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Version 2

Golden Gate Cloning LVL 0 V.2

DOI

<https://dx.doi.org/10.17504/protocols.io.uvhew36>

Daniel Marchal¹

¹iGEM Team Marburg 2018



Daniel Marchal

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Protocol status: Working

We use this protocol and it's working

Created: October 21, 2018

Last Modified: October 21, 2018

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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**



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