Jan 06, 2020 Version 1

Homemade Gibson Mastermix V.1

Forked from Homemade Gibson Mastermix

DOI
dx.doi.org/10.17504/protocols.io.ba2yigfw

Anna Behle[1], Tatsuya Sakaguchi[2]

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

Tatsuya Sakaguchi
Kurume University

DOI: dx.doi.org/10.17504/protocols.io.ba2yigfw

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

Protocol Citation: Anna Behle, Tatsuya Sakaguchi 2020. Homemade Gibson Mastermix. **protocols.io https://dx.doi.org/10.17504/protocols.io.ba2yigfw**

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

Created: January 06, 2020

Last Modified: January 06, 2020

Protocol Integer ID: 31544

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
- 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 × 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_2O		173.4 µL (to 300 µL)	
Total volume		**375 µL**	

Preparation of 1.33x Assembly Mastermix

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

7 Prepare 25 × 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.