Oct 21, 2018 Version 2

Golden Gate Cloning LVL 0 V.2

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

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

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

Created: October 21, 2018

Last Modified: October 21, 2018

Protocol Integer ID: 17033

Keywords: golden gate cloning lvl, cloning, lvl

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 µL of 70 ng Template DNA. 0 °C

2 2. Add three fold excess of PCR-Fragment. 0 °C

3 3. Add 0.5 µL Esp3I. 0 °C

4 4. Add 0.5 µL T7-Ligase. 0 °C

5 5. Add 1 µL T4-Ligase Buffer. 0 °C

6 6. Fill with Nuclease-free water to 10 µL. 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