USER cloning

Introduction

Assembly of Bio-Bricks or PCR products and linear plasmid with USER overhangs.

Materials

- 1 µL Linearized vector (concentration between 50 and 100 ng/µL)
- 2 µL each biobrick (with USER overhangs)
- 0.5 µL USER enzyme mix
- 1 uL CutSmart buffer for every 10µL volume
- MQ water until 10µL

Procedure

1. In a PCR tube combine all reagents. Water should be added up to a total volume of 10 µL. If the plasmid has a low concentration, more than 1 µL can be added and water is adjusted thereafter (keeping the total volume of 10 µL).
2. Incubate reaction mix in a thermocycler for 30 minutes at 37 ºC followed by 30 minutes at room temperature (25 ºC in a thermocycler).
3. Transform the whole reaction in *E. coli* immediately.