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 Mg2+ 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 Mg2+ 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 Mg2+ stock
20.33g MgCl2 ·6H2O
24.65g MgSO4 ·7H2O
Add distilled water to 100ml. Filter sterilize.

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

60mM Tris-HCl (pH 7.8)
20mM MgCl2
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.

[ng of vector x kb size of insert / kb size of vector] x insert:vector molar ratio = 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?

[50ng vector x 0.5kb insert/ 3.0kb vector] x 3/1 = 25ng insert