

Golden Gate Cloning LVL 0

1. Reaction Setup on ice:

- 1.1. Add 1 μL of 70 ng Template DNA.
- 1.2. Add three fold excess of PCR-Fragment.
- 1.3. Add 0.5 μL BsmBI.
- 1.4. Add 0.5 μL T7-Ligase.
- 1.5. Add 1 μL T4-Ligase Buffer.
- 1.6. Fill with Nuclease-free water to 10 μL .

2. Thermocycling conditions:

3. 30 Cycles of step 3.1 and 3.2:
 - 3.1. 2 min. 98°C.
 - 3.2. 5 min. 16°C.
4. 30 min. 37°C.
5. 10 min. 80°C.
6. Hold 20°C.