

CLAIMS

1. A method of analysing ribonucleic acid-ribonucleic acid (RNA-RNA) interactions comprising:

5 cross-linking base-paired nucleotides of at least one RNA molecule and/or at least one pair of RNA molecules using a biotin-tagged, reversible cross-linking agent to produce at least one cross-linked RNA molecule and/or at least one pair of cross-linked RNA molecules;

10 fragmenting the said cross-linked RNA molecule and/or pair of cross-linked RNA molecules to produce a plurality of fragments of cross-linked RNA molecule and/or pair of cross-linked RNA molecules;

using said biotin to extract said cross-linked RNA molecule and/or pair of cross-linked RNA molecules obtained from the preceding step;

15 ligating the said cross-linked RNA fragment(s) to produce cross-linked ligated RNA chimera(s);

reversing the cross-linking of the said agent to the said RNA molecule and/or pair of RNA molecules to produce a ligated RNA chimera molecule(s) and/or RNA chimera pair(s);

20 preparing a sequence library by sequencing the ligated RNA chimera molecule(s) or pair(s); and

analysing the sequence library to determine RNA-RNA interactions.

2. The method according to claim 1 wherein said RNA is present in a cell and said crosslinking using said biotin-tagged, reversible cross-linking agent involves the use of a cellular uptake agent, such as a detergent.

3. The method according to claims 1 or 2 wherein the step of using said biotin to extract said cross-linked RNA molecule and/or pair of cross-linked RNA molecules is undertaken before the step of fragmenting the said cross-linked RNA molecule and/or pair of cross-linked RNA molecules.

4. The method according to any one of claims 1-3 wherein said cross-linking agent comprises a furocoumarin compound.

5. The method according to claim 4 wherein the cross-linking agent comprises psoralen.
- 5 6. The method according to of any one of claims 1-5 wherein said biotin of said cross-linking agent comprises a first member of a binding pair selected from biotin/streptavidin.
- 10 7. The method according to any one of claims 1-6 wherein the step of cross-linking said RNA molecule(s) with a cross-linking agent to produce cross-linked RNA molecule(s) is carried out using ultraviolet irradiation at wavelengths in the range of about 300nm to about 400nm.
- 15 8. The method according to any one of claims 1-7 wherein the step of reversing the cross-linking of the cross-linked ligated RNA molecule(s) is carried out using ultraviolet irradiation at wavelengths in the range of about 200nm to no more than about 300nm.
- 20 9. The method according to any one of claims 1-8 wherein the step of preparing a sequence library by sequencing the ligated RNA chimera molecule or pairs comprises the use of at least one or more of the following: adaptor ligation, reverse transcription, cDNA circularization or polymerase chain reaction (PCR).
- 25 10. The method according to any one of claims 1-9 wherein the step of fragmenting the cross-linked RNA molecule and/or pair of RNA molecules to produce a plurality of fragments comprises producing fragments having an average size in the range of 100 to 500 base pairs in length.
- 30 11. The method according to any one of claims 1-10 wherein the concentration of cross-linking agent used crosslinks at approximately one in every 150 bases.
12. The method according to any one of claims 1-11 wherein continuous pairwise interactions or those spaced apart by less than 50 bases are removed to focus the analysis on the long-range intramolecular and intermolecular interactions.

- 5 13. The method according to any one of claims 1-12 wherein said RNA molecule and/or at least one member of said pair of RNA molecules is ascribed a "circularization score" defined as the average base pair interaction distance within each molecule, normalized by the length of said RNA molecule or the length of said member of said pair of RNA molecules.
- 10 14. The method according to claim 13 wherein said RNA molecule and/or said at least one member of said pair of RNA molecules are classified into groups according to their "circularization score".
- 15 15. The method according to any one of claims 2-13 wherein the cell is mammalian, human, bacterial or yeast.
- 15 16. A kit for analysing ribonucleic acid-ribonucleic acid (RNA-RNA) interactions comprising:
- 20 a biotin-tagged, reversible cross-linking agent for reversibly cross-linking base paired nucleotides of at least one RNA molecule and/or at least one pair of RNA molecules to produce at least one cross-linked RNA molecule and/or at least one pair of cross-linked RNA molecules;
- 20 a fragmentation buffer for fragmenting the cross-linked RNA molecule and/or pair of cross-linked RNA molecules to produce a plurality of fragments;
- an RNA ligase for ligating the cross-linked RNA fragment(s) to produce cross-linked ligated RNA chimeras;
- 25 a binding partner for said biotin on said agent; and
- optionally, instructions on how to use the kit.
17. The kit according to claim 16 further comprising reagents for sequencing the cross-linked ligated RNA chimeras to prepare a sequence library.
- 30 18. The kit according to claim 17 wherein the reagents for sequencing the ligated RNA chimeras comprises at least one of: a RNA ligase, reverse transcription primers and DNA polymerase.
- 35 19. The kit according to any one of claims 16-18 wherein the cross-linking agent comprises a furocoumarin compound.

20. The kit according to claim 19 wherein the cross-linking agent comprises psoralen.

21. The kit according to any one of claims 16-20 wherein said biotin of said cross-linking agent comprises a first member of a binding pair selected from biotin/streptavidin.

22. The kit according to claim 21 wherein said binding partner is the other member of a binding pair selected from biotin/streptavidin.

23. The kit of according to any one of claims 16-21 further comprising a detergent to facilitate cellular uptake of the cross-linking agent into a cell.

24. A method of studying a subject, the method comprising:

obtaining a cell sample from a subject;

cross-linking base-paired nucleotides of at least one RNA molecule and/or at least one pair of RNA molecules using a biotin-tagged, reversible cross-linking agent to produce at least one cross-linked RNA molecule and/or at least one pair of cross-linked RNA molecules;

fragmenting the said cross-linked RNA molecule and/or pair of cross-linked RNA molecules to produce a plurality of fragments of cross-linked RNA molecule and/or pair of cross-linked RNA molecules;

using said biotin to extract said cross-linked RNA molecule and/or pair of cross-linked RNA molecules obtained from the preceding step;

ligating the said cross-linked RNA fragment(s) to produce cross-linked ligated RNA chimera(s);

reversing the cross-linking of the said agent to the said RNA molecule and/or pair of RNA molecules to produce ligated RNA chimera molecule(s) and/or RNA chimera pair(s);

preparing a sequence library by sequencing the ligated RNA chimera molecule(s) or pair(s);

analysing the sequence library to determine RNA-RNA interactions in the cell sample; and

comparing the determined RNA-RNA interactions with a set of pre-existing data to attribute a clinical picture to the subject.

25. The method of claim 23 wherein the method of studying a subject comprises at least one of: diagnosing the subject of a clinical condition, predicting the risk of the subject having a clinical condition, screening the subject for suitability for a particular treatment or determining the efficacy of a drug candidate on the subject.

26. A drug discovery method, the method comprising:

exposing RNA to a drug;

cross-linking base-paired nucleotides of at least one RNA molecule and/or at least one pair of RNA molecules using a biotin-tagged, reversible cross-linking agent to produce at least one cross-linked RNA molecule and/or at least one pair of cross-linked RNA molecules;

fragmenting the said cross-linked RNA molecule and/or pair of cross-linked RNA molecules to produce a plurality of fragments of cross-linked RNA molecule and/or pair of cross-linked RNA molecules;

using said biotin to extract said cross-linked RNA molecule and/or pair of cross-linked RNA molecules obtained from the preceding step;

ligating the said cross-linked RNA fragment(s) to produce cross-linked ligated RNA chimera(s);

reversing the cross-linking of the said agent to the said RNA molecule and/or pair of RNA molecules to produce a ligated RNA chimera molecule(s) and/or RNA chimera pair(s);

preparing a sequence library by sequencing the ligated RNA chimera molecule(s) or pair(s);

analysing the sequence library to determine RNA-RNA interactions; and attributing an efficacy score to the drug based on the determined RNA-RNA interactions.