

Claims

1. A poly(N)polymerase characterized in that the poly(N)polymerase is a bacterial poly(N)polymerase and immobilized onto a solid support.
- 5 2. The poly(N)polymerase according to claim 1, wherein the poly(N)polymerase is selected from the group consisting of poly(A)polymerase, poly(U)polymerase, poly(G)polymerase and poly(C)polymerase.
3. The poly(N)polymerase according to claim 1, wherein the poly(N)polymerase is
10 poly(A)polymerase.
4. The poly(N)polymerase according to claim 1, wherein the poly(N)polymerase is immobilized onto the solid support by covalent binding, affinity binding, or physical
15 adsorption.
5. The poly(N)polymerase according to claim 1, wherein the poly(N)polymerase is immobilized onto the solid support by covalent binding.
6. The poly(N)polymerase according to claim 1, wherein, the poly(N)polymerase is
20 immobilized by covalent binding to a thiol-activated solid support, haloacetyl functionalized solid support, epoxy-functionalized solid support, pyridyl disulfide-functionalized solid support, maleimide-activated solid support or a mixture thereof, preferably the poly(N)polymerase is immobilized by covalent binding to a thiol-activated solid support, haloacetyl functionalized solid support, pyridyl disulfide-functionalized solid support,
25 maleimide-activated solid support or a mixture thereof.
7. The poly(N)polymerase according to claim 1, wherein the poly(N)polymerase is immobilized via a thiol group of at least one cysteine residue.
8. The poly(N)polymerase according to claim 4, wherein the covalent binding is a
30 disulfide bridge, thioester bond or a thioether bond, preferably the covalent binding is a disulfide bridge or a thioether bond.

9. The poly(N)polymerase according to claim 1, wherein the solid support comprises a member selected from the group consisting of sepharose, thiopropyl-sepharose, sephadex, agarose, silica, magnetic beads, methacrylate beads, and nanoparticles, preferably the solid support comprises a member selected from the group consisting of sepharose, thiopropyl-sepharose, sephadex, agarose, silica, magnetic beads, and nanoparticles.
10. The poly(N)polymerase according to claim 1, wherein the solid support comprises a reactive group selected from the group consisting of thiol, haloacetyl, pyridyl disulfide, epoxy, maleimide and mixtures thereof.
11. The poly(N)polymerase according to claim 1, wherein the solid support is selected from the group consisting of activated thiol sepharose, thiopropyl-sepharose, thiol-activated sephadex, thiol-activated agarose, silica-based thiol-activated matrix, silica-based thiol-activated magnetic beads, pyridyl disulfide-functionalized nanoparticles, maleimide-activated agarose and mixtures thereof.
12. The poly(N)polymerase according to claim 1, wherein the poly(N)polymerase is derived from *Escherichia coli*, *Streptomyces coelicolor*, *Meiothermus silvanus*, *Bacillus subtilis*, *Thermus aquaticus*, *Shigella flexneri*, *Shigella dysenteriae*, *Citrobacter koseri*, *Salmonella bongori*, *Salmonella enterica*, *Trabulsiella guamensis*, *Kluyvera ascorbata*, *Citrobacter freundii*, *Enterobacter cloacae*, *Enterococcus gallinarum*, *Grimontia indica*, or *Salinivibrio costicola*.
13. The poly(N)polymerase according to claim 1, wherein the poly(N)polymerase comprises an amino acid sequence being at least 80% identical to an amino acid sequence as depicted in any one of SEQ ID NOs: 1 to 22, 24 to 155, or 203, preferably comprises an amino acid sequence being at least 80% identical to any one of SEQ ID NOs: 1, 2, 3, 16 to 22, 24 to 155, or 203, more preferably at least 80% identical to any one of SEQ ID NOs: 17 to 22, 32 to 83, 85-111, 113-139, 141-145, 146-148, 150-152, 154-155, or 203, even more preferably at least 80% identical to any one of SEQ ID NOs: 18, 58-83, 85-111, 113-139, or 203, and most preferably at least 80% identical to SEQ ID NO: 113.
14. The poly(N)polymerase according to claim 1, wherein the poly(N)polymerase comprises at least one newly introduced cysteine residues compared to a native poly(N)polymerase,

preferably the poly(N)polymerase comprises an amino acid sequence as depicted in any one of SEQ ID NOs: 17-22, 32-155, or 203.

15 15. The poly(N)polymerase according to claim 1, wherein the poly(N)polymerase comprises only one cysteine residue or is mutated to comprise only one cysteine residue, preferably the poly(N)polymerase comprises an amino acid sequence as depicted in any one of SEQ ID NOs: 2, 16-22, 24-83, 85-111, 113-139, 141-144, 146-148, 150-152, 154-155, or 203.

10 16. The poly(N)polymerase according to claim 1, wherein the poly(N)polymerase comprises a linker element as depicted in SEQ ID Nos: 156-180.

17. The poly(N)polymerase according to claim 1, wherein the poly(N)polymerase comprises a purification tag as depicted in SEQ ID NOs: 181-201.

15 18. A method for producing the poly(N)polymerase, preferably being a poly(A)polymerase, characterized in that the poly(N)polymerase is a bacterial poly(N)polymerase and immobilized onto a solid support, comprising a step of contacting the poly(N)polymerase with a solid support under conditions suitable for immobilizing the poly(N)polymerase to the solid support by covalent binding, affinity binding, or physical adsorption.

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19. A poly(N)polymerase, preferably being a poly(A)polymerase, being immobilized onto a solid support for producing polynucleotidylated ribonucleic acid (poly(N)RNA) molecules, preferably polyadenylated ribonucleic acid (poly(A)RNA) molecules, for use in gene therapy, immunotherapy, protein replacement therapy and/or vaccination.

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20. An enzyme reactor comprising a poly(N)polymerase being immobilized onto a solid support or comprising a poly(N)polymerase according to any one of claims 1 to 16 or comprising a poly(N)polymerase obtainable by the method according to claim 18, preferably the poly(N)polymerase is a poly(A)polymerase.

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21. A kit comprising a poly(N)polymerase characterized in that the poly(N)polymerase is immobilized onto a solid support, a poly(N)polymerase reaction buffer, and nucleoside monophosphates, optionally a nucleotide mixture, further optionally an RNA polymerase, and optionally an RNA *in vitro* transcription buffer.

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