Standardized Cloning and Curing of Plasmids

Plasmids are highly useful tools for studying living cells and for heterologous expression of genes and pathways in cell factories. Standardized tools and operating procedures for handling such DNA vectors are core principles in synthetic biology. Here, we describe protocols for molecular cloning and exchange of genetic parts in the Standard European Vectors Architecture (SEVA) vector system. Additionally, to facilitate rapid testing and iterative bioengineering using different vector designs, we provide a one-step protocol for a universal CRISPR-Cas9-based plasmid curing system (pFREE) and demonstrate the application of this system to cure SEVA constructs (all vectors are available at SEVA/Addgene).

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