VERSION 2

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

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Golden Gate lvl 0 V.2

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ABSTRACT

Golden Gate reaction protocol for lvl 0

MATERIALS

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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

Pipetting scheme for assembly reaction

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 Esp3I (NEB)

6 Water to 10 µL

Thermocycler Rapid Protocol

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

Alternative Thermocycler Improved Protocol

13 37 °C 00:01:30

14 16 °C 00:03:00

15 Cycle step 13 and 14 15x

16 50 °C 00:05:00

17

80 °C

00:10:00

Transformation

18

Add 2 μ L - 5 μ L of each assembly reaction to 50 μ L competent cells.

19

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