

Claims

1. A method for the synthesis of a ~~desired~~-nucleic acid molecule of pre-determined 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 of pre-determined sequence;
 - 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 yeast host cells with a mixture of fragments of DNA comprising 10 or more subsets of from about 15 kb to about 200 kb or 50-500 kb in size and assembling the fragments of DNA comprising the 10 or more subsets *in vivo* to obtain the desired nucleic acid molecule of pre-determined sequence, wherein one of the fragments of DNA includes an origin of replication operable in yeast.
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.~~
- ~~4.3.~~ 4.3. The method of ~~claim 3~~claim 1, wherein said ~~desired~~-nucleic acid molecule of step c) includes a centromere and/or a selectable marker.
- ~~5.4.~~ 5.4. The method of claim 1, wherein one of the fragments of DNA is a vector and said vector comprises the origin of replication.

6.5. The method of ~~claim 5~~claim 4, wherein said vector comprises the centromere and/or a selectable marker.

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

8.7. The method of claim 1, wherein the fragments are double-stranded and contain 100 - 5 x 10⁶ base pairs.

9.8. 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.~~

9. The method of claim 1 wherein the nucleic acid molecule of pre-determined sequence is a synthetic genome.

10. The method of claim 1 wherein two of the fragments each comprises a telomere at one terminus.