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pGEM®-T Vector Systems Troubleshooting Guide

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The pGEM-T Vector System allows direct cloning of PCR products without restriction digestion, modification of primers, or purification of amplified DNA.*

Introduction

Of the several methods currently employed for cloning PCR* products, most require extra manipulation of the DNA after amplification. One method involves using oligonucleotide primers that contain restriction sites within their sequence. This approach not only increases the cost of primer synthesis but is also inefficient - cutting with restriction enzymes near the end of PCR products has proven to be very difficult (1). Another approach is to enzymatically treat the PCR product to produce blunt ends and then ligate the fragment into a blunt-ended vector. PCR products do not have blunt ends because *Taq* DNA Polymerase** catalyzes the addition of nucleotides, almost exclusively adenosines, in a non-template directed manner to the 3´-termini of the double stranded products (2). In addition to requiring extra steps, blunt-end ligations are inefficient and so this approach requires a high concentration of blunt-ended PCR product.

*The polymerase chain reaction (PCR) process for amplifying nucleic acid is covered by U.S. Pat. Nos. 4,683,195 and 4,683,202, assigned to Hoffmann-LaRoche. Patents pending in other countries.

** This product has not been licensed for use in the polymerase chain reaction (PCR).

The pGEM®-T Vector Systems take advantage of the non-template dependent addition of a single deoxyadenosine to the 3´-end of PCR products by using a vector with ends that complement those of the amplified fragment. The pGEM-T Vector is prepared by digesting Promega's pGEM-5Zf(+) Vector with *Eco*R V to generate blunt ends and then adding a terminal thymidine to the 3´-strand at both ends. These single 3´-T overhangs at the insertion site greatly improve the efficiency of ligation of a PCR product into the plasmid by providing ends complementary to those of the insert and reducing self-ligation. The system requires very little starting material, and in most cases, fragments can be cloned directly without further manipulations.

Controls assure product performance

To assure good performance of the system, use only the components provided with the system. The T4 DNA Ligase supplied has been quality tested by ligating the Control DNA, a 469 base pair PCR product, into the pGEM-T Vector. Low quality or nuclease contaminated ligase may promote the loss of the overhanging T's in the pGEM-T Vector, which in turn favors the blunt-end self-ligation of the vector over the ligation of the vector with the insert. The competent cells supplied with the system should be handled as recommended to preserve their high efficiency: 10^8 cfu/µg of DNA. When used as

recommended, the percentage of white colonies with the system's Control DNA insert should be at least 15%, of which 80% are usually true recombinants. The percentage of recombinant colonies obtained when cloning other inserts (in a size range of 500 to 1000 base pairs) may be as high as 80%, depending on the composition and purity of the fragment. Several important factors that should be considered when cloning in the pGEM-T Vector are described in Table 1.

Table 1. Troubleshooting Guide for the Use of pGEM-T Vector.

Results	Possible Causes	Recommendations
Ligating the Control DNA Insert		
Few or no colonies.	Cells have lost competency.	Test the efficiency of the cells by transforming an uncut plasmid that encodes antibiotic selection, for example pGEM-5Zf(+).
High background of blue colonies.	Vector self-ligated without T's.	Use only the T4 DNA Ligase provided with the kit. It has been quality tested for use in the pGEM-T Vector system.
Ligating a PCR insert		
Low percentage of white colonies.	Vector self-ligated without T's.	Use only the T4 DNA Ligase provided with the kit.
	Insufficient amount of insert available.	Adjust the vector:insert ratio.
	PCR product less than 500 base pairs long or a multiple of 3 base pairs long.	These inserts do not efficiently disrupt the *lac*Z gene. Screen blue or pale blue colonies for the presence of inserts.
	Multiple PCR products.	Gel purify the fragment of interest if multiple bands were generated during amplification or if there is a large amount of dimer present.
Unexpected inserts in white colonies.	Secondary products or primers are cloned in the pGEM-T vector.	Gel purify the PCR product (see above).
Insert in blue or pale blue colonies.	Cloned in a short PCR insert which maintained *lac*Z in reading frame.	PCR products is less than 500 base pairs long or a multiple of 3 base pairs long .

Method to quickly screen pGEM®-T vector recombinants

To determine the percentage of true recombinants, rapidly perform a screen by picking a number of white colonies and resuspending each one in 50µl of sterile water. Boil for 5-10 minutes and spin in a microcentrifuge for 2-3 minutes to pellet cell debris. Use 5-10µl of this supernatant as the template for small PCR reactions. Amplify with the same primers used to generate the insert or primers within the vector such as the T7 Promoter and SP6 Promoter Primers (Cat.# Q5021 and Q5011). Alternatively, screen potential recombinants by growing 1ml liquid cultures for 6 hours to overnight. Extract the DNA using the Wizard(TM) Minipreps DNA Purification System and digest each sample with a restriction enzyme that is characteristic of the insert (ideally, one with a unique restriction site in the insert and no more than one site in the vector). Under certain circumstances, pale blue colonies may contain inserts as well; small inserts and certain reading frames are less efficient in disrupting the *lacZ* gene, thus the blue/white color differentiation is less pronounced (3).

Summary

The pGEM-T Vector Systems provide a convenient method for the direct cloning of PCR fragments. The T4 DNA Ligase included with the systems has been quality controlled to provide the maximum percentage of recombinant clones. The blue/white cloning assay allows quick screening and identification of recombinant clones.

References

1. Kaufman, D. L. and Evans, G. A. (1990) *BioTechniques* **9**, 304.
2. Clark, J. M. (1988) *Nucl. Acids Res.* **16**, 9677.
3. Murray, E., Singer, K., Cash K., and Williams R. (1993) *Promega Notes* **41**, 1.

Ordering Information

Product	Cat.#
pGEM®-T Vector System I	A3600
The system includes the pGEM®-T Vector, Ligase, 10X Buffer and a Positive Control.	

Product	Cat.#
pGEM®-T Vector System II	A3610
The system includes the same components as System I plus high efficiency JM109 Competent Cells.	

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