

There is therefore a need for a process that can be used to synthesise DNA and like molecules at significant scale.

Summary

5 The present disclosure relates to a process for cell-free production of DNA. The process may allow for enhanced production of DNA compared to current methodologies involving closed batch processes. This significantly increases productivity whilst reducing the cost of synthesising DNA, particularly on a large scale. The present disclosure relates generally to isothermal methods of amplifying DNA that do not require temperature to be cycled via heating and cooling.

10 Accordingly there is provided a cell-free process for synthesising DNA comprising contacting a DNA template with at least one polymerase in the presence of one or more primers and nucleotides to form a reaction mixture, under conditions promoting amplification of said template by displacement of replicated strands through strand-displacement replication of another strand, wherein further nucleotides are supplied in a controlled manner to the reaction mixture.

15 The cell-free process thus involves amplification of the template via strand displacement replication. There is provided a cell-free process for synthesising DNA comprising contacting a DNA template with at least one polymerase in the presence of one or more primers and nucleotides to form a reaction mixture, under conditions promoting amplification of said template by strand displacement replication, wherein further nucleotides are supplied in a controlled manner to the
20 reaction mixture

 Accordingly, there is also provided a cell-free process for synthesising DNA comprising contacting a DNA template with at least one polymerase in the presence of nucleotides to form a reaction mixture, under conditions promoting amplification of said template by displacement of replicated strands through strand-displacement replication of another strand, wherein further
25 nucleotides are supplied in a controlled manner to the reaction mixture.

 In one aspect of the present disclosure, there is provided a cell-free process for synthesising DNA comprising contacting a DNA template with at least one polymerase in the presence of a starting concentration of nucleotides to form a reaction mixture, wherein isothermal amplification of the DNA template occurs via strand-displacement replication and wherein a supply of further
30 nucleotides is required for continued synthesis of DNA by the polymerase, and the amount of further nucleotides supplied to the reaction mixture is controlled.

In another aspect of the present disclosure, there is provided a cell-free process for synthesising DNA comprising contacting a DNA template with at least one polymerase in the presence of a starting concentration of nucleotides and at least one primer to form a reaction mixture, wherein isothermal amplification of the DNA template occurs via strand-displacement replication and wherein a supply of further nucleotides is required for continued synthesis of DNA by the polymerase, and the amount of further nucleotides supplied to the reaction mixture is controlled.

The cell-free process thus involves amplification of the template via strand displacement replication. There is provided a cell-free process for synthesising DNA comprising contacting a DNA template with at least one polymerase in the presence of nucleotides to form a reaction mixture, under conditions promoting amplification of said template by strand displacement replication, wherein further nucleotides are supplied in a controlled manner to the reaction mixture.

Thus, the provision of a controlled supply of further nucleotides to the reaction mixture is advantageous, since this rational control strategy can allow for improvements in the rate of synthesis of the DNA and/or an improved DNA yield. These improvements are compared to an analogous reaction mixture where all the nucleotides are supplied in the reaction mixture at the

Claims

1. A cell-free process for synthesising DNA comprising contacting a DNA template with at least one polymerase in the presence of a starting concentration of nucleotides to form a reaction mixture, wherein isothermal amplification of the DNA template occurs via strand-displacement replication and wherein a supply of further nucleotides is required for continued synthesis of DNA by the polymerase, and the amount of further nucleotides supplied to the reaction mixture is controlled.
2. A process according to claim 1 wherein said reaction mixture further comprises one or more primers.
3. A cell-free process for synthesising DNA comprising contacting a DNA template with at least one polymerase in the presence of a starting concentration of nucleotides and at least one primer to form a reaction mixture, wherein isothermal amplification of the DNA template occurs via strand-displacement replication and wherein a supply of further nucleotides is required for continued synthesis of DNA by the polymerase, and the amount of further nucleotides supplied to the reaction mixture is controlled.
4. A cell-free process for synthesising DNA according to any one of claims 1 to 3 wherein the reaction mixture further comprises one or more metal cations and the supply of further metal cations to the reaction mixture is controlled, optionally independently to the supply of the further nucleotides.
5. The cell-free process of any one of claims 1 to 4 wherein the final yield of DNA from the reaction mixture is large scale, optionally 0.2 – 10 g/l.

6. The cell-free process of claim 5 wherein the starting concentration of nucleotides in the reaction mixture is not sufficient to achieve the final yield.
7. The cell-free process of any one of claims 1 to 6 wherein the further nucleotides are supplied as required by the polymerase.
8. The cell-free process of claim 7 wherein the supply of further nucleotides is controlled to the reaction mixture based upon theoretical calculations of enzyme kinetics for the polymerase.
9. The cell-free process of claim 7 wherein the supply of further nucleotides is controlled to the reaction mixture based upon measureable parameters of the reaction mixture.
10. The cell-free process of claim 9 wherein the measurable parameters of the reaction mixture include the rate of DNA synthesis, the concentration of DNA synthesised, the viscosity of the reaction mixture or the volume of the reaction mixture.
11. The cell-free process of any one of claims 1 to 7 wherein the concentration of DNA synthesised is monitored.
12. The cell-free process of claim 10 or claim 11 wherein the concentration of the DNA in the reaction mixture is monitored by measuring the difference in pressure of the reaction mixture or a part or portion thereof.
13. The process according to any one of claims 1 to 12 wherein said DNA template is circular, and amplification of said DNA template is by rolling circle amplification.
14. The process according to any one of the preceding claims, wherein said DNA template is a closed linear DNA, preferably wherein said DNA template is incubated under denaturing conditions to form a closed circular single stranded DNA.

15. The process according to any one of the preceding claims wherein said DNA template comprises at least one processing enzyme target sequence, preferably a recombinase targeting sequence or a protelomerase target sequence.

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16. The process according to claim 15 wherein a processing enzyme is supplied to the reaction mixture, and said supply is controlled.
- 5 17. The process according to any one of claims 1 to 8 wherein said further nucleotides are supplied in a plurality of aliquots to the reaction mixture, preferably said further nucleotides are supplied as aliquots at regular intervals throughout the duration of the process, optionally at least every 30 minutes.
- 10 18. The process according to claim 4 wherein said further metal ions are supplied as aliquots at regular intervals throughout the duration of the process, optionally at least every 30 minutes.
- 15 19. The process according to any one of the preceding claims wherein said nucleotides or further nucleotides comprise biologically inactive nucleotides, and preferably are supplied to the reaction mixture via activation.
- 20 20. The process according to any one of the preceding claims wherein said process is performed in a synthesis apparatus, said apparatus comprising:
a reaction vessel having at least a first port and a second port;
a temperature control device for controlling the temperature of the reaction vessel;
a plurality of reservoirs for holding reaction components, preferably comprising said further nucleotides and/or further metal ions;
a plurality of conduits, each of the plurality of conduits connecting the first port of the reaction vessel with a respective one of the plurality of reservoirs;
25 at least one pressure sensor located proximate the first port or second port of the reaction vessel;
supply means for selectively supplying controlled amounts of reaction components held in the reservoirs to the reaction vessel;
agitation means for selectively withdrawing an amount of content from the reaction vessel
30 through the second port thereof and selectively returning the contents of the reaction vessel to the reaction vessel through the second port thereof; and
control means for controlling the supply means and the agitation means, wherein, the pressure sensor detects a change in pressure externally of the reaction vessel related to the

concentration of the DNA and sends a signal to the control means and the control means controls the supply means and/or agitation means in response to a change in observed pressure.

- 5 21. The process according to claim 20 wherein said signal indicates the concentration of DNA, and is generated by measuring a pressure difference created by a reciprocating pump that removes and returns a portion of the reaction mixture over the pressure sensor.

22. The process according to claim 4 or claim 20 wherein said metal cations comprise one or more metals selected from the list consisting of: magnesium (Mg^{2+}), manganese (Mn^{2+}), calcium (Ca^{2+}), beryllium (Be^{2+}), zinc (Zn^{2+}) and strontium (Sr^{2+}), lithium (Li^+), sodium (Na^+) or potassium (K^+), preferably Mg^{2+} .

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23. The process according to claim 4, or claim 22, wherein said metal cation is a divalent cation and the ratio between said divalent metal cations and the nucleotides is maintained at about 3:1 in the reaction mixture.

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24. The process according to any preceding claim wherein the concentration of nucleotides in the reaction mixture is maintained between 0.001mM and 6mM, optionally at about 3mM.

25. The process according to any one of the preceding claims wherein said nucleotides and/or further nucleotides are deoxyribonucleoside triphosphates (dNTPs), or a derivative or modified version thereof, preferably wherein said nucleotides and/or further nucleotides are one or more of deoxyadenosine triphosphate (dATP), deoxyguanosine triphosphate (dGTP), deoxycytidine triphosphate (dCTP), deoxythymidine triphosphate (dTTP) and derivatives thereof.

26. The process according to any one of the preceding claims wherein said nucleotides are provided as one or more of free acids, their salts or chelates thereof, optionally wherein said salts or chelates includes one or more of the following metal ions: Mg^{2+} , Be^{2+} , Ca^{2+} , Sr^{2+} , Li^{+} , Na^{+} , K^{+} , Mn^{2+} or Zn^{2+} .