



Oct 17, 2019

Golden Gate lvl 0 V.1

Vinca Seiler¹, René Inckemann¹

¹iGEM Team Marburg 2019

1 Works for me dx.doi.org/10.17504/protocols.io.8d3hs8n



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

- 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

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).



This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited