

Competent Cells

10 mL of antibiotic free LB medium was deposited in a 15 mL conical tube, which was then left cultivating for 24 hrs at 37 °C with an agitation of 225 rpm. After that 1.5 mL of culture was diluted in 40 mL of LB medium preheated to 37 °C and incubated for 2-3 hrs with an agitation of 225 rpm or until the OD reaches from 0.4 to 0.6. The cells were left to incubate for 20 min on ice. The suspension was centrifugated for 15 min at 3000 rpm at 4 °C, after which the supernatant was discarded. The cells were resuspended with sterile 20 mL of 50 mM CaCl₂. The suspension was left to cool on ice for 20 min before being centrifugated once more for 15 min at 3000 rpm and 4 °C. The supernatant was discarded and the cells were resuspended with 4ml of sterile 100 mM CaCl₂. The cells were then put on ice and refrigerated until they were needed.