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Sampling the parameter space for heterologous bacterial production of intracellular domain of TLR1 reveals multiple optima

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Motivation and Aim: The T7 expression system is widely used for the bacterial production of recombinant proteins for structural and functional analysis and therapeutic applications. Despite that many successful approaches are known, high level expression of soluble target proteins in *Eschericia coli* cells often requires a systematic optimization of wide range of cultivation parameters, particularly if heterologous protein expression takes place. Here we investigate the effect of three key cultivation parameters (concentration of the chemical inductor, temperature and time of postinductional culturing) on the expression level of TIR domain of TLR1 (TIR_{TLR1}) in a soluble form. We also study the role of mild detergent Triton X-100 and the ionic strength of the cell lysis solution in the target protein solubility and the final yield.

Methods and Algorithms: Basic protocols of genetic engineering, protein chemistry and *Eschericia coli* cells cultivation using M9 minimal salts and LB reach growth media were implemented. A small-scale expression in BL21(DE3) / BL21(DE3)pLysS bacterial strain cells followed by an SDS-PAGE was chosen as the main strategy to analyze the expression level of the TIR_{TLR1} under various cultivation conditions. The correct folding of the heterologously expressed target protein was biophysically characterized by CD and NMR spectroscopies.

Results: The results of the extensive screening of bacterial cell cultivation and lysis parameters used for the production of TIR_{TLR1} domain are presented. Fine-tuning of all the parameters yielded about 30 mg/l M9 of correctly folded soluble protein. We showed that similar maximal yields may be obtained through several various combinations of the parameters, allowing to select the appropriate time consumption and reproducibility of the protocol.

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