

Claims

1. A method for the synthesis of a desired nucleic acid molecule, comprising:
 - a) providing a plurality of cassettes, each cassette containing the nucleotide sequence of a portion of the desired nucleic acid, wherein the cassettes are 2-10 kb in length and contain overlapping portions of the nucleotide sequence of the desired nucleic acid molecule and wherein the cassettes, if combined according to the overlapping portions, provide the complete nucleotide sequence of the desired nucleic acid molecule;
 - b) ~~combining-joining~~ said cassettes *in vitro* to obtain a plurality of resulting subsets wherein said subsets contain overlapping portions of the desired nucleic acid molecule of at least 80 bp sequence and wherein the subsets, if assembled according to the overlapping portions, would provide the complete nucleotide sequence of the desired nucleic acid molecule; and
 - c) transfecting host cells with a mixture of fragments of DNA comprising 10 or more subsets and assembling the fragments of DNA comprising 10 or more the subsets in vivo in a host culture to obtain the desired nucleic acid molecule, wherein one of the fragments of DNA said assembly further includes an origin of replication.
2. ~~The method of 1, wherein the overlapping portions comprise at least 20 bases each.~~
32. The method of claim 1, wherein a step b) and/or step c) is repeated at least once.
43. The method of claim 1, wherein step c) is conducted in yeast, and the origin of replication is operable in yeast.
54. The method of claim 43, wherein the desired nucleic acid molecule~~said~~ assembly of step c) includes a centromere and/or a selectable marker.

65. The method of claim 1, wherein one of the fragments of DNA is a the origin of replication is contained on an additional vector and said vector comprises an origin of replication.

76. The method of claim 5, wherein said vector comprises the centromere and/or a selectable marker are contained on an additional vector.

87. The method of claim 1, wherein one or more cassettes in step a) contains a watermark.

9. ~~A method to assemble 10 or more DNA fragments into a single DNA molecule, which method comprises:~~

~~(a) transfecting a culture of host cells with a mixture containing a sufficient number of copies of each of said 10 fragments to result in uptake of copies amounting to at least about double the number of different fragments by the average cell in said culture, wherein said fragments contain overlapping sequences of at least 10 base pairs, and wherein the overlapping sequences are arranged so that the sequences overlap to provide a desired order of assembly, and~~

~~wherein an origin of replication is included among said fragments;~~

~~(b) permitting sufficient time for assembly of said fragments in said yeast culture; and~~

~~(c) either~~

~~(i) culturing said host to obtain sufficient copies of the assembled nucleic acid molecule for recovery, or~~

~~(ii) extracting DNA from said host and transfecting an alternative culture allowing said alternative culture to replicate sufficient copies of said assembled nucleic acid molecule for recovery.~~

10. The method of claim 9, which further includes the step of isolating the assembled DNA molecule from the host culture or alternative culture.

~~11. The method of claim 9, wherein at least two target DNA molecules are assembled in said host culture.~~

~~12. The method of claim 9, wherein the origin of replication is operable in the host and a centromere operable in the host is included among said fragments.~~

~~13. The method of claim 9, wherein an origin of replication operable in an alternative system is included among said fragments.~~

~~14. The method of claim 9, wherein a selectable marker is included in said fragments and wherein said culturing is under selection conditions.~~

~~158. The method of claim 91, wherein the fragments are double-stranded and contain 100-5 x 10⁶ base pairs.~~

~~16. The method of claim 9, wherein the fragments are single-stranded and contain 40-1,000 nucleotides.~~

~~17. The method of claim 9, wherein two of said fragments each comprises a telomere at one terminus.~~

~~189. The method of claim 91, wherein the assembled DNA molecule represents a naturally occurring genome.~~

~~19. The method of claim 9, wherein the assembled DNA molecule includes a desired metabolic pathway.~~

~~20. The method of any of claims 9-19, wherein the host culture is yeast.~~

~~2110. Isolated DNA molecules assembled by the method of any of claims 1-19.~~

~~2211. A host culture or alternative culture which comprises a DNA molecule assembled according to the method of any of claims 1-19.~~

12. The host culture of claim 1 which is yeast

2313. The host cultures of claim 22-12 which wherein the host culture is yeast and the an alternative culture of claim 22 which is an *E. coli* or *Bacillus* culture.

14 The method of claim 1, wherein each subset is about 50-500 kb in length.

24. A method to assess the criticality of a gene which method comprises deleting or disrupting a candidate gene from the synthetic nucleic acid molecules of claim 21 transfected into a cell culture and evaluating the effect of said deleting or disrupting on the survival of the culture.

Claims

1. A method for the synthesis of a desired nucleic acid molecule sequence, comprising:
 - a) providing a plurality of cassettes, each cassette containing a nucleotide sequence of a portion of the desired nucleic acid molecule, wherein the cassettes are 2-10 kb in length and contain overlapping portions of the nucleotide sequence of the desired nucleic acid molecule and wherein the cassettes, if combined according to the overlapping portions, provide the complete nucleotide sequence of the desired nucleic acid molecule;
 - b) joining said cassettes *in vitro* to obtain a plurality of resulting subsets wherein said subsets contain overlapping portions of the desired nucleic acid molecule of at least 80 bp and wherein the subsets, if assembled according to the overlapping portions, would provide the complete nucleotide sequence of the desired nucleic acid molecule; and
 - c) transfecting host cells with a mixture of fragments of DNA comprising 10 or more subsets and assembling the fragments of DNA comprising 10 or more subsets *in vivo* to obtain the desired nucleic acid molecule, wherein one of the fragments of DNA includes an origin of replication.
2. The method of claim 1, wherein step b) and/ or step c) is repeated at least once.
3. The method of claim 1, wherein step c) is conducted in yeast, and the origin of replication is operable in yeast.
4. The method of claim 3, wherein said desired nucleic acid molecule of step c) includes a centromere and/or a selectable marker.
5. The method of claim 1, wherein one of the fragments of DNA is a vector and said vector comprises the origin of replication.

6. The method of claim 5, wherein said vector comprises the centromere and/or a selectable marker.
7. The method of claim 1, wherein one or more cassettes in step a) contains a watermark.
8. The method of claim 1, wherein the fragments are double-stranded and contain 100 - 5 x 10⁶ base pairs.
9. The method of claim 1, wherein the assembled DNA molecule represents a naturally occurring genome.
10. Isolated DNA molecules assembled by the method of claim 1.
11. A host culture which comprises a DNA molecule assembled according to the method of claim 1.
12. The host culture of claim 1 which is yeast.
13. The cultures of claim 12 wherein the host culture is yeast and an alternative culture is *E. coli* or *Bacillus*.
14. The method of claim 1, wherein each subset is about 50-500 kb in length.