



(86) Date de dépôt PCT/PCT Filing Date: 2014/08/22	(51) Cl.Int./Int.Cl. <i>C07H 21/00</i> (2006.01), <i>C07H 21/04</i> (2006.01), <i>C12P 19/34</i> (2006.01), <i>C40B 50/06</i> (2006.01)
(87) Date publication PCT/PCT Publication Date: 2015/02/26	
(45) Date de délivrance/Issue Date: 2019/11/05	
(85) Entrée phase nationale/National Entry: 2016/02/11	(72) Inventeurs/Inventors: SANDBERG, RICKARD, SE; PICELLI, SIMONE, SE; FARIDANI, OMID R., SE
(86) N° demande PCT/PCT Application No.: US 2014/052233	(73) Propriétaire/Owner: LUDWIG INSTITUTE FOR CANCER RESEARCH LTD, CH
(87) N° publication PCT/PCT Publication No.: 2015/027135	
(30) Priorité/Priority: 2013/08/23 (US61/869,220)	(74) Agent: PIASETZKI NENNIGER KVAS LLP

(54) Titre : PROCEDES ET COMPOSITIONS POUR LA SYNTHÈSE D'ADN-C ET LE PROFILAGE DE
TRANSCRIPTOME D'UNE CELLULE UNIQUE AU MOYEN DE REACTION PAR PERMUTATION DE MATRICE
(54) Title: METHODS AND COMPOSITIONS FOR CDNA SYNTHESIS AND SINGLE-CELL TRANSCRIPTOME
PROFILING USING TEMPLATE SWITCHING REACTION

(57) **Abrégé/Abstract:**

This application discloses methods for cDNA synthesis with improved reverse transcription, template switching and preamplification to increase both yield and average length of cDNA libraries generated from individual cells. The new methods include exchanging a single nucleoside residue for a locked nucleic acid (LNA) at the TSO 3' end, using a methyl group donor, and/or a MgCb concentration higher than conventionally used. Single-cell transcriptome analyses incorporating these differences have full-length coverage, improved sensitivity and accuracy, have less bias and are more amenable to cost-effective automation. The invention also provides cDNA molecules comprising a locked nucleic acid at the 3'-end, compositions and cDNA libraries comprising these cDNA molecules, and methods for single-cell transcriptome profiling.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property
Organization
International Bureau



WIPO | PCT



(10) International Publication Number

WO 2015/027135 A1

(43) International Publication Date
26 February 2015 (26.02.2015)

(51) International Patent Classification:

C12P 19/34 (2006.01) C07H 21/04 (2006.01)

(21) International Application Number:

PCT/US2014/052233

(22) International Filing Date:

22 August 2014 (22.08.2014)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

61/869,220 23 August 2013 (23.08.2013) US

(71) Applicant: LUDWIG INSTITUTE FOR CANCER RESEARCH [US/US]; 666 Third Avenue, 28th Floor, New York, NY 10017 (US).

(72) Inventors: SANDBERG, Rickard; C/o Ludwig Institute For Cancer Research, Karolinska Institutet, S-171 77 Stockholm (SE). PICELLI, Simone; C/o Ludwig Institute For Cancer Research, Karolinska Institutet, S-171 77 Stockholm (SE). FARIDANI, Omid, R.; Karolinska Institutet, S-171 77 Stockholm (SE).

(74) Agents: MACLEOD, Janet, M. et al.; Fox Rothschild LLP, 997 Lenox Drive, Building 3, Lawrenceville, NJ 08648-2311 (US).

(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, BN, 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, IR, IS, JP, KE, KG, 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, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, 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, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, 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, KM, ML, MR, NE, SN, TD, TG).

Published:

- with international search report (Art. 21(3))
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))

(54) Title: METHODS AND COMPOSITIONS FOR CDNA SYNTHESIS AND SINGLE-CELL TRANSCRIPTOME PROFILING USING TEMPLATE SWITCHING REACTION

(57) Abstract: This application discloses methods for cDNA synthesis with improved reverse transcription, template switching and preamplification to increase both yield and average length of cDNA libraries generated from individual cells. The new methods include exchanging a single nucleoside residue for a locked nucleic acid (INA) at the TSO 3' end, using a methyl group donor, and/or a MgCb concentration higher than conventionally used. Single-cell transcriptome analyses incorporating these differences have full-length coverage, improved sensitivity and accuracy, have less bias and are more amendable to cost-effective automation. The invention also provides cDNA molecules comprising a locked nucleic acid at the 3'-end, compositions and cDNA libraries comprising these cDNA molecules, and methods for single-cell transcriptome profiling.



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METHODS AND COMPOSITIONS FOR cDNA SYNTHESIS AND SINGLE-CELL
TRANSCRIPTOME PROFILING USING TEMPLATE SWITCHING REACTION

5

FIELD OF THE INVENTION

The present invention relates to methods for synthesis of double stranded
10 cDNA with improved yield and average length, and cDNA molecules synthesized
and cDNA libraries generated from individual cells.

BACKGROUND OF THE INVENTION

Single-cell gene expression analyses hold promise to characterize cellular
heterogeneity, but current methods sacrifice either the coverage, sensitivity or
15 throughput. Several methods exist for full-length cDNA construction from large
amounts of RNA, including cap enrichment procedures (Maruyama, K. & Sugano,
S., *Gene* **138**, 171-174 (1994); Carninci, P. & Hayashizaki, Y., *Meth. Enzymol.* **303**,
19-44 (1999); Das, M., *et al.*, *Physiol. Genomics* **6**, 57-80 (2001)), but it is still
challenging to obtain full-length coverage from single-cell amounts of RNA.
20 Existing methods use either 3' end polyA-tailing of cDNA (e.g., Tang, F. *et al.*, *Nat.*
Methods **6**, 377-382 (2009); Sasagawa, Y. *et al.*, *Genome Biol.* **14**, R31 (2013)) or
template switching (Zhu, Y. Y., *et al.*, *BioTechniques* **30**, 892-897 (2001);
Ramsköld, D. *et al.*, *Nat. Biotechnol.* **30**, 777-782 (2012)), whereas other
methods sacrifice full-length coverage altogether for early multiplexing (Islam, S.
25 *et al.*, *Genome Res.* (2011). doi:10.1101/gr.110882.110; Hashimshony, T., *et al.*,
Cell Rep. **2**, 666-673 (2012)). It has recently been shown that Smart-Seq, which
relies on template switching, has more even read coverage across transcripts
than polyA-tailing methods (Ramsköld, D. *et al.*, *Nat. Biotechnol.* **30**, 777-782
(2012)), consistent with the common use of template switching in applications
30 designed to directly capture RNA 5' ends, including nanoCAGE (Plessy, C. *et al.*,
Nat. Methods **7**, 528-534 (2010)) and STRT (Islam, S. *et al.*, *Genome Res.* (2011).

doi:10.1101/gr.110882.110). Single-cell applications utilizing template switching are dependent upon the efficiency of the reverse transcription, the template switching reaction, and a uniform polymerase chain reaction (PCR) preamplification to obtain representative cDNA in sufficient amounts for
5 sequencing. Despite the widespread use of these reactions, no systematic efforts to improve cDNA library yield and average length from single-cell amounts have been reported.

SUMMARY OF THE INVENTION

The present invention provides improved methods for synthesis of cDNA,
10 in particular, in the reverse transcription, template switching and preamplification of single cell applications utilizing template switching reactions, to increase both yield and average length of cDNA libraries generated from individual cells. Single-cell transcriptome analyses incorporating these differences have improved sensitivity and accuracy, and are less biased and
15 more amenable to cost-effective automation.

Specifically, to improve full-length transcriptome profiling from single cells, this application discloses evaluation of a large number of variations to reverse transcription, template switching oligonucleotides (TSO) and PCR preamplification, and comparison of the results to commercial Smart-Seq
20 (hereafter called SMARTer®) in terms of cDNA library yield and length. The modifications disclosed herein surprisingly and significantly increased both the yield and length of the cDNA obtained from as little as 1 ng of starting total RNA.

In one embodiment, the present invention provides a method for preparing DNA that is complementary to an RNA molecule, the method
25 comprising conducting a reverse transcription reaction in the presence of a template switching oligonucleotide (TSO) comprising a locked nucleic acid residue.

In another embodiment, the present invention provides a method of increasing the yield of cDNA, comprising use of an additive, such as a methyl
30 group donor, in the cDNA synthesis. In one embodiment, the methyl group donor is betaine.

In another embodiment, the present invention provides a method of increasing the yield of cDNA, comprising use of an increased concentration of metal salt, for example, $MgCl_2$, in the synthesis of cDNA.

5 In a preferred embodiment, the method comprises use of a methyl group donor in combination with an increased concentration of $MgCl_2$ in the cDNA synthesis. In a particularly preferred embodiment, the method comprises use of methyl group donor betaine in combination with an increased concentration of $MgCl_2$, which has shown a significant positive effect on the yields of cDNA.

10 In another embodiment, the present invention provides a method of increasing the average length of a preamplified cDNA, comprising administering dNTPs prior to the RNA denaturation rather than in the reverse transcriptase (RT) master mix.

In another embodiment, the present invention provides a cDNA library produced by a method according to any of the embodiments disclosed herein.

15 In another embodiment, the present invention provides use of a cDNA library produced according to any of the embodiments disclosed herein for single-cell transcriptome profiling.

20 In another embodiment, the present invention provides a method for analyzing gene expression in a plurality of single cells, comprising the steps of preparing a cDNA library according to a method according to any embodiment disclosed herein; and sequencing the cDNA library.

It has been demonstrated in accordance with the present invention that these methods performed on purified RNA are applicable to individual metazoan cells, including for example mammalian cells.

25 In another embodiment, the present invention provides a template switching oligonucleotide (TSO) comprising an LNA at its 3'-end.

In another embodiment, the present invention provides use of a TSO according to any of the embodiments disclosed herein for synthesis of double stranded cDNA.

These and other aspects of the present invention will be better appreciated by reference to the following drawings and detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates improvements in cDNA library yield and length. **(A)** Median yield of preamplified cDNA from 1 ng total RNA using different template switching oligonucleotides, relative to those obtained using the rG3 oligo. All oligo sequences are found in Table 1. **(B)** Median yield of preamplified cDNA from 1 ng total RNA in reactions with betaine (black) or without (gray) and as a function of increasing Mg^{2+} concentration, relative to cDNA yields obtained using SMARTer[®]-like conditions (betaine and 6mM Mg^{2+}). **(C)** Length of preamplified cDNA generated from 1 ng total mouse brain RNA in reactions that deployed dNTPs prior to RNA denaturation (early) or in RT master mix (late). **(D)** Median yield of preamplified cDNA from HEK293T cells using the LNA-G and SMARTer[®] IIA template switching oligos with the optimized protocol. Dashed lines indicate median yield from commercial SMARTer[®] reactions. **(E)** Median yield of preamplified cDNA from DG-75 cells in reactions with or without betaine. **(F)** Lengths of cDNA libraries generated from single HEK293T cells in reactions with or without bead extraction. Note that brain mRNAs are naturally longer than cell line mRNAs. (A-F) The replicate measurements are represented as boxplots with the numbers of replicates per condition indicated in parenthesis. Significant differences in mean yield or length were determined using the Student's *t*-test.

Figure 2 illustrates sensitive full-length transcriptome profiling in single cells. **(A)** Percentage of genes reproducibly detected in replicate cells, binned according to expression level. All pair-wise comparisons were performed within replicates for the optimized protocol and SMARTer[®] and reported as the mean and 90% confidence interval. **(B)** Standard deviation in gene expression estimates within replicates in bins of genes sorted according to expression levels. Error bars, s.e.m. ($n \geq 4$). **(C)** The mean numbers of genes detected in HEK293T cells using SMARTer[®] and optimized protocol, at different RPKM cut-offs. Significant increase in gene detection in the optimized protocol was obtained at all RPKM thresholds (all with $p < 0.5$; Student's *t*-test). **(D)** The mean fraction of genes detected as expressed (RPKM > 1) in bins of genes sorted according to their

GC content. The mRNA-Seq data from a human tissue was included as a no-preamplification control. Error bars denote SEM ($n \geq 4$), and the lower panel shows the GC range for genes in each bin. (E) The mean fraction of reads aligning to the 3' most 20% of the genes, 5' most 20% and the middle 60% for single-cell data generated using different protocols. (F) Principal component analyses of single-cell gene expression data showing the two most significant components. Cells are colored according to preamplification enzyme and protocol variant.

Figure 3 shows cDNA yields using LNA bases in template switching oligonucleotides.

Figure 4 illustrates single-cell RNA-Seq sensitivity and variability. (A) Percentage of genes reproducibly detected in replicate cells. (B) Standard deviation in gene expression estimates.

Figure 5 illustrates validation of single-cell RNA-Seq results using another analysis pipeline. (A) Percentage of genes reproducibly detected in replicate cells. (B) Standard deviation in gene expression estimates. (C) The mean numbers of genes detected. (D) The mean fraction of genes detected as expressed. (E) The mean fraction of reads aligning to the 3' most 20% of the genes, 5' most 20% and the middle 60% for single-cell data generated using different protocols.

Figure 6 illustrates comparison of single-cell transcriptomic data generated with Smart-Seq2, Quartz-Seq and SMARTer. (A) Percentage of genes reproducibly detected in replicate cells. All pair-wise comparisons were performed with Smart-Seq2 and Quartz-seq, and reported as the mean and 90% confidence interval. (B) Standard deviation in gene expression estimates in (A). (C) Percentage of genes reproducibly detected in replicate cells. All pair-wise comparisons were performed with Smart-Seq2 and SMARTer®, and reported as the mean and 90% confidence interval. (D) Standard deviation in gene expression estimates in (C). (E) Percentage of genes reproducibly detected in replicate cells. All pair-wise comparisons were performed with Quartz-seq, and reported as the mean and 90% confidence interval. (F) Standard deviation in gene expression estimates in (E).

Figure 7 illustrates mapping statistics for single-cell libraries generated using SMARTer, optimized Smart-Seq and variants of the optimized protocol. (A) Fraction of uniquely aligned reads with 1 to 9 mismatches for each single-cell RNA-Seq library. (B) Percentage of reads that aligned uniquely, aligned to multiple genomic coordinates, or did not align for all single-cell RNA-Seq libraries. (C) Fraction of uniquely aligned reads that mapped to exonic, intronic or intergenic regions. (D) Number of sequenced reads per cell and library preparation protocol.

Figure 8 illustrates gene expression and GC levels in single-cell RNA-Seq protocols.

Figure 9 illustrates single-cell RNA-Seq sensitivity and variability. (A) Percentage of genes reproducibly detected in replicate cells. (B) Standard deviation in gene expression estimates.

Figure 10 illustrates read coverage across genes in single-cell RNA-Seq data.

Figure 11 illustrates read coverage across transcripts. (A) Mean fraction coverage read for all genes. (B)-(F) Transcripts grouped by length into 5 equal-sized bins.

Figure 12 illustrates read peaks in single-cell RNA-Seq data. (A) Number of genes with one or more high density peaks per single-cell RNA-Seq library. (B) Heatmaps of read densities across genes with peaks in the highest number of libraries.

Figure 13 illustrates assessment of the technical and biological variability in single-cell transcriptomics using Smart-Seq2. (A) Percentage of genes reproducibly detected in dilutions of HEK cells. (B) Standard deviation in gene expression estimates for (A). (C) Percentage of genes reproducibly detected in dilutions of HEK total RNA. (D) Standard deviation in gene expression estimates for (D). (E) Standard deviation in gene expression estimates for (D) with pair-wise comparisons of individual cells.

Figure 14 illustrates comparison of libraries generated with commercial Tn5 (Nextera) to in-house produced Tn5. (A) Percentage of genes reproducibly detected using in-house conditions. (B) Standard deviation in gene expression

estimates for (A). (C) Percentage of genes reproducibly detected using commercial buffers and conditions. (D) Standard deviation in gene expression estimates for (D). (E) Differences in reactions carried out in (A).

DETAILED DESCRIPTION OF THE INVENTION

5 This application discloses methods for cDNA synthesis with improved reverse transcription, template switching and preamplification to increase both yield and average length of cDNA libraries generated from individual cells.

10 In one embodiment, the present invention provides a method for preparing DNA that is complementary to an RNA molecule, comprising the steps of:

 annealing a cDNA synthesis primer to the RNA molecule and synthesizing a first cDNA strand to form an RNA-cDNA intermediate; and

15 conducting a reverse transcriptase reaction by contacting the RNA-cDNA intermediate with a template switching oligonucleotide (TSO), wherein the TSO comprises a locked nucleic acid (LNA) at its 3'-end, under conditions suitable for extension of the first DNA strand that is complementary to the RNA molecule, rendering it additionally complementary to the TSO.

20 In another embodiment of the present invention, the reverse transcription reaction is conducted in the presence of a methyl group donor and a metal salt.

 In another embodiment of the present invention, the methyl group donor is betaine.

 In another embodiment of the present invention, the metal salt is a magnesium salt.

25 In another embodiment of the present invention, the magnesium salt has a concentration of at least 7 mM, at least 8 mM, or at least 9 mM.

 In another embodiment of the present invention, the template switching oligonucleotide optionally comprises one or two ribonucleotide residues.

In another embodiment of the present invention, the template switching oligonucleotide comprises at least one or two ribonucleotide residues and an LNA residue.

In another embodiment of the present invention, the at least one or two
5 ribonucleotide residues are riboguanine.

In another embodiment of the present invention, the locked nucleic acid residue is selected from the group consisting of locked guanine, locked adenine, locked uracil, locked thymine, locked cytosine, and locked 5-methylcytosine.

In another embodiment of the present invention, the locked nucleic acid
10 residue is locked guanine.

In another embodiment of the present invention, the locked nucleic acid residue is at the 3'-most position.

In another embodiment of the present invention, the template switching oligonucleotide comprises at the 3'-end two ribonucleotide residues and one
15 locked nucleotide residue characterized by formula $rGrG+N$, wherein $+N$ represents a locked nucleotide residue.

In another embodiment of the present invention, the template switching oligonucleotide comprises $rGrG+G$.

In another embodiment of the present invention, the methyl group donor
20 is betaine, and the metal salt is $MgCl_2$ at a concentration of at least 9 mM.

In another embodiment of the present invention, the method further comprises amplifying the DNA strand that is complementary to the RNA molecule and the template switching oligonucleotide using an oligonucleotide primer.

25 In another embodiment of the present invention, the template switching oligonucleotide is selected from the oligonucleotides in Table S2 .

In another embodiment of the present invention, the cDNA synthesis primer is an oligo-dT primer.

In another embodiment of the present invention, the cDNA is synthesized
30 on beads comprising an anchored oligo-dT primer.

In another embodiment of the present invention, the oligo-dT primer comprises a sequence of 5'-AAGCAGTGGTATCAACGCAGAGTACT₃₀VN-3', wherein "N" is any nucleoside base, and "V" is selected from the group consisting of "A", "C" and "G".

5 In another embodiment of the present invention, the method further comprises PCR preamplification, tagmentation, and final PCR amplification.

In another embodiment of the present invention, the PCR preamplification is conducted without purifying the cDNA obtained from reverse transcription reaction.

10 In another embodiment of the present invention, the RNA is total RNA in a cell.

In another embodiment, the present invention provides a cDNA library produced by the method according to any embodiment disclosed herein.

15 In another embodiment, the present invention provides use of a cDNA library produced by the method according to any embodiment disclosed herein for single-cell transcriptome profiling.

In another embodiment, the present invention provides a method for analyzing gene expression in a plurality of single cells, the method comprising the steps of: preparing a cDNA library produced by the method according to any
20 embodiment disclosed herein; and sequencing the cDNA library.

In another embodiment, the present invention provides a template switching oligonucleotide (TSO) comprising a locked nucleotide residue at the 3'-end. The TSOs of the present invention can be used in the synthesis of cDNA to improve yield and length.

25 In another embodiment, the TSO comprises three nucleotide residues at the 3'-end, wherein said three nucleotide residues are selected from the group consisting of +N+N+N, N+N+N, NN+N, rN+N+N, and rNrN+N, wherein N at each occurrence is independently a deoxyribonucleotide residue, rN at each occurrence is independently a ribonucleotide residue, and +N at each occurrence
30 is independently a locked nucleotide residue.

In one embodiment, the portion of the TSO that is on the 5' side of the three nucleotide residues at the 3'-end, also referred to herein as the 5'-portion, comprises an arbitrary nucleotide sequence comprised of ribonucleotides, deoxyribonucleotides, or mixtures thereof. In one preferred embodiment, the 5'-portion of the TSO comprises all ribonucleotides. In another preferred embodiment, the 5'-portion of the TSO comprises all deoxyribonucleotides.

In another embodiment, the locked nucleotide residue in the TSOs is selected from the group consisting of locked guanine, locked adenine, locked uracil, locked thymine, locked cytosine, and locked 5-methylcytosine

In another embodiment, the three nucleotide residues at the 3'-end of the TSOs are NN+G or rNrN+G, wherein N at each occurrence is independently a deoxyribonucleotide residue, and rN at each occurrence is independently a ribonucleotide residue.

In another embodiment, the three nucleotide residues at the 3'-end of the TSOs are rGrG+N, wherein +N is locked nucleotide residue.

In another embodiment, the three nucleotide residues at the 3'-end of the TSOs are rGrG+G.

The TSOs preferably have a length of from about 10 to about 50 nucleotides, or from about 15 to about 45 nucleotides, or from about 20 to about 40 nucleotides, or from about 24 to about 35 nucleotides, or about 30 nucleotides.

In another embodiment, the present invention provides use of a TSO according to any one of the embodiments disclosed herein in the synthesis of a cDNA.

Examples of metal cations useful for the present invention include, but are not limited to, Mg^{2+} and Mn^{2+} , with Mg^{2+} preferred; and their concentrations can be in the range of 0- 30 μM , inclusive, with a preferred range of 3-20 μM , and a more preferred range of 9-12 μM .

In addition to methyl donor betaine, other additives that may be added in the cDNA synthesis of the present invention include, but are not limited to, trehalose, sucrose, glucose, maltose, DMSO(dimethyl sulfoxide), formamide, non-

ionic detergents, TMAC(tetramethylammonium chloride), 7-deaza-2'-deoxyguanosine (dC⁷GTP), bovine serum albumin (BSA), and T4 gene 32 protein.

The present invention is applicable to reactions using all reverse transcriptases that are MMLV-related and have template switching activity. MMLV-related reverse transcriptases include wild-type Moloney murine leukemia virus and its variants, including for example derivatives lacking RNase H activity such as SUPER-SCRIPT II (Invitrogen), POWER SCRIPT (BD Biosciences) and SMART SCRIBE (Clontech). TSOs useful for the present invention may comprise barcodes, including but not limited to molecular barcodes or sample barcodes.

The cDNA synthesized according to the present invention may have applications as cDNA synthesized according to any literature methods, including but not limited to construction of small quantity cDNA library, single-cell cDNA analyses, single-cell gene expression analyses, few-cell cDNA analyses, few-cell gene expression analyses, single-cell qPCR analyses (that use this preamplification step), and cap capturing based amplification.

The following non-limiting examples illustrate certain aspects of the present invention.

EXAMPLES

EXAMPLE 1

Methods

Experiments using total RNA

RNA experiments were performed using the Control Total RNA supplied with the SMARTer[®] Ultra Low RNA Kit for Illumina Sequencing (Clontech), extracted from mouse brain. One microliter of a 1 ng/μl solution was used in each experiment and mixed with 1 μl of anchored oligo-dT primer (10 mM, 5'-AAGCAGTGGTATCAACGCAGAGTACT₃₀VN-3', where "N" is any base and "V" is either "A", "C" or "G") and 1 μl of dNTP mix (10 mM, Fermentas), denatured at 72°C for 3 min and immediately placed on ice afterwards. Seven μl of the first strand reaction mix containing 0.50 μl SuperScript II RT (200 U ml⁻¹, Invitrogen), 0.25 μl RNase inhibitor (20 U ml⁻¹, Clontech), 2 μl Superscript II First-Strand

Buffer (5x, Invitrogen), 0.25 μ l DTT (100 mM, Invitrogen), 2 μ l betaine (5 M, Sigma), 0.9 μ l $MgCl_2$ (100 mM, Sigma), 1 μ l TSO (10 μ M, the complete list of the oligos can be found in Table S1) and 0.1 μ l Nuclease-free water (Gibco) were added to each sample. Reverse transcription reaction was carried out by incubating at 42°C for 90 min, followed by 10 cycles of (50°C for 2 min, 42°C for 2 min). Finally, the RT was inactivated by incubation at 70°C for 15 min.

PCR pre-amplification

In the original Smart-Seq protocol purification with Ampure XP beads is performed after first strand cDNA synthesis. PCR is then carried out directly on the cDNA immobilized on the beads, after adding 2 μ l Advantage 2 Polymerase Mix (50X, Clontech), 5 μ l Advantage 2 PCR Buffer (10X, Clontech), 2 μ l dNTP mix (10 mM, Clontech), 2 μ l IS PCR primer (12 μ M, Clontech) and 39 μ l nuclease-free water to a final reaction volume of 50 μ l. In the present examples the cDNA was not purified after RT but just added the same PCR master mix, taking into account that the volume after first strand cDNA synthesis is 10 μ l and adjusting the amount of water accordingly. Reaction was incubated at 95°C 1 min, then cycled 15 times between (95°C 15 sec, 65°C 30 sec, 68°C 6 min), with a final extension at 72°C for 10 min.

A second modification that significantly improved cDNA yield was the replacement of Advantage 2 Polymerase mix with KAPA HiFi HotStart ReadyMix (KAPA Biosystems). Purification after first strand cDNA synthesis was omitted also in this case. The PCR master mix had the following composition: 25 μ l KAPA HiFi HotStart ReadyMix (2X, KAPA Biosystems), 1 μ l IS PCR primers (10 mM, 5'-AAGCAGTGGTATCAACGCAGAGT-3') and 14 μ l nuclease-free water (Gibco). The program used was as follows: 98°C 3 min, then 15 cycles of (98°C 15 sec, 67°C 20 sec, 72°C 6 min), with a final extension at 72°C for 5 min.

Regardless of the PCR protocol used, PCR was purified using a 1:1 ratio of AMPure XP beads (Beckman Coulter), performing the final elution in 15 μ l of EB solution (Qiagen). Library size distribution was checked on a High-Sensitivity DNA chip (Agilent Bioanalyzer) after a 1:5 dilution. The expected average size should be around 1.5-2.0 kb and the fraction of fragments below 300 bp should be negligible. To evaluate the performance of the different modifications

introduced in the protocol, the amount of cDNA comprised in the interval 300-9000 bp in the Agilent Bioanalyzer plot was assessed.

Tagmentation reaction and final PCR amplification

Five nanograms of cDNA were then used for the tagmentation reaction
5 carried out with Nextera® DNA Sample Preparation kit (Illumina), adding 25 µl of
2X Tagment DNA Buffer and 5 µl of Tagment DNA Enzyme, in a final volume of 50
µl. Tagmentation reaction was incubated at 55°C for 5 min, followed by
purification with DNA Clean & Concentrator™-5 kit (Zymo Research) with a final
elution in 20 µl Resuspension Buffer (RSB) from the Nextera® kit. The whole
10 volume was then used for limited-cycle enrichment PCR, along with 15 µl of
Nextera® PCR Primer Mix (NPM), 5 µl of Index 1 primers (N7xx), 5 µl of Index 2
primers (N5xx) and 5 µl of PCR Primer Cocktail (PPC). A second amplification
round was performed as follows: 72°C 3 min, 98°C 30 sec, then 5 cycles of (98°C
10 sec, 63°C 30 sec, 72°C 3 min). Purification was done with a 1:1 ratio of AMPure
15 XP beads and samples were loaded on a High-Sensitivity DNA chip to check the
quality of the library, while quantification was done with Qubit High-Sensitivity
DNA kit (Invitrogen). Libraries were diluted to a final concentration of 2 nM,
pooled and sequenced on Illumina HiSeq 2000.

Single-cell cDNA isolation

20 Single HEK293T (human), DG-75 (human), C2C12 (mouse) and MEF
(mouse) cells were manually picked under the microscope after resuspension in
PBS. Volume of liquid was kept as low as possible, usually below 0.5 µl and
preferably below 0.3 µl. Cells were then transferred to a 0.2 ml thin-wall PCR tube
containing 2 µl of a mild hypotonic lysis buffer composed of 0.2% Triton® X-100
25 (Sigma) and 2 U/µl of RNase inhibitor (Clontech). Cells already picked were kept
on ice throughout the process or stored at -80°C if not used immediately. All the
downstream steps were the same as when using total RNA (see above), with the
only exception of the quality control with the High Sensitivity DNA chip, where
samples were loaded pure (without dilution), due to the limited amount of cDNA
30 obtained from RT in single cells.

When working with total RNA it was observed that cDNA yield could be
increased using a double amount of TSO or different combinations of TSOs and

PCR enzymes (data not shown). To validate this finding, some experiments on HEK293T cells were repeated using different amounts of TSO (1 or 2 μ l of a 10 μ M solution), TSO types (rGrGrG, rGrG+G or rGrG+N) or PCR enzymes (KAPA HiFi or Advantage 2). Sequencing results for the most significant comparisons are reported in Figures S2-S7. The final protocol (i.e. "optimized") refers to the one using only 1 μ l of the 10 μ M rGrG+G TSO and KAPA HiFi HotStart ReadyMix as enzyme in the first PCR (without AMPure XP bead purification).

Smart-Seq experiments

To evaluate and compare the performance of the present method, cDNA libraries were generated with the same total RNA and single cells using the Smart-Seq protocol, following manufacturer's instructions (see Clontech manual). After PCR pre-amplification, 5 ng of cDNA were used for the tagmentation reaction and processed exactly in the same way as described above.

Statistical analyses of cDNA yield and length

Performances of the different protocols were evaluated with regard to cDNA yield and average cDNA length according to the Bioanalyzer in the range of 3009,000 bp. For mouse brain total RNA samples, each experimental variable was evaluated in a pairwise manner selecting a set of experiments where all other variables are identical. Within that set of experiments, the significance for a change in yield or length, between the two variables, was evaluated using Student's t-test and Wilcoxon rank sum test (Table 1, sheet B).

In the HEK293T cell experiments each optimized experimental setting was compared to each other, as well as to the SMARTer[®] protocol, using Student's t-test and Wilcoxon rank sum test (Table 3, sheet B). All analyses and figures were produced with using R.

Read alignments and gene expression estimation

Single-cell libraries were sequenced with Nextera dual indexes (i7+i5) on an Illumina HiSeq 2000, giving 43bp insert reads after demultiplexing and removing cellular barcodes. The reads were aligned to human (hg19) or mouse (mm10) genomes using STAR v2.2.0 (Dobin et al. Bioinformatics 2013 29(1): 15-

21) with default settings and filtered for uniquely mapping reads. Gene expression values were calculated as RPKM values for each transcript in Ensembl release 69 and RefSeq (February 2013) using *rpkmforgenes* (Ramsköld et al. PLoS Comp Biol., 5, e1000598, 2009).

5 Single-cell RNA-Seq sensitivity and variability

Analyses of gene detection in single HEK293T cells (Fig. 2A, Fig. 5A, and 7A) were calculated over all possible pairs of technical replicates from each experimental setting. Genes were binned by expression level in the two samples, and were considered detected if the RPKM was above 0.1 in both samples. The
 10 mean for all possible pairs of technical replicates within a group was used together with standard deviation using the adjusted Wald method. Analyses of variation (Fig. 2B and Fig. 5B & 7B) were also calculated on pairs of samples, binning genes by the mean of log expression, excluding genes below 0.1 RPKM in either sample. As gene expression levels across single cells are often log normally
 15 distributed (Bengtsson Genome Res 2005 15(10): 1388-1392), absolute difference in $\log_{10}$ expression values and s.d. were calculated by multiplying mean difference in a bin with 0.886.

Analyses of read coverage and GC tolerance

Gene body coverage was calculated using the RSeQC-2.3.4 package (Wang,
 20 Wang and Li. Bioinformatics 2012 ;28(16):2184-5) for the longest transcript of all protein coding genes (Fig. 2E and Fig. 8). Gene detection at different GC-content was calculated using longest transcript for all protein coding RefSeq genes that were binned by GC-content into 10 equal sized bins, and the numbers of genes with no detection, or detection at different RPKM cutoffs were
 25 calculated (Fig. 2D and Fig. 6).

Read peak analyses

Some genes displayed unexplained peaks with high density of reads within the gene body. To identify these regions, the gene bodies of each gene were divided into 101 equally sized bins and each gene with at least one bin with
 30 >5 standard deviation read density over the mean read distribution within that gene. In these analyses genes with low expressed genes (those with fewer reads

than around 2,000---10,000 reads depending on the sequencing depth per cell) were discarded. The number of such genes in each cell is represented in Fig. 9A. And the genes with peaks in the highest number of HEK239T cells are displayed as heatmaps in Fig. 9B illustrating that the peaks are consistently found at the same position in all experiments.

EXAMPLE 2

To improve full-length transcriptome profiling from single cells, a large number of variations to reverse transcription, template switching oligonucleotide (TSO) and PCR preamplification (in total 457 experiments) were evaluated, and the results were compared to commercial Smart-Seq (hereafter called SMARTer[®]) in terms of cDNA library yield and length (**Table 1**). Importantly, modifications were identified that significantly increased both cDNA yield and length obtained from 1 ng of starting total RNA (**Table 1**).

In particular, exchanging only a single guanylate for a locked nucleic acid (LNA) guanylate at the TSO 3' end (rGrG+G), led to a 2-fold increase in cDNA yield relative to the SMARTer[®] IIA oligo used in commercial Smart-Seq ($p=0.003$, Student's *t*-test; **Fig. 1a**, **Table 2** and **Fig. 3**).

Additionally, it was discovered that the methyl group donor betaine in combination with higher $MgCl_2$ concentrations had a significant positive effect on yield (2-4 fold increase, $p=0.0012$, Student's *t*-test, for all comparisons) (**Fig. 1b**). The commercial Smart-Seq buffer has a final concentration of 6 mM $MgCl_2$, but it was found herein that higher yield is obtained when increasing the concentration to 9 mM or beyond. Finally, the average length of the preamplified cDNA increased with 370 nts when administering dNTPs prior to the RNA denaturation rather than in the RT master mix ($p=7.8 \times 10^{-9}$, Student's *t*-test; **Fig. 1c**).

It was further demonstrated that these improvements obtained with purified RNA extended to cDNA reactions performed directly in lysates of individual human and mouse cells. To this end, single-cell cDNA libraries were generated from a total of 262 individual human or mouse cells (159 HEK293T,

34 DG-75, 30 C2C12 and 39 MEF cells) spanning different cell sizes and total
RNA contents (**Table 3**). Analyses of the single-cell cDNA libraries demonstrated
higher cDNA yields both with the use of the LNA-containing TSO (3-fold increase,
p<0.001, Student's *t*-test; **Fig. 1d**) and with betaine together with high Mg²⁺
5 concentrations (4-fold increase, p=3.7x10⁻⁶, Student's *t*-test; **Fig. 1e**).

The sensitivity and accuracy of single-cell methods are limited by the
efficiency of each sample-processing step. The SMARTer[®] protocol uses bead
purification to remove unincorporated adaptors from the first strand cDNA
reaction before the preamplification with Advantage 2 Polymerase (Adv2).
10 However, performing bead purification in small volumes poses a significant
recovery challenge for liquid handling automation. It was determined herein that
KAPA HiFi Hot Start (KAPA) DNA Polymerase efficiently amplified first-strand
cDNA directly after reverse transcription, with no need for prior bead
purification. Libraries preamplified without bead purification had no reduction
15 in yield, but the average cDNA length increased with 450 nts (p=2.6x10⁻¹²,
Student's *t*-test; **Fig. 1f**) demonstrating that KAPA preamplification improves
cDNA generation and offers a viable approach for Smart-Seq automation.

To demonstrate the significance of the improved cDNA generation on
downstream applications, its impact on single-cell transcriptome profiling was
20 assessed. To this end, single HEK293T cell libraries generated both according to
the commercial SMARTer (n=4) and using variations of the present protocol
were sequenced (Smart-Seq2, n=35) (**Table 4**).

The improved conversion of RNA to cDNA should improve gene
expression profiling as more original RNA molecules are accessible for
25 sequencing. Indeed, both a significant increase in the ability to detect gene
expression (**Fig. 2a**) and lowered technical variation for low and medium
abundance transcripts were observed (**Fig. 2b** and **Fig. 4**). The improved
sensitivity of the optimized protocol led to the average detection of 2,372 more
genes in each cell (p<0.05 Student's *t*-test; **Fig. 2c**). All these improvements were
30 independently validated using an alternative RNA-Seq alignment and analyses
strategy (**Fig. 5**). Moreover, both better sensitivity and lower variability in
single-cell transcriptome data generated with Smart-Seq2 than for data available

for Quartz-Seq were obtained (Fig. 6). Although the sequenced libraries had similar mappings characteristics as SMARTer libraries, a 7% increase in unmapped reads was noted (Fig. 7).

Several preamplification enzymes have lower GC bias than the Advantage2 (Adv2) that is used with SMARTer[®], indicating that single-cell profiling could also improve with cDNA preamplifications using KAPA. Indeed, the single-cell libraries preamplified with KAPA detected more genes at higher GC levels (Fig. 2d and Fig. 8) and improved sensitivity and accuracy (Fig. 9). When compared with the low coverage of 5' regions in single-cell data generated through 3' end polyA-tailing of cDNA, single-cell RNA-Seq libraries that had been preamplified with KAPA had significantly better coverage across the full length of transcripts ($p < 10^{-5}$, 1.6×10^{-3} for 5' and 3' ends respectively, Student's *t*-test), as they approached the expected fraction of reads at the 5' and 3' ends (Fig. 2e and Figs. 10-11). Importantly, global gene expression profiles from cells preamplified with KAPA and Adv2 separated on the first principal component (Fig. 2f), demonstrating that preamplification bias had significant impact on absolute expression levels. Regions with artificially large number of reads aligned (i.e. peak) appearing systematically in Smart-Seq irrespectively of preamplification enzyme that necessitated filtering were sequenced (Fig. 12). Together, the data show that preamplification using KAPA improved GC tolerance and read coverage across transcripts.

To determine the extent of technical variability in the single-cell transcriptome profiling with Smart-Seq2, sequencing libraries were generated from dilution series of HEK293T cells (100, 50 and 10 cells) and total RNA (1ng, 100pg, 10pg). Technical losses and variations were small when analyzing 10 cells or more, but considerable variability exists at single-cell levels, as previously observed. It is informative to contrast the technical variability with the biological variability present in cells of the same or different cell type origin (Fig. 13A-D). To this end, additional single-cell transcriptomes were sequenced from DG-75 (n=7), C2C12 (n=6) and MEF (n=7) cells. Analyzing the biological variability between and within cell populations revealed that biological variability associated with cell type specific expression exceeded technical

variability at around 50 RPKM, but the exact threshold will depend on the RNA content present in the cell types studied (Fig. 13E).

This invention provides a new protocol that improves sensitivity, accuracy and coverage across transcripts and is more amenable to automation.
5 Moreover, the new protocol costs less than 12% of the current cost per reaction and only 3% when using in-house produced Tn5 (Fig. 14).

Although these results were reached in the context of Smart-Seq single-cell gene expression analyses, these modifications are applicable other single-cell methods that rely on template switching, including those carried out on
10 microfluidic chips (e.g. Fluidigm C1) or inside emulsion droplets.

The foregoing examples and description of the preferred embodiments should be taken as illustrating, rather than as limiting the present invention as defined by the claims. As will be readily appreciated, numerous variations and combinations of the features set forth above can be utilized without departing
15 from the present invention as set forth in the claims. Such variations are not regarded as a departure from the spirit and script of the invention, and all such variations are intended to be included within the scope of the following claims.

TABLE S1. Tables of cDNA library yield and length starting with purified total RNA

Worksheet A lists all 457 cDNA libraries generated from mouse brain total RNA. The general protocol followed for each sample is indicated in the "general protocol" column, with specific information on the template switching oligonucleotide, RT enzyme, PCR enzyme, MgCl₂ concentration, betaine, bead purification and dNTPs administration timing detailed in separate columns. **Worksheet B** contains a list of direct comparisons of variables that effect cDNA library yield and average length using replicate groups that have identical reaction parameters except for the experimental variable evaluated.

Entry	amount (ng)	PCR cycles	ul elution	dil. Bioanalyzer	additional description	conc (pg/ul)	avg size (bp)	TSO	amount TSO (ul of 10 uM in 10 ul RT rxn)	RT enzyme	MgCl ₂ (mM)	RT protocol	betaine (M)	purification after RT	PCR enzyme	PCR rxn vol (ul)	dNTPs added in the beginning?	other additives
1	1	15	10	1:5	SMARTer kit	318	1669	SMARTer Oligo IIA	1	SMARTscrib e	6	90' @ 42°C	-	yes	Advantag e	50	-	-
2	1	15	10	1:5	SMARTer kit but replacing ISPCR primers	263	1857	SMARTer Oligo IIA	1	SMARTscrib e	6	90' @ 42°C	-	yes	Advantag e	50	-	-
3	1	15	10	1:5	SMARTer kit but replacing oligo dT	172	1784	SMARTer Oligo IIA	1	SMARTscrib e	6	90' @ 42°C	-	yes	Advantag e	50	-	-
4					SMARTer kit but replacing FSB	FAILE D		SMARTer Oligo IIA	1	SMARTscrib e	6	90' @ 42°C	-	yes	Advantag e	50	-	3 mM MnCl ₂
5	1	15	10	1:5	modified SMARTer kit	393	1683	SMARTer Oligo IIA	1	SSRTII	6	90' @ 42°C	-	yes	Advantag e	50	-	-
6	1	15	NA	NA	modified SMARTer kit	121	1780	rG5	1	SMARTscrib e	6	90' @ 42°C	-	yes	Advantag e	50	-	-
7	1	15	NA	NA	modified SMARTer kit	1038	1346	rGrGrG	1	SMARTscrib e	6	90' @ 42°C	-	yes	Advantag e	50	-	-
8	1	15	NA	NA	modified SMARTer kit	90	1652	rGrGrGp	1	SMARTscrib e	6	90' @ 42°C	-	yes	Advantag e	50	-	-
9	1	15	NA	NA	modified SMARTer kit	84	1608	ISO	1	SMARTscrib e	6	90' @ 42°C	-	yes	Advantag e	50	-	-
10	1	15	NA	NA	SMARTer kit	3628	183	SMARTer	1	SMARTscrib	6	90' @	-	yes	Advantag e	50	-	-

11	1	15	NA	NA	modified SMARTer kit		210	8	650	650	1	SMARTscrib e	6	90' @ 42°C	-	ye s	Advantag e	50	-	-
12	1	15	NA	NA	modified SMARTer kit		185	2	6423	6423	1	SMARTscrib e	6	90' @ 42°C	-	ye s	Advantag e	50	-	-
13	1	15	NA	NA	modified SMARTer kit		218	7	666	666	1	SMARTscrib e	6	90' @ 42°C	-	ye s	Advantag e	50	-	-
14	1	15	NA	NA	modified SMARTer kit		218	1	525	525	1	SMARTscrib e	6	90' @ 42°C	-	ye s	Advantag e	50	-	-
15	1	15	NA	NA	SMARTer kit but replacing ISPCR primers		178	2	3366	3366	1	SMARTscrib e	6	90' @ 42°C	-	ye s	Advantag e	50	-	-
16	1	15	NA	NA	modified SMARTer kit		197	0	435	435	1	SMARTscrib e	6	90' @ 42°C	-	ye s	Advantag e	50	-	-
17	1	15	NA	NA	SMARTer kit		144	5	1143	1143	1	SMARTscrib e	6	90' @ 42°C	-	ye s	Advantag e	50	-	-
18	1	15	NA	NA	SMARTer kit but replacing ISPCR primers		151	9	1504	1504	1	SMARTscrib e	6	90' @ 42°C	-	ye s	Advantag e	50	-	-
19	1	15	NA	NA	SMARTer kit but replacing ISPCR primers		172	8	1984	1984	1	SMARTscrib e	6	90' @ 42°C	-	ye s	Advantag e	50	-	-
20	1	15	NA	NA	modified SMARTer kit		154	3	1346	1346	1	SSRTII	6	90' @ 42°C	-	ye s	Advantag e	50	-	-
21	1	15	NA	NA	modified SMARTer kit		168	3	1191	1191	1	SSRTII	6	90' @ 42°C	-	ye s	Advantag e	50	-	-
22	1	15	NA	NA	SMARTer kit but using Superscript II FSB (3 mM MgCl2)		142	0	103	103	1	SMARTscrib e	3	90' @ 42°C	-	ye s	Advantag e	50	-	-
23	1	15	NA	NA	SMARTer kit but using Superscript II FSB (3 mM MgCl2)		160	9	192	192	1	SMARTscrib e	3	90' @ 42°C	-	ye s	Advantag e	50	-	-
24					SMARTer kit but using STRT buffer		FAIL		FAIL	FAIL	1	SMARTscrib e	-	90' @ 42°C	-	ye s	Advantag e	50	-	6 mM MnCl2
25	1	15	NA	NA	SMARTer kit		237	8	1573	1573	1	SMARTscrib e	6	90' @ 42°C	-	ye s	Advantag e	50	-	-
26	1	15	NA	NA	modified SMARTer kit		202	4	246	246	1	SMARTscrib e	3	90' @ 42°C	-	ye s	Advantag e	50	-	-
27	1	15	NA	NA	modified SMARTer kit		194	5	1393	1393	1	SMARTscrib e	3	90' @ 42°C	-	ye s	Advantag e	50	-	-
28	1	15	NA	NA	modified SMARTer kit		218	0	259	259	1	SMARTscrib e	3	90' @ 42°C	-	ye s	Advantag e	50	-	-
29	1	15	NA	NA	modified SMARTer kit		195	8	776	776	1	SMARTscrib e	6	90' @ 42°C	1	ye s	Advantag e	50	-	-
30	1	15	NA	NA	modified SMARTer kit		180	8	731	731	1	SMARTscrib e	6	90' @ 42°C	2	ye s	Advantag e	50	-	-
31	1	15	NA	NA	modified SMARTer kit		196	5	1936	1936	1	SMARTscrib e	6	90' @ 42°C	1	ye s	Advantag e	50	-	-
32					modified SMARTer kit		FAIL		FAIL	FAIL	1	SMARTscrib e	6	90' @ 42°C	1	ye s	Advantag e	50	-	3 mM MnCl2
33					modified SMARTer kit		FAIL		FAIL	FAIL	1	SMARTscrib e	6	90' @ 42°C	-	ye s	Advantag e	50	-	3 mM MnCl2

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58	1	15	20	-	SMARTer kit	6525	2295	SMARTer Oligo IIA	1	SMARTscrib e	6	90' @ 42°C	-	yes	Advantage	50	-	-
59	1	15	20	-	SMARTer kit	6806	2270	SMARTer Oligo IIA	1	SMARTscrib e	6	90' @ 42°C	-	yes	Advantage	50	-	-
60	1	15	20	-	in house prot, processed immediately after adding lysis buffer (LB)	5922	1861	rGrGrG	1	SSRTII	6	90' @ 42°C	1	yes	Advantage	50	-	-
61	1	15	20	-	in house prot, processed immediately after adding lysis buffer (LB)	5419	1892	rGrGrG	1	SSRTII	6	90' @ 42°C	1	yes	Advantage	50	-	-
62	1	15	20	-	in house prot, processed immediately after adding lysis buffer (LB), 60' after adding lysis buffer (LB), stored at RT	5664	1806	rGrGrG	1	SSRTII	6	90' @ 42°C	1	yes	Advantage	50	-	-
63	1	15	20	-	in house prot, processed immediately after adding lysis buffer (LB), 45' after adding lysis buffer (LB), stored at RT	5554	1800	rGrGrG	1	SSRTII	6	90' @ 42°C	1	yes	Advantage	50	-	-
64	1	15	20	-	in house prot, processed immediately after adding lysis buffer (LB), 30' after adding lysis buffer (LB), stored at RT	5552	1772	rGrGrG	1	SSRTII	6	90' @ 42°C	1	yes	Advantage	50	-	-
65	1	15	20	-	in house prot, processed immediately after adding lysis buffer (LB), 10' after adding lysis buffer (LB), stored at RT	6335	1824	rGrGrG	1	SSRTII	6	90' @ 42°C	1	yes	Advantage	50	-	-
66	1	15	20	-	in house prot, processed immediately after adding lysis buffer (LB), 60' after adding lysis buffer (LB), stored in the fridge	4904	1700	rGrGrG	1	SSRTII	6	90' @ 42°C	1	yes	Advantage	50	-	-
67	1	15	20	-	in house prot, processed	3938	1544	rGrGrG	1	SSRTII	6	90' @ 42°C	1	yes	Advantage	50	-	-

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79	10	10	15	1:5	in house prot	1942	1286	rGrGrG	1	SSRTII	6	90'@ 42°C	1	ye s	Advantag e	50	-	-
80	10	12	15	1:5	in house prot	1635	1891	2OMe	1	SSRTII	6	90'@ 42°C	1	ye s	Advantag e	50	-	-
81	10	12	15	1:5	in house prot	1230	1705	2OMe	1	SSRTII	6	90'@ 42°C	1	ye s	Advantag e	50	-	-
82	10	12	15	1:5	in house prot	4700	1602	rGrG+G	1	SSRTII	6	90'@ 42°C	1	ye s	Advantag e	50	-	-
83	10	12	15	1:5	in house prot	4051	1717	rGrG+G	1	SSRTII	6	90'@ 42°C	1	ye s	Advantag e	50	-	-
84	10	12	15	1:5	in house prot	733	1904	ddC	1	SSRTII	6	90'@ 42°C	1	ye s	Advantag e	50	-	-
85	10	12	15	1:5	in house prot	637	1897	ddC	1	SSRTII	6	90'@ 42°C	1	ye s	Advantag e	50	-	-
86	10	12	15	1:5	in house prot	1104	1556	rGrGrG	1	SSRTII	6	90'@ 42°C	1	ye s	Advantag e	50	-	-
87	1	15	15	1:5	in house prot	923	1734	2OMe	1	SSRTII	6	90'@ 42°C	1	ye s	Advantag e	50	-	-
88	1	15	15	1:5	in house prot	842	1620	2OMe	1	SSRTII	6	90'@ 42°C	1	ye s	Advantag e	50	-	-
89	1	15	15	1:5	in house prot	3387	1426	rGrG+G	1	SSRTII	6	90'@ 42°C	1	ye s	Advantag e	50	-	-
90	1	15	15	1:5	in house prot	3609	1473	rGrG+G	1	SSRTII	6	90'@ 42°C	1	ye s	Advantag e	50	-	-
91	1	15	15	1:5	in house prot	401	1463	ddC	1	SSRTII	6	90'@ 42°C	1	ye s	Advantag e	50	-	-
92	1	15	15	1:5	in house prot	470	1661	ddC	1	SSRTII	6	90'@ 42°C	1	ye s	Advantag e	50	-	-
93	1	15	15	1:5	in house prot	1550	1341	rGrGrG	1	SSRTII	6	90'@ 42°C	1	ye s	Advantag e	50	-	-
94	1	15	15	1:5	in house prot	1267	1483	rGrGrG	1	SSRTII	6	90'@ 42°C	1	ye s	Advantag e	50	-	-
95	1	15	15	1:5	in house prot	2176	1438	rGrG+G	1	SSRTII	6	90'@ 42°C	1	ye s	Advantag e	50	-	-
96	1	15	15	1:5	in house prot	1469	1705	rGrGrG	1	SSRTII	9	90'@ 42°C	1	ye s	Advantag e	50	-	-
97	1	15	15	1:5	in house prot	2383	1596	rGrG+G	1	SSRTII	9	90'@ 42°C	1	ye s	Advantag e	50	-	-
98	1	15	15	1:5	in house prot	1431	1807	rGrGrG	1	SSRTII	12	90'@ 42°C	1	ye s	Advantag e	50	-	-
99	1	15	15	1:5	in house prot	3630	1623	rGrG+G	1	SSRTII	12	90'@ 42°C	1	ye s	Advantag e	50	-	-
100	1	15	15	1:5	in house prot	1277	1610	rGrGrG	1	SSRTII	15	90'@ 42°C	1	ye s	Advantag e	50	-	-
101	1	15	15	1:5	in house prot	1884	1462	rGrG+G	1	SSRTII	15	90'@ 42°C	1	ye s	Advantag e	50	-	-
102	1	15	15	1:5	in house prot	1123	1652	SMARTer Oligo IIA	1	SSRTII	6	90'@ 42°C	1	ye s	Advantag e	50	-	-
103	1	15	15	1:5	in house prot	935	1593	SMARTer Oligo IIA	1	SSRTII	15	90'@ 42°C	1	ye s	Advantag e	50	-	-
104	1	15	15	1:5	in house prot, KAPA	2156	176	rGrG+G	1	SSRTII	12	90'@ 42°C	1	ye s	KAPA	50	-	-

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126	1	15	15	1:5	in house prot	1090	175 2	rGrG+G	1	SSRTII	-	60'@42° C, then 90'@60° C	-	ye s	Advantag e	50	-	0.3M trehalose
127	1	15	15	1:5	in house prot	863	156 5	rGrG+G	1	SSRTII	-	60'@42° C, then 90'@60° C	-	ye s	Advantag e	50	-	0.6M trehalose
128	1	15	15	1:5	in house prot	896	165 2	rGrG+G	1	SSRTII	-	60'@42° C, then 90'@60° C	-	ye s	Advantag e	50	-	0.6M trehalose
129	1	15	15	1:5	in house prot	1078	165 5	rGrG+G	1	SSRTII	-	60'@42° C, then 90'@60° C	1	ye s	Advantag e	50	-	0.3M trehalose
130	1	15	15	1:5	in house prot	562	151 7	rGrG+G	1	SSRTII	-	60'@42° C, then 90'@60° C	1	ye s	Advantag e	50	-	0.3M trehalose
131	1	15	15	1:5	in house prot	998	159 4	rGrG+G	1	SSRTII	-	60'@42° C, then 90'@60° C	0. 6	ye s	Advantag e	50	-	0.3M trehalose
132	1	15	15	1:5	in house prot	925	154 5	rGrG+G	1	SSRTII	-	60'@42° C, then 90'@60° C	0. 6	ye s	Advantag e	50	-	0.3M trehalose
133	1	15	15	1:5	in house prot	1155	161 8	rGrG+G	1	SSRTII	3	60'@42° C, then 90'@60° C	1	ye s	Advantag e	50	-	0.3M trehalose
134	1	15	15	1:5	in house prot	603	143 3	rGrG+G	1	SSRTII	3	60'@42° C, then 90'@60° C	1	ye s	Advantag e	50	-	0.3M trehalose
135	1	15	15	1:5	in house prot	1561	171 6	rGrG+G	1	SSRTII	3	60'@42° C, then 90'@60° C	0. 6	ye s	Advantag e	50	-	0.3M trehalose
136	1	15	15	1:5	in house prot	445	157 5	rGrG+G	1	SSRTII	-	60'@50° C, then 90'@42° C	-	ye s	Advantag e	50	-	0.3M trehalose
137	1	15	15	1:5	in house prot	1466	169 8	rGrG+G	1	SSRTII	-	90'@42° C, 30'@60° C, then 30'@42° C	-	ye s	Advantag e	50	-	0.3M trehalose
138	1	15	15	1:5	in house prot	703	158 0	rGrG+G	1	SSRTII	-	60'@50° C, then	-	ye s	Advantag e	50	-	0.6M trehalose

139	1	15	15	1:5	in house prot	1397	1740	rGrG+G	1	SSRTII	-	90'@42° C, 30'@60° C, then 30'@42° C	-	Advantage	50	-	0.6M trehalose
140	1	15	15	1:5	in house prot	355	1425	rGrG+G	1	SSRTII	-	60'@50° C, then 90'@42° C	-	Advantage	50	-	0.6M trehalose
141	1	15	15	1:5	in house prot	1654	1598	rGrG+G	1	SSRTII	-	90'@42° C, 30'@60° C, then 30'@42° C	-	Advantage	50	-	0.6M trehalose
142	1	15	15	1:5	in house prot	1470	1480	rGrG+G	1	SSRTII	12	60'@50° C, then 90'@42° C	-	Advantage	50	-	0.6M trehalose
143	1	15	15	1:5	in house prot	1389	1480	rGrG+G	1	SSRTII	12	90'@42° C, 30'@60° C, then 30'@42° C	-	Advantage	50	-	0.6M trehalose
144	1	15	15	1:5	in house prot	3959	1641	rGrG+G	1	SSRTII	12	90'@42° C, then 10X (2'@50°C - 2'@42°C)	-	Advantage	50	-	-
145	1	15	15	1:5	in house prot	3816	1732	rGrG+G	1	SSRTII	12	90'@42° C, then 10X (2'@60°C - 2'@42°C)	-	Advantage	50	-	-
146	1	15	15	1:5	in house prot	3179	1769	rGrG+G	1	SSRTII	12	90'@42° C, then 10X (2'@50°C - 2'@42°C)	-	Advantage	50	-	0.3M trehalose
147	1	15	15	1:5	in house prot	3271	1828	rGrG+G	1	SSRTII	12	90'@42° C, then 10X (2'@60°C - 2'@42°C)	-	Advantage	50	-	0.3M trehalose

148	1	15	15	1:5	in house prot	4081	1706	rGrG+G	1	SSRTII	12	90'@42° C, then 5x (2'@50°C) 2'@42°C)	1	yes	Advantage	50	-	-
149	1	15	15	1:5	in house prot	3858	1771	rGrG+G	1	SSRTII	12	90'@42° C, then 5x (2'@50°C) 2'@42°C)	1	yes	Advantage	50	-	-
150	1	15	15	1:5	in house prot	3711	1781	rGrG+G	1	SSRTII	12	90'@42° C, then 5x (2'@50°C) 2'@42°C)	1	yes	Advantage	50	-	-
151	1	15	15	1:5	in house prot	4015	1773	rGrG+G	1	SSRTII	12	90'@42° C, then 10x (2'@50°C) 2'@42°C)	1	yes	Advantage	50	-	-
152	1	15	15	1:5	in house prot	3671	1753	rGrG+G	1	SSRTII	12	90'@42° C, then 10x (2'@50°C) 2'@42°C)	1	yes	Advantage	50	-	-
153	1	15	15	1:5	in house prot	3498	1708	rGrG+G	1	SSRTII	12	90'@42° C, then 10x (2'@50°C) 2'@42°C)	1	yes	Advantage	50	-	-
154	1	15	15	1:5	in house prot	3804	1610	rGrG+G	1	SSRTII	12	90'@42° C, then 15x (2'@50°C) 2'@42°C)	1	yes	Advantage	50	-	-
155	1	15	15	1:5	in house prot	3613	1679	rGrG+G	1	SSRTII	12	90'@42° C, then 15x (2'@50°C) 2'@42°C)	1	yes	Advantage	50	-	-

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171	1	15	15	1:5	in house prot	2152	1455	rGrG+G	1	SSRTII	12	2'@42°C 90'@42°C, then 10x (2'@50°C)	1	yes	Advantage	50	-	-
172	1	15	15	1:5	in house prot	1454	1084	rGrG+G	1	SSRTII	12	2'@42°C 90'@42°C, then 10x (2'@50°C)	1	yes	Advantage	50	-	0.816M 1,2 propandi ol
173	1	15	15	1:5	in house prot	1331	1106	rGrG+G	1	SSRTII	12	2'@42°C 90'@42°C, then 10x (2'@50°C)	1	yes	Advantage	50	-	0.816M 1,2 propandi ol
174	1	15	15	1:5	in house prot	1373	1089	rGrG+G	1	SSRTII	12	2'@42°C 90'@42°C, then 10x (2'@50°C)	1	yes	Advantage	50	-	0.816M 1,2 propandi ol
175	1	15	15	1:5	in house prot	1904	1453	rGrG+G	1	SSRTII	12	2'@42°C 90'@42°C, then 10x (2'@50°C)	1	yes	Advantage	50	-	1.075M ethylene glycol
176	1	15	15	1:5	in house prot	2855	1390	rGrG+G	1	SSRTII	12	2'@42°C 90'@42°C, then 10x (2'@50°C)	1	yes	Advantage	50	-	1.075M ethylene glycol
177	1	15	15	1:5	in house prot	2571	1670	rGrG+G	1	Maxima H-	4	90'@50°C	1	yes	Advantage	50	-	-
178	1	15	15	1:5	in house prot	2549	1722	rGrG+G	1	Maxima H-	4	90'@50°C	1	yes	Advantage	50	-	-
179	1	15	15	1:5	in house prot	288	1599	rGrG+G	1	Revertaid Premium	4	90'@50°C	1	yes	Advantage	50	-	-
180	1	15	15	1:5	in house prot	129	1608	rGrG+G	1	Revertaid Premium	4	90'@50°C	1	yes	Advantage	50	-	-

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214	1	15	15	1:5	in house prot	1279	1919	rGrG+G	1	SSRTII	12	90' @42° C, then 10x (2' @50°C) (2' @42°C)	1	yes	Advantage	50	-	-
215	1	15	15	1:5	in house prot	1739	1959	rGrG+G	1	SSRTII	12	90' @42° C, then 10x (2' @50°C) (2' @42°C)	1	yes	Advantage	50	-	-
216	1	15	15	1:5	in house prot	1861	2063	rGrG+G	1	SSRTII	12	90' @42° C, then 10x (2' @50°C) (2' @42°C)	1	yes	Advantage	50	-	-
217	1	15	15	1:5	in house prot (40u SSRTII)	2329	2260	rGrG+G	1	SSRTII	12	90' @42° C, then 10x (2' @50°C) (2' @42°C)	1	yes	Advantage	50	yes	-
218	1	15	15	1:5	in house prot (40u SSRTII)	2273	2218	rGrG+G	1	SSRTII	12	90' @42° C, then 10x (2' @50°C) (2' @42°C)	1	yes	Advantage	50	yes	-
219	1	15	15	1:5	in house prot (40u SSRTII, with dNTPs+betaine added in the beginning)	2013	2268	rGrG+G	1	SSRTII	12	90' @42° C, then 10x (2' @50°C) (2' @42°C)	1	yes	Advantage	50	yes	-
220	1	15	15	1:5	in house prot (40u SSRTII, with dNTPs+betaine added in the beginning)	1932	2122	rGrG+G	1	SSRTII	12	90' @42° C, then 10x (2' @50°C) (2' @42°C)	1	yes	Advantage	50	yes	-

221	1	15	15	1:5	in house prot (40u SSRTII, with dNTPs+betaine+Mg Cl2 added in the beginning)	1877	200 9	rGrG+G	1	SSRTII	12	90'@42° C, then 10x (2'@50°C)	1	yes	Advantage	50	yes	-
222	1	15	15	1:5	in house prot (40u SSRTII, with dNTPs+betaine+Mg Cl2 added in the beginning)	989	196 3	rGrG+G	1	SSRTII	12	90'@42° C, then 10x (2'@50°C)	1	yes	Advantage	50	yes	-
223	1	15	15	1:5	in house prot (40u SSRTII, with all reagents except SSRTII added in the beginning)	2269	131 6	rGrG+G	1	SSRTII	12	90'@42° C, then 10x (2'@50°C)	1	yes	Advantage	50	yes	-
224	1	15	15	1:5	in house prot (40u SSRTII, with all reagents except SSRTII added in the beginning)	1926	129 2	rGrG+G	1	SSRTII	12	90'@42° C, then 10x (2'@50°C)	1	yes	Advantage	50	yes	-
225	1	15	15	1:5	in house prot (40u SSRTII, with all reagents except SSRTII added in the beginning)	1536	129 7	rGrG+G	1	SSRTII	12	90'@42° C, then 10x (2'@50°C)	1	yes	Advantage	50	yes	-
226	1	15	15	1:5	in house prot	1604	192 5	rGrG+G	1	SSRTII	12	90'@42° C, then 10x (2'@50°C)	1	yes	Advantage	50	yes	-
227	1	15	15	1:5	in house prot	1493	191 0	rGrG+G	1	SSRTII	12	90'@42° C, then 10x (2'@50°C)	1	yes	Advantage	50	yes	-
228	1	15	15	1:5	in house prot	1540	192 4	rGrG+G	1	SSRTII	12	90'@42° C, then 10x (2'@50°C)	1	yes	Advantage	50	yes	-

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236	1	15	15	1:5	in house prot (no denaturation step @72°C for oligo-dT (incubated 5'@RT))	1332	179 2				1	SSRTII	12	2'@42°C) 90'@42° C, then 10x (2'@50°C - 2'@42°C)	1	yes	Advantage	50	yes	-
237	1	15	15	1:5	in house prot	1728	221 4				1	SSRTII	12	90'@42° C, then 10x (2'@50°C - 2'@42°C)	1	yes	Advantage	50	yes	-
238	1	15	15	1:5	in house prot	1620	228 3				1	SSRTII	12	90'@42° C, then 10x (2'@50°C - 2'@42°C)	1	yes	Advantage	50	yes	-
239	1	15	15	1:5	in house prot	1563	226 0				1	SSRTII	12	90'@42° C, then 10x (2'@50°C - 2'@42°C)	1	yes	Advantage	50	yes	-
240	1	15	15	1:5	in house prot	1485	217 1				1	SSRTII	12	90'@42° C, then 10x (2'@55°C - 2'@42°C)	1	yes	Advantage	50	yes	-
241	1	15	15	1:5	in house prot	1444	228 1				1	SSRTII	12	90'@42° C, then 10x (2'@55°C - 2'@42°C)	1	yes	Advantage	50	yes	-
242	1	15	15	1:5	in house prot	1308	223 1				1	SSRTII	12	90'@42° C, then 10x (2'@55°C - 2'@42°C)	1	yes	Advantage	50	yes	-
243	1	15	15	1:5	in house prot	1618	217 6				1	SSRTII	12	90'@42°	1	yes	Advantage	50	yes	-

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262	1	15	15	1:5	in house prot (+extra DTT)	2209	183 7	rGrG+G	1	Maxima H-	12	90'@ 50°C	1	yes	Advantag e	50	yes	-
263	1	15	15	1:5	in house prot (NO extra DTT)	647	211 7	rGrG+G	1	Maxima H-	4	90'@ 50°C	-	yes	Advantag e	50	yes	-
264	1	15	15	1:5	in house prot (NO extra DTT)	613	214 7	rGrG+G	1	Maxima H-	4	90'@ 50°C	-	yes	Advantag e	50	yes	-
265	1	15	15	1:5	in house prot	1442	198 9	rGrG+G	1	SSRTII	12	90'@42° C, then 10x (2'@50°C - 2'@42°C)	1	yes	Advantag e	50	yes	-
266	1	15	15	1:5	in house prot	1496	210 5	rGrG+G	1	SSRTII	12	90'@42° C, then 10x (2'@50°C - 2'@42°C)	1	yes	Advantag e	50	yes	-
267	1	15	15	1:5	in house prot	1228	267 7	rGrG+G	1	Maxima H-	4	90'@ 50°C	-	-	KAPA HiFi HS	50	yes	-
268	1	15	15	1:5	in house prot	882	228 3	rGrG+G	1	Maxima H-	4	90'@ 50°C	-	-	Advantag e	50	yes	-
269	1	15	15	1:5	in house prot	2475	255 8	rGrG+G	1	Maxima H-	4	90'@ 50°C	1	-	KAPA HiFi HS	50	yes	-
270	1	15	15	1:5	in house prot	1557	220 1	rGrG+G	1	Maxima H-	4	90'@ 50°C	1	-	Advantag e	50	yes	-
271	1	15	15	1:5	in house prot	4074	218 5	rGrG+G	1	Maxima H-	12	90'@ 50°C	1	-	KAPA HiFi HS	50	yes	-
272	1	15	15	1:5	in house prot	2927	233 0	rGrG+G	1	Maxima H-	12	90'@ 50°C	1	-	Advantag e	50	yes	-
273	1	15	15	1:5	in house prot	3213	252 4	rGrG+G	1	SSRTII	12	90'@42° C, then 10x (2'@50°C - 2'@42°C)	1	-	KAPA HiFi HS	50	yes	-
274	1	15	15	1:5	in house prot	3359	266 2	rGrG+G	1	SSRTII	12	90'@42° C, then 10x (2'@50°C - 2'@42°C)	1	-	Advantag e	50	yes	-
275	1	15	15	1:5	in house prot	819	206 9	rGrG+G	1	Maxima H-	4	90'@ 50°C	-	yes	Advantag e	50	yes	-
276	1	15	15	1:5	in house prot	737	220 2	rGrG+G	1	Maxima H-	4	90'@ 50°C	-	yes	Advantag e	50	yes	-
277	1	15	15	1:5	in house prot	1375	193 7	rGrG+G	1	Maxima H-	4	90'@ 50°C	1	yes	Advantag e	50	yes	-

278	1	15	15	1:5	in house prot	1426	2008	rGrG+G	1	Maxima H-	4	90' @ 50°C	1	yes	Advantage	50	yes	-
279	1	15	15	1:5	in house prot	2773	1848	rGrG+G	1	Maxima H-	12	90' @ 50°C	1	yes	Advantage	50	yes	-
280	1	15	15	1:5	in house prot	2987	1916	rGrG+G	1	Maxima H-	12	90' @ 50°C	1	yes	Advantage	50	yes	-
281	1	15	15	1:5	in house prot	2155	2266	rGrG+G	1	SSRTII	12	90' @ 42°C, then 10x (2' @ 50°C) 2' @ 42°C	1	yes	Advantage	50	yes	-
282	1	15	15	1:5	in house prot	2240	2271	rGrG+G	1	SSRTII	12	90' @ 42°C, then 10x (2' @ 50°C) 2' @ 42°C	1	yes	Advantage	50	yes	-
283	1	15	15	1:5	in house prot	694	2244	rGrG+G	1	SSRTII	6	90' @ 42°C, then 10x (2' @ 50°C) 2' @ 42°C	-	yes	Advantage	50	yes	1M proline
284	1	15	15	1:5	in house prot	682	2361	rGrG+G	1	SSRTII	6	90' @ 42°C, then 10x (2' @ 50°C) 2' @ 42°C	-	yes	Advantage	50	yes	1M proline
285	1	15	15	1:5	in house prot	745	2264	rGrG+G	1	SSRTII	6	90' @ 42°C, then 10x (2' @ 50°C) 2' @ 42°C	-	yes	Advantage	50	yes	1M proline
286	1	15	15	1:5	in house prot	766	2311	rGrG+G	1	SSRTII	12	90' @ 42°C, then 10x (2' @ 50°C) 2' @ 42°C	-	yes	Advantage	50	yes	1M proline
287	1	15	15	1:5	in house prot	685	2304	rGrG+G	1	SSRTII	12	90' @ 42°C, then 10x	-	yes	Advantage	50	yes	1M proline

288	1	15	15	1:5	in house prot	642	2226	rGrG+G	1	SSRTII	12	(2' @50°C 2' @42°C)	-	yes	Advantage	50	yes	1M proline
289	1	15	15	1:5	in house prot	1133	1873	rGrG+G	1	SSRTII	12	90' @42° C, then 10x (2' @50°C 2' @42°C)	1	yes	Advantage	50	yes	
290	1	15	15	1:5	in house prot	1443	2148	rGrG+G	1	SSRTII	12	90' @42° C, then 10x (2' @50°C 2' @42°C)	1	yes	Advantage	50	yes	
291	1	15	15	1:5	in house prot	1147	2066	rGrG+G	1	SSRTII	12	90' @42° C, then 10x (2' @50°C 2' @42°C)	1	yes	Advantage	50	yes	
292	1	15	15	1:5	in house prot	1322	2084	rGrG+G	1	SSRTII	12	90' @42° C, then 10x (2' @50°C 2' @42°C)	1	yes	Advantage	50	yes	
293	1	15	15	1:5	in house prot	1013	2095	rGrG+G	1	SSRTII	12	90' @42° C, then 10x (2' @50°C 2' @42°C)	1	yes	Advantage	50	yes	
294	1	15	15	1:5	in house prot	960	2125	rGrG+G	1	SSRTII	12	90' @42° C, then 10x (2' @50°C 2' @42°C)	1	yes	Advantage	50	yes	

295	1	15	15	1:5	in house prot with SMARTer dT30 (unanchored oligo)	1296	212 9	rGrG+G	1	SSRTII	12	90' @42° C, then 10x (2' @50°C	1	yes	Advantage	50	yes	
296	1	15	15	1:5	in house prot with SMARTer dT30 (unanchored oligo)	1390	219 4	rGrG+G	1	SSRTII	12	90' @42° C, then 10x (2' @50°C	1	yes	Advantage	50	yes	
297	1	15	15	1:5	in house prot with SMARTer dT30 (unanchored oligo)	1419	208 4	rGrG+G	1	SSRTII	12	90' @42° C, then 10x (2' @50°C	1	yes	Advantage	50	yes	
298	1	18	15	1:2 0	in house prot	5456	229 3	rGrG+G	2	SSRTII	12	90' @42° C, then 10x (2' @50°C	1	yes	Advantage	50	yes	
299	1	18	15	1:2 0	in house prot	5753	233 4	rGrG+G	2	SSRTII	12	90' @42° C, then 10x (2' @50°C	1	yes	Advantage	50	yes	
300	1	18	15	1:2 0	in house prot	5341	236 5	rGrG+G	2	SSRTII	12	90' @42° C, then 10x (2' @50°C	1	yes	Advantage	50	yes	
301	1	18	15	1:2 0	in house prot	2266	175 0	rGrG+G	2	SSRTII	12	90' @42° C, then 10x (2' @50°C	1	yes	Advantage	50	yes	
302	1	18	15	1:2 0	in house prot	1530	185 0	rGrG+G	2	SSRTII	12	90' @42° C, then	1	yes	Advantage	50	yes	

303	1	18	15	1:20	in house prot	1883	1703	rGrG+G	2	SSRTII	12	10x (2'@50°C 2'@42°C)	1	yes	Advantage	50	yes		
304	1	18	15	1:20	in house prot	2856	1731	rGrG+G	2	SSRTII	12	90'@42°C, then 10x (2'@50°C 2'@42°C)	1	yes	Advantage	50	yes		
305	1	18	15	1:20	in house prot	3150	2371	rGrG+N	1	SSRTII	12	90'@42°C, then 10x (2'@50°C 2'@42°C)	1	yes	Advantage	50	yes		
306	1	18	15	1:20	in house prot	2889	2393	rGrG+N	1	SSRTII	12	90'@42°C, then 10x (2'@50°C 2'@42°C)	1	yes	Advantage	50	yes		
307	1	18	15	1:20	in house prot	3773	2302	rGrG+N	2	SSRTII	12	90'@42°C, then 10x (2'@50°C 2'@42°C)	1	yes	Advantage	50	yes		
308	1	18	15	1:20	in house prot	3744	2318	rGrG+N	2	SSRTII	12	90'@42°C, then 10x (2'@50°C 2'@42°C)	1	yes	Advantage	50	yes		
309	1	18	15	1:20	in house prot	4113	2390	rGrG+N	2	SSRTII	12	90'@42°C, then 10x (2'@50°C 2'@42°C)	1	yes	Advantage	50	yes		

310	1	18	15	1:20	in house prot	3903	2309	rGrG+N	2	SSRTII	12	2'@42°C 90'@42°C, then 10x (2'@50°C)	1	yes	Advantage	50	yes	
311	1	18	15	1:20	in house prot	2649	2436	rGrG+N	2	SSRTII	12	2'@42°C 90'@42°C, then 10x (2'@50°C)	1	yes	Advantage	50	yes	
312	1	18	15	1:20	in house prot	2856	2422	rGrG+N	2	SSRTII	12	2'@42°C 90'@42°C, then 10x (2'@50°C)	1	yes	Advantage	50	yes	
313	1	18	15	1:20	in house prot	2674	2412	rGrG+N	2	SSRTII	12	2'@42°C 90'@42°C, then 10x (2'@50°C)	1	yes	Advantage	50	yes	
314	1	18	15	1:20	in house prot	2641	2427	rGrG+N	2	SSRTII	12	2'@42°C 90'@42°C, then 10x (2'@50°C)	1	yes	Advantage	50	yes	
315	1	18	15	1:20	in house prot	5251	2272	rGrG+G	2	SSRTII	12	2'@42°C 90'@42°C, then 10x (2'@50°C)	1	yes	Advantage	50	yes	
316	1	15	15	1:5	in house prot	1255	2057	rGrG+G	1	SSRTII	12	2'@42°C 90'@42°C, then 10x (2'@50°C)	1	-	KAPA HiFi HS	50	yes	
317	1	15	15	1:6	in house prot	1135	2123	rGrG+G	1	SSRTII	12	90'@42°C	1	-	KAPA HiFi HS	50	yes	

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347	1	15	15	1:5	in house prot	37	1918	rGrG+G	1	SSRTII	3	90'@42° C, then 10x (2'@50°C)	1	-	KAPA HiFi HS	25	yes	
348	1	15	15	1:5	in house prot	65	1888	rGrG+G	1	SSRTII	3	90'@42° C, then 10x (2'@50°C)	1	-	KAPA HiFi HS	25	yes	
349	1	15	15	1:5	in house prot	46	1976	rGrG+G	1	SSRTII	3	90'@42° C, then 10x (2'@50°C)	1	-	KAPA HiFi HS	25	yes	
350	1	15	15	1:5	in house prot	39	1966	rGrG+G	1	SSRTII	3	90'@42° C, then 10x (2'@50°C)	1	-	KAPA HiFi HS	25	yes	
351	1	15	15	1:5	in house prot	44	1784	rGrG+G	1	SSRTII	3	90'@42° C, then 10x (2'@50°C)	1	-	KAPA HiFi HS	25	yes	
352	1	15	15	1:5	in house prot	95	1791	rGrG+G	1	SSRTII	3	90'@42° C, then 10x (2'@50°C)	1	-	KAPA HiFi HS	25	yes	
353	1	15	15	1:5	in house prot	80	1897	rGrG+G	1	SSRTII	3	90'@42° C, then 10x (2'@50°C)	1	-	KAPA HiFi HS	25	yes	
354	1	15	15	1:5	in house prot	281	1685	rGrG+G	1	SSRTII	6	90'@42° C, then	1	-	KAPA HiFi HS	25	yes	

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392	1	15	15	1:5	in house prot	1713	1596	rGrG+G	1	SSRTII	25	(2' @50°C 2' @42°C)	1	-	KAPA HiFi HS	25	yes		
393	1	15	15	1:5	in house prot	1428	1667	rGrG+G	1	SSRTII	30	90' @42° C, then 10x (2' @50°C	1	-	KAPA HiFi HS	25	yes		
394	1	15	15	1:5	in house prot	1274	1711	rGrG+G	1	SSRTII	30	90' @42° C, then 10x (2' @50°C	1	-	KAPA HiFi HS	25	yes		
395	1	15	15	1:5	in house prot	1471	1651	rGrG+G	1	SSRTII	30	90' @42° C, then 10x (2' @50°C	1	-	KAPA HiFi HS	25	yes		
396	1	15	15	1:5	in house prot	1362	1653	rGrG+G	1	SSRTII	30	90' @42° C, then 10x (2' @50°C	1	-	KAPA HiFi HS	25	yes		
397	1	15	15	1:5	in house prot	86	1996	rGrG+G	1	SSRTII	6	90' @42° C, then 10x (2' @50°C	1	-	KAPA HiFi HS	25	yes		
398	1	15	15	1:5	in house prot	66	2002	rGrG+G	1	SSRTII	6	90' @42° C, then 10x (2' @50°C	1	-	KAPA HiFi HS	25	yes		

399	1	15	15	1:5	in house prot	92	1941	rGrG+G	1	SSRTII	6	90' @42° C, then 10x (2' @50°C) 2' @42°C	1	-	KAPA HiFi HS	25	yes	
400	1	15	15	1:5	in house prot	86	1898	rGrG+G	1	SSRTII	6	90' @42° C, then 10x (2' @50°C) 2' @42°C	1	-	KAPA HiFi HS	25	yes	
401	1	15	15	1:5	in house prot	85	2071	rGrG+G	1	SSRTII	9	90' @42° C, then 10x (2' @50°C) 2' @42°C	1	-	KAPA HiFi HS	25	yes	
402	1	15	15	1:5	in house prot	75	2078	rGrG+G	1	SSRTII	9	90' @42° C, then 10x (2' @50°C) 2' @42°C	1	-	KAPA HiFi HS	25	yes	
403	1	15	15	1:5	in house prot	120	2081	rGrG+G	1	SSRTII	9	90' @42° C, then 10x (2' @50°C) 2' @42°C	1	-	KAPA HiFi HS	25	yes	
404	1	15	15	1:5	in house prot	150	1984	rGrG+G	1	SSRTII	9	90' @42° C, then 10x (2' @50°C) 2' @42°C	1	-	KAPA HiFi HS	25	yes	
405	1	15	15	1:5	in house prot	132	2088	rGrG+G	1	SSRTII	12	90' @42° C, then 10x (2' @50°C) 2' @42°C	1	-	KAPA HiFi HS	25	yes	
406	1	15	15	1:5	in house prot	101	2139	rGrG+G	1	SSRTII	12	90' @42° C, then (2' @50°C)	1	-	KAPA HiFi HS	25	yes	

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414	1	15	15	1:5	in house prot	136	2042	rGrG+G	1	SSRTII	20	2' @42°C) 90' @42° C, then 10x (2' @50°C	1	-	KAPA HiFi HS	25	yes		
415	1	15	15	1:5	in house prot	153	2008	rGrG+G	1	SSRTII	20	90' @42° C, then 10x (2' @50°C	1	-	KAPA HiFi HS	25	yes		
416	1	15	15	1:5	in house prot	166	2111	rGrG+G	1	SSRTII	20	90' @42° C, then 10x (2' @50°C	1	-	KAPA HiFi HS	25	yes		
417	1	15	15	1:5	in house prot	259	2484	rGrG+G	1	SSRTII	6	90' @42° C, then 10x (2' @50°C	-	-	KAPA HiFi HS	25	yes		
418	1	15	15	1:5	in house prot	255	2505	rGrG+G	1	SSRTII	6	90' @42° C, then 10x (2' @50°C	-	-	KAPA HiFi HS	25	yes		
419	1	15	15	1:5	in house prot	231	2493	rGrG+G	1	SSRTII	6	90' @42° C, then 10x (2' @50°C	-	-	KAPA HiFi HS	25	yes		
420	1	15	15	1:5	in house prot	258	2469	rGrG+G	1	SSRTII	6	90' @42° C, then 10x (2' @50°C	-	-	KAPA HiFi HS	25	yes		
421	1	15	15	1:5	in house prot	226	2529	rGrG+G	1	SSRTII	6	90' @42°	-	-	KAPA HiFi HS	25	yes		

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429	1	15	15	1:5	in house prot	585	2409	rGrG+G	1	SSRTII	9	2'@42°C 90'@42°C, then 10x (2'@50°C)	-	-	KAPA HiFi HS	25	yes		
430	1	15	15	1:5	in house prot	502	2591	rGrG+G	1	SSRTII	9	2'@42°C 90'@42°C, then 10x (2'@50°C)	-	-	KAPA HiFi HS	25	yes		
431	1	15	15	1:5	in house prot	541	2533	rGrG+G	1	SSRTII	9	2'@42°C 90'@42°C, then 10x (2'@50°C)	-	-	KAPA HiFi HS	25	yes		
432	1	15	15	1:5	in house prot	554	2555	rGrG+G	1	SSRTII	9	2'@42°C 90'@42°C, then 10x (2'@50°C)	-	-	KAPA HiFi HS	25	yes		
433	1	15	15	1:5	in house prot	677	2368	rGrG+G	1	SSRTII	12	2'@42°C 90'@42°C, then 10x (2'@50°C)	-	-	KAPA HiFi HS	25	yes		
434	1	15	15	1:5	in house prot	792	2287	rGrG+G	1	SSRTII	12	2'@42°C 90'@42°C, then 10x (2'@50°C)	-	-	KAPA HiFi HS	25	yes		
435	1	15	15	1:5	in house prot	842	2325	rGrG+G	1	SSRTII	12	2'@42°C 90'@42°C, then 10x (2'@50°C)	-	-	KAPA HiFi HS	25	yes		
436	1	15	15	1:5	in house prot	737	2312	rGrG+G	1	SSRTII	12	2'@42°C	-	-	KAPA	25	yes		

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451	1	15	15	1:5	in house prot	128	2111	rGrG+G	1	SSRTII	15	90' @42° C, then 10x (2' @50°C 2' @42°C)	-	-	KAPA HiFi HS	25	yes	
452	1	15	15	1:5	in house prot	147	2049	rGrG+G	1	SSRTII	15	90' @42° C, then 10x (2' @50°C 2' @42°C)	-	-	KAPA HiFi HS	25	yes	
453	1	15	15	1:5	in house prot	101	2127	rGrG+G	1	SSRTII	15	90' @42° C, then 10x (2' @50°C 2' @42°C)	-	-	KAPA HiFi HS	25	yes	
454	1	15	15	1:5	in house prot	108	2251	rGrG+G	1	SSRTII	15	90' @42° C, then 10x (2' @50°C 2' @42°C)	-	-	KAPA HiFi HS	25	yes	
455	1	15	15	1:5	in house prot	128	2007	rGrG+G	1	SSRTII	20	90' @42° C, then 10x (2' @50°C 2' @42°C)	-	-	KAPA HiFi HS	25	yes	
456	1	15	15	1:5	in house prot	86	2104	rGrG+G	1	SSRTII	20	90' @42° C, then 10x (2' @50°C 2' @42°C)	-	-	KAPA HiFi HS	25	yes	
457	1	15	15	1:5	in house prot	98	2147	rGrG+G	1	SSRTII	20	90' @42° C, then 10x (2' @50°C 2' @42°C)	-	-	KAPA HiFi HS	25	yes	
458	1	15	15	1:5	in house prot	94	1961	rGrG+G	1	SSRTII	20	90' @42° C, then	-	-	KAPA HiFi HS	25	yes	

10x	
(2' @ 50°C)	
-	
2' @ 42°C)	

B. Analyses of experimental variables effecting cDNA library yield and length

To evaluate the effect on cDNA yield and length of each component of the protocol, we compiled groups of replicates from different experiments that only differed in the experimental variable evaluated (column A). These experiments were used to compute Student t-test p-values and Wilcoxon rank sum test p-values for both cDNA yield and cDNA average length.

Experim ental Variable Tested	Variant 1	Variant 2	cDNA Yield			cDNA length					Numb er of replic ates
			t-test Yield	Wilco xon-test Yield	mean yield (Var1)	mean yield (Var2)	t-test cDNA length	wilcox on-test cDNA length	mean length (Var1)	mean length (Var2)	
elution volume (ul)	20	30	2.69E-02	3.33E-01	166.79	56.535	2.43E-01	3.33E-01	2230.5	1930	2
TSO	2rGrG+G	3rGrG+G	3.01E-02	3.33E-01	83.025	138.225	1.50E-01	3.33E-01	1887	1722.5	2
TSO	2rGrG+G	C6 amino	4.43E-02	3.33E-01	83.025	36.1875	3.11E-01	3.33E-01	1887	1747	2
TSO	2rGrG+G	rGrG+G	2.56E-04	3.33E-01	83.025	299.025	1.71E-01	3.33E-01	1887	1707	2
TSO	2rGrG+G	phosphate	1.12E-02	3.33E-01	83.025	44.0625	7.13E-01	1.00E+00	1887	1907	2
TSO	2OMe	ddC	2.00E-01	9.52E-02	117.39	62.7	8.95E-01	8.41E-01	1779.2	1795.8	5
TSO	2OMe	rGrG+G	2.59E-02	7.94E-03	117.39	369.615	7.72E-02	1.51E-01	1779.2	1597	5
TSO	2OMe	rGrGrG	7.68E-01	8.86E-01	123.675	109.9313	8.19E-03	2.86E-02	1797.75	1416.5	4
TSO	2OMe	SMARTer Oligo IIA	-	-	69.225	84.225	-	-	1734	1652	1
TSO	3rGrG+G	C6 amino	4.11E-03	3.33E-01	138.225	36.1875	8.17E-01	1.00E+00	1722.5	1747	2

TSO	3rGrG+G	rGrG+G	1.36E-03	1.00E-01	130.375	285.775	6.09E-01	1.00E+00	1691.3333	1646.3333	3
TSO	3rGrG+G	phosphate	2.63E-02	3.33E-01	138.225	44.0625	3.09E-02	3.33E-01	1722.5	1907	2
TSO	C6 amino	rGrG+G	3.25E-03	3.33E-01	36.1875	299.025	7.44E-01	6.67E-01	1747	1707	2
TSO	C6 amino	phosphate	3.42E-01	3.33E-01	36.1875	44.0625	2.97E-01	3.33E-01	1747	1907	2
TSO	ddC	rGrG+G	1.36E-02	7.94E-03	62.7	369.615	1.53E-01	2.22E-01	1795.8	1597	5
TSO	ddC	rGrGrG	2.16E-01	2.00E-01	66.4313	109.9313	6.64E-02	1.14E-01	1770.5	1416.5	4
TSO	ddC	SMARTer Oligo IIA	-	-	30.075	84.225	-	-	1463	1652	1
TSO	dGCGGG	dGCGGGp	2.63E-03	1.00E-01	47.3067	14.2533	9.78E-04	1.00E-01	2104.6667	2309.3333	3
TSO	dGCGGG	rGrGrG	2.21E-02	1.00E-01	47.3067	150.6733	9.85E-01	7.00E-01	2104.6667	2107.3333	3
TSO	dGCGGG	rGrGrGp	1.32E-01	3.33E-01	48.33	35.64	2.38E-03	3.33E-01	2117.5	2588.5	2
TSO	dGCGGGp	rGrGrG	1.37E-02	1.00E-01	14.2533	150.6733	2.41E-01	1.00E-01	2309.3333	2107.3333	3
TSO	dGCGGGp	rGrGrGp	2.47E-03	3.33E-01	14.48	35.64	5.93E-02	3.33E-01	2317	2588.5	2
TSO	rGrG+G	phosphate	2.41E-03	3.33E-01	299.025	44.0625	1.89E-01	3.33E-01	1707	1907	2
TSO	rGrG+G	rGrGrG	6.55E-03	4.96E-04	235.7125	82.075	1.71E-01	2.14E-01	1588.8333	1713	12
TSO	rGrG+G	SMARTer Oligo IIA	2.74E-01	3.33E-01	197.6625	77.175	5.20E-02	3.33E-01	1444	1622.5	2
TSO	rGrGrG	rGrGrGp	4.64E-04	2.86E-02	154.565	32.765	2.56E-05	2.86E-02	2256.5	2583.5	4
TSO	rGrGrG	SMARTer Oligo IIA	1.62E-01	3.33E-01	106.0125	77.175	4.67E-01	6.67E-01	1475.5	1622.5	2
MgCl2 concentr ation (mM)	0	3	5.27E-01	4.00E-01	65.95	82.975	9.97E-01	1.00E+00	1588.6667	1589	3
MgCl2 concentr ation	0	12	6.31E-01	1.00E+00	75.3375	107.2125	7.78E-01	1.00E+00	1511.5	1480	2

(mM)																					
MgCl2 concentr ation (mM)	4	6	3.46E- 01	6.67E- 01	102.3	124.05								2.28E- 01	3.33E- 01	1517.5	1398			2	
MgCl2 concentr ation (mM)	4	9	2.52E- 02	3.33E- 01	102.3	211.87 5								5.08E- 01	6.67E- 01	1517.5	1563.5			2	
MgCl2 concentr ation (mM)	4	12	9.44E- 05	1.30E- 04	91.395	198.79 5								3.07E- 01	2.47E- 01	1918.8	1753.9			10	
MgCl2 concentr ation (mM)	4	15	3.32E- 01	3.33E- 01	102.3	130.87 5								3.81E- 01	6.67E- 01	1517.5	1360			2	
MgCl2 concentr ation (mM)	6	9	5.94E- 01	6.86E- 01	154.59 38	178.16 25								3.80E- 03	2.86E- 02	1390.75	1607			4	
MgCl2 concentr ation (mM)	6	12	8.77E- 01	7.30E- 01	127.01 67	132.94 17								4.77E- 01	2.58E- 01	1709.77 78	1845.888 9			9	
MgCl2 concentr ation (mM)	6	15	4.50E- 01	8.41E- 01	140.52	113.79								7.04E- 01	5.48E- 01	1443	1477			5	
MgCl2 concentr ation (mM)	9	12	8.71E- 01	6.86E- 01	178.16 25	170.81 25								8.05E- 01	8.86E- 01	1607	1582.25			4	
MgCl2 concentr ation (mM)	9	15	1.17E- 01	2.00E- 01	178.16 25	124.70 62								1.24E- 01	2.00E- 01	1607	1448			4	
MgCl2 concentr ation	12	15	2.94E- 01	3.43E- 01	170.81 25	124.70 62								2.87E- 01	3.43E- 01	1582.25	1448			4	

(mM)																			
betaine concentration (M)	0	0.6																	
betaine concentration (M)	0	1																	
betaine concentration (M)	0.6	1																	
betaine concentration (M)	1	1.5																	
RT enzyme	Maxima H-	Revertaid Premium																	
RT enzyme	Revertaid H-	SSRTII																	
RT enzyme	SMARTscribe	SSRTII																	
RT enzyme	SSRTII	SSRTIII																	
RT protocol	60@42C, then 90@60C	60@50C, then 90@42C																	
RT protocol	60@42C, then 90@60C	90@42C, 30@60C, then 30@42C																	
RT protocol	60@50C, then 90@42C	90@42C, 30@60C, then 30@42C																	
RT protocol	90@42C	90@42C, then 10x (2@50C-2@42C)																	
RT protocol	90@42C	90@42C, then 10x (2@55C-2@42C)																	

RT protocol	90@42C	90@42C, then 10x (2@60C- 2@42C)	8.90E- 01	4.00E- 01	158.45	170.47 5	6.57E- 01	4.00E- 01	1924.33 33	2025.333 3	3
RT protocol	90@42C	90@42C, then 15x (2@50C- 2@42C)	4.30E- 01	6.67E- 01	244.76 25	278.13 75	6.57E- 01	6.67E- 01	1680	1644.5	2
RT protocol	90@42C	90@42C, then 20x (2@50C- 2@42C)	8.51E- 01	6.67E- 01	244.76 25	237.22 5	1.20E- 01	3.33E- 01	1680	1467	2
RT protocol	90@42C	90@42C, then 5x (2@50C- 2@42C)	2.86E- 01	3.33E- 01	244.76 25	297.71 25	4.87E- 01	6.67E- 01	1680	1738.5	2
RT protocol	90@42C, then 10x (2@50C- 2@42C)	90@42C, then 10x (2@55C- 2@42C)	5.04E- 03	1.00E- 01	165.37 5	105.92 5	5.96E- 01	1.00E +00	2248.66 67	2227.666 7	3
RT protocol	90@42C, then 10x (2@50C- 2@42C)	90@42C, then 10x (2@60C- 2@42C)	5.23E- 01	4.21E- 01	206.29 5	175.17	9.27E- 01	5.48E- 01	2031.2	2015.4	5
RT protocol	90@42C, then 10x (2@50C- 2@42C)	90@42C, then 15x (2@50C- 2@42C)	7.32E- 01	1.00E +00	291.12 5	300.3	1.71E- 01	2.00E- 01	1722.33 33	1639.666 7	3
RT protocol	90@42C, then 10x (2@50C- 2@42C)	90@42C, then 20x (2@50C- 2@42C)	4.97E- 01	4.00E- 01	246.87 5	196.37 5	7.32E- 02	1.00E- 01	1714	1521	3
RT protocol	90@42C, then 10x (2@50C- 2@42C)	90@42C, then 5x (2@50C- 2@42C)	9.92E- 01	1.00E +00	291.12 5	291.25	5.65E- 01	7.00E- 01	1722.33 33	1752.666 7	3
RT protocol	90@42C, then 10x (2@55C- 2@42C)	90@42C, then 10x (2@60C- 2@42C)	2.69E- 01	4.00E- 01	105.92 5	114.77 5	2.23E- 01	4.00E- 01	2227.66 67	2172.333 3	3
RT	90@42C,	90@42C,	-	-	286.2	285.3	-	-	1732	1610	1

protocol	then 10x (2@60C- 2@42C)	then 15x (2@50C- 2@42C)																		
RT protocol	90@42C, then 10x (2@60C- 2@42C)	90@42C, then 20x (2@50C- 2@42C)	-	286.2	259.27 5	-	-	1732	1525											1
RT protocol	90@42C, then 10x (2@60C- 2@42C)	90@42C, then 5x (2@50C- 2@42C)	-	286.2	306.07 5	-	-	1732	1706											1
RT protocol	90@42C, then 15x (2@50C- 2@42C)	90@42C, then 20x (2@50C- 2@42C)	2.94E- 01	278.13 75	237.22 5	3.33E- 01	1.47E- 01	1644.5	1467	3.33E- 01	1.47E- 01	1644.5	1467							2
RT protocol	90@42C, then 15x (2@50C- 2@42C)	90@42C, then 5x (2@50C- 2@42C)	7.35E- 01	300.3	291.25	1.00E +00	2.30E- 02	1639.66 67	1752.666 7	1.00E- 01	2.30E- 02	1639.66 67	1752.666 7							3
RT protocol	90@42C, then 20x (2@50C- 2@42C)	90@42C, then 5x (2@50C- 2@42C)	1.90E- 01	237.22 5	297.71 25	3.33E- 01	8.13E- 02	1467	1738.5	3.33E- 01	8.13E- 02	1467	1738.5							2
RT protocol	90@50C	90@55C	3.50E- 03	62.595	12.24	7.94E- 03	2.09E- 01	2146.2	2051	1.51E- 01	2.09E- 01	2146.2	2051							5
RT protocol	90@50C	90@60C	-	164.4	6	-	-	1858	1933	-	-	1858	1933							1
PCR enzyme	Advantage 2 Pol.	KAPA HiFi HS	6.44E- 01	186.63 75	171.01 25	7.55E- 01	5.18E- 01	1929.41 67	2029.5	5.90E- 01	5.18E- 01	1929.41 67	2029.5							12
PCR enzyme	Advantage 2 Pol.	Phusion HS	4.33E- 01	212.36 25	182.68 13	4.86E- 01	8.11E- 01	1701	1722.75	3.43E- 01	8.11E- 01	1701	1722.75							4
PCR enzyme	Advantage 2 Pol.	Q5 NEB	5.85E- 02	212.36 25	133.85 62	2.86E- 02	6.29E- 01	1701	1743.75	3.43E- 01	6.29E- 01	1701	1743.75							4
PCR enzyme	KAPA HiFi HS	Phusion HS	2.60E- 01	223.72 5	182.68 13	3.43E- 01	7.49E- 01	1702	1722.75	8.86E- 01	7.49E- 01	1702	1722.75							4
PCR enzyme	KAPA HiFi HS	Q5 NEB	2.99E- 02	223.72 5	133.85 62	2.86E- 02	4.93E- 01	1702	1743.75	8.86E- 01	4.93E- 01	1702	1743.75							4
PCR enzyme	Phusion HS	Q5 NEB	1.25E- 01	182.68 13	133.85 62	1.14E- 01	5.99E- 01	1722.75	1743.75	8.86E- 01	5.99E- 01	1722.75	1743.75							4
purificati on.	0	yes	6.77E- 01	186.85	203.22 5	6.05E- 01	3.44E- 01	2022.22 22	1875.444 4	3.87E- 01	3.44E- 01	2022.22 22	1875.444 4							9

dNTPs added in the beginning	0	yes	4.55E-03	1.37E-02	193.6781	134.3812		3.44E-04	5.55E-05	1709.4167	2008.0833	24
other additives	0	0.816M 1,2 propandiol	6.89E-04	1.00E-01	291.125	103.95		3.50E-03	1.00E-01	1722.3333	1093	3
other additives	0	1.075M ethylene glycol	1.82E-01	3.33E-01	299.025	178.4625		9.93E-02	3.33E-01	1707	1421.5	2
other additives	0	3 mM MnCl2	1.10E-01	3.33E-01	56.535	28.095		1.13E-01	3.33E-01	1930	1479.5	2
other additives	0	6 mM MnCl2	1.05E-01	3.33E-01	56.535	16.95		1.03E-01	3.33E-01	1930	1213	2
other additives	0.3M trehalose	0.6M trehalose	8.18E-01	6.86E-01	77.0625	72.3563		4.45E-01	4.86E-01	1679	1634.25	4
other additives	0.816M 1,2 propandiol	1.075M ethylene glycol	2.82E-01	3.33E-01	104.4375	178.4625		3.99E-02	3.33E-01	1095	1421.5	2
other additives	3 mM MnCl2	6 mM MnCl2	1.45E-01	3.33E-01	28.095	16.95		1.46E-01	3.33E-01	1479.5	1213	2

TABLE S2. List of all template switching oligonucleotides tested.

TSO name	Sequence (5' → 3')	5'-end blocking groups	5'-end modifications	3'-end modifications	3'-end blocking groups
2OMe	AAGCAGTGGTATCAACGCAGAGT ACrGrGrGmG	-	-	3 Riboguanosines + 1 Guanosine	2O'-Methyl
C6	AAGCAGTGGTATCAACGCAGAGT ACATrGrGrG	-	-	3 Riboguanosines	Aminolink C6
ddC	AAGCAGTGGTATCAACGCAGAGT ACrGrGrGrGddC	-	-	4 Riboguanosines + 1 Dideoxycytosine	-
dGCG	AAGCAGTGGTATCAACGCAGAGT ACGCGGG	-	-	1 Guanosine + 1 Cytosine + 3 Guanosines	-
dGCG	AAGCAGTGGTATCAACGCAGAGT ACGCGGG	-	-	1 Guanosine + 1 Cytosine + 3 Guanosines	Phosphate
ISO	iGiCiGAAGCAGTGGTATCAACGCA GAGTACrGrCrGrG	Methyl C5	isoGuanosine- isoCytosine- isoGuanosine	1 Riboguanosine + 1 Ribocytosine + 3 Riboguanosines	Phosphate
rGrG+	AAGCAGTGGTATCAACGCAGAGT ACrGrG+G	-	-	2 Riboguanosines + 1 LNA-modified Guanosine	-
rGrG+	AAGCAGTGGTATCAACGCAGAGT ACrGrG+N	-	-	2 Riboguanosines + 1 LNA-modified nucleotide (any)	-
+G+G	AAGCAGTGGTATCAACGCAGAGT AC+G+G+G	-	-	3 LNA-modified Guanosines	-
rG+G	AAGCAGTGGTATCAACGCAGAGT ACrG+G+G	-	-	2 LNA-modified Guanosines	-
rGrGr	AAGCAGTGGTATCAACGCAGAGT ACATrGrGrG	-	-	3 Riboguanosines	-
rG3p	AAGCAGTGGTATCAACGCAGAGT ACATrGrGrGp	-	-	3 Riboguanosines	Phosphate
rG5	AAGCAGTGGTATCAACGCAGAGT ACrGrGrGrGrG	-	-	5 Riboguanosines	-

sample	cell type	species	conc (ng/ul)	avg size (bp)	TSO	amount TSO (ul) or 10uM solution in	RT enzyme	oligo dT	MgCl2 (mM)	betaine (M)	purification after RT?	PCR enzyme	PCR rxn vol (ul)	notes
HEK_2	HEK293 T	<i>H.sapiens</i>	10.778	1099	rGrG+G	1	SSRTII	SMARTer dT30VN	13	1	yes	Advantage	50	Could be a cell aggregate
HEK_3	HEK293 T	<i>H.sapiens</i>	9.596	1142	rGrG+G	1	SSRTII	SMARTer dT30VN	14	1	yes	Advantage	50	Could be a cell aggregate
HEK_4	HEK293 T	<i>H.sapiens</i>	1.160	1098	rGrG+G	1	SSRTII	SMARTer dT30VN	15	1	yes	Advantage	50	
HEK_5	HEK293 T	<i>H.sapiens</i>	0.590	1203	rGrG+G	1	SSRTII	SMARTer dT30VN	16	1	yes	Advantage	50	
HEK_6	HEK293 T	<i>H.sapiens</i>	2.210	1175	rGrG+G	1	SSRTII	SMARTer dT30VN	17	1	yes	Advantage	50	
HEK_7	HEK293 T	<i>H.sapiens</i>	0.410	1136	rGrG+G	1	SSRTII	SMARTer dT30VN	18	1	yes	Advantage	50	
HEK_8	HEK293 T	<i>H.sapiens</i>	4.184	1146	rGrG+G	1	SSRTII	SMARTer dT30VN	19	1	yes	Advantage	50	Could be a cell aggregate
HEK_9	HEK293 T	<i>H.sapiens</i>	0.840	1141	rGrG+G	1	SSRTII	SMARTer dT30VN	20	1	yes	Advantage	50	
HEK_10	HEK293 T	<i>H.sapiens</i>	1.130	1088	rGrG+G	1	SSRTII	SMARTer dT30VN	21	1	yes	Advantage	50	
HEK_12	HEK293 T	<i>H.sapiens</i>	9.095	1106	rGrG+G	1	SSRTII	SMARTer dT30VN	23	1	yes	Advantage	50	Could be a cell aggregate
HEK_13	HEK293 T	<i>H.sapiens</i>	0.490	1017	rGrG+G	1	SSRTII	SMARTer dT30VN	24	1	yes	Advantage	50	
HEK_14	HEK293 T	<i>H.sapiens</i>	3.640	1046	rGrG+G	1	SSRTII	SMARTer dT30VN	25	1	yes	Advantage	50	Could be a cell aggregate
HEK_16	HEK293 T	<i>H.sapiens</i>	17.225	1072	rGrG+G	1	SSRTII	SMARTer dT30VN	27	1	yes	Advantage	50	Could be a cell aggregate
HEK_SMRT_1	HEK293 T	<i>H.sapiens</i>	0.760	1429	SMARTer Oligo IIA	1	SMARTscribe	SMARTer dT30VN	6	1	yes	Advantage	50	
HEK_SMRT_2	HEK293 T	<i>H.sapiens</i>	2.304	1411	SMARTer Oligo IIA	1	SMARTscribe	SMARTer dT30VN	6	1	yes	Advantage	50	Could be a cell aggregate
HEK_SMRT_3	HEK293 T	<i>H.sapiens</i>	1.544	1424	SMARTer Oligo IIA	1	SMARTscribe	SMARTer dT30VN	6	1	yes	Advantage	50	
HEK_SMRT_4	HEK293 T	<i>H.sapiens</i>	0.422	1457	SMARTer Oligo IIA	1	SMARTscribe	SMARTer dT30VN	6	1	yes	Advantage	50	
HEK_18	HEK293 T	<i>H.sapiens</i>	0.208	1384	rGrGrG	1	SSRTII	SMARTer dT30VN	12	1	yes	Advantage	50	40u SSRT II

HEK_19	HEK293 T	<i>H.sapiens</i>	0.512	154 1	rGrGrG	1	SSRTII	SMARTer dT30VN	12	1	ye s	Advantage	50	40u SSRT II
HEK_20	HEK293 T	<i>H.sapiens</i>	0.550	161 4	rGrGrG	1	SSRTII	SMARTer dT30VN	12	1	ye s	Advantage	50	40u SSRT II
HEK_21	HEK293 T	<i>H.sapiens</i>	0.240	149 2	rGrGrG	1	SSRTII	SMARTer dT30VN	12	1	ye s	Advantage	50	40u SSRT II
HEK_22	HEK293 T	<i>H.sapiens</i>	0.957	122 2	rGrGrG	1	SSRTII	SMARTer dT30VN	12	1	ye s	Advantage	50	40u SSRT II
HEK_23	HEK293 T	<i>H.sapiens</i>	0.872	139 2	rGrGrG	1	SSRTII	SMARTer dT30VN	12	1	ye s	Advantage	50	40u SSRT II
HEK_24	HEK293 T	<i>H.sapiens</i>	0.315	132 6	rGrGrG	1	SSRTII	SMARTer dT30VN	12	1	ye s	Advantage	50	40u SSRT II
HEK_25	HEK293 T	<i>H.sapiens</i>	0.462	147 4	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	ye s	Advantage	50	
HEK_26	HEK293 T	<i>H.sapiens</i>	0.248	144 1	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	ye s	Advantage	50	
HEK_27	HEK293 T	<i>H.sapiens</i>	0.244	148 7	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	ye s	Advantage	50	
HEK_28	HEK293 T	<i>H.sapiens</i>	0.262	149 6	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	ye s	Advantage	50	
HEK_29	HEK293 T	<i>H.sapiens</i>	0.761	140 2	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	ye s	Advantage	50	
HEK_31	HEK293 T	<i>H.sapiens</i>	0.594	158 3	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	ye s	Advantage	50	
HEK_32	HEK293 T	<i>H.sapiens</i>	0.457	134 7	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	ye s	Advantage	50	
HEK_33	HEK293 T	<i>H.sapiens</i>	0.473	150 7	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	ye s	Advantage	50	
HEK_34	HEK293 T	<i>H.sapiens</i>	0.315	150 1	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	ye s	Advantage	50	
HEK_35	HEK293 T	<i>H.sapiens</i>	0.744	191 7	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	Advantage	50	
HEK_37	HEK293 T	<i>H.sapiens</i>	1.531	162 6	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	Advantage	50	
HEK_38	HEK293 T	<i>H.sapiens</i>	0.344	187 0	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	Advantage	50	
HEK_39	HEK293 T	<i>H.sapiens</i>	0.453	196 8	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	Advantage	50	
HEK_40	HEK293 T	<i>H.sapiens</i>	0.580	195 8	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	Advantage	50	
HEK_41	HEK293 T	<i>H.sapiens</i>	0.382	120 2	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	ye s	Advantage	50	
HEK_42	HEK293 T	<i>H.sapiens</i>	0.392	128 8	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	ye s	Advantage	50	
HEK_43	HEK293 T	<i>H.sapiens</i>	0.844	131 9	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	ye s	Advantage	50	
HEK_44	HEK293 T	<i>H.sapiens</i>	0.582	145 2	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	ye s	Advantage	50	
HEK_45	HEK293 T	<i>H.sapiens</i>	0.682	131 2	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	ye s	Advantage	50	
HEK_46	HEK293	<i>H.sapiens</i>	0.692	141	rGrG+G	1	SSRTII	SMARTer	12	1	ye s	Advantage	50	

HEK_47	HEK293 T	<i>H.sapiens</i>	0.716	133	1	rGrGrG	1	SSRTII	SMARTer dT30VN	12	1	ye s	Advantage	50	
HEK_48	HEK293 T	<i>H.sapiens</i>	0.578	139	1	rGrGrG	1	SSRTII	SMARTer dT30VN	12	1	ye s	Advantage	50	
HEK_49	HEK293 T	<i>H.sapiens</i>	2.681	144	0	rGrGrG	1	SSRTII	SMARTer dT30VN	12	1	ye s	Advantage	50	Could be a cell aggregate
HEK_50	HEK293 T	<i>H.sapiens</i>	1.158	150	7	rGrGrG	1	SSRTII	SMARTer dT30VN	12	1	ye s	Advantage	50	
HEK_51	HEK293 T	<i>H.sapiens</i>	0.710	138	9	rGrGrG	1	SSRTII	SMARTer dT30VN	12	1	ye s	Advantage	50	
HEK_52	HEK293 T	<i>H.sapiens</i>	1.050	146	3	rGrGrG	1	SSRTII	SMARTer dT30VN	12	1	ye s	Advantage	50	
HEK_53	HEK293 T	<i>H.sapiens</i>	0.714	138	4	rGrGrG	1	SSRTII	SMARTer dT30VN	12	1	ye s	Advantage	50	
HEK_54	HEK293 T	<i>H.sapiens</i>	0.637	150	9	rGrGrG	1	SSRTII	SMARTer dT30VN	12	1	ye s	Advantage	50	
HEK_55	HEK293 T	<i>H.sapiens</i>	1.338	187	3	rGrGrG	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
HEK_56	HEK293 T	<i>H.sapiens</i>	1.258	184	6	rGrGrG	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
HEK_57	HEK293 T	<i>H.sapiens</i>	3.012	179	8	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
HEK_58	HEK293 T	<i>H.sapiens</i>	1.763	173	4	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
HEK_59	HEK293 T	<i>H.sapiens</i>	2.837	183	3	rGrGrG	1	SSRTII	SMARTer dT30VN	12	1	-	Advantage	50	
HEK_60	HEK293 T	<i>H.sapiens</i>	1.889	175	8	rGrGrG	1	SSRTII	SMARTer dT30VN	12	1	-	Advantage	50	
HEK_61	HEK293 T	<i>H.sapiens</i>	3.733	184	1	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	Advantage	50	
HEK_62	HEK293 T	<i>H.sapiens</i>	2.430	184	9	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	Advantage	50	
HEK_63	HEK293 T	<i>H.sapiens</i>	0.758	184	5	rGrGrG	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
HEK_64	HEK293 T	<i>H.sapiens</i>	0.948	190	7	rGrGrG	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
HEK_65	HEK293 T	<i>H.sapiens</i>	1.364	183	7	rGrGrG	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
HEK_66	HEK293 T	<i>H.sapiens</i>	1.897	182	1	rGrGrG	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
HEK_67	HEK293 T	<i>H.sapiens</i>	2.721	174	6	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
HEK_68	HEK293 T	<i>H.sapiens</i>	5.123	169	9	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	Could be a cell aggregate
HEK_69	HEK293 T	<i>H.sapiens</i>	2.499	189	2	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
HEK_70	HEK293 T	<i>H.sapiens</i>	1.632	171	5	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
HEK_71	HEK293 T	<i>H.sapiens</i>	2.614	143	8	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	ye s	Advantage	50	

HEK_72	HEK293 T	<i>H.sapiens</i>	1.343	153 5	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	ye s	Advantage	50	
HEK_73	HEK293 T	<i>H.sapiens</i>	2.618	157 0	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	ye s	Advantage	50	
HEK_74	HEK293 T	<i>H.sapiens</i>	2.037	161 1	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	ye s	Advantage	50	
HEK_75	HEK293 T	<i>H.sapiens</i>	1.862	149 4	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	ye s	Advantage	50	
HEK_76	HEK293 T	<i>H.sapiens</i>	0.560	156 7	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	ye s	Advantage	50	
HEK_77	HEK293 T	<i>H.sapiens</i>	1.609	160 4	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	ye s	Advantage	50	
HEK_78	HEK293 T	<i>H.sapiens</i>	0.748	139 2	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	ye s	Advantage	50	
HEK_79	HEK293 T	<i>H.sapiens</i>	2.876	209 3	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	Advantage	50	
HEK_80	HEK293 T	<i>H.sapiens</i>	6.017	197 5	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	Advantage	50	Could be a cell aggregate
HEK_81	HEK293 T	<i>H.sapiens</i>	5.110	183 4	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	Advantage	50	Could be a cell aggregate
HEK_82	HEK293 T	<i>H.sapiens</i>	2.659	191 2	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
HEK_83	HEK293 T	<i>H.sapiens</i>	3.820	184 8	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	Could be a cell aggregate
HEK_84	HEK293 T	<i>H.sapiens</i>	1.875	194 1	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
HEK_85	HEK293 T	<i>H.sapiens</i>	4.477	200 3	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	Could be a cell aggregate
HEK_86	HEK293 T	<i>H.sapiens</i>	4.162	178 4	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	Advantage	50	Could be a cell aggregate
HEK_87	HEK293 T	<i>H.sapiens</i>	0.539	196 4	rGrG+G	1	Maxima H minus	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
HEK_88	HEK293 T	<i>H.sapiens</i>	0.526	186 0	rGrG+G	1	Maxima H minus	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
HEK_89	HEK293 T	<i>H.sapiens</i>	0.288	188 1	rGrG+G	1	Maxima H minus	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
HEK_90	HEK293 T	<i>H.sapiens</i>	0.650	156 0	rGrG+G	1	Maxima H minus	SMARTer dT30	12	1	-	KAPA HiFi HS	50	
HEK_91	HEK293 T	<i>H.sapiens</i>	0.341	185 2	rGrG+G	1	Maxima H minus	SMARTer dT30	12	1	-	KAPA HiFi HS	50	
HEK_92	HEK293 T	<i>H.sapiens</i>	0.354	178 3	rGrG+G	1	Maxima H minus	SMARTer dT30	12	1	-	KAPA HiFi HS	50	
HEK_93	HEK293 T	<i>H.sapiens</i>	1.520	193 9	rGrG+N	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
HEK_94	HEK293 T	<i>H.sapiens</i>	1.722	174 0	rGrG+N	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
HEK_95	HEK293 T	<i>H.sapiens</i>	0.485	174 0	rGrG+N	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
HEK_96	HEK293 T	<i>H.sapiens</i>	1.090	183 3	rGrG+N	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
HEK_97	HEK293	<i>H.sapiens</i>	2.856	173	rGrG+N	2	SSRTII	SMARTer	12	1	-	KAPA HiFi	50	

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HEK_124	HEK293 T	<i>H.sapiens</i>	2.301	161 7	rGrG+N	2	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
HEK_135	HEK293 T	<i>H.sapiens</i>	0.784	181 0	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	2x dCTP
HEK_136	HEK293 T	<i>H.sapiens</i>	1.311	199 0	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	2x dCTP
HEK_137	HEK293 T	<i>H.sapiens</i>	0.895	182 9	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	2x dCTP
HEK_138	HEK293 T	<i>H.sapiens</i>	1.318	194 8	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	2x dCTP
HEK_139	HEK293 T	<i>H.sapiens</i>	1.853	170 4	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	2x dCTP
HEK_140	HEK293 T	<i>H.sapiens</i>	4.009	183 3	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	2x dCTP (NOT SINGLE CELL)
HEK_141	HEK293 T	<i>H.sapiens</i>	5.230	182 0	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	Could be a cell aggregate
HEK_144	HEK293 T	<i>H.sapiens</i>	4.803	176 6	rGrG+G	2	SSRTII	SMARTer dT30VN	12	1	-	Advantage	50	Could be a cell aggregate
HEK_145	HEK293 T	<i>H.sapiens</i>	2.166	161 9	rGrG+G	2	SSRTII	SMARTer dT30VN	12	1	-	Advantage	50	
HEK_146	HEK293 T	<i>H.sapiens</i>	2.283	155 2	rGrG+G	2	SSRTII	SMARTer dT30VN	12	1	-	Advantage	50	
HEK_147	HEK293 T	<i>H.sapiens</i>	2.099	131 6	rGrG+G	2	SSRTII	SMARTer dT30VN	12	1	-	Advantage	50	
HEK_148	HEK293 T	<i>H.sapiens</i>	2.191	133 9	rGrG+G	2	SSRTII	SMARTer dT30VN	12	1	-	Advantage	50	HEDGEHOG PATTERN
HEK_149	HEK293 T	<i>H.sapiens</i>	1.560	142 6	rGrG+G	2	SSRTII	SMARTer dT30VN	12	1	-	Advantage	50	
HEK_150	HEK293 T	<i>H.sapiens</i>	2.830	150 8	rGrG+G	2	SSRTII	SMARTer dT30VN	12	1	-	Advantage	50	
HEK_151	HEK293 T	<i>H.sapiens</i>	2.220	122 0	rGrG+G	2	SSRTII	SMARTer dT30	12	1	-	Advantage	50	
HEK_152	HEK293 T	<i>H.sapiens</i>	2.654	153 4	rGrG+G	2	SSRTII	SMARTer dT30	12	1	-	Advantage	50	HEDGEHOG PATTERN
HEK_153	HEK293 T	<i>H.sapiens</i>	2.039	165 5	rGrG+G	2	SSRTII	SMARTer dT30	12	1	-	Advantage	50	
HEK_154	HEK293 T	<i>H.sapiens</i>	3.001	183 7	rGrG+G	2	SSRTII	SMARTer dT30	12	1	-	Advantage	50	
HEK_155	HEK293 T	<i>H.sapiens</i>	1.378	184 6	rGrG+G	2	SSRTII	SMARTer dT30	12	1	-	Advantage	50	
HEK_156	HEK293 T	<i>H.sapiens</i>	1.065	185 3	rGrG+G	2	SSRTII	SMARTer dT30	12	1	-	Advantage	50	
HEK_157	HEK293 T	<i>H.sapiens</i>	1.621	177 2	rGrG+G	2	SSRTII	SMARTer dT30	12	1	-	Advantage	50	
HEK_158	HEK293 T	<i>H.sapiens</i>	2.418	170 6	rGrG+G	2	SSRTII	SMARTer dT30	12	1	-	Advantage	50	
HEK_SMRT_5	HEK293 T	<i>H.sapiens</i>	0.128	149 5	SMARTer Oligo IIA	1	SMARTscribe	SMARTer dT30VN	6	1	yes	Advantage	50	
HEK_SMRT_6	HEK293 T	<i>H.sapiens</i>	0.042	146 9	SMARTer Oligo IIA	1	SMARTscribe	SMARTer dT30VN	6	1	yes	Advantage	50	
HEK_SMRT	HEK293	<i>H.sapiens</i>	0.207	135	SMARTer Oligo	1	SMARTscribe	SMARTer	6	1	yes	Advantage	50	

7	T			9	IIA		dt30VN		s		
HEK_SMRT_8	HEK293 T	<i>H.sapiens</i>	0.246	1340	SMARTer Oligo IIA	1	SMARTscribe	6	ye	Advantage	50
HEK_SMRT_9	HEK293 T	<i>H.sapiens</i>	0.152	1455	SMARTer Oligo IIA	1	SMARTscribe	6	ye	Advantage	50
HEK_SMRT_10	HEK293 T	<i>H.sapiens</i>	0.256	1228	SMARTer Oligo IIA	1	SMARTscribe	6	ye	Advantage	50
HEK_SMRT_11	HEK293 T	<i>H.sapiens</i>	0.072	1238	SMARTer Oligo IIA	1	SMARTscribe	6	ye	Advantage	50
HEK_SMRT_12	HEK293 T	<i>H.sapiens</i>	0.256	1292	SMARTer Oligo IIA	1	SMARTscribe	6	ye	Advantage	50
C_1	C2C12	<i>M.musculus</i>	3.003	1696	rGrG+G	1	SSRTII	12	-	KAPA HiFi HS	25
C_2	C2C12	<i>M.musculus</i>	1.110	1772	rGrG+G	1	SSRTII	12	-	KAPA HiFi HS	25
C_3	C2C12	<i>M.musculus</i>	1.157	1660	rGrG+G	1	SSRTII	12	-	KAPA HiFi HS	25
C_4	C2C12	<i>M.musculus</i>	1.965	1674	rGrG+G	1	SSRTII	12	-	KAPA HiFi HS	25
C_5	C2C12	<i>M.musculus</i>	2.884	1708	rGrG+G	1	SSRTII	12	-	KAPA HiFi HS	25
C_6	C2C12	<i>M.musculus</i>	2.325	1646	rGrG+G	1	SSRTII	12	-	KAPA HiFi HS	25
C_7	C2C12	<i>M.musculus</i>	1.941	1577	rGrG+G	1	SSRTII	12	-	KAPA HiFi HS	25
C_8	C2C12	<i>M.musculus</i>	2.248	1666	rGrG+G	1	SSRTII	12	-	KAPA HiFi HS	25
C_9	C2C12	<i>M.musculus</i>	1.379	1614	rGrG+G	1	SSRTII	12	-	KAPA HiFi HS	50
C_10	C2C12	<i>M.musculus</i>	1.832	1809	rGrG+G	1	SSRTII	12	-	KAPA HiFi HS	50
C_11	C2C12	<i>M.musculus</i>	3.644	1735	rGrG+G	1	SSRTII	12	-	KAPA HiFi HS	50
C_12	C2C12	<i>M.musculus</i>	1.245	1735	rGrG+G	1	SSRTII	12	-	KAPA HiFi HS	50
C_13	C2C12	<i>M.musculus</i>	1.913	1896	rGrG+G	1	SSRTII	12	-	KAPA HiFi HS	50
C_14	C2C12	<i>M.musculus</i>	1.703	1836	rGrG+G	1	SSRTII	12	-	KAPA HiFi HS	50
C_15	C2C12	<i>M.musculus</i>	1.588	1937	rGrG+G	1	SSRTII	12	-	KAPA HiFi HS	50
C_16	C2C12	<i>M.musculus</i>	1.730	1937	rGrG+G	1	SSRTII	12	-	KAPA HiFi HS	50
MEF_1	MEF	<i>M.musculus</i>	0.999	1397	rGrG+G	1	SSRTII	12	-	KAPA HiFi HS	25
MEF_2	MEF	<i>M.musculus</i>	2.862	1710	rGrG+G	1	SSRTII	12	-	KAPA HiFi HS	25
MEF_3	MEF	<i>M.musculus</i>	1.571	1577	rGrG+G	1	SSRTII	12	-	KAPA HiFi HS	25
MEF_4	MEF	<i>M.musculus</i>	1.120	1489	rGrG+G	1	SSRTII	12	-	KAPA HiFi HS	25

MEF_5	MEF	M.muscul us	2.121	167 2	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	25	
MEF_6	MEF	M.muscul us	5.025	161 0	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	25	Could be a cell aggregate
MEF_7	MEF	M.muscul us	5.874	106 5	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	25	Could be a cell aggregate
MEF_8	MEF	M.muscul us	1.272	?	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	25	
MEF_9	MEF	M.muscul us	1.650	?	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	25	
MEF_10	MEF	M.muscul us	2.071	?	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	25	
MEF_11	MEF	M.muscul us	0.755	?	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	25	
MEF_12	MEF	M.muscul us	7.528	?	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	25	Could be a cell aggregate
MEF_13	MEF	M.muscul us	2.271	?	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	25	
MEF_14	MEF	M.muscul us	2.327	?	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	25	
MEF_15	MEF	M.muscul us	3.149	?	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	25	Could be a cell aggregate
MEF_16	MEF	M.muscul us	2.045	?	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	25	
MEF_17	MEF	M.muscul us	0.497	180 3	rGrGrG	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
MEF_18	MEF	M.muscul us	0.427	187 9	rGrGrG	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
MEF_19	MEF	M.muscul us	0.406	187 3	rGrGrG	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
MEF_20	MEF	M.muscul us	0.680	198 4	rGrGrG	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
MEF_21	MEF	M.muscul us	0.429	173 8	rGrGrG	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
MEF_22	MEF	M.muscul us	0.634	208 9	rGrGrG	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
MEF_23	MEF	M.muscul us	0.722	201 9	rGrGrG	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
MEF_24	MEF	M.muscul us	0.397	188 1	rGrGrG	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
MEF_25	MEF	M.muscul us	0.981	200 7	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	Advantage	50	
MEF_26	MEF	M.muscul us	0.626	182 7	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	Advantage	50	
MEF_28	MEF	M.muscul us	0.656	197 9	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	Advantage	50	
MEF_29	MEF	M.muscul us	1.356	214 3	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	Advantage	50	
MEF_30	MEF	M.muscul us	1.242	213 7	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	Advantage	50	
MEF_31	MEF	M.muscul	2.240	201	rGrG+G	1	SSRTII	SMARTer	12	1	-	Advantage	50	

MEF_32	MEF	<i>M.muscul</i> <i>us</i>	0.781	181 7	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	Advantage	50	
MEF_33	MEF	<i>M.muscul</i> <i>us</i>	0.603	178 1	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
MEF_34	MEF	<i>M.muscul</i> <i>us</i>	5.014	189 4	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	Could be a cell aggregate
MEF_35	MEF	<i>M.muscul</i> <i>us</i>	1.651	206 4	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
MEF_36	MEF	<i>M.muscul</i> <i>us</i>	1.017	177 3	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
MEF_37	MEF	<i>M.muscul</i> <i>us</i>	1.119	178 3	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
MEF_38	MEF	<i>M.muscul</i> <i>us</i>	0.681	165 9	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
MEF_39	MEF	<i>M.muscul</i> <i>us</i>	1.444	146 9	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
MEF_40	MEF	<i>M.muscul</i> <i>us</i>	0.845	140 1	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
SMRT_1	C2C12	<i>M.muscul</i> <i>us</i>	0.063	117 0	SMARTer Oligo IIA	1	SMARTscribe	SMARTer dT30VN	12	1	ye s	Advantage	50	
SMRT_2	C2C12	<i>M.muscul</i> <i>us</i>	0.165	112 7	SMARTer Oligo IIA	1	SMARTscribe	SMARTer dT30VN	12	1	ye s	Advantage	50	
SMRT_3	C2C12	<i>M.muscul</i> <i>us</i>	0.130	116 9	SMARTer Oligo IIA	1	SMARTscribe	SMARTer dT30VN	12	1	ye s	Advantage	50	
SMRT_4	C2C12	<i>M.muscul</i> <i>us</i>	0.091	132 5	SMARTer Oligo IIA	1	SMARTscribe	SMARTer dT30VN	12	1	ye s	Advantage	50	
SMRT_5	C2C12	<i>M.muscul</i> <i>us</i>	0.036	128 2	SMARTer Oligo IIA	1	SMARTscribe	SMARTer dT30VN	12	1	ye s	Advantage	50	
SMRT_6	C2C12	<i>M.muscul</i> <i>us</i>	0.135	137 3	SMARTer Oligo IIA	1	SMARTscribe	SMARTer dT30VN	12	1	ye s	Advantage	50	
SMRT_7	C2C12	<i>M.muscul</i> <i>us</i>	0.161	152 5	SMARTer Oligo IIA	1	SMARTscribe	SMARTer dT30VN	12	1	ye s	Advantage	50	
SMRT_8	C2C12	<i>M.muscul</i> <i>us</i>	0.178	131 4	SMARTer Oligo IIA	1	SMARTscribe	SMARTer dT30VN	12	1	ye s	Advantage	50	
SMRT_9	C2C12	<i>M.muscul</i> <i>us</i>	0.036	136 3	SMARTer Oligo IIA	1	SMARTscribe	SMARTer dT30VN	12	1	ye s	Advantage	50	
SMRT_10	C2C12	<i>M.muscul</i> <i>us</i>	0.129	126 1	SMARTer Oligo IIA	1	SMARTscribe	SMARTer dT30VN	12	1	ye s	Advantage	50	
SMRT_11	C2C12	<i>M.muscul</i> <i>us</i>	0.080	123 4	SMARTer Oligo IIA	1	SMARTscribe	SMARTer dT30VN	12	1	ye s	Advantage	50	
SMRT_12	C2C12	<i>M.muscul</i> <i>us</i>	0.111	144 7	SMARTer Oligo IIA	1	SMARTscribe	SMARTer dT30VN	12	1	ye s	Advantage	50	
SMRT_14	C2C12	<i>M.muscul</i> <i>us</i>	0.081	140 8	SMARTer Oligo IIA	1	SMARTscribe	SMARTer dT30VN	12	1	ye s	Advantage	50	
SMRT_16	C2C12	<i>M.muscul</i> <i>us</i>	0.074	128 0	SMARTer Oligo IIA	1	SMARTscribe	SMARTer dT30VN	12	1	ye s	Advantage	50	
BC_1	B-cells	<i>H.sapiens</i>	0.826	165 7	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	

BC_3	B-cells	<i>H. sapiens</i>	1.091	165 9	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
BC_4	B-cells	<i>H. sapiens</i>	0.401	164 9	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
BC_5	B-cells	<i>H. sapiens</i>	0.501	154 7	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
BC_6	B-cells	<i>H. sapiens</i>	0.573	153 4	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
BC_7	B-cells	<i>H. sapiens</i>	0.328	134 3	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
BC_8	B-cells	<i>H. sapiens</i>	0.661	160 7	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
BC_9	B-cells	<i>H. sapiens</i>	0.500	160 5	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
BC_10	B-cells	<i>H. sapiens</i>	0.840	178 2	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
BC_11	B-cells	<i>H. sapiens</i>	0.829	181 3	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
BC_12	B-cells	<i>H. sapiens</i>	0.648	164 2	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
BC_13	B-cells	<i>H. sapiens</i>	0.297	181 5	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
BC_15	B-cells	<i>H. sapiens</i>	0.982	182 5	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
HEK_159	HEK293 T	<i>H. sapiens</i>	3.057	146 4	rGrG+G	1	SSRTII	SMARTer dT30VN	15	1	-	KAPA HiFi HS	25	Could be a cell aggregate
HEK_160	HEK293 T	<i>H. sapiens</i>	3.506	179 9	rGrG+G	1	SSRTII	SMARTer dT30VN	15	1	-	KAPA HiFi HS	25	Could be a cell aggregate
HEK_161	HEK293 T	<i>H. sapiens</i>	2.027	161 1	rGrG+G	1	SSRTII	SMARTer dT30VN	15	1	-	KAPA HiFi HS	25	
HEK_162	HEK293 T	<i>H. sapiens</i>	2.513	171 4	rGrG+G	1	SSRTII	SMARTer dT30VN	15	1	-	KAPA HiFi HS	25	
HEK_163	HEK293 T	<i>H. sapiens</i>	0.558	909	rGrG+G	1	SSRTII	SMARTer dT30VN	20	1	-	KAPA HiFi HS	25	
HEK_164	HEK293 T	<i>H. sapiens</i>	1.490	139 7	rGrG+G	1	SSRTII	SMARTer dT30VN	20	1	-	KAPA HiFi HS	25	
HEK_165	HEK293 T	<i>H. sapiens</i>	0.606	133 4	rGrG+G	1	SSRTII	SMARTer dT30VN	20	1	-	KAPA HiFi HS	25	
HEK_166	HEK293 T	<i>H. sapiens</i>	1.203	151 5	rGrG+G	1	SSRTII	SMARTer dT30VN	20	1	-	KAPA HiFi HS	25	
BC_17	B-cells	<i>H. sapiens</i>	0.393	160 3	rGrG+G	1	SSRTII	SMARTer dT30VN	12	-	-	KAPA HiFi HS	50	
BC_19	B-cells	<i>H. sapiens</i>	0.216	173 0	rGrG+G	1	SSRTII	SMARTer dT30VN	12	-	-	KAPA HiFi HS	50	
BC_20	B-cells	<i>H. sapiens</i>	0.074	113 1	rGrG+G	1	SSRTII	SMARTer dT30VN	12	-	-	KAPA HiFi HS	50	
BC_21	B-cells	<i>H. sapiens</i>	0.126	144 5	rGrG+G	1	SSRTII	SMARTer dT30VN	12	-	-	KAPA HiFi HS	50	
BC_22	B-cells	<i>H. sapiens</i>	0.325	170 2	rGrG+G	1	SSRTII	SMARTer dT30VN	12	-	-	KAPA HiFi HS	50	
BC_23	B-cells	<i>H. sapiens</i>	0.183	175	rGrG+G	1	SSRTII	SMARTer	12	-	-	KAPA HiFi	50	

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Table S4. Detailing variants of the Smart-seq2 protocol

Protocol name	TSO	amount TSO (ul)	Bead purification before pre-amplification?	DNA Polymerase
Smart-seq2	rGrG+G	1 ul (10 uM)	no	KAPA HiFi HotStart ReadyMix
Variant 1	rGrG+G	2 ul (10 uM)	no	KAPA HiFi HotStart ReadyMix
Variant 2	rGrG+N	2 ul (10 uM)	no	KAPA HiFi HotStart ReadyMix
Variant 3	rGrG+G	1 ul (10 uM)	no	Advantage 2
Variant 4	rGrGrG	1 ul (10 uM)	no	KAPA HiFi HotStart ReadyMix
SMARTer	SMARTer Oligo IIA	1 ul (12 uM)	yes	Advantage 2

CLAIMS

1. A method for preparing DNA that is complementary to an RNA molecule, comprising the steps of:

annealing a cDNA synthesis primer to said RNA molecule and synthesizing a first cDNA strand to form an RNA-cDNA intermediate; and

conducting a reverse transcriptase reaction by contacting said RNA-cDNA intermediate with a template switching oligonucleotide (TSO), wherein the 3' most nucleotide of said TSO is a locked nucleic acid (LNA), under conditions suitable for extension of the first DNA strand that is complementary to the RNA molecule, rendering it additionally complementary to the TSO.

2. The method of claim 1, wherein said reverse transcription reaction is conducted in the presence of a methyl group donor and a metal salt.

3. The method of claim 2, wherein said methyl group donor is betaine.

4. The method of claim 2, wherein said metal salt is magnesium salt.

5. The method of claim 4, wherein said magnesium salt has a concentration of at least 7 mM, at least 8 mM, or at least 9 mM.

6. The method of any one of claims 1 to 5, wherein said template switching oligonucleotide comprises at least one or two ribonucleotide residues and said LNA residue.

7. The method of claim 6, wherein said at least one or two ribonucleotide residues are riboguanine.

8. The method of any one of claims 1 to 7, wherein said locked nucleic acid residue is selected from the group consisting of locked guanine, locked adenine, locked uracil, locked thymine, locked cytosine, and locked 5-methylcytosine.

9. The method of any one of claims 1 to 8, wherein said locked nucleic acid residue is locked guanine.

10. The method of any one of claims 1 to 9, wherein said template switching oligonucleotide comprises at the 3'-end three nucleotide residues characterized by formula rGrG+N, wherein +N represents a locked nucleotide residue.

11. The method of claim 10, wherein said template switching oligonucleotide comprises rGrG+G.

12. The method of any one of claims 2 to 11, wherein said methyl group donor is betaine, and said metal salt is MgCl₂ at a concentration of at least 9 mM.

13. The method of any one of claims 1 to 12, further comprising amplifying said DNA strand that is complementary to said RNA molecule and said template switching oligonucleotide using an oligonucleotide primer.

14. The method of any one of claims 1 to 13, wherein said template switching oligonucleotide is selected from the oligonucleotides in Table below.

Entry	TSO name	Sequence (5' -> 3')
1	rGrG+G	AAGCAGTGGTATCAACGCAGAGTACrGrG+G
2	rGrG+N	AAGCAGTGGTATCAACGCAGAGTACrGrG+N
3	+G+G+G	AAGCAGTGGTATCAACGCAGAGTAC+G+G+G
4	rG+G+G	AAGCAGTGGTATCAACGCAGAGTACrG+G+G

15. The method of any one of claims 1 to 14, wherein the cDNA is synthesized on beads comprising an anchored oligo-dT primer.

16. The method of claim 15, wherein said oligo-dT primer comprises a sequence of 5'-AAGCAGTGGTATCAACGCAGAGTACT₃₀VN-3', wherein "N" is any nucleoside base, and "V" is selected from the group consisting of "A", "C" and "G".

17. The method of any one of claims 12 to 16, further comprising PCR preamplification, tagmentation, and final PCR amplification.

18. The method of claim 17, wherein the PCR preamplification is conducted without purifying the cDNA obtained from reverse transcription reaction.

19. The method of any one of claims 1 to 18, wherein said RNA is total RNA in a cell.

20. A method for analyzing gene expression in a plurality of single cells, the method comprising the steps of: preparing a cDNA library according to the method of any one of claims 1 to 19; and sequencing the cDNA library.

21. Use of a template switching oligonucleotide (TSO), wherein the 3' most nucleotide of said TSO is a locked nucleic acid, in the synthesis of a double strand cDNA according to the method of any one of claims 1 to 19.

FIGURE 1

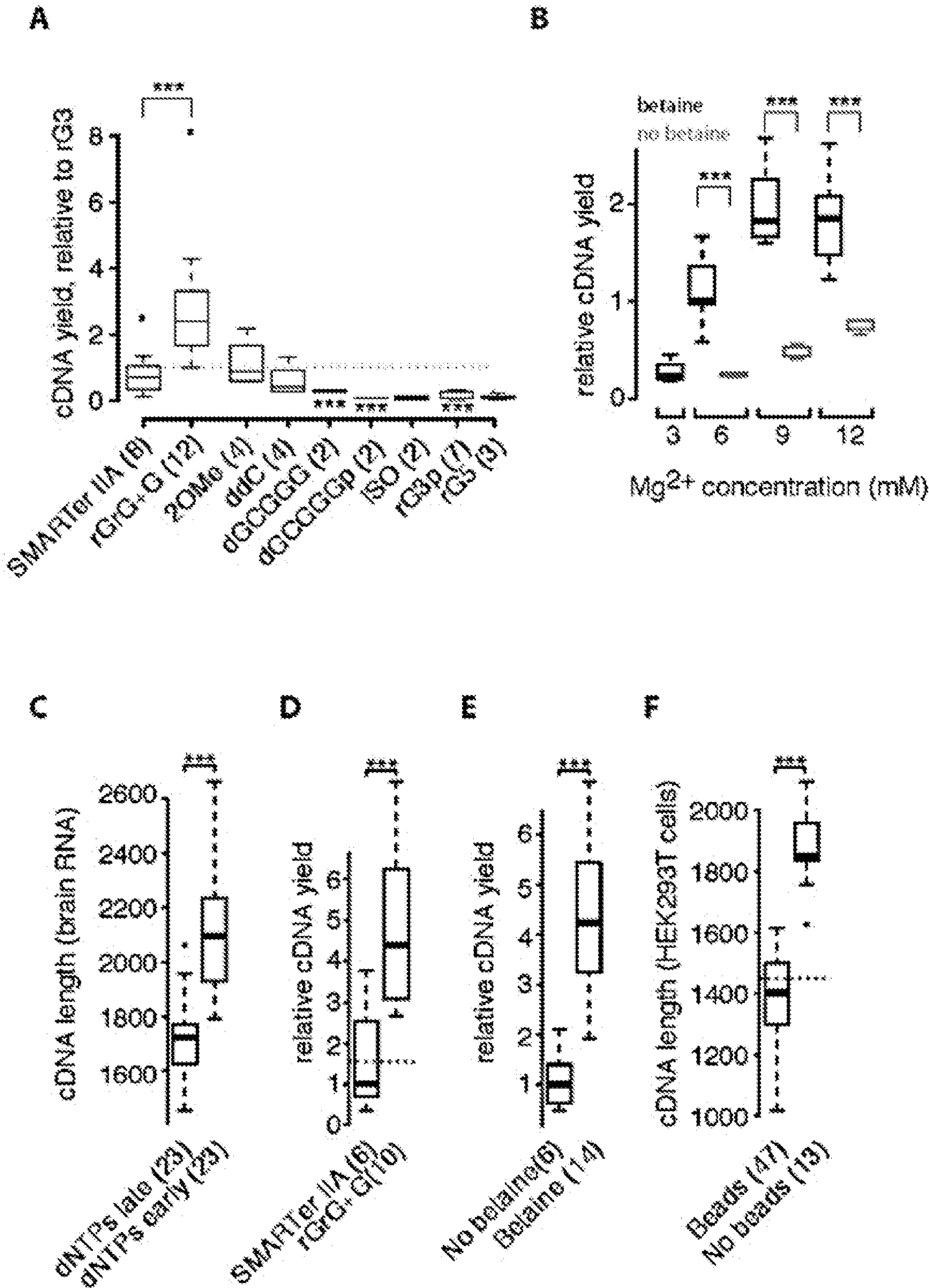


FIGURE 2

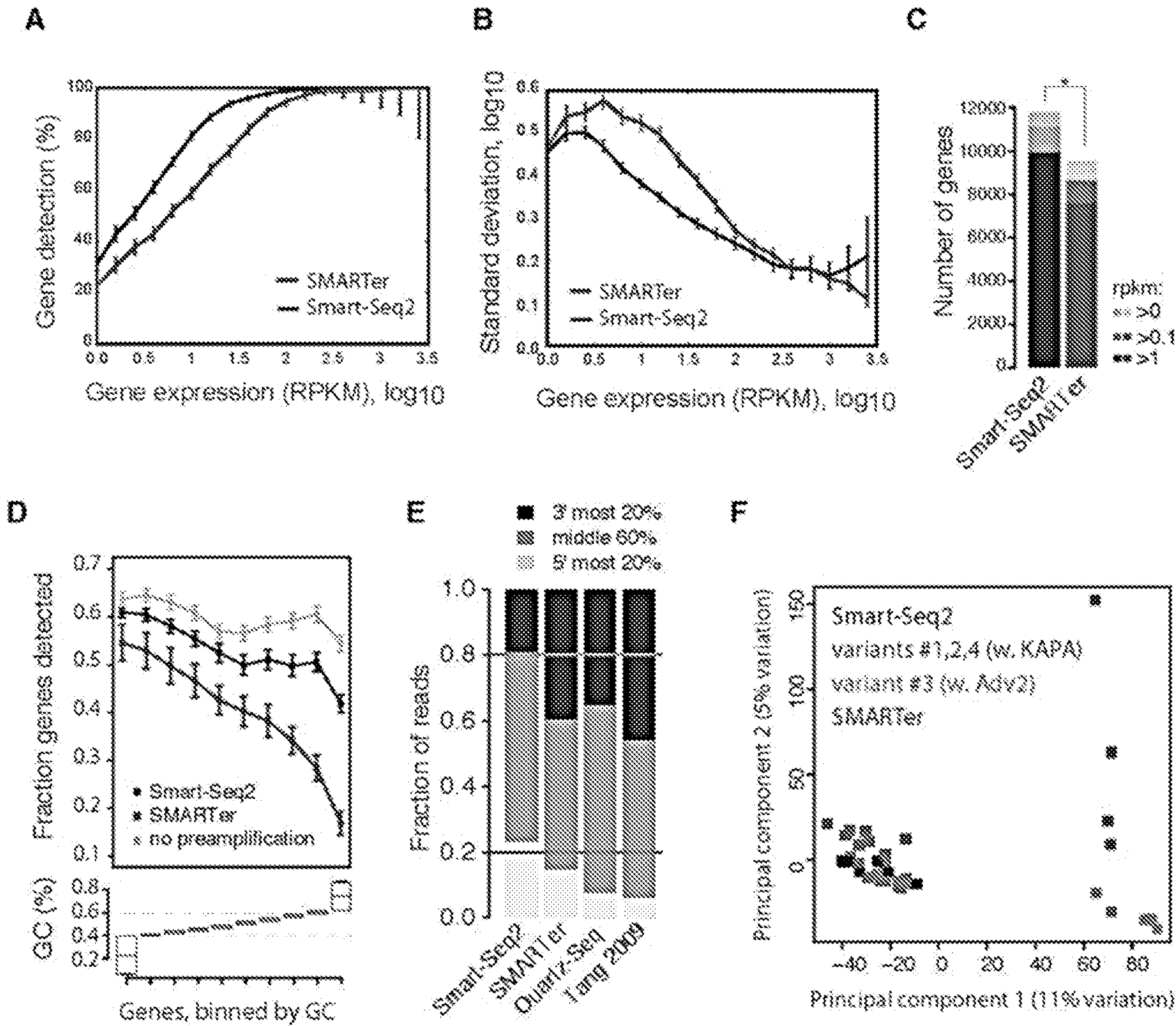


FIGURE 3

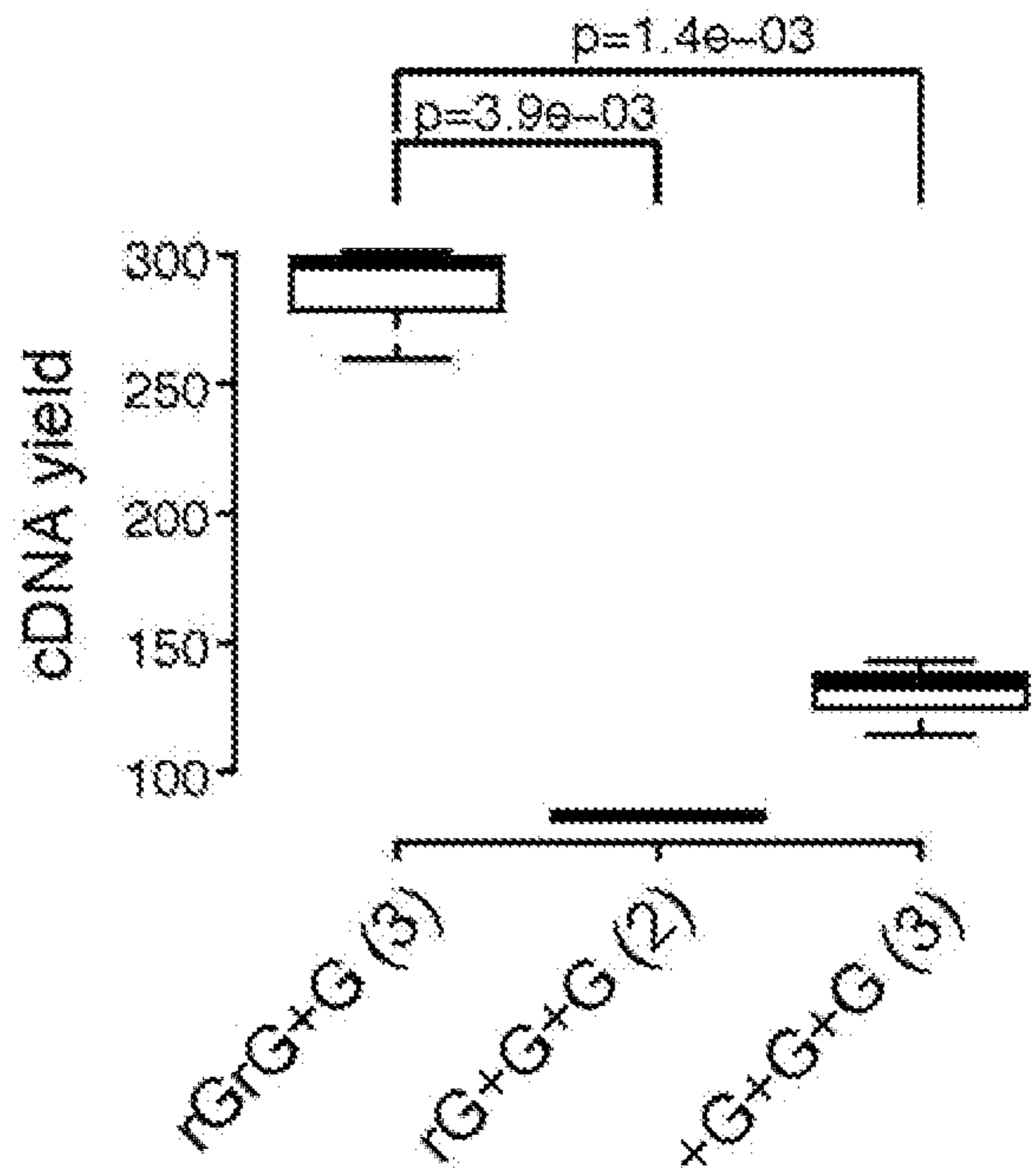
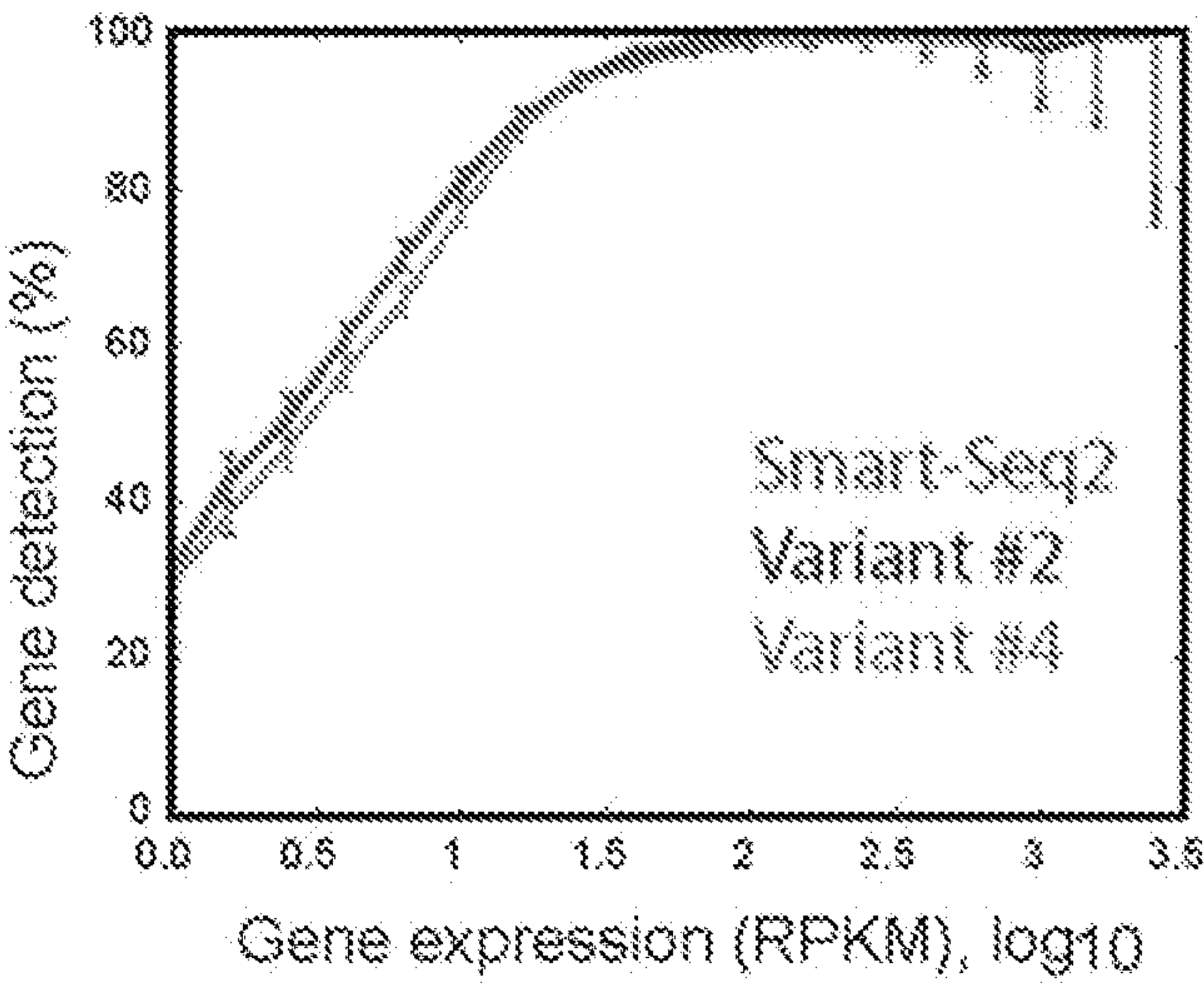


FIGURE 4

A



B

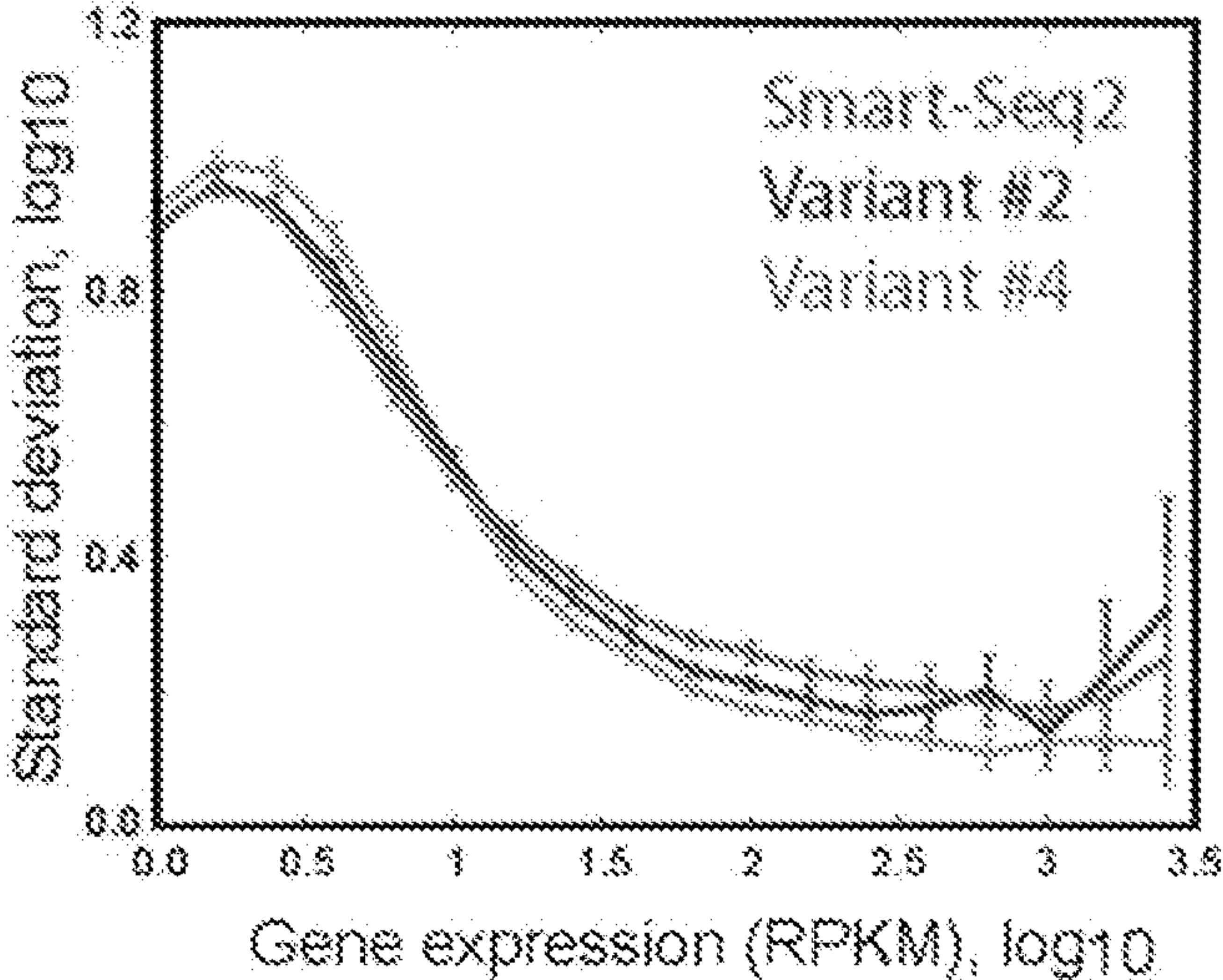


FIGURE 5

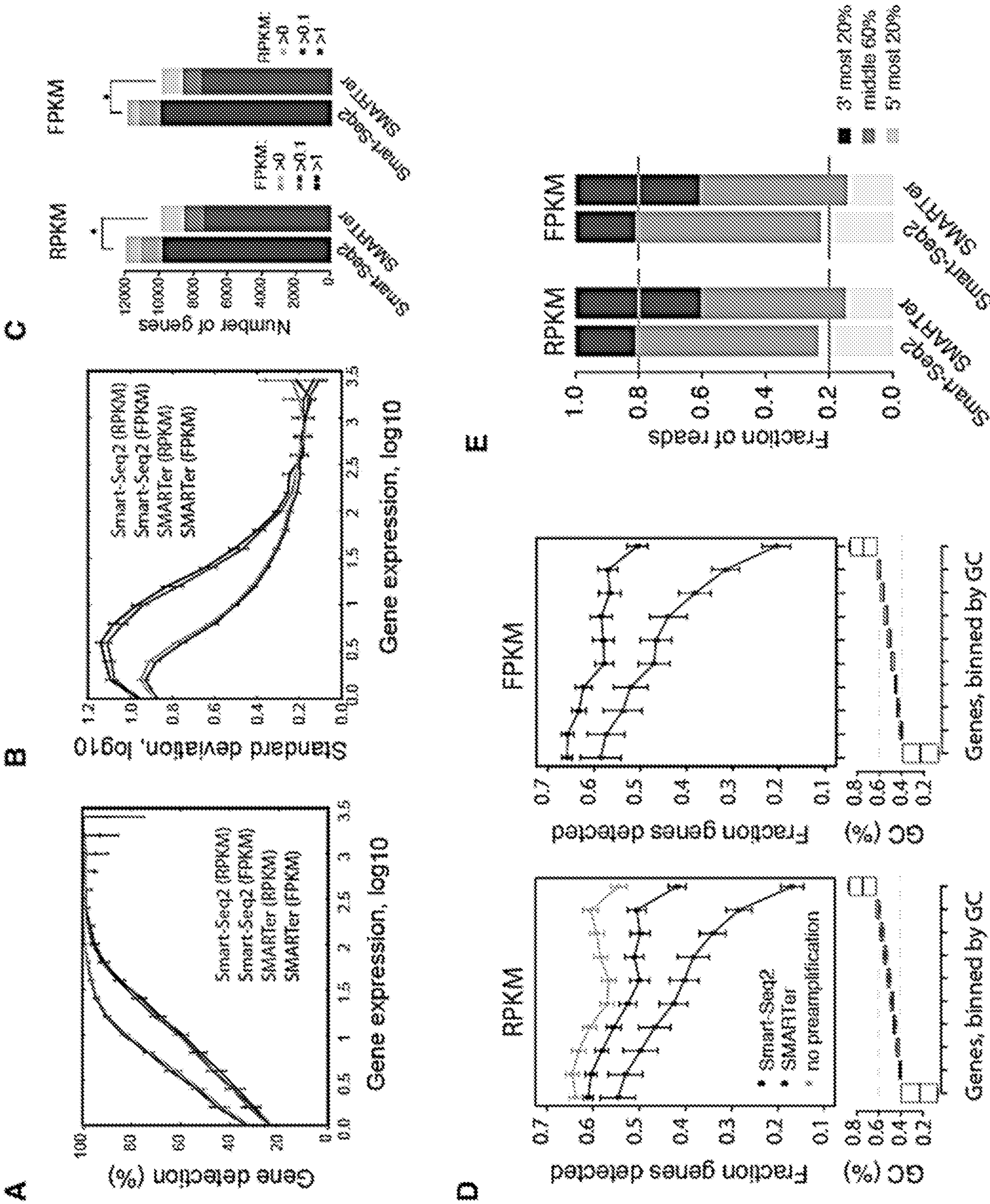


FIGURE 6

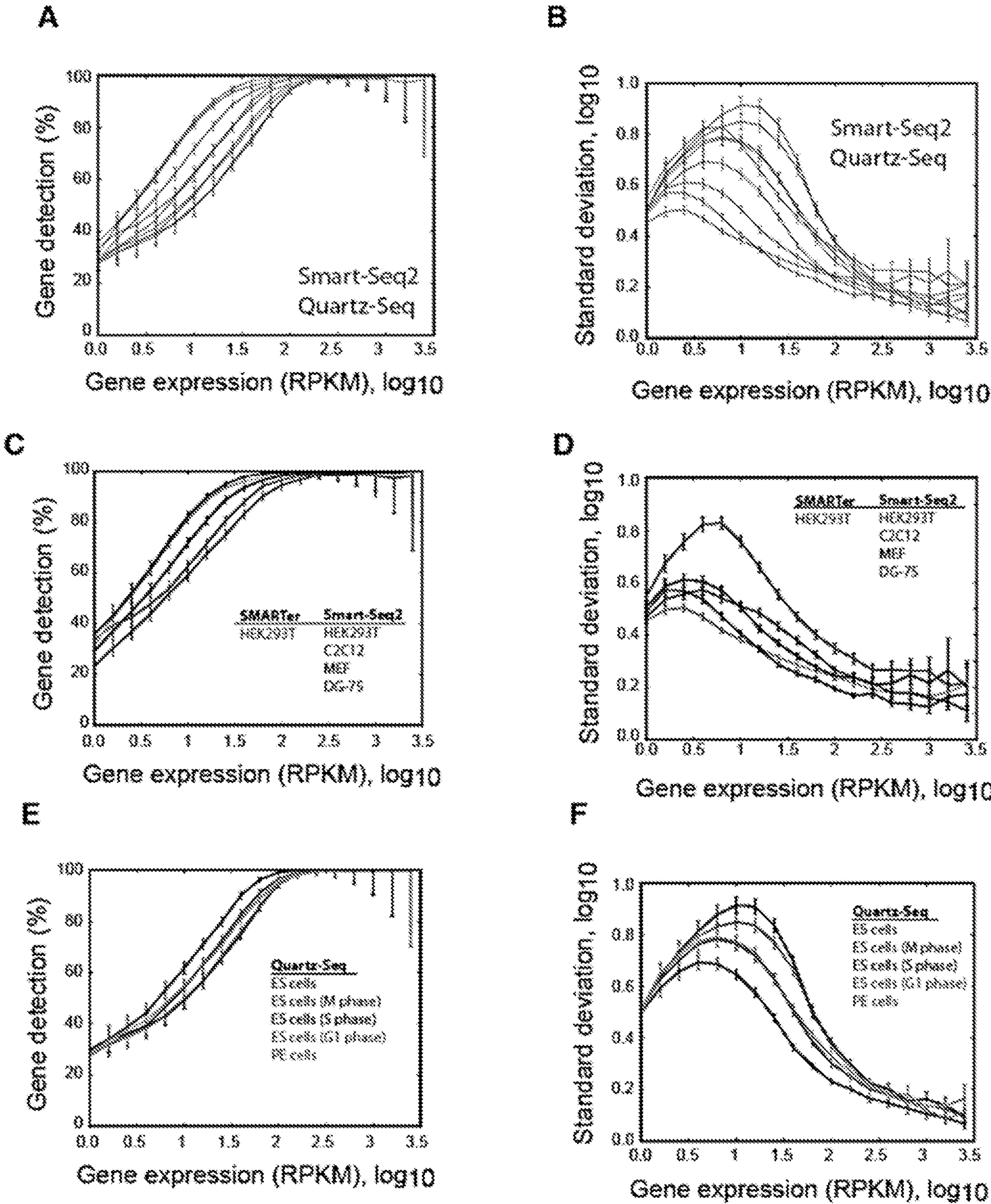


FIGURE 7

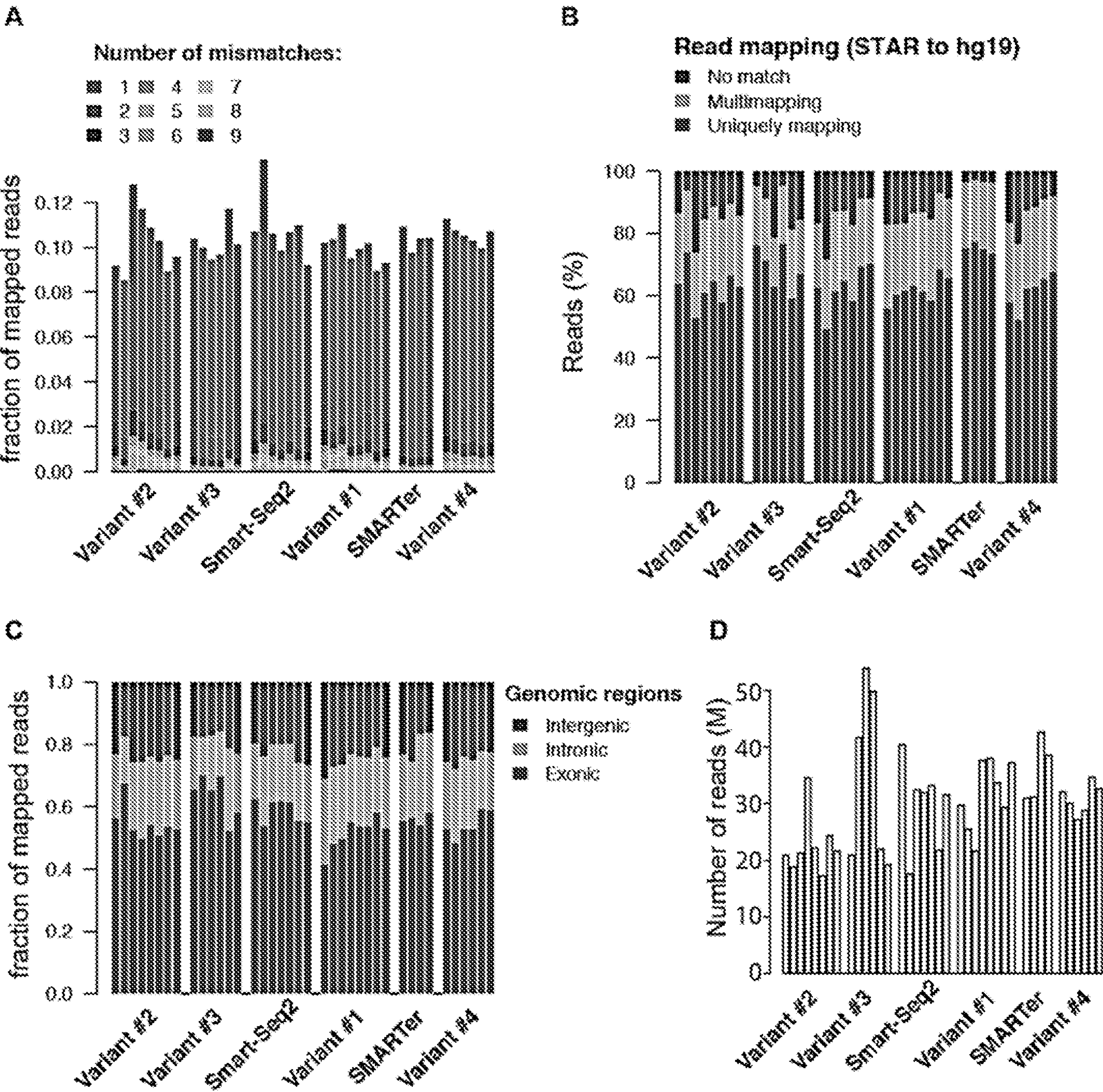


FIGURE 8

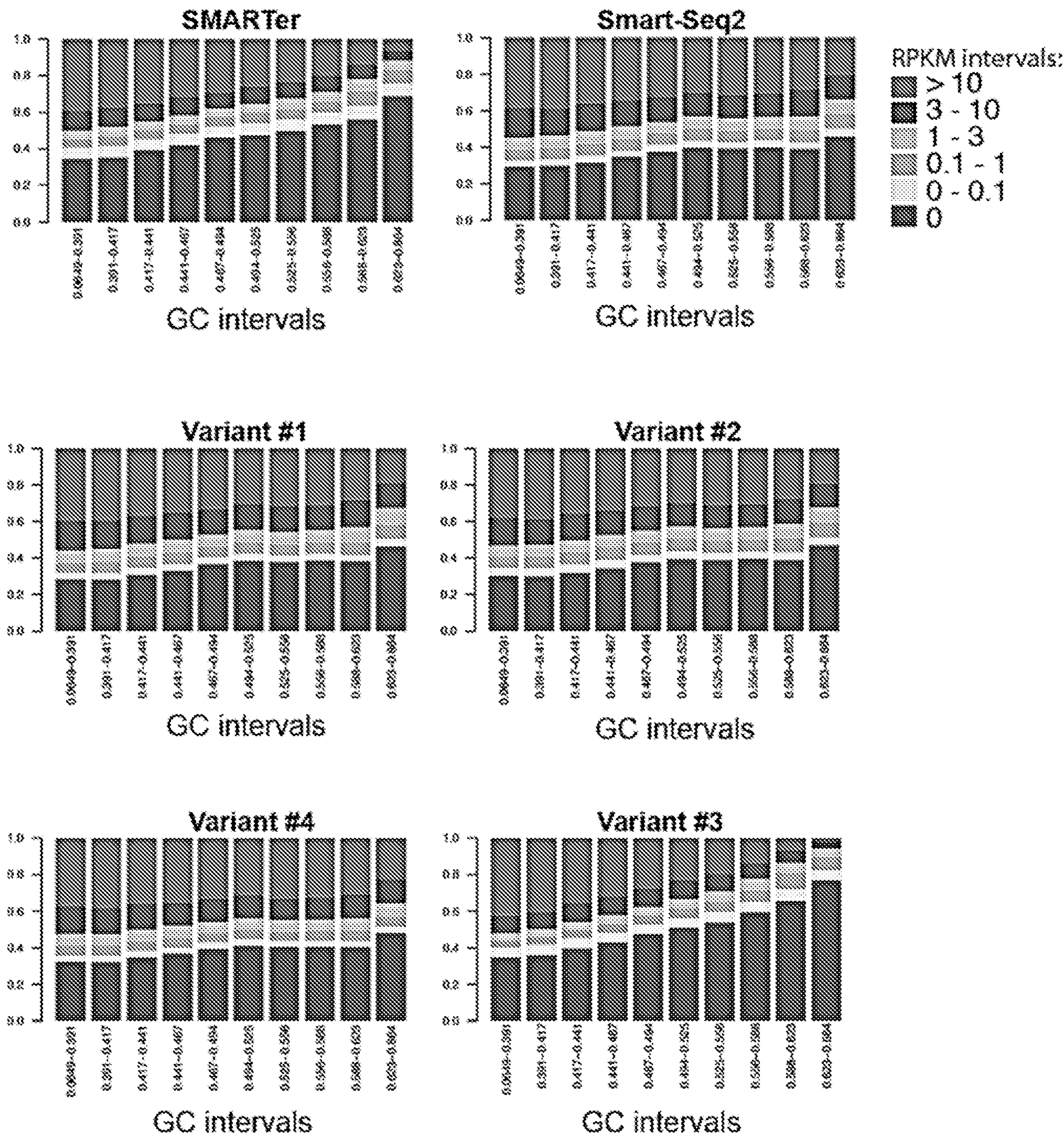
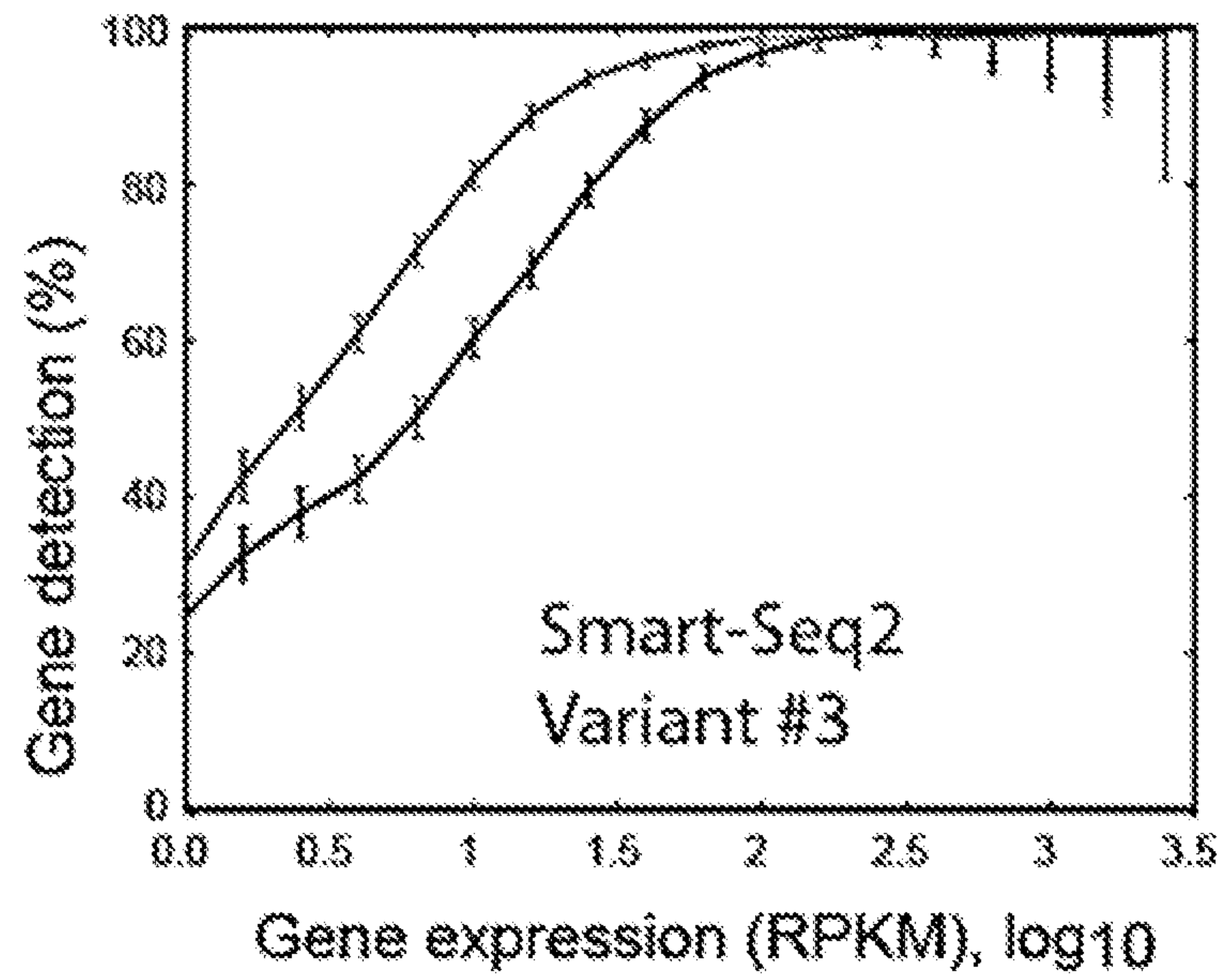
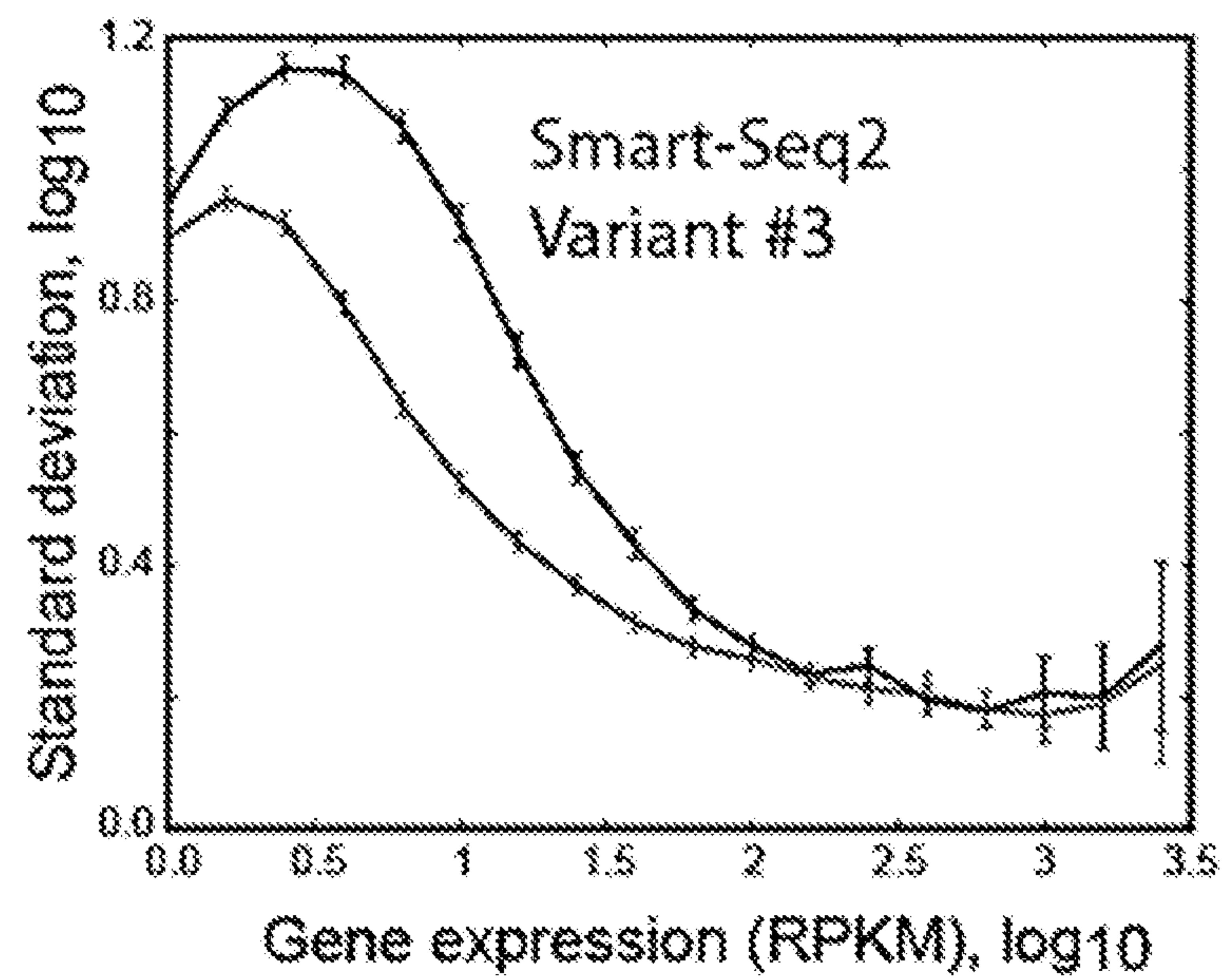


FIGURE 9**A****B**

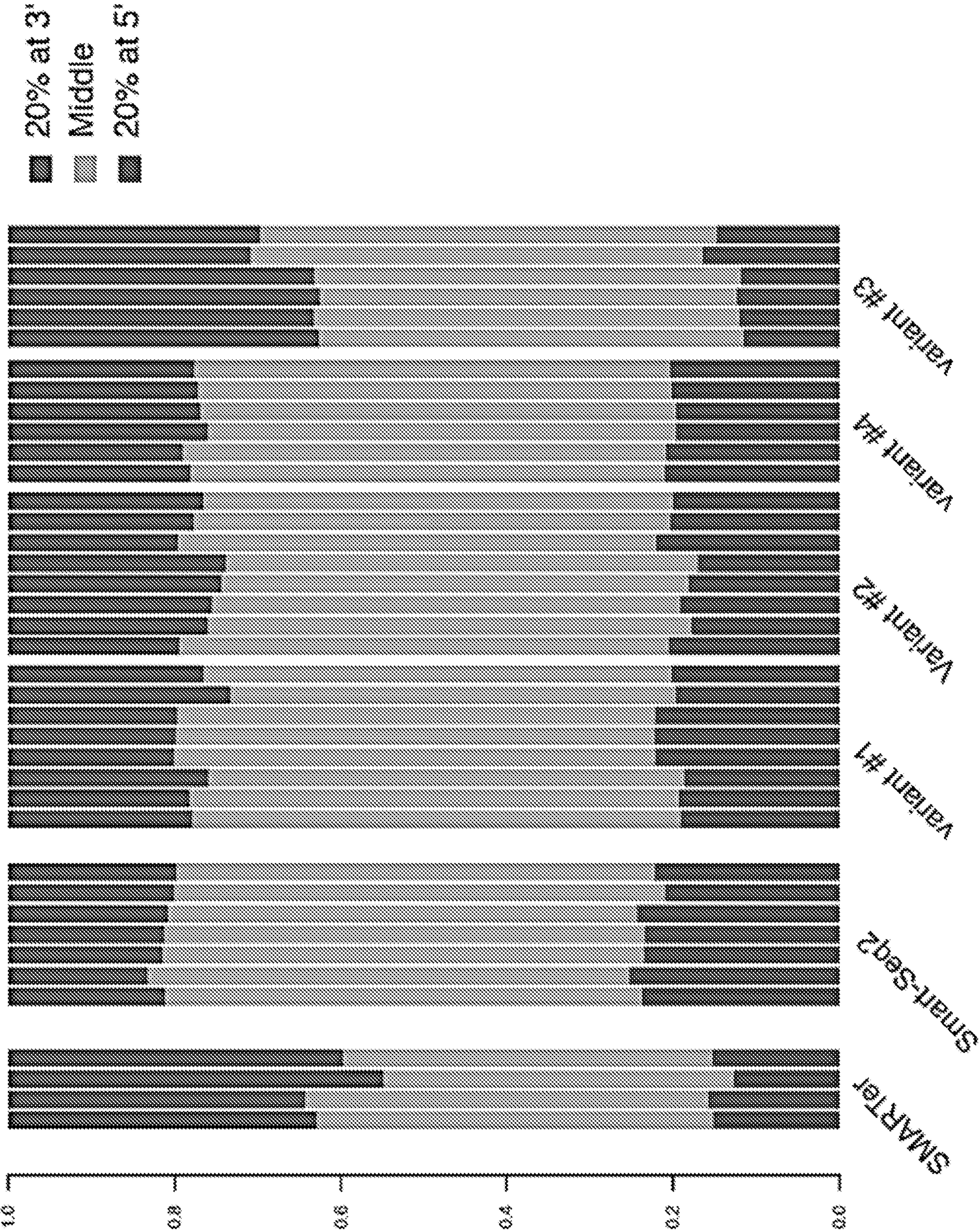


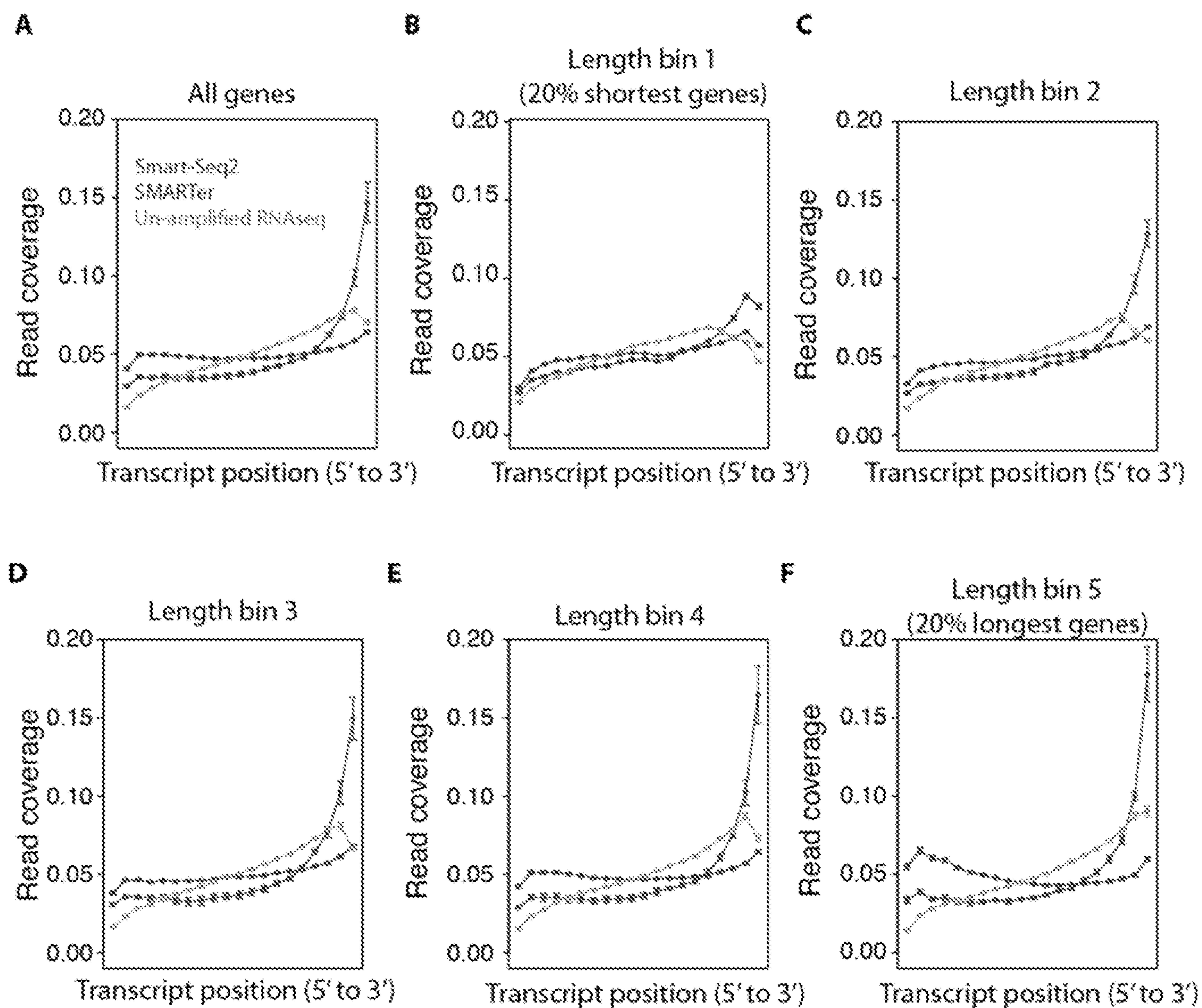
FIGURE 11

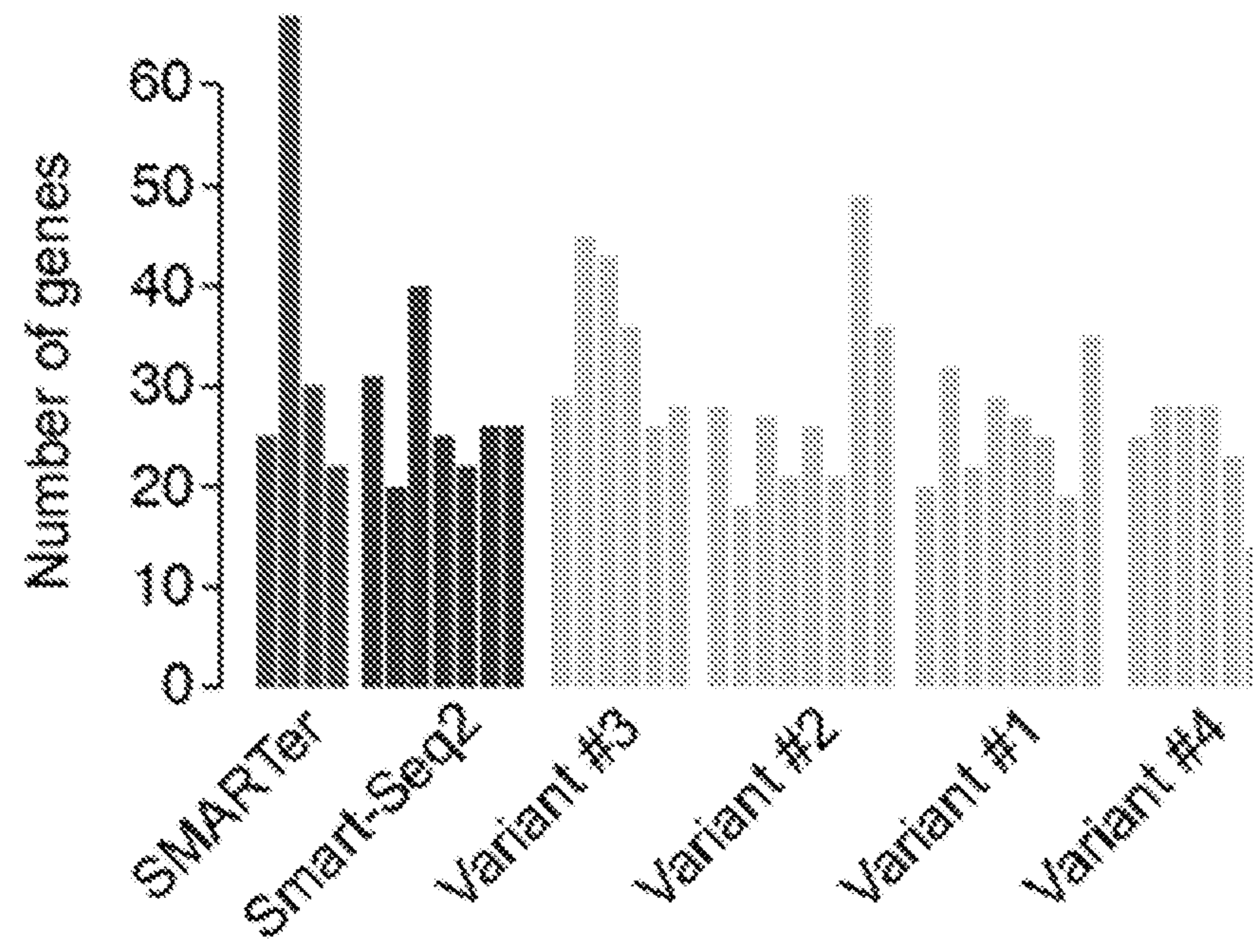
FIGURE 12A**A**

FIGURE 12 (Cont'd)

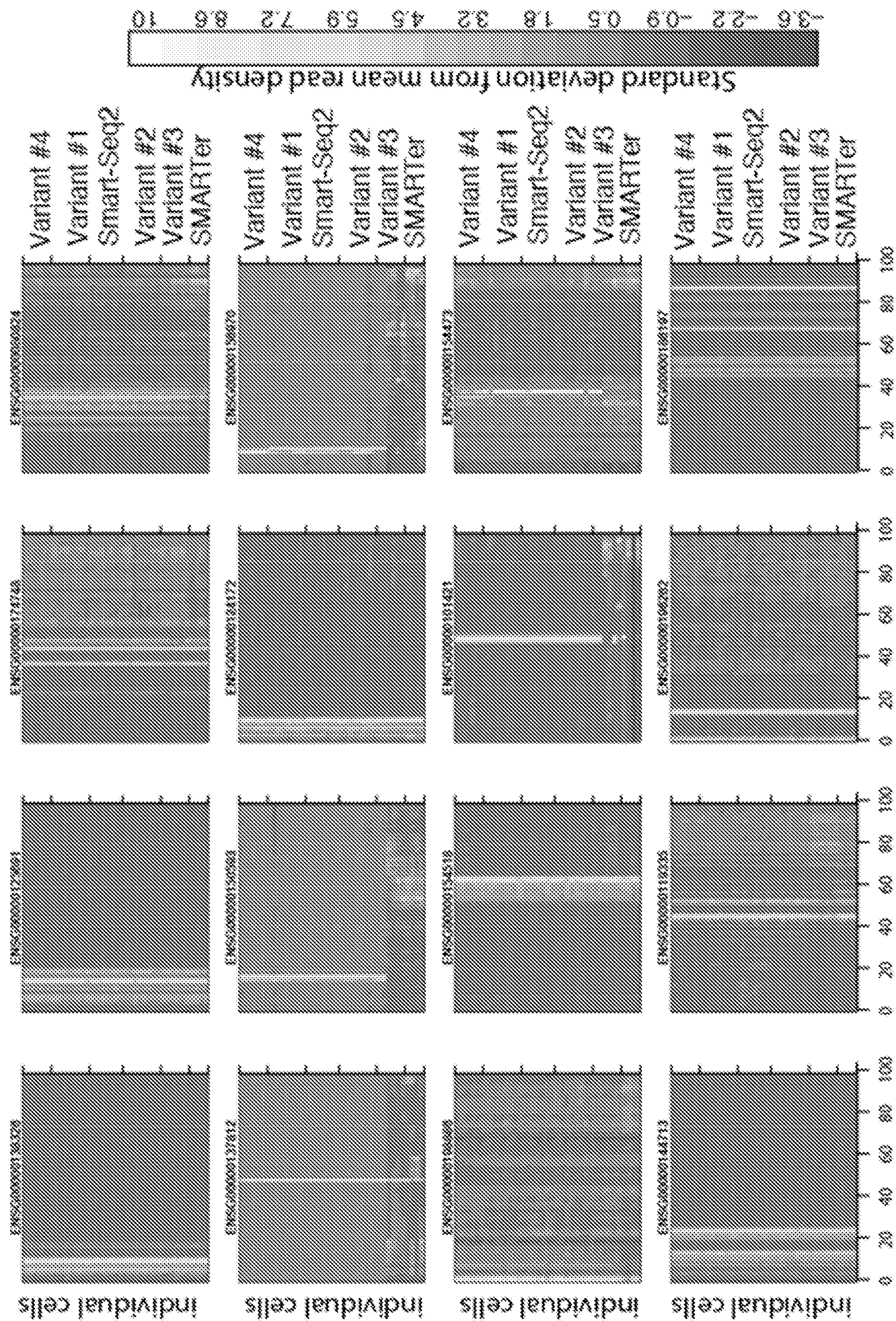


FIGURE 13

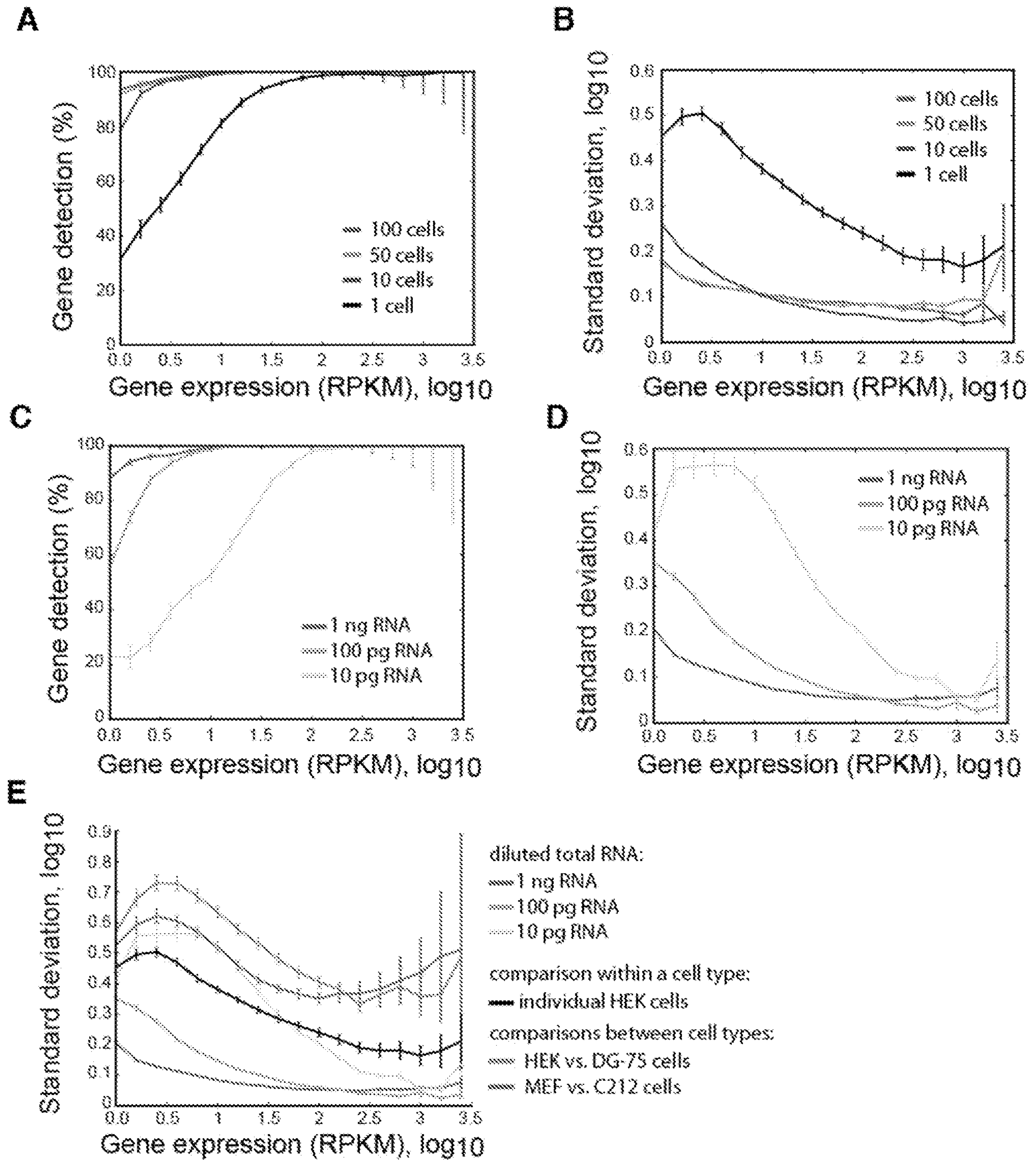
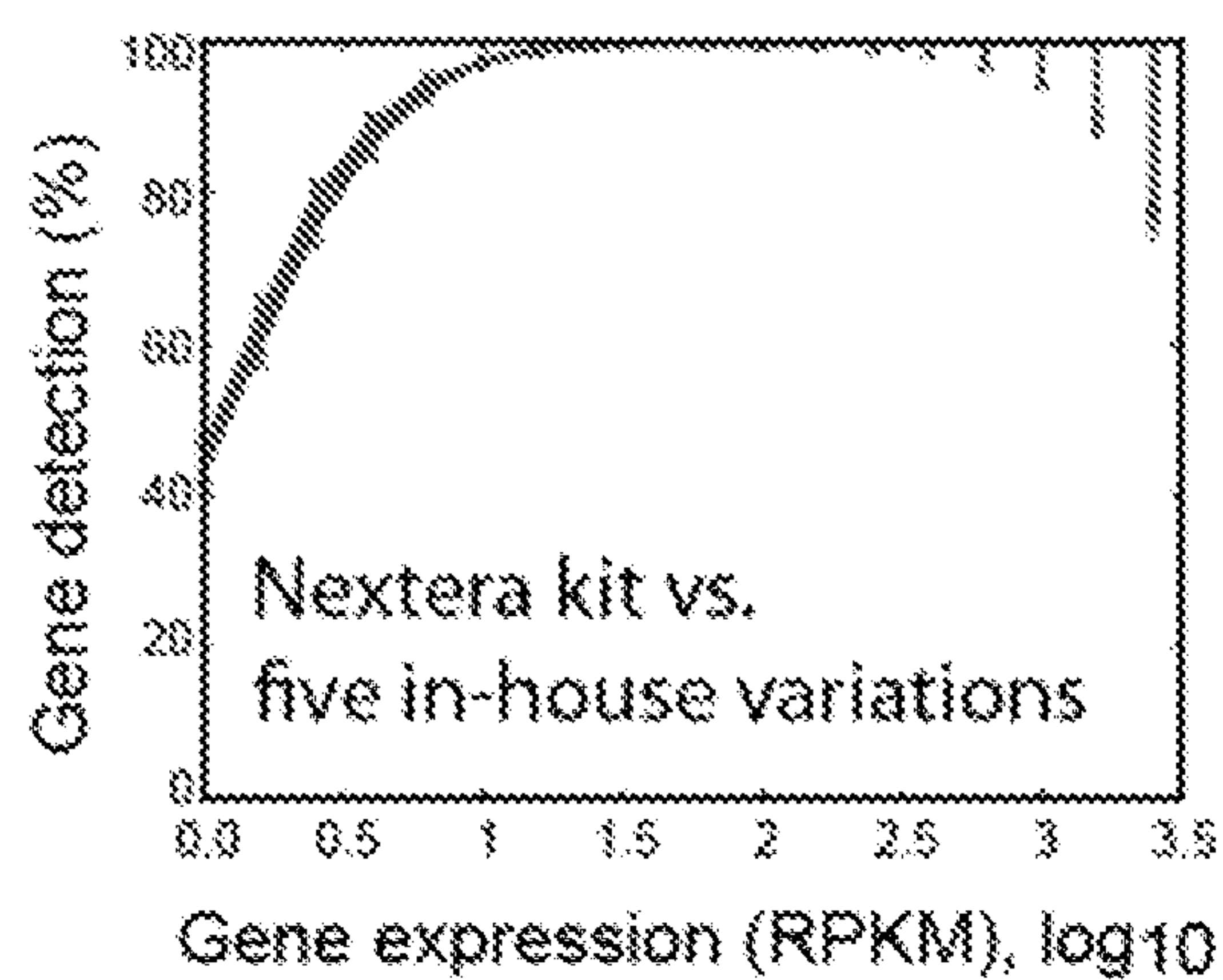
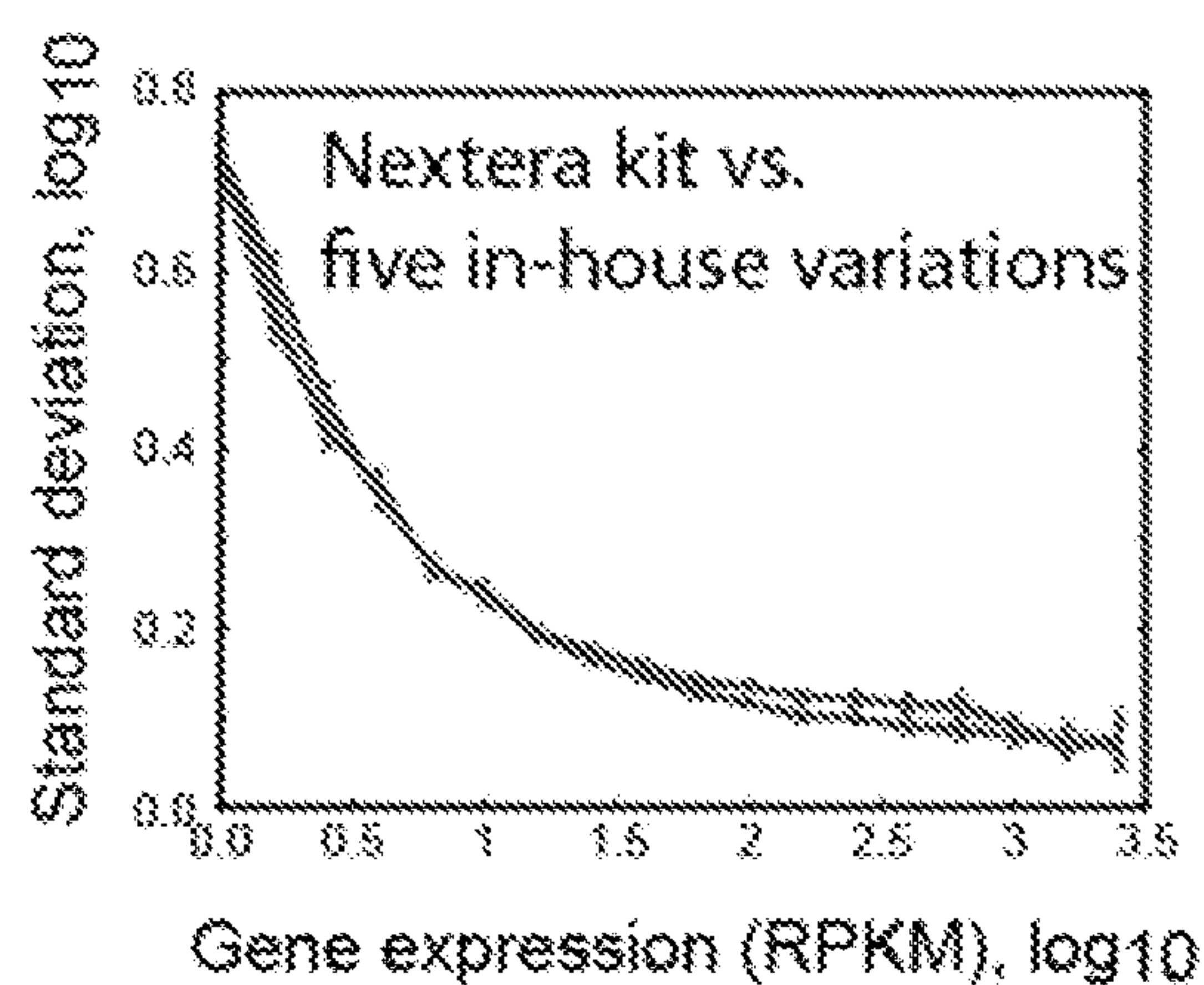
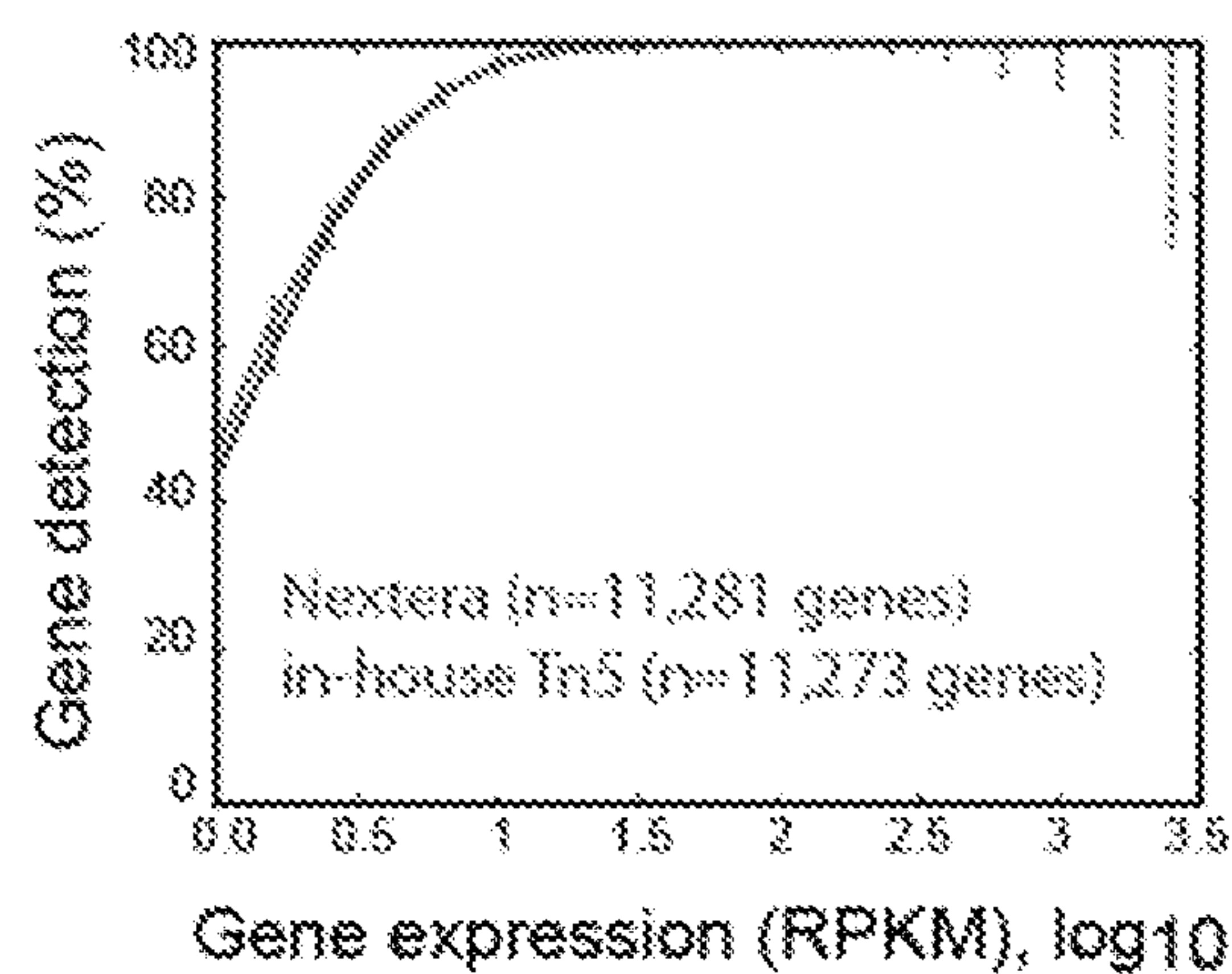
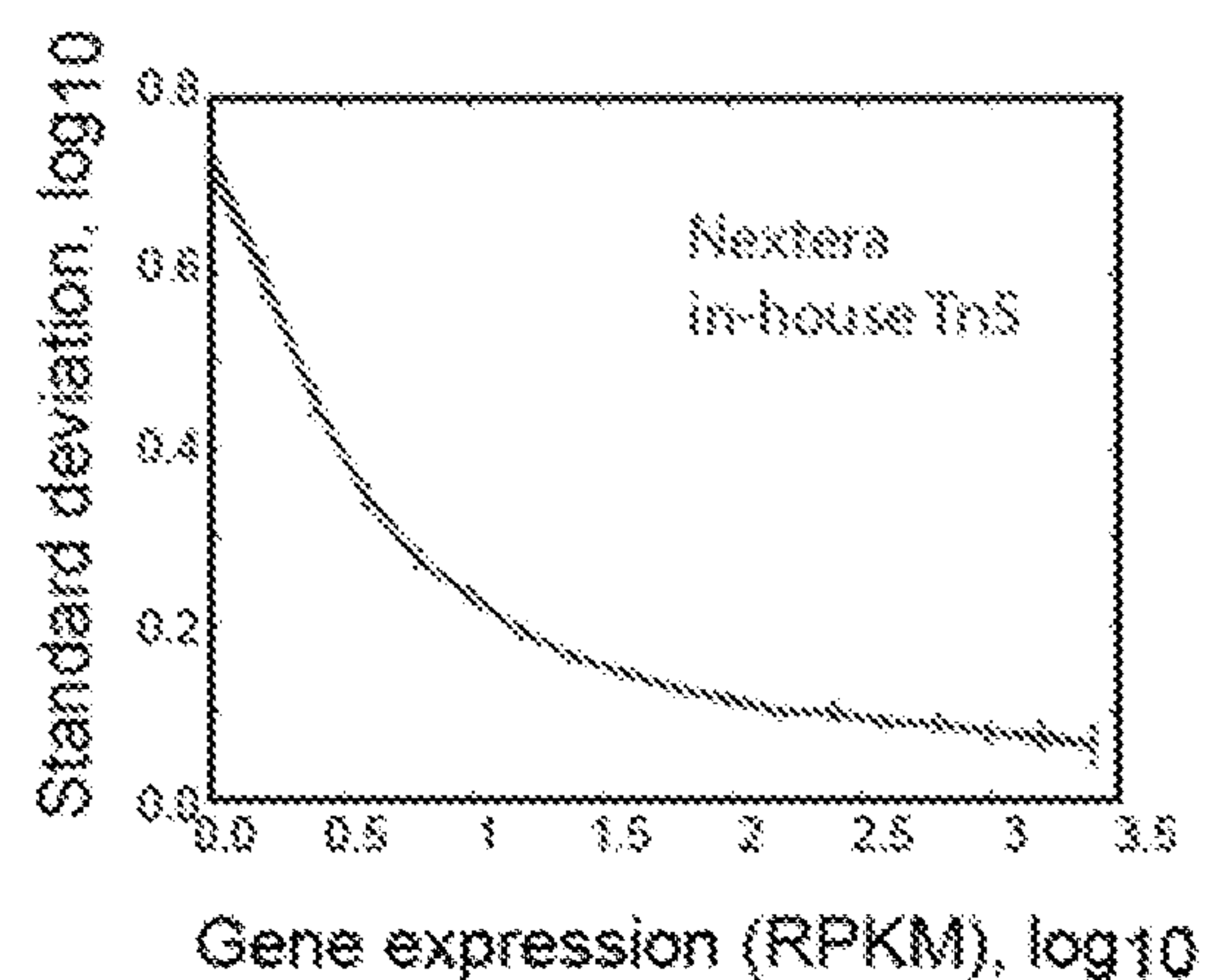


FIGURE 14

A**B****C****D****E**

Tn5 variant	Tagmentation volume (μl)	Purification after tagmentation	PCR enzyme
Reference	50	Clean & Concentrator kit	Nextera kit
1	20	NT buffer (Nextera XT kit)	Nextera kit
2	20	NT buffer (Nextera XT kit)	KAPA HiFi DNA Pol.
3	20	0.01% SDS	KAPA HiFi DNA Pol.
4	20	0.001% SDS	KAPA HiFi DNA Pol.
5	20	water	KAPA HiFi DNA Pol.

Percentage SDS refers to its final concentration in the 50 μl PCR reaction