Sonication

Aim

To lyse cells and extract intracellular components, often with the goal of acquiring recombinant proteins.

Procedure

1. Resuspend the cells in chilled lysis buffer. Whereas the preferred volume differs, 5 ml can be used as a starting point when working with overnight cultures of up to 50 ml.

2. Cool the cell suspension on ice for 10 min.

3. Sonicate the cells using the following set up:
    - PULSE: ON Cycle 3 sec, OFF Cycle 3 sec
    - TIMER: 1 min 30 sec
    - AMPLITUDE: 21 %

CAUTION! Keep the suspension on ice and avoid foaming.

4. Remove cell debris by ultracentrifugation at 4 ◦C for 30 min at 4000 rpm.

5. Collect the supernatant in a new tube. Resuspend the pellet in PBS if insoluble proteins are of interest.

Lysis Buffer:

- 50 mM Tris-HCl pH 7.5
- 50-200 mM NaCl (200 mM if ion exchange chromatography won't be used as first purification step)
- 5 mM DTT

Lab protocol

Updated: October 28th 2017

iGEM Stockholm

Sources

Extraction and Clarification

Preparation and Clarification by sonication by European Molecular Biology Laboratory(EMBL)

https://www.embl.de/pepcore/pepcore_services/protein_purification/extraction_clarification/cell_lysates_ecoli/sonica (received: 20/07/2016)

Modifications were made to the amplitude to suit the sonicator used by iGEM Stockholm.