



# Stability of pGEM<sup>®</sup>-T Vectors

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PCR<sup>\*</sup> products are frequently cloned into plasmid vectors for subsequent manipulations, such as sequencing and mutagenesis. The pGEM<sup>®</sup>-T Vector Systems<sup>(a)\*\*</sup>, and other T-vectors available from Promega, allow direct cloning of PCR products without restriction digestion, modification of primers or purification of amplified DNA. The thymidine overhangs of the pGEM<sup>®</sup>-T Vectors are stable even when the DNA is subjected to freezing and heating.

<sup>\*</sup>The PCR process is covered by patents issued and applicable in certain countries. Promega does not encourage or support the unauthorized or unlicensed use of the PCR process.

<sup>(a)</sup>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.

<sup>\*\*</sup>Licensed under one or both of U.S. Pat. No. 5,487,993 and European Pat. No. 0 550 693.

## INTRODUCTION

Nonproofreading thermostable DNA polymerases catalyze the addition of nucleotides, preferably adenosines, to the 3'-ends of the double-stranded DNA products in a nontemplate-directed manner (1). The pGEM<sup>®</sup>-T Vectors possess thymidines at the 3'-ends to exploit the complementary, amplification-generated adenosines of most PCR products. We constructed the pGEM<sup>®</sup>-T Vector by digesting Promega's pGEM<sup>®</sup>-5Zf(+) Vector with *EcoR* V within the multiple cloning site to generate blunt ends. Terminal thymidines are added at both ends to create a "T-vector" (1). The single 3'-T overhangs at the insertion site greatly improve PCR product ligation efficiency by providing complementary ends to those of the insert. The 3'-T overhangs provide the added benefit of greatly reducing self-ligation of the linearized vector.

A frequent misconception regarding T-vectors is that the T-overhangs are not stable. Some are concerned that the vector will lose the added Ts when the DNA is stored or handled improperly. Likewise, there is a belief that the A-overhangs of a PCR product will be lost if the fragment is not ligated immediately into a vector. Loss of the T- or A-overhangs are unlikely and uncommon events in the absence of contaminating nucleases. The T- and A-overhangs of the vector and insert are attached to the DNA molecules covalently and should, therefore, be as stable as any other type of base overhang. Nevertheless, we decided to verify the integrity of the ends of both vector and insert molecules by incubating each at elevated temperatures and subjecting them to multiple freeze-thaw cycles.

## VECTOR STABILITY

Vector and insert DNAs were subjected to conditions that tested the stability of the overhangs. Aliquots of pGEM<sup>®</sup>-T Vector DNA were incubated as indicated in [Table 1](#).

Following incubation, the vector DNA was used in two ligation reactions with or without the pGEM<sup>®</sup>-T Vector System Control Insert. The ligation reactions were performed according to the instructions recommended in the [pGEM<sup>®</sup>-T Vector System Technical Manual #TM042](#).

After each ligation, a portion of the reaction was used to transform JM109 Competent Cells, High Efficiency (Cat.# L2001). The following day, the colonies were counted and screened for the presence of inserts using PCR and the same primers as those used to generate the insert. The results are listed in [Table 1](#).

The results from these treatments indicate that the incubation conditions have no effect on the percentage of white colonies generated. Inserts were identified in most of the white colonies; only treatment by incubation at room temperature for seven days showed a decline in recombinants.

**Table 1. Generation of Positive Recombinants Following Differential Incubation and Ligation of Vector DNA.**

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	Incubation		White Colonies per Plate	Recombinants (+insert only)
	Temperature	Duration		
1	20°C	24 hours	+ Insert: 93% - Insert: 0%	80%
2	20°C	seven days	+Insert: 95% -Insert: 7%	90%
3	4°C	seven days	+Insert: 95% -Insert: 22%	89%
4	RT	24 hours	+Insert: 92% -Insert: 5%	100%
5	RT	seven days	+Insert: 92% -Insert: 0%	70%
6	37°C	24 hours	+Insert: 98% -Insert: 17%	100%
7 Five freeze-thaw cycles*			+Insert: 97% -Insert: 10%	80%
8 20 freeze-thaw cycles*			+Insert: 91% -Insert: 0%	80%

*Abbreviation: RT, room temperature.*  
\*Freeze-thaws were performed successively using a dry ice/ethanol bath and a heat block.

## INSERT STABILITY

A second misconception is that a PCR product must be cloned into a T-vector as soon as possible after amplification. To test this hypothesis, we generated a 360bp DNA fragment by PCR amplification. The PCR product was divided into two aliquots. One half was purified using Promega's Wizard<sup>®</sup> PCR Preps DNA Purification System (Cat.# A7170). PCR product from the second, unpurified reaction mix was ligated into the pGEM<sup>®</sup>-T Vector directly. The second half was not purified to assess any effect of primers, dNTPs, magnesium and *Taq* DNA polymerase on insert stability or ligation efficiency.

Portions of the PCR products were analyzed by agarose gel electrophoresis to verify the absence of secondary amplification products and primer dimers (data not shown). These molecules can ligate to the pGEM<sup>®</sup>-T Vector and prevent ligation of the desired PCR fragment.

Both the purified and unpurified PCR products were divided further into smaller aliquots. Each aliquot was subjected to different storage conditions as indicated in [Table 2](#).

**Table 2. Generation of Positive Recombinants Following Differential Incubation and Ligation of Insert DNA.**

	Incubation		White Colonies per Plate		Recombinants (+ insert only)	
	Temperature	Duration	Purified DNA	Unpurified DNA	Purified DNA	Unpurified DNA
1	-20°C	seven days	89%	92%	80%	90%
2	4°C	seven days	92%	90%	100%	100%
3	RT	seven days	94%	90%	80%	90%
4	37°C	seven days	92%	90%	100%	80%
5	Five freeze-thaw cycles*		93%	91%	90%	100%
6	20 freeze-thaw cycles*		95%	86%	100%	100%
	Positive Control (+ Insert)			96%		100%
	Negative Control (- Insert)			12%		NA

*Abbreviations: NA, not applicable; RT, room temperature.*  
\*Freeze-thaws were performed successively.

Portions of each DNA aliquot were then ligated into the pGEM<sup>®</sup>-T Vector as recommended. In addition, a positive control ligation with the pGEM<sup>®</sup>-T Vector Control Insert was assembled. Finally, a negative control ligation with no insert DNA was included. Following the overnight ligation incubation, the ligation products were transformed into JM109 Competent Cells, High Efficiency, as recommended in the system. The results are listed in [Table 2](#); white colonies were screened for the presence of insert as described previously.

The results after incubation of the insert show that the PCR product is stable. In addition, comparison of purified and unpurified insert show that post-PCR purification is not critical for successful T-vector cloning.

## SUMMARY

In conclusion, this work demonstrates the stability of both the T-overhangs of the pGEM<sup>®</sup>-T Vector and the A-overhangs of insert DNA generated by PCR amplification. Although the T-overhangs of the pGEM<sup>®</sup>-T Vectors and the A-overhangs of insert DNA are robust, we recommend that the DNA be stored as suggested in the product literature provided with the system. The concern over storing a PCR fragment prior to ligation into a suitable vector seems unfounded. In all cases, proper procedures should be maintained to ensure both the vector and insert are not contaminated by exonucleases, which will affect cloning efficiency.

## REFERENCE

1. Mezei, L.M. and Storts, D.R. (1994) In: *PCR Technology: Current Innovations*, Griffin, H.G. and Griffin, A.M., eds., CRC Press, Boca Raton, FL.

Ordering Information		
Product	Size	Cat.#
pGEM <sup>®</sup> -T Vector System I	20 reactions	A3600
pGEM <sup>®</sup> -T Vector System II*	20 reactions	A3610
pGEM <sup>®</sup> -T Easy Vector System I	20 reactions	A1360
pGEM <sup>®</sup> -T Easy Vector System II*	20 reactions	A1380
Wizard <sup>®</sup> PCR Preps DNA Purification System	50 preps	A7170
JM109 Competent Cells, High Efficiency	1ml	L2001

\*Each System II provides JM109 Competent Cells, High Efficiency.

Related Products		
Product	Size	Cat.#
pTARGET <sup>™</sup> Mammalian Expression Vector System <sup>(b)**</sup>	20 reactions	A1410
PinPoint <sup>™</sup> Xa-1 T-Vector System I <sup>(c)**</sup>	20 reactions	V2610
PinPoint <sup>™</sup> Xa-1 T-Vector System II <sup>(c)**</sup>	20 reactions	V2850

The pTARGET<sup>™</sup> System and PinPoint<sup>™</sup> System II provide JM109 Competent Cells, High Efficiency.

**Editor's Note:** pGEM<sup>®</sup>-T Easy Vector is a newer version of the pGEM<sup>®</sup>-T Vector. The pGEM<sup>®</sup>-T Easy Vector contains the same features as the original vector but has two restriction sites each for *EcoR* I and *Not* I in the multiple cloning region allowing for insert isolation with single restriction digests.

<sup>(b)</sup>The CMV vector technology is the subject of U.S. Pat. No. 5,168,062 assigned to the University of Iowa Research Foundation.

*(c) For research purposes only. Not for diagnostic or therapeutic use. For nonresearch uses of the portion of the vector encoding the biotinylation sequence, please contact Promega Corporation for licensing information.*

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