Exposition assay in bacteria

1. Selected clones that had certain exposition protein coding constructs were grown in 5ml of LB medium with ampicilin (100 mg/L) for 16 hours in a shaking water bath at 37°C temperature. 500 µl of grown cell culture was then mixed with glycerol (1:1) and put in a -70°C for stocking. 50 µl of grown cell culture was mixed with 5 mL of fresh LB medium with ampicilin (100 mg/L) to start to grow a day culture.
2. Day cultures were grown until the OD at the lenght of 600nm reached 0.4.
3. Then the culture was supplied with 0.5mM of IPTG.
4. After induction, the cells were cultured for an additional 4 h at 30°C.
5. After the cultivation cells were harvested by centrifugation at 3500g for 5 min at 4°C.
6. Cells were washed with PBS (7.2 pH) and incubated with monoclonal 6xHis-tag antibody for 4 h at 4°C or with GFP for 24 h at 4°C.
7. Cells that were incubated with monoclonal with 6xHis-tag antibody were washed with PBS (7.2 pH) 3 times and incubated with IgG conjugated with peroxidase for 16 h at 4°C.
8. After the incubation both cells (incubated with either 6xHis-tag antibody or GFP) were washed with PBS 3 times.
9. OD at 600nm was measured in order to load same amount of cells in microplate wells.
10. Cells that were incubated with igG conjugated with peroxidase were harvested by centrifugation at 3500g for 5 min at 4° and mixed with 100 µl of TMB and incubated for 15min then 100 µl of 2M H_2SO_4 was added to stop the reaction.
11. The absorbsance of TMB after the peroxidase reaction was measured at 450nm and the fluorescence of GFP was measured by performing excitation at 480nm and detecting the emission at 530nm.