

CLAIMS

1. A method of characterising a cell, the method comprising:
 - determining the structure of RNA molecules in the cell according to a method comprising:
 - contacting the RNA molecules with a modifying agent comprising 2-methylnicotinic acid imidazolidine (NAI) or 2-methylnicotinic acid imidazolidine-azide (NAI-N3) to obtain modified RNA molecules;
 - reverse transcribing the modified RNA molecules;
 - sequencing the product obtained from the preceding step to generate sequencing reads; and
 - analysing the sequencing reads to determine the structure of the RNA molecules.
2. A method of classifying cells into one or more cell populations, the method comprising:
 - determining the structure of RNA molecules in the cell according to a method comprising:
 - contacting the RNA molecules with a modifying agent comprising 2-methylnicotinic acid imidazolidine (NAI) or 2-methylnicotinic acid imidazolidine-azide (NAI-N3) to obtain modified RNA molecules;
 - reverse transcribing the modified RNA molecules;
 - sequencing the product obtained from the preceding step to generate sequencing reads; and
 - analysing the sequencing reads to determine the structure of the RNA molecules; and
 - classifying the cells into one or more cell populations based on similarity in the structure of their RNA molecules.
3. The method according to claim 2, wherein the method is a method of classifying cells into different cell types and/or different stages of development.

4. The method according to any one of claims 1-3, wherein the modifying agent comprises 2-methylnicotinic acid imidazolidine-azide (NAI-N3).
5. The method according to any one of claims 1-4, wherein the reverse transcribing step is carried out in a manganese-containing medium, optionally wherein the reverse transcribing step is carried out at a temperature of less than 50°C.
6. The method according to any one of claims 1-5, wherein the reverse transcribing step is carried out using Moloney murine leukemia virus (MMLV) reverse transcriptase, optionally a genetically modified MMLV reverse transcriptase.
7. The method according to any one of claims 1-6, wherein the reverse transcribing step is carried out for least about 2 hours, optionally at least about 4 hours, further optionally at least about 6 hours, further optionally at least about 8 hours.
8. The method according to any one of claims 1-7, wherein the method further comprises fragmenting the modified RNA molecules prior to the reverse-transcribing step.
9. The method according to claim 8, wherein fragmenting the modified RNA molecules comprises heating the modified RNA molecules.
10. The method according to claim 9, wherein the modified RNA molecules is heated in the presence of deoxynucleoside triphosphate (dNTP).
11. The method according to any one of claims 8-10, wherein the fragmenting step is carried out in a medium that is substantially free of magnesium.
12. The method according to any one of claims 8-11, wherein the fragmenting step is carried out in the same vessel as the reverse transcribing step.

13. The method according to any one of claims 1-12, wherein the method further comprises amplifying the reverse transcribed product prior to the sequencing step.
14. The method according to claim 13, wherein the method further comprises purifying the amplicons, further optionally purifying the amplicons for at least two times.
15. The method according to any one of claims 1-14, wherein the RNA molecules consist of RNA molecules of a single cell.