

*Technically Speaking*

## pGEM®-T Vector System

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PCR products are frequently cloned into plasmid vectors in order to facilitate subsequent manipulations, such as sequencing and subcloning. The pGEM®-T Vector System provides a convenient method for the direct cloning of these amplification products. Here are answers to some of the most frequently asked questions regarding Promega's pGEM®-T Vector Cloning System.

### **Q: What is the pGEM®-T Vector System?**

The pGEM®-T Vector System facilitates the direct cloning of PCR\* products into a convenient plasmid vector (1). The system includes Positive Control Insert DNA, T4 DNA Ligase, Ligase 10X Buffer and linearized pGEM®-5Zf(+)\*\* vector DNA containing 3'-terminal deoxythymidine overhangs.

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

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

### **Q: What are the advantages of using the pGEM®-T Vector System?**

Most other strategies require an enzymatic treatment of the PCR product prior to ligation. One common approach enzymatically treats the PCR product to produce blunt ends and then ligates it into a plasmid vector linearized with a blunt end cut. This enzymatic treatment is necessary because *Taq*\*\*\* DNA polymerase adds a nucleotide, almost exclusively deoxyadenosine, to the 3'-termini of the amplification product (2). In addition to being more cumbersome than direct cloning, this technique requires blunt-end ligation, which is less efficient than cohesive-end ligation, and requires higher concentrations of PCR product in the ligation reaction.

\*\*\*This product has not been licensed for use in the polymerase chain reaction (PCR). The PCR process for amplifying nucleic acids is covered by U.S. Patent Nos. 4,683,195 and 4,683,202, assigned to Hoffmann-LaRoche. Patents pending in other countries.

Another common strategy is to incorporate restriction sites into the amplification primers. This strategy not only increases the cost of oligonucleotide synthesis but is also inefficient. Restriction sites close to the ends of PCR products can be very difficult to cleave (3), resulting in reduced cloning efficiency. Unlike these strategies, the pGEM®-T Vector System requires no enzymatic treatment prior to ligation.

### **Q: How does the system work?**

The pGEM®-T Vector Cloning System takes advantage of the non-template dependent addition of deoxyadenosine onto the 3'-end of PCR products by providing pGEM®-5Zf(+) plasmid DNA that has been cleaved at the *EcoR* V site and had a single 3'-terminal deoxythymidine added to both ends. By using this vector, PCR products can be directly cloned without prior enzymatic manipulation while also taking advantage of the higher efficiency of a cohesive-end ligation.

### **Q: How can I select for recombinants?**

Potential recombinants can be selected by an initial blue/white colony screen (4). Clones may be analyzed further by techniques such as restriction enzyme analysis and small scale PCR screening (5).

### **Q: Are there limitations to the blue/white colony selection strategy?**

The blue/white colony screen relies on the efficient disruption of the *lacZ* gene. Although the pGEM®-T Control Insert will produce recombinants that generate white colonies, the insertion of other DNA fragments into the *lacZ* coding sequence will not result in white colonies unless the fragments disrupt the *lacZ* reading frame. When attempting to determine if a reading frame disruption will occur, remember to include the terminal deoxyadenosine residues in your calculations.

Although it tends to occur most frequently with PCR products of 500bp or less, inserts of up to 2kb have been reported to result in blue colonies (6). Some insert DNAs can also result in pale blue colonies (7). For this reason it is often best to perform a control ligation without insert DNA. By comparing numbers of blue colonies between no insert control and PCR ligations, the likelihood of having generated in-frame recombinants may be assessed.

**Q: Why is the plasmid only sold with ligase?**

The T4 DNA Ligase provided in the system has been tested for its ability to ligate the Control DNA, a 468 base pair PCR product, into the pGEM®-T Vector. Other ligase preparations may contain exonuclease activity or other contaminants that could remove overhanging terminal nucleotides and result in higher levels of non-recombinants.

**Q: Do I need to gel purify my PCR product?**

If a single amplification product is generated, as determined by agarose gel analysis, gel purification may not be necessary. However, even if no extraneous bands are visualized, there may be a small quantity of primer-dimer present in the reaction. Even a very small mass of primer-dimer corresponds to a large molar quantity that can result in unacceptably high levels of background colonies. For this reason, gel purification should be considered. A faster alternative is to use the Wizard™ PCR Preps DNA Purification System (Cat.# A7170). The resin in this system will exclude primers and primer-dimers while efficiently purifying PCR products greater than 200 base pairs in size.

**Q: Can I use my own competent cells with this system?**

The pGEM®-T Vector System is sold either with (Cat.# A3610) or without (Cat.#A3600) high efficiency JM109 competent cells, allowing the customer the option of providing their own high efficiency competent cells. It is *critical*, however, that the competent cells have an efficiency of at least 10<sup>8</sup> cfu/μg DNA. Host strains other than JM109 may be used but they should be compatible with blue/white color screening and standard ampicillin selection.

**Q: What are the optimal ligation conditions for cloning my product?**

The optimal insert:vector ratio for a particular construct must be determined empirically. Although a 1:1 molar ratio is often optimal, molar ratios ranging from 1:8 to 8:1 have been successfully employed. A range of ratios should be tested for each experiment. Typical ligation conditions use 1μl of Ligase 10X Buffer, 50ng of plasmid DNA, 1 Weiss unit of T4 DNA Ligase, and insert DNA, in a 10μl total volume. The ligation is incubated at 15°C for 3 hours to overnight.

**Q: Are there steps that can be taken to further enhance system performance?**

Several methods can be used to increase the number of clones obtained. Caution should be exercised to avoid overexposing DNA to UV light if viewing PCR products in an agarose gel prior to gel purification. Prior to transformation, a quick heat inactivation (65°C, 10 minutes, followed by incubation on ice) will result in increased ligation efficiency. Additionally, the use of SOC medium instead of LB after heat shock may enhance system performance.

**References**

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