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Golden Gate lvl 0 V.2

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Works for me

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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

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 µl - 5 µl of each assembly reaction to 50 µl competent cells.

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



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