Competency test

Introduction

When you make a new batch of competent cells , it is recommended to test before transformation that they are not contaminated with plasmidic DNA and their overall efficiency.To test for contamination, you can perform a negative control where you do not add DNA to your bacteria : if your chemiocompetent bacteria are not contaminated you will get no colony using the Transformation of chemiocompetent cells (https://benchling.com/fxcamval/f/0Td2PzNY-modified-protocols/prt-PxmeWRtv-transformation-of-chemiocompetent-cells/edit)protocol. The overall efficiency of your chemiocompetent cells can be estimated by performing a positive control. In this case you transform 10 ng of a supercoiled plasmid that confer resistance to ampicilin or another relevant antibiotic.

Materials

- Positive control
  - 100 uL competent cells
  - 1 uL of 10 ng/uL plasmidic DNA
  - 1010 ul LB broth
- Negative control
  - 100 uL competent cells
  - 1000 uL LB broth

Procedure

Positive control

1. Add:

   100 ul chemiocompetent cells
   1 uL of 10 ng/uL plasmidic DNA
   1010 uL LB broth

Negative control

2. Add:

   100 uL chemiocompetent cells
   1000 uL LB broth

Transformation of competent cells

3. Transform as indicated in Transformation of chemiocompetent cells (https://benchling.com/fxcamval/f/0Td2PzNY-modified-protocols/prt-PxmeWRtv-transformation-of-chemiocompetent-cells/edit) protocol.

4. Use same antibiotic for both controls and for the actual ligation you want to transform.

5. For the positive control, plate 1/20 of a sample on one plate, 1/10 in another one and the rest of it in a third plate. Incubate overnight at 37°C. For the negative control plate all the sample on a plate with the same antibiotic(s).

Effieciency calculation

6. The next day, count the number of colonies obtained. Good competent cells should yield ≥ $1x10^6$ colony-forming units (CFU)/µg of plasmidic DNA in your positive control. If your chemiocompetent bacteria are not contaminated you will get no colony in the negative control.

   ***Note**: Report the efficiency of your competent cells in CFU/µg of plasmidic DNA. the number will vary depending on the size of your plasmide.*
   Use the following formula to calculate the efficiency:

   Efficiency =(number of colonies x 100) / dilution factor