

# PacI+Nt.BbvCI digest for USER linearisation

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## Introduction

This protocol is for linearising backbones with the PacI+Nt.BbvCI USER cassette. This will allow assembly of PCR product with USER tails to be assembled into this linearised plasmid

## Materials

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

## Procedure

### Digest I

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

### Digest II

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