

PacI+Nt.BbvCI Digestion for USER linearization

Introduction

This protocol is for linearizing plasmids with the PacI+Nt.BbvCI USER cassette. This will allow assembly of PCR products with USER tails into this linearized plasmid.

Materials

- For digest I
 - Plasmid: 40 μ L
 - PacI: 2 μ L
 - Cutsmart buffer (NEB): 10 μ L
 - MQ water: 48 μ L
- For digest II
 - Finished digest I
 - Nt.BbvCI: 2 μ L

Procedure

Digest I

1. Mix all the reagents mentioned above and incubate at 37 °C for overnight.

Digest II

2. Take the digest I reaction from the 37 °C incubator, add in the 2 μ L Nt.BbvCI to the reaction mixture, and incubate for 2 hours at 37 °C.
3. Heat inactivate the samples at 80 °C for 30 minutes.