Golden Gate lvl 0 V.1

Vinca Seiler, René Inckemann

iGEM Team Marburg 2019

ABSTRACT

Golden Gate reaction protocol for lvl 0

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 |

DOI: dx.doi.org/10.17504/protocols.io.8d3hs8n

Protocol Citation: Vinca Seiler, René Inckemann 2019. Golden Gate lvl 0. protocols.io

https://dx.doi.org/10.17504/protocols.io.8d3hs8n

License: This is an open access protocol distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working

Created: Oct 17, 2019
PROTOCOL integer ID: 28827

1. 0.5 µL of DNA insert (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

7. Thermocycler conditions:
   - 37 °C
   - 00:20:00

protocols.io | https://dx.doi.org/10.17504/protocols.io.8d3hs8n
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).