



Oct 21, 2018

Working

Golden Gate Cloning LVL 1

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ABSTRACT

This protocol refers to LVL 1 cloning with the Marburg Collection

PROTOCOL STATUS





Working

We use this protocol in our group and it is working

GUIDELINES

This protocol refers to LVL 1 cloning with the Marburg Collection

MATERIALS

NAME	CATALOG #	VENDOR
 T7 DNA Ligase - 100,000 units	M0318S	New England Biolabs
 nuclease free water		Contributed by users
 BsaI-HFv2	R3733L	New England Biolabs
 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 5 μ l of Golden Gate Mix into *V. natriegens*



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