OCT 21, 2018

🌐 Golden Gate Cloning LVL 2

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ABSTRACT

This cloning protocol refers to the Marburg Collection

GUIDELINES

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MATERIALS

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

✂ nuclease free water **Contributed by users**

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

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

BEFORE START INSTRUCTIONS

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

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.uview4e

Protocol Citation: Daniel Marchal 2018. Golden Gate Cloning LVL 2. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.uview4e>

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Protocol status: Working
 We use this protocol and it's working

Created: Oct 21, 2018

Last Modified: Oct 21, 2018

PROTOCOL integer ID:
 17034

Predigestion

- 1 Before start, digest the Resistance and Ori plasmide with BsaI.

- 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.