

(51) International Patent Classification:
C12Q 1/68 (2006.01)(21) International Application Number:
PCT/SG2011/000210(22) International Filing Date:
13 June 2011 (13.06.2011)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/354,683 14 June 2010 (14.06.2010) US(71) Applicant (for all designated States except US): **NATIONAL UNIVERSITY OF SINGAPORE** [SG/SG];
21 Lower Kent Ridge Road, Singapore 119077 (SG).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **TOO, Heng, Phon** [MY/SG]; c/o National University of Singapore, Department of Biochemistry, 21 Heng Mui Keng Terrace, Level 5, Singapore 119613 (SG). **ANWAR, Azlinda, B.** [SG/SG]; c/o National University of Singapore, Department of Biochemistry, 21 Heng Mui Keng Terrace, Level 5, Singapore 119613 (SG).(74) Agent: **YU SARN AUDREY & PARTNERS**; 24 Raffles Place, #27-01 Clifford Centre, Singapore 048621 (SG).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

[Continued on next page]

(54) Title: MODIFIED STEM-LOOP OLIGONUCLEOTIDE MEDIATED REVERSE TRANSCRIPTION AND BASE-SPACING CONSTRAINED QUANTITATIVE PCR

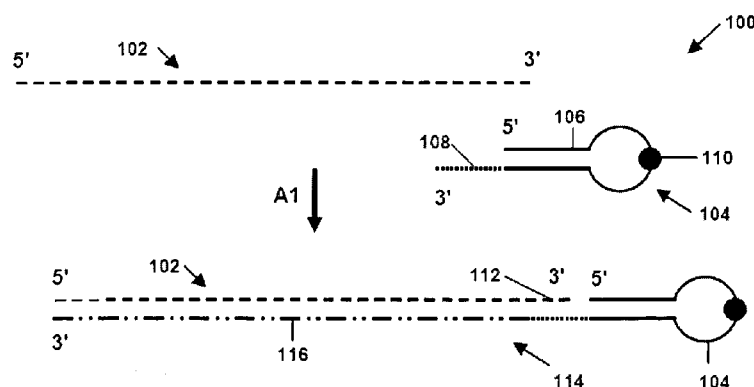


FIGURE 1

(57) Abstract: There is provided a method for detecting a target RNA molecule in a sample. The method comprises reverse transcribing the target RNA contained in the sample using an RT oligonucleotide, the RT oligonucleotide comprising a stem-loop portion containing one or more nucleotides modified or modifiable to block DNA polymerase extension and a target annealing portion that is complementary to a downstream portion of the target RNA, the target annealing portion located 3' to the stem-loop portion, to produce a reverse transcription product that comprises the RT oligonucleotide and a 3' extended region; amplifying the reverse transcription product using (i) a first amplification primer that anneals to a downstream portion of the 3' extended region of the reverse transcription product and (ii) a second amplification primer that anneals to an interface portion of a DNA strand complementary to the reverse transcription product, the interface portion comprising a region that is complementary to a 3' portion of the RT oligonucleotide and a 5' portion of the 3' extended region in the reverse transcription product, to produce an amplification product; and detecting the amplification product; wherein the stem-loop portion adopts a stem-loop structure under conditions used for said reverse transcribing but does not adopt the stem-loop structure under conditions used for said amplifying and wherein when the stem-loop portion contains one or more nucleotides that are modifiable to block DNA polymerase extension, the method further comprises modifying the modifiable nucleotide prior to said amplifying.





Published:

— with international search report (Art. 21(3))

— with sequence listing part of description (Rule 5.2(a))