

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. Add 1 μ L of 70 ng Template DNA. 0 °C
2. Add three fold excess of PCR-Fragment. 0 °C
3. Add 0.5 μ L BsmBI. 0 °C
4. Add 0.5 μ L T7-Ligase. 0 °C
5. Add 1 μ L T4-Ligase Buffer. 0 °C
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



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