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Golden Gate Cloning LVL 2

DOI

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Protocol status: Working

We use this protocol and it's working

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Abstract

This cloning protocol refers to the Marburg Collection

Guidelines

This cloning protocol refers to the Marburg Collection

Materials

MATERIALS

✕ T7 DNA Ligase - 100,000 units **New England Biolabs Catalog #M0318S**

✕ nuclease free water

✕ Esp3I **New England Biolabs Catalog # R0734S**

✕ 10X NEB T4 DNA ligase buffer **New England Biolabs**

Before start

Before start, the resistance and ori parts have to be digested with BsaI and purified!



Predigestion

- 1 Before start, digest the Resistance and Ori plasmide with Bsal.
- 2 Purify the Fragments with PCR Cleanup.

Reaction Setup on ice:

- 3 1. Add 20 fmol of TU's.
- 4 2. Add 0.5 μ L BsmBI.
- 5 3. Add 0.5 μ L T7-Ligase.
- 6 4. Add 1 μ L T4-Ligase Buffer.
- 7 5. Fill with Nuclease-free water to 10 μ L.

Thermocycling conditions

- 8 30 Cycles of 5min 37°C / 10min 16°C
- 9 30 min. 37°C.
- 10 10 min. 80°C.



11 Hold 20°C.