

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
5 with between 0.2 and 2 divalent cations and between 0.2 and 2.5 monovalent cations per nucleotide and adding a nucleotidyltransferase.
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.
- 10 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.
- 15 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+} .
- 20 8. 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.
9. A cell-free process according to claim 8 wherein said monovalent cations may be independently selected from a polyatomic ion such as an oxonium ion, preferably
25 ammonium or a derivative thereof.
10. A cell-free process according to claim 8 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^+).

11. 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, preferably wherein the divalent and monovalent cations are present at a ratio of less than 4:1 cation:
5 nucleotide, optionally 3.5:1, 3:1 or 2.5:1 or below.
12. A cell-free process according to claim 11 wherein said nucleotide complex with divalent cations is poorly soluble until mixing with nucleotides complexed with monovalent cations.
13. A cell-free process according to claims 11 and 12 wherein the nucleotide complex is
10 soluble.
14. A cell-free process according to any preceding claim wherein said nucleotide complex is obtained at a concentration of at least 30mM.
15. A cell-free process according to any preceding claim wherein said nucleotide complex is obtained at a concentration of at least 40mM.
- 15 16. A cell-free process according to any preceding claim wherein said nucleotide complex and nucleotidyltransferase form a reaction mixture.
17. A cell-free process according to claim 16 wherein further components are added to the reaction mixture, including but not limited to any one or more of the following:
- 20 a) template nucleic acid;
- 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

18. A cell-free process according to claim 17 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.
19. A cell-free process according to any preceding claim wherein said nucleotidyltransferase is a DNA polymerase, preferably a strand-displacing DNA polymerase.
20. A cell-free process according to claim 19 wherein said nucleotidyltransferase is capable of isothermal DNA synthesis.
21. A cell- free process according to any one of claims 1-18 wherein said nucleotidyltransferase does not require a template.