



Oct 21, 2018

Working

## Golden Gate Cloning LVL 2

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### ABSTRACT

This cloning protocol refers to the Marburg Collection

### PROTOCOL STATUS

#### Working

We use this protocol in our group and it is working

### GUIDELINES

This cloning protocol refers to the Marburg Collection

### MATERIALS

NAME	CATALOG #	VENDOR
T7 DNA Ligase - 100,000 units	M0318S	New England Biolabs
nuclease free water		Contributed by users
Esp3I	R0734S	New England Biolabs
10X NEB T4 DNA ligase buffer		New England Biolabs

### BEFORE STARTING

Before start, the resistance and ori parts have to be digested with BsaI and purified!

#### Predigestion

- 1 Before start, digest the Resistance and Ori plasmide with BsaI.
- 2 Purify the Fragments with PCR Cleanup.

#### Reaction Setup on ice:

- 3 1. Add 20 fmol of TU's.
- 4 2. Add 0.5 µL BsmBI.

- 5 3. Add 0.5  $\mu$ L T7-Ligase.
- 6 4. Add 1  $\mu$ L T4-Ligase Buffer.
- 7 5. Fill with Nuclease-free water to 10  $\mu$ L.

#### Thermocycling conditions

- 8 30 Cycles of 5min 37°C / 10min 16°C
- 9 30 min. 37°C.
- 10 10 min. 80°C.
- 11 Hold 20°C.



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