CLAIMS

[Claim 1]

A method of amplifying a complementary DNA strand, the method comprising:

a target RNA-capturing step of capturing a target RNA with a target RNA-capture oligo comprising a target capture portion, said target RNA-capture oligo being a free oligo not bound to a solid-phase carrier;

a complementary-strand synthesis step of carrying out reverse transcription reaction using a reverse transcriptase having terminal transferase activity, to synthesize a complementary DNA strand whose sequence is complementary to the captured RNA, and to add an additional sequence consisting of several arbitrary bases to the 3'-end of at least part of the complementary DNA molecules;

an unreacted-oligo removal step of removing a target RNA-capture oligo that has not captured the target RNA by nucleic acid size fractionation;

an RNA degradation step of degrading RNA using a ribonuclease;

a homopolymer addition step of carrying out a reaction using terminal deoxynucleotidyl transferase in the presence of dATP, dTTP, dCTP or dGTP and a chain-terminating nucleotide triphosphate, to add a nucleotide homopolymer to the 3'-end of the complementary DNA strand;

a second-strand synthesis step of synthesizing a second strand for the complementary DNA strand using a second-strand-synthesizing primer which comprises a primer comprising a complementary homopolymer portion whose sequence is complementary to the nucleotide homopolymer, to generate a DNA double strand composed of the complementary DNA strand and the second strand; and

a nucleic acid amplification step of carrying out nucleic acid amplification reaction using the DNA double strand as a template.

[Claim 2]

The method according to claim 1, wherein: the target RNA-capture oligo comprises a first adapter portion on the 5'-side of the target capture portion; the second-strand-synthesizing primer comprises a long primer comprising a second adapter portion on the 5'-side of the complementary homopolymer portion; and in the nucleic acid amplification step, nucleic acid amplification reaction is carried out using a primer targeting the first adapter and a primer targeting the second adapter.

[Claim 3]

The method according to claim 1, wherein: the second-strand-synthesizing primer comprises a long primer comprising a second adapter portion on the 5'-side of the complementary homopolymer portion; and, in the nucleic acid amplification step, nucleic acid amplification reaction is carried out using a primer targeting the second adapter and a primer targeting a desired region in the complementary DNA strand.

[Claim 4]

The method according to claim 2 or 3, wherein the long primer comprises, between the second adapter portion and the complementary homopolymer portion, a molecular barcode portion consisting of a random sequence.

[Claim 5]

The method according to claim 2 or 3, wherein the chain length of the complementary homopolymer portion of the long primer is 6 to 15 bases.

[Claim 6]

The method according to claim 1, wherein the target RNA-capture oligo comprises, from the 5'-side to the 3'-side, a first adapter portion, a cell identification barcode portion, and a target capture portion.

[Claim 7]

The method according to claim 1, wherein: the target RNA-capture oligo comprises a target poly(A) RNA-capture oligo comprising a poly(T) portion as a target capture portion; and the target RNA comprises a poly(A) RNA.

[Claim 8]

A method of amplifying a complementary DNA strand, the method comprising:

a target RNA-capturing step of capturing a target RNA with a target RNA-capture oligo comprising a target capture portion;

a complementary-strand synthesis step of carrying out reverse transcription reaction using a reverse transcriptase having terminal transferase activity, to synthesize a complementary DNA strand whose sequence is complementary to the captured RNA, and to add an additional sequence consisting of several arbitrary bases to the 3'-end of at least part of the complementary DNA molecules;

an unreacted-oligo removal step of removing a target RNA-capture oligo that has not captured the target RNA;

an RNA degradation step of degrading RNA using a ribonuclease;

a homopolymer addition step of carrying out a reaction using terminal deoxynucleotidyl transferase in the presence of dATP, dTTP, dCTP or dGTP and a chain-terminating nucleotide triphosphate, to add a nucleotide homopolymer to the 3'-end of the complementary DNA strand;

a second-strand synthesis step of synthesizing a second strand for the complementary DNA strand using a second-strand-synthesizing primer which comprises a primer comprising a complementary homopolymer portion whose sequence is complementary to the nucleotide homopolymer, to generate a DNA double strand composed of the complementary DNA strand and

the second strand; and

a nucleic acid amplification step of carrying out nucleic acid amplification reaction using the DNA double strand as a template,

wherein: the target RNA-capture oligo comprises a target poly(A) RNA-capture oligo comprising a poly(T) portion as a target capture portion; and the target RNA comprises a poly(A) RNA; and

wherein in the complementary-strand synthesis step, reverse transcription reaction is carried out in the presence of a template-switching oligo comprising: a sequence capable of hybridizing with the additional sequence, at its 3'-end; and a switching sequence on the 5'-side of said sequence capable of hybridizing with the additional sequence; to hybridize the template-switching oligo with the complementary DNA strand having the additional sequence added thereto, and to further add a sequence complementary to the switching sequence to the 3'-end of the complementary DNA strand.

[Claim 9]

The method according to claim 8, wherein the template-switching oligo comprises an oligonucleotide comprising, at its 3'-end, GGG, GUG, or NGG (wherein G and U are bases of ribonucleotides, and N is a base of any ribonucleotide selected from A, U, G, and C; and wherein one or more nucleotide analogues are optionally included) as the sequence capable of hybridizing with the additional sequence, and the switching sequence comprises a second adapter portion.

[Claim 10]

The method according to claim 9, wherein the switching sequence comprises a molecular barcode portion consisting of a random sequence on the 3'-side of the second adapter portion.

[Claim 11]

The method according to claim 8, wherein the target RNA-capture oligo is a free oligo not bound to a solid-phase carrier, and wherein the unreacted-oligo removal step comprises removal of a target RNA-capture oligo that has not captured the target RNA and a template-switching oligo that has not hybridized with the complementary DNA strand by nucleic acid size fractionation.

[Claim 12]

The method according to claim 8, wherein: the target RNA-capture oligo is a solid-phased oligo bound to a solid-phase carrier; and the unreacted-oligo removal step is carried out by exonuclease treatment.

[Claim 13]

The method according to claim 12, wherein: the solid-phase carrier is a bead; and the target RNA-capture oligo comprises, from the 5'-side to the 3'-side, a first adapter portion, a bead identification barcode portion, and a target capture portion.

[Claim 14]

The method according to claim 12, wherein: the solid-phase carrier is a plate; and the target RNA-capture oligo is immobilized on a plurality of compartments on the plate, and comprises, from the 5'-side to the 3'-side, a first adapter portion, a compartment identification barcode portion, and a target capture portion.

[Claim 15]

The method according to claim 8, wherein the second-strand-synthesizing primer comprises: a long primer comprising a second adapter portion on the 5'-side of the complementary homopolymer portion; and a short primer comprising no complementary homopolymer portion and comprising a second adapter portion at its 3'-side region.

[Claim 16]

The method according to claim 15, wherein the long primer comprises, between the second adapter portion and the complementary homopolymer portion, a molecular barcode portion consisting of a random sequence.

[Claim 17]

The method according to claim 8, wherein in the complementary-strand synthesis step, reverse transcription reaction is carried out using a reverse transcriptase further having strand displacement activity in the presence of the template-switching oligo, to carry out second-strand synthesis for the complementary DNA strand with which the template-switching oligo has been hybridized.

[Claim 18]

The method according to claim 1 or 8, wherein the chain-terminating nucleotide triphosphate is ddNTP, a ddNTP derivative, 3'-dNTP, or 3'-deoxy-5-methyluridine-5'-triphosphate.

[Claim 19]

The method according to claim 18, wherein the ddNTP derivative is ddNTP in which the 3'-position is modified with an atomic group comprising no OH group.

[Claim 20]

The method according to claim 18, wherein the chain-terminating nucleotide triphosphate is ddNTP.

[Claim 21]

The method according to claim 1 or 8, wherein in the homopolymer addition step, dCTP and

chain-terminating CTP are added to carry out poly(C) addition.

[Claim 22]

The method according to claim 1 or 8, wherein the RNA degradation step and the homopolymer addition step are carried out simultaneously.