Source: Landry wiki

Gibson cloning

Gibson cloning is used to clone one or more fragments in a vector of interest. In the lab, we generally use PCR amplified vector and fragments. Here is the general procedure in that specific case...

Amplification of vector:

Use a high fidelity polymerase (like the Kapa HiFi hotstart polymerase) to amplify your vector of interest. Your oligos should be around 20-24 nt (it depends on the Tm of the oligo - see following section) and should be designed as to amplify your vector from each side of the insertion site. PCR reaction must then be digested with DpnI to get rid of parental plasmid. Purify on column.

(A vector can also be digested and directly used for cloning. In that case, make a double-digestion and make sure it is complete.)

Amplification of fragments (inserts):

Use a high fidelity polymerase (like the Kapa HiFi hotstart polymerase) to amplify your fragments of interest (from gDNA or plasmid). Design a forward primer with an overlap region (≥ 16 nt - usually 20 to 24 nt) at the 5´-end, which is complementary to the reverse primer of the vector. The 3´-end of the forward primer (around 20 nt - it depends on the Tm, try to have a 50% GC content) should contain insert-specific sequences to amplify the target fragment. The "≥ 16 nt" overlap region of the forward primer should be complementary to the 5´-end sequence of the vector which corresponds to the insertion site. The size of the overlap region is determined by the number of nucleotides needed to reach a Tm > 48°C. If necessary, one can also add nucleotides between the overlap region and gene-specific sequence region to ensure, for example, that the expressed protein is in frame. Similarly, the reverse primer should also contain an overlap region (≥ 16 nt - usually 20 to 24 nt) at the 5-end and an insert-specific sequence at the 3´end.

If the amplification is made from a plasmid, digest the PCR reaction with DpnI and purify on column.

Manufacture Gibson mix:

1) Prepare 6 ml of 5X ISO Buffer in a 15 ml falcon tube as follows:

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3 ml 1 M Tris-HCl pH 7.5
+ 150µl 2 M MgCl2
+ 240µl 100 mM dNTP mix (25 mM each: dGTP, dCTP, dATP, dTTP)
+ 300µl 1 M DTT
+ 1.5 g PEG-8000
+ 300µl 100 mM NAD
+ dH20 to 6 ml
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- Store at -20°C in 320µl aliquots.

2) Prepare 1.2 ml of Gibson assembly master mix as follows:

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320µl 5X ISO Buffer
+ 0.64µl 10 U/µl T5 exonuclease (This is optimized for 20-150 bp sequence
homology overlaps)
+ 20µl 2 U/µl Phusion polymerase
+ 160µl 40 U/µl Taq ligase
+ 700µl dH20
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- Store at -20°C in 7.5µl aliquots in PCR tubes (or strips).

Gibson cloning:

1) Thaw a 7.5µl aliquot of the Gibson assembly master mix, and keep on ice until use.

2) Measure the DNA concentration (ng/µl) of each assembly piece (on an agarose gel).

3) Add 50ng of the vector backbone (PCR or linearized) and a 1:2 (vector:insert) molar ratio amounts of the other assembly pieces to the thawed 7.5µl master mix in a 10µl total volume assembly reaction mixture as follows:

```
vector backbone (50ng)
each insert (to a 1:2 molar ratio with backbone)
7.5µl Gibson assembly master mix
ddH20 to 10µl
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- The vector:insert ratio must be adapted to the size of the fragments to clone. For smaller fragments, more inserts should be put in the reaction.

4) Incubate the assembly reaction at 50°C for 60 minutes, and then place on ice.

5) Transform 5µl of the assembly reaction into 100µl of competent E.coli and plate 100µl and 900µl on 2YT plates with the appropriate antibiotic(s).

- Gibson cloning is hard to acheive with repeated sequences.
- See the original brochure for more information about the general protocol and the procedure to design the fragments for assembly: File:Manual from NEB for Gibson cloning.pdf