

Claims

1. 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 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 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 same reaction mixture.

2. The method according to claim 1, wherein the single cell is selected from the group consisting of: a cell from a pre-implantation embryo (such that the embryo is not destroyed), a stem cell, a suspected cancer cell, a suspected tumour-derived cell, a suspected embryonic cell, a cell from a pathogenic organism, and a cell obtained from a crime scene.

3. The method according to claim 1 or 2, wherein

a) the forward primer of the first or second primer pair is selected from the group consisting of SEQ ID NOs 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85 and 87;

b) the reverse primer of the first or second primer pair is selected from the group consisting of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86 and 88; or

c) the first or second primer pair is selected from the group consisting of SEQ ID NOs 1 and 2; SEQ ID NOs 3 and 4; SEQ ID NOs 5 and 6; SEQ ID NOs 7 and 8; SEQ ID NOs 9 and 10;

SEQ ID NOs 11 and 12; SEQ ID NOs 13 and 14; SEQ ID NOs 15 and 16; SEQ ID NOs 17 and 18; SEQ ID NOs 19 and 20; SEQ ID NOs 21 and 22; SEQ ID NOs 23 and 24; SEQ ID

NOs 25 and 26; SEQ ID NOs 27 and 28; SEQ ID NOs 29 and 30; SEQ ID NOs 31 and 32; SEQ ID NOs 33 and 34; SEQ ID NOs 35 and 36; SEQ ID NOs 37 and 38; SEQ ID NOs 39

and 40; SEQ ID NOs 41 and 42; SEQ ID NOs 43 and 44; SEQ ID NOs 45 and 46; SEQ ID

NOs 47 and 48; SEQ ID NOs 49 and 50; SEQ ID NOs 51 and 52; SEQ ID NOs 53 and 54; SEQ ID NOs 55 and 56; SEQ ID NOs 57 and 58; SEQ ID NOs 59 and 60; SEQ ID NOs 61

and 62; SEQ ID NOs 63 and 64; SEQ ID NOs 65 and 66; SEQ ID NOs 67 and 68; SEQ ID

NOs 69 and 70; SEQ ID NOs 71 and 72; SEQ ID NOs 73 and 74; SEQ ID NOs 75 and 76; SEQ ID NOs 77 and 78; SEQ ID NOs 79 and 80; SEQ ID NOs 81 and 82; SEQ ID NOs 83

and 84; SEQ ID NOs 85 and 86; and SEQ ID NOs 87 and 88;

wherein the second primer pair comprises primers that bind to intronic regions of the target

DNA region;

wherein

a) the forward primer for semi-nested or nested PCR is selected from the group consisting of

SEQ ID NOs: 89, 90, 91, 92, 93, 94, 95, 15, 17, 19, 99, 23, 101, 102, 104, 105, 106, 107, 108, 110, 111, 43, 45, 115, 116, 117, 118, 55, 120, 59, 61, 123, 124, 125, 126, 71, 127, 75, 77, 130, 81, 132, 133 and 134;

b) the reverse primer for semi-nested or nested PCR is selected from the group consisting of

SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 96, 97, 98, 22, 100, 26, 103, 30, 32, 34, 36, 109, 40, 112, 113, 114, 48, 50, 52, 54, 119, 58, 121, 122, 64, 66, 68, 70, 72, 74, 128, 129, 80, 131, 84, 86

and 88; or

c) the primers for semi-nested or nested PCR is selected from the group consisting of SEQ ID

NOs 89 and 2; SEQ ID NOs 90 and 4; SEQ ID NOs 91 and 6; SEQ ID NOs 92 and 8; SEQ ID

NOs 93 and 10; SEQ ID NOs 94 and 12; SEQ ID NOs 95 and 14; SEQ ID NOs 15 and 96;

SEQ ID NOs 17 and 97; SEQ ID NOs 19 and 98; SEQ ID NOs 99 and 22; SEQ ID NOs 23

and 100; SEQ ID NOs 101 and 26; SEQ ID NOs 102 and 103; SEQ ID NOs 104 and 30; SEQ

ID NOs 105 and 32; SEQ ID NOs 106 and 34; SEQ ID NOs 107 and 36; SEQ ID NOs 108

and 109; SEQ ID NOs 110 and 40; SEQ ID NOs 111 and 112; SEQ ID NOs 43 and 113; SEQ

ID NOs 45 and 114; SEQ ID NOs 115 and 48; SEQ ID NOs 116 and 50; SEQ ID NOs 117

127 and 74; SEQ ID NOs 75 and 128; SEQ ID NOs 77 and 129; SEQ ID NOs 130 and 80; SEQ ID NOs 81 and 131; SEQ ID NOs 132 and 84; SEQ ID NOs 133 and 86; and SEQ ID NOs 134 and 88;

wherein the analysis in step (d) comprises analyzing the amplified target cDNA for gene expression;

and wherein the analysis in step (d) comprises analyzing the amplified target DNA for mutations.

4. The method according to any one of the preceding claims, wherein step (b) comprises about 1 to about 50 cycling steps,

wherein optionally step (b) comprises 3 cycling steps; wherein optionally each cycling step comprises about 1 to about 50 cycles of denaturation, annealing and elongation,

wherein optionally each cycling step comprises 6 cycles of denaturation, annealing and elongation;

wherein optionally the annealing and/or elongation temperature in a cycle is about 40°C to about 75°C;

wherein optionally the annealing and/or elongation is carried out for about 10 seconds to about 10 minutes;

wherein optionally the denaturation is carried out at a temperature of about 75°C to about 120°C;

wherein optionally the denaturation is carried out for about 1 second to about 10 minutes; and wherein optionally

step (b) comprises:

6 cycles of 60°C for 4 minutes followed by 95°C for 1 minute,

6 cycles of 55°C for 4 minutes followed by 95°C for 1 minute, and

6 cycles of 50°C for 4 minutes followed by 95°C for 1 minute.

5. The method according to claim 1, wherein the nested primer in step (c) is one that matches or corresponds to the reverse primer in step (a) or the forward primer in step (b)(i); wherein the pre-amplification in step (b) does not include a final extension step.

6. The method according to claim 5, wherein

a) the forward primer of the first or second primer pair is selected from the group consisting of SEQ ID NOs 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85 and 87;

b) the reverse primer of the first or second primer pair is selected from the group consisting of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86 and 88; or

c) the first or second primer pair is selected from the group consisting of SEQ ID NOs 1 and 2; SEQ ID NOs 3 and 4; SEQ ID NOs 5 and 6; SEQ ID NOs 7 and 8; SEQ ID NOs 9 and 10; SEQ ID NOs 11 and 12; SEQ ID NOs 13 and 14; SEQ ID NOs 15 and 16; SEQ ID NOs 17 and 18; SEQ ID NOs 19 and 20; SEQ ID NOs 21 and 22; SEQ ID NOs 23 and 24; SEQ ID

NOs 25 and 26; SEQ ID NOs 27 and 28; SEQ ID NOs 29 and 30; SEQ ID NOs 31 and 32; SEQ ID NOs 33 and 34; SEQ ID NOs 35 and 36; SEQ ID NOs 37 and 38; SEQ ID NOs 39 and 40; SEQ ID NOs 41 and 42; SEQ ID NOs 43 and 44; SEQ ID NOs 45 and 46; SEQ ID NOs 47 and 48; SEQ ID NOs 49 and 50; SEQ ID NOs 51 and 52; SEQ ID NOs 53 and 54; SEQ ID NOs 55 and 56; SEQ ID NOs 57 and 58; SEQ ID NOs 59 and 60; SEQ ID NOs 61 and 62; SEQ ID NOs 63 and 64; SEQ ID NOs 65 and 66; SEQ ID NOs 67 and 68; SEQ ID NOs 69 and 70; SEQ ID NOs 71 and 72; SEQ ID NOs 73 and 74; SEQ ID NOs 75 and 76; SEQ ID NOs 77 and 78; SEQ ID NOs 79 and 80; SEQ ID NOs 81 and 82; SEQ ID NOs 83 and 84; SEQ ID NOs 85 and 86; and SEQ ID NOs 87 and 88;

wherein optionally

a) the forward primer for semi-nested or nested PCR is selected from the group consisting of SEQ ID NOs: 89, 90, 91, 92, 93, 94, 95, 15, 17, 19, 99, 23, 101, 102, 104, 105, 106, 107, 108, 110, 111, 43, 45, 115, 116, 117, 118, 55, 120, 59, 61, 123, 124, 125, 126, 71, 127, 75, 77, 130, 81, 132, 133 and 134;

b) the reverse primer for semi-nested or nested PCR is selected from the group consisting of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 96, 97, 98, 22, 100, 26, 103, 30, 32, 34, 36, 109, 40, 112, 113, 114, 48, 50, 52, 54, 119, 58, 121, 122, 64, 66, 68, 70, 72, 74, 128, 129, 80, 131, 84, 86 and 88; or

c) the primers for semi-nested or nested PCR is selected from the group consisting of SEQ ID NOs 89 and 2; SEQ ID NOs 90 and 4; SEQ ID NOs 91 and 6; SEQ ID NOs 92 and 8; SEQ ID NOs 93 and 10; SEQ ID NOs 94 and 12; SEQ ID NOs 95 and 14; SEQ ID NOs 15 and 96; SEQ ID NOs 17 and 97; SEQ ID NOs 19 and 98; SEQ ID NOs 99 and 22; SEQ ID NOs 23 and 100; SEQ ID NOs 101 and 26; SEQ ID NOs 102 and 103; SEQ ID NOs 104 and 30; SEQ ID NOs 105 and 32; SEQ ID NOs 106 and 34; SEQ ID NOs 107 and 36; SEQ ID NOs 108 and 109; SEQ ID NOs 110 and 40; SEQ ID NOs 111 and 112; SEQ ID NOs 43 and 113; SEQ ID NOs 45 and 114; SEQ ID NOs 115 and 48; SEQ ID NOs 116 and 50; SEQ ID NOs 117 and 52; SEQ ID NOs 118 and 54; SEQ ID NOs 55 and 119; SEQ ID NOs 120 and 58; SEQ ID NOs 59 and 121; SEQ ID NOs 61 and 122; SEQ ID NOs 123 and 64; SEQ ID NOs 124 and 66; SEQ ID NOs 125 and 68; SEQ ID NOs 126 and 70; SEQ ID NOs 71 and 72; SEQ ID NOs 127 and 74; SEQ ID NOs 75 and 128; SEQ ID NOs 77 and 129; SEQ ID NOs 130 and 80; SEQ ID NOs 81 and 131; SEQ ID NOs 132 and 84; SEQ ID NOs 133 and 86; and SEQ ID NOs 134 and 88.

7. The method according to any one of the preceding claims, wherein the simultaneous amplification in step (b) is a pre-amplification step.

8. The method according to any one of the preceding claims, wherein the method is performed simultaneously for one or more target RNA regions, and/or one or more target cDNA regions, and/or one or more target DNA regions.

9. Use of the method according to any one of the preceding claims for determining the DNA profile and/or the gene expression profile of a single cell.

10. Use of the method according to any one of claims 1-8 for validation of large-scale transcriptomics data sets.