VERSION 1

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Protocol status: Working
 We use this protocol and it's working

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PROTOCOL integer ID:
 17031

🌐 Golden Gate Cloning LVL 0 V.1

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MATERIALS

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⌘ T7 DNA Ligase - 100,000 units New England Biolabs Catalog #M0318S

⌘ nuclease free water Contributed by users

⌘ Bsal-HFv2 New England Biolabs Catalog # R3733L

⌘ 10X NEB T4 DNA ligase buffer New England Biolabs

Reaction Setup on ice

- 1 1. Add 1 μ L of 70 ng Template DNA. 0 °C
- 2 2. Add three fold excess of PCR-Fragment. 0 °C
- 3 3. Add 0.5 μ L BsmBI. 0 °C
- 4 4. Add 0.5 μ L T7-Ligase. 0 °C
- 5 5. Add 1 μ L T4-Ligase Buffer. 0 °C
- 6 6. Fill with Nuclease-free water to 10 μ L. 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