

process or a continuous flow process. The batch may be a closed batch (i.e. all of the reaction components are provided at the start of the DNA synthesis) or further components can be supplied to the reaction as required during the process, such as described in WO2016/034849, incorporated herein by reference. Should further additions be required, this will dilute the concentration of the nucleotide or nucleotide complexes, unless further nucleotide complexes are added to replenish or increase the concentration.

The present inventors have found that each of the different counter-ions may add a particular characteristic to the enzymatic DNA synthesis reaction. For example the use of ammonium ions in nucleotide complexes may result in the use of some higher concentrations of nucleotides.

The enzymatic cell-free synthesis of DNA with such ions can be carried out in minimal buffering agents, in which no additional salts which have been shown to enhance DNA synthesis or assist in primer binding, or detergents, are added. This minimal buffer may comprise an agent (a buffering agent) to stabilise the pH. The minimal buffering agent may contain a small amount of cations provided by the presence of a chemical used to denature the template, such as sodium, potassium or ammonium hydroxide. In the Examples, 5mM sodium or potassium hydroxide was used as a denaturant, but this concentration may be altered to suit the conditions of the reaction, within the skill of those used to performing DNA denaturation, and amounts of sodium, potassium or ammonium hydroxide could be provided from 2.5mM, up to 5mM, up to 10mM, 15mM, 20mM or 25mM or more may be employed dependent upon the nature of the template. Therefore, the reaction mixture may contain a small or minimal amount of cations and anions which were not originally associated with the nucleotide complex.

Thus, in one aspect, the present disclosure provides a cell-free process for the enzymatic synthesis of DNA in solution comprising obtaining a nucleotide complex, where said nucleotide complex comprises a nucleotide associated with between 0.2 and 2 divalent cations and between 0.2 and 2.5 monovalent cations per nucleotide and adding a nucleotidyltransferase, wherein said nucleotide complex is obtained at a concentration of at least 40 mM.

Further advantages are described below.

Brief description of the Drawings

The present invention will be described further below with reference to exemplary embodiments and the accompanying drawings, in which:

Figures 1A to 1B are plots showing results obtained with experiments using varied starting concentrations of nucleotide salts for either sodium alone (dNTP:4Na^+)(continuous line) or a mixed sodium/magnesium dNTP ($\text{dNTP:2Na}^+/\text{Mg}^{2+}$)(dotted line). The plot depicts the reaction yields in g/l (A) and the reaction efficiency as a % (B) for each type of dNTP plotted vs the start dNTP concentration used. Figure 1C shows DNA produced after 1 week of RCA, with more viscous DNA solutions corresponding to higher yields remaining attached to the base of the Eppendorf tube when inverted.

Claims

1. A cell-free process for the enzymatic synthesis of DNA in solution comprising obtaining a nucleotide complex, where said nucleotide complex comprises a nucleotide associated with between 0.2 and 2 divalent cations and between 0.2 and 2.5 monovalent cations per nucleotide and adding a nucleotidyltransferase, wherein said nucleotide complex is obtained at a concentration of at least 40 mM.
2. A cell-free process according to claim 1 wherein the nucleotide complex is a salt.
3. A cell-free process according to any one of claims 1 or 2 wherein said nucleotide complex is electrically neutral.
4. A cell-free process according to claim 3 wherein in order to obtain electrical neutrality the complex associates with one or more hydrogen or hydronium ions.
5. A cell-free process according to any preceding claim wherein the complex is associated with between about 0.5 and 1.5 divalent cations, preferably 1 divalent cation per nucleotide entity.
6. A cell-free process according to any preceding claim wherein the complex is associated with between about 0.2 and 2 monovalent cations.
7. A cell-free process according to any preceding claim wherein said divalent cations are independently selected from: magnesium (Mg^{2+}), beryllium (Be^{2+}), calcium (Ca^{2+}), strontium (Sr^{2+}), manganese (Mn^{2+}) or zinc (Zn^{2+}), preferably Mg^{2+} or Mn^{2+} .
8. A cell-free process according to any one of claims 1 to 6, wherein said divalent cations are independently selected from magnesium (Mg^{2+}) or manganese (Mn^{2+}).
9. A cell-free process according to any preceding claim wherein said monovalent cations are independently selected from: an alkali metal, a transition metal, or a polyatomic ion.
10. A cell-free process according to claim 9 wherein said monovalent cations may be independently selected from a polyatomic ion such as an oxonium ion.
11. A cell-free process according to claim 10, wherein said oxonium ion is ammonium or a derivative thereof.

12. A cell-free process according to claim 9 wherein said monovalent cations may be an alkali metal, independently selected from : lithium (Li^+), sodium (Na^+), potassium (K^+), rubidium (Rb^+), caesium (Cs^+) or francium (Fr^+).
- 5 13. A cell-free process according to any preceding claim, wherein said nucleotide complex in solution is obtained by mixing nucleotides complexed with divalent cations and a solution of nucleotides complexed with monovalent cations.
14. A cell-free process according to claim 13, wherein the divalent and monovalent cations are present at a ratio of less than 4:1 cation: nucleotide, optionally 3.5:1, 3:1 or 2.5:1 or below.
- 10 15. A cell-free process according to claim 13 or 14 wherein said nucleotide complex with divalent cations is poorly soluble until mixing with nucleotides complexed with monovalent cations.
16. A cell-free process according to any one of claims 13 to 15 wherein the nucleotide complex is soluble.
- 15 17. A cell-free process according to any preceding claim wherein said nucleotide complex and nucleotidyltransferase form a reaction mixture.
18. A cell-free process according to claim 17 wherein further components are added to the reaction mixture, including but not limited to any one or more of the following:
 - a) template nucleic acid;
 - 20 b) primer;
 - c) primase;
 - d) denaturing agent, such as sodium or ammonium hydroxide;
 - e) buffering agents; including buffering salts;
 - f) pyrophosphatase; and/or
 - 25 g) magnesium or manganese salts
19. A cell-free process according to claim 18 wherein magnesium or manganese salts are added to the reaction mixture as a co-factor for the nucleotidyltransferase, such that the total ratio of the magnesium and/or manganese to nucleotide does not exceed 2:1.

20. A cell-free process according to any preceding claim wherein said nucleotidyltransferase is a DNA polymerase.
21. A cell-free process according to any one of claims 1 to 19, wherein said nucleotidyltransferase is a strand-displacing DNA polymerase.
- 5 22. A cell-free process according to claim 20 or 21 wherein said nucleotidyltransferase is capable of isothermal DNA synthesis.
23. A cell- free process according to any one of claims 1 to 19 wherein said nucleotidyltransferase does not require a template.