

CLAIMS

1. A method for processing RNA comprising:
 - (i) contacting a first RNA sample with an oligonucleotide array, in which the oligonucleotide array comprises two or more oligonucleotides linked to a surface, and in which two or more RNA molecules from the first RNA sample anneal to the oligonucleotides of the oligonucleotide array;
 - (ii) extending two or more of the oligonucleotides by reverse transcription using the annealed RNA molecules as templates to generate a DNA array comprising two or more cDNA molecules;
 - (iii) disassociating the annealed RNA molecules from the cDNA molecules;
 - (iv) removing the first RNA sample from the surface;
 - (v) contacting a second RNA sample with the DNA array, in which one or more RNA molecules from the second RNA sample anneal to the cDNA molecules; and
 - (vi) extracting the unannealed RNA molecules thereby generating processed RNA;
- in which the first RNA sample and the second RNA sample are derived from the same RNA sample.
2. A method according to Claim 1, in which the oligonucleotides comprise oligo-dT sequences.
3. A method according to Claim 1 or 2, in which the surface is one or more magnetic beads.
4. A method according to Claim 1, 2 or 3, in which the method is carried out in a microfluidic flowcell.
5. A method according to any preceding claim, in which the first RNA sample and/or the second RNA sample comprises full length RNA.
6. A method according to any preceding claim, in which the oligonucleotides are optimally spaced so that the cDNA molecules of the DNA array do not interact with each other.
7. A method according to any preceding claim, further comprising sequencing the processed RNA.

8. A method according to any preceding claim, further comprising, prior to step (i):
 - (a) contacting a biological sample with an oligonucleotide array, in which the oligonucleotide array comprises two or more oligonucleotides linked to a surface, and in which one or more RNA molecules from the biological sample anneal to the oligonucleotides of the oligonucleotide array;
 - (b) removing the unannealed sample from the surface; and
 - (c) disassociating the annealed RNA molecule(s) from the oligonucleotides to obtain an RNA sample.
9. A method according to any preceding claim, further comprising, following step (vi) disassociating the annealed RNA molecules from the cDNA molecules and removing the disassociated RNA molecules from the surface and, optionally, repeating steps (v) and (vi) with a further RNA sample.
10. A method according to Claim 8 or 9, in which the biological sample comprises a biological fluid or a fluid or lysate generated from a biological material.
11. Use of an RNA processing device for producing processed RNA from a biological sample in a method according to any preceding claim, the device comprising:
 - (i) a first module to receive the biological sample, in which the first module comprises:
 - (a) two or more oligonucleotides capable of annealing to one or more RNA molecules in the biological sample;
 - (b) a first waste outlet to remove unannealed sample;
 - (c) a sample outlet through which a portion of the sample comprising the one or more RNA molecules is capable of flowing following disassociation from the oligonucleotides; and
 - (ii) a second module to receive the one or more RNA molecules from the first module, in which the second module comprises:
 - (a) two or more oligonucleotides capable of annealing to one or more RNA molecules in the sample;
 - (b) a processed RNA outlet through which the processed RNA can be obtained;

- (c) a waste RNA outlet to remove one or more RNA molecules;

in which the first module and the second module together define a flow path along which a sample is capable of flowing.

12. Use according to Claim 11 in which:

- 5 (i) the oligonucleotides of the first module are linked to a first surface and the oligonucleotides of the second module are linked to a second surface;
- (ii) the first module further comprises a sample inlet through which the biological sample is capable of entering the first module;
- 10 (iii) the first module further comprises a first reagent inlet through which reagents are capable of entering the first module and/or in which the second module further comprises a second reagent inlet through which reagents are capable of entering the second module;
- (iv) the RNA processing device further comprises temperature control means for adjusting the temperature of the first module and/or the second module;
- 15 (v) the first module comprises a flow cell and/or the second module comprises a flow cell; and/or
- (vi) the oligonucleotides are optimally spaced.

13. Use of a kit for processing an RNA sample in a method according to any of Claims 1 to 10, the kit comprising:

- 20 (a) one or more magnetic beads, in which two or more oligo-dT molecules are linked to the one or more magnetic beads;
- (b) a hybridization buffer; and
- (c) a reverse transcriptase.

14. Use according to Claim 13, in which the kit further comprises one or more, up to all, of
25 dinucleotide triphosphates (dNTPs), $MgCl_2$ and a buffer.

15. A method of analysing a biological sample from a subject, the method comprising:

- (a) extracting RNA from the biological sample;
- (b) preparing a processed RNA sample; and

(c) sequencing the processed RNA;

in which preparing a processed RNA sample comprises taking a portion of the extracted RNA to be a first RNA sample and a portion of the extracted RNA to be a second RNA sample and following the steps of a method according to any of Claims 1 to 7.

5 16. A method according to Claim 15 in which:

(i) the RNA is full length RNA;

(ii) the biological sample comprises a biological fluid or a fluid or lysate generated from a biological material, optionally in which the biological fluid comprises a blood sample; and/or

10 (iii) the RNA extracted from the sample comprises cell-free RNA.

17. A method according to Claim 15 or 16, in which the method comprises diagnosing a disease in the subject, optionally in which the presence or absence of one or more RNA molecules in the processed RNA sample is used to identify whether the subject has the disease.

15 18. A method according to Claim 15, 16 or 17, in which extracting RNA from the biological sample comprises:

(a) contacting the biological sample with an oligonucleotide array in which the oligonucleotide array comprises two or more oligonucleotides linked to a surface and in which one or more RNA molecules from the sample anneals to the oligonucleotides of
20 the oligonucleotide array;

(b) removing the unannealed sample from the surface; and

(c) disassociating the annealed RNA molecules from the oligonucleotides thereby generating an RNA sample.

19. A method according to Claim 18, in which the oligonucleotides comprise one or more
25 oligo-dT sequences.

20. A method according to any of Claims 15 to 19 comprising use of a RNA processing device according to Claim 11 or 12 or a kit according to Claim 13 or 14.