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

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

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

Ligation Using 2X Rapid Ligation Buffer

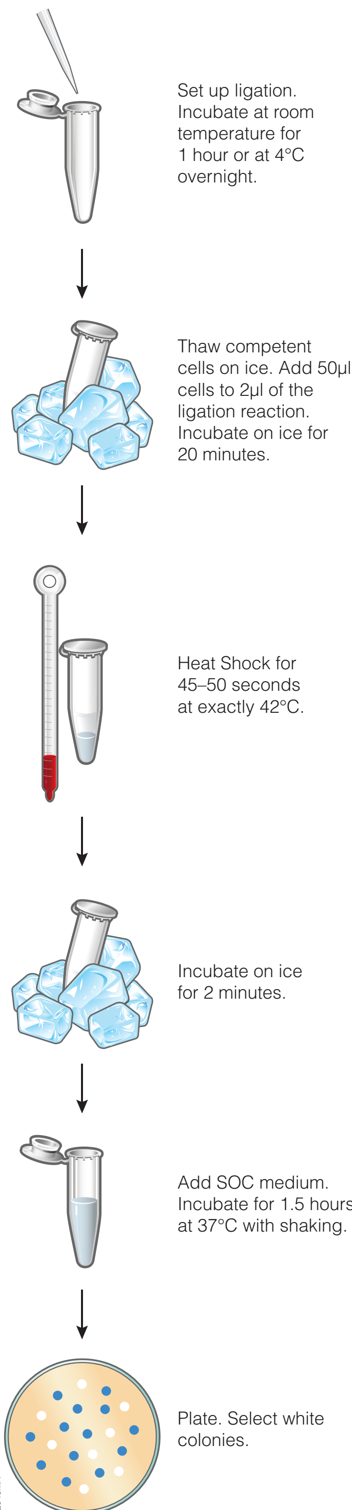
1. Briefly centrifuge the pGEM®-T or pGEM®-T Easy Vector and Control Insert DNA tubes to collect contents at the bottom of the tube.
2. Set up ligation reactions as described below. Vortex the 2X Rapid Ligation Buffer vigorously before each use. Use 0.5ml tubes known to have low DNA-binding capacity.

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

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 2µl 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 50µl of cells to the ligation reaction tubes from Step 2. Use 100µl 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 950µl room temperature SOC medium to the ligation reaction transformations and 900µl to the uncut DNA control tube. Incubate for 1.5 hours at 37°C with shaking (~150rpm).
7. Plate 100µl 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.



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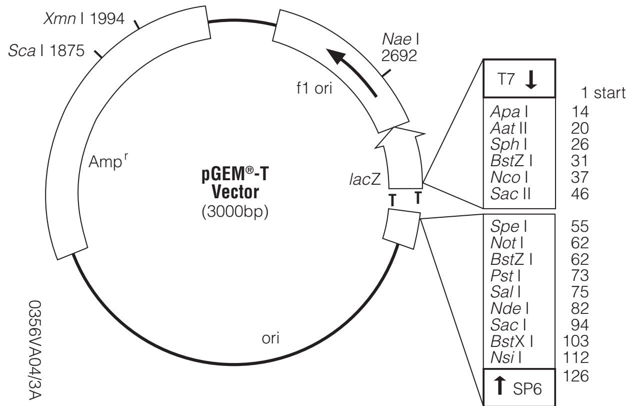
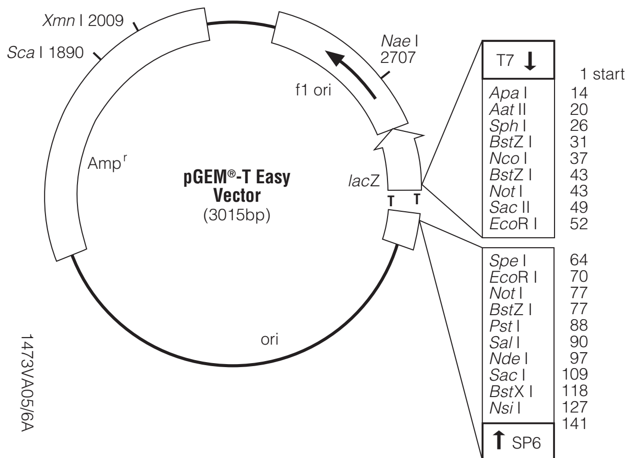
pGEM®-T Vector Circle Map and Sequence Reference Points



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
lacZ start codon	165
lac operator	185–201
β-lactamase coding region	1322–2182
phage f1 region	2365–2820
lac 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
lacZ start codon	180
lac operator	200–216
β-lactamase coding region	1337–2197
phage f1 region	2380–2835
lac 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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