USER cloning

Introduction

This is a protocol to do USER cloning based on the standard PacI/Nt.BBvCI casette plasmid
It's adapted from protocols by Rasmus Frandsen and Kresten J. Olsen (DTU Bioengineering) respectively

Materials

Reagents

- USER-linearized insert(s)
- Linearised plasmid backbone (Plasmid digested with PacI/Nt.BBvCI)
- USER enzyme
- Cutsmart buffer
- Milli-Q water

Consumables

- PCR strips

Procedure

Preparing USER-reaction

1. In a PCR-tube, combine;

   200 ng USER-linearized insert(s),
   100 ng USER-linearized vector backbone,
   1 uL 10X CutSmart buffer,
   1 uL USER enzyme,
   H2O to 10 uL

2. Incubate reaction mix as described below

   37C for 25 min
   25C for 10 min
   20C for 10 min,
   15C for 10 min
   10C until further use

3. Transform reaction mixture in E. coli as described in the Hifi assembly protocol