

Mg²⁺, Ca²⁺, Sr²⁺, Ba²⁺, Mn²⁺, Co²⁺, Fe²⁺, Ni²⁺, Cu²⁺, Zn²⁺, and their combinations thereof. In an exemplary embodiment, the metal cofactor is Mg²⁺. In another embodiment, the metal cofactor is a combination of Mg²⁺ and Mn²⁺.

According to the present disclosure, the nucleotide monomer may have a removable blocking moiety. Examples of the removable blocking moiety include, but are not limited to, a 3'-O-blocking moiety, a base blocking moiety, and a combination thereof.

The nucleotide monomer having a removable blocking moiety is also referred to as a reversible terminator. Therefore, the nucleotide monomer having the 3'-O-blocking moiety is also referred to as 3'-blocked reversible terminator or a 3'-O-modified reversible terminator, and the nucleotide monomer having the base blocking moiety is also referred to as a 3'-unblocked reversible terminator or a 3'-OH unblocked reversible terminator.

As used herein, the term "reversible terminator" refers to a chemically modified nucleotide monomer. When such a reversible terminator is incorporated into a growing nucleic acid by a polymerase, it blocks the further incorporation of a nucleotide monomer by the polymerase. Such "reversible terminator" base and a nucleic acid can be deprotected by chemical or physical treatment, and following such deprotection, the nucleic acid can be further extended by a polymerase.

Examples of the 3'-O-blocking moiety include, but are not limited to, O-azidomethyl, O-amino, O-allyl, O-phenoxyacetyl, O-methoxyacetyl, O-acetyl, O-(p-toluene)sulfonate, O-phosphate, O-nitrate, O-[4-methoxy]-tetrahydrothiopyranyl, O-tetrahydrothiopyranyl, O-[5-methyl]-tetrahydrofuranyl, O-[2-methyl,4-methoxy]-tetrahydropyranyl, O-[5-methyl]-tetrahydropyranyl, and O-tetrahydrothiofuranyl, θO -2-nitrobenzyl, θO -methyl, and O-acyl.

Examples of the 3'-unblocked reversible terminators include, but are not limited to, 7-[(S)-1-(5-methoxy-2-nitrophenyl)-2,2-dimethyl-propyloxy]methyl-7-deazadATP, 5-[(S)-1-(5-methoxy-2-nitrophenyl)-2,2-dimethyl-propyloxy]methyl-dCTP, 1-(5-methoxy-2-nitrophenyl)-2,2-dimethyl-propyloxy]methyl-7-deaza-dGTP, 5-[(S)-1-(5-methoxy-2-nitrophen-yl)-2,2- dimethyl-propyloxy]methyl-dUTP, and 5-[(S)-1-(2-nitrophenyl)-2,2-dimethyl-propyloxy]methyl-dUTP.

According to the present disclosure, the base blocking moiety may be a reversible dye-terminator. Examples of the reversible dye-terminator include, but are not limited to, a reversible dye-terminator of Illumina NovaSeq, a reversible dye-terminator of Illumina NextSeq, a reversible dye-terminator of Illumina MiSeq, a reversible dye-terminator of

gene construct were subjected to site-directed mutagenesis using Q5 Site-directed Mutagenesis Kit (New England Biolabs, Ipswich, MA, USA). The resulting mutagenized gene construct was expressed in BL21(DE3) cells, and the expressed protein was purified using Akta Pure FPLC system (GE Healthcare Life Sciences, Marlborough, MA, USA) through HisTrap Q and Heparin columns sequentially. KOD1^{exo-} DNA polymerase thus obtained has an amino acid sequence of SEQ ID NO: 2.

10 μ L of the nucleic acid synthesis reaction mixture was preincubated for 2 minutes at one of the following temperatures: 10°C, 20°C, 30°C, 35°C, 40°C, 45°C, 50°C, 55°C, 60°C, 70°C, 80°C, and 90°C. Subsequently, a suitable amount of Mg²⁺ serving as metal cofactors were added into the respective reaction mixture to initiate the template-independent nucleic acid synthesis, which was allowed to proceed for 5 minutes. The synthesis was terminated by adding 10 μ L of 2X quench solution (containing 95% de-ionized formamide and 25 mM ethylenediaminetetraacetic acid (EDTA)).

The resulting synthesis reaction products were subjected to denaturation at 98°C for 10 minutes. Subsequently, the synthesis reaction products were analyzed by a 15% denaturing urea-polyacrylamide gel. The synthesis reaction products on the gel were

that the efficiency of template-independent nucleic acid synthesis with a family-B DNA polymerase can be enhanced using a combination of different types of divalent cations.

All patents and references cited in this specification are incorporated herein in their entirety as reference. Where there is conflict, the descriptions in this case, including the definitions, shall prevail.

While the disclosure has been described in connection with what are considered the exemplary embodiments, it is understood that this disclosure is not limited to the disclosed embodiments but is intended to cover various arrangements included within the spirit and scope of the broadest interpretation so as to encompass all such modifications and equivalent arrangements.

polyformaldehyde, cellulose, cellulose acetate, paper, a ceramic, a metal, a metalloid, a semiconductor material, a magnetic particle, a plastic, a gel-forming material, a gel, a nanostructured surface, a nanotube, and a nanoparticle.

5. The method of Claim 1, wherein the initiator is exposed to the nucleotide monomer at a temperature ranging from 10°C to 90°C.
6. The method of Claim 1, wherein the initiator is exposed to the nucleotide monomer at a pH of not less than 8.0.
7. The method of Claim 1, wherein the metal cofactor is selected from the group consisting of Mg^{2+} , Ca^{2+} , Sr^{2+} , Ba^{2+} , Mn^{2+} , Co^{2+} , Fe^{2+} , Ni^{2+} , Cu^{2+} , Zn^{2+} and combinations thereof.
8. The method of Claim 1, wherein the nucleic acid polymerase further has a 3' to 5' exonuclease domain and is a family-B DNA polymerase selected from the group consisting of a bacterial family-B DNA polymerase, a eukaryotic family-B DNA polymerase, an archaeal family-B DNA polymerase, and a viral family-B DNA polymerase.
9. The method of Claim 8, wherein the family-B DNA polymerase is selected from the group consisting of a family-B DNA polymerase of *Thermococcus kodakaraensis* (KOD1), a family-B DNA polymerase of