Version 2

Feb 02, 2021

Homemade Gibson Mastermix V.2

Version 1 is forked from Homemade Gibson Mastermix

Anna Behle[1], Tatsuya Sakaguchi[2]

[1]Institute for Synthetic Microbiology; [2]Kurume University

1 Works for me dx.doi.org/10.17504/protocols.io.br2nm8de

Tatsuya Sakaguchi
Kurume University

ABSTRACT

Recipe for homemade 2 x Gibson Mastermix.

EXTERNAL LINK

https://openwetware.org/wiki/Gibson_Assembly

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

FORK NOTE

FORK FROM

Forked from Homemade Gibson Mastermix, Anna Behle

CREATED

Feb 02, 2021

LAST MODIFIED

Feb 02, 2021

PROTOCOL INTEGER ID

46894

Citation: Anna Behle, Tatsuya Sakaguchi (02/02/2021). Homemade Gibson Mastermix. https://dx.doi.org/10.17504/protocols.io.br2nm8de

MATERIALS TEXT

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_2O	-	to final volume of 4 mL	

2 Mix dNTPs, NAD^+, Tris-HCl, $MgCl_2$ and DTT.

3 Slowly add PEG-8000 to mixture and mix well, until completely dissolved. Add H_2O 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:

Citation: Anna Behle, Tatsuya Sakaguchi (02/02/2021). Homemade Gibson Mastermix. https://dx.doi.org/10.17504/protocols.io.br2nm8de

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_2O		173.4 µL (to 300 µL)	

A	B	C	D
Total volume		**300 µL**	

Preparation of 1.33x Assembly Mastermix

6 Work on ice. Mix H_2O and 5x buffer, then add enzymes.

7 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

8 After addition of DNA, incubate Gibson assembly mix at 50 ˚C for 2 hours.
00:00:00 Gibson assembly

Transformation

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

Citation: Anna Behle, Tatsuya Sakaguchi (02/02/2021). Homemade Gibson Mastermix. https://dx.doi.org/10.17504/protocols.io.br2nm8de