Golden Gate Cloning LVL 0 Version 2

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Version 2
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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
Esp3I	R0734S	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 Esp3I. 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