Subcloning using DH5 Competent Cells

Invitrogen Cat. No. 18265-017

Modified from Invitrogen's DH5 insert:

General Guidelines
Follow these guidelines when using Subcloning Efficiency DH5 competent *E. coli*.

• Handle competent cells gently as they are highly sensitive to changes in temperature or mechanical lysis caused by pipetting.

• Thaw competent cells on ice, and transform cells immediately following thawing. After adding DNA, mix by swirling or tapping the tube gently. **Do not mix cells by pipetting.**

• DH5 cells **do not** require IPTG to induce expression from the *lac* promoter. To select transformants using blue/white screening, make sure that selective plates contain 50 µg/ml X-gal.

Transforming Competent Cells
Use this procedure to transform Subcloning Efficiency DH5 Competent Cells. We recommend verifying the transformation efficiency of the cells using the pUC19 control DNA supplied with the kit. **Do not** use these cells for electroporation.

1. Thaw on ice one tube of DH5 cells. Place 1.5 ml microcentrifuge tubes on wet ice.

2. Gently mix cells with the pipette tip and aliquot 50 µl of cells for each transformation into a 1.5 ml microcentrifuge tube.

3. Refreeze any unused cells in the dry ice/ethanol bath for 5 minutes before returning to the -80°C freezer. **Do not use liquid nitrogen.**

4. Add 1 to 5 µl (1-10 ng) of DNA to the cells and mix gently. **Do not mix by pipetting up and down.** For the pUC19 control, add 2.5 µl (250 pg) of DNA to the cells and mix gently.

5. Incubate tubes on ice for 30 minutes.

6. Heat shock cells for 20 seconds in a 42°C water bath without shaking.

7. Place tubes on ice for 2 minutes.

8. Add 950 µl of pre-warmed medium of choice to each tube.

9. Incubate tubes at 37°C for 1 hour at 225 rpm.

10. Spread 20 µl to 200 µl from each transformation on pre-warmed selective plates. We recommend plating two different volumes to ensure that at least one plate will have well-spaced colonies. For the pUC19 control, plate 100 µl on an LB plate containing 100 µg/ml ampicillin.

11. Store the remaining transformation reaction at +4°C. Additional cells may be plated out the next day, if desired.

12. Incubate plates overnight at 37°C.