

# USER reaction v1.0

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## Introduction

When USER fragments has been constructed, this protocol can be used to create the USER overhangs, anneal the fragments and transform the construct into chemically competent *E. coli*.

## Materials

- › Digested USER backbone or backbone with USER tails
- › USER fragments
- › 1µL USER enzyme
- › 1µL T4 buffer

## Procedure

### Mix

1. The fragments and backbone are mixed in a PCR tube to a total volume of 10µL with a molar majority of inserts to avoid self-ligation of the backbone.

Use <http://nebiocalculator.neb.com/#!/ligation> to calculate the desired amounts

2. CRITICAL      **REMEMBER EMPTY VECTOR CONTROL**

Substitute inserts with MilliQ

3. Add USER enzyme and T4 buffer

### Reaction

4. Incubate in a thermocycler @ 37°C for 25 min
5. Incubate in a thermocycler @ 25°C for 25 min
6. Add 8µL MilliQ water to the reaction to a final volume of 20µL
7. Use the reaction mix directly to transform chemically competent *E. coli* cells