaq® Green Master Mix	100 reactions	M7112[1], M7122[2]
	1,000 reactions	M7113[1], M7123[2]
GoTaq® Colorless Master Mix	100 reactions	M7142[1], M7132[2]
	1,000 reactions	M7143[1], M7133[2]

GoTaq® Master Mixes are premixed solutions containing GoTaq® DNA Polymerase, GoTaq® Reaction Buffer (Green or Colorless), dNTPs and Mg^{2+}.
[1]Cat.#s M7112, M7113, M7142 & M7143 are available in Europe or through Distributors supported by Promega European Branch Offices.
[2]Cat.#s M7122, M7123, M7132 & M7133 are available in all other countries, including the United States. For Laboratory Use.

Product	Size	Cat.#
GoTaq® DNA Polymerase	100u	M3171[1], M3001[2]

Available in additional sizes.
[1]Cat.# M3171 is available in Europe or through Distributors supported by Promega European Branch Offices.
[2]Cat.# M3001 is available in all other countries, including the United States. For Laboratory Use.

RT-PCR Systems

Product	Size	Cat.#
Access RT-PCR System	20 reactions	A1260
	100 reactions	A1250
	500 reactions	A1280
AccessQuick™ RT-PCR System	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	50 preps	A9281
	250 preps	A9282
Wizard® SV 96 PCR Clean-Up System	1 × 96 preps	A9340
	4 × 96 preps	A9341
	8 × 96 preps	A9342

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

Competent Cells

Product	Size	Cat.#
JM109 Competent Cells*, >10^8cfu/µg	5 × 200µl	L2001
Single Step (KRX) Competent Cells	5 × 200µl	L3001

*For Laboratory Use.

Accessory Products

Product	Size	Cat.#
X-Gal	100mg (50mg/ml)	V3941
IPTG, Dioxane-Free	1g	V3955
	5g	V3951

For Laboratory Use.

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