

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

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

Quick
PROTOCOL

Cloning PCR Products with pGEM[®]-T and pGEM[®]-T Easy Vectors

Ligation Using 2X Rapid Ligation Buffer

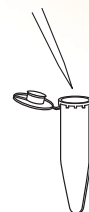
1. Briefly centrifuge the pGEM[®]-T or pGEM[®]-T Easy Vector and Control Insert DNA tubes to collect contents at the bottom of the tube.
2. Set up ligation reactions as described below. Vortex the 2X Rapid Ligation Buffer vigorously before each use. Use 0.5ml tubes known to have low DNA-binding capacity.

Reagents	Standard Reaction	Positive Control	Background Control
2X Rapid Ligation Buffer, T4 DNA Ligase	5 μ l	5 μ l	5 μ l
pGEM [®] -T or pGEM [®] -T Easy Vector (50ng)	1 μ l	1 μ l	1 μ l
PCR product	X μ l	—	—
Control Insert DNA	—	2 μ l	—
T4 DNA Ligase (3 Weiss units/ μ l)	1 μ l	1 μ l	1 μ l
Deionized water to a final volume of	10 μ l	10 μ l	10 μ l

3. Mix the reactions by pipetting. Incubate the reactions 1 hour at room temperature. Alternatively, incubate the reactions overnight at 4°C for the maximum number of transformants.

Transformation of JM109 High Efficiency Competent Cells

1. Prepare LB/ampicillin/IPTG/X-Gal plates.
2. Centrifuge the ligation reactions briefly. Add 2 μ l of each ligation reaction to a sterile 1.5ml tube on ice. Prepare a control tube with 0.1ng of uncut plasmid.
3. Place the JM109 High Efficiency Competent Cells in an ice bath until just thawed (5 minutes). Mix cells by **gently** flicking the tube.
4. Carefully transfer 50 μ l of cells to the ligation reaction tubes from Step 2. Use 100 μ l of cells for the uncut DNA control tube. **Gently** flick the tubes and incubate on ice for 20 minutes.
5. Heat-shock the cells for 45–50 seconds in a water bath at exactly 42°C. DO NOT SHAKE. Immediately return the tubes to ice for 2 minutes.
6. Add 950 μ l room temperature SOC medium to the ligation reaction transformations and 900 μ l to the uncut DNA control tube. Incubate for 1.5 hours at 37°C with shaking (~150rpm).
7. Plate 100 μ l of each transformation culture onto duplicate LB/ampicillin/IPTG/X-Gal plates. For the uncut DNA control, a 1:10 dilution with SOC is recommended.
8. Incubate plates overnight at 37°C. Select white colonies.



Set up ligation. Incubate at room temperature for 1 hour or at 4°C overnight.



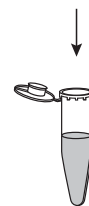
Thaw competent cells on ice. Add 50 μ l cells to 2 μ l of the ligation reaction. Incubate on ice for 20 minutes.



Heat Shock for 45–50 seconds at exactly 42°C.



Incubate on ice for 2 minutes.



Add SOC medium. Incubate for 1.5 hours at 37°C with shaking.

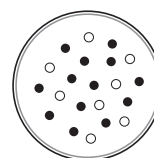


Plate. Select white colonies.

Additional protocol information in Technical Manual #TM042, available online at: www.promega.com

ORDERING/TECHNICAL INFORMATION:

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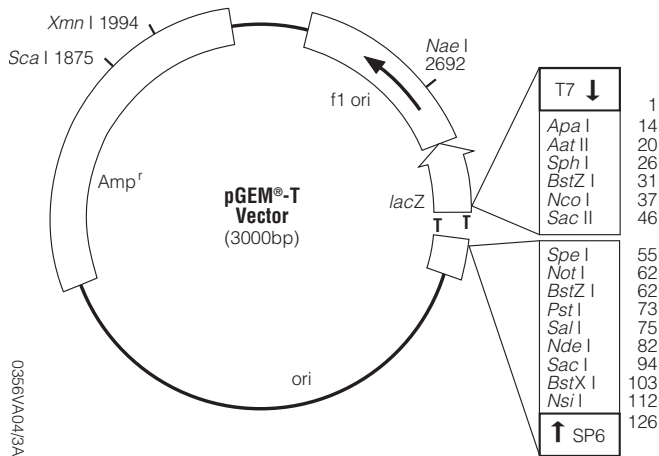
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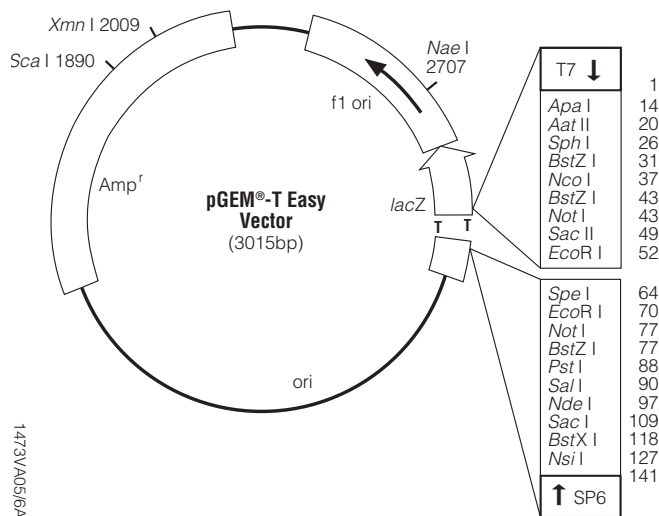
pGEM®-T Vector Circle Map and Sequence Reference Points



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
<i>lacZ</i> start codon	165
<i>lac</i> operator	185–201
β -lactamase coding region	1322–2182
phage f1 region	2365–2820
<i>lac</i> operon sequences	2821–2981, 151–380
pUC/M13 Forward Sequencing Primer binding site	2941–2957
T7 RNA Polymerase promoter (–17 to +3)	2984–3

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
<i>lacZ</i> start codon	180
<i>lac</i> operator	200–216
β -lactamase coding region	1337–2197
phage f1 region	2380–2835
<i>lac</i> operon sequences	2836–2996, 166–395
pUC/M13 Forward Sequencing Primer binding site	2949–2972
T7 RNA Polymerase promoter (–17 to +3)	2999–3

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