

CLAIMS

1. A method of selective amplification of single stranded cDNA, the method comprising:

(i) providing a cDNA sample comprising double stranded cDNA templates, each template having a known 5' pre-attached adapter and a known 3' pre-attached adapter;

5 (ii) denaturing the cDNA sample to produce single stranded cDNA templates;

(iii) re-associating the cDNA sample to produce a mixture of post-association single stranded cDNA templates and post-association double stranded cDNA templates;

(iv) annealing a 5' adapter complex to the 5' pre-attached adapter of at least one post-association single stranded cDNA template, and annealing a 3' adapter complex to the
10 3' pre-attached adapter of the same post-association single stranded cDNA template, in which each adapter complex comprises at least one oligonucleotide;

(v) ligating an oligonucleotide from the 5' adapter complex to the 5' pre-attached adapter of the post-association single stranded cDNA template and ligating an oligonucleotide from the 3' adapter complex to the 3' pre-attached adapter of the same
15 post-association single stranded cDNA template; and

(vi) selectively amplifying the cDNA sample using primers specific to the ligated oligonucleotides, in which:

(A) the 5' adapter complex is a front oligonucleotide dimer comprising:

(i) a front lig-oligonucleotide for ligating to the 5' pre-attached adapter of
20 the (post-association) single stranded cDNA template; and

(ii) a front link-oligonucleotide for annealing to the 5' pre-attached adapter and the front lig-oligonucleotide, the front link-oligonucleotide comprising a region complementary to the 5' pre-attached adapter and a region complementary to the front lig-oligonucleotide,

25 such that, on annealing, an end of the front lig-oligonucleotide is adjacent an end of the 5' pre-attached adapter to enable ligation of the front lig-oligonucleotide to the 5' pre-attached adapter at a ligation site; and

(B) the 3' adapter complex is a back oligonucleotide dimer comprising:

(i) a back lig-oligonucleotide for ligating to the 3' pre-attached adapter of the (post-association) single stranded cDNA template; and

(ii) a back link-oligonucleotide for annealing to the 3' pre-attached adapter and the back lig-oligonucleotide, the back link-oligonucleotide comprising a region complementary to the 3' pre-attached adapter and a region complementary to the back lig-oligonucleotide,

such that, on annealing, an end of the back lig-oligonucleotide is adjacent an end of the 3' pre-attached adapter to enable ligation of the back lig-oligonucleotide to the 3' pre-attached adapter at a ligation site; and in which:

(C) the front link-oligonucleotide comprises:

(i) a template overhang region at an end of the front link-oligonucleotide proximal the region complementary to the 5' pre-attached adapter, the template overhang region being non-complementary to a corresponding region of the (post-association) single stranded cDNA template; and/or

(ii) a lig-oligonucleotide overhang region at an end of the front link-oligonucleotide proximal the region complementary to the front lig-oligonucleotide, the lig-oligonucleotide overhang region being non-complementary to a corresponding region of the front lig-oligonucleotide; and/or

(D) the back link-oligonucleotide comprises:

(i) a template overhang region at an end of the back link-oligonucleotide proximal the region complementary to the 3' pre-attached adapter, the template overhang region being non-complementary to a corresponding region of the (post-association) single stranded cDNA template; and/or

(ii) a lig-oligonucleotide overhang region at an end of the back link-oligonucleotide proximal the region complementary to the back lig-oligonucleotide, the lig-oligonucleotide overhang region being non-complementary to a corresponding region of the back lig-oligonucleotide.

2. A method of selective amplification of cDNA comprising known adapter sequences, the method comprising:

(i) providing a cDNA sample comprising double stranded cDNA templates, a portion of the templates having a known 5' pre-attached adapter and a known 3' pre-attached adapter;

(ii) denaturing the cDNA sample to produce single stranded cDNA templates;

(iii) annealing a 5' adapter complex to the 5' pre-attached adapter of at least one single stranded cDNA template, and annealing a 3' adapter complex to the 3' pre-attached adapter of the same single stranded cDNA template, in which each adapter complex comprises at least one oligonucleotide;

(iv) ligating an oligonucleotide from the 5' adapter complex to the 5' pre-attached adapter of the single stranded cDNA template and ligating an oligonucleotide from the 3' adapter complex to the 3' pre-attached adapter of the same single stranded cDNA template; and

(v) selectively amplifying the cDNA sample using primers specific to the ligated oligonucleotides, in which:

(A) the 5' adapter complex is a front oligonucleotide dimer comprising:

(i) a front lig-oligonucleotide for ligating to the 5' pre-attached adapter of the (post-association) single stranded cDNA template; and

(ii) a front link-oligonucleotide for annealing to the 5' pre-attached adapter and the front lig-oligonucleotide, the front link-oligonucleotide comprising a region complementary to the 5' pre-attached adapter and a region complementary to the front lig-oligonucleotide,

such that, on annealing, an end of the front lig-oligonucleotide is adjacent an end of the 5' pre-attached adapter to enable ligation of the front lig-oligonucleotide to the 5' pre-attached adapter at a ligation site; and

(B) the 3' adapter complex is a back oligonucleotide dimer comprising:

(i) a back lig-oligonucleotide for ligating to the 3' pre-attached adapter of the (post-association) single stranded cDNA template; and

(ii) a back link-oligonucleotide for annealing to the 3' pre-attached adapter and the back lig-oligonucleotide, the back link-oligonucleotide comprising a region complementary to the 3' pre-attached adapter and a region complementary to the back lig-oligonucleotide,

5 such that, on annealing, an end of the back lig-oligonucleotide is adjacent an end of the 3' pre-attached adapter to enable ligation of the back lig-oligonucleotide to the 3' pre-attached adapter at a ligation site; and in which:

(C) the front link-oligonucleotide comprises:

10 (i) a template overhang region at an end of the front link-oligonucleotide proximal the region complementary to the 5' pre-attached adapter, the template overhang region being non-complementary to a corresponding region of the (post-association) single stranded cDNA template; and/or

15 (ii) a lig-oligonucleotide overhang region at an end of the front link-oligonucleotide proximal the region complementary to the front lig-oligonucleotide, the lig-oligonucleotide overhang region being non-complementary to a corresponding region of the front lig-oligonucleotide; and/or

(D) the back link-oligonucleotide comprises:

20 (i) a template overhang region at an end of the back link-oligonucleotide proximal the region complementary to the 3' pre-attached adapter, the template overhang region being non-complementary to a corresponding region of the (post-association) single stranded cDNA template; and/or

25 (ii) a lig-oligonucleotide overhang region at an end of the back link-oligonucleotide proximal the region complementary to the back lig-oligonucleotide, the lig-oligonucleotide overhang region being non-complementary to a corresponding region of the back lig-oligonucleotide.

3. A method according to Claim 1 or 2, in which the template overhang and/or lig-oligonucleotide overhang is between 1 bp and 20 bp in length.

4. A method according to Claim 1, 2 or 3, in which a combined length of the front link-oligonucleotide and the front lig-oligonucleotide is less than 300 bp and/or a combined length of the back link-oligonucleotide and the back lig-oligonucleotide is less than 300 bp.
5. A method according to any preceding claim, in which the front and/or back link-oligonucleotide has a length of less than 200 bp.
6. A method according to any preceding claim, in which the front oligonucleotide dimer and/or the back oligonucleotide dimer has at least one non-blunt end.
7. A method according to any preceding claim, in which the front link-oligonucleotide and/or the back link-oligonucleotide provides at least 5 bp of complementary binding either side of the ligation site.
8. A method according to any preceding claim, in which a nucleotide sequence of the front oligonucleotide dimer is different and non-complementary to a nucleotide sequence of the back oligonucleotide dimer.
9. A method according to any preceding claim, in which at least one of the front oligonucleotide dimer and the back oligonucleotide dimer is annealable to the (post-association) single stranded cDNA template at a temperature of over 30°C.
10. A method according to any preceding claim, in which a concentration of the front oligonucleotide dimer and/or a concentration of the back oligonucleotide dimer exceeds a concentration of a predicted total single stranded cDNA concentration or total cDNA in the cDNA sample.
11. An oligonucleotide dimer composition for use in a method according to any preceding claim for selective amplification of single stranded cDNA by ligation of an oligonucleotide to a 5' and a 3' end of a post-association single stranded cDNA template having known 5' and 3' pre-attached adapters, in which the composition comprises:
 - (A) a front oligonucleotide dimer comprising:
 - (i) a front lig-oligonucleotide for ligating to the 5' pre-attached adapter of the post-association single stranded cDNA template; and
 - (ii) a front link-oligonucleotide for annealing to the 5' pre-attached adapter and the front lig-oligonucleotide, the front link-oligonucleotide comprising a region

complementary to the 5' pre-attached adapter and a region complementary to the front lig-oligonucleotide,

such that, on annealing, an end of the front lig-oligonucleotide is adjacent an end of the 5' pre-attached adapter to enable ligation of the front lig-oligonucleotide to the 5' pre-attached adapter at a ligation site; and

(B) a back oligonucleotide dimer comprising:

(i) a back lig-oligonucleotide for ligating to the 3' pre-attached adapter of the post-association single stranded cDNA template; and

(ii) a back link-oligonucleotide for annealing to the 3' pre-attached adapter and the back lig-oligonucleotide, the back link oligonucleotide comprising a region complementary to the 3' pre-attached adapter and a region complementary to the back lig-oligonucleotide,

such that, on annealing, an end of the back lig-oligonucleotide is adjacent an end of the 3' pre-attached adapter to enable ligation of the back lig-oligonucleotide to the 3' pre-attached adapter at a ligation site,

in which:

(C) the front link-oligonucleotide comprises:

(i) a template overhang region at an end of the front link-oligonucleotide proximal the region complementary to the 5' pre-attached adapter, the template overhang region being non-complementary to a corresponding region of the post-association single stranded cDNA template; and/or

(ii) a lig-oligonucleotide overhang region at an end of the front link-oligonucleotide proximal the region complementary to the front lig-oligonucleotide, the lig-oligonucleotide overhang region being non-complementary to a corresponding region of the front lig-oligonucleotide; and/or

(D) the back link-oligonucleotide comprises:

(i) a template overhang region at an end of the back link-oligonucleotide proximal the region complementary to the 3' pre-attached adapter, the template overhang region being non-complementary to a corresponding region of the post-association single stranded cDNA template; and/or

(ii) a lig-oligonucleotide overhang region at an end of the back link-oligonucleotide proximal the region complementary to the back lig-oligonucleotide, the lig-oligonucleotide overhang region being non-complementary to a corresponding region of the back lig-oligonucleotide.

- 5 12. An oligonucleotide dimer composition according to Claim 11, in which the template overhang and/or lig-oligonucleotide overhang is between 1 bp and 20 bp in length.
13. An oligonucleotide dimer composition according to Claim 11 or 12, in which a combined length of the front link-oligonucleotide and the front lig-oligonucleotide is less than 300 bp and/or a combined length of the back link-oligonucleotide and the back lig-oligonucleotide is less than 300 bp.
- 10 14. An oligonucleotide dimer composition according to Claim 11, 12 or 13, in which the front and/or back link-oligonucleotide has a length of less than 200 bp.
- 15 15. An oligonucleotide dimer composition according to any of Claims 11 to 14, in which the front oligonucleotide dimer and/or the back oligonucleotide dimer has at least one non-blunt end.
16. An oligonucleotide dimer composition according to any of Claims 11 to 15, in which, in use of the composition, the front link-oligonucleotide and/or the back link-oligonucleotide provides at least 5 bp of complementary binding either side of the ligation site.
17. An oligonucleotide dimer composition according to any of Claims 11 to 16, in which a nucleotide sequence of the front oligonucleotide dimer is different and non-complementary to a nucleotide sequence of the back oligonucleotide dimer.
- 20 18. An oligonucleotide dimer composition according to any of Claims 11 to 17, in which the front oligonucleotide dimer and/or the back oligonucleotide dimer is annealable to the post-association single stranded cDNA template at a temperature of over 30°C.
- 25 19. Use of a method according to any of Claims 1 to 10 or an oligonucleotide dimer composition according to any of Claims 11 to 18 in a process of RNA or DNA sequencing.
20. Use of a method according to any of Claims 1 to 10 or an oligonucleotide dimer composition according to any of Claims 11 to 18 in a process of metagenomic sequencing for discovery of new microbes and/or detection of low abundance microbes.

21. Use of a method according to any of Claims 1 to 10 or an oligonucleotide dimer composition according to any of Claims 11 to 18 in a process of screening DNA or RNA samples, or screening genetic samples for the presence of infectious diseases.

22. Use of a method according to any of Claims 1 to 10 or an oligonucleotide dimer composition according to any of Claims 11 to 18 in a process of detecting a nucleic acid biomarker.

23. A selective amplification kit for selectively amplifying low abundance cDNA from a cDNA sample, the cDNA sample comprising cDNA templates having known 5' and 3' pre-attached adapters, the kit comprising means for preparing an oligonucleotide dimer composition according to any of Claims 11 to 18 and means for implementing the method of selective amplification according to any of Claims 1 to 10, in which the means for preparing an oligonucleotide dimer composition comprise:

(i) a front lig-oligonucleotide for ligating to the 5' pre-attached adapter;

(ii) a front link-oligonucleotide for annealing to the 5' pre-attached adapter and the front lig-oligonucleotide, the front link-oligonucleotide comprising a region complementary to the 5' pre-attached adapter and a region complementary to the front lig-oligonucleotide,

such that, on annealing, an end of the front lig-oligonucleotide is adjacent an end of the 5' pre-attached adapter to enable ligation of the front lig-oligonucleotide to the 5' pre-attached adapter at a ligation site;

(iii) a back lig-oligonucleotide for ligating to the 3' pre-attached adapter; and

(iv) a back link-oligonucleotide for annealing to the 3' pre-attached adapter and the back lig-oligonucleotide, the back link oligonucleotide comprising a region complementary to the 3' pre-attached adapter and a region complementary to the back lig-oligonucleotide,

such that, on annealing, an end of the back lig-oligonucleotide is adjacent an end of the 3' pre-attached adapter to enable ligation of the back lig-oligonucleotide to the 3' pre-attached adapter at a ligation site,

in which:

the front link-oligonucleotide comprises:

(i) a template overhang region at an end of the front link-oligonucleotide proximal the region complementary to the 5' pre-attached adapter, the template overhang region being non-complementary to a corresponding region of the cDNA template; and/or

5 (ii) a lig-oligonucleotide overhang region at an end of the front link-oligonucleotide proximal the region complementary to the front lig-oligonucleotide, the lig-oligonucleotide overhang region being non-complementary to a corresponding region of the front lig-oligonucleotide; and/or

the back link-oligonucleotide comprises:

10 (i) a template overhang region at an end of the back link-oligonucleotide proximal the region complementary to the 3' pre-attached adapter, the template overhang region being non-complementary to a corresponding region of the cDNA template; and/or

15 (ii) a lig-oligonucleotide overhang region at an end of the back link-oligonucleotide proximal the region complementary to the back lig-oligonucleotide, the lig-oligonucleotide overhang region being non-complementary to a corresponding region of the back lig-oligonucleotide.