the sample to provide full length cDNA(s) of the at least one RNA,

c) purifying the sample at least from excess nucleotides

d) adding a dideoxy nucleotide, which dideoxy nucleotide is modified at the 3‘-position to include a first partner of a pair of azide and alkyne molecules, and a template-independent polymerase to attach a single 3‘-azide- or 3‘-alkyne modified dideoxy nucleotide at the 3‘-end of the cDNA(s) obtained in step b),

e) purifying the sample at least from excess modified dideoxy nucleotide added in step d),

f) adding an adapter molecule, the adapter molecule comprising a polynucleotide sequence and, attached to its 5‘ end, a second partner of said pair of azide and alkyne molecules, under conditions to perform a click reaction and to ligate the adapter molecule to the 3‘-modified cDNA(s) obtained in step d) under formation of a triazole linkage,

g) adding a second primer, the second primer comprising a nucleotide sequence which is complementary to at least 6 of the nucleotides at the 5‘-end of the adapter molecule and contains at its 3‘-end a nucleotide which is complementary to the dideoxy nucleotide at the 3‘-end of the cDNA(s), to effect hybridization and binding of the second primer to the ligated adapter/cDNA molecule obtained in step f) at a position overlapping the triazole linkage,

and alternatively

h) adding a DNA polymerase to achieve chain extension of the second primer to produce second strand DNA(s) including sequences complementary to the whole cDNA(s) obtained in step b), and either

i) sequencing the second strand DNA(s) of step h), or

Claims

1. Method for amplifying and / or sequencing at least one RNA, preferably mRNA, contained in a sample, the method comprising:
   a) providing at least one first primer, which first primer includes a sequence which is complementary to a sequence which is located at or near the 3'-end of the at least one RNA to be amplified, and adding said at least one first primer to the sample under conditions which allow for hybridization of at least one first primer to at least one RNA,
   b) reverse transcribing the at least one RNA under conditions including addition of a reverse transcriptase and nucleotides to the sample to provide full length cDNA(s) of the at least one RNA,
   c) purifying the sample at least from excess nucleotides
   d) adding a dideoxy nucleotide, which dideoxy nucleotide is modified at the 3'-position to include a first partner of a pair of azide and alkyne molecules, and a template-independent polymerase to attach a single 3'-azide- or 3'-alkyne modified dideoxy nucleotide at the 3'-end of the cDNA(s) obtained in step b),
   e) purifying the sample at least from excess modified dideoxy nucleotide added in step d),
   f) adding an adapter molecule, the adapter molecule comprising a polynucleotide sequence and, attached to its 5' end, a second partner of said pair of azide and alkyne molecules, under conditions to perform a click reaction and to ligate the adapter molecule to the 3'-modified cDNA(s) obtained in step d) under formation of a triazole linkage,
   g) adding a second primer, the second primer comprising a nucleotide sequence which is complementary to at least 6 of the nucleotides at the 5'-end of the adapter molecule and contains at its 3'-end a nucleotide which is complementary to the dideoxy

nucleotide at the 3‘-end of the cDNA(s), to effect hybridization and binding of the second primer to the ligated adapter/cDNA molecule obtained in step f) at a position overlapping the triazole linkage,

and alternatively

h) adding a DNA polymerase to achieve chain extension of the second primer to produce second strand DNA(s) including sequences complementary to the whole cDNA(s) obtained in step b), and either

i) sequencing the second strand DNA(s) of step h), or

j) adding a third primer which is identical to at least a part of the first primer, and amplifying the full-length cDNA(s) to obtain a full length RNA library,

or

h’) adding a DNA polymerase to achieve chain extension of the second primer and simultaneously determining the sequence of the second strand DNA(s) and the whole cDNA(s).

2. Method for amplifying and or sequencing at least one RNA, preferably mRNA contained in a sample, the method comprising:

a) providing at least one first primer, which first primer includes a sequence which is complementary to a sequence which is located at or near the 3’-end of the at least one mRNA to be amplified, and which primer contains a modification by a first partner of a pair of azide and alkyne molecules at its 5’ end, and adding said at least one first primer to the sample under conditions which allow for hybridization of at least one first primer to at least one mRNA,

b) reverse transcribing the at least one mRNA under conditions including addition of a reverse transcriptase and nucleotides to

the sample to provide full length cDNA(s) of the at least one mRNA,

c) purifying the sample at least from excess nucleotides

d) adding a dideoxy nucleotide, which dideoxy nucleotide is modified at the 3‘-position to include a second partner of a pair of azide and alkyne molecules, and a template-independent polymerase to attach a single 3‘-azide- or 3‘-alkyne modified dideoxy nucleotide at the 3‘-end of the cDNA(s) obtained in step b),

e) purifying the sample at least from excess modified dideoxy nucleotide added in step d),

f) performing a click ligation reaction to generate circular single stranded cDNA(s) under formation of a triazole linkage,

g) adding a second primer, the second primer comprising a nucleotide sequence which is complementary to at least 6 of the nucleotides at the 5‘-end of first primer and contains at its 3‘-end a nucleotide which is complementary to the dideoxy nucleotide at the 3‘-end of the cDNAs, to effect hybridization and binding of the second primer to the ligated circular first primer/cDNA molecule(s) obtained in step f) at a position overlapping the triazole linkage, and either

h) adding a DNA polymerase to achieve chain extension of the second primer and subsequently amplify the full-length cDNA(s) or

h’) adding a DNA polymerase to achieve chain extension of the second primer and simultaneously determining the sequence of the circular single stranded DNA(s) including the whole cDNA(s).

3. Method according to claim 1, wherein in step a) as the at least one first primer a poly(dT) primer, preferably including a 5‘-extension of 2 to 50 nucleotides as anchor sequence, is provided and added to the sample

under conditions which allow for hybridization to the poly(A) tail(s) of the at least one mRNA present in the sample.

4. Method according to claim 1,2 or 3, wherein in step d) 3‘-alkyne- or 3‘-azide-modified ddGTP or 3‘-alkyne- or 3‘-azide-modified ddCTP, preferably 3‘-azide-modified ddGTP, most preferably 3‘-azido-2‘,3‘-dideoxy GTP (AzddGTP) is used as the dideoxy nucleotide.

5. Method according to anyone of claims 1 to 4, wherein in step g) a second primer is added which at the 3‘-end contains dC or dG, preferably dC.

6. Method according to anyone of claims 1 to 5, wherein in step g) a second primer is added which at the second position on the 3‘-end of the nucleotide sequence contains dC or dG, preferably dC.

7. Method according to anyone of claims 1 to 6, wherein in step d) terminal deoxynucleotidyl transferase (TdT) is used as the template-independent polymerase.

8. Method according to anyone of claims 1 and 3 to 7, wherein in step f) an alkyne is preferably attached to the 5‘-end of the adapter molecule.

9. Method according to anyone of claims 1 to 8, wherein in step f) the click reaction comprises a copper catalyzed azide-alkyne cycloaddition (CuAAC) or a strain-promoted copper-free click ligation (SPAAC).

10. Use of a method according to anyone of claims 1 to 9 for preparing a full-length mRNA library from a sample containing a plurality of mRNA molecules.

11. Use according to claim 10, wherein the sample containing a plurality of mRNA molecules comprises the total mRNA of one or more types of cells or the whole exome of anorganism.

12. Method for sequencing a plurality of mRNAs contained in a sample, the total mRNA of one or more types of cells or the whole exome of an individual, the method comprising providing a sample containing such plurality of mRNAs, such total cell mRNA or exome of an individual, preparing a library of full-length mRNA by performing a method according to anyone of claims 1 to 9 or in accordance with the use of claim 10, and determining the sequence of the amplified mRNA or obtained mRNA library.

13. Method according to claim 12, wherein long-read sequencing technology is applied.

14. Method according to claim 12 or 13 for variant mapping in complex disorders, investigation of genetic aberrations.

15. Kit for use in amplifying at least one RNA, preferably mRNA, contained in a sample, the kit comprising:
    a) a first primer which primer includes a sequence which is complementary to a sequence which is located at or near the 3'-end of the at least one mRNA to be amplified,
    b) a reverse transcriptase,
    c) a dideoxy nucleotide which is modified at the 3‘ position to include a first partner of a pair of azide and alkyne molecules,
    d) a template-independent polymerase,
    e) an adapter molecule comprising a polynucleotide sequence and attached to its 5‘-end a second partner of said pair of azide and alkyne molecules

f) a second primer comprising a nucleotide sequence which is complementary to at least 6 of the nucleotides at the 5‘-part of the adapter molecule and which contains at its 3‘-end a nucleotide which is complementary to the dideoxy nucleotide of c),

g) a third primer which is identical to at least a part of the first primer.

16. Kit for use in amplifying and/or sequencing at least one RNA, preferably mRNA, contained in a sample, the kit comprising:

a) a first primer which primer includes a sequence which is complementary to a sequence which is located at or near the 3’-end of the at least one mRNA to be amplified and which primer contains a modification by a first partner of a pair of azide and alkyne molecules at its 5’ end,

b) a reverse transcriptase,

c) a dideoxy nucleotide which is modified at the 3‘ position to include a second partner of a pair of azide and alkyne molecules,

d) a template-independent polymerase,

e) a second primer comprising a nucleotide sequence which is complementary to at least 6 of the nucleotides at the 5‘-part of the first primer and which contains at its 3‘-end a nucleotide which is complementary to the dideoxy nucleotide of c),

17. Kit according to claim 15 or 16, which further comprises at least one of:

h) an RNase,

i) all four kinds of naturally occurring nucleotides,

j) reagents for performing a click reaction,

k) buffers and solvents,

l) reagents for performing cDNA amplification

m) reagents for purification.