Golden Gate lvl 1/2

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1 Works for me dx.doi.org/10.17504/protocols.io.8d4hs8w

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ABSTRACT

Golden Gate reaction protocol for lvl 1/2

MATERIALS

NAME	CATALOG #	VENDOR
T4 DNA Ligase	M0202	New England Biolabs
Esp3I	R0734L	New England Biolabs
BsaI-HFv2	R3733S	New England Biolabs
10X NEB T4 DNA ligase buffer		New England Biolabs

Pipetting scheme for assembly reaction

1 **0.5 µl** of each DNA insert. For an improved assembly efficiency, the amount of DNA inserts can optionally be normalized to equimolar concentrations (~ **20 fmol** each) or use **75 ng** of each insert (antibiotic resistance part should be diluted 1:10, **7.5 ng** - **10 ng**).

2 **1 µl** T4 DNA Ligase buffer (NEB)

3 **0.5 µl** T4 DNA Ligase (NEB)

4 **0.5 µl** BsaI-HF®v2(NEB) for **lvl 1** / EspI3 (NEB) for **lvl 2**

5 Water to **10 µl** . (**20 µl** also possible) **0.5 µl** of each DNA insert

Thermocycler Improved Protocol

6 **37 °C** **00:01:30**

7 **16 °C** **00:03:00**

8 Cycle step 6 and 7 15x

9 50 °C 00:05:00

10 80 °C 00:10:00

Alternative Thermocycler Troubleshoot/Overnight Protocol

11 37 °C 00:02:00

12 16 °C 00:05:00

13 Cycle steps 11 & 12 x 50

14 50 °C 00:10:00

15 80 °C 00:10:00

Transformation

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

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