

of proofreading activity in reverse transcriptases. This is particularly problematic where the RNA-seq experiments are performed on a low number of cells.

Accordingly, there is a need to provide a method to simultaneously analyse RNA and DNA from a single cell or limited amounts of nucleic acids that overcomes, or at least ameliorates one or more of the disadvantages described above.

There is a need to provide such a method in a cost-effective way, with minimum procedural steps.

Summary

In a first example, there is provided a method of simultaneously analyzing RNA and DNA in a sample, the method comprising the steps of:

- 10 (a) contacting the sample with a reverse primer from a first primer pair, the reverse primer from the first primer pair being directed to a target RNA region, and a reverse transcriptase to effect reverse transcription of the RNA into cDNA;
- (b) subsequently contacting the sample with:
 - 15 (i) a forward primer from the first primer pair, the forward primer from the first primer pair being directed to a target cDNA region,
 - (ii) a reverse primer and a forward primer from a second primer pair, the reverse primer and forward primer from the second primer pair being directed to a target DNA region, and
 - (iii) a DNA polymerase
- 20 (c) analyzing the amplified target cDNA region and/or the amplified target DNA region.

One example of the method of the first example further comprises the step of subjecting the sample from step (b) to a semi-nested PCR using the reverse primer in step (a) or the forward primer in step (b)(i), and a nested primer that binds within the amplified target cDNA region.

- 25 In one aspect, the present disclosure refers to a method of simultaneously analyzing RNA and DNA in a sample, the method comprising the steps of: (a) contacting the sample with a reverse primer from a first primer pair, the reverse primer from the first primer pair being directed to a target RNA region, and a reverse transcriptase to effect reverse transcription of the RNA into cDNA; (b) subsequently contacting the sample with: (i) a forward primer from the first primer pair, the forward primer from the first primer pair being directed to a target cDNA region, (ii) a
- 30 reverse primer and a forward primer from a second primer pair, the reverse primer and forward primer from the second primer pair being directed to a target DNA region, and (iii) a DNA polymerase to simultaneously amplify the target cDNA region and the target DNA region; (c) (A) subjecting the sample from step (b) to a semi-nested PCR using the reverse primer in step (a) or the forward primer in step (b)(i), and a nested primer that binds within the amplified target cDNA
- 35 region; and (B) subjecting the sample from step (b) to a nested PCR using a nested primer pair that binds within the amplified target DNA region; and (d) analyzing the amplified target cDNA region and the amplified target DNA region; wherein the sample is a single cell; and wherein the first primer pair comprises primers that span exon-exon boundaries or are separated by at least one intron on the corresponding DNA region; and wherein steps (a) and (b) are conducted in the
- 40 same reaction mixture.

In a second example, there is provided a method of simultaneously analyzing RNA and DNA in a sample, the method comprising the steps of:

- (a) lysing a cell in the sample;
- (b) contacting the lysed cell sample with a reverse primer from a first primer pair, the reverse
- 45 primer from the first primer pair being directed to a target RNA region, and a reverse transcriptase to effect reverse transcription of the RNA into cDNA;
- (c) subsequently contacting the lysed cell sample with:

(i) a forward primer from the first primer pair, the forward primer from the first primer pair being directed to a target cDNA region,

(ii) a reverse primer and a forward primer from a second primer pair, the reverse primer and forward primer from the second primer pair being directed to a target DNA region, and

5 (iii) a DNA polymerase

to simultaneously amplify the target cDNA region and the target DNA region in a pre-amplification step;

10 (d) subjecting the sample from step (c) to a semi-nested PCR using the reverse primer in step (b) or the forward primer in step (c)(i), a nested primer that binds within the pre-amplified target cDNA region, and a DNA polymerase to further amplify the target cDNA region, and/or subjecting the sample from step (c) to a nested PCR using a nested primer pair that binds within the pre-amplified target DNA region and a DNA polymerase to further amplify the target DNA region; and

15 (e) analyzing the further amplified target cDNA region and/or the further amplified target DNA region.

In a third example, there is provided use of the method according to the first or second example for determining the DNA profile and/or the gene expression profile of a single cell or a plurality of cells, or cell-free RNA and/or DNA derived from a single cell or a plurality of cells.

20 In one aspect, the present disclosure refers to use of the method disclosed herein for determining the DNA profile and/or the gene expression profile of a single cell.

In a fourth example, there is provided use of the method according to the first or second example for validation of large-scale transcriptomics data sets.

25 In one aspect, the present disclosure refers to use of the method disclosed herein for validation of large-scale transcriptomics data sets.

In a fifth example, there is provided a kit for performing the method according to the first or second example, or for use according to the third or fourth example, wherein the kit comprises:

(a) a primer selected from the group consisting of:

30 i. the reverse primer of step (a) of the method of the first example or step (b) of the method of the second example,

ii. the forward primer of step (b)(i) of the method of the first example or step (c)(i) of the method of the second example,

35 iii. the primer pair of step (b)(ii) of the method of the first example or step (c)(ii) of the method of the second example, and

iv. the nested primer and nested primer pair of the method of the first example or step (d) of the method of the second example;

(b) one or more reagents, selected from the group consisting of:

- i. a reverse transcriptase and one or more suitable reaction buffers for the reverse transcription in step (a) of the method of the first example or step (b) of the method of the second example,
 - 5 ii. a DNA polymerase and one or more suitable reaction buffers for the amplification in step (b) or the semi-nested or nested PCR of the method of the first example, or step (c) or (d) of the method of the second example, and
 - iii. one or more labelled or unlabelled deoxyribonucleotides selected from the group consisting of dATP, dCTP, dGTP, and dTTP or dUTP; and
- (c) instructions for performing the method according to the first or second example, or for use
10 according to the third or fourth example.

Brief Description of Drawings

The invention will be better understood with reference to the detailed description when considered in conjunction with the non-limiting examples and the accompanying drawings, in which:

15 Fig.1

[Fig. 1] provides an outline of the scrmPCR method of the disclosure. This method can be used to detect and quantify RNA transcripts and to sequence DNA from the same single cell. A schematic of the method is enclosed as Figure 1 and is composed of the following steps:

- 20 **1)** Primer design: Primer sets are designed for semi-nested PCR (RNA transcripts) or nested PCR (genomic DNA). In some cases, primers were derived from the literature (Table 1). **2)** Reverse transcription: Multiplex reverse primers are added to the lysed single cell, together with a reverse transcriptase enzyme (step II in Fig. 1). **3)** Pre-amplification: Multiplex forward primers are added to the mixture, together with primers
25 specific for DNA sequences and a pre-amplification step is performed using an optimized annealing temperature (Step III in Fig. 1). **4)** Cleanup: Unused primers are removed by spin column or enzymatic digestion. **5)** Dilution of the sample. **6)** For RNA analysis, perform qPCR using a semi-nested approach, where a primer is designed to bind a sequence internal to the pre-amplified product and the other primer has been
30 previously used during the pre-amplification step (step IV in Fig.1) **7)** For DNA analysis: PCR using a nested approach where both primers are designed to bind a sequence internal to the pre-amplified product (step IV in Fig.1).

Fig.2

- 35 [Fig. 2] shows that nested PCR is required for specific amplification of PCR products from single cells. In (a), primers for TP53 exon 6 were optimized to amplify tissue DNA