Golden Gate Cloning LVL 2

Daniel Marchal[1]

[1]iGEM Team Marburg 2018

dx.doi.org/10.17504/protocols.io.uview4e

Oct 21, 2018

Working

iGEM Marburg 2018

Daniel Marchal

ABSTRACT

This cloning protocol refers to the Marburg Collection

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

GUIDELINES

This cloning protocol refers to the Marburg Collection

MATERIALS

NAME	CATALOG #	VENDOR
T7 DNA Ligase - 100,000 units	M0318S	New England Biolabs
nuclease free water		Contributed by users
Esp3I	R0734S	New England Biolabs
10X NEB T4 DNA ligase buffer		New England Biolabs

BEFORE STARTING

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.