

For white-blue colony screening by **pGEM-T easy vector** (a high copy number plasmid), you need to use LB/Amp plate supplied by IPTG/X-GAL

**Prepare 1L of LB plates as follows:**

10g Tryptone  
5g Yeast extract  
5g NaCl (instead of 10g)  
15g Agar  
NaOH to adjust pH to 7.0

Autoclave, let it cool to 50°C and add Ampicillin (to final conc 100µg/ml).

**Amp, LB plates supplied with 0.1M IPTG and 50mg/ml X-Gal** (5-bromo-4-chloro-3-indolyl-b-D-galactoside):

Spread **100µl of 100mM IPTG** on the above plate (LB/Amp) and then spread **20ul of 50mg/ml X-Gal**. Let the plate absorb the chemicals for 30min/37C before using it to spread your transformant bacteria.

**SOC medium (100ml)**

2.0g Bacto®-tryptone  
0.5g Bacto®-yeast extract  
1ml 1M NaCl  
0.25ml 1M KCl  
1ml 2M Mg<sup>2+</sup> stock, filter sterilized (as prepared below)  
1ml 2M glucose, filter sterilized

Add Bacto®-tryptone, Bacto®-yeast extract, NaCl and KCl to 97ml distilled water. Stir to dissolve. Autoclave and cool to room temperature. Add 2M Mg<sup>2+</sup> stock and 2M glucose, each to a final concentration of 20mM. Bring to 100ml with sterile, distilled water. Filter the complete medium through a 0.2µm filter unit. The final pH should be 7.0.

**2M Mg<sup>2+</sup> stock**

20.33g MgCl<sub>2</sub> · 6H<sub>2</sub>O  
24.65g MgSO<sub>4</sub> · 7H<sub>2</sub>O  
Add distilled water to 100ml. Filter sterilize.

### **2X Rapid Ligation Buffer, T4 DNA Ligase (provided)**

60mM Tris-HCl (pH 7.8)  
20mM MgCl<sub>2</sub>  
20mM DTT  
2mM ATP  
10% polyethylene glycol (MW8000, ACS Grade)

Store in single-use aliquots at -20°C. Avoid multiple freeze/thaw cycles.

### **Optimizing Insert:Vector Molar Ratios**

The pGEM®-T and pGEM®-T Easy Vector Systems have been optimized using a 1:1 molar ratio of the Control Insert DNA to the Vectors. However, ratios of 8:1 to 1:8 have been successfully used. If initial experiments with your PCR product are suboptimal, ratio optimization may be necessary. Ratios from 3:1 to 1:3 provide good initial parameters. The concentration of PCR product should be estimated by comparison to DNA mass standards on a gel or by using a fluorescent assay (5). The pGEM®-T and pGEM®-T Easy Vectors are approximately 3kb and are supplied at 50ng/ml.

To calculate the appropriate amount of PCR product (insert) to include in the ligation reaction, use the following equation.

$$[\text{ng of vector} \times \text{kb size of insert} / \text{kb size of vector}] \times \text{insert:vector molar ratio} = \text{ng of insert}$$

Sufficient pGEM®-T or pGEM®-T Easy Vector is provided to vary insert:vector ratios as recommended and to perform control reactions.

### **Example of insert:vector ratio calculation:**

How much 0.5kb PCR product should be added to a ligation in which 50ng of 3.0kb vector will be used if a 3:1 insert:vector molar ratio is desired?

$$[50\text{ng vector} \times 0.5\text{kb insert} / 3.0\text{kb vector}] \times 3/1 = 25\text{ng insert}$$