Promega Notes **Number 68, 1998, p. 31**

Q & A Technically Speaking

By Terri Sundquist and Mary Doers
Promega Corporation

Corresponding author: e-mail to tsundqui@promega.com

Optimized Cloning with T Vectors

Promega offers a number of T-Vectors designed specifically for the cloning and expression of PCR[a] *products (1-3). Many researchers have used these vectors to clone a wide range of amplified DNAs. This article presents suggestions for optimizing cloning conditions with any T-Vector system, including handling competent cells and addressing common performance issues.*

Q What results can I expect using the pGEM®-T and pGEM®-T Easy Vector Systems?

Use of the recommended protocol, the Control Insert DNA and the high efficiency competent cells (>1 x 10^8cfu/µg) supplied with the pGEM®-T and pGEM®-T Easy Vector Systems II[b,c] should yield at least 100 colonies. Out of 100 control colonies, greater than 60% should be white, containing plasmid with Control Insert DNA. A background control ligation with no insert DNA will generally yield 10-30 blue colonies (uncut vector) if the competent cells are >10^8cfu/µg.

Q What is the proper way to handle competent cells?

Several factors are critical in maintaining the high efficiency level of competent cells:

1. The cells should not be subjected to multiple freeze-thaw cycles; the efficiency will drop with each thaw. After thawing a vial containing 200µl of competent cells and performing transformation reactions, discard any unused volume rather than refreezing.
2. The cells should be thawed on ice and used immediately upon thawing.
3. Because competent cells are fragile, vortexing can decrease the transformation efficiency. Use gentle pipetting to mix the cells and DNA before transformation.
4. Certain tubes used for transformations can reduce the efficiency up to ~50% due to inefficient heat shock treatment. If this is a problem, use of sterile 17 x 100mm polypropylene culture tubes can increase efficiency.
5. The addition of SOC medium (4) instead of LB medium after heat shock can increase the number of bacterial colonies obtained.

Q How competent must the cells be to ensure good results with T-Vectors?

Promega recommends the use of high efficiency competent cells (>10^8cfu/µg) to maximize the number of colonies obtained and to increase the chances of identifying a colony containing the insert of interest. Promega supplies chemically competent, high efficiency JM109 competent cells (>10^8cfu/µg) with the pGEM®-T Vector System II, pGEM®-T Easy Vector System II, and pTARGET™ Mammalian Expression Vector System[c,d]. If using cells prepared in your laboratory, you should check the efficiency of the cells just before use. The use of competent cells with a transformation efficiency of less than 10^8cfu/µg is not recommended.

Q How can I determine the efficiency of competent cells?

A control transformation reaction is often performed to determine the efficiency of competent cells. Competent cells are transformed with a small amount (0.1-1ng) of circular plasmid DNA. The number of colony forming units (cfu) are counted after overnight growth on LB plates containing the appropriate antibiotic. The equation for transformation efficiency (cfu/µg) is:

(cfu on control plate) (1 x 10^3ng)

x x (dilution plated)

(ng of control plasmid DNA) (µg)

Q	What factors can affect ligation efficiency?

Components of a typical PCR amplification, such as salts, dNTPs, primers, and the thermostable DNA polymerase, can have adverse effects on ligation efficiency. Thus, removal of these reagents prior to ligation using size exclusion chromatography, Promega's Wizard® PCR Preps DNA Purification System(e), or other methods (5), can be beneficial.

Like the desired PCR product, primer-dimer and nonspecific amplification products will also contain a 3´-A overhang and can be ligated into a T-Vector. Removal of these products will increase the percent of white colonies that contain the insert of interest.

Finally, all ligations can be optimized by altering the molar ratio of vector to insert DNA. Molar ratios of 1:3, 1:1 and 3:1 insert:vector DNA are routinely tested for optimal ligation efficiency. In rare cases, molar ratios up to 10:1, insert:vector, are necessary for the ligation to be successful.

Q	What other factors can affect the overall cloning efficiency of T-Vectors?

The cloning efficiency of a given insert cannot be predicted but will depend upon a number of factors, including insert size or sequence and purity of PCR product. These factors can be as critical to the success of T-Vector cloning as the competent cells. For example, large inserts are prone to deletions and rearrangements, which can decrease the cloning efficiency of the correct insert (**Note:** Inserts of up to 7.5kb have been successfully cloned into Promega's pGEM®-T and pGEM®-T Easy Vector Systems). For problematic inserts, it may be necessary to use a strain designed to minimize DNA rearrangement. In addition, smaller inserts that do not disrupt the reading frame of the *lac*Z gene may not produce white colonies. Many of the resulting blue colonies will contain recombinant vector with the insert of interest. A higher number of blue colonies in the sample than in the background control would be indicative of this problem.

The purity of the PCR product can affect cloning efficiency if nucleases are a contaminant. Removal of the "T" overhang of the vector and the "A" overhang of the PCR product can greatly decrease cloning efficiency due to the reduced efficiency of blunt end ligations. If the thermostable DNA polymerase used in PCR possesses 3´-->5´ exonuclease "proofreading activity" (see Table 1), the majority of the PCR products will also be blunt-ended. Reference 7 provides details on restoring the "A" overhangs by a simple A-tailing protocol. The *pGEM®-T and pGEM®-T Easy Vector Systems Technical Manual* #TM042, provided with these products, gives further details on important cloning efficiency considerations.

REFERENCES

1. Johnson, R. (1995) *Promega Notes* **51**, 26.
2. Lesley, S.A. *et al.* (1995) *Promega Notes* **54**, 8.
3. Marcus, L., Hartnett, J. and Storts, D.R. (1996) *Promega Notes* **58,** 36.
4. Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual,* Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
5. Frohman, M.A. (1994) In: *The Polymerase Chain Reaction*, Mullis, K.B., Ferré, F. and Gibbs, R.A., eds. Birhäuser Boston, Cambridge, MA
6. Newton, C.R. and Graham, A. (1994) *PCR*, BIOS Scientific Publishers Ltd., Oxford, UK.
7. Kobs, G. (1997) *Promega Notes* **62**, 15.

Ordering Information

Product	Size	Cat.#
pGEM®-T Easy Vector System I	20 reactions	A1360
pGEM®-T Easy Vector System II	20 reactions	A1380
pGEM®-T Vector System I	20 reactions	A3600
pGEM®-T Vector System II	20 reactions	A3610
	20 reactions	A1410

pTARGET™ Mammalian Expression Vector System		
JM109 Competent Cells, High Efficiency (>10^8cfu/µg)	5 x 200µl	L2001
Wizard® PCR Preps DNA Purification System	50 preps	A7170

Editor's Note: *pGEM®-T Easy offers all the features of the original pGEM®-T Vector but includes recognition sites for both* Eco*R I and* Not *I flanking the multiple cloning site for single digestion release of the cloned insert. Also, the System II products provide High Efficiency (>10^8cfu/µg) JM109 Competent Cells.*

Table 1. Comparison of Thermostable Polymerases (6).

Property	*Taq*(f)/ AmpliTaq®	*Tfl*	Stoffel Fragment	*Tth*(f)	Vent$_R$® (exo-)	Vent$_R$®/*Tli* (f)	DeepVent$_R$™	*Pfu*(f)	*Pwo*	*UlTma*®
5´-->3´ Exonuclease Activity	Yes	Yes	No	Yes	No	No	No	No	No	No
3´-->5´ Exonuclease Activity	No	No	No	No	No	Yes	Yes	Yes	Yes	Yes
Reverse Transcription Activity	Weak	Yes	Weak	Yes	Weak	No	NA	NA	NA	Weak
Resulting DNA Ends	3´A	3´A	3´A	3´A	70% Blunt, 30% Single base overhang	>95% Blunt	Blunt	Blunt	Blunt	>95% Blunt

NA; not available.

[a] *The PCR process is covered by patents issued applicable in certain countries. Promega does not encourage or support the unauthorized or unlicensed use of the PCR process.*

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

[c] *Licensed under one or both of U.S. Pat. No. 5,487,993 and European Pat. No. 0 550 693.*

[d] *Covered under U.S. Pat. No. 5,168,062 assigned to the University of Iowa Research Foundation.*

[e] *Licensed under U.S. Pat. No. 5,075,430.*

[f] *Some applications in which this product may be used are covered by patents issued and applicable in certain countries. Because purchase of this product does not include a license to perform any patented application, users of this product may be required to obtain a patent license depending upon the particular application and country in which the product is used. For more specific information, please contact Promega.*

© 1998 Promega Corporation. All Rights Reserved.

pGEM and Wizard are trademarks of Promega Corporation and are registered with the U.S. Patent and Trademark Office. pTARGET is a trademark of Promega Corporation.

AmpliTaq and UlTma are registered trademarks of Roche Molecular Systems, Inc. Deep Vent$_R$ is a trademark of, and Vent$_R$ is a registered trademark of New England Biolabs, Inc.

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