Isolation of monoclonal microcarriers colonized by fluorescent E. coli.

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Microencapsulation gains increasing importance for processing of bacterial libraries and especially in high-throughput (HT) environments where >10(6) samples per day are studied. As a rule, a one-to-one relationship between an individual cell and analytical results is of key importance. Ideally, each microcarrier would therefore contain exactly one cell or colony. However, synthesis of larger numbers of capsules containing exactly one cell is not feasible as cells are randomly distributed during carrier-production. The dilemma is that high dilution conditions will yield a satisfactory degree of monoclonality, but also a very large fraction of empty compartments, whereas distribution under low dilution generates unacceptable numbers of polyclonal compartments for whose removal no satisfactory technologies exist. Hydrogel carriers with a volume of 35 nL were used as growth compartments for individual microbial colonies. E. coli cells expressing green fluorescent protein (GFP) were encapsulated at low dilution thereby intentionally producing a considerable amount of polyclonal microcarrieres. Empty and polyclonal microcarriers were then removed from the desired monoclonal fraction by a COPAS Plus particle analyzer. The results were compared with model predictions in order to investigate possible limitations in the analysis and sorting of monoclonal microcarriers by COPAS. Fluorescent E. coli cells (GFP) distributed randomly throughout the microcarrier population. Cells were successfully propagated to colonies in the microcarriers and enriched to 95% monoclonality by a COAPS sorter. Enrichment-efficiency was found to mainly depend on the colony diameter. With increasing colony size two contrary effects were observed: First, improved sorting efficiency due to increased fluorescence intensity and therefore higher detection efficiency, and second, deterioration of sorting efficiency due to occlusion occurring in polyclonal carriers. The combination of microencapsulation under low dilution conditions followed by HT sorting procedures is an efficient way for isolating larger amount of monoclonal carriers from bacterial libraries while concomitantly keeping the amounts of empty carriers at a moderate level.