Gibson Assembly

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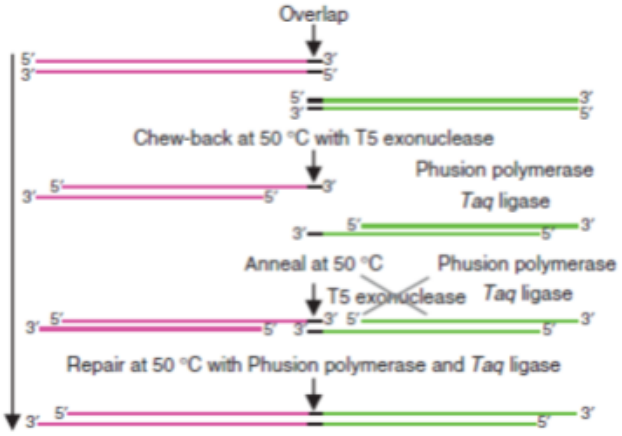
Modified from original Gibson cloning protocol: http://www.natureprotocols.com/2009/04/16/onestep_enzymatic_assembly_of.php

This protocol is useful for cloning without having to use restriction enzymes. It can be used for traditional vector/insert cloning, cloning multiple inserts into a vector, combing multiple DNA fragments together, and multi-site-directed mutagenesis. It is faster and more efficient than traditional cloning.

Reaction summary:

Gibson, D. G., Young, L., Chuang, R. Y., Venter, J. C., Hutchison, C. A., 3rd, and Smith, H. O. (2009) *Nature methods* **6**, 343-345



Reaction Summary- Gibson Assembly

Materials

1. 5X isothermal (ISO) reaction buffer (25% PEG-8000, 500 mM Tris-HCl pH 7.5, 50

mM $MgCl_2$, 50 mM DTT, 1 mM each of the 4 dNTPs, and 5 mM NAD). This is prepared as described below.

2. T5 exonuclease (Epicentre)

3. Phusion DNA polymerase (New England Biolabs)

4. Taq DNA ligase (New England Biolabs)

Equipment

1. Heat block or thermocycler with PCR tubes.

Reaction Stock Preparation

1. Prepare 5X ISO buffer. Six ml of this buffer can be prepared by combining the following:

- 3 ml of 1 M Tris-HCl pH 7.5
- 150 µl of 2 M $MgCl_2$
- 60 µl of 100 mM dGTP
- 60 µl of 100 mM dATP
- 60 µl of 100 mM dTTP
- 60 µl of 100 mM dCTP
- 300 µl of 1 M DTT

- 1.5 g PEG-8000
- 300 µl of 100 mM NAD
- Add water to 6 ml
- Aliquot 100 µl and store at -20 °C

2. Prepare master mixture. Combine the following:

- 320 µl 5X ISO buffer
- 0.64 µl of 10 U/µl T5 exonuclease
- 20 µl of 2 U/µl Phusion polymerase
- 160 µl of 40 U/µl Taq ligase
- Add water to 1.2 ml
- Aliquot 15 µl and store at -20 °C.

This assembly mixture can be stored at -20 °C for at least one year.

The enzymes remain active following at least 10 freeze-thaw cycles.

Primer Design

Gibson assembly primers are broken down in two parts: primer sequence and overlap sequence. The primer sequence should be designed using traditional characteristics in mind (i.e. Tm° values, G/C ratio, and G/C anchors). The overlap sequence needs to have between 20 – 150 bp homology to insert or vector.

My Gibson assembly primers are typically 60 bp in length (30 bp of vector and 30 bp of insert). Staying under 60 bp allows you to order normal oligos from IDT without having to pay for megamers. I order the reverse compliment to simplify primers design so I can amplify both vector and insert sequences. Digested products can be used too if the 5' and 3' overlaps are homologous between vector and insert (e.g. if a digested vector were to be used). For DNA molecules overlapping by larger than 150 bp, prepare the assembly mixture by using 3.2 µl of 10 U/ µl T5 exo.

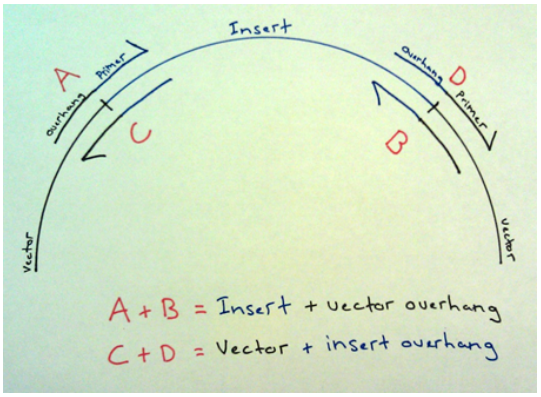
Primer design schematic:

My primers typically look like this:

A: 5' – (30 bp vector/ 30 bp insert) – 3' and D: 5' – (30 bp insert/ 30 bp vector) – 3'

C: 3' – (30 bp vector/ 30 bp insert) – 5' B: 3' – (30 bp insert/ 30 bp vector) – 5'

If amplifying vector, digest with DpnI prior to Gibson assembly to remove parental vector. Clean-up is not required.



Primer Design- Gibson

Reaction Procedure

1. Thaw a 15 µl assembly mixture aliquot and keep on ice until ready to be used.

2. Add 5 µl of DNA to be assembled to the master mixture. The DNA should be in equimolar amounts. Total DNA to be assembled should range between 20-200 ng. For large DNA segments, increasingly proportionate amounts of DNA should be added (e.g. 250 ng of each 150 kb DNA segment).

3. Thoroughly mix gently.

5. Incubate at 50 °C for 15 to 60 min.

6. If cloning is desired, transform 1 – 5 µL. Dialyze prior to electroporation.

More information about Gibson and related cloning methods, visit: **The SLIC, Gibson and CPEC assembly methods (and GeneArt® Seamless Cloning)**

Gibson limitations and obstacles

A major limitation (especially in high GC rich templates such as in P. aeruginosa) to Gibson assembly is that the termini of the DNA sequence fragments to be assembled should not have stable single stranded DNA secondary structure, such as a hairpin or a stem loop, or repeated sequences, as this would directly compete with the required single-stranded annealing/priming of neighboring assembly fragments. So make sure to run your sequences in Scitools to look for such nuisance areas (Delta G for hairpins should be more than negative 10 and with a lower annealing temperature than that of primer:primer duplex. *Comment by DonHK*).

Posted by Hemantha Kulasekara at 9:58 am