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Golden Gate lvi 1/2

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Protocol status: Working

We use this protocol and it's working

Created: October 17, 2019

Last Modified: October 20, 2019

Protocol Integer ID: 28828

Abstract

Golden Gate reaction protocol for lvi 1/2



Materials

MATERIALS












⊗ 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**

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 (\sim  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 Bsal-HF[®]v2(NEB) for **lvi 1** / EspI3 (NEB) for **lvi 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 × 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).