Gibson assembly

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Gibson assembly is a DNA assembly method which allows for the joining of multiple DNA fragments in a single, isothermal reaction. It was invented in 2009 by Daniel Gibson while he was at the J. Craig Venter Institute (JCVI).[1]

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Process

The entire Gibson assembly reaction requires a small number of components with very few manipulations.[1][2]

The method can simultaneously combine numerous (>10) DNA fragments based on sequence identity. It requires that the DNA fragments contain ~20-40 base pair overlap with adjacent DNA fragments. These DNA fragments are mixed with a cocktail of three enzymes, along with other buffer components.

The three required enzyme activities are: exonuclease, DNA polymerase, and DNA ligase.

- The exonuclease chews back DNA from the 5' end. The resulting single-stranded regions on adjacent DNA fragments can anneal.
- The DNA polymerase incorporates nucleotides to fill in any gaps.
- The DNA ligase covalently joins the DNA of adjacent segments, thereby removing any nicks in the DNA.

The entire mixture is incubated at 50°C for up to one hour. The resulting product is different DNA fragments joined into one.

Advantages of Gibson assembly

This DNA assembly method has many advantages compared to conventional restriction enzyme/ligation cloning of recombinant DNA.

- No restriction digest of the DNA fragments after PCR is necessary. The backbone vector can be digested, or synthesized by PCR.
- It is far simpler than conventional cloning schemes, as it requires fewer steps and fewer reagents.

The process also takes less time.
- No restriction site scar remains between two DNA fragments (a.k.a, "scarless").
- Multiple DNA fragments can be combined simultaneously in a single-tube reaction.

References

1. ^ *a* *b* Gibson DG, Young L, Chuang RY, Venter JC, Hutchison CA 3rd, Smith HO. (2009). "Enzymatic assembly of DNA molecules up to several hundred kilobases". *Nature Methods* **6** (5): 343–345. doi:10.1038/nmeth.1318 (http://dx.doi.org/10.1038%2Fnmeth.1318). PMID 19363495 (https://www.ncbi.nlm.nih.gov/pubmed/19363495).
2. ^ Gibson DG. (2011). "Enzymatic assembly of overlapping DNA fragments". *Methods in Enzymology* **498**: 349–361. doi:10.1016/B978-0-12-385120-8.00015-2 (http://dx.doi.org/10.1016%2FB978-0-12-385120-8.00015-2). PMID 21601685 (https://www.ncbi.nlm.nih.gov/pubmed/21601685).

Further information

- A Guide to Gibson Assembly from the University of Cambridge, UK (http://www.synbio.org.uk/dna-assembly/guidetogibsonassembly.html)

- Gibson Assembly - A tutorial from supplier (http://www.youtube.com/watch?v=tlVbf5fXhp4&feature=share&list=PLQl3WvQSRR-tsJEzvaVpsfe1tixg9Bzk3&index=2) New England Biolabs
- Perkel, Jeffrey M. (January 2014). "Seamlessly rewriting the lab cloning manual" (http://www.biotechniques.com/BiotechniquesJournal/2014/January/Seamlessly-rewriting-the-lab-cloning-manual/biotechniques-349455.html). Tech News. *BioTechniques* **56** (1): 12–14. "Gibson says he no longer even bothers with standard restriction enzyme-based cloning in his lab."

Categories: DNA
