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Golden Gate lvl 0 V.2

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Protocol status: Working

We use this protocol and it's working

Created: October 18, 2019

Last Modified: October 18, 2019

Protocol Integer ID: 28837

Abstract

Golden Gate reaction protocol for lvl 0




Materials

MATERIALS

 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 ( 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).