VERSION 2

FEB 02, 2021

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.br2nm8de

External link:
https://openwetware.org/wiki/Gibson_Assembly

Protocol Citation: Anna Behle, Tatsuya Sakaguchi 2021. Homemade Gibson Mastermix. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.br2nm8de> Version created by Tatsuya Sakaguchi

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

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

Created: Feb 02, 2021

Last Modified: Feb 02, 2021

PROTOCOL integer ID:
 46894

Homemade Gibson Mastermix V.2

Version 1 is forked from [Homemade Gibson Mastermix](#)

Anna Behle¹, Tatsuya Sakaguchi²

¹Institute for Synthetic Microbiology; ²Kurume University

Tatsuya Sakaguchi
 Kurume University

ABSTRACT

Recipe for homemade 2 x Gibson Mastermix.

MATERIALS

MATERIALS

- ⊗ beta-Nicotinamide adenine dinucleotide (NAD⁺) - 0.2 ml **New England Biolabs Catalog #B9007S**
- ⊗ Taq DNA Ligase - 2,000 units **New England Biolabs Catalog #M0208S**
- ⊗ T5 Exonuclease - 5,000 units **New England Biolabs Catalog #M0363L**
- ⊗ PEG-8000 **Contributed by users**
- ⊗ Phusion high-fidelity PCR kit **Thermo Scientific Catalog #F553S**
- ⊗ DTT (Dithiothreitol) (> 99% pure) Protease free **Gold Biotechnology Catalog #DTT**
- ⊗ Deoxynucleotide Solution Set - 25 umol of each **New England Biolabs Catalog #N0446S**

Preparation of 5x isothermal reaction buffer

1 Recipe for 4 mL:

Component	Molarity / Concentration	Amount	Final concentration
Tris-HCl, pH 7.5	1 M	2 mL	500 mM
MgCl ₂	1 M	200 µL	50 mM
dATP	100 mM	40 µL	1 mM
dCTP	100 mM	40 µL	1 mM
dGTP	100 mM	40 µL	1 mM
dTTP	100 mM	40 µL	1 mM
DTT	1 M	200 µL	50 mM
PEG-8000	-	1 g	25 %
NAD ⁺	100 mM	200 µL	5 mM
H ₂ O	-	to final volume of 4 mL	

2 Mix dNTPs, NAD⁺, Tris-HCl, MgCl₂ and DTT.

3 Slowly add PEG-8000 to mixture and mix well, until completely dissolved. Add H₂O to a final volume of 4 mL.

4 Prepare aliquots of the 5x isothermal buffer as required, e.g. 100 µL. Store at -20 °C.

Preparation of 1,33x Assembly Mastermix

5 Recipe for 25 x 15 µL aliquots:

Component	Concentration	Amount	Final concentration (after adding DNA)
5x isothermal rxn buffer	5x	80 µL	1x
Taq DNA Ligase	40 U/µL	40 µL	4 U/µL
T5 Exonuclease	1 U/µL	1.6 µL	4 U/mL
Phusion High-Fidelity DNA Polymerase	2 U/µL	5 µL	25 U/mL
H ₂ O		173.4 µL (to 300 µL)	

A	B	C	D
Total volume		300 µL	

Preparation of 1.33x Assembly Mastermix

- Work on ice. Mix H₂O and 5x buffer, then add enzymes.
- Prepare 20 x 15 µL aliquots in PCR tubes. Store at -20 °C.
These aliquots are concentrated 1.33 x - add your DNA in a volume of 5 µL to a final volume/concentration of 20 µL / 1x.

Gibson assembly

- After addition of DNA, incubate Gibson assembly mix at 50 °C for 2 hours.

00:00:00 Gibson assembly

Transformation

- Transform chemically competent cells with an aliquot of your assembly mix.