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🌐 Golden Gate lvl 1/2

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ABSTRACT

Golden Gate reaction protocol for lvl 1/2

MATERIALS

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✂ T4 DNA Ligase **New England Biolabs Catalog #M0202**

✂ Esp3I **New England Biolabs Catalog # R0734L**

✂ BsaI-HFv2 **New England Biolabs Catalog # R3733S**

✂ 10X NEB T4 DNA ligase buffer **New England Biolabs**

OPEN ACCESS

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protocols.io

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

Created: Oct 17, 2019

Last Modified: Oct 20, 2019

PROTOCOL integer ID:
28828

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).