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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

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 $MgCl_2$
- 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 tot he 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 µl. Add this mixture to one 15 µl 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 µl of the assembly reaction for a bacterial transformation.