

CLAIMS

What is claimed is:

1. A B-family DNA polymerase variant modified from a wild-type B-family DNA polymerase having an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, and 17, wherein
an amino acid L at position 408 of SEQ ID NOs: 2, 3, or 4 is substituted with F, H, I, Q, S, W, or Y;
an amino acid L at position 409 of SEQ ID NO: 5 is substituted with F, H, I, Q, S, W, or Y;
an amino acid L at position 411 of SEQ ID NO: 6 is substituted with F, H, I, Q, S, W, or Y;
an amino acid L at position 485 of SEQ ID NO: 7 is substituted with F, H, I, Q, S, W, or Y;
an amino acid M at position 426 of SEQ ID NO: 8 is substituted with F, H, I, Q, S, W, or Y;
an amino acid L at position 518 of SEQ ID NO: 9 is substituted with F, H, I, Q, S, W, or Y;
an amino acid L at position 417 of SEQ ID NO: 10 is substituted with F, H, I, Q, S, W, or Y;
an amino acid L at position 606 of SEQ ID NO: 11 is substituted with F, H, I, Q, S, W, or Y;
an amino acid L at position 612 of SEQ ID NO: 12 is substituted with F, H, I, Q, S, W, or Y;
an amino acid L at position 425 of SEQ ID NO: 13 is substituted with F, H, I, Q, S, W, or Y;

an amino acid L at position 423 of SEQ ID NO: 14 is substituted with F, H, I, Q, S, W, or Y;

an amino acid L at position 415 of SEQ ID NO: 15 is substituted with F, H, I, Q, S, W, or Y;

an amino acid L at position 412 of SEQ ID NO: 16 is substituted with F, H, I, Q, S, W, or Y; or

an amino acid L at position 253 of SEQ ID NO: 17 is substituted with F, H, I, Q, S, W, or Y.

2. The B-family DNA polymerase variant of claim 1, wherein the B-family DNA polymerase variant is modified from a wild-type B-family DNA polymerase having an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 3, 4, 5, 6, 7, 8, and 9.
3. The B-family DNA polymerase variant of claim 1, wherein the wild-type B-family DNA polymerase is *Thermococcus gorgonarius* DNA polymerase (Tgo), *Thermococcus kodakarensis* DNA polymerase (Kod1), *Thermococcus* sp. (strain 9°N-7) DNA polymerase (9°N), *Pyrococcus furiosus* DNA polymerase (Pfu), *Thermococcus litoralis* DNA polymerase (Vent), *Methanococcus maripaludis* DNA polymerase (Mma), *Methanosarcina acetivorans* DNA polymerase (Mac), human DNA polymerase delta catalytic p125 subunit (hPOLD), *Saccharomyces cerevisiae* DNA polymerase delta catalytic subunit (ScePOLD), *Pyrobaculum islandicum* DNA polymerase (Pis), *Sulfolobus solfataricus* DNA polymerase (Sso), *Pseudomonas aeruginosa* DNA polymerase II (Pae), *Escherichia coli* DNA polymerase II (Eco), *Escherichia* phage RB69 DNA polymerase (RB69), *Escherichia* phage T4 DNA polymerase (T4), or *Bacillus* phage Phi29 DNA polymerase (Phi29).
4. The B-family DNA polymerase variant of claim 3, wherein the B-family DNA

polymerase variant has deficient 3' to 5' exonuclease activity.

5. The B-family DNA polymerase variant of claim 4, wherein the B-family DNA polymerase variant having deficient 3' to 5' exonuclease activity is derived from *Thermococcus gorgonarius* DNA polymerase (Tgo) having a wild-type amino acid sequence of SEQ ID NO: 2; and wherein
 - i. an amino acid Y at position 409 of SEQ ID NO: 2 is not substituted or substituted with A, C, D, F, G, H, I, K, L, M, N, or Q; and
 - ii. an amino acid P at position 410 of SEQ ID NO: 2 is not substituted or substituted with A, G, S, or T.
6. The B-family DNA polymerase variant of claim 4, wherein the B-family DNA polymerase variant having deficient 3' to 5' exonuclease activity is derived from *Thermococcus gorgonarius* DNA polymerase (Tgo) having a wild-type amino acid sequence of SEQ ID NO: 2; and wherein
 - i. an amino acid Y at position 409 of SEQ ID NO: 2 is not substituted or substituted with A, C, D, F, G, H, I, K, L, M, N, or Q;
 - ii. an amino acid P at position 410 of SEQ ID NO: 2 is not substituted or substituted with A, G, S, or T; and
 - iii. an amino acid A at position 485 of SEQ ID NO: 2 is substituted with C, D, E, F, G, H, K, L, R, T, or Y.
7. The B-family DNA polymerase variant of claim 4, wherein the B-family DNA polymerase variant having deficient 3' to 5' exonuclease activity is derived from *Thermococcus kodakarensis* DNA polymerase (Kod1) having a wild-type amino acid sequence of SEQ ID NO: 3; and wherein:
 - i. an amino acid Y at position 409 of SEQ ID NO: 3 is not substituted or substituted with A, C, D, F, G, H, I, K, L, M, N, or Q; and
 - ii. an amino acid P at position 410 of SEQ ID NO: 3 is not substituted or

substituted with A, G, S, or T.

8. The B-family DNA polymerase variant of claim 4, wherein the B-family DNA polymerase variant having deficient 3' to 5' exonuclease activity is derived from *Thermococcus kodakarensis* DNA polymerase (Kod1) having a wild-type amino acid sequence of SEQ ID NO: 3; and wherein
 - i. an amino acid Y at position 409 of SEQ ID NO: 3 is not substituted or substituted with A, C, D, F, G, H, I, K, L, M, N, or Q;
 - ii. an amino acid P at position 410 of SEQ ID NO: 3 is not substituted or substituted with A, G, S, or T; and
 - iii. an amino acid A at position 485 of SEQ ID NO: 3 is substituted with C, D, E, F, G, H, K, L, R, T, or Y.
9. The B-family DNA polymerase variant of claim 4, wherein the B-family DNA polymerase variant having deficient 3' to 5' exonuclease activity is derived from *Thermococcus sp.* (strain 9°N-7) DNA polymerase (9°N) having a wild-type amino acid sequence of SEQ ID NO: 4; and wherein
 - i. an amino acid Y at position 409 of SEQ ID NO: 4 is not substituted or substituted with A, C, D, F, G, H, I, K, L, M, N, or Q; and
 - ii. an amino acid P at position 410 of SEQ ID NO: 4 is not substituted or substituted with A, G, S, or T.
10. The B-family DNA polymerase variant of claim 4, wherein the B-family DNA polymerase variant having deficient 3' to 5' exonuclease activity is derived from *Thermococcus sp.* (strain 9°N-7) DNA polymerase (9°N) having a wild-type amino acid sequence of SEQ ID NO: 4; and wherein
 - i. an amino acid Y at position 409 of SEQ ID NO: 4 is not substituted or substituted with A, C, D, F, G, H, I, K, L, M, N, or Q;
 - ii. an amino acid P at position 410 of SEQ ID NO: 4 is not substituted or

- substituted with A, G, S, or T; and
- iii. an amino acid A at position 485 of SEQ ID NO: 4 is substituted with C, D, E, F, G, H, K, L, R, T, or Y.
11. The B-family DNA polymerase variant of claim 4, wherein the B-family DNA polymerase variant having deficient 3' to 5' exonuclease activity is derived from *Pyrococcus furiosus* DNA polymerase (Pfu) having a wild-type amino acid sequence of SEQ ID NO: 5; and wherein
- i. an amino acid Y at position 410 of SEQ ID NO: 5 is not substituted or substituted with A, C, D, F, G, H, I, K, L, M, N, or Q; and
 - ii. an amino acid P at position 411 of SEQ ID NO: 5 is not substituted or substituted with A, G, S, or T.
12. The B-family DNA polymerase variant of claim 4, wherein the B-family DNA polymerase variant having deficient 3' to 5' exonuclease activity is derived from *Pyrococcus furiosus* DNA polymerase (Pfu) having a wild-type amino acid sequence of SEQ ID NO: 5; and wherein
- i. an amino acid Y at position 410 of SEQ ID NO: 5 is not substituted or substituted with A, C, D, F, G, H, I, K, L, M, N, or Q;
 - ii. an amino acid P at position 411 of SEQ ID NO: 5 is not substituted or substituted with A, G, S, or T; and
 - iii. an amino acid A at position 486 of SEQ ID NO: 5 is substituted with C, D, E, F, G, H, K, L, R, T, or Y.
13. The B-family DNA polymerase variant of claim 4, wherein the B-family DNA polymerase variant having deficient 3' to 5' exonuclease activity is derived from *Thermococcus litoralis* DNA polymerase (Vent) having a wild-type amino acid sequence of SEQ ID NO: 6; and wherein
- i. an amino acid Y at position 412 of SEQ ID NO: 6 is not substituted or

- substituted with A, C, D, F, G, H, I, K, L, M, N, or Q; and
- ii. an amino acid P at position 413 of SEQ ID NO: 6 is not substituted or substituted with A, G, S, or T.
14. The B-family DNA polymerase variant of claim 4, wherein the B-family DNA polymerase variant having deficient 3' to 5' exonuclease activity is derived from *Thermococcus litoralis* DNA polymerase (Vent) having a wild-type amino acid sequence of SEQ ID NO: 6; and wherein
- i. an amino acid Y at position 412 of SEQ ID NO: 6 is not substituted or substituted with A, C, D, F, G, H, I, K, L, M, N, or Q;
 - ii. an amino acid P at position 413 of SEQ ID NO: 6 is not substituted or substituted with A, G, S, or T; and
 - iii. an amino acid A at position 488 of SEQ ID NO: 6 is substituted with C, D, E, F, G, H, K, L, R, T, or Y.
15. The B-family DNA polymerase variant of claim 4, wherein the B-family DNA polymerase variant having deficient 3' to 5' exonuclease activity is derived from *Methanosarcina acetivorans* DNA polymerase (Mac) having a wild-type amino acid sequence of SEQ ID NO: 7; and wherein
- i. an amino acid Y at position 486 of SEQ ID NO: 7 is not substituted or substituted with A, C, D, F, G, H, I, K, L, M, N, or Q; and
 - ii. an amino acid P at position 487 of SEQ ID NO: 7 is not substituted or substituted with A, G, S, or T.
16. The B-family DNA polymerase variant of claim 4, wherein the B-family DNA polymerase variant having deficient 3' to 5' exonuclease activity is derived from *Methanosarcina acetivorans* DNA polymerase (Mac) having a wild-type amino acid sequence of SEQ ID NO: 7; and wherein
- i. an amino acid Y at position 486 of SEQ ID NO: 7 is not substituted or

- substituted with A, C, D, F, G, H, I, K, L, M, N, or Q;
 - ii. an amino acid P at position 487 of SEQ ID NO: 7 is not substituted or substituted with A, G, S, or T; and
 - iii. the amino acid A at position 565 of SEQ ID NO: 7 is substituted with C, D, E, F, G, H, K, L, R, T, or Y.
17. The B-family DNA polymerase variant of claim 4, wherein the B-family DNA polymerase variant having deficient 3' to 5' exonuclease activity is derived from *Pyrobaculum islandicum* DNA polymerase (Pis) having a wild-type amino acid sequence of SEQ ID NO: 8; and wherein
- i. an amino acid Y at position 427 of SEQ ID NO: 8 is not substituted or substituted with A, C, D, F, G, H, I, K, L, M, N, or Q; and
 - ii. an amino acid P at position 428 of SEQ ID NO: 8 is not substituted or substituted with A, G, S, or T.
18. The B-family DNA polymerase variant of claim 4, wherein the B-family DNA polymerase variant having deficient 3' to 5' exonuclease activity is derived from *Pyrobaculum islandicum* DNA polymerase (Pis) having a wild-type amino acid sequence of SEQ ID NO: 8; and wherein
- i. an amino acid Y at position 427 of SEQ ID NO: 8 is not substituted or substituted with A, C, D, F, G, H, I, K, L, M, N, or Q;
 - ii. an amino acid P at position 428 of SEQ ID NO: 8 is not substituted or substituted with A, G, S, or T; and
 - iii. an amino acid A at position 508 of SEQ ID NO: 8 is substituted with C, D, E, F, G, H, K, L, R, T, or Y.
19. The B-family DNA polymerase variant of claim 4, wherein the B-family DNA polymerase variant having deficient 3' to 5' exonuclease activity is derived from *Sulfolobus solfataricus* DNA polymerase (Sso) having a wild-type amino acid

sequence of SEQ ID NO: 9; and wherein

- i. an amino acid Y at position 519 of SEQ ID NO: 9 is not substituted or substituted with A, C, D, F, G, H, I, K, L, M, N, or Q; and
- ii. an amino acid P at position 520 of SEQ ID NO: 9 is not substituted or substituted with A, G, S, or T.

20. The B-family DNA polymerase variant of claim 4, wherein the B-family DNA polymerase variant having deficient 3' to 5' exonuclease activity is derived from *Sulfolobus solfataricus* DNA polymerase (Sso) having a wild-type amino acid sequence of SEQ ID NO: 9; and wherein

- i. an amino acid Y at position 519 of SEQ ID NO: 9 is not substituted or substituted with A, C, D, F, G, H, I, K, L, M, N, or Q;
- ii. an amino acid P at position 520 of SEQ ID NO: 9 is not substituted or substituted with A, G, S, or T; and
- iii. an amino acid A at position 601 of SEQ ID NO: 9 is substituted with C, D, E, F, G, H, K, L, R, T, or Y.

21. The B-family DNA polymerase variant of claim 4, wherein the B-family DNA polymerase variant exhibits an activity of synthesizing nucleic acids in a template-independent manner by adding at least one nucleotide selected from the group of naturally occurring nucleotide, nucleotide analogue, or a mixture thereof, to an extendible initiator.

22. The B-family DNA polymerase variant of claim 21, wherein the extendible initiator comprises a single-stranded oligonucleotide initiator, a blunt-ended double-stranded oligonucleotide initiator, or a mixture thereof.

23. The B-family DNA polymerase variant of claim 21, wherein the extendible initiator is free form nucleic acid to be reacted in a liquid phase.

24. The B-family DNA polymerase variant of claim 21, wherein the extendible initiator

is immobilized on a solid support, wherein the solid support comprises a particle, bead, slide, array surface, membrane, flow cell, well, microwell, nano-well, chamber, microfluidic chamber, channel, microfluidic channel, or any other surfaces.

25. The B-family DNA polymerase variant of claim 21, wherein the at least one nucleotide is linked with a detectable label.

26. The B-family DNA polymerase variant of claim 21, wherein the B-family DNA polymerase variant exhibits the activity at reaction temperatures from 10°C to 100°C.

27. A kit for performing *de novo* enzymatic nucleic acid synthesis, comprising a B-family DNA polymerase variant derived from a wild-type B-family DNA polymerase having an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, and 17, wherein the B-family DNA polymerase variant exhibits activity of synthesizing nucleic acids in a template-independent manner by adding at least one nucleotide selected from the group of naturally occurring nucleotide, nucleotide analogue, or a mixture thereof, to an extendible initiator, thereby synthesizing a desired nucleic acid sequence, and wherein

an amino acid L at position 408 of SEQ ID NOs: 2, 3, or 4 is substituted with F, H, I, Q, S, W, or Y;

an amino acid L at position 409 of SEQ ID NO: 5 is substituted with F, H, I, Q, S, W, or Y;

an amino acid L at position 411 of SEQ ID NO: 6 is substituted with F, H, I, Q, S, W, or Y;

an amino acid L at position 485 of SEQ ID NO: 7 is substituted with F, H, I, Q, S, W, or Y;

an amino acid M at position 426 of SEQ ID NO: 8 is substituted with F, H, I, Q, S, W, or Y;

an amino acid L at position 518 of SEQ ID NO: 9 is substituted with F, H, I, Q, S, W, or Y;

an amino acid L at position 417 of SEQ ID NO: 10 is substituted with F, H, I, Q, S, W, or Y;

an amino acid L at position 606 of SEQ ID NO: 11 is substituted with F, H, I, Q, S, W, or Y;

an amino acid L at position 612 of SEQ ID NO: 12 is substituted with F, H, I, Q, S, W, or Y;

an amino acid L at position 425 of SEQ ID NO: 13 is substituted with F, H, I, Q, S, W, or Y;

an amino acid L at position 423 of SEQ ID NO: 14 is substituted with F, H, I, Q, S, W, or Y;

an amino acid L at position 415 of SEQ ID NO: 15 is substituted with F, H, I, Q, S, W, or Y;

an amino acid L at position 412 of SEQ ID NO: 16 is substituted with F, H, I, Q, S, W, or Y; or

an amino acid L at position 253 of SEQ ID NO: 17 is substituted with F, H, I, Q, S, W, or Y.