

Claims

1. A cell-free process for the enzymatic synthesis of DNA comprising the use of nucleotide salts at a concentration greater than 10 mM,

wherein said nucleotide salts comprise a monovalent cation with an ionic radius that is greater than that of the sodium ion, wherein said monovalent cation is selected from potassium, caesium, rubidium, francium, ammonium, or derivatives of ammonium, and wherein the cell-free process further comprises the use of one or more divalent metal cations.

2. A cell-free process for the enzymatic synthesis of DNA comprising the use of nucleotide salts, wherein said nucleotide salts are present at a concentration of at least 10mM and are either:

(a) a nucleotide salt comprising a monovalent cation whose ionic radius is greater than that of the sodium ion, or

(b) two or more nucleotide salts, each salt comprising different monovalent cations, wherein at least one of the cations has an ionic radius greater than that of the sodium ion, wherein said monovalent cation is independently selected from potassium, caesium, rubidium, francium, ammonium, or derivatives of ammonium, and wherein the cell-free process further comprises the use of one or more divalent metal cations.

3. ~~A~~The cell-free process according to any preceding claim wherein said nucleotide salts are present at a concentration of at least 15mM, at least 20 mM, at least 25mM, at least 30mM, at least 35mM or at least 40mM.

~~4. —A cell-free process according to any preceding claim wherein said monovalent cation or cations are independently selected from an alkali earth metal, a transition metal, or a polyatomic ion.~~

~~5. A cell-free process according to claim 4 wherein said monovalent cation or cations are independently selected from the list comprising potassium, ammonium, derivatives of ammonium, rubidium, caesium, or francium.~~

5 ~~64.~~ A-The cell-free process according to any preceding claim wherein said cell-free process further comprises the use of one or more primers or a primase.

~~75.~~ A-The cell-free process according to any preceding claim wherein said ~~cell~~-free process further comprises the use of a template.

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~~86.~~ -The cell-free process ~~for synthesising DNA~~ according to any preceding claim wherein the ~~cell-free process further comprises the use of~~ one or more divalent metal cations are, preferably selected from ~~the list comprising~~ magnesium, manganese, calcium, beryllium, zinc, ~~and or~~ strontium.

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~~7.~~ The cell-free process according to any preceding claim wherein the ratio between said divalent metal cations and the nucleotide salts is 1.5:1 or less.

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~~98.~~ A-The cell-free process according to claim ~~8-6~~ wherein the ratio between said divalent metal cations and the nucleotide salts is equal to or less than 1:1 in the reaction mixture, preferably less than 1:1.

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~~109.~~ A-The cell-free process according to any preceding claim wherein the process further uses a maximum concentration of 10mM sodium and/or lithium nucleotide salts.

~~1110.~~ A-The cell-free process according to any preceding claim wherein the process further comprises the use of a chemical denaturing agent, preferably sodium hydroxide, potassium hydroxide or ammonium hydroxide, and a pyrophosphatase.

~~12~~11. A-The cell-free process according to claim ~~11-10~~ wherein no pH buffering agents are added to the process, and preferably no additional salts or detergents are added.

5 ~~13~~12. The cell-free process according to claim ~~12-11~~ wherein the nucleotide salts comprise caesium ions.

~~14~~13. A-The cell-free process according to claim ~~11-10~~ wherein pH buffering agents, but no additional salts or detergents are added.

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~~15~~14. A-The cell-free process according to claim ~~14-13~~ wherein the nucleotide salts comprise ammonium ions.

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~~16~~15. The cell-free process according to any preceding claim wherein the process is for the synthesis of DNA on a large scale, preferably at least 3g/l.

~~17~~16. The use of nucleotide salts comprising caesium cations in the enzymatic cell-free synthesis of DNA.

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~~18.~~ ~~The use of claim 17~~ wherein the enzymatic cell-free synthesis of DNA takes place in the presence of ~~low levels of~~ divalent metal cations, optionally at a ratio of 0.2:1 to 0.8:1 divalent metal cation to nucleotide salt, preferably 0.2:1 to 0.5:1, wherein the nucleotide salts are present at a concentration greater than 10 mM.

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~~19~~17. The use of claim ~~17-16~~ wherein the enzymatic cell-free synthesis of DNA takes place in minimal buffering agents, optionally comprising a pH buffer alone, without detergent or additional salts.

~~2018~~. A method of amplifying a DNA template using a DNA polymerase wherein maintaining a ratio of divalent metal cations to nucleotides in the reaction mixture of 0.5:1 -or below is required, comprising the use of nucleotide salts comprising caesium ~~ions~~cations, wherein the nucleotide salts are present at a concentration greater than 10 mM.

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~~2119~~. The use of nucleotide salts comprising rubidium cations in the enzymatic cell-free synthesis of DNA, wherein the enzymatic cell-free synthesis of DNA takes place in the presence of divalent metal cations, and wherein the nucleotide salts are present at a concentration greater than 10 mM.

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~~2220~~. A cell-free method of amplifying a DNA template, said method comprising contacting said template and a DNA polymerase -with nucleotides in the form of a salt in an amount equal to or greater than 40mM, preferably greater than 60mM or optionally greater than 80mM, wherein said salt comprises ammonium ions, wherein the method further comprises contacting said template and DNA polymerase with divalent metal cations.

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~~2321~~. An enzymatic DNA synthesis ~~which is performed under conditions of reduced concentrations of divalent cations, preferably magnesium,~~ comprising the use of nucleotides in the form of salts comprising a monovalent cation -with an ionic radius greater than that of the sodium ion, and further comprising the addition of divalent metal cations, wherein the ratio between said divalent metal cations and the nucleotide salts is equal to or less than 1:1 in the reaction mixture, and wherein the nucleotide salts are present at a concentration greater than 10 mM.

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~~2422~~. A cell-free process, use, method, cell-free method or enzymatic DNA synthesis of any preceding claim wherein the enzyme is a DNA polymerase, optionally a strand-displacement type polymerase.