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Golden Gate lvl 0 V.1

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Vinca Seiler[1], René nckemann[1]

[1]iGEM Team Marburg 2019

Igem Marburg
iGEM Marburg 2019

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

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Abstract

Golden Gate reaction protocol for lvl 0

Materials

MATERIALS

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

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 EspI3 (NEB)

6 Water to 10 µL

Thermocycler conditions

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

Transformation

13 Add 2 µL - 5 µL of each assembly reaction to 50 µL competent cells.

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