

Claims

1. A method for the synthesis of a 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 nucleic acid molecule, wherein the cassettes are 2-10 kb in length and contain overlapping portions of the nucleotide sequence of the nucleic acid molecule and wherein the cassettes, if combined according to the overlapping portions, provide the complete nucleotide sequence of the 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 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 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 said nucleic acid molecule of step c) includes a centromere and/or a selectable marker.
4. The method of claim 1, wherein one of the fragments of DNA is a vector and said vector comprises the origin of replication.
5. The method of claim 4, wherein said vector comprises the centromere and/or a selectable marker.

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

~~7. The method of claim 1, wherein the fragments are double-stranded and contain 100–5 × 10⁶ base pairs.~~

~~78.~~ The method of claim 1, wherein the assembled DNA molecule represents a naturally occurring genome.

~~89.~~ The method of claim 1 wherein the nucleic acid molecule of pre-determined sequence is a synthetic genome.

~~940.~~ The method of claim 1 wherein two of the fragments each comprises a telomere at one terminus.

Claims

1. A method for the synthesis of a 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 nucleic acid molecule, wherein the cassettes are 2-10 kb in length and contain overlapping portions of the nucleotide sequence of the nucleic acid molecule and wherein the cassettes, if combined according to the overlapping portions, provide the complete nucleotide sequence of the 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 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 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 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 said nucleic acid molecule of step c) includes a centromere and/or a selectable marker.
4. The method of claim 1, wherein one of the fragments of DNA is a vector and said vector comprises the origin of replication.
5. The method of claim 4, wherein said vector comprises the centromere and/or a selectable marker.

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

7. The method of claim 1, wherein the assembled DNA molecule represents a naturally occurring genome.

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

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