Oct 21, 2018

Golden Gate Cloning LVL 2

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

Daniel Marchal[1]

[1]iGEM Team Marburg 2018

Daniel Marchal

OPEN ACCESS

DOI: https://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

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

Created: October 21, 2018

Last Modified: October 21, 2018

Protocol Integer ID: 17034

Keywords: golden gate cloning lvl, cloning protocol, cloning, marburg collection, lvl

Abstract

This cloning protocol refers to the Marburg Collection

Guidelines

This cloning protocol refers to the Marburg Collection

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**

Before start

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

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 µL BsmBI.

5 3. Add 0.5 µL T7-Ligase.

6 4. Add 1 µL T4-Ligase Buffer.

7 5. Fill with Nuclease-free water to 10 µL.

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.