What is claimed is:

1. A method for detecting a target nucleic acid sequence from a DNA or a mixture of nucleic acids by a PTOCE (PTO Cleavage and Extension) assay, comprising:

(a) hybridizing the target nucleic acid sequence with an upstream oligonucleotide and a PTO (Probing and Tagging Oligonucleotide); wherein the upstream oligonucleotide comprises a hybridizing nucleotide sequence complementary to the target nucleic acid sequence; the PTO comprises (i) a 3'-targeting portion comprising a hybridizing nucleotide sequence complementary to the target nucleic acid sequence and (ii) a 5'-tagging portion comprising a nucleotide sequence non-complementary to the target nucleic acid sequence; wherein the 3'-targeting portion is hybridized with the target nucleic acid sequence and the 5'-tagging portion is not hybridized with the target nucleic acid sequence; the upstream oligonucleotide is located upstream of the PTO;

(b) contacting the resultant of the step (a) to an enzyme having a 5' nuclease activity under conditions for cleavage of the PTO; wherein the upstream oligonucleotide or its extended strand induces cleavage of the PTO by the enzyme having the 5' nuclease activity such that the cleavage releases a fragment comprising the 5'-tagging portion or a part of the 5'-tagging portion of the PTO;

(c) hybridizing the fragment released from the PTO with a CTO (Capturing and Templating Oligonucleotide); wherein the CTO comprises in a 3' to 5' direction (i) a capturing portion comprising a nucleotide sequence complementary to the 5'-tagging portion or a part of the 5'-tagging portion of the PTO and (ii) a templating portion comprising a nucleotide sequence non-complementary to the 5'-tagging portion and the 3'-targeting portion of the PTO; wherein the fragment released from the PTO is hybridized with the capturing portion of the CTO;

(d) performing an extension reaction using the resultant of the step (c) and a template-dependent nucleic acid polymerase; wherein the fragment hybridized with the capturing portion of the CTO is extended and an extended duplex is

formed; wherein the extended duplex has a T_m value adjustable by (i) a sequence and/or length of the fragment, (ii) a sequence and/or length of the CTO or (iii) the sequence and/or length of the fragment and the sequence and/or length of the CTO;

(e) melting the extended duplex over a range of temperatures to give a target signal indicative of the presence of the extended duplex; wherein the target signal is provided by (i) at least one label linked to the fragment and/or the CTO, (ii) a label incorporated into the extended duplex during the extension reaction, (iii) a label incorporated into the extended duplex during the extension reaction and a label linked to the fragment and/or the CTO, or (iv) an intercalating label; and

(f) detecting the extended duplex by measuring the target signal; whereby the presence of the extended duplex indicates the presence of the target nucleic acid sequence.

2. The method according to claim 1, wherein the presence of the extended duplex is detected by melting analysis.

3. The method according to claim 1, wherein the melting of the step (e) is followed by hybridization to give the target signal indicative of the presence of the extended duplex.

4. The method according to claim 3, wherein the presence of the extended duplex is detected by hybridization analysis.

5. The method according to claim 1, wherein the target signal is provided by at least one label linked to the fragment and/or the CTO.

6. The method according to claim 5, wherein the fragment or the CTO has an interactive dual label comprising a reporter molecule and a quencher molecule;

wherein the melting of the extended duplex in the step (e) induces change of a signal from the interactive dual label to give the target signal in the step (e).

7. The method according to claim 5, wherein the fragment has one of an interactive dual label comprising a reporter molecule and a quencher molecule and the CTO has the other of the interactive dual label; wherein the melting of the extended duplex in the step (e) induces change of a signal from the interactive dual labels to give the target signal in the step (e).

8. The method according to claim 5, wherein the fragment or the CTO has a single label, and the melting of the extended duplex in the step (e) induces change of a signal from the single label to give the target signal in the step (e).

9. The method according to claim 5, wherein the labels are positioned to the extent that when a hybrid between an uncleaved PTO and the CTO is formed, the hybrid does not give a non-target signal in the step (e).

10. The method according to claim 5, wherein the labels are positioned to the extent that when a hybrid between an uncleaved PTO and the CTO is formed, the hybrid gives a non-target signal in the step (e); wherein the T_m value of the extended duplex is higher than that of the hybrid between the uncleaved PTO and the CTO.

11. The method according to claim 1, wherein the target signal is provided by a single label incorporated into the extended duplex during the extension reaction; wherein the incorporated single label is linked to a nucleotide incorporated during the extension reaction; wherein the melting of the extended duplex in the step (e) induces change of a signal from the single label to give the target signal in the step (e).

12. The method according to claim 11, wherein the nucleotide incorporated during the extension reaction has a first non-natural base and the CTO has a nucleotide having a second non-natural base with a specific binding affinity to the first non-natural base.

13. The method according to claim 1, wherein the target signal is provided by a label incorporated into the extended duplex during the extension reaction and a label linked to the fragment and/or the CTO, and the incorporated label is linked to a nucleotide incorporated during the extension reaction; wherein the two labels are an interactive dual label of a reporter molecule and a quencher molecule; wherein the melting of the extended duplex in the step (e) induces change of a signal from the interactive dual label to give the target signal in the step (e).

14. The method according to claim 13, wherein the nucleotide incorporated during the extension reaction has a first non-natural base and the CTO has a nucleotide having a second non-natural base with a specific binding affinity to the first non-natural.

15. The method according to claim 1, wherein the PTO and/or CTO is blocked at its 3'-end to prohibit its extension.

16. The method according to claim 1, wherein the upstream oligonucleotide is an upstream primer or an upstream probe.

17. The method according to claim 1, wherein the upstream oligonucleotide has a partial-overlapped sequence with the 3'-targeting portion of the PTO.

18. The method according to claim 1, wherein the upstream oligonucleotide is located adjacently to the PTO to the extent that the upstream oligonucleotide induces

cleavage of the PTO by the enzyme having the 5′ nuclease activity.

19. The method according to claim 16, wherein the upstream primer induces through its extended strand the cleavage of the PTO by the enzyme having the 5′ nuclease activity.

20. The method according to claim 1, wherein the capturing portion comprises at its 5′-end part a nucleotide sequence complementary to a 5′-end part of the 3′-targeting portion of the PTO.

21. The method according to claim 20, wherein the nucleotide sequence complementary to the 5′-end part of the 3′-targeting portion of the PTO is 1-5 nucleotides in length.

22. The method according to claim 1, wherein the method further comprises repeating the steps (a)-(b), (a)-(d) or (a)-(f) with denaturation between repeating cycles.

23. The method according to claim 1, wherein the steps (a)-(f) are performed in a reaction vessel or in separate reaction vessels.

24. The method according to claim 1, wherein the method is performed to detect at least two types of target nucleic acid sequences; wherein the upstream oligonucleotide comprises at least two types of oligonucleotides, the PTO comprises at least two types of the PTOs and the CTO comprises at least one type of the CTOs.

25. The method according to claim 24, wherein the 5′-tagging portions of the at least two types of PTOs have the identical sequence to each other.

26. The method according to claim 24, wherein the extended duplexes corresponding to the at least two types of the target nucleic acid sequences have different T_m values from each other.

27. The method according to claim 24, wherein the at least two types of the target signals corresponding to the at least two types of the target nucleic acid sequences are provided from different types of labels from each other.

28. The method according to claim 24, wherein the at least two types of the target signals corresponding to the at least two types of the target nucleic acid sequences are provided from the same type of labels.

29. The method according to claim 28, wherein the at least two type of the target signals corresponding to the at least two types of the target nucleic acid sequences are provided from the same type of labels; wherein the extended duplexes corresponding to the at least two types of the target nucleic acid sequences have different T_m values from each other.

30. The method according to claim 1, wherein the upstream oligonucleotide is an upstream primer and the step (b) uses a template-dependent nucleic acid polymerase for the extension of the upstream primer.

31. The method according to claim 1, wherein the enzyme having the 5' nuclease activity is a thermostable DNA polymerase having a 5' nuclease activity or FEN nuclease.

32. The method according to claim 1, wherein the target nucleic acid sequence comprises a nucleotide variation.

33. The method according to claim 1, wherein the CTO is immobilized through its 5'-end or its 3'-end onto a solid substrate.

34. The method according to claim 33, wherein the target signal is provided by a single label linked to the fragment or by a single label incorporated into the extended duplex during the extension reaction.

35. The method according to any one of claims 1-34, wherein the method is performed in the presence of a downstream primer.

36. A method for detecting a target nucleic acid sequence from a DNA or a mixture of nucleic acids by a PTOCE (PTO Cleavage and Extension) assay, comprising:

(a) hybridizing the target nucleic acid sequences with a primer pair comprising an upstream primer and a downstream primer and a PTO (Probing and Tagging Oligonucleotide); wherein each of the upstream primer and the downstream primer comprise a hybridizing nucleotide sequence complementary to the target nucleic acid sequences; the PTO comprises (i) a 3'-targeting portion comprising a hybridizing nucleotide sequence complementary to the target nucleic acid sequence and (ii) a 5'-tagging portion comprising a nucleotide sequence non-complementary to the target nucleic acid sequence; wherein the 3'-targeting portion is hybridized with the target nucleic acid sequence and the 5'-tagging portion is not hybridized with the target nucleic acid sequence; the PTO is located between the upstream primer and the downstream primer; wherein the PTO is blocked at its 3'-end to prohibit its extension;

(b) contacting the resultant of the step (a) to a template-dependent nucleic acid polymerase having a 5' nuclease activity under conditions for extension of the primers and for cleavage of the PTO; wherein when the PTO is hybridized with the target nucleic acid sequence, the upstream primer is extended and the extended strand induces cleavage of the PTO by the template-dependent nucleic acid

polymerase having the 5′ nuclease activity such that the cleavage releases a fragment comprising the 5′-tagging portion or a part of the 5′-tagging portion of the PTO;

(c) hybridizing the fragment released from the PTO with a CTO (Capturing and Templating Oligonucleotide); wherein the CTO comprises in a 3′ to 5′ direction (i) a capturing portion comprising a nucleotide sequence complementary to the 5′-tagging portion or a part of the 5′-tagging portion of the PTO and (ii) a templating portion comprising a nucleotide sequence non-complementary to the 5′-tagging portion and the 3′-targeting portion; wherein the fragment released from the PTO is hybridized with the capturing portion of the CTO;

(d) performing an extension reaction using the resultant of the step (c) and the template-dependent nucleic acid polymerase; wherein the fragment hybridized with the capturing portion of the CTO is extended and an extended duplex is formed; wherein the extended duplex has a T_m value adjustable by (i) a sequence and/or length of the fragment, (ii) a sequence and/or length of the CTO or (iii) the sequence and/or length of the fragment and the sequence and/or length of the CTO;

(e) melting the extended duplex over a range of temperatures to give a target signal indicative of the presence of the extended duplex; wherein the target signal is provided by (i) at least one label linked to the fragment and/or the CTO, (ii) a label incorporated into the extended duplex during the extension reaction, (iii) a label incorporated into the extended duplex during the extension reaction and a label linked to the fragment and/or the CTO, or (iv) intercalating label; and

(f) detecting the extended duplex by measuring the target signal; whereby the presence of the extended duplex indicates the presence of the target nucleic acid sequence.

37. The method according to claim 36, wherein the method further comprise repeating the steps (a)-(b), (a)-(d) or (a)-(f) with denaturation between repeating

cycles.

38. The method according to claim 36, wherein the method is performed to detect at least two types of target nucleic acid sequences.

39. The method according to claim 38, wherein the at least two type of the target signals corresponding to the at least two types of the target nucleic acid sequences are provided from the same type of labels; wherein the extended duplexes corresponding to the at least two types of the target nucleic acid sequences have different T_m values from each other.

40. A method for detecting a target nucleic acid sequence from a DNA or a mixture of nucleic acids by a PTOCE (PTO Cleavage and Extension) assay, comprising:

(a) hybridizing the target nucleic acid sequence with an upstream oligonucleotide and a PTO (Probing and Tagging Oligonucleotide); wherein the upstream oligonucleotide comprises a hybridizing nucleotide sequence complementary to the target nucleic acid sequence; the PTO comprises (i) a 3′-targeting portion comprising a hybridizing nucleotide sequence complementary to the target nucleic acid sequence and (ii) a 5′-tagging portion comprising a nucleotide sequence non-complementary to the target nucleic acid sequence; wherein the 3′-targeting portion is hybridized with the target nucleic acid sequence and the 5′-tagging portion is not hybridized with the target nucleic acid sequence; the upstream oligonucleotide is located upstream of the PTO;

(b) contacting the resultant of the step (a) to an enzyme having a 5′ nuclease activity under conditions for cleavage of the PTO; wherein the upstream oligonucleotide or its extended strand induces cleavage of the PTO by the enzyme having the 5′ nuclease activity such that the cleavage releases a fragment comprising the 5′-tagging portion or a part of the 5′-tagging portion of the PTO;

(c) hybridizing the fragment released from the PTO with a CTO (Capturing and Templating Oligonucleotide); wherein the CTO comprises in a 3′ to 5′ direction (i) a

capturing portion comprising a nucleotide sequence complementary to the 5'-tagging portion or a part of the 5'-tagging portion of the PTO and (ii) a templating portion comprising a nucleotide sequence non-complementary to the 5'-tagging portion and the 3'-targeting portion of the PTO; wherein the fragment released from the PTO is hybridized with the capturing portion of the CTO;

(d) performing an extension reaction using the resultant of the step (c) and a template-dependent nucleic acid polymerase; wherein the fragment hybridized with the capturing portion of the CTO is extended and a extended duplex is formed; wherein the extended duplex has a T_m value adjustable by (i) a sequence and/or length of the fragment, (ii) a sequence and/or length of the CTO or (iii) the sequence and/or length of the fragment and the sequence and/or length of the CTO; wherein the extended duplex provides a target signal by (i) at least one label linked to the fragment and/or CTO, (ii) a label incorporated into the extended duplex during the extension reaction, (iii) at least one label linked to the fragment and/or CTO and a label incorporated into the extended duplex during the extension reaction or (iv) intercalating label; and

(e) detecting the extended duplex by measuring the target signal at a predetermined temperature that the extended duplex maintains its double-stranded form, whereby the presence of the extended duplex indicates the presence of the target nucleic acid sequence.

41. The method according to claim 40, wherein the target signal provided by the extended duplex is given during the extension of the step (d); wherein a hybrid between an uncleaved PTO and the CTO does not provides a non-target signal.

42. The method according to claim 40, wherein the target signal provided by the extended duplex is given by the hybridization of the fragment and the CTO in the step (c) and the formation of the extended duplex maintains the target signal in the step (d); wherein a hybrid between an uncleaved PTO and the CTO does provides a non-

target signal; wherein the predetermined temperature is higher than the hybrid's T_m value minus 10°C.

43. The method according to claim 40, wherein the target signal is provided by at least one label linked to the fragment and/or the CTO.

44. The method according to claim 43, wherein the fragment has an interactive dual label comprising a reporter molecule and a quencher molecule; wherein the hybridization of the fragment and the CTO in the step (c) induces change of a signal from the interactive dual label to give the target signal and the extended duplex maintains the target signal.

45. The method according to claim 43, wherein the CTO has an interactive dual label comprising a reporter molecule and a quencher molecule; wherein the hybridization of the fragment and the CTO in the step (c) induces change of a signal from the interactive dual label to give the target signal and the extended duplex maintains the target signal.

46. The method according to claim 43, wherein the CTO has an interactive dual labels comprising a reporter molecule and a quencher molecule; wherein the extension of the fragment in the step (d) induces change of a signal from the interactive dual label to give the target signal.

47. The method according to claim 43, wherein the fragment has one of an interactive dual label comprising a reporter molecule and a quencher molecule and the CTO has the other of the interactive dual label; wherein the hybridization of the fragment and the CTO in the step (c) induces change of a signal from the interactive dual label to give the target signal and the extended duplex maintains the target signal.

48. The method according to claim 43, wherein the fragment or the CTO has a single label and the hybridization of the fragment and the CTO in the step (c) induces change of a signal from the interactive dual label to give the target signal and the extended duplex maintains the target signal.

49. The method according to claim 43, wherein the CTO has a single label and the extension of the fragment in the step (d) induces change of a signal from the single label to give the target signal.

50. The method according to claim 43, wherein the labels are positioned to the extent that when a hybrid between an uncleaved PTO and the CTO is formed, the hybrid does not give a non-target signal in the step (d).

51. The method according to claim 43, wherein the labels are positioned to the extent that when a hybrid between an uncleaved PTO and the CTO is formed, the hybrid gives a non-target signal in the step (d); wherein the T_m value of the extended duplex is higher than that of the hybrid between the uncleaved PTO and the CTO.

52. The method according to claim 40, wherein the target signal is provided by a single label incorporated into the extended duplex during the extension reaction; wherein the incorporated single label is linked to a nucleotide incorporated during the extension reaction; wherein the extension of the fragment in the step (d) induces change of a signal from the single label to give the target signal in the step (d).

53. The method according to claim 52, wherein the nucleotide incorporated during the extension reaction has a first non-natural base and the CTO has a nucleotide having a second non-natural base with a specific binding affinity to the first non-natural base.

54. The method according to claim 40, wherein the target signal is provided by a label incorporated into the extended duplex during the extension reaction and a label linked to the fragment and/or the CTO; wherein the label incorporated is linked to a nucleotide incorporated during the extension reaction; wherein the two labels are an interactive dual label of a reporter molecule and a quencher molecule; wherein the extension of the fragment in the step (d) induces change of a signal from the interactive dual label to give the target signal.

55. The method according to claim 54, wherein the nucleotide incorporated during the extension reaction has a first non-natural base and the CTO has a nucleotide having a second non-natural base with a specific binding affinity to the first non-natural.

56. The method according to claim 40, wherein the PTO and/or CTO is blocked at its 3'-end to prohibit its extension.

57. The method according to claim 40, wherein the upstream oligonucleotide is an upstream primer or an upstream probe.

58. The method according to claim 40, wherein the upstream oligonucleotide is located adjacently to the PTO to the extent that the upstream oligonucleotide induces cleavage of the PTO by the enzyme having the 5' nuclease activity.

59. The method according to claim 57, wherein the upstream primer induces through its extended strand the cleavage of the PTO by the enzyme having the 5' nuclease activity.

60. The method according to claim 40, wherein the method further comprises

repeating the steps (a)-(b), (a)-(d) or (a)-(e) with denaturation between repeating cycles.

61. The method according to claim 40, wherein the steps (a)-(e) are performed in a reaction vessel or in separate reaction vessels.

62. The method according to claim 40, wherein the method is performed to detect at least two types of target nucleic acid sequences, wherein the upstream oligonucleotide comprises at least two types of oligonucleotides, the PTO comprises at least two types of the PTOs, and the CTO comprises at least one type of the CTO.

63. The method according to claim 40, wherein the upstream oligonucleotide is an upstream primer and the step (b) uses a template-dependent nucleic acid polymerase for the extension of the upstream primer.

64. The method according to claim 40, wherein the CTO is immobilized through its 5'-end or 3'-end onto a solid substrate.

65. The method according to claim 64, wherein the target signal is provided by a single label linked to the fragment or by a sing label incorporated into the extended duplex during the extension reaction.

66. The method according to any one of claims 40-65, wherein the method is performed in the presence of a downstream primer.

67. A kit for detecting a target nucleic acid sequence from a DNA or a mixture of nucleic acids by a PTOCE (PTO Cleavage and Extension) assay, comprising:

(a) an upstream oligonucleotide comprising a hybridizing nucleotide sequence complementary to the target nucleic acid sequence;

(b) a PTO (Probing and Tagging Oligonucleotide) comprising (i) a 3'-targeting portion comprising a hybridizing nucleotide sequence complementary to the target nucleic acid sequence and (ii) a 5'-tagging portion comprising a nucleotide sequence non-complementary to the target nucleic acid sequence, wherein the 3'-targeting portion is hybridized with the target nucleic acid sequence and the 5'-tagging portion is not hybridized with the target nucleic acid sequence; the upstream oligonucleotide is located upstream of the PTO; wherein the upstream oligonucleotide or its extended strand induces cleavage of the PTO by an enzyme having a 5' nuclease activity such that the cleavage releases a fragment comprising the 5'-tagging portion or a part of the 5'-tagging portion of the PTO; and

(c) a CTO (Capturing and Templating Oligonucleotide) comprising in a 3' to 5' direction (i) a capturing portion comprising a nucleotide sequence complementary to the 5'-tagging portion or a part of the 5'-tagging portion of the PTO and (ii) a templating portion comprising a nucleotide sequence non-complementary to the 5'-tagging portion and the 3'-targeting portion of the PTO; wherein the fragment released from the PTO is hybridized with the capturing portion of the CTO; and the fragment hybridized with the capturing portion of the CTO is extended by a template-dependent nucleic acid polymerase to form an extended duplex.

68. The kit according to claim 67, wherein the kit further comprises an enzyme having a 5' nuclease activity.

69. The kit according to claim 67, wherein the kit further comprises a template-dependent nucleic acid polymerase.

70. The kit according to claim 67, wherein the PTO and/or the CTO has at least one label.

71. The kit according to claim 67, wherein the kit further comprises a label to be incorporated into the extended duplex during the extension reaction.

72. The kit according to claim 67, wherein the kit further comprises a label to be incorporated into the extended duplex during the extension reaction and the PTO and/or the CTO has at least one label.

73. The kit according to claim 67, wherein the kit further comprises an intercalating label.

74. The kit according to claim 70, wherein the label is a single label or interactive dual label.

75. The kit according to claim 67, wherein the kit is used for detection of at least two types of nucleic acid sequences wherein the upstream oligonucleotide comprises at least two types of oligonucleotides, the PTO comprises at least two types of the PTO and the CTO comprises at least two types of the CTO.

76. The kit according to claim 67, wherein the CTO is immobilized through its 5'-end or 3'-end onto a solid substrate.

77. The method according to any one of claims 67-76, wherein the kit further comprises a downstream primer.

What is claimed is:

1. A method for detecting a target nucleic acid sequence from a DNA or a mixture of nucleic acids by a PTOCE (PTO Cleavage and Extension) assay, comprising:

(a) hybridizing the target nucleic acid sequence with an upstream oligonucleotide and a PTO (Probing and Tagging Oligonucleotide); wherein the upstream oligonucleotide comprises a hybridizing nucleotide sequence complementary to the target nucleic acid sequence; the PTO comprises (i) a 3'-targeting portion comprising a hybridizing nucleotide sequence complementary to the target nucleic acid sequence and (ii) a 5'-tagging portion comprising a nucleotide sequence non-complementary to the target nucleic acid sequence; wherein the 3'-targeting portion is hybridized with the target nucleic acid sequence and the 5'-tagging portion is not hybridized with the target nucleic acid sequence; the upstream oligonucleotide is located upstream of the PTO;

(b) contacting the resultant of the step (a) to an enzyme having a 5' nuclease activity under conditions for cleavage of the PTO; wherein the upstream oligonucleotide or its extended strand induces cleavage of the PTO by the enzyme having the 5' nuclease activity such that the cleavage releases a fragment comprising the 5'-tagging portion or a part of the 5'-tagging portion of the PTO;

(c) hybridizing the fragment released from the PTO with a CTO (Capturing and Templating Oligonucleotide); wherein the CTO comprises in a 3' to 5' direction (i) a capturing portion comprising a nucleotide sequence complementary to the 5'-tagging portion or a part of the 5'-tagging portion of the PTO and (ii) a templating portion comprising a nucleotide sequence non-complementary to the 5'-tagging portion and the 3'-targeting portion of the PTO; wherein the fragment released from the PTO is hybridized with the capturing portion of the CTO;

(d) performing an extension reaction using the resultant of the step (c) and a template-dependent nucleic acid polymerase; wherein the fragment hybridized with the capturing portion of the CTO is extended and an extended duplex is formed; wherein the extended duplex has a T_m value adjustable by (i) a sequence and/or length of the fragment, (ii) a sequence and/or length of the CTO or (iii) the sequence and/or length of the fragment and the sequence and/or length of the CTO;

(e) melting the extended duplex over a range of temperatures to give a target signal indicative of the presence of the extended duplex; wherein the target signal is provided by (i) at least one label linked to the fragment and/or the CTO, (ii) a label incorporated into the extended duplex during the extension reaction, (iii) a label incorporated into the extended duplex during the extension reaction and a label linked to the fragment and/or the CTO, or (iv) an intercalating label; and

(f) detecting the extended duplex by measuring the target signal; whereby the presence of the extended duplex indicates the presence of the target nucleic acid sequence.

2. The method according to claim 1, wherein the presence of the extended duplex is detected by melting analysis.

3. The method according to claim 1, wherein the melting of the step (e) is followed by hybridization to give the target signal indicative of the presence of the extended duplex.

4. The method according to claim 3, wherein the presence of the extended duplex is detected by hybridization analysis.

5. The method according to claim 1, wherein the target signal is provided by at least one label linked to the fragment and/or the CTO.

6. The method according to claim 5, wherein the fragment or the CTO has an interactive dual label comprising a reporter molecule and a quencher molecule; wherein the melting of the extended duplex in the step (e) induces change of a signal from the interactive dual label to give the target signal in the step (e).

7. The method according to claim 5, wherein the fragment has one of an interactive dual label comprising a reporter molecule and a quencher molecule and the CTO has the other of the interactive dual label; wherein the melting of the extended duplex in the step (e) induces change of a signal from the interactive dual labels to give the target signal in the step (e).

8. The method according to claim 5, wherein the fragment or the CTO has a single label, and the melting of the extended duplex in the step (e) induces change of a signal from the single label to give the target signal in the step (e).

9. The method according to claim 5, wherein the labels are positioned to the extent that when a hybrid between an uncleaved PTO and the CTO is formed, the hybrid does not give a non-target signal in the step (e).

10. The method according to claim 5, wherein the labels are positioned to the extent that when a hybrid between an uncleaved PTO and the CTO is formed, the hybrid gives a non-target signal in the step (e); wherein the T_m value of the extended duplex is higher than that of the hybrid between the uncleaved PTO and the CTO.

11. The method according to claim 1, wherein the target signal is provided by a single label incorporated into the extended duplex during the extension reaction; wherein the incorporated single label is linked to a nucleotide incorporated during the extension reaction; wherein the melting of the extended duplex in the step (e) induces change of a signal from the single label to give the target signal in the step (e).

12. The method according to claim 11, wherein the nucleotide incorporated during the extension reaction has a first non-natural base and the CTO has a nucleotide having a second non-natural base with a specific binding affinity to the first non-natural base.

13. The method according to claim 1, wherein the target signal is provided by a label incorporated into the extended duplex during the extension reaction and a label linked to the fragment and/or the CTO, and the incorporated label is linked to a nucleotide incorporated during the extension reaction; wherein the two labels are an interactive dual label of a reporter molecule and a quencher molecule; wherein the melting of the extended duplex in the step (e) induces change of a signal from the interactive dual label to give the target signal in the step (e).

14. The method according to claim 13, wherein the nucleotide incorporated during the extension reaction has a first non-natural base and the CTO has a nucleotide having a second non-natural base with a specific binding affinity to the first non-natural.

15. The method according to claim 1, wherein the upstream oligonucleotide is an upstream primer or an upstream probe.

16. The method according to claim 1, wherein the upstream oligonucleotide has a partial-overlapped sequence with the 3'-targeting portion of the PTO.

17. The method according to claim 1, wherein the capturing portion comprises at its 5'-end part a

nucleotide sequence complementary to a 5'-end part of the 3'-targeting portion of the PTO.

18. The method according to claim 1, wherein the method further comprises repeating the steps (a)-(b), (a)-(d) or (a)-(f) with denaturation between repeating cycles.

19. The method according to claim 1, wherein the method is performed to detect at least two types of target nucleic acid sequences; wherein the upstream oligonucleotide comprises at least two types of oligonucleotides, the PTO comprises at least two types of the PTOs and the CTO comprises at least one type of the CTOs.

20. The method according to claim 19, wherein the extended duplexes corresponding to the at least two types of the target nucleic acid sequences have different T_m values from each other.

21. The method according to claim 1, wherein the enzyme having the 5' nuclease activity is a thermostable DNA polymerase having a 5' nuclease activity or FEN nuclease.

22. The method according to claim 1, wherein the target nucleic acid sequence comprises a nucleotide variation.

23. The method according to claim 1, wherein the CTO is immobilized through its 5'-end or its 3'-end onto a solid substrate.

24. The method according to claim 23, wherein the target signal is provided by a single label linked to the fragment or by a single label incorporated into the extended duplex during the extension reaction.

25. The method according to any one of claims 1-24, wherein the method is performed in the presence of a downstream primer.

26. A method for detecting a target nucleic acid sequence from a DNA or a mixture of nucleic acids by a PTOCE (PTO Cleavage and Extension) assay, comprising:

(a) hybridizing the target nucleic acid sequences with a primer pair comprising an upstream primer and a downstream primer and a PTO (Probing and Tagging Oligonucleotide); wherein each of the upstream primer and the downstream primer comprise a hybridizing nucleotide sequence complementary to the target nucleic acid sequences; the PTO comprises (i) a 3'-targeting portion comprising a hybridizing nucleotide sequence complementary to the target nucleic acid sequence and (ii) a 5'-tagging portion comprising a nucleotide sequence non-complementary to the target nucleic acid sequence; wherein the 3'-targeting portion is hybridized with the target nucleic acid sequence and the 5'-tagging portion is not hybridized with the target nucleic acid sequence; the PTO is located between the upstream primer and the downstream primer; wherein the PTO is blocked at its 3'-end to prohibit its extension;

(b) contacting the resultant of the step (a) to a template-dependent nucleic acid polymerase having a 5' nuclease activity under conditions for extension of the primers and for cleavage of the PTO; wherein when the PTO is hybridized with the target nucleic acid sequence, the upstream primer is extended and the extended strand induces cleavage of the PTO by the template-dependent nucleic acid polymerase having the 5' nuclease activity such that the cleavage releases a fragment comprising the 5'-tagging portion or a part of the 5'-tagging portion of the PTO;

(c) hybridizing the fragment released from the PTO with a CTO (Capturing and Tempiating Oligonucleotide); wherein the CTO comprises in a 3' to 5' direction (i) a capturing portion comprising a nucleotide sequence complementary to the 5'-tagging portion or a part of the 5'-tagging portion of the PTO and (ii) a templating portion comprising a nucleotide sequence non-complementary to the 5'-

tagging portion and the 3'-targeting portion; wherein the fragment released from the PTO is hybridized with the capturing portion of the CTO;

(d) performing an extension reaction using the resultant of the step (c) and the template-dependent nucleic acid polymerase; wherein the fragment hybridized with the capturing portion of the CTO is extended and an extended duplex is formed; wherein the extended duplex has a T_m value adjustable by (i) a sequence and/or length of the fragment, (ii) a sequence and/or length of the CTO or (iii) the sequence and/or length of the fragment and the sequence and/or length of the CTO;

(e) melting the extended duplex over a range of temperatures to give a target signal indicative of the presence of the extended duplex; wherein the target signal is provided by (i) at least one label linked to the fragment and/or the CTO, (ii) a label incorporated into the extended duplex during the extension reaction, (iii) a label incorporated into the extended duplex during the extension reaction and a label linked to the fragment and/or the CTO, or (iv) intercalating label; and

(f) detecting the extended duplex by measuring the target signal; whereby the presence of the extended duplex indicates the presence of the target nucleic acid sequence.

27. The method according to claim 26, wherein the method further comprise repeating the steps (a)-(b), (a)-(d) or (a)-(f) with denaturation between repeating cycles.

28. A method for detecting a target nucleic acid sequence from a DNA or a mixture of nucleic acids by a PTOCE (PTO Cleavage and Extension) assay, comprising:

(a) hybridizing the target nucleic acid sequence with an upstream oligonucleotide and a PTO (Probing and Tagging Oligonucleotide); wherein the upstream oligonucleotide comprises a hybridizing nucleotide sequence complementary to the target nucleic acid sequence; the PTO comprises (i) a 3'-targeting portion comprising a hybridizing nucleotide sequence complementary to the target nucleic acid sequence and (ii) a 5'-tagging portion comprising a nucleotide sequence non-complementary to the target nucleic acid sequence; wherein the 3'-targeting portion is hybridized with the target nucleic acid sequence and the 5'-tagging portion is not hybridized with the target nucleic acid sequence; the upstream oligonucleotide is located upstream of the PTO;

(b) contacting the resultant of the step (a) to an enzyme having a 5' nuclease activity under conditions for cleavage of the PTO; wherein the upstream oligonucleotide or its extended strand induces cleavage of the PTO by the enzyme having the 5' nuclease activity such that the cleavage releases a fragment comprising the 5'-tagging portion or a part of the 5'-tagging portion of the PTO;

(c) hybridizing the fragment released from the PTO with a CTO (Capturing and Templating Oligonucleotide); wherein the CTO comprises in a 3' to 5' direction (i) a capturing portion comprising a nucleotide sequence complementary to the 5'-tagging portion or a part of the 5'-tagging portion of the PTO and (ii) a templating portion comprising a nucleotide sequence non-complementary to the 5'-tagging portion and the 3'-targeting portion of the PTO; wherein the fragment released from the PTO is hybridized with the capturing portion of the CTO;

(d) performing an extension reaction using the resultant of the step (c) and a template-dependent nucleic acid polymerase; wherein the fragment hybridized with the capturing portion of the CTO is extended and a extended duplex is formed; wherein the extended duplex has a T_m value adjustable by (i) a sequence and/or length of the fragment, (ii) a sequence and/or length of the CTO or (iii) the sequence and/or length of the fragment and the sequence and/or length of the CTO; wherein the extended duplex provides a target signal by (i) at least one label linked to the fragment and/or CTO, (ii) a label incorporated into the extended duplex during the extension reaction, (iii) at least one label linked to the fragment and/or CTO and a label incorporated into the extended duplex during the extension reaction or (iv) intercalating label; and

(e) detecting the extended duplex by measuring the target signal at a predetermined temperature that the extended duplex maintains its double-stranded form, whereby the presence of the extended duplex indicates the presence of the target nucleic acid sequence.

29. The method according to claim 28, wherein the target signal provided by the extended duplex is given during the extension of the step (d); wherein a hybrid between an uncleaved PTO and the CTO does not provides a non-target signal.

30. The method according to claim 28, wherein the target signal provided by the extended duplex is given by the hybridization of the fragment and the CTO in the step (c) and the formation of the extended duplex maintains the target signal in the step (d); wherein a hybrid between an uncleaved PTO and the CTO does provides a non-target signal; wherein the predetermined temperature is higher than the hybrid's T_m value minus 10 °C sufficient to dissociate the hybrid to remove the non-target signal.

31. The method according to claim 28, wherein the target signal is provided by at least one label linked to the fragment and/or the CTO.

32. The method according to claim 31, wherein the fragment has an interactive dual label comprising a reporter molecule and a quencher molecule; wherein the hybridization of the fragment and the CTO in the step (c) induces change of a signal from the interactive dual label to give the target signal and the extended duplex maintains the target signal.

33. The method according to claim 31, wherein the CTO has an interactive dual label comprising a reporter molecule and a quencher molecule; wherein the hybridization of the fragment and the CTO in the step (c) induces change of a signal from the interactive dual label to give the target signal and the extended duplex maintains the target signal.

34. The method according to claim 31, wherein the CTO has an interactive dual labels comprising a reporter molecule and a quencher molecule; wherein the extension of the fragment in the step (d) induces change of a signal from the interactive dual label to give the target signal.

35. The method according to claim 31, wherein the fragment has one of an interactive dual label comprising a reporter molecule and a quencher molecule and the CTO has the other of the interactive dual label; wherein the hybridization of the fragment and the CTO in the step (c) induces change of a signal from the interactive dual label to give the target signal and the extended duplex maintains the target signal.

36. The method according to claim 31, wherein the fragment or the CTO has a single label and the hybridization of the fragment and the CTO in the step (c) induces change of a signal from the interactive dual label to give the target signal and the extended duplex maintains the target signal.

37. The method according to claim 31, wherein the CTO has a single label and the extension of the fragment in the step (d) induces change of a signal from the single label to give the target signal.

38. The method according to claim 31, wherein the labels are positioned to the extent that when a hybrid between an uncleaved PTO and the CTO is formed, the hybrid does not give a non-target signal in the step (d).

39. The method according to claim 31, wherein the labels are positioned to the extent that when a hybrid between an uncleaved PTO and the CTO is formed, the hybrid gives a non-target signal in the step (d); wherein the T_m value of the extended duplex is higher than that of the hybrid between the

uncleaved PTO and the CTO.

40. The method according to claim 28, wherein the target signal is provided by a single label incorporated into the extended duplex during the extension reaction; wherein the incorporated single label is linked to a nucleotide incorporated during the extension reaction; wherein the extension of the fragment in the step (d) induces change of a signal from the single label to give the target signal in the step (d).

41. The method according to claim 40, wherein the nucleotide incorporated during the extension reaction has a first non-natural base and the CTO has a nucleotide having a second non-natural base with a specific binding affinity to the first non-natural base.

42. The method according to claim 28, wherein the target signal is provided by a label incorporated into the extended duplex during the extension reaction and a label linked to the fragment and/or the CTO; wherein the label incorporated is linked to a nucleotide incorporated during the extension reaction; wherein the two labels are an interactive dual label of a reporter molecule and a quencher molecule; wherein the extension of the fragment in the step (d) induces change of a signal from the interactive dual label to give the target signal.

43. The method according to claim 42, wherein the nucleotide incorporated during the extension reaction has a first non-natural base and the CTO has a nucleotide having a second non-natural base with a specific binding affinity to the first non-natural.

44. The method according to claim 28, wherein the upstream oligonucleotide is an upstream primer or an upstream probe.

45. The method according to claim 28, wherein the method further comprises repeating the steps (a)-(b), (a)-(d) or (a)-(e) with denaturation between repeating cycles.

46. The method according to claim 28, wherein the method is performed to detect at least two types of target nucleic acid sequences; wherein the upstream oligonucleotide comprises at least two types of oligonucleotides, the PTO comprises at least two types of the PTOs, and the CTO comprises at least one type of the CTO.

47. The method according to claim 28, wherein the CTO is immobilized through its 5'-end or 3'-end onto a solid substrate.

48. The method according to claim 47, wherein the target signal is provided by a single label linked to the fragment or by a sing label incorporated into the extended duplex during the extension reaction.

49. The method according to any one of claims 28-48, wherein the method is performed in the presence of a downstream primer.

50. A kit for detecting a target nucleic acid sequence from a DNA or a mixture of nucleic acids by a PTOCE (PTO Cleavage and Extension) assay, comprising:

(a) an upstream oligonucleotide comprising a hybridizing nucleotide sequence complementary to the target nucleic acid sequence;

(b) a PTO (Probing and Tagging Oligonucleotide) comprising (i) a 3'-targeting portion comprising a hybridizing nucleotide sequence complementary to the target nucleic acid sequence and (ii) a 5'-tagging portion comprising a nucleotide sequence non-complementary to the target nucleic acid sequence, wherein the 3'-targeting portion is hybridized with the target nucleic acid sequence and the

5'-tagging portion is not hybridized with the target nucleic acid sequence; the upstream oligonucleotide is located upstream of the PTO; wherein the upstream oligonucleotide or its extended strand induces cleavage of the PTO by an enzyme having a 5' nuclease activity such that the cleavage releases a fragment comprising the 5'-tagging portion or a part of the 5'-tagging portion of the PTO; and

(c) a CTO (Capturing and Templating Oligonucleotide) comprising in a 3' to 5' direction (i) a capturing portion comprising a nucleotide sequence complementary to the 5'-tagging portion or a part of the 5'-tagging portion of the PTO and (ii) a templating portion comprising a nucleotide sequence non-complementary to the 5'-tagging portion and the 3'-targeting portion of the PTO; wherein the fragment released from the PTO is hybridized with the capturing portion of the CTO; and the fragment hybridized with the capturing portion of the CTO is extended by a template-dependent nucleic acid polymerase to form an extended duplex.

51. The kit according to claim 50, wherein the kit further comprises an enzyme having a 5' nuclease activity.

52. The kit according to claim 50, wherein the PTO and/or the CTO has at least one label.

53. The kit according to claim 50, wherein the kit further comprises a label to be incorporated into the extended duplex during the extension reaction.

54. The kit according to claim 50, wherein the kit further comprises a label to be incorporated into the extended duplex during the extension reaction and the PTO and/or the CTO has at least one label.

55. The kit according to claim 50, wherein the kit further comprises an intercalating label.

56. The kit according to claim 50, wherein the CTO is immobilized through its 5'-end or 3'-end onto a solid substrate.

57. The method according to any one of claims 50-56, wherein the kit further comprises a downstream primer.