

Transformation

Use the ligation mixture to transform into competent *E. coli* cells.

6.1 Prewarm culture plates to increase the drying rate of plated cells. The culture plates should contain the appropriate antibiotic for the transforming plasmid.

6.2 Store Z-Competent™ cells at -70°C or colder. Thaw a 100- μL tube of Z-Competent™ cells for 5 min on ice. At the same time, cool the tubes containing the ligation mixtures on ice.

6.3 Very gently add 25 – 50 μL of Z-Competent™ cells to each ligation mixture of 10 μL .

6.4 Let the mixtures incubate on ice for 5 min.

6.5 Add SOC media with no antibiotic to a final volume of 60 – 100 μL /tube. For plasmids using the ampicillin resistance marker, the cells will begin repairing their cell walls immediately and are ready to be plated. For plasmids with other antibiotic resistance markers, incubate without shaking for 20 min before plating.

6.6 Spread the cells on culture plates containing the appropriate antibiotic. Let the plates incubate overnight until colonies are visible and large enough to pick individually.

6.7 If transformation with Z-Competent™ cells is unsuccessful, traditional heat-shock transformation or electroporation with a different brand of competent cells may yield higher efficiencies.