# Golden Gate Cloning LVL 0 Version 1

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Working

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PROTOCOL STATUS

**Working**
We use this protocol in our group and it is working

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 1. Add 1 µL of 70 ng Template DNA. 0 °C

2 2. Add three fold excess of PCR-Fragment. 0 °C

3 3. Add 0.5 µL BsmBI. 0 °C

4 4. Add 0.5 µL T7-Ligase. 0 °C

5 5. Add 1 µL T4-Ligase Buffer. 0 °C

6 6. Fill with Nuclease-free water to 10 µL. 0 °C

7 Thermocycling conditions:

Thermocycling conditions

8 30 Cycles of 2min 98°C / 5min 16°C

9 30 min. 37°C. 37 °C

10 10 min. 80°C. 80 °C

11 Hold 20°C. 20 °C