VERSION 2

OCT 21, 2018

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.uvhew36

Protocol Citation: Daniel Marchal 2018. Golden Gate Cloning LVL 0. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.uvhew36>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

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

Created: Oct 21, 2018

Last Modified: Oct 21, 2018

PROTOCOL integer ID:
 17033

🌐 Golden Gate Cloning LVL 0 V.2

Daniel Marchal¹

¹iGEM Team Marburg 2018

Daniel Marchal

MATERIALS

MATERIALS

⌘ 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

Reaction Setup on ice

- 1 1. Add 1 μ L of 70 ng Template DNA. 0 °C
- 2 2. Add three fold excess of PCR-Fragment. 0 °C
- 3 3. Add 0.5 μ L Esp3I. 0 °C
- 4 4. Add 0.5 μ L T7-Ligase. 0 °C
- 5 5. Add 1 μ L T4-Ligase Buffer. 0 °C
- 6 6. Fill with Nuclease-free water to 10 μ L. 0 °C
- 7 Thermocycling conditions:

Thermocycling conditions

- 8 30 Cycles of 2min 98°C / 5min 16°C

9 30 min. 37°C. 37 °C

10 10 min. 80°C. 80 °C

11 Hold 20°C. 20 °C