Patent Literature

[0006]

Patent Literature 1: National Publication of International Patent Application No. 2005-528121

Patent Literature 2: Japanese Patent Laid-Open No. 2002-291490

Patent Literature 3: National Publication of International Patent Application No. 2008-535518

Summary

[0006a] In one aspect of the present disclosure, there is provided a method for designing a primer having a mutation introduced thereinto for use in a nucleic acid amplification method, said method comprising:

a basic sequence-design step for designing, as a basic primer sequence, a nucleotide sequence completely complementary to a template DNA; and

a mutation introduction site-selection step for selecting, as a mutation introduction site, one or more nucleotide residues, said nucleotide residue(s) being contained in the basic primer sequence and satisfying one or more requirements selected from the group consisting of the following requirements (1) to (4);

wherein the aforesaid mutation is not recognized as a nucleotide residue by DNA polymerase:

(1) a nucleotide residue possibly contributing to the formation of a primer dimer;
(2) a nucleotide residue possibly contributing to the formation of a loop structure in a single primer molecule;
(3) when it is predicted that a primer comprising the aforesaid basic primer sequence forms a primer dimer, a nucleotide residue positioned in a region other than a region to which the primer is complementarily or non-complementarily hybridizable; and
(4) when it is predicted that a primer comprising the aforesaid basic primer sequence forms a loop structure in a single primer molecule, a nucleotide residue positioned in a region other than a region which forms the loop structure.

[0006b] In another aspect of the present disclosure, there is provided a primer designed by a design method disclosed herein.

[0006c] In a further aspect of the present disclosure, there is provided a nucleic acid amplification method comprising using a primer disclosed herein.

Technical Problem

[0007] The method described in Patent Literature 1 cannot be applied to a system of detecting an amplified nucleic acid using an intercalator, though this approach is effective for quantitative PCR, which involves detecting

the presence or absence or abundance of a target nucleic acid by reading out fluorescence emitted from an amplification product. Furthermore, the method cannot suppress the occurrence of a primer dimer itself and therefore, cannot prevent the exhaustion of primers ascribable to the formation of a nonspecific amplification product caused by the primer dimer.

[0008] The methods described in Patent Literatures 2 and 3 suppress the occurrence itself of a primer dimer responsible for a nonspecific amplification. These literatures, however, merely abstractly describe "within three 3'-terminal nucleotide positions" or "within 4 nucleotides from the 3' end," etc. as to a position to

Claims

[Claim 1]

A method for designing a primer having a mutation introduced thereinto for use in a nucleic acid amplification method, said method comprising:

a basic sequence-design step for designing, as a basic primer sequence, a nucleotide sequence completely complementary to a template DNA; and

a mutation introduction site-selection step for selecting, as a mutation introduction site, one or more nucleotide residues, said nucleotide residue(s) being contained in the basic primer sequence and satisfying one or more requirements selected from the group consisting of the following requirements (1) to (4);

wherein the aforesaid mutation is not recognized as a nucleotide residue by DNA polymerase:

(1) a nucleotide residue possibly contributing to the formation of a primer dimer;

(2) a nucleotide residue possibly contributing to the formation of a loop structure in a single primer molecule;

(3) when it is predicted that a primer comprising the aforesaid basic primer sequence forms a primer dimer, a nucleotide residue positioned in a region other than a region to which the primer is complementarily or non-complementarily hybridizable; and

(4) when it is predicted that a primer comprising the aforesaid basic primer sequence forms a loop structure in a single primer molecule, a nucleotide residue positioned

in a region other than a region which forms the loop structure.

[Claim 2]

The design method according to claim 1, wherein the aforesaid mutation is one or more mutations selected from the group consisting of the following mutations (A) to (D):

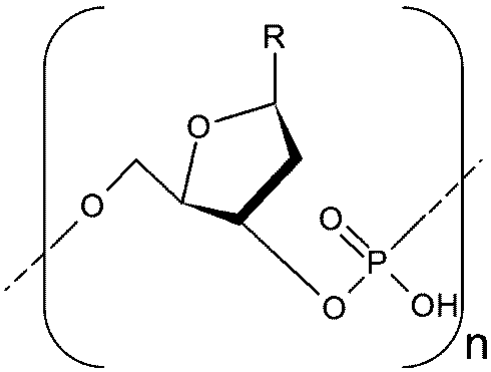
(A) a nucleotide residue or a polynucleotide bound at its 5' end and 3' end with the 5' end and 3' end, respectively, of nucleotide residues flanking the aforesaid mutation introduction site;

(B) a spacer chain consisting of a carbon chain or a PEG chain;

(C) a spacer chain consisting of a tetrahydrofuran derivative represented by the general formula 1:

General formula 1

[Formula 1]



wherein R represents H or a hydroxy group, and n represents a natural number; and

(D) a spacer chain that has undergone photodegradable modification.

[Claim 3]

A primer designed by a design method according to any one of claims 1 or 2.

[Claim 4]

A nucleic acid amplification method comprising using a primer according to claim 3.

[Claim 5]

The nucleic acid amplification method according to claim 4, wherein the nucleic acid amplification method is an isothermal amplification method.