

Cell Transformation

The competent cells were thawed on ice for 15 min from a 260 μ l stock. Then 50 μ l of competent cells were put into 2 ml tube, while being kept on ice during most of the manipulation. Next 1 μ l of resuspended DNA was pipetted into a 2 ml tube. Consequently the tubes were incubated on ice for 30 min. After that the tubes were set in water bath for 1 min and 42°C. Again the tubes were put on ice, but this time for 5 min. 200 μ l of LB media were added to each tube transformation. Subsequently the cells were incubated at 37 °C for 2 hours and 200 RPM. Once the 2 hours passed, the cells were inoculated into two petri plates for a 20 μ l and 200 μ l plating, to ensure single colonies were spread sterilized glass beads were used to spread the culture. Lastly the plates where incubate for 18 hrs.