EasyClone: method for iterative chromosomal integration of multiple genes in Saccharomyces cerevisiae

Development of strains for efficient production of chemicals and pharmaceuticals requires multiple rounds of genetic engineering. In this study, we describe construction and characterization of EasyClone vector set for baker's yeast Saccharomyces cerevisiae, which enables simultaneous expression of multiple genes with an option of recycling selection markers. The vectors combine the advantage of efficient uracil excision reaction-based cloning and Cre-LoxP-mediated marker recycling system. The episomal and integrative vector sets were tested by inserting genes encoding cyan, yellow, and red fluorescent proteins into separate vectors and analyzing for co-expression of proteins by flow cytometry. Cells expressing genes encoding for the three fluorescent proteins from three integrations exhibited a much higher level of simultaneous expression than cells producing fluorescent proteins encoded on episomal plasmids, where correspondingly 95% and 6% of the cells were within a fluorescence interval of \( \text{Log}_{10} \) mean ± 15% for all three colors. We demonstrate that selective markers can be simultaneously removed using Cre-mediated recombination and all the integrated heterologous genes remain in the chromosome and show unchanged expression levels. Hence, this system is suitable for metabolic engineering in yeast where multiple rounds of gene introduction and marker recycling can be carried out.

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