

Protocol

- Grow 10 mL cultures overnight in LB media with 10 uL of Chloramphenicol
- Transfer an aliquot of the overnight cultures to fresh culture LB media at a ratio of 1:200.
- Grow cultures until log phase (OD₆₀₀ 0.4-0.8).
- Induce the expression of the genes with 1 mM IPTG (final concentration) and let grow for 4 additional hours
- Measure OD again and transfer approximately 10⁸ cells to 1.5 mL microcentrifuge tubes
- Spin the tubes down at 4,000 g for 20 min and remove supernatant.
- Wash cells with water and re-pellet at the same conditions
- Remove water easily with a pipette and dry pellets overnight with a SpeedVac
- Rehydrate dry pellets adding 1 mL of culture media and vortex
- Transfer cells to chloramphenicol plates and grow overnight at 37 °C
- The following day, count the colonies and report the survival as colony forming units/10⁸cells plated.

Additional information

For our assays on stabilization of proteins we were drying overnight in the SpeedVac (Eppendorf Vacufuge Concentrator, at a temperature of 30 °C and the non-spinning mode for aqueous solutions). Therefore, it would be interesting if you could apply the same conditions for the drying.

Succes/Good luck/Suerte !

TU Delft iGEM team 2017

PS: Should you encounter any difficulties in the realisation of the experiments or was anything not clear enough, please feel free to contact me back to:

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