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Abstract

“A high-throughput microtiter plate-based screening method for the detection of full-length recombinant proteins”

Escherichia coli is an important host for the (heterologous) production of recombinant proteins. The development and optimization of a protocol to overproduce a desired protein in *E. coli* is often tedious. A novel high-throughput screening method based on the Luminex® xMAP™ bead technology was developed allowing a rapid evaluation of a certain expression strategy. A variant of green fluorescent protein (GFPuv) from *Aequorea victoria* was used as a reporter to establish the methodology. The N-terminus and the C-terminus of GFPuv were engineered to contain a His₆- and an HA-tag (YPYDVPDYA), respectively. The double-tagged protein was loaded onto Luminex microspheres *via* its His₆-tag, the presence of the HA-tag was verified using an anti-HA antibody. High throughput detection of full-length proteins (containing both tags) on the beads was performed using an automated Luminex 100IS analyzer. The results were compared to results obtained by classical Western-blot analysis. Comparison of the two methods revealed, that the Luminex-based method is faster and more economical in detecting full-length (intact) soluble recombinant protein, allowing one to routinely screen a high number of parameters in gene expression experiments. As proof-of-concept, different protocols to overproduce double-tagged model eucaryotic proteins in *E. coli* were monitored using the new approach. Relevant parameters for optimizing gene expression of the corresponding genes were rapidly identified. The new method was also applied to compare the yeast expression systems *Pichia pastoris* and *Pichia angusta*.

Biography

Matthias Mack studied biology and biochemistry and did his PhD in the field of molecular microbiology/enzyme chemistry. He worked as a scientist (metabolic engineering) for Hoffmann-La Roche in Basel (Switzerland) and BASF-LYNX Bioscience in Heidelberg (Germany). In 2000 he was appointed professor for microbiology at Mannheim University of Applied Sciences.