Transformation of E. coli cells of strain DH5α

Hanna Eriksson

Abstract

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Protocol

Step 1.
Retrieve competent cells from -80 °C freezer and thaw on ice for roughly 20 minutes.

Step 2.
Add 5 ul ligation mixture and incubate on ice for 30 minutes. Flick the tube gently every other minute to mix. While waiting, prepare 800 ul LB medium per transformation and place on 37 °C for preheating in preparation for step 4.

Step 3.
Heat shock the cells for 45 seconds at 42 °C and place back on ice for another 5 minutes.

Step 4.
Add approximately 800 ul of preheated (37 °C) LB medium without antibiotics and incubate for 1-1.5 hours at 37°C with shaking. During this time, agar plates with appropriate antibiotics can be made in preparation of step 6.

Step 5.
Concentrate by centrifugation at 2000 g for 3 minutes, followed by pouring off the majority of the supernatant (leaving approximately 100-200 ul) and resuspending the cells in the remaining supernatant.

Step 6.
Spread cells on previously prepared agar plates with appropriate antibiotics. Allow to grow over night in 37 °C.

Step 7.
Pick colonies, e.g. with a pipette tip and restreak on a fresh LB agar plate with appropriate antibiotics.