Monitoring plasmid stability in agar plate cultures of recombinant protein producing Escherchia coli expressing using GFP-based flow cytometry.

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Abstract

Flow cytometry is ideally suited to analysing cultures that display high degrees of heterogeneity, such as cultures with both plasmid-containing and plasmid-free cells. Autofluorescent proteins, being genetically encoded, relatively benign and without the additional costs of fluorescent dyes, are perfect for the analysis of plasmid retention in bacteria. Previous studies have used GFP-FCM to monitor plasmid loss in both E. coli[1] and Saccharomyces cerevisiae[2] during growth in liquid culture. We used GFP-FCM to determine the rate of plasmid loss in E. coli containing an expression vector encoding a recombinant protein-GFP fusion[3] on agar plates under normal laboratory storage conditions at 4∘C. Over time the proportion of cells in lower fluorescence populations increase, correlating with plasmid loss tested by replica plating. Overnight growth in liquid medium containing antibiotic caused a decrease in the proportion of low-fluorescence cells, presumably due to re-selection for the presence of the plasmid. There was a positive relationship between the growth rate of the overnight cultures and the proportion of fluorescent cells on the plate. FCM was also useful for initial screening of transformants, having identified an atypical transformant that displayed markedly reduced culturability. It is suggested that using FCM to screen candidate transformants might play an important role in applications that require high levels of consistency and reliability, such as in bioprocesses or in producing a cell bank. References: 1. Patkar A, Vijayasankaran N, Urry DW & Srienc F (2002) Flow cytometry as a useful tool for process development: rapid evaluation of expression systems. J Biotech 93:217-229 2. Ishii J, Izawa K, Matsumura S, Wakamura K, Tanino T, Tanaka T, Ogino C, Fukuda H & Kondo A (2009) A Simple and Immediate Method for Simultaneously Evaluating Expression Level and Plasmid Maintenance in Yeast. J Biochem 145(6):701-708 3. Sevastyanovich Y, Alfasi S, Overton T, Hall R, Jones J, Hewitt C & Cole J (2009) Exploitation of GFP fusion proteins and stress avoidance as a generic strategy for the production of high-quality recombinant proteins. FEMS Microbiol Lett 299:86-94

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