Homemade Gibson Mastermix

Anna Behle

Abstract

Recipe for homemade 1.33 x Gibson Mastermix.

Citation: Anna Behle Homemade Gibson Mastermix. **protocols.io**
dx.doi.org/10.17504/protocols.io.n9xdh7n
Published: 06 Apr 2018

Materials

- beta-Nicotinamide adenine dinucleotide (NAD+) - 0.2 ml B9007S by New England Biolabs
- Q5 High-Fidelity DNA Polymerase - 100 units M0491S by New England Biolabs
- Taq DNA Ligase - 2,000 units M0208S by New England Biolabs
- T5 Exonuclease - 5,000 units M0363L by New England Biolabs
- PEG-8000 by Contributed by users
- DTT (Dithiothreitol) (> 99% pure) Protease free DTT by Gold Biotechnology
- Deoxynucleotide (dNTP) Solution Set N0446S by New England Biolabs

Protocol

Preparation of 5x isothermal reaction buffer

Step 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

Preparation of 5x isothermal reaction buffer

Step 2.

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

Preparation of 5x isothermal reaction buffer

Step 3.

Slowly add PEG-8000 to mixture and mix well, until completely dissolved. Add H_2O to a final volume of 4 mL.

Preparation of 5x isothermal reaction buffer

Step 4.

Prepare aliquots of the 5x isothermal buffer as required, e.g. 100 µL.

Store at -20 ˚C.

Preparation of 1,33x Assembly Mastermix

Step 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/µL	6.25 µL	25 U/mL
H_2O		216.75	
Total volume		**375 µL**	

Preparation of 1.33x Assembly Mastermix

Step 6.

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

Preparation of 1.33x Assembly Mastermix

Step 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

Step 8.

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

Transformation

Step 9.

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