

BBF RFC 62: Fast multiple gene fragment ligation method based on homologous recombination

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1. Purpose

With the established BioBrick Assembly standards, ligation of different parts has to be accomplished step by step. It can be time-consuming when dealing with multiple fragment ligation.

BBF RFC 62 is developed aimed at completing the ligation of multiple fragments quickly and efficiently based on homologous recombination.

2. Relation to other BBF RFCs

This BBF RFC updates BBF RFC 10.

3. Copyright Notice

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4. Description

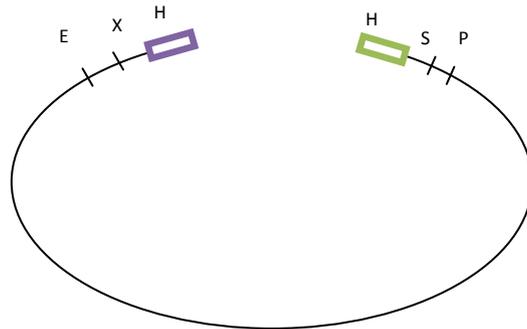
This method depends on sequence and ligation-independent cloning (SLIC), which allows the assembly of multiple DNA fragments in a single reaction using in vitro homologous recombination and single-strand annealing. SLIC mimics in vivo homologous recombination by relying on exonuclease-generated ssDNA overhangs in insert and vector fragments, and the assembly of these fragments by recombination in vitro.

Homologous recombination in vivo depends upon a double-stranded break, generation of ssDNA by exonucleases, homology searching by recombinases, annealing of homologous stretches, and repair of overhangs and gaps by enzymes that include resolvases, nucleases and polymerases.

It is possible to generate recombination intermediates in vitro and introduce these into cells to allow the cell endogenous repair machinery to finish the repair to generate recombinant DNA.

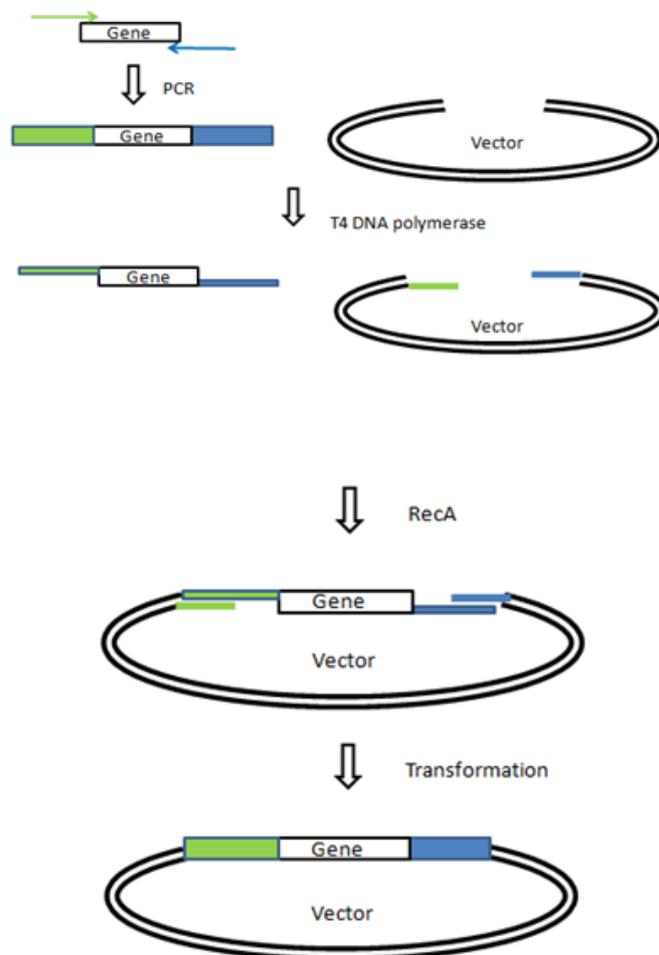
The experiment generally includes the following steps:

- a) Introduce restriction sites and homologous recombination sites to the vector.



“H” stands for “Homologous Recombination Site”

- b) Generate the vector by cleavage with a restriction enzyme
- c) Generate the insert by PCR.
- d) Treat both the vector and the insert with T4 DNA polymerase in the absence of dNTPs to chew back 5' strand to reveal ssDNA overhangs.
- e) Incubate vector and insert with RecA protein and ATP to promoter recombination.
- f) Transform the products into E.coli.



When dealing with multiple gene fragments, these fragments can be introduced with different homologous recombination sites, then mixed with the vector. Different fragments can be generated as this:

Fragment A : (Homology)₁-(n₁n₁n₁...n₁n₁n₁)-(Homology)₂

Fragment B: (Homology)₂-(n₂n₂n₂...n₂n₂n₂)-(Homology)₂

Fragment C: (Homology)₂-(n₃n₃n₃...n₃n₃n₃)-(Homology)₂

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6. References

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[2] **Sambrook J, Maniatis T, Fritsch E F.** Molecular Cloning: a Laboratory Manual. Cold Spring

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