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Version 1

Golden Gate lvl 0 V.1

DOI

<https://dx.doi.org/10.17504/protocols.io.8d3hs8n>

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Protocol status: Working

We use this protocol and it's working

Created: October 17, 2019



Last Modified: October 17, 2019

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Keywords: golden gate reaction protocol for lvi, golden gate reaction protocol, golden gate lvi, lvi, protocol

Abstract

Golden Gate reaction protocol for lvi 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**



1  0.5 μL of DNA insert ($[1\text{M}]$ 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 EspI3 (NEB)

6 Water to  10 μL

Thermocycler conditions

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

Transformation

13

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

14

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