Evaluation of ultra-low input RNA sequencing for the study of human T cell transcriptome

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Figure S1

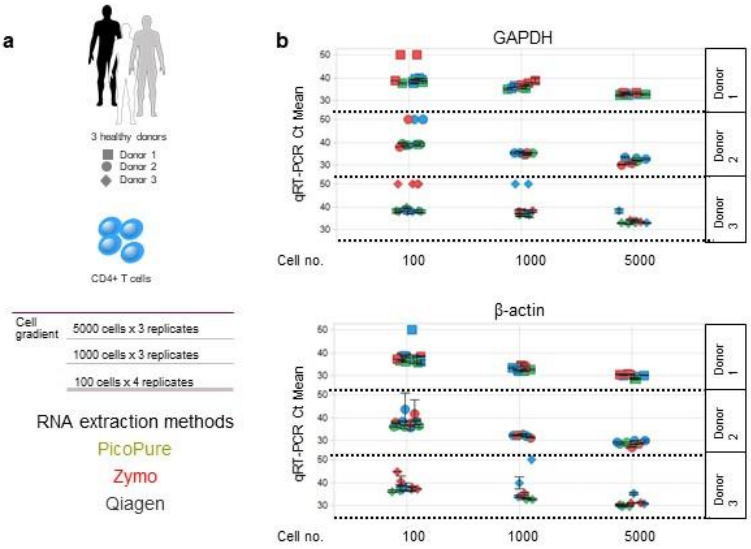

a
3 healthy donors
Donor 1
Donor 2
Donor 3
CD4+ T cells
Cell gradient
5000 cells x 3 replicates
1000 cells x 3 replicates
100 cells x 4 replicates
RNA extraction methods
PicoPure
Zymo
Qiagen
b
GAPDH
qRT-PCR Ct Mean
Donor 1
Donor 2
Donor 3
Cell no.
100
1000
5000
β-actin
qRT-PCR Ct Mean
Donor 1
Donor 2
Donor 3
Cell no.
100
1000
5000

Figure S2

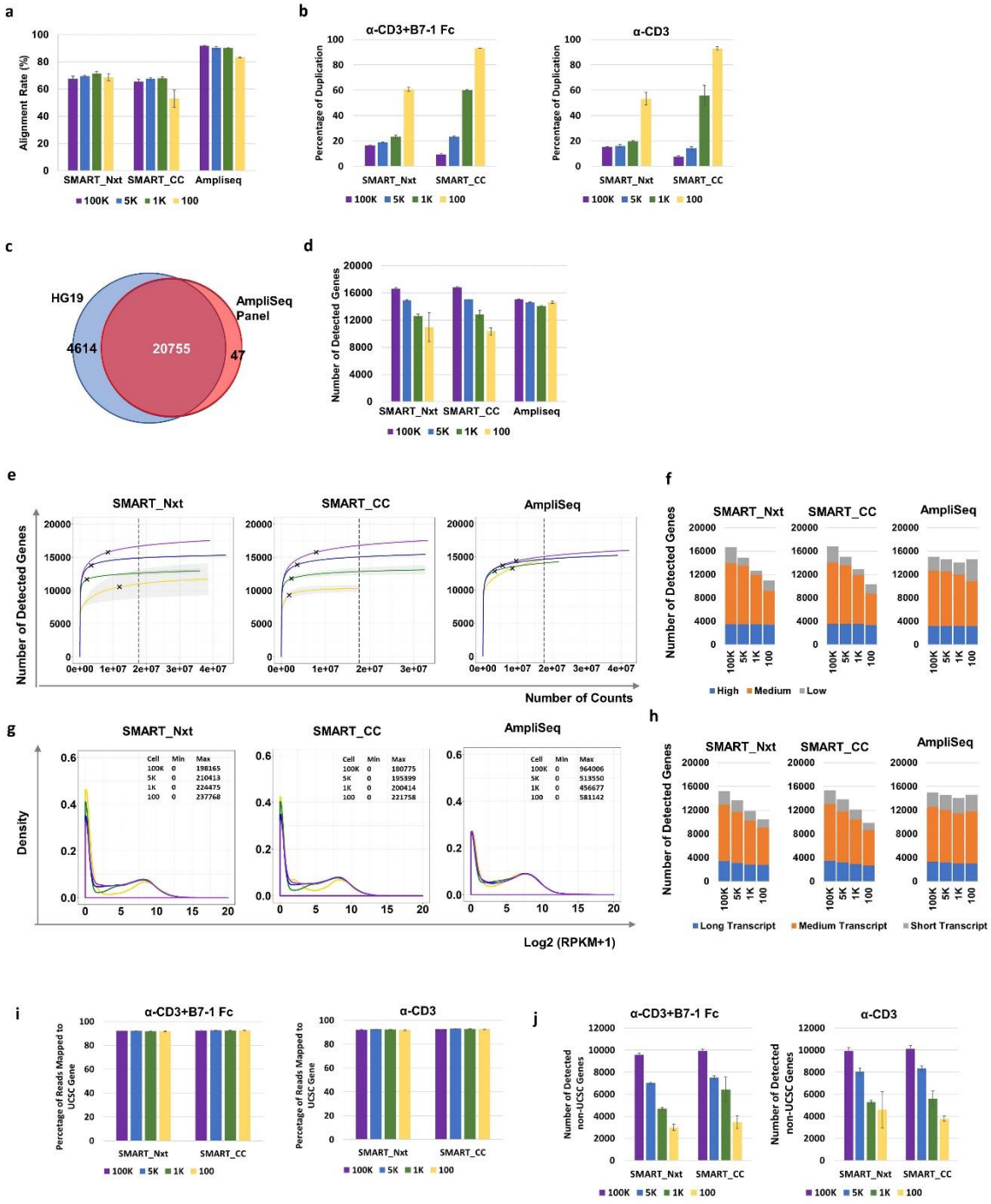

a
Alignment Rate (%)
SMART_Nxt SMART_CC Ampliseq
100K 5K 1K 100
b
α-CD3+B7-1 Fc
α-CD3
Percentage of Duplication
SMART_Nxt SMART_CC
100K 5K 1K 100
c
HG19
AmpliSeq Panel
4614
20755
47
d
Number of Detected Genes
SMART_Nxt SMART_CC Ampliseq
100K 5K 1K 100
e
SMART_Nxt
SMART_CC
AmpliSeq
Number of Detected Genes
Number of Counts
f
SMART_Nxt
SMART_CC
AmpliSeq
Number of Detected Genes
High Medium Low
g
SMART_Nxt
SMART_CC
AmpliSeq
Density
Log2 (RPKM+1)
h
SMART_Nxt
SMART_CC
AmpliSeq
Number of Detected Genes
Long Transcript Medium Transcript Short Transcript
i
α-CD3+B7-1 Fc
α-CD3
Percentage of Reads Mapped to UCSC Gene
SMART_Nxt SMART_CC
100K 5K 1K 100
j
α-CD3+B7-1 Fc
α-CD3
Number of Detected non-UCSC Genes
SMART_Nxt SMART_CC
100K 5K 1K 100

Figure S3

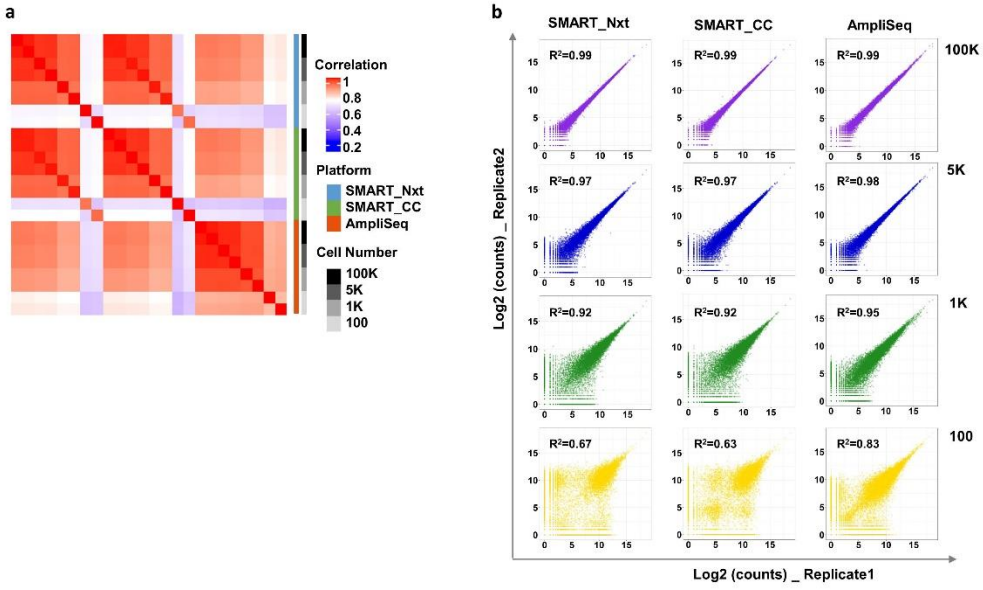

a
Correlation
1
0.8
0.6
0.4
0.2
Platform
SMART_Nxt
SMART_CC
AmpliSeq
Cell Number
100K
5K
1K
100
b
SMART_Nxt
SMART_CC
AmpliSeq
100K
5K
1K
100
R²=0.99
R²=0.99
R²=0.99
R²=0.97
R²=0.97
R²=0.98
R²=0.92
R²=0.92
R²=0.95
R²=0.67
R²=0.63
R²=0.83
Log2 (counts) _ Replicate2
Log2 (counts) _ Replicate1

Figure S4

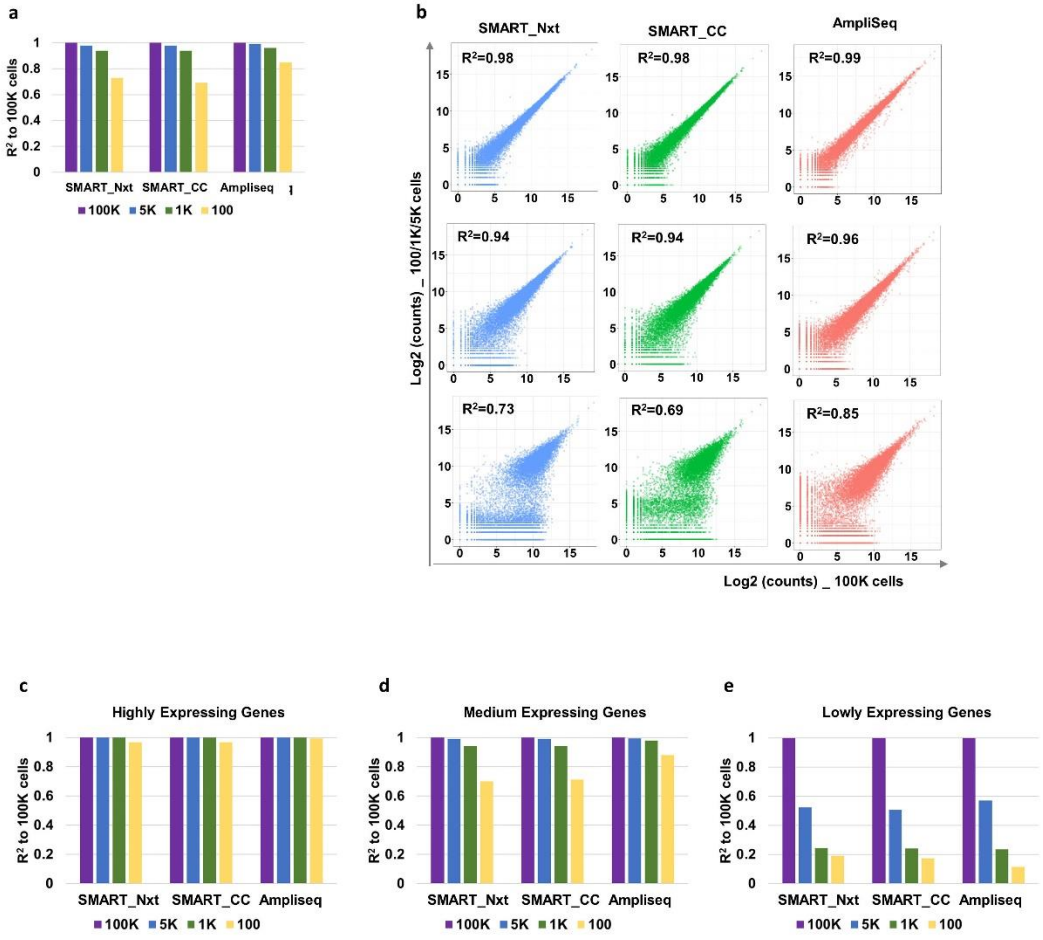
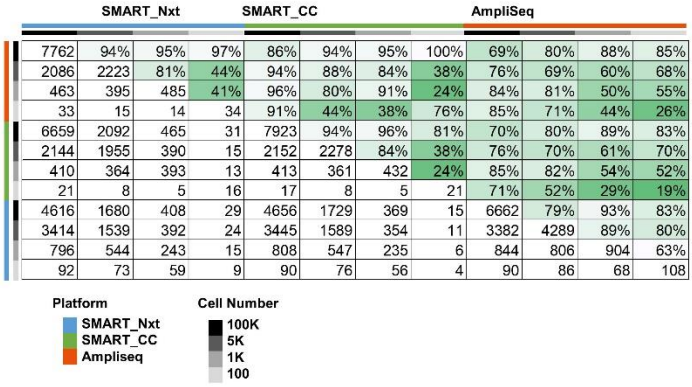

a
R² to 100K cells
SMART_Nxt
SMART_CC
Ampliseq
100K
5K
1K
100
b
SMART_Nxt
SMART_CC
AmpliSeq
R²=0.98
R²=0.98
R²=0.99
R²=0.94
R²=0.94
R²=0.96
R²=0.73
R²=0.69
R²=0.85
Log2 (counts) _ 100/1K/5K cells
Log2 (counts) _ 100K cells
c
Highly Expressing Genes
d
Medium Expressing Genes
e
Lowly Expressing Genes

Figure S5

a


SMART_Nxt
SMART_CC
AmpliSeq
7762 94% 95% 97% 86% 94% 95% 100% 69% 80% 88% 85%
2086 2223 81% 44% 94% 88% 84% 38% 76% 69% 60% 68%
463 395 485 41% 96% 80% 91% 24% 84% 81% 50% 55%
33 15 14 34 91% 44% 38% 76% 85% 71% 44% 26%
6659 2092 465 31 7923 94% 96% 81% 70% 80% 89% 83%
2144 1955 390 15 2152 2278 84% 38% 76% 70% 61% 70%
410 364 393 13 413 361 432 24% 85% 82% 54% 52%
21 8 5 16 17 8 5 21 71% 52% 29% 19%
4616 1680 408 29 4656 1729 369 15 6662 79% 93% 83%
3414 1539 392 24 3445 1589 354 11 3382 4289 89% 80%
796 544 243 15 808 547 235 6 844 806 904 63%
92 73 59 9 90 76 56 4 90 86 68 108
Platform
SMART_Nxt
SMART_CC
Ampliseq
Cell Number
100K
5K
1K
100

b

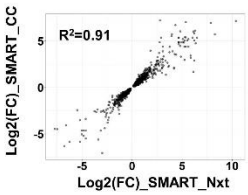

R²=0.91
Log2(FC)_SMART_CC
Log2(FC)_SMART_Nxt
5
0
-5
-5
0
5
10

Figure S6

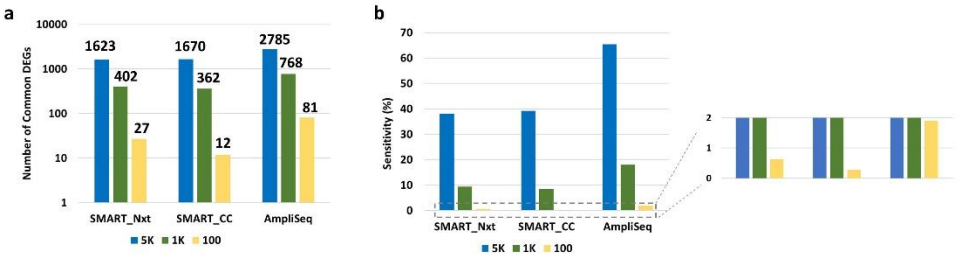

a
Number of Common DEGs
10000
1000
100
10
1
1623
402
27
1670
362
12
2785
768
81
SMART_Nxt
SMART_CC
AmpliSeq
5K
1K
100
b
Sensitivity (%)
70
60
50
40
30
20
10
0
2
1
0
SMART_Nxt
SMART_CC
AmpliSeq
5K
1K
100

Figure Legend

Figure S1. Comparing RNA extraction methods with different cell inputs

(a) RNA extraction protocol comparison. CD4+ T cells were extracted from three healthy donors. Cells gradients of 100 to 5,000 were used with 2-4 replicates each per RNA extraction method. (b) qRT-PCR showed consistent amplification of 1000 and 5000 cell input. At 100 cells input, the ct mean values for Qiagen remained consistent among replicates, across donors for both housekeeping genes, GAPDH and β-actin. Zymo extraction could not detect GAPDH in the majority of samples, while multiple replicates failed to amplify GAPDH with PicoPure extracted RNA.

Figure S2. Number of detected genes decreased with reduced input in SMART technology, while it remained constant for AmpliSeq technology

(a) Alignment rates for samples at the four input cell gradients (100, 1K, 5K, 100K) for the three technologies. Bar plot shows mean +/- standard deviation of the replicates. (b). PCR duplication rates for samples at the four input cell gradients (100, 1K, 5K, 100K) for SMART_Nxt and SMART_CC from samples of α-CD3+B7-1 Fc (left) or α-CD3 (right) treatment. Bar plot shows mean +/- standard deviation of the replicates. (c) Venn diagram showing overlap of genes detected in the AmpliSeq panel and those annotated in UCSC hg19 database. (d) Number of gene detection (count>0) for samples at the four input cell gradients (100, 1K, 5K, 100K) for the three technologies. Bar plot shows the mean mapping percentage +/- standard deviation of the two replicates. (e) Collection curves showing the number of detected genes at different sequencing depths. Solid lines indicate the mean and shading regions indicate standard deviation. Black crossings indicate, for each sample, the sequencing depth where 90% of the

genes were detected. Dashed black lines indicate sampled library size for downstream analysis. (f) Number of detected genes, grouped into high, medium and low expressing genes. (g) Density plot showing the distribution of the log2 transformed RPKM values in each cell input in SMART_Nxt (left), SMART_CC (middle), AmpliSeq (right). Minimum and maximum RPKM values at each cell input were also listed on the upper right of the plot. (h) Number of detected genes, grouped into short, medium and long transcripts. (i) Percentage of the reads that mapped to UCSC genes at the four input cell gradients (100, 1K, 5K, 100K) for SMART_Nxt and SMART_CC from samples of α-CD3+B7-1 Fc (left) or α-CD3 (right) treatment. Bar plot shows mean +/- standard deviation of the two replicates. (j) Number of detected non-UCSC genes at the four input cell gradients (100, 1K, 5K, 100K) for SMART_Nxt and SMART_CC from samples of α-CD3+B7-1 Fc (left) or α-CD3 (right) treatment. Bar plot shows mean +/- standard deviation of the two replicates. For Figure (a, d-h), samples from α-CD3 treatment were used.

Figure S3. **Consistency between technical replicates was high at cell input equal to or above 1K, and there was increased variability at 100 cell input**

(a) Heatmap showing the correlation of log2 transformed count values (Blue indicates low correlation and red indicates high correlation). (b) Scatter plots showing the correlation between the two replicates for each cell gradient in each platform. R^2 indicates coefficient of determination. Samples from α-CD3 treatment were used.

Figure S4. Consistency between different gradients wass high at cell input equal to or above 1K, and the greatest impact was observed on the loss of low expressing genes at 100 cell input

(a) Bar plot showing the correlation between samples from the 5K, 1K and 100 cell input to samples from the 100K cells for each platform. Replicate 1 was used for the calculation. R^2 indicates coefficient of determination. (b) Scatter plot showing the correlation between samples from the 5K (top), 1K (middle), 100 (bottom) cells to samples from the 100K cells for each platform. Replicate 1 was used for the calculation. R2 indicates coefficient of determination. (c) Bar plot showing the correlation between samples from the 5K, 1K and 100 cell gradient to samples from the 100K cells for each platform, splitting into high, (d) medium and (e) low expressing genes. Replicate 1 was used for the calculation. R^2 indicates coefficient of determination. In each figure, samples from α-CD3 treatment were used.

Figure S5. Three different platforms detected common differentially expressed genes; however platform specific detection was also observed

(a) Percentage (upper triangle) and the absolute number (lower triangle) of differentially expressed genes that overlap between each pair of comparisons. The percentages were calculated as the number of overlapped DEGs divided by the number of DEGs from the comparison with a smaller number DEGs. (b). Fold correlation for SMART_CC and SMART_Nxt common DEGs

Figure S6. Number of differentially expressed genes decreased with reduced input

(a) Number of detected DEGs between α-CD3 and α-CD3 + B7-1-Fc at different cell inputs in each protocol using the common DEGs detected by all three protocols at 100K cells as reference (FDR<0.05). The absolute number of detected genes were shown

above each bar in the plot. (b) Sensitivity for detecting common DEGs at low input samples.

Supplemental Table 1-6

Supplemental Table 1: qRT_PCR validation for AmpliSeq specific DEGs
Supplemental Table 2: qRT_PCR validation for SMART_CC and AmpliSeq DEGs
Supplemental Table 3: qRT_PCR validation for SMART_CC specific DEGs
Supplemental Table 4: qRT_PCR validation for SMART_Nxt and AmpliSeq DEGs
Supplemental Table 5: qRT_PCR validation for SMART_Nxt and SMART_CC DEGs
Supplemental Table 6: qRT_PCR validation for SMART_Nxt specific DEGs

Supplemental Table 1

	LogFC_2				
GeneID	Ampliseq	Clontech	Nextera	Type	Fluidgm Fold change
ARHGEF26	-4.451	-0.237	-0.174	ampliseq_only	4.729711104
ARHGEF35	2.778	-0.116	-0.214	ampliseq_only	0.070400418
CACNA2D2	-2.01	-0.319	-0.609	ampliseq_only	0.216487808
CACNA2D4	-2.725	-0.255	-0.345	ampliseq_only	0.403306269
CCL3L3	8.098	0	0	ampliseq_only	3547.935556
CRLF2	2.799	0	0.028	ampliseq_only	1575.137645
ENTPD1	2.176	0.231	0.075	ampliseq_only	1734.013322
FARP1	2.616	-0.102	0.28	ampliseq_only	0.126832463
FCRL3	-2.07	-0.509	-0.652	ampliseq_only	2.53225905
GRK4	5.384	-0.508	-0.547	ampliseq_only	0.975671779
IKBKG	4.02	0.196	0.12	ampliseq_only	0.555741
IL3RA	2.027	0.149	0.657	ampliseq_only	157.587791
KIR3DL2	2.769	0.648	0.566	ampliseq_only	35.34443274
LGALS9C	-3.26	0.024	-0.884	ampliseq_only	0.304111852
MAK	2.316	-0.013	0.464	ampliseq_only	195.6126115
MDP1	-4.833	-0.123	-0.126	ampliseq_only	0.714284957
PPP1R2P3	-4.368	0.005	0.071	ampliseq_only	0.000318083
PTPN14	4.884	0.325	-0.197	ampliseq_only	2416.565431
RGS5	-2.841	-0.234	0.002	ampliseq_only	12.36557016
SPDYA	-4.62	-0.333	-0.337	ampliseq_only	33.51900148
TERT	-4.619	0.366	0.035	ampliseq_only	8.2107227
USP17L5	-2.736	0	0	ampliseq_only	0.018428531

~59% match

Supplemental Table 2

	LogFC_2				
GeneID	**Ampliseq**	**Clontech**	**Nextera**	**Type**	**Fluidgm Fold change**
ABCA6	-2.057	-2.543	0.348	clontech_ampliseq	157.5708718
CCR5	3.558	2.346	0.668	clontech_ampliseq	0.223984855
HIPK4	-2.229	-3.013	-0.365	clontech_ampliseq	0.312898872
LIPH	6.74	2.686	0.673	clontech_ampliseq	2471.645148
SRGAP1	-3.562	-2.298	-0.459	clontech_ampliseq	0.126832463
TEX29	-2.321	-2.183	-0.458	clontech_ampliseq	0.910199088

~66% match

Supplemental Table 3

	LogFC_2				
GeneID	Ampliseq	Clontech	Nextera	Type	Fluidgm Fold change
ARSD	-0.578	-2.412	-0.813	clontech_only	2.041292716
CKAP2L	0.965	-2.686	-0.178	clontech_only	7.534788593
ENPP3	-0.663	2.513	0.899	clontech_only	73.92026442
FAM64A	-0.6	-5.197	-0.784	clontech_only	0.126832463
FTH1P3	0.327	-2.068	-0.084	clontech_only	0.083361652
GALNT8	-0.508	4.87	0.035	clontech_only	0.927793753
IQCA1	0.049	-2.173	-0.951	clontech_only	6.110590983
JAKMIP2	-0.743	-2.364	-0.538	clontech_only	26.64622903
NOSTRIN	-0.857	-3.014	0.033	clontech_only	0.775658733
PHKA1	-0.723	-3.464	-0.949	clontech_only	0.05186038
POTEE	0	3.092	0.033	clontech_only	1.132802248
SEMA5A	-0.448	-2.656	-0.944	clontech_only	8.172515188
SLC45A3	-0.191	-2.061	-0.908	clontech_only	15.82384763
TSPAN1	-0.472	-5.345	-0.658	clontech_only	0.126832463

~50% match

Supplemental Table 4

	LogFC_2				
GeneID	**Ampliseq**	**Clontech**	**Nextera**	**Type**	**Fluidgm Fold change**
ACOT12	-2.671	-0.376	-3.206	nextera_ampliseq	4.126932578
CSF2RA	3.9	0.869	4.875	nextera_ampliseq	20167.20544
GPR156	-2.18	-0.659	-2.394	nextera_ampliseq	255.6361657
MPL	3.079	0.337	2.722	nextera_ampliseq	87.21847306
PLOD2	3.34	0.995	2.264	nextera_ampliseq	707.0014277
PROC	4.669	0.924	3.542	nextera_ampliseq	0.126832463
TUBB2B	-6.632	-0.592	-2.921	nextera_ampliseq	0.015163991

~57% match

Supplemental Table 5

	LogFC_2				
GeneID	**Ampliseq**	**Clontech**	**Nextera**	**Type**	**Fluidgm Fold change**
CCR8	-0.894	-3.764	-2.444	nextera_clontech	0.328639365
CLDN5	0	6.726	4.329	nextera_clontech	59.04443075
DDAH1	0.05	-2.64	-2.729	nextera_clontech	350.542172
G0S2	0.21	3.315	4.875	nextera_clontech	223.0889748
GNG4	1.691	2.48	2.61	nextera_clontech	11.82291923
IGF2BP3	1.424	2.136	1.668	nextera_clontech	168.6752421
LRRC32	0	5.728	8.27	nextera_clontech	627.3610374
MPP3	-0.869	-3.241	-2.333	nextera_clontech	0.700955433
MYLK	0.047	3.345	3.141	nextera_clontech	12595.5048
NPC1L1	-1.639	-2.894	-2.57	nextera_clontech	1975.647765
PDGFRA	0	5.74	3.978	nextera_clontech	1223.588505
SORBS1	0	-2.471	-2.833	nextera_clontech	0.158461889
TBC1D8B	-1.459	-2.841	-1.292	nextera_clontech	24.85857359
TMSB4Y	-0.561	-4.458	-7.717	nextera_clontech	0.029909375
TNFSF14	0	5.407	5.274	nextera_clontech	11204.03815
TNS1	1.017	2.963	5.241	nextera_clontech	0.0463417
ZBTB32	0.042	2.522	2.653	nextera_clontech	1.535469451

~76% match

Supplemental Table 6

	LogFC_2				
GeneID	Ampliseq	Clontech	Nextera	Type	Fluidgm Fold change
CPLX3	-0.447	0.022	-4.375	nextera_only	0.531113727
NDUFA4L2	0.723	0.554	4.877	nextera_only	8.528112234
PHEX	-0.488	0.911	2.932	nextera_only	126.586083
RDH16	0.477	0.307	-2.904	nextera_only	176.3005933
RPS26P11	-0.482	0.355	-5.027	nextera_only	0.095360996
RUSC2	0.675	-0.27	-2.48	nextera_only	3.515482612
SIGLEC9	-0.196	0.478	2.402	nextera_only	2284.503158
SNORA58	0.23	0.715	3.324	nextera_only	0.992248372
YY2	0.103	0.985	2.22	nextera_only	1.936806054
ZBTB8A	-0.356	-0.875	2.357	nextera_only	4.974363037
ZNF221	0.577	0.705	2.234	nextera_only	1.208235059

~72% match