Oct 18, 2019

Golden Gate lvl 0 V.2

Vinca Seiler[1], René Inckemann[1]

[1]iGEM Team Marburg 2019

1 Works for me dx.doi.org/10.17504/protocols.io.8edhta6

iGEM Marburg
iGEM Marburg 2019

ABSTRACT

Golden Gate reaction protocol for lvl 0

MATERIALS

NAME	CATALOG #	VENDOR
BsmBI - 1,000 units	R0580L	New England Biolabs
T4 DNA Ligase	M0202	New England Biolabs
Esp3I	R0734L	New England Biolabs
10X NEB T4 DNA ligase buffer		New England Biolabs

Pipetting scheme for assembly reaction

1 **0.5 µl** of DNA insert ([M] **60 ng/µl**)

2 **0.5 µl** of entry Vector (15 ng/ µL)

3 **1 µl** T4 DNA Ligase buffer (NEB)

4 **0.5 µl** T4 DNA Ligase (NEB)

5 **0.5 µl** Esp3I (NEB)

6 Water to **10 µl**

Thermocycler Rapid Protocol

7

37 °C 00:20:00

8 37 °C 00:01:30

9 16 °C 00:03:00

10 Cycle step 8 and 9 5-10x

11 50 °C 00:05:00

12 80 °C 00:10:00

Alternative Thermocycler Improved Protocol

13 37 °C 00:01:30

14 16 °C 00:03:00

15 Cycle step 13 and 14 15x

16 50 °C 00:05:00

17 80 °C 00:10:00

Transformation

18 Add 2 µl - 5 µl of each assembly reaction to 50 µl competent cells.

19 Cells should be recovered for 01:00:00 (Amp) to 02:00:00 (Kan, Chloramphenicol).