Golden Gate Cloning LVL 1

Daniel Marchal[1]

[1]iGEM Team Marburg 2018

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

Oct 21, 2018

Working

iGEM Marburg 2018

Daniel Marchal

ABSTRACT

This protocol refers to LVL 1 cloning with the Marburg Collection

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

GUIDELINES

This protocol refers to LVL 1 cloning with the Marburg Collection

MATERIALS

NAME	CATALOG #	VENDOR
T7 DNA Ligase - 100,000 units	M0318S	New England Biolabs
nuclease free water		Contributed by users
BsaI-HFv2	R3733L	New England Biolabs
10X NEB T4 DNA ligase buffer		New England Biolabs

Reaction Setup on ice

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

2 Add 1 µL, 37.5 ng of Promoter plasmide.

3 Add 1 µL, 37.5 ng of RBS plasmide.

4 Add 1 µL, 37.5 ng of CDS plasmide.

5 Add 1 µL, 37.5 ng of Terminator plasmide.

6 Add 1 µL, 37.5 ng of 3'Connector plasmide.

7 Add 1 µL, 37.5 ng of ORI plasmide.

8 Add 1 µL, 37.5 ng of Antibiotic Resistance plasmide.

9 Add 0.5 µL BsaI.

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