Accurate DNA assembly and genome engineering with optimized uracil excision cloning - DTU Orbit (09/11/2017)

Accurate DNA assembly and genome engineering with optimized uracil excision cloning

Simple and reliable DNA editing by uracil excision (a.k.a. USER cloning) has been described by several research groups, but the optimal design of cohesive DNA ends for multigene assembly remains elusive. Here, we use two model constructs based on expression of gfp and a four-gene pathway that produces β-carotene to optimize assembly junctions and the uracil excision protocol. By combining uracil excision cloning with a genomic integration technology, we demonstrate that up to six DNA fragments can be assembled in a one-tube reaction for direct genome integration with high accuracy, greatly facilitating the advanced engineering of robust cell factories.

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