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Homemade Gibson Mastermix

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ABSTRACT

Recipe for homemade 1.33 x Gibson Mastermix.

MATERIALS

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- ⊗ beta-Nicotinamide adenine dinucleotide (NAD⁺) - 0.2 ml **New England Biolabs Catalog #B9007S**
- ⊗ Q5 High-Fidelity DNA Polymerase - 100 units **New England Biolabs Catalog #M0491S**
- ⊗ 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**
- ⊗ DTT (Dithiothreitol) (> 99% pure) Protease free **Gold Biotechnology Catalog #DTT**
- ⊗ Deoxynucleotide Solution Set - 25 umol of each **New England Biolabs Catalog #N0446S**

OPEN ACCESS

DOI:

dx.doi.org/10.17504/protocols.io.n9xdh7n

External link:

https://openwetware.org/wiki/Gibson_Assembly

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protocols.io

<https://dx.doi.org/10.17504/protocols.io.n9xdh7n>

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Protocol status: Working

We use this protocol and it's working

Created: Apr 05, 2018

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PROTOCOL integer ID:

11287

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	100 µL	1x
Taq DNA Ligase	40 U/µL	50 µL	4 U/µL
T5 Exonuclease	1 U/µL	2 µL	4 U/mL

Q5 Hi-Fi DNA Polymerase	2 U/ μ L	6.25 μ L	25 U/mL
H ₂ O		216.75	
Total volume		375 μL	

Preparation of 1.33x Assembly Mastermix

- 6 Work on ice. Mix H₂O and 5x buffer, then add enzymes.

- 7 Prepare 25 x 15 μ L aliquots in PCR tubes. Store at -20 °C.
These aliquots are concentrated 1.33 x - add your DNA in a volume of 5 μ L to a final volume/concentration of 20 μ L / 1x.

Gibson assembly

- 8 After addition of DNA, incubate Gibson assembly mix at 50 °C for 45 min.

00:45:00 Gibson assembly

Transformation

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