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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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Abstract

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

Protocol materials

☒ Poly(ethylene glycol) 8000 [PEG 8000] **Merck MilliporeSigma (Sigma-Aldrich) Catalog #89510**

☒ Tris Base **Merck MilliporeSigma (Sigma-Aldrich) Catalog #T1503**

☒ Dithiothreitol (DTT) **Melford Catalog # MB1015**

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

☒ 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**



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 Poly(ethylene glycol) 8000 [PEG 8000] **Merck MilliporeSigma (Sigma-Aldrich) Catalog #89510**

 Tris Base **Merck MilliporeSigma (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 MgCl₂
- 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 water

Freeze 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 μ l. Add this mixture to one 15 μ 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 μ l of the assembly reaction for a bacterial transformation.