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Golden Gate Cloning LVL 1

DOI

<https://dx.doi.org/10.17504/protocols.io.uvgeu3w>

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Protocol status: Working

We use this protocol and it's working

Created: October 21, 2018



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Keywords: golden gate cloning lvi, cloning, lvi, marburg collection

Abstract

This protocol refers to LVL 1 cloning with the Marburg Collection

Guidelines

This protocol refers to LVL 1 cloning with the Marburg Collection

Materials

MATERIALS

⊗ T7 DNA Ligase - 100,000 units **New England Biolabs Catalog #M0318S**

⊗ nuclease free water

⊗ BsaI-HFv2 **New England Biolabs Catalog # R3733L**

⊗ 10X NEB T4 DNA ligase buffer **New England Biolabs**



Reaction Setup on ice

- 1 Add 1 μ L, 37.5 ng of 5'Connector plasmide.
- 2 Add 1 μ L, 37.5 ng of Promoter plasmide.
- 3 Add 1 μ L, 37.5 ng of RBS plasmide.
- 4 Add 1 μ L, 37.5 ng of CDS plasmide.
- 5 Add 1 μ L, 37.5 ng of Terminator plasmide.
- 6 Add 1 μ L, 37.5 ng of 3'Connector plasmide.
- 7 Add 1 μ L, 37.5 ng of ORI plasmide.
- 8 Add 1 μ L, 37.5 ng of Antibiotic Resistance plasmide.
- 9 Add 0.5 μ L Bsal.
- 10 Add 0.5 μ L T4-Ligase.
- 11 Add 1 μ L T4-Ligase Buffer

Thermocycling conditions

- 12 60 Cycles of 2min 37°C / 5min 16°C



13 60 min. 60°C.

14 10min. 80°C.

15 Hold 20°C.

Transformation

16 Transform 5µl of Golden Gate Mix into *V. natriegens*