iGEM Experiment 3 – Construction of CIChE strains

Strategy

Transform plasmids constructed in experiment 2 in the E. coli strain constructed in experiment 1

SOP

Make *E. coli* DendA DE3 Ch1.ccdA-Pmb1GFP-CmFRT electrocompetent and transform with plasmids pSB#X#T7ccdB

1. Incubate *E. coli* MG1655 ΔendA DE3 Ch1.ccdA-Pmb1GFP-CmFRT in 3ml LB+Cm o/n @ 37 °C
2. Add around 500 µl of the overnight culture to 10 ml LB+Cm (in 50ml falcon tube) in order to have an $OD_{660\ nm}$ between 0.05 and 0.1. Also add 500 µl 1mM L-arabinose solution.
3. Incubate at 37 °C till OD 0.6
4. Place falcon tube on ice during 30 min.

   From now on it's **important** to keep everything **cold**! Use chilled tips and recipients!
5. Centrifuge 4 min at 1250rcf (4 ºC)
6. Resuspend the pellet in 45 ml cold MQ-water and centrifuge again during 5 min at 1250rcf (4 ºC)
7. Resuspend the pellet in 1 ml cold MQ-water and transfer into a cold eppendorf
8. Centrifuge at 12000g during 20 - 30 seconds (4 ºC)
9. Resuspend the pellet in 50 µl cold MQ-water by moving the pipet point around (do not pipet up and down!)
10. Transfer 50 µl of the suspension into a new cold eppendorf, what 's left over will be used as the control
11. Add 3 µl of the plasmid DNA (100ng-200ng) and mix gently by turning:
    a. pSB3T5-T7ccdB
    b. pSB4A5-T7ccdB
    c. pSB6A1-T7ccdB
12. Transform the strain by electroporation with the Gene Pulser:
    a. Transfer the sample to the gene Pulser Cuvette using a cold tip
    b. Tap gently to make sure that the sample is evenly distributed between the sides of the Cuvette. Also take care that everything is on the bottom of the cuvette.
    c. Insert the cuvette into the pulse slider and close slide [pay attention to the correct orientation of the cuvette]

d. Pulse once by pressing the two red button until the beep

13. Add immediately 1 ml SOC to the cells when the Gene Pulser beeps. Remove the cuvette from the pulse chamber and invert several times to mix. Transfer into a new culture tube.
14. Also add 1 ml SOC to what is left of the washed cells (= control, see above)
15. Incubate the transformed cells and the control during 2 h at 37 °C on a shaker
16. Transfer 50 µl of the culture + 50 µl SOC on a LB+Tet/Amp (340 µg/ml) plate.
17. Inoculate single colony in 3 ml LB+Tet/Amp and incubate o/n @ 37 °C
18. Archive strains
    a. Create new strain number in sMEMO strain list
    b. Add 0.5 ml o/n culture to 0.5 ml sterile 70% glycerol
    c. Mix thoroughly and store @ -80°C