Type IIS cloning (MoClo)

Introduction

Protocol adapted from "A Modular Cloning System for Standardized Assembly of Multigene Constructs" by Weber *et al.* by Dorottya Marko. Contributions from Björn Ancker Persson and Núria Garriga Alonso.

Materials

- 2uL Reaction mixture
  - T4 ligase buffer 10x 1 uL
  - BSA (1mg/mL) 0.5 uL
  - BsaI or SapI (5U/uL) 0.25 uL
  - T4 ligase (5U/uL) 0.25 uL
- DNA mixture
  - 15 fmol/uL insert (each insert) -> Length(bp)/100 = total ng needed for 10uL reaction
  - 7.5 fmol/uL -> Length(bp)/200 = total ng needed for 10uL reaction
- H_20 until 10uL

Procedure

Temperature Cycle

1. 37ºC 3min Restriction
2. 16ºC 4min Ligation
3. Go to step 1 x26
4. 50ºC 5min
5. 80ºC 10min Enzyme deactivation
6. 4ºC Forever