

CLAIMS

1. A linear double stranded DNA comprising a coding strand and a non-coding strand, wherein said DNA comprises (i) a coding sequence element encoded by the coding strand in the direction of from 5' to 3' of the coding strand and (ii) an RNA polymerase promotor sequence element upstream of the coding sequence element, wherein said non-coding strand is coupled at its 3' end to a support or a tag, and wherein said support or tag is the only support or tag coupled to said DNA.
2. The linear double stranded DNA according to claim 1, wherein said non-coding strand is coupled at its 3' end to a support or a tag via a triazole.
3. The linear double stranded DNA according to claim 1 or 2, wherein said tag is biotin.
4. The linear double stranded DNA according to claim 3, wherein said biotin is associated with streptavidin, preferably a streptavidin coated bead, most preferably a streptavidin coated magnetic bead.
5. The linear double stranded DNA according to any one of claims 1 to 4, wherein the coding sequence element is flanked by a 5' UTR and/or a 3' UTR element.
6. A method for producing linear double stranded DNA comprising a coding strand and a non-coding strand, wherein said non-coding strand is coupled at its 3' end to a support or a tag, comprising the steps of:
 - (a) providing linear double stranded DNA comprising a coding sequence element encoded by the coding strand, followed at the 3' end by a restriction site element;
 - (b) incubating said DNA with (i) a modified deoxynucleotide and (ii) an enzyme capable of adding said modified deoxynucleotide at a 3' end of a strand in order to provide linear double stranded DNA with a modified deoxynucleotide at the 3' end of each strand;
 - (c) incubating the DNA obtained in step (b) with a restriction endonuclease recognizing said restriction site element in order to obtain linear double stranded DNA with a modified deoxynucleotide at the 3' end of the non-coding strand;
 - (d) coupling the DNA obtained in step (c) via its modified deoxynucleotide to a support or a tag in order to provide linear double stranded DNA, wherein the non-coding strand of said DNA is coupled at its 3' end to a support or a tag.
7. A method for producing linear double stranded DNA comprising a coding strand and a non-coding strand, wherein said non-coding strand is coupled at its 3' end to a support or a tag, comprising the steps of:
 - (a) providing linear double stranded DNA comprising a coding sequence element encoded by the coding strand, followed at the 3' end by a restriction site element;
 - (b) incubating said DNA with (i) a modified deoxynucleotide and (ii) an enzyme capable of adding said modified deoxynucleotide at a 3' end of a strand in order to provide linear double stranded DNA with a modified deoxynucleotide at the 3' end of each strand;

- (c) coupling the DNA obtained in step (b) via the modified deoxynucleotide at the 3' end of each strand to a support or a tag;
- (d) incubating the DNA obtained in step (c) with a restriction endonuclease recognizing said restriction element in order to provide linear double stranded DNA, wherein the non-coding strand of said DNA is coupled at its 3' end to a support or a tag.
- 5
8. The method according to claim 6 or 7, wherein the modified deoxynucleotide is selected from the group consisting of an alkyne deoxynucleotide, an azide deoxynucleotide, an azadibenzocyclooctyne deoxynucleotide, a *trans*-cyclooctene deoxynucleotide, and a vinyl deoxynucleotide.
- 10
9. The method according to any one of claims 6 to 8, wherein the enzyme capable of adding a modified deoxynucleotide at the 3' end of a strand in step (b) is a DNA polymerase.
10. The method according to claim 9, wherein the DNA polymerase is selected from the group consisting of a *Thermus aquaticus* DNA polymerase, an *Escherichia coli* DNA polymerase, a *Saccharomyces cerevisiae* DP1 DNA polymerase, a mammalian DNA β polymerase, an engineered DNA polymerase, a DNA polymerase I large (Klenow) fragment and a terminal transferase.
- 15
11. The method according to claim 10, wherein the DNA polymerase is a *Thermus aquaticus* DNA polymerase and wherein the linearized DNA provided in step (a) comprises a blunt end at the 5' end of the coding sequence element.
- 20
12. The method according to any one of claims 6 to 11, wherein the support is selected from the group consisting of a magnetic bead, a nanoparticle, agarose, glass, poly(methyl methacrylate), a microchip, sepharose, sephadex and silica and wherein the tag is selected from the group consisting of biotin and PEG.
- 25
13. The method according to any one of claims 6 to 12, wherein the support or the tag used in the coupling step is an activated support or an activated tag.
- 30
14. The method according to claim 13, wherein the activated support or tag is selected from the group consisting of an alkyne-activated support or tag, an azide-activated support or tag, an azadibenzocyclooctyne-activated support or tag, a tetrazine-activated support or tag, and a *trans*-cyclooctene-activated support or tag.
- 35
15. The method according to claim 13 or 14, wherein the modified deoxynucleotide is coupled to the activated support or tag via CuAAC, SPAAC or tetrazine-alkene ligation.
16. The method according to any one of claims 13 to 15, wherein the modified deoxynucleotide is an alkyne deoxynucleotide and wherein the activated support or tag is an azide-activated support or tag.
- 40
17. The method according to any one of claims 13 to 15, wherein the modified deoxynucleotide is an azide deoxynucleotide and wherein the activated support or tag is an alkyne-activated support or tag.

18. The method according to any one of claims 13 to 15, wherein the modified deoxynucleotide is an azadibenzocyclooctyne deoxynucleotide and wherein the activated support or tag is an azide-activated support or tag.
- 5
19. The method according to any one of claims 13 to 15, wherein the modified deoxynucleotide is an azide deoxynucleotide and wherein the activated support or tag is an azadibenzocyclooctyne-activated support or tag.
- 10
20. The method according to any one of claims 13 to 15, wherein the modified deoxynucleotide is a *trans*-cyclooctene deoxynucleotide and wherein the activated support or tag is a tetrazine-activated support or tag.
- 15
21. The method according to any one of claims 13 to 15, wherein the modified deoxynucleotide is a vinyl deoxynucleotide and wherein the activated support or tag is a tetrazine-activated support or tag.
22. The method according to any one of claims 13 to 15, wherein the modified deoxynucleotide is an ethynyl-dNTP and wherein the activated support or tag is an azide-activated support or tag.
- 20
23. The method according to claim 22, wherein the modified deoxynucleotide is an ethynyl-dATP and wherein the activated tag is an azide-activated biotin.
24. The method according to claim 22 or 23, wherein the coupling step is carried out in the presence of Cu(I).
- 25
25. The method according to claim 24, wherein the coupling step is performed in the presence of Cu(I)-TBTA or Cu(I)-THPTA.
26. The method according to claim 24 or 25, wherein an additional washing step is performed in order to remove Cu(I) via complexation to EDTA after the coupling step.
- 30
27. The method according to any one of claims 6 to 26, wherein said method comprises an additional step after the step where said DNA is incubated with a restriction endonuclease, namely an additional step of separating the linear double stranded DNA with a modified deoxynucleotide or with a support or a tag at the 3' end of the non-coding strand from linear double stranded DNA with a modified deoxynucleotide or with a support or a tag at the 3' end of the coding strand.
- 35
28. The method according to claim 27, wherein said separating is achieved via size of the DNA, preferably using AMPure XP beads.
- 40
29. The method according to any one of claims 6 to 28, wherein the restriction site element is an EcoRI site and wherein the restriction endonuclease is EcoRI.

30. A method for producing linear double stranded DNA comprising a coding strand and a non-coding strand, wherein said non-coding strand is coupled at its 3' end to a tag, comprising the steps of:
- 5 (a) providing linear double stranded DNA comprising a coding sequence element encoded by the coding strand, followed at the 3' end by a restriction site element;
- (b) incubating said DNA with (i) a tag-linked deoxynucleotide and (ii) an enzyme capable of adding a tag-linked deoxynucleotide at a 3' end of a strand in order to provide linear double stranded DNA with a tag-linked deoxynucleotide at the 3' end of each strand;
- 10 (c) incubating the DNA obtained in step (b) with a restriction endonuclease recognizing said restriction site element in order to obtain linear double stranded DNA, wherein the non-coding strand of said DNA is coupled at its 3' end to a tag.
31. The method according to claim 30, wherein the tag-linked deoxynucleotide is selected from the group consisting of a biotin-deoxynucleotide and a PEG-deoxynucleotide.
- 15 32. The method according to claim 30 or 31, wherein the enzyme capable of adding a tag-linked deoxynucleotide at the 3' end of a strand in step (b) is selected from the group of *Thermus aquaticus* DNA polymerase and terminal transferase.
- 20 33. The method according to any one of claims 29 to 32, wherein said method comprises an additional step after the step where said DNA is incubated with a restriction endonuclease, namely an additional step of separating the linear double stranded DNA with a tag-linked deoxynucleotide at the 3' end of the non-coding strand from linear double stranded DNA with a tag-linked deoxynucleotide at the 3' end of the coding strand.
- 25 34. The method according to claim 33, wherein said separating is achieved via size of the DNA, preferably using AMPure XP beads.
- 35 35. The method according to any one of claims 30 to 34, wherein the restriction site element is an EcoRI site and wherein the restriction endonuclease is EcoRI.
- 30 36. A method for producing linear double stranded DNA comprising a coding strand and a non-coding strand, wherein said non-coding strand is coupled at its 3' end to a support or a tag, comprising the steps of:
- 35 (c) providing linear double stranded DNA comprising a coding sequence element encoded by the coding strand, wherein said DNA has a blunt end 5' of said coding element and a sticky end 3' of said coding element;
- (d) incubating said DNA with (i) a modified deoxynucleotide and (ii) an enzyme capable of adding said modified deoxynucleotide at a blunt end to the 3' end of a single strand and not at a sticky end in order to provide linear double stranded DNA with a modified deoxynucleotide at the 3' end of the non-coding strand;
- 40 (e) coupling the DNA obtained in step (b) via its modified deoxynucleotide to a support or a tag in order to provide linear double stranded DNA, wherein the non-coding strand of said DNA is coupled at its 3' end to a support or a tag.

37. A method for producing linear double stranded DNA comprising a coding strand and a non-coding strand, wherein said non-coding strand is coupled at its 3' end to a tag, comprising the steps of:
- 5 (a) providing linear double stranded DNA comprising a coding sequence element encoded by the coding strand, wherein said DNA has a blunt end 5' of said coding element and a sticky end 3' of said coding element;
- 10 (b) incubating said DNA with (i) a tag-linked deoxynucleotide and (ii) an enzyme capable of adding a tag-linked deoxynucleotide at a blunt end to the 3' end of a single strand and not at a sticky end in order to provide linear double stranded DNA with a tag-linked deoxynucleotide at the 3' end of the non-coding strand.
38. The method according to claim 36 or 37, wherein the enzyme capable of adding a modified deoxynucleotide or a tag-linked deoxynucleotide at a blunt end to the 3' end of a single strand is *Thermus aquaticus* DNA polymerase.
- 15 39. Use of the linear double stranded DNA according to any one of claims 1 to 5 in an RNA *in vitro* transcription reaction.
40. A method for producing RNA *in vitro* comprising the steps of:
- 20 (a) providing the double stranded linear DNA according to any one of claims 1 to 5 as template DNA;
- (b) providing (i) ribonucleoside triphosphates and (ii) a DNA-dependent RNA polymerase;
- (c) incubating the DNA provided in step (a) with (i) and (ii) provided in step (b) under suitable conditions in order to produce RNA.
- 25 41. The method according to claim 40, wherein the DNA-dependent RNA polymerase is a bacteriophage RNA polymerase, preferably a T3, T7 or SP6 DNA-dependent RNA polymerase.
42. The method according to claim 40 or 41, wherein a cap analogue is additionally provided in step (b).
- 30 43. The method according to any one of claims 40 to 42, wherein a ribonuclease inhibitor is additionally provided in step (b).
44. The method according to any one of claims 40 to 43, wherein pyrophosphatase is additionally provided in step (b).
- 35 45. The method according to any one of claims 40 to 44, wherein $MgCl_2$ is additionally provided in step (b).
46. The method according to any one of claims 40 to 45, wherein the DNA is incubated in step (c) in a buffer suitable for producing RNA *in vitro*.
- 40 47. The method according to any one of claims 40 to 46, wherein the DNA provided in step (a) is re-used in at least one further RNA *in vitro* production cycle.

48. A bioreactor for RNA *in vitro* transcription comprising
- 5 (a) a reaction vessel (13) comprising the linear double stranded DNA according to any one of claims 1 to 5;
- (b) a vessel (14) comprising ribonucleoside triphosphates and DNA-dependent RNA polymerase, wherein said vessel is connected to the reaction vessel; and
- (c) a product vessel (15) for collecting the RNA product, wherein said vessel is also connected to the reaction vessel.
- 10 49. The bioreactor according to claim 48, wherein the reaction vessel (13) comprises the linear double stranded DNA associated with a streptavidin coated magnetic bead according to claim 4.
50. The bioreactor according to claim 49, wherein a magnet is surrounding the reaction vessel (13) from the outside.
- 15 51. The bioreactor according to claim 50, wherein the magnet is capable of oscillating in order to mix a reaction mixture comprising said linear double stranded DNA.
52. The bioreactor according to claim 50 or 51, wherein the magnet is capable of attracting the linear double stranded DNA in order to separate it from the RNA product, which may be collected in the product vessel (15).
- 20 53. The bioreactor according to claim 48, wherein the support or the tag of the linear double stranded DNA according to any one of claims 1 to 5 is linked to said reaction vessel (13).
- 25 54. The bioreactor according to any one of claims 48 to 53, wherein the vessel (14) further comprises at least one of the following independently selected from the group consisting of a buffer suitable for *in vitro* transcription, a cap analogue, modified ribonucleoside triphosphates, a ribonuclease inhibitor, a pyrophosphatase, MgCl₂, an antioxidant and a polyamine.
- 30 55. The bioreactor according to any one of claims 48 to 54, wherein the reaction vessel (13) comprises at least one means for measuring and/or adjusting pH, salt concentration, magnesium concentration, phosphate concentration, temperature, pressure, flow velocity, RNA concentration and/or ribonucleotide triphosphate concentration.
- 35 56. The bioreactor according to any one of claims 48 to 55, wherein the bioreactor comprises a filtration membrane between the reaction vessel (13) and the product vessel (15), preferably an ultrafiltration membrane for separating the RNA product from the reaction mix.
- 40 57. The bioreactor according to claim 56, wherein the filtration or ultrafiltration membrane has a molecular cut-off in a range from 10 to 100 kDa, 10 to 75 kDa, 10 to 50 kDa, 10 to 25 kDa or 10 to 15 kDa.

58. The bioreactor according to claim 56 or 57, wherein the filtration or ultrafiltration membrane is selected from the group consisting of regenerated cellulose, modified cellulose, polysulfone, polyethersulfone, polyacrylonitrile, polymethylmethacrylate, polyvinyl alcohol and polyarylethersulfone.
- 5 59. The bioreactor according to any one of claims 48 to 58, wherein the product vessel (15) comprises a resin to capture the produced RNA and in order to separate the RNA product from other soluble components of the reaction mix.
- 10 60. The bioreactor according to any one of claims 48 to 59, wherein said bioreactor operates in a batch, semi-batch or in a continuous mode.
61. Use of the bioreactor according to any one of claims 48 to 60 in a method according to any one of claims 40 to 47.
- 15 62. A kit comprising
- (a) a modified deoxynucleotide;
 - (b) a *Thermus aquaticus* DNA polymerase capable of adding said modified deoxynucleotide to the 3' end of a strand at a blunt DNA end;
 - 20 (c) an activated support or tag;
 - (d) a counterpart of said support or tag associating in a highly specific manner with said support or tag.
63. A kit comprising
- 25 (a) a tag-linked deoxynucleotide;
 - (b) a *Thermus aquaticus* DNA polymerase capable of adding said tag-linked deoxynucleotide to the 3' end of a strand at a blunt DNA end;
 - (c) a counterpart of said tag associating in a highly specific manner with said tag.