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

(57) Abstract: This application discloses methods for cDN'A 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.

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

CROSS REFERENCE TO RELATED APPLICATIONS

The present application claims priority to U.S. Provisional Patent Application Serial No. 61/869,220, filed August 23, 2014, the contents of which are hereby incorporated by reference.

FIELD OF THE INVENTION

The present invention relates to methods for synthesis of double stranded 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 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. 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. *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 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 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, 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 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 (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 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 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.

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.

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.

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.

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.

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≥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 alinged 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 commerical 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

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.

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

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.

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.

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 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 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 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 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.

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 on beads comprising an anchored oligo-dT primer.

In another embodiment of the present invention, the oligo-dT primer comprises a sequence of 5′-AAGCAGTGGTATCAACGCAGAGTACT$_{30}$VN-3′, wherein "N" is any nucleoside base, and "V" is selected from the group consisting of "A", "C" and "G".

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.

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.

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 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.

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 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 (dC^7GTP), 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′-AAGCAGTGGTATCAACGCAGAGTACT$_{30}$VN-3′, where "N" is any base and "V" is either "A", "C" or "G") and 1 μl of dNTP mix (10 mM, Fermentas), denaturated 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 μl DTT (100 mM, Invitrogen), 2 μl betaine (5 M, Sigma), 0.9 μl $MgCl_2$ (100 mM, Sigma), 1 μl TSO (10 μM, the complete list of the oligos can be found in Table S1) and 0.1 μl 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 μl Advantage 2 Polymerase Mix (50X, Clontech), 5 μl Advantage 2 PCR Buffer (10X, Clontech), 2 μl dNTP mix (10 mM, Clontech), 2 μl IS PCR primer (12 μM, Clontech) and 39 μl nuclease-free water to a final reaction volume of 50 μl. 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 μl 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 μl KAPA HiFi HotStart ReadyMix (2X, KAPA Biosystems), 1 μl IS PCR primers (10 mM, 5'-AAGCAGTGGTATCAACGCAGAGT-3') and 14 μl 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 μl 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 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 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 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

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 (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 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 µl of a 10 µM 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 µl of the 10 µM 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).

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 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 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, 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 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 >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 ($p=7.8\times10^{-9}$, 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 Mg^{2+} 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). 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 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 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 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 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[dg], 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 ($p<10^{-5}$, $1.6x10^{-3}$ 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. 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 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 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.

All references cited herein are incorporated herein by reference in their entireties.

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	MgCl2 (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	SMARTscribe	6	90'@ 42°C	-	yes	Advantage	50	-	-
2	1	15	10	1:5	SMARTer kit but replacing ISPCR primers	263	1857	SMARTer Oligo IIA	1	SMARTscribe	6	90'@ 42°C	-	yes	Advantage	50	-	-
3	1	15	10	1:5	SMARTer kit but replacing oligo dT	172	1784	SMARTer Oligo IIA	1	SMARTscribe	6	90'@ 42°C	-	yes	Advantage	50	-	-
4					SMARTer kit but replacing FSB	FAILED		SMARTer Oligo IIA	1	SMARTscribe	6	90'@ 42°C	-	yes	Advantage	50	-	3 mM MnCl2
5	1	15	10	1:5	modified SMARTer kit	393	1683	SMARTer Oligo IIA	1	SSRTII	6	90'@ 42°C	-	yes	Advantage	50	-	-
6	1	15	NA	NA	modified SMARTer kit	121	1780	rG5	1	SMARTscribe	6	90'@ 42°C	-	yes	Advantage	50	-	-
7	1	15	NA	NA	modified SMARTer kit	1038	1346	rGrGrG	1	SMARTscribe	6	90'@ 42°C	-	yes	Advantage	50	-	-
8	1	15	NA	NA	modified SMARTer kit	90	1652	rGrGrGp	1	SMARTscribe	6	90'@ 42°C	-	yes	Advantage	50	-	-
9	1	15	NA	NA	modified SMARTer kit	84	1608	ISO	1	SMARTscribe	6	90'@ 42°C	-	yes	Advantage	50	-	-
10	1	15	NA	NA	SMARTer kit	3628	183	SMARTer	1	SMARTscrib	6	90'@	-	ye	Advantag	50	-	-

11	1	15	NA	NA	modified SMARTer kit	650	210 8	rG5	1	SMARTscribe	6	90 '@ 42°C	-	yes	Advantage	50	-	-
12	1	15	NA	NA	modified SMARTer kit	6423	185 2	rGrGrG	1	SMARTscribe	6	90 '@ 42°C	-	yes	Advantage	50	-	-
13	1	15	NA	NA	modified SMARTer kit	666	218 7	rGrGrGp	1	SMARTscribe	6	90 '@ 42°C	-	yes	Advantage	50	-	-
14	1	15	NA	NA	modified SMARTer kit	525	218 1	ISO	1	SMARTscribe	6	90 '@ 42°C	-	yes	Advantage	50	-	-
15	1	15	NA	NA	SMARTer kit but replacing ISPCR primers	3366	178 2	SMARTer Oligo IIA	1	SMARTscribe	6	90 '@ 42°C	-	yes	Advantage	50	-	-
16	1	15	NA	NA	modified SMARTer kit	435	197 0	ds oligos	1	SMARTscribe	6	90 '@ 42°C	-	yes	Advantage	50	-	-
17	1	15	NA	NA	SMARTer kit	1143	144 5	SMARTer Oligo IIA	1	SMARTscribe	6	90 '@ 42°C	-	yes	Advantage	50	-	-
18	1	15	NA	NA	SMARTer kit but replacing ISPCR primers	1504	151 9	SMARTer Oligo IIA	1	SMARTscribe	6	90 '@ 42°C	-	yes	Advantage	50	-	-
19	1	15	NA	NA	SMARTer kit but replacing ISPCR primers	1984	172 8	SMARTer Oligo IIA	1	SMARTscribe	6	90 '@ 42°C	-	yes	Advantage	50	-	-
20	1	15	NA	NA	modified SMARTer kit	1346	154 3	SMARTer Oligo IIA	1	SSRTII	6	90 '@ 42°C	-	yes	Advantage	50	-	-
21	1	15	NA	NA	modified SMARTer kit	1191	168 3	SMARTer Oligo IIA	1	SSRTII	6	90 '@ 42°C	-	yes	Advantage	50	-	-
22	1	15	NA	NA	SMARTer kit but using Superscript II FSB (3 mM MgCl2)	103	142 0	SMARTer Oligo IIA	1	SMARTscribe	3	90 '@ 42°C	-	yes	Advantage	50	-	-
23	1	15	NA	NA	SMARTer kit but using Superscript II FSB (3 mM MgCl2	192	160 9	SMARTer Oligo IIA	1	SMARTscribe	3	90 '@ 42°C	-	yes	Advantage	50	-	-
24					SMARTer kit but using STRT buffer	FAILED		SMARTer Oligo IIA	1	SMARTscribe	-	90 '@ 42°C	-	yes	Advantage	50	-	6 mM MnCl2
25	1	15	NA	NA	SMARTer kit	1573	237 8	SMARTer Oligo IIA	1	SMARTscribe	6	90 '@ 42°C	-	yes	Advantage	50	-	-
26	1	15	NA	NA	modified SMARTer kit	246	202 4	rG5	1	SMARTscribe	3	90 '@ 42°C	-	yes	Advantage	50	-	-
27	1	15	NA	NA	modified SMARTer kit	1393	194 5	rGrGrG	1	SMARTscribe	3	90 '@ 42°C	-	yes	Advantage	50	-	-
28	1	15	NA	NA	modified SMARTer kit	259	218 0	rGrGrGp	1	SMARTscribe	3	90 '@ 42°C	-	yes	Advantage	50	-	-
29	1	15	NA	NA	modified SMARTer kit	776	195 8	SMARTer Oligo IIA	1	SMARTscribe	6	90 '@ 42°C	1	yes	Advantage	50	-	-
30	1	15	NA	NA	modified SMARTer kit	731	180 8	SMARTer Oligo IIA	1	SMARTscribe	6	90 '@ 42°C	2	yes	Advantage	50	-	-
31	1	15	NA	NA	modified SMARTer kit	1936	196 5	SMARTer Oligo IIA	1	SMARTscribe	6	90 '@ 42°C	1	yes	Advantage	50	-	-
32					modified SMARTer kit	FAILED		SMARTer Oligo IIA	1	SMARTscribe	6	90 '@ 42°C	1	yes	Advantage	50	-	3 mM MnCl2
33					modified SMARTer	FAILE		SMARTer	1	SMARTscrib	6	90 '@	-	yes	Advantag	50	-	3 mM

					kit	D		Oligo IIA		e		42°C		s	e			MnCl2, 5% DMSO
34	1	15	NA	NA	SMARTer kit	580	2634	SMARTer Oligo IIA	1	SMARTscribe	6	90´@ 42°C	-	yes	Advantage	50	-	-
35	1	15	NA	NA	modified SMARTer kit	166	2208	SMARTer Oligo IIA	1	SMARTscribe	3	90´@ 42°C	1	yes	Advantage	50	-	-
36	1	15	NA	NA	modified SMARTer kit	204	2016	SMARTer Oligo IIA	1	SMARTscribe	3	90´@ 42°C	1	yes	Advantage	50	-	-
37	1	15	NA	NA	modified SMARTer kit	547	2171	SMARTer Oligo IIA	1	SMARTscribe	6	90´@ 42°C	1	yes	Advantage	50	-	-
38	1	15	NA	NA	modified SMARTer kit	640	2028	SMARTer Oligo IIA	1	SMARTscribe	6	90´@ 42°C	1	yes	Advantage	50	-	-
39	1	15	NA	NA	modified SMARTer kit	145	1938	rGrGrG	1	SMARTscribe	3	90´@ 42°C	1	yes	Advantage	50	-	-
40	1	15	NA	NA	modified SMARTer kit	224	1841	rGrGrG	1	SMARTscribe	3	90´@ 42°C	1	yes	Advantage	50	-	-
41	1	15	NA	NA	modified SMARTer kit	496	1863	rGrGrG	1	SMARTscribe	6	90´@ 42°C	1	yes	Advantage	50	-	-
42	1	15	NA	NA	modified SMARTer kit	493	1911	rGrGrG	1	SMARTscribe	6	90´@ 42°C	1	yes	Advantage	50	-	-
43	1	15	20	-	in house prot	8431	2226	rGrGrG	1	SSRTII	6	90´@ 42°C	1	yes	Advantage	50	-	-
44	1	15	20	-	in house prot	8248	2235	rGrGrG	1	SSRTII	6	90´@ 42°C	1	yes	Advantage	50	-	-
45	1	15	20	-	in house prot	7672	2317	rGrGrG	1	SSRTII	6	90´@ 42°C	1.5	yes	Advantage	50	-	-
46	1	15	20	-	in house prot	6562	2248	rGrGrG	1	SSRTII	6	90´@ 42°C	1.5	yes	Advantage	50	-	-
47	1	15	20	-	SMARTer kit	8156	2298	SMARTer Oligo IIA	1	SMARTscribe	6	90´@ 42°C	-	yes	Advantage	50	-	-
48	1	15	20	-	in house prot	1744	2592	rGrGrGp	1	SSRTII	6	90´@ 42°C	1	yes	Advantage	50	-	-
49	1	15	20	-	in house prot	1820	2585	rGrGrGp	1	SSRTII	6	90´@ 42°C	1	yes	Advantage	50	-	-
50	1	15	20	-	in house prot	1591	2541	rGrGrGp	1	SSRTII	6	90´@ 42°C	1.5	yes	Advantage	50	-	-
51	1	15	20	-	in house prot	1398	2616	rGrGrGp	1	SSRTII	6	90´@ 42°C	1.5	yes	Advantage	50	-	-
52	1	15	20	-	in house prot	2265	2110	dGCGGG	1	SSRTII	6	90´@ 42°C	1	yes	Advantage	50	-	-
53	1	15	20	-	in house prot	2568	2125	dGCGGG	1	SSRTII	6	90´@ 42°C	1	yes	Advantage	50	-	-
54	1	15	20	-	in house prot	2263	2079	dGCGGG	1	SSRTII	6	90´@ 42°C	1	yes	Advantage	50	-	-
55	1	15	20	-	in house prot	691	2290	dGCGGGp	1	SSRTII	6	90´@ 42°C	1	yes	Advantage	50	-	-
56	1	15	20	-	in house prot	757	2344	dGCGGGp	1	SSRTII	6	90´@ 42°C	1	yes	Advantage	50	-	-
57	1	15	20	-	in house prot	690	2294	dGCGGGp	1	SSRTII	6	90´@ 42°C	1	yes	Advantage	50	-	-

58	1	15	20	-	SMARTer kit	6525	2295	SMARTer Oligo IIA	1	SMARTscribe	6	90´@ 42°C	-	yes	Advantage	50	-	-
59	1	15	20	-	SMARTer kit	6806	2270	SMARTer Oligo IIA	1	SMARTscribe	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	-	-

68	1	15	20	-	immediately after adding lysis buffer (LB), 45´ after adding lysis buffer (LB), stored in the fridge	3793	158 5	rGrGrG	1	SSRTII	6	90´@ 42°C	1	yes	Advantage	50	-	-				
69	1	15	20	-	in house prot, processed immediately after adding lysis buffer (LB), 10´ after adding lysis buffer (LB), stored in the fridge	5312	182 4	rGrGrG	1	SSRTII	6	90´@ 42°C	1	yes	Advantage	50	-	-				
70	1	15	30	-	in house prot, RT for 1h @42°C, then added 3mM MnCl2 and incubated for 15'	1024	154 7	rGrGrG	1	SSRTII	6	90´@ 42°C	1	yes	Advantage	50	-	3 mM MnCl2				
71	1	15	30	-	in house prot, RT for 1h @42°C, then added 3mM MnCl2 and incubated for 15'	849	141 2	rGrGrG	1	SSRTII	6	90´@ 42°C	1	yes	Advantage	50	-	3 mM MnCl2				
72	1	15	30	-	in house prot, RT for 1h @42°C, then added 6mM MnCl2 and incubated for 15'	558	122 7	rGrGrG	1	SSRTII	6	90´@ 42°C	1	yes	Advantage	50	-	6 mM MnCl2				
73	1	15	30	-	in house prot, RT for 1h @42°C, then added 6mM MnCl2 and incubated for 15'	572	119 9	rGrGrG	1	SSRTII	6	90´@ 42°C	1	yes	Advantage	50	-	6 mM MnCl2				
74	1	15	30	-	in house prot	2105	205 1	rGrGrG	1	SSRTII	6	90´@ 42°C	1	yes	Advantage	50	-	-				
75	1	15	30	-	in house prot	1664	180 9	rGrGrG	1	SSRTII	6	90´@ 42°C	1	yes	Advantage	50	-	-				
76	10 0	10	15	1:5	in house prot	3196	194 6	2OMe	1	SSRTII	6	90´@ 42°C	1	yes	Advantage	50	-	-				
77	10 0	10	15	1:5	in house prot	8894	176 7	rGrG+G	1	SSRTII	6	90´@ 42°C	1	yes	Advantage	50	-	-				
78	10 0	10	15	1:5	in house prot	1939	205 4	ddC	1	SSRTII	6	90´@ 42°C	1	yes	Advantage	50	-	-				

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

					HiFi after washing beads 1		7					42°C		s	HiFi HS			
105	1	15	15	1:5	in house prot, KAPA HiFi after washing beads 1	352	2151	rGrGrG	1	SSRTII	12	90´@ 42°C	1	yes	KAPA HiFi HS	50	-	-
106	1	15	15	1:5	in house prot, KAPA HiFi after washing beads 2	1729	1611	rGrG+G	1	SSRTII	12	90´@ 42°C	1	yes	KAPA HiFi HS	50	-	-
107	1	15	15	1:5	in house prot, KAPA HiFi after washing beads 2	203	2073	rGrGrG	1	SSRTII	12	90´@ 42°C	1	yes	KAPA HiFi HS	50	-	-
108	1	15	15	1:5	in house prot, KAPA HiFi after washing beads 1+elution	1108	1480	rGrG+G	1	SSRTII	12	90´@ 42°C	1	yes	KAPA HiFi HS	50	-	-
109	1	15	15	1:5	in house prot, KAPA HiFi after washing beads 1+elution	301	1903	rGrGrG	1	SSRTII	12	90´@ 42°C	1	yes	KAPA HiFi HS	50	-	-
110	1	15	15	1:5	in house prot, KAPA HiFi after washing beads 2+elution	1337	1522	rGrG+G	1	SSRTII	12	90´@ 42°C	1	yes	KAPA HiFi HS	50	-	-
112	1	15	15	1:5	in house prot, KAPA HiFi after washing beads 2+elution	383	1935	rGrGrG	1	SSRTII	12	90´@ 42°C	1	yes	KAPA HiFi HS	50	-	-
113	1	15	15	1:5	in house prot	2897	1737	rGrG+G	1	SSRTII	12	90´@ 42°C	1	yes	Advantage	50	-	-
114	1	15	15	1:5	in house prot	1853	1706	rGrGrG	1	SSRTII	12	90´@ 42°C	1	yes	Advantage	50	-	-
115	1	15	15	1:5	in house prot	1492	1563	rGrG+G	1	Revertaid H-	4	90´@ 42°C	1	yes	Advantage	50	-	-
116	1	15	15	1:5	in house prot	1236	1472	rGrG+G	1	Revertaid H-	4	90´@ 42°C	1	yes	Advantage	50	-	-
117	1	15	15	1:5	in house prot	1843	1450	rGrG+G	1	Revertaid H-	6	90´@ 42°C	1	yes	Advantage	50	-	-
118	1	15	15	1:5	in house prot	1465	1346	rGrG+G	1	Revertaid H-	6	90´@ 42°C	1	yes	Advantage	50	-	-
119	1	15	15	1:5	in house prot	2996	1597	rGrG+G	1	Revertaid H-	9	90´@ 42°C	1	yes	Advantage	50	-	-
120	1	15	15	1:5	in house prot	2654	1530	rGrG+G	1	Revertaid H-	9	90´@ 42°C	1	yes	Advantage	50	-	-
121	1	15	15	1:5	in house prot	2159	1487	rGrG+G	1	Revertaid H-	12	90´@ 42°C	1	yes	Advantage	50	-	-
122	1	15	15	1:5	in house prot	1890	1412	rGrG+G	1	Revertaid H-	12	90´@ 42°C	1	yes	Advantage	50	-	-
123	1	15	15	1:5	in house prot	1504	1246	rGrG+G	1	Revertaid H-	15	90´@ 42°C	1	yes	Advantage	50	-	-
124	1	15	15	1:5	in house prot	1986	1474	rGrG+G	1	Revertaid H-	15	90´@ 42°C	1	yes	Advantage	50	-	-
125	1	15	15	1:5	in house prot	1109	1691	rGrG+G	1	SSRTII	-	60´@42° C, then 90´@60° C	-	yes	Advantage	50	-	0.3M trehalose

126	1	15	15	1:5	in house prot	1090	1752	rGrG+G	1	SSRTII	-	60´@42° C, then 90´@60° C	-	yes	Advantage	50	-	0.3M trehalose
127	1	15	15	1:5	in house prot	863	1565	rGrG+G	1	SSRTII	-	60´@42° C, then 90´@60° C	-	yes	Advantage	50	-	0.6M trehalose
128	1	15	15	1:5	in house prot	896	1652	rGrG+G	1	SSRTII	-	60´@42° C, then 90´@60° C	-	yes	Advantage	50	-	0.6M trehalose
129	1	15	15	1:5	in house prot	1078	1655	rGrG+G	1	SSRTII	-	60´@42° C, then 90´@60° C	1	yes	Advantage	50	-	0.3M trehalose
130	1	15	15	1:5	in house prot	562	1517	rGrG+G	1	SSRTII	-	60´@42° C, then 90´@60° C	1	yes	Advantage	50	-	0.3M trehalose
131	1	15	15	1:5	in house prot	998	1594	rGrG+G	1	SSRTII	-	60´@42° C, then 90´@60° C	0.6	yes	Advantage	50	-	0.3M trehalose
132	1	15	15	1:5	in house prot	925	1545	rGrG+G	1	SSRTII	-	60´@42° C, then 90´@60° C	0.6	yes	Advantage	50	-	0.3M trehalose
133	1	15	15	1:5	in house prot	1155	1618	rGrG+G	1	SSRTII	3	60´@42° C, then 90´@60° C	1	yes	Advantage	50	-	0.3M trehalose
134	1	15	15	1:5	in house prot	603	1433	rGrG+G	1	SSRTII	3	60´@42° C, then 90´@60° C	1	yes	Advantage	50	-	0.3M trehalose
135	1	15	15	1:5	in house prot	1561	1716	rGrG+G	1	SSRTII	3	60´@42° C, then 90´@60° C	0.6	yes	Advantage	50	-	0.3M trehalose
136	1	15	15	1:5	in house prot	445	1575	rGrG+G	1	SSRTII	-	60´@50° C, then 90´@42° C	-	yes	Advantage	50	-	0.3M trehalose
137	1	15	15	1:5	in house prot	1466	1698	rGrG+G	1	SSRTII	-	90´@42° C, 30´@60° C, then 30´@42° C	-	yes	Advantage	50	-	0.3M trehalose
138	1	15	15	1:5	in house prot	703	1580	rGrG+G	1	SSRTII	-	60´@50° C, then	-	yes	Advantage	50	-	0.6M trehalose

139	1	15	15	1:5	in house prot	1397	1740	rGrG+G	1	SSRTII	-	90´@42° C		ye s	Advantage	50	-	0.6M trehalose
												90´@42° C, 30´@60° C, then 30´@42° C						
140	1	15	15	1:5	in house prot	355	1425	rGrG+G	1	SSRTII	-	60´@50° C, then 90´@42° C	0.6	ye s	Advantage	50	-	0.6M trehalose
												90´@42° C, 30´@60° C, then 30´@42° C						
141	1	15	15	1:5	in house prot	1654	1598	rGrG+G	1	SSRTII	-	60´@50° C, then 90´@42° C	0.6	ye s	Advantage	50	-	0.6M trehalose
												90´@42° C, 30´@60° C, then 30´@42° C						
142	1	15	15	1:5	in house prot	1470	1480	rGrG+G	1	SSRTII	12	90´@42° C	0.6	ye s	Advantage	50	-	0.6M trehalose
												90´@42° C, 30´@60° C, then 30´@42° C						
143	1	15	15	1:5	in house prot	1389	1480	rGrG+G	1	SSRTII	12	90´@42° C	0.6	ye s	Advantage	50	-	0.6M trehalose
												90´@42° C, then 10X (2´@50°C - 2´@42°C)	1	ye s	Advantage	50	-	-
144	1	15	15	1:5	in house prot	3959	1641	rGrG+G	1	SSRTII	12							
												90´@42° C, then 10X (2´@60°C - 2´@42°C)	1	ye s	Advantage	50	-	-
145	1	15	15	1:5	in house prot	3816	1732	rGrG+G	1	SSRTII	12							
												90´@42° C, then 10X (2´@50°C - 2´@42°C)	0.5	ye s	Advantage	50	-	0.3M trehalose
146	1	15	15	1:5	in house prot	3179	1769	rGrG+G	1	SSRTII	12							
												90´@42° C, then 10X (2´@60°C - 2´@42°C)	0.5	ye s	Advantage	50	-	0.3M trehalose
147	1	15	15	1:5	in house prot	3271	1828	rGrG+G	1	SSRTII	12							

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 -	1	yes	Advantage	50	-	-

156	1	15	15	1:5	in house prot	4595	1630	rGrG+G	1	SSRTII	12	90´@42° C, then 15x (2´@50°C - 2´@42°C)	1	yes	Advantage	50	-	-						
157	1	15	15	1:5	in house prot	3457	1525	rGrG+G	1	SSRTII	12	90´@42° C, then 20x (2´@50°C - 2´@42°C)	1	yes	Advantage	50	-	-						
158	1	15	15	1:5	in house prot	2869	1409	rGrG+G	1	SSRTII	12	90´@42° C, then 20x (2´@50°C - 2´@42°C)	1	yes	Advantage	50	-	-						
159	1	15	15	1:5	in house prot	1529	1629	3rGrG+G	1	SSRTII	12	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	yes	Advantage	50	-	-						
160	1	15	15	1:5	in house prot	1901	1728	3rGrG+G	1	SSRTII	12	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	yes	Advantage	50	-	-						
161	1	15	15	1:5	in house prot	1785	1717	3rGrG+G	1	SSRTII	12	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	yes	Advantage	50	-	-						
162	1	15	15	1:5	in house prot	1086	1928	2rGrG+G	1	SSRTII	12	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	yes	Advantage	50	-	-						
163	1	15	15	1:5	in house prot	1128	1846	2rGrG+G	1	SSRTII	12	90´@42°	1	yes	Advantage	50	-	-						

164	1	15	15	1:5	in house prot	596	1892	phosphate	1	SSRTII	12	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	yes	Advantage	50	-	-					
165	1	15	15	1:5	in house prot	579	1922	phosphate	1	SSRTII	12	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	yes	Advantage	50	-	-					
166	1	15	15	1:5	in house prot	546	1830	C6 amino	1	SSRTII	12	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	yes	Advantage	50	-	-					
167	1	15	15	1:5	in house prot	419	1664	C6 amino	1	SSRTII	12	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	yes	Advantage	50	-	-					
168	1	15	15	1:5	in house prot	3076	1567	rGrG+G	1	SSRTII	12	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	yes	Advantage	50	-	-					
169	1	15	15	1:5	in house prot	2889	1465	rGrG+G	1	SSRTII	12	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	yes	Advantage	50	-	-					
170	1	15	15	1:5	in house prot	3653	1735	rGrG+G	1	SSRTII	12	90´@42° C, then 10x (2´@50°C	1	yes	Advantage	50	-	-					

												- 2´@42°C)					
171	1	15	15	1:5	in house prot	2152	1455	rGrG+G	1	SSRTII	12	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	yes	Advantage	50	-	-
172	1	15	15	1:5	in house prot	1454	1084	rGrG+G	1	SSRTII	12	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	yes	Advantage	50	-	0.816M 1,2 propandiol
173	1	15	15	1:5	in house prot	1331	1106	rGrG+G	1	SSRTII	12	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	yes	Advantage	50	-	0.816M 1,2 propandiol
174	1	15	15	1:5	in house prot	1373	1089	rGrG+G	1	SSRTII	12	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	yes	Advantage	50	-	0.816M 1,2 propandiol
175	1	15	15	1:5	in house prot	1904	1453	rGrG+G	1	SSRTII	12	90´@42° C, then 10x (2´@50°C - 2´@42°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	90´@42° C, then 10x (2´@50°C - 2´@42°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	-	-

181					in house prot	FAILED		rGrG+G	1	Maxima H-	12	90´@50°C	1	yes	Advantage	50	-	-
182					in house prot	FAILED		rGrG+G	1	Maxima H-	12	90´@50°C	1	yes	Advantage	50	-	-
183	1	15	15	1:5	in house prot	2633	1341	rGrG+G	1	Revertaid Premium	12	90´@50°C	1	yes	Advantage	50	-	-
184	1	15	15	1:5	in house prot	2662	1325	rGrG+G	1	Revertaid Premium	12	90´@50°C	1	yes	Advantage	50	-	-
185	1	15	15	1:5	in house prot	2814	1674	rGrG+G	1	SSRTII	12	90´@42°C, then 10x (2´@50°C - 2´@42°C)	1	yes	Advantage	50	-	-
186					in house prot	FAILED		rGrG+G	1	SSRTII	12	90´@42°C, then 10x (2´@50°C - 2´@42°C)	1	yes	Long PCR Enzyme mix	50	-	-
187	1	15	15	1:5	in house prot, PCR w/o purif in 100ul	3155	1764	rGrG+G	1	SSRTII	12	90´@42°C, then 10x (2´@50°C - 2´@42°C)	1	-	Phusion HS	100	-	-
188	1	15	15	1:5	in house prot, PCR w/o purif in 100ul	2740	1793	rGrG+G	1	SSRTII	12	90´@42°C, then 10x (2´@50°C - 2´@42°C)	1	-	Phusion HS	100	-	-
189	1	15	15	1:5	in house prot, PCR w/o purif in 100ul	1857	1739	rGrG+G	1	SSRTII	12	90´@42°C, then 10x (2´@50°C - 2´@42°C)	1	-	Q5 NEB	100	-	-
190	1	15	15	1:5	in house prot, PCR w/o purif in 100ul	1697	1697	rGrG+G	1	SSRTII	12	90´@42°C, then 10x (2´@50°C - 2´@42°C)	1	-	Q5 NEB	100	-	-
191	1	15	15	1:5	in house prot, PCR w/o purif in 100ul	3278	1639	rGrG+G	1	SSRTII	12	90´@42°	1	-	KAPA HiFi HS	100	-	-

192	1	15	15	1:5	in house prot, PCR w/o purif in 100ul	3719	1590	rGrG+G	1	SSRTII	12	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	-	KAPA HiFi HS	100	-	-
193	1	15	15	1:5	in house prot, PCR w/o purif in 50ul	3816	1630	rGrG+G	1	SSRTII	12	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	-	Advantage	50	-	-
194	1	15	15	1:5	in house prot, PCR w/o purif in 50ul	2462	1653	rGrG+G	1	SSRTII	12	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	-	Advantage	50	-	-
195	1	15	15	1:5	in house prot, PCR w/o purif in 50ul	2848	1588	rGrG+G	1	SSRTII	12	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	-	Advantage	50	-	-
196	1	15	15	1:5	in house prot, PCR w/o purif in 50ul	2012	1669	rGrG+G	1	SSRTII	12	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	-	Phusion HS	50	-	-
197	1	15	15	1:5	in house prot, PCR w/o purif in 50ul	1836	1665	rGrG+G	1	SSRTII	12	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	-	Phusion HS	50	-	-
198	1	15	15	1:5	in house prot, PCR w/o purif in 50ul	2542	1771	rGrG+G	1	SSRTII	12	90´@42° C, then 10x (2´@50°C	1	-	KAPA HiFi HS	50	-	-

												- 2´@42°C)						
199	1	15	15	1:5	in house prot, PCR w/o purif in 50ul	2393	1808	rGrG+G	1	SSRTII	12	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	-	KAPA HiFi HS	50	-	-
200	1	15	15	1:5	in house prot, PCR w/o purif in 50ul	2200	1933	rGrG+G	1	SSRTII	12	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	-	Advantage	50	-	-
201	1	15	15	1:5	in house prot, PCR w/o purif in 50ul	2371	1920	rGrG+G	1	SSRTII	12	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	-	Advantage	50	-	-
202	1	15	15	1:5	in house prot, PCR w/o purif in 50ul	1717	1780	rGrG+G	1	SSRTII	12	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	-	Q5 NEB	50	-	-
203	1	15	15	1:5	in house prot, PCR w/o purif in 50ul	1868	1759	rGrG+G	1	SSRTII	12	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	-	Q5 NEB	50	-	-
204	1	15	15	1:5	in house prot	40	1772	rGrG+G	1	SSRTIII	12	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	yes	Advantage	50	-	-
205	1	15	15	1:5	in house prot	16	1017	rGrG+G	1	SSRTIII	12	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	yes	Advantage	50	-	-
206	1	15	15	1:5	in house prot	88	175	rGrG+G	1	SSRTIII	12		1	ye	Advantag	50	-	-

							3					90´@42°C, then 10x (2´@50°C - 2´@42°C)		s	e			
207	1	15	15	1:5	in house prot	61	1557	rGrG+G	1	SSRTIII	12	90´@42°C, then 10x (2´@50°C - 2´@42°C)	1	yes	Advantage	50	-	-
208	1	15	15	1:5	in house prot	182	1766	rGrG+G	1	SSRTIII	12	90´@42°C, then 10x (2´@50°C - 2´@42°C)	1	yes	Advantage	50	-	-
209	1	15	15	1:5	in house prot	136	1742	rGrG+G	1	SSRTIII	12	90´@42°C, then 10x (2´@50°C - 2´@42°C)	1	yes	Advantage	50	-	-
210	1	15	15	1:5	in house prot	279	1701	rGrG+G	1	SSRTIII	12	90´@42°C, then 10x (2´@50°C - 2´@42°C)	1	yes	Advantage	50	-	-
211	1	15	15	1:5	in house prot	233	1643	rGrG+G	1	SSRTIII	12	90´@42°C, then 10x (2´@50°C - 2´@42°C)	1	yes	Advantage	50	-	-
212	1	15	15	1:5	in house prot	447	1602	rGrG+G	1	SSRTII	12	90´@42°C, then 10x (2´@50°C - 2´@42°C)	1	yes	Advantage	50	-	-
213	1	15	15	1:5	in house prot	363	1541	rGrG+G	1	SSRTII	12	90´@42°C, then 10x	1	yes	Advantage	50	-	-

214	1	15	15	1:5	in house prot	1279	1919	rGrG+G	1	SSRTII	12	(2´@50°C - 2´@42°C)	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	2009	rGrG+G	1	SSRTII	12	90´@42° C, then 10x (2´@50°C - 2´@42°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	1963	rGrG+G	1	SSRTII	12	90´@42° C, then 10x (2´@50°C - 2´@42°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	1316	rGrG+G	1	SSRTII	12	90´@42° C, then 10x (2´@50°C - 2´@42°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	1292	rGrG+G	1	SSRTII	12	90´@42° C, then 10x (2´@50°C - 2´@42°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	1297	rGrG+G	1	SSRTII	12	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	yes	Advantage	50	yes	-		
226	1	15	15	1:5	in house prot	1604	1925	rGrG+G	1	SSRTII	12	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	yes	Advantage	50	yes	-		
227	1	15	15	1:5	in house prot	1493	1910	rGrG+G	1	SSRTII	12	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	yes	Advantage	50	yes	-		
228	1	15	15	1:5	in house prot	1540	1924	rGrG+G	1	SSRTII	12	90´@42° C, then	1	yes	Advantage	50	yes	-		

												10x (2´@50°C - 2´@42°C)						
229	1	15	15	1:5	in house prot	1946	2143	rGrG+G	1	SSRTII	12	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	yes	Advantage	50	yes	-
230	1	15	15	1:5	in house prot	1620	2100	rGrG+G	1	SSRTII	12	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	yes	Advantage	50	yes	-
231	1	15	15	1:5	in house prot	1724	2059	rGrG+G	1	SSRTII	12	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	yes	Advantage	50	yes	-
232	1	15	15	1:5	in house prot (no denaturation step @72°C for oligo-dT (incubated 5´@RT))	1355	1813	rGrG+G	1	SSRTII	12	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	yes	Advantage	50	-	-
233	1	15	15	1:5	in house prot (no denaturation step @72°C for oligo-dT (incubated 5´@RT))	1330	1810	rGrG+G	1	SSRTII	12	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	yes	Advantage	50	-	-
234	1	15	15	1:5	in house prot (no denaturation step @72°C for oligo-dT (incubated 5´@RT))	1319	1795	rGrG+G	1	SSRTII	12	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	yes	Advantage	50	-	-
235	1	15	15	1:5	in house prot (no denaturation step @72°C for oligo-dT (incubated 5´@RT))	1493	1842	rGrG+G	1	SSRTII	12	90´@42° C, then 10x (2´@50°C -	1	yes	Advantage	50	yes	-

236	1	15	15	1:5	in house prot (no denaturation step @72°C for oligo-dT (incubated 5´@RT))	1332	1792	rGrG+G	1	SSRTII	12	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	2214	rGrG+G	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	2283	rGrG+G	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	2260	rGrG+G	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	2171	rGrG+G	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	2281	rGrG+G	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	2231	rGrG+G	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	2176	rGrG+G	1	SSRTII	12	90´@42°	1	yes	Advantage	50	yes	-							

244	1	15				in house prot	1385	2168	rGrG+G	1	SSRTII	12	90´@42° C, then 10x (2´@60°C - 2´@42°C)	1	yes	Advantage	50	yes	-				
245	1	15	15	1:5		in house prot	1588	2173	rGrG+G	1	SSRTII	12	90´@42° C, then 10x (2´@60°C - 2´@42°C)	1	yes	Advantage	50	yes	-				
246	1	15	15	1:5		in house prot	1332	2096	rGrG+G	1	SSRTII	12	90´@ 42°C	1	yes	Advantage	50	yes	-				
247	1	15	15	1:5		in house prot	1376	2054	rGrG+G	1	SSRTII	12	90´@ 42°C	1	yes	Advantage	50	yes	-				
248	1	15	15	1:5		in house prot	15	2251	rGrG+G	1	Maxima H-	4	90´@ 55°C	-	yes	Advantage	50	yes	-				
249	1	15	15	1:5		in house prot	22	1988	rGrG+G	1	Maxima H-	4	90´@ 55°C	-	yes	Advantage	50	yes	-				
250	1	15	15	1:5		in house prot	62	2084	rGrG+G	1	Maxima H-	4	90´@ 55°C	1	yes	Advantage	50	yes	-				
251	1	15	15	1:5		in house prot	84	1867	rGrG+G	1	Maxima H-	4	90´@ 55°C	1	yes	Advantage	50	yes	-				
252	1	15	15	1:5		in house prot	633	2065	rGrG+G	1	Maxima H-	4	90´@ 55°C	-	yes	Advantage	50	yes	-				
253						in house prot (+extra DTT)	FAILED		rGrG+G	1	Maxima H-	4	90´@ 60°C	-	yes	Advantage	50	yes	-				
254						in house prot (+extra DTT)	FAILED		rGrG+G	1	Maxima H-	4	90´@ 60°C	1	yes	Advantage	50	yes	-				
255						in house prot (+extra DTT)	FAILED		rGrG+G	1	Maxima H-	12	90´@ 60°C	1	yes	Advantage	50	yes	-				
256	1	15	15	1:5		in house prot (+extra DTT)	80	1933	rGrG+G	1	Maxima H-	12	90´@ 60°C	1	yes	Advantage	50	yes	-				
257	1	15	15	1:5		in house prot (+extra DTT)	634	2177	rGrG+G	1	Maxima H-	4	90´@ 50°C	-	yes	Advantage	50	yes	-				
258	1	15	15	1:5		in house prot (+extra DTT)	684	2195	rGrG+G	1	Maxima H-	4	90´@ 50°C	-	yes	Advantage	50	yes	-				
259	1	15	15	1:5		in house prot (+extra DTT)	1013	2142	rGrG+G	1	Maxima H-	4	90´@ 50°C	1	yes	Advantage	50	yes	-				
260	1	15	15	1:5		in house prot (+extra DTT)	1195	2100	rGrG+G	1	Maxima H-	4	90´@ 50°C	1	yes	Advantage	50	yes	-				
261	1	15	15	1:5		in house prot (+extra DTT)	2192	1858	rGrG+G	1	Maxima H-	12	90´@ 50°C	1	yes	Advantage	50	yes	-				

262	1	15	15	1:5	in house prot (+extra DTT)	2209	1837	rGrG+G	1	Maxima H-	12	90´@ 50°C	1	yes	Advantage	50	yes	-
263	1	15	15	1:5	in house prot (NO extra DTT)	647	2117	rGrG+G	1	Maxima H-	4	90´@ 50°C	-	yes	Advantage	50	yes	-
264	1	15	15	1:5	in house prot (NO extra DTT)	613	2147	rGrG+G	1	Maxima H-	4	90´@ 50°C	-	yes	Advantage	50	yes	-
265	1	15	15	1:5	in house prot	1442	1989	rGrG+G	1	SSRTII	12	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	yes	Advantage	50	yes	-
266	1	15	15	1:5	in house prot	1496	2105	rGrG+G	1	SSRTII	12	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	yes	Advantage	50	yes	-
267	1	15	15	1:5	in house prot	1228	2677	rGrG+G	1	Maxima H-	4	90´@ 50°C	-	-	KAPA HiFi HS	50	yes	-
268	1	15	15	1:5	in house prot	882	2283	rGrG+G	1	Maxima H-	4	90´@ 50°C	-	-	Advantage	50	yes	-
269	1	15	15	1:5	in house prot	2475	2558	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	2201	rGrG+G	1	Maxima H-	4	90´@ 50°C	1	-	Advantage	50	yes	-
271	1	15	15	1:5	in house prot	4074	2185	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	2330	rGrG+G	1	Maxima H-	12	90´@ 50°C	1	-	Advantage	50	yes	-
273	1	15	15	1:5	in house prot	3213	2524	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	2662	rGrG+G	1	SSRTII	12	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	-	Advantage	50	yes	-
275	1	15	15	1:5	in house prot	819	2069	rGrG+G	1	Maxima H-	4	90´@ 50°C	-	yes	Advantage	50	yes	-
276	1	15	15	1:5	in house prot	737	2202	rGrG+G	1	Maxima H-	4	90´@ 50°C	-	yes	Advantage	50	yes	-
277	1	15	15	1:5	in house prot	1375	1937	rGrG+G	1	Maxima H-	4	90´@ 50°C	1	yes	Advantage	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

												(2´@50°C - 2´@42°C)				
288	1	15	15	1:5	in house prot	642	222 6	rGrG+G	1	SSRTII	12	90´@42° C, then 10x (2´@50°C - 2´@42°C)	-	ye s	Advantag e	50	yes	1M proline
289	1	15	15	1:5	in house prot	1133	187 3	rGrG+G	1	SSRTII	12	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	ye s	Advantag e	50	yes	
290	1	15	15	1:5	in house prot	1443	214 8	rGrG+G	1	SSRTII	12	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	ye s	Advantag e	50	yes	
291	1	15	15	1:5	in house prot	1147	206 6	rGrG+G	1	SSRTII	12	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	ye s	Advantag e	50	yes	
292	1	15	15	1:5	in house prot	1322	208 4	rGrG+G	1	SSRTII	12	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	ye s	Advantag e	50	yes	
293	1	15	15	1:5	in house prot	1013	209 5	rGrG+G	1	SSRTII	12	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	ye s	Advantag e	50	yes	
294	1	15.	15	1:5	in house prot	960	212 5	rGrG+G	1	SSRTII	12	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	ye s	Advantag e	50	yes	

295	1				in house prot with SMARTer dT30 (unanchored oligo)	1296	2129	rGrG+G	1	SSRTII	12	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	yes	Advantage	50	yes				
296	1	15	15	1:50	in house prot with SMARTer dT30 (unanchored oligo)	1390	2194	rGrG+G	1	SSRTII	12	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	yes	Advantage	50	yes				
297	1	15	15	1:50	in house prot with SMARTer dT30 (unanchored oligo)	1419	2084	rGrG+G	1	SSRTII	12	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	yes	Advantage	50	yes				
298	1	18	15	1:20	in house prot	5456	2293	rGrG+G	2	SSRTII	12	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	yes	Advantage	50	yes				
299	1	18	15	1:20	in house prot	5753	2334	rGrG+G	2	SSRTII	12	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	yes	Advantage	50	yes				
300	1	18	15	1:20	in house prot	5341	2365	rGrG+G	2	SSRTII	12	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	yes	Advantage	50	yes				
301	1	18	15	1:20	in house prot	2266	1750	rGrG+G	2	SSRTII	12	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	yes	Advantage	50	yes				
302	1	18	15	1:20	in house prot	1530	1850	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	90´@42°C, then 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 -	1	yes	Advantage	50	yes							

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

318					in house prot	925	222 4	rGrG+G	1	SSRTII	12	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	-	KAPA HiFi HS	50	yes					
319	1	15	15	1:8	in house prot	790	232 6	rGrG+G	1	SSRTII	12	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	-	KAPA HiFi HS	50	yes						
320	1	15	15	1:9	in house prot	9	286 6	rGrG+G	1	SSRTII	12	30´@42° C, 10´@50° C, 10´@55° C	1	-	KAPA HiFi HS	50	yes	1M sorbitol + 0.3M trehalose					
321	1	15	15	1:10	in house prot	17	264 8	rGrG+G	1	SSRTII	12	30´@42° C, 10´@50° C, 10´@55° C	1	-	KAPA HiFi HS	50	yes	1M sorbitol + 0.3M trehalose					
322	1	15	15	1:11	in house prot	18	274 4	rGrG+G	1	SSRTII	12	30´@42° C, 10´@50° C, 10´@55° C	1	-	KAPA HiFi HS	50	yes	1M sorbitol + 0.3M trehalose					
323	1	15	15	1:12	in house prot	14	284 4	rGrG+G	1	SSRTII	12	30´@42° C, 10´@50° C, 10´@55° C	1	-	KAPA HiFi HS	50	yes	1M sorbitol + 0.3M trehalose					
324	1	15	15	1:13	in house prot	24	254 6	rGrG+G	1	SSRTII	12	30´@42° C, 10´@50° C,	1	-	KAPA HiFi HS	50	yes	0.75M sorbitol + 0.15M trehalose					

325	1	15	15	1:14	in house prot	30	2478	rGrG+G	1	SSRTII	12	30´@42° C, 10´@50° C, 10´@55° C	1	-	KAPA HiFi HS	50	yes	0.75M sorbitol + 0.15M trehalose							
326	1	15	15	1:15	in house prot	40	2609	rGrG+G	1	SSRTII	12	30´@42° C, 10´@50° C, 10´@55° C	1	-	KAPA HiFi HS	50	yes	0.75M sorbitol + 0.15M trehalose							
327	1	18	15	1:10	in house prot	3197	2061	rGrG+G	1	SSRTII	15	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	-	KAPA HiFi HS	50	yes								
328	1	18	15	1:10	in house prot	3085	2065	rGrG+G	1	SSRTII	15	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	-	KAPA HiFi HS	50	yes								
329	1	18	15	1:10	in house prot	2627	2134	rGrG+G	1	SSRTII	15	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	-	KAPA HiFi HS	50	yes								
330	1	18	15	1:10	in house prot	2750	2035	rGrG+G	1	SSRTII	15	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	-	KAPA HiFi HS	50	yes								
331	1	18	15	1:10	in house prot	980	1920	rGrG+G	1	SSRTII	3	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	-	KAPA HiFi HS	50	yes								
332	1	18	15	1:1	in house prot	997	186	rGrG+G	1	SSRTII	3		1	-	KAPA	50	yes								

				0			3					90´@42° C, then 10x (2´@50°C - 2´@42°C)			HiFi HS			
333	1	18	15	1:1 0	in house prot	1055	192 5	rGrG+G	1	SSRTII	3	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	-	KAPA HiFi HS	50	yes	
334	1	18	15	1:1 0	in house prot	938	193 1	rGrG+G	1	SSRTII	3	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	-	KAPA HiFi HS	50	yes	
335	1	18	15	1:1 0	in house prot	2192	211 9	rGrG+G	1	SSRTII	6	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	-	KAPA HiFi HS	50	yes	
336	1	18	15	1:1 0	in house prot	2122	204 6	rGrG+G	1	SSRTII	6	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	-	KAPA HiFi HS	50	yes	
337	1	18	15	1:1 0	in house prot	2408	207 9	rGrG+G	1	SSRTII	6	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	-	KAPA HiFi HS	50	yes	
338	1	18	15	1:1 0	in house prot	1836	210 9	rGrG+G	1	SSRTII	6	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	-	KAPA HiFi HS	50	yes	
339	1	18	15	1:1 0	in house prot	2861	209 8	rGrG+G	1	SSRTII	9	90´@42° C, then 10x	1	-	KAPA HiFi HS	50	yes	

340	1	18	15	1:10	in house prot	2518	2086	rGrG+G	1	SSRTII	9	90´@42°C, then 10x (2´@50°C - 2´@42°C)	1	-	KAPA HiFi HS	50	yes						
341	1	18	15	1:10	in house prot	2289	2135	rGrG+G	1	SSRTII	9	90´@42°C, then 10x (2´@50°C - 2´@42°C)	1	-	KAPA HiFi HS	50	yes						
342	1	18	15	1:10	in house prot	2553	2168	rGrG+G	1	SSRTII	9	90´@42°C, then 10x (2´@50°C - 2´@42°C)	1	-	KAPA HiFi HS	50	yes						
343	1	18	15	1:10	in house prot	2571	2134	rGrG+G	1	SSRTII	12	90´@42°C, then 10x (2´@50°C - 2´@42°C)	1	-	KAPA HiFi HS	50	yes						
344	1	18	15	1:10	in house prot	2691	2115	rGrG+G	1	SSRTII	12	90´@42°C, then 10x (2´@50°C - 2´@42°C)	1	-	KAPA HiFi HS	50	yes						
345	1	18	15	1:10	in house prot	2348	2121	rGrG+G	1	SSRTII	12	90´@42°C, then 10x (2´@50°C - 2´@42°C)	1	-	KAPA HiFi HS	50	yes						
346	1	18	15	1:10	in house prot	2046	2162	rGrG+G	1	SSRTII	12	90´@42°C, then 10x (2´@50°C - 2´@42°C)	1	-	KAPA HiFi HS	50	yes						

347	1	15	15	1:5	in house prot	37	1918	rGrG+G	1	SSRTII	3	90´@42° C, then 10x (2´@50°C - 2´@42°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 - 2´@42°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 - 2´@42°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 - 2´@42°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 - 2´@42°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 - 2´@42°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 - 2´@42°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						

355	1	15	15	1:5	in house prot	348	1753	rGrG+G	1	SSRTII	6	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	-	KAPA HiFi HS	25	yes								
356	1	15	15	1:5	in house prot	204	1734	rGrG+G	1	SSRTII	6	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	-	KAPA HiFi HS	25	yes								
357	1	15	15	1:5	in house prot	210	1840	rGrG+G	1	SSRTII	6	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	-	KAPA HiFi HS	25	yes								
358	1	15	15	1:5	in house prot	207	1853	rGrG+G	1	SSRTII	6	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	-	KAPA HiFi HS	25	yes								
359	1	15	15	1:5	in house prot	285	1801	rGrG+G	1	SSRTII	6	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	-	KAPA HiFi HS	25	yes								
360	1	15	15	1:5	in house prot	120	1942	rGrG+G	1	SSRTII	6	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	-	KAPA HiFi HS	25	yes								
361	1	15	15	1:5	in house prot	201	1732	rGrG+G	1	SSRTII	6	90´@42° C, then 10x (2´@50°C -	1	-	KAPA HiFi HS	25	yes								

362	1	15	15	1:5	in house prot	395	1808	rGrG+G	1	SSRTII	9	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	-	KAPA HiFi HS	25	yes							
363	1	15	15	1:5	in house prot	558	1887	rGrG+G	1	SSRTII	9	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	-	KAPA HiFi HS	25	yes							
364	1	15	15	1:5	in house prot	429	1792	rGrG+G	1	SSRTII	9	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	-	KAPA HiFi HS	25	yes							
365	1	15	15	1:5	in house prot	340	1779	rGrG+G	1	SSRTII	9	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	-	KAPA HiFi HS	25	yes							
366	1	15	15	1:5	in house prot	511	1797	rGrG+G	1	SSRTII	9	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	-	KAPA HiFi HS	25	yes							
367	1	15	15	1:5	in house prot	353	1783	rGrG+G	1	SSRTII	9	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	-	KAPA HiFi HS	25	yes							
368	1	15	15	1:5	in house prot	365	1785	rGrG+G	1	SSRTII	9	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	-	KAPA HiFi HS	25	yes							
369	1	15	15	1:5	in house prot	333	1840	rGrG+G	1	SSRTII	9	90´@42°	1	-	KAPA HiFi HS	25	yes							

												C, then 10x (2´@50°C - 2´@42°C)						
370	1	15	15	1:5	in house prot	477	1818	rGrG+G	1	SSRTII	12	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	-	KAPA HiFi HS	25	yes	
371	1	15	15	1:5	in house prot	547	1754	rGrG+G	1	SSRTII	12	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	-	KAPA HiFi HS	25	yes	
372	1	15	15	1:5	in house prot	265	1917	rGrG+G	1	SSRTII	12	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	-	KAPA HiFi HS	25	yes	
373	1	15	15	1:5	in house prot	349	1815	rGrG+G	1	SSRTII	12	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	-	KAPA HiFi HS	25	yes	
374	1	15	15	1:5	in house prot	255	1763	rGrG+G	1	SSRTII	12	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	-	KAPA HiFi HS	25	yes	
375	1	15	15	1:5	in house prot	388	1760	rGrG+G	1	SSRTII	12	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	-	KAPA HiFi HS	25	yes	
376	1	15	15	1:5	in house prot	387	1816	rGrG+G	1	SSRTII	12	90´@42° C, then 10x (2´@50°C	1	-	KAPA HiFi HS	25	yes	

377	1	15	15	1:5	in house prot	635	1928	rGrG+G	1	SSRTII	15	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	-	KAPA HiFi HS	25	yes						
378	1	15	15	1:5	in house prot	515	1873	rGrG+G	1	SSRTII	15	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	-	KAPA HiFi HS	25	yes						
379	1	15	15	1:5	in house prot	658	1989	rGrG+G	1	SSRTII	15	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	-	KAPA HiFi HS	25	yes						
380	1	15	15	1:5	in house prot	602	1905	rGrG+G	1	SSRTII	15	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	-	KAPA HiFi HS	25	yes						
381	1	15	15	1:5	in house prot	412	1896	rGrG+G	1	SSRTII	15	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	-	KAPA HiFi HS	25	yes						
382	1	15	15	1:5	in house prot	476	1958	rGrG+G	1	SSRTII	15	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	-	KAPA HiFi HS	25	yes						
383	1	15	15	1:5	in house prot	422	1853	rGrG+G	1	SSRTII	15	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	-	KAPA HiFi HS	25	yes						
384	1	15	15	1:5	in house prot	551	1876	rGrG+G	1	SSRTII	15		1	-	KAPA	25	yes						

												90´@42° C, then 10x (2´@50°C - 2´@42°C)			HiFi HS			
385	1	15	15	1:5	in house prot	1736	1698	rGrG+G	1	SSRTII	20	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	-	KAPA HiFi HS	25	yes	
386	1	15	15	1:5	in house prot	1294	1750	rGrG+G	1	SSRTII	20	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	-	KAPA HiFi HS	25	yes	
387	1	15	15	1:5	in house prot	1160	1736	rGrG+G	1	SSRTII	20	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	-	KAPA HiFi HS	25	yes	
388	1	15	15	1:5	in house prot	1245	1786	rGrG+G	1	SSRTII	20	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	-	KAPA HiFi HS	25	yes	
389	1	15	15	1:5	in house prot	1234	1733	rGrG+G	1	SSRTII	25	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	-	KAPA HiFi HS	25	yes	
390	1	15	15	1:5	in house prot	1654	1684	rGrG+G	1	SSRTII	25	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	-	KAPA HiFi HS	25	yes	
391	1	15	15	1:5	in house prot	1327	1696	rGrG+G	1	SSRTII	25	90´@42° C, then 10x	1	-	KAPA HiFi HS	25	yes	

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

407	1	15	15	1:5	in house prot	131	2221	rGrG+G	1	SSRTII	12	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	-	KAPA HiFi HS	25	yes						
408	1	15	15	1:5	in house prot	149	2083	rGrG+G	1	SSRTII	12	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	-	KAPA HiFi HS	25	yes						
409	1	15	15	1:5	in house prot	169	2105	rGrG+G	1	SSRTII	15	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	-	KAPA HiFi HS	25	yes						
410	1	15	15	1:5	in house prot	195	2020	rGrG+G	1	SSRTII	15	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	-	KAPA HiFi HS	25	yes						
411	1	15	15	1:5	in house prot	141	2042	rGrG+G	1	SSRTII	15	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	-	KAPA HiFi HS	25	yes						
412	1	15	15	1:5	in house prot	196	2077	rGrG+G	1	SSRTII	15	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	-	KAPA HiFi HS	25	yes						
413	1	15	15	1:5	in house prot	123	2133	rGrG+G	1	SSRTII	20	90´@42° C, then 10x (2´@50°C - 2´@42°C)	1	-	KAPA HiFi HS	25	yes						

																	2´@42°C)						
414	1	15	15	1:5	in house prot	136	2042	rGrG+G	1	SSRTII	20	90´@42° C, then 10x (2´@50°C - 2´@42°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 - 2´@42°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 - 2´@42°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 - 2´@42°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 - 2´@42°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 - 2´@42°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 - 2´@42°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						

422	1	15	15	1:5	in house prot	280	2402	rGrG+G	1	SSRTII	6	90´@42° C, then 10x (2´@50°C - 2´@42°C)	-	-	KAPA HiFi HS	25	yes		
423	1	15	15	1:5	in house prot	270	2449	rGrG+G	1	SSRTII	6	90´@42° C, then 10x (2´@50°C - 2´@42°C)	-	-	KAPA HiFi HS	25	yes		
424	1	15	15	1:5	in house prot	249	2557	rGrG+G	1	SSRTII	6	90´@42° C, then 10x (2´@50°C - 2´@42°C)	-	-	KAPA HiFi HS	25	yes		
425	1	15	15	1:5	in house prot	423	1680	rGrG+G	1	SSRTII	9	90´@42° C, then 10x (2´@50°C - 2´@42°C)	-	-	KAPA HiFi HS	25	yes		
426	1	15	15	1:5	in house prot	433	2537	rGrG+G	1	SSRTII	9	90´@42° C, then 10x (2´@50°C - 2´@42°C)	-	-	KAPA HiFi HS	25	yes		
427	1	15	15	1:5	in house prot	504	2571	rGrG+G	1	SSRTII	9	90´@42° C, then 10x (2´@50°C - 2´@42°C)	-	-	KAPA HiFi HS	25	yes		
428	1	15	15	1:5	in house prot	483	2002	rGrG+G	1	SSRTII	9	90´@42° C, then 10x (2´@50°C	-	-	KAPA HiFi HS	25	yes		

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

437	1	15	15	1:5	in house prot	831	2233	rGrG+G	1	SSRTII	12	90´@42° C, then 10x (2´@50°C - 2´@42°C)	-	-	KAPA HiFi HS	25	yes			
438	1	15	15	1:5	in house prot	780	2468	rGrG+G	1	SSRTII	12	90´@42° C, then 10x (2´@50°C - 2´@42°C)	-	-	KAPA HiFi HS	25	yes			
439	1	15	15	1:5	in house prot	41	2536	rGrG+G	1	SSRTII	6	90´@42° C, then 10x (2´@50°C - 2´@42°C)	-	-	KAPA HiFi HS	25	yes			
440	1	15	15	1:5	in house prot	28	2646	rGrG+G	1	SSRTII	6	90´@42° C, then 10x (2´@50°C - 2´@42°C)	-	-	KAPA HiFi HS	25	yes			
441	1	15	15	1:5	in house prot	44	2702	rGrG+G	1	SSRTII	6	90´@42° C, then 10x (2´@50°C - 2´@42°C)	-	-	KAPA HiFi HS	25	yes			
442	1	15	15	1:5	in house prot	30	2504	rGrG+G	1	SSRTII	6	90´@42° C, then 10x (2´@50°C - 2´@42°C)	-	-	KAPA HiFi HS	25	yes			
443	1	15	15	1:5	in house prot	47	2664	rGrG+G	1	SSRTII	9	90´@42° C, then 10x	-	-	KAPA HiFi HS	25	yes			

444	1	15	15	1:5	in house prot	54	2652	rGrG+G	1	SSRTII	9	90´@42° C, then 10x (2´@50°C - 2´@42°C)	-	-	KAPA HiFi HS	25	yes		
445	1	15	15	1:5	in house prot	70	2502	rGrG+G	1	SSRTII	9	90´@42° C, then 10x (2´@50°C - 2´@42°C)	-	-	KAPA HiFi HS	25	yes		
446	1	15	15	1:5	in house prot	80	2555	rGrG+G	1	SSRTII	9	90´@42° C, then 10x (2´@50°C - 2´@42°C)	-	-	KAPA HiFi HS	25	yes		
447	1	15	15	1:5	in house prot	122	2374	rGrG+G	1	SSRTII	12	90´@42° C, then 10x (2´@50°C - 2´@42°C)	-	-	KAPA HiFi HS	25	yes		
448	1	15	15	1:5	in house prot	138	2368	rGrG+G	1	SSRTII	12	90´@42° C, then 10x (2´@50°C - 2´@42°C)	-	-	KAPA HiFi HS	25	yes		
449	1	15	15	1:5	in house prot	105	2441	rGrG+G	1	SSRTII	12	90´@42° C, then 10x (2´@50°C - 2´@42°C)	-	-	KAPA HiFi HS	25	yes		
450	1	15	15	1:5	in house prot	108	2377	rGrG+G	1	SSRTII	12	90´@42° C, then 10x (2´@50°C - 2´@42°C)	-	-	KAPA HiFi HS	25	yes		

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.

			cDNA Yield				cDNA length				
Experimental Variable Tested	**Variant 1**	**Variant 2**	**t-test Yield**	**Wilcoxon-test Yield**	**mean yield (Var1)**	**mean yield (Var2)**	**t-test cDNA length**	**wilcoxon-test cDNA length**	**mean length (Var1)**	**mean length (Var2)**	**Number of replicates**
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 concentration (mM)	0	3	5.27E-01	4.00E-01	65.95	82.975	9.97E-01	1.00E+00	1588.6667	1589	3
MgCl2 concentration	0	12	6.31E-01	1.00E+00	75.3375	107.2125	7.78E-01	1.00E+00	1511.5	1480	2

(mM)												
MgCl2 concentration (mM)	4	6	3.46E-01	6.67E-01	102.3	124.05		2.28E-01	3.33E-01	1517.5	1398	2
MgCl2 concentration (mM)	4	9	2.52E-02	3.33E-01	102.3	211.875		5.08E-01	6.67E-01	1517.5	1563.5	2
MgCl2 concentration (mM)	4	12	9.44E-05	1.30E-04	91.395	198.795		3.07E-01	2.47E-01	1918.8	1753.9	10
MgCl2 concentration (mM)	4	15	3.32E-01	3.33E-01	102.3	130.875		3.81E-01	6.67E-01	1517.5	1360	2
MgCl2 concentration (mM)	6	9	5.94E-01	6.86E-01	154.5938	178.1625		3.80E-03	2.86E-02	1390.75	1607	4
MgCl2 concentration (mM)	6	12	8.77E-01	7.30E-01	127.0167	132.9417		4.77E-01	2.58E-01	1709.7778	1845.8889	9
MgCl2 concentration (mM)	6	15	4.50E-01	8.41E-01	140.52	113.79		7.04E-01	5.48E-01	1443	1477	5
MgCl2 concentration (mM)	9	12	8.71E-01	6.86E-01	178.1625	170.8125		8.05E-01	8.86E-01	1607	1582.25	4
MgCl2 concentration (mM)	9	15	1.17E-01	2.00E-01	178.1625	124.7062		1.24E-01	2.00E-01	1607	1448	4
MgCl2 concentration	12	15	2.94E-01	3.43E-01	170.8125	124.7062		2.87E-01	3.43E-01	1582.25	1448	4

(mM)												
betaine concentration (M)	0	0.6	7.74E-01	6.86E-01	80.6063	73.725		3.70E-02	1.14E-01	1690.75	1540.5	4
betaine concentration (M)	0	1	1.61E-01	1.65E-01	51.93	81.2025		3.55E-01	2.18E-01	2127.8	2006.9	10
betaine concentration (M)	0.6	1	4.49E-01	1.00E+00	87.1	69.875		7.58E-01	1.00E+00	1618.3333	1596.6667	3
betaine concentration (M)	1	1.5	7.73E-01	3.43E-01	101.215	86.115		8.82E-01	6.86E-01	2409.5	2430.5	4
RT enzyme	Maxima H-	Revertaid Premium	1.92E-02	3.33E-01	192	15.6375		1.66E-01	3.33E-01	1696	1603.5	2
RT enzyme	Revertaid H-	SSRTII	2.47E-02	4.11E-02	148.2125	222.375		9.03E-02	1.32E-01	1423	1552.8333	6
RT enzyme	SMARTscribe	SSRTII	-	-	15.9	19.65		-	-	1669	1683	1
RT enzyme	SSRTII	SSRTIII	1.21E-06	1.55E-04	252.3094	9.7031		8.59E-01	6.74E-01	1637.125	1618.875	8
RT protocol	60@42C, then 90@60C	60@50C, then 90@42C	1.47E-01	3.33E-01	73.95	43.05		5.70E-01	1.00E+00	1628	1577.5	2
RT protocol	60@42C, then 90@60C	90@42C, 30@60C, then 30@42C	1.50E-01	3.33E-01	73.95	107.3625		3.70E-01	3.33E-01	1628	1719	2
RT protocol	60@50C, then 90@42C	90@42C, 30@60C, then 30@42C	5.90E-02	2.00E-01	55.7437	110.7375		1.58E-01	1.46E-01	1515	1629	4
RT protocol	90@42C	90@42C, then 10x (2@50C-2@42C)	3.09E-01	3.43E-01	173.1562	235.8		6.43E-01	4.86E-01	1877.5	1973	4
RT protocol	90@42C	90@42C, then 10x (2@55C-2@42C)	6.72E-02	3.33E-01	101.55	109.8375		1.90E-01	3.33E-01	2075	2226	2

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	259.275		-	-	1732	1525	1
RT protocol	90@42C, then 10x (2@60C-2@42C)	90@42C, then 5x (2@50C-2@42C)	-	-	286.2	306.075		-	-	1732	1706	1
RT protocol	90@42C, then 15x (2@50C-2@42C)	90@42C, then 20x (2@50C-2@42C)	2.94E-01	3.33E-01	278.1375	237.225		1.47E-01	3.33E-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	1.00E+00	300.3	291.25		2.30E-02	1.00E-01	1639.6667	1752.6667	3
RT protocol	90@42C, then 20x (2@50C-2@42C)	90@42C, then 5x (2@50C-2@42C)	1.90E-01	3.33E-01	237.225	297.7125		8.13E-02	3.33E-01	1467	1738.5	2
RT protocol	90@50C	90@55C	3.50E-03	7.94E-03	62.595	12.24		2.09E-01	1.51E-01	2146.2	2051	5
RT protocol	90@50C	90@60C	-	-	164.4	6		-	-	1858	1933	1
PCR enzyme	Advantage 2 Pol.	KAPA HiFi HS	6.44E-01	7.55E-01	186.6375	171.0125		5.18E-01	5.90E-01	1929.4167	2029.5	12
PCR enzyme	Advantage 2 Pol.	Phusion HS	4.33E-01	4.86E-01	212.3625	182.6813		8.11E-01	3.43E-01	1701	1722.75	4
PCR enzyme	Advantage 2 Pol.	Q5 NEB	5.85E-02	2.86E-02	212.3625	133.8562		6.29E-01	3.43E-01	1701	1743.75	4
PCR enzyme	KAPA HiFi HS	Phusion HS	2.60E-01	3.43E-01	223.725	182.6813		7.49E-01	8.86E-01	1702	1722.75	4
PCR enzyme	KAPA HiFi HS	Q5 NEB	2.99E-02	2.86E-02	223.725	133.8562		4.93E-01	8.86E-01	1702	1743.75	4
PCR enzyme	Phusion HS	Q5 NEB	1.25E-01	1.14E-01	182.6813	133.8562		5.99E-01	8.86E-01	1722.75	1743.75	4
purification.	0	yes	6.77E-01	6.05E-01	186.85	203.225		3.44E-01	3.87E-01	2022.2222	1875.4444	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 Amino	AAGCAGTGGTATCAACGCAGAGT ACATrGrGrG	-	-	3 Riboguanosines	Aminolink C6
ddC	AAGCAGTGGTATCAACGCAGAGT ACrGrGrGrGddC	-	-	4 Riboguanosines + 1 Dideoxycytosine	-
dGCG GG	AAGCAGTGGTATCAACGCAGAGT ACGCGGG	-	-	1 Guanosine + 1 Cytosine + 3 Guanosines	-
dGCG GGp	AAGCAGTGGTATCAACGCAGAGT ACGCGGG	-	-	1 Guanosine + 1 Cytosine + 3 Guanosines	Phosphate
ISO	iGiCiGAAGCAGTGGTATCAACGCA GAGTACrGrCrGrGrG	Methyl C5	isoGuanosine-isoCytosine-isoGuanosine	1 Riboguanosine + 1 Ribocytosine + 3 Riboguanosines	Phosphate
rGrG+G	AAGCAGTGGTATCAACGCAGAGT ACrGrG+G	-	-	2 Riboguanosines + 1 LNA-modified Guanosine	-
rGrG+N	AAGCAGTGGTATCAACGCAGAGT ACrGrG+N	-	-	2 Riboguanosines + 1 LNA-modified nucleotide (any)	-
+G+G+G	AAGCAGTGGTATCAACGCAGAGT AC+G+G+G	-	-	3 LNA-modified Guanosines	-
rG+G+G	AAGCAGTGGTATCAACGCAGAGT ACrG+G+G	-	-	2 LNA-modified Guanosines	-
rGrGrG	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 of 10uM solution in)	RT enzyme	oligo dT	MgCl2 (mM)	betaine (M)	purification after RT?	PCR enzyme	PCR rxn vol (ul)	notes
HEK_2	HEK293T	*H.sapiens*	10.778	109	rGrG+G	1	SSRTII	SMARTer dT30VN	13	1	yes	Advantage	50	Could be a cell aggregate
HEK_3	HEK293T	*H.sapiens*	9.596	114	rGrG+G	1	SSRTII	SMARTer dT30VN	14	1	yes	Advantage	50	Could be a cell aggregate
HEK_4	HEK293T	*H.sapiens*	1.160	109	rGrG+G	1	SSRTII	SMARTer dT30VN	15	1	yes	Advantage	50	
HEK_5	HEK293T	*H.sapiens*	0.590	120	rGrG+G	1	SSRTII	SMARTer dT30VN	16	1	yes	Advantage	50	
HEK_6	HEK293T	*H.sapiens*	2.210	117	rGrG+G	1	SSRTII	SMARTer dT30VN	17	1	yes	Advantage	50	
HEK_7	HEK293T	*H.sapiens*	0.410	113	rGrG+G	1	SSRTII	SMARTer dT30VN	18	1	yes	Advantage	50	
HEK_8	HEK293T	*H.sapiens*	4.184	114	rGrG+G	1	SSRTII	SMARTer dT30VN	19	1	yes	Advantage	50	Could be a cell aggregate
HEK_9	HEK293T	*H.sapiens*	0.840	114	rGrG+G	1	SSRTII	SMARTer dT30VN	20	1	yes	Advantage	50	
HEK_10	HEK293T	*H.sapiens*	1.130	108	rGrG+G	1	SSRTII	SMARTer dT30VN	21	1	yes	Advantage	50	
HEK_12	HEK293T	*H.sapiens*	9.095	110	rGrG+G	1	SSRTII	SMARTer dT30VN	23	1	yes	Advantage	50	Could be a cell aggregate
HEK_13	HEK293T	*H.sapiens*	0.490	101	rGrG+G	1	SSRTII	SMARTer dT30VN	24	1	yes	Advantage	50	
HEK_14	HEK293T	*H.sapiens*	3.640	104	rGrG+G	1	SSRTII	SMARTer dT30VN	25	1	yes	Advantage	50	Could be a cell aggregate
HEK_16	HEK293T	*H.sapiens*	17.225	107	rGrG+G	1	SSRTII	SMARTer dT30VN	27	1	yes	Advantage	50	Could be a cell aggregate
HEK_SMRT_1	HEK293T	*H.sapiens*	0.760	142	SMARTer Oligo IIA	1	SMARTscribe	SMARTer dT30VN	6	1	yes	Advantage	50	
HEK_SMRT_2	HEK293T	*H.sapiens*	2.304	141	SMARTer Oligo IIA	1	SMARTscribe	SMARTer dT30VN	6	1	yes	Advantage	50	Could be a cell aggregate
HEK_SMRT_3	HEK293T	*H.sapiens*	1.544	142	SMARTer Oligo IIA	1	SMARTscribe	SMARTer dT30VN	6	1	yes	Advantage	50	
HEK_SMRT_4	HEK293T	*H.sapiens*	0.422	145	SMARTer Oligo IIA	1	SMARTscribe	SMARTer dT30VN	6	1	yes	Advantage	50	
HEK_18	HEK293T	*H.sapiens*	0.208	138	rGrGrG	1	SSRTII	SMARTer dT30VN	12	1	yes	Advantage	50	40u SSRT II

HEK_19	HEK293T	*H.sapiens*	0.512	154 1	rGrGrG	1	SSRTII	SMARTer dT30VN	12	1	yes	Advantage	50	40u SSRT II
HEK_20	HEK293T	*H.sapiens*	0.550	161 4	rGrGrG	1	SSRTII	SMARTer dT30VN	12	1	yes	Advantage	50	40u SSRT II
HEK_21	HEK293T	*H.sapiens*	0.240	149 2	rGrGrG	1	SSRTII	SMARTer dT30VN	12	1	yes	Advantage	50	40u SSRT II
HEK_22	HEK293T	*H.sapiens*	0.957	122 2	rGrGrG	1	SSRTII	SMARTer dT30VN	12	1	yes	Advantage	50	40u SSRT II
HEK_23	HEK293T	*H.sapiens*	0.872	139 2	rGrGrG	1	SSRTII	SMARTer dT30VN	12	1	yes	Advantage	50	40u SSRT II
HEK_24	HEK293T	*H.sapiens*	0.315	132 6	rGrGrG	1	SSRTII	SMARTer dT30VN	12	1	yes	Advantage	50	40u SSRT II
HEK_25	HEK293T	*H.sapiens*	0.462	147 4	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	yes	Advantage	50	
HEK_26	HEK293T	*H.sapiens*	0.248	144 1	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	yes	Advantage	50	
HEK_27	HEK293T	*H.sapiens*	0.244	148 7	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	yes	Advantage	50	
HEK_28	HEK293T	*H.sapiens*	0.262	149 6	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	yes	Advantage	50	
HEK_29	HEK293T	*H.sapiens*	0.761	140 2	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	yes	Advantage	50	
HEK_31	HEK293T	*H.sapiens*	0.594	158 3	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	yes	Advantage	50	
HEK_32	HEK293T	*H.sapiens*	0.457	134 7	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	yes	Advantage	50	
HEK_33	HEK293T	*H.sapiens*	0.473	150 7	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	yes	Advantage	50	
HEK_34	HEK293T	*H.sapiens*	0.315	150 1	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	yes	Advantage	50	
HEK_35	HEK293T	*H.sapiens*	0.744	191 7	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	Advantage	50	
HEK_37	HEK293T	*H.sapiens*	1.531	162 6	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	Advantage	50	
HEK_38	HEK293T	*H.sapiens*	0.344	187 0	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	Advantage	50	
HEK_39	HEK293T	*H.sapiens*	0.453	196 8	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	Advantage	50	
HEK_40	HEK293T	*H.sapiens*	0.580	195 8	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	Advantage	50	
HEK_41	HEK293T	*H.sapiens*	0.382	120 2	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	yes	Advantage	50	
HEK_42	HEK293T	*H.sapiens*	0.392	128 8	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	yes	Advantage	50	
HEK_43	HEK293T	*H.sapiens*	0.844	131 9	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	yes	Advantage	50	
HEK_44	HEK293T	*H.sapiens*	0.582	145 2	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	yes	Advantage	50	
HEK_45	HEK293T	*H.sapiens*	0.682	131 2	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	yes	Advantage	50	
HEK_46	HEK293	*H.sapiens*	0.692	141	rGrG+G	1	SSRTII	SMARTer	12	1	yes	Advantage	50	

	T			1				dT30VN			s			
HEK_47	HEK293 T	*H.sapiens*	0.716	1337	rGrGrG	1	SSRTII	SMARTer dT30VN	12	1	yes	Advantage	50	
HEK_48	HEK293 T	*H.sapiens*	0.578	1391	rGrGrG	1	SSRTII	SMARTer dT30VN	12	1	yes	Advantage	50	
HEK_49	HEK293 T	*H.sapiens*	2.681	1440	rGrGrG	1	SSRTII	SMARTer dT30VN	12	1	yes	Advantage	50	Could be a cell aggregate
HEK_50	HEK293 T	*H.sapiens*	1.158	1507	rGrGrG	1	SSRTII	SMARTer dT30VN	12	1	yes	Advantage	50	
HEK_51	HEK293 T	*H.sapiens*	0.710	1389	rGrGrG	1	SSRTII	SMARTer dT30VN	12	1	yes	Advantage	50	
HEK_52	HEK293 T	*H.sapiens*	1.050	1463	rGrGrG	1	SSRTII	SMARTer dT30VN	12	1	yes	Advantage	50	
HEK_53	HEK293 T	*H.sapiens*	0.714	1384	rGrGrG	1	SSRTII	SMARTer dT30VN	12	1	yes	Advantage	50	
HEK_54	HEK293 T	*H.sapiens*	0.637	1509	rGrGrG	1	SSRTII	SMARTer dT30VN	12	1	yes	Advantage	50	
HEK_55	HEK293 T	*H.sapiens*	1.338	1873	rGrGrG	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
HEK_56	HEK293 T	*H.sapiens*	1.258	1846	rGrGrG	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
HEK_57	HEK293 T	*H.sapiens*	3.012	1798	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
HEK_58	HEK293 T	*H.sapiens*	1.763	1734	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
HEK_59	HEK293 T	*H.sapiens*	2.837	1833	rGrGrG	1	SSRTII	SMARTer dT30VN	12	1	-	Advantage	50	
HEK_60	HEK293 T	*H.sapiens*	1.889	1758	rGrGrG	1	SSRTII	SMARTer dT30VN	12	1	-	Advantage	50	
HEK_61	HEK293 T	*H.sapiens*	3.733	1841	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	Advantage	50	
HEK_62	HEK293 T	*H.sapiens*	2.430	1849	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	Advantage	50	
HEK_63	HEK293 T	*H.sapiens*	0.758	1845	rGrGrG	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
HEK_64	HEK293 T	*H.sapiens*	0.948	1907	rGrGrG	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
HEK_65	HEK293 T	*H.sapiens*	1.364	1837	rGrGrG	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
HEK_66	HEK293 T	*H.sapiens*	1.897	1821	rGrGrG	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
HEK_67	HEK293 T	*H.sapiens*	2.721	1746	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
HEK_68	HEK293 T	*H.sapiens*	5.123	1699	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	Could be a cell aggregate
HEK_69	HEK293 T	*H.sapiens*	2.499	1892	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
HEK_70	HEK293 T	*H.sapiens*	1.632	1715	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
HEK_71	HEK293 T	*H.sapiens*	2.614	1438	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	yes	Advantage	50	

HEK_72	HEK293T	*H.sapiens*	1.343	1535	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	yes	Advantage	50	
HEK_73	HEK293T	*H.sapiens*	2.618	1570	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	yes	Advantage	50	
HEK_74	HEK293T	*H.sapiens*	2.037	1611	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	yes	Advantage	50	
HEK_75	HEK293T	*H.sapiens*	1.862	1494	rGrGrG	1	SSRTII	SMARTer dT30VN	12	1	yes	Advantage	50	
HEK_76	HEK293T	*H.sapiens*	0.560	1567	rGrGrG	1	SSRTII	SMARTer dT30VN	12	1	yes	Advantage	50	
HEK_77	HEK293T	*H.sapiens*	1.609	1604	rGrGrG	1	SSRTII	SMARTer dT30VN	12	1	yes	Advantage	50	
HEK_78	HEK293T	*H.sapiens*	0.748	1392	rGrGrG	1	SSRTII	SMARTer dT30VN	12	1	yes	Advantage	50	
HEK_79	HEK293T	*H.sapiens*	2.876	2093	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	Advantage	50	
HEK_80	HEK293T	*H.sapiens*	6.017	1975	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	Advantage	50	Could be a cell aggregate
HEK_81	HEK293T	*H.sapiens*	5.110	1834	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	Advantage	50	Could be a cell aggregate
HEK_82	HEK293T	*H.sapiens*	2.659	1912	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
HEK_83	HEK293T	*H.sapiens*	3.820	1848	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	Could be a cell aggregate
HEK_84	HEK293T	*H.sapiens*	1.875	1941	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
HEK_85	HEK293T	*H.sapiens*	4.477	2003	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	Could be a cell aggregate
HEK_86	HEK293T	*H.sapiens*	4.162	1784	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	Advantage	50	Could be a cell aggregate
HEK_87	HEK293T	*H.sapiens*	0.539	1964	rGrG+G	1	Maxima H minus	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
HEK_88	HEK293T	*H.sapiens*	0.526	1860	rGrG+G	1	Maxima H minus	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
HEK_89	HEK293T	*H.sapiens*	0.288	1881	rGrG+G	1	Maxima H minus	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
HEK_90	HEK293T	*H.sapiens*	0.650	1560	rGrG+G	1	Maxima H minus	SMARTer dT30	12	1	-	KAPA HiFi HS	50	
HEK_91	HEK293T	*H.sapiens*	0.341	1852	rGrG+G	1	Maxima H minus	SMARTer dT30	12	1	-	KAPA HiFi HS	50	
HEK_92	HEK293T	*H.sapiens*	0.354	1783	rGrG+G	1	Maxima H minus	SMARTer dT30	12	1	-	KAPA HiFi HS	50	
HEK_93	HEK293T	*H.sapiens*	1.520	1939	rGrG+N	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
HEK_94	HEK293T	*H.sapiens*	1.722	1740	rGrG+N	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
HEK_95	HEK293T	*H.sapiens*	0.485	1740	rGrG+N	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
HEK_96	HEK293T	*H.sapiens*	1.090	1833	rGrG+N	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
HEK_97	HEK293	*H.sapiens*	2.856	173	rGrG+N	2	SSRTII	SMARTer	12	1	-	KAPA HiFi	50	

	T			1				dT30VN				HS		
HEK_98	HEK293 T	*H.sapiens*	2.776	188 1	rGrG+N	2	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
HEK_99	HEK293 T	*H.sapiens*	1.954	173 0	rGrG+N	2	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
HEK_100	HEK293 T	*H.sapiens*	2.836	190 9	rGrG+N	2	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
HEK_101	HEK293 T	*H.sapiens*	2.266	175 0	rGrG+G	2	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
HEK_102	HEK293 T	*H.sapiens*	1.530	185 0	rGrG+G	2	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
HEK_103	HEK293 T	*H.sapiens*	1.883	170 3	rGrG+G	2	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
HEK_105	HEK293 T	*H.sapiens*	4.105	192 9	rGrG+G	2	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	Could be a cell aggregate
HEK_106	HEK293 T	*H.sapiens*	2.580	200 9	rGrG+G	2	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
HEK_107	HEK293 T	*H.sapiens*	2.572	191 0	rGrG+G	2	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
HEK_108	HEK293 T	*H.sapiens*	1.496	197 3	rGrG+G	2	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
HEK_109	HEK293 T	*H.sapiens*	3.459	190 8	rGrG+G	2	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	Could be a cell aggregate
HEK_110	HEK293 T	*H.sapiens*	4.591	192 1	rGrG+G	2	SSRTII	SMARTer dT30VN	12	1	-	Advantage	50	Could be a cell aggregate
HEK_111	HEK293 T	*H.sapiens*	3.841	197 2	rGrG+G	2	SSRTII	SMARTer dT30VN	12	1	-	Advantage	50	Could be a cell aggregate
HEK_112	HEK293 T	*H.sapiens*	4.279	191 6	rGrG+G	2	SSRTII	SMARTer dT30VN	12	1	-	Advantage	50	Could be a cell aggregate
HEK_113	HEK293 T	*H.sapiens*	2.123	164 8	rGrG+G	2	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
HEK_114	HEK293 T	*H.sapiens*	2.526	184 5	rGrG+G	2	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
HEK_115	HEK293 T	*H.sapiens*	2.084	156 9	rGrG+N	2	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
HEK_116	HEK293 T	*H.sapiens*	0.511	162 8	rGrG+N	2	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
HEK_117	HEK293 T	*H.sapiens*	2.690	182 3	rGrG+N	2	SSRTII	SMARTer dT30VN	12	1	-	Advantage	50	
HEK_118	HEK293 T	*H.sapiens*	1.454	177 4	rGrG+N	2	SSRTII	SMARTer dT30VN	12	1	-	Advantage	50	
HEK_119	HEK293 T	*H.sapiens*	0.361	159 8	rGrG+N	2	SSRTII	SMARTer dT30VN	12	1	-	Advantage	50	
HEK_120	HEK293 T	*H.sapiens*	1.215	193 5	rGrG+N	2	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
HEK_121	HEK293 T	*H.sapiens*	0.434	168 2	rGrG+N	2	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
HEK_122	HEK293 T	*H.sapiens*	0.739	155 3	rGrG+N	2	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
HEK_123	HEK293 T	*H.sapiens*	1.599	168 8	rGrG+N	2	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	

HEK_124	HEK293T	*H.sapiens*	2.301	1617	rGrG+N	2	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
HEK_135	HEK293T	*H.sapiens*	0.784	1810	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	2x dCTP
HEK_136	HEK293T	*H.sapiens*	1.311	1990	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	2x dCTP
HEK_137	HEK293T	*H.sapiens*	0.895	1829	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	2x dCTP
HEK_138	HEK293T	*H.sapiens*	1.318	1948	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	2x dCTP
HEK_139	HEK293T	*H.sapiens*	1.853	1704	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	2x dCTP
HEK_140	HEK293T	*H.sapiens*	4.009	1833	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	2x dCTP (NOT SINGLE CELL)
HEK_141	HEK293T	*H.sapiens*	5.230	1820	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	Could be a cell aggregate
HEK_144	HEK293T	*H.sapiens*	4.803	1766	rGrG+G	2	SSRTII	SMARTer dT30VN	12	1	-	Advantage	50	Could be a cell aggregate
HEK_145	HEK293T	*H.sapiens*	2.166	1619	rGrG+G	2	SSRTII	SMARTer dT30VN	12	1	-	Advantage	50	
HEK_146	HEK293T	*H.sapiens*	2.283	1552	rGrG+G	2	SSRTII	SMARTer dT30VN	12	1	-	Advantage	50	
HEK_147	HEK293T	*H.sapiens*	2.099	1316	rGrG+G	2	SSRTII	SMARTer dT30VN	12	1	-	Advantage	50	
HEK_148	HEK293T	*H.sapiens*	2.191	1339	rGrG+G	2	SSRTII	SMARTer dT30VN	12	1	-	Advantage	50	HEDGEHOG PATTERN
HEK_149	HEK293T	*H.sapiens*	1.560	1426	rGrG+G	2	SSRTII	SMARTer dT30VN	12	1	-	Advantage	50	
HEK_150	HEK293T	*H.sapiens*	2.830	1508	rGrG+G	2	SSRTII	SMARTer dT30VN	12	1	-	Advantage	50	
HEK_151	HEK293T	*H.sapiens*	2.220	1220	rGrG+G	2	SSRTII	SMARTer dT30	12	1	-	Advantage	50	
HEK_152	HEK293T	*H.sapiens*	2.654	1534	rGrG+G	2	SSRTII	SMARTer dT30	12	1	-	Advantage	50	HEDGEHOG PATTERN
HEK_153	HEK293T	*H.sapiens*	2.039	1655	rGrG+G	2	SSRTII	SMARTer dT30	12	1	-	Advantage	50	
HEK_154	HEK293T	*H.sapiens*	3.001	1837	rGrG+G	2	SSRTII	SMARTer dT30	12	1	-	Advantage	50	
HEK_155	HEK293T	*H.sapiens*	1.378	1846	rGrG+G	2	SSRTII	SMARTer dT30	12	1	-	Advantage	50	
HEK_156	HEK293T	*H.sapiens*	1.065	1853	rGrG+G	2	SSRTII	SMARTer dT30	12	1	-	Advantage	50	
HEK_157	HEK293T	*H.sapiens*	1.621	1772	rGrG+G	2	SSRTII	SMARTer dT30	12	1	-	Advantage	50	
HEK_158	HEK293T	*H.sapiens*	2.418	1706	rGrG+G	2	SSRTII	SMARTer dT30	12	1	-	Advantage	50	
HEK_SMRT_5	HEK293T	*H.sapiens*	0.128	1495	SMARTer Oligo IIA	1	SMARTscribe	SMARTer dT30VN	6	1	yes	Advantage	50	
HEK_SMRT_6	HEK293T	*H.sapiens*	0.042	1469	SMARTer Oligo IIA	1	SMARTscribe	SMARTer dT30VN	6	1	yes	Advantage	50	
HEK_SMRT_	HEK293	*H.sapiens*	0.207	135	SMARTer Oligo	1	SMARTscribe	SMARTer	6	1	ye	Advantage	50	

7	T			9	IIA			dT30VN			s			
HEK_SMRT_8	HEK293T	*H.sapiens*	0.246	1340	SMARTer Oligo IIA	1	SMARTscribe	SMARTer dT30VN	6	1	yes	Advantage	50	
HEK_SMRT_9	HEK293T	*H.sapiens*	0.152	145 5	SMARTer Oligo IIA	1	SMARTscribe	SMARTer dT30VN	6	1	yes	Advantage	50	
HEK_SMRT_10	HEK293T	*H.sapiens*	0.256	122 8	SMARTer Oligo IIA	1	SMARTscribe	SMARTer dT30VN	6	1	yes	Advantage	50	
HEK_SMRT_11	HEK293T	*H.sapiens*	0.072	123 8	SMARTer Oligo IIA	1	SMARTscribe	SMARTer dT30VN	6	1	yes	Advantage	50	
HEK_SMRT_12	HEK293T	*H.sapiens*	0.256	129 2	SMARTer Oligo IIA	1	SMARTscribe	SMARTer dT30VN	6	1	yes	Advantage	50	
C_1	C2C12	*M.musculus*	3.003	169 6	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	25	Could be a cell aggregate
C_2	C2C12	*M.musculus*	1.110	177 2	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	25	
C_3	C2C12	*M.musculus*	1.157	166 0	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	25	
C_4	C2C12	*M.musculus*	1.965	167 4	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	25	
C_5	C2C12	*M.musculus*	2.884	170 8	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	25	Could be a cell aggregate
C_6	C2C12	*M.musculus*	2.325	164 6	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	25	
C_7	C2C12	*M.musculus*	1.941	157 7	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	25	
C_8	C2C12	*M.musculus*	2.248	166 6	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	25	
C_9	C2C12	*M.musculus*	1.379	161 4	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
C_10	C2C12	*M.musculus*	1.832	180 9	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
C_11	C2C12	*M.musculus*	3.644	173 5	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	Could be a cell aggregate
C_12	C2C12	*M.musculus*	1.245	173 5	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
C_13	C2C12	*M.musculus*	1.913	189 6	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
C_14	C2C12	*M.musculus*	1.703	183 6	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
C_15	C2C12	*M.musculus*	1.588	193 7	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
C_16	C2C12	*M.musculus*	1.730	193 7	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
MEF_1	MEF	*M.musculus*	0.999	139 7	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	25	
MEF_2	MEF	*M.musculus*	2.862	171 0	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	25	
MEF_3	MEF	*M.musculus*	1.571	157 7	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	25	
MEF_4	MEF	*M.musculus*	1.120	148 9	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	25	

MEF_5	MEF	*M.musculus*	2.121	1672	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	25	
MEF_6	MEF	*M.musculus*	5.025	1610	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	25	Could be a cell aggregate
MEF_7	MEF	*M.musculus*	5.874	1065	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	25	Could be a cell aggregate
MEF_8	MEF	*M.musculus*	1.272	?	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	25	
MEF_9	MEF	*M.musculus*	1.650	?	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	25	
MEF_10	MEF	*M.musculus*	2.071	?	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	25	
MEF_11	MEF	*M.musculus*	0.755	?	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	25	
MEF_12	MEF	*M.musculus*	7.528	?	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	25	Could be a cell aggregate
MEF_13	MEF	*M.musculus*	2.271	?	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	25	
MEF_14	MEF	*M.musculus*	2.327	?	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	25	
MEF_15	MEF	*M.musculus*	3.149	?	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	25	Could be a cell aggregate
MEF_16	MEF	*M.musculus*	2.045	?	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	25	
MEF_17	MEF	*M.musculus*	0.497	1803	rGrGrG	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
MEF_18	MEF	*M.musculus*	0.427	1879	rGrGrG	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
MEF_19	MEF	*M.musculus*	0.406	1873	rGrGrG	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
MEF_20	MEF	*M.musculus*	0.680	1984	rGrGrG	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
MEF_21	MEF	*M.musculus*	0.429	1738	rGrGrG	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
MEF_22	MEF	*M.musculus*	0.634	2089	rGrGrG	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
MEF_23	MEF	*M.musculus*	0.722	2019	rGrGrG	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
MEF_24	MEF	*M.musculus*	0.397	1881	rGrGrG	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
MEF_25	MEF	*M.musculus*	0.981	2007	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	Advantage	50	
MEF_26	MEF	*M.musculus*	0.626	1827	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	Advantage	50	
MEF_28	MEF	*M.musculus*	0.656	1979	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	Advantage	50	
MEF_29	MEF	*M.musculus*	1.356	2143	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	Advantage	50	
MEF_30	MEF	*M.musculus*	1.242	2137	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	Advantage	50	
MEF_31	MEF	*M.musculus*	2.240	2011	rGrG+G	1	SSRTII	SMARTer	12	1	-	Advantage	50	

		us		5				dT30VN						
MEF_32	MEF	*M.musculus*	0.781	1817	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	Advantage	50	
MEF_33	MEF	*M.musculus*	0.603	1781	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
MEF_34	MEF	*M.musculus*	5.014	1894	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	Could be a cell aggregate
MEF_35	MEF	*M.musculus*	1.651	2064	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
MEF_36	MEF	*M.musculus*	1.017	1773	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
MEF_37	MEF	*M.musculus*	1.119	1783	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
MEF_38	MEF	*M.musculus*	0.681	1659	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
MEF_39	MEF	*M.musculus*	1.444	1469	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
MEF_40	MEF	*M.musculus*	0.845	1401	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	
SMRT_1	C2C12	*M.musculus*	0.063	1170	SMARTer Oligo IIA	1	SMARTscribe	SMARTer dT30VN	12	1	yes	Advantage	50	
SMRT_2	C2C12	*M.musculus*	0.165	1127	SMARTer Oligo IIA	1	SMARTscribe	SMARTer dT30VN	12	1	yes	Advantage	50	
SMRT_3	C2C12	*M.musculus*	0.130	1169	SMARTer Oligo IIA	1	SMARTscribe	SMARTer dT30VN	12	1	yes	Advantage	50	
SMRT_4	C2C12	*M.musculus*	0.091	1325	SMARTer Oligo IIA	1	SMARTscribe	SMARTer dT30VN	12	1	yes	Advantage	50	
SMRT_5	C2C12	*M.musculus*	0.036	1282	SMARTer Oligo IIA	1	SMARTscribe	SMARTer dT30VN	12	1	yes	Advantage	50	
SMRT_6	C2C12	*M.musculus*	0.135	1373	SMARTer Oligo IIA	1	SMARTscribe	SMARTer dT30VN	12	1	yes	Advantage	50	
SMRT_7	C2C12	*M.musculus*	0.161	1525	SMARTer Oligo IIA	1	SMARTscribe	SMARTer dT30VN	12	1	yes	Advantage	50	
SMRT_8	C2C12	*M.musculus*	0.178	1314	SMARTer Oligo IIA	1	SMARTscribe	SMARTer dT30VN	12	1	yes	Advantage	50	
SMRT_9	C2C12	*M.musculus*	0.036	1363	SMARTer Oligo IIA	1	SMARTscribe	SMARTer dT30VN	12	1	yes	Advantage	50	
SMRT_10	C2C12	*M.musculus*	0.129	1261	SMARTer Oligo IIA	1	SMARTscribe	SMARTer dT30VN	12	1	yes	Advantage	50	
SMRT_11	C2C12	*M.musculus*	0.080	1234	SMARTer Oligo IIA	1	SMARTscribe	SMARTer dT30VN	12	1	yes	Advantage	50	
SMRT_12	C2C12	*M.musculus*	0.111	1447	SMARTer Oligo IIA	1	SMARTscribe	SMARTer dT30VN	12	1	yes	Advantage	50	
SMRT_14	C2C12	*M.musculus*	0.081	1408	SMARTer Oligo IIA	1	SMARTscribe	SMARTer dT30VN	12	1	yes	Advantage	50	
SMRT_16	C2C12	*M.musculus*	0.074	1280	SMARTer Oligo IIA	1	SMARTscribe	SMARTer dT30VN	12	1	yes	Advantage	50	
BC_1	B-cells	*H.sapiens*	0.826	1657	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50	

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

				6				dT30VN				HS	
BC_24	B-cells	*H.sapiens*	0.097	133 8	rGrG+G	1	SSRTII	SMARTer dT30VN	12	-	-	KAPA HiFi HS	50
BC_25	B-cells	*H.sapiens*	0.826	165 7	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50
BC_26	B-cells	*H.sapiens*	1.091	165 9	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50
BC_27	B-cells	*H.sapiens*	0.401	164 9	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50
BC_28	B-cells	*H.sapiens*	0.501	154 7	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50
BC_29	B-cells	*H.sapiens*	0.573	153 4	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50
BC_30	B-cells	*H.sapiens*	0.328	134 3	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50
BC_31	B-cells	*H.sapiens*	0.661	160 7	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50
BC_32	B-cells	*H.sapiens*	0.500	160 5	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50
BC_33	B-cells	*H.sapiens*	0.840	178 2	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50
BC_34	B-cells	*H.sapiens*	0.829	181 3	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50
BC_35	B-cells	*H.sapiens*	0.648	164 2	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50
BC_36	B-cells	*H.sapiens*	0.297	181 5	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50
BC_37	B-cells	*H.sapiens*	0.982	182 5	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50
BC_38	B-cells	*H.sapiens*	0.853	179 5	rGrG+G	1	SSRTII	SMARTer dT30VN	12	1	-	KAPA HiFi HS	50

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 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.

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 locked nucleic acid residue is at the 3'-most position.

11. The method of any one of claims 1 to 10, 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.

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

13. The method of any one of claims 2 to 12, wherein said methyl group donor is betaine, and said metal salt is $MgCl_2$ at a concentration of at least 9 mM.

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

15. The method of any one of claims 1 to 14, wherein said template switching oligonucleotide is selected from the oligonucleotides in Table S2 .

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

17. The method of claim 16, wherein said oligo-dT primer comprises a sequence of 5′-AAGCAGTGGTATCAACGCAGAGTACT$_{30}$VN-3′, wherein "N" is any nucleoside base, and "V" is selected from the group consisting of "A", "C" and "G".

18. The method of any one of claims 13 to 17, further comprising PCR preamplification, tagmentation, and final PCR amplification.

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

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

21. A cDNA library produced by the method of any one of claims 1 to 20.

22. Use of a cDNA library of claim 21 for single-cell transcriptome profiling.

23. 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 20; and sequencing the cDNA library.

24. A template switching oligonucleotide (TSO) comprising a locked nucleotide residue at its 3'-end.

25. The TSO of claim 24, comprising three nucleotide residues at the 3'-end 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 is independently a locked nucleotide residue.

26. The TSO of claim 24 or 25, wherein said locked nucleotide residue is selected from the group consisting of locked guanine, locked adenine, locked uracil, locked thymine, locked cytosine, and locked 5-methylcytosine.

27. The TSO of claim 25, wherein said three nucleotide residues are NN+G or rNrN+G.

28. The TSO of claim 25, wherein said three nucleotide residues are rGrG+N or GG+N.

29. The TSO of claim 25, wherein said three nucleotide residues are rGrG+G or GG+G.

30. Use of a TSO of any one of claims 24-29 in the synthesis of a double strand cDNA according to the method of any one of claims 1-20.

FIGURE 1

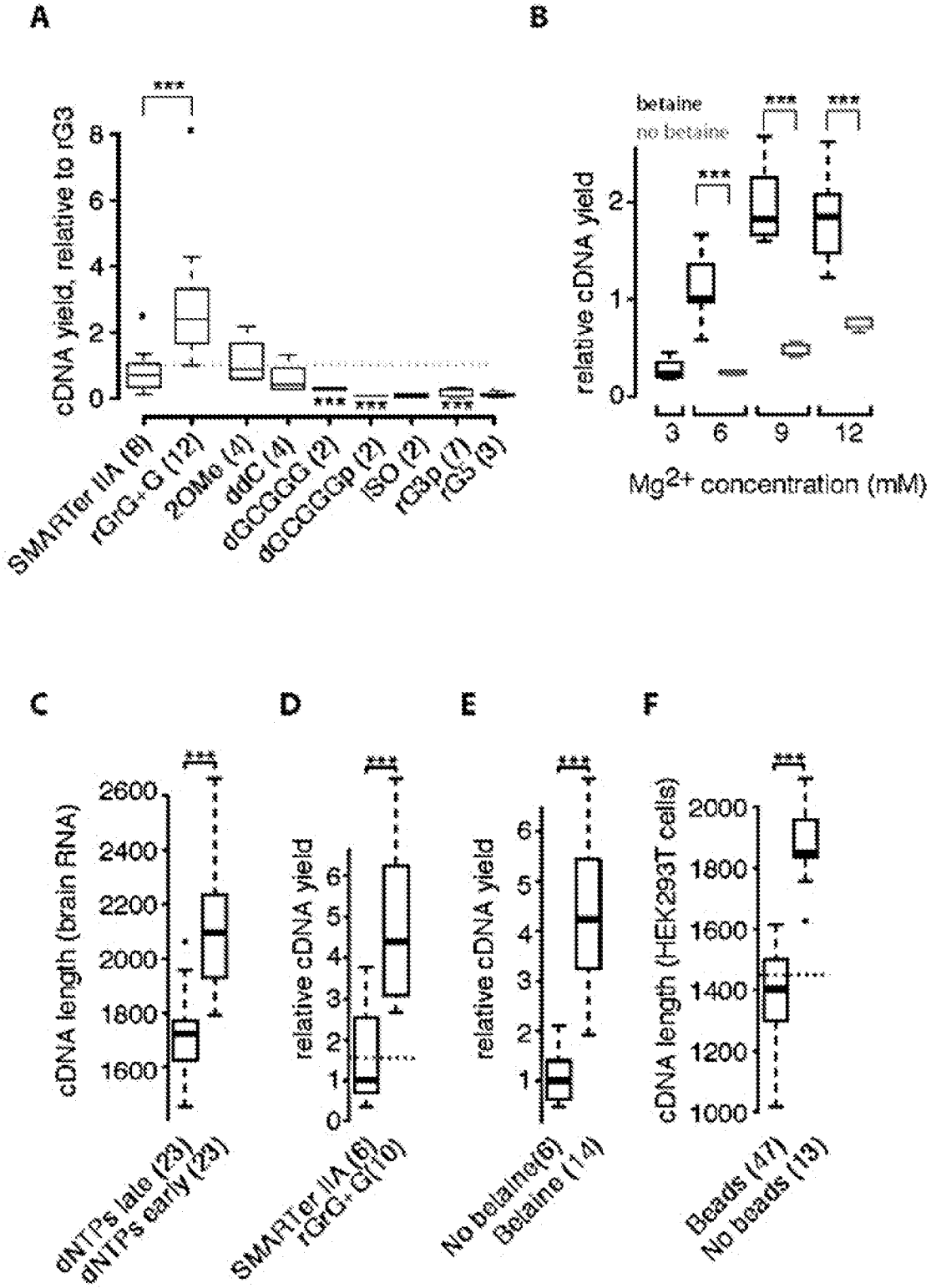

A
B
C
D
E
F
cDNA yield, relative to rG3
SMARTer IIA (8)
rGrG+G (12)
2OMe (4)
ddC (4)
dGCGGGG (2)
dGCGGGGp (2)
iSO (2)
rG3p (1)
rG5 (3)
relative cDNA yield
betaine
no betaine
Mg2+ concentration (mM)
cDNA length (brain RNA)
dNTPs late (23)
dNTPs early (23)
relative cDNA yield
SMARTer IIA (6)
rGrG+G(10)
relative cDNA yield
No betaine(6)
Betaine (14)
cDNA length (HEK293T cells)
Beads (47)
No beads (13)

FIGURE 2

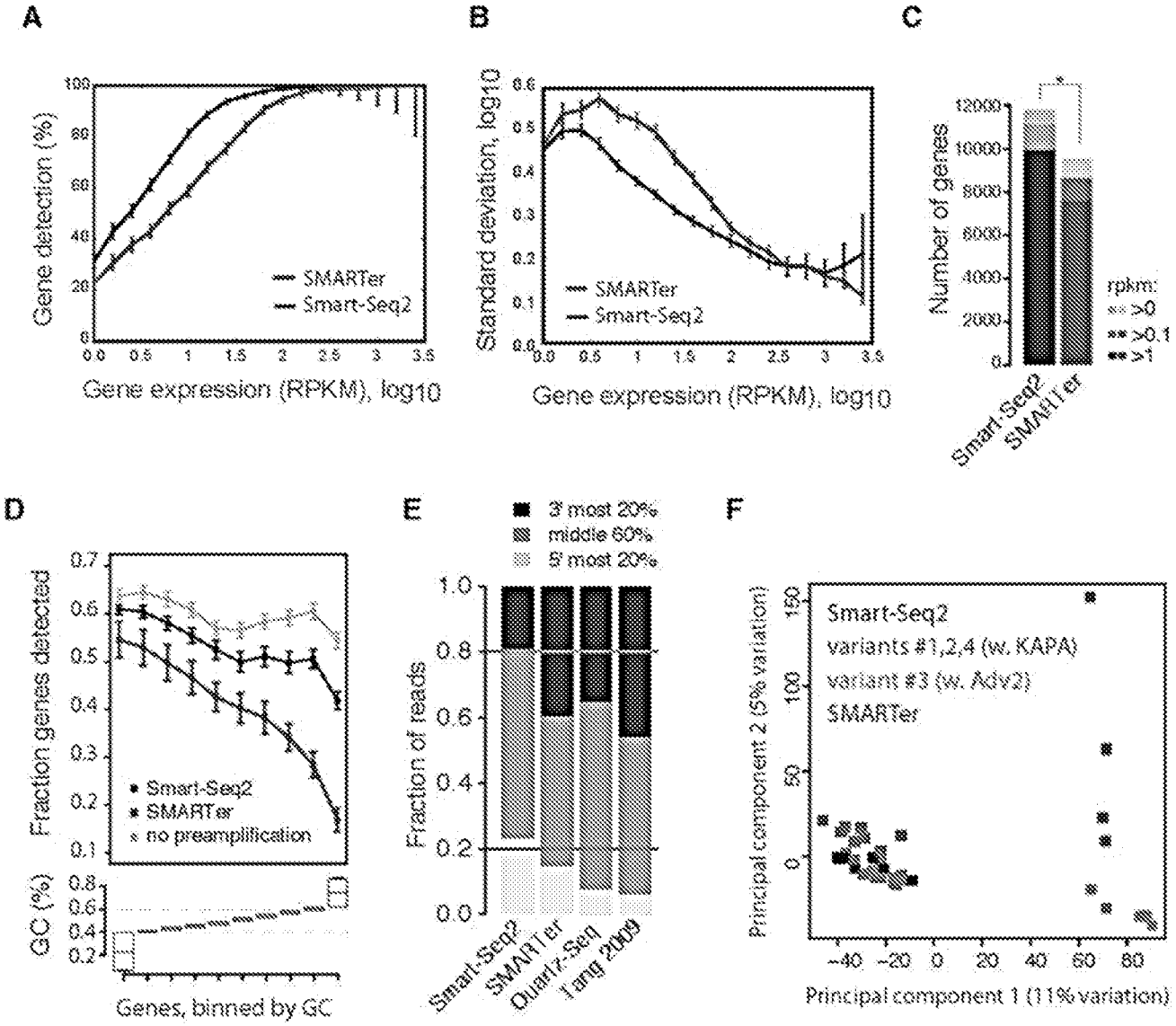

A
Gene detection (%)
SMARTer
Smart-Seq2
Gene expression (RPKM), log10
B
Standard deviation, log10
SMARTer
Smart-Seq2
Gene expression (RPKM), log10
C
Number of genes
rpkm:
>0
>0.1
>1
Smart-Seq2
SMARTer
D
Fraction genes detected
Smart-Seq2
SMARTer
no preamplification
GC (%)
Genes, binned by GC
E
3' most 20%
middle 60%
5' most 20%
Fraction of reads
Smart-Seq2
SMARTer
Quartz-Seq
Tang 2009
F
Smart-Seq2
variants #1,2,4 (w. KAPA)
variant #3 (w. Adv2)
SMARTer
Principal component 2 (5% variation)
Principal component 1 (11% variation)

FIGURE 3

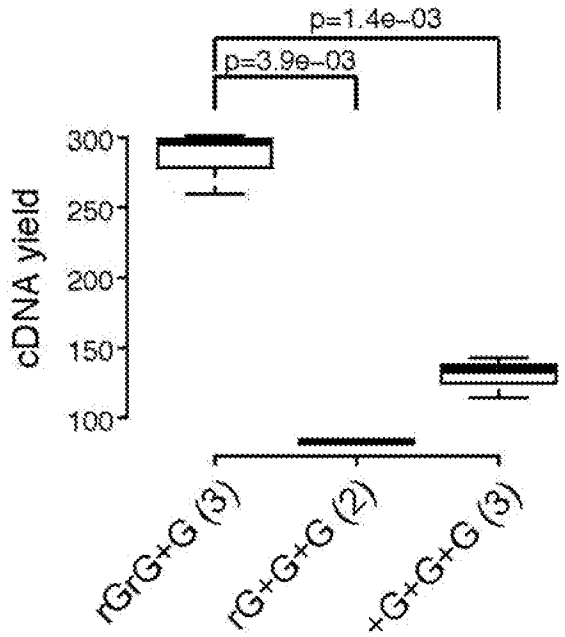


FIGURE 4

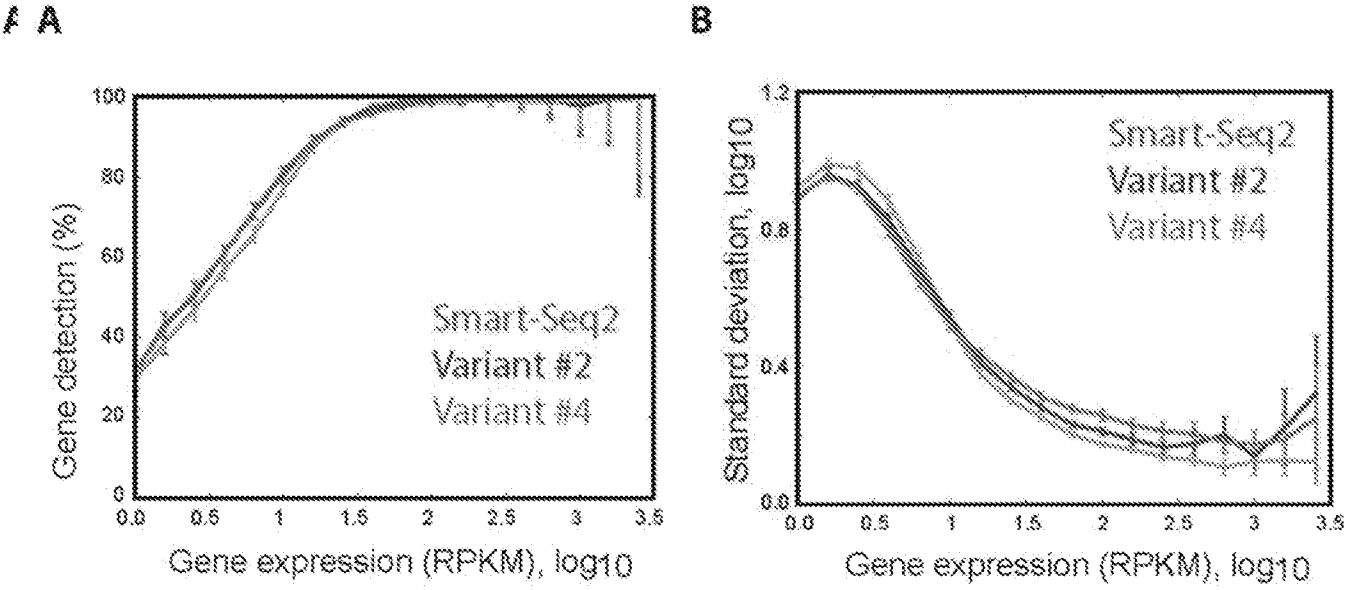


FIGURE 5

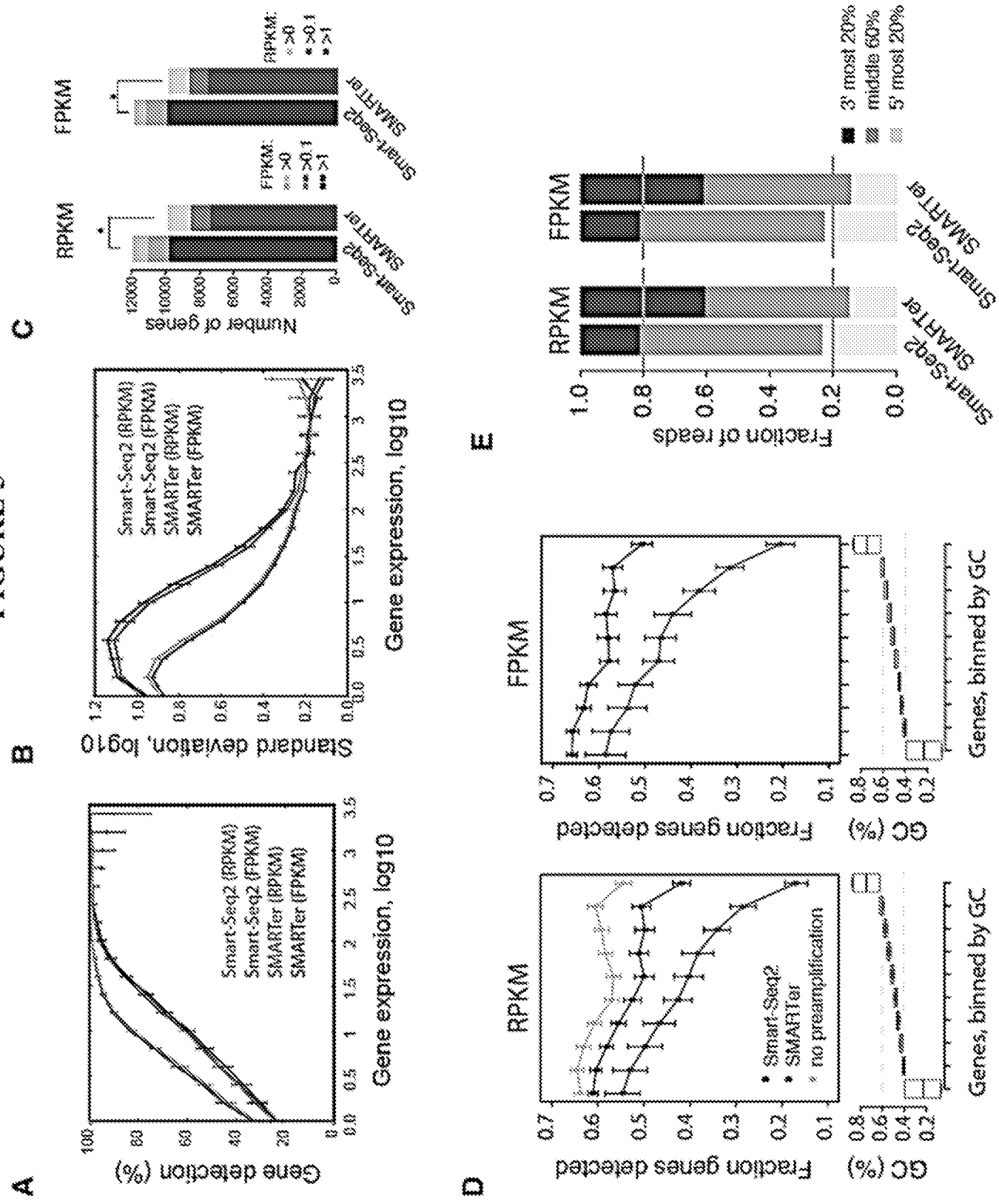

A
Gene detection (%)
Gene expression, log10
Smart-Seq2 (RPKM)
Smart-Seq2 (FPKM)
SMARTer (RPKM)
SMARTer (FPKM)
B
Standard deviation, log10
Gene expression, log10
C
Number of genes
RPKM
FPKM
Smart-Seq2
SMARTer
RPKM:
>0
>0.1
>1
D
RPKM
FPKM
Fraction genes detected
Smart-Seq2
SMARTer
no preamplification
GC (%)
Genes, binned by GC
E
Fraction of reads
3' most 20%
middle 60%
5' most 20%

FIGURE 6

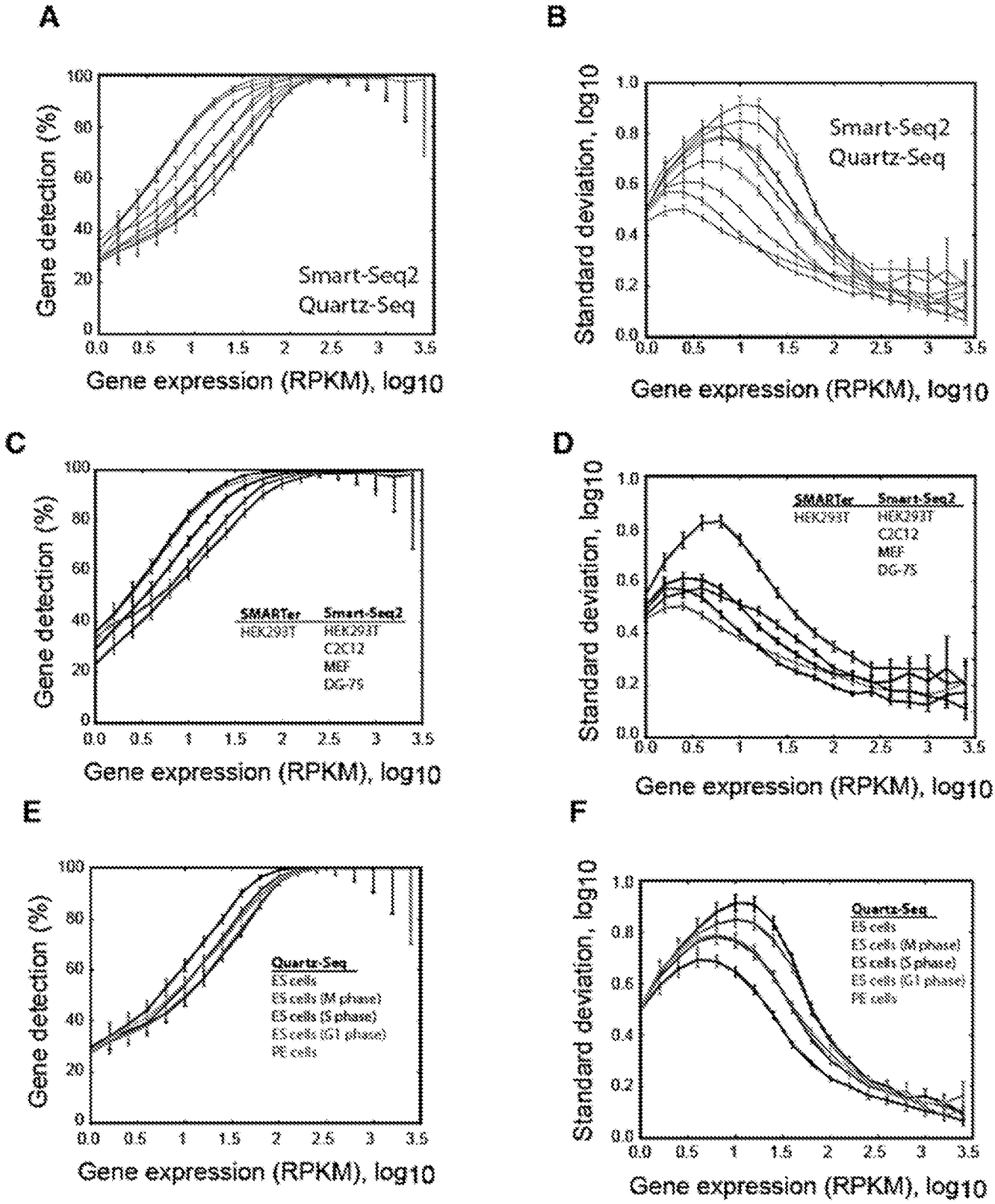

A
Gene detection (%)
Smart-Seq2
Quartz-Seq
Gene expression (RPKM), log10
B
Standard deviation, log10
Smart-Seq2
Quartz-Seq
Gene expression (RPKM), log10
C
Gene detection (%)
SMARTer
Smart-Seq2
HEK293T
HEK293T
C2C12
MEF
DG-75
Gene expression (RPKM), log10
D
Standard deviation, log10
SMARTer
Smart-Seq2
HEK293T
HEK293T
C2C12
MEF
DG-75
Gene expression (RPKM), log10
E
Gene detection (%)
Quartz-Seq
ES cells
ES cells (M phase)
ES cells (S phase)
ES cells (G1 phase)
PE cells
Gene expression (RPKM), log10
F
Standard deviation, log10
Quartz-Seq
ES cells
ES cells (M phase)
ES cells (S phase)
ES cells (G1 phase)
PE cells
Gene expression (RPKM), log10

FIGURE 7

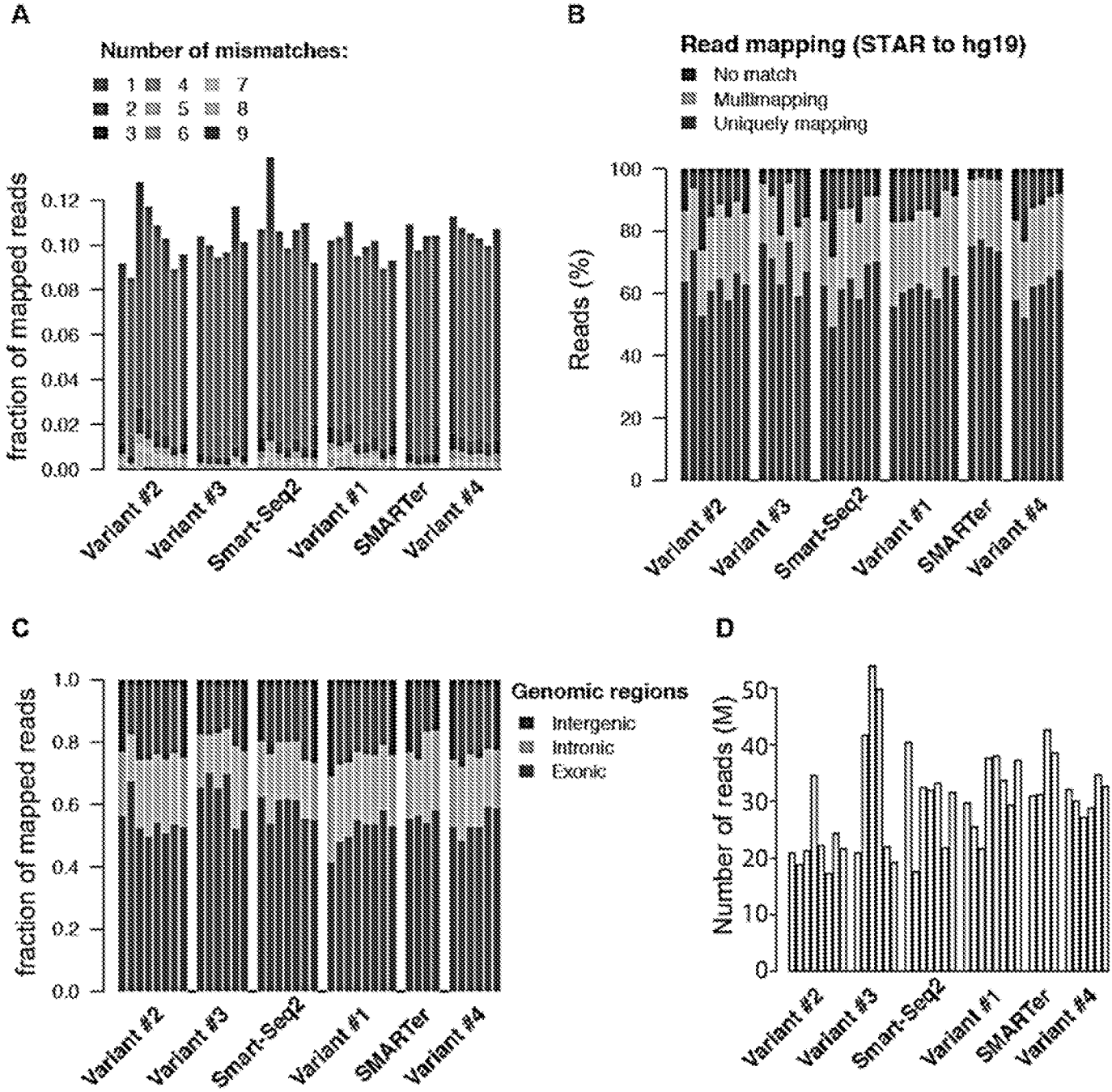

A
Number of mismatches:
1 4 7
2 5 8
3 6 9
fraction of mapped reads
0.00
0.02
0.04
0.06
0.08
0.10
0.12
Variant #2
Variant #3
Smart-Seq2
Variant #1
SMARTer
Variant #4
B
Read mapping (STAR to hg19)
No match
Multimapping
Uniquely mapping
Reads (%)
0
20
40
60
80
100
Variant #2
Variant #3
Smart-Seq2
Variant #1
SMARTer
Variant #4
C
fraction of mapped reads
0.0
0.2
0.4
0.6
0.8
1.0
Genomic regions
Intergenic
Intronic
Exonic
Variant #2
Variant #3
Smart-Seq2
Variant #1
SMARTer
Variant #4
D
Number of reads (M)
0
10
20
30
40
50
Variant #2
Variant #3
Smart-Seq2
Variant #1
SMARTer
Variant #4

FIGURE 8

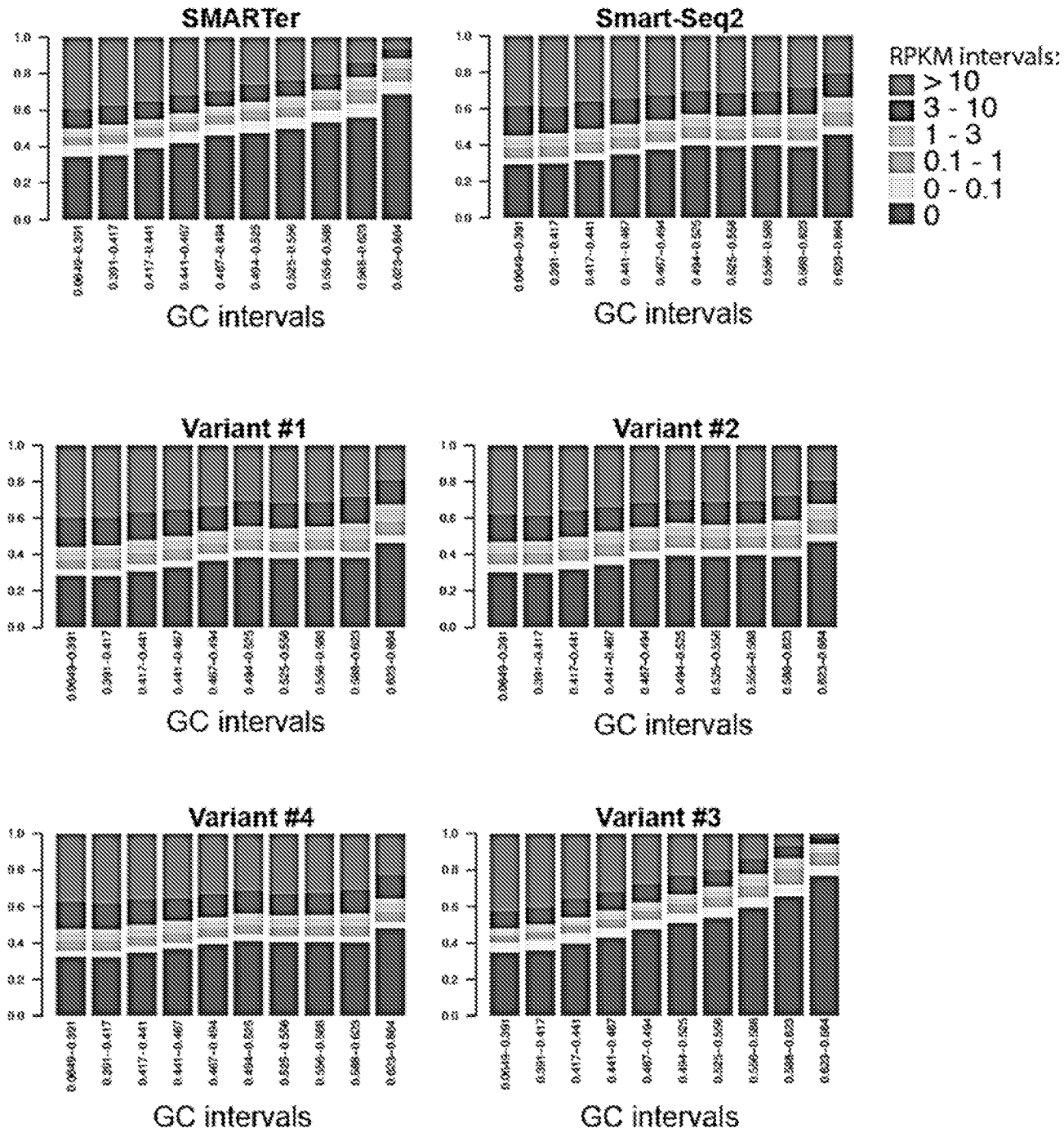
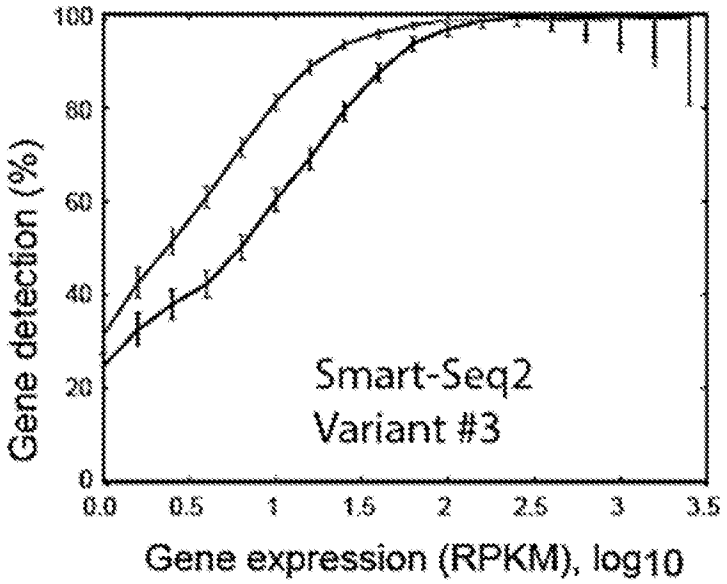

SMARTer
Smart-Seq2
RPKM intervals:
> 10
3 - 10
1 - 3
0.1 - 1
0 - 0.1
0
GC intervals
Variant #1
Variant #2
Variant #4
Variant #3

FIGURE 9

A


Gene detection (%)
100
80
60
40
20
0
0.0
0.5
1
1.5
2
2.5
3
3.5
Smart-Seq2
Variant #3
Gene expression (RPKM), log10

B

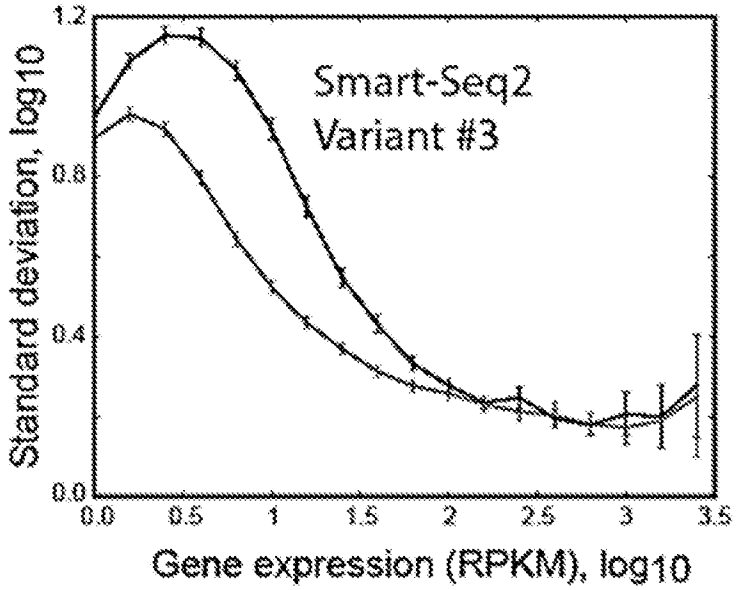

Standard deviation, log10
1.2
0.8
0.4
0.0
0.0
0.5
1
1.5
2
2.5
3
3.5
Smart-Seq2
Variant #3
Gene expression (RPKM), log10

FIGURE 10

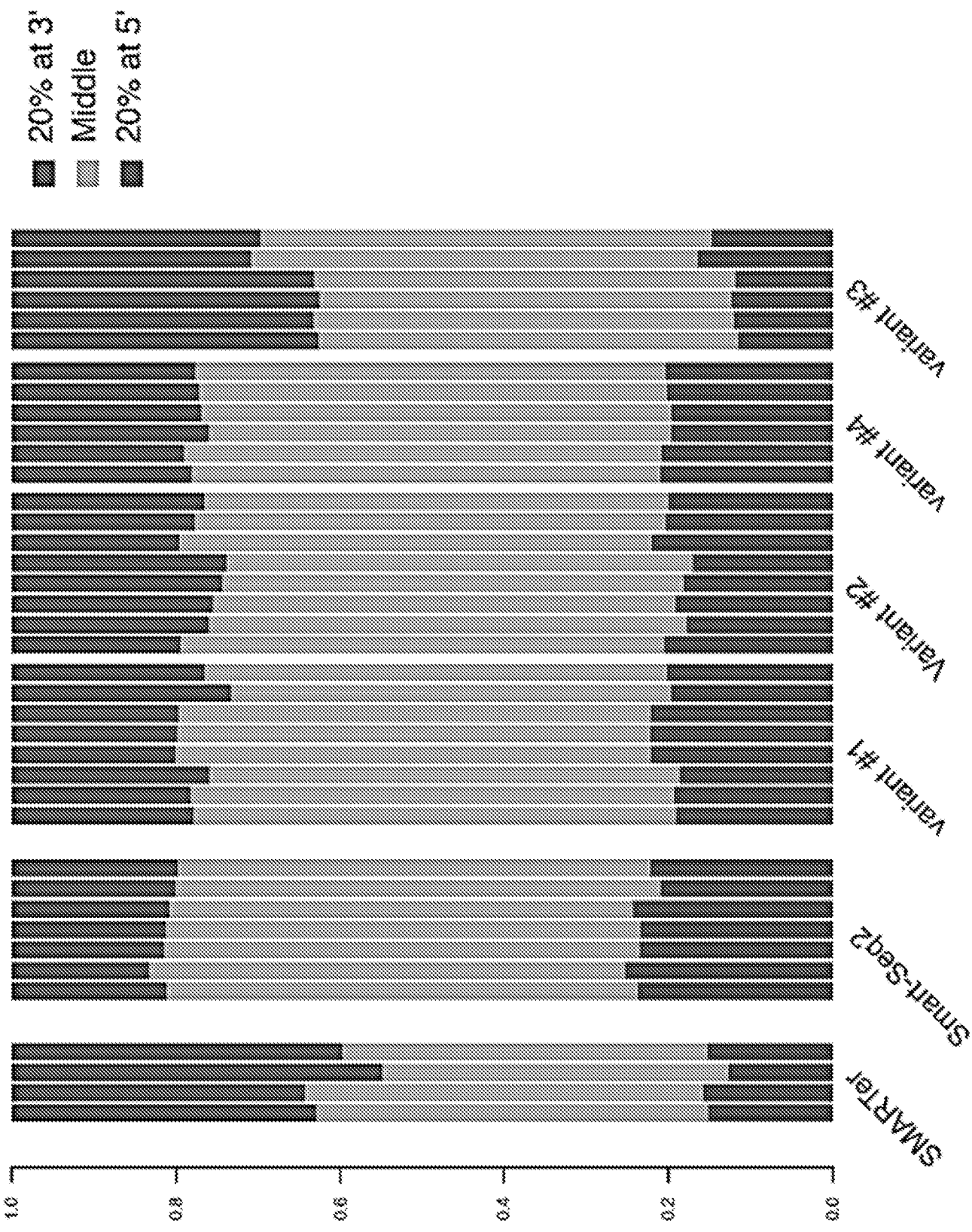

20% at 3'
Middle
20% at 5'
SMARTer
Smart-Seq2
Variant #1
Variant #2
Variant #4
Variant #3
1.0
0.8
0.6
0.4
0.2
0.0

FIGURE 11

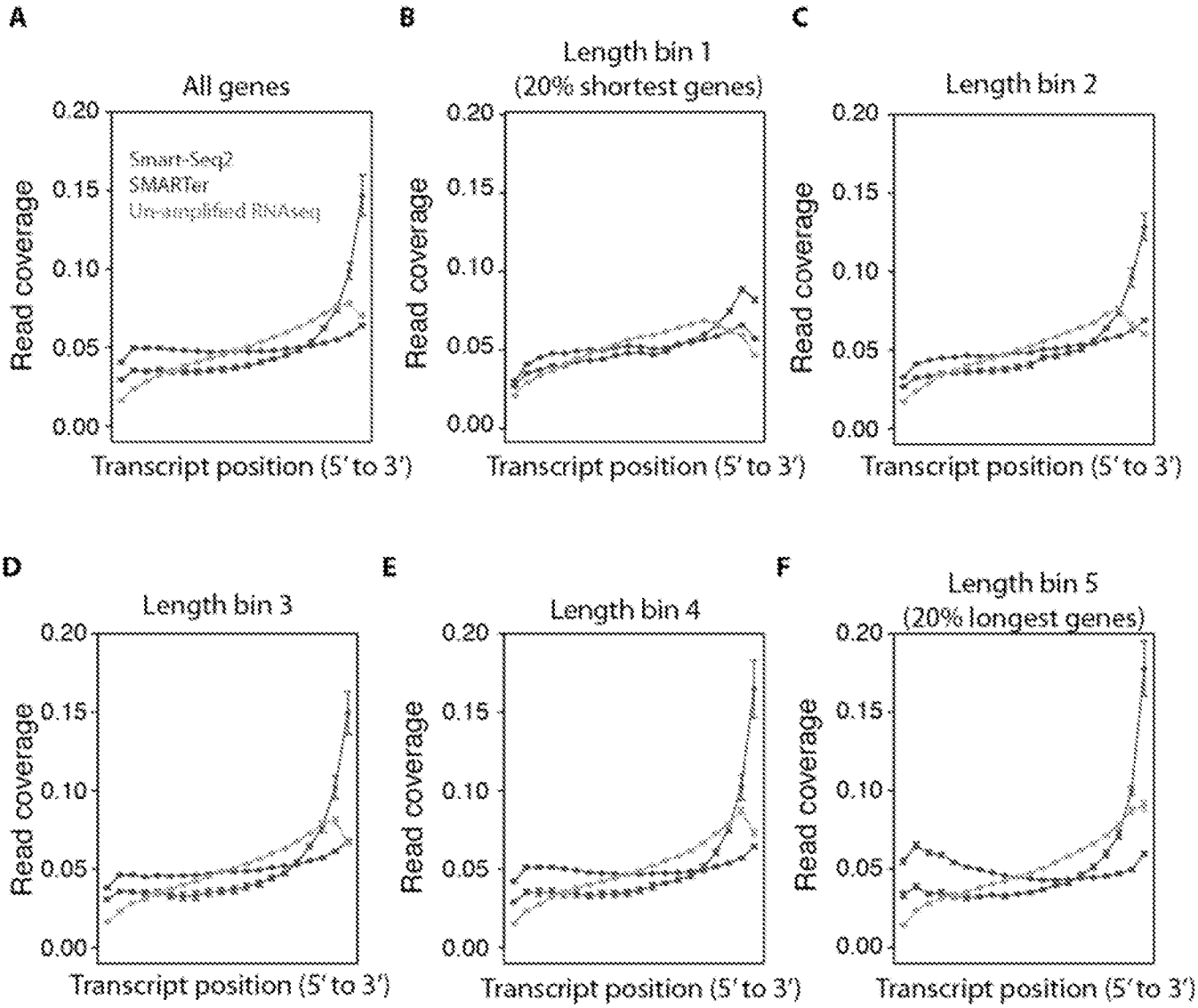

A
All genes
Smart-Seq2
SMARTer
Un-amplified RNAseq
Read coverage
0.20
0.15
0.10
0.05
0.00
Transcript position (5' to 3')
B
Length bin 1
(20% shortest genes)
C
Length bin 2
D
Length bin 3
E
Length bin 4
F
Length bin 5
(20% longest genes)

FIGURE 12A

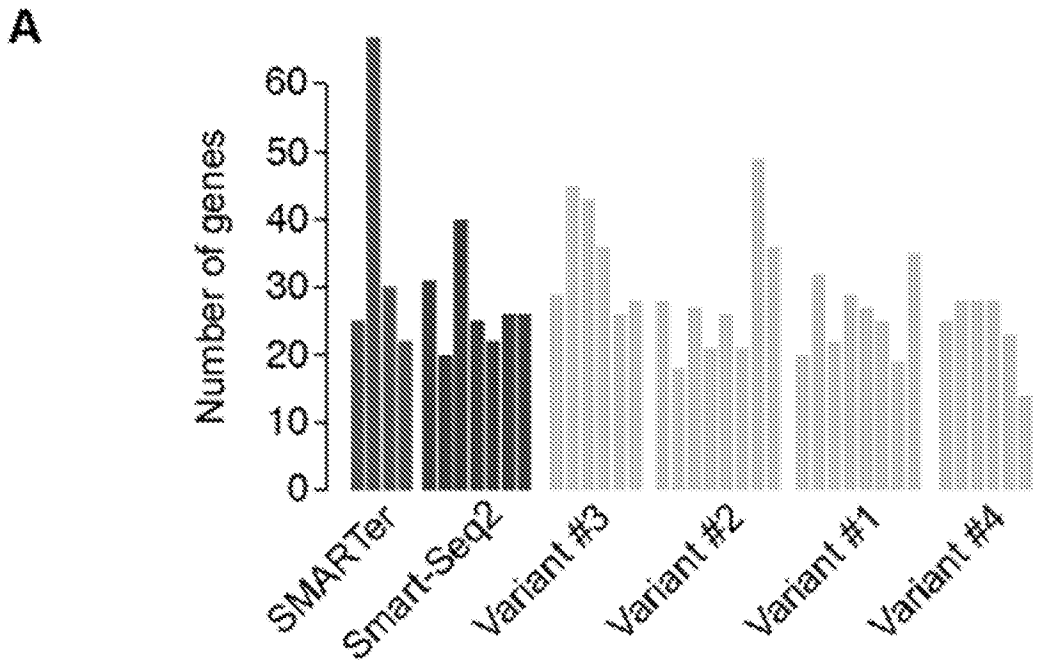

A
Number of genes
0
10
20
30
40
50
60
SMARTer
Smart-Seq2
Variant #3
Variant #2
Variant #1
Variant #4

FIGURE 12 (Cont'd)

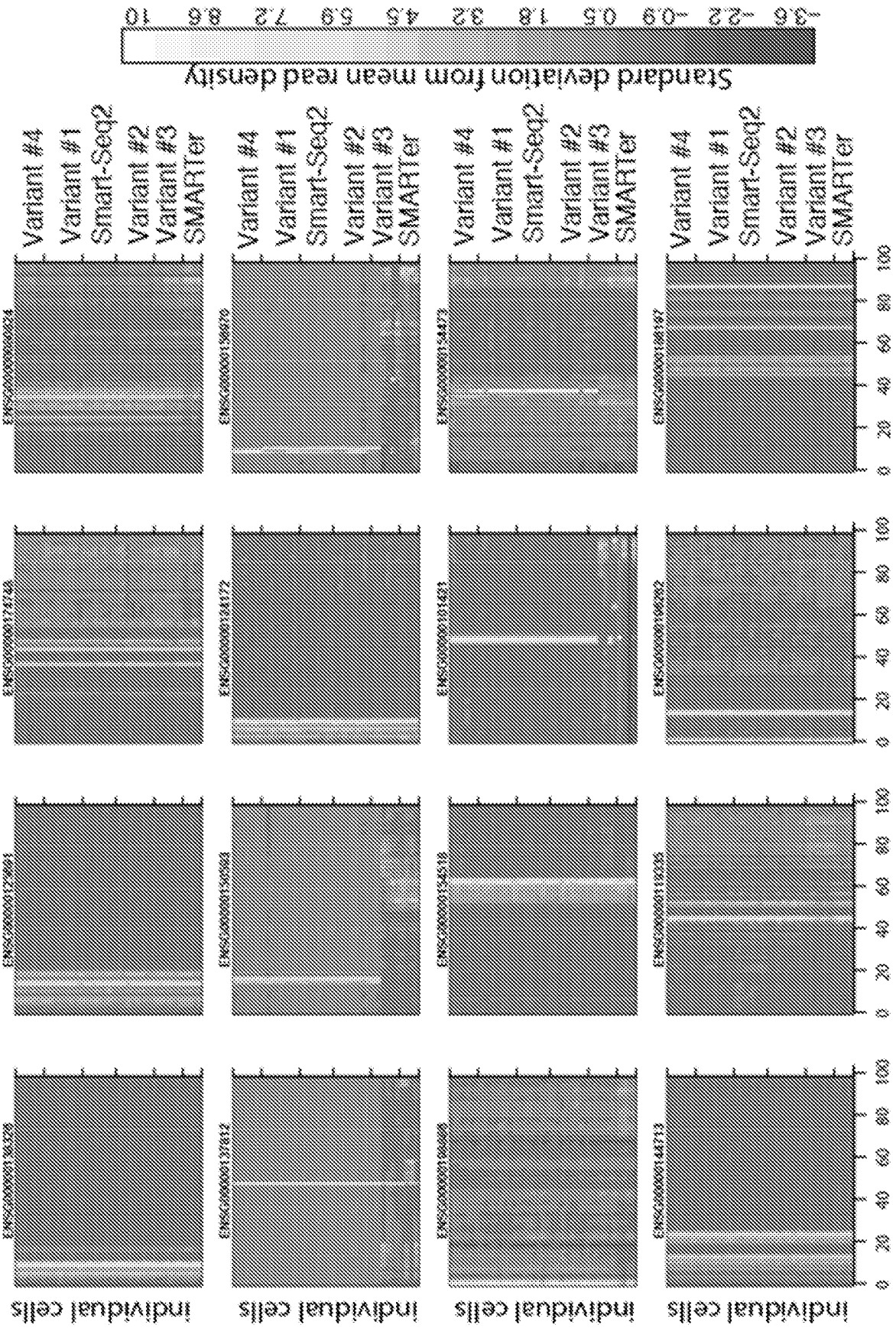


FIGURE 13

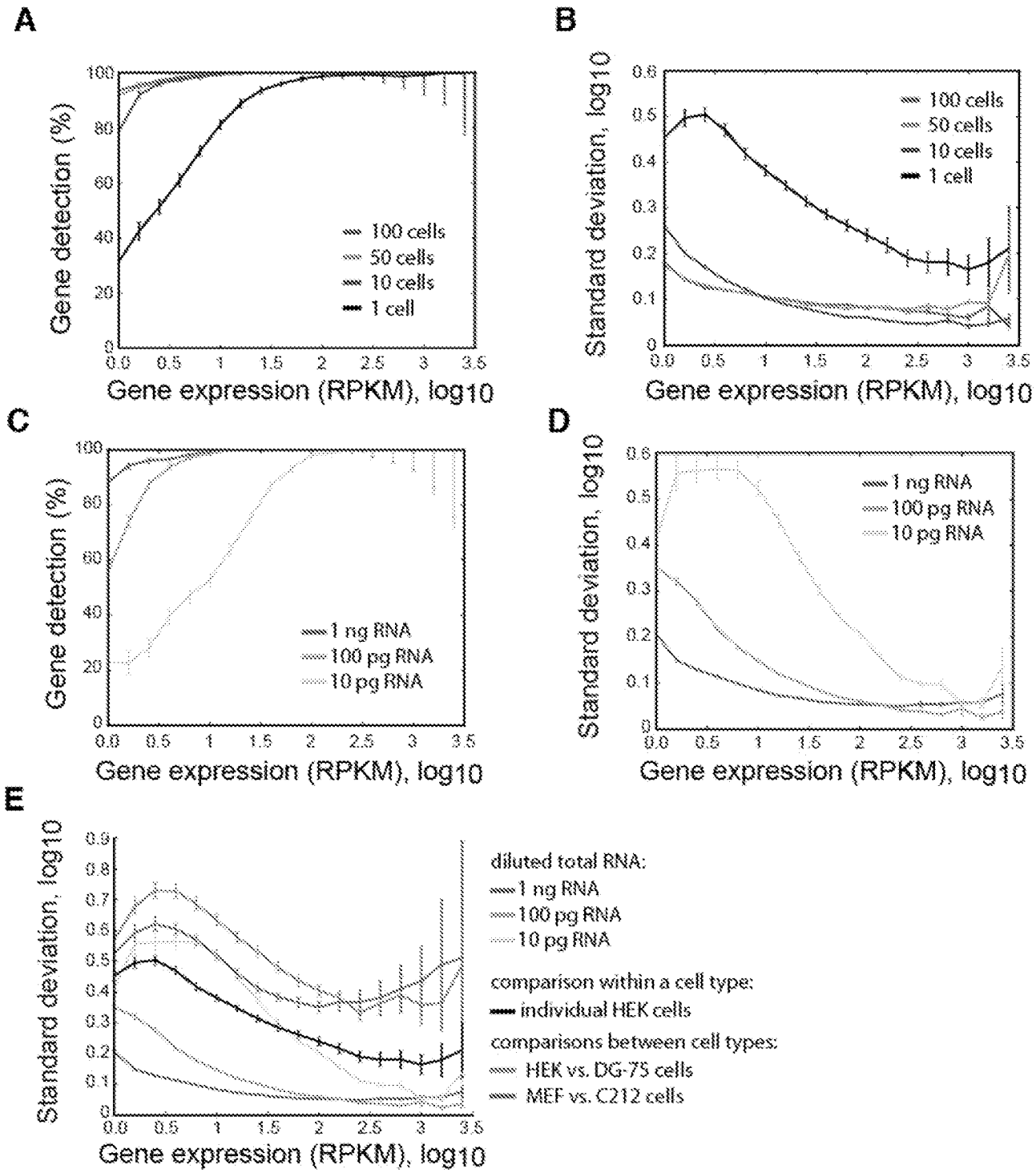
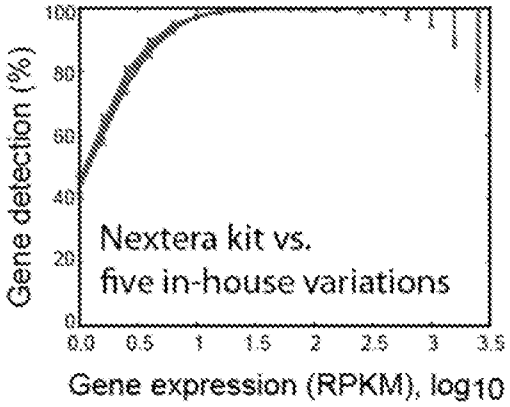
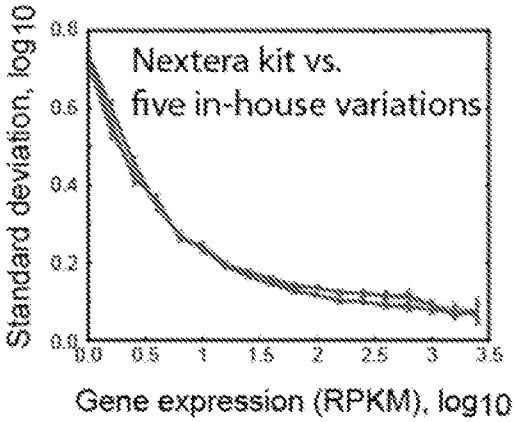

A
Gene detection (%)
100 cells
50 cells
10 cells
1 cell
Gene expression (RPKM), log10
B
Standard deviation, log10
100 cells
50 cells
10 cells
1 cell
Gene expression (RPKM), log10
C
Gene detection (%)
1 ng RNA
100 pg RNA
10 pg RNA
Gene expression (RPKM), log10
D
Standard deviation, log10
1 ng RNA
100 pg RNA
10 pg RNA
Gene expression (RPKM), log10
E
Standard deviation, log10
Gene expression (RPKM), log10
diluted total RNA:
1 ng RNA
100 pg RNA
10 pg RNA
comparison within a cell type:
individual HEK cells
comparisons between cell types:
HEK vs. DG-75 cells
MEF vs. C212 cells

FIGURE 14

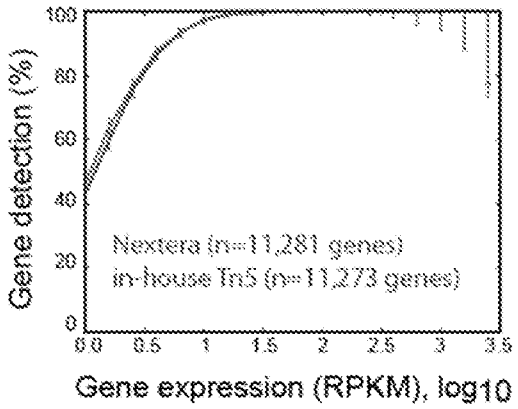
A



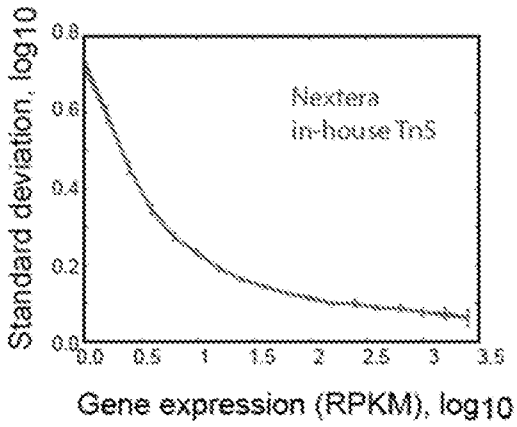
B



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

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 14/52233

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12P 19/34; C07H 21/04 (2014.01)
CPC - C12Q 1/686; C12Q 1/6865; C07H 21/00
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
CPC: C12Q 1/686; C12Q 1/6865; C07H 21/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
CPC: C12Q 1/686; C12Q 1/6865; C07H 21/00 (text search)
USPC: 435/91.2; 435/91.21; 536/23.1 (text search)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Electronic data bases: PatBase; Google Scholar; Google Patent
Search terms: template switch oligonucleotide (TSO), 3' hybridization domain and 5' adapter, locked nucleic acid (LNA), "SMART" template switching, full-length cDNA synthesis, betaine, destabilizing secondary RNA structure,

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ----- Y	US 2012/0010091 A1 (LINNARSON) 12 January 2012 (12.01.2012). Especially para [0023], 0051], [0056], [0057], [0087], [0089], [0124].	1, 6/1, 7/1, 24-29 ---------- 2-5, (6-7)/(2-5)
Y	SPEISS et al. A highly efficient method for long-chain cDNA synthesis using trehalose and betaine. 15 February 2002 vol 301 no 2 Pages 168-174. Especially abstract, pg 169 col 1 para 2.	2-5, (6-7)/(2-5)
A	KAPTEYN et al. Incorporation of non-natural nucleotides into template-switching oligonucleotides reduces background and improves cDNA synthesis from very small RNA samples. BMC Genomics 2 July 2010 Vol 11 No 413 Pages 1-9. Especially abstract.	1, 24

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 November 2014 (18.11.2014)	11 DEC 2014
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 14/52233

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing filed or furnished:

   a. (means)

   ☐ on paper

   ☒ in electronic form

   b. (time)

   ☐ in the international application as filed

   ☐ together with the international application in electronic form

   ☒ subsequently to this Authority for the purposes of search

2. ☒ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 14/52233

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 8-23, 30
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.