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Golden Gate lvl 0 V.1

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

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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 ([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).