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## Recombining DNA by Gibson Assembly

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**Protocol status:** Working

**We use this protocol and it's working**

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## Disclaimer








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## Abstract

Our protocol for producing recombinant DNA via Gibson Assembly, based on a home-made Gibson Master Mix.

## Protocol materials


-  Dithiothreitol (DTT) **Melford Catalog # MB1015** Step 1
-  Deoxynucleotide Solution Set - 25 umol of each **New England Biolabs Catalog #N0446S** Step 2
-  Taq DNA Ligase - 2,000 units **New England Biolabs Catalog #M0208S** Step 3
-  T5 Exonuclease - 1,000 units **New England Biolabs Catalog #M0363S** Step 3
-  Phusion DNA polymerase **New England Biolabs** Step 3
-  Poly(ethylene glycol) 8000 [PEG 8000] **Merck MilliporeSigma (Sigma-Aldrich) Catalog #89510** Step 1
-  Tris Base **Merck MilliporeSigma (Sigma-Aldrich) Catalog #T1503** Step 1



- 1
  -  Poly(ethylene glycol) 8000 [PEG 8000] **Sigma Aldrich Catalog #89510**
  -  Tris Base **Sigma Aldrich Catalog #T1503**
  -  Dithiothreitol (DTT) **Melford Catalog # MB1015**


## Preparation of 5x Isothermal Buffer


- 2 Mix the following to give 1 ml of 5x Isothermal Buffer:
  - 450 µl 50% PEG 8000
  - 250 µl 2M Tris-HCl pH 7.5
  - 100 µl 500 mM MgCl<sub>2</sub>
  - 50 µl 1M DTT
  - 100 µl 50 mM NAD
  - 10 µl each of 100 mM ATP, CTP, GTP and TTP (PCR grade)
  - 10 µl sterile water


 Deoxynucleotide Solution Set - 25 umol of each **New England Biolabs Catalog #N0446S**

## Preparation of Gibson Master Mix

- 3 Mix the following reagents:
  - 160 µl of 5x Isothermal Buffer
  - 3.2 µl of 1U/µl T5 exonuclease (diluted 1:10 in water from the 10 U/µl stock)
  - 10 µl of 2U/µl Phusion polymerase
  - 80 µl of 40 U/µl Taq ligase
  - 346.8 µl deionised waterFreeze in 15 µl aliquots (one 15 µl aliquot is ready-to-use for one Gibson assembly reaction)

 Taq DNA Ligase - 2,000 units **New England Biolabs Catalog #M0208S**

 T5 Exonuclease - 1,000 units **New England Biolabs Catalog #M0363S**

 Phusion DNA polymerase **New England Biolabs**

## Gibson Assembly

- 4 Preparation: You need to have prepared two or more fragments of DNA which you wish to recombine, and which have 25-40 nucleotide homologous regions at their ends. For example, this could be a linearised vector and a PCR product which has sequences corresponding to the ends of the linearised vector introduced via the primers.



1. Mix the fragments to be assembled in equimolar ratios in a total volume of 5  $\mu$ l. Add this mixture to one 15  $\mu$ l aliquot of Gibson Master Mix.
2. Incubate the reaction in a 50°C water bath or in a PCR machine at 50°C for one hour.
3. Use 2-10  $\mu$ l of the assembly reaction for a bacterial transformation.