

Claims

1. A method for assembling a nucleic acid molecule in a single step from a set of overlapping oligonucleotides, the method comprising:
 - (a) contacting a set of overlapping oligonucleotides with
 - a DNA polymerase;
 - a mixture of dNTPs; and
 - polyethylene glycol;to form an assembly mixture;
 - (b) subjecting the assembly mixture to multiple cycles, each cycle comprising one or more of an annealing phase, an extension phase, a denaturation phase,
 - (c) thereby assembling the nucleic acid molecule from a set of overlapping oligonucleotides in a single step.
2. The method of claim 1, wherein the set of oligonucleotides comprises end oligonucleotides and non-end oligonucleotides, and the end oligonucleotides are provided in the assembly mixture at a higher concentration than the non-end oligonucleotides.
3. The method of claim 1 or claim 2, wherein at least one annealing phase occurs at a temperature of between 50 °C and 77 °C.
4. The method of any one of claims 1 to 3, wherein the extension phase of a cycle is increased in time relative to the extension phase of the previous cycle.
5. The method of any one of claims 1 to 4, wherein the DNA polymerase is a modified DNA polymerase from *Pyrococcus furiosus*.
6. The method of any one of claims 1 to 5, wherein the set of oligonucleotides is assembled into a gene.
7. The method of any one of claims 1 to 6, wherein the polyethylene glycol is PEG 8000.
8. The method of claim 7, wherein the concentration of PEG is 0.025% or greater.
9. The method of claim 7, wherein the concentration of PEG is 0.375% or greater.
10. The method of any one of claims 1 to 9, wherein the annealing phase occurs at 67 °C.
11. The method of any one of claims 1 to 9, wherein the annealing phase and the extension phase occur at 67 °C.

12. The method of any one of claims 9 to 11, wherein the nucleic acid molecule is greater than 1 kb in length.
13. The method of claim 12, wherein the nucleic acid molecule is greater than 2 kb in length.
14. The method of claim 12, wherein the nucleic acid molecule is greater than 3 kb in length.
15. The method of any one of claims 1 to 14, wherein the set of overlapping oligonucleotides comprises at least 5 oligonucleotides.
16. The method of claim 15, wherein the set of overlapping oligonucleotides comprises at least 60 oligonucleotides.
17. The method of claim 16, wherein the set of overlapping oligonucleotides comprises at least 75 oligonucleotides.
18. The method of claim 17, wherein the nucleic acid molecule assembled is greater than 2 kb, the initial extension phase is between 5 minutes and 7 minutes, and subsequent extension phases are time varied phases.
19. The method of claim 18 wherein the nucleic acid molecule assembled is greater than 3 kb, the initial extension phase is between 5 minutes and 7 minutes, and subsequent extension phases are progressively increased in time relative to the initial extension phase.
20. The method of claim 19 wherein the set of overlapping nucleotides comprises more than 100 oligonucleotides.
21. The method of any one of claims 1 to 20, wherein one or more phases are time varied phases.
22. The method of claim 21, wherein the extension phase is a time varied phase.
23. The method of claim 22, wherein the extension phase is cumulatively extended by about 15 seconds per cycle.
24. The method of any one of claims 1 to 23, wherein the multiple cycles comprise at least 25 cycles.
25. The method of any one of claims 1 to 24, wherein the nucleic acid molecule assembled comprises one or more AT rich sequences.