Efficient in vivo assembly of DNA fragments for biosynthesis

Technology #m09-048

Current approaches for the cloning of large sized DNA fragments include in vitro DNA ligation, yeast homologous recombination using several selective markers and inchworm elongation. While these techniques can all be effective, they require significant laboratory resources and manpower and can be quite inefficient. Furthermore, these techniques are not necessarily suited for the introduction of DNA fragment sizes corresponding to biosynthetic pathways, which are typically between 3 and 20 genes. This technology offers an efficient approach for the in vivo assembly of large DNA fragments in yeast; it is a robust and high-yielding process with applications across many areas of synthetic biology.

Endonuclease-induced homologous recombination for robust and efficient sequential in vivo assembly of an infinite number of DNA fragments

This technology allows for the sequential in vivo assembly of an infinite number of DNA fragments by using a technically simple strategy that employs endonuclease-induced homologous recombination. Rather than relying on many PCR amplification steps, as do most in vitro methods, this technology induces double-stranded breaks in a core fragment of DNA to trigger the attachment of additional DNA fragments. As such, it relies on two separate DNA constructs: (1) an acceptor module in the chromosome of the yeast host cell and (2) an introduced donor module. Attachment of the donor DNA to the acceptor DNA is triggered by activating cleavage of a specific endonuclease site on the acceptor molecule. Subsequent donor DNA fragments can be added by iterating the process.

The technology has been demonstrated to efficiently assemble large libraries of multigene DNA constructs.

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Applications:

• Synthesis of DNA sequences for multi-gene biosynthetic pathways
• Synthesis of large DNA libraries for protein/metabolic engineering and synthetic biology applications
• Biosynthesis of complex natural products

**Advantages:**

• Produces high yielding recombinants and efficiently creates large libraries in vivo
• Eliminates the use of many PCR amplification steps by employing the use of double-stranded DNA breaks to trigger in vivo homologous recombination
• Applicable to both small and large DNA fragment assembly
• The cycling of only two selectable markers and the use of orthogonal endonucleases allows for repeated rounds of assembly of many DNA fragments
• The assembled DNA is in a known location of the yeast genome and can thus be easily transferred from one strain to another

**Patent Information:**

Patent Pending (US 20120202251)

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**Related Publications:**


**Inventors**

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