iGEM Pasteur Paris 2016
Protocols

A CUSTOMIZABLE BIODETECTION SYSTEM

PURIFICATION by FAST PROTEIN LIQUID CHROMATOGRAPHY

Aim: To check if the His-tag works and if our protein has really been produced

Materials:

- Microbiology equipment: 37 °C incubator (static, and shaking), Bunsen burner, sterile loop, petri dishes with appropriate antibiotic on agar, timer, Akta prime (GE lifesciences) Antibiotics stock (carbenicillin 50 mg/ml, chloramphenicol 34 mg/ml)
    - Culture (stored at -80°C)
    - Protease inhibition Phenyl methyl sulfoxide 100 mM (PMSF) in ethanol
    - Tris-Cl 1M
    - NaCl 5M
    - Imidazole 1.5 M
    - Buffer A (Tris-Cl 50 mM pH 7.4, NaCl 150 mM)
    - Buffer B (Buffer A + 250 mM Imidazole)
    - Lysis buffer (Buffer A + 15 µM PMSF)

Protocol:

1. Lysis of bacteria : add 10-15 ml of lysis buffer (to the pellet of bacteria)
2. Let pellet thaw under cold running water during at approx. 5 min.
3. Preparation of buffer A: in a 500 ml bottle, put 25 ml of Tris-Cl 1M, 15 ml of NaCl 5M
4. Measure the pH and correct it by adding NaOH or HCl to reach 7.4. In our case, the solution was too acid, so we added droplets of concentrated NaOH 10N.
5. Fill the bottle with reversed-osmosed Milli-Q water (Millipore A10)
   Final concentrations: Tris-Cl 50 mM pH 7.4, 150 mM NaCl
6. Preparation of buffer B (elution): in a 500 ml bottle add 25 ml of Tris, 15 ml of NaCl and 125 ml of imidazole
7. Correct the pH to reach 7.4 and fill with water to 500 ml
   Final concentrations: Tris-Cl 50 mM pH 7.4, NaCl 150 mM, imidazole 250 mM

8. As our samples were too sticky we sonicated them on ice, during 3 cycles of 1 min sonication (Branson 450, medium tip, power set to just before limit of microtip, duty cycle 60%), and 1 min cooling.

9. Centrifuge the samples for 20 min at 16 000 RPM (30966g), in 50 ml polycarbonate tubes, equilibrated on a precision balance, in a Beckman Avanti X26-J centrifuge equipped with JA-25.50 rotor.

10. Filter our buffers to eliminate impurities though a vacuum equipped filter device with 0.45 µm Whatman filters.

11. The supernatant is put in clean 50 ml Falcon

12. Purification of protein with AKTA prime FPLC from GE lifesciences (Pharmacia)
13. The column used is a BioRad Bioscale Nuvia 5 ml IMAC –fitted with M6 connectors to the luer system.
14. Collect 1.0 ml fractions, and follow absorbance at 280 nm

15. All the tubes are stored at 4°C