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Homemade Gibson Mastermix

Forked from Homemade Gibson Mastermix

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1 Works for me dx.doi.org/10.17504/protocols.io.ba2yigfw

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ABSTRACT

Recipe for homemade 2 x Gibson Mastermix.

EXTERNAL LINK

https://openwetware.org/wiki/Gibson_Assembly

MATERIALS

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

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.

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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:

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 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.

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