Antibody Microarray for Recruitment of Metabolic Regulator Proteins in *L*-Threonine Overproducing *Escherichia coli*

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Abstract

We assessed the performance of antibody microarray in profiling of protein expression in *Escherichia coli* using a two-color fluorescence method, and developed the procedure for routine implementation of analyzing data from antibody microarray. Twenty eight *E. coli* proteins involved in L-threonine biosynthetic pathway were over-expressed, purified to homogeneity, and used for production of respective polyclonal antibodies from rabbits. The antibody microarray was constructed with the affinity-purified antibodies, and employed for profiling of relevant protein expression between the prototropic *E. coli* W3110 and the L-threonine-producing *E. coli* TF5015. From the analysis of the factors affecting the performance of antibody microarray, the cross-reactivities of used polyclonal antibodies were found to most critical. To compensate the deviations in the observed ratios from the known ratios in the protein concentration, the correction factors were introduced and determined for each antibody based on the calibration experiments. By employing the correction factors in analyzing data, the antibody microarray identified 11 proteins to be up-regulated, and one protein was down-regulated. The result from the antibody microarray was validated by ana-

lysed using western blotting and two-dimensional gel electrophoresis. Finally for evaluating their influence on improvement the production level of L-threonine, three proteins comprising THR operon and PPC protein, of identified proteins by antibody microarray, were individually amplified in TF5015, resulting in a significant increase of L-threonine production. The approach developed here can enable the antibody microarray to be practically implemented to discovery of biomarkers, diagnosis of disease, and profiling of protein expression.