OCT 21, 2018

🌐 Golden Gate Cloning LVL 1

Daniel Marchal¹

¹iGEM Team Marburg 2018

Daniel Marchal

ABSTRACT

This protocol refers to LVL 1 cloning with the Marburg Collection

GUIDELINES

This protocol refers to LVL 1 cloning with the Marburg Collection

MATERIALS

MATERIALS

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

✂ nuclease free water **Contributed by users**

✂ BsaI-HFv2 **New England Biolabs Catalog # R3733L**

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

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.uvgew3w

Protocol Citation: Daniel Marchal 2018. Golden Gate Cloning LVL 1. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.uvgew3w>

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:
 17032

Reaction Setup on ice

1 Add 1 µL, 37.5 ng of 5'Connector plasmide.

- 2 Add 1 μ L, 37.5 ng of Promoter plasmide.
- 3 Add 1 μ L, 37.5 ng of RBS plasmide.
- 4 Add 1 μ L, 37.5 ng of CDS plasmide.
- 5 Add 1 μ L, 37.5 ng of Terminator plasmide.
- 6 Add 1 μ L, 37.5 ng of 3'Connector plasmide.
- 7 Add 1 μ L, 37.5 ng of ORI plasmide.
- 8 Add 1 μ L, 37.5 ng of Antibiotic Resistance plasmide.
- 9 Add 0.5 μ L Bsal.

10 Add 0.5 µL T4-Ligase.

11 Add 1 µL T4-Ligase Buffer

Thermocycling conditions

12 60 Cycles of 2min 37°C / 5min 16°C

13 60 min. 60°C.

14 10min. 80°C.

15 Hold 20°C.

Transformation

16 Transform 5µl of Golden Gate Mix into *V. natriegens*